Efficacy of *Pseudomonas fluorescens* and organic amendments against black root rot disease caused by *Rhizoctonia solani in strawberry (Fragaria x ananassa Duch.)*

ABSTRACT

Black root rot of strawberry caused by *Rhizoctonia solani* is one of the devastating soil borne disease. An experiment was conducted to evaluate the efficacy of organic amendments, PGPR (*Pseudomonas Fluorescens*) and botanical treatment for the management of *R. solani* on strawberry. The study was laid out in Randomized Block design (RBD) including 4 replications with 8 treatments in pot condition at the greenhouse of Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, Uttar Pradesh, during *Rabi* season 2023-2024. The treatments consisted of combination of FYM, vermicompost, biomix compost, neem cake, cocopeat and botanical neem leaf extract were used as soil amendments and *P. fluorescens* as root treatment for evaluation on the plant growth, yield, physicochemical parameters (TSS and total ascorbic acid), disease intensity (%) and benefit cost ratio of strawberry. The present investigation results revealed that T_7 *P. fluorescens* (0.3%) + FYM @ 100g + biomix compost @ 15g + vermicompost @ 15g + neem leaf extract @10% had the most promising results in term of maximum plant height (14.9 cm), maximum leaf number(15.75), days taken to first flowering (86 days), berry length (4.08 cm), berry diameter (2.84 cm), yield (38.87 qha^{-1}), TSS (8.80 °Brix), total ascorbic acid (51.60mg/100g) and B:C ratio (1:2.24). It was also observed that T_7 superior over other treatments giving least per cent disease intensity (30.75 %) followed by T_6 FYM @100g + *P. fluorescens* (0.3%) + biomix compost @15g + neem cake @15g + neem leaf extract @10% (35.50%). These findings highlight the potential of organic amendments, PGPR (*P. fluorescens*) and botanical treatments, as effective alternatives for managing black root rot disease caused by *R. solani* in strawberry cultivation.

Keywords: Black root rot, Rhizoctonia solani, Pseudomonas fluorescens, FYM, vermicompost, biomix compost, neem cake, neem leaf extract

1. INTRODUCTION

Strawberry (*Fragaria* × *ananassa* Duch.) cv. Chandler is an important fruit that belongs to the family Rosaceae and genus *Fragaria*. It occupies an important place among the small fruit plants and it is grown throughout the world. It is a good source of essential nutrients, antioxidants, bioactive compounds and beneficial phytochemicals, which have relevant biological activity in human **(Nishu *et al*., 2021)**.

Strawberries are grown worldwide in approximately 80 countries and grows on 397,603 ha with a worldwide production of more than 9.5 million tons. Leading strawberry-producing countries are the China (3.3 million tons), USA (1.2 million tons)) and Tukey (0.7 million tons) **(FAO STAT, 2023)** In India encompasses an estimated area of around 3310 ha with total production of 19.84 metric tonnes **(Horticultural statistics at a glance, 2021)**.

Strawberry is one of the important temperate fruits of India Its successful cultivation requires an optimum day temperature of 22-23°C and night temperature of 7-13°C in India **(Tripathi *et al.,* 2015)**. The Panchgani-Mahabaleshwer region of Maharashtra grows more than 85 percent of country's strawberries. Presently, strawberry cultivation is spreading in plain of Indian climatic condition **(Meena *et al.,* 2018)**.

Strawberry plants are affected by a large number of diseases caused by fungi, bacteria, viruses, nematodes and arthropods. These pathogens cause damage on the leaves, roots, crowns and fruits. Among different diseases, black root rot is a common, yield-limiting and serious disease complex that adversely affects strawberry production in many regions of the world. It is considered to be a complex disease, caused by the several pathogens (*Rhizoctonia* spp., *Pythium* spp., *Fusarium* spp. and *Cylindrocarpon* spp.), environmental factors and nematodes on strawberry **(Matsumoto and Yoshida, 2006)**. The organism that is most commonly associated with the black root rot complex is *Rhizoctonia solani* and *R. fragariae*. These pathogens, single or in combination fill the vascular system of strawberries, resulting in the prevention of nutrient and water uptake **(Demir *et al.,* 2023)**.
This disease can cause yield losses of 30-50% in strawberries, decline in vigour and productivity of the plant stand causing damage to the host and considerable reduction in the yield **(Ahmed *et al.,* 2017**). Black root rot disease of strawberry characterised by stunted growth, brittle, wilt under heat stress and blackened root systems. They produced fewer crowns of reduced diameter compared with unaffected plants and produced less fruit of reduced quality. Affected plants leaves are generally smaller and few numbers of runners are produced **(Kumari and Thakur, 2022)**.
The major approach to control crown and root-rot in strawberry cultivation has dependent on injecting artificial fungicides **(Coque *et al.*, 2020).** However, the usage application of fungicides raises a number of problems, including the buildup of resistance in the diseases that are being targeted, harmful residue on fruits and adverse outcomes on the earth as well as society **(Mheidi *et al.*, 2023).** In recent years, plant extracts derivatives become the importance for the control of the plant diseases due to their antifungal and antibacterial properties. The application of botanical extracts for disease management could be less expensive, less polluting and ecofriendly **(Rahila *et al.*, 2020).**
Use of organic by-products as amendments to reduce soilborne plant diseases is gaining the interest of plant pathologists, manufacturing and processing industries, regulators, consumers and growers. The potentiality of Plant growth-promoting rhizobacteria in agriculture is steadily increased as it offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements. Biocontrol of strawberry root rot triggered by the *Rhizoctonia* genus can be accomplished by either boosting native antagonists like those present in organic manure like vermicompost, FYM etc. to a density adequate for suppression of pathogen(s) or inserting alien antagonists. Amid the many antagonists investigated by scientists are the *Bacillus* in addition to *Pseudomonas* genera, which have been proven to be beneficial for lowering the spread of numerous soil-borne diseases **(Attia, 2019)**. Thus, the present study aimed to evaluate the effect of selected organic amendments and PGPR (*Pseudomonas fluorescens)* for the management of black root rot caused by *Rhizoctonia solani* in strawberry. Keeping the above in view the study was undertaken during *Rabi* season 2023, at the green house, Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj.

