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# SCREENING AND CHARACTERIZATION OF PGPRs COLLECTED FROM RHIZOSPHERE SOILS OF SORGHUM CROP GROWN VERTISOLS

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## ABSTRACT

Different plant growth promoting rhizo bacteria were isolated from sorghum growing vertisols of different districts of Andhra Pradesh during the year 2018-19. Isolations were carried out in microbiology lab at Agricultural Research Station, ANGRAU, Amaravathi. A total of sixty isolates were obtained from 20 different rhizospheric soil samples.The PGP bacteria such as *Azotobacter*, *Azospirillium*, PSB, KRB, ZnSB and PGP isolates were isolated. The isolated PGPRs were screened for their morphological, physiological and biochemical characteristics. All the 60 isolates *i.e*. *Azotobacter*, *Azospirillium*, PSB, KRB, ZnSB and PGP isolate performed differently based on the strain specificity.

**Keywords:** *Azospirillium*, *Azotobacter*, Morphology, PGPR, Physiology, Sorghum

## INTRODUCTION

A diverse array of bacteria, including species of *Rhizobium, Bradyrhizobium, Pseudomonas, Azospirillum, Azotobacter, Bacillus, Klebsiella, Enterobacter, Xanthomonas* and many others, have been shown to facilitate plant growth by various mechanisms. These microorganisms are the potential tools for sustainable agriculture because they not only ensure the availability of essential nutrients to plants but also enhance the nutrient use efficiency. Several authors have reported significant increase in growth and yield of agricultural crops in response to microbial inoculants both under greenhouse and field conditions (Naiman *et al*., 2009).

The use of plant growth promoting rhizobacteria (PGPR), including phosphorus mobilizing bacteria, potassium solubilizing bacteria, zinc solubilizing bacteria as biofertilizers had been suggested as a sustainable alternative to chemical fertilizers (Castro *et al.,* 2003). Moreover, Potassium solubilizing bacteria (KSB) were well known for their capability to solubilize potassium from mica, illite. Therefore, application of K minerals with inoculation of bacteria, that dissolve them, may provide continuous supply of soluble K useful for increasing K uptake and enhanced soil fertility.

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## MATERIALS AND METHODS

### Characterization of different PGPR isolates

During the year 2018-19, isolation of PGPRs was carried out in microbiology lab at Agricultural Research Station, Amaravathi, ANGRAU. The bacterial isolates were identified on the basis of their morphological, physiological and biochemical characteristics according to the standard methods described in Bergey's manual of systematic bacteriology (1993). All isolates were checked for their purity and then studied for the colony morphology and pigmentation. The cell shape and gram reaction were also recorded as per the standard procedures given by Barthalomew and Mittewar (1950). Morphological characteristics of the colony of each isolate was examined on nutrient agar and selective medium and incubated for specific period of time. Cultural morphology of isolates was observed by different characteristics of colonies such as shape, size, elevation, surface, margin, color, etc., were recorded.

### Gram's staining

A drop of sterile distilled water was placed in the center of glass slide. A loopful of inoculum from young culture was taken, mixed with water, and placed in the center of the slide. The suspension was spread out on slide using the inoculation needle to make a thin suspension. The smear was dried in air and fixed through mild heating by passing the slide 3 to 4 times over the flame. The smear was then flooded with crystal violet solution for 1 min and washed gently with flow of tap water. Then the slide was flooded with iodine solution. After incubation at room temperature for 1 min, iodine solution was drained out followed by washing with 95 per cent ethanol. After that, it was washed with water within 15 to 30 seconds and blotted carefully. The smear was incubated with safranin solution for 1 min. The slide was washed gently in flow of tap water and dried in air. The slide was examined under microscope at 100× power with oil immersion and data was recorded.

### Biochemical and physiological characterization of effective Isolates

**Starch hydrolysis:** The ability of the isolates to hydrolyse starch was examined by the procedure of Eckford (1927).

**Hydrogen sulphide test:** Sterilized Hydrogen sulfide- Indole-Motility agar stabs were inoculated along the wall of the tubes with overnight cultures of the isolates and incubated for 48 h at 28 ± 2 ° C. Visualization of black colour along the line of inoculation indicated a positive reaction for the test. (Cowan and Steel, 1970)

**Indole production:** Sterilized SIM agar slants were inoculated with the overnight cultures of the isolates and incubated for 48 h at 28 ± 2 ° C. Following incubation, 10 drops of Kovac's indole reagent were added to each tube. The isolates showing production of red colour were recorded as positive for indole production (Isenberg and Sundheim, 1958).

**Catalase test:** This test was performed to study the presence of catalase enzyme in bacterial colonies. Fresh cultures of pure isolates were taken on glass slides and one drop of $H_2O_2$ (30%) was added. Appearance of gas bubble indicated the presence of catalase enzyme (Rangaswami and Bagyaraj, 1993).

**Oxidase test:** The overnight cultures of the test isolates were spotted on plates poured with sterile trypicase soy agar and the plates were incubated for 24 h at 28 ± 2 °C. After incubation, 2-3 drops of N, N, N', N'-tetramethyl-p-phenylenediamine dihydrochloride (Wurster's reagent) were added onto the surface of each test organism. The isolates showing change of colour to

maroon were observed as oxidase positive. (Cappuccino and Sherman, 1992)

**Gelatine liquification:** The overnight cultures of the test isolates were inoculated to sterilized nutrient gelatin deep tubes and incubated for 24 h at 28 ± 2 °C. Then, the tubes were kept in the refrigerator for 30 minutes at 4 °C. The isolates showing liquefied gelatin were taken as positive and those which resulted in solidification of gelatin on refrigeration were recorded as negative for the test. (Blazevic and Ederer, 1975)

**Methyl red test:** Sterilized glucose-phosphate broth tubes were inoculated with the test culture and incubated at 28 ± 2 °C for 48 h. After incubation five drops of methyl red indicator was added to each tube and gently shaken. Red colour production was taken as positive and yellow colour production was taken as negative for the test. (Seeley *et al.,* 1990).

**Voges Prausker's test:** Sterilized tubes containing MR-VP broth were inoculated with test cultures. The tubes were incubated for 48 hours at 37 °C. After incubation 10 drops of Barritt's reagent A was added and gently shaken followed by addition of ten drops of Barritt's reagent B. The development of rose colour in the broth was taken as positive for the test. (Seeley *et al.,*1990)

**Citrate utilization:** Isolates were streaked on Simmon's citrate agar slants and incubated at 28 ± 2 °C for 24 h. Change in colour from green to blue indicates the positive reaction for citrate utilization (Mac Faddin, 2000).

**Ammonia production:** The isolates were tested for ammonia production by inoculating the isolates in to 10 ml of sterilized peptone water in the test tubes. The tubes were incubated for 48-72 hrs at 36 ± 2 °C. Nessler's reagent (0.5 ml) was added in each tube. Change in color of the medium from brown to yellow colour was taken as positive test for ammonia production.

**Carbohydrate utilization test:** All pure bacterial isolates were screened for the carbohydrate fermentation abilities using different carbohydrates (lactose, sucrose, dextrose) in Peptone broth medium. Bacterial

**Table 1. Coding of PGPR isolates according to their geographical position**

| S.No. | PGPR organisms | No. of isolates | Isolate code |
|---|---|---|---|
| 1 | *Azotobacter* | 18 | KAA-1, KPM-1, KNB-1, KSS-1, AKK-1, ATT-1, AYK-1, AYY-1, CCC-1, CII-1, CTT-1, PGG-1, PMM-1, PVV-1, GAA-1, GBB-1, GPP-1, GRR-1 |
| 2 | *Azospirillium* | 14 | KAA-2, KPM-2, KNB-2, KSS-2, ATT-2, AYY-2, CII-2, CPP-2, CTT-2, PGG-2, PVV-2, GBB-2, GPP-2, GRR-2 |
| 3 | PSB | 12 | KAA-3, KPM-3, KNB-3, KSS-3, AKK-3, CCC-3, CPP-3, CTT-3, PCC-3, GAA-3, GPP-3, GRR-3 |
| 4 | KRB | 6 | KAA-4, KNB-4, AKK-4, CII-4, CTT-4, GAA-4 |
| 5 | ZnSB | 6 | KPM-5, KSS-5, CPP-5, PCC-5, GBB-5, GRR-1 |
| 6 | PGPR Isolate | 4 | KPM-6, KSS-6, CPP-6, GAA-6 |

isolates were inoculated in broth containing specific carbohydrate. The change in colour of Peptone broth was observed for utilization of particular carbohydrate present in broth (MacFaddin, 2000).

## RESULTS AND DISCUSSION

Soil samples were collected from different sorghum grown vertisols of Andhra Pradesh state (Table 8). Rhizospheric soil is rich in PGPR organisms due to secretion of exudates from plant roots. Different PGPR organisms isolated were *Azotobacter*, *Azospirillum*, phosphorus solubilizing bacteria, potassium releasing bacteria, zinc solubilising bacteria and PGPR isolate (*Pseudomonas fluorescence*). Isolated PGPR organisms were purified by streak plate method and the cultures were preserved in slants and stabs for further screening and characterization of the isolates.

### Morphological and cultural characteristics of *Azotobacter* isolates

A total of 18 isolates of *Azotobacter* were screened. All the isolates showed good growth, flat, entire and wrinkled at the edge of the colony. All the isolates were gram –ve and rod shaped by microscopic observation. Sandeep *et al*. (2011) isolated *Azotobacter* sp. and identified based on morphological and biochemical characteristics and carried out all the biochemical tests.

**Table 2. Morphological and cultural characterization of *Azotobacter* isolates**

| S.No. | Isolate name | Gram reaction | Cell shape | Colony morphology |
|---|---|---|---|---|
| 1 | KAA-1 | -ve | Rods | Good growth, gummy, flat, entire, wrinkled at the edge of the colony |
| 2 | KPM-1 | -ve | Rods | Good growth, flat, entire, wrinkled at the edge of the colony |
| 3 | KNB-1 | -ve | Rods | Good growth, flat, entire, wrinkled at the edge of the colony |
| 4 | KSS-1 | -ve | Rods | Good growth, flat, entire, wrinkled at the edge of the colony |
| 5 | AKK-1 | -ve | Rods | Good growth, flat, entire, wrinkled at the edge of the colony |

**Table 3. Morphological and cultural characteristics of *Azospirillum* isolates**

| S.No. | Isolate name | Gram reaction | Cell shape | Colony morphology |
|---|---|---|---|---|
| 1 | KAA-2 | -ve | Vibroid | White dense small, undulate margins |
| 2 | KPM-2 | -ve | Vibroid | Pale yellow, dense small, undulated |
| 3 | KNB-2 | -ve | Vibroid | Pale yellow, dense small, undulated |
| 4 | KSS-2 | -ve | Vibroid | White dense small, undulate margins |
| 5 | ATT-2 | -ve | Vibroid | Pale yellow, dense small, undulated |

**Morphological and cultural characteristics of phosphate solubilizing bacterial isolates**

Out of the 12 isolates, four isolates KAA-3, KNB-3, CCC-3 and CTT-3 were yellowish green, six isolates KPM-3, KSS-3, AKK-3, CPP-3, GAA-3 and GRR-3 were dull white and remaining two isolates were off white. By making similar studies twenty eight bacterial cultures were isolated by Preeti *et al.* (2011) from Korea and four isolates were identified as *Pseudomonas* sp. and others were *Bacillus subtilis*.

**Morphological and cultural characteristics of potassium releasing bacterial isolates**

Out of the six isolates, KAA-4 and GAA-4 were showing white colour colonies, KNB-4 was greyish white in colour and AKK-4, CII-4 and CTT-4 were showing creamy white colonies. KAA-4, KNB-4 and GAA-4 were slimy in nature. Maurya *et al*. (2014) studied characteristics of K solubilizing bacteria from Inceptisol (KI) and Alfisol (KA), respectively. Majority of the isolates were entire smooth margin, raised, translucent, gram +ve rods and whitish to creamy in appearance.

**Table 4. Morphological and cultural characteristics of PSB isolates**

| S.No. | Isolate name | Gram reaction | Cell shape | Colony morphology |
|---|---|---|---|---|
| 1 | KAA-3 | -ve | Rods | Yellowish, green, irregular, spreading, glistening, convex, opaque, viscid colony |
| 2 | KPM-3 | -ve | Rods | Dull white, round, non spreading, glistening, convex, opaque, viscid colony |
| 3 | KNB-3 | -ve | Rods | Yellowish, green, irregular, spreading, glistening, convex, opaque, viscid colony |
| 4 | KSS-3 | -ve | Rods | Dull white, round, non spreading, glistening, convex, opaque, viscid colony |
| 5 | AKK-3 | -ve | Rods | Dull white, irregular, spreading, glistening, convex, opaque, viscid colony |

**Table 4. Morphological and cultural characteristics of PSB isolates**

| S.No. | Isolate name | Gram reaction | Cell shape | Colony morphology |
|---|---|---|---|---|
| 1 | KAA-4 | +ve | Rods | White, smooth, small, slimy spreading |
| 2 | KNB-4 | +ve | Rods | Greyish white, smooth, slimy, large, widely spreading |
| 3 | AKK-4 | +ve | Rods | Creamy white, small, regular, opaque |
| 4 | CII-4 | +ve | Rods | Creamy white, smooth, regular, widely spreading |
| 5 | CTT-4 | +ve | Rods | Creamy white, small, irregular, opaque |
| 6 | GAA-4 | +ve | Rods | White, smooth, slimy, large |

**Morphological and cultural characteristics of zinc solubilizing bacterial isolates**

Out of the six isolates, KPM-5, KSS-5, PCC-5 and GBB-5 were dull white in colour, while CPP-5 and GRR-5 were off white in colour. All the isolates show irregular, smooth, flat, opaque and viscid colonies.

Sharma *et al.* (2014) characterized bacterial isolates with Zn solubilization efficiency for their biochemical studies. Biochemical characterization revealed 19D positive for citrate and nitrate reduction and negative for IAA production.

**Morphological and cultural characteristics of PGPR isolate (*Pseudomonas fluorescence*)**

Out of the four isolates, KPM-6 and GAA-6 were yellowish green in colour while KSS-6 and CPP-6 were dull white in colour. KPM-6 CPP-6 were spreading while KSS-6 and GAA-6 showed non-spreading colonies. KSS-

**Table 6. Morphological and cultural characteristics of ZnSB isolates**

| S.No. | Isolate name | Gram reaction | Cell shape | Colony morphology |
|---|---|---|---|---|
| 1 | KPM-5 | +ve | Rods | Dull white, irregular, non spreading, smooth, flat, opaque, viscid colony |
| 2 | KSS-5 | +ve | Rods | Dull white, irregular, spreading, smooth, flat, opaque, viscid colony |
| 3 | CPP-5 | +ve | Rods | Off white, irregular, spreading, smooth, flat, opaque, viscid colony |
| 4 | PCC-5 | +ve | Rods | Dull white, irregular, spreading, smooth, flat, opaque, viscid colony |
| 5 | GBB-5 | +ve | Rods | Dull white, irregular, spreading, smooth, flat, opaque, viscid colony |
| 6 | GRR-5 | +ve | Rods | Off white, irregular, spreading, smooth, flat, opaque, viscid colony |

**Table 7. Morphological and cultural characterization of PGPR isolates**

| S.No. | Isolate name | Gram reaction | Cell shape | Colony morphology |
|---|---|---|---|---|
| 1 | KPM-6 | -ve | Rods | Yellowish, green, irregular, spreading, glistening, convex, opaque, viscid colony |
| 2 | KSS-6 | -ve | Rods | Dull white, round, non spreading, glistening, convex, opaque, viscid colony |
| 3 | CPP-6 | -ve | Rods | Dull white, irregular, spreading, glistening, convex, opaque, viscid colony |
| 4 | GAA-6 | -ve | Rods | Yellowish, green, irregular, non spreading, glistening, convex, opaque, viscid colony |

**Table 8. Details of soil samples collected from different districts of Andhra Pradesh state**

| S.No. | District | Mandal | Village | Geographical location | | Sample Code | Soil Type | Cropping Pattern |
|---|---|---|---|---|---|---|---|---|
| | | | | Latitude | Longitude | | | |
| 1 | Kurnool | Allagadda | Allagadda | 15°8' 0.96''N | 78° 29'51''E | KAA | Black soil | Rice -Sorghum |
| 2 | Kurnool | Panyam | Madduru | 15° 24' 59.719" N | 78° 23' 18.977" E | KPM | Black soil | Rice - Sorghum |
| 3 | Kurnool | Nandyal | Brahmanapalle | 15°25' 4.485" N | 78° 23'23.921" E | KNB | Black soil | Rice - Sorghum |
| 4 | Kurnool | Sirivella | Sirivella | 15°50' 4.2216 N | 78° 3' 0.7272" E | KSS | Black soil | Rice - Sorghum |
| 5 | Anantapuramu | Kalyandurg | Kalyandurg | 14°33' 7.92" N | 77°6' 39.6" E | AKK | Black soil | Sorghum-Sorghum |
| 6 | Anantapuramu | Tadipatri | Tadipatri | 14°31' 31.8" N | 77°46' 21.756" E | ATT | Black soil | Sorghum-Sorghum |
| 7 | Anantapuramu | Yadiki | Kamalapur | 15°2' 57.876" N | 77°52' 23.016" E | AYK | Black soil | Sorghum-Sorghum |
| 8 | Anantapuramu | Yadiki | Yadiki | 13°48' 43.632" N | 77°30' 50.652" E | AYY | Black soil | Sorghum-Sorghum |
| 9 | Chittoor | Chandragiri | Chandragiri | 13°35' 13.848" N | 79°18' 56.448" E | CCC | Black soil | Rice -Sorghum |
| 10 | Chittoor | Pileru | Pileru | 13°35' 47.256" N | 78°59' 10.788" E | CII | Black soil | Sorghum-Sorghum |
| 11 | Chittoor | Punganur | Punganur | 13°21' 52.668" N | 78°34' 26.22" E | CPP | Black soil | Sorghum-Sorghum |
| 12 | Chittoor | Tirupati | Tirupati | 13°37' 43.428" N | 79°25' 8.652" E | CTT | Black soil | Sorghum-Sorghum |
| 13 | Prakasam | Chirala | Chirala | 15°49' 48.72" N | 80°21' 13.68" E | PCC | Black soil | Sorghum-Sorghum |
| 14 | Prakasam | Giddalur | Giddalur | 15°22' 56.136" N | 78°55' 23.052" E | PGG | Black soil | Sorghum-Sorghum |
| 15 | Prakasam | Markapuram | Markapuram | 15°2' 15.936" N | 78°59' 10.5072" E | PMM | Black soil | Sorghum-Sorghum |
| 16 | Prakasam | Vetapalem | Vetapalem | 15°46' 44.328" N | 80°18' 19.98" E | PVV | Black soil | Sorghum-Sorghum |
| 17 | Guntur | Amaravathi | Amaravathi | 16°22' 9.372" N | 80°26' 1.86" E | GAA | Black soil | Sorghum-Sorghum |
| 18 | Guntur | Bapatla | Bapatla | 15°54' 29.888" N | 80°28' 7.32" E | GBB | Black soil | Rice -Sorghum |
| 19 | Guntur | Ponnuru | Ponnuru | 16°3' 59.4" N | 80°33' 5.04" E | GPP | Black soil | Rice -Sorghum |
| 20 | Guntur | Repalle | Repalle | 16°1' 8.04" N | 80°49' 42.96" E | GRR | Black soil | Rice -Sorghum |

**Table 9. Biochemical and physiological characterization of *Azotobacter* isolates collected from different soil samples**

| S.No. | Biochemical and Physiological characterization | *Azotobacter* isolates | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 |
| | | KA A1 | KP M1 | KN B1 | KS S1 | AK K1 | AT T1 | AY K 1 | AY Y1 | C C C1 | CI I 1 | CT T1 | PG G1 | PM M1 | PV V1 | GA A1 | GB B1 | GP P1 | GR R1 |
| 1 | Starch hydrolysis | + | + | + | + | + | + | - | - | + | + | + | + | + | - | + | + | + | + |
| 2 | Hydrogen Sulphide test | + | - | - | + | - | + | - | - | + | - | + | - | + | + | + | + | - | + |
| 3 | Indole Production | + | + | - | + | + | + | + | - | + | + | + | - | + | + | - | + | + | - |
| 4 | Catalase test | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 5 | Oxidase test | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 6 | Gelatin liquefaction | + | - | - | - | - | - | + | - | - | - | + | - | - | - | + | - | - | - |
| 7 | Methyl red test | + | - | + | - | + | - | + | + | + | + | + | + | + | + | + | + | + | + |
| 8 | Voges Prauskers test | + | - | - | + | - | - | - | + | + | - | - | - | + | - | + | - | - | - |
| 9 | Citrate Utilization | + | + | - | - | - | + | - | + | - | - | + | + | - | + | + | - | + | + |
| 10 | Ammonia Production | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 11 | Carbohydrate Utilization a)Dextrose | + | + | + | + | + | - | + | + | + | - | + | - | + | + | + | + | + | - |
| | b) Lactose | - | - | - | - | - | - | - | - | - | - | + | + | - | - | - | + | - | - |
| | c) Sucrose | + | - | - | + | - | - | - | + | + | - | - | - | + | - | + | - | - | - |

**Table 10. Biochemical and physiological characterization of *Azospirillium* isolates collected from different soil samples**

| S.No. | Biochemical and Physiological characterization | *Azospirillium* Isolates | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
| | | KA A2 | KP M2 | KN B2 | KS S2 | AT T2 | AY Y2 | CI I2 | CP P2 | CT T2 | PG G2 | PV V2 | GB B2 | GP P2 | GR R2 |
| 1 | Starch hydrolysis | + | - | + | - | - | - | + | - | + | - | + | + | - | + |
| 2 | Hydrogen Sulphide test | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | Indole Production | + | - | - | - | + | - | - | - | + | - | - | - | - | - |
| 4 | Catalase test | + | + | + | + | + | - | + | + | + | - | + | + | - | + |
| 5 | Oxidase test | + | + | + | + | + | + | - | + | - | + | + | - | + | - |
| 6 | Gelatin liquefaction | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 7 | Methyl red test | + | - | + | + | - | - | - | + | + | + | + | + | - | - |
| 8 | Voges Prauskers test | + | - | - | - | + | + | - | - | - | - | - | - | - | - |
| 9 | Citrate Utilization | + | + | + | + | + | - | + | - | + | - | + | + | + | + |
| 10 | Ammonia Production | + | + | + | - | + | + | + | + | - | - | - | + | + | + |
| 11 | Carbohydrate Utilization a)Dextrose | + | + | + | + | - | - | - | + | + | + | + | - | - | - |
| | b) Lactose | + | + | + | + | + | + | + | - | - | - | - | + | + | + |
| | c) Sucrose | + | + | - | - | - | - | + | - | + | - | - | - | - | - |

**Table 11. Biochemical and physiological characterization of the PSB and PGPR isolates collected from different soil samples**

| S.No. | Biochemical and Physiological characterization | PSB Isolates | | | | | | | | | | | | PGPR Isolates | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 1 | 2 | 3 | 4 |
| | | KA A3 | KP M3 | KN B3 | KS S3 | AK K3 | CC C3 | CP P3 | CT T3 | PC C3 | GA A3 | GP P3 | GR R3 | KP M6 | KS S6 | CP P6 | GA A6 |
| 1 | Starch hydrolysis | + | + | - | + | + | - | + | + | + | + | - | - | - | - | + | - |
| 2 | Hydrogen Sulphide test | + | + | - | + | + | - | + | + | + | + | - | - | - | - | - | - |
| 3 | Indole Production | + | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| 4 | Catalase test | + | + | - | + | - | + | - | + | + | + | + | - | + | + | + | + |
| 5 | Oxidase test | + | + | - | + | - | - | - | + | + | + | - | - | + | + | + | + |
| 6 | Gelatin Liquefaction | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | - |
| 7 | Methyl red test | + | + | - | - | - | + | + | - | - | - | - | - | + | - | - | + |
| 8 | Voges Prauskers test | + | + | - | - | - | + | - | - | + | - | - | + | - | - | - | - |
| 9 | Citrate Utilization | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + | + |
| 10 | Ammonia Production | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 11 | Carbohydrate Utilization a)Dextrose | + | - | - | + | - | - | + | + | + | - | - | - | + | - | + | - |
| | b) Lactose | + | - | - | - | + | - | + | + | + | + | - | - | - | - | + | - |
| | c) Sucrose | + | - | - | - | + | + | - | - | + | - | - | - | + | + | + | - |

**Table 12. Biochemical and physiological characterization of KRB and ZnSB isolates collected from different soil samples**

| S.No. | Biochemical and P | KRB Isolates | | | | | | ZnSB Isolates | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | hysiological | 1 | 2 | 3 | 4 | 5 | 6 | 1 | 2 | 3 | 4 | 5 | 6 |
| | characterization | KAA | KNB | AKK | CII | CTT | GAA | KPM | KSS | CPP | PCC | GBB | GRR |
| | | 4 | 4 | 4 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 5 |
| 1 | Starch hydrolysis | + | + | - | + | + | - | + | + | + | + | - | + |
| 2 | Hydrogen Sulphide test | - | - | - | - | - | - | - | - | - | - | - | - |
| 3 | Indole Production | + | + | - | + | - | + | + | + | + | - | - | - |
| 4 | Catalase test | + | + | + | + | + | - | + | + | + | + | + | + |
| 5 | Oxidase test | + | + | + | - | + | + | + | + | + | - | - | + |
| 6 | Gelatin Liquefaction | + | + | + | - | + | - | + | + | - | + | - | + |
| 7 | Methyl red test | + | + | - | - | - | + | + | + | - | + | - | + |
| 8 | Voges Prauskers test | - | - | - | - | - | - | - | + | - | - | - | - |
| 9 | Citrate Utilization | + | + | + | + | - | + | + | + | + | + | - | - |
| 10 | Ammonia Production | - | - | - | - | - | - | + | - | - | + | - | - |
| 11 | Carbohydrate Utilization a)Dextrose | + | + | + | + | + | - | + | + | + | + | + | - |
| | b) Lactose | + | - | + | + | - | - | + | + | - | - | - | - |
| | c) Sucrose | + | + | - | + | - | + | + | + | - | - | - | - |

6 isolate showed round colonies while other 5 isolates showed irregular colonies (Table 7).The isolated bacteria observed under UV light showed yellowish green and majority of isolates had light green fluorescence. Exhibition of fluorescence was the peculiar character of fluorescent *Pseudomonas* hence the isolates were confirmed.

Similar results were observed with Basha *et al.* (2014) isolated fifty *Pseudomonas fluorescens* strains from rhizospheric soil and root nodules of pigeonpea, biochemically characterized and identified as *Pseudomonas fluorescens*.

**Biochemical and physiological characterization of *Azotobacter* and *Azospirillum* isolates**

The characteristics of isolates of *Azotobacter* and *Azospirillum* were represented in the Tables 9 and 10. Sakthivel and Karthikeyan (2012) obtained 30 bacterial isolates from *C. forskohlii* rhizospheric soil. The isolates were identified as *Azospirillum* sp., *Bacillus* spp., *Pseudomonas* spp., and *Azotobacter* spp. These bacterial strains were tested for morphological, biochemical characteristics and screened for their direct growth promoting activities (IAA production, production of Ammonia and Phosphate solubilization) and indirect growth promoting activities (HCN production and Siderophore production).

**Biochemical and physiological characterization of phosphate solubilizing bacterial isolates**

The characteristics of isolates of PSB were represented in Table 11. Shahab and Ahmed (2008) tested 10 rhizospheric bacteria (PSB) for their utilization of carbon sources such as Glucose, Fructose, Sucrose and Lactose. Glucose was the most favorable carbon source for solubilization while lactose is the least favorable carbon source.

**Biochemical and physiological characterization of potassium releasing bacterial isolates**

The characteristics of isolates of KRB were represented in Table 12. Maurya *et al.* (2014) studied characteristics of the nine and four isolates of K solubilizing bacteria from Inceptisol (KI) and Alfisol (KA), respectively. Majority of the isolates were entire smooth margin, raised, translucent, gram +ve rods and whitish to creamy in appearance. Isolate KI16 and KA59 produced high slime.

**Biochemical and physiological characterization of other PGPR organisms (*Pseudomonas fluorescence*)**

The characteristics of *Pseudomonas fluorescence* isolates of were represented in Table 11. Maleki *et al*. (2010) isolated 144 bacteria from cucumber rhizosphere, out of them eight isolates were identified as *Pseudomonas fluorescens*. Among them, CV-6 strain showed positive reactions for catalase, protease and phosphatase.

**CONCLUSIONS**

All the sixty isolates *i.e*., *Azotobacter*, *Azospirillium*, PSB, KRB, ZnSB and PGP isolates were tested for their morphological, physiological and biochemical characterization. All the isolates performed differently based on their strain specificity for the different tests performed. Out of them, *Azotobacter*, *Azospirillium* and few PSB isolates were gram negative, while the remaining KRB and ZnSB isolates were gram positive. Cyst formation is the major characteristic feature of *Azotobacter* isolates, while pellicle formation is the characteristic feature for *Azospirillium* isolates, nutrient solubilization is the characteristic

feature of PSB and KRB isolates and finally antagonistic activity is the specific character for PGP isolates. Gelatin liquefaction and citrate utilization was the characteristic feature of PSB, KRB and PGPR isolates. Hence, almost 99% of PSB, KRB and PGPR isolates reacted positively to the above activity.

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# EVALUATION OF HERMETIC STORAGE BAGS FOR THE MANAGEMENT OF PULSE BEETLE, *Callosobruchus maculatus* (FAB.) IN CHICKPEA

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## ABSTRACT

Studies on management of pulse bruchid, *Callosobruchus maculatus* (Fab.) on stored chickpea (*Cicer arietinum* L.) through hermetic storage was carried out in the laboratory of Department of Entomology, Agricultural College, Bapatla during 2021-22.Three hermetic storage bags *viz.*, super bag, polythene bag and triple layer plastic bag were evaluated along with conventional storage bags against pulse beetle damage and seed quality parameters of stored chickpea for six months storage period. Chickpea grains stored in super bags recorded zero number of adult beetles of *C. maculatus*, zero grain damage, no moisture change, and above 90 percent viability of chickpea grains upto six months storage period followed by triple layer plastic bags and found to be the most effective when compared to other types of bags tested.

**Keywords:** *Callosobruchus maculatus*, Chickpea, Hermetic storage, Pulse beetle, Super bag

## INTRODUCTION

Pulse beetle, *Callosobruchus maculatus* (Fab.) is a worldwide insect pest that can cause significant losses in stored pulses, even up to cent percent, rendering the grain unfit for consumption or seed within 4-6 months (Jha *et al.*, 2015). Chickpea (*Cicer arietinum* L.) is widely grown in *rabi* season under dry and rainfed areas of India and play an important role in the nutritional security of millions of people as it constitutes a significant portion of the diet with enriched protein and minerals (Mohanapure *et al.*, 2021). India is the world's largest producer of chickpea, accounting for roughly 70 and 67 percent of total area and production, respectively (Dixit *et al.*, 2017). The considerable damage and economic losses by bruchids are realized mostly during storage of chickpea and other pulses where bruchids complete a major part of their life cycle (Revanasidda *et al.*, 2021) and multiply rapidly in favourable environmental conditions, like optimal temperature and high humidity.

Hermetic storageis a technique in which the air is prevented from entering or leaving from an air tight storage bag, where the respiratory metabolism of organisms and grain itself lowers the oxygen content and raise the carbondioxide content of the inter-granular atmosphere to a level where aerobic

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respiration is no longer possible for the insect growth. The conditions inside the tightly sealed bag control the growth and multiplication of insects and fungi, hence no need of any fumigation (Prasanthi *et al*., 2017). It is also one of the alternative methods to chemical control. Hence, different hermetic storage bags were evaluated against the pulse beetle on stored chickpea.

## MATERIALS AND METHODS

The experiment was carried out in the laboratory of Department of Entomology, Agricultural College, Bapatla, Andhra Pradesh during 2021-22. Different hermetic storage bags were evaluated for the management of *C.maculatus* in chickpea along with conventional storage bags as detailed below (Figure 3).

**Super bag:** The bag was made of polyethylene and thickness was about 80 ì, supplied by Grainpro Inc., Hyderabad, Telangana, India.

**Polythene bag:** The bag was made of polyethylene and thickness was 100 ì (400 Gauge), purchased from local market, Vijayawada, Andhra Pradesh, India.

**Triple layer plastic bag:**This bag consists of three layers (as per the technical specifications of the Purdue Improved Crop Storage (PICS) bags developed by Purdue University, USA); inner and middle layers were made up of 80 ì thickness high density polyethylene (HDPE) material and do not allow diffusion of gases (Oxygen and Carbon dioxide) while the outermost layer was a normal woven sac made up of polypropylene that provides strength for handling. It was supplied by ICRISAT, Hyderabad, Telangana, India.

Nylon bag, Cloth bag, Thick netted jute bag, Thin netted jute bags were purchased from local market, Bapatla, Andhra Pradesh, India. All the types of bags were taken in 22 cm × 15 cm size (500 g capacity) and filled with 500 g of disinfested chickpea grains of JG-11 variety and newly emerged beetles (one day old) were released into the bags @ 10 pairs per bag. Then, the bags were closed air tight by tying/ stitching with a wire. The treatments were made in six sets and replicated thrice to know the influence of hermetic storage at monthly interval upto six months. The first set was opened after one month likewise second, third, fourth, fifth and sixth sets opened after 2, 3, 4, 5 and 6 months, respectively, to record the data.

### Observations recorded

**Adult Emergence:** The emerged beetles were anesthetized with the help of ethyl acetate and counted from each replication.

**Grain Damage (by count):** A representative sample of 50 g from each treatment in three replications was separated after opening of bags at every month interval and counted for calculating percent grain damage.

**Moisture Percentage:** It was measured by using electronic moisture balance (MOC-120H, Shimadzu Corporation, Japan) which determines the moisture content of samples by heating under infrared illumination and measures changes in mass due to evaporation (drying loss method). Ten grams sample of treated grains were taken in the pan and allowed them to illuminate with infrared rays at 120 °C. The moisture percentage was displayed after 30-35 min.

**Viability Percentage:** Fifty seeds from each treatment of un-infested grains were separately tested for viability by tetrazolium test (0.5% tetrazolium solution). The number of seeds turned red colour (embryonal axis region) were recorded as viable seeds and converted to percentage.

### Statistical Analysis

The recorded data was subjected to suitable transformations and then subjected to

**Table 1. Adult emergence of pulse beetle, *C. maculatus* from chickpea grains stored in different types of bags and grain damage**

| S. No. | Storage bag | No. of adults emerged[#] | | | | | | Grain damage (by count) (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 30 DAS | 60 DAS | 90 DAS | 120 DAS | 150 DAS | 180 DAS | 30 DAS[*] | 60 DAS[$] | 90 DAS[$] | 120 DAS[$] | 150 DAS[$] | 180 DAS[$] |
| 1. | Super bag | 0.0 (0.0)$^{d}$ | 0.0 (0.0)$^{e}$ | 0.0 (0.0)$^{c}$ | 0.0 (0.0)$^{d}$ | 0.0 (0.0)$^{c}$ | 0.0 (0.0)$^{e}$ | 0.0 (1.0)$^{d}$ | 0.0 (0.40)$^{d}$ | 0.0 (0.40)$^{b}$ | 0.0 (0.40)$^{b}$ | 0.0 (0.40)$^{b}$ | 0.0 (0.40)$^{b}$ |
| 2. | Polythene bag | 1.33 (0.36)$^{c}$ | 3.0 (0.59)$^{c}$ | 3.67 (0.65)$^{b}$ | 4.0 (0.69)$^{c}$ | 4.33 (0.72)$^{b}$ | 5.33 (0.79)$^{c}$ | 0.0 (1.00)$^{d}$ | 0.16 (1.60)$^{d}$ | 0.16 (1.60)$^{b}$ | 0.16 (1.60)$^{b}$ | 0.16 (1.60)$^{b}$ | 0.33 (2.16)$^{b}$ |
| 3. | Triple layer plastic bag | 1.33 (0.36)$^{c}$ | 2.0 (0.46)$^{d}$ | 3.33 (0.62)$^{b}$ | 3.33 (0.63)$^{c}$ | 3.67 (0.65)$^{b}$ | 4.00 (0.69)$^{d}$ | 0.0 (1.00)$^{d}$ | 0.16 (1.60)$^{d}$ | 0.16 (1.60)$^{b}$ | 0.16 (1.60)$^{b}$ | 0.16 (1.60)$^{b}$ | 0.16 (1.60)$^{b}$ |
| 4. | Nylon bag | 243.0 (2.39)$^{a}$ | 1075.67 (3.03)$^{a}$ | 2114.67 (3.33)$^{a}$ | 2198.33 (3.34)$^{a}$ | 2077.67 (3.32)$^{a}$ | 2046.67 (3.31)$^{a}$ | 6.50 (2.74)$^{b}$ | 92.03 (73.64)$^{b}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ |
| 5. | Cloth bag | 247.67 (2.40)$^{a}$ | 1336.33 (3.13)$^{a}$ | 2105.0 (3.32)$^{a}$ | 2109.33 (3.32)$^{a}$ | 2071.0 (3.32)$^{a}$ | 2012.0 (3.30)$^{a}$ | 7.32 (2.86)$^{b}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ |
| 6. | Thick netted jute bag | 77.0 (1.89)$^{b}$ | 141.33 (2.15)$^{b}$ | 1473.0 (3.17)$^{a}$ | 1491.33 (3.17)$^{b}$ | 1570.67 (3.20)$^{a}$ | 1574.00 (3.20)$^{b}$ | 4.39 (2.32)$^{c}$ | 49.11 (44.47)$^{c}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ |
| 7. | Thin netted jute bag | 264.67 (2.42)$^{a}$ | 997.0 (3.0)$^{a}$ | 2085.67 (3.32)$^{a}$ | 2082.67 (3.32)$^{a}$ | 2025.33 (3.31)$^{a}$ | 2042.67 (3.31)$^{a}$ | 11.71 (3.56)$^{a}$ | 89.59 (71.17)$^{b}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ | 100.0 (89.56)$^{a}$ |
| **SEm(±)** | | **0.037** | **0.043** | **0.048** | **0.023** | **0.043** | **0.030** | **0.102** | **0.881** | **0.641** | **0.641** | **0.641** | **0.803** |
| **CD (5% LOS)** | | **0.113** | **0.132** | **0.144** | **0.069** | **0.131** | **0.090** | **0.308** | **2.672** | **1.945** | **1.945** | **1.945** | **2.436** |

Values in the parenthesis are [#] log, * square root and $ angular transformed values; DAS- Days After Storage; The values in each column with similar alphabet do not vary significantly at p=0.05; LOS- Level of Significance.

**Table 2. Grain moisture content and viability of chickpea grains stored in different types of bags**

| S. No. | Storage bag | Moisture content (%) (Mean ±SE) | | | | | | Viability (%) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 30 DAS | 60 DAS | 90 DAS | 120 DAS | 150 DAS | 180 DAS | 30 DAS* | 60 DAS$ | 90 DAS$ | 120 DAS $ | 150 DAS $ | 180 DAS $ |
| 1. | Super bag | 7.77 ±0.01 e | 7.78 ±0.02 f | 7.78 ±0.05 e | 7.78 ±0.02 e | 7.78 ± 0.01 e | 7.78 ± 0.02 e | 99.33 (10.02)a | 99.33 (86.99)a | 97.33 (80.70)a | 97.33 (80.70)a | 92.00 (73.62)a | 92.00 (73.62)a |
| 2. | Polythene bag | 7.95 ±0.03 e | 7.97 ±0.01 e | 7.97 ±0.01 e | 7.96 ±0.02 e | 7.96 ± 0.01 e | 7.96 ± 0.02 e | 98.67 (9.98)a | 96.00 (78.69)b | 96.67 (79.57)a | 90.00 (71.59)b | 88.00 (69.75)b | 82.67 (65.43)b |
| 3. | Triple layer plastic bag | 7.95 ±0.05 e | 7.93 ±0.03 e | 7.96 ±0.03 e | 7.96 ±0.03 e | 7.96 ± 0.03 e | 7.97 ± 0.01 e | 98.67 (9.98)a | 96.67 (79.57)b | 96.00 (78.69)a | 93.33 (75.25)b | 89.33 (71.03)ab | 86.00 (68.03)b |
| 4. | Nylon bag | 10.52 ±0.19 b | 13.84 ±0.09 a | 19.48 ±0.51 a | 25.61 ±0.25 a | 27.01 ±0.06 a | 28.57 ±0.26 a | 89.33 (9.50)c | 51.33 (45.75)d | 48.00 (43.83)c | 0.00 (0.40)e | 0.00 (0.40)e | 0.00 (0.40)d |
| 5. | Cloth bag | 10.92 ±0.03 a | 13.66 ±0.04 b | 16.70 ±0.09 b | 18.30 ±0.16 b | 18.73 ±0.13 b | 18.85 ±0.05 b | 91.33 (9.61)bc | 48.67 (44.22)d | 46.67 (43.07)c | 39.33 (38.82)d | 0.00 (0.40)e | 0.00 (0.40)d |
| 6. | Thick netted jute bag | 8.50 ±0.17 d | 9.14 ±0.07 d | 13.45 ±0.13 d | 14.31 ±0.13 d | 15.02 ±0.02 d | 15.32 ±0.13 d | 94.00 (9.75)b | 80.67 (63.97)c | 68.67 (55.99)b | 66.67 (54.75)c | 47.33 (43.45)c | 16.67 (24.03)c |
| 7. | Thin netted jute bag | 9.88 ±0.10 c | 12.05 ±0.03 c | 15.21 ±0.07 c | 17.43 ±0.22 c | 17.27 ±0.13 c | 17.32 ±0.14 c | 90.00 (9.54)bc | 54.67 (47.66)d | 51.33 (45.75)c | 44.67 (41.92)d | 16.00 (23.54)d | 0.00 (0.40)d |
| **SEm(±)** | | **0.108** | **0.050** | **0.203** | **0.147** | **0.075** | **0.123** | **0.074** | **1.599** | **1.347** | **1.299** | **1.001** | **0.931** |
| **CD (5% LOS)** | | **0.327** | **0.152** | **0.617** | **0.446** | **0.228** | **0.374** | **0.224** | **4.850** | **4.087** | **3.940** | **3.037** | **2.823** |

Values in the parenthesis are * square root and $ angular transformed values; DAS- Days After Storage; SE- Standard error; The values in each column with similar alphabet do not vary significantly at p=0.05; LOS- Level of Significance

ANOVA in a completely Randomized Design (CRD).

## RESULTS AND DISCUSSION

### Adult emergence and grain damage

Chickpea grains stored in super bags gave complete protection against *C. maculatus* where in the number of beetles of *C. maculatus* and the grain damage upto six months storage period was nil. However, triple layer plastic bags and polythene bags have recorded very few counts of adults (1.33 to 4.0 and 1.33 to 5.33, respectively), less percent grain damage by count (0.0 to 0.16% and 0.0 to 0.33%, respectively) upto six months of storage period. The conventional storage bags differed significantly with the hermetic bags by recording a greater number of adult pulse beetles (>2100) and resulted in 100% grain damage (Table 1).

### Percentage of moisture and viability of chickpea seed

The initial moisture content and viability of grains before conducting experiment was 7.72 and 99.67 percent, respectively. There was no much variation in the moisture content of grains stored in super bags (7.77 to 7.78%), triple layer plastic bags (7.95% to 7.97%) and polythene bags (7.95% to 7.96%) upto six months (Table 2).

However, a slight raise in moisture content was observed in super bag (0.05 to 0.06%) followed by polythene bag (0.23 to 0.24%) and triple layer plastic bag (0.23 to 0.25%) upto 180 DAS. The content of moisture was raised from 2.80% at the end of first month to 20.85% by the end of sixth month in chickpea grains stored in nylon bag (2.80 to 20.85%) followed by cloth bag (3.20 to 11.13%), thin netted jute bag (2.16 to 9.60%) and thick netted jute bag (0.78 to 7.60%) upto 180 DAS (Figure 1).

Above 90 percent viability of chickpea grains was maintained by super bags up to 180 days, whereas, the triple layer plastic bags and polythene bags maintained only up to 120 days of storage (Table 2). There was a slight decrease in percent viability of chickpea grains stored in super bag (0.0 to 7.38%) followed by triple layer plastic bag (0.67 to 13.42%) and polythene bag (0.67 to 16.78%) upto 180 DAS. The decrease in viability percentage was

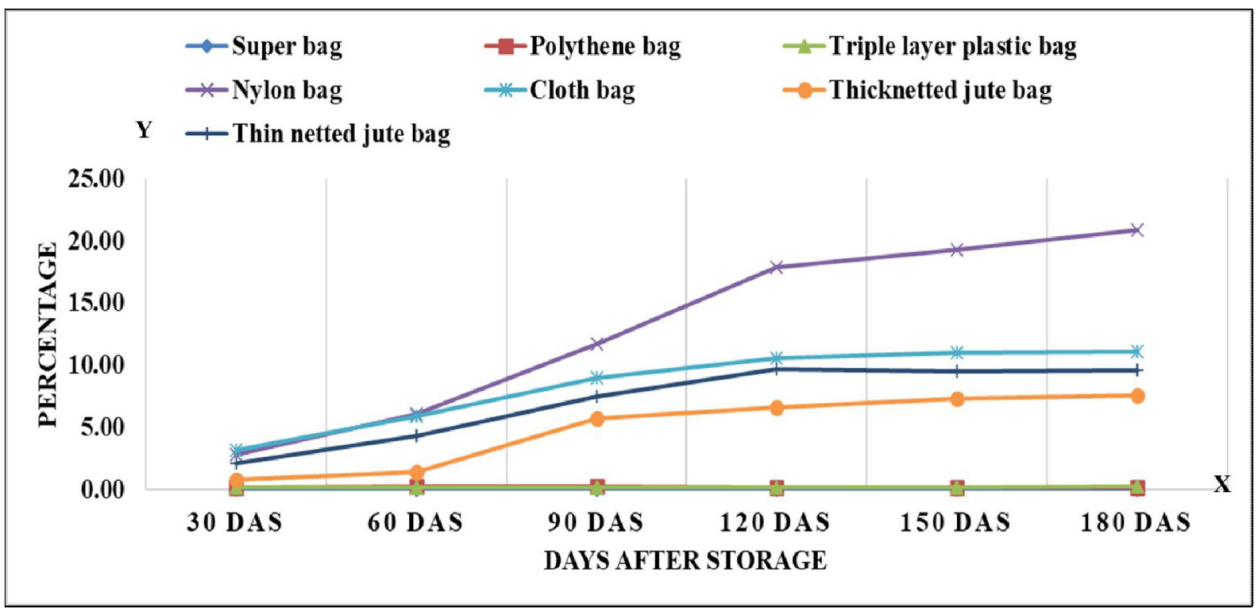


**Figure 1. Percent increase in grain moisture content of chickpea stored in different types of bags**

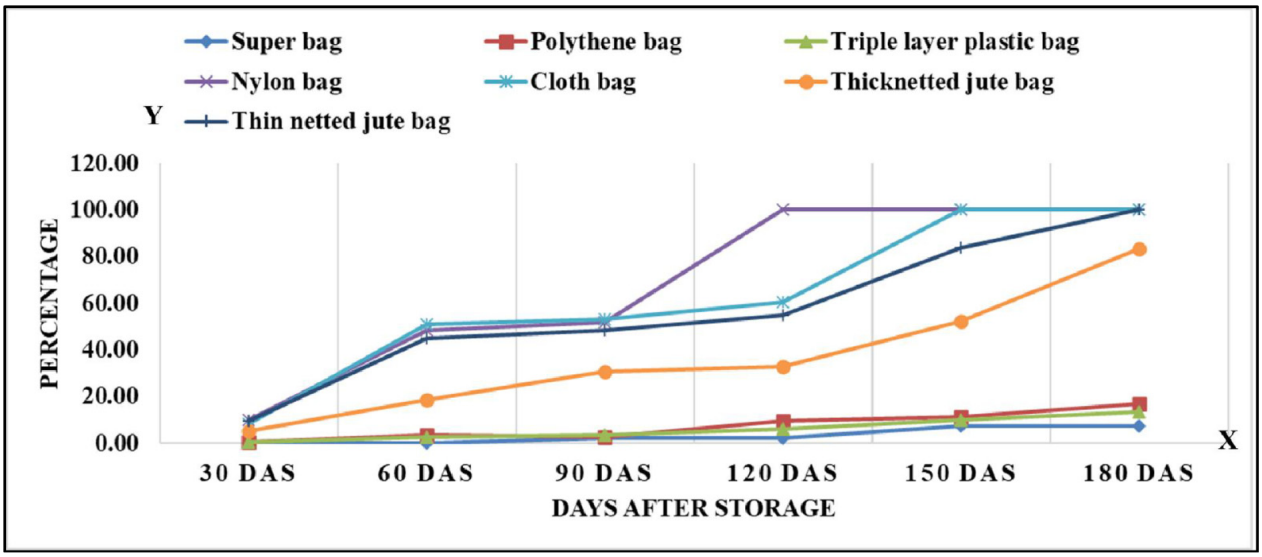


**Figure 2. Percent decrease in viability of chickpea grains stored in different types of bags**

observed in chickpea grains stored in nylon bag (10.07 to 100.0%) followed by thin netted jute bag (9.39 to 100.0%), cloth bag (8.05 to 100.0%), and thick netted jute bag (5.37 to 83.23%) upto 180 DAS (Figure 2).

According to this study findings, the storage of chickpea grains using super bags prevented the development of *C. maculatus* population, grain damage, moisture loss and viability loss upto six months of storage compared to conventional storage bags *viz.*, nylon, cloth, thick netted and thin netted jute bags. There was no adult emergence of *C. maculatus* and grain damage of chickpea grains stored in super bag upto six months, which may be due to hypoxia and hypercarbia conditions created inside the bags that lead to the inhibition of growth and development of pulse beetle. The percent increase in moisture content and decrease in viability of chickpea grains stored in super bags were very low compared to remaining bags up to six months, which may be due to inhibition of growth of pulse beetle and maintenance of stability in moisture levels. The triple layer plastic bag and polythene bags were also found to give better protection with respect to grain damage (<0.5%) and adult emergence (<10) up to six months, whereas, the increase in moisture content and decrease in viability were slightly more when compared to super bag.

Experimental findings of the study were in accordance with the results of Babu *et al.* (2020), who reported that the triple layer bags working on the principle of hermetic storage created hypoxia and hypercarbia conditions within a short period of time, effectively reducing insect development and reducing grain damage of stored grains, experimental results from the study were consistent with their findings. The percent seed viability of different pulses stored in different hermetic bags *viz.*, super bags, polythene bags, triple layer plastic bags and other hermetic containers was maintained below 90 percent and moisture remained unchanged or slightly changed (increased or decreased) from the initial moisture contents up to several months (Silva *et al.*, 2018; Atilaw *et al.*, 2021; Sharma *et al.*, 2022 and Yewle *et al.*, 2022).

## CONCLUSIONS

Among the hermetic bags tested, super bags offered complete protection to the chickpea grains up to six months without any infestation by *C.maculatus* and adult emergence and also maintained constant moisture levels with more than 90 percent seed viability, followed by triple layer plastic bags.

a) Super bag

b) Polythene bag

c) Triple layer plastic bag

d) Nylon bag

e) Cloth bag

f) Thick netted jute bag

g) Thin netted jute bag

**Figure 3. Different storage bags used in the evaluation of hermetic storage technique in chickpea against pulse beetle, *C. maculatus***

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# ENHANCING THE PRODUCTIVITY OF CHICKPEA THROUGH FOLIAR NUTRITION UNDER RAINFED CONDITIONS

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## ABSTRACT

The study was carried out at Regional Agricultural Research Station, Lam for three years (2020-21, 2021-22, 2022-23) for assessing the productivity of chickpea using foliar nutrition under rainfed conditions of the Krishna Agro Climatic Zone of South Coastal Andhra Pradesh.The experiment was laid out in Randomized Block Design and was replicated thrice. The experiment consisting of eight treatments *viz*., control (RDF:N20:P50:K0 kg $ha^{-1}$), application of water soluble NPK, Zn, Fe, soluble monophosphate and soluble potassium nitrate at different stages alone and in combinations. Pooled analysis (2020-2023) of the experimental results revealed that along with recommended dose of fertilizers (N20:P50:K0 kg $ha^{-1}$) foliar application of Zn @ 0.2% at pre flowering, water soluble monophosphate @1% at flowering and water soluble potassium nitrate @1% at pod filling stage recorded significantly higher seed yield (1871 kg $ha^{-1}$) and protein content (22.90%) which was on par with foliar application of Fe @ 0.5% at pre flowering, monophosphate@1% at flowering and potassium nitrate @ 1% at pod filling stage with seed yield (1821 kg $ha^{-1}$) and (22.63%) protein content, both the treatments recorded BCR of 1.9.

**Keywords:** Chickpea, Flowering and pod filling, Foliar nutrition, Potassium Nitrate, Pre-flowering and Protein content

## INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a hardy, dry land crop sown on marginal lands, which can grow to full maturity on conserved moisture. Seasonal variability in available moisture is the major constraint to crop production under rainfed farming (Mandakini *et al.*, 2022). The erratic and low rainfall along with high temperatures in the rainfed farming induces periods of water stress during crop growth. Chickpea is grown during *rabi* under reducing soil moisture conditions without any irrigation. As a result, there was a water deficit for crops at critical growth stages which affects the nutrient pool in soiland its uptake ultimately causing yield reduction. To increase the yield of chickpea under rainfed conditions, we have to take into consideration not only the normalization of the plant water regime but also the normalization of plant feeding and elimination of deficiencies of nutrients during crop growth. Zinc plays a significant role in various enzymatic and physiological activities of plants. It stabilizes the structure of

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membranes and cellular components and catalysis the process of oxidation in plant cells. Chickpea is widely considered a zinc-deficiency-sensitive crop. Zinc deficiency affects plant-water relationships, such as stomatal closure and reduced transpiration. Zinc-deficient plants appear stunted, size of leaflets reduces and delays cropmaturity. The younger leaves turn a pale green color first, then a reddish-brown discoloration appears on the leaflet margins and the lower part of the stem. The upper portion of the leaflet becomes bronzed and necrotic. Iron acts as a co-factor for various enzymes performing basic functions in the human body. Inadequate supply of iron leads to disability, anemia and stunted mental growth. Its malnutrition may be reduced by enhancing the bio-available iron content through iron supplementation and food fortification. Foliar application of watersoluble mono phosphate has risen as a result of many advantages such as quick and efficient utilization of nutrients, elimination of losses through leaching, fixation and regulating the uptake of nutrients by plants (Bhavya *et al.,* 2020). Application of potassium nitrate to the soil is made before the growing season or as a supplement during the growing season by foliar spray. Foliar nutrition is a process that feeds plants by applying liquid fertilizer just to their leaves. Through their stomata, nutrients are absorbed more quickly, yet overall absorption may be just as high. Keeping these in view, an investigation was initiated to know the response of chickpea to foliar nutrition under residing soil moisture under the rainfed situation in the agro-climatic zone of south coastal Andhra Pradesh.

## MATERIALS AND METHODS

The field experiment was conducted at Regional Agricultural Research Station, Lam, located at Guntur (Latitude:$16^0$ $18^1$, Longitude: $80^0$ $29^1$, Altitude:33 MSL) during 2020-21, 2021-22, 2022-23. The climate is sub-tropical with mean annual rainfall of 950 mm. The soil of the experimental field was clay loam in texture, slightly alkaline in reaction (pH 8.5), non-saline and medium in available N (312.5 kg $ha^{-1}$), high in $P_2O_5$ (185.4.1 kg $ha^{-1}$) and $K_2O$ (1016 kg $ha^{-1}$) and very low in organic carbon (0.20%), respectively. The micronutrients Zn (0.7 ppm), Fe (4.5 ppm), Mn (9.5 ppm) and Cu (1.7 ppm) were boarder to critical limits at the experimental site before experimentation.

The experiment was laid out in RBD and replicate thrice and the experiment consisted eight treatments *viz.*, $T_1$: Recommended Dose of Fertilizers(20: 50 : 0 NPK kg $ha^{-1}$) $T_2$: RDF+Water soluble NPK fertilizer @ 1% (19:19:19) at vegetative stage $T_3$: RDF+ Foliar application of Zn @ 0.2 % at pre-flowering stage, $T_4$: RDF+ Foliar application of Fe @ 0.5% at pre-flowering stage, $T_5$:RDF+Water soluble monophosphate @ 1% (00:52:34) at flowering stage $T_6$: RDF+Water soluble potassium nitrate@1% (13:0:45) at pod filling stage, $T_7$:$T_1$+$T_3$+$T_5$+$T_6$, and$T_8$: $T_1$+$T_4$+$T_5$+$T_6$.The variety used was JG-11 and adopted a recommended package of practices of ANGRAU for its cultivation.

At harvest, the data on growth parameters and yield attributes such as plant height (cm), number of branches per plant, number of pods per plant, number of seeds per pod and test weight (g) were collected from ten randomly selected plants. The seed and straw yield of each plot were determined at harvest and then converted to kg $ha^{-1}$. Protein content (%) was calculated by multiplying N content by 6.25 (Chapman and Pratt, 1978).The total nitrogen content in the seed was determined according to the Micro-kjeldahl method of AOAC (1996). The data obtained on growth parameters, yield, yield attributes and seed protein% of chickpea were analyzed statistically. The significance of treatmental

effects was tested with the help of F test at 5% level of significance to compare the treatments.Pooled analysis of three years data was presented in Table 1 and the results were discussed.

