

Effect of rhizome priming with *Bacillus subtilis* and soil amendments with organic matter and microalgae against *Fusarium equiseti* of Turmeric (*Curcuma longa* L.)

ABSTRACT

Rhizome rot of turmeric caused by *Fusarium equiseti* emerge as a significant soil-borne threat to crop health. An experiment was conducted to evaluate the effect of rhizome priming with *Bacillus subtilis* and soil amendments with compost and microalgae against *Fusarium equiseti* of Turmeric (*Curcuma longa* L.). Experiment was conducted under field condition at the courtyard of department of Plant Pathology, SHUATS, Prayagraj, during *kharif* season of 2022. Various soil treatments including Farm yard manure (FYM), Spent mushroom compost (SMC), and Microalgae were used, along with rhizome treatment utilizing *Bacillus subtilis*, to evaluate their efficacy against *Fusarium equiseti* in Turmeric. The experiment was carried out in Randomized Block design (RBD) with three (3) replications. Results revealed that in the soil application, (T₅) i.e., the combination of all the treatments recorded minimum disease intensity (59.26%) which was significant over other treatments and control (T₀) (33.86%). To evaluate the radial growth (mm) of *Fusarium equiseti* in turmeric, seven botanicals from Manipur viz., *Zanthoxylum acanthopodium*, *Phlogacanthus thyrsoformis*, *Solanum xanthocarpum*, *Centella asiatica*, *Drymaria cordata*, *Persicaria chinensis* and *Gynuracus imbuwa* were investigated *in vitro* at 10% and 30%. The antagonistic effects of various botanicals were evaluated *in vitro*, demonstrating inhibition of mycelial growth. Among the tested botanicals, *Zanthoxylum acanthopodium* inhibited the highest inhibition rate (86.27%), followed by *Phlogacanthus thyrsoformis* (77.45%), *Solanum xanthocarpum* (75.48%), *Persicaria chinensis* (68.62%), *Drymaria cordata* (65.68%), *Gynuracus imbuwa* (62.74%) with *Centella asiatica* displaying the lowest inhibition rate (57.84%), all at a concentration of 30%.

Keywords: Turmeric, Rhizome rot, *Fusarium equiseti*, Botanicals, *In vitro*

1. INTRODUCTION

Turmeric (*Curcuma longa* L.), a member of the Zingiberaceae family, is a perennial plant characterized by its erect growth habit and abundant foliage [16]. With a maximum height of up to 1 meter, it features a compact stem, oblong leaves with pointed tips, and vibrant funnel-shaped yellow flowers. Widely distributed across tropical and subtropical regions, turmeric is predominantly cultivated in Asian nations, notably India and China [12]; [19]. Dry turmeric is comprised of approximately 69.43% carbohydrates, 6.3% proteins, 5.1% oils, and 3.5% minerals, alongside other essential elements. Thus far, about 235 compounds, predominantly phenolics and terpenoids, have been discovered in different turmeric species. [20]

The global turmeric production stands at approximately 11 lakh tonnes annually, with India leading as the largest producer and exporter, accounting for 80% of the world's output. The distribution of global production consists of 78% from various regions, with China contributing 8%, Myanmar 4%, and Nigeria and Bangladesh together contributing 6%. Other significant contributors include China, Myanmar, Nigeria, Bangladesh, Pakistan, Sri Lanka, Taiwan, Burma, Indonesia, Malaysia, Vietnam, Thailand, and Central America. India's turmeric industry alone contributes about 78% to global production and 60% to turmeric exports [7]. Telangana emerges as the primary state for both turmeric cultivation, covering 51 thousand hectares, and production, yielding 294 thousand metric tons, contributing 27.84% to the nation's total output in the 2016-17 period. Other notable turmeric-producing states include Maharashtra (177.85 thousand MT), Tamil Nadu (112.59 thousand MT), and Andhra Pradesh (79.73 thousand MT) [10]. Turmeric holds significant economic value in the North Eastern Hill Region (NEHR), with a cultivation area of 38.6 thousand hectares and a production of 93.16 thousand tons, although lower than the national average [15]. Among NEHR states, Assam leads in turmeric cultivation area (17.63 thousand ha), followed by Mizoram and Sikkim. In terms of production, Mizoram leads with 29.51 thousand tons, followed by Assam and Sikkim [18].

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Turmeric is prone to many fungal, bacterial, viral and nematode diseases. Turmeric is vulnerable to various diseases including leaf spot, anthracnose, and rhizome rot [2]. *Fusarium* species are recognized as significant soil-borne plant pathogens, widely prevalent in diverse sources including air, soil, plants, marine ecosystems, and freshwater. Among these species, *F. equiseti* is responsible for causing wilt diseases in a variety of plant hosts such as grafted watermelon, grape, cucumber, tomato, cowpea, bean, and potato[9];[4].