2. MATERIAL AND METHODS

The experiment was carried out in pot condition at the green house, Department of Plant Pathology, Sam Higginbottom University of Agriculture,Technology and Sciences, Prayagraj during *Rabi* season 2023. The study was laid-out in Randomized Block Design (RBD) with four replications. The treatments include the combination of different organic amendments like FYM, vermicompost (VC), biomix compost (BC) – organic compost mixed with bio fertilizers like PSB and *Azotobacter chroococcum*, neem cake (NC) and cocopeat (CP) were applied as soil amendments (SA) during the soil preparation, botanical neem leaf extract (NLE) was applied as soil drenching (SD) and liquid formulation *Pseudomonas fluorescens* (Pf) in broth was applied as root dip (RD) of the runners for 30 mins before planting of the runners. 8 treatment combinations *viz.,*T_0 – control (untreated check), T_1 - FYM @100g (SA) + Pf @ 0.3% (RD) + VC @15g (SA) + NLE @ 10% (SD), T_2 - FYM @100g + Pf @ 0.3% (RD) + BC @15g (SA) + NLE @10% (SA), T_3 - FYM @100g + Pf @ 0.3% (RD) + CP @ 15g (SA) + NLE @10% (SA), T_4 - FYM @100g + Pf @ 0.3% (RD) + NC @15g (SA) + NLE @10% (SD). T_5 –FYM @100g + Pf @ 0.3% (RD) + BC @15g (SA) + CP @15g (SA) + NLE @10%(SD), T_6 – FYM @100g + Pf @0.3% (RD) + BC @15g (SA) + NC @15g (SA) + NLE @10% (SD) and T_7 - FYM @100g + Pf @ 0.3% (RD) + BC @15g (SA) + VC @15g (SA) + NLE @10% (SD) were adopted. The data recorded of different parameters during experiment were pooled and analyzed. Observation on plant height and numbers of leaves were recorded at 30. 60, 90 and 120 DAT. At each picking, data of berry weight and yield were recorded. The length and width of five randomly selected berries were by using vernier callipers.

2.1 Isolation and identification of the pathogen

Diseased plants were collected from different places during the cropping season and isolation of pathogen was carried out in the laboratory. Firstly, collected plant diseased samples (roots) washed thoroughly with water, cut off to the part with the symptom of 2 mm, surface sterilized with the 1 per cent sodium hypochlorite (NaOCl) solution for 1 min, then washed with sterilized distilled water thrice to remove any sodium hypochlorite traces and dried by sterilized filter paper and then transferred to the petri plates with potato dextrose agar media (1 pieces for each plate) with the help of sterilized needle and incubated in incubator at 28 ± 2°C for 2-3 days and examined at frequent intervals to check the growth of the fungal pathogen..
The fungal colony culture was initially white, cottony and abundant in aerial mycelium, but it progressively turned into brown colour. Sclerotia of the cultural type were scattered separately or sometimes joined laterally on PDA. They were dark brown to black, subspherical to irregular and 0.6 -6.0 mm in diameter. The hyphae are typically wide (6-12 μm) and exhibit right-angled branching with a characteristic constriction at the point of branching and a septum near the branch. The cells are

multinucleate and the hyphal walls are generally smooth. The fungus does not produce spores, which is unusual for many fungal pathogens, but instead forms hard sclerotia, darkly pigmented structures that allow it to survive in harsh conditions. The fungus was identified as *R. solani* based on cultural appearance on media, sclerotial morphology and other physiological characteristics of mycelium **(Kim *et al.,* 1994)**.

2.1.1 Purification and maintenance of the pathogen

The cultures of the fungus were sub-cultured on petri plates and kept in laboratory at 28 ± 1 °C for 15 days. Such mother cultures were preserved at 4 °C in refrigerator. Further, these cultures were sub-cultured once in a month and used for future purpose **(Toussoun and Nelson, 1976)**.

2.2 Preparation of Phyto-extracts

Fresh and healthy neem leaves were collected and washed with running tap water followed by sterile distilled water and air dry at 27 ℃. The plant materials and water were used at a ratio of 1:1 (weight: volume) and ground the mixture by using pestle and mortar to obtained extract. The extracts were then filtered through double-layered muslin cloth and then with filter paper. The concentrated filtrated neem leaf extract was considered as standard solution (100%). The neem leaf extract at 10% prepared from standard was drenched at the rhizosphere region of the plant **(Bambode and Shukla, 1973)**.

2.3 Measurement of total soluble solids (TSS) along with total ascorbic acid (vitamin C)

A digital hand refractometer was utilized to figure out the total soluble solids content of the strawberry **(Paul *et al.,* 2010).** Ascorbic acid content was calculated by using 2, 6–Dicholorophenol indophenol visual titration method. Dye solution was prepared by dissolving 42 mg of $NaHCO_3$ in a small amount of distilled water and added 52g of 2,6- dichlorophenol indophenols and diluted with distilled water to 200ml. Standard vitamin C solution was prepared by taking 100 mg of vitamin C mixed with 100 ml of 3 percent oxalic acid in 100 ml volumetric flask. 10 ml of standard vitamin C solution was taken in a conical flask and it was titrated with prepared dye from burette until the solution turn into light pink and recorded dye used while titration process (V1 ml). 5 g of strawberry sample was taken and grinded into strawberry juice and filterd by using double layering muslin cloth and added 4 per cent oxalic acid to a known volume volume (100ml), then pipetted 5 ml of the supernatant and added 10 ml 4 per cent oxalic acid and titrating against dye (V_2 ml). The content of vitamin C in strawberry was calculated using the formula **(Sadasivam and Balasubraminan, 1987)** which is given below

$$\text{Content of vitamin C (mg/100g)} = \frac{\text{V1ml x 5ml x weight of the sample}}{\text{0.05 x V2ml x 100ml}} \text{x 100}$$

2.4 Disease assessment

Plants per treatment per replication were regularly watched for first appearance of disease. The observation on disease intensity was recorded using a progressive 0-5 scale, as showed in (Table 1 and Plate1). Numerical rating scale was given on the basis of percentage of area infected by pathogen on the root **(Fang *et al.,* 2011)** as described below

