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Effect of *Pseudomonas fluorescens*, Organic Amendments and Botanicals against *Fusarium culmorum* on Black Turmeric (*Curcuma caesia*)

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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

Rhizome rot is a soil-borne disease that severely affects black turmeric and is caused by *Fusarium culmorum*. An experiment was conducted at the Department of Plant Pathology in SHUATS, Prayagraj, to evaluate the effectiveness of *Pseudomonas fluorescens*, organic amendments, and botanicals against *Fusarium culmorum*. The experiment was carried out in the *kharif* season of 2022-2023, and various soil treatments were used, such as farmyard manure (FYM), spent mushroom compost (SMC), mustard cake, and neem cake. *Pseudomonas fluorescens* was used as a rhizome treatment. The results showed that the combination of all treatments (T9) had the least disease incidence (16.35%) compared to other treatments and the control (T0) (44.27%). To

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assess the radial growth of *Fusarium culmorum* on black turmeric, seven different botanicals from Manipur were tested at concentrations of 10% and 30%. These botanicals were *Ageratina adenophora*, *Bidens pilosa*, *Centella asiatica*, *Plantago major*, *Strobilanthes crispus, Saurauia napaulensis*, and *Artemesia vulgaris*. The antagonistic effect of these botanicals was evaluated *in vitro*, and the results indicate that the most significant reduction in biomass was observed with *Ageratina adenophora* at a 30% concentration, which showed a 78.5% inhibition rate with a radial growth of 2.66 cm. This was followed by *Artemesia vulgaris,* which exhibited a 75.3% inhibition rate with a radial growth of 3.16 cm compared to the control and other botanicals tested.

Keywords: Rhizome rot; Fusarium culmorum; botanicals; In vitro; black turmeric; kali haldi; Curcuma caesia; chemical compounds.

1. INTRODUCTION

The Zingiberaceae family comprises over 70 species of rhizomatous herbs, including Curcuma, which is known for its medicinal properties*. Curcuma caesia* (Roxb.), also known as Kali Haldi, is a perennial herb with a bluish-black rhizome. Its therapeutic properties have been recognized by various tribal communities for centuries. The rhizome of black turmeric is highly aromatic due to the presence of volatile oil components, and its color is significantly darker blue than that of *C. aeruginosa*. The *Curcuma caesia* plant contains a total of thirty significant components, representing 97.48% of the volatile oil, with camphor (28.3%), ar-turmerone (12.3%), (Z)-β-ocimene (8.2%), ar-cur cumene (6.8%), 1,8-cineole (5.3%), β-element (4.8%), borneol (4.4%), bornyl acetate (3.3%), and γ-cur cumene (2.82%) as the major constituents [1].

Black turmeric is a plant species that is native to North-East and Central India. It grows best in areas with moist deciduous forests and rich, humid, clayey soils. This plant can be found in several Indian states, including Chhattisgarh, Madhya Pradesh, Odisha, Uttar Pradesh, and West Bengal [2].

Fusarium species are responsible for causing severe economic losses in the agricultural sector worldwide. Diseases caused by species of this genus are difficult to manage, leading to significant losses. The Fusarium genus, specifically the *species F. oxysporum, F. solani, F. incarnatum,* and *F. musae*, causes postharvest diseases in fruits such as orange, muskmelon, banana, and kiwifruit. [3].

Dry rot disease caused by *Fusarium* species is a major concern globally, with more than 13 species reported as causal agents [4]. During storage, the losses due to dry rot disease can range from 25 to 60%. In Gansu province of China, dry rot disease was estimated to cause around 88% of total post-harvest losses. In Michigan state of the USA, approximately 50% of seed lots were reported to have infections of *Fusarium* species*. F. sambucinum* is the most aggressive fungus species causing dry rot in Europe and North America [5]. Losses of up to 50% have been reported in crops such as bananas, pineapples, peas, tomatoes, and lentils [6].

In 2006, Leslie and Summerell [7] published the first report identifying *Fusarium culmorum* as the cause of stalk rot in maize crops in China. This fungus has also been found to cause ear rot and produce mycotoxins. As a result, it is important to monitor the occurrence of maize stalk and ear rot caused by *Fusarium culmorum*, as it poses a significant risk for crop loss and mycotoxin contamination. A recent study by Xia et al. in [8] emphasizes the need for continued vigilance in this regard.

The use of chemical compounds has had a great impact on the environment and posed a health hazard. Therefore, plant-based pesticides are emerging as an important alternative to synthetic chemicals as they do not pose a threat to the natural environment, or human, and animal health. Plants contribute 75% of molecular medicines either directly or indirectly [9] *Lantana camara* is a rich source of bioactive compounds such as flavones, isoflavones, flavonoids, and anthocyanins. The antifungal activities of *L. camara* extracts can cause cell lysis and alter the membrane integrity. The extracts from its leaves significantly inhibit the radial growth of *Rhizoctonia solani* and *Fusarium oxysporum*. Spraying the leaf extract drastically reduces plant mortality [10].

