



Effect of Organic Manures and Biocapsules on Growth, Yield and Quality of Strawberry (*Fragaria x annanasa* cv. Winter Dawn)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

The experiment was carried out at Research Field of Horticulture Research Farm, Naini Agricultural Institute, Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj (U.P.) India, during October 2022-2023 to investigate the effect of organic manures and bio capsules on growth, yield and quality of strawberry (*Fragaria annanasa–Winter dawn*) thirteen treatments with their combinations, replicated thrice in a Randomized Block Design. In strawberry use the application of bio-capsules and organic manures hasten early flowering along with the expanded duration of blossoming, harvesting by increasing the growth, yield and quality of strawberry. The

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integrated nutrient management maximized plant height (cm), number of leaves per plant, plant spread (cm), number of flowers per plant, number of fruit per plant, fruit weight (g), fruit length (cm), fruit diameter (cm), fruit yield/ha, total soluble solids, ascorbic acid (mg/100g). The result obtained in the present investigation, thus, indicates the possibility of successful use of application of organic sources of like FYM, Vermicompost, poultry manures, goat manures along with bio capsules and it might prove to be a better option for enhancing growth, yield and quality of strawberry. It is concluded that the treatment T11 (Poultry manure (20 t/ha.) + Bio capsule (150% of optimum) was found best in terms of vegetative growth and yield characters of strawberry. The economics of the treatment T11 was found best in terms of B:C ratio 3.50 of strawberry. So, application of this organic manures and bio capsules combination can be recommended to growers after few more conjunctive trials.

Keywords: Bio capsules; organic manure's; vermicompost; strawberry.

1. INTRODUCTION

"The commercially important strawberries (Fragaria x ananassa Duch.) belongs to the family Rosaceae and the genus Fragaria, which comprises of 23 species" Rousseau-Gueutin et al. [1]. "It is one of the most delicious, delicate flavoured, refreshing and attractive red fruit of the world" [2]. "With its high nutritional value, the strawberry is one of the most popular among berry fruits in the world. All of these strawberries have seven basic types of chromosomes. However, they exhibit a series of ploidy levels, ranging from diploid species such as Fragaria vesca (2n = 2x = 14), to decaploid species, such as some accessions of Fragaria iturupensis (2n = 10x = 70). The cultivated strawberry, Fragaria x ananassa is an octoploid (2n = 8x = 56)" Nathewet et al. [3]. "Strawberry (Fragaria x ananassa Duch.), the fleshy fruit of strawberry is classified as an aggregate fruit. Strawberries mainly consist of water (91%), carbohydrates (7.7%) and only minor amounts of fat (0.7%). The taste of fruit mainly depends on three different compounds viz., sugars, acids and aromatic compounds. The strawberry fruit contains 0.5 per cent total sugar and 0.90 per cent to 1.85 per cent acidity and also prominently mallic and citric acids" Watt and Merril, (1959). "As compared to other berry fruits, strawberries contain a higher percentage of vitamin C, phenolics and flavonoids" Hakkinen and Torronen [4]. "They are also rich in phenolic compounds, including anthocyanins, hydrolyzable tannins and phenolic acids" Giampieri et al. [5]. "The current global scenario of firmly emphasizes the need to adopt ecofriendly agricultural practices for sustainable food production. The use of organic manures in such situation is, therefore practically a paying proposal. Organic farming is a holistic way of farming with the aim of conserving the natural

resources. Organic manures like Goat manure, Poultry manure, Vermicompost and FYM etc. have been utilized in agriculture as a significant source of organic manure. Organic manures improve the physical properties of soil (water holding capacity, soil aeration, drainage and water retention capacity) and increase important beneficial microorganism population. Organic agriculture has grown during the last decade and continues to grow as consumers become increasingly concerned about the negative impacts of conventional farming practices on human health and the environment" [6].

