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Influence of Entomopathogenic Fungi *Beauveria bassiana* on Growth and Yield of Tomato (*Solanum lycopersicum* L.)

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ABSTRACT

Two experiments were conducted on the influence of entomopathogenic fungi *Beauveria bassiana* UHSB-END1 (University of Horticultural Sciences Bagalkot – Endophytic1)on growth and yield of tomato (*Solanum lycopersicum L.)* during December 2023 to May 2024 at the vegetable science block, University of Horticultural Sciences, Bagalkot. The experiment I was laid out in a completely randomized design having five treatments (T_1 - Soaking seeds for 2 hrs, T_2 - Soaking seeds for 6 hrs, T_3 - Soaking seeds for 12 hrs, T_4 - Soaking seeds for 24 hrs, T_5 – Control) with four replications. The experiment II was laid out in randomized complete block design having ten treatments (T_1 - Seed treatment, T_2 - Seedling root dip, T_3 - Soil drenching, T_4 - Foliar spray, T_5 - Seed treatment + Seedling root dip + Soil drenching, T_6 - Seed treatment + Seedling root dip + Foliar spray, T_7 - Seed treatment + Soil drenching + Foliar spray, T_8 - Seedling root dip + Soil drenching + Foliar spray, T_9 - Seed treatment + Seedling root dip + Soil drenching + Foliar spray, T_{10} – Control) with three replications. The experiments were conducted to study the impact of *B. bassiana* UHSB-END1 on growth and development of tomato seedlings and fruit yield. The result showed that the seeds soaked with *B. bassiana* for 12 hrs (T_3) showed the best results. They had a 100 per cent germination rate and seedling survivability with desirable seedlings length and seedling dry matter in experiment I. The highest plant height (48.92, 57.30, 63.82 and 67.34 cm) and leaf chlorophyll (52.51, 50.52, 39.82 and 29.04 SPAD) content showed in the treatment T_7- seed treatment + soil drenching + foliar spray and total dry matter (3878.33 kg/ha) at final harvest, leaf area (29.75, 32.87, 35.04 and 35.50 cm^2) in the treatment T_6 - seed treatment + seedling root dip treatment + foliar spray at 30, 60, 90 DAT and at final harvest of tomato respectively. The maximum yield attributes like fruit volume (67.34 cm^3), fruit diameter (69.17 mm) and fruit weight (70.23 g), the highest yield per ha (56.32 t) showed notable results in the treatment T_6- seed treatment + seedling root dip treatment + foliar spray were observed in experiment II.

Keywords: *Beauveria bassiana*, Chlorophyll, Dry matter, Entomopathogenic, Fruit volume, Fruit diameter, Fruit weight, Leaf area, Survivability, Seed germination, Seedling length, Tomato, Yield.

INTRODUCTION

Beauveria bassiana, a fungus that generates an array of toxic secondary metabolites, including beauvericin, bassianin, bassianolide, beauverolides, tenellin, oosporein, and oxalic acid. These compounds play a key role in enabling the fungus to infect and ultimately eliminate its hosts (Wang *et al*, 2021). It also plays an important role in other aspects like colonizers of internal plant tissues (Wakil *et al*, 2020), as colonizers of the rhizosphere (Nelly *et al*, 2019), as promoters of plant growth and plant fitness (Jaber and Enkerli, 2017), act as enhancers of tolerance to environmental challenges, like drought tolerance and promoting overall growth as biofertilizers (Vega *et al*, 2009).

Many plants maintain symbiotic relationships with endophytic fungi (Card *et al*, 2016), which inhabit plant tissues without causing any apparent harm

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Experiment I. Effect of *Beauveria bassiana* UHSB-END1 seed treatment on the growth characteristics of tomato seedlings in different hours.