## RESULTS AND DISCUSSION

### Growth Parameters

The plant height and number of branches per plant influenced by foliar nutrition on chickpea were presented in Table 1 and found that no significant difference was observed with regard to plant height. Whereasthe number of branches per plant differed significantly. At harvest significantly higher number of branches per plant (4.6) was recorded with the treatment that received foliar application of Zn @ 0.2% at pre flowering, soluble monophosphate @ 1% at flowering and soluble potassium nitrate @ 1% at pod filling stage in addition to the RDF (20:50:0 kg $ha^{-1}$) as basal applicationwhich is on par with the $T_8$ and that might be due to the increased availability of zinc, soluble monophosphate and soluble potassium nitrate through foliar application at different growth stages as the soil may not supply the sufficient quantity of these nutrients under limited moisture conditionand further these nutrients met the peak nutrient demand requirement of chickpea at different stages *i.e.,* pre flowering, flowering and pod filling stages. The foliar applied nutrients involved in the process of efficient absorption and translocation in the plant and helped inactivating the metabolic synthesis of Indole Acetic Acid, Auxins and biological stimulation of enzymatic activity and photosynthetic pigmentation which in turn encourage the chlorophyll formation, photosynthetic efficiency and thereby accumulates more photosynthates which might be resulted in more number of branches under rainfed conditions (Gowthami and Ananda, 2015) and Nandaniya *et al. (*2016). A significant increase in number of branches $plant^{-1}$ following the foliar application of different micronutrients in chickpea had been reported by Saini and Singh (2017), Jadhav *et al. (*2019) and Vinod *et al. (*2020).

### Yield and Yield Parameters

The study on foliar nutrition in chickpea under rainfed conditions was carried and the data pertaining to yield and yield parameters were analyzed and that the results indicated that number of pods per plant (50.3) and test weight (242.5 g) were significantly influenced by the foliar application of Zn @ 0.2% at pre flowering, soluble monophosphate @ 1% at flowering and soluble potassium nitrate @ 1% at pod filling stage in addition to the RDF applied as basal($T_7$) which was on par with foliar application of Fe @ 0.5% at pre flowering, soluble monophosphate @ 1% and foliar application of soluble potassium nitrate @ 1% at pod filling stage in addition to RDF ($T_8$) over the rest of the treatments and the lowest number of pods per plant (30.3), test weight (234.9 g) were recorded with alone basal application of RDF (20:50:0 kg $ha^{-1}$). Zinc application regulates the nutrient balance and stomatal opening thereby maintaining the adverse effects of water deficit stress under rainfed conditions. Similar results were found by Saini and Singh (2017), Vinod *et al*. (2020) and Mandakini *et al. (*2022). Soluble monophosphate applied at the flowering stage might be resulted in balanced supply of phosphate and met the peak nutrient demand at the flowering stage of the crop thereby improved the flowering cycle of the crop under rainfed conditions Ma *et al*. (2021). Foliar application of potassium during pod formation and filling stage often coincides with high potassium demands during the time of declining root activity and resulted in more number of pods with more pod weight. Membrane and chlorophyll degradation are

**Table 1. Yield and yield attributes as influenced by foliar nutrition of chickpea at different growth stages (Pooled data of three years**

| S.No | Treatments | Plant height at harvest (cm) | No. of branches plant$^{-1}$ | No. of pods plant $^{-1}$ | No. of seeds pod$^{-1}$ | Test wt (g) | Seed yield (kg ha$^{-1}$) | Straw Yield (kg ha$^{-1}$) | Protein Content (%) | BCR |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | $T_1$: RDF (20:50:0 kg ha$^{-1}$) | 44.9 | 3.5 | 30.3 | 1.1 | 238.1 | 975 | 775 | 21.10 | 1.1 |
| 2 | $T_2$: RDF + Water soluble NPK fertilizer @ 1% (19:19:19) at vegetative stage | 46.6 | 3.6 | 33.2 | 1.1 | 234.9 | 1085 | 787 | 22.19 | 1.1 |
| 3 | $T_3$:RDF+ Foliar application of Zn @ 0.2 % at pre flowering stage | 46.1 | 3.9 | 42.0 | 1.2 | 238.4 | 1373 | 917 | 22.13 | 1.7 |
| 4 | $T_4$:RDF+ Foliar application of Fe @ 0.5 % at pre flowering stage | 46.2 | 3.9 | 38.1 | 1.1 | 236.8 | 1385 | 841 | 21.78 | 1.5 |
| 5 | $T_5$:RDF+Water soluble monophosphate @ 1 % (00:52:34) at flowering stage | 48.4 | 4.1 | 36.8 | 1.2 | 235.2 | 1445 | 820 | 22.23 | 1.4 |
| 6 | $T_6$:RDF+Water soluble potassium nitrate @ 1 % (00:52:34) at pod filling stage | 46.5 | 4.1 | 41.1 | 1.1 | 239.5 | 1523 | 912 | 22.24 | 1.6 |
| 7 | $T_7$: $T_1+T_3+T_5+T_6$ | 48.8 | 4.6 | 50.3 | 1.2 | 242.5 | 1871 | 1019 | 22.90 | 1.9 |
| 8 | $T_8$: $T_1+T_4+T_5+T_6$ | 47.3 | 4.6 | 47.0 | 1.2 | 240.0 | 1821 | 980 | 22.63 | 1.9 |
| SEm ± | | 1.07 | 0.14 | 1.05 | 0.02 | 2.75 | 94.2 | 51.7 | 0.1 | - |
| CD @ 5% | | NS | 0.43 | 3.19 | NS | 8.34 | 285.99 | 120.9 | 0.34 | - |
| CV (%) | | 3.96 | 6.01 | 4.58 | 3.68 | 2.00 | 11.38 | 15.77 | 12.8 | - |

common in potassium deficient plants. Hence, it might be considered as one of the alternatives to reduce such problems. The results of this research are in agreement with the findings of Bhavya *et al.* (2020), and Bibek *et al*. (2020).

Furthermore, the study indicated that seedand straw yield were significantly influenced by the foliar application of different nutrients under rainfed situations and the results indicated that significantly higher seed yield (1871 kg $ha^{-1}$) and straw yield (1019 kg $ha^{-1}$) were noticed with foliar application of Zn @0.2% at pre flowering, soluble monophosphate @ 1% at flowering and soluble potassium nitrate @ 1% at pod filling stagein addition to the RDF (20:50:0 kg $ha^{-1}$) as basal application ($T_7$) which was on par with foliar application of Fe @ 0.5% at pre flowering, soluble monophosphate @ 1% and foliar application of soluble potassium nitrate @ 1% at pod filling stage in addition to the RDF ($T_8$). The lowest seed yield (975 kg $ha^{-1}$) and straw yield(775 kg $ha^{-1}$) were recorded in treatment control ($T_1$) and presented (Table 1). This increment in the yield might be due to foliar application of nutrients that directly absorbed by the plants increase the metabolic activities of plants resulted in synthesis of photosynthetic products and their efficient translocation from source to sink under limited moisture conditions thereby increasing number of pods, test weight ultimately resulting in higher seed yield and straw yield. These findings are in conformation with the findings of Shivanand *et al.* (2017), Sanjay (2017) and Yashona *et al*. (2020).

**Protein Content**

The influence of foliar nutrients on seed protein content of chickpea was presented in Table 1 and the highest seed protein content (22.9 %) was recorded in treatment ($T_7$) that received RDF along with foliar application of Zn @0.2% at pre flowering, soluble monophosphate @ 1% at flowering and soluble potassium nitrate @ 1% at pod filling stage which was on par with Fe combination in addition to RDF ($T_8$) and this might be due to increase in micronutrient availability enhances N uptake by plants through nodule formation, which increases the amino acids synthesis and it helps in conversion of amino acids to high quality protein. However, the lowest value of seed protein content (21.1%) was obtained from the $T_1$ (control treatment). The results of the present research are in agreement with the findings of Shivanand *et al.* (2017), Sanjay (2017), Islam *et al*.(2018), Kachave *et al.* (2018) and Kobaraee (2019).

**Economics**

To know the cost effectiveness of each treatment and its combinations of different foliar nutrients combinations were presented in Table 1 and the highest B:C ratio of (1.9) was arrived with the combination application of Zn @0.2% at pre flowering, soluble monophosphate @ 1% at flowering and soluble potassium nitrate @ 1% at pod filling stage in addition to the RDF as basal application ($T_7$) which is onpar with Fe @ 0.5% in combination of monophosphate @ 1% at flowering and soluble potassium nitrate @ 1% at pod filling stage in addition to the RDF as basal application ($T_8$) and this might be due to nutrient demand of the crop at all the growth stages was met by foliar nutritionthat resulted in increased seed and straw yield. The requirement and amount incurred for foliar spray were comparatively less than soil application in chickpea under rainfed situation of Krishna zone. These findings were in accordance with Santosh *et al.* (2020) and Bahure *et al.* (2016).

**CONCLUSIONS**

Along with RDF, foliar application of Zn @ 0.2%, monophosphate @ 1% at flowering

and potassium nitrate @ 1% at pod filling stage in chickpea, respectively recorded significantly higher growth, yield and protein content under rainfed situation of Krishna agro climatic zone of Andhra Pradesh.

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# RESPONSE OF PIGEONPEA TO ZINC SULPHATE AND POTASSIUM UNDER RAINFED CONDITIONS IN WESTERN MANDALS OF CHITTOOR DISTRICT

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## ABSTRACT

On-Farm Trials (OFTs) on yield enhancement in pigeonpea through basal application of Potassium and Zinc Sulphate were conducted at Krishi Vigyan Kendra, Kalikiri under rainfed conditions in selected areas of Chittoor district between 2017-18 and 2019-20 in field of five farmers in 2.0 ha during each year. Soil type was sandy loams. Treatments comprised Basal application of Zinc Sulphate (25 kg $ha^{-1}$) + MOP (60 kg $ha^{-1}$) + Nitrogen (20 kg $ha^{-1}$), Phosphorus (50 kg $ha^{-1}$) as Technology Option 1 and Basal application of FYM @ 20 q $ac^{-1}$ as farmers practice. In TO1 Nitrogen @ 20 kg $ha^{-1}$, Phosphorus @ 50 kg $ha^{-1}$, Potash @ 60 kg $ha^{-1}$ and Zinc Sulphate @ 25 kg $ha^{-1}$ were applied to pigeonpea. In TO2 (Farmers practice) FYM @ 20 q $ac^{-1}$ was applied.Yield attributes *viz.,* number of pods per plant (223), 100 seed weight (11.1 g), yield (4.2 q $ha^{-1}$) and B: C ratio (1.1) were significantly higher in case of treatment plot compared to check plot with number of pods per plant (194.3), 100 seed weight (9.2 g), yield (3.3 q $ha^{-1}$) and B: C ratio (1.0).

**Keywords:** Economics, Potassium, Pigeonpea Yield, Zinc Sulphate

## INTRODUCTION

Pigeonpea (*Cajanus cajan* L.) is one of the important pulse crops in India which ranks second after chickpea. It is highly rich in protein and suitable for growing in tropical countries and sub-tropical regions(Sahaja *et al.,* 2019). World-wide pigeonpea is grown in an area of 60.96 lakh ha, with a production of 50.12 lakh tonnes and with productivity of 822.2 kg $ha^{-1}$(FAO STAT, 2020). In India during 2020-21, pigeonpea was grown in an area of 48.24 lakh ha, production of 38.8. lakh tonnes and productivity of 804 kg $ha^{-1}$ and it ranks first in pigeonpea production (agricoop.nic.in). In India, pigeonpea takes second position in total pulse production after chickpea. In Andhra Pradesh, pigeonpea was grown in area of 2.33 lakh ha during 2020-21 and produced1.16 lakh tonnes with productivity of 496 kg $ha^{-1}$ productivity (http://indiastat.com). In Chittoor district, pigeonpea was grown in an area of 7501 ha during 2020-21. Pigeonpea is rich source of proteins. In pigeonpea, reduction in productivity is due to high incidence of pests and diseases during flowering and pod formation. Availability of potassium and zinc to crop will improve yields of pigeonpea along with nitrogen and phosphorus as these are

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**Table 1. Monthly rainfall data during crop growth period**

| S.No. | Month and Year | Normal (mm) | Actual (mm) | Deviation (%) | Month and Year | Normal (mm) | Actual (mm) | Deviation (%) | Month and Year | Normal (mm) | Actual (mm) | Deviation (%) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | June, 2017 | 83.09 | 85.0 | 2.29 | June, 2018 | 78.7 | 75.4 | -11.18 | June , 2019 | 83.09 | 26.3 | -68.34 |
| 2 | July, 2017 | 92.29 | 46.4 | - 49.72 | July, 2018 | 102 | 49 | -51.96 | July , 2019 | 92.29 | 65.6 | -28.9 |
| 3 | August , 2017 | 108.06 | 194.0 | 79.52 | August, 2018 | 117.4 | 57.5 | -51.02 | August,2019 | 108.06 | 84.9 | -21.43 |
| 4 | Septembe r, 2017 | 145.4 | 154.0 | 5.91 | September, 2018 | 141.4 | 129.1 | -8.70 | September,2 019 | 145.4 | 152.2 | 4.6 |
| 5 | October, 2017 | 146.6 | 257.1 | 75.37 | October, 2018 | 162.7 | 69.5 | -57.3 | October, 2019 | 146.6 | 75.3 | -48.63 |
| 6 | November , 2017 | 97.58 | 45.8 | - 53.06 | November, 2018 | 162.6 | 103.0 | -36.7 | November,20 19 | 97.58 | 22 | -77.45 |
| 7 | December , 2017 | 48.3 | 27.2 | - 43.68 | December, 2018 | 70.1 | 16.4 | -76-60 | December, 2019 | 40.94 | 0 | -100 |

essential nutrients for pigeonpea. Potassium helps in increasing root growth, improving drought and disease resistance. Zinc increases crude protein content, energy value, amino acids, lipid content and crop yield in pulses. (Chalak *et al*., 2018). Soils in Chittoor district are in general deficit in micro nutrients. Moreover redgram is being cultivated in sandy loams recording low yields. Research results at RARS, Tirupati showed that application of zinc sulphate and potassium as basal dose will increase yield of pigeonpea by about 23 percent.

## MATERIALS AND METHODS

On-Farm Trials on Yield enhancement in pigeonpea through basal application of Potassium and Zinc Sulphate were conducted in rainfed condition in sandy loam soils in western mandals of chittoor district between 2017-18 and 2019-20 in the fields of five farmers in 2.0 ha during each year. Sowings were done with seed drill with spacing of 180 cm between rows and intercropped with pigeonpea. Treatments comprised of Basal application of Zinc Sulphate (25 kg $ha^{-1}$) + MOP (60 kg $ha^{-1}$) + Nitrogen (20 kg $ha^{-1}$), Phosphorus (50 kg $ha^{-1}$) as Technology Option 1 and Basal application of FYM @ 20 q $ac^{-1}$as farmers practice. In TO1 Nitrogen @ 20 kg $ha^{-1}$, Phosphorus @ 50 kg $ha^{-1}$, Potash @ 60 kg $ha^{-1}$ and Zinc Sulphate @ 25 kg $ha^{-1}$ were applied to pigeonpea. In TO2 (Farmers practice) FYM @ 20 q $ac^{-1}$ was applied. Irrigations were not given as it was grown under rainfed conditions. Monthly rainfall data of the study area is depicted (Table 1). Five plants were selected in each field and data was recorded on number of pods per plant, number of seeds per pod and 100 seed weight which were statistically analyzed. Yield was recorded in 5 sq.m in five locations after threshing of the produce. Economics was calculated as shown below by considering prevailing market prices of inputs and output.

**Table 2. Yield attributes of treatment and check plots of pigeonpea**

| S.No. | Year | No. of pods per plant | | No. of seeds per pod | | 100 seed weight (g) | |
|---|---|---|---|---|---|---|---|
| | | TO1 | TO2 | TO1 | TO2 | TO1 | TO2 |
| 1 | 2017-18 | 254 | 213 | 4.0 | 4.0 | 11.3 | 9.5 |
| 2 | 2018-19 | 205 | 190 | 4.0 | 4.0 | 11.1 | 9.2 |
| 3 | 2019-20 | 210 | 180 | 4.0 | 4.0 | 11.0 | 9.0 |
| | **Mean** | 223.0 | 194.3 | 4.0 | 4.0 | 11.1 | 9.2 |

**TO1:** Basal application of Zinc Sulphate(25 kg $ha^{-1}$) + MOP (60 kg $ha^{-1}$) + Nitrogen (20 kg $ha^{-1}$), Phosphorus (50 kg $ha^{-1}$); **TO2**: Basal application of FYM @ 20 q $ac^{-1}$

**Table 3. Yield and economics of treatment and check plots of pigeonpea**

| S.No. | Year | Yield (q $ha^{-1}$) | | Percent increase in yield over check | Gross returns (Rs. $ha^{-1}$) | | Net returns (Rs. $ha^{-1}$) | | B: C ratio | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | TO1 | TO2 | | TO1 | TO2 | TO1 | TO2 | TO1 | TO2 |
| 1 | 2017-18 | 5.0 | 3.75 | 33 | 20000 | 15000 | 1000 | -1000 | 1.05 | 0.94 |
| 2 | 2018-19 | 3.7 | 3.15 | 17.5 | 20350 | 17325 | 1850 | 825 | 1.10 | 1.05 |
| 3 | 2019-20 | 3.9 | 2.95 | 8.5 | 21450 | 16225 | 2950 | -275 | 1.16 | 0.98 |
| | **Mean** | 4.2 | 3.3 | 19.7 | 20600 | 16183 | 1933 | -150.0 | 1.1 | 1.0 |

**TO1:**Basal application of Zinc Sulphate(25kg $ha^{-1}$) + MOP (60 kg $ha^{-1}$) + Nitrogen (20 kg $ha^{-1}$), Phosphorus (50 kg $ha^{-1}$); **TO2**: Basal application of FYM @ 20 q $ac^{-1}$

## RESULTS AND DISCUSSION

**Yield attributes:** On an average, no. of pods per plant in treatment and check plots were223 and 194.3, respectively. 100 seed weight of treatment and check plots were 11.1 and 9.2, respectively (Table 2).The highest 100 seed weight was due to application of potassium and zinc sulphate. Similar results were also reported by Chalak *et al.* (2018). It has been concluded that there was significant difference between treatment and check plot with regard to number of pods per plant and 100 seed weight (Table 4)

**Yield and Economics:** Perusal of the data presented in the Tables 3 and 4 and Fig.1 and 2 revealed that in demo plot, yield and net returns were found to be significantly higher than in control (farmers practice) during all the years (2017-18 to 2019-20). In treatment plot, mean yield of 4.2 q $ha^{-1}$ was recorded. Whereas, in control plot 3.3 q $ha^{-1}$ yield was recorded. Net returns of treatment and check plot were 1933 and -150 Rs. $ha^{-1}$, respectively. Mean B: C ratio of treatment and check plots were 1.1 and 1.0, respectively (Table 3).The

**Table 4. Summary of t-test in comparing no. of pods per plant, 100 seed weight, yield and net returns in treatment and farmers practice for three years**

| S.No. | Particulars | Treatments | N | Mean | Std.Deviation | t-value | p-value |
|---|---|---|---|---|---|---|---|
| 1 | No. of pods | TO1 | 5 | 223.0 | 0.38 | 2.36* | 0.003 |
| 2 | per plant | TO2 | 5 | 194.3 | 0.25 | 2.36* | 0.003 |
| 3 | 100 seed | TO1 | 5 | 11.1 | 0.12 | 2.31* | 0.004 |
| 4 | weight | TO2 | 5 | 9.2 | 0.10 | 2.31* | 0.004 |
| 5 | Yield | TO1 | 5 | 4.2 | 0.16 | 2.31* | 0.002 |
| 6 | | TO2 | 5 | 3.3 | 0.16 | 2.31* | 0.002 |
| 7 | Net returns | TO1 | 5 | 1933 | 1.6 | 2.36* | 0.004 |
| 8 | | TO2 | 5 | -150 | 1.0 | 2.36* | 0.004 |

***Significant at 5% level**

**TO1:** Basal application of Zinc Sulphate(25 kg $ha^{-1}$) **+** MOP (60 kg $ha^{-1}$) **+** Nitrogen (20 kg $ha^{-1}$), Phosphorus (50 kg $ha^{-1}$); **TO2**: Basal application of FYM @ 20 q $ac^{-1}$

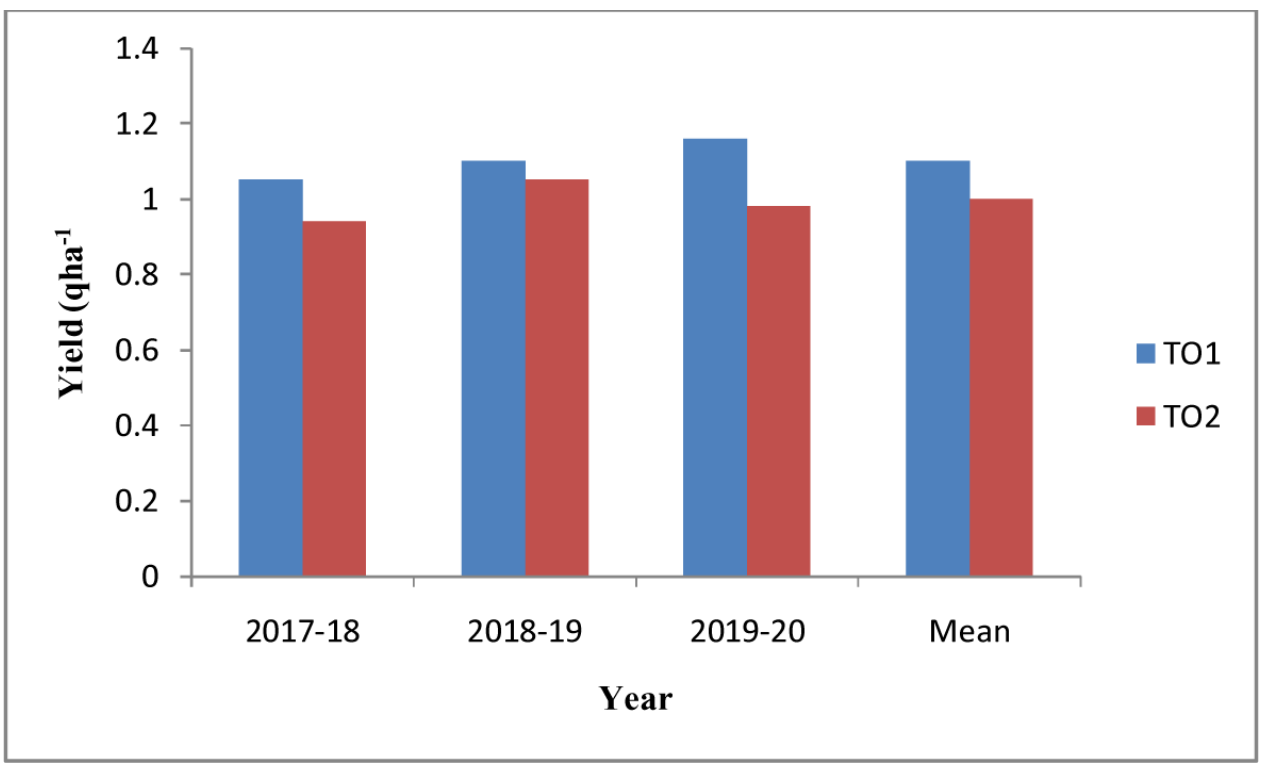


**Fig 1. Comparision of treatment and check plots in terms of yield**

higher yield resulted due to more number of pods per plant and 100 seed weight as it was one of the important yields attributing character.The positive effect of K on crop yield might also be due to improved nodulation, higher shoot growth and improved yield

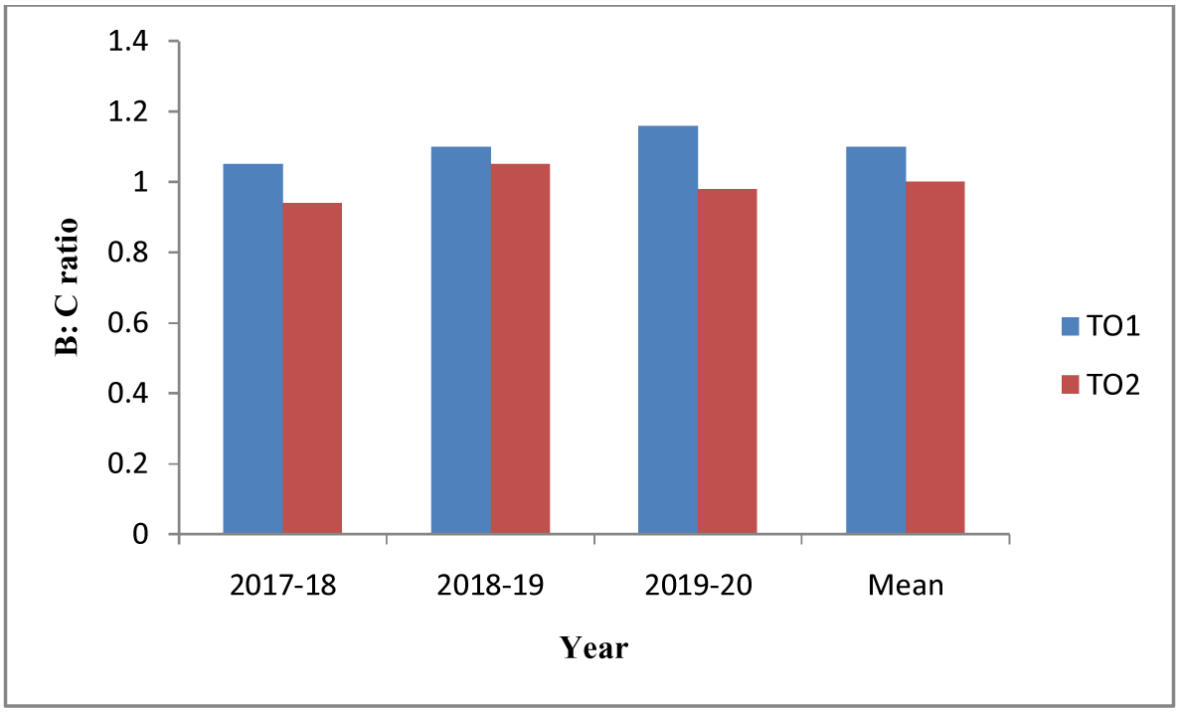


**Fig 2. Comparision of Treatment and Check plots in terms of B:C ratio**

attributing characters (Chalak *et al*., 2018). The positive response of the pigeon pea to zinc fertilization have been reported by Shah *et al*. (2016) and Purushottam *et al*. (2018). Similar results are in compliance with the findings of Mukundgowda *et al.* (2015) and Buriro *et al.* (2015). Balpande *et al*. (2016) reported that application of potassium along with nitrogen and Phosphorus increases the nutrient uptake, yield and quality of pigeon pea.Yashona *et al*. (2020) reported that application of zinc sulphate gave 7-25 percent higher yields compared to control.

**CONCLUSIONS**:

Basal application of Nitrogen, Phosphorus, Potash supplying fertilizers and zinc sulphate proved best in terms of increasing pods per plant, test weight and giving higher yields (4.2 q $ha^{-1}$) when compared to check plot (3.3 q $ha^{-1}$) in which only FYM was applied. It was due to fulfilling of the nutrient requirements necessary to the crop. Hence, application of required fertilizers was proved beneficial in giving higher yields and net returns in pigeonpea.

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# IMPACT OF ORGANIC WEED MANAGEMENT PRACTICES ON WEEDS CONTROL, GROWTH AND YIELD OF MAIZE (*Zea mays* L.)

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## ABSTRACT

This field experiment on organic weed managament practices was conducted during *rabi*, 2021-22 at wetland farm of S.V. Agricultural College, Tirupati, with 10 management practices in a randomized block design and replicated thrice. Among all the organic weed management practices, lower weed density and dry weight with higher weed control efficiency and weed control index was recorded with corn gluten meal 3.5 t $ha^{-1}$ as pre-emergence (PE) followed by hand weeding (HW) at 30 days after sowing (DAS), however, it was statistically comparable with HW twice at 15 DAS and 30 DAS. Significantly higher growth parameters, yield attributes and kernel and stover yield of maize were recorded with corn gluten meal 3.5 t $ha^{-1}$ as pre-emergence followed by HW at 30 DAS over rest of the treatments. The next best treatment in reducing density and dry weight of weedswith higher growth parameters, yield attributes and yield was HW twice at 15 DAS and 30 DAS, which was however, at par with groundnut shells mulch 12.5 t $ha^{-1}$ and mango leaves mulch 5 t $ha^{-1}$.

**Key words:**Corn gluten meal, Live mulch, Maize, Organic weed management

## INTRODUCTION

Maize (*Zea mays* L.) is one of the world's major cereal crop, ranking third in importance after wheat and rice. It is grown on 194 m ha area, in more than 170 countries across the globe with 1148 mt of production. In India, it is grown on 9.89 m ha area with production of 31.65 mt and productivity of 3199 kg $ha^{-1}$ (*www.indiastat.com*, 2021). The actual yield of maize produced in India is below the world average and this is mainly due to poor weed management practices coupled with low resource inputs.

Weeds constitute one of the major economic problem for maize growers and it can reduce yield up to 86 percent. The magnitude of yield loss largely depends upon the composition of weed flora, period of crop-weed competition and their intensity. Weeds compete with the crop plants forvital growth resources. Corn is a wide spaced crop and heavy weed interference from 3 to 6 WAS significantly depressed the growth parameters and kernel yield of maize.Modern agriculture is productivity oriented and weed management is primarily focused on curative control, as herbicides are highly effective and relatively cheap. Continuous use of herbicides for weed management leads to loss of bio-diversity, environmental pollution and develops herbicide

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resistance in weeds. In organic farming, the weed problem was intense mainly due to application of organic manures, mulches and on farm biomass which exacerbates the weed multiplication and growth. In view of this, to minimise the chemical usage and to maintain biodiversity, use of ecological weed management strategies is a typical component comprising of cultural, mechanical,biological and physical practices forweed suppression without use of synthetic herbicides. Hand Weeding (HW) is the most popular method to remove weeds.However, it is tedious, time consuming and labour demanding. Mulching is an effective method of weed control without using chemicals. The use of biomass from trees such as mango, shells of groundnut can be used as a mulch swhich acts as a physical barrier to impede weeds emergence (Choudhary and Kumar, 2014). Live mulch involves growing of smother crop between the rows of the main crop. It is very important to kill and till in, or manage live mulch so that it does not compete with the actual crop. Use of extracts ofallelopathic plants as natural herbicides is one of the prime areas of research to suppress the weed growth (Naeem *et al*., 2016). Keeping these in view, the investigation was carried out to study the effect of various non-chemical weed management practices on weed population, dry weight andproductivity of maize.

## MATERIALS AND METHODS

This field experiment was conducted during *rabi* 2021-22 at wetland farm, S. V. Agricultural College, Tirupati, located at 13.5 °N latitude and 79.5 °E longitude with an altitude of 182.9 m above mean sea level in the Southern Agro-Climatic Zone of Andhra Pradesh, India. The soil was sandy clay loam in texture, neutral in soil reaction, low in organic carbon (0.26) and available nitrogen (249 kg $ha^{-1}$) and medium in available phosphorus (37 kg $ha^{-1}$) and potassium (285 kg $ha^{-1}$). A total rainfall of 801.0 mm was received during the crop growth period in 34 rainy days. The experiment was laidoutin Randomized Block Design with 10 organic weed management practices and replicated thrice. Organic weed management practices includes hand weeding twice at 15 DAS and 30 DAS ($W_1$), groundnut shells mulch 12.5 t $ha^{-1}$ ($W_2$), saw dust mulch 5 t $ha^{-1}$ ($W_3$), mango leaves mulch 5 t $ha^{-1}$ ($W_4$), live mulching with two rows of cowpea ($W_5$), live mulching with two rows of sunhemp($W_6$), eucalyptus leaf extract spray 15 l $ha^{-1}$ at 15 DAS and 30 DAS ($W_7$), sunflower extract spray 18 l $ha^{-1}$ at 15 DAS and 30 DAS ($W_8$), corn gluten meal 3.5 t $ha^{-1}$ as pre-emergence followed by HW at 30 DAS ($W_9$) and weedy check ($W_{10}$). Maize hybrid '*DHM-117"* was raised with recommended package of practices except for the weed management. The crop was fertilized with 240 kg N, 80 kg $P_2O_5$ and 80 kg $K_2O$ $ha^{-1}$. Nitrogen was applied in the form of urea in three equal splits at 1/3 as basal, 1/3 at knee high stage and the remaining 1/3 at tasseling stage. The entire dose of phosphorus as single super phosphate and potassium as muriate of potash was applied basally at the time of sowing. Weed management practices were imposed as per the treatments. Different organic mulches were applied on the day of sowing in between the rows of maize. Live mulches were grown upto 40 DAS and are uprooted and spread on the soil surface. The required quantities of filtered concentrated plant water extracts were sprayed at 15 DAS and 30 DAS. Weed population was counted with the help of 0.5 $m^2$ quadrat thrown randomly at two places in each plot and same samples were used for estimation of weed dry weight by drying the weed samples in a hot air oven at 65 ºC till constant dry weight was attained. Due to large variation in values of weed density and biomass, the corresponding data was subjected to square root

transformation $\left(\sqrt{x+0.5}\right)$ and the corresponding transformed values were used for statistical analysis.Five randomly selected plants were tagged in each treatment and from each replication in the net plot area and used for making observations on growth parameters and yield attributes at harvest of maize. Kernel and stover yield of maize were recorded based on the yield obtained from net plot.

Weed Control Efficiency (WCE) was worked out by using the following formula

$$\text{Weed Control Efficiency} = \frac{WP_C - WP_T}{WP_C} \times 100$$

Where,

$WP_C$ = Weed population in control (unweeded) plot.

$WP_T$= Weed population in treated plot.

Weed Control Index (WCI) : It was calculated by the formula as given below

$$\text{Weed Control Index} = \frac{W_C - W_T}{W_C} \times 100$$

Where,

$W_C$ = Weed dry weight in control (unweeded) plot.

$W_T$ = Weed dry weight in treated plot.

## RESULTS AND DISCUSSION

### Weed flora

The weed flora associated with maize belonged to thirteen different taxonomic families, of which the predominant weed species were *Dactyloctenium aegyptium* (L.) Willd (36%), *Cyperus rotundus* L. (22%), *Digitarias-anguinalis* (L.) Scop. (18%), *Boerhaviae recta* L. (11%), *Commelina benghalensis* L. (6%), *Euphorbia hirta* L. (3%) and others (4%).

### Weed density, dry weight and weed control efficiency

All the organic weed management practices significantly influenced weed density, biomass and weed control efficiency at 60 DAS and 80 DAS of maize (Table 1). Among the different organic weed management practices, significantly lower density and dry weight of total weeds with higher weed control efficiency and weed persistence index at 60 DAS and 80 DAS were recorded with corn gluten meal @ 3.5 t $ha^{-1}$ as post-emergence followed by HW at 30 DAS, which was however comparable withhand weeding twice at 15 DAS and 30 DAS. The lowest weed density in corn gluten meal treatment might be due to the pre-emergence herbicidal activity that efficiently reduced the germination of weed seeds (Yang and Lu, 2010). Hand weeding performed at 15 DAS and 30 DAS might have effectively reduced the density and biomass of total weeds by removing the weed flushes in early as well as later stages of crop growth. Similar results were also reported by Patil *et al*. (2013). Among the different organic mulches and live mulches, lower weed density and biomass of total weeds coupled with higher weed control efficiency at 60 DAS was recorded with live mulching with two rows of cowpea, which was statistically at par with live mulching with two rows of sunhemp, groundnut shells mulch @ 12.5 t $ha^{-1}$ and mango leaves mulch @ 5 t $ha^{-1}$. Whereas, at 80 DAS groundnut shells mulch @ 12.5 t $ha^{-1}$, was the next best treatment with reduced density and dryweight of weeds and higher weed control efficiency, but was at par with mango leaves mulch @ 5 t $ha^{-1}$, live mulching with two rows of cowpea and live mulching with two rows of sunhemp in the order of descent without any significant disparity among them. Similar results of reduced density and dry weight of weeds with mulches was also noticed by Saimaheswari *et al*. (2022).The highest density and dry weight of weeds with

**Table 1. Weed dynamics of maizeas influenced by various organic weed management practices during 2021-22**

| S.No. | Treatment | Weed density (No. $m^{-2}$)* | | Weed dry weight (g $m^{-2}$)* | | Weed control efficiency (%) | | Weed control index(%) | |
|---|---|---|---|---|---|---|---|---|---|
| | | 60 DAS | 80 DAS | 60 DAS | 80 DAS | 60 DAS | 80 DAS | 60 DAS | 80 DAS |
| 1. | $W_1$ : Hand weeding twice at 15 and 30 DAS | 8.48 (71.34) | 7.07 (49.54) | 6.33 (39.60) | 6.04 (36.09) | 78.21 | 78.37 | 79.50 | 80.67 |
| 2. | $W_2$ : Groundnut shells mulch @ 12.5 t $ha^{-1}$ | 11.54 (132.67) | 8.93 (79.33) | 7.95 (62.79) | 7.65 (58.16) | 67.32 | 68.96 | 61.88 | 69.05 |
| 3. | $W_3$ : Saw dust mulch @ 5 t $ha^{-1}$ | 16.19 (262.34) | 13.90 (193.33) | 11.44 (130.51) | 11.35 (128.36) | 28.66 | 29.41 | 24.61 | 24.58 |
| 4. | $W_4$ : Mango leaves mulch @ 5 t $ha^{-1}$ | 11.84 (139.66) | 9.47 (89.33) | 8.17 (66.23) | 7.94 (62.59) | 65.44 | 66.82 | 59.87 | 65.15 |
| 5. | $W_5$ : Live mulching with 2 rows of cowpea | 10.65 (112.99) | 9.75 (94.67) | 7.57 (56.92) | 8.04 (64.19) | 70.20 | 65.92 | 67.53 | 63.07 |
| 6. | $W_6$ : Live mulching with 2 rows of sunhemp | 10.98 (120.00) | 9.91 (97.70) | 7.74 (59.46) | 8.26 (67.79) | 69.50 | 63.80 | 65.52 | 61.89 |
| 7. | $W_7$ : Eucalyptus leaf extract spray @ 15 l $ha^{-1}$ at 15 and 30 DAS | 14.85 (220.67) | 12.79 (163.67) | 10.81 (116.50) | 10.96 (119.77) | 36.32 | 34.13 | 36.59 | 36.15 |
| 8. | $W_8$ : Sunflower extract spray @ 18 l $ha^{-1}$ at 15 and 30 DAS | 15.47 (239.33) | 13.40 (179.67) | 11.10 (122.72) | 11.05 (121.62) | 32.92 | 33.11 | 31.23 | 29.91 |
| 9. | $W_9$ : Corn gluten meal @ 3.5 t $ha^{-1}$ as PE *fb* HW at 30 DAS | 7.73 (59.33) | 6.47 (41.37) | 6.02 (35.70) | 5.44 (29.17) | 80.48 | 83.4 | 82.95 | 83.86 |
| 10. | $W_{10}$ : Weedy check (control) | 18.65 (348.00) | 16.01 (256.33) | 13.54 (182.96) | 13.50 (181.83) | - | - | - | - |
| | SEm ± | 0.512 | 0.547 | 0.394 | 0.350 | - | - | - | - |
| | CD @ 5 % | 1.58 | 1.54 | 1.04 | 1.04 | - | - | - | - |

*Data in parentheses are original values, which were square root transformed and analysed statistically.

**Table 2. Growth and yield attributes and yield of *rabi*maize as influenced by various organic weed management practices during 2021-22**

| S.No. | Treatment | Plant height (cm) | Leaf area index | Dry matter production (t/ha) | SPAD chlorophyll meter readings | Cob length (cm) | Cob girth (cm) | No. of kernel rows $cob^{-1}$ | No. of kernels $cob^{-1}$ | Hundred kernel weight (g) | Kernel yield (kg $ha^{-1}$) | Stover yield (kg $ha^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1. | $W_1$ : Hand weeding twice at 15 and 30 DAS | 228 | 2.60 | 13.32 | 36.7 | 17.3 | 15.2 | 15.3 | 293 | 30.3 | 6412 | 7029 |
| 2. | $W_2$ : Groundnut shells mulch @ 12.5 t $ha^{-1}$ | 223 | 2.56 | 13.11 | 35.8 | 17.2 | 15.0 | 15.2 | 285 | 29.7 | 6272 | 6918 |
| 3. | $W_3$ : Saw dust mulch @ 5 t $ha^{-1}$ | 179 | 1.75 | 8.67 | 30.9 | 13.1 | 11.5 | 12.5 | 184 | 23.1 | 3558 | 4951 |
| 4. | $W_4$ : Mango leaves mulch @ 5 t $ha^{-1}$ | 222 | 2.54 | 13.01 | 36.9 | 16.9 | 14.8 | 15.1 | 278 | 29.4 | 6225 | 6846 |
| 5. | $W_5$ : Live mulching with 2 rows of cowpea | 204 | 2.28 | 11.32 | 33.2 | 15.4 | 13.6 | 14.0 | 248 | 27.5 | 4916 | 6208 |
| 6. | $W_6$ : Live mulching with 2 rows of sunhemp | 200 | 2.21 | 11.02 | 32.8 | 15.1 | 13.4 | 13.8 | 235 | 26.6 | 4847 | 5901 |
| 7. | $W_7$ : Eucalyptus leaf extract spray @ 15 l $ha^{-1}$ at 15 and 30 DAS | 183 | 1.83 | 9.31 | 31.5 | 13.5 | 12.1 | 12.8 | 204 | 24.6 | 4036 | 5192 |
| 8. | $W_8$: Sunflower extract spray @ 18 l $ha^{-1}$ at 15 and 30 DAS | 180 | 1.77 | 9.04 | 31.2 | 13.4 | 11.8 | 12.7 | 195 | 23.8 | 3952 | 5097 |
| 9. | $W_9$ : Corn gluten meal @ 3.5 t $ha^{-1}$ as PE *fb* HW at 30 DAS | 249 | 2.87 | 14.41 | 39.5 | 18.6 | 15.6 | 16.0 | 328 | 32.2 | 7289 | 7616 |
| 10. | $W_{10}$ : Weedy check (control) | 163 | 1.48 | 6.87 | 25.1 | 11.6 | 10.1 | 11.4 | 161 | 20.3 | 2653 | 4211 |
| | SEm ± | 5.1 | 0.081 | 0.025 | 0.79 | 0.44 | 0.36 | 0.31 | 7.4 | 0.56 | 195.4 | 189.6 |
| | CD @ 5 % | 15 | 0.24 | 0.73 | 2.4 | 1.2 | 1.0 | 0.9 | 21 | 1.7 | 586 | 563 |

lowest weed control efficiency and weed persistence index were noticed with weedy check due to heavy weed infestation throughout the crop growing period (Barad *et al*., 2016).

**Yield attributes and yield**

The results revealed that different organic weed control measures significantly improved the growth, yield attributes and yield of maize (Table 2). Growth parameters of maize, *viz.*, plant height, leaf area index and drymatter production and yield attributes, *viz.*, cob length, cob girth, kernel weight cob$^{-1}$ and hundred kernel weight and kernel and stover yield were significantly higher with corn gluten meal 3.5 t ha$^{-1}$ as pre-emergence followed by HW at 30 DAS over rest of the treatments. Pre-emergence application of corn gluten meal followed by hand weeding might have effectively controlled the weeds throughout the crop growing season which inturn accelerated the plant growth and drymatter production which finally reflected in the form of higher yield attributes and yield. The next best treatment was HW twice at 15 DAS and 30 DAS, however, it was at par with groundnut shells mulch 12.5 t ha$^{-1}$ and mango leaves mulch 5 t ha$^{-1}$. This might be due to lower weed density and biomass at early and later stages of crop growth which inturn led to better translocation of photosynthates from source to developing sink and thereby produced higher yield attributes and yield (Mahto *et al*., 2020).

Live mulching with two rows of cowpea, was comparable with live mulching with two rows of sunhemp in reducing weed growth but reduced the maize kernel yield due to competition between crop and live mulch during the initial stages of the crop growth.The latter two treatments were statistically superior over eucalyptus leaf extract spray @ 15 l ha$^{-1}$ at 15 DAS and 30 DAS ($W_7$) and sunflower extract spray @ 18 l ha$^{-1}$ at 15 DAS and 30 DAS ($W_8$). SPAD chlorophyll meter reading was highest with corn gluten meal @ 3.5 t ha$^{-1}$ as pre-emergence-emergence followed by HW at 30 DAS, which was significantly superior over rest of the weed management practices. Increase in leaf chlorophyll content might be due to efficient weed control throughout the crop growing period and increased nitrogen availability as corn gluten meal contains 10 percent nitrogen content by weight. Mango leaves mulch @ 5 t ha$^{-1}$ was statistically at par with hand weeding twice at 15 DAS and 30 DAS and groundnut shells mulch @ 12.5 t ha$^{-1}$. Higher SPAD readings in the above treatments might be due to effective weed control and better availability of nutrients, moisture to the crop that in turn enhanced the photosynthetic rate of crop resulted in increased supply of carbohydrates leading to increased leaf greenness. Similar results were also reported by Rani *et al*. (2019). Growth parameters, yield attributes, kernel and stover yield of maize were significantly lowest with weedy check due to reduced drymatter production and poor translocation of assimilates from the source to the developing cobs because of severe weed competition.

## CONCLUSIONS

The study revealed that corn gluten meal 3.5 t ha$^{-1}$ as pre-emergence followed by HW at 30 DAS and hand weeding twice at 15 DAS and 30 DAS realized higher kernel yield. The next best organic weed management practice was mango leaves mulch 5 t ha$^{-1}$ or groundnut shells mulch 12.5 t ha$^{-1}$ is considered to be the most effective for obtaining broad-spectrum weed control and to maximize the productivity of maize.

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# RHIZOSPHERE MICROBIOME, SOIL ENZYMES AND GROUNDNUT YIELD AS INFLUENCED BY ADDITION OF BIOCHAR AND FERTILIZERS IN IRRIGATED ALFISOL OF NORTH COASTAL ANDHRA PRADESH

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## ABSTRACT

The field experiment was conducted in irrigated Alfisols of North coastal Andhra Pradesh to study the effect of biochar and chemical fertilizers on rhizosphere microbiome, soil enzymes and yield performance of groundnut crop (variety K-6) during *rabi*, 2018-19. Biochar application to soil significantly increased soil bacterial, fungal and actinomycetes population. At pod development stage, the highest number of bacterial count (39.0 x$10^6$ CFU $g^{-1}$ soil) was observed in $T_5$ (100% RDF + biochar @ 6 t $ha^{-1}$) which was on par with all the biochar applied treatments ($T_3$, $T_4$, $T_6$, $T_7$, $T_8$). The Biochar applied @ 6 t $ha^{-1}$ ($T_5$ & $T_8$) significantly increased the bacterial population as compared to non-biochar applied treatments ($T_1$ & $T_2$). Fungal and actinomycetes population followed the similar trend of bacterial count. Soil urease activity was significantly superior in biochar applied treatments ($T_3$, $T_4$, $T_5$, $T_6$, $T_7$, $T_8$) compared to non biochar applied treatments ($T_1$ and $T_2$). With increased rates of biochar application the urease activity markedly increased in soil. Similar trend was noticed with respect to dehydrogenase, acid phosphatase and alkaline phosphatase enzymes in soil in response to addition of biochar. At pod development stage, the highest leaf area index (3.16) was recorded with 100% RDF + biochar @ 6 t $ha^{-1}$($T_5$) which was significantly higher than $T_1$ (control), $T_2$ (100% RDF) and $T_6$ (75% RDF + biochar @ 2 t $ha^{-1}$). The highest drymatter accumulation of 2951 kg $ha^{-1}$ at peg penetration and 6428 kg $ha^{-1}$ at pod development was observed in $T_5$ (100% RDF + biochar @ 6 t $ha^{-1}$) which was on par with $T_3$ (100% RDF + biochar @ 2 t $ha^{-1}$), $T_4$ (100% RDF + biochar @ 4 t $ha^{-1}$), $T_8$ (75% RDF + biochar @ 6 t $ha^{-1}$) treatments. Groundnut pod yield was highest (4019.58 kg $ha^{-1}$) in treatment received 100% RDF + biochar @ 6 t $ha^{-1}$, which was on par with $T_4$ (100% RDF + biochar @ 6 t $ha^{-1}$) and $T_8$ (75% RDF + biochar @ 6 t $ha^{-1}$).

**Keywords:** Alfisol; Biochar; Groundnut; Rhizosphere microbiome; Soil enzymes

## INTRODUCTION

Biochar is the charcoal obtained by the pyrolysis of biomass, *i.e.*, by incomplete thermal decomposition of organic material under low oxygen conditions. Unlike charcoal and similar materials, biochar is produced with

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the aim of being used as a soil amendment (Lehmann and Joseph, 2009). However, biochar is a more stable solid than the common organic conditioners, due to its very low degradation rate which is estimated as several hundred years for total degradation. Thus, its potential effects on the chemical, physical and biological properties of the soil may extend over a long period of time (Atkinson *et al.,* 2010). Most of the available studies focus on the biochemical effects of biochar on amended soil, including the nutrients that it makes available, as well as on its impact on CEC, pH, vegetative growth, crop yield and its carbon sequestration potential (Atkinson *et al.,* 2010 and Mukherjee and Lal, 2013). Incorporation of biochar into the soil may modify biological and enzymatic activities of soil and till to date, little attention has been paid to investigate the biochar-induced changes on biological properties of sandy loam soils.

Several studies have been carried out throughout the world to identify the effects of incorporating organic matter into the soil, and the resulting advantages for its biological properties are well known (Castellini *et al.*, 2014). In recent years there has been increased use of biochar as an addition to agricultural soils, since it is potentially improving both crop productivity and soil quality (Vaccari *et al.*, 2011; Mukherjee and Lal, 2013). It is an alternative that may be potentially integrated into sustainable agricultural systems. However, an accurate evaluation of the biochar effects on the biological properties and enzymatic activities of the soil is highly essential, since the effects of excessively high inputs are difficult to remedy. There is only very limited information available on impact of biochar on biological properties and enzymatic activities in sandy loam soils, hence the investigation was taken up.

## MATERIALS AND METHODS

The study was carried out during *rabi*, 2018-19. The experimental plot geographically lies between $83^0$ 56.602$^l$ E longitude and $18^0$ 22.752$^l$ N latitude and at an altitude of 12 m above MSL in the Agricultural College Farm, North Coastal Andhra Pradesh. The experimental soil was red sandy loam (Alfisol), neutral in reaction and low in organic carbon. Soil pH was 6.13, EC was 0.27 dS $m^{-1}$ at 25 $^0$C, organic carbon content was 0.36 %, CEC was 14.62 c mol (p+) $kg^{-1}$. Soil available nitrogen 131 kg $ha^{-1}$, available $P_2O_5$ 15.67 kg $ha^{-1}$ and available $K_2O$ 195 kg $ha^{-1}$, respectively. Biochar was prepared under the low oxygen conditions by pyrolysis process with dried crop residue of Mesta (*Hibiscus sabdariffa var. altissima* L., a bast fibre crop of Malvaceae family) with 29.4 percent recovery. The biochar was alkaline in reaction (pH 8.38), slightly saline ( EC 4.39 $dSm^{-1}$), organic carbon 35.04%, CEC 54.26 c mol (p+) $kg^{-1}$ soil, bulk density 0.42 g $cm^{-3}$, water retention 128% on dry weight basis. The field experiment was laid in Randomized block design with eight treatments using peanut (variety - Kadiri 6) as a test crop. $T_1$ - Control; $T_2$ - 100% Recommended dose of fertilizers (RDF of 30 – 40 - 50 kg N- $P_2O_5$- $K_2O$ $ha^{-1}$) only; $T_3$ - 100% RDF + biochar @ 2 t $ha^{-1}$; $T_4$ - 100% RDF + biochar @ 4 t $ha^{-1}$; $T_5$ - 100% RDF + biochar @ 6 t $ha^{-1}$; $T_6$ - 75% RDF + biochar @ 2 t $ha^{-1}$; $T_7$ - 75% RDF + biochar @ 4 t $ha^{-1}$; $T_8$ - 75% RDF + biochar @ 6 t $ha^{-1}$.

Organic carbon content of the soil samples was estimated by wet digestion method (Walkley and Black, 1934). Microbial biomass was estimated by fumigation extraction technique (Sparling and West,1988). Bacteria, fungi and actinomycetes

populations in soil were estimated using the standard procedures (Kapoor and Paroda, 2007).

Enzymatic activity was also determined by using the standard procedures *viz*., Urease activity (µg $NH_4^+$ released $g^{-1}$ soil 2 $hr^{-1}$) by adopting Tris hydroxyl amino methane (THAM) method (Tabatabai and Bremner, 1972); Acid phosphatase and Alkaline phosphatase activity (µg of p- nitrophenol released $g^{-1}$ soil $h^{-1}$) by adopting p-nitro phenol method (Tabatabai and Bremner, 1969), Dehydrogenase activity (µg of TPF produced $g^{-1}$soil $day^{-1}$) by 2,3,5-Triphenyl tetrazolium chloride (TTC) method (Castellini *et al*., 2014).

Plant height (cm) was measured from the base of the plant to the top of the main shoot of the five labeled plants in each plot. Leaf area was measured by using leaf area meter and was expressed as leaf area index (LAI) using the formula suggested by Watson (1952). Plant samples for drymatter study were collected at peg penetration, pod development and harvest stages. At each sampling, five plants were uprooted at random in each treatment in the sampling row. These samples were shade dried followed by oven dried at 65 °C till a constant weight was recorded. The dry weight of these samples was recorded. Later drymatter production was computed on hectare basis and expressed in kg $ha^{-1}$.

## RESULTS AND DISCUSSION

### Rhizosphere microbiome

Biochar application to soil significantly increased soil bacterial, fungal and actinomycetes population (Table 1). In general the bacterial population increased from peg penetration to pod development and then decreased towards harvest. At pod development stage the highest number of bacterial count (39.0 $x10^6$ CFU $g^{-1}$ soil) was observed in $T_5$ (100% RDF + biochar @ 6 t $ha^{-1}$) which was on par with all the biochar applied treatments ($T_3$, $T_4$, $T_6$, $T_7$, $T_8$) and were significantly ($P \leq 0.05$) superior to $T_1$ (control) and $T_2$ (100% RDF alone). The lowest bacterial population (at pod development) of 24.0 $x10^6$ CFU $g^{-1}$ soil was found in $T_1$ (control) at pod development stage. Biochar application to soil encouraged the development of bacteria in

**Table 1. Effect of Biochar and fertilizer addition on Rhizosphere Microbiome (CFU $g^{-1}$ soil) in soil**

| Treatments | Bacteria (×$10^6$) | | | Fungi (×$10^3$) | | | Actinomycetes (×$10^5$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Peg penetration | Pod development | Harvest | Peg penetration | Pod development | Harvest | Peg penetration | Pod development | Harvest |
| $T_1$ | 21.33 | 24.00 | 21.00 | 3.00 | 3.00 | 2.67 | 8.33 | 7.33 | 6.67 |
| $T_2$ | 22.67 | 25.33 | 24.00 | 3.67 | 4.67 | 4.00 | 9.67 | 10.33 | 9.67 |
| $T_3$ | 32.00 | 35.67 | 32.67 | 4.33 | 4.67 | 4.33 | 14.00 | 15.67 | 15.00 |
| $T_4$ | 33.67 | 37.67 | 35.33 | 4.67 | 6.33 | 5.67 | 15.33 | 17.00 | 15.67 |
| $T_5$ | 37.00 | 39.00 | 37.33 | 6.33 | 8.33 | 7.00 | 17.00 | 19.67 | 19.33 |
| $T_6$ | 30.67 | 34.33 | 31.33 | 4.00 | 5.33 | 4.67 | 12.33 | 13.67 | 12.67 |
| $T_7$ | 32.33 | 35.33 | 34.00 | 4.67 | 5.67 | 5.33 | 13.33 | 15.00 | 14.00 |
| $T_8$ | 34.00 | 38.66 | 36.33 | 5.00 | 7.33 | 6.00 | 15.33 | 17.33 | 17.00 |
| SEm± | 2.17 | 1.87 | 1.92 | 0.37 | 0.43 | 0.36 | 0.75 | 1.09 | 0.92 |
| CD @ 5% | 6.60 | 5.77 | 5.83 | 1.12 | 1.33 | 1.11 | 2.28 | 3.33 | 2.79 |
| CV (%) | 12.39 | 10.97 | 10.57 | 14.37 | 13.40 | 12.93 | 10.02 | 13.13 | 11.70 |

biochar treated soil as compared to control (Baronti *et al.*, 2014). Higher bacterial abundance in biochar added soils was due to higher availability of organic carbon for bacterial proliferation (Ming *et al.*, 2016).