Presently, the widespread use of chemical compounds without discrimination has had a profound effect on the environment and has created health hazards. Consequently, plant-based pesticides have gained importance as an alternative to synthetic chemicals due to their lack of threat to the natural environment, human health, and animal welfare. Plants contribute to 75% of molecular medicines either directly or indirectly [8];[3].

2. MATERIALS AND METHODS

The experiment was carried out at the courtyard of the Department of Plant Pathology, Sam Higginbottom University of Agriculture, Technology and Sciences, during the kharif season of 2022-2023. Organic amendments such as Farm yard manure (FYM), spent mushroom compost (SMC), microalgae and combination of all three were used during experiment. Experimental plot of size 2m² was prepared. Before transplanting, the rhizomes were first treated with *Bacillus subtilis* @ 0.01g in 1 liter of water. The organic amendments i.e. FYM and SMC were applied to soil and mixed at different dosage before sowing. Microalgae @ 4g/2m² was mixed with water and was applied after germination of plant at rhizosphere area of the plant. Microalgae was applied after 15 days interval for the second and third application.

The appliances and materials used are ingredient of media, turmeric rhizomes, diseased samples, chromic acid, antibiotic, non-absorbent cotton, HgCl₂, alcohol etc., glassware like petri dishes, beakers, funnels, pipettes, conical flasks, culture, test tubes, measuring cylinder and equipment's namely Hot air oven, LPG gas burner, autoclave, B.O.D. incubator, laminar air flow, instruments like cork-borer, inoculation needle, inoculation loop, forceps, spirit lamp, Bunsen burner, enamel tray etc. For *in vitro*, seven botanicals from Manipur viz., *Xanthoxylum acanthopodium*, *Phlogacanthusthysiformis*, *Solanum xanthocarpum*, *Persicaria chinensis*, *Drymaria cordata*, *Gynuracusimbua*, *Centella asiatica* were used.

3. Symptoms

Fig. 1. Symptoms of disease on turmeric plants and rhizome.



In early stage, the central portion of the leaves retains its green color, whereas the edges turn yellow. Mild yellowing occurs at the tips of lower leaves, accompanied by foliage drying, indicative of the crop reaching

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maturity. Upon cutting open infected rhizomes, affected areas usually exhibit a dull brown or dark appearance.

4. Preparation of Botanical extract:

The fresh selected samples were collected and cleansed thoroughly with clean water and air dried. The dried leaves were then blended into powder and sterile distilled water was added to it in equal amount. The soaked medicinal powder was first filtered with muslin cloth, then with whatman filter paper and further centrifuged at 1500rpm for 20 minutes. The clean suspended solution was transferred into 100ml conical flask and sterilized in an autoclave under 15lbs pressure for 20 minutes to create a stock solution. Further each botanical stock solution was used at two concentrations i.e., 10% and 30%, were tested on the radial growth of *F. equiseti* at 24hrs, 48 hrs and 72hrs after inoculation [5];[17].

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5. Isolation of *Fusarium equiseti*:

The collected plant samples (rhizome) were washed with water, sections with symptoms measuring 2mm were cut off, and then surface sterilized using 0.1% mercuric chloride for 5-10 seconds. Subsequently, they were rinsed twice with sterilized distilled water to eliminate any residues of mercuric chloride, dried using sterile filter paper, and transferred onto petri plates containing potato dextrose agar media (four pieces per plate). The plates were then incubated for seven days at a temperature of 25±1°C [6]. The fungus cultures were inoculated onto sterilized petri plates and PDA slants, and maintained in the laboratory at 28±1 °C for a period of 15 days. These mother culture slants were preserved at 4°C in a refrigerator. Furthermore, they were sub-cultured monthly and utilized for future experiments.

6. Identification and morphological characteristics of *Fusarium equiseti*:

Using a sterile needle, a small segment of the culture was extracted and placed onto a sterile glass slide. Subsequent staining with lactophenol and cotton blue facilitated the microscopic examination of fungal structures' morphology and culture traits. Initially, a dense white mycelium was formed, later transitioning to a yellowish to buff brown coloration. After 6 days in culture, macroconidia were observed, presenting 3–7 septa with tapered and elongated apical cells and distinctive foot-shaped cells. Chlamydospores appeared thick, intercalary, and abundant in chains or clusters, exhibiting ellipsoidal or globose shapes. Microconidia were absent. Based on morphological and pathological characteristics, the fungus was identified as *Fusarium equiseti* (Corda) Sacc [1].