The excessive and careless use of chemicals has been discouraged due to their toxic effects on non-target organisms and the environment.

Additionally, the development of resistant strains of pathogens against various chemical fungicides has been observed. In light of these facts and the importance of addressing the damage caused by diseases, a study was undertaken to screen the anti-fungal activity of some indigenous plants of Manipur state against *Fusarium culmorum* on black turmeric.

2. MATERIALS AND METHODS

2.1 Application of Organic Amendments

Forty pots were chosen for the experiment, and each one was filled with 15 kg of sterilized soil. The selected pots were well-prepared and marked according to the layout plan. Organic amendments such as farmyard manure (FYM), spent mushroom compost, neem cake (NC), and mustard cake (MC), were used during the experiment.

2.2 Seeding of Rhizomes

The healthy rhizomes were treated with *Pseudomonas fluorescens* in 1 liter of water for 24 hours before planting in the pots.

2.3 Collection of Disease Samples

The infected plants that displayed typical symptoms were gathered from the field, specifically the courtyard of the Department of Plant Pathology at SHUATS, Allahabad during the *kharif* season of 2023 to be identified. The initial symptoms were characterized by leaf yellowing, slight wilting, and stunting. As the infection progressed, the leaves would yellow and dry up, which are typical signs of crop maturity. Upon cutting open the infected rhizomes, the affected areas typically appear dull brown and dark in color. The affected rhizomes appear soft and shrunken, indicating the severity of the infection.

2.4 Isolation of *Fusarium culmorum* [11]

The rhizome samples were collected and washed with water. The part of the rhizome with symptoms was cut into 2mm pieces and then surface sterilized with mercuric chloride (0.1%) for 5-10 seconds. After that, the samples were washed thrice with sterilized distilled water to remove any remaining traces of mercuric chloride. The samples were then dried using sterilized filter paper and transferred to Petri plates with potato dextrose agar media (one piece per plate). The plates were then incubated in an incubator for 7 days at 25°C ± 10°C.

2.5 Identification and Morphological Characteristics of *Fusarium culmorum*

Fungal isolates are purified and then transferred from the end of the isolated fungal culture using a sterile needle that is mounted on the slide. The slide is then stained with lactophenol and cotton blue and examined under a microscope. The identification of the morphology of the fungal species is based on the shape of the macroconidia that are formed on sporodochia. The conidiophores are branched monophialides, which are short and wide. The macroconidia are relatively short and stout and have an apical cell that is usually blunt or slightly papillate, while the basal cell is foot-shaped or notched. Macroconidia are generally thick-walled and curved and usually have 3 to 5 septa. There are no microconidia present. The chlamydospores, which are oval to globose in shape, are formed intercalary in the hyphae and can be solitary, in chains, or clumps. They can also be formed from macroconidia [7].

The pathogen species was identified and confirmed by the Indian Type Culture Collection (ITCC), which is a division of Plant Pathology at ICAR-Indian Agricultural Research Institute. The identification was based on morphological characteristics and sequences of ITS-rDNA. The sequence data was deposited in GenBank with accession number PP345604.

2.6 Maintenance of Culture

The fungus cultures were transferred to Petri plates and PDA slants and incubated in the laboratory at a temperature of 28± °C for 7 days. The original cultures were preserved at a temperature of 4°C in a refrigerator. Additionally, these cultures were sub-cultured once a month and saved for future use.

2.7 Preparation of Botanical Extracts

The botanicals that were gathered were washed with running tap water and then with sterile water. After this, they were air-dried for a day to get rid of any moisture on the surface. The leaves were then turned into powder by blending them. After processing, the powder was carefully placed into plastic bags, which were then labeled and sealed tightly to prevent air from getting in.


Fig. 1. Infected rhizomes and leaves

Fig. 2. Pure culture of *Fusarium culmorum* in Petri plates and slants

To create aqueous extracts of botanicals at both 10% and 30% concentrations, we began by soaking the powdered botanicals in sterile distilled water. The soaked botanicals were then filtered first with muslin cloth and then with Whatman filter paper. After that, they were centrifuged at 1500 rpm for 20 minutes. The resulting suspended solution was transferred into a 100ml conical flask, sterilized in an autoclave at 15 lbs pressure for 20 minutes, and kept as a stock solution. Each botanical stock solution was tested at two concentrations, and its effect on the radial growth of *Fusarium culmorum* was measured at 24, 48, and 72 hours after inoculation using the method outlined by Odey et al. [12].