Table 1 Disease rating scale

Scale	Description
0	root well developed, no discolouration
1	<25% root discoloured
2	≥25%, <50% root discoloured
3	≥50%, <75% root discoloured
4	≥75% root discoloured
5	all root discoloured (rotted)

Plate 1. Disease rating scale

2.4.1 Per Cent Disease Intensity (PDI)

Per cent disease intensity was recorded at 150 DAT at the end of the experiment. Per cent disease intensity was calculated in accordance with following formula **(Mckinney, 1923)** which was given below

$$\text{Disease intensity (\%)} = \frac{\text{Sum of all individual disease ratings}}{\text{Total no. of plants observed x Maximum disease grade}} \text{ x } 100$$

2.5 Benefit cost ratio

Cost of cultivation, gross return, net return and benefit cost ratio was worked out to evaluate the economics of each treatment, based on the existing market prices of input and output **(Reddy and Reddi, 2004).** The benefit cost ratio was calculated by using the following formula

$$\text{B: C ratio} = \frac{\text{Gross return (Rs/ha)}}{\text{Cost of cultivation (Rs/ha)}}$$

3. RESULTS AND DISCUSSION

Under greenhouse condition, plant height and numbers of leaves were recorded at 30. 60, 90 and 120 DAT. At each picking, data of berry weight and yield were recorded. The length and width of five randomly selected berries were by using vernier callipers.

3.1 Plant height (cm) of strawberry

The data presented in the table 2 and depicted in figure 1 revealed that plant height (cm) of strawberry significantly increasedl n treatment T_7 - FYM @100g +Pf @0.3% BC @15g + VC @15g + NLE @10% (14.90 cm) followed T_6 – FYM @100g + Pf @0.3% + BC @15g + NC @15g + NLE @10% (13.97 cm), T_1- FYM @100g + Pf @0.3% + VC @15g + NLE @10% (13.22 cm), T_5- FYM @100g + Pf @0.3% + BC @15g+ CP @15g + NLE @10% (13.10 cm), T_2 - FYM @100g + Pf @0.3% + BC @15g + NLE @10% (12.42 cm), T_4 - FYM @100g + Pf @0.3% + NC @15g + NLE @10% (11.70 cm), T_3- FYM @100g + Pf @0.3% + CP @15g + NLE @10% (11.00 cm) as compared to (untreated checked) T_0 - control (8.60 cm). Comparing the treatments with the CD value (0.66) all the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) were found significant over (untreated checked) T_0 - control. Among the treatments (T_1 and T_5) were found non-significant to each other, (T_2, T_3, T_4, T_6 and T_7) were found to be significant over all the other treatments.

As per finding from this study, the maximum plant height (cm) was observed in T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10%. The probable reasons for this result may be due to the soil nitrogen contents increased with the use of FYM and other organic fertilizer. Nitrogen is the constituent of chlorophyll, more chlorophyll contents result in more photosynthesis, thus may have increased plant height in strawberry **(Zahid *et al.,* 2022)**. Vermicompost may have attributed to better availability of plant growth regulator and humic acid which is produced by the increased activity of microbes. The microbes like fungi, bacteria, yeast, actinomycetes, algae etc. are capable to produce auxin, gibberellin **(Singh *et al.,* 2008)**. *Pseudomonas fluorescens* generates indole acetic acid as a growth regulator which may have helped in strawberry plant growth **(Badawy, 2023)**. The increment in growth parameter due to the biofertilizers application might be due to the vital role and promoting effect of bacteria present in the applied biofertilizers on morphology and physiological of the root system, thus favouring the growth parameter and contributing some hormones like gibberellin, auxin and cytokinin. Similar findings are consistant with research conducted on *Moringa oleifera* Lam. plants **(Youssef, 2016).** Compounds from neem leaf such as isoprenoids (azadirone, gedunin, vilasinin, azadirachtin) and non-isoprenoids (sulphurous compounds, poly phenolics like flavonoids, dihydrochalcone, coumarin, tannins) were reported to be fungistatic in nature. Different parts of neem have demonstrated great biological activities against microbes through the inhibition of growth and breakdown of their cell walls. Similar findings have been reported by **Bhattarai *et al.,* (2022)** and **Adusei and Azupio (2022)**. So, neem leaf extract significantly inhibits the pathogen and may have leads to better health of the plant which in turn may have helped the plant in attaining maximum plant height.

3.2 Numbers of leaves of strawberry

The data presented in the table 2 and depicted in figure 1 revealed that number of leaves of strawberry significantly increased in treatment T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10% (15.75) followed T_6 FYM @100g + Pf @0.3% + BC @15g + NC@15g + NLE @10% (14.37), T_5 - FYM@100g + Pf @0.3% + BC @15g + CP @15g + NLE @10% (13.00), T_1 - FYM @100g + Pf @0.3% + VC @15g + NLE @10% (12.50), T_2 - FYM @100g + Pf @0.3% + BC @15g + NLE @10% (11.87), T_4 - FYM @100g + Pf @0.3% + NC @15g + NLE @10% (10.37), T_3 - FYM @100g + Pf @0.3% + CP @15g + NLE @10% (9.00) as compared to (untreated checked) T_0 - control (7.50).

Comparing the treatments with the CD value (1.25) all the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) were found significant over (untreated checked) T_0 - control. Among the treatments (T_1, T_2 and T_5) were found non-significant to each other, (T_3, T_4, T_6 and T_7) were found to be significant over all the other treatments.

As per finding from this study, the maximum numbers of leaves were observed in T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10%. The probable reasons for this result may be due to the soil nitrogen contents increased with the use of FYM and other organic fertilizer. Nitrogen is the constituent of chlorophyll, more chlorophyll contents result in more photosynthesis, thus may have increased numbers of leaves in strawberry **(Zahid *et al.,* 2022)**. Application of PSB contain in biomix compost may have helped in cell elongation and cell division in merismatics region of plant, this may be due to the production of plant growth regulator such as IAA and GA **(Kumar *et al.,* 2015)**. *Pseudomonas fluorescens* generates indole acetic acid as a growth regulator which may have helped in strawberry plant growth **(Badawy, 2023)**. Compounds from neem leaf such as isoprenoids and non-isoprenoids were reported to be fungistatic in nature. Different parts of neem have demonstrated great biological activities against microbes through the inhibition of growth and breakdown of their cell walls. Similar findings have been reported by **Bhattarai *et al.* (2022)** and **Adusei and Azupio (2022)**. So, neem leaf extract significantly inhibits the pathogen and may have leads to better health of the plant.