Identification of the pathogen species was confirmed through National Fungal Culture Collection of India (NFCCI) located at Agharkar Research Institute, Pune and identified as *Fusarium equiseti*. Further it was molecular characterized by simple sequencing method. >TR-1 *Fusarium equiseti* genes for ITS1

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TCCGTAGGTGAACCTGCGGAGGGATCATTACCGAGTTTACAACCTCCCAAACCCCTGTGAACAT
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AAATCGATTGGCGGTACGTCGAGCTTCCATAGCGTAGTAATCATACCTCGTTACTGGTAAT
CGTCGCGGCCACGCCGTAACCCCAACTTCTGAATGTTGACCTCGGATCAGGTAGGAATACCC
GCTGAACTTAAGCATATCAATAAGCGGAGGA
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Fig. 2. Microscopic view of *Fusarium equiseti*

7. Maintenance of Culture

The fungus cultures were sub-cultured on sterilized petri plates and PDA slants and maintained in the laboratory at a temperature of $28 \pm 1^\circ\text{C}$ for a period of 15 days. These mother culture slants were preserved in a refrigerator at 4°C . Furthermore, they were sub-cultured monthly and utilized for future experiments.



Fig. 3. Pure culture of *Fusarium equiseti* in petriplate and slants

8. Poison Food technique:

The antifungal activity of plant extract is to be tested against the pathogen in the laboratory. The experiment will be carried out in a completely randomized design (RBD) by poisoned food technique. A requisite amount of the filtrate will be mixed in PDA just before pouring to achieve the desired concentrations of 10% and 30% and gently shaken to ensure thorough mixing of the extract. The PDA plates containing the plant extracts were inoculated under sterile conditions with the pathogen by transferring a 5 mm diameter agar disc from the fresh cultures. Three replications should be maintained for each treatment. The basal medium (PDA) without any phytoextract served as the control. All the Petri dishes that were inoculated were subjected to incubation at $25 \pm 1^\circ\text{C}$. The radial growth of the test fungus will be measured in all the treatments after 24hrs, 48hrs and 72hrs and compared with the control.

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The percentage of fungal growth inhibition will be calculated using the formula:[21]

$$I = \frac{C-T}{C} \times 100$$

Where,
I = per cent inhibition
C = Colony diameter in control
T = Colony diameter in treatment

9. RESULT AND DISCUSSION

9.1 Disease Intensity of *Fusarium equiseti* in Turmeric at different DAS

The data provided in Table 1 and Figure 1 revealed minimum disease intensity in T₅- Microalgae @ 1% + FYM @ 1kg + SMC @ 125gm (15.05%, 20.96%, 22.92% respectively) followed by T₃- SMC @ 250gm + Microalgae 2% (18.44%, 23.03%, 26.85% respectively), T₄- FYM @ 2kg + Microalgae @ 2% (19.76%, 26.78%, 29.43% respectively), T₂- Microalgae @ 4% (21.18%, 27.01%, 30.18% respectively), T₁- SMC @ 500gm (23.29%, 29.38%, 32.90% respectively) T₆- FYM @ 4kg (25.22%, 30.72%, 35.32% respectively) and untreated checked T₀- control (27.08%, 32.26%, 38.25% respectively). However, at 75 DAS, treatment (T₃, T₄) were found non-significant and statistically at par with each other. At 90 and 105 DAS all the treatments were found significant over control.

9.2 Plant growth parameters of Turmeric

9.2.1 Plant height (cm)

The data provided in Table 2 and Figure 2 revealed maximum plant height in T₅- Microalgae @ 1% + FYM @ 1kg + SMC @ 125gm (57.06 cm, 59.26 cm, 64.53cm respectively) followed by T₃- SMC @ 250gm + Microalgae 2% (54.00 cm, 55.86 cm, 61.33cm respectively), T₄- FYM @ 2kg + Microalgae @ 2% (51.66 cm, 53.80 cm, 58.06 cm respectively), T₂- Microalgae @ 4% (50.26 cm, 52.40 cm, 56.06 cm respectively), T₁- SMC @ 500gm (47.66 cm, 49.93 cm, 53.46 cm respectively) T₆- FYM @ 4kg (33.40 cm, 35.26 cm, 39.66 cm respectively) and untreated checked T₀- control (32.00 cm, 33.86 cm, 37.53 cm respectively). However, at 75 DAS, treatment (T₃, T₄); (T₄, T₂); (T₂, T₁) and (T₆, T₀) were found non-significant and statistically at par with each other. At 90 and 105 DAS all the treatments were found significant over control.