2.8 In vitro Efficacy of Botanicals Against *Fusarium culmorum*

The effectiveness of a plant extract in preventing fungal growth was tested against a particular pathogen in a laboratory. The experiment followed a completely randomized design (RBD) using the poisoned food technique. To achieve the desired concentrations of 10% and 30%, a certain amount of the extract filtrate was mixed with PDA and shaken thoroughly to ensure proper mixing. The PDA plates containing the plant extracts were then inoculated aseptically with the pathogen by transferring a 5mm diameter agar disc from fresh cultures. Each treatment was replicated three times, while the control was represented by the basal medium (PDA) without any phytoextract. All Petri dishes were incubated at 25±1°C, and the radial growth (in millimeters) of the fungus was measured and compared to the control.

The percent inhibition of fungal growth was estimated using the following formula [13]:

$$I = C-T/\ C \times 100$$

Where

I = percent inhibition
C = Colony diameter in control
T = Colony diameter in treatment

3. RESULTS AND DISCUSSION

Minimum disease intensity was recorded in T9(FYM + SMC+NC+MC-3.20%) followed by T8 (NC +SMC-3.90%), T2(SMC-6.45%), T7(NC+SMC- 9.17%), T3(NC-10.60%), T4(MC-11.70%), T5(NC+FYM-11.92%), T6(MC+FYM-14.57%), T1(FYM-14.67%) as compared to untreated checked T0-Control (21.27%). At 90 and 105 DAT all the treatments were found significant over control. The results are in agreement with the findings of Sreegayathri et al. [14] soil application of neem cake @ 250 kg ha^{-1} + *Streptomyces reticuli* @ 10 ml/ lit + *Pseudomonas fluorescens* @ 2.5 kg ha^{-1} + *Trichoderma viride* @ 2.5 kg ha^{-1} recorded a significantly lower wilt incidence of 14.4 percent as against 42.5 percent in the control, which was found to be 66.1 percent reduction over control and gall index was also significantly reduced to 1.6 as against 5.0 in control.

Result based on field experiment, the effectiveness of the soil amendments and biocontrol agent (Spent mushroom compost, farm yard manure, neem cake, mustard cake, and *Pseudomonas fluorescens*) on the plant growth parameters increases significantly as compared to the control. The maximum plant height was observed in the treatment in T9-(FYM+ SMC+NC+MC- 53.25cm) followed T6(MC+FYM-48.75cm), T8(NC+SMC-48.25cm), T2-(SMC-47.50cm), T5 (NC+FYM-47.50cm), T7(NC+SMC-47.50cm), T4-(MC-46.25cm), T3-(NC-40.00cm), T1(FYM-37.00cm) while minimum plant height was observed in untreated T0-Control (28.75cm). The above results are in agreement with the findings of Zeeshan et al. [15] who observed that button mushroom compost was more effective against height, number of fruits per plant, fresh weight of plant, number of shoots per plant, root length, root weight, and dry plants.

In case of number of leaves/plant the maximum number was recorded in treatments T9(FYM+ SMC+NC @3g+MC-6.00%) followed by T8(NC+SMC-5.75%), T2(SMC-5.50%), T7 (NC+SMC-5.50%), T3(NC-5.25%), T5(NC+FYM-5.00%), T1(FYM-4.75%), T4(MC -4.75%), T6(MC+FYM-4.75%), as compared to untreated T0-Control (4.25%). At 90 and 105 DAT all the treatments were found significant over control. The above findings are in agreement with Datta et al. [16] who concluded that the application of green leaf manure and application of farm yard manure @ 30 tonnes/ha treatments for dry yield and quality of turmeric. Similar findings by Umar et al. [17] observed that the effect of two organic amendments viz: Bitter leaf and Cashew seed kernel used two weeks after germination, indicated superior growth parameters.

The data presented on Table 1, at 75 days of sowing, reveals that the highest rhizome weight of black turmeric plants was recorded in T9(FYM+ SMC+NC+MC-190g) followed by T2(SMC- 170g), T4(MC-125g), T8(NC+SMC-97.25), T5(NC+FYM-69.50), T7(NC+SMC-66.25g), T6(MC+FYM-61.50g), T3(NC-60g), T1(FYM-40g), as compared to untreated checked T0- Control (35.50g). Among the treatments (T5, T7), (T6, T3) were found non-significant to each other. The results above are in agreement with the similar findings of Altindal et al. (2015).

Nuket Altindal [18] that the use of spent mushroom compost (SMC) will result in the highest tuber weight and the yield of tuber i,e, 50% from the application of SMC. Similarly, Mishra and Singh [19].

Latiffah Zakaria [6] observed that the foliar destruction due to leaf spot reduces the yield considerably when the disease starts in its early stages of crop growth.

3.1 Evaluation of Botanicals Against *Fusarium culmorum In vitro*

The botanical extracts were screened for their efficacy against *Fusarium culmorum* on PDA amended with their 10% and 30% concentrations. The data on the radial growth(mm) of the colony and the percent inhibition of mycelial growth recorded have been presented here.