Strawberry (Fragaria ananassa Duch.) is a widely grown hybrid species of the genus Fragaria belonging to the family Rosaceae. Strawberry occupies a unique place amongest cultivated berry fruit. It is one of the most popular soft fruits and is cultivated in plains as well as in the hills up to an elevation of 3000m MSL. Among the fruits, strawberry gives the guickest returns in the shortest possible time. The strawberry is a low-growing, herbaceous plant with prostrate growth habit, which behaves as an annual in sub-tropical region and perennial in temperate region having fibrous root system and a crown from which arise basal leaves. The leaves are compound with three leaflets, saw tooth-edged and hairy. The flowers- generally white, rarely reddish, are borne in small clusters on slender stalks arising from the axils of the leaves. Leaves and flowers are spring from almost the ground both with the similar length. Flowering stems with no leaves holding flower have five petals, five sepals and numerous stamens. At the end of each hairy leaf stalk, the leaves divided in three toothed oval leaflets. As a plant ages, the root system becomes woody, and the "mother" crown sends out runners that touch the ground and root, thus enlarging the plant.

The strawberry (Fragaria x ananassa Duch.), an aggregate fruit, has attained the status of being one of the most important soft fruits of the world. The Romans were believed to cultivate strawberries before the Christian era. However, the cultivated strawberry (Fragaria x ananassa) is an octaploid hybrid of two native American species; F. chiloensis and F.virginiana was first developed in France in the 17th century. The wild species F. vesca was found throughout the hemisphere northern lona before the development of cultivated strawberry. Most of the countries in Europe had been growing strawberry commercially since the 18th century, and today nearly 50% of the world's strawberry production is in Europe. Fragaria species has a basic chromosomal number x = 7. The name strawberry has been derived from the practice of straw as mulch for effective cultivation of crop since many years ago.

Bio-capsules could be used to deliver additional nutrients, improve major elements deficiency in strawberries Beniwal M. et al. [7]. "Biofertilizers are considered as economically attractive and ecologically sound route for reducing external inputs augmenting nutrient supply and to improve the quality and quantity of internal resources with maximum outputs. These biofertilizers act as carriers containing beneficial micro-organisms in a viable state, intended for seed or soil application, designed to improve soil fertility status and also helps in plant growth by increasing the number and biological activity of desired microorganisms in root environment" Gabr et al. [8]. In the study of Singh et al. [9] they concluded that the treatment combination with azotobacter azospirillum and plant growth regulators shows maximum growth and yield in strawberry.

Manures are organic substances made from leftover animal, human, and plant faces that have complex organic structures containing plant nutrients. Farmyard manure (FYM) refers to the decomposed mixture of dung and urine of farm animals along with litter and left over material from roughages or fodder fed to the cattle. On an average well decomposed farmyard manure contains 0.5 per cent Nitrogen (N), 0.2 per cent Phosphate (P2O5) and .0.5 per cent Potassium (K2O).

"Vermicompost is a product of biodegradation and stabilization of organic materials by interaction between earthworms and microorganisms. It is a finely-divided, peat-like material, with high porosity, aeration, drainage, water holding capacity and microbial activity, which make it an excellent soil conditioner" [10,11]. "Addition of different vermicomposts, produced from different sources, like cattle manure, pig manure, food waste etc., increases the rate of germination and growth, and yield of many high value crops" Atiyeh et al. [11].

Trichoderma have been known since at least the 1920s for their ability to act as biocontrol agents against plant pathogens. Until recently, the principal mechanisms for control have been assumed to be those primarily acting upon the included pathogens and mycoparasitism, antibiosis, and competition for resources and space. These fungi colonize the root epidermis and outer cortical layers and release bioactive molecules that cause walling off of the Trichoderma thallus. At the same time, the transcriptome and the proteome of plants are substantially altered. Consequently, in addition to induction of pathways for resistance in plants, increased plant growth and nutrient uptake occur. The world first bio capsule technology, where one capsule is equal to 1kg /litre of Trichoderma. In sustainable crop production, beneficial microorganisms are applied to control diseases and for growth promotion. Trichoderma harzianum a biocontrol agent encapsulated in trichocap promotes growth, triggers defense, and imparts direct action on the pathogen.

2. MATERIALS AND METHODS

2.1 Data Analysis

The data was analyzed using STAR.

The details of the various materials used and methods adopted in carrying out the experiment are presented below:

2.2 Geographical Location of the Experimental Site

The experimental site is located at a latitude of 25.41° North and longitude of 81.84 ° East, with an altitude of 98 meters above the mean sea level (MSL).