The fungal population increased from peg penetration to pod development and then decreased towards harvest. At pod development stage the highest fungal count (8.33 x$10^3$ CFU $g^{-1}$ soil) was observed in $T_5$ (100% RDF + biochar @ 6 t $ha^{-1}$) which was on par with $T_8$ (7.33 x$10^3$ CFU $g^{-1}$ soil) where 75% RDF + biochar @ 6 t $ha^{-1}$ was applied and both $T_5$ and $T_8$ were significantly (P d" 0.05) superior to $T_1$ (control), $T_2$ (100% RDF alone), $T_3$ (100% RDF + biochar @ 2 t $ha^{-1}$), $T_4$ (100% RDF + biochar @ 4 t $ha^{-1}$), $T_6$ (75% RDF + biochar @ 2 t $ha^{-1}$) and $T_7$ (75% RDF + biochar @ 4 t $ha^{-1}$). Biochar application to soil led to increased soil organic carbon which may serve as an energy source to fungi and secretion of flavanoids, sesquiterpenes and strigolactones by plant roots might have resulted in increased colonization of plant roots by AM fungi and increased spore germination and hyphal branching of AM fungi.

At pod development the highest number of actinomycetes population (19.67 x$10^5$ CFU $g^{-1}$ soil) was observed in $T_5$ (100% RDF + biochar @ 6 t $ha^{-1}$) which was on par with $T_8$ (75% RDF + biochar @ 6 t $ha^{-1}$) and T4 (100% RDF+ Biochar 4 t $ha^{-1}$), both $T_5$ (100% RDF+ Biochar 6 t $ha^{-1}$) and $T_8$ (75% RDF+ Biochar 6 t $ha^{-1}$) were significantly (P d" 0.05) superior to $T_1$ (control), $T_2$ (100% RDF), $T_3$ (100% RDF + biochar @ 2 t $ha^{-1}$), $T_6$ (75% RDF + biochar @ 2 t $ha^{-1}$) and $T_7$ (75% RDF + biochar @ 4 t $ha^{-1}$). The lowest actinomycetes population (7.33 x$10^5$ CFU $g^{-1}$ soil) was found in $T_1$ (control) at pod development stage. Increased soil pH due to biochar application, caused increased actinomycetes population in soil (Watzinge *et al.*, 2014). Ability of actinomycetes to degrade persistant and complex substrates like biochar could be a reason for increased actinomycetes population (Vaccari *et al.*, 2011 and Yun *et al.*, 2017)

**Organic carbon and microbial biomass carbon of soil**

The effect of biochar on soil organic carbon content (Table 2) indicated that significant increase in organic carbon of soil in 4 t $ha^{-1}$ and 6 t $ha^{-1}$ biochar applied treatments ($T_4$, $T_5$, $T_7$ & $T_8$) than no biochar added treatments ($T_1$ and $T_2$). Increasing trend of organic carbon was noticed from peg penetration to harvest stage. At harvest stage, the highest organic carbon (0.54%) was observed in $T_8$ (75% RDF + biochar @ 6 t $ha^{-1}$) treatment which was on par with $T_5$ (100% RDF + biochar @ 6 t $ha^{-1}$) treatment (0.53%) and both the treatments were significantly higher to $T_1$ (control) and $T_2$ (100% RDF). The increased rates of application of biochar to soil significantly increased soil organic carbon content. Biochar being high organic carbon source, up on its application to the soil releases carbon into the soil system and also due to the mineralization of biochar adsorbed organic matter in soil system resulted in increased organic carbon content in the soil (Abrishamkesh *et al.*, 2015). Furthermore, biochar itself is a matrix of organic complex and its application to soil system increases soil organic carbon content (Elangovan and Chandrasekharan, 2014).

Microbial biomass significantly influenced by biochar addition. The highest microbial biomass of 326.5 µg $g^{-1}$ soil at pod development stage and 349.4 µg $g^{-1}$ at harvest were noticed when biochar applied @ 6 t $ha^{-1}$+ 75% RDF ($T_8$) which is on par with $T_5$ (biochar @ 6 t $ha^{-1}$+ 100% RDF). The lowest microbial

**Table 2. Effect of biochar and fertilizer addition on oxidisable organic carbon and microbial biomass carbon in soil**

| Treatments | Soil oxidizable organic carbon (%) | | | Soil microbial biomass carbon (µg $g^{-1}$) | | |
|---|---|---|---|---|---|---|
| | Peg penetration | Pod development | Harvest | Peg penetration | Pod development | Harvest |
| $T_1$ | 0.30 | 0.30 | 0.32 | 116.8 | 156.1 | 182.6 |
| $T_2$ | 0.32 | 0.34 | 0.34 | 112.6 | 166.8 | 178.9 |
| $T_3$ | 0.39 | 0.41 | 0.43 | 185.2 | 252.4 | 277.3 |
| $T_4$ | 0.45 | 0.46 | 0.48 | 203.9 | 280.3 | 291.8 |
| $T_5$ | 0.51 | 0.52 | 0.53 | 231.3 | 323.9 | 335.7 |
| $T_6$ | 0.40 | 0.41 | 0.44 | 178.5 | 236.2 | 271.5 |
| $T_7$ | 0.45 | 0.47 | 0.48 | 195.3 | 269.7 | 290.2 |
| $T_8$ | 0.52 | 0.51 | 0.54 | 239.1 | 326.5 | 349.4 |
| **SEm±** | 0.03 | 0.04 | 0.04 | 14.9 | 17.1 | 13.9 |
| **CD @ 5%** | 0.09 | 0.12 | 0.13 | 45.2 | 51.5 | 41.9 |
| **CV (%)** | 10.82 | 10.22 | 11.36 | 9.96 | 9.92 | 11.05 |

biomass observed in control ($T_1$). Microbial biomass markedly increased with increasing rates of biochar from 2 to 6 t $ha^{-1}$.

**Soil enzymes**

The impact of biochar addition on soil enzymes activity indicated significant ($P \leq 0.05$) influence from peg penetration to harvest. The

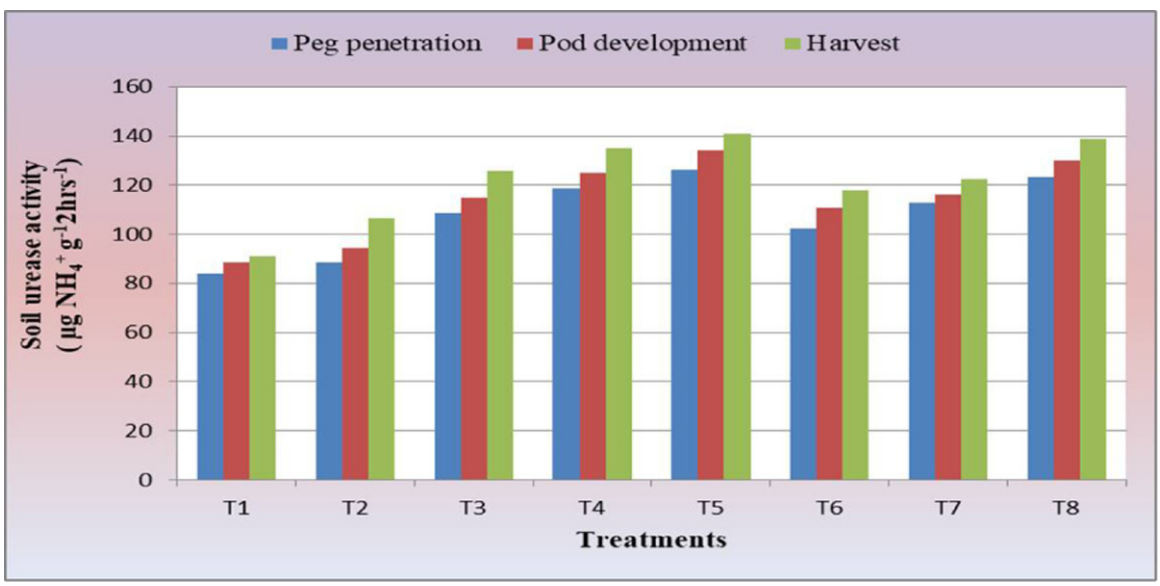


**Fig.1. Soil Urease activity (µg $NH_4^+$ $g^{-1}2hr^{-1}$) in soil at different stages of crop growth ingroundnut**

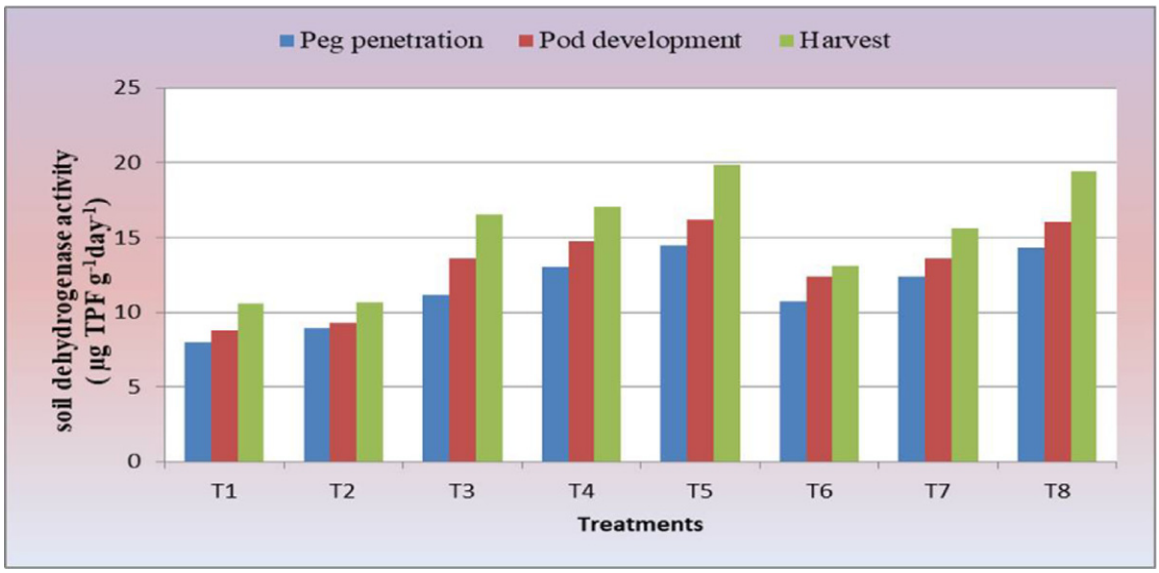


**Fig.2. Soil dehydrogenase activity (µg TPF $g^{-1}day^{-1}$) in soil at different stages of crop growth in groundnut**

highest soil urease enzyme activity (Fig. 1) of 126.3 µg $NH_4^+$ $g^{-1}$ 2 $h^{-1}$, 134.3 µg $NH_4^+$ $g^{-1}$ 2 $hs^{-1}$ and 140.67 µg $NH_4^+$ $g^{-1}$ 2 $hr^{-1}$, at peg penetration, pod development and harvest stages of peanut respectively was found in $T_5$ (100% RDF + biochar addition @ 6 t $ha^{-1}$) which was significantly higher than control ($T_1$) and 100% RDF treatment ($T_2$). Urease activity increased with increased rates of biochar application from 2 t $ha^{-1}$ to 6 t $ha^{-1}$. Increased urease activity in soil by addition of biochar in wheat growing soils was reported by Du *et al.*

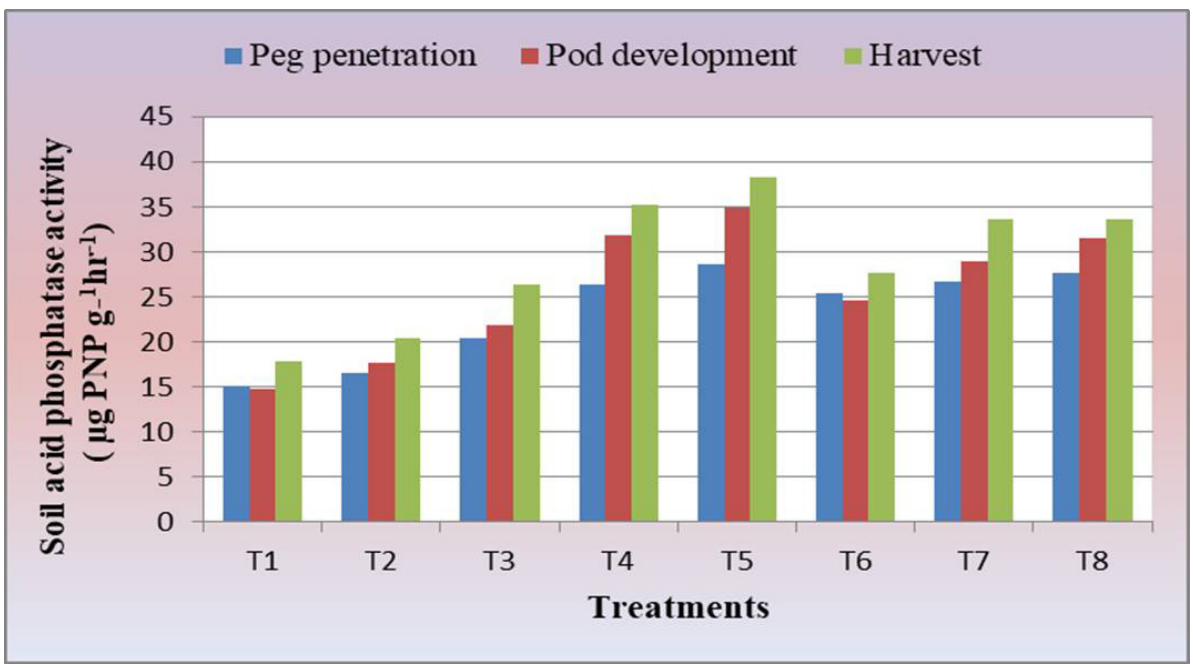


**Fig.3. Soil acid phosphatase activity (µg PNP $g^{-1}hr^{-1}$) in soil at different stages of crop growth in groundnut**

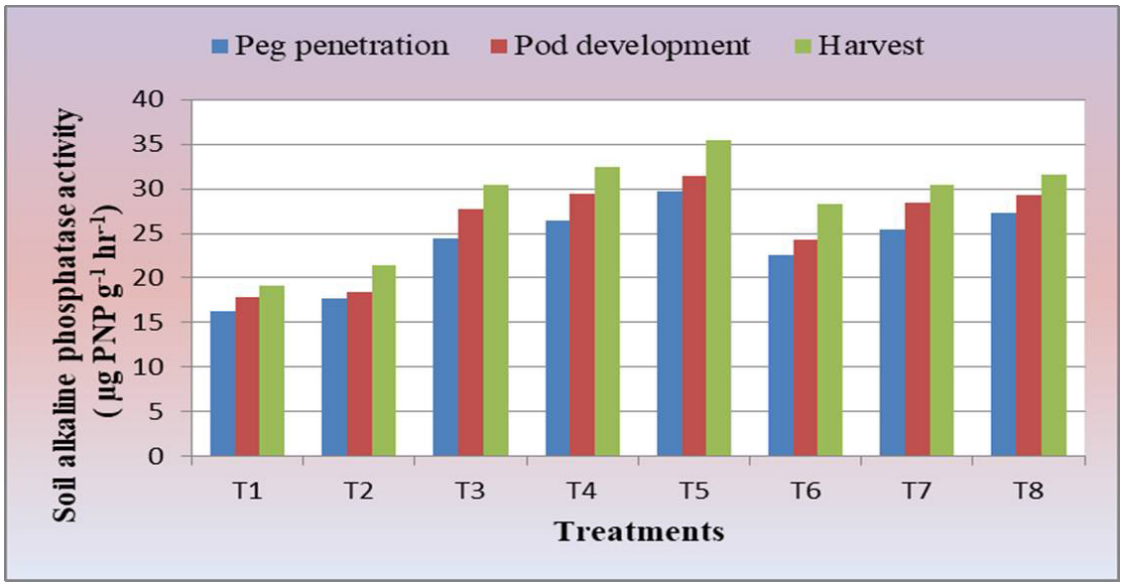


**Fig.4. Soil alkaline phosphatase activity (µg PNP g$^{-1}$hr$^{-1}$) in soil at different stages of crop growth in groundnut**

(2014). Highest dehydrogenase enzyme activity (Fig. 2) of 14.48 µg TPF g$^{-1}$ day$^{-1}$ was recorded in $T_5$ (100% RDF + biochar @ 6 t ha$^{-1}$) which was on par with $T_8$ and $T_4$ and significantly (P d" 0.05) higher to $T_1$ (control), $T_2$ (100% RDF only) and $T_3$ (100% RDF + biochar @ 2 t ha$^{-1}$) and $T_6$ (75% RDF + biochar @ 2 t ha$^{-1}$) and $T_7$ (75% RDF + biochar @ 4 t ha$^{-1}$) at peg penetration stage. Volatile matter content in biochar might have led to higher dehydrogenase activity with higher rates of its addition (Ouyang *et al.*, 2014).

Biochar application significantly (P d" 0.05) influenced the acid and alkaline phosphatase activities (Fig. 3 & 4) in soil. The highest acid and alkaline phosphatase activities of 38.23 µg PNP g$^{-1}$ hr$^{-1}$ and 29.77 µg PNP g$^{-1}$ hr$^{-1}$ were observed in $T_5$ (100% RDF + biochar @ 6 t ha$^{-1}$). The increase in acid and alkaline phosphatase activity by biochar addition could be due to enhancement of enzyme function caused by interaction with biochar (Jindo *et al.*, 2012 and Chen *et al.*, 2013).

**Plant growth and yield**

Biochar application to soil caused significant variations in leaf area index (LAI) of groundnut (Table 3). The LAI increased from peg penetration to pod development. At pod development stage, the highest LAI (3.16) was recorded in $T_5$ treatment (100% RDF + biochar @ 6 t ha$^{-1}$) which was significantly higher than $T_1$ (control), $T_2$ (100% RDF) and $T_6$ (75% RDF + biochar @ 2 t ha$^{-1}$). Drymatter accumulation increased from peg penetration to harvest (Table 3). Highest drymatter accumulation of 2951 kg ha$^{-1}$ and 6428 kg ha$^{-1}$ at peg penetration and pod development stage, respectively was observed in $T_5$ (100% RDF + biochar @ 6 t ha$^{-1}$) which was on par with $T_3$ (100% RDF + biochar @ 2 t ha$^{-1}$), $T_4$ (100% RDF + biochar @ 4 t ha$^{-1}$), T7 (75% RDF + biochar @ 4 t ha$^{-1}$) and $T_8$ (75% RDF + biochar @ 6 t ha$^{-1}$) treatments and significantly superior to $T_6$ (75% RDF + biochar @ 2 t ha$^{-1}$), $T_2$ (100% RDF) and $T_1$ (control). Application of biochar resulted in better soil physical environment and also increased availability of

**Table 3. Effect of biochar and fertilizer addition on growth parameters, dry matter accumulation and yield parameters of groundnut**

| Treatments | Leaf area index | | | Dry matter accumulation (kg ha$^{-1}$) | | Pod yield (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|
| | Peg penetration | Pod development | Harvest | Peg penetration | Pod development | |
| $T_1$ | 1.62 | 2.40 | 2.33 | 2135 | 4973 | 2877 |
| $T_2$ | 1.85 | 2.68 | 2.45 | 2545 | 5643 | 3437 |
| $T_3$ | 2.01 | 2.85 | 2.61 | 2669 | 5858 | 3539 |
| $T_4$ | 2.05 | 2.93 | 2.69 | 2835 | 6214 | 3887 |
| $T_5$ | 2.19 | 3.16 | 2.73 | 2951 | 6428 | 4020 |
| $T_6$ | 1.83 | 2.61 | 2.40 | 2507 | 5473 | 3393 |
| $T_7$ | 1.96 | 2.75 | 2.42 | 2732 | 5611 | 3613 |
| $T_8$ | 1.98 | 2.83 | 2.58 | 2784 | 5706 | 3783 |
| **SEm±** | 0.07 | 0.09 | 0.07 | 117 | 244 | 157.0 |
| P ≤ 0.05 | 0.21 | 0.26 | 0.23 | 356 | 641 | 476.3 |
| CV (%) | 6.51 | 6.63 | 5.32 | 7.67 | 7.36 | 7.60 |

nutrients by improving biological activity which resulted in higher plant growth and biomass production (Rao *et al*., 2017). Biochar addition to soil improved microbial activity, nutrients availability and plant growth (Lehmann and Joseph, 2009).

Effect of biochar on groundnut pod yield (Table 3) revealed that highest pod yield (4020 kg ha$^{-1}$) in $T_5$ (100% RDF + biochar @ 6 t ha$^{-1}$) which was on par with $T_4$ (3887 kg ha$^{-1}$), $T_8$ (3783 kg ha$^{-1}$), $T_7$ (3613 kg ha$^{-1}$). However, the pod yield of groundnut in $T_5$ was significantly higher than that of $T_1$ (control), $T_2$ (100% RDF), $T_3$ (100% RDF + biochar @ 2 t ha$^{-1}$) and $T_6$ (75% RDF + biochar @ 2 t ha$^{-1}$). The increase in pod yield yield with the biochar addition was due to increased retension of water and nutrients in soil, availability of soil bounded nutrients through chelation with concomitant absorption by the plants (Agegnehu *et al*., 2015 and Balasubramanian *et al*., 2021).

## CONCLUSIONS

Significant increase of rhizosphere microbiome *viz*., microbial population and soil enzymes (urease, dehydrogenase, acid and alkaline phosphatase) was noticed with addition of biochar to soil compared to the treatments which did not receive biochar in sandy loam soils. Biochar addition to soil @ 6 t ha$^{-1}$ resulted in marked increment in microbial population and enzyme activities compared to 2 t ha$^{-1}$ and control treatments. Application of biochar significantly increased growth, biomass production and groundnut pod yield. Higher pod yields of groundnut was recorded when biochar applied @ 6 t ha$^{-1}$ + 100% RDF, 4 t ha$^{-1}$ + 100% RDF, and 6 t ha$^{-1}$ + 75% RDF which were significantly higher to control and RDF alone applied treatments. This research provided information on the effect of biochar in combination of inorganic fertilsiers on soil biological properties and performance of peanut.

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# SEASONAL INCIDENCE OF *Rhopalosiphum maidis* (Fitch) ON MAIZE CROP IN GUNTUR AND KRISHNA DISTRICTS OF ANDHRA PRADESH

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## ABSTRACT

The seasonal occurrence of *Rhopalosiphum maidis* (Fitch) on maize was carried out in the Guntur and Krishna districts of Andhra Pradesh during *rabi,* 2021-2022 and *rabi,* 2022-2023. A total of 30 farmer fields were selected from six mandals of maize growing areas *viz.,* Bapatla, Tsunduru, Ponnur, Karlapalem in Guntur district and two mandals *viz*., Mopidevi and Avanigadda in Krishna district.The population of aphids was recorded at weekly intervals on randomly selected 25 plants of each farmer field from germination to harvesting stage of the crop. The aphids were counted as number of aphids on 1 $cm^2$ leaf area from three leaves (top, middle, lower) of each plant. The data was statistically analyzed using SPSS for Windows (version 20.0) and Microsoft Excel 2016. In *rabi* 2021-22 the initial incidence of aphids was commenced from SMW5 (0.21 aphids sq. $cm^{-1}$) and the highest mean population was recorded in SMW 9 (3.64 aphids sq. $cm^{-1}$). In *rabi* 2022-23 the initial incidence was first noticed during SMW4 (1.71 aphids sq. $cm^{-1}$) and the peak incidence was noticed during SMW 6(4.96 aphids sq. $cm^{-1}$).The population of aphids showed a positive significant correlation with maximum temperature in both the seasons *i.e.*, *rabi*, 2021-22 (r=0.685**) and *rabi,* 2022-23 (r=0.612*).

**Keywords:** Abiotic factors, Aphid, Maize, Peak incidence, Seasonal occurrence

## INTRODUCTION

Maize (*Zea mays* L.) is an important cereal crop in the world's agricultural economy. Worldwide, it is popular because of its high genetic yield potential and wider adaptability (Kalyan *et al*., 2019). In India, maize is emerging as third most important crop after rice and wheat. In 2021-2022, maize was grown in area of 2.17 m.ha during *rabi* and 7.78 m. ha during *kharif* with a productivity of 2914 kg $ha^{-1}$ and 5084 kg $ha^{-1,}$ respectively. Whereas, in Andhra Pradesh maize was grown in an area of 2.12 mha in *rabi* and 1.30 m. ha in *kharif*, with a productivity of 6880 kg $ha^{-1}$ and 3390 kg $ha^{-1}$, respectively (GOI, 2022). In addition as a staple food for human being and quality feed for animals, it serves as a basic raw material for thousands of industrial products that

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includes starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceutical, cosmetic, film, textile, gum, packageand paper industries.The yield potential of a maize cultivar when grown in field, experiences various biotic (diseases, insects and weeds) and abiotic (temperature, moisture, wind) constraints, which reduces quantity and quality of the produce, resulting in crop losses (Ravindren *et al*., 2020). Among biotic factors, insect pests are one of the major limitations for low yield of maize. In India, nearly 32.1 percent of the actual produce is lost due to insect pests. As many as 141 insect pests cause varying degree of damage to maize crop right from sowing till harvest. Corn leaf aphid (*Rophalosiphum maidis* Fitch), *Rhopalosiphum maidis* is a major agricultural pest and polyphagous attacking more than 182 plant species (Alam *et al*., 2014).

Global climate change signifies an increase in average temperature, change in the rainfall pattern and enormous climatic events. These seasonal and long-term variations would influence the fauna, flora and population of insect-pests (Bhamare *et al.,* 2023). The studies on seasonal incidence of aphid on maize crop and their correlation with the weather parameters provide basic information about seasonal occurrence of insect pest. This would facilitate to execute proper development of spray schedule management strategies for the control of the pest.

## MATERIALS AND METHODS

### Sampling procedure

The study was carried out in the Guntur and Krishna districts of Andhra Pradesh during *rabi,* 2021-2022 and *rabi,* 2022-2023. Based on the statistical data obtained from the Department of Agriculture, mandals with the highest area under maize cultivation were selected. Four mandals *viz.,* Bapatla, Tsunduru, Ponnur, Karlapalem in Guntur district and two mandals *viz.*, Mopidevi and Avanigadda in Krishna district, with a total of 12 villages were selected.During *rabi,* 2021-2022 and *rabi,* 2022-2023, the data on the seasonal incidence of aphid was collected at weekly intervals in 30 farmer fields in the maize ecosystem through a roving survey, where the sampling was done randomly in fixed plots in each farmer field.The data was collected on 25 randomly selected maize plantsin hypothetically assumed and marked with Z or W path (Kalyan *et al.,* 2019) leaving five meters distance from the field boarders.

### Meterological Data

The meterological data for the study was obtained from two meterological observatories *i.e.,* Agricultural Research Station (ARS) Bapatla and Agricultural Research Station (ARS), Machilipatnam.

## OBSERVATIONS RECORDED

### *Rhopalosiphum maidis*

The population of aphids was recorded at weekly intervals on randomly selected 25 plants of each farmer field from germination to harvesting stage of the crop. The aphids were counted as number of aphids on 1 $cm^2$ oleaf area from three leaves (top, middle, lower) of each plant(Lal *et al*., 2017).

The statistical analysis of the data on aphid population on *rabi* maize and weather parameters were carried out by simple correlation and multiple linear regression using SPSS for Windows (version 20.0) and Microsoft Excel 2016.

## RESULTS AND DISCUSSION

### Incidence of aphid during *rabi,* 2021-22

The data on aphid incidence (Fig.1) revealed that the initial mean population of aphids commenced from Standard Meterological Week 5 (SMW5) *i.e*., 0.21

aphidssq. cm$^{-1}$and gradually increased causing the highest mean population recorded in February during SMW 9 *i.e.,* 3.64 aphids sq. cm$^{-1}$, where the crop is at silking to cob development stage. The above findings were in parallel with Mahmoud *et al.* (2021) who reported peak infestation of aphid at 97 days after germination.

The incidence of aphids was highest during SMW 9 *i.e.,* 3.64 aphidssq. cm$^{-1}$leaf area, when favourable abiotic conditions prevailed *i.e.,* 32.71 °C of maximum temperature, 18.23 °C of minimum temperature, 84.71% morning relative humidity, 57.00% evening relative humidity. The correlation between aphid infestation with abiotic factors (Table 1) revealed a significant positive correlation with maximum temperature (0.685**) and a positive correlation with minimum temperature (0.238), while evening relative humidity (-0.340), morning relative humidity (-0.294), and rainfall (-0.274) showed a negative non-significant correlation.Bhamare *et al.* (2023) also reported thatevening relative humidity (r= -0.011) showed a negative, non-significant correlation with the population of aphids. Patil *et al*. (2015) and Kumar *et al*. (2016) noticed a negative, non-significant correlation betweenrainfall and population of aphids.

The multiple linear regression analysis revealed that all the weather parameterscould influence the population of *R. maidis* to the extent of 81 percent *i.e*., $R^2$ =0.813 (Table 2).

**Incidence of aphid during *rabi,* 2022-23**

The incidence of aphid was first noticed in 4$^{th}$ week of January *i.e*., SMW 4 (1.71 aphids sq.cm$^{-1}$ leaf area) and continued till harvest *i.e.,* SMW13 (26$^{th}$ Mar- 01 Apr 2022). The peak incidence of aphid was recorded in second week of February *i.e*., SMW 6 (4.96 aphids sq. cm$^{-1}$ leaf area), when the crop is at silking to maturity stage (Fig 2).The results of were also in agreement with the findings of Waleed *et al*. (2020). Choudhary *et al*. (2017) who illustrated that population of *R. maidis* appeared in the first week of January and reached to peak in the first week of February.

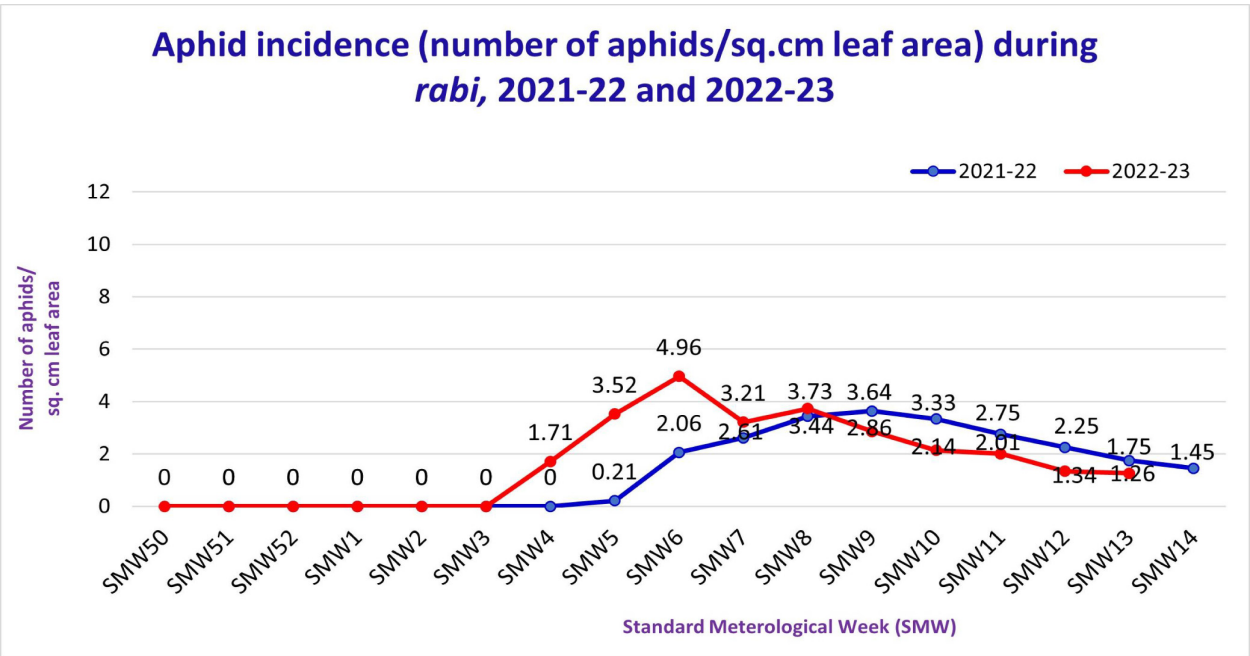


**Figure 1. Seasonal incidence of *Rhopalosiphum maidis* on maize during *rabi*,2021-'22 and 2022-'23**

**Figure 2. Infestation of *Rhopalosiphum maidis* on maize during *rabi,* 2021-22 and 2022-23**

**Table 1. Correlation of *Rhopalosiphummaidis* population with weather parameters on maize during *rabi,* 2021-22 and 2022-23**

| S.No. | Season and Year | Correlation coefficient (r) | | | | |
|---|---|---|---|---|---|---|
| | | Temperature °C | | Relative humidity (%) | | Rainfall (mm) |
| | | Maximum | Minimum | Morning | Evening | |
| 1. | *Rabi* 2021-2022 | 0.685** | 0.238 | -0.294 | -0.340 | -0.274 |
| 2. | *Rabi* 2022-2023 | 0.612* | 0.003 | -0.019 | -0.396 | -0.273 |

**Correlation is significant at 0.01 level (2-tailed)

*Correlation is significant at 0.05 level (2-tailed)

**Table 2. Multiple linear regression equation for *Rhopalosiphum maidis* on maize with weather parameters during *rabi*, 2021-22 and 2022-23**

| S.No. | Season and Year | Regression equation | $R^2$ |
|---|---|---|---|
| 1. | *Rabi* 2021-2022 | Y= -57.274+1.104 X1-0.270 X 2+0.351 X3-0. 002X4+0.005X5 | 0.813 |
| 2. | *Rabi* 2022-2023 | Y= -3.679+1.197X1-0.411X2-0.335X3+0.062X4+0.042X5 | 0.513 |

Note:$X_1$ = Maximum temperature (Mean), $X_2$ = Minimum temperature (Mean), $X_3$= Morningrelativehumidity(Mean), $X_4$ = Eveningrelativehumidity(Mean), $X_5$ = Rainfall (Mean)

The incidence of aphids was highest during SMW 6 *i.e.,* 4.96 aphids sq.cm$^{-1}$ leaf area, when favourable abiotic conditions prevailed *i.e.,* 32.3 $^{o}$C of maximum temperature, 18.2 $^{o}$C of minimum temperature, 85.9% morning relative humidity, 72.3% evening relative humidity.Similar to the findings, Kumar *et al*.(2016) also reported highest population of aphids on maize was found in the month of February. The results of the correlation between aphid and abiotic factors (Table 1) revealed a positive significant correlation with maximum temperature (0.612*) and a non-significant positive correlation with minimum temperature (0.003), whereas, morning relative humidity (-0.019), evening relative humidity (-0.396) and rainfall (-0.273) showed non-significant negative correlation. Lal *et al*. (2017) also conducted a similar study and reported that maximum temperature (0.88*) had a significant positive correlation.

The multiple linear regression analysis showed that all the weather parameters together responsible for a variationin the population of *R. maidis* to the extent of 51 percent *i.e*., $R^2$ =0.513 (Table 2).

## CONCLUSIONS

The initial occurrence of *R. maidis* was observed during SMW 5 (0.21 aphidssq. cm$^{-1}$ leaf area and reached peak population in SMW9 (3.64 aphids sq. cm$^{-1}$ leaf area) during *rabi* 2021-22. In *rabi,* 2022-23, the initial incidence was first noticed in SMW 4 (1.71 aphids sq.cm$^{-1}$ leaf area) and highest population of aphid was noticed during SMW 6(4.96 aphids sq. cm$^{-1}$ leaf area). The correlation between aphids and abiotic factors revealed a positive significant correlation with maximum temperature during *rabi* 2021-22 (r=0.685**) and *rabi* 2022-23 (r=0.612*). The multiple linear regression analysis revealed weather parameters could influence the population of *R. maidis* on maize to the extent of 81 percent ($R^2$ =0.813) and 51 percent ($R^2$ =0.513) during *rabi,* 2021-22 and 2022-23, respectively. This study provides an opportunity for the development of management strategies significant for the control of the pest. The results are benefitting to promote Economic Threshold Level (ETL) among farmers as a result of which unnecessary and irrational usage of pesticides can be reduced.

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# EFFECT OF CULTURE MEDIA ON GROWTH AND SPORULATION OF *Colletotrichum lindemuthianum* (SACC AND MAGN) ISOLATES CAUSING ANTHRACNOSE OF FIELD BEAN

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## ABSTRACT

Anthracnose (*Colletotrichum lindemuthianum*) is the most important fungal disease affecting field bean. Growth and sporulation of thirty six isolates of *C. lindemuthianum* collected from five agro-climatic zones of Andhra Pradesh was evaluated in the year 2017-18 on four different solid and liquid culture media. Results revealed that potato dextrose agar was the best for both radial growth (66.74 mm) and dry mycelial weight (264.74 mg), whereas, host leaf extract agar significantly supported more sporulation 7.3 x$10^4$. The least mycelia growth of 39.32 mm, dry mycelial weight of 234.70 mg and sporulation 2.9 x$10^4$ were observed on Sabourband's agar. Among the different isolates, CI-33 grew fast with mycelial growth and sporulation (78.86 mm and 8.4x$10^4$) and CI-32 produced the highest dry mycelial weight 266.75 mg). The least radial growth (31.35 mm), the lowest sporulation (4.1 x$10^4$) and lowest mycelial dry weight (227.75 mg) was recorded in CI-28, CI-1 and CI 17, respectively. Mean conidial length of 36 isolates ranged from 11.87 to 13.03 µm with the maximum length (13.03 µm) recorded in host leaf extract agar, while minimum was recorded in oat meal agar. Width of conidia varied from 3.59 to 4.11 µm on different media and 3.53 to 4.23 µm in different isolates.The growth phase study revealed that increase in the dry mycelial weight of fungus upto 15 days of incubation and growth declined from 17$^{th}$ day onwards.

**Keywords:** *Colletotrichum lindemuthianum,* Culture media, Field bean, Radial growth, Sporulation

## INTRODUCTION

Field bean, *Lablab purpureus* L. (Sweet), (*Dolichos hyacinth bean*), is an ancient herbaceous multipurpose legume crop. It is used mainly in animal feeding, in the form of fresh forage, hay, forage meal, grain and straw, grazing and browsing, and for human consumption, as fresh leaves, immature pods, immature grains and mature grains (Mihailovic *et al*., 2010). It is also used for green manuring, erosion control, nitrogen fixation and drought tolerance, cover crop in orchards and also as a weed smothering crop (Murphy and Colucci, 1999). Plant residues are used either as animal fodder or chopped into the soil as a green manure and known as poor man's bean (Ismunadji and Arsyad, 1990) and is known by different names in different parts of India. Anthracnose caused by *Colletotrichum*

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*lindemuthianum* is one of the major fungal diseases and has been reported from all regions of India on various beans in mild to severe form. The pathogen is often present in or on the seed produced in infected pods. Infected seed may show yellowish to brown sunken lesions.It causes considerable damage by reducing seed quality and yield (Sharma *et al*., 2008). Every living being requires food for its growth and reproduction; fungi are not an exception to it. Fungi secure food and energy from the substrate upon which they live in the nature. In order to culture the fungi in the laboratory, it is necessary to supplement those essential elements and compounds in the medium which are required for their growth and other life processes. Neither all media are equally good for all fungi nor can be a universal substrate or artificial medium on which all fungi can grow and reproduce well. Variation in the growth and sporulation of *Colletotrichum* isolates from different crops was reported by earlier workers (Krishna *et al.,* 2011, Kumar *et al*., 2017). Fungal isolates from different geographical locations may behave differently to utilize different media for their growth and sporulation. Therefore laboratory studies were conducted using different media to identify suitable medium for growth and sporulation of *C. lindemuthianum* isolated from field bean.

## MATERIALS AND METHODS

### Isolation of fungi

Field bean growing areas were surveyed at flowering and pod formation stages and about 96 diseased samples from different plant parts showing anthracnose symptoms were collected from Andhra Pradesh in the year 2016-17. The diseased samples showing typical symptoms were thoroughly washed repeatedly in tap water. Thereafter, small pieces of about half centimetre were cut using the sterilized blade for isolation. Care was taken to ensure that each cut portion had some healthy part as well. The pieces were then surface sterilized with 0.1% mercuric chloride for 20-30 seconds and rinsed thoroughly three times with sterile distilled water to remove disinfectants and traces of chemical. Leaf bits were dried using blotter paper to remove excess moisture and transferred aseptically onto PDA plates. The Petri plates were incubated at 25±1 °C. After 48-72 h of incubation, the marginal mycelial growth was aseptically removed for further sub-culturing on PDA plates. Single spore isolation technique was used for getting pure culture of the fungus. Pathogen causing the anthracnose of field bean was identified based on morpho-taxonomic characters (Baxter *et al*., 1983). The pure cultures were preserved at low temperature (4±1 °C) in refrigerator for further use.

### Preparation of culture media

Potato Dextrose Agar (PDA), Oat Meal Agar (OMA), Host Leaf Extract Agar (HLEA) and Sabouraud's agar (SA) both solid as well as liquid form, were prepared to culture fungus. The composition of liquid media was same as in solid media except omission of agar agar. The pH of the media was adjusted to 7.0 before sterilization by adding 0.1N HCl or 0.1N NaOH. The standard composition and technique for media preparation were adapted from Dhingra and Sinclair (2012).

### Growth and sporulation of *C. lindemuthianum* isolates on different solid media

Twenty ml of respective medium mentioned above was poured into 90 mm diameter Petri plates. After solidification, 5 mm discs of *C. lindemuthianum* isolate from seven

days old actively growing culture were cut using a cork borer and a single disc was placed upside down at the centre of Petri plate. The experiments were conducted using a completely randomized design with two replications and the plates were incubated at 25±1 °C. The measurements of the colony diameter were taken.

**Fungus growth measurement technique**

Radial growth of the fungus was determined directly by measuring the diameter of colonies from underside the culture plate against light in the perpendicular axis when the maximum growth was attained in any one of the media tested.

**Assessment of sporulation**

Sporulation of individual isolates on different media was assessed 15 days after incubation. At the end of incubation period, five mm mycelial discs of each isolate was cut with a sterile cork borer from the centre to the margin at three different equidistant places along the radius of the growth. Three discs were placed in a sterile test tube containing 15 ml of sterile distilled water and the conidia were brought into the suspension by crushing with a glass rod. The suspension was shaken in a vortex mixer to separate the conidia. From this spore suspension, 0.01 ml was transferred on to the counting chamber of the haemocytometer and the number of spores in a small square at the centre was counted to calculate the total number of spores in 1 ml of the spore suspension. Three replications were maintained for each isolate and average number of spores per ml was determined.

**Measurement of spore size**

Micrometric measurement of spores was done by standardized ocular micrometer. Average and range of 20 spores were taken per each isolate.

**Growth on different liquid media**

One hundred ml of different liquid broth media were added separately into each of 250 ml conical flasks. These flasks were then sterilized at 1.1 kg $cm^{-2}$ pressure for 20 min. The flasks were inoculated with five mm mycelial discs obtained from seven days old culture and incubated at 25±1 °C. Each treatment was replicated twice. Fifteen days after incubation the cultures were harvested separately at a time by filtration through previously weighed Whatman No. 42 filter paper of 12.5 cm diameter, which were dried to a constant weight at 60 °C in an electric oven prior to filtration. The mycelial mat on the filter paper was thoroughly washed with sterile distilled water to get rid of the salts likely to be associated with the mycelial mass. The filter paper along with the mycelial mat were dried to a constant weight at 60 °C for 48 h, cooled in desiccators and weighed immediately on an analytical balance. The difference between final and the initial weight of filter discs were taken as the weight of the mycelia.

**RESULTS AND DISCUSSION**

The pathogen causing anthracnose of field bean was identified as *Colletotrichum lindemuthianum.* Based on the similarities of morpho-taxonomic characters, 36 isolates representing one or two samples for each major field bean growing area were selected. Wide range of media was used for isolation of different groups of fungi that influence the mycelial growth, colony morphology, pigmentation and sporulation depending upon the composition of specific culture medium. Type of culture media and their chemical compositions significantly affected the mycelial growth and conidial production of *C. lindemuthianum.*

**Effect of solid media on growth and sporulation**

Considerable differences were recorded with respect to macroscopic and microscopic characteristics of different isolates of *C. lindemuthianum* on four solid media *viz*., growth rate, colony diameter, spore size and sporulation capacity *etc*. All the four culture media supported the mycelial growth of all the isolates of *C. lindemuthianum* (Table 1). Potato dextrose agar (66.74 mm) and host leaf extract agar (66.20 mm) were found to be the best and supported vegetative growth of different isolates of *C. lindemuthianum* and were statistically on par with each other. Oat meal agar medium (62.88 mm) was next best and the least growth was obtained on Sabourband's agar with an average diameter of only 39.32 mm. The effect of different media on the growth of isolates, difference in the growth of different isolates on these media and their interaction was found significant.

Significant differences were noticed with respect to growth of different isolates on solid media, CI-33 grew fast with mycelial growth of 78.86 mm followed by CI-34 (77.38 mm) and CI-32 (76.69 mm) and they were at par. Isolates CI-35 (75.50 mm) and CI-25 (72.50 mm) both were statistically at par. The least growth was observed in CI-28 with an average diameter of only 31.35 mm.

The interaction of media and isolates was also significant, isolate CI-32 produced maximum radial growth of 90.00 mm on host leaf extract agar and which was superior to the other isolates. The next best isolates grew fast in the media were CI-33 (89.75 mm) on HLEA and PDA (88.70 mm); CI-32 on PDA (87.75 mm); CI-34 on PDA (87.75 mm) and HLEA (87.25 mm); CI-35 on PDA and HLEA (86.00 mm); CI-33 and CI-34 on OMA (85.50 and 84.00 mm); CI-25 on PDA (85.50 mm) and HLEA (84.50 mm), which were on par with each other.

Similar trend was noticed with respect to growth rate of different isolates on four solid media. The effect of media and isolates and their interaction were significant on relative growth rate. Fast growth rate of 4.45 and 4.41 mm was noticed in potato dextrose agar and host leaf extract agar,respectively, which were statistically on par with each other. Medium growth rate of 4.19 mmwas found in oat meal agar medium and slow growth of 2.62 mm was observed on Sabourband's agar medium. The effect of four solid media on growth pattern and colony colour varied among the isolates and most of the colonies had cottony or fluffy mycelial growth with regular to irregular margin.

On potato dextrose agar, some of the isolates resembled with each other in growth pattern. Mycelial growth on PDA medium was fluffy, thick with regular or irregular margin, while on OMA,growth was sparse and submerged. In case of Sabourband's agar mycelial growth was compact with irregular margin (Fig.1). Variation in the growth pattern of *C. lindemuthianum* isolate of french bean in different culture media was observed by Sonali Bhagat *et al*. (2022).

Differences were also noticed among the isolates of *C. lindemuthianum* with respect to morphological characters including conidia size, sporulation (Table 2).The mean conidial length of 36 isolates ranged from 11.87 to 13.03 µm with maximum length of 13.03 µm recorded in host leaf extract agar and it was the minimum in oat meal agar. Among the isolates,conidial length varied from 11.41 µm to 13.63 µm. Isolate CI-11 produced conidia with maximum length, whereas, isolate CI-4 expressed minimum conidial length. No significant difference was noticed with respect

to width of conidia and it varied from 3.59 to 4.11 µm on different media and 3.53 to 4.23 µm in different isolates. Maximum width was recorded in the isolate, CI-9 and conidia of minimum width were observed in CI-33.

With respect to growth of various isolates of *C. lindemuthianum* on four solid media, maximum colony diameter was recorded in PDA medium after 15 days of incubation followed by host leaf extract media. Rajesha and Mantur (2014) and Sardhara *et al.* (2016) reported PDA asthe best culture medium for growth, while Pria *et al.* (1997) and Pereira *et al.* (1998) found that host extract agar was the best for sporulation of *C.lindemuthianum*. Chaudhary and Singh (2016) also observed that PDA was the best culture medium for growth of *C.capsici* f. sp. *cyamopsicola.*

The effect of different solid media, isolates and their interaction on spore production was found significant (Table 2). The data revealed that spore production varied widely from 2.9 $x10^4$ to 7.3 $x10^4$ on different media. Among them, host leaf extract agar significantly superior and supported more sporulation 7.3 $x10^4$. The next best medium for spore production was PDA with 6.7 $x10^4$ conidia/5.00 $mm^2$ colony area. The least spore production was noticed with Sabourband's agar medium. Similarly, spore production of different isolates varied from 4.1 to 8.4$x10^4$. Among the isolates, CI-33 recorded the highest spore production (8.4$x10^4$) followed by CI-32 (7.6 $x10^4$) and CI-35 (7.5$x10^4$) which were found on par with each other; the least was recorded in CI-1 with the production capacity of 4.1 $x10^4$ conidia/5.00 $mm^2$ colony area. Host leaf extract agar medium favoured the production of more number of conidia in various isolates due to disintegration of host contents within few days of incubation favour the spore production. Host leaf extract agar medium promoted the highest radial growth and the high growth rate in case of *Corynespora* (Siva Prasad *et al*., 2021). Despite the less vegetative growth of isolates observed in Sabourband's agar medium, it supported dense, compact early sporulation in comparision to other media. The non-uniformity of distribution and different rates of conidial production of *C. lindemuthianum* on different media were analysed by Mendes Costa (1996).

The differences in colony colour, mycelial growth, spore size and spore production in different isolates of *C. lindemuthianum* is attributed to the influence of various nutrients present in different media. Bean leaf extract was the best among all media for sporulation. Better growth and sporulation of *C. gloeosporioides* were reported on PDA earlier by Rajesha and Mantur (2014) in Dolichos bean, Kumar *et al* (2015) in chillies, Sanjeev Leharwan *et al.*(2018) in mango. Maximum mycelia growth and excellent sporulation of *C. gloeosporioides* isolate from aonla was obtained with Richard's agar medium followed by PDA and corn meal agar (Asalkar *et al*., 2019). Sonali Bhagat *et al.* (2022) reported PDA as the best for *C. lindemuthianum* from French bean. Similar observations were reported for other fungi (Siva Prasad *et al*., 2021).