Table 1. Treatment details of experiment 1

T_1	Soaking seeds for 2 hrs duration with 5 g/L *Beauveria bassiana*
T_2	Soaking seeds for 6 hrs duration with 5 g/L *Beauveria bassiana*
T_3	Soaking seeds for 12 hrs duration with 5 g/L *Beauveria bassiana*
T_4	Soaking seeds for 24 hrs duration with 5 g/L *Beauveria bassiana*
T_5	Control (soaking seeds only with water)

Experiment – II Effect of *Beauveria bassiana* UHSB-END 1 on growth and yield of tomato.

Table 2. Treatment details of experiment 2

T_1	Tomato seeds treated with 5 g/L *Beauveria bassiana* for 12 hrs duration
T_2	Seedling root dip treatment with 5 g/L *B. bassiana* for 15 min. (While transplanting)
T_3	Soil drenching with 10 g/L *B. bassiana* 15 days after transplanting of tomato seedlings
T_4	Foliar spray with 4 g/L *B. bassiana* 30, 45, 60 days after transplanting of tomato seedlings.
T_5	Seed treatment + Seedling root dip treatment + Soil drenching ($T_1 + T_2 + T_3$)
T_6	Seed treatment + Seedling root dip treatment + Foliar spray ($T_1 + T_2 + T_4$)
T_7	Seed treatment + Soil drenching + Foliar spray ($T_1 + T_3 + T_4$)
T_8	Seedling root dip treatment + Soil drenching + Foliar spray ($T_2 + T_3 + T_4$)
T_9	Seed treatment + Seedling root dip treatment + Soil drenching + Foliar spray ($T_1 + T_2 + T_3 + T_4$)
T_{10}	Standard check (Control) – Untreated tomato seedlings.

(Bamisile *et al,* 2018). Once established, these fungi form mutualistic partnerships with their hosts (Sasan and Bidochka, 2012) enhancing plant growth and increasing resilience to both biotic and abiotic stresses (Zimmermann,2007) Endophytes are notable for their phylogenetic diversity and wide range of lifestyles, including variations in colonization patterns, transmission modes, host specificity, and tissue preference within plants (Behie *et al*. 2015).There is growing interest in utilizing entomopathogenic fungi (EPFs) as endophytes due to their unique advantages over traditional applications. One such fungus, *Beauveria bassiana*, has demonstrated the ability to colonize numerous plant species, including wheat, soybean, rice, beans, onion, tomato, palm, grape, potato, and cotton (Vega, 2018). It can establish local or systemic presence within roots, stems, leaves, and internal plant tissues. Inoculation methods such as seed treatment, foliar spraying, and soil irrigation have been shown to successfully introduce endophytic fungi into plants (Akello and Sikora, 2012). For instance, applying *B. bassiana* and *Metarhizium anisopliae* to seeds led to effective colonization and significant plant growth improvements as indicated by increased stem height, root length, and biomass (Jaber, 2018 ; Shao-Fang *et al*. 2017). In particular, soil and foliar applications of *B. bassiana* have been widely used to enhance corn seedling development (Yuhong *et al*. 2017). This study aims to assess the effects of *B. bassiana* UHSB-END1 on the growth and yield of tomato (*Solanum lycopersicum*), focusing on its potential as a growth-promoting endophytic fungus.

MATERIALS AND METHODS

The experiment - I was conducted using a completely randomized design with five treatments and four replications (Table 1). The crop used in the study was tomato (*Solanum lycopersicum L.*), specifically the hybrid variety Arka Rakshak from Indian Institute of Horticultural Research, Hesaragatta, Bangalore. The experiment - II was conducted using Randomized Complete Block Design with ten treatments and three replications (Table 2). The best tomato seedlings of experiment - 1 were used for experiment - II. The experiment - II was executed under irrigated conditions using a drip system, with planting gaps of 90 cm × 45 cm. Each plot contained 44 plants, with a total of 30 plots. The plot proportion was 10.16 m by 2 m, resulting in a net plot size of 610 m^2. The experiment took place during the spring-summer season. Seeds were sown in protrays on December 12, 2023, and transplanted on January 13, 2024. The final harvest occurred on May 7, 2024. The recommended dose of fertilizers (RDF) for tomatoes was 120kg N, 80kg P_2O_5 and 50 kg K_2O per hectare. The experiment took place at the vegetable science block, College of Horticulture in Bagalkot, during the 2023-24 period. This location falls within the Northern Dry Zone of