2.3 Climatic Conditions of the Experimental Area

The area of Prayagraj comes under humid subtropical climate, which experiences warm humid monsoon, hot dry summer and cold dry winter. The annual mean temperature is 26.1°C while monthly mean temperatures are 18-29°C. The daily average maximum temperature is about 22°C and the minimum temperature is 9°C. The average annual rainfall received is 1042.2 mm. At this location, the temperature reaches up to 46°C-48°C and the minimum temperature recorded is 4°C-5°C. The relative humidity ranges in this location ranges between 20-94%.

2.4 Layout and Treatment Combinations

Studies on Effect of organic manures and biocapsules on growth, yield and quality of strawberry carried out on cv. Winter dawn during 2023- 24 at Horticulture Research Farm of Naini Agricultural Institute, SHUATS, Prayagraj, (Uttar Pradesh). The experiment was laid out in RBD viz. Treatments at 13 levels viz. T0: control Control (N:P:K – 100:60:40 kg/ha). T1: Vermicompost (10 t/ha) + Bio capsule (optimum), T2: FYM (20 t/ha.) + Bio capsule (optimum), T3: Poultry manure (20 t/ha.) + Bio capsule (optimum), T4: Goat manure (20 t/ha) + Bio capsule (optimum), T5: Vermicompost (10 t/ha) + Bio capsule (50% of optimum), T6: FYM (20 t/ha.) + Bio capsule (50% of optimum), T7: Poultry manure (20 t/ha.) + Bio capsule (50% of optimum), T8: Goat manure (20 t/ha) + Bio capsule (50% of optimum), T9: Vermicompost (10 t/ha) + Bio capsule (150% of optimum), T10: FYM (20 t/ha.) + Bio capsule (150% of optimum), T11: Poultry manure (20 t/ha.) + Bio capsule (150% of optimum), T12 Goat manure (20 t/ha) + Bio capsule (150% of optimum).

2.5 Running Status

1. Growth Parameter

- Plant Height (cm)
- Plant Spread (cm)

2. Leaf Parameter

• Number of leaves per plant

3. Flower Parameter

- Days taken to 1st flowering
- No. of flowers per plant
- Days to maturity

4. Fruit Parameter

- Days taken to 1st fruiting
- Number of fruits per plant
- Fruit weight (g)
- Fruit Length (cm)

Fruit diameter (cm)

5. Yield Parameter

- Yield per plant (g)
- Yield per plot (kg)
- Yield per hectare (q)

6. Quality Parameter

- Total Soluble Solids (Brix)
- Ascorbic acid content (mg/100 g of fresh fruit)
- Acidity

7. Economics

• Cost of cultivation (Rs)

3. RESULTS AND DISCUSSION

3.1 Effect of Organic Manures and Bio Capsules on Plant Height (cm) and Number of Leaves of Strawberry cv. Winter Dawn

Strawberry plants in treatment T₁₁ recorded the maximum plant height (14.43 cm) and number of leaves per plant (19.04). On the other hand, the minimum plant height (11.00 cm) and number of leaves per plant (13.00) were observed in plants grown with treatment control. The increased plant height of strawberry may be due to the increased nitrogen fixation, organic nitrogen utilization, development of root system Beer et al. [12]. Similar results were also reported by Tripathi et al. [13] in strawberry. Higher number of leaves & leaf area might be due to higher cell division caused by cytokinins & also due to higher supply of assimilate mediated by biocapsules application. Increased number of leaves might have increased the photosynthetic activity resulting in higher accumulation of carbohydrates. The findings are in close agreement with the findings of Dwivedi et al. (1999) in strawberry.

3.2 Plant Spread (cm) and Flower Parameter

The maximum spread of plant (24.02 cm) was recorded in plants in the treatment T_{11} - Poultry manure (20 t/ha.) + Bio capsule (150% of optimum), whereas, minimum plant spread (19.63 cm) were observed in treatment T0 control. Beer et al. [12], reported increase in plant spread might be due to increased growth of plant in the form of height, which accumulated more photosynthesis & thereby increases leaf area per plant. These findings are corroborating with the finding of Umar et al. [14].