There were differences in mycelial growth and sporulation of different isolates even in the same media. Similar observations were made by Denobys and Baudry (1995) and Siva Prasad *et al*. (2021). It can be argued that variation in the isolates may be inherent since isolates were collected from different localities; hence, the morphological and physiological characters are influenced by environmental conditions through natural

**Table 1. Effect of culture media on growth of *Colletotrichum lindemuthianum* isolates causing anthracnose of field bean**

| Isolate code | Solid media | | | | | | | | | | Liquid media | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mycelial growth (mm) | | | | Mean | Growth rate (mm/day) | | | | Mean | Dry mycelial weight (mg) | | | | Mean |
| | PDA | OMA | HLEA | SA | | PDA | OMA | HLEA | SA | | PDA | OMA | HLEA | SA | |
| CI-1 | 74.35 | 68.35 | 73.50 | 43.50 | 64.93 | 4.96 | 4.56 | 4.90 | 2.90 | 4.33 | 276.00 | 260.50 | 235.50 | 204.50 | 244.13 |
| CI-2 | 63.85 | 64.00 | 65.50 | 43.85 | 59.30 | 4.26 | 4.27 | 4.37 | 2.92 | 3.95 | 251.00 | 240.50 | 255.25 | 222.50 | 242.31 |
| CI-3 | 74.45 | 71.50 | 72.50 | 46.00 | 66.11 | 4.97 | 4.77 | 4.84 | 3.07 | 4.41 | 254.35 | 240.25 | 250.50 | 214.00 | 239.78 |
| CI-4 | 73.90 | 72.00 | 75.50 | 43.00 | 66.10 | 4.93 | 4.80 | 5.04 | 2.87 | 4.41 | 280.70 | 260.50 | 239.25 | 233.50 | 253.49 |
| CI-5 | 36.80 | 37.10 | 40.00 | 16.80 | 32.68 | 2.46 | 2.48 | 2.67 | 1.12 | 2.18 | 235.00 | 220.25 | 243.00 | 243.85 | 235.53 |
| CI-6 | 74.00 | 71.00 | 71.50 | 34.50 | 62.75 | 4.93 | 4.73 | 4.77 | 2.30 | 4.18 | 280.50 | 250.25 | 258.00 | 251.50 | 260.06 |
| CI-7 | 75.10 | 71.50 | 71.55 | 43.50 | 65.41 | 5.01 | 4.77 | 4.77 | 2.90 | 4.36 | 270.50 | 258.00 | 258.00 | 242.50 | 257.25 |
| CI-8 | 65.60 | 62.50 | 69.00 | 35.50 | 58.15 | 4.38 | 4.17 | 4.60 | 2.37 | 3.88 | 280.70 | 240.25 | 245.50 | 232.85 | 249.83 |
| CI-9 | 37.10 | 35.50 | 40.50 | 17.10 | 32.55 | 2.47 | 2.37 | 2.70 | 1.14 | 2.17 | 250.50 | 247.50 | 245.50 | 231.50 | 243.75 |
| CI-10 | 67.65 | 63.50 | 63.00 | 47.65 | 60.45 | 4.51 | 4.23 | 4.20 | 3.18 | 4.03 | 252.25 | 235.25 | 230.50 | 220.00 | 234.50 |
| CI-11 | 57.70 | 54.35 | 55.05 | 37.70 | 51.20 | 3.85 | 3.63 | 3.67 | 2.52 | 3.41 | 260.50 | 250.50 | 230.50 | 222.75 | 241.06 |
| CI-12 | 74.15 | 70.35 | 73.00 | 44.00 | 65.38 | 4.94 | 4.69 | 4.87 | 2.93 | 4.36 | 267.85 | 245.50 | 230.50 | 210.50 | 238.59 |
| CI-13 | 69.35 | 66.00 | 66.00 | 39.50 | 60.21 | 4.63 | 4.40 | 4.40 | 2.64 | 4.02 | 278.00 | 265.50 | 250.50 | 245.00 | 259.75 |
| CI-14 | 68.15 | 48.00 | 70.50 | 48.15 | 58.70 | 4.54 | 3.20 | 4.70 | 3.21 | 3.91 | 270.65 | 262.75 | 245.50 | 255.00 | 258.48 |
| CI-15 | 73.50 | 71.00 | 71.00 | 43.50 | 64.75 | 4.90 | 4.73 | 4.74 | 2.90 | 4.32 | 248.00 | 235.50 | 217.85 | 222.50 | 230.96 |
| CI-16 | 76.00 | 72.00 | 73.00 | 46.00 | 66.75 | 5.07 | 4.80 | 4.87 | 3.07 | 4.45 | 260.50 | 255.25 | 240.25 | 240.50 | 249.13 |
| CI-17 | 73.50 | 68.00 | 74.50 | 41.35 | 64.34 | 4.90 | 4.54 | 4.97 | 2.76 | 4.29 | 240.00 | 235.50 | 225.50 | 210.00 | 227.75 |
| CI-18 | 71.00 | 66.00 | 65.50 | 45.50 | 62.00 | 4.74 | 4.40 | 4.37 | 3.03 | 4.13 | 265.10 | 260.50 | 245.50 | 230.00 | 250.28 |
| CI-19 | 47.00 | 43.00 | 43.00 | 27.00 | 40.00 | 3.14 | 2.87 | 2.87 | 1.80 | 2.67 | 270.40 | 250.00 | 258.00 | 244.50 | 255.73 |
| CI-20 | 63.15 | 58.15 | 61.50 | 43.15 | 56.49 | 4.21 | 3.88 | 4.10 | 2.88 | 3.77 | 279.00 | 270.50 | 245.50 | 233.85 | 257.21 |
| CI-21 | 44.50 | 40.50 | 46.50 | 24.50 | 39.00 | 2.97 | 2.70 | 3.10 | 1.64 | 2.60 | 235.50 | 243.00 | 235.50 | 243.00 | 239.25 |
| CI-22 | 56.65 | 53.00 | 55.00 | 36.65 | 50.33 | 3.78 | 3.54 | 3.67 | 2.45 | 3.36 | 290.50 | 275.50 | 250.50 | 241.50 | 264.50 |
| CI-23 | 61.50 | 59.50 | 63.50 | 41.50 | 56.50 | 4.10 | 3.97 | 4.24 | 2.77 | 3.77 | 259.00 | 245.50 | 230.25 | 220.00 | 238.69 |

*Table 1 Contd....*

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| Isolate code | Solid media | | | | | | | | | | Liquid media | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mycelial growth (mm) | | | | Mean | Growth rate (mm/day) | | | | Mean | Dry mycelial weight (mg) | | | | Mean |
| | PDA | OMA | HLEA | SA | | PDA | OMA | HLEA | SA | | PDA | OMA | HLEA | SA | |
| CI-24 | 64.00 | 61.55 | 60.00 | 44.00 | 57.39 | 4.27 | 4.10 | 4.00 | 2.93 | 3.83 | 250.50 | 253.00 | 245.50 | 258.00 | 251.75 |
| CI-25 | 85.50 | 81.00 | 84.50 | 39.00 | 72.50 | 5.70 | 5.40 | 5.64 | 2.60 | 4.83 | 240.50 | 243.00 | 210.50 | 200.50 | 223.63 |
| CI-26 | 58.00 | 52.50 | 57.00 | 38.00 | 51.38 | 3.87 | 3.50 | 3.80 | 2.53 | 3.43 | 275.50 | 255.50 | 240.50 | 237.00 | 252.13 |
| CI-27 | 71.45 | 68.00 | 69.50 | 35.50 | 61.11 | 4.76 | 4.53 | 4.64 | 2.37 | 4.07 | 258.00 | 255.50 | 250.50 | 233.00 | 249.25 |
| CI-28 | 36.70 | 33.50 | 38.50 | 16.70 | 31.35 | 2.45 | 2.24 | 2.57 | 1.12 | 2.09 | 255.50 | 255.50 | 240.50 | 250.50 | 250.50 |
| CI-29 | 63.50 | 62.00 | 65.00 | 43.50 | 58.50 | 4.24 | 4.14 | 4.33 | 2.90 | 3.90 | 260.50 | 258.00 | 240.50 | 240.50 | 249.88 |
| CI-30 | 71.50 | 68.00 | 73.00 | 44.00 | 64.13 | 4.77 | 4.54 | 4.87 | 2.93 | 4.27 | 270.50 | 270.50 | 260.50 | 248.00 | 262.38 |
| CI-31 | 72.00 | 67.50 | 71.50 | 37.50 | 62.13 | 4.80 | 4.50 | 4.77 | 2.50 | 4.14 | 280.50 | 255.50 | 240.50 | 248.00 | 256.13 |
| CI-32 | 87.75 | 81.50 | 90.00 | 47.50 | 76.69 | 5.85 | 5.44 | 6.00 | 3.17 | 5.11 | 295.50 | 275.50 | 250.50 | 245.50 | 266.75 |
| CI-33 | 88.70 | 85.50 | 89.75 | 51.50 | 78.86 | 5.92 | 5.70 | 5.99 | 3.44 | 5.26 | 285.50 | 275.25 | 255.25 | 241.00 | 264.25 |
| CI-34 | 87.75 | 84.00 | 87.25 | 50.50 | 77.38 | 5.85 | 5.60 | 5.82 | 3.37 | 5.16 | 285.50 | 250.50 | 251.25 | 252.50 | 259.94 |
| CI-35 | 86.00 | 83.00 | 86.00 | 47.00 | 75.50 | 5.73 | 5.54 | 5.74 | 3.14 | 5.03 | 280.50 | 265.50 | 260.50 | 240.50 | 261.75 |
| CI-36 | 51.00 | 48.65 | 50.50 | 31.00 | 45.29 | 3.40 | 3.25 | 3.37 | 2.07 | 3.02 | 235.50 | 240.25 | 235.50 | 238.50 | 237.44 |
| Mean | 66.74 | 62.88 | 66.2 | 39.32 | | 4.45 | 4.19 | 4.41 | 2.62 | | 264.74 | 252.84 | 243.02 | 234.70 | |
| **Factors** | **C.D.** | **SE(d)** | **SE(m)** | | | **C.D.** | **SE(d)** | **SE(m)** | | | **CD** | **SE (d)** | **SE(m)** | | |
| Media | 1.09 | 0.55 | 0.39 | | | 0.07 | 0.04 | 0.03 | | | 3.13 | 1.58 | 1.12 | | |
| Isolates | 3.27 | 1.65 | 1.17 | | | 0.22 | 0.1 | 0.08 | | | 9.39 | 4.74 | 3.35 | | |
| Interaction | 6.54 | 3.31 | 2.34 | | | 0.44 | 0.22 | 0.16 | | | 18.77 | 9.49 | 6.71 | | |

**Table 2. Effect of culture media on conidial characters and sporulation of *Colletotrichum lindemuthianum* isolates causing anthracnose of field bean**

| Isolate code | Length of conidia (µm) | | | | | Width of conidia (µm) | | | | | Sporulation ($10^4$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | PDA | OMA | HLEA | SA | Mean | PDA | OMA | HLEA | SA | Mean | PDA | OMA | HLEA | SA | Mean |
| CI-1 | 12.15 | 10.50 | 13.00 | 11.35 | 11.75 | 3.85 | 3.65 | 3.20 | 3.45 | 3.54 | 5.00 | 4.00 | 6.00 | 1.50 | 4.10 |
| CI-2 | 10.20 | 12.00 | 13.45 | 10.90 | 11.64 | 4.15 | 3.95 | 3.00 | 3.20 | 3.58 | 5.00 | 5.50 | 7.00 | 3.00 | 5.10 |
| CI-3 | 12.95 | 12.50 | 13.00 | 10.70 | 12.29 | 3.50 | 4.00 | 3.35 | 4.30 | 3.79 | 7.50 | 5.00 | 6.00 | 4.00 | 5.60 |
| CI-4 | 11.30 | 11.00 | 13.05 | 10.30 | 11.41 | 4.15 | 3.40 | 3.65 | 3.20 | 3.60 | 10.00 | 2.50 | 8.50 | 2.50 | 5.90 |
| CI-5 | 11.95 | 11.50 | 13.00 | 11.00 | 11.86 | 3.60 | 3.85 | 3.55 | 3.25 | 3.56 | 6.50 | 4.50 | 9.50 | 2.00 | 5.60 |
| CI-6 | 12.95 | 10.00 | 13.80 | 12.85 | 12.40 | 3.70 | 3.90 | 3.85 | 3.70 | 3.79 | 7.50 | 4.50 | 8.50 | 2.50 | 5.80 |
| CI-7 | 11.35 | 12.50 | 13.50 | 13.10 | 12.61 | 3.70 | 4.30 | 3.55 | 3.10 | 3.66 | 5.50 | 2.50 | 7.00 | 2.00 | 4.30 |
| CI-8 | 11.30 | 13.50 | 12.45 | 12.90 | 12.54 | 4.00 | 4.30 | 3.20 | 3.85 | 3.84 | 3.50 | 5.00 | 6.50 | 2.50 | 4.40 |
| CI-9 | 14.50 | 12.50 | 12.95 | 12.90 | 13.21 | 4.15 | 4.50 | 4.70 | 3.60 | 4.23 | 4.00 | 5.00 | 7.00 | 2.00 | 4.50 |
| CI-10 | 14.00 | 11.95 | 13.35 | 14.00 | 13.33 | 4.60 | 3.95 | 4.15 | 3.60 | 4.08 | 4.00 | 5.00 | 7.50 | 4.00 | 5.10 |
| CI-11 | 14.85 | 12.50 | 13.45 | 13.70 | 13.63 | 3.95 | 3.75 | 4.05 | 4.00 | 3.94 | 4.50 | 3.50 | 6.00 | 3.00 | 4.30 |
| CI-12 | 11.20 | 13.50 | 13.20 | 13.50 | 12.85 | 4.00 | 3.25 | 4.40 | 3.95 | 3.90 | 8.50 | 4.00 | 7.00 | 4.00 | 5.90 |
| CI-13 | 12.00 | 12.50 | 13.35 | 12.55 | 12.60 | 4.35 | 3.75 | 4.35 | 4.00 | 4.11 | 6.50 | 3.50 | 7.50 | 1.00 | 4.60 |
| CI-14 | 14.05 | 13.00 | 13.50 | 13.00 | 13.39 | 3.65 | 3.35 | 4.20 | 3.50 | 3.68 | 7.50 | 4.00 | 4.00 | 2.50 | 4.50 |
| CI-15 | 13.30 | 10.00 | 14.35 | 12.55 | 12.55 | 3.60 | 3.15 | 3.85 | 3.85 | 3.61 | 9.00 | 4.50 | 8.00 | 3.50 | 6.30 |
| CI-16 | 12.60 | 10.50 | 13.70 | 13.40 | 12.55 | 3.85 | 4.00 | 3.90 | 4.00 | 3.94 | 4.50 | 5.50 | 8.00 | 2.50 | 5.10 |
| CI-17 | 12.15 | 11.50 | 13.70 | 11.95 | 12.33 | 3.75 | 4.30 | 3.95 | 3.75 | 3.94 | 6.50 | 4.50 | 5.50 | 3.50 | 5.00 |
| CI-18 | 13.10 | 11.50 | 13.00 | 13.50 | 12.78 | 3.65 | 4.00 | 3.30 | 3.25 | 3.55 | 3.50 | 6.00 | 6.50 | 3.50 | 4.90 |
| CI-19 | 12.85 | 11.00 | 13.50 | 13.65 | 12.75 | 3.90 | 3.60 | 3.40 | 3.60 | 3.63 | 5.50 | 6.00 | 8.50 | 4.00 | 6.00 |
| CI-20 | 12.80 | 12.00 | 13.30 | 12.55 | 12.66 | 4.25 | 4.00 | 3.95 | 3.25 | 3.86 | 7.50 | 2.50 | 6.00 | 2.50 | 4.60 |
| CI-21 | 11.00 | 12.50 | 13.40 | 13.15 | 12.51 | 4.40 | 3.90 | 3.70 | 3.30 | 3.83 | 4.00 | 4.50 | 6.50 | 5.00 | 5.00 |
| CI-22 | 9.85 | 12.00 | 12.95 | 12.60 | 11.85 | 4.60 | 3.95 | 3.65 | 3.85 | 4.01 | 7.00 | 4.50 | 6.50 | 2.50 | 5.10 |
| CI-23 | 10.55 | 11.50 | 12.20 | 12.95 | 11.80 | 4.40 | 3.70 | 3.45 | 4.10 | 3.91 | 6.00 | 4.50 | 8.50 | 3.00 | 5.50 |

*Table 2 Contd....*

*Table 2 Contd....*

| Isolate code | Length of conidia (µm) | | | | | Width of conidia (µm) | | | | | Sporulation ($10^4$) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **PDA** | **OMA** | **HLEA** | **SA** | **Mean** | **PDA** | **OMA** | **HLEA** | **SA** | **Mean** | **PDA** | **OMA** | **HLEA** | **SA** | **Mean** |
| CI-24 | 10.50 | 11.00 | 12.90 | 13.35 | 11.94 | 4.00 | 3.85 | 3.80 | 3.30 | 3.74 | 8.50 | 5.50 | 6.50 | 2.00 | 5.60 |
| CI-25 | 10.95 | 11.50 | 13.00 | 13.30 | 12.19 | 3.70 | 4.00 | 3.65 | 3.65 | 3.75 | 7.50 | 4.50 | 9.50 | 3.50 | 6.30 |
| CI-26 | 12.00 | 13.00 | 12.45 | 13.45 | 12.73 | 3.45 | 4.00 | 3.65 | 3.35 | 3.61 | 4.50 | 4.50 | 7.50 | 2.50 | 4.80 |
| CI-27 | 11.30 | 10.50 | 13.50 | 13.40 | 12.18 | 3.65 | 3.35 | 3.55 | 3.65 | 3.55 | 5.50 | 6.50 | 6.00 | 4.50 | 5.60 |
| CI-28 | 12.20 | 12.00 | 11.85 | 13.60 | 12.41 | 3.65 | 3.50 | 3.55 | 3.50 | 3.55 | 8.50 | 5.50 | 7.00 | 2.50 | 5.90 |
| CI-29 | 12.80 | 13.50 | 12.00 | 13.50 | 12.95 | 4.35 | 3.50 | 4.00 | 3.55 | 3.85 | 8.00 | 6.00 | 5.50 | 2.50 | 5.50 |
| CI-30 | 13.95 | 11.50 | 13.00 | 13.00 | 12.86 | 4.65 | 3.55 | 4.05 | 3.40 | 3.91 | 8.50 | 7.00 | 6.00 | 2.50 | 6.00 |
| CI-31 | 10.60 | 11.50 | 12.00 | 12.95 | 11.76 | 4.45 | 3.60 | 3.95 | 3.70 | 3.93 | 4.50 | 6.00 | 7.50 | 3.50 | 5.40 |
| CI-32 | 11.05 | 11.00 | 12.40 | 12.50 | 11.74 | 4.45 | 3.65 | 4.20 | 3.95 | 4.06 | 10.00 | 9.50 | 8.50 | 2.50 | 7.60 |
| CI-33 | 10.30 | 13.50 | 13.00 | 11.85 | 12.16 | 4.05 | 3.45 | 3.40 | 3.20 | 3.53 | 10.50 | 8.50 | 11.50 | 3.00 | 8.40 |
| CI-34 | 11.80 | 12.00 | 12.35 | 13.00 | 12.29 | 4.30 | 3.90 | 3.85 | 3.25 | 3.83 | 8.50 | 7.00 | 9.00 | 3.00 | 6.90 |
| CI-35 | 11.35 | 13.00 | 13.50 | 11.85 | 12.43 | 4.40 | 3.90 | 3.80 | 3.30 | 3.85 | 8.50 | 6.50 | 10.50 | 4.50 | 7.50 |
| CI-36 | 12.05 | 11.50 | 12.00 | 12.30 | 11.96 | 4.60 | 4.15 | 3.30 | 3.60 | 3.91 | 7.50 | 6.50 | 7.00 | 2.00 | 5.80 |
| Mean | 12.05 | 11.87 | 13.03 | 12.70 | | 4.04 | 3.80 | 4.11 | 3.59 | | 6.70 | 5.10 | 7.30 | 2.90 | |
| **Factors** | **C.D.** | **SE(d)** | **SE(m)** | | | **C.D.** | **SE(d)** | **SE(m)** | | | **C.D.** | **SE(d)** | **SE(m)** | | |
| Media -A | 0.32 | 0.16 | 0.17 | | | N/A | 0.29 | 0.21 | | | 0.40 | 0.2 | 0.1 | | |
| Isolates -B | 0.97 | 0.49 | 0.35 | | | N/A | 0.87 | 0.62 | | | 1.10 | 0.6 | 0.4 | | |
| Interaction | 1.94 | 0.98 | 0.69 | | | N/A | 1.75 | 1.23 | | | 2.20 | 1.1 | 0.8 | | |

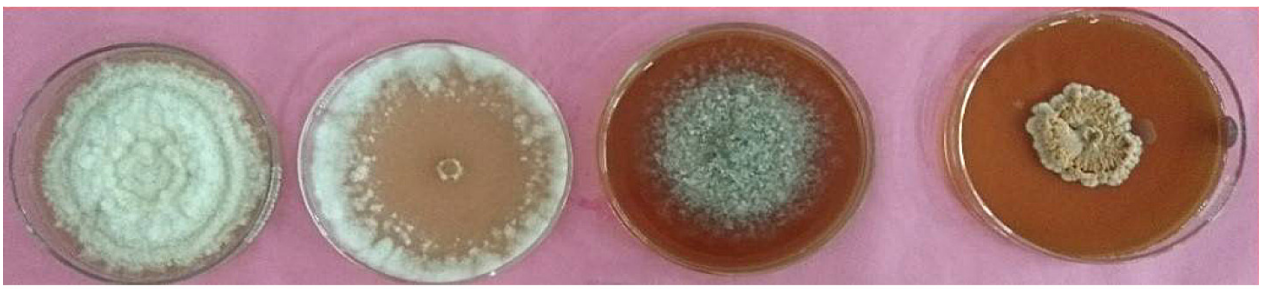


**Figure 1. Growth of isolate CI 4 on different solid media after 15 days of incubation**

chance mutations which may be responsible for such variability.

**Effect of on liquid media on growth**

The growth responses were also studied in four liquid media. The dry mycelial weight of each isolate obtained after 15 days of incubation was recorded (Table 1). The effect of different media on growth and mycelial dry weight of different isolates in different media and their interaction was found significant. Among the four liquid media tested, dry mycelial weight varied from 234.70 to 264.74 mg with the highest dry weight of 264.74 mgrecorded in PDA which was significantly superior to other media. The next best medium in supporting the growth was HLEA (243.02 mg) and the least was observed in Sabourband's agar medium (234.70 mg). Dry mycelial weight varied from 227.75 to 266.75 mg in different isolates. The isolate CI-32 recorded the highest dry weight of 266.75 mg, followed by CI-22 (264.50 mg) and CI-33 (264.25 mg) which were on par, while the lowest was recorded in CI- 17. The growth phase study on *C. lindemuthianum* revealed that the fungus produced maximum dry mycelial weight of 264.74 mg on 15$^{th}$ day in potato dextrose broth, beyond which autolysis occurred.

## CONCLUSIONS

The pathogenic isolates associated with bean anthracnose were identified as *C. lindemuthianum* based on the spore size and shape of conidia as well as other characters described in the literature. Culture media significantly influenced the growth, colony character and sporulation and spore size of the different isolates of *C. lindemuthianum* as affected by the geographical location and nutrition. Potato dextrose agar was found the best culture medium for growth and visible colony morphology where as host leaf extract agar, for excellent sporulation. The liquid Potato dextrose broth also showed the highest dry mycelial weight and excellent sporulation.

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# SPECIES DIVERSITY OF PULSE BEETLE IN FOUR MAJOR GRAIN LEGUMES IN ANDHRA PRADESH

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## ABSTRACT

Studies were carried in *kharif*, 2022 to know the species diversity of pulse beetles infesting stored grain legumes in Krishna zone of Andhra Pradesh.The pulse beetles were identified as *Callosobruchus maculatus* and *C. chinensis* based on their morphological characters. A total of 1391 adults of *C. maculatus* were observed in blackgram. About 737 and 473 individuals of *C. maculatus* were recorded in greengram and chickpea, respectively. A total of 566 individuals of *C. maculatus* and 241 individuals of *C. chinensis* were found in pigeonpea grains collected from the different storage godowns. The diversity indices for pulse beetle species in pigeonpea were calculated. The Shannon- Wiener index was 2.712, indicating moderate diversity.The species evenness was 0.6273 suggesting that the evenness among the species was zero and the Margalef index was 3.436 indicating relatively high species richness.

**Keywords** : Diversity indices, Legumes, Morphological characters,Pulse beetle,Storage godowns

## INTRODUCTION

Grain legumes are significant source of protein and play an important role in the balanced diet of the majority of the Indian population. Pulses are cultivated in an area of 1.23 million ha with a production of 1.09 million tonnes in Andhra Pradesh during 2021-22 (Division of Economics and Statistics, 2022). Chickpea, pigeon pea, black gram and greengram are the major grain legume crops grown in Andhra Pradesh. Pulses are stored at various levels in public or private warehouses in various quantities for various purposes and for shorter or longer periods. However, stored grain legumes suffer great damage due to insect attack (Negamo *et al.,* 2007).The pulse beetle *Callosobruchus* spp. (Family: Chrysomelidae, order: Coleoptera) is responsible for about 24% of the damage (Stojanova *et al*., 2011). Sometimes, in cases of severe infestation, the damage can even reach 100% (Pruthi and Singh, 1950).

Species of pulse beetles include *C. chinensis* Linnaeus, *C. maculatus* Fabricius, *C. analis* Linnaeus, *C. rhodesianus* Pic, *C. dolichosi* Gyllenhal*, C. nigripennis* Allard, *C. subinnotatus* Pic*, C. phaseoli* Gyllenhal, *Acanthoscelides obtectus* Say and *Zabrotes subfasciatus* Bohemann(Tuda *et al*., 2006). *Callosobruchus chinensis, C. maculatus* and *C. analis* are the most common species of pulse beetle found in India (Raina, 1970). Recently, Harish *et al.* (2018) reported that

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*C. analis*is the dominant species found on stored pulses in Hyderabad and Karnataka region.

Diversity is a kind of equilibrium maintained by nature that decides the carrying capacity of an ecosystem which helps to understand the relationship between the host, insect-pests and their natural enemies in an ecosystem.The diversity index is a statistic measure that provides information about the composition of a community, it measures the species richness taking into account the relative abundance of species or evenness. Species richness is the number of species per sample. More the number of species present in a sample, the richer will be the sample. The Shannon-Weiner index represents both the abundance and evenness of the species present (Madhumathi, 2022). With this in view, studies were conducted to know the species diversity of pulse beetle infesting stored grain legumes in Krishna zone of Andhra Pradesh.

## MATERIALS AND METHODS

The Krishna zone of Andhra Pradesh comprises of six districts *viz.,* Krishna, NTR, Guntur, Bapatla, Palanadu and Prakasam. Backgram and greengram are grown under rice fallow conditions in Krishna and Bapatla districts;chickpea is the major pulse crop in Prakasam district while pigeonpea is also predominantly cultivated in Guntur, Palanadu and Prakasam districts. Infested samples of these four major pulses *viz*., blackgram, greengram, chickpea, and pigeonpea were collected from the storage godowns located in Krishna zone during *kharif*, 2022 (Fig. 1). A minimum quantity of 250 g of each sample was collected in tightly secured plastic jars (750 ml capacity) directly from the site and kept in the Entomology laboratory at Post Harvest Technology Centre, Bapatla up to 10 days after collection to allow the emergence of adult insects from the immature stages, if any present.Thus, a total of 96 samples (24

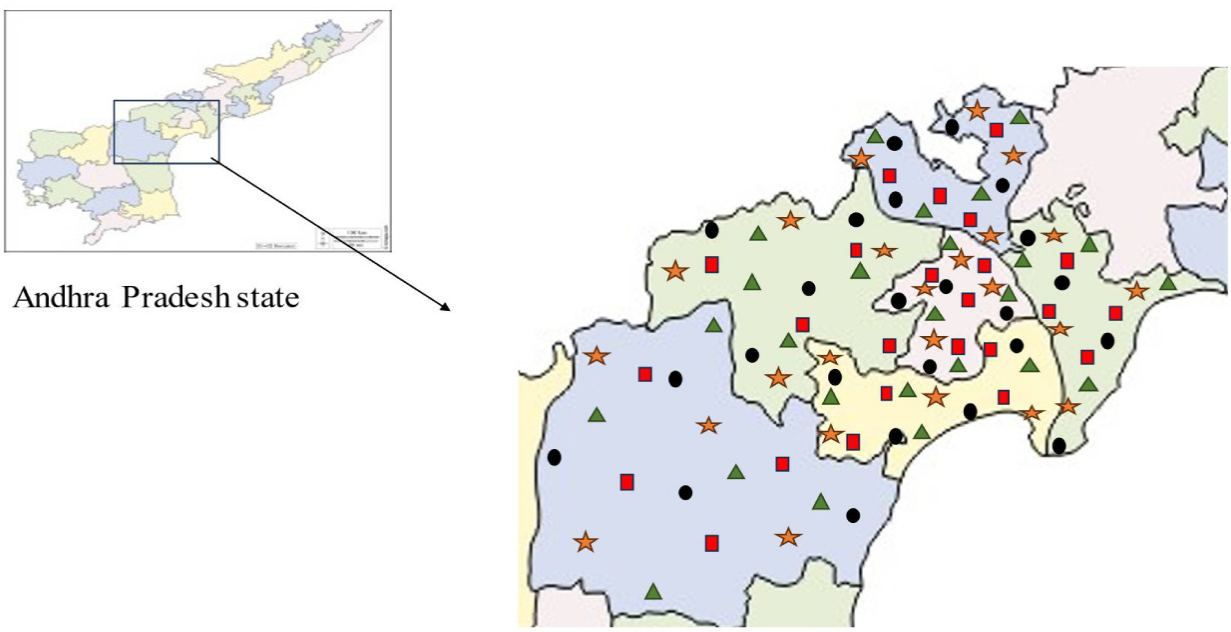



**Fig. 1.** Map showing the locations of the sample collection from different districts of Krishna Zone (blackgram: ●;greengram: ▲; chickpea: ★; pigeonpea: ■).

samples for each crop) were collected and observed.

**Identification of pulse beetles:** After the emergence, adult beetles were collected and the genus *Callosobruchus* and different species of *Callosobruchus* were identified preliminarily under the digital stereo binocular microscope (M/s Leica Microsystems, Model: S9i) using the taxonomic keys suggested by Khare (1993) and Mike Hackston (2016). They were then sent to the Taxonomist of ICAR-National Bureau of Agricultural Insect Resources (NBAIR), Bangalore for further confirmation.

**Species diversity among the pulse beetles:** After the emergence of insects in the plastic jar, the adult insects were separated and counted according to the species to study their diversity and it was explained through the use of these indices; Shannon Wiener index (Vanitha *et al.,* 2019) and Evenness and Richness (Margalef index).

Shannon Wiener index (H) = $\sum_{i=1}^{n} pi \ ln \, pi$

Where, pi = ni/N

ni = Sample size

N = Total number of individuals in a sample

*ln* = Natural logarithm

The higher the Shannon Wiener index, the greater is the diversity in the species.

Species Evenness (J) = $(J) = \frac{H}{H_{max}}$

Where, $H_{max}$ = *ln* (N)

H = Shannon Wiener index

*ln* = Natural logarithm

N = Total number of individuals in a sample.

If the value of 'J' is zero, it indicates that there is no evenness and if the value is one, it indicates complete evenness.

Species richness (Margalef index), $D = \frac{S-1}{\log N}$

Where D = Species richness

S = Total number of species

N = Total number of individuals

If the value of 'D' is 0.5, it indicates that the number of individuals are less and if the value is closer or > 1, it indicates that the number of individuals are more.

## RESULTS AND DISCUSSION

**Identification of pulse beetles:** After the emergence of pulse beetle adults, the morphological study was done under the stereo-zoom binocular microscope for identification of the species through the use of taxonomic keys. The antennae, hind legs and elytra observations revealed that the insect species belonged to the genus *Callosobruchus*, Family Chrysomelidae, Subfamily- Bruchinae and Order- Coleoptera.

**Genus identification:** The subfamily Bruchinae consists of several genera. Based on the morphological characters of the pulse beetles *i.e.,* a double-raised area covered with pale hairs in the middle at the base of the pronotum and the hind femur with a tooth on the inner and outer surface, it was confirmed that the insects belong to the genus *Callosobruchus*.

**Species identification:** The morphological characters such as antenna, hind femora, sternite region and elytrum of the species in the genus *Callosobruchus* were observed under the microscope using the taxonomic keys. The study revealed that there were variations between the species present in that genus. The species identified were *Callosobruchus chinensis* and *C. maculatus* which were later confirmed at ICAR- NBAIR, Bangalore in ensuring the accuracy of our species identification. The morphological

differences between these two species of the genus *Callosobruchus* are detailed below.

***Callosobruchus maculatus:*** The antennae of both sexes are slightly serrated from the 4th segment to the apical segment. The segments of the abdominal sternites are indistinct in males, while they are distinct and separate in females. A tooth like structure is observed on the hind femur. The tooth in the outer carina is large and blunt, while in both sexes there is a sharp tooth of same size in the inner carina. The colour of the elytra is dull. The elytra of the female is reddish-brown with two medio-lateral black spots and a conspicuous white pubescence that forms two C-shaped markings with their bases facing each other. Whereas, the male had a mediolateral black spot that is barely noticeable and did not have that such C-shaped markings. In females, a pair of black posterior lateral spots are observed on the pygidium. Both sexes have dense white patches on the caudal margin of the meta episternum (Fig 2 and 2a).

***Callosobruchus chinensis*:**From the fourth to the apical segments, the male's antennae are pectinate to heavily pectinate. In females, serrate type of antenna is found. The segments of the abdominal sternites are indistinct in males,and are distinct and separate in females. Tooth like structures are seen on the inner and outer carina on the hind femur in both sexes. Near the body's midsection, white, ivory-like specks can be seen. Brown, black, and white dots combine to form bands near the tip of the elytra. The pygidium of males and females is covered with white or silver setae, however, it is exposed and not completely covered by elytra. Both sexes are observed with dense white patches on the 2-5 sternite segments of the meta episternum (Fig 3 and 3a).

The observations made in this study are in conformity with earlier studies by Applebaum *et al.* (1968) who reported that the Japanese strain of male *C. chinensis* had pectinate antennae.Hu *et al.* (2009)also reported that antennae of the female and male *C. maculatus* and of the female *C. chinensis*have a serrated in shape, while those of the male *C. chinensis* are pectinate. Fatima *et al.* (2016) described that male and female adults of *C. maculatus* have a smooth inner carina of the hind femur and an inner tooth typically as long as or very slightly longer than outer tooth, while *C. chinensis* has an inner tooth of the hind femur with sides more or less parallel that converge near apex. Seram *et al.* (2022) observed sexual dimorphism of antennae in *C. chinensis*. In males, the fourth apical segments are pectinate to highly pectinate while in females, the segments are serrate. In *C. maculatus*, the antennae are slightly serrated across the apical segments in both males and females.

**Species diversity of pulse beetles in different stored grain legumes**

A total of 1391 adult insects were recorded in the blackgram samples collected from different storage godowns located in the Krishna zone belonging to the Class Insecta, Order Coleoptera, Family Chrysomelidae, Genus *Callosobruchus* and the species *maculatus* (Tables 1&2). From the greengram samples as many as 737 individuals of *C. maculatus* were recorded. Similarly, upto 473 individuals of *C. maculatus* were also recorded in the chickpea samples.While, in the Pigeon pea samples obtained from the various godowns in the Krishna Zone, a total of 807 individuals belonging to the same genus *Callosobruchus* were found. However, two different species namely *C. maculatus* (566) and *C. chinensis* (241) were found coexisting. The Shannon- Wiener index was 2.712, the species evenness was 0.6273 which indicates that the species evenness was zero and the

**Table 1. Pulse beetle species identified in the pulse grain samples in Krishna zone**

| Classificatior | Blackgram | Greengram | Chickpea | Pigeon pea |
|---|---|---|---|---|
| **Class** | Insecta | Insecta | Insecta | Insecta |
| **Order** | Coleoptera | Coleoptera | Coleoptera | Coleoptera |
| **Family** | Chrysomelidae | Chrysomelidae | Chrysomelidae | Chrysomelidae |
| **Genus** | *Callosobruchus* | *Callosobruchus* | *Callosobruchus* | *Callosobruchus* |
| **Species** | *maculatus* | *maculatus* | *maculatus* | *maculatus, chinensis* |

Margalef index was 3.436 which indicates that the species richness was higher because more than one species was present (Table 3). Thus, except Pigeonpea in the remaining three pulses there was only one insect species *i.e.,C. maculatus.*Similarly, to the investigation, Mounika *et al.* (2022) reported that Coochbehar district in West Bengal had the highest diversity with species evenness (0.49), Brillouin index (1.17), Simpsons index (0.68), Shannon index (1.23), Hill index (0.49) and effective diversity (3.43) followed by Jalpaiguri with Species evenness (0.46), Brillouin index (1.04), Simpson index (0.64), Shannon index (1.09), hill index (0.48) and Effective diversity (2.99). Species diversity indices were lowest in Alipurduar district.

Many previous studies on pulse beetle diversity in stored pulse reported similar results. Aidbhavi*et al*. (2023) identified five bruchid species infesting edible stored pulses in India. *Callosobruchus analis* was distributed in 50% of the samples and locations, followed by *C. maculatus* and *C. chinensis*. The overall species diversity index was 1.1, while the effective number of species was three. Mounika *et al.* (2022) conducted a survey in three districts of West Bengal to find the diversity of species of pulse beetle and species abundance in stored chickpea and recorded the species namely, *C. chinensis*, *C. maculatus* and *C. analis.* Harish *et al.* (2018) observed that *C. analis, C. maculatus, C. chinensis* and *T. castaneum* are the major insect pests of stored pulses in six districts of Hyderabad-Karnataka region.

## CONCLUSIONS

The pulse beetles specifically, *C. maculatus* and *C. chinensis* (Coleoptera: Chyrsomelidae) were identified in various grain legumes including blackgram, greengram, chickpea and pigeonpea. A total of 1391 adult insects were recorded in the blackgram samples belonging to *C. maculatus*. About 737 individuals of *C. maculatus* were recorded from the greengram samples. From the samples of chickpea, 473 individuals of *C.maculatus* were recorded. In pigeonpea samples, both *C. maculatus* (566 individuals) and *C. chinensis* (241 individuals) of were found. Since the Pigeonpea samples contained both the species, the Shannon- Wiener index was 2.712, indicating moderate diversity. The species evenness was 0.6273 indicates that the evenness among the species was zero and the Margalef index was 3.436 indicating relatively high species richness.The study has achieved the primary testing of pulse beetle abundance and diversity in various grain legumes collected from processing facilities in the Krishna Zone of Andhra Pradesh.

**Table 2. Pulse beetle population in stored pulses and diversity indices**

| Sample No. | District | Insect population (No./ 250 g sample) at 10 DAC | | | | |
|---|---|---|---|---|---|---|
| | | Blackgram | Greengram | Chickpea | eon pea | |
| | | *C. maculatus* | *C. maculatus* | *C. maculatus* | *C. maculatus* | *C. chinensis* |
| 1 | Krishna | 68 | 6 | 9 | 7 | - |
| 2 | Krishna | 35 | 73 | 6 | 9 | - |
| 3 | Krishna | 256 | 69 | 123 | 154 | - |
| 4 | Krishna | 59 | 43 | 23 | 5 | - |
| 5 | NTR | 18 | 38 | 36 | 16 | - |
| 6 | NTR | 58 | 41 | 0 | 7 | - |
| 7 | NTR | 24 | 2 | 12 | 11 | - |
| 8 | NTR | 64 | 7 | 8 | 4 | - |
| 9 | Guntur | 74 | 223 | 32 | 32 | - |
| 10 | Guntur | 8 | 36 | 6 | 8 | - |
| 11 | Guntur | 79 | 12 | 10 | 54 | - |
| 12 | Guntur | 13 | 43 | 17 | 31 | - |
| 13 | Bapatla | 26 | 23 | 22 | 69 | - |
| 14 | Bapatla | 16 | 25 | 18 | - | 72 |
| 15 | Bapatla | 159 | 16 | 16 | - | 43 |
| 16 | Bapatla | 21 | 9 | 6 | 21 | - |
| 17 | Palanadu | 61 | 8 | 8 | - | 108 |
| 18 | Palanadu | 19 | 3 | 0 | - | 18 |
| 19 | Palanadu | 31 | 7 | 24 | 3 | - |
| 20 | Palanadu | 14 | 0 | 27 | 11 | - |
| 21 | Prakasam | 96 | 5 | 19 | 56 | - |
| 22 | Prakasam | 30 | 9 | 35 | 12 | - |
| 23 | Prakasam | 116 | 13 | 8 | 35 | - |
| 24 | Prakasam | 46 | 26 | 8 | 21 | - |
| Total number of individuals | | 1391 | 737 | 473 | 566 | 241 |
| Shannon Wiener Diversity index | | - | - | - | 2.712 | |
| Species Evenness | | - | - | - | 0.6273 | |
| Species Richness | | - | - | - | 3.436 | |

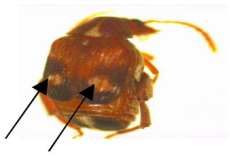
| Morphological character | Female | Male |
|---|---|---|
| **Antennae** | lightly serrate from 4th- apical segment  | Slightly serrate from 4th- apical segment  |
| **Abdominal sternites** | Distinct  | ndistinct  |
| **Elytra** | White C-shaped marks onthe elytra  | No white C-shaped marks on the elytra  |

**Fig.2. Morphological differences among the identified species of the genus: *Callosobruchusmaculatus***

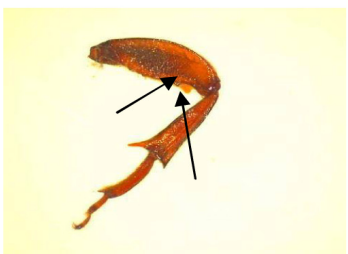
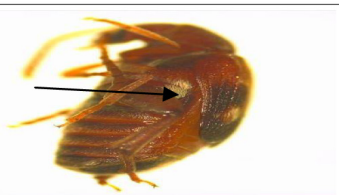
| | |
|---|---|
| **lind femur:** In both sexes, teeth are present on both inner and outer carina of the hind femur |  |
| **Pygidium**: In females a pair of black posterior lateral spots are observed on the pygidium |  |
| **Sternite region:** In both sexes, white patches on caudal margin of metaepisternum |  |

**Fig. 2a. Distinct morphological characters of *Callosobruchus maculatus***

| Morphological characters | Female | Male |
|---|---|---|
| Antennae | rate  | inate  |
| bdominal nites | Distinct  | Indistinct  |

**Fig. 3. Morphological differences among the identified species of the genus: *Callosobruchus chinensis***

**Hind femur:** Tooth like structure on each inner and outer carina of hind inboth sexes

**Elytra:** White ivory like specks near the body's midsection.

**Sternite region:** Dense white patches on 2-5 sternite segments of meta episternum in both sexes.

**Pygidium:** In both sexes it is covered with white or silver setae, and not entirely covered by elytra.

**Fig. 3a. Distinct morphological characters of *Callosobruchus chinensis***

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We would like to express our sincere gratitude to the Principal Scientist (Agricultural Entomology) at the Division of Germplasm Collection and Characterization, ICAR-National Bureau of Agricultural Insect Resources, Bengaluru for her invaluable assistance in the identification of insect species sampled in this study. Their expertise and the facilities provided were instrumental in ensuring the accuracy and reliability of our research. We acknowledge their significant contribution to our work and extend our appreciation for their support in advancing our understanding of different species of pulse beetle.

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# PERFORMANCE OF LIQUID BIOFERTILIZERS IN ENHANCING PRODUCTIVITY, NUTRIENT AVAILABILITY AND UPTAKE BY FODDER SORGHUM

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## ABSTRACT

The experiment was conducted during *rabi*, 2022-23 on sandy clay loam soils of wetland farm, S.V. Agricultural College, Tirupati. The experiment was laid in the randomized block design and replicated thrice. The results showed that the maximum numerical values of N, P and K uptake by plants at harvest as well as available N, $P_2O_5$ and $K_2O$ in the soil at harvest and significantly higher green and dry fodder yield were obtained with the application of 75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) compared to all other treatments.

**Keywords:** *Azospirillum*, Fodder sorghum,Liquid biofertilizers, Nutrient uptake, Productivity, Sandy clay loam soils

## INTRODUCTION

The livestock population in India plays a crucial role in the Agricultural sector, contributing significantly to the GDP. However, the livestock industry faces challenges due to the scarcity and low quality of fodder. On an average, there is a 40 percent gap in the dry and green fodder supply; by 2025, this deficit might rise to 45 percent. To meet the high quality forage demand of India's large animal population,there is a need to increase the production and productivity of forage crops while maintaining soil health and environmental sustainability. Sorghum (*Sorghum bicolor* (L.) *Moench*) is a vital fodder crop in India, because this is the most significant, versatile and widely cultivated andsupplies 60 percent of India's livestock needs. Unlike other fodder crops, it can endure heat, drought and waterlogging better but its yield is relatively modest and requires proper nutrient management (Singh *et al*., 2016).

In nutrient management practices biofertilizer is one of the components that can enhance crop growth and yield. Chemical fertilizers alone are not sustainable and the use of biofertilizers can be a practical solution to improve soil health and nutrient availability for higher crop productivity (Sneha *et al*., 2018). The use of biofertilizers can potentially enhance agricultural productivity, improve soil health and offer a sustainable alternative to chemical fertilizers.

Liquid biofertilizers are formulations that include favourable bacteria/fungi/algae that promote the growth of plants, enhance soil fertility and boost nutrient uptake. Compared

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to conventional fertilizers, liquid biofertilizers are more affordable, environmentally friendly and sustainable.

## MATERIALS AND METHODS

The investigation was carried out during *rabi*, 2022-23 on sandy clay loam soils of wetland farm, S.V. Agricultural College, Tirupati, Acharya N.G. Ranga Agricultural University, Andhra Pradesh. The soil was neutral in reaction (6.9 pH),low in available nitrogen (115 kg $ha^{-1}$), medium in available phosphorus (29 kg $ha^{-1}$) and low in available potassium (156 kg $ha^{-1}$) status. The field experiment was conducted with randomised block design consisting of eight treatments and replicated thrice. Treatments includes $T_1$: Absolute control, $T_2$: 100% RDF, $T_3$: 75% RDF + *Azospirillum* + PSB + KSB (Seed treatment@10 ml $kg^{-1}$), $T_4$: 75% RDF + *Azospirillum* + PSB + KSB (Soil application@1.25 l $ha^{-1}$), $T_5$: 75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application), $T_6$: 50% RDF + *Azospirillum* + PSB + KSB (Seed treatment@10 ml $kg^{-1}$), $T_7$: 50% RDF + *Azospirillum* + PSB + KSB (Soil application@1.25 l $ha^{-1}$), $T_8$: 50% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application). The crop was sown at 30 cm × 10 cm spacing with a seed rate of 25 kg $ha^{-1}$. Recommended dose of fertilizer was 60 - 40 - 30 N, $P_2O_5$, $K_2O$ kg $ha^{-1}$. Soil application of biofertilizers was done by mixing 1.25 l $ha^{-1}$ of each bio inoculant in 500 kg of well decomposed FYM and applied as basal dose (applied 24 h before sowing). Seed treatment of biofertilizers was done by mixing 10 ml of each bio inoculant with 1 kg of seed and drying for 10-15 minutes under shade before sowing. Irrigation and weeding were done as and when required. At 50% flowering, harvesting was completed.The data collected on various crop parameters were statistically evaluated using the randomised block design method recommended by Panse and Sukhatme (1985).

Green fodder yield offorage sorghum from the net plot area was taken separately by leaving 5 cm stubbles from ground surface and expressed in t $ha^{-1}$.After harvesting fodder sorghum from net plot area, heaps were left in the field for one week for sun drying. Then dry fodder yield of sorghum from net plot area was weighed and total dry fodder yield was expressed in t $ha^{-1}$.

Nutrient uptake was estimated by taking the whole plant at harvesting stage, then driedand analysed for nutrient content.The nutrient uptake was calculated using the following formula:

$$\text{Nutrient uptake by plant } \left(\text{kg ha}^{-1}\right) = \frac{\text{Nutrient content } (\%) \times \text{Weight of dry matter } \left(\text{kg ha}^{-1}\right)}{100}$$

Post-harvest soil nutrient status was estimated by taking the soil samples at 0-15 cm depth from each treatmentplot after harvest of the crop and analysed for chemical properties by using Alkaline potassium permanganate method (Subbiah and Asija, 1956) for available nitrogen, Olsen's method (Olsen *et al*., 1954) for available phosphorous and Flame photometry (Jackson, 1973) for available potassium in the soil.

## RESULTS AND DISCUSSION

Data pertaining to yield, N, P and K uptake by fodder sorghumand post-harvest soil nutrient statusat harvest as influenced by application of liquid biofertilizers at different levels of recommended dose of fertilizers was discussed in different sections.

### Green and dry fodder yield

Critical observation of the data revealed that higher green and dry fodder yield of fodder sorghum was recorded with the application of 75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) ($T_5$) which was on par with 100% RDF ($T_2$). These two

**Table 1. Green and dry fodder yield of fodder sorghum (t ha$^{-1}$) as influenced by application of liquid biofertilizers at different levels of recommended dose of fertilizers**

| Treatment | | Green fodder yield(t ha$^{-1}$) | Dry fodder yield(t ha$^{-1}$) |
|---|---|---|---|
| $T_1$ :Control | | 13.9 | 6.0 |
| $T_2$ : 100% RDF | | 30.6 | 12.8 |
| $T_3$ : 75% RDF + *Azospirillum* + PSB + KSB (Seed treatment @10 ml kg$^{-1}$) | | 26.5 | 11.3 |
| $T_4$ : 75% RDF + *Azospirillum* + PSB + KSB (Soil application @1.25 l ha$^{-1}$) | | 27.3 | 11.3 |
| $T_5$ : 75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) | | 32.1 | 13.0 |
| $T_6$ : 50% RDF + *Azospirillum* + PSB + KSB (Seed treatment @10 ml kg$^{-1}$) | | 21.9 | 9.7 |
| $T_7$ : 50% RDF + *Azospirillum* + PSB + KSB (Soil application @1.25 l ha$^{-1}$) | | 22.2 | 9.8 |
| $T_8$ : 50% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) | | 25.5 | 11.2 |
| | SEm± | 1.03 | 0.41 |
| | CD @ 5% | 3.1 | 1.3 |

treatments are inturn significantly superior over remaining treatments.Statistically lower green and dry fodder yieldswere noticed in absolute control ($T_1$) (Table 1). Enhanced mineral and water uptake, root development and vegetative growth together with the conversion of unavailable plant nutrients into available form by biofertilizers all contribute to higher nutrient uptake, which eventually increases green and dry fodder yield. These results are supported by findings of Yadahalli *et al*. (2022),Patel *et al*. (2018) and Verma *et al*. (2014).

**Nutrient uptake**

The highest N, P and K uptake at harvest by fodder sorghum was observed with 75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) ($T_5$) which was on par with 100% RDF ($T_2$). These two treatments were significantly superior over rest of treatments, significantly the lowest N, P and K uptake was recorded in absolute control($T_1$) of fodder sorghum crop (Table 2). The results demonstrated that the combination of chemical and biofertilizers *i.e*.,75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) ($T_5$) improved the uptake of N, P and K might be due to addition of biofertilizers which created a favourable regime of soil microclimate thereby releasing large quantities and a wide range of organic acids during the solubilization process and chelation o f complex intermediate organic molecules produced by microbial activity. Consequently, the use of biofertilizers made all the nutrients more

**Table 2. Nutrient uptake of fodder sorghum at harvest as influenced by application of liquid biofertilizers at different levels of recommended dose of fertilizers**

| Treatment | Nutrient uptake (kg ha$^{-1}$) | | |
|---|---|---|---|
| | N | P | K |
| $T_1$ : Control | 48.8 | 6.8 | 28.4 |
| $T_2$ : 100% RDF | 132.1 | 33.8 | 93.4 |
| $T_3$ : 75% RDF + *Azospirillum* + PSB + KSB (Seed treatment @10 ml kg$^{-1}$) | 109.4 | 25.4 | 75.0 |
| $T_4$ : 75% RDF + *Azospirillum* + PSB + KSB (Soil application @1.25 l ha$^{-1}$) | 112.1 | 26.8 | 76.2 |
| $T_5$ : 75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) | 144.1 | 37.5 | 96.3 |
| $T_6$ : 50% RDF + *Azospirillum* + PSB + KSB (Seed treatment @10 ml kg$^{-1}$) | 88.9 | 13.2 | 54.7 |
| $T_7$ : 50% RDF + *Azospirillum* + PSB + KSB (Soil application @1.25 l ha$^{-1}$) | 91.6 | 16.7 | 61.3 |
| $T_8$ : 50% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) | 108.1 | 23.3 | 70.1 |
| SEm± | 4.26 | 1.28 | 3.57 |
| CD @ 5% | 13.0 | 3.9 | 10.9 |

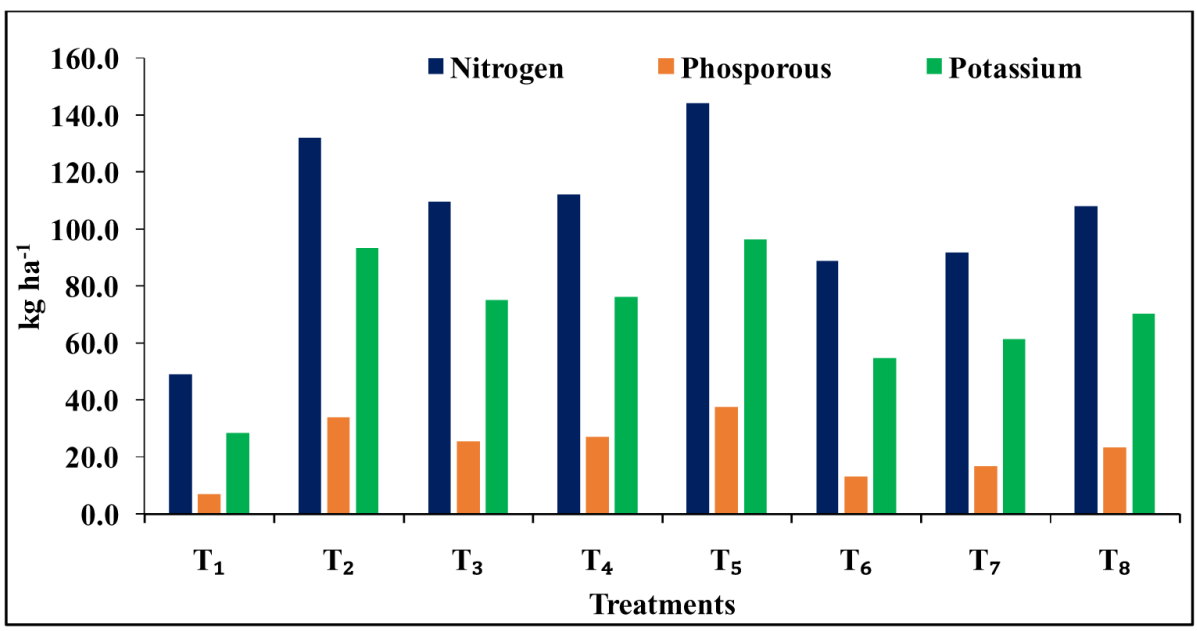


**Fig. 1. Nutrient uptake (kg ha$^{-1}$) of fodder sorghum at harvest as influenced by application ofliquid biofertilizers at different levels of recommended dose of fertilizers**

**Table 3. Post harvest soil nutrient status as influenced by application of liquid biofertilizers at different levels of recommended dose of fertilizers**

| Treatment | Post harvest soil nutrient status (kg ha$^{-1}$) | | |
|---|---|---|---|
| | Available N | Available $P_2O_5$ | Available $K_2O$ |
| $T_1$ :Control | 52.6 | 37.6 | 152.7 |
| $T_2$ : 100% RDF | 130.8 | 47.2 | 230.8 |
| $T_3$ : 75% RDF + *Azospirillum* + PSB + KSB (Seed treatment @10 ml kg$^{-1}$) | 120.7 | 46.6 | 224.8 |
| $T_4$ : 75% RDF + *Azospirillum* + PSB + KSB (Soil application @1.25 l ha$^{-1}$) | 150.5 | 52.6 | 252.1 |
| $T_5$ : 75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) | 164.0 | 55.6 | 275.2 |
| $T_6$ : 50% RDF + *Azospirillum* + PSB + KSB (Seed treatment @10 ml kg$^{-1}$) | 100.0 | 41.2 | 202.2 |
| $T_7$ : 50% RDF + *Azospirillum* + PSB + KSB (Soil application @1.25 l ha$^{-1}$) | 109.9 | 43.6 | 210.5 |
| $T_8$ : 50% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) | 120.5 | 46.6 | 214.2 |
| SEm± | 6.23 | 1.59 | 10.26 |
| CD @ 5% | 19.1 | 4.9 | 31.4 |

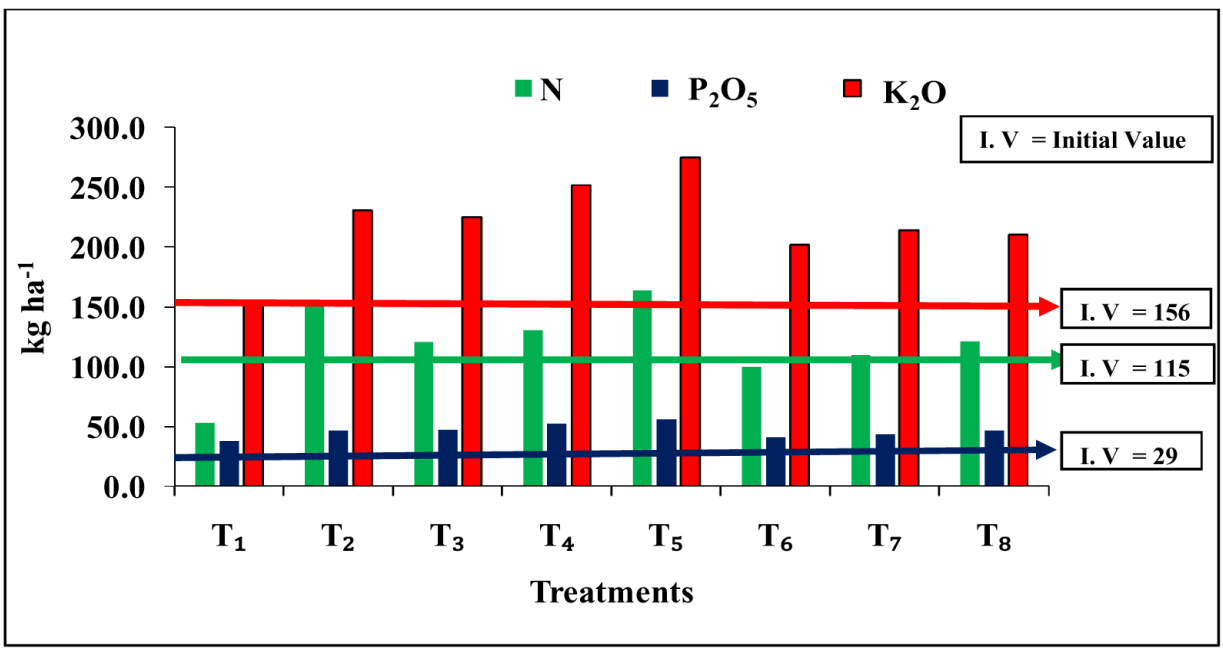


**Fig. 2. Post harvest soil nutrient status (kg ha$^{-1}$) as influenced by application of liquid biofertilizers at different levels of recommended dose of fertilizers**

readily available by extracting them from unavailable forms and appropriate nutrient absorption, translocation and as similation will ultimately result in an increase in the dry matter accumulation suggesting a greater intake of essential nutrients. These are in confirmation with findings of Goutami and Prasuna Rani (2018) and Patil *et al*. (2020).

**Post-harvesting soil nutrient status**

The data presented on post-harvest soil nutrient status revealed that application of 75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) ($T_5$) recorded the highest soil available N, $P_2O_5$and $K_2O$,which was however on par with the application of 75% RDF + *Azospirillum* + PSB + KSB (Soil application @1.25 l $ha^{-1}$) ($T_4$). Significantly lowest available N, $P_2O_5$ and $K_2O$ were observed with absolute control ($T_1$). Maximum availability of N, $P_2O_5$,$K_2O$ in soil with application of 75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) ($T_5$) was due to application of inorganic fertilizers along with bio fertilizers containing *Azospirillum* increased the nitrogen fixation capacity of plant and thereby the availability of N. Application of PSB resulted in solubilization and mineralization of unavailable P that increases $P_2O_5$ content in soil. Potassium solubilizing bacteria increase the available soil K pool due to result of clay's interaction with organic acids, which reduces K fixation there by releases K and disintegrate potassium minerals as a result of organic acids being released by bioinoculants containing KSB. The results are in conformity with the findings of Rekha *et al.* (2018), Kant *et al*. (2017), Khambalkar *et al.* (2017) and Raja *et al*. (2017).

## CONCLUSIONS

Combined application of 75% RDF + *Azospirillum* + PSB + KSB (Both seed & soil application) is a viable and sustainable nutrient management practice for achieving higher productivity of fodder sorghum in the Southern Agroclimatic Zone of Andhra Pradesh.

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# EVALUATION OF NEW OIL PALM CROSSES FOR THEIR FRESH FRUIT BUNCH YIELD AT ANDHRA PRADESH

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## ABSTRACT

Vegetable oil demands are ever-increasing globally. India is one of the world's major vegetable oil importers and second largest consumer. There is scope to raise the productivity of oil palm to two folds with the current land use and with the development of new hybrids. In this regard, new oil crosses (D x P) were evaluated for 12 years at Horticultural Research Station, Vijayarai, Andhra Pradesh, India. The number of bunches produced per palm per year has risen from 4 to 6 Years after planting and decreased from 7 to 10 YAP, where these crosses recorded an average bunch weight of 2 to 3 kg in the first harvest year, while it was 20 to 24 kg, by palms reaching 11 to 12 years after planting (8$^{th}$ and 9$^{th}$ harvest years). Out of all the 9 production years (4 YAP to 12 YAP), NRCOP-37 produced the highest number of bunches in 5 production years and in the remaining 4 years also it recorded on par bunch production with high yielding cross of that particular year. In the yield stabilization period (9 to 12 YAP), NRCOP-37 recorded the highest yield of 200.13 kg (9 YAP), 203.97 kg (10 YAP), and 206.09(12 YAP) kg/palm/year in the above 3 harvesting years. While the cross NRCOP-39 recorded 263.73 kg/palm/year of FFB at 11 years after planting. The cross NRCOP-37 recorded a higher FFB yield of 28.61 t/ha/year by the 6th harvest year (9 YAP) and this cross recorded FFB yields of 29.16, 34.37, and 29.47 t/ha/year at the 7th,8th and 9th harvest years. This is the one cross where annual yield fluctuation (FFB production) is very less, which is recording stable FFB yield (t/ha/year) at yield stabilization period.

## INTRODUCTION

Oil palm is one such good crop, which yields very high oil for a relatively small area of land use. In the world, palm oil is set to remain the most important vegetable oil in terms of production and consumption. Palm oil singly contributes over 36 % of global vegetable oil production. In the 1990s, the world's palm oil production was only 11.45 million tons, whereas now in 2023 it is 79.4 million tons (USDA,2023). In these 32 years, its production rose to 69 % globally. The rise in oil palm production was all because of high-yielding new hybrids, area expansion, and also ever-increasing vegetable oil demands in the world. Even though the production rise has been good in the last 3 decades, an exponential increase in productivity was not recorded with the current tenera hybrids as they have not reached the oil palm full potential productivity. It has the potential of producing oil at 8-14 t/ha/year (Corley and

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Tinker,2016). Now the world's average productivity has stagnated at 3-4 t/ha/year. In India, productivity was only 4 to 4.5 t/ha/year under assured irrigated conditions. A country like India which is densely populated demands huge vegetable oil imports and needs to increase oil palm production with the development of new hybrids having the potential of producing 8 to 10 tons of oil per hectare per year.It is suggested to study the hybrid/clone performance in local conditions before taking decision on order for planting material (Rethinam and Murugesan,2018). On the other hand, there is scope to increase the area under oil palm cultivation in India. The country has got a potential of two million ha for cultivation of oil palm crop against the present area coverage of 2.6 lakh ha (Rethinam,2014). So that, India can save the import duty on vegetable oils in future years. Efficient use of natural resources with good input management also accounts for increasing productivity in oil palm.