Table 2. Effect of selected treatments on plant height (cm), number of leaves, days taken to first flowering (days), berry length (cm) and berry diameter (cm)

Treatments	Plant Height (cm)	Numbers of leaves	Days taken to first flowering (days)	Berry length (cm)	Berry diameter (cm)
T_0- Control (untreated checked)	8.60	7.5	105.50	2.02	1.72
T_1- FYM @100g + Pf @0.3% + VC @15g + NLE @10%	13.22^{a}	12.50^{a}	91.00^{b}	3.80^{a}	2.54^{a}
T_2- FYM @100g + Pf @0.3% + BC @15g+ NLE @10%	12.42	11.87^{a}	93.75^{a}	3.42	2.52^{a}
T_3- FYM @100g + Pf @0.3% + CP @15g + NLE @10%	11.00	9.00	96.75	3.18	2.30^{b}
T_4- FYM @100g + Pf @0.3% + NC @15g + NLE @10%	11.70	10.37	93.50^{a}	3.62	2.28^{b}
T_5- FYM @100g + Pf @0.3% + BC @15g + CP @15g +NLE@10%	13.15^{a}	13.00^{a}	91.12^{b}	3.82^{a}	2.58^{a}
T_6- FYM @100g + Pf @0.3% + BC @15g + NC @15g +NLE@10%	13.97	14.37	88.00	3.86^{a}	2.60^{a}
T_7- FYM @100g + Pf @0.3%+ BC @15g + VC @15g +NLE@10%	14.90	15.75	86.00	4.08	2.84
CD at 5%	0.66	1.25	1.67	0.17	0.20

Table 3. Effect of selected treatments on TSS(°Brix), total ascorbic acid (mg/100g), yield (q/ha), disease intensity (%) and B:C ratio

Treatments	TSS (°Brix)	Total ascorbic acid (mg/100g)	Yield (q/ha)	Disease intensity (%) at 150 DAT	B:C ratio
T_0- Control (untreated checked)	6.50	46.00	20.60	50.75	1:1.63
T_1- FYM @100g + Pf @0.3% + VC @15g + NLE @10%	8.13^{a}	49.66^{a}	33.86	35.75^{a}	1:2.11
T_2- FYM @100g + Pf @0.3% + BC @15g + NLE @10%	8.03	49.00^{b}	31.99^{a}	40.50^{b}	1:1.93
T_3- FYM @100g + Pf @0.3% + CP @15g + NLE @10%	7.16	47.17	30.25	45.25	1:1.86
T_4- FYM @100g + Pf @0.3% + NC @15g + NLE @10%	7.36	48.57^{b}	31.84^{a}	42.75	1:1.87
T_5- FYM @100g + Pf @0.3% + BC@15g + CP@15g + NLE@10%	8.16^{a}	50.00^{a}	32.06^{a}	39.25^{b}	1:1.82
T_6- FYM @100g + Pf @0.3% + BC @15g + NC @15g + NLE@10%	8.43^{a}	50.93	35.86	35.50^{a}	1:2.14
T_7- FYM @100g + Pf @0.3% + BC @15g + VC@15g + NLE@10%	8.80	51.60	38.87	30.75	1:2.24
CD at 5%	0.18	0.55	1.46	2.23	

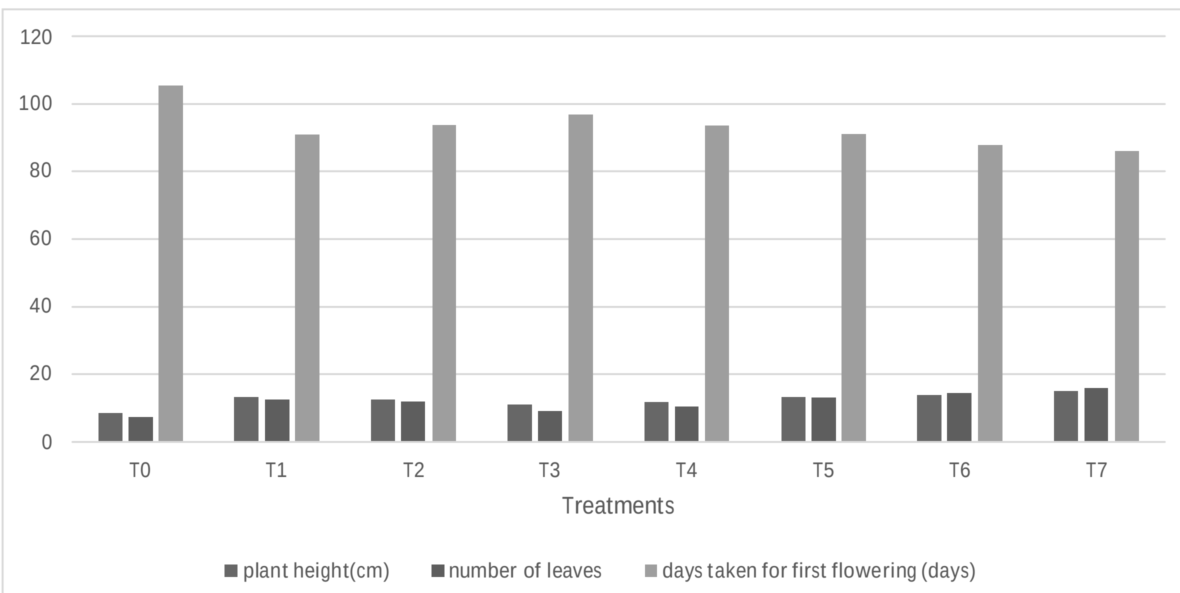

Fig. 1. Effect of selected treatments on plant height (cm), number of leaves and days taken to first flowering (days)