Treatment		Plant height (cm)	Numbe	Number of leaves per plant			
	60 Days	90 Days	120 Days	60 Days	90 Days	120 Days		
T0	7.12	9.56	11	5.93	10.4	13		
T1	8.13	10.53	12.66	6.66	11.2	15.2		
T2	8	10.53	12.6	6.6	10.66	14.38		
Т3	8.75	11.3	13.4	7.86	12.6	16.64		
T4	8.6	10.96	13	7.6	12.1	16.55		
T5	8.31	10.66	12.76	7.06	11.6	15.96		
T6	8.26	10.54	12.66	6.86	11.4	15.6		
T7	9.41	11.51	13.86	8.02	12.66	17.73		
T8	9.23	11.5	13.76	7.91	12.6	16.66		
Т9	8.48	10.93	12.86	7.33	11.8	16.53		
T10	8.36	10.8	12.76	7.17	11.73	16.33		
T11	10.2	12.26	14.43	9.26	13.4	19.04		
T12	9.5	12.1	13.9	8.13	12.86	18.26		
F-test	S	S	S	S	S	S		
SE(m) <u>+</u>	0.6	0.75	0.63	0.55	0.57	0.86		
CD 5%	1.37	2.18	1.83	1.62	1.68	2.5		
CV	12.05	11.93	8.4	12.93	8.43	9.1		

 Table 1. Effect of organic manures and bio capsules on plant height (cm) number of leaves of

 Strawberry cv. Winter Dawn

 Table 2. Effect of organic manures and bio capsules on plant spread (cm) and Flower parameter of Strawberry fruits cv. Winter Dawn

Treatment	Plant spread (cm)			No. of	No. of	No. of days	Days to
	60	90	120 Days	days to	flowers	Taken to	fruit
	Days	Days	-	first	per plant	first fruit	Maturity
	-	-		flowering		set	-
Т0	13.76	16.5	18.83	111	21	4.15	25.97
T1	14.6	17.7	20	109.46	23.73	4.26	26.97
T2	14.13	16.96	19.1	109.73	23.32	4.23	26.73
Т3	17.2	20	22.09	103.04	25.36	5.6	28.46
Τ4	16.73	19.33	21.76	103.4	25.02	4.73	28.15
T5	16.46	18.62	21.18	106.53	24.06	4.36	27.2
Т6	16.46	18.33	21.02	109.11	23.8	4.3	27.15
T7	17.93	20.76	22.3	102.33	26.06	5.79	30
Т8	17.4	20.26	22.27	102.75	25.4	5.68	28.61
Т9	16.73	19.2	21.51	105.72	24.48	4.56	27.63
T10	16.73	18.83	21.36	105.81	24.36	4.53	27.52
T11	18.6	21.62	24.2	101	27.45	5.9	31.58
T12	18.02	20.86	23.17	101.13	26.26	5.8	31.33
F-test	S	S	S	S	S	S	S
SE(m) <u>+</u>	0.89	1.01	0.09	1.96	0.8	0.17	0.5
CD 5%	2.6	2.94	2.77	3.22	2.32	0.52	1.45
CV	9.26	9.14	7.66	5.73	5.65	6.36	3.46

Minimum days taken first flowering and fruiting was recorded with T_{11} (Poultry manure (20 t/ha.) + Bio capsule (150% of optimum), (101.00). Closely followed by T_{12} (101.13). Maximum days taken for first flowering and fruiting was recorded in T_0 (111.00). These outcomes have got the support of the findings of Kumar et al. [15] who also recorded earliest flowering and fruiting with the application of cent per cent bio-capsules and organic fertilizers, Singh D. et al. [16], who recorded earliest flowering. Tripathi et al. [13] also recorded advancement in flowering. Number of days required minimum to produce first flower might be due to balanced supply of vermicompost & bio-fertilizers, which provides all the necessary elements to plant to become early flowering and fruiting. Maximum number of flowers per plant was recorded with T_{11} (Poultry manure (20 t/ha.) + Bio capsule (150% of optimum)) 27.45. Closely followed by T_{12} (26.26). Minimum number of flowers per plant was recorded in T_0 control (21.00). This might due to the effect of organic manures and bio-capsules obtained from the cell elongation and division. Soluble bio capsules increase photosynthetic activities of leaves, which leading to development of primary flowers, production of viable flowers. Similar results were also obtained by Tripathi et al. [13] and Singh A.K. et al. [17] in strawberry.