Karnataka (Agroclimatic Zone-3), situated at a latitude of 16º 10' and a longitude of 74º 42' with an elevation of 542 meters above sea level. The experimental site featured a nearly flat topography with sufficient surface drainage. The data in respect of all the above parameters were tabulated and subjected to the statistical analysis by adopting Completely Randomized Design at critical difference values with 1% level of significance of the corresponding degree of freedom for experiment I and the Randomized Complete Block Design were analyzed at a 5% probability level using Fischer's method of variance analysis for experiment II. Whenever the 'F test' indicated significance, the critical difference at the same level was determined (Panse and Sukhatme, 1954).

Germination percentage (%) : Tomato seeds began to sprout within 3–4 days of sowing. The germination percentage was verified by dividing the number of seeds that successfully germinated by the total number of seeds sown, then multiplying the result by 100 to express it as a percentage

Radicle length (cm): Radicle is a plant tissue which grows from the micropyle of the seed and develops further down into the soil. The radicle length observation was carried in 3 to 4 days old tomato seedlings.

Plumule length (cm): Plumule is the part of seed embryo that grows into a shoot by the process of seed germination. Plumule length observation was carried in 3 to 4 days old tomato seedlings.

Seedling height (cm) : Five randomly chosen and tagged plants were measured for seedling height at 30 days post sowing using a scale from the ground to the tip of the main stem. The average height of five plants was computed and expressed in centimeters.

Plant height (cm): Five randomly chosen and tagged plants were measured at 30, 60, 90 and at the final harvest, after transplantation. It was measured by using a scale/tape from the ground to the tip of the main stem. The average height of five plants was computed and illustrated in centimeters.

Leaf area (cm^2) : Five randomly chosen and tagged plants were evaluated at 30, 60, 90 and at the final harvest, after transplantation. It was measured by multiplying correction factors with length into breadth of base, bottom and top of the leaves of plant. The average of five plants was computed and expressed in centimeter square (cm^2).

Leaf chlorophyll content (SPAD): Five randomly chosen and tagged plants were quantified at 30, 60, 90 and at the final harvest, after transplantation. It was measured by using SPAD meter from the base, bottom and top of the leaves of plant.

Seedling Survivability (%): The seedling survivability rate was determined by summing the number of healthy living seedlings, dividing that total by the overall number of seedlings planted and then multiplying the result by 100 to express it as a percentage.

Seedling dry matter (g): Seedling dry matter was calculated by subtraction of freshly weighed seedling by the oven dried weighed seedling. It was achieved by 30 days old seedlings.

Volume of fruit (cm^3): Five fruits from each treatment were collected and examined in the laboratory, where volume was assessed by water displacement method and expressed in cubic centimeters (cm^3). The average volume of these five fruits was calculated and used for statistical analysis.

Fruit diameter (mm) : Five fruits per treatment were collected and fruit diameter was recorded by using vernier caliper, then average value of five fruits was taken for statistical analysis.

Fruit weight (g) : The mean weight of tomato fruit was derived by averaging the weight of 5 randomly selected fruits from the tagged plants and weighed individually using digital scale and was expressed in grams.

Fruit yield (kg/plant): Fruit yield of five erratically tagged plants was estimated by the summation of weight of each picking per plant and the average yield per plant was calculated and stated in kg per plant.

Fruit yield (kg/plot) : It was computed by multiplying the total yield per plant with the total number of plants per plot and it was expressed in kg per plot.