9.3 Average number of leaves/plant

The data depicted in Table 3 and Figure 3 reveal that the total number of leaves significantly increased in T₅- Microalgae @ 1% + FYM @ 1kg + SMC @ 125gm (6.53%, 6.93%, 7.60% respectively) followed by T₃- SMC @ 250gm + Microalgae 2% (6.26%, 6.60%, 7.40% respectively), T₄- FYM @ 2kg + Microalgae @ 2% (6.00%, 6.53%, 7.20% respectively), T₂- Microalgae @ 4% (5.80%, 6.20%, 7.00% respectively), T₁- SMC @ 500gm (5.53%, 6.00%, 6.60% respectively) T₆- FYM @ 4kg (5.20%, 5.73%, 6.40% respectively) and untreated checked T₀- control (5.00%, 5.53%, 6.20% respectively). However, at 75 DAS, treatment, (T₅, T₃); (T₃, T₄); (T₄, T₂); (T₂, T₁) and (T₆, T₀) were found non-significant and statistically at par with each other. At 90 and 105 DAS, all the treatments were found significant over control.

9.4 Yield component of Turmeric

9.4.1 Weight of rhizome

The data depicted in the table 4 and figure 4 reveals that the rhizome weight of turmeric significantly increased in T₅- Microalgae @ 1% + FYM @ 1kg + SMC @ 125gm (188.33g) followed by T₃- SMC @ 250gm + Microalgae 2% (123.06g), T₄- FYM @ 2kg + Microalgae @ 2% (98.66g), T₂- Microalgae @ 4% (97.46g), T₁- SMC @ 500gm (69.06g) T₆- FYM @ 4kg (45.40g) and untreated checked T₀- control (22.46g). However, all the treatments were found significant over control.

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Table 1: Effect of organic matter and microalgae on disease intensity (%) of turmeric at 75, 90 and 105 DAS

Treatment No.	Treatments	Diseaseintensity (%)		
		75DAT	90 DAT	105DAT
T ₀	Control	27.08	32.26	38.25
T ₁	SMC	23.29	29.38	32.90
T ₂	Microalgae	21.18	27.01	30.18
T ₃	SMC + Microalgae	18.44	23.03	26.85
T ₄	FYM + Microalgae	19.76	26.78	29.43
T ₅	Microalgae + FYM + SMC	15.05	20.96	22.92
T ₆	FYM	25.22	30.72	35.32
	CD (5%)	0.89	1.74	1.33
	SE d±	0.40	0.79	0.60
	C.V	2.32	3.57	2.40

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Table 2: Effect of organic matter and microalgae on plant height (cm) of turmeric at 75, 90 and 120 DAS

Treatment No.	Treatments	Plant height(cm)		
		75DAT	90 DAT	120DAT
T ₀	Control	32.00	33.86	37.53
T ₁	SMC	47.66	49.93	53.46
T ₂	Microalgae	50.26	52.40	56.06
T ₃	SMC + Microalgae	54.00	55.86	61.33
T ₄	FYM + Microalgae	51.66	53.80	58.06
T ₅	Microalgae + FYM + SMC	57.06	59.26	64.53
T ₆	FYM	33.40	35.26	39.66
	CD (5%)	2.71	2.65	1.89
	SE d±	1.23	1.20	0.86
	C.V	3.24	3.03	1.98

Table 3: Effect of organic matter and microalgae on total number of leaves of turmeric at 75, 90 and 105 DAS.

Treatment No.	Treatments	Total no. of leaves		
		75DAT	90 DAT	120DAT
T ₀	Control	5.00	5.53	6.20
T ₁	SMC	5.53	6.00	6.60
T ₂	Microalgae	5.80	6.20	7.00
T ₃	SMC + Microalgae	6.26	6.60	7.40
T ₄	FYM + Microalgae	6.00	6.53	7.20
T ₅	Microalgae + FYM + SMC	6.53	6.93	7.60
T ₆	FYM	5.20	5.73	6.40
	CD (5%)	0.33	0.36	0.19
	SE d±	0.15	0.16	0.08
	C.V	3.19	3.21	1.54

Table 4: Effect of organic matter and microalgae on the weight (gm) of turmeric rhizomes.