**Key words:** Average bunch weight, fresh fruit bunch yield, hybrids in oil palm, tenera crosses.

## MATERIALS AND METHODS

Ten new oil palm (D X P) cross combinations (NRCOP-31 to NRCOP-40) were planted during the year 2011 to evaluate their production performance. The experiment was laid out in Randomized Block Design (RBD) with three replications, at Horticultural Research Station, Vijayarai, Eluru District of Andhra Pradesh, India (16.81$^0$N, 81.03$^0$E). The normal average annual rainfall of the region was in the range of 800-900 mm, dry period prevails from February to May. All 10 crosses in this evaluation trial were developed at the Indian Institute of Oil Palm Research (IIOPR), Pedavegi, Eluru District of Andhra Pradesh. The research trial was planted with 6 palms at 9 x 9 x 9 m spacing accommodating 143 palms per hectare. The plantation was under the irrigated condition with 1200:600:1200 g of N: P: K application in four splits at quarterly intervals and 500 g of magnesium sulfate and 100 g of boron application in two splits in a year. The data was recorded in palms for their growth and yield right from planting to 12 years after planting (YAP). All the mature fruit bunches were harvested at 11-14 day intervals, starting from 4 years after planting to 12 years (9 harvest years). Every bunch was weighed together with loose fruits using a balance mounted on a tripod. Fresh Fruit Bunch(FFB) yield recorded per palm per year was obtained by adding the weight of all bunches produced by the palm in that year. FFB yield per hectare was obtained by the total bunch weight per palm which was then multiplied by the stand per hectare to obtain total bunch weight (FFB yield) per hectare per year. All the data was summarized according to treatments and subjected to statistical analysis, using WASP (Web Agri Stat Package 2) developed by Ashok Kumar Jangam and PranjaliNinadWadekar at ICAR Research Complex, Goa.403402.India.

## RESULTS AND DISCUSSION

In the present study, bunch production of 10 oil palm crosses (NRCOP-31 to NRCOP-40) varied widely among the crosses and across all nine production years (4 to 12 YAP). In the first year of production, *i.e.* 4 years after planting (YAP), the number of bunches produced per palm per year did not differ significantly in all the 10 crosses, they recorded bunch production in the range from 3.89 to 6.55 per year per palm. The number of bunches produced per palm per year has risen in the next two harvest years, i.e. 5 YAP and 6 YAP. Variation in the number of harvested bunches is mainly determined by changes in the rate of inflorescence development.From the first harvest year (4 YAP) to the second harvest year (5 YAP) there was a wide increase in bunch production potential, it was 48%, 180%, 40%, 71%, 94%, 65%, 78%, 91%, 46%, and 134 % and again in the next year

(5 YAP to 6 YAP), the rise in bunch production was 68%, 31%, 150%, 80%, 24%, 80%, 64%, 114%, 102% and 33% in respective crosses from NRCOP-31 to NRCOP-40. In the active growth phase 4 to 6 YAP, competition for assimilates was less with minimum stress in early age of palms, both vegetative and generative growth is a source limited in oil palm, and competition occurs between different sinks (Corley and Tinker, 2016). So, the number of bunches produced in 2nd and 3rd harvest year was more in all the 10 crosses.

The number of bunches produced per palm per year in all the crosses decreased from 7 to 10 YAP, where inflorescence abortions during the early stages of inflorescence development may be more in this period.Abortion of female inflorescence before anthesis, failure of developing bunches between anthesis and bunch ripeness was the reason for less bunch production in palms (LotteWoittiez et al,2017).At 7 years after planting, there was a 10 % reduction in bunch production when compared to the 6th year after planting. There was a 23 % reduction in the 8 years after planting compared to the 7th year (Table:1). There was a 29 % reduction for bunch production in palms at 9 years after planting compared to the 8th year and at 10 years after planting, it was 17.27% reduction compared to the 9th year. At 11 years after planting palms recorded a 43 % rise in bunch production compared to the 10th year.In the 12th year, i.e. in the 9th harvest year, again a decrease was noticed among all the 10 crosses. It was a 25 % reduction. Raise and fall in bunch production in every alternate year was the trend up to the 9th harvest year (MadhaviLathaet al,2020). From 7 to 10 years after planting (the above 4-year period), the bunches produced per palm per year decreases due to equal distribution of assimilates for both vegetative and reproductive growth, and trunk development will be more in this period, so there will be competition for assimilates for the development of both vegetative and reproductive growth. The number of ripe bunches available for harvest is determined by the number of inflorescences initiated, sex differentiation *i.e.,* development of inflorescence into male or female takes place 16-21 months before anthesis in different clones (Corley and Tinker, 2016).

Out of all the 9 production years (4 to 12 YAP), NRCOP-37 produced the highest number of bunches in 5 production years (Table:1), and in the remaining 4 years it recorded on par bunch production with the high yielding cross of that particular year. While NRCOP-31 recorded the highest bunch production in 3 production years (2015-16,2019-20 and 2022-23). The Cross NRCOP-34 recorded the highest number of bunches (19.23) in one year (2016-17) out of 9 production years. The two crosses NRCOP-33 and NRCOP-40 recorded the lowest bunch production in all the 9 production years. The trend for annual rise and fall for bunch production was the same for all 10 crosses.

The average bunch weight recorded in all 9 production years (4 YAP to 12 YAP) shows a gradual increase in bunch weight(Fig:1). In the first harvest year (4YAP), the average bunch weight was 2-3 kg, while rose from 6 to 7 kg in the third harvest year and it was almost the same as in the fourth and fifth harvest years. In the eighth harvest year, it rose to 12 to 14 kg, in the 9th and 10th harvest years it was 16 to 20 kg. By reaching 11 to 12 years after planting (8th and 9th harvest years), the average bunch weight reached, 20 to 24 kg (Fig:1). In oil palm with an increase in the age of palms the sex ratio decreases thereby the number of bunches produced per palm per year are reduced, which in turn increase the average bunch weight Madhavilathaet al. (2016). The trend for an increase in bunch weight was the same in all the crosses, like bunch production. The cross, where they produce the lowest number of bunches in a production year, recorded the highest average bunch weight. There was an inverse relationship

between the number of bunches produced per palm per year and the average bunch weight of that harvest year, MadhaviLathaet al. (2021). In the high-yielding cross NRCOP-37, the average bunch weight was 18-20 kg in the yield-stabilizing period (9 to 12 YAP). Even though the cross NRCOP-31, had more bunches in 3 production years its yield was low because in this cross the average bunch weight was only 14-16 kg as against 18-20 kg recorded in high yielding crosses NRCOP-37 and NRCOP-39(Fig:1).

In the yield incremental period (4 to 8 YAP) all the 10 crosses showed significant differences in their FFB yield production except at four years after planting. (Table:2). While in the yield stabilization period (9th,10th,11th, and 12th YAP), FFB production did not differ significantly among the 10 crosses. These results are in agreement with the results of MadhaviLathaet al.(2021).

Five years after planting, NRCOP-32 recorded the highest yield of 93.36 kg/palm/year, which was on par with the crosses NRCOP-34,35,37 and 39. On the 6th YAP, NRCOP-39 recorded the highest yield of 165.63 kg/palm/year when it recorded on par yield with the crosses NRCOP-32,33,34,36,37 and 38.The crosses recording FFB yield of more than 22 t/ha at sixth harvest year have better prospects for cultivation in Andhra Pradesh (Sanjeevraddi et al., 2016). On the 7th YAP, NRCOP-34 recorded the highest yield of 179.06 kg/palm/year and it was on par with 4 crosses NRCOP-32,35,37 and 38 (Table:2). Oil palm yields depends on weather parameters and genetic potential of hybrids (Manorama et al., 2020). In the 8th year after planting again, NRCOP-39 recorded its highest yield of 186.05 kg/palm/year. It was on par with all the crosses in that production year except for 3 crosses, NRCOP-31,36, and 40. These results are in agreement with the results of MadhaviLathaet al.(2020).

In the yield stabilization period (9 to 12 YAP), NRCOP-37 recorded the highest yield of 200.13 kg, 203.97 kg, and 206.09 kg/palm/year at the 9th,10th, and 12th years after planting. while the cross NRCOP-39 recorded 263.37 kg at 11 years after planting (Table:2), but in this 4-year yield stabilization period, all the 10 crosses did not differ significantly for their FFB production. Significant correlation between two age group palms were observed for their FFB yield variation(MadhaviLatha et al., 2022)

The steep FFB yield increase was recorded in all the 10 crosses from 4 to 6 YAP, it was 14.4 to 143.55 kg/palm/year. Corley and Tinker 2016, reported that although the vegetative growth and development are continuous and constant there were strong seasonal variations of reproductive growth in oil palm. The palms recorded a stable FFB yield from 7 to 10 years after planting, so it was observed that it was the yield stabilization period of these crosses, with a recorded average yield of 10 crosses at 140 to 160 kg/palm/year and in the 11th year after planting, the yield raised to 212.63 kg/palm/year and again at 12 years after planting it was reduced to 167.07 kg/palm/year (Fig:2). Maybe the palms show a decline in yield from the 12th year onwards.Annual yield data recorded for 6 years at IIOPR indicated a production trend of on and off-year production which is comparable to that of alternate bearing in other fruit crops (Naveen Kumar *et al.,*2017). The crosses NRCOP-32,34,37,38 and 39 recorded 20 t/ha/year of FFB yield by 6 years after planting (Fig:3). The high-yielding cross NRCOP-37 recorded 28.61 t/ha/year by 6 years after planting. At the 7th,8th, and 9th harvest years, this is the one cross where palms recorded stable of 29.16,34.37 and 29.47 t/ha/year in their respective harvesting years. Yield fluctuation (FFB production) was very less (Fig:3) in this cross NRCOP-37.An increase of more than 30% oil yield was realized with switching over from dura to the tenera planting material (Kushairi and Mohad Din Amiruddin,2020).

**Table 1. Number of bunches produced per palm per annum in new oil palm crosses (Gen 8D)**

| Year | 4th year after planting | 5th year after planting | 6th year after planting | 7th year after planting | 8th year after planting | 9th year after planting | 10th year after planting | 11th year after planting | 12th year after planting |
|---|---|---|---|---|---|---|---|---|---|
| **Crosses** | **2014-15** | **2015-16** | **2016-17** | **2017-18** | **2018-19** | **2019-20** | **2020-21** | **2021-22** | **2022-23** |
| NRCOP-31 | 6.19 | 9.26 | 15.60 | 14.30 | 12.37 | 11.06 | 8.69 | 11.66 | 11.02 |
| NRCOP-32 | 4.14 | 11.61 | 15.23 | 15.00 | 12.33 | 9.06 | 6.50 | 10.55 | 8.50 |
| NRCOP-33 | 3.89 | 5.44 | 13.63 | 11.40 | 10.23 | 6.15 | 4.94 | 9.16 | 5.12 |
| NRCOP-34 | 6.22 | 10.67 | 19.23 | 17.00 | 12.80 | 10.18 | 8.27 | 10.41 | 8.08 |
| NRCOP-35 | 5.45 | 10.61 | 13.23 | 14.40 | 11.65 | 7.11 | 7.61 | 10.61 | 8.44 |
| NRCOP-36 | 5.87 | 9.72 | 17.50 | 15.70 | 11.33 | 9.72 | 7.28 | 7.66 | 8.72 |
| NRCOP-37 | 6.55 | 11.67 | 19.16 | 16.30 | 13.45 | 9.83 | 10.83 | 12.27 | 10.33 |
| NRCOP-38 | 4.32 | 8.28 | 17.76 | 16.20 | 10.68 | 8.93 | 8.82 | 11.40 | 8.76 |
| NRCOP-39 | 5.90 | 8.67 | 17.56 | 16.00 | 12.58 | 9.33 | 7.24 | 11.81 | 8.99 |
| NRCOP-40 | 3.95 | 9.28 | 12.43 | 13.10 | 10.57 | 6.50 | 6.50 | 8.96 | 6.83 |
| CD ( 0.05 ) | NS | 3.39 | 3.81 | 1.98 | 2.75 | 2.89 | 2.64 | NS | 2.97 |
| CV % | 17.98 | 20.80 | 13.77 | 7.67 | 18.05 | 19.02 | 20.10 | 19.13 | 20.45 |

**Table 2. Fresh Fruit Bunch (FFB) yield produced per palm per annum in new oil palm crosses (Gen 8D)**

| Year | 4th year after planting | 5th year after planting | 6th year after planting | 7th year after planting | 8th year after planting | 9th year after planting | 10th year after planting | 11th year after planting | 12th year after planting |
|---|---|---|---|---|---|---|---|---|---|
| **Crosses** | **2014-15** | **2015-16** | **2016-17** | **2017-18** | **2018-19** | **2019-20** | **2020-21** | **2021-22** | **2022-23** |
| NRCOP-31 | 18.82 | 56.54 | 100.40 | 128.80 | 126.69 | 162.33 | 125.02 | 194.45 | 185.13 |
| NRCOP-32 | 12.01 | 93.36 | 158.03 | 171.44 | 175.97 | 165.54 | 139.02 | 234.91 | 163.30 |
| NRCOP-33 | 9.84 | 52.90 | 159.63 | 154.87 | 174.22 | 112.64 | 112.38 | 228.02 | 117.41 |
| NRCOP-34 | 20.22 | 78.23 | 159.43 | 179.06 | 174.51 | 165.20 | 145.36 | 196.28 | 146.95 |
| NRCOP-35 | 12.54 | 88.79 | 121.90 | 173.67 | 159.48 | 123.59 | 163.04 | 222.49 | 169.46 |
| NRCOP-36 | 14.62 | 58.32 | 131.43 | 157.29 | 145.52 | 152.39 | 118.18 | 144.84 | 170.89 |
| NRCOP-37 | 19.00 | 83.26 | 161.46 | 170.56 | 168.32 | 200.13 | 203.97 | 239.50 | 206.09 |
| NRCOP-38 | 10.37 | 54.97 | 163.16 | 176.44 | 158.69 | 161.93 | 176.49 | 238.83 | 180.39 |
| NRCOP-39 | 14.63 | 54.21 | 165.63 | 157.45 | 186.05 | 164.35 | 146.52 | 263.73 | 199.04 |
| NRCOP-40 | 12.01 | 70.84 | 114.50 | 159.65 | 136.02 | 103.30 | 120.35 | 163.37 | 132.07 |
| CD ( 0.05 ) | NS | 24.70 | 39.80 | 8.85 | 40.36 | NS | NS | NS | NS |
| CV % | 17.30 | 20.83 | 16.16 | 9.43 | 19.15 | 23.22 | 24.97 | 24.64 | 25.12 |

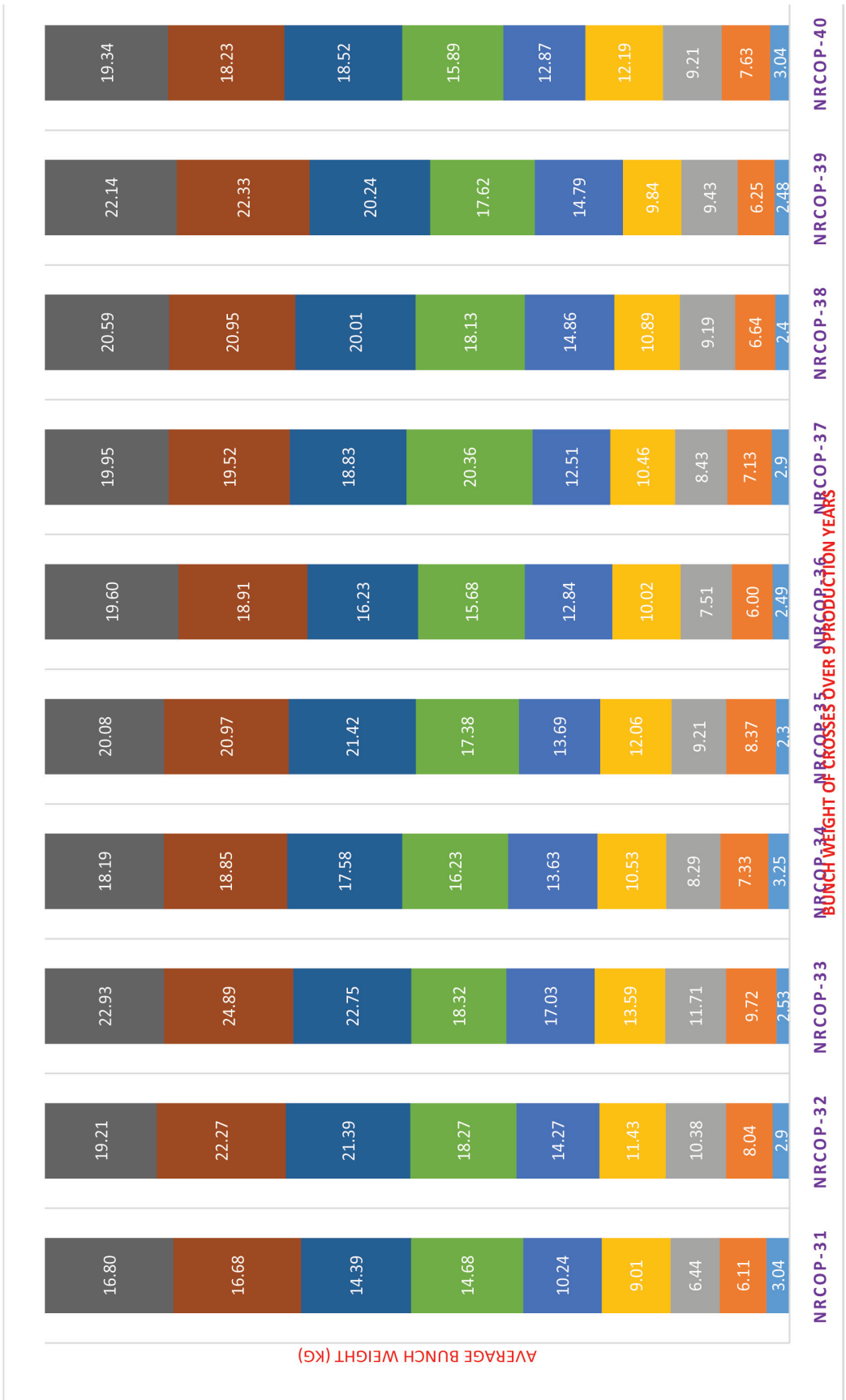


Figure 1. Average bunch weight of new oil palm cross over the evaluation period (kg) 2014-15 to 2022-23 (9 years)

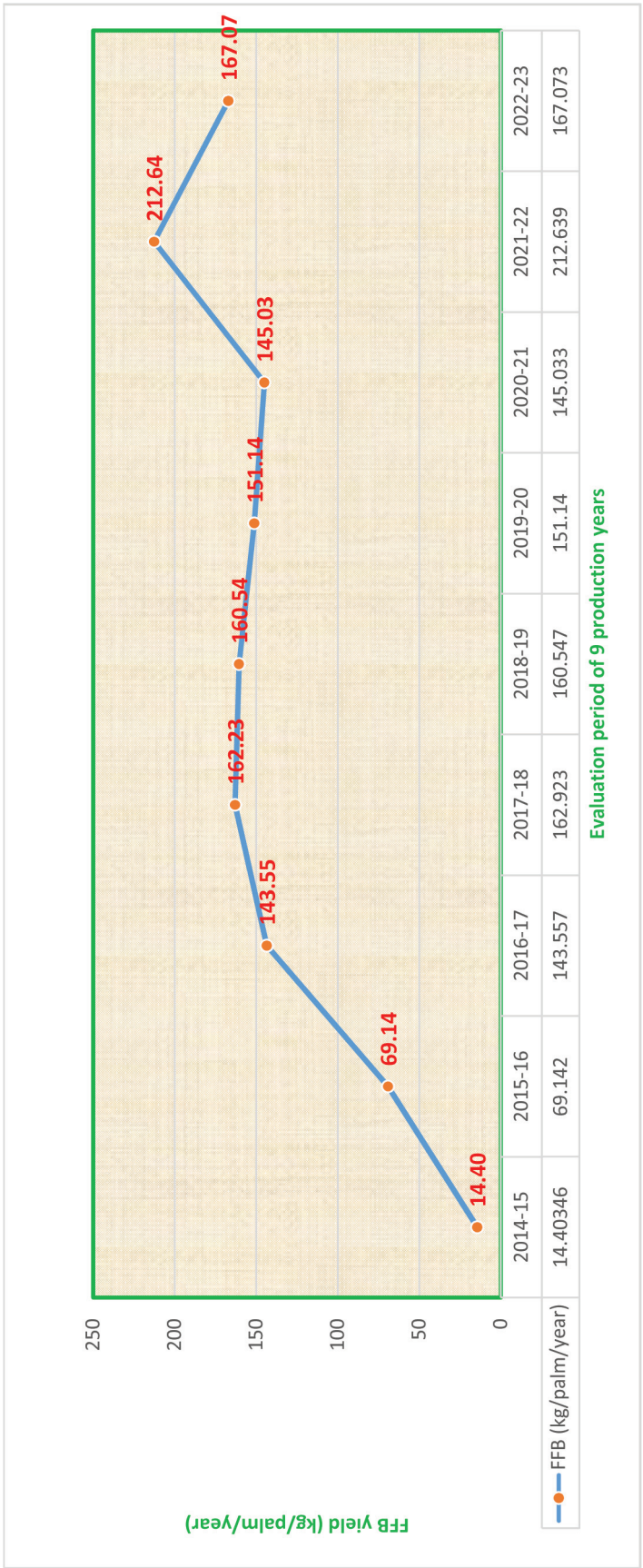


Figure 2. Average yield potential of oil palm crosses from 4th year after planting to 12th year after planting

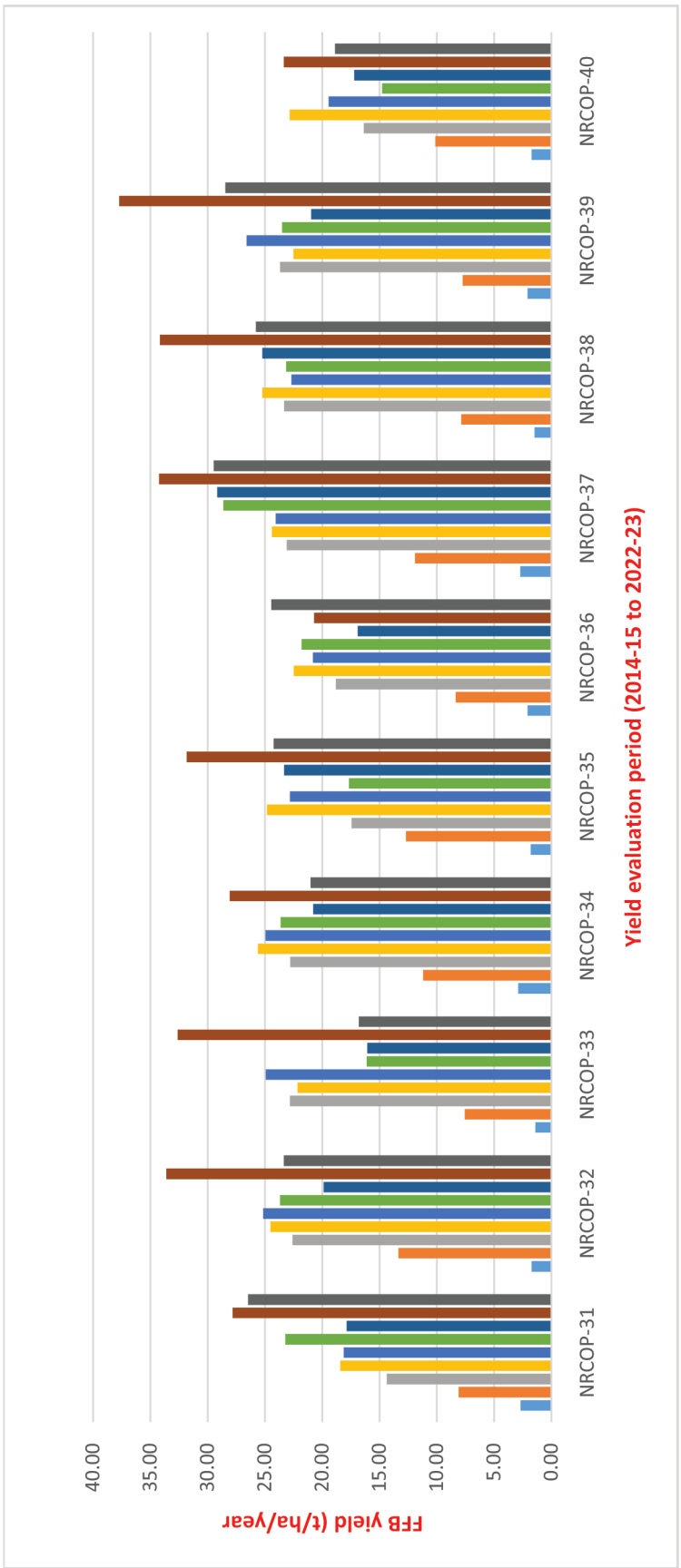


Figure 3. Annual FFB yield potential of oil palm crosses from 4th year after planting to 12th year after planting

## CONCLUSION

In oil palm, bunch production was higher from 4 years after planting to 6 years after planting and then decrease from seven years after planting to 12 YAP, meanwhile with an increase in the age of palms average bunch weight increases up to 12 years after planting. In high-yielding palms like NRCOP-37 and NRCOP-39 maximum yield of 28 t/ha/year was recorded by 6 years after planting. These two crosses are reaching a high yield in the early years and recorded stable FFB yield for seven production years with very less annual yield variations in FFB production. These results show that NRCOP-37 was a good potential cross suitable for cultivation in Andhra Pradesh conditions.

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# PERFORMANCE EVALUATION OF HEXACOPTER UAV (ANGRAU - PUSHPAK) SPRAYER FOR MANAGEMENT OF LATE LEAF SPOT IN GROUNDNUT CROP

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## ABSTRACT

This study was conducted to assess the efficacy of low volume UAV sprayer (25 L $ha^{-1}$) for controlling late leaf spot, (*Nothopassalora personata)* disease of groundnut in Krishna agro-climatic zone of Andhra Pradesh. The experiment was conducted during late *rabi* season between 2021-2022 and 2022-23. To comprehend the suitability of UAV spraying in groundnut to control late leaf spot, restricted randomised block design (RRBD) was imposed in the field of five acres with five treatments *viz.*, T1-100%, T2-75% and T3-50% RDP with UAV spraying and T4-100% RDP with human back power sprayer and T5-control plot with only water spraying with UAV. To control late leaf spot, CIB & RC recommended chemicals carbendazim 12% + mancozeb 63% WP at 61 DAS and followed by hexaconazole 5% EC at 68 DAS were sprayed as per the experimental treatment protocols. The analysis of the data revealed that spray with UAV sprayer is more efficient and precise in application and resulting in better control in late leaf spot foliar damage as the lowest percent disease incidence (PDI) was observed in T1 (24.58) followed by T2 (25.73) and then by T4 (25.88) which were on par with each other and PDI reduction percentages with respect to T5 are of 48.87, 44.65, 44.65 were observed in 2021-22, respectively. Similar results were found during 2022-23, *i.e*. the lowest PDI was observed in T1 (25.53) followed by T4 (27.75) and then T2 (28.33) which were on par with each other and reduction percentages of 51.20, 50.12, 48.51 were observed, respectively. The economic analysis through incremental cost-benefit ratio (ICBR) revealed that the highest ICBR ratio of 7.99 (T2) and 6.81 (T1) were realised and no phytotoxicity was observed in UAV spraying of fungicides in groundnut at low volume spraying of 25 L $ha^{-1}$ without compromising the bio-efficacy.

**Key Words:** Carbendazim, Groundnut, Hexaconazole, Mancozeb, Late leaf spot, UAV sprayer

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## INTRODUCTION

Unmanned aerial vehicle (UAV) can help to meet the demand of national food security by timely crop protection in efficient and effective manner to large areas in the shortest time span. It can menace the farmers problems by reducing high cost of labours, health problems by coming in contact with chemicals (fertilizers and pesticides, etc.). It saves time, money and reduce drudgery in field applications.

Groundnut is a very important food and cash crop of India. It has many benefits for human health, nutrition, soil fertility and environment. It occupies pre-eminent position in national edible oil economy after food grains in earning the foreign exchange and national food security in edible oil demands. Groundnut is a tropical legume crop grown in around 120 countries in the world in a total area of 327 lakh ha with the production of 539 lakh tonnes. India tops the world in area under cultivation and second largest producer with 101 lakh tonnes in 2021-2022 contributing 19% of global production. In Andhra Pradesh, it is grown in Anantapur, Chittoor, Kurnool, Kadapa, Nellore, Guntur and Prakasam districts. Guntur is highest in productivity with 4282 kg $ha^{-1}$ in 2021-22 (www.indiastat.com).Groundnut is popular summer crop and valuable cash crop for millions of small- scale farmers and rainfed farmers in Andhra Pradesh, where it is grown in the coastal agro-ecosystem.

Groundnut is attacked by various diseases throughout the year and yield losses due to leaf spot and rust were 50 percent and 27 percent, respectively (Kumar and Thirumalaisamy, 2016). In recent years, drone spraying used to reduce drudgery in plant protection and fertilizer application. Keeping this in view, study was carried to manage late leaf spot using UAV sprayer.

## MATERIALS AND METHODS

The research was conducted at operational research project (ORP) fields in P.G. Palem, Bapatla District, Andhra Pradesh during late *rabi,* 2021-22 and 2022-23. The scope of the study included to study on the bio-efficacy, phytotoxicity, plant growth and yield under UAV spraying envelope in groundnut when sprayed with the recommended chemical fungicides to manage late leaf spot (*Nothopassalora personata)*.

### Design of Plant Protection for UAV Spraying

A hexacopter UAV was designed and standardized specifically for plant protection spraying in agricultural crops by Acharya N G Ranga Agricultural University. The UAV built by ANGRAU with the specifications (Table 1), was designated as “ANGRAU-Pushpak-01”, a model RPAS (Remotely Piloted Aircraft System). ANGRAU – PUSHPAK drones are registered as model remotely piloted aircraft systems as per the DGCA rules and regulations. The specifications pertaining to technical parameters and payload data are detailed below (Figure 1 & Table 1).

Standardization of UAV weight, spraying height above crop canopy, flight speed, spray


**Figure 1. ANGRAU PUSHPAK- 01 – a10 L capacity hexacopter UAV standardized for agricultural spraying**

**Table 1. Technical parameters of Remotely Piloted Aircraft System (RPAS) "ANGRAU-Pushpak-01" for Plant Protection research**

| S.No. | Classification | | Parameters |
|---|---|---|---|
| 1. | Official Designation | : | ANGRAU – PUSHPAK-01 series Agricultural drone |
| 2. | Size (mm) | : | 1495 mm × 1308 mm × 500 mm (Arms unfolded with motor and without propellers) |
| 3. | Category of drone | : | Rotorcraft |
| 4. | Sub-Category | ; | Model Remotely Piloted Aircraft System |
| 5. | Class of the Drone | : | Small - All up weight (AUW) of 24.8kg |
| 6. | Motors Type and Specification | : | BLDC (Brushless Direct Current) with 180 KV rating; Input Current: 80A; IPX7 |
| 7. | Maximum Thrust of each Motor | : | 12kg/Axis (48V, Sea Level) |
| 8. | Battery Specification | : | 16000 mAh capacity with charging C rating 1C and discharging C rating of 15 C and Burst Discharge rating of 30C; 6S1P; 22.2 V and 355.2 Wh |
| 9. | Spray width | : | 2.8 m |
| 10. | Pay Load capacity | : | 10-12 kg or Litres |
| 11. | Field Capacity | : | 2.5 ha/hr |
| 12. | Spray system | : | Hydraulic |
| 13. | Flight mode | : | Autonomous |
| 14. | Navigation System | : | GNSS |
| 15. | Forward speed of the UAV | : | 5.5 m/s |
| 16. | Nozzle type | | Flat fan (VP 110015) |
| 17. | Number of nozzles | : | 4 Numbers |
| 18. | Nozzle flow rate | : | 0.42 to 0.45 lpm |
| 19. | Spraying direction | : | Vertically down |
| 20. | Spray angle | : | 110 degrees |
| 21. | Spray fluid volume | : | 25 L $ha^{-1}$ (Low volume and high concentration) |
| 22. | Radio communication frequency | : | 2.40 GHz - 2.4833 GHz |

width, direction of flight travel and standardization of parameters for aerial spraying with drone operational envelope like ambient temperature, optimal ambient temperature, humidity, wind speeds, wind direction, etc. and there by optimal time for spraying a unit of land duly considering the endurance of the batteries, spray fluid volume and uniformity of droplet distribution and their deposition (vertical and horizontal distribution) with ANGRAU Pushpak-01-UAV were done duly following the procedures given by DGCA, New Delhi.

**Details of crop, soil, climate characteristics and experimental design**

The fungicides were tested for their efficacy against late leaf spot by imposing five treatments with different dosages (Table 2). The treatments were imposed on the groundnut crop of TAG 24 variety of erect, semi dwarf, early (102 days crop duration), high harvest index (> 50%) and high water use efficiency during late *rabi* 2021-22, 2022-23. The CIB & RC recommended fungicides to control late leaf spot were carbendazim 12% +

**Table 2. Details of the crop, soil, disease, recommended chemicals sprayed using UAV to control *late leaf spot* and spraying envelope**

| **Treatment** | Spray fluid Volume, L / acre | UAV forward Speed, m/s | Drone Flying height, m above crop canopy, m | Carbendazim 12% + Mancozeb 63% WP, ml/acre | Hexaconazole 5% EC, ml/acre |
|---|---|---|---|---|---|
| Dates of Sowing (DAS) | | | | 17.01.2022 & 08.01.2023 | |
| Treatment Imposed on | | | | 61 DAS | 68 DAS |
| T1 | 10 | 5.5 | 0.6-1.0 | 200 | 600 |
| T2 | 10 | 5.5 | 0.6-1.0 | 150 | 450 |
| T3 | 10 | 5.5 | 0.6-1.0 | 100 | 300 |
| T4 | 200 | 0.20 | Lancer height | 200 | 600 |
| T5 | 10 | 5.5 | 0.6-1.0 | Only Water | Only Water |
| **Meteorological Data at the time of UAV Spraying** | | | | | |
| Temperature, $^{0}$C | | | | 33.5 | 32 |
| Relative Humidity, % | | | | 72 | 74 |
| Wind Speed, kmph | | | | 8.5 | 10 |
| Wind Direction | | | | W-E | W-E |
| UAV Flight Direction | | | | W-E | W-E |

mancozeb 63% WP at dosage of 200 g acre$^{-1}$ at 61 DAS followed by Hexaconazole 5% EC at dosage of 600 ml acre$^{-1}$ at 68 DAS. The groundnut seeds were sown in the month of January in both the years with a seed rate of 86 kg acre$^{-1}$ using tractor trailed seed drill in sandy loam soil with good irrigation water source available. In total, 5 treatments were imposed with four replications in restricted randomised block design (RRBD) with a buffer zone of 5m between the treatment plots of 1.0 acre each. Two spray technologies tested for the bio-efficacy of the chemicals sprayed are UAV and knapsack sprayer (Human back pack sprayer).

**Bio efficacy studies on late leaf spot (*Nothopassalora personata*)**

These chemicals were sequentially applied at seven days interval from 61 days after sowing (DAS) and 68 DAS, when late leaf spot damage appearance was observed on groundnut leaves (Figure 2). To ascertain the bio-efficacy, percent disease incidence, phytotoxicity, NDVI data, plant and yield data.

The disease incidence was worked out as below to calculate percent disease incidence by using following formula:

$$\%\ PDI = \frac{Sum\ of\ all\ numerical\ ratings}{Total\ plants\ observed\ X\ Maximum\ grade} X100$$

Rating of PDI (percent disease incidence) is described as follows:

1. No disease and 0% disease severity

2. Lesions present largely on lower leaves, no defoliation and 1-5% disease severity

3. Lesions present largely on lower leaves, very few on middle leaves, defoliation of some leaflets evident on lower leaves Observations to be recorded and 6-10% disease severity

4. Lesions on lower and middle leaves but severe on lower leaves; defoliation of some leaflet evident on lower leaves and 11-20% disease severity

5. Lesions present on all lower leaves and middle leaves, over 50% defoliation on lower Leaves and 21-30% disease severity

**Figure 2. A UAV spraying of recommended chemicals for bio-efficacy and phytotoxicity studies**

6. Severe lesions present on lower leaves and middle leaves, lesions present, but less severe on top leaves; extensive defoliation of lower leaves, defoliation of some leaflets evident on middle leaves and 31-40% disease severity

7. Lesions on all leaves but less severe on top leaves, defoliation of all lower and some middle Leaves and 41- 60% disease severity

8. Defoliation of all lower and middle leaves, severe lesions on top leaves, some defoliation of top leaves evident and 61-80% disease severity

9. Almost all leaves defoliated, leaving bare stems, some leaflets may remain but show sever leaf spots and 81- 100% disease severity.

Furthermore, obtained data were converted into per cent reduction over control through the following formula. The per cent reduction of population over control (% ROC) was calculated by modified Abbott's formula followed by Flemming and Retnakaran (1985).

Percentage reduction of late leaf spot foliar damage over control =

$$\%\ ROC = 1 - \frac{Post\ Treatment\ population\ in\ the\ treatment}{Pre - treatment\ population\ in\ the\ treatment} X \frac{pre\ treatment\ population\ in\ the\ untreated\ check}{post\ treatment\ population\ in\ the\ untreated\ check} X\ 100$$

**Statistical Analysis:**The data on the percent disease incidence were transformed to arcsine values before analysis and subjected to one way ANOVA using OP STAT software package. The treatments effect was compared by following Duncan's Multiple Range Test (DMRT).

**Yield**: Pod yield was recorded treatment wise and expressed in kg $ha^{-1}$.

Incremental Cost-Benefit ratio: This was calculated separately for each treatment as per following formulae:

Incremental Cost-benefit ratio: Net Return / Cost of plant protection or each treatment.

**Phytotoxicity Studies:** Phytotoxicity symptoms on plants were recorded one week after application of chemicals. Observations for specific parameters like chlorosis, necrosis, wilting, vein clearing, hyponasty and epinasty were taken using the 0-9 scale as 0 - No phytotoxicity; 1 – 0 to 10%, 2 – 11 to 20%, 3 – 21 to 30%, 4 – 31 to 40%, 5 – 41 to 50%, 6 – 51 to 60%, 7 – 61 to 70%, 8 – 71 to 80%, 9 – 81 to 90%, 10 – 91 to 100% phytotoxicity.

**Overall health of the crop due to drone spraying**

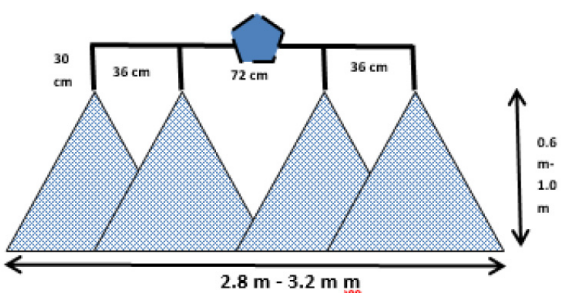
The overall health of the plant under UAV spraying and human back pack spraying technology is ascertained from the NDVI values were used to monitor the crop growth, health and to identify potential diseased parts in the fields. The NDVI readings were measured using Green Seeker™ handheld sensor which is easy to use optical sensor that instantly measures plant health and vigour. The sensor was held 24-48" (60-120 cm) above the crop canopy and observed the reading on the display. The higher the NDVI value the greater plants density and health.

## RESULTS AND DISCUSSION

### Standard Operating Procedures (SOPs)

The standard operating procedures (SOP's) (Table 4) for drone spraying in groundnut using ANGRAU – PUSHPAK -01 (Figure 2) developed as the part of this experiment from vegetative stage to harvesting stage were used for imposing the spraying treatments. At the time of imposing the

**Table 4. SOP's of drone spraying in groundnut**

| S.No. | UAV Spraying Parameter / Condition | SOP's | Results Observed |
|---|---|---|---|
| **A. Standardised parameters for UAV and spraying system** | | | |
| 1. | Weight of the Drone | Small Category – 24.8 for spraying | The plants did not lodge, leaves, flowers and fruits did not drop |
| 2. | Spray Fluid Volume | 25 L $ha^{-1}$ or 10 L $acre^{-1}$ (Low Volume Spraying) | This volume is arrived based on relative reduction of the droplet size (100-250 µm) from the conventional technology (300-750 µm) and phytotoxicity studies. |
| 3. | Nozzle Type & Specifications | Flat Fan & VP 110015 | Manufacturer specifications |
| 4. | Droplet size of the Nozzle | 100-250µm | Through water sensitive paper studies |
| 5. | Discharge of each nozzle | 0.42 to 0.45 lpm | Measured through Discharge test |
| 6. | No. of Nozzles in Hexacopter | 4 Nozzles | |
| 7. | Arrangement of Nozzles | Beneath the rotors and strictly avoid boom arrangement to avoid formation of vertices which cause non-uniform spraying. | |
| 8. | Spacing, vertical distance and arrangement of 4 nozzles on the drone. (Under 5 Kmph wind speed and $35^0$c- static condition at 0.6-1.0 m height above crop canopy). | * Beneath the rotors at 30 cm from the arms axis.<br>* Distance between nozzles from each other on either side at 36 cm.<br>* The nozzle flow fan should be vertically downwards without any obliqueness.<br>* The nozzles should be adjusted accordingly.<br>* All the nozzle tips must be aligned to be in horizontally parallel<br>  <br> | |

**B Standard Flight parameters**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 9. | Optimal flight height (above crop canopy), Optimal drone forward speed and corresponding width of spray coverage | S.No. | Item | Drone Flight Height, m above crop canopy | Width of Spraying, m | Forward Speed of UAV, m/s | Arrived by studying the iterative operations to match 10 L delivery with speeds, widths and corresponding heights of spraying with no crop damage and with 10-15% drift losses |
| | | 1. | Vegetative stage to Before Flowering stage | 0.6-1.0 | 2.8 | 5.5 | |
| | | 2 | From flowering stage onwards | 1.0-1.5 | 3.2 | 4.5 | |
| 10. | Recommended diurnal Schedule of drone spraying | 8-11 A.M. & 3.00 P.M. to 6.00 P.M. (Avoid Rainy periods) | | | | | Arrived based on the wind speeds & prevailing temperatures and corresponding spray coverage |
| 11. | Optimum wind speeds recommended | 3-10 kmph wind speeds are good for insecticides, fungicides and foliar nutrients, PGR, PGI's etc. and for herbicides, <5 kmph wind speeds are recommended with reduced flight height. | | | | | |
| 12. | Drone flight direction for spraying | The direction of drone travel must be parallel to the longest side of the field. During exigencies, if wind speeds >10 kmph are prevailing, direction of drone travel must be parallel to the direction of the wind. | | | | | |
| 13. | Drift | 0.5-1.5 m effective drift on either side at 5-10 kmph wind speeds and temperature of about 35-40 $^0$C is found. | | | | | |
| 14. | Optimal ambient temperature envelope | 35-40 $^0$C. Drone spraying above 40 $^0$C results in excessive vapour drift causes low bio-efficacy achieved due to poor deposition. | | | | | |
| 15. | Time for Drone spraying | 1. Net Time of 15 min. ha$^{-1}$ and<br>2. Gross Time of 24.71 min. ha$^{-1}$ (Including chemical filling, nozzle checking, battery replacement and pilot instructions to the farmers). | | | | | |
| 16. | Field Capacity of recommended 10L payload spraying drone | 2.43 ha h$^{-1}$ | | | | | |

treatments, the groundnut is at pegging and fruiting stage with an average plant height of 30 cm at 61 DAS and 35 cm at 68 DAS, respectively (Sambaiah *et al.*, 2023)

**Biometric Observations (Plant heights and NDVI values)**

Plant heights were in range of 26.65 cm to 18.45 cm at before spray with 26.65 cm (T1), 24.35 cm (T2), 25.80 cm (T4) on par with each other except 19.4 cm (T3), 18.45 cm (T5) and at the end of the two sprays, similar trend was followed with respect to plant heights were in range of 15.56 cm – 30.90 cm with the highest 30.90 cm (T1), 29.20 cm (T2), 29.00 cm (T4) which were at par with each other and 25.0 (T3) and 15.56 cm (T5) showing there is no change in plant growths at before and after sprays in 2021-22. In 2022-23, Plant heights were in range of 25.25 cm to 17.50 cm at before spray and at the end of the two sprays plant heights were in range of 15.80 cm – 31.50 cm with the highest 31.50 cm (T1), 29.50 cm (T2), 28.50 cm (T4) which were at par with each other and 22.50 (T3) and 15.80 cm (T5) representing there is no change in plant growths. NDVI values which are representation of phytotoxicity values were taken at before spray and after spray and were in the range of 0.68 - 0.85 except 0.75 (T3), 0.68 (T5) in before spray during 2021-22 and after two sprays similar trend was noticed which were in range of 0.74 (T1), 0.74 (T2), 0.71 (T4) at par with each other and 0.66 (T3), 0.38 (T5) representing no change in chlorophyll content and growth after two sprays. In 2022-23, after two sprays also no phytotoxicity and no change in chlorophyll content represented in Table 5.

**Bio-efficacy of UAV sprayer against Late leaf spot, *Nothopassalora personata***

The data on the late leaf spot disease PDI was found in the range of 22.10 -23.00 and 18.89 - 21.1 at 1DBS during2021-22 and

**Table 5. Biometric parameters in different treatments in drone spraying in Groundnut (*rabi*, 2021-22 & *rabi*, 2022-23)**

| Treatment | Plant height (cm) | | | | NDVI (indicators of phytotoxicity) | | | |
|---|---|---|---|---|---|---|---|---|
| | *Rabi,* 2021-22 | | *Rabi,* 2022-23 | | *Rabi,* 2021-22 | | *Rabi,* 2022-23 | |
| | 1 DBS | 7 DASS | 1 DBS | 7 DASS | 1 DBS | 7 DASS | 1 DBS | 7 DASS |
| T1 | 26.65 [a] | 30.90 [a] | 25.25 [a] | 31.50 [a] | 0.85 [a] | 0.74 [a] | 0.80 [a] | 0.74 [a] |
| T2 | 24.35 [a] | 29.20 [a] | 23.35 [a] | 29.50 [a] | 0.84 [a] | 0.74 [a] | 0.79 [a] | 0.73 [a] |
| T3 | 19.4 [b] | 25.0 [b] | 17.50 [b] | 22.50 [b] | 0.75 [b] | 0.66 [b] | 0.70 [b] | 0.65 [b] |
| T4 | 25.80 [a] | 29.00 [a] | 24.50 [a] | 28.50 [a] | 0.84 [a] | 0.71 [a] | 0.78 [a] | 0.72 [a] |
| T5 | 18.45 [b] | 15.56 [c] | 15.05 [c] | 15.80 [c] | 0.68 [c] | 0.38 [c] | 0.63 [c] | 0.35 [c] |
| C D @ 5% | 2.73 | 1.80 | 2.05 | 2.50 | 0.01 | 0.04 | 0.02 | 0.05 |
| C V % | 7.68 | 4.80 | 5.68 | 4.80 | 1.28 | 5.46 | 1.58 | 5.26 |

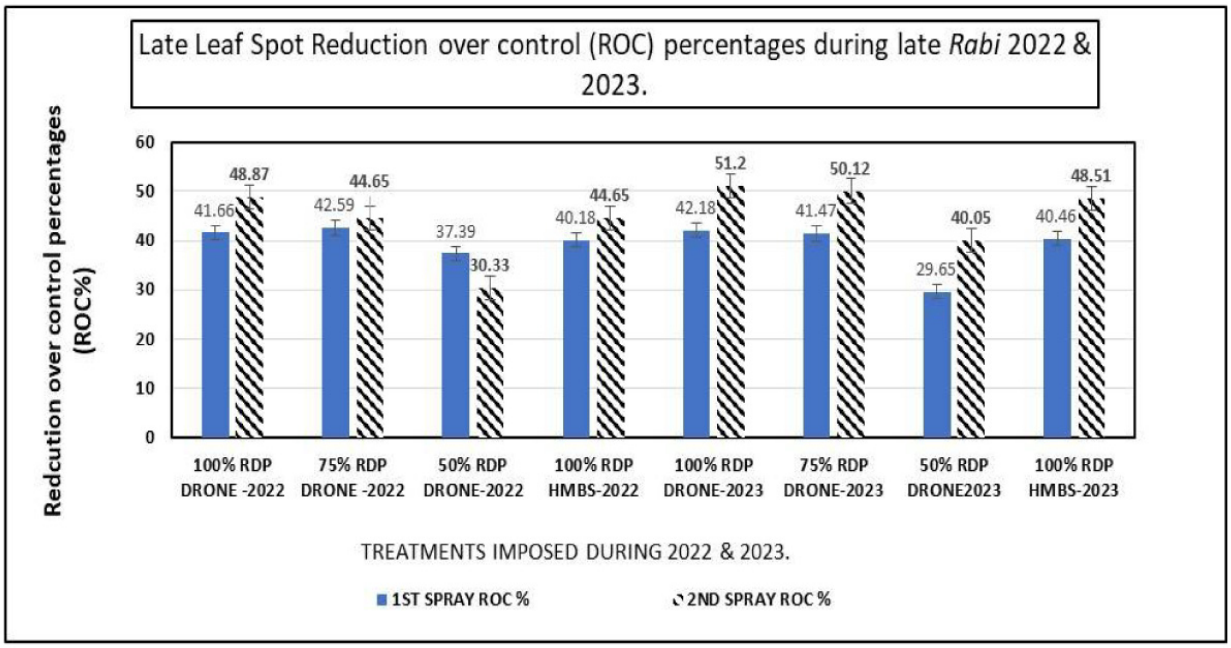



**Figure 3. Late leaf spot reduction over control (ROC %) during late *rabi* 2021-22 & and late *rabi* 2022-23 in groundnut**

**Table 6. Efficacy of different treatments against late leaf spot % in groundnut (late *rabi,* 2022 & 2023)**

| Treatment | *Rabi,* 2021-22 | | | Rabi, 2022-23 | | |
|---|---|---|---|---|---|---|
| | Carbendazim 12% + Mancozeb 63% WP | | Hexaconazole 5% EC | Carbendazim 12% + Mancozeb 63% WP | | Hexaconazole 5% EC |
| | 1 DBS | 7 DAFS | 7 DASS | 1 DBS | 7 DAFS | 7 DASS |
| T1 | 22.90 | 22.36 (28.19) [a] | 24.58 (29.70) [a] | 18.89 | 23.86 (29.16) [a] | 25.53 (30.33) [a] |
| T2 | 22.10 | 23.51 (28.99) [a] | 25.73 (30.46) [a] | 19.4 | 26.37 (31.06) [a] | 28.33 (32.81) [a] |
| T3 | 23.00 | 27.33 (31.49) [b] | 33.73 (35.47) [b] | 21.1 | 32.83 (34.92) [b] | 35.05 (37.07) [b] |
| T4 | 22.36 | 24.04 (29.33) [a] | 25.88 (30.55) [a] | 20.55 | 25.30 (30.18) [a] | 27.75 (32.54) [a] |
| T5 | 23.23 | 35.88 (40.80) [c] | 49.04 (44.42) [c] | 19.44 | 42.78 (40.84) [c] | 53.95 (47.25) [c] |
| CD @ 5% | NS | 1.55 | 2.12 | NS | 3.35 | 2.50 |
| CV % | 2.22 | 3.21 | 3.99 | 4.06 | 6.47 | 5.55 |

Figures in parentheses are arcsine transformed values

**Table 7. Economics of UAV spraying for the management of late leaf spot in groundnut**

| | Actual Yield (kg ha$^{-1}$) | Incremental yield over Control (kg ha$^{-1}$) | Incremental yield over control (` ha$^{-1}$) (A) | Cost of plant protection `ha$^{-1}$ (B) | Net returns (` ha$^{-1}$) (A-B)=C | ICBR Ratio (C/B) | ICBR Rank |
|---|---|---|---|---|---|---|---|
| T1 | 3800 | 3600 | 2,52,000 | 32,257.50 | 2,19,742.5 | 6.81 | II |
| T2 | 3500 | 3300 | 2,31,000 | 25,693.13 | 2,05,306.87 | 7.99 | I |
| T3 | 1992 | 1792 | 1,25,440 | 19,128.75 | 1,06,311.3 | 5.55 | IV |
| T4 | 3480 | 3280 | 2,29,600 | 33,757.50 | 1,958,42.5 | 5.80 | III |
| T5 | 200 | 0 | 0 | 0 | 0 | | |

1. Labour charges for one spray per acre @ Rs.500 with human back pack sprayer and with UAV, it is Rs.400 per acre and 2. Minimum support price of groundnut is Rs.70 per kg of pod yield in 2023

2022-23. During 2021-22, foliar spray with carbendazim 12% + mancozeb 63% WP, showed percent disease incidence was in range of 22.36 – 27.88 was against 35.88 in control, the lowest PDI was observed in T1 (22.36) followed by 23.51 (T2), 24.04 (T4) which were significant on par with each other. After second spray with hexaconazole 5% SC, percent disease incidence was in range of 24.58– 33.73 was against 49.04 in control, the lowest PDI was observed in T1 (24.58) followed by 25.73 (T2), 25.88 (T4) which were significant on par with each other.

In 2021-22, percent disease reduction was in range of 30.33 – 48.87 in two sprays with the highest reduction was observed in (T1) with the range of 41.66- 48.87 followed by 42.59 – 44.65 (T2), 40.18 – 44.65 (T4) which were on par with each other and the lowest reduction was observed in T3 (30.33 -37.39).

During 2022-23, foliar spray with carbendazim 12% + mancozeb 63% WP, showed percent disease incidence was in range of 23.86 – 32.83 was against 42.78 in control, lowest PDI was observed in T1 (23.86) followed by 25.30 (T4), 26.37 (T2) which were significant on par with each other. After second spray with hexaconazole 5% SC, percent disease incidence was in range of 25.53– 35.05 was against 53.95 in control, lowest PDI was observed in T1 (51.20) followed by 50.12 (T2), 48.51 (T4) which were significant on par with each other.

In 2022-23, percent disease reduction was in range of 29.65 – 51.20 in two sprays with the highest reduction was observed in (T1) with the range of 42.18- 51.20 followed by 41.47 – 50.12 (T2), 40.46 – 48.51 (T4) which were on par with each other and lowest reduction was observed in T3 (29.65 – 40.05).

Gadhiya *et al.* (2018) obtained 32.60% control of late leaf spot with foliar application of carbendazim 12% + mancozeb 63% WP and 31.00 % control with hexaconazole 5% EC.Patel *et al.* (2022) observed 27.96 % in carbendazim 12% + mancozeb 63% WP and 25.32% in hexaconazole 5 % EC with human back-sprayer. Sharma *et al.* (2020) also reported hexaconazole 5% EC has 33.37 % reduction over control and gave better pod and haulm yields. AH *et al.* (2021) they noticed that

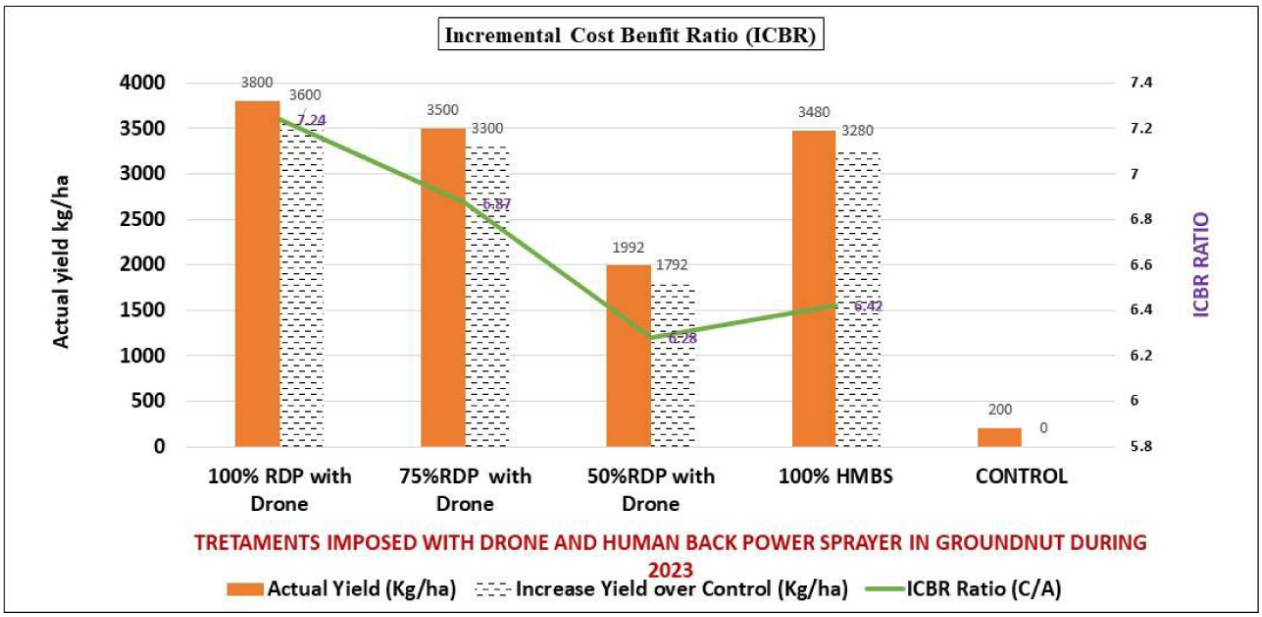



**Figure 4. Incremental cost benefit ratio in response to imposed treatments with drone during late *Rabi* 2023 in groundnut**

53.48% and 67.91% control was observed with hexaconazole 5% EC.