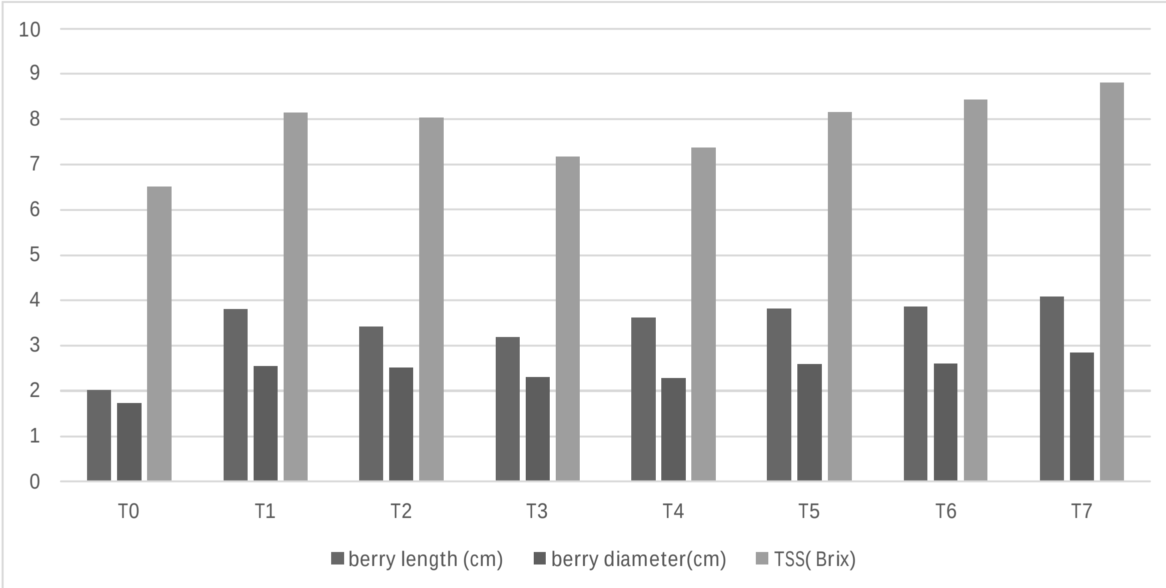

Fig. 2. Effect of selected treatments on berry length (cm), berry diameter (cm) and TSS (°Brix)

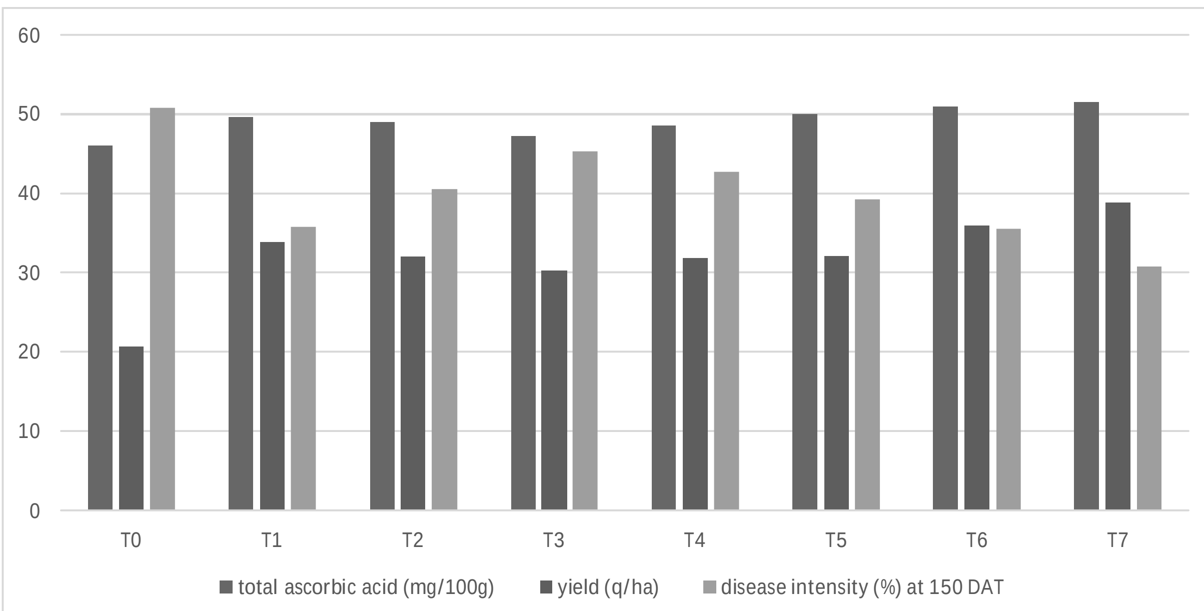

Fig. 3. Effect of selected treatments on total ascorbic acid (mg/100g), yield (q/ha) and disease intensity (%) at 150 DAT