Treatment No.	Treatment Name	Rhizome Weight (gm)			
		R1	R2	R3	Mean
T ₀	Control	20.40	24.00	23.00	22.46
T ₁	SMC	70.00	69.00	68.20	69.06
T ₂	Microalgae	92.80	108.00	91.60	97.46
T ₃	SMC+microalgae	119.40	133.20	116.60	123.06
T ₄	FYM+microalgae	112.20	90.80	93.00	98.66
T ₅	Microalgae+FYM+SMC	190.00	190.00	185.00	188.33
T ₆	FYM	39.40	50.60	46.20	45.40
	CD at 5%		12.34		
	SE.d(±)		5.60		
	C.V		7.45		

10. Evaluation of botanicals against *Fusarium equiseti* in vitro:

The botanicals extracts were screened for their efficacy against *Fusarium equiseti* on PDA amended with their 10% and 30% concentration. The data on the radial growth of the colony (mm) and percent inhibition of mycelial growth recorded have been presented here. The data presented in table 5 reveals that at 10% concentration, after 24hrs, 48hrs and 72hrs incubation, the least radial growth of *Fusarium equiseti* was observed in T₁-*Xanthoxylum acanthopodium* (3.66mm), followed by T₂- *Phlogacanthusthysiformis* (5.33mm), T₃- *Solanum xanthocarpum* (5.83mm), T₆- *Persicaria chinensis* (6.33mm), T₅- *Drymaria cordata* (7.00mm), T₇- *Gynuracusimbua* (7.66mm), T₄-*Centella asiatica* (7.83mm) and T₀-Untreated (12.66mm). All the botanical treatments exhibited significance over control. The result showed that maximum percentage of inhibition was observed in T₁-*Xanthoxylum acanthopodium* (77.54%), followed by T₂- *Phlogacanthusthysiformis* (67.34%), T₃- *Solanum xanthocarpum* (64.28%), T₆- *Persicaria chinensis* (61.22%), T₅- *Drymaria cordata* (57.14%), T₇- *Gynuracusimbua* (53.05%), T₄-*Centella asiatica* (52.04%) and T₀-Untreated (0.00%).

At 30% concentration, after 24hrs, 48hrs and 72hrs incubation, the least radial growth of *Fusarium equiseti* was observed in in T₁-*Xanthoxylum acanthopodium* (2.33mm), followed by T₂- *Phlogacanthusthysiformis* (3.83mm), T₃- *Solanum xanthocarpum* (4.16mm), T₆- *Persicaria chinensis* (5.33mm), T₅- *Drymaria cordata* (5.83mm), T₇- *Gynuracusimbua* (6.33mm), T₄-*Centella asiatica* (7.16mm) and T₀-Untreated (17.00mm). All the botanical treatments exhibited significance over control. The result showed that maximum percentage of inhibition was observed in T₁-*Xanthoxylum acanthopodium* (86.27%), followed by T₂- *Phlogacanthusthysiformis* (77.45%), T₃- *Solanum xanthocarpum* (75.48%), T₆- *Persicaria chinensis* (68.62%), T₅- *Drymaria cordata* (65.68%), T₇- *Gynuracusimbua* (62.74%), T₄-*Centella asiatica* (57.84%) T₀-Untreated (0.00%).

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Table 5: Effect of botanicals at a concentration of 10% on radial growth(mm) of *Fusarium equisetii* in vitro at 24hrs, 48hrs and 72hrs.

Treatments	Mean of radial growth (mm) at			Mean of percent of inhibition (%) over control		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
T0	5.66	9.83	16.33	0.00	0.00	0.00
T1	2.16	2.66	3.66	61.76	72.87	77.54
T2	2.83	4.33	5.33	50.00	55.93	67.34
T3	3.33	4.83	5.83	41.18	50.84	64.28
T4	4.83	6.16	7.83	14.71	37.28	52.04
T5	4.50	5.50	7.00	20.59	44.06	57.14
T6	3.83	5.33	6.33	32.36	45.76	61.22
T7	4.66	5.83	7.66	17.64	40.67	53.05
C.D	0.49	0.84	1.61			
C.V	6.99	8.63	12.17			

Table 6: Effect of botanicals at a concentration of 30% on the radial growth (mm)

Treatments	Mean of radial growth (mm) at			Mean of percent inhibition (%) over control at		
	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
T0	6.16	10.83	17.00	0.00	0.00	0.00
T1	1.16	1.66	2.33	81.07	84.61	86.27
T2	1.33	2.33	3.83	78.38	78.46	77.45
T3	1.83	2.83	4.16	70.27	73.84	75.48
T4	3.83	5.33	7.16	37.84	50.77	57.84
T5	2.83	4.16	5.83	54.06	61.53	65.68
T6	2.33	3.83	5.33	62.16	64.61	68.62
T7	3.66	5.16	6.33	40.53	52.30	62.74
C.D	0.87	1.54	1.00			
C.V	17.11	19.32	8.70			

of Fu sariu m equis eti in vitro at 24 hours, 48 hours, and 72 hours.