### Effect on yield and economics

Yield was highest (3800 kg ha$^{-1}$) with T1 followed by T2 (3500 kg ha-$^{1}$), T4 (3480 kg ha$^{-1}$) and the next best yield was presented in T3 (1992 kg ha$^{-1}$) and in control (200 kg ha$^{-1}$). Net return was higher with 100% recommended application (Rs.219742.5 ha$^{-1}$) followed by 75% RDP with drone (Rs.205307 ha$^{-1}$) and with T4 with (Rs.195842.5 ha$^{-1}$) over untreated plot. The cost effectiveness was higher with ICBR ratio of (7.99) with 75% RDP with drone followed by (6.81) with 100% dosage with drone (T1).

## CONCLUSIONS

The control efficiencies of leaf spot of groundnut with 100% and 75% RDP with UAV sprayer and human back power sprayer showed PDI reduction by 48.87%, 44.65%, 44.65% in 2021-22 and 51.20%, 50.12%, 48.51% in 2022-23, respectively and Incremental Cost-Benefit Ratio (ICBR) was the highest in T2 (7.99). The recommended doses of carbendazim 12% + mancozeb 63% WP and Hexaconazole 5% EC for UAV spraying are 150 g per acre and 350 ml per acre, respectively in groundnut for controlling late leaf spot effectively.

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# QUALITY ASSESSMENT AND NUTRIENT ANALYSIS OF FREEZE-DRIED GRAPE SEED AND PEEL POWDERS

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## ABSTRACT

The study conducted in the year 2023 aimed to develop freeze-dried grape seed and peel powder and evaluate its quality parameters and nutrients. Sun-dried grape seed and peel powder were also developed with a significant focus on comparision with freeze-dried samples, taking into account the economic feasibility and popularity of sun drying as compared to freeze-drying. Grape seed and peel were separated manually. The seed and peel were freeze-dried and stored in a freezer and sun-dried samples were kept in airtight containers. The quality parameters, nutrient composition, and sensory characteristics of both the freeze-dried and sun-dried grape peel and seed powder were examined. Quality parameters assessed included colour, solubility, pH and sensory attributes. Nutrients analysed included ascorbic acid by dye method, iron, phosphorus and carbohydrates by colorimetry. It was observed that freeze-dried grape seed and peel powders retained more colour, while freeze-dried grape peel powder had the highest solubility (67.22%). Both freeze-dried and sun-dried grape seed and peel powder exhibited an acidic pH (3.97,5.25,4.23 and 5.06). Freeze-dried grape peel powder was found to be rich in ascorbic acid (113.49 mg). Sun-dried grape seed and freeze-dried grape peel powders were found to have high phosphorus content (123.33 mg) and 108.33 mg, respectively), whereas,sun-dried grape seed featured the highest iron content (25.83 mg). Freeze-dried grape seed and peel powders were superior to sun-dried powder in quality and nutrients and hence can be used for manufacturing nutraceuticals.

**Keywords:** Freeze-dried grape peel, Freeze-dried grape seed, Grape, Sun-dried grape peel, Sun-dried grape seed, *Vitis vinifera* L.

## INTRODUCTION

Grape (*Vitis vinifera* L.) is a fruit considered botanically as a berry and an evergreen, woody vine with a blistering bark and among the most extensively consumed fruits in the world. It is a member of the Vitaceae family and several varieties are known, among which one of the most popular species is the *Vitis vinifera* L. which outnumbers all the other species by 90 percent (Parihar and Sharma, 2021). They can be consumed raw, in fresh form, and can also be consumed by processing them into products like jam,wine, jelly, extract, raisins, vinegar, etc.

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The skin, stem, seeds, and juice of the grape have also been utilized in the manufacturing of nutritional supplements and grape extract. Several pharmacological and therapeutic advantages of grape seed extract have been demonstratedanti-oxidative, antibacterial activity, anti-inflammatory, neuroprotective hepatoprotective, and cardioprotective effects (Martin *et al*., 2020). The by-products of grapes have numerous applications in the food industry. Many products obtained from grape seed like nutraceuticals are sold in the market such as grape seed extract capsules and grape seed oil. A wide spectrum of biological activities such as antioxidant, anti-inflammatoryand antibacterial characteristics are present in grape-derived nutraceuticals (Georgiev *et al.*, 2016).

Grape is an exceptional source of bioactive compounds, particularly polyphenols which bestow grapes with a variety of biological activities. Anthocyanins, flavanols, flavonols and resveratrol are the most important grape polyphenols (Cosme *et al.*, 2018). According to studies,grape pulp has a total polyphenol content that is 130 times lower on average than the seed (Kupe *et al*.,2021). They are an excellent source of antioxidants especially red grape varieties which provide more antioxidants than white or bluish grape varieties (Zhou *et al.,*2022). Grapes have several health benefits and the whole fruit, skin, leaves, and seed of the grape plant are used as medicine. The entire fruit, peel, leaves, and seed of the grape plant are utilized in medicine, and grapes have several health benefits (Sabra *et al*., 2021).

Foods may be dried in several ways. One of the most effective method for drying is “freeze-drying” and among the most widely used modes is “Sun drying”. Freeze-drying is a technique for eliminating water by sublimation of ice crystals from frozen material (Bhambere *et al*.,2015). Although freeze-drying is seen to be an expensive procedure, it is employed because it makes transporting food, especially to space, simple (Bhatta *et al*.,2020). Sun has been used for preserving food through the process of drying since time immemorial. Sun drying is one of the most popular ways of preservation that is still widely practiced in several regions of the world. Sun drying essentially entails relying solely on the strength of the sun and natural breeze. It is simple for regular people to use without any special skills or training. Sun drying is sustainable and considered to be cost-efficient (Mohammed *et al*., 2020)

Although studies on the qualities and nutritional worth of fresh grapes have been conducted, none have yet been done on freeze-dried and sun-dried grape peel and seed powder. The study aimed to prepare freeze-dried grape seed and peel powder, evaluate its quality and determine its nutritive and organoleptic properties. The study also focused on comparison the of the freeze-dried grape seed and peel powder with sun-dried grape seed and peel powders.

## MATERIALS AND METHODS

### Sample collection

The study was conducted at Avinashilingam Institute for Home Science and Higher Education for Women in the year 2023. Grapes (*Vitis vinifera* L.) were collected by checking the freshness, size, colour and maturity of the fruit from Madampatti Grape Growers’ Association, Coimbatore.

### Sample preparation

The grapes were washed thoroughly. The seed, peel and pulp of cleaned grapes were separated. The pulp that was obtained was discarded. Both freeze-drying and sun drying of grape seed and peel were carried

out. Freeze-drying of seed and peel was carried out in a freeze drier at 48 °C in a vacuum pressure of 0.17-0.20 mbar. The powdered seed and peel were dried in the sun for seven days. After drying, the seed and peel were ground to make a powder. 358 g of freeze-dried grape peel powder,132 g of freeze-dried grape seed powder, 551 g of sun-dried grape peel powder and 252 g of sun-dried grape seed powder were acquired from 10 kg of grapes. Freeze-dried grape seed and peel powder were stored in the freezer and sun-dried grape seed and peel powder were stored in airtight containers during the study period.

**Quality assessment**

Quality assessment of the freeze-dried and sun-dried grape seed and peel powderswere assessed by its colour, solubility, pH, and sensory attributes.

**Colour**

Colour assessments were carried out using a C-10 portable colour reader. The instrument used to measure the colour was based on the L*a*b* colour system. Initially, the instrument was calibrated using a black and white calibration block and further the colour of grape seed and peel powder was assessed.

**Solubility**

The solubility of the grape seed and peel powder was tested as per Trimedona *et al.* (2022) method. The solubility was tested by adding 1 g of a powder sample to a beaker containing 10 ml of distilled water. The mixture was kept at room temperature for 5 min. The supernatant was poured onto a pre-weighed petri dish and dried in an oven at 105 °C for 2 h. The solubility of the powder in percentage was determined by calculating the difference between the weight of an empty petri dish and the weight of a petri dish with obtained solids content and was divided by the initial sample weight and multiplied by 100 to calculate the percentage of solubility.

$$\text{Solubility} = \frac{\text{Wt. of petri dish with solids (after drying)(g)} - \text{Wt. of the empty petri dish(g)} \times 100}{\text{Initial wt. of the sample(g)}}$$

**pH**

The pH was tested using a pH meter. Two g of grape seed and peel powder were dissolved in 10 ml of distilled water which is neutral in pH in a beaker. The measurement electrodes were immersed into the solution in a beaker and the pH was observed.

**Sensory evaluation**

Sensory evaluation of the freeze-dried and sun-dried grape seed and peel powder was carried out in the Food Sensory Laboratory, Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore. The samples were evaluated for their sensory attributes – Appearance, colour, flavour, taste and overall acceptability using a 9-point hedonic scale. Thirty semi-trained panel members were involved in the organoleptic evaluation and they were asked to assign a score of 1 (minimum) to 9 (maximum) for the sensory characteristics of freeze-dried and sundried *Vitis vinifera* L. seed and peel powders.

**Nutrient analysis**

The phosphorus and iron content was estimated colorimetrically by measuring the intensity of colour developed at 660 millimicrons and 540 millimicrons respectively. Proximate analysis of nutrients such as carbohydrates and Vitamin C was carried out. Approximation of carbohydrates was done by the anthrone method and vitamin C was estimated by titrating against standardized 2,6 dichlorophenol indophenol dye. The analysis

was done for triplicate samples and conducted in the Nutrition Research Laboratory, Department of Food Science and Nutrition, Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore.

**Statistical analysis**

The statistical data of sensory scores were obtained using Sigma Plot 14.5 version and one-way Analysis of Variance (ANOVA) was applied to obtain the p-value and to test whether any significant difference was present or absent.

## RESULTS AND DISCUSSION

**Quality assessment**

**Colour**

In this study, it was found that freeze-dried grape seed and peel powder retained more colour compared tosun-dried grape seed and peel powder. The l*a*b values measured using the colour reader were +49.43, -1.96, and -6.86 for freeze-dried grape peel powder, +6.86, -3.95 and -10.04 for freeze-dried grape seed powder, +49.5, -2.89, -7.39 for sun-dried grape peel powder and +48.87, -3.53 and -8.67 for sun-dried grape seed powder. ÄE value for freeze-dried and sun-dried peel powder was 1.07 and ÄE value for freeze-dried and sun-dried seed powder was 42.03.

Freeze-dried seed and peel powder were found to be better in quality in terms of colour than sun-dried seed and peel powder. On comparing the freeze-dried and sun-dried peel powder, the colour difference was perceptible through close observation and for freeze-dried and sun-dried seed powder the colours were more or less similar.

**Solubility**

Freeze-dried grape peelpowder was assessed to have a solubility of 67.22% followed by sun-dried grape peel powder (61.46%) freeze-dried grape seedpowder (30.76%) and sundried grape seed powder (29.63%). Freeze-dried grape peel powder readily dissolved in distilled water with only a small amount of deposit compared to freeze-dried grape seed powder, sun-dried grape peel and seed powders. Freeze-dried grape peel powder was assessed to have the highest solubility, followed by sun-dried grape peel powder, freeze-dried grape seed powder, and sundried grape seed powder. Freeze-dried grape peel powder readily dissolved in water.

**Table 1. Sensory evaluation scores of freeze-dried and sun-dried seed and peel powders of grape**

| S.No. | Sample | Appearance | Colour | Flavour | Texture | Taste | Overall acceptability |
|---|---|---|---|---|---|---|---|
| 1 | Freeze-dried grape peel powder | 7.9 ± 1.29 | 8.06±1.22 | 7.7±1.20 | 8.03±0.99 | 7.63±1.32 | 7.9 ± 0.95 |
| 2 | Freeze-dried grape seed powder | 7.66 ± 0.88 | 7.7±0.98 | 6.86± 1.79 | 7.23±1.22 | 6.43±1.83 | 6.8 ± 1.52 |
| 3 | Sun-dried grape peel powder | 8.03 ± 0.80 | 8.23±0.81 | 7.73± 1.01 | 7.73±1.28 | 7.56±1.73 | 7.8 ± 1.37 |
| 4 | Sun-dried grape seed powder | 7.66 ± 1.02 | 7.79±1.37 | 6.86± 1.79 | 7.1±1.56 | 6.8 ±1.64 | 7.2 ± 1.56 |

**Table 2. Overall acceptability of freeze-dried and sun-dried seed and peel powders of grape**

| S.No. | Sample | Mean Scores | SD | Standard error | P value |
|---|---|---|---|---|---|
| 1 | Freeze-dried grape peel powder | 7.90 | 0.960 | 0.175 | <0.005 |
| 2 | Freeze-dried grape seed powder | 6.87 | 1.525 | 0.278 | |
| 3 | Sun-dried grape peel powder | 7.80 | 1.375 | 0.251 | |
| 4 | Sun-dried grape seed powder | 7.20 | 1.562 | 0.285 | |

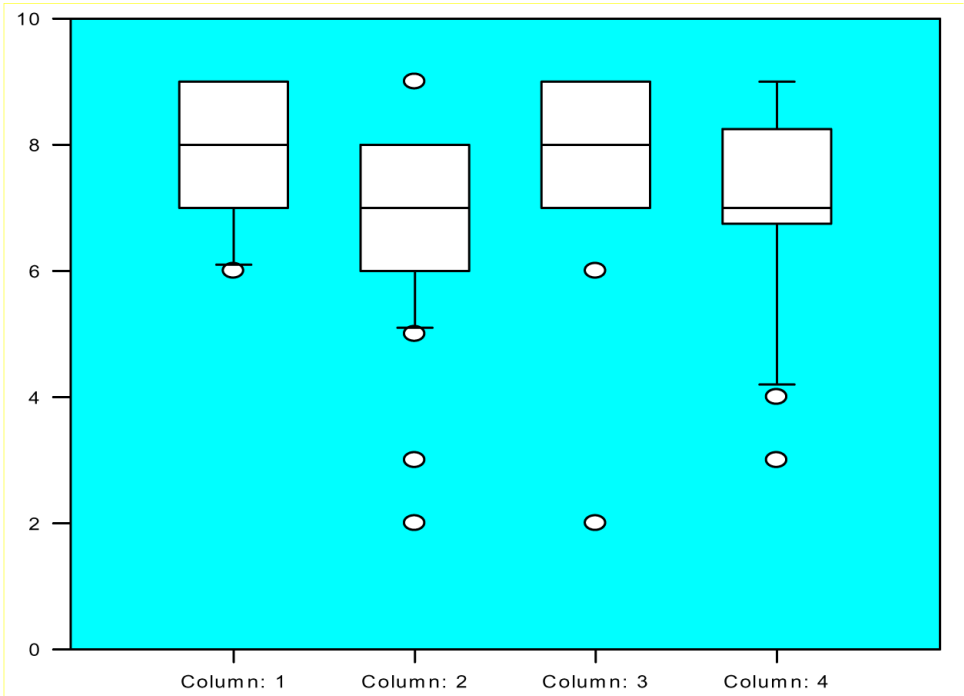


**Fig 1. Overall acceptability of freeze-dried and sun-dried seed and peel powders of grape**

**pH**

The pH was observed as 3.97, 5.25, 4.23, and 5.06 for freeze-dried grape peel and seed and sun-dried grape peel and seed powders, respectively.The pH of freeze-dried grape peel and seed and sun-dried grape peel and the seed powders were determined to be acidic (pH<7).

**Sensory evaluation**

The average sensory evaluation scores were obtained from 30 semi-trained panel members and are represented inTable 1.

While freeze-dried grape peel powder was determined to be the best in terms of texture and flavour, sun-dried grape peel powder was shown to be superior in terms of appearance, colour and taste (Table 1). Freeze-dried grape peel powder was concluded as the sample with the highest overall acceptability.

The statistical data obtained using the Sigma plot 14.5 version is presented as mean, standard deviation, standard error and p-value in Table 3 and Figure 1.

**Table 3. Nutrient content of freeze-dried and sun-driedpeel and seed powders per 100 g**

| S. No. | Sample | Vitamin C (mg) | Phosphorous (mg) | Iron (mg) | Carbohydrate (g) |
|---|---|---|---|---|---|
| 1 | Freeze-dried grape peel powder | 113.49 ±2.50 | 85 ±2.5 | 14.16 ±2.88 | 0.3 ±0.01 |
| 2 | Freeze-dried grapeseed powder | 63.8 ±8.70 | 123.33 ±3.81 | 23.33 ±1.44 | 0.66 ±0.01 |
| 3 | Sun-dried grape peel powder | 81.57 ±3.07 | 123.33 ±1.44 | 19.16 ±2.88 | 0.66 |
| 4 | Sun-dried grape seed powder | 46.10 ±6.14 | 108.33 ±2.88 | 25.83 ±3.81 | 0.34 |

The p-value obtained <0.005, represents a significant difference between the four samples-freeze dried grape peel powder, freeze-dried grape seed powder,sun-dried grape peel powder and sun-dried grape seed powder. From the graph, it can be interpreted that there is a significant difference between column 2 and column 4 (Fig 1) *i.e.* freeze-dried grape seed powder and sun-dried grape seed powder.

**Nutrient analysis**

The nutrient content of freeze-dried and sun-dried grape peel and seed powders is presented inTable 2.

Freeze-dried grape peel powder consisted of 113.49 mg per 100 g of Vitamin C. Vitamin C estimated in freeze-dried grape seed powder was 63.8 mg/100 g, the sun-dried grape peel powder was 81.57 mg/100 g and the sun-dried grape seed powder was 46.10 mg/100 g.The phosphorus content/100 g was estimated as follows- 85 mg in the freeze-dried grape peel powder, 123.33 mg in grape seed powder,123.33 mg in sun-dried grape peel powder and 108.33 mg in sun-dried grape seed powder. The iron content /100 g was found to be 14.16 mg in the freeze-dried grape peel powder, 23.33 mg in freeze-dried grape seed powder, 19.16 mg in sun-dried grape peel powder and 25.83 mg in sun-dried grape seed powder. The carbohydrate was determined to be 0.3 g in the freeze-dried grape peel powder,0.66 g in freeze-dried grape seed powder, 0.66 g in sun-dried grape peel powder and 0.34 g in sun-dried grape seed powder.

According to Sharma *et al*. (2018), pomegranate peel had the highest levels of vitamin C compared to the seed and whole fruit powder. Freeze-dried grape peel powder was observed to be rich in vitamin C. It was concluded that freeze-dried grapes had a greater amount of ascorbic acid compared to sun-dried grapes. On comparing seed and peels, it was concluded that grape peel powder had greater vitamin C content than freeze-dried grape seed powder. The phosphorus content/100 g was estimated to be similar in freeze-dried grape seed and sun-dried grape peel powders. The study concluded that freeze-dried grape seed and sun-dried grape peel powder had high phosphorus content, the iron content /100 g was found to be 14.16 mg in the freeze-dried grape peel powder, 23.33 mg in freeze-dried grape seed powder, 19.16 mg in sun-dried grape peel powder and 25.83 mg in sun-dried grape seed powder. The findings showed sun-dried grape seed powder was greatest in iron followed by freeze-dried grape seed powder. It was concluded that grape seed powder had higher iron content than grape peel powder. It was found that the freeze-dried and sun-dried grape seed and peel powders had negligible amounts of carbohydrates.

## CONCLUSIONS

Overall, freeze-dried grape peel and seed powders were found to have better quality and nutrients than sun-dried grape

seed and peel powders and may therefore the former products can be used to make nutraceuticals.

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# COMPARATIVE STUDY OF INTERIOR DESIGN AND NON – INTERIOR DESIGN STUDENTS PREFERENCES *VIS-À-VIS* HOME FURNISHINGS

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## ABSTRACT

This study was conducted among college students at Avinashilingam Institute for Home Science and Higher Education for Women, Coimbatore in the year 2023 to examine the design and colour preferences for home furnishings. The researcher selected 200 students from different disciplines to assess their preferences for home furnishings. One hundred samples were taken from the discipline of interior design, and another hundred from Homescience Extension Education, English, and Tamil disciplines. The results recommended that the interior design students preferred light colours for curtains and beddings.The statistical analysis revealed that at a significance level of 5%, the selection of curtain had the highest value, 15.560. Personal tastes significantly influenced 63.5% of the students in their selection of the furnishings.

**Keywords:** Colour, Consumer perception, Design, Home furnishing, Interior

## INTRODUCTION

A home is more than just a location for family members to reside; it is also a place for the owner to unwind, relax, and express his or her individuality. Therefore, interior design and decoration of living spaces must be more aesthetically appealing and varied. The interior design plays a significant role in decorating theinteriors (Anita *et al*., 2017). The term “Interior furnishing” means any type of furnishing made in whole or in part of fabric or related material and intended for use or which may reasonably be expected to be used, in homes, offices, or other places of assembly or accommodation. Furnishings include decorative items such as cushion covers, curtains and draperies, tablecloths, cushions, bedspreads, blankets, pillows, and linens, as well as accessories used to decorate a home or room (Tugba *et al*., 2020).

In the modern world, the necessity and use of furnishings are not new; rather, they have evolved and developed in tandem with human needs and demands. The designs, colours, and prices of fabrics for home furnishings were mostly favored by consumers (Devi *et al*., 2019). The design entails a significant social transformation that extends beyond the creation of a new or improved product. It provides an object its identity, and its form is a potent tool for creativity. To generalize what consumers want, other than

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colour choice, it will be necessary to take into account individual perception, and consumer behavior regarding home furnishings. Colour preference is also an essential factor in architecture and interior design, as people prefer to reside in environments with an appropriate colour scheme, where they feel comfortable (Wenzel *et al*., 2012).

Raizada (2012) opined that colour is an important determinant in consumers' product selection decisions, and it may also impact their perceptions of product quality. The colours are extremely important because they bring happiness, festivity, and vitality into our lives. Increasingly, colour influences the sales of a wide variety of products today. The manufacturer must ascertain the colours that consumers prefer or the factors that influence their colour selection.

This study aimed to investigate the design and colour preferences of the 200 students in relation to home furnishings. In addition, it examines the factors that influence the choice of design and colour for home furnishings that students prefer. This study revealed that personal preference, knowledge, experience, and functional factors influence students' selection of design and colour, as depicted by the study.

## MATERIALS AND METHODS

The purpose of the study was to determine the design and colour preferences of students regarding household furnishings. The research was conducted between January and April 2023. This study employed techniques of purposive sampling for its methodology. The interview schedule was meticulously devised to assess demographic variables such as age, major subject, level of study programme, knowledge-based questions pertaining to interior design and selection of design, colour for home furnishings, and selection factors. For the study, curtains, bedding, carpets, table linen, and placemats were chosen as home furnishings. For the analysis, colour harmonies such as monochromatic, analogous, complementary, and triad harmony were utilized, and for home furnishings, natural, geometric, and basic designs were chosen**.**Two hundred female students from the Avinashilingam Institute of Home Science and Higher Education for Women in Coimbatore, Tamil Nadu, were selected from the Interior design (ID), Non –

**Table 1. Demographic characteristics of the selected students (n=200)**

| S.No. | Demographic characteristics | Number of respondents | Percentage (%) |
|---|---|---|---|
| **1** | **Age (years)** | | |
| | 18 or below | 27 | 13.5 |
| | 19 to 20 | 158 | 79.0 |
| | Above 21 | 15 | 7.5 |
| **2** | **Discipline** | | |
| | Interior Design | 100 | 50 |
| | Non – Interior Design | 100 | 50 |
| **3** | **Under-graduation** | | |
| | II yr. | 53 | 26.5 |
| | III yr. | 147 | 73.5 |

Interior design such as English, Home Science Extension and Tamil in their second and third years of undergraduate study. The primary data were used to acquire information, while secondary data were gathered from books and scholarly articles to enhance the study. The frequency distribution of respondents' responses was determined through descriptive statistical analysis. The interview schedule was used to select respondents who would interpret the variables in depth. To analyze the interpretation of the data, percentage and Chi-square test analyses were utilized as statistical tools.

## RESULTS AND DISCUSSION

### Demographic characteristics of the selected students

The purpose of the survey was to determine the preferences of female college students on a variety of home furnishings that could be used in the home. The students chosen were in their second and third years of study. The vast majority of the respondents (79.0%) belonged to the age range between 19 and 20 years (Table 1). One hundred of the selected respondents were studying interior design as their primary field of study and hundred studentswere pursuing disciplines other than interior design, including Home Science Extension, English and Tamil. A majority of 73.5 percent of students selected were from third - year under graduation.

### Knowledge of the fundamentals of interior decoration, design, and colour selection for home furnishings

The level of student knowledge regarding interior decoration, colour harmony, and selection of design for home furnishings

**Table 2. Students' level of knowledge on interior decoration, colour harmony and design selections (n=200)**

| S.No. | Level of Understanding | Interior Design Percentage (%) | Non- Interior Design Percentage (%) |
|---|---|---|---|
| 1. | **Fundamentals of interior decoration** | | |
| | Greater | 80 | - |
| | Medium | 20 | 14 |
| | Vague | - | 20 |
| | Poor | - | 66 |
| 2. | **Colour harmonies** | | |
| | Greater | 60 | - |
| | Medium | 32 | - |
| | Vague | 02 | 24 |
| | Poor | 06 | 76 |
| 3. | **Selection of design for home furnishings** | | |
| | Greater | 75 | - |
| | Medium | 25 | 09 |
| | Vague | | 45 |
| | Poor | - | 46 |

was assessed by administering study-specific knowledge-related queries. The following Table depicts the students' level of knowledge regarding these topics.

Table 2 shows that a maximum 80 percent of the respondents from interior design department stated that they had greater understanding on fundamentals of decorating the interiors. A total of only 20 percent of the respondents had medium understanding on decorating the interiors and 66 percent of the respondents from other disciplines stated poor understanding on decorating the interiors. Regarding the knowledge on selection of colour harmony 60 percent respondents from interior design had greater understanding on selection of colour harmony for interiors and 76 percent of respondents from other disciplines had poor understanding on selection of colour for interiors. Furthermore, 75 percent of the respondents from interior design had greater understanding on selection of design for home furnishings. Nearly 46 percent respondents from other disciplines had poor understanding in selection of design for home furnishings,

**Table 3. Most preferred colours for selected home furnishings among students (n=200)**

| S.No. | Selected Furnishing Items | Selected Colours | Interior Design n=100 | Non- Interior Design n=100 | Total n=200 | Perce-ntage (%) |
|---|---|---|---|---|---|---|
| 1 | Curtains | Light | 57 | 47 | 104 | 52.0 |
| | | Bright | 20 | 29 | 49 | 24.5 |
| | | Dull* | 19 | 10 | 29 | 14.5 |
| | | Dark | 04 | 14 | 18 | 9.0 |
| 2 | Beddings | Light | 66 | 60 | 126 | 63.0 |
| | | Dark | 21 | 20 | 41 | 20.5 |
| | | Bright | 08 | 17 | 25 | 12.5 |
| | | Dull* | 05 | 03 | 08 | 4.0 |
| 3 | Carpet | Dark | 57 | 51 | 108 | 54.0 |
| | | Multi | 28 | 26 | 54 | 27.0 |
| | | Light | 15 | 23 | 38 | 19.0 |
| 4 | Sofa and | Light | 49 | 42 | 91 | 45.5 |
| | Cushion covers | Dark | 30 | 28 | 58 | 29.0 |
| | | Multi | 21 | 16 | 37 | 18.5 |
| 5 | Table Linen | Light | 21 | 06 | 27 | 13.5 |
| | | Multi | 20 | 05 | 25 | 12.5 |
| | | Dark | 17 | 04 | 21 | 10.5 |
| 6 | Table mat | Dark | 34 | 12 | 46 | 23.0 |
| | | Light | 16 | 03 | 19 | 9.5 |
| | | Multi | 12 | 02 | 14 | 7.0 |

**Note:** Dull colour indicates,colour with low intensity

and 45 percent of students stated on vague understanding in the selection of design for home furnishings.

**Preferences of design and color for home furnishing**

Design and colour play an increasingly essential part in the sales of furnishings. The maker must identify the designs and colours that people like or discover. This will give them a better understanding of the needs and wants of consumers (Kennamer, 1986). The study outcome showed that the preferences of students on design and colour preferences for home furnishings to decorate the interiors. The colour choices for home furnishings are given in the Table 3.

In the course of this research, consideration was given to curtains, bedding, carpets, table linen, and table mats. Regardless of the field of study that the students are pursuing, a maximum of 52 percent and 63 percent of the students who were chosen expressed their preference for light colours for curtains and bedding respectively. This is due to the fact that lighter colours will make the space appear more open and brighter (Savavibool and Moorapun, 2017). Darker hues were picked for carpets by more than half of the students because of the high likelihood that they will become soiled

**Table 4. Highly preferred design by the students for home furnishings (n=200)**

| S.No. | Selected Furnishing Items | Selected design | Interior Design n=100 | Non-Interior Design n=100 | Total n=200 | Perce-ntage(%) |
|---|---|---|---|---|---|---|
| 1 | Curtains | Natural | 56 | 49 | 105 | 52.5 |
| | | Plain & simple | 29 | 34 | 63 | 31.5 |
| | | Geometric | 15 | 17 | 32 | 16.0 |
| 2 | Sofa and | Plain & simple | 41 | 58 | 99 | 49.5 |
| | cushion- | Natural | 33 | 31 | 64 | 32.0 |
| | covers | Geometric | 26 | 11 | 37 | 18.5 |
| 3 | Carpet | Natural | 30 | 40 | 70 | 35.0 |
| | | Geometric | 43 | 32 | 75 | 37.5 |
| | | Plain& simple | 27 | 28 | 55 | 27.5 |
| 4 | Beddings | Natural | 41 | 41 | 82 | 41.0 |
| | | Plain & simple | 42 | 39 | 81 | 40.5 |
| | | Geometric | 17 | 20 | 37 | 18.5 |
| 5 | Table linen | Plain & simple | 55 | 42 | 97 | 48.5 |
| | | Natural | 37 | 50 | 87 | 43.5 |
| | | Geometric | 08 | 08 | 16 | 8.0 |
| 6 | Table mat | Natural | 47 | 32 | 79 | 39.5 |
| | | Plain & simple | 40 | 27 | 67 | 33.5 |
| | | Geometric | 13 | 26 | 39 | 19.5 |

quickly, whereas multi- coloured options were chosen by 27 percent of the students for the same. As a result of the fact that the students from other disciplines were not particularly knowledgeable about the other furnishings, the response to these items was extremely limited. According to Yu *et al*. (2018), many practitioners confess to a lack of knowledge upon which to base their colour decisions. While the vast majority of students majoring in interior design favored light or white table linen, dark colours were preferred for table mats.The Table 4 displays the results of the design preferences of surveyed students.

Regardless of the field of study that the students are pursuing, a maximum of 52.5 percent of students favored conventional design with the natural motif for curtains, while 49.5 percent selected plain colours for sofa and cushion coverings. Among interior design students, 42 percent proposed solid colours for bedding, 55 percent for table linen, and 47 percent of the students preferred natural design for table mats. The largest percentage of interior design students who offered geometric patterns for carpet was 43 percent. On the whole irrespective of the major they study, 40 percent and 41 percent of respondents liked a conventional design of natural motif for their carpet and bedding. 48.5 percent of the student's preferred plain or simple design for their table linen. Table 5 displays the colour and colour harmony preferences of the students for living and bedroom decoration.

Despite the fact that the students of interior design were aware of colour harmonies that the other students were not aware of, just a few students of interior design responded for the most favoured colour harmony and other students from different disciplines did not answer the questions. When asked about the reason why they did not respond to the question, they responded that when selecting colour harmony for their interiors, they will pay emphasis to the available design and colour in the stores rather than a particular colour harmony. The students who responded offered a monochromatic colour harmony (the hue pink) for the living room, and they suggested blue colour for the bedroom since they considered it should reflect a cool shade. As these students are female students, they would have preferred pink hues for the living room

**Table 5. Interior design students' preferences on colour and colour harmony for living and bedrooms (n=100)**

| S.No. | Colour harmony | Living room | Perce-ntage | Bed-room | Perce-ntage |
|---|---|---|---|---|---|
| 1 | Monochromatic | Pink | 29.0 | Blue | 34.0 |
| 2 | Triad | Red, yellow, blue | 22.0 | Yellow-orange, blue-green, Red-violet | 22.0 |
| 3 | Complementary | Blue and orange | 19.0 | Yellow and purple | 14.0 |
| 4 | Split Complementary | Yellow orange, blue, purple | 14.0 | Yellow. Purple, blue purple | 15.0 |
| 5 | Analogous | Violet, blue-violet, blue | 16.0 | Blue-violet, blue, blue-green | 15.0 |

and blue for the bedroom. Jiang *et al*. (2020) stated that girls prefer pink and purple colours whereas, boys preferred red, and both genders preferred blue. Furthermore, a few interior design students suggested direct complimentary colour schemes of blue, orange, yellow and purple for the living room and the bedroom, respectively. The students recommended that a primary colour triad could be used in the living room while an intermediate colour triad could be used in the bedroom. The responses for the split complementary and analogous colour harmonies are extremely limited compared with other colour harmonies.

**Influencing factors for the selection of design and colour in home furnishings**

The decision to purchase home furnishings was influenced by a variety of factors, including design, brand, colour, consumer awareness, and convenience (Nigar, 2021). This study examined how much of an impact the choice of design and colour may have on the final product of a home furnishing project. For the purpose of this study, several influencing elements, such as knowledge, personal taste, and experience with selection, ideas contributed by members of the family and friends, and functional factors such as colour fastness, durability, washability, and abrasion resistance were chosen. Table 6 outlines the elements that are considered when making decisions on the selection of design and colour of home furnishings.

More weightage was given to their own personal taste when choosing the furnishings for their homes by 63.5 percent of the students, regardless of the academic field in which they are enrolled. It was noteworthy to observe that just 43 percent of students who pursue Interior design placed priority on applying their knowledge in the process of selecting home furnishings for their homes. It is encouraging to see that the thoughts and opinions of members of the family were taken into consideration when selecting furnishings for the home by 26 percent of the respondents from the other disciplines. When it comes to the selection of furnishings for interiors, it is disheartening to learn that functional factors were not accorded a great deal of importance. As a result, it is essential to raise students' levels of awareness regarding the functional factors that should be addressed while

**Table 6. The factors that influence selection of design and colour for home furnishings**

**(n=200)**

| S.No. | Influencing Factors | Students in different disciplines | | | |
|---|---|---|---|---|---|
| | | Interior design =100 | Non-Interior Design =100 | Total =200 | Percentage (%) |
| 1 | Personal taste | 66 | 61 | 127 | 63.5 |
| 2 | Knowledge | 43 | 14 | 57 | 28.5 |
| 3 | Opinions of family members and friends | 16 | 26 | 42 | 21.0 |
| 4 | Experience | 07 | 09 | 16 | 8.0 |
| 5 | Functional factors | 01 | 02 | 03 | 1.5 |

*Multiple responses

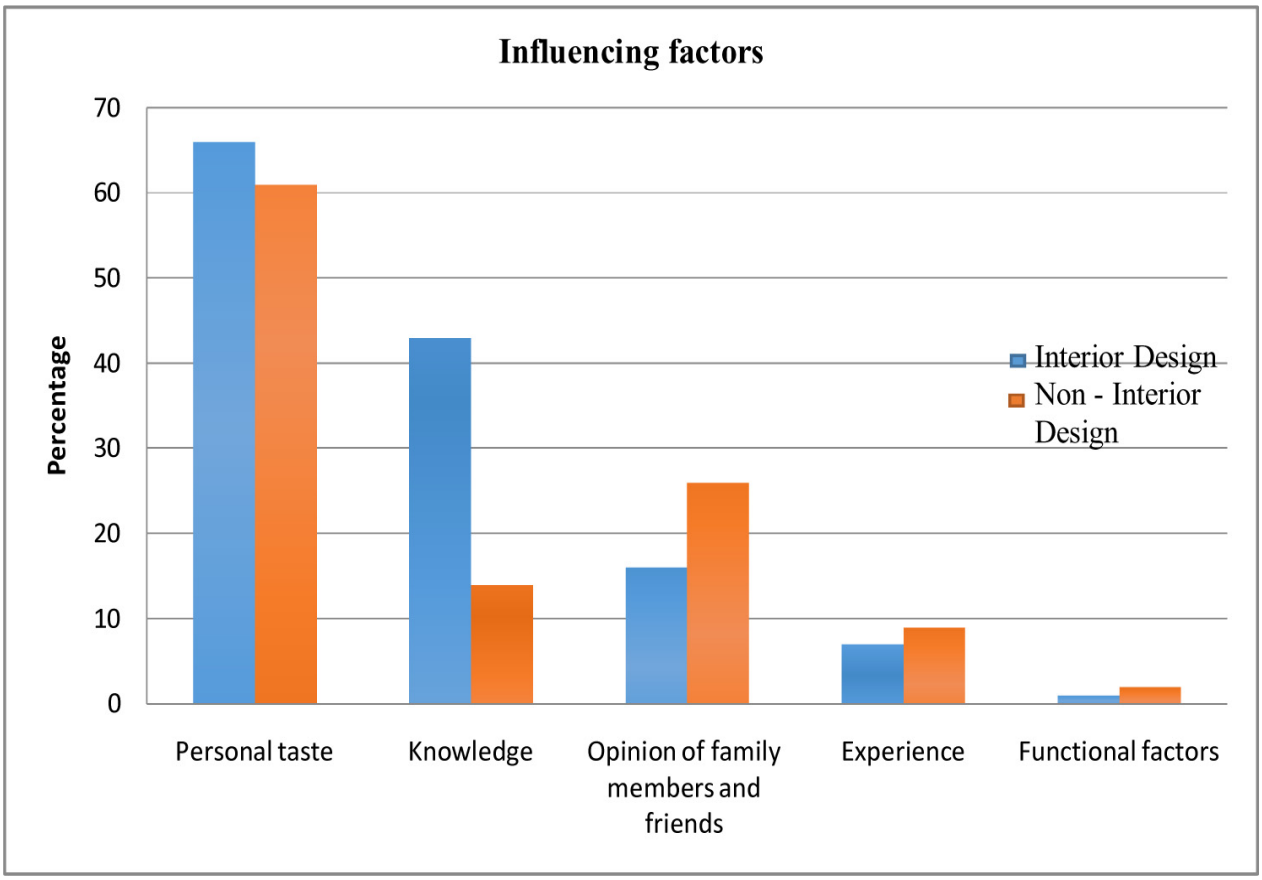


**Figure 1. Graphical representation of influencing factors on selection of design and colour for home furnishings**

**Table 7. Chi-Square test analysis of student preferences for interior furnishing $X^2$ value**

| S.No. | Preferences | Furnishing Items | $X^2$ value | df | Significance |
|---|---|---|---|---|---|
| 1 | Colour | Curtains | 15.560 | 6 | * |
| | | Beddings | 5.543 | 9 | NS |
| | | Carpets | 9.683 | 6 | NS |
| 2 | Design | Curtains | 7.346 | 6 | NS |
| | | Sofa and cushion covers | 19.992 | 9 | * |
| | | Carpets | 21.597 | 9 | ** |
| | | Beddings | 15.309 | 12 | NS |
| | | Table linen | 6.176 | 6 | NS |
| | | Table mat | 9.479 | 6 | NS |

*:Significant at 5% level, **:Significant at 1% level, NS-Not significant, df – Degrees of freedom

choosing home furnishings. A graphical representation of influencing factors is shown in Figure 1.

**Chi-square test analysis of student's preferences for interior furnishing**

Furnishings are one of the most demanding and emerging fields, full of scope of innovation and creativity. Interior furnishings, in particular convey a message to make space unique and reflect the personalities of users (Anita *et al*., 2017). The findings of the statistical study of the student's preferences for interior decoration and colour, as well as design selections of selected home furnishings are presented in Table 7.

Table 7 represents the results of the Chi-Square test for the student's preferences of design and colours for home furnishings. In the analysis of the colour preferences of students, the colour selection for curtains had the highest value, 15.560, with a difference of 6 and a significance level of 5%. In addition, the selection of colours for carpets and rugs has not reached a noteworthy level. Next, to evaluate the students' choice of design for sofa and cushion covers, a significant level of 5% and a value of 19.992 were obtained. Carpet design reached a significant level of 1%, with a value of 21.597. The selection of designs for bed covers, table linens, and table mats has not reached a significant level.

## CONCLUSIONS

Design and colour are of utmost importance when selecting home furnishings for interior design. Consequently, this study was conducted to examine the design and colour preferences of students with regard to home furnishings. The study revealed that the majority of interior design students prefer monochromatic colour harmony with pink and blue for living room and bedroom decoration respectively. In selecting colours for domestic furnishings, the colour palette reflects the psychological influences of adolescent girls. The study also revealed that students do not adequately consider functional factors. Therefore, it is essential to educate the students on functional factors while selecting furnishings. This study facilitated consumer-centric design and colour applications in design activities, putting consumer contentment at the forefront.

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# MOLECULAR IDENTIFICATION OF NOVEL PROBIOTIC BACTERIA ISOLATED FROM COW MILK AND ITS ANTIBACTERIAL APPLICATIONS

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## ABSTRACT

The purpose of the study conducted in the year 2021 was to isolate and identify *Lactobacillus* species through biochemical characterization and to find antibacterial potential of the species. Researchers collected milk sample from local milk vendor, Eachanari in December, 2021. In an Enriching MRS broth the colonies were isolated and loop full of isolate were streaked on MRS agar plate. By morphological characteristics determined through gram staining method the isolated bacteria were identified as *Lactobacillus* species. Through different identification methods such as casein digestion, acid and salt tolerance the isolate was characterized for probiotic properties. In a skim milk agar plate the isolated strain digested casein. This indicated production of protease enzyme which is a significant probiotic property. In MRS broth, an acid tolerance test was conducted at pH levels 3, 4, 5, 6, 7 and 8. The isolate was able to endure both an extremely acidic pH and alkaline media. NaCl tolerance test was done in MRS broth at concentrations of 3%, 4%, 5%, 6%, 7%, and 8% NaCl. The isolate survived well in solutions containing 3%, 4%, and 6% NaCl. After biochemical studies, molecular identification was carried out to identify the probiotic starin. The isolated probiotic was identified as *Limosilactobacillus fermentum* strain BTFSN. The isolated strain was then checked for antibacterial activity against three pathogens. Zone of inhibition were observed. The study indicated that *Lactobacillus* species from milk sample have potential probiotic properties and possess antimicrobial property.

**Keywords:** Antibacterial, Isolate, *Limosilactobacillus fermentum,* Lactobacillus sp, LAB, Probiotic.

## INTRODUCTION

Probiotics is currently a rapidly expanding market for food producers, particularly in the dairy food sector, with impressive growth potential. Species of *Lactobacillus, Bifidobacterium*, Streptococcus, and Enterococcus make up the majority of the probiotic bacteria (Tachedjian *et al.,* 2017). The oldest techniques for food preservation are fermentation-based. Before microbes were discovered, people used fermented foods including kefir, cheese, curd, miso, and nato.Consuming fermented food products may extend a person's lifespan. The theory was supported by observations made by Bulgarian peasants. The ingestion of fermented milk products containing lactic acid bacteria, which replaces harmful gut bacteria, is said to have

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contributed to the longevity of Bulgarian peasants. According to Dj, B. (1988), L. bulgaricus destroys putrefactive intestinal bacteria, which improves the host's general health (Rodrigues *et al.* 2020). The probiotic notion was first established by Lilly and Stillwell in 1965 as advantageous microbes that aid in the growth of other microorganisms (Abd El-Hack *et al.,* 2020). Probiotics are defined by Fuller (1989) as living

Probiotic organisms predominantly establish themselves in the human host's intestinal tract, with the commensal microbiome in the intestines playing a crucial role. This microbiome contributes significantly to enhancing resistance against infections, distinguishing the host's immune system, and manufacturing essential nutrients (Ubeda & Pamer, 2012). Evidence suggests that probiotics could play a role in both treating and preventing infectious diseases (Yang *et al.*, 2020). Presently, infectious diseases are commonly tackled through the administration of antibiotics. Nevertheless, the indiscriminate use of antibiotics can lead to individual-level issues such as specific drug-related side effects, as well as broader public health concerns such as the emergence of multidrug-resistant bacteria (Yang et al., 2020). As a result, there's a pressing need to explore new avenues in antimicrobial therapy, particularly focusing on treatments derived from natural products (Silva *et al.*, 2019). Numerous clinical trials have exhibited the efficacy of probiotics in addressing an array of medical conditions, including constipation, diarrhea, polycystic ovary syndrome, ulcerative colitis, stress and anxiety, inflammatory bowel disease, breast cancer, and diabetes. Despite the well-established biological properties of probiotics, such as their antimicrobial effects, research in this realm remains relatively nascent and requires further in-depth examination.

Therefore, keeping the above in mind researcher have taken this study to isolate and identify a novel probiotic using both biochemical and molecular identification techniques. This will involve analyzing various biochemical markers by utilizing molecular tools such as DNA sequencing to accurately identify and classify the probiotic strain and aimed to analyze the presence of anti-bacterial properties of the strain.

## MATERIALS AND METHODS

The study was conducted in the year 2021.

### Sample collection

Milk sample was collected from a local milk vendor from Eachanari (Dec 2021) and was used for isolation of possible probiotic strain.

### Isolation of probiotic strain

Collected samples were used for serial dilution up to $10^7$. A 100 $\mu$ *l* sample from the $10^4$ were spread in the nutrient agar plates by spread plate technique and kept for incubation for 24 h at 37 $^0$ C.

After incubation, nutrient plate was observed for growth and a loop full of colony was inoculated in a MRS broth for subculture.

### Gram staining

Gram staining is one of the common methods for characterization of a bacterial strain. The bacterial isolate was studied for gram characters by gram's staining. Using the standard procedure on clean glass slide a single colony was smeared and let to air dry and then heat fixed. The heat-fixed smear was covered with a crystal violet solution, following which it was rinsed with water and the slide was covered with mordant gram's iodine. With 95% ethyl alcohol and water, the smear on the slide was decolored. Safranin was applied as a counterstain for 60 seconds, washed with

water, and the slides were examined under 100x oil immersion at 100x magnification (Mannan *et al.,* 2017).

**pH tolerance test**

The pH of the MRS broth was adjusted with 10 N HCL and 1N NaOH to 3, 4, 5, 6, 7, and 8. The cultures were put into the respect MRS broth in an eppendorf tube and incubated for two days at 37 $^{0}$ C.. The turbidity will determine the growth of the LAB Mokoena(2017).

**NaCl tolerance test**

For salt tolerance test the isolate were inoculated in MRS broth with 3%, 4%, 5%, 6%, 7% and 8% of NaCl concentration. Bacterial culture was inoculated and then incubated at 30 $^{0}$C for 2 days *i.e.*, 48 h (Busto *et al.,* 2018)

**Casein digestion**

The casein digestion activity was carried out in MRS agar plate having 1% of skim milk powder. Protease activity indicates casein digestion. Fresh culture was inoculated in the plate and was kept for incubation for 48 hours at 37 °C Clear zone formation.

in the vicinity of the culture indicate protease activity (Mannan *et al.,* 2017) Distilled water and Protease enzyme were used as negative and positive control, respectively.

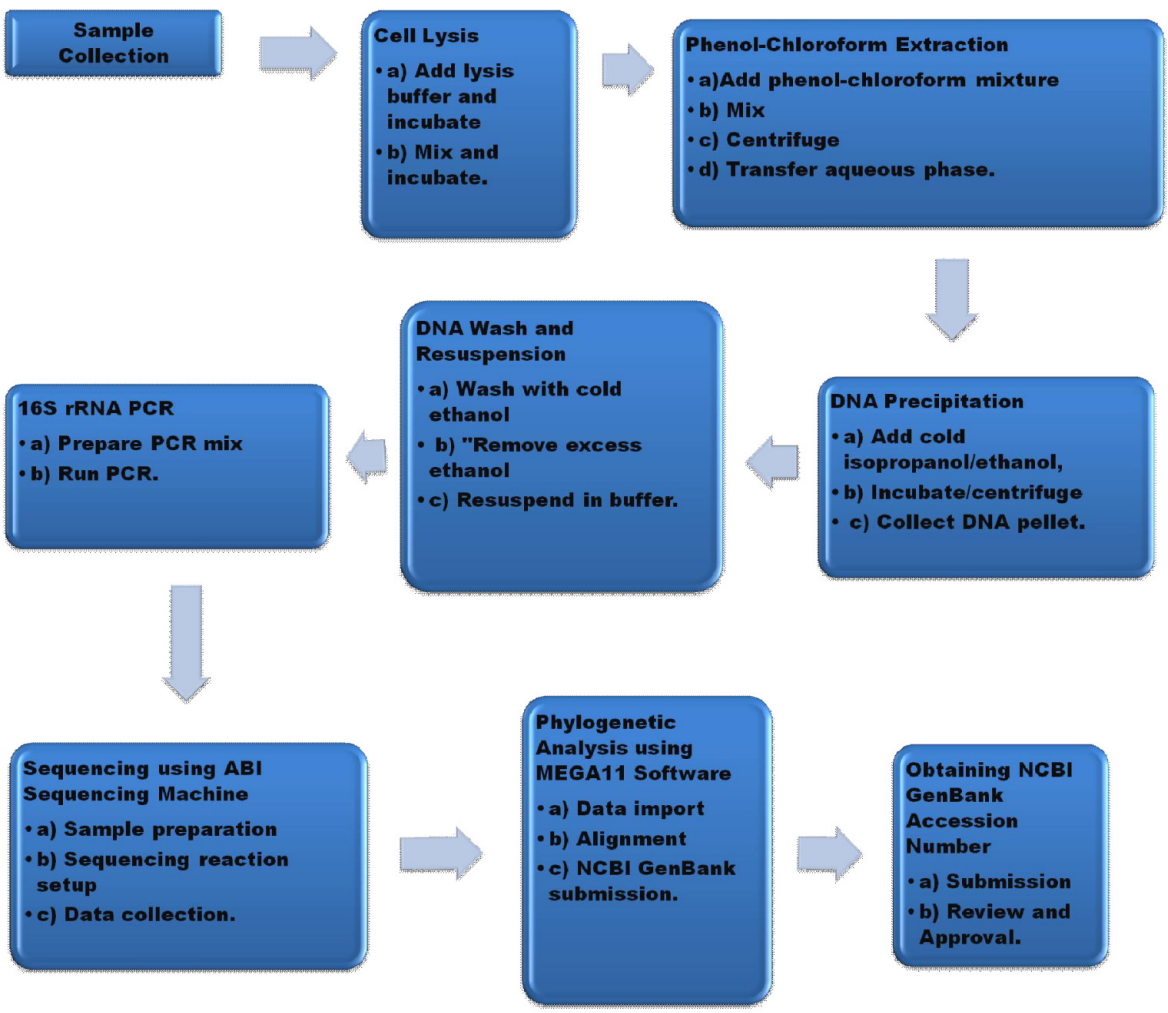


**Figure 1. Flowchart of the steps involved in DNA isolation**

**Molecular identification**

The phenol chloroform technique was used to isolate the DNA. The 16s rRNA PCR was carried out as follows (Ahmed *et. al.,* 2021) After PCR reaction the Amplified product was used for sequencing using ABI sequencing machine (ABI 3599 x L Genetic analyzer). The phylogenetic analysis was performed using MEGA11 software and NCBI gen bank accession number was obtained (OP051100).

**Steps involved in DNA isolation**

The process of DNA isolation and subsequent 16S rRNA gene sequencing involves several essential steps to extract and analyze genetic information from a biological sample. Firstly, collection of the sample containing the target DNA, following this step, the process of cell lysis ensues. A lysis buffer is added to the sample, initiating cell membrane disruption and protein denaturation through incubation. Next, a phenol-chloroform extraction step separates the aqueous phase containing the DNA from the organic phase. The DNA is then precipitated using cold isopropanol or ethanol, and the resulting pellet is washed and resuspended in an appropriate buffer.

To specifically target the 16S rRNA gene, a Polymerase Chain Reaction (PCR) is performed. This involves preparing a PCR reaction mix with DNA template, gene-specific primers, nucleotides, polymerase, and buffer, followed by running the PCR program to amplify the 16S rRNA gene. The amplified product is then prepared for sequencing reaction using an ABI sequencing machine, which generates raw sequencing data.

Subsequently, the obtained sequencing data is imported into analysis software (MEGA11) where sequences are aligned for accurate ordering. The resulting 16S rRNA gene sequence is then submitted to NCBI GenBank, undergoing review and approval. Upon approval, an accession number is assigned, and NCBI may provide a phylogenetic tree, depicting the strain's position within the standard phylogenetic tree based on its 16S rRNA gene sequence. This comprehensive process enables researchers to explore and classify microorganisms at a molecular level.A phylogenetic tree is provided by the NCBI which unable the researchers to point were the strain lies in the standard Phylogenetic tree

**Antibacterial activity**

The antimicrobial activity were tested against *Escherichia coli, Salmonella typhi and Klebsiella pneumonia and* Moxifloxacin was placed as a negative control by well diffusion technique on the MRS agar plate spread with the bacteria isolate andzone of inhibition was used to determine the antibacterial activity after being incubated at 37 °C for 24 hours (Ahmed *et al.,* 2021).

**RESULTS AND DISCUSSION**

The isolate procured from the cow's milk were identified using different identification methods such as gram staining, casein digestion, acid and salt tolerance and molecular identification for probiotic properties. Their morphology, characteristic and on the aspect of catalases negative and gram positive rod shape the isolate were analysed . According to Houque *et al.* (2010) and Rao *et al*. (2015), the strain was identified and confirmed as catalase negative and gramme positive. Lactobacillus is a genetically and physiologically diverse group of rod-shaped, gram- positive, catalase-negative bacteria.

pH is an is essential aspect for the survival of the culture and probiotic strain survived well in acidic environment (Pundir *et al.,*2013). In the study, the strain was analyzed in both acidic and alkaline conditions at

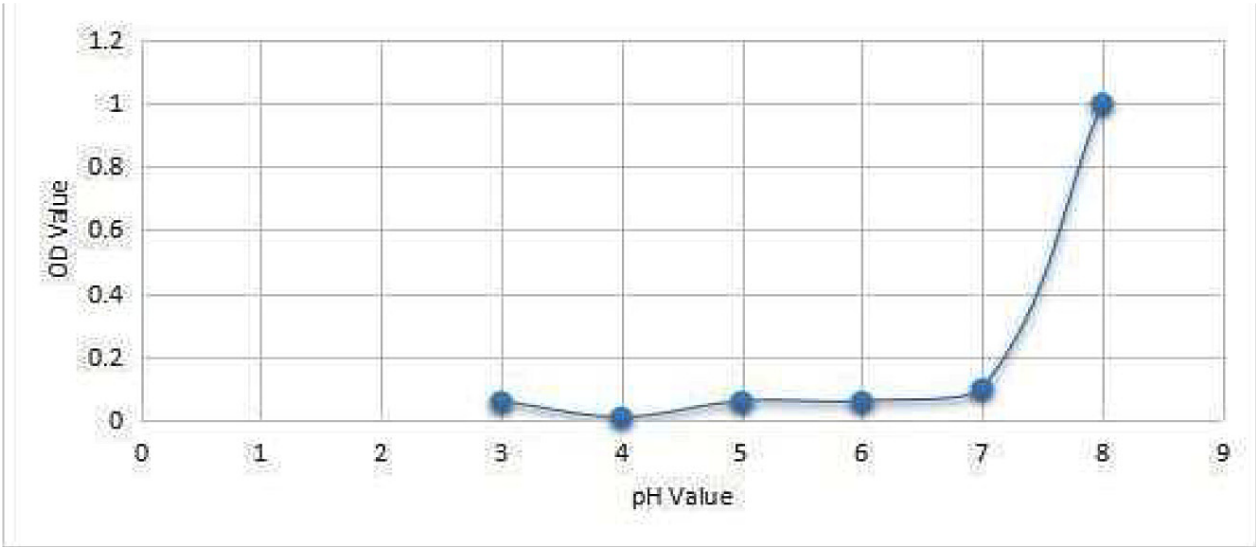


**Figure 2. Graph showing survival of the isolate in different pH**

different range of pH 3 to 8. The readings were observed with the help of UV spectrophotometer. The results showed isolated *Lactobacillus* thrived in highly acidic pH yet it showed maximum growth at pH 8 as shown in Fig 1.

According to Huan Xiang *et al.* (2019) in the presence of NaCl, growth of some bacteria is inhibited. For the analysis of NaCl tolerance of *Lactobacillus* spp.it was performed at 3%, 4%, 5%, 6%, 7%and 8% of NaCl concentration. The results were recorded using a UV Spectrophotometer. The isolates survived well in 3%, 4% and 6%. The concentration 5%, 7% and 8% the value was recorded less than0.15 OD, indicating less survival of bacteria (Fig 2.) LAB does not grow well in high NaCl concentration (Rahman *et al.*, 2015).