Fruit yield (t/ha): Total yield from each replication was noted and multiplied on the basis of per ha population of plants and expressed in t ha^{-1}.

Dry matter yield (kg/ha) : Five plants in each net plot were arbitrarily uprooted at the final phase of experiment, washed with tap water and wiped-out extra moisture by sponging with dry cotton wool and immediately their fresh weight. To determine the dry weight of different parts of plant roots, stem, leaves and fruits were separated and they were chopped separately into small pieces to enable drying and were dried at 65-

70 °C until stable mass was obtained and multiplied on the basis of per ha population of plants and expressed in kg ha^{-1}.

Cost of cultivation (Rs. /ha): Cost incurred from land preparation to harvest of crops including cost of all the inputs, cost of all the operations carried out, interest on the working capital, supervision charge and pre-farm to marketing charge.

Gross returns (Rs. /ha): Gross return per ha was calculated by considering the economic yield obtained per ha and price of the product prevailing in the market at the time of harvest.

Net returns (Rs. /ha): Net returns (Rs. /ha) = Gross returns (Rs. / ha) – Cost of cultivation (Rs. / ha)

Benefit: cost ratio : Benefit: Cost ratio for different treatments were worked out based on the expenditure and income to study the economics of tomato production by using different methods of application of *Beauveria bassiana*.

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

RESULTS AND DISCUSSION

Growth parameters

The most coveted observations noticed in the treatment involving seeds soaked for 12 hrs (T_3) yielded the best results across various parameters (Table 3). Specifically, this treatment achieved a 100 per cent germination rate, with the radicle length reaching 3.43 cm and the plumule length extending to 4.43 cm. The overall seedling height was 23.38 cm with 100 per cent seedling survivability. Additionally, the seedlings had a dry weight of 0.37 g.

These outcomes are attributed to the treatment with *Beauveria bassiana*. This fungus helps in breaking the seed dormancy and enhances germination rates. When seeds are soaked, they absorb water, which is crucial for initiating the germination process. The presence of *B. bassiana* further promotes healthier and more vigorous seedlings, leading to better overall growth and development. However, the less than 12 hrs-soaked seeds were failed due to insufficient colonization of *B. bassiana* and more than 12 hrs-soaked seeds were failed due to over succulents of seeds (Akello and Sikora, 2012).

Plant height

The data concerning to plant height at 30, 60, 90 days after transplanting (DAT) and at final harvest showed significant differences among the treatments owing to different methods of application with *B. bassiana* UHSB - END1. Compared to other treatments the highest plant height was observed in the treatment of T_7- seed treatment + soil drenching + foliar spray (48.92, 57.30, 63.82 and 67.34 cm) at 30, 60, 90 days after transplanting (DAT) and at final harvest, respectively (Table 4). This could be a result of higher seedling vigour and colonization rate showed by *B. bassiana* which significantly boosted the plant height (Dara *et al,* 2017; Afandhi *et al*, 2019).

Leaf area

The data concerning to leaf area at 30, 60, 90 days after transplanting (DAT) and at final harvest revealed notable variations among the treatments, inspired by different methods of application with *B. bassiana* UHSB - END1. Compared to other treatments the maximum leaf area was noticed in the treatment of T_6 - seed treatment + seedling root dip treatment + foliar spray (29.75, 32.87, 35.04 and 35.50 cm^2) at 30, 60, 90 days DAT and at final harvest respectively (Table 5). This may be result of combination of different application methods likely facilitates the easy colonization of *B. bassiana* on leaf tissue of plant, shows positive impact on the leaf area. These observations align with the outcomes of various studies (Krell *et al,* 2018; Pal and Ghosh, 2018).