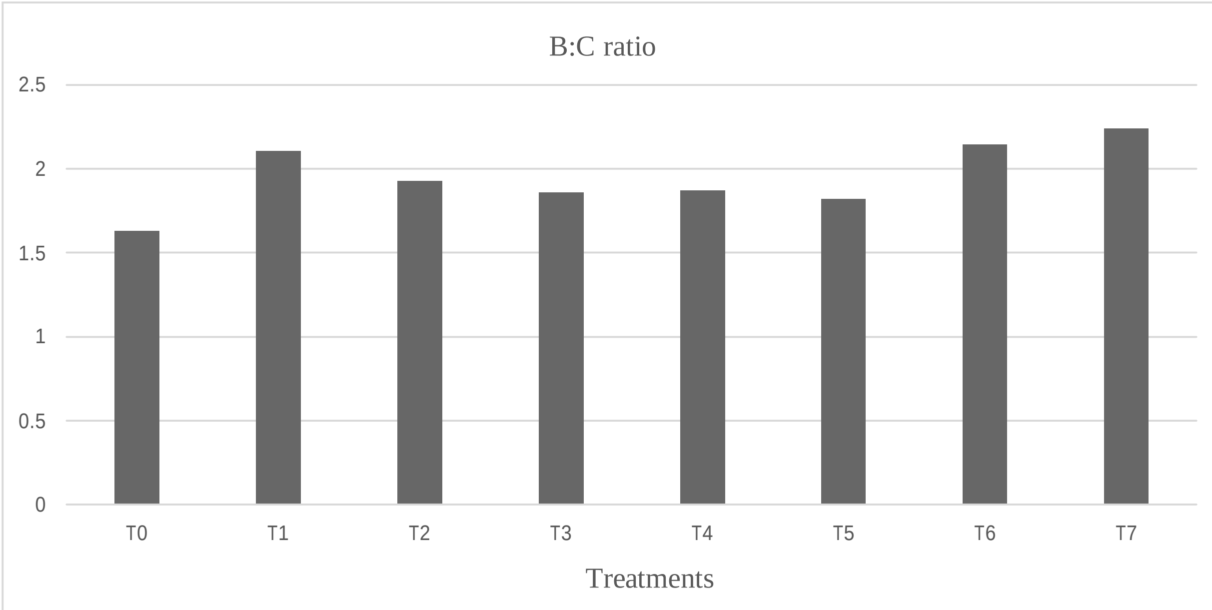

Fig. 4. Effect of selected treatments on B:C ratio

Days taken to first flowering of strawberry

The data presented in the table 2 and depicted in figure 1 revealed that minimum days taken to first flowering was observed in treatment T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10% (86.00 days) followed T_6 – FYM @100g + Pf @0.3% + BC @15g + NC @15g + NLE @10% (88.00 days), T_1 - FYM @100g + Pf @0.3% + VC @15g + NLE @10% (91 days), T_5 – FYM @100g + Pf @0.3% + BC @15g + CP @15g + NLE @10% (91.25 days), T_4 - FYM @100g + Pf @0.3% + NC @15g + NLE @10% (93.50 days), T_2 - FYM @100g + Pf @0.3% + BC @15g + NLE @10% (93.75 days), T_3 - FYM @100g + Pf @0.3% + CP @15g + NLE @10% (95.25 days) as compared to (untreated checked) T_0 - control (105.5 days). Comparing the treatments with the CD value (1.67) all the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) were found significant over (untreated checked) T_0 - control.

Among the treatments (T_2 and T_4); (T_1 and T_5) were found non-significant to each other, (T_3, T_6 and T_7) were found to be significant over all the other treatments.

As per finding from this study, the days taken to first flowering was observed in T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10%. The probable reasons for this result may be due to application of FYM and organic fertilizers play vital role in the production of gibberellic acid in roots which may have helped in breaking bud dormancy, which may have resulted in enhanced bud production and increase flowering site in strawberry **(Zahid *et al.,* 2022)**. The release of nutrient and primary nitrogen from the vermicompost may have conceded with the period of flowering differentiation **(Cabilovski *et al.,* 2023)**. Application of biofertilizer like PSB may have helped in increasing the biological nitrogen fixation and availability of phosphorus which may have helped strong vegetative growth in strawberry **(Kumar *et al.,* 2015)**. Compounds from neem leaf such as azadirone, gedunin, vilasinin, azadirachtin, sulphurous compounds and polyphenolics like flavonoids, dihydrochalcone, coumarin, tannins were reported to be fungistatic in nature. Different parts of neem have demonstrated great biological activities against microbes through the inhibition of growth and breakdown of their cell walls. Similar findings have been reported by **Bhattarai *et al.* (2022)** and **Adusei and Azupio (2022)**. So, neem leaf extract significantly inhibits the pathogen and may have leads to better health of the plant.

Berry length (cm) of strawberry

The data presented in the table 2 and depicted in figure 2 revealed that berry length (cm) `of strawberry significantly increased in treatment T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10% (4.08 cm) followed T_6 FYM @100g + Pf @0.3% + BC @15g + NC @15g + NLE @10% (3.86 cm), T_5 – FYM @100g + Pf @0.3% + BC @15g + CP @15g + NLE @10% (3.82 cm), T_1 - FYM @100g + Pf @0.3% + VC @15g + NLE @10% (3.80 cm), T_4 - FYM @100g + Pf @0.3% + NC @15g + NLE @10% (3.62 cm), T_2 - FYM @100g + Pf @0.3% (0.3%) + BC @15g + NLE @10% (3.42 cm), T_3 - FYM @100g + Pf @0.3% + CP @15g + NLE @10% (3.18 cm) as compared to (untreated checked) T_0 - control (2.02 cm).

Comparing the treatments with the CD value (0.17) all the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) were found significant over (untreated checked) T_0 - control. Among the treatments (T_1, T_5 and T_6) were found non-significant to each other, (T_2, T_3, T_4 and T_7) were found to be significant over all the other treatments.

As per finding from this study, the maximum berry length (cm) was observed in T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE@10%. The probable reasons for this result may be due to the soil nitrogen contents increased with the use of FYM and other organic fertilizer. Nitrogen is the constituent of chlorophyll, more chlorophyll contents result in more photosynthesis, thus may have increased in fruits weight and fruits diameter in strawberry **(Zahid *et al.,* 2022)**. Vermicompost and *Azotobacter chrooroccum* present in biomix compost may have increased the accumulation of dry matter. Berry size, weight and volume are highly co-related with the dry matter content and balanced level of hormone. **(Tripathi *et al.,* 2015)**. PSB and vermicompost may have extended photosynthetic capacity of plant which may have turned in accumulation of dry matter in strawberry **(Yashasvi *et al.,* 2021)**. Compounds from neem leaf such as isoprenoids (azadirone, gedunin, vilasinin, azadirachtin) and non-isoprenoids (sulphurous compounds, polyphenolics like flavonoids, dihydrochalcone, coumarin, tannins) were reported to be fungistatic in nature. Different parts of neem have demonstrated great biological activities against microbes through the inhibition of growth and breakdown of their cell walls. Similar findings have been reported by **Bhattarai *et al.* (2022)** and **Adusei and Azupio (2022)**. So, neem leaf extract significantly inhibits the pathogen and may have leads to better health of the plant.