Lactobacillus digest casein to grow in milk and use the degradation products afterwards

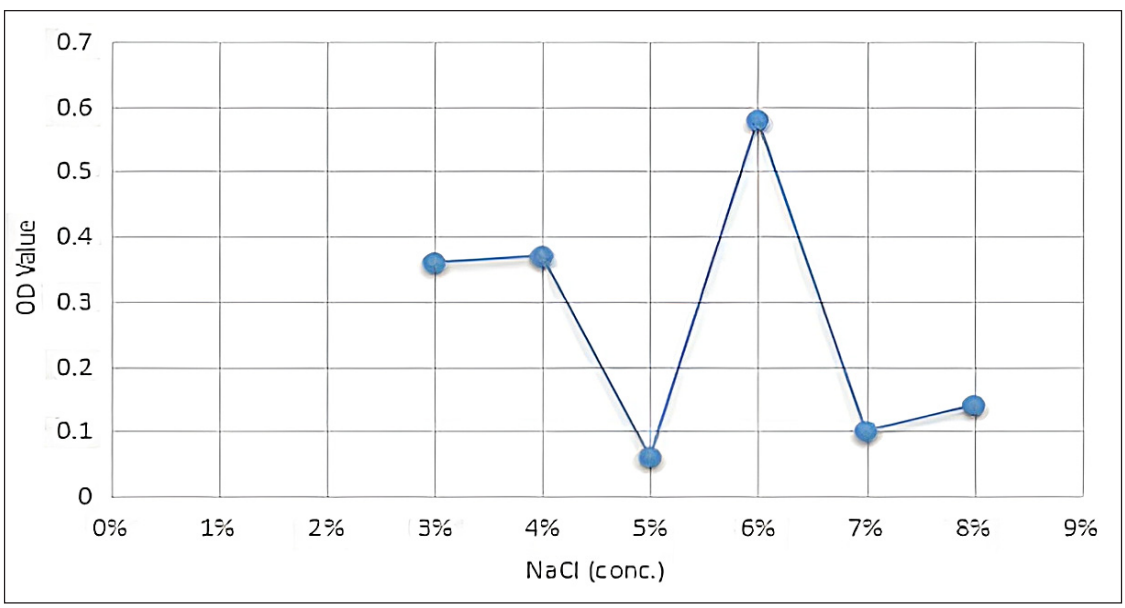


**Figure 3. Graph showing survival of isolate in different NaCl concentration**

**Figure 4. Skim milk agar plate with casein digestion activity around the well poured with the isolate and protease enzyme**

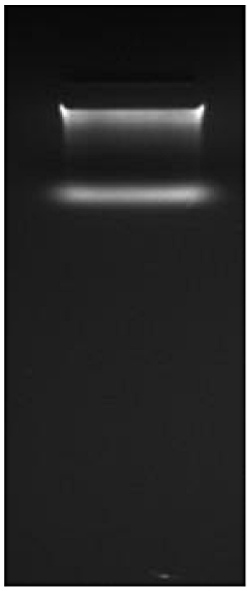

**Figure 5**

**Figure 5. PCR Amplicon (~1.5kb) Loaded on 1.5% Agarose Gel**

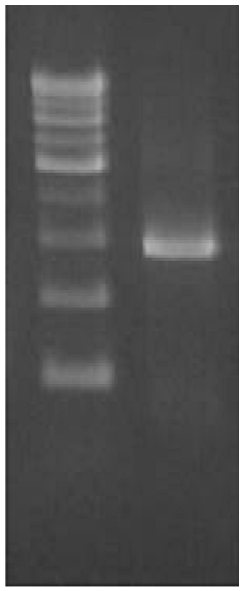

**Figure 6**

**Figure 6. Lane 1 : 500bp DNA ladder ; Lane 2: PCR amplified product**

(Mannan *et al.,* 2017) The isolate was able to create a zone of inhibition, suggesting that casein was digested and that a protease enzyme was produced (Atanasova *et al.,* 2014). Both the positive control *i.e.,* protease enzyme and the isolate were forming zone of inhibition as shown in Fig 3.

The DNA was isolated using phenol chloroform method. The 16sRNA PCR was carried out as follows. After PCR reaction the

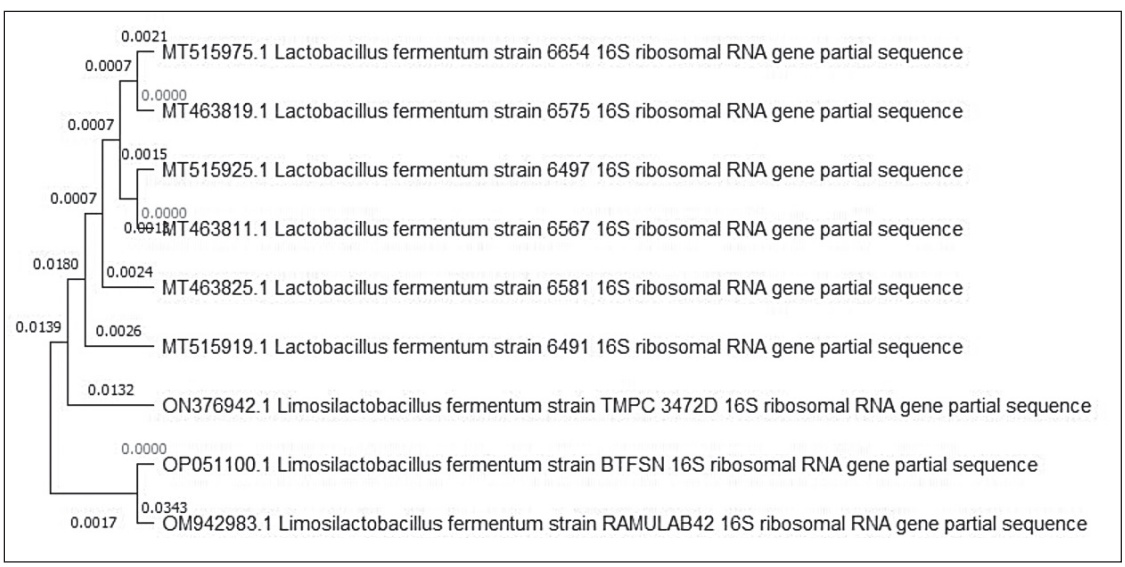


**Figure 7. Phylogenetic tree provided by the NCBI**


**Figure 8. Plate spread with *E. coli* 8mm and 6mm inhibitory zone observation**


**Figure 9. Plate spread with *S. typhi.* 7 mm and 5 mm inhibitory zone observation**


**Figure 10. Plate spread with *K. pneumonae.* 8 mm and 3 mm inhibitory zone was observation**

Amplified product was used for sequencing using ABI sequencing machine (ABI 3599 x L Genetic analyzer) represented in Fig 5. The phylogenetic analysis was performed using MEGA 11 software and NCBI gen bank accession number was obtained (OP051100), the isolated probiotic was identified as *Limosilactobacillus fermentum* strain BTFSN and phylogenetic tree was provided by the NCBI gen bank. The BLAST result showed similarity of the isolate is 99.56% ( Fig 7).

**Antibacterial activity**

The formation of the zone indicated the antimicrobial property of the isolate. In the study, 5 mm, 3 mm and 6 mm diameters of zone was observed for *S.typhi, K. pneumonae* and *E.coli* respectively in 10 ul isolate and 7 mm, 8 mm, 8 mm diameter of zone was observed for *S.typhi, K. pneumonia* and *E.coli* respectively for 20ul of isolate (Fig. 7, Fig. 8,Fig. 9.)

**CONCLUSIONS**

The study indicated that *Lactobacillus* species from milk sample showed characteristics properties of a probiotic. The isolates were able to survive in very acidic media and also able to survive higher concentration of alkaline condition. The protease enzyme activity was observed and it

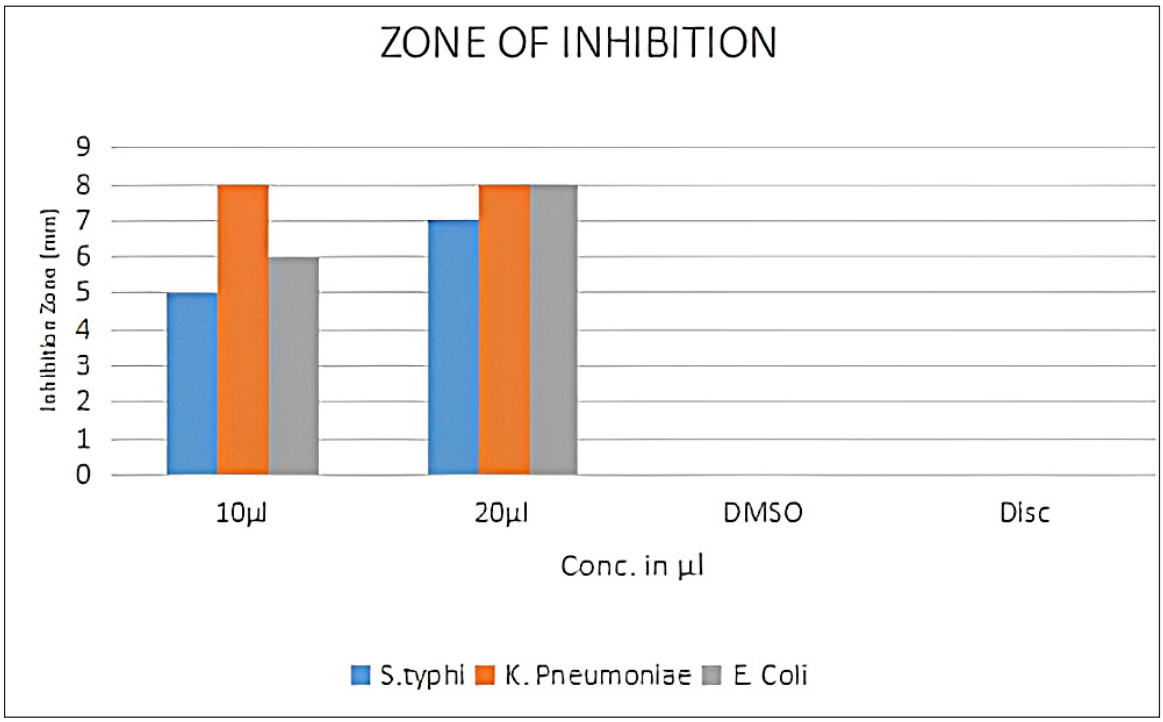


**Figure 11. This graph reflects the inhibitory zone**

indicates the digestion of casein by the isolates which is a probiotic property and the isolates possess antibacterial property. Further studies are being conducted to apply the isolated strain in incorporation of fruit juice to enhance the nutraceutical properties and increase shelf life with the isolates acting as bio preservative.

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# EFFECT OF PROCESSING ON THE PHYSICO-CHEMICAL PARAMETERS AND PROXIMATE COMPOSITION OF QUALITY PROTEIN MAIZE FLOUR

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## ABSTRACT

The purpose of the study (2022) is to compare QPM maize flour's physico-chemical composition before and after processing. A bag of 12 kg of QPM of the Shaktiman-5 variety was purchased. The gathered maize grains were cleaned by sifting out any remaining foreign material and sick or damaged seeds. In case of processing, the remaining 10 kg of maize grains were split into four equal sets in duplicate. There were 2.5 kg of corn grains in each replica. One set was preserved as the control (in triplicate) out of the four sets (in triplicate) for the study. In case of processing, the other three sets (each in triplicate) were retained. Boiling, roasting, and processing with alkali were the processing techniques used.The highest percentage of moisture content was found in the QPM control samples (13.06%), followed by samples of boiled maize (8.21%), alkali-treated samples (4.80%), and samples that had been roasted (3.65%). In terms of fat, it was discovered that roasted samples (3.92%) had the highest concentration, followed by alkali-treated maize samples (3.40%), boiling samples (3.28%), and control samples (3.23%). Ash level was highest in roasted samples (1.25%), then boiling samples (1.24%), untreated samples (1.14%), and samples treated with alkali (0.93%). The control samples had the highest percentage of fibre content (3.87%), followed by samples of boiled maize (3.79%), alkali-treated samples (3.30%), and samples that had been roasted (2.64%). Alkali treated sample had the greatest protein content percentage (10.85%), followed by control maize. In terms of carbohydrates, roasted samples (77.77%), alkali-treated samples (76.72%), boiling samples (73.13%), and control samples (67.87%) showed the highest amount.While it dropped in alkali-treated maize samples (18.42%), the proportion of ash concentration increased in boiled (8.77%) and roasted (9.65%) samples. After processing the QPM of the Shaktiman-5 variety, there were unquestionably both qualitative and quantitative modifications.

**Keywords:** Nutrition, Physico-Chemical, Processing, Proximate composition, Shaktiman-5 variety QPM

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## INRTODUCTION

The most fundamental human requirement is food. Individual health and nutritional well-being are essential for community growth. Malnutrition is a significant issue on a global scale. Today, micronutrient deficiency is a major concern. A significant public health risk is micronutrient deficiency. In India, micronutrient deficiencies affect more than 80% of the population, which lowers immunity. (Brown *et al.*, 2020)

Malnutrition, also known as malnourishment, is a condition brought on by consuming a diet that is either deficient in nutrients or excessively rich in them, leading to health issues. Malnutrition can also result from a deficiency in minerals. It occurs when our body does not receive enough nutrients in the form of carbohydrates, proteins, lipids, and vitamins. Micro minerals are those found in the human body at concentrations lower than 0.05 percent. Trace elements are also present in the microminerals. Chromium, cobalt, copper, fluorine, iodine, iron, manganese, molybdenum, selenium, and zinc are some of these trace elements. Zinc is one of these significant trace minerals. Micronutrient deficiencies affect more than 80% of Indians, which can lead to poor health immunity. (Chauhan *et al.*, 2021)

More than a billion people consume maize on a daily basis, both in its entire form and in various processed forms. The nutritional profile of products made from maize is altered by various processing techniques, which has a significant impact on the population that relies on this crop for a significant amount of their caloric demands. Processing techniques that lower phytic acids, including as soaking, fermenting, boiling, and nixtamalization, can increase the bioavailability of minerals. Changes in processing techniques and promoting the consumption of whole-grain maize products versus degermed, refined goods can reduce the loss of micronutrients during processing. (Akinlolu *et al.*, 2014)

The nutritional value of cereals is primarily determined by their chemical make-up and the presence of anti-nutritional elements like phytic acid. Phytates can be found in whole grain cereals, lentils, and other plants in trace amounts. They have a high affinity for binding divalent cations, and human tests have shown that they inhibit the absorption of zinc. (Sandstorm, 2019).

Around the world, maize is a significant cereal crop for both human nourishment and animal feed. Maize has earned a well-deserved reputation as a nutritional cereal thanks to its high concentration of carbohydrates, lipids, proteins, and several crucial vitamins and minerals. Maize is the source of protein and calories for several million individuals, mostly in poor nations. (Prasanna, *et al*, 2018).

A total of 14.9% moisture, 11.1% protein, 3.6% fat, 2.7% fibre, 66.2% carbs, and 1.5% mineral matter made up maize's composition. Zinc makes up 2.8 mg/100 g of the total mineral matter, the majority of which is located in the germ. Additionally, maize contains 1 g of phytic acid per 100 grammes concentrated in the germ, which binds to zinc and produces insoluble complexes that lower zinc's bioavailability. By lowering the amount of phytic acid in the maize, the zinc can be made more bioavailable. This can be accomplished both by processing and by adding phytase enzyme, which aids in hydrolyzing phytate to reduce Inositol phosphates and boost zinc absorption. (Kayode, 2018).

The balanced amino acid makeup of the Quality Protein Maize variety has increased the protein quality. The term "Quality Protein Maize" (QPM) refers to a form of maize with

greater nutritional and biological value that is practically identical to regular maize in terms of cultivation and kernel phenotype. For individuals who rely on maize for their energy, protein, and other nutrient needs, the nutritional advantages of QPM are in fact rather substantial. Other nutritional advantages of QPM include better calcium absorption, higher glucose and carotene utilisation, and higher niacin availability due to higher tryptophan and lower leucine concentration. Furthermore, the quality or acceptability of high protein maize can be preserved while being converted into consumable goods.

## MATERIALS AND METHODS

In the year 2022, the study was carried out in the labs of the College of Community Science, OUAT, Bhubaneswar. The goal of the study was to process maize to make flour and determine its proximate composition and physico-chemical characteristics. The methods and means of achieving the objectives have been categorised under various headings:

### Selection of raw materials

The most prevalent normal QPM maize variety that is grown and well-liked by consumers of maize (Shaktiman-5 variety) was chosen for the investigation.

### Procurement and processing of raw materials

Grain from the farmer's field that had just been harvested was of the QPM variety. A total of 10 kg of QPM variety maize grains were required for the study. To get rid of dust and other undesired elements, the grains were cleansed thoroughly.

The cleaned maize grains were separated into 4 lots, with one lot serving as the "control" and the other 3 lots being processed by roasting, boiling, and lime treatment. For the investigation, each batch was further divided into 5 equal sections. There was therefore 20 parts made from QPM maize. Fourty parts in total were available for conducting the analytical work.

### Analysis of maize grains for physico-chemical parameters

A precision balance was used to measure 100 grains weight of wheat and maize.Grain volume was calculated using the displacement method. The mass/volume method was used to determine the density of grains.

### Determination of proximate composition

The hot air oven method was used to determine the moisture content. The conventional method of analysis was used to estimate the amount of ash. (AOAC, 2000.). The amount of crude fibre was calculated using a common analytical technique.(AOAC, 2000.).By calculating the product's total nitrogen content using the Micro-Kjeldhal Method, crude protein was estimated (AOAC, 2000.).Using the accepted method and procedure, the fat was determined.(AOAC, 2000.). The quantity of total carbohydrate content is generally represented by the number that results from deducting the sum of the percentages of moisture, fat, ash, crude fibre, and crude protein from 100 (AOAC, 2000.).

### Comparative efficacy of different parameters in maize

Standard statistical techniques like Mean, Standard Deviation, and Paired 't' test were used to evaluate the parameters' comparative efficacy.

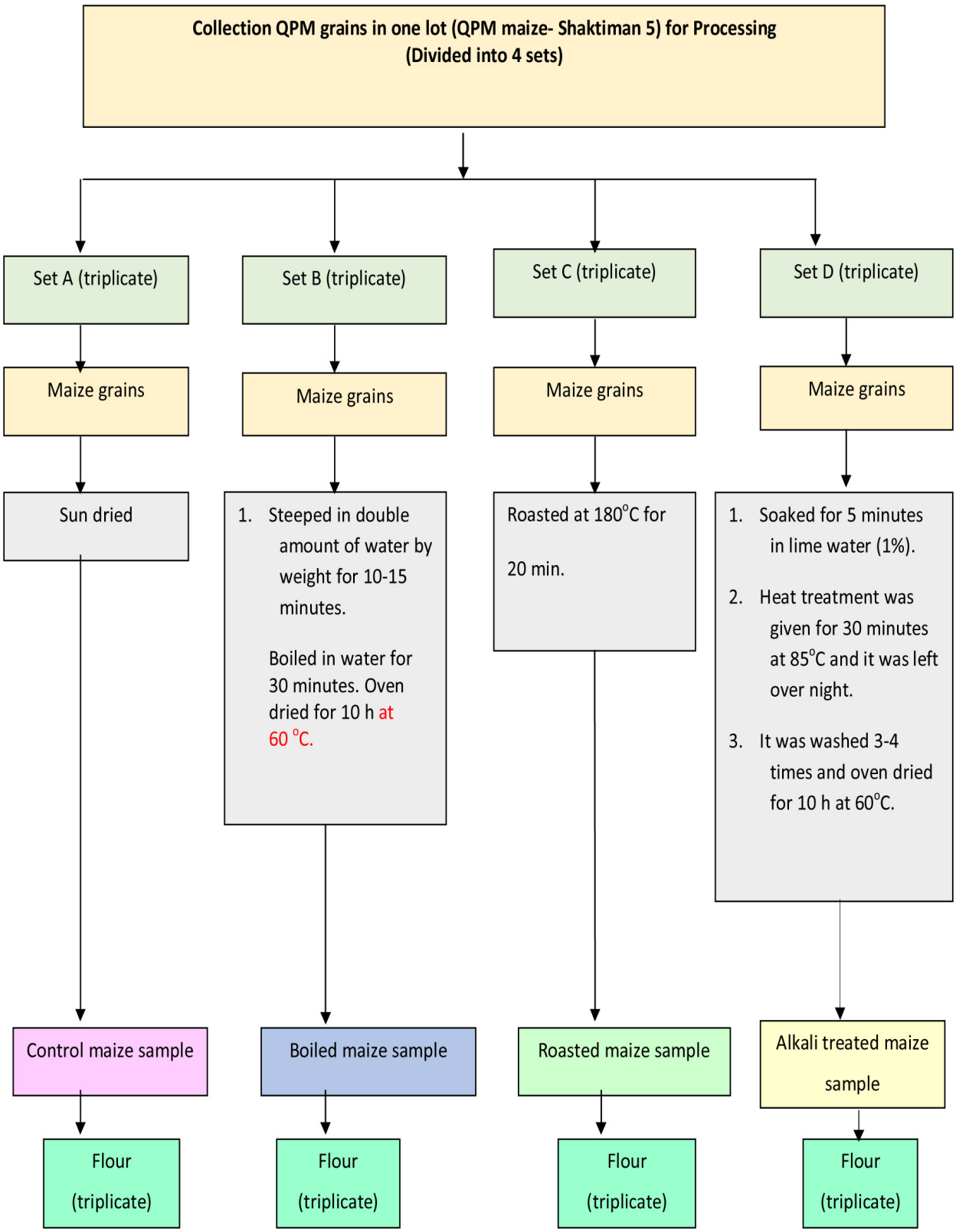


**Fig. 1. Preparation of QPM maize grains and flour sample**

**Table 1. Physico-chemical parameters of freshly harvested QPM grains**

| S.No. | Parameters | Observations |
|---|---|---|
| 1. | Weight (gm) | 32.21 ± 0.77 |
| 2. | Volume (cc) | 25 |
| 3. | Density (g/cc) | 1.61 ± 0.03 |

Each value is the mean of six observations.

## RESULTS AND DISCUSSION

### Physico- chemical parameters of freshly harvested QPM grains

Freshly harvested QPM grains' physico-chemical characteristics Weight, volume, and density measurements of the product were taken in order to evaluate the physico-chemical qualities of QPM maize grains. The gathered information is shown in Table 1 and graphically represented in Fig. 2. Freshly harvested QPM maize grains' weight was determined to be 32.21 ± 0.77 g, while their volume was measured at 25 cc. 1.61 ± 0.03 g/cc was the density.

### Proximate composition of freshly harvested QPM flour before and after processing

It was discovered what the proximate composition of QPM flour (control and processed) was. Moisture, fat, ash, fibre, protein, and carbohydrates were the components of the proximate composition. Tables 2 and 3 and Fig. 3 provide data on the nutritional quality of flour and quantitative changes in nutritional quality following application of various processing techniques.

Table 2 shows the approximate composition of QPM flour made from recently harvested QPM grains before and after processes like boiling, roasting, and alkali processing. The control sample was taken from the raw, recently harvested maize grains. The other samples consisted of alkali-treated, roasted, and boiled maize grains. According to Table 2, the control flour sample had 67.87 percent carbohydrates, 10.83 percent protein, 3.23 percent fat, 3.87 percent fibre, and 1.14 percent ash. The percentages of moisture, fat, ash, fibre, protein, and carbohydrates in a sample of boiled maize flour were 8.21, 3.28, 1.24, 3.79, 10.35, and 73.13, respectively. A total of 3.65 percent moisture, 3.92 percent fat, 1.25 percent ash, 2.64 percent fibre, 10.77 percent protein, and 77.77 percent carbohydrates made up the sample of roasted maize flour. The percentages of moisture, fat, ash, fibre, protein, and carbohydrates in a sample of alkali-treated maize flour were 4.80, 3.40, 0.93, 3.30, 10.85, and 76.72, respectively.

According to Sefa-Dedeh *et al.* (2004), nixtamalization of the maize enhanced the pH, lipid content, moisture content, water absorption capacity, and yellowing of the end products. The protein content of the nixtamalized maize products was enhanced by alkaline cooking.

Table 2 shows that the control samples had the highest percentage of moisture content (13.06%), followed by the boiled maize sample (8.21%), the alkali-treated sample (4.80%), and the roasted sample (3.65%). Thirteen percent moisture content is regarded as a safe level. As a result, the moisture content in the control sample was close to the safe range, or 13%.

The mechanical drying of the maize sample was the cause of the reduced moisture

content in the boiled and alkali treated corn sample. The loss of moisture owing to the application of heat in the case of roasting was the cause of the lowest moisture content (3.65%). In terms of fat, it was discovered that roasted samples (3.92%) had the highest concentration, followed by alkali-treated maize samples (3.40%), boiling samples (3.28%), and control samples (3.23%). Ash level was highest in roasted samples (1.25%), then boiling samples (1.24%), untreated samples (1.14%), and samples treated with alkali (0.93%). The control samples had the highest percentage of fibre content (3.87%), followed by samples of boiled maize (3.79%), alkali-treated samples (3.30%), and samples that had been roasted (2.64%).

The alkali treated sample had the highest proportion of protein (10.85%), followed by the control maize sample (10.83%), the roasted sample (10.77%), and the boiled sample (10.35%). In terms of carbohydrates, roasted samples (77.77%), alkali-treated samples (76.72%), boiling samples (73.13%), and control samples (67.87%) showed the highest amounts.

At the 1% level of probability, the statistical analysis clearly demonstrated that the moisture content of the control maize samples was higher than that of the boiling, roasted, and alkali-treated samples ('t' values of 7.006, 9.274, and 9.916, respectively). Additionally, at the 0.1% level of significance, the moisture content of boiled maize samples was higher than that of roasted maize samples and alkali-treated maize samples ('t' value 12.715), whereas the moisture content of roasted maize samples was significantly lower than that of alkali-treated maize samples ('t' value -5.974).

According to Ayatse *et al.* (1983), heat processing—such as roasting, nixtamalization, extrusion, boiling, baking, or microwaving—improves the colour, texture, flavour, and nutritional value of food products when done under the right conditions.

No discernible change was seen between the control and boiling maize samples in terms of fat. But significant difference at 1% level of probability can be observed between control and roasted maize samples ('t' value -25.02), control and alkali treated maize samples ('t' value -7.164), boiled and roasted samples ('t' value -25.66) and roasted and alkali treated maize samples ('t' value 39.401), whereas, the difference between fat content of boiled and alkali treated maize samples ('t; value -3.585) was found to be significant at 5% level of probability.

The difference in ash content between the control and boiled samples was not statistically significant, although it was considerably different from the roasted and alkali-treated maize samples ('t' values of -2.944 and 6.346, respectively) in the control samples.

Each value is the mean of six observations; $^{NS}$ Not significant; *Significant at 5% level of probability; **Significant at 1% level of probabilityAdditionally, the ash content of boiled maize samples was higher than that of alkali-treated maize samples ('t' value 25.802 at 1% level of significance), but the difference between ash content of roasted and boiled maize samples was determined to be non-significant. Roasted and alkali treated maize samples showed a significant difference at the 1% level of probability ('t' value 23.833).

When comparing control and roasted maize samples for fibre, a significant difference ('t' value of 5.137) was found at the 1% level of probability. The ash content of the control maize samples did not differ significantly from the boiled maize samples or the alkali-treated maize samples. At the 1% level of significance, the fibre content of the boiling maize samples

**Table 2. Proximate composition of QPM flour from freshly harvested maize grains before and after processing.**

| S.No. | Maize grain | Parameters (g/100 g) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Sample | Moisture | FatAshFibre | Protein | Carbohydrate | |
| | | (Mean ± S.D) | (MeanS.D) | (MeanS.D) | (MeanS.D) | (MeanS.D) | (MeanS.D) |
| 1. | Control (A) | 13.06±2.39 | 3.23±1.232 | 1.14±0.437 | 3.87±1.566 | 10.83±0.260 | 67.87±2.738 |
| 2. | Boiled (B) | 8.21±3.195 | 3.28±1.229 | 1.24±0.015 | 3.79±0.094 | 10.35±0.405 | 73.13±0.839 |
| 3. | Roasted (C) | 3.65±0.292 | 3.92±0.032 | 1.25±0.027 | 2.64±0.060 | 10.77±0.236 | 77.77±0.446 |
| 4. | Alkali treated (D) | 4.80±0.465 | 3.40±0.042 | 0.93±0.013 | 3.30±0.083 | 10.85±0.286 | 76.72±0.267 |
| **'t' value among maize samples** | | | | | | | |
| 1. | A×B | 7.006** | (-) 1.219$^{NS}$ | (-) 2.501$^{NS}$ | 0.333$^{NS}$ | 2.903* | (-) 5.990** |
| 2. | A×C | 9.274** | (-) 25.02** | (-) 2.944* | 5.137** | 0.778$^{NS}$ | (-) 8.331** |
| 3. | A×D | 9.916** | (-) 7.164** | 6.346** | 2.206$^{NS}$ | (-) 0.275$^{NS}$ | (-) 8.340** |
| 4. | B×C | 12.715** | (-) 25.66** | (-) 0.439$^{NS}$ | 25.214** | (-) 2.094$^{NS}$ | (-) 10.26** |
| 5. | B×D | 19.145** | (-) 3.585* | 25.802** | 10.144** | (-) 4.797** | (-) 13.82** |
| 6. | C×D | (-) 5.974** | 39.401** | 23.833** | (-) 12.68** | (-) 0.595$^{NS}$ | 4.362** |

**Table 3. Changes in proximate composition of QPM flour after processing as compared to control sample**

| | Parameter | Percentage change in maize grains | | |
|---|---|---|---|---|
| | (g/100g) | Boiled (B) | Roasted (C) | Alkali treated (D) |
| 1. | Moisture | 37.13 ↓ | 72.05↓ | 63.24 ↓ |
| 2. | Fat | 1.58 | 21.36 | 5.26 |
| 3. | Ash | 8.77 | 9.65 | 18.42 ↓ |
| 4. | Fibre | 2.06 ↓ | 31.78↓ | 14.72 ↓ |
| 5. | Protein | 4.43 ↓ | 0.55 ↓ | 0.18 |
| 6. | Carbohydrate | 7.75 | 14.59 | 13.04 |

**Note: Indicates decreasing trend**

was higher than that of the roasted maize samples ('t' value 25.214) and the alkali-treated maize samples ('t' value 10.144). At 1% level of significance, the fibre content of the roasted maize samples was substantially lower than that of the alkali-treated samples ('t' value: -12.68).

In terms of protein content, boiled maize samples and control samples showed a significant difference ('t' value 2.903) at the 5% level of probability, whereas the difference between boiled maize samples and alkali-treated samples showed a significant difference ('t' value -4.797) at the 1% level of probability. Although the protein level in the roasted maize samples was lower than it was in the control samples, the difference was not substantial. Similar to the protein content, the difference between alkali-treated and control maize samples was not statistically significant. Once more, the difference in protein content between roasted and boiling maize samples and between alkali-treated maize samples and roasted maize samples.

All the maize samples had increased carbohydrate content after processing, but the increase in boiled maize samples was significant at 1% level of probability ('t' value -5.990), whereas the carbohydrate content of control maize samples was significantly lower than that of roasted maize samples ('t' value -8.331) and alkali treated maize samples ('t' value -8.340).

At 1% level of significance, the carbohydrate content of the boiling maize samples was lower than that of the roasted maize samples ('t' value -10.26) and the alkali-treated maize samples ('t' value -13.82). On the other hand, at the 1% level of probability, the carbohydrate content of the roasted maize samples was substantially larger than that of the alkali-treated maize samples ('t' value 4.362).

**Changes in proximate composition of QPM flour after processing as compared to control sample**

Table 3 and Fig. 2 show the variations in the proximate composition of QPM flour following the application of various processing techniques in comparison to the control sample. The largest percentage loss in moisture content was 72.05 percent in the case of the roasted sample, followed by 63.24 percent in the alkali treated sample and 37.13 percent in the boiling sample, as the moisture content was lost in grains acquired after

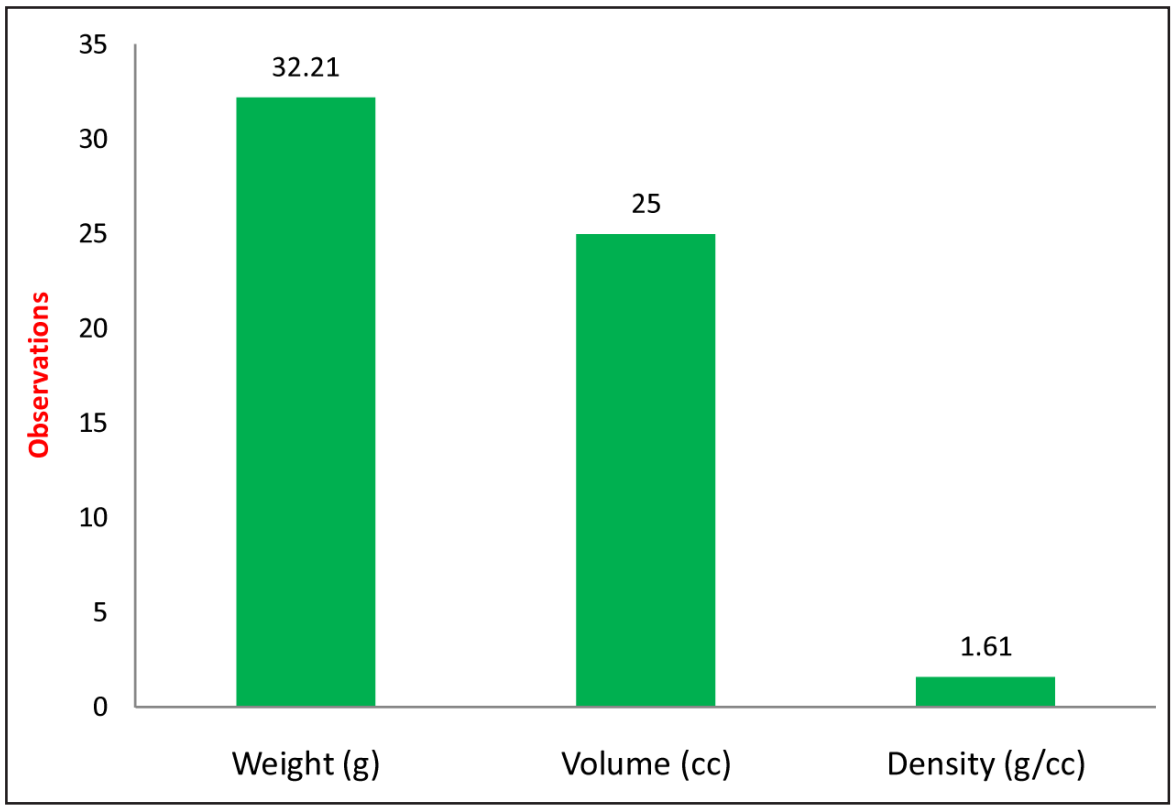


**Figure 2. Physico- chemical parameters of freshly harvested QPM grains**

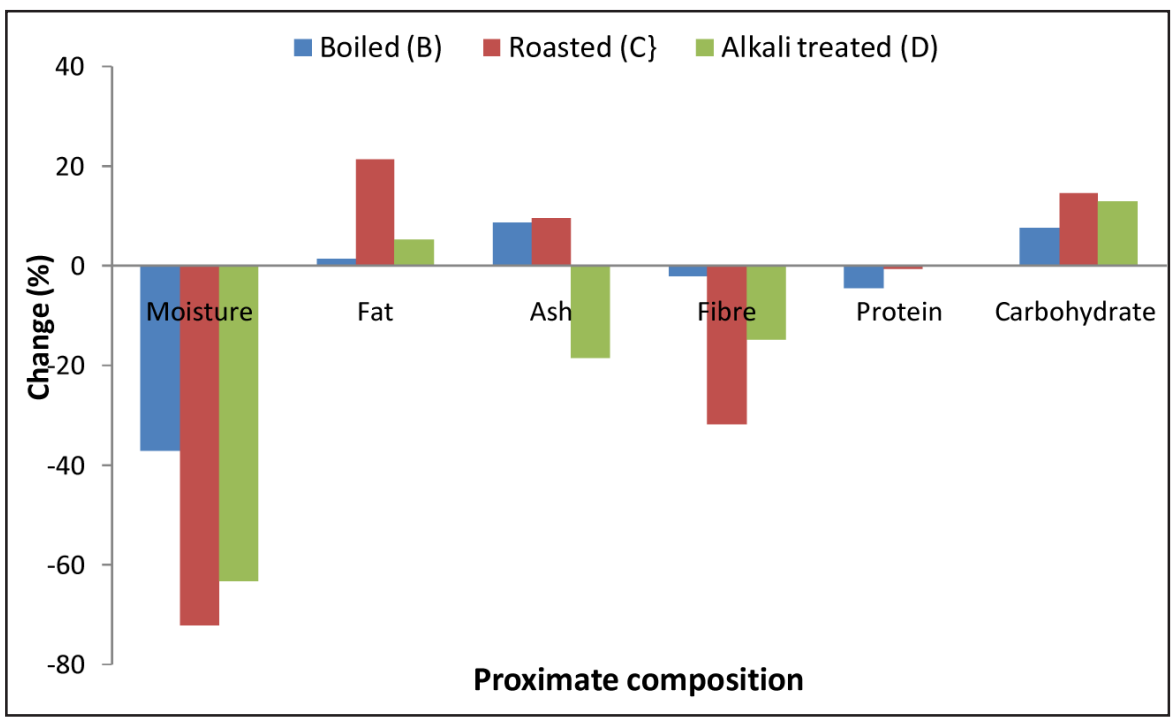


**Figure 3. Changes in proximate composition in QPM flour after processing as compared to control sample**

processing either due to mechanical drying or roasting.

In all three treated samples, the level of fat increased. Roasted maize samples (21.36%) showed the greatest increase in fat content, followed by alkali-treated samples (5.26%) and boiling samples (1.58%).The ash concentration increased in samples of roasted and boiling corn by 9.65 and 8.77 percent, respectively, whereas, it dropped in samples of alkali-treated maize by 18.42 percent.All three of the treated samples showed a decrease in the level of fibre. Samples of roasted maize showed the greatest drop in fibre content (31.78%), followed by samples of alkali-treated maize (14.72%) and samples of boiling maize (2.06%). Boiling and roasting samples both resulted in a 4.43 percent and 0.55 percent drop in the protein level, respectively. In contrast, it went up by 0.18 percent in samples of alkali-treated corn.All of the maize samples showed an increase in carbohydrate content. Carbohydrate content increased by the most in roasted samples (by 14.59%), alkali-treated samples (by 13.04%), and boiling samples (by 7.75%).

From Tables 2 and 3 & Figure 3, it can be inferred that aside from boiling, the changes in protein content were not substantial. The protein content of samples of boiling maize was substantially lower. Contrarily, following processing, the carbohydrate content rose. While the fibre level in the roasted maize samples decreased, the ash content was dramatically reduced after roasting and alkali treatment. After boiling and roasting, the fat content considerably rose. After processing, there are certainly changes in both quality and quantity.

## CONCLUSIONS

Boiled, roasted, and alkali treated QPM of Shaktiman-5 variety maize grain samples showed a change in proximate composition in processed maize grains. In case of samples of boiling, roasted, and alkali-treated maize, respectively, the percentages of moisture and fibre content fell by 37.13 and 2.06, 72.05 and 31.78, and 63.24 and 14.72, while the percentages of fat and carbohydrate increased by 1.58 and 7.75, 21.36 and 14.59, and 5.26 and 13.04, respectively. While it dropped in alkali-treated maize samples (18.42%), the proportion of ash concentration increased in boiled (8.77%) and roasted (9.65%) samples. Alkali-treated samples of maize had a higher percentage of protein (0.18%), but samples of the grain that had been boiled (4.43%) and then roasted (0.55%) had a lower percentage of protein.

With the exception of boiling, processing did not significantly alter the protein content. The protein content of samples of boiling maize was substantially lower. Contrarily, following processing, the carbohydrate content rose. While the fibre level in the roasted maize samples decreased, the ash content was dramatically reduced after roasting and alkali treatment. After boiling and roasting, the fat content considerably rose. After processing, there are certainly changes in both quality and quantity.

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# PREPARATION OF A NOVEL CARBONATED FERMENTED PROBIOTIC DAIRY BEVERAGE USING *Lacticaseibacillus rhamnosus* AND *Candida krusei* as CO-CULTURES

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## ABSTRACT

The study aimed to create a novel carbonated fermented probiotic dairy beverage by employing a co-culture of *Lacticaseibacillus rhamnosus* ADMH2 and *Candida krusei* ADMY 29. The resilience of both strains to acidic and bile environment along with gelatinase activity and cell hydrophobicity were assessed to ensure their suitability for probiotic applications. Optimal co-culturing was achieved with a 1:3 ratio of *Lacticaseibacillus rhamnosus* ADMH2 to *Candida krusei* ADMY 29 revealing remarkable attributes like protease activity, extracellular polysaccharide production and antimicrobial activity with minimal setting time. This synergistic co-culture was employed to create a carbonated probiotic dairy beverage in a delightful vanilla flavor. The product showed sustained probiotic and yeast counts (above 6.0 logCFU/mL).

**Keywords:** Carbonated dairy beverage, Fermented milk, Lactic acid bacteria, Probiotics, Yeast

## INTRODUCTION

The rising popularity of carbonated fermented beverages can be linked to their thirst-quenching and refreshing properties, which are accentuated by the addition of carbon dioxide ($CO_2$). $CO_2$ is considered safe and is a natural component found in fresh milk, making it a cost-effective method to improve the shelf life of dairy products (Silva *et al.,* 2018). In recent times, utilization of mixed-culture fermentations using Lactic acid bacteria (LAB) and yeasts aim to leverage the synergistic effects of both microbes to enhance the fermentation process and enhance the overall quality of the resulting products (Tangyu *et al.*, 2019). Building on this knowledge, the study aimed to develop a carbonated fermented probiotic dairy beverage using a co-culture of *Lacticaseibacillus rhamnosus* ADMH 2 and yeast *Candida krusei* ADMY 29.

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## MATERIALS AND METHODS

Lactic acid bacteria (LAB) *Lacticaseibacillus rhamnosus* ADMH 2 and yeast *Candida krusei* ADMY 29 were obtained from the culture stock of the Department of Dairy Microbiology, CDST, Pookode. Activation of LAB *L. rhamnosus* ADMH 2 and yeast *Candida krusei* ADMY 29 was done by inoculating 1% inoculum in de Man, Rogosa and Sharpe (MRS) broth and Yeast Extract Peptone Glucose (YEPG) growth medium, respectively. The tubes containing growth medium were incubated at appropriate temperatures for 24 h.

**Evaluation of probiotic properties: Bile and acid tolerance, gelatinase activity and microbial adhesion to solvents assay**

*L. rhamnosus* ADMH 2 and *Candida krusei* ADMY 29 were tested for their capacity to endure bile salts (BS) and low pH as per Shokryazdan *et al.* (2014). To test tolerance to bile salt, the LAB and yeast strains were inoculated 1% (v/v) in MRS broth and YEPG medium respectively with or without BS at 0.3 and 0.6% and incubated at 37 °C for 24 h. Samples were taken immediately, after 4 h to assess the initial response and 24 h to evaluate the long term persistence of strains in the presence of bile salts by observing the optical density at 600 nm. To test their resistance to low pH the strains were inoculated 1% (v/v) in respective medium acidified to pH 1.5, 2 and 3.5 with 1 N HCl and growth was observed in the beginning and after 24 h of incubation at 37 °C. Growth was monitored by measuring absorbance at 600 nm using a spectrophotometer (Agilent, Cary, 60 UV-Vis Spectrophotometer) (Galli *et al*., 2022). Gelatinase activity and adhesive attributes were evaluated as per Jain *et al.* (2017) with some modifications. To check gelatinase activity, active cultures were stabbed into sterilized nutrient gelatin tubes. After incubation at 25 $^{o}$C for seven days, tubes were observed for liquefied gelatin. To determine microbial adhesion to solvents, microbial cells were harvested by centrifuging active cultures at 12000 rpm for 10 minutes, washed thrice in phosphate buffered saline and resuspended in the same buffer. The absorbance of the cell suspension was recorded at 600 nm ($A_0$). Five mL of xylene was added into 5 mL cell suspension and vortexed. Two-phase system was developed and the aqueous phase was removed after 1 h of incubation at room temperature and its absorbance was measuredat 600 nm ($A_1$).The percentage of microbial adhesion to solvent was computed as $(1-A_1/A_0)100$.

**Selection of co-culture ratio for the preparation of fermented milk**

Five different proportional combination of *Lacticaseibacillus rhamnosus* ADMH 2 and yeast *Candida krusei* ADMY 29 were used as co- cultures as per Dan *et al.* (2023). A 100 ml of homogenized toned milk were inoculated with co- cultures in varying proportions (100:0, 75:25, 50:50, 25:75, 0:100) at one percent level followed by incubation at 37°C until the formation of a good coagulum. Combination that gave the best activity and least setting time was used for the preparation of carbonated fermented probiotic dairy drink. Activity of test cultures was evaluated by Horrell Elliker test. Milk samples were inoculated with 3% of the cultures and incubated at 37.7 °C for 3.5 h. At end of incubation period, the entire contents of each tube were titrated with sodium hydroxide and phenolphthalein indicator. Setting time was determined by observing the incubated milk samples at regular intervals for coagulum formation. pH was measured using calibrated digital pH meter (EsicopHmeter) and

acidity was measured by titrating against 0.1N NaOH with phenolphthalein as indicator and expressed as % Lactic acid/LA per mL of the samples prepared.

### Evaluation of functional properties

Protease and extracellular polysaccharide (EPS) production potential and antimicrobial properties of the LAB and yeast individually and the selected yeast-lactic acid bacteria co-culture were evaluated. Protease and extracellular polysaccharide (EPS) production potential were evaluated by streaking the cultures on skimmed milk agar and congo red agar respectively followed by incubation at 37 °C for 24 h (Amrutha *et al*., 2019). Antimicrobial activity was tested by well assay method against pathogenic *E.coli, S. aureus*, *Klebsiella* spp. and *Salmonella typhi* (Adetoye *et al*., 2018).

### Preparation of carbonated fermented probiotic dairy beverage (FPDB)

FPDB was prepared using homogenized toned milk, sugar, stabilizer and the selected LAB and yeast co-culture ratio. Homogenized toned milk was heated at 85 °C for 30 min and cooled down to 30 °C . Inoculum was added at the rate of 2% and incubated at 30 °C for 14 h. Sugar syrup (15%) was added along with stabilizer carboxy methyl cellulose (0.3%) and vanilla flavor. It was then homogenized at 150 psi and cooled to 7± 1 °C. Finally it was carbonated after filling in sanitized glass bottles of 200 mL capacity and stored at 7 °C . The FPDB was carbonated and evaluated for shelf life stability and probiotic viability (Ravindra *et al*., 2014).

### Shelf life studies

Carbonated FPDB was evaluated for its shelf life under refrigerated conditions for sevendays. Changes in sensory attributes, acidification (measured by pH values and titratable acidity) and viability of probiotics were analyzed both at the end of the fermentation process and throughout storage period. For sensory evaluation refrigerated carbonated samples and uncarbonated control were served to sensory panel of sixpanelists to evaluate its sensory attributes using a9- point hedonic scale. Viability of *Lacticaseibacillus rhamnosus* ADMH 2 and *Candida krusei* ADMY 29 were determined according to standard methods using MRS agar and yeast extract glucose chloramphenicol agar (YGCA), respectively.

## RESULTS AND DISCUSSION

### Evaluation of probiotic properties

The results of evaluation of bile and acid tolerance are presented in Fig.1. For probiotic strains to have a positive impact on the human gut, it is essential that they possess the ability to withstand bile salts and low pH levels (2-2.5) of human gut (Archana*et al*., 2022). Both *Lacticaseibacillus rhamnosus* ADMH 2 and *Candida krusei* ADMY 29 were found to tolerate the bile concentrations (0.3% and 0.6%) and acidic pH of 1.5, 2 and 3. *Candida krusei* ADMY 29 showed more tolerance to bile salts and gastric acidic pH compared to *Lacticaseibacillus rhamnosus* ADMH 2. The protease enzyme, gelatinase is one of the virulence factors that can hydrolyze bioactive peptides and able to breakdown casein, collagen and haemoglobin. Both *Lacticaseibacillus rhamnosus* ADMH 2 and *Candida krusei* ADMY 29 exhibited no positive gelatinase activity proving their safety for food applications as potent probiotic starter culture. Microbial adhesion to xylene is considered as a marker for evaluating adhesiveness of microbial cells to host epithelium and enhances competition and colonization in the

gastrointestinal tract against pathogens and minimum 40% hydrophobicity is required for a probiotic strain for adhesiveness (Jain *et al.,* 2017).*Lacticaseibacillus rhamnosus* ADMH 2 and *Candida krusei* ADMY 29 showed cell hydrophobicity 87.15±0.72% and 65.48±0.86%, respectively in xylene. Thus both *Lacticaseibacillus rhamnosus* ADMH 2 and *Candida krusei* ADMY 29 used in the study satisfy the prerequisite of a probiotic such as acid and bile tolerance, negative gelatinase activity and excellent adhesive attributes and can be further analyzed for clinical and biotherapeutic applications to confirm these in vitro safety findings. Consistent with this study, several other investigators also reported the probiotic potential of different strains of *Lacticaseibacillus rhamnosus* and *Candida krusei* (Chelliah *et al.,* 2016; Jain *et al.,* 2017).

**Selection of co-culture ratio for the preparation of fermented milk**

Five different combinations of fermented milk were prepared with varying LAB-Yeast ratios. Results of activity, setting time, pH and acidity were determined(Table 1).During the Horrell-Elliker test, it was observed that the acidity remained below 0.30% LA when milk was fermented solely with LAB. However, when LAB and yeast were combined in ratios of 3:1 and 1:1, a gradual culture activity was noted, resulting in acidities of 0.30% and 0.306% LA, respectively. Combination of LAB and Yeast in the ratio 1:3 gave the best active culture with acidity 0.410 % LA and the least setting time (7h). Thus, co-culture of LAB and yeast in the ratio 1:3 was used for further study.

**Evaluation of functional properties**

The yeast culture used in the study was found to be proteolytic in nature producing zone of clearance around the streak due to production of protease enzyme. LAB used exhibited no proteolytic activity. However the co-cultures showed proteolytic activity (Fig. 2). Anh *et al*. (2021) also documented increased

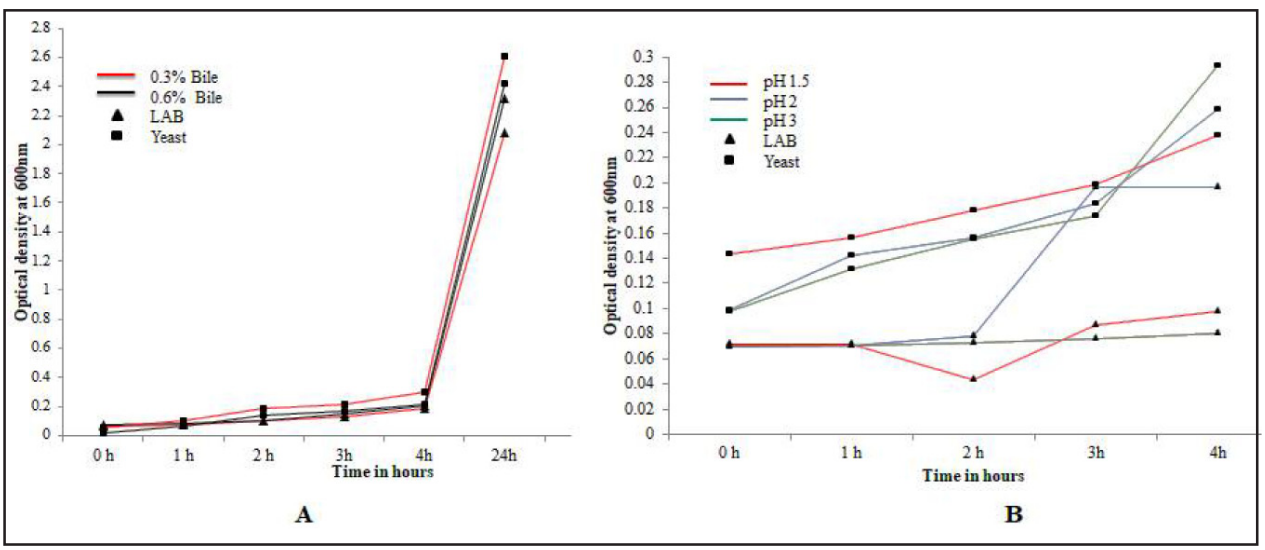


**Figure 1. (A) Line graph representing growth of LAB and yeast determined by measuring the absorbance in MRS broth and YEPG medium respectively with or without BS at 0.3 and 0.6 %. (B) Differences in growth of LAB and yeast cells between the initial concentration and after 4 h in acidic conditions**

**Table 1. Results of activity test, setting time, pH and acidity determined for fermented milk prepared with varying LAB: Yeast ratios**

| S. No. | Sample (LAB : Yeast ratio) | Acidity in Horrell- Elliker test (% LA) | Setting time (h) | pH | Titratable acidity (% LA) |
|---|---|---|---|---|---|
| 1 | A (100:0) | 0.23 | >15 | 4.2 | 0.945 |
| 2 | B (75:25) | 0.30 | 8 | 4.6 | 0.936 |
| 3 | C (50:50) | 0.306 | 8 | 4.6 | 0.936 |
| 4 | D (25:75) | 0.410 | 7 | 4.6 | 0.855 |
| 5 | E (0:100) | 0.35 | 8 | 4.8 | 0.720 |

protease activity (1.8–2.4 times) in their study using co-culture technology. In congo red assay, both strains of LAB and yeast and their co-culture showed extracellular polysaccharide production which was indicated by black shiny colonies on congo red agar (Fig. 3). Exopolysaccharides from LAB are reported to have antimicrobial properties and probiotic effects on the intestinal health and immune system (Yang *et al.,* 2023). Peng *et al*. (2023) highlighted the use of co-culture strategy to enhance the production of microbial polysaccharides or generate novel polysaccharides through various mechanisms, such as leveraging symbiotic, antagonistic, or chemo-sensitive interactions between different microorganisms. LAB and co-culture used in the study showed antimicrobial activity against target organisms except *Salmonella typhi***.** The diameter of zone of inhibition was measured using a ruler (Table 2). Yeast showed antimicrobial activity only against *S.aureus*.

**Shelf life studies of carbonated fermented probiotic dairy beverage (FPDB)**

Carbonated vanilla flavoured FPDB was prepared and evaluated for its shelf life under refrigerated conditions (7 °C).The sensory quality of the carbonated and uncarbonated product under refrigerated storage was judged using a 9-point hedonic scale at daily intervals


**Figure 2. Protease production test: A) LAB with no zone of clearance B) Yeast culture and C) Co-culture showing zone of clearance in skimmed milk agar plates**

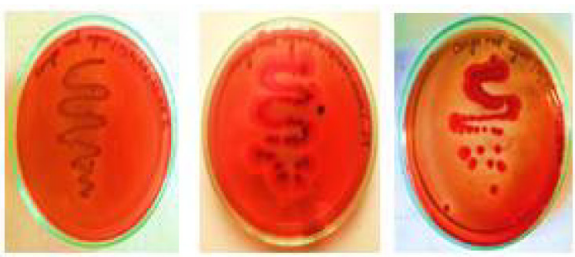


**Figure 3. Congo red assay: Formation of black shiny colonies on congo red agar streaked with A) LAB culture B) Yeast culture and C) Co-culture**

**Table 2. Results of antimicrobial activity measured as diameter of zone of inhibition**

| S. No. | Target organism | Zone of clearance measured in mm | | |
|---|---|---|---|---|
| | | LAB | Co-culture | Yeast |
| 1 | *S.aureus* | 14 mm | 12 mm | 30 mm |
| 2 | *E.coli* | 18 mm | 15 mm | No zone |
| 3 | *Klebsiella spp.* | 15 mm | 12 mm | No zone |
| 4 | *S.typhi* | No zone | No zone | No zone |

and the scores obtained are represented (Table 3). The scores obtained for sensory analysis were statistically analyzed using Kruskal Wallis test followed by independent t test in which a comparison was made between the carbonated and uncarbonated samples on each day of storage. The results revealed a significant difference between the flavor and overall acceptability scores of the uncarbonated sample throughout the storage period. However, the scores obtained for all its sensory parameters were found to have a non-significant difference during the period of storage. In case of carbonated sample, the scores obtained for overall acceptability were found to have significant difference during the storage period. All other scores obtained for the same was found to have no significant difference. A slight bitterness was felt for uncarbonated one but it was less in carbonated sample. In sensory analysis of the product, it was clear that the carbonation of this product had a better acceptance among the sensory panelists. Both the samples were acceptable up to seven days of storage.