Chlorophyll content

The leaf chlorophyll content at final harvest ranged between 21.11 SPAD to 29.04 SPAD (Table 6). The significantly higher leaf chlorophyll content was recorded in treatment T_7 - seed treatment + soil drenching + foliar spray (29.04 SPAD), followed by T_6 - seed treatment + seedling root dip treatment + foliar spray (27.83 SPAD). The minimal leaf chlorophyll content was recorded in the treatment T_{10} (21.11 SPAD) of standard check (control) This shows that, bioactive compounds produced by the B. bassiana boost the photosynthetic activity which enhances the chlorophyll content in plants (Jaber, 2018 ; Shao-Fang *et al*. 2017).

Total dry matter

The data on total dry matter (kg/ha) of tomato crop after the final harvest showed significant differences among the treatments, persuaded by different methods of application with *B. bassiana* UHSB - END1 (Table 6). Compared to other treatments the maximum total dry matter was obtained in the treatment T_6 - seed treatment + seedling root dip

Table 3. Growth characteristics of tomato seedlings as influenced by duration of soaking seed treatment with *Beauveria bassiana* UHSB - END1 (experiment -1)

Treatment No.	Germination (%)	Radicle length (cm)	Plumule length (cm)	Seedling height (cm)	Seedling survivability (%)	Seedling dry matter (g)
T_1	92.31	2.23	2.88	15.15	92.31	0.30
T_2	92.31	2.85	3.60	20.30	92.31	0.31
T_3	100.00	3.43	4.43	23.38	100.00	0.37
T_4	84.62	3.00	3.65	20.98	84.62	0.27
T_5	92.31	2.00	3.98	15.73	92.31	0.16
S. Em ±	1.20	0.33	0.33	0.70	1.31	0.07
C.D. at 1%	5.02	1.37	1.38	2.91	5.46	0.30

Table 4. Plant height (cm) at various growth stages of tomato as influenced by different methods of *Beauveria bassiana* UHSB – END1 application

Treatment No.	Treatment	30 DAT	60 DAT	90 DAT	At final harvest
T_1	Seed treatment	40.21	46.40	58.91	63.21
T_2	Seedling root dip treatment	39.33	43.72	55.43	58.83
T_3	Soil drenching	37.70	40.51	48.92	60.40
T_4	Foliar Spray	39.91	50.11	57.30	59.94
T_5	$T_1+T_2+T_3$	40.84	49.32	54.52	64.23
T_6	$T_1+T_2+T_4$	42.31	53.74	57.41	62.72
T_7	$T_1+T_3+T_4$	48.92	57.30	63.82	67.34
T_8	$T_2+T_3+T_4$	43.73	52.23	60.70	64.11
T_9	$T_1+T_2+T_3+T_4$	45.13	55.51	62.10	65.80
T_{10}	Standard check	35.12	40.23	47.81	52.33
S. Em ±		0.64	0.82	0.63	0.81
C.D. at 5%		1.91	2.45	1.88	2.42

DAT – Days after transplanting

treatment + foliar spray (3878.33 kg/ha). This is because of the faster growth rate and photosynthetic assimilation rate brought by these fungi improved availability and translocation of nutrients, which encouraged greater partitioning of photosynthates for dry matter yield (Jirakkakul *et al* 2015).

Yield attributes and yield

The yield attributes such as fruit volume, fruit diameter and fruit weight found to be the highest in treatment T_6 - seed treatment + seedling root dip treatment + foliar spray (67.34 cm^3, 69.17 mm and 70.23 g), respectively (Table 7). The increase in yield attributing characters leads to increase the yield of crop. Thereby, T_6 recorded the highest yield per plant, per plot and per hectare (2.56 kg, 112.64 kg and 56.32 t) respectively. This can be attributed to the enhanced translocation of photo assimilates to the fruits when various methods of *B. bassiana* were applied combinedly induces the yield (El-Gepaly, 2021; Sánchez-Rodríguez *et al,* 2018).