3.5 Berry width (cm) of strawberry

The data presented in the table 2 and depicted in figure 2 revealed that berry diameter of strawberry significantly increased in treatment T_7 - FYM @100g + Pf @0.3% **+** BC @15g + VC @15g + NLE @10% (2.84 cm) followed T_6 – FYM @100g + Pf @0.3% + BC @15g + NC @15g + NLE @10% (2.60 cm), T_5 – FYM @100g + Pf @0.3% + BC @15g + CP @15g + NLE @10% (2.58 cm), T_1 - FYM @100g + Pf @0.3% + VC @15g + NLE @10% (2.54 cm), T_2 - FYM @100g + Pf @0.3% + BC @15g + NLE @10% (2.52 cm), T_3 - FYM @100g + Pf @0.3% + CP @15g + NLE @10% (2.30 cm), T_4 - FYM @100g + Pf @0.3% + NC @15g + NLE @10% (2.28 cm), as compared to (untreated checked) T_0 - control (1.72 cm).
Comparing the treatments with the CD value (0.20) all the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) were found significant over (untreated checked) T_0 - control. Among the treatments (T_1, T_2, T_5 and T_6); (T_3 and T_4,) were found non-significant to each other, T_7 was found to be significant over all the other treatments.
As per finding from this study, the maximum berry diameter (cm) was observed in T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10%. The probable reasons for this result may be due to the soil nitrogen contents increased with the use of FYM and other organic fertilizer. Nitrogen is the constituent of chlorophyll, more chlorophyll contents result in more photosynthesis, thus may have increased in fruits weight and fruits diameter in strawberry **(Zahid *et al.,* 2022)**. Vermicompost and *Azotobacter chroococcum* may have increased the accumulation of dry matter. Berry size, weight and volume are highly co-related with the dry matter content and balanced level of hormone **(Tripathi *et al.,* 2015)**. PSB and vermicompost may have extended photosynthetic capacity of plant which may have turned in accumulation of dry matter in strawberry **(Yashasvi *et al.,* 2021)**.

3.6 TSS (°Brix) of strawberry

The data presented in the table 3 and depicted in figure 2 revealed that total soluble solids (°Brix) of strawberry significantly increased in treatment T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10% (8.80 °Brix) followed T_6 FYM @100g + Pf @0.3% + BC @15g + NC @15g + NLE @10% (8.43 °Brix), T_5 – FYM @100g + Pf @0.3% + BC @15g + CP @15g + NLE @10% (8.16 °Brix), T_1 - FYM @100g + Pf @0.3% + VC @15g + NLE @10% (8.13 °Brix), T_2 - FYM @100g + Pf @0.3% + BC @15g + NLE @10% (8.03 °Brix), T_4 - FYM @100g + Pf @0.3% + NC @15g + NLE @10% (7.36 °Brix), T_3 - FYM @100g + Pf @0.3% + CP @15g + NLE @10% (7.16 °Brix), as compared to (untreated checked) T_0 - control (6.5 °Brix).
Comparing the treatments with the CD value (0.18) all the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) were found significant over (untreated checked) T_0 - control. Among the treatments (T_1, T_2 and T_5) were found non-significant to each other, (T_3, T_4, T_7 and T_6) were found to be significant over all the other treatments.
As per finding from this study, the maximum TSS (°Brix) value was observed in T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10%. The probable reasons for this result may be due to the application of FYM in soils resulted in an increased chlorophyll production and more chlorophyll contents may have increased photosynthesis, thus may have helped in increased soluble solid contents **(Zahid *et al.,* 2022)**. Application *Azotobacter* spp. and vermicompost may have increased TSS and sugar contents due to the quick metabolic transformation of starch and pectin into soluble compound and rapid translocation of sugar from leaves to developing fruits in strawberry **(Tripathi *et al.,* 2015)**.

3.7 Total ascorbic acid (mg/100g) of strawberry

The data presented in the table 3 and depicted in figure 3 revealed that total ascorbic acid (mg/100g) of strawberry significantly increased in treatment T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10% (51.60 mg/100g) followed T_6 – FYM @100g + Pf @0.3% + BC @15g + NC @15g + NLE @10% (50.93 mg/100g), T_5 FYM @100g + Pf @0.3% + BC @15g + CP @15g + NLE @10% (50 mg/100g), T_1 - FYM @100g + Pf @0.3% + VC @15g + NLE @10% (49.67 mg/100g), T_2 - FYM @100g + Pf @0.3% + BC @15g + NLE @10% (49 mg/100g), T_4 - FYM @100g + Pf @0.3% + NC @15g + NLE @10% (48.57 mg/100g), T_3 - FYM @100g + Pf @0.3% + CP @15g + NLE @10% (47.17 mg/100g), as compared to (untreated checked) T_0 - control (46.00 mg/100g).

Comparing the treatments with the CD value (0.55) all the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) were found significant over (untreated checked) T_0 - control. Among the treatments (T_1 and T_5); (T_2 and T_4) were found non-significant to each other, (T_3, T_6 and T_7) were found to be significant over all the other treatments.
As per finding from this study, the maximum total ascorbic acid (mg/100g) was observed in T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10%. The probable reasons for this result may be due to the fact that microbial inoculant like *Azotobacter* may have increased in availability of phosphorus and secrete of growth promoting substance which may have accelerated the physiological process like carbohydrate synthesis and may have helped in increased ascorbic acid content **(Tripathi *et al.,* 2015)**. The soil nitrogen contents increased with the use of FYM and other organic fertilizer. Nitrogen is the constituent of chlorophyll, more chlorophyll contents result in more photosynthesis, thus may have increased in total ascorbic acid and potassium present in the soil also may have helped in increased vitamin C content in fruits **(Zahid *et al.,* 2022)**.