Maximum the number of days to taken first fruit set per plant was recorded with T_{11} (Poultry manure (20 t/ha.) + Bio capsule (150% of optimum)) 5.90. Closely followed by T_{12} (5.80). Minimum the number of days to taken first fruit set per plant was recorded in T_0 control (4.15). The data presented in the revealed the number of days to fruit maturity was significantly influenced by different treatments. Maximum the number of days to fruit maturity was recorded with T_{11} (Poultry manure (20 t/ha.) + Bio capsule (150% of optimum)) 31.58. Closely followed by T_{12} (31.33). Minimum the number of days to taken fruit maturity was recorded in T_0 control (25.97).

3.3 Fruit Parameter

The data presented in the revealed the number of fruits per plant was significantly influenced by different treatments. Maximum number of fruits per plant and was recorded with T₁₁ (Poultry manure (20 t/ha.) + Biocapsule (150% of optimum)) with 19.66. Closely followed by T_{12} (17.67). Minimum number of fruits per plant was recorded in T_0 control (12.33). Maximum fruit weight (g) was recorded with T11 (Poultry manure (20 t/ha.) + Bio capsule (150% of optimum)) 38.04 g closely followed by T₁₂ (37.26 g). Minimum fruit weight (g) was recorded in T₀ control (32.00 g). Maximum fruit length (cm) was recorded with T₁₁ (Poultry manure (20 t/ha.) + Bio capsule (150% of optimum)) 3.89 cm closely followed by T₁₂ (3.66 cm). Minimum fruit length (cm) was recorded in T_0 control (2.17 cm). Maximum fruit diameter of fruit was recorded with T_{11} (Poultry manure (20 t/ha.) + Bio capsule (150% of optimum)) 3.84 cm closely followed by T₁₂ (3.58 cm). Minimum fruit diameter of fruit was recorded in T₀ control (2.71 cm). The results are consistent with those of Singh *et al.* (2006) in strawberry, and they are similar to those of Sardoei et al. [18]. Arancon et al. [19] also reported increased fruit volume with the application of organic fertilizer and biocapsules in strawberry.

3.4 Yield Parameter and Quality Parameter

The maximum yield per plant (747.78 g) was recorded in plants in the treatment T₁₁- Poultry manure (20 t/ha.) + Bio capsule (150% of optimum). The minimum number of fruits per plant (394.56 g) was recorded in treatment T0control. The maximum yield per plot (6.00 kg) was recorded in plants in the treatment T₁₁-Poultry manure (20 t/ha.) + Bio capsule (150% of optimum). The minimum number of fruits per plant (2.90 kg) was recorded in treatment T0control. The maximum number of fruits per plant (51.66 q) was recorded in plants in the treatment T₁₁- Poultry manure (20 t/ha.) + Bio capsule (150% of optimum). The minimum number of fruits per plant (30.67 q) was recorded in treatment T0- control. The strawberry plants in treatment T₁₁- Poultry manure (20 t/ha.) + Bio capsule (150% of optimum) had fruits with the maximum TSS (11.40 °B) while the minimum (7.64 °B) was recorded in treatment T0- Control. The maximum titratable acidity (0.52 per cent) was observed in fruits in treatment T₁₁ while the minimum (0.33 per cent) was found in fruits in treatment T0. The maximum vitamin C (58.94 mg100g-1) was recorded in treatment T_{11} , while minimum (47.21 mg100g-1) was recorded in treatment T0. The results are in line with Nowsheen et al. [20] and Umar et al. [14] in the strawberry who described maximum yield in the strawberry. Increment in the number of runners per plant may be because of enhanced growth of vegetative and leaves number that gathered more photo- synthates and hence increased runners and the leaf area per plant. PGR and micronutrients might have an effect on runner production because of its ability to increase the nutrient availability in the soil as well as provide better environment for better and healthy root development [21,22].

3.5 Economics of Strawberry Cultivation

The maximum Cost of cultivation (Rs.), gross return, net return and benefit cost ratio was recorded in the Treatment T_{11} with cost of cultivation Rs.249700, Gross return Rs. 1033200 and Net Return Rs 783500 and Benefit cost ratio Rs 3.14.