Economics

The maximum gross return (Rs. 5,63,200/ha), net return (Rs. 3,90,389/ha) with B: C (3.26) ratio was found in the treatment T_6 - seed treatment + seedling root dip treatment + foliar spray compared to other treatments. This might be due to effective utilization of nutrients through different methods of *B. bassiana* application yield attributes were increased. So, economics of tomato in the treatment T_6 was amplified, followed by different studies who have shown that *B.*

Table 5. Leaf area (cm^2) at various growth stages of tomato as inspired by different methods of *Beauveria bassiana* UHSB – END 1 application

Treatment No.	Treatment	30 DAT	60 DAT	90 DAT	At final harvest
T_1	Seed treatment	22.51	29.02	29.99	33.47
T_2	Seedling root dip treatment	19.77	21.53	25.26	32.78
T_3	Soil drenching	21.30	27.44	29.13	32.38
T_4	Foliar Spray	23.55	29.61	29.94	31.47
T_5	$T_1+T_2+T_3$	19.23	20.66	27.86	28.37
T_6	$T_1+T_2+T_4$	29.75	32.87	35.04	35.50
T_7	$T_1+T_3+T_4$	25.63	24.94	28.88	30.96
T_8	$T_2+T_3+T_4$	20.77	25.62	26.14	28.77
T_9	$T_1+T_2+T_3+T_4$	23.27	24.94	25.51	28.83
T_{10}	Standard check	17.97	19.79	24.46	25.66
S. Em ±		1.31	2.24	2.60	2.20
C.D. at 5%		3.90	6.66	7.72	6.52

DAT – Days after transplanting

Table 6. Leaf chlorophyll content (SPAD) of tomato at various growth stages and total dry matter at final harvest as inspired by different methods of *Beauveria bassiana* UHSB – END 1 application

Treatment No.	Treatment	30 DAT	60 DAT	90 DAT	At final harvest	TDM at final harvest (kg/ha)
T_1	Seed treatment	40.41	34.73	34.13	25.42	3580.23
T_2	Seedling root dip treatment	45.31	42.31	32.20	26.21	3698.01
T_3	Soil drenching	39.20	38.42	31.32	24.74	3601.22
T_4	Foliar Spray	49.92	42.23	35.81	26.53	3623.31
T_5	$T_1+T_2+T_3$	44.30	38.41	31.40	26.94	3811.19
T_6	$T_1+T_2+T_4$	52.13	42.80	36.93	27.83	3878.33
T_7	$T_1+T_3+T_4$	52.51	50.52	39.82	29.04	3830.24
T_8	$T_2+T_3+T_4$	42.52	37.43	31.04	23.52	3729.13
T_9	$T_1+T_2+T_3+T_4$	45.81	42.71	35.43	22.42	3740.07
T_{10}	Standard check	35.63	32.74	28.12	21.11	3564.11
S. Em ±		0.48	0.61	0.54	0.31	64.12
C.D. at 5%		1.43	1.81	1.61	0.93	190.50

DAT – Days after transplanting
TDM – Total dry matter

Table 7. Yield attributes and yield of tomato as influenced by different methods of *Beauveria bassiana* UHSB – END 1 application

Treatment No.	Treatment	Fruit volume (cm^3)	Fruit diameter (mm)	Fruit weight (g)	Fruit yield (kg/plant)	Fruit yield (kg/plot)	Fruit yield (t/ha)
T_1	Seed treatment	47.16	51.33	56.39	2.09	91.96	45.98
T_2	Seedling root dip treatment	52.83	55.16	57.50	2.01	88.44	44.22
T_3	Soil drenching	51.40	53.03	55.69	1.99	87.56	43.78
T_4	Foliar Spray	52.06	53.49	55.83	2.16	95.04	47.52
T_5	$T_1+T_2+T_3$	58.44	60.63	62.19	2.47	108.68	54.34
T_6	$T_1+T_2+T_4$	67.34	69.17	70.23	2.56	112.64	56.32
T_7	$T_1+T_3+T_4$	61.69	63.33	66.51	2.51	110.44	55.22
T_8	$T_2+T_3+T_4$	57.78	59.25	61.89	2.43	106.92	53.46
T_9	$T_1+T_2+T_3+T_4$	53.88	55.81	61.16	2.38	104.72	52.36
T_{10}	Standard check	44.50	48.56	54.87	1.87	82.28	41.14
S. Em ±		**2.58**	**2.25**	**2.74**	**0.03**	**0.97**	**0.64**
C.D. at 5%		**7.67**	**6.69**	**8.14**	**0.10**	**2.87**	**1.90**