Effect of carbonation on acidification of vanilla flavoured FPDB measured as pH values and acidity during storage is presented (Table 4). The initial pH of the beverage was not noticeably affected by carbonation. pH values of the carbonated and uncarbonated beverages were 5.2 and 5.19, respectively. When $CO_2$ reacts with milk in a water-based environment, it forms carbonic acid and releases $H^+$ ions, which would normally increase the beverage's acidity.The absence of a notable pH and acidity shift was probably attributed to the milk proteins' buffering capacity. However, a marginal decrease in pH and increase in acidity was observed in control after seven days of storage. The slight formation of lactic acid in fermented products during storage is associated with the continuing enzyme activity of the starter microorganisms, despite their reduced metabolic rate during storage.However, $CO_2$ interferes with this process bydisrupting internal enzymatic equilibrium, especially at refrigeration temperatures where gassolubility is increased. This could be the reason for absence of significant increase in acidity in carbonated FPDB throughout the storage period. The slight decrease in pH and increase in acidity during storage is due to post-acidification as a result of residual activity of both LAB and yeast culture (Mani-López *et al*., 2014). Similar trend in pH and acidity shift has been reported by Ravindra *et al*. (2014) in carbonated fermented dairy drink. Carbonated FPDB was also analyzed during the storage in refrigerated conditions at 7 °C for seven days to monitor microbial viability. Probiotic LAB and yeasts count did not differ between the carbonated and uncarbonated samples

**Table 3. Effect of carbonation on sensory scores of FPDB**

| S. No. | Attributes | Sample | Days of storage | | | | | | | Kruskal-Wallis H |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | 1 | 2 | 3 | 4 | 5 | 6 | 7 | |
| 1 | Flavour | Uncarbonated | 6.0±0.365 | 5.91±0.200 | 5.83±0.166 | 5.66±0.166 | 5.25±0.170 | 5.16±0.105 | 5.1±0.125 | 15.443* |
| | | Carbonated | 7.0±0.223 | 7.0±0.00 | 7.0±0.129 | 6.91±0.083 | 6.58±0.153 | 6.25±0.170 | 6.08±0.083 | 3.548ns |
| | | T value | 2.015* | 3.140* | 3.047* | 3.017* | 2.884* | 2.994* | 3.052* | |
| 2 | Consistency | Uncarbonated | 6.58±0.523 | 6.55±0.523 | 6.5±0.374 | 6.41±0.454 | 6.16±0.421 | 6.0±0.341 | 6.0±0.365 | 3.650ns |
| | | Carbonated | 7.0±0.00 | 7.0±0.00 | 7.0±0.00 | 7.0±0.00 | 6.91±0.083 | 6.60±0.152 | 6.41±0.153 | 25.038ns |
| | | T value | 2.300ns | 5.832** | 2.262ns | 5.945** | 7.101** | 2.977* | 6.416** | |
| 3 | Acidity | Uncarbonated | 6.83±0.401 | 6.83±0.401 | 6.66±0.421 | 6.75±0.359 | 6.66±0.357 | 6.41±0.327 | 6.38±0.327 | 3.299ns |
| | | Carbonated | 7.16±0.278 | 7.08±0.271 | 7.0±0.258 | 7.0±0.258 | 6.85±0.206 | 6.66±0.210 | 6.75±0.170 | 4.523ns |
| | | T value | 1.002ns | 0.447ns | 0.096ns | 0.191ns | 0.171ns | 0.540ns | 0.71 7ns | |
| 4 | Colour and appearance | Uncarbonated | 7.08±0.454 | 7.0±0.447 | 6.83±0.477 | 6.83±0.401 | 6.75±0.359 | 6.66±0.421 | 6.50±0.341 | 2.884ns |
| | | Carbonated | 7.41±0.200 | 7.33±0.210 | 7.2±0.210 | 7.16±0.166 | 7.08±0.083 | 7.0±0.258 | 6.83±0.166 | 6.759ns |
| | | T value | 0.698ns | 0.365ns | 0.254ns | 0.527ns | 0.538ns | 0.527ns | 0.738ns | |
| 5 | Container and closure | Uncarbonated | 8±0.075 | 7.9±0.166 | 7.8±0.013 | 7.8±0.113 | 7.8±0.134 | 7.8±0.15 | 7.8±0.153 | 12.42 ns |
| | | Carbonated | 8±0.121 | 7.8±0.0.100 | 7.8±0.112 | 7.83±0.106 | 7.83±0.126 | 7.8±0.166 | 7.8±0.116 | 12.27 ns |
| | | T value | 0.956 ns | 0.222ns | 0.212 ns | 0.289 ns | 1.306 ns | 1.527 ns | 1.256 ns | |
| 6 | Overall acceptability | Uncarbonated | 7.4±0.169 | 7.14±0.101 | 7±0.158 | 6.94±0.203 | 6.63±0.185 | 6.50±0.266 | 6.34±0.069 | 14.17** |
| | | Carbonated | 7.72±0.243 | 7.01±0.171 | 7.0±0.2170 | 7±0.170 | 6.67±0.190 | 6.77±0.080 | 6.58±0.146 | 17.132* |
| | | T value | 1.343 ns | 1.116 ns | 0.597 ns | 0.377 ns | 0.546 ns | 0.049 ** | 0.183 ** | |

**Figures are mean ± standard error of three replications, *-Significant at five per cent level (p<0.05), **-Significant at one per cent level (p<0.01), ns-non-significant (p>0.05).**

Table 4. Changes in pH, acidity and microbial profile of carbonated FPDB and control during storage at 7 °C

| S. No. | Parameters | Carbonated FPDB | | | Uncarbonated control | | |
|---|---|---|---|---|---|---|---|
| | | Day 0 | Day 3 | Day 7 | Day 0 | Day 3 | Day 7 |
| 1 | pH | 5.2±0.57 | 5.09±0.13 | 5±0.52 | 5.19±0.15 | 5±0.53 | 4.7±0.16 |
| 2 | Acidity | 0.47±0.52 | 0.54±0.13 | 0.62±0.12 | 0.49±0.02 | 0.58±0.71 | 0.75±0.12 |
| 3 | LAB count | 10.40±0.12 | 10.34±0.17 | 9.13±0.02 | 10.40±0.13 | 10.33±0.12 | 9.00±0.03 |
| 4 | Yeast count | 9.36±0.15 | 9.43±0.14 | 7.60±0.15 | 9.34±0.13 | 9.40±0.02 | 7.60±0.17 |

* Values are the means ± standard deviations

throughout the storage period indicating that the presence of $CO_2$ did not notably impact the viability of the probiotics. LAB and yeast reached concentrations in the order of $10^9$ and $10^7$ cells $mL^{-1}$ respectively from an initial concentration of $10^{10}$ and $10^9$ cells $mL^{-1}$ in both carbonated and uncarbonated FPDB in seven days of storage (Table 4).The count of probiotic bacteria and yeasts were still above 6.0 log CFU/mL in all samples until the end period of refrigerated storage. Similar results were reported by Haddad (2017) indicated a minor reduction in the probiotic count from the initial concentration, but this count remained above 6.0 log CFU/g in commercially available probiotic fermented dairy products in the Jordanian market throughout the 14-day refrigerated storage period. Additionally, the research highlighted that the probiotic bacteria counts in certain fermented dairy products marketed as probiotic products in Jordan did not consistently meet or exceed the therapeutic threshold of 6.0 log CFU/g. Parrella *et al.* (2017) reported a similar observation stating the co-incubation of lactobacilli with the yeast results in improvement of the viability of the bacteria. As per the recommendations of the FAO CODEX standard, fermented milk require a minimum concentration of $10^7$ CFU/g for bacteria and $10^4$ CFU/g for yeasts and the developed FPDB is in conformity with the recommendations. Moreover both the carbonated and uncarbonated FPDB contained concentrations exceeding $10^6$ CFU/mL for both LAB and yeast, meeting the minimum requirement for the product to be labeled as probiotic at the time of consumption. The concentration and viability of microorganisms towards the end of storage play a vital role in evaluating the probiotic potential of the fermented milk. It is essential that their viability remains above a certain threshold level throughout the product's shelf

life to ensure the preservation of their health-promoting properties (Galli *et al.,* 2022).

## CONCLUSIONS

The strains *Lacticaseibacillus rhamnosus* ADMH 2 and *Candida krusei* ADMY 29 used in the study showed potential probiotic properties such as acid and bile tolerance, negative gelatinase activity and excellent adhesive attributes and also their co-culture exhibited functional properties like proteolytic activity, exopolysachharide production and antimicrobial activity. Moreover, both the starter cultures maintained recommended therapeutic levels of probiotics for seven days in carbonated and non-carbonated FPDB when stored at regular refrigerated temperatures. However, sensory panelists showed greater acceptance for the carbonated FPDB and the carbonation process did not significantly alter the product's pH, acidity and starter culture viability of the product. By combining the benefits of potential probiotic microbes and carbonation, this research represents a promising initial step towards developing a novel and appealing carbonated probiotic dairy beverage. However, further investigation is needed to confirm these *in vitro* probiotic potentials and safety findings in clinical and biotherapeutic applications.

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# AN ECONOMIC ANALYSIS OF WHEAT PRODUCTION IN THE LIGHT OF CLIMATE CHANGE IN INDIA

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## ABSTRACT

India is the second-largest producer of wheat in the world. Uttar Pradesh, Punjab, Haryana, Madhya Pradesh, Rajasthan, Bihar, and Gujarat are the major wheat-producing states. Out of all of these states, Uttar Pradesh produces the larger quantity of wheat. Wheat is exported by India to Bangladesh, Sri Lanka, the United Arab Emirates, Yemen, the Philippines, and Indonesia. This study aims to analyze the effects of climate change and other non-climatic factors on wheat production in India between 1991 and 2021. The researcher used climate and non-climate change variables: mean, maximum, minimum temperature, rainfall, production, net MSP, and cultivation area. This work tested long-run cointegration using an Auto-Regressive Distributed Lag (ARDL) bound test cointegration technique to evaluate short-run relationships among modeled variables. CUSUM and CUSUMQ tests assure model reliability and validity. The study's findings demonstrated that the cultivated area has a major role in enhancing wheat output in the short and long term, while precipitation favors wheat production. In India, a 1% increase in cultivation area can increase 1.3% in wheat production in the long run. In the short run, a 1% increase in cultivation area increased 0.58% wheat production in India. Furthermore, although the temperature has little immediate effect, it is crucial for boosting wheat output over the long term.

**Keywords:** ARDL Approach, Climate Change, India, Wheat Production

## INTRODUCTION

Over 3000 scientists of the IPCC (Intergovernmental Panel on Climate Change) said that climate change would drastically reduce rice and wheat output in India. High temperatures, rainfall, and human-caused greenhouse gas (GHG) concentrations, including carbon dioxide, methane, and nitrous oxide, have changed the climate, and cyclones affect agriculture's production, distribution, and food prices. Agriculture is the most critical origin of income in an emergent nation and the economic foundation of South Asian countries. South Asia provides food for 20% of the world's population despite only having 5% of the accessible agricultural land. Because such a huge percentage of South Asia's population lives in rural areas and relies on agriculture as their primary means of subsistence, the agricultural sector is critically essential to the region (Chandio *et al.,* 2022). Wheat is a key crop for sustaining global food security. India is the world's second-largest wheat producer,

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accounting for 14% of global wheat production (FAO, 2021). Wheat is the Indian population's principal staple crop (1.35 billion people). In India, 29.6 million hectares of wheat were harvested in 2018, producing 99.7 million tonnes of wheat (FAO, 2021). In addition to producing food, the wheat harvest provides a living for many people in rural regions. As a result, understanding current problems and risks to wheat productivity is critical for developing present and future sustainable wheat production methods that ensure continued food supply. Climate change has been shown to influence agricultural production considerably, and wheat is no exception.

According to estimates, global climate change has little effect on Pakistani wheat yield (Janjua *et al.,* 2014). The production of wheat in Henan Province is negatively impacted by environmental factors like as temperature and rainfall. Climate change has a greater impact on Henan Province. Climate (as measured by temperature and rainfall) has been demonstrated to have a positive influence on wheat yield in Hebei Province. Furthermore, the temperature had a detrimental impact on wheat output in Shandong Province, although rainfall had a favorable impact (Zhang *et al.*, 2022). The potential vulnerability of Kazakhstan's wheat yields to future precipitation declines and rises in the frequency and severity of drought (Karatayev *et al.,* 2022). Wheat and energy prices are rising in the long term. Climate change will reduce rainfed wheat output in East England by 5.4% to 32.9%, more remarkable in the low-emission scenario. Rainfed winter wheat crop is estimated to lose 43.6% to 100.0% economically (El Chami and Daccache, 2015).

Wheat production was significantly impacted by temperatures, potential evapotranspiration, and vapor pressure, more so than by precipitation and wet days (Madhukar *et al.*, 2021). Depending on the location investigated, the direct influence of climate change on temperature and precipitation changes leads to losses in wheat output ranging from 1% to 8%. Following that, the indirect effect of climate change is explored, taking into consideration the impact of climate change on water availability, which leads to a decrease in irrigation. Non-climatic variables such as inputs, number of tractors, wheat area, tube wells number, and usage of fertilizer in each district all had an impact on wheat output. The fixed effect model indicated that a temperature rise has a considerable influence on November and January but has a negative impact on the month of April. The findings also revealed a non-linear association between precipitation and the months of April and November (Raza *et al.*, 2019). In this case, production losses are higher, ranging from 4% to 36%, depending on the region and irrigation regime. They contend that compared to the direct repercussions of climate change, the indirect effects may be more detrimental to future wheat harvests (Daloz *et al.*, 2021).

According to recent studies, climate change is having a substantial impact on wheat output throughout the globe (Yu *et al.*, 2020). However, there has been little research on this issue in India. Wheat is the most significant food crop. The empirical study of the effects of climate change and non-climatic variables on wheat production requires further investigation since it is crucial for food security. Given the significance of agriculture to the Indian economy, studying the impact of climate change on wheat production is essential. This study investigates the effects of climatic and non-climatic factors on wheat output in India. This study adds to the current body of information in various ways. First, the ARDL technique and the Granger causality framework of the vector error correction model (VECM) are seldom utilized to analyze the short- and long-term impacts of CC on wheat

**Table 1. Descriptive Statistics**

| S.No. | Variables | Mean | Standard deviation | Minimum | Maximum | Skewness | Kurtosis | Jarque-Bera | Probability |
|---|---|---|---|---|---|---|---|---|---|
| 1 | Wheat Production | 78.75 | 15.76 | 55.13 | 108.56 | 0.35 | 2.0 | 1.94 | 0.37 |
| 2 | Max temp | 30.82 | 0.30 | 30.08 | 31.52 | -0.04 | 3.51 | 0.34 | 0.84 |
| 3 | Min Temp | 18.90 | 0.26 | 18.35 | 19.49 | 0.17 | 2.93 | 0.15 | 0.92 |
| 4 | Mean Temp | 24.84 | 0.28 | 24.19 | 25.48 | 0.07 | 3.24 | 0.10 | 0.94 |
| 5 | Precipitation | 1111.51 | 91.27 | 908.10 | 1320.91 | 0.09 | 2.82 | 0.90 | 0.95 |
| 6 | Cultivation | 276.94 | 23.40 | 233 | 314 | 0.05 | 1.85 | 1.71 | 0.42 |
| 7 | MSP | 939.35 | 539.97 | 225 | 1975 | 0.48 | 1.92 | 2.72 | 0.25 |

**Source: Authors' Calculation**

output in India. This research addresses that gap by examining the short- and long-term impacts of climatic or non-climatic parameters (max, min, mean temperature, precipitation, cultivation area, and MSP) on wheat output using the ARDL model. Previous research in India does not consider non-climatic variables like cultivation area and minimum support price. Most of the studies consider only climatic variables. And this study has used recent data. The second distinctive feature of this research is the use of the VECM Granger Causality framework to identify short-run causal relationships between the variables. Third, the CUSUM test estimates causal associations between variables.

## MATERIALS AND METHODS

### Data

The current study used time series data (annual) for India from 1991 to 2021. This research used climatic and non-climatic variables for an estimate. These variables include annual minimum, maximum, and mean temperatures, yearly rainfall, MSP, and cultivated area. The Table has reported descriptive statistics of the variables.

Table 1 reported summary statistics of the variables. The mean value for wheat production was 78.75, with minimum and maximum values of 55.13, 108.56, and the corresponding standard deviation of 15.76. The mean values for climatic variables (max, min, mean temp, precipitation) are 30.82, 18.90, 24.84, and 1111.51, with minimum and maximum values being 30.08, 18.35, 24.19, 908.10, 31.52, 19.49, 25.48, and 1320.91 respectively. Standard deviations were 0.30, 0.26, 0.28, and 91.27. The mean values for non-climatic variables (MSP, cultivation area) were 939.35, 276.94 with minimum and maximum values of 225, 233, 1975, 314 and corresponding standard deviations of 539.97, 23.40. Precipitation, MSP, cultivation, Minimum temperature, Mean temperature, and wheat production were positively skewed. On the other hand, the maximum temperature was negatively skewed. All the variables were platykurtic (Lower Peak) because their kurtosis value was less than 3 except for maximum and mean temperature. These variables were leptokurtic (high peak) because the values exceeded 3. The Jarque-Bera probability values of wheat production, max, mean, min temperature, precipitation, cultivation area,

**Table 2. Correlation Summary**

| S.No. | Variables | LWhat produ-ction | LCulti-vation | LMax temp | LMean temp | LMin temp | LMSP | LPre-cipita-tion |
|---|---|---|---|---|---|---|---|---|
| 1 | LWheatproduction | 1 | | | | | | |
| 2 | LCultivation | 0.958 | 1 | | | | | |
| 3 | LMax temp | 0.464 | 0.4796 | 1 | | | | |
| 4 | LMean temp | 0.481 | 0.4983 | 0.9946 | 1 | | | |
| 5 | LMin Temp | 0.516 | 0.5216 | 0.9781 | 0.992 | 1 | | |
| 6 | LMSP | 0.973 | 0.9462 | 0.5689 | 0.594 | 0.6149 | 1 | |
| 7 | LPrecipitation | 0.408 | 0.4350 | 0.0394 | 0.075 | 0.1182 | 0.4143 | 1 |

**Source- Authors' Computation**

and MSP were 0.37, 0.84, 0.94, 0.92, 0.95, 0.42 and 0.25, respectively. The Jarque-Bera p values of all the variables were greater than 10%, which means data was normally distributed, so the researcher accepted the null hypothesis.

The absolute value of r lies between 0.3 and 0.7. The value of r less than 0.3 showed a low degree of association, and 0.7 showed a high degree of association. Table 2 displayed the correlation results, indicating that cultivation area, Max temperature, precipitation, cultivation area, Mean, Min temperature, and MSP were positively but strongly correlated and dependent on each other with wheat production due to 'r' values being greater than 0.30.

## ECONOMETRIC MODEL

**Methodology**- The researcher employed the Autoregressive Distributed Lag (ARDL) model, a bound testing cointegration approach developed by Pesaran et al. (M.H.Pesaran & Shin, Econometrics and Economic theory 20 th century). There were logic-based justifications for using this method. While some variables in our investigation remain unchanging at the level they are examined, others are integrated in the first order. Therefore, compared to other econometric methods, the ARDL model was the superior method in this particular circumstance. In addition, since our goal is to examine the influence of several independent factors on a single dependent variable (wheat production) in both the short and long run, this method is preferable to others because it allows us to do so in both time frames. Small data samples fit ARDL. It calculates the short-run and long-run coefficients concurrently and uses OLS for cointegration among variables (Janjua *et al.,* 2014). ARDL provides flexibility regarding the order of integration of the variables. Unlike other methodologies like the Johansen cointegration technique, this technique does not require pre-testing the variables integrated into the model for unit roots. It is appropriate irrespective of whether the regressors in the model are purely I(0), purely I(1), or mutually cointegrated. In contrast, this method's viability is questioned whenever a variable of second-order difference is present. The ARDL model of wheat production is shown in its general form.

Wheat Production = f (Max, Mean, Min Temp, Precip, Cultivation area, MSP) (1)

The variables in Equation (1) are $WP_t$ for wheat production, $T_1$, $T_2$, and $T_3$ for max, min, and annual mean temperature. PPt for precipitation; $Cul_t$ for Cultivation area; emissions; and MSP for minimum support price.

Equation (1) can be expressed as follows:

$$WP_t = \lambda_0 + \lambda_1 T_1 + \lambda_2 T_2 + \lambda_3 T_3 + \lambda_4 PP_t + - \lambda_5 Cul_t + \lambda_6 MSP + \mu_t$$

This research used natural logarithmic variables to minimize multicollinearity and volatility in yearly time series data. Using Equation (2) and natural logarithm, the following log model is described:

$$LnWP_t = \lambda_0 + \lambda_1 LnT_1 + \lambda_2 LnT_3 + \lambda_3 LnT_3 + \lambda_4 LnPP_t + \lambda_5 LnCul_t + \lambda_6 LnMSP + \mu_t$$

The ARDL model consists of two primary parts. The first thing that has to be done is to determine whether or not there is a connection that lasts through time between the study variables. The following is how the ARDL model specification is described by Equation (4):

where $\alpha_0$ shows the intercept; the order of lag is indicated by p; Ä represents the first difference operator, and the error term is shown by $\varepsilon_t$. The long-run equilibrium relationship between LnWP, LnT1, LnT2, LnT3, LnPP, LnCul, and LnMSP was examined in this study using the F-test. The null hypothesis states that LnWP, LnT1, LnT2, LnT3, LnPP, LnCul, and LnMSP do not cointegrate {$H_0$: $\delta_1 = \delta_2 = \delta_3 = \delta_4 = \delta_5 = 0$; $H_1$: $\delta 1 \neq \delta 2 \neq \delta 3 \neq \delta 4 \neq \delta 5 \neq 0$}. The estimated F-test or Wald-test is compared to the lower and upper limit values (Pesaran *et al*., 2001). The null hypothesis of no cointegration between LnWP, LnT1, LnT2, LnT3, LnPP, LnCul, and LnMSP is denied if the computed F-test is greater than the upper level bound. If the estimated F-test is less than the upper bound, the null hypothesis of no cointegration between LnWP, LnT1, LnT2, LnT3, LnPP, LnCul, and LnMSP cannot be rejected. The null hypothesis of no cointegration of LnWP, LnT1, LnT2, LnT3, LnPP, LnCul, and LnMSP becomes inconclusive. However, if the computed F-test falls between the lower and upper levels of the bands. The second step is to examine the short-term relationship between min, mean, max temperature, precipitation, cultivation, minimum support price, and wheat production in India using the following ECM in ARDL formulation:

## RESULTS AND DISCUSSION

**Unit Root Test Results**- Before using the ARDL bound testing approach; we first analyze each variable's consistency. To correctly compute F-statistics, the bound testing approach mandates that all variables must be integrated according to nature I(0), nature I(1), or both natures. However, the researcher was bound by the limitation that none of the variables in the study must be integrated of order two when employing the unit root test. In the bound testing approach,

$$\Delta(LnWP)_t = \alpha_0 + \sum_{i=1}^{p} \delta 1 LnWP_{t-k} + \sum_{i=0}^{p} \delta 2\ LnT1_{t-k} + \sum_{i=0}^{p} \delta 3\ LnT2_{t-k} + \sum_{i=1}^{p} \delta 4\ LnT3_{t-k} + \sum_{i=1}^{p} \delta 5\ LnPP_{t-k} + \sum_{i=1}^{p} \delta 5\ LnCul_{t-k} + \sum_{i=1}^{p} \delta 6\ LnMSP_{t-k} + \lambda 1 LnWP_{t-1} + \lambda 2 LnT1_{t-1} + \lambda 3 LnT2_{t-1} + \lambda 4 LnT3_{t-1} + \lambda 5 LnPP_{t-1} + \lambda 6 LnCul_{t-1} + \lambda 7 LnMSP_{t-1} + \varepsilon t \quad (4)$$

$$\Delta(LnWP)_t = \alpha_0 + \sum_{i=1}^{p} \delta 1\ LnWP_{t-k} + \sum_{i=0}^{p} \delta 2\ LnT1_{t-k} + \sum_{i=1}^{p} \delta 3\ LnT2_{t-k} + \sum_{i=0}^{p} \delta 4\ LnT3_{t-k} + \sum_{i=0}^{p} \delta 5\ LnPP_{t-k} + \sum_{i=1}^{p} \delta 6\ LnCul_{t-k} + \sum_{i=1}^{p} \delta 7\ LnMSP_{t-k} + \alpha ECM_{t-1} + \varepsilon t \quad (5)$$

**Table 3. Unit root test table**

| S.No. | Variables | ADF test statistics | | | | P.P. test statistics | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Levels t-stat prob. | | 1st Differences t-sta prob. | | Levels t-sta prob. | | 1st Differences t-stat prob. | | 1%level of significance | 5% Level of significance |
| 1 | LnProduction | -0.53 | *0.8702* | -8.45 | *0.0000*** | -0.52 | *0.8717* | -8.56 | *0.0000*** | I(1) | I(1) |
| 2 | LnCultivation | -1.16 | *0.6779* | -7.15 | *0.0000*** | -0.93 | *0.7628* | -7.21 | *0.0000*** | I(1) | I(1) |
| 3 | LnMax temp | -3.30 | *0.0236*** | -5.97 | *0.0000*** | -3.24 | *0.0270*** | -10.89 | *0.0000*** | I(1) | I(0) |
| 4 | LnMeanTemp | -3.26 | *0.0260*** | -4.00 | *0.0055*** | -3.14 | *0.0336*** | -12.12 | *0.0000*** | I(1) | I(0) |
| 5 | LnMinTemp | -3.17 | *0.0316*** | -4.21 | *0.0034*** | -3.11 | *0.0363*** | -10.41 | *0.0000*** | I(1) | I(0) |
| 6 | LnMSP | -0.97 | *0.7494* | -4.18 | *0.0029*** | -2.08 | *0.2509* | -4.98 | *0.0004*** | I(1) | I(1) |
| 7 | LnPrecipitation | -4.32 | *0.0020**** | -5.99 | *0.0000*** | -4.32 | *0.0020**** | -10.25 | *0.0000*** | I(0) | I(0) |

(**) significant at 5% (***) significant at 1% level

variables from level (2) would provide incorrect findings. We employed the Augmented Dickey-Fuller (ADF) and Phillips Perron (P.P.) unit root tests to investigate the sequence of integration of each variable.

Table 3 includes the results of ADF and P.P., both of the tests that were taken. These tests indicated the stationary and non-stationary status of variables. According to the findings in the Table, not a single variable in our investigation is integrated at the order 2 level. The results indicated that some variables such as max, mean, min temperature, and precipitation are stationary at level, while others (cultivation, MSP) were stationary at first difference. The null hypothesis for ADF is that 'The variable has a unit root.' This showed the variable is non-stationary. Wheat production was stationary at the first difference, and p values for ADF and P.P. are 0.00*** , 0.00***. Cultivation was stationary at first, and the p-value for ADF and P.P. are 0.00***,0.00***. The maximum temperature was stationary at a level, and the p-value for ADF and P.P. are 0.02**, 0.02**. The mean temperature was stationary at a level, and p values for ADF and P.P. are 0.02**, 0.03**. The minimum temperature was stationary at a level, and p values for ADF and P.P. are 0.03**, 0.03**. MSP was stationary at the first difference, and p values for ADF and P.P. were 0.00***,0.00***. The fact that the I(2) variable was not present lends credence to using the ARDL bound testing method.

**Approach to Bound Testing for Cointegration Testing-**

**Source- Authors' calculation**

The bound test was used to examine variable relationships across time. The Akike Information Criterion (AIC) and the Schwarz Bayesian Criterion (SBC) both suggested that the researcher utilize the reduced lag length one, so we did that. The findings make it

**Table 4. Result of the F-test for Cointegration**

| S.No. | Test statistics | Value | Significance | I(0) | I(1) |
|---|---|---|---|---|---|
| 1 | F-statistics | 6.518734 | 10% | 2.12 | 3.23 |
| 2 | K | 6 | 5% | 2.45 | 3.61 |
| | | | 2.5% | 2.75 | 3.99 |
| | | | 1% | 3.15 | 4.43 |

**Table 5. Long-Run ARDL Analysis of dependent and independent variables**

| S.No. | Variables | Coefficient | Standard Error | t-statistics | p-value |
|---|---|---|---|---|---|
| 1 | LCultivation | 1.32 | 0.59 | 2.20 | 0.0462 |
| 2 | LMax temp | 78.90 | 80.45 | 0.98 | 0.3446 |
| 3 | LMean temp | -157.14 | 134.01 | -1.17 | 0.2620 |
| 4 | LMin temp | 74.48 | 53.90 | 1.38 | 0.1903 |
| 5 | LMSP | 0.06 | 0.10 | 0.63 | 0.5357 |
| 6 | LPrecipitation | 0.41 | 0.27 | 1.46 | 0.1664 |

**Source- Authors' Calculation**

abundantly evident that the computed value of F-statistics was higher than the upper bound values (Fu) of 4.43, 3.61, and 3.23 at 1%, 5%, and 10%, respectively. This was shown (Table 4) by the fact that the value is 6.518734. The Fcal> Fu concludes that there was, in the long term, a connection between the variables.

The result of the long-run ARDL model was demonstrated in Table 5, which indicated that the cultivation coefficient value was significant in the long run. It shows a positive relation between wheat production and cultivation area. It means that if the cultivation area was increased by 1%, the production would increase by 1.3% in the long run. The coefficient values for maximum, minimum, mean temperature, precipitation, and MSP were insignificant but positively related to wheat production except for mean temperature. The mean temperature has a negative relation with wheat production in the long term.

The following Table 6 provides the error-correcting model's short-run dynamic outcomes. The ECM coefficient's sign was negative, indicating that it has substantial value. The short-term value of ECM showed the transition rate from disequilibrium to equilibrium. The more excellent value of ECM indicated a more rapid process of adjustment. The findings showed that the value of the ECM term requires that wheat output from the short run to the long run be adjusted by about 58% each year with high significance. Shock-induced disequilibrium takes slightly more than a year to reach equilibrium. The findings suggest that cultivated area, Maximum, Minimum, and Mean temperature values were significantly associated in the short term. But, the Cultivation area was positively associated with while maximum and mean temperature were negatively correlated with wheat production. The fact that the wheat production R-square value is 0.91 indicated that 91% of

**Table 6. Short-Run ARDL Analysis**

| S.No. | Variables | Coefficient | Standard Error | t-stati-stics | Proba-bility |
|---|---|---|---|---|---|
| 1 | C | 5.27 | 0.64 | 8.19 | 0.0000 |
| 2 | D(LCULTVSN) | 0.58 | 0.14 | 3.98 | 0.0015 |
| 3 | D(LCULTVSN(-1)) | 0.76 | 0.13 | -5.51 | 0.0001 |
| 4 | D(LMAX_TEMP) | -24.83 | 13.37 | 1.85 | 0.0862 |
| 5 | D(LMAX_TEMP(-1)) | -21.77 | 4.35 | 5.00 | 0.0002 |
| 6 | D(LMEAN_TEMP) | -44.69 | 21.83 | -2.04 | 0.0614 |
| 7 | D(LMEAN_TEMP(-1)) | -19.93 | 3.76 | -5.29 | 0.0001 |
| 8 | D(LMIN_TEMP) | 18.86 | 8.81 | 2.14 | 0.0518 |
| 9 | D(LPRECI) | 0.00 | 0.04 | 0.00 | 0.9957 |
| 10 | CointEq(-1)* | -0.58 | 0.07 | -8.16 | 0.0000 |
| 11 | R-squared | 0.91 | Mean dependent var | | 0.023020 |
| 12 | Adjusted R-squared | 0.87 | S.D. dependent var | | 0.060079 |
| 13 | S.E. of regression | 0.02 | Akaike info criterion | | -4.603361 |
| 14 | Sum squared resid | 0.00 | Schwarz criterion | | -4.131880 |
| 15 | Log likelihood | 0.00 | Hannan-Quinn criter | | -4.455699 |
| 16 | F-statistic | 22.88 | Durbin-Watson stat | | 1.706491 |
| 17 | Prob(F-statistic) | 0.00 | | | |

**Source- Authors' computation**

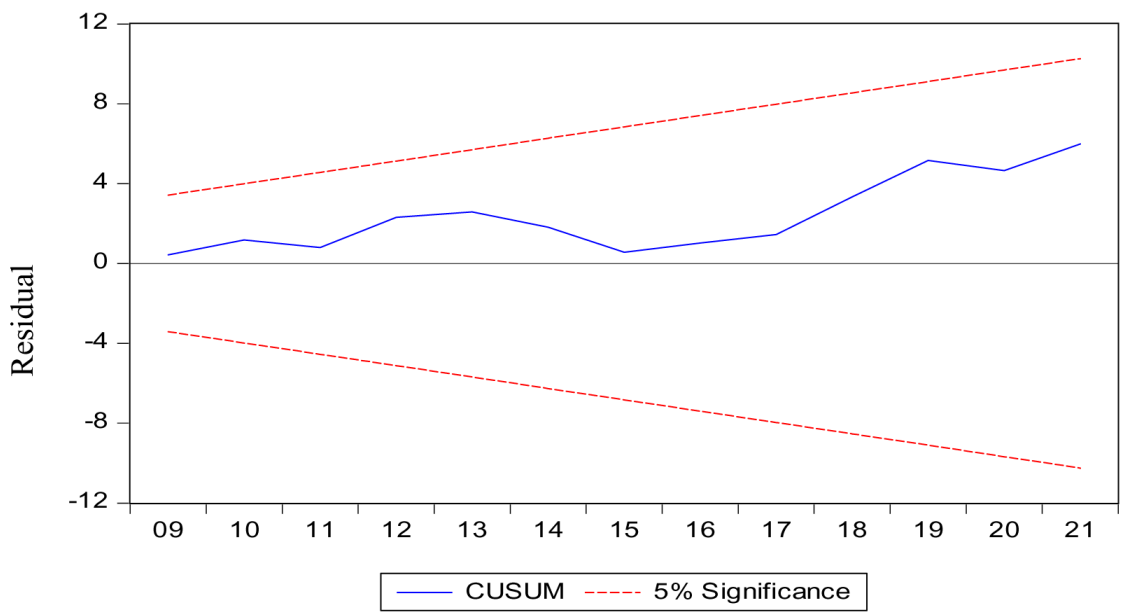


**Figure 1. Time Series Data**

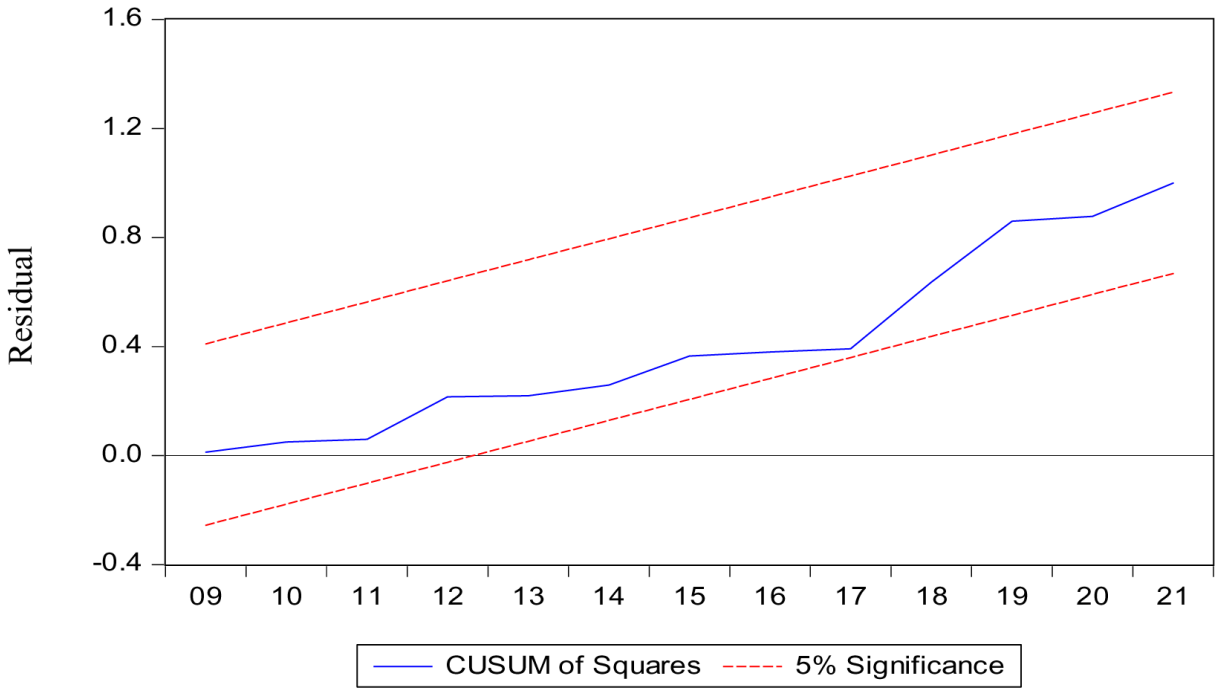


**Figure 2. Time Series**

the variance in wheat output can be attributed to changes in independent variables. Because R-square was larger than 60% and F-statistics were statistically significant at 5%, model goodness of fit was excellent.

### Model Stability

CUSUM and CUSUMSQ tests were utilized by the researcher to evaluate ECM stability. Graphical representations of the outcomes of the tests were provided here. The lines CUSUM and CUSUMSQ were shown to fall inside the crucial band of the 5% significance level over time by the graphs. The graphical findings validate the hypothesis that the ECM model was reliable.

## CONCLUSIONS

The results showed that increasing cultivation area was the way to increase wheat production in long term. Wheat production and cultivation areas have a favorable association. The coefficient values for the maximum, minimum, mean temperature, precipitation, and MSP are insignificant but positively related to wheat production except for mean temperature. The mean temperature has a negative relation with wheat production in the long run. On the other hand, the cultivation area was positively associated, while maximum and mean temperatures were negatively associated and correlated with wheat output in the short term.

The research demonstrated that increasing wheat output depends critically on wheat cultivation areas, both in the short and long term. A change in temperature, on the other hand, has no immediate influence on wheat output but may have a long-term impact on Indian wheat. This study's findings are consistent with those of previous research (Kiani *et al.,* 2018; Zhai *et al*., 2017).

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# TEMPORAL INSIGHTS: ASSESSING THE GROWTH PERFORMANCE OF RICE IN INDIA

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## ABSTRACT

The study analyzed the growth, decomposition and instability of rice with regard to parameters such as area, production and yield bewtween 1970-'71 and 2019-'20 through the utilization of time series data. The findings indicated a notable expansion in the cultivation area of rice over the decades, except during the periods of 1980-'90 and 2010-'20. There was a declining trend in the area during the 2000-'10 decade. Production displayed a substantial increase throughout all decades and the overall study period. Yield demonstrated a minimal increase in the initial decade, followed by a substantial increase in all subsequent decades. The decomposition analysis revealed that the influence of the cultivation area on rice production was more prominent compared to the impact of yield during the studied time intervals. Yield's influence was more pronounced during the 1970-'80 and 1990-'00 decades than in all other decades, while the interaction effect was relatively less significant. The stability of rice production was found to be the most precarious factor, followed by yield, with area exhibiting the least amount of instability. Policymakers need to prioritize research to boost rice yield, ensuring sustained production growth and implementing diversified cultivation strategies and resilient practices to counteract production instability.

**Key words:** Decomposition, Growth, Instability, Production, Rice

## INTRODUCTION

Agriculture remains the dominant driving force of the Indian economy and constitutes the fundamental pillar of the nation's socio-economic advancement. It contributes to approximately 19% of the GDP and supports a majority of the population, encompassing about two-thirds of its inhabitants. India holds the world's second-highest position in rice production after China (GoI, 2022). Rice serves as a staple sustenance for over 3.5 billion individuals globally, especially across Asia, Latin America, and certain parts of Africa (National Geographic Society, 2023). Encompassing an area exceeding 160 million hectares, rice cultivation thrives across diverse climatic conditions. Rice stands as a staple food for its 800 million citizens in India, contributing nearly 40% to the overall food grain yield. The country dedicates 43 million hectares to rice cultivation, resulting in the production of 112 million tons of milled rice, with an average yield of 2.6 tons per hectare (Pathak *et al.,* 2020). Rice cultivation is distributed across nearly every state in India. In the fiscal year 2018-19, the primary rice-producing states contributed to the nation's

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total rice output as follows: West Bengal was the major state with 13.79%, followed by Uttar Pradesh with 13.34%, Andhra Pradesh with 12.84% and Punjab (11.01 %) (Singh *et al.,* 2021). Rice contribution accounts for approximately 10 percent of the agricultural Gross Domestic Product (GDP), and its cultivation leads to the generation of 3.5 billion days of employment in India (Ahmad *et al.,* 2017, Kumar *et al.,* 2018). Over time, India has witnessed a notable rise in its rice production. Nevertheless, the nation has recently encountered notable challenges linked to environmental deterioration and shifts in climate patterns. Projections indicated a potential decline in cereal yield ranging between 10% and 40% by the year 2100. Current evidence already indicates adverse effects on wheat and paddy yields in certain Indian regions, attributable to rising temperatures, escalating water stress, and a decrease in the frequency of rainy days (Mahato, 2014). Economic instability refers to the deviation from a consistent and predictable progression over time. This concept quantifies the potential risk and uncertainty arising from fluctuations in factors such as production, trade, income, and prices. The measurement of instability, particularly concerning agricultural production is pertinent to matters related to food security and the impacts of output fluctuations on agricultural prices and producers' returns (FAO, 1998). The study has undertaken an analysis to explore the growth, breakdown, and volatility of rice in terms of area, production, and yield across different decades.

## MATERIALS AND METHODS

The research aimed to evaluate the performance of rice in India across five distinct sub-periods: Period I (1970-'71 to 1979-'80), Period II (1980-'81 to 1989-'90), Period III (1990-'91 to 1999-2000), Period IV (2000-'01 to 2009-'10), Period V (2010-'11 to 2019-'20) and the Overall Period (1970-'71 to 2019-'20). Data sourced from the Department of Economics and Statistics (DES), Government of India analysed to assess the Compound Annual Growth Rates (CAGR) and to estimate the impact of area, yield, and their interaction on total rice production. This was achieved through the utilization of log-linear growth models and a decomposition approach. Additionally, Cuddy Della Valle Index (CDVI) and the coefficient of variation (CV) were calculated to gauge the variability in area, production and yield. The collected secondary data underwent statistical analysis employing the subsequent analytical tools and techniques.

### Compound Annual Growth Rates (CAGR) analysis

In order to obtain an estimate of the compound annual growth rate (CAGR), the exponential time trend equation, which takes the following form was utilized:

$$Y=a\ b^{t} \quad \text{——————————————} \quad (1)$$

When expressed in logarithmic form, the equation becomes linear.

$$\ln(Y)=\ln a + t \ln(b_{1)} \quad \text{————————————} \quad (2)$$

Y: The variable whose rate of increase was being calculated

t: Chronological time (1, 2…n)

The coefficients a and b were estimated using regression.

This formulation presupposed a sustained, unchanging growth rate as time progressed. When 'b' was a negative value, it implied a continuous deceleration. If 'b' equaled zero, it signified the absence of any discernible trend, whereas, a positive 'b' value suggested a persistent acceleration in growth. In the context of estimating the Compound Annual Growth Rate (CAGR) using the

exponential time trend equation, Dandekar (1980) pointed out that employing the parameter 'B' which was equivalent to the natural logarithm of 'B' as the annual growth rate was an incorrect approach. Instead, the formula for finding growth rate ($e^B$-1) was derived using the compounding formula. Thus, the CAGR (percent) was given by ($e^B$ – 1) X 100, using the compounding formula.

$Y_t = Y_0(1+r)^t$ ———————————— —(a)

or

$Ln(Y_{t)} = Ln(Y_{0)} + t\ Ln(1+r)$ ————————(b)

or

$Ln(Y_{t)} = A + tb$ where ————————————(c)

$A = Ln(Y_{0)}$ and $B = Ln(1+r)$ ————————(d)

This equation is the log linear form of the exponential function and gives CAGR when differentiated with respect to t as follows:

$1/Y_t.dY_t/dt = Ln(1+r)$————————————(e)

$e^B = 1 + r$ ————————————————(f)

$r = e^B – 1$ ————————————————(g)

Thus, the CAGR (percent) was given by ($e^B$ – 1) X 100

Y represents the area or production or yield of the crop.

**Decomposition analysis**

The initial structured method of breaking down the growth trend was introduced by Minhas and Vaidyanathan (1965). Several scholars (Rehman and Salam, 2011; Sharma *et al.,* 2017; Devegowda *et al.,* 2019) have adapted and modified this model, presenting it in the following format:

$P_o = A_o * Y_o$

$P_n = Y_o * Y_n$

$P = P_n - P_o$ (Production change)

$A_o$ =Area (Base year)

$A_n$ =Area (Current year)

$Y_o$ =Yield (Base year)

$Y_n$=Yield (Current year)

"A=Change in Area ($A_n$-$A_o$)

"Y=Change in Yield ($Y_n$-$Y_o$)

Finally,

$$P = P_n - P_o = \underbrace{A_o * \Delta Y}_{\text{Yield effect}} + \underbrace{Y_o * \Delta A}_{\text{Area effect}} + \underbrace{\Delta A * \Delta Y}_{\text{Interaction effect}}$$

When a more significant alteration in production primarily results from the yield effect, it indicates that technological advancements, particularly in yield, have played a crucial role in driving the changes in production. This research assessed the impacts of cultivation area, yield and their combined interaction on the variations in crop production over five distinct time periods. To minimize or remove potential biases by employing triennium averages of area, production and yield for both the reference and current years of the respective crops. We subjected the data related to production aspects of the chosen crops to statistical analysis using the following analytical tools and methods.

**Cuddy Della Valle Index**

Extent and type of instability within the area, production, and yield of the rice crop across India, was calculated using coefficient of variation (CV). However, the basic CV does not adequately capture the underlying trend present within the time series data. To address this issue, a measure of instability was determined using the Cuddy Della Valle Index (CDVI) was originally developed by Cuddy and Valle (1978), which rectifies the shortcomings of the coefficient of variation. This index is represented by the following equation:

**Table 1. Compound annual growth rates in the area, production and yield of rice in India**

| S.No | Time Period | Area (%) | Production (%) | Yield (%) |
|---|---|---|---|---|
| 1 | Period I (1970 - '80) | 0.89 *** | 1.90 * | 1.00 NS |
| 2 | Period II (1980 - '90) | 0.41 NS | 3.62 *** | 3.20 *** |
| 3 | Period III (1990 - '00) | 0.68 *** | 2.00 *** | 1.32 *** |
| 4 | Period IV (2000 - '10) | -0.02 NS | 1.59 * | 1.61 *** |
| 5 | Period V (2010 - '20) | 0.17 NS | 1.87 *** | 1.69 *** |
| 6 | Overall Period (1970 - '20) | 0.32 *** | 2.20 *** | 1.88 *** |

*** Statistically significant at 1% percent level; ** Statistically significant at 5% level

* Statistically significant at 10% level; NS - Statistically non-significant

Coefficient of Variation (CV) = SD / Mean *100

where, SD = Standard deviation of area/ production/ yield

CDVI = CV* "1 - $\bar{R}^2$

where,

$\bar{R}^2$ is the adjusted $R^2$ from a time trend regression.

## RESULTS AND DISCUSSION

### Compound annual growth rate (CAGR) in the area, production and yield in rice

During period I, the yield exhibited a positive yet non-significant growth rate, whereas both area and production experienced positive and significant growth rates at the one percent and 10 percent levels, as shown in Table 1. The growth rate of the area was positive but non-significant in periods II and V. During period IV, it was negative and statistically non-significant but, in the period III, and the entire observation period, the growth rates of area, production and yield were positive and statistically significant at the one percent level. However, during periods II, IV and V, the growth rates of production and yield were positive and statistically significant at the one percent and 10 percent levels.

The growth rate of the cultivated area was at its lowest during period IV (-0.02 percent). Conversely, period I recorded the highest growth rate in area (0.89 percent), outperforming period III (0.68 percent), period II (0.41 percent), the entire time span (0.32

**Table 2. Average in the area, production and yield of rice in India**

| S. No. | Time Period | Average Area (Lakh ha) | Average Production (Lakh tonnes) | Average Yield (kg $ha^{-1}$) |
|---|---|---|---|---|
| 1 | Period I (1970 - '80) | 386.48 | 447.60 | 1158.15 |
| 2 | Period II (1980 - '90) | 406.58 | 597.73 | 1470.16 |
| 3 | Period III (1990 - '00) | 432.15 | 799.96 | 1851.15 |
| 4 | Period IV (2000 - '10) | 434.13 | 891.91 | 2054.46 |
| 5 | Period V (2010 - '20) | 436.95 | 1080.86 | 2473.63 |
| 6 | Overall Period (1970 - '20) | 419.26 | 763.61 | 1821.35 |

percent) and period V (0.17 percent). As for the production growth rate, period II exhibited the highest increase (3.62 percent), followed by the overall period (2.20 percent), period III (2.00 percent), period I (1.90 percent), period V (1.87 percent) and period IV (1.59 percent) respectively.

The growth rate of yield was found to be highest in period II (3.20 percent), followed by overall period (1.88 percent), period V (1.69 percent), period IV (1.61 percent), period - III (1.32 percent) and period I (1.00 percent). The decline in area in period - IV, could be attributed to drought, dry spells, unremunerative prices, low farm level technical efficiency, lower profitability. Results obtained are in conformity with the findings of Kumar (2019) and Pathak *et al*. (2020).

Table 2 showed gradual increase in average area, production and yield of rice over the decades. Overall period noticed average increase in 419.26 lakh ha, with the average production 763.61 lakh tonnes and average yield 1821.35 kg $ha^{-1}$ indicated in Figure 1. The highest average area (436.95 lakh ha), production (1080.86 lakh tonnes) and yield (2473.63 kg $ha^{-1)}$ was recorded in Period V compared to other periods.

**Decomposition annual of area, production and yield of rice in India**

Table 3 indicated the instability in area, production and yield of rice in India. Production change of 80.78% was noticed for the Period I subsequently 156.43% for Period II, 121.56% for Period III, 116% for Period IV and 138.61% for Period V, respectively. Whereas, for the overall period it was 745.22%. Area effect of 57.87% for Period I that was least among all, Period II noticed 87.62%, 66.55% for Period III, 96.40% for Period IV, 87.47% for Period V, whereas, for the overall period 76.86%. Yield effect was highest for Period I (37.86%), 9.77 % for Period II, second highest of 30.15% found in Period III, Period IV found

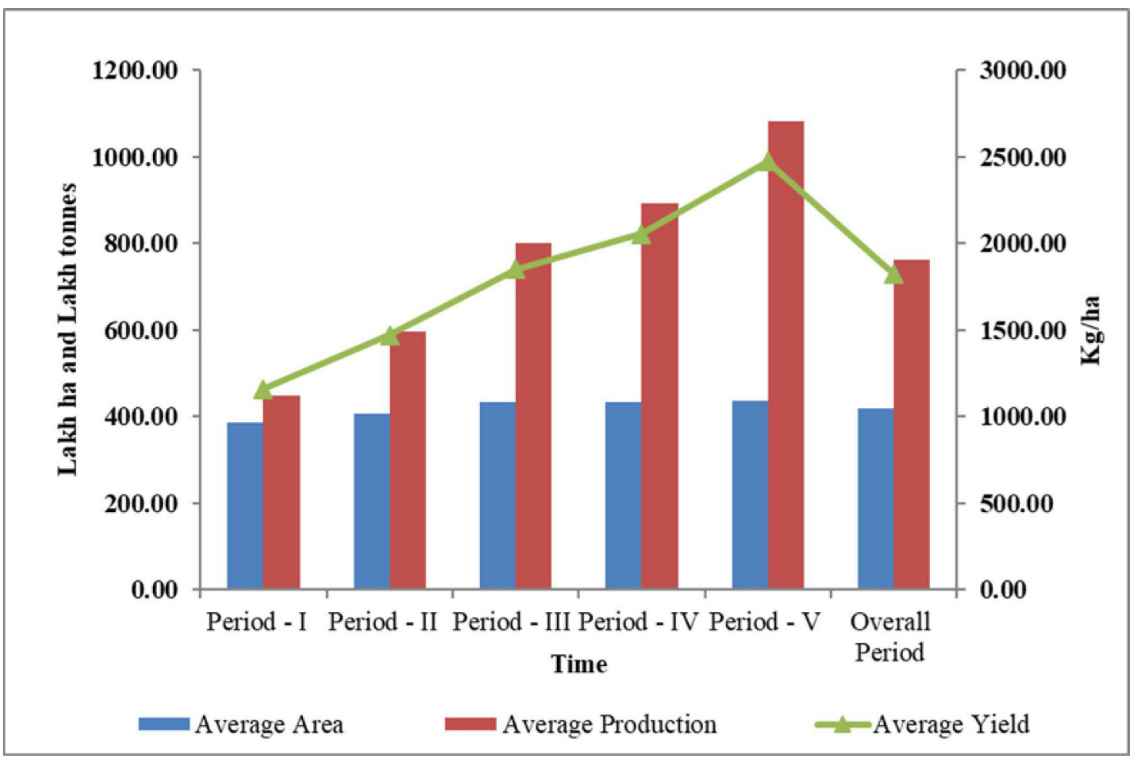


**Figure 1. Average area, production and yield of Rice in Pan-India**

**Table 4. Instability in area, production and yield of rice in India**

| S.No. | Particulars | Area (Lakh ha) | Production (Lakh tonnes) | Yield (kg ha$^{-1}$) | Area (Lakh ha) | Production (Lakh tonnes) | Yield (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|---|
| | | **Period I (1970 - '80)** | | | | | **Period IV (2000 - '10)** |
| 1 | Mean | 386.48 | 447.60 | 1155.86 | 434.13 | 891.91 | 2052.41 |
| 2 | SD | 12.51 | 51.71 | 100.45 | 14.56 | 78.47 | 139.93 |
| 3 | CV | 3.24 | 11.55 | 8.69 | 3.35 | 8.80 | 6.82 |
| 4 | CDVI | 1.92 | 10.49 | 8.55 | 3.56 | 7.93 | 5.21 |
| | | **Period II (1980 - '90)** | | | | **Period V (2010 - '20)** | |
| 1 | Mean | 406.58 | 597.73 | 1466.98 | 436.95 | 1080.86 | 2473.13 |
| 2 | SD | 12.44 | 79.82 | 161.43 | 5.15 | 66.33 | 140.16 |
| 3 | CV | 3.06 | 13.35 | 11.00 | 1.18 | 6.14 | 5.67 |
| 4 | CDVI | 2.97 | 8.15 | 5.64 | 1.12 | 2.66 | 2.62 |
| | | **Period III (1990 - '00)** | | | | **Overall Period (1970 - '20)** | |
| 1 | Mean | 432.15 | 799.96 | 1849.69 | 419.26 | 763.61 | 1799.62 |
| 2 | SD | 10.46 | 53.96 | 86.29 | 22.72 | 233.02 | 478.01 |
| 3 | CV | 2.42 | 6.74 | 4.67 | 5.42 | 30.52 | 26.56 |
| 4 | CDVI | 1.35 | 3.19 | 2.64 | 3.00 | 6.53 | 5.21 |

**Table 3. Decomposition analysis in area, production and yield of rice in India**

| S.No. | Time Periods | Production Change (%) | Area Effect (%) | Yield Effect (%) | Interaction Effect (%) |
|---|---|---|---|---|---|
| 1 | Period - I (1970 - '80) | 80.78 | 57.87 | 37.86 | 4.26 |
| 2 | Period - II (1980 - '90) | 156.43 | 87.62 | 9.77 | 2.61 |
| 3 | Period - III (1990 - '00) | 121.56 | 66.55 | 30.15 | 3.30 |
| 4 | Period - IV (2000 - '10) | 116.11 | 96.40 | 3.17 | 0.43 |
| 5 | Period - V (2010 - '20) | 138.61 | 87.47 | 11.20 | 1.33 |
| 6 | Overall Period (1970 -'20) | 745.22 | 76.86 | 9.73 | 13.42 |

lowest among all with 3.17%, 11.20% for Period IV, 9.73 % found for overall period. Interaction followed similar pattern followed in area effect where it highest interaction observed in Period I (4.26%) subsequently by Period III (3.30%), Period II (2.61%), Period V (1.33%) lowest among all found in Period IV (0.43%). Overall period observed 13.42% interaction effect.

The decomposition analysis revealed that change in production was more influenced by area effect in period I, while in period II, period III, period IV, period V and overall period respectively. Similar results were reported by Kumar (2019) and Pathak *et al*. (2020) in the crop.

**Instability of area, production and yield in rice in India**

Table 4 presents variations in stability across different factors, specifically area, production and yield. In the initial period (I), the highest level of variability was identified in production, accounting for 11.55%, followed by yield with 8.69 % and then area with 3.24 %. Notably, a consistent pattern was observed when examining the Cuddy Della Valle Index (CDVI), with production at 10.49, yield at 8.55 and area at 1.92. During the subsequent period (II), a similar pattern persisted, with production displaying the greatest instability at 13.35%, followed by yield at 11% and area at 3.06%. CDVI values for this period stood at 8.15 for production, 5.64 for yield and 2.97 for area. Period III exhibited the highest instability in production, standing at 6.74%, while yield showed a variation of 4.67%, and area exhibited the lowest instability with a coefficient of variation (CV) of 2.42%. CDVI values for this period were 3.19 for production, 2.64 for yield and 1.35 for area. Transitioning to the fourth period (IV), instability percentages were 8.80% for production, 6.82% for yield and 3.56% for area. Correspondingly, CDVI values were highest for production at 7.93, followed by yield at 5.21 and area at 3.56. In the most recent decade, period V, the trend remained consistent with the previous periods, showing instability percentages of 6.14% for production, 5.67% for yield and 1.18% for area. In general instability found more in production followed by yield but less instability found in area. Instability was highest in the Period II among all. Overall period also noticed instability highest for production subsequently by yield and area. CDVI also followed same pattern for all it was highest for area followed by yield and production. Results were confirmatory with Kumar (2019) and Pathak *et al*. (2020).

**CONCLUSIONS**

Rice crop was noticed with significant increase growth in the area over the period, non-significant increase for the period 1980-'90 and 2010-'20 but negative trend in area

was observed for rice area in the 2000-‘10 decade attributed to drought, dry spells, unremunerative prices, low farm level technical efficiency, lower profitability. Production observed significant growth for all the three decades and overall period. Yield found to be non-significant increase in the first decade and significant in all other periods. Decomposition analysis confirmed area effect more in the rice for the periods compared to yield effect 1970-‘80 decade and 1990-‘00 decade sowed more of yield effect compared to other decades but interaction effect was less for rice. Production has the most instable, followed by yield, but area has the least. 1980-‘90 decade had the most instable over all the periods. Increase in area affected the production positively. There is a need for policy emphasis on bolstering rice yield through focused research, enabling consistent production growth. Also, to address fluctuations in cultivation area and production instability, adopting diversified cultivation strategies, resilient practices, and efficient early warning systems becomes imperative for sustainable rice sector management.

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