Treatment	No. of fruit per	Fruit Weight (g)	Fruit length	Fruit Diameter
	plant		(cm)	(cm)
Т0	12.33	32	2.17	2.71
T1	13.66	34.2	2.84	2.73
T2	12.33	33.38	2.63	2.98
Т3	16.66	35.64	3.5	3.38
Τ4	16.33	35.55	3.47	3.03
T5	14.66	34.96	3.31	3.24
Т6	14.33	34.6	3.14	3.59
T7	17.33	36.73	3.65	3.81
Т8	17	35.66	3.61	3.58
Т9	16	35.53	3.41	3.62
T10	15	35.33	3.32	3.72
T11	19.66	38.04	3.89	3.84
T12	17.66	37.26	3.66	3.58
F-test	S	S	S	S
SE(m) <u>+</u>	1.06	0.38	0.14	0.38
CD 5%	3.08	0.24	0.42	0.19
CV	12.06	3.84	7.58	0.42

Table 3. Fruit Parameter of plants

Table 4. Yield parameter and quality parameter

Treatment	Yield per Plant(g)	Yield per Plot (kg)	Yield per Hectare(q)	Total titrabe acidiy %	TSS (°Brix)	Ascorbic acids (mg/100g of fruit)
Т0	394.56	2.9	30.67	0.33	7.64	47.21
T1	467.17	3.35	37.28	0.36	8.33	48.66
T2	411.58	3.15	35.01	0.34	7.66	47.06
Т3	593.76	4.99	40.51	0.44	10.16	56.2
Τ4	580.53	4.87	39.13	0.43	10.1	58.42
T5	512.51	4.05	30.06	0.38	9.05	50.86
Т6	495.82	3.45	23.39	0.37	9	56.63
T7	636.53	5.31	44.09	0.45	11.4	53.7
Т8	606.22	5.25	43.8	0.45	10.66	51.14
Т9	568.48	4.8	38.33	0.4	10	57.36
T10	529.95	4.17	31.4	0.39	10	57.36
T11	747.87	6	51.66	0.52	11.41	58.94
T12	658.01	5.49	46.06	0.45	12.41	54.53
F-test	S	S	S	S	S	S
SE(m) <u>+</u>	23.19	0.41	4.52	0.03	0.74	3.47
CD 5%	15.08	1.2	13.18	0.09	2.15	10.14
CV	16.35	16.31	16.23	13.64	6.4	8.92

Table 5. Economics of different treatment combi	nation of Cluster bean
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Notation	Fruit yield(q)	Selling rate (Rs. /q)	Gross return (Rs.)	Cost of cultivation	Net return (Rs.)	B/C ratio
Т0	30.67	20000	613400	211700	401700	1.9
T1	37.28	20000	745600	214700	530900	2.47
T2	35.01	20000	700200	206700	493500	2.39
Т3	40.51	20000	810200	213700	596500	2.79
T4	39.13	20000	782600	225200	557400	2.48
T5	30.06	20000	601200	207700	393500	1.89
Т6	23.39	20000	467800	209700	258100	1.23

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Notation	Fruit yield(q)	Selling rate (Rs. /q)	Gross return (Rs.)	Cost of cultivation	Net return (Rs.)	B/C ratio
T7	44.09	20000	881800	219200	662600	3.02
Т8	43.8	20000	876000	221200	654800	2.96
Т9	38.33	20000	766600	225200	541400	2.4
T10	31.4	20000	628000	207700	420300	2.02
T11	51.66	20000	1033200	249700	783500	3.14
T12	46.06	20000	921200	219200	702000	3.2

4. CONCLUSION

The result obtained in the present investigation, thus, indicates the possibility of successful use of application of organic sources of like FYM, Vermicompost, poultry manures, goat manures along with bio capsules and it might prove to be a better option for enhancing growth, yield and quality of strawberry. It is concluded that the treatment T₁₁ (Poultry manure (20 t/ha.) + Bio capsule (150% of optimum) was found best in terms of vegetative growth and yield characters of strawberry. The economics of the treatment T₁₁ was found best in terms of B:C ratio 3.50 of strawberry. So, application of this organic manures and bio capsules combination can be recommended to growers after few more conjunctive trials.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declares that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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