UNDER PEER REVIEW

Fig 4: Effect of organic matter and microalgae on disease intensity of turmeric at 75, 90 and 120 DAS

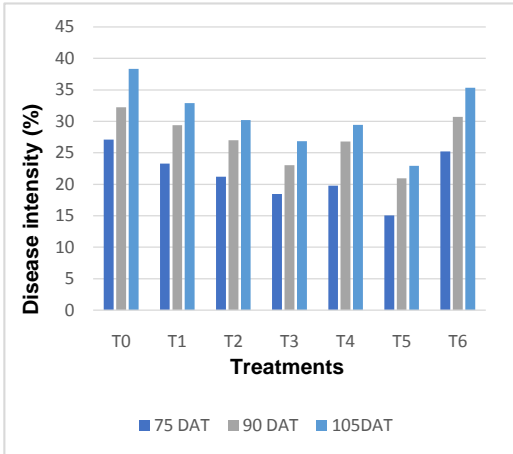


Fig 5: Effect of organic matter and microalgae on plant height of turmeric at 75, 90 and 105 DAS.

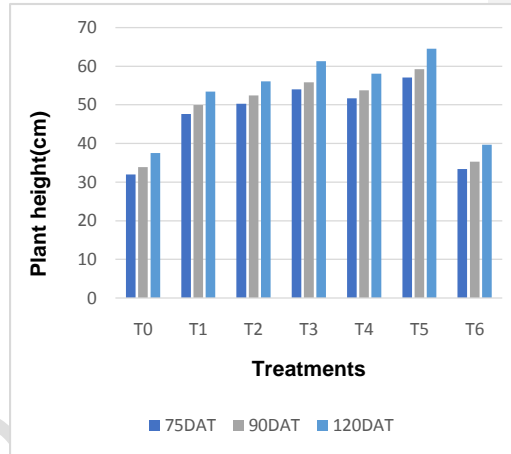


Fig 6: Effect of organic matter and microalgae on total number of leaves of turmeric at 75, 90 and 120 DAS.

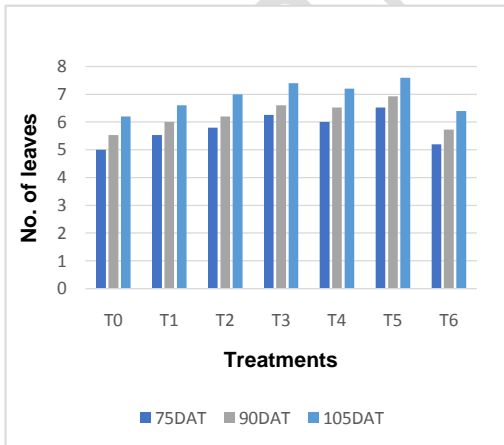


Fig 7: Effect of organic matter and microalgae on the weight (mm) of turmeric rhizomes.