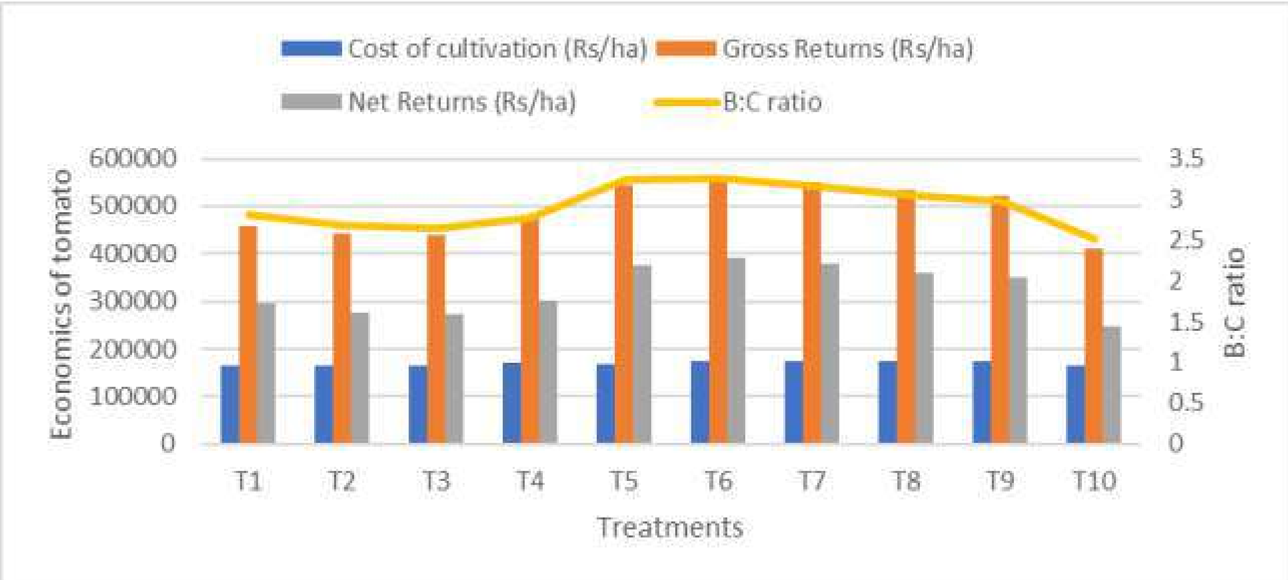

Fig 1. Economics (Rs. / ha) of tomato plants as influenced by different methods of *Beauveria bassiana* UHSB – END1 application

bassiana treated seeds can lead to an increase in plant biomass and fruit production, contributing to a better B:C ratio (Krell *et al*, 2018; Pal and Ghosh, 2018).

CONCLUSION

Based on a comprehensive evaluation of the results, it can be inferred that the application of treatment T_6 - seed treatment + seedling root dip treatment + foliar spray had a notably positive effect on tomato growth and yield, influenced by the entomopathogenic microbe *Beauveria bassiana* UHSB-END1. Beyond its role as an entomopathogen, *B. bassiana* offers significant advantages, which enhances overall crop growth and development. This beneficial effect can serve as an indirect supplement to traditional fertilizers, providing a sustainable alternative to commercial fertilization methods.

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