3.8 Yield (q ha^{-1}) of strawberry

The data presented in the table 3 and depicted in figure 3 revealed that yield (qha^{-1}) of strawberry significantly increased in treatment T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10% (38.87 q ha^{-1}) followed T_6 FYM @100g + Pf @0.3% + BC @15g + NC @15g + NLE @10% (35.86 q ha^{-1}), T_1 - FYM @100g + Pf @0.3% + VC @15g + NLE @10% (33.86 q ha^{-1}), T_5 FYM @100g + Pf @0.3% + BC @15g + CP @15g + NLE @10% (32.06 q ha^{-1}), T_2 - FYM @100g + Pf @0.3% + BC @15g + NLE @10% (31.99 q ha^{-1}), T_4 - FYM @100g + Pf @0.3% + NC @15g + NLE @10% (31.84 q ha^{-1}), T_3 - FYM @100g + Pf @0.3% + CP @15g + NLE @10% (30.25 q ha^{-1}), as compared to (untreated checked) T_0 - control (20.60 q ha^{-1}).
Comparing the treatments with the CD value (1.46) all the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) were found significant over (untreated checked) T_0 - control. Among the treatments (T_2, T_4 and T_5) were found non-significant to each other, (T_1, T_3, T_6 and T_7) were found to be significant over all the other treatments.
As per finding from this study, the maximum yield (q ha^{-1}) was observed in T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10%. The probable reasons for this result may be due to the soil nitrogen contents increased with the use of FYM and other organic fertilizer. Nitrogen is the constituent of chlorophyll, more chlorophyll contents result in more photosynthesis thus may have increased yield in strawberry **(Zahid *et al.,* 2022)**. Vermicompost may have attributed to better availability of plant growth regulator and humic acid which is produced by the increased activity of microbes **(Singh *et al.,* 2008)**. *Pseudomonas fluorescens* generates indole acetic acid as a growth regulator which may have helped in strawberry yield **(Badawy, 2023)**. The increment in yield due to the biofertilizers application might be due to the vital role and promoting effect of bacteria present in the applied biofertilizers on morphology and physiological of the root system, thus favouring the growth parameter as well as capable of contributing some hormones like gibberellin, auxin and cytokinin. Similar findings are consistant with research conducted on *Moringa oleifera* Lam. plants **(Youssef, 2016).**

3.9 Disease intensity (%) at 150 DAT

The data presented in the table 3 and depicted in figure 3 revealed that disease intensity (%) of strawberry significantly minimum in treatment T_7 - FYM @100g + Pf @0.3%+ BC @15g + VC @15g + NLE @10% (30.75%) followed T_6 FYM @100g + Pf @0.3% + BC @15g + NC @15g + NLE @10% (35.50%), T_1 - FYM @100g + Pf @0.3% + VC @15g + NLE @10% (35.75%), T_5 – FYM @100g + Pf @0.3% + BC @15g + CP @15g + NLE @10% (39.25%), T_2 - FYM @100g + Pf @0.3% + BC @15g + NLE @10% (40.50%), T_4 - FYM @100g + Pf @0.3% + NC @15g + NLE @10% (42.75%), T_3 - FYM @100g + Pf @0.3% + CP @15g + NLE @10% (45.25%), as compared to (untreated checked) T_0 - control (50.75%).
Comparing the treatments with the CD value (2.23) all the treatments (T_1, T_2, T_3, T_4, T_5, T_6 and T_7) were found significant over (untreated checked) T_0 - control. Among the treatments (T_1 and T_6); (T_2 and T_5) were found non-significant to each other, (T_3, T_4 and T_6) were found to be significant over all the other treatments.
As per finding from this study, the least disease intensity (%) was observed in T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10%. The probable reasons for this result may be due to PGPR can create several kinds of antibiotics, which may have related to the bacterial capacity to suppress plant pathogen development. A variety of PGPRs can produce enzymes like proteases, chitinases, glucanases, and lipases, which can lyse a section of the cell walls of numerous dangerous fungi. The activities of *Pseudomonas fluoresces* and *Azotobacter chroococcum* may have the abilities to produce several antibiotics such as catalase, siderophores oomycine A and pyrrolnitrin. Similar findings have been reported by **Badawy (2023)** and **Juber *et al.* (2016)**. Compounds from neem leaf such as isoprenoids (azadirone, gedunin, vilasinin, azadirachtin) and non-isoprenoids (sulphurous compounds, poly phenolics like flavonoids, dihydrochalcone, coumarin, tannins) were reported to be fungistatic in nature. Different parts of neem have demonstrated great biological activities against microbes through the inhibition of growth and breakdown of their cell walls. Similar, findings have been reported by **Bhattarai *et al.* (2022)** and **Adusei and Azupio (2022)**. So, neem leaf extract significantly inhibits the pathogen which may have leads to minimum disease intensity per cent.

3.10 Benefit cost ratio

The treatment wise economics of strawberry production were estimated and the results have been presented in table 3 and figure 4 the economics analysis of the data over the session that (treated check) T_7 - FYM @100g + Pf @0.3% + BC @15g + VC @15g + NLE @10% was recorded highest gross returns Rs. 971750, net returns Rs.539200 with C:B ratio 1:2.24 followed with T_6 – FYM @100g + Pf @0.3% + BC @15g + NC @15g + NLE @10% recorded gross returns Rs 896500, net returns Rs. 478950 with B:C ratio 1:2.14 as compared to (untreated checked) T_0 - Control gross returns Rs. 515000, net returns Rs. 199200 with B:C ratio 1:1.63.

4. CONCLUSION

The findings of this study identified the causal organism responsible for black root rot of strawberry (*Fragaria x ananassa* duch.) was *Rhizoctonia solani*. Among the treatments, T_7 - FYM at 100g + *Pseudomonas fluorescens* (0.3%) + biomix compost at 15g + vermicompost at 15g + neem leaf extract at 10 per cent per pot recorded the highest plant height (cm), total leaf number, berry length (cm), berry diameter (cm), total soluble solid (°brix), ascorbic acid (g/100mg), yield (q ha^{-1}), benefit cost ratio and recorded the lowest days taken to first flowering (days) as well as per cent disease intensity (%) of strawberry. It is worth mentioning that the conclusions drawn from this study are based on observations made during a specific cropping season spanning december 2023 to may 2024, within the agro climatic conditions of Prayagraj, U.P. As such, further research and more experimentation over many seasons should be conducted in future for further recommendations.

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