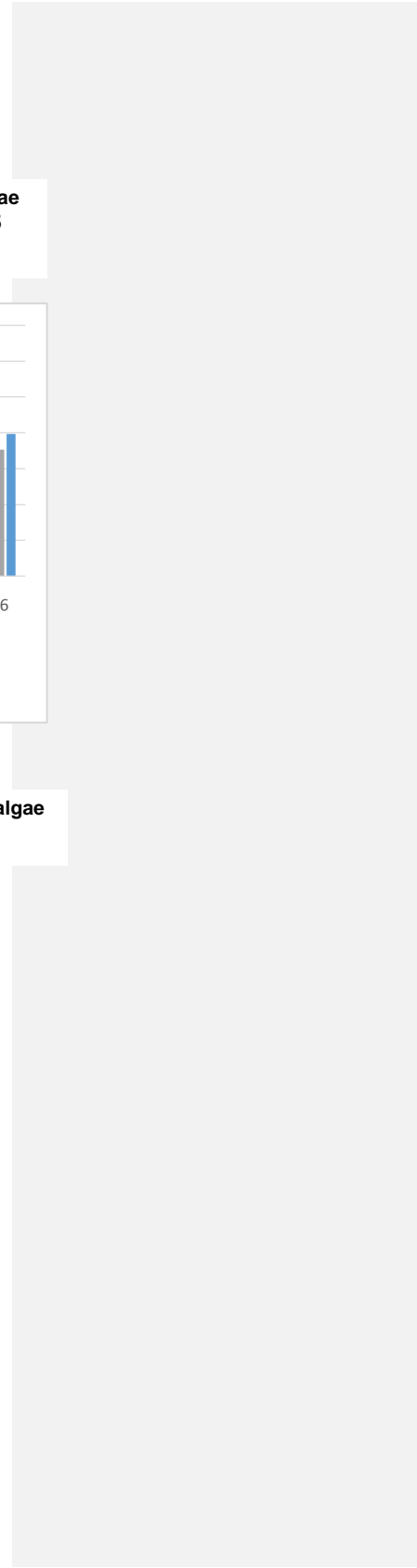


Fig 8: Effect of botanicals at a concentration of 10% on the radial growth (mm) of *Fusarium equiseti* in vitro at 24 hours, 48 hours, and 72 hours.

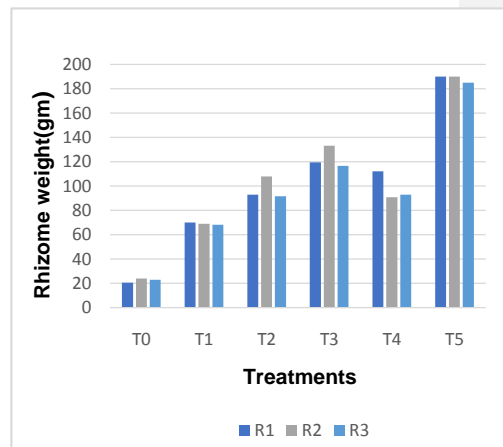
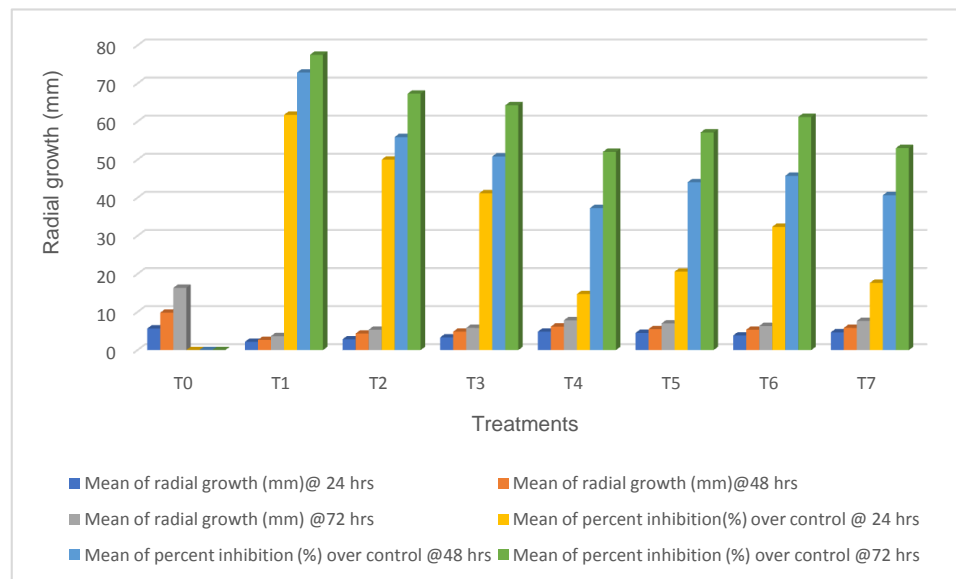
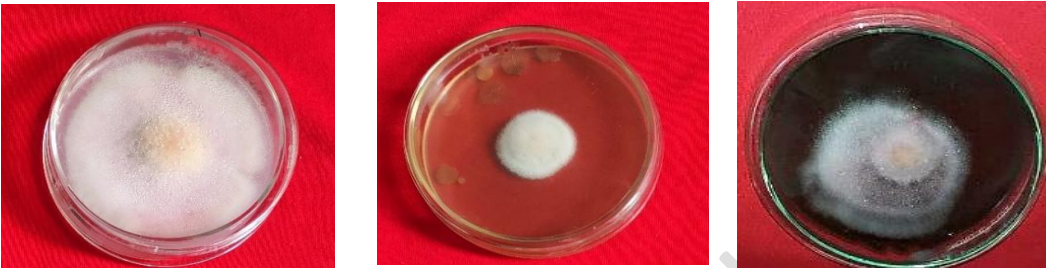


Fig 9: Effect of botanicals at a concentration of 30% on the radial growth (mm) of *Fusarium equiseti* in vitro at 24 hours, 48 hours, and 72 hours.

Fig. 10. In vitro evaluation of botanicals on radial growth (mm) of *Fusarium equiseti* at 10 % concentration.



T₀- Control

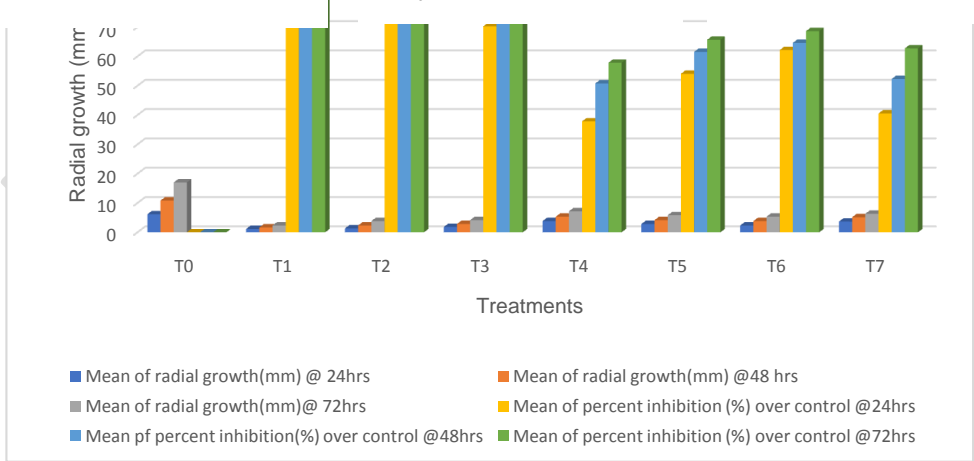
T₁- *Zanthoxylum acanthopodium*

T₂ - *Phlogacanthusthysiformis*

T₃- *Solanum xanthocarpum*

T₆ - *Persicaria chinensis*

T₅ - *Drymaria cordata*



T₇ – *Gynuracusimbua*



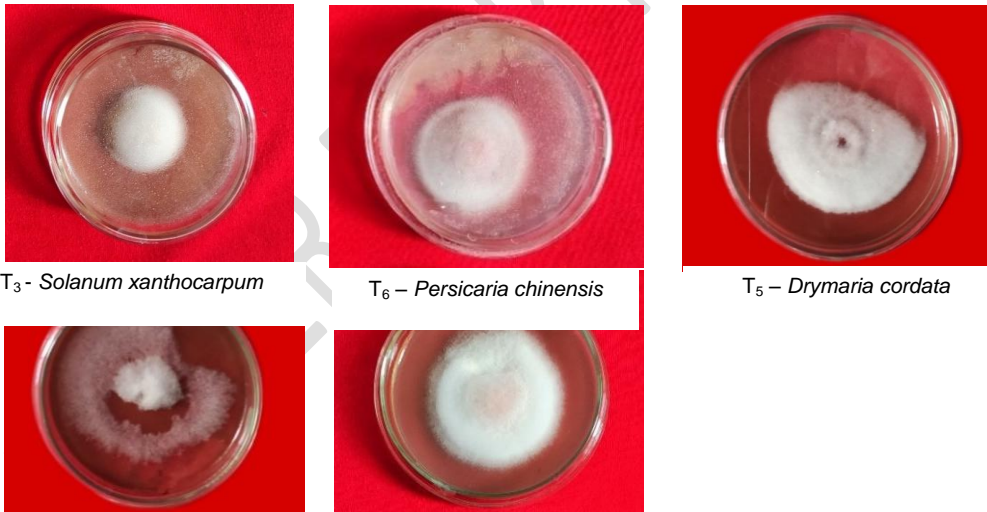
T₄ – *Centella asiatica*



Fig. 11. *In vitro* evaluation of botanicals on radial growth (mm) of *Fusarium equisetiat*



30% concentration.



11. CONCLUSION

The present study clearly reveals that among the selected treatments, T₅(Microalgae + FYM + SMC) significantly reduced the disease intensity of *Fusarium equiseti*, increased plant height, number of leaves and rhizome weight. The evaluation of botanicals against *Fusarium equiseti* *in vitro* at concentrations of 10% and 30% revealed effectiveness particularly in T₁, involving *Xanthoxylum acanthopodium*. This shows the ecofriendly application of bio-fertilizers in safeguarding plant health. Hence, the utilization of organic enhancements and biocontrol agents can be cost-effective, sustainable, and devoid of residual side effects. Consequently, these environmentally friendly treatments emerge as superior alternatives to fungicides, given their minimal adverse effects on the ecosystem, easy accessibility, and economic feasibility.

Comment [ML34]: Authors should include the research future prospects

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