The results data presented in Table 2, of selected botanicals at 10% concentration, after 24hrs, 48hrs, and 72hrs incubation, all the selected botanicals significantly inhibit radial growth of *Fusarium culmorum,* and among the botanicals the least radial growth of *Fusarium culmorum* was observed in T5-*Ageratina adenophora*(17.4 cm) followed by T2- *Artemesia vulgaris* (23.1cm),) with percent growth reduction of (54.5) and (39.6) respectively over control. Similarly, at 30% concentration, the least radial growth of *Fusarium culmorum* was observed in T5-*Ageratina adenophora* (2.7cm), followed by T2- *Artemesia vulgaris* (3.1cm), with a percent growth reduction of (78.5) and (75.3) respectively over untreated control.

Table 1. Effect of organic amendments and *Pseudomonas fluorescens* on disease intensity (%), plant growth parameters and yield of black turmeric

Symbol	Name of treatment	Disease intensity (%) @			Plant height(cm) @			No. of leaves/plant @			Rhizome weight(g)
		75DAS	90DAS	105DAS	75DAS	90DAS	120DAS	75DAS	90DAS	120DAS	Mean
T0	Control	21.27	31.47	44.27	28.75	35.00	43.75	4.25	4.75	5.50	35.00
T1	FYM	14.67	25.62	38.67	37.00	50.00	57.50	4.75	5.50	6.25	40.00
T2	SMC	6.45	12.10	25.35	46.00	60.00	69.50	5.50	6.00	6.25	170.00
T3	Neem cake (NC)	10.60	20.50	32.07	40.00	56.25	63.75	5.25	6.00	6.75	60.00
T4	Mustard cake (MC)	11.70	20.75	32.12	42.50	58.75	66.25	4.75	5.50	6.00	125.00
T5	Neem cake + FYM	11.92	22.82	32.32	45.00	60.00	68.75	5.00	5.50	6.50	69.00
T6	Mustard cake+ FYM	14.57	22.30	32.55	48.25	61.25	72.50	4.75	5.00	6.00	61.00
T7	Neem cake +SMC	9.17	18.32	27.25	43.00	60.00	67.50	5.50	6.00	6.75	66.00
T8	Mustard cake+ SMC	3.90	11.50	19.45	46.25	61.25	71.25	5.75	6.25	7.00	97.25
T9	FYM+SMC+NC+MC	3.20	10.15	16.35	53.25	68.00	78.25	6.00	6.50	7.50	190.00
	C.D. (5%)	2.90	2.71	4.09	6.06	5.41	6.68	0.91	0.91	0.98	14.45
	SE d±	1.40	1.31	1.98	2.94	2.62	2.75	0.44	0.44	0.44	7.00
	C.V	18.52	9.62	9.34	9.62	6.41	5.91	12.11	10.83	10.30	10.83

Table 2. *In vitro* effects of botanicals of different conc. on radial growth (mm) of *Fusarium culmorum* at 24 hrs, 48 hrs, and 72 hrs

Symbol	Treatment	Average radial growth of mycelium (10% conc.) @			% growth inhibition (10%conc.) @			Average radial growth of mycelium (30% conc.) @			% growth inhibition (30%conc.) @		
		24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs	24hrs	48hrs	72hrs
T0	**Control**	10.5	34.7	38.3	0.00	0.00	0.00	7.16	11.00	12.66	0.00	0.00	0.00
T1	*Bidens pilosa*	9.5	31.6	37.3	9.52	8.93	2.61	2.16	3.16	5.50	69.8	71.2	56.3
T2	*Artemesia vulgaris*	5.67	21.1	23.1	46.0	39.1	39.6	0.83	1.83	3.16	88.2	83.2	75.3
T3	*Strobilanthes crispus*	7.5	24.0	27.0	28.5	30.8	29.5	1.33	2.50	3.83	81.2	78.7	69.5
T4	*Plantago major*	8.16	25.7	28.4	22.2	25.9	25.8	1.83	2.33	4.33	79.05	77.2	65.6
T5	*Ageratina adenophora*	5.34	14.8	17.4	49.1	57.3	54.5	0.66	1.66	2.66	90.6	84.8	78.5
T6	*Centella asiatica*	9.34	26.1	33.1	11.0	24.7	13.5	1.50	2.83	4.83	74.3	74.1	62.9
T7	*Saurauia napaulensis*	6.50	22.1	25.1	38.0	36.3	34.4	1.00	2.00	3.50	86.03	81.8	72.3
	C.D. (5%)	1.05	1.10	0.56				0.45	0.48	0.47			
	C.V.	7.63	2.50	1.11				12.54	8.06	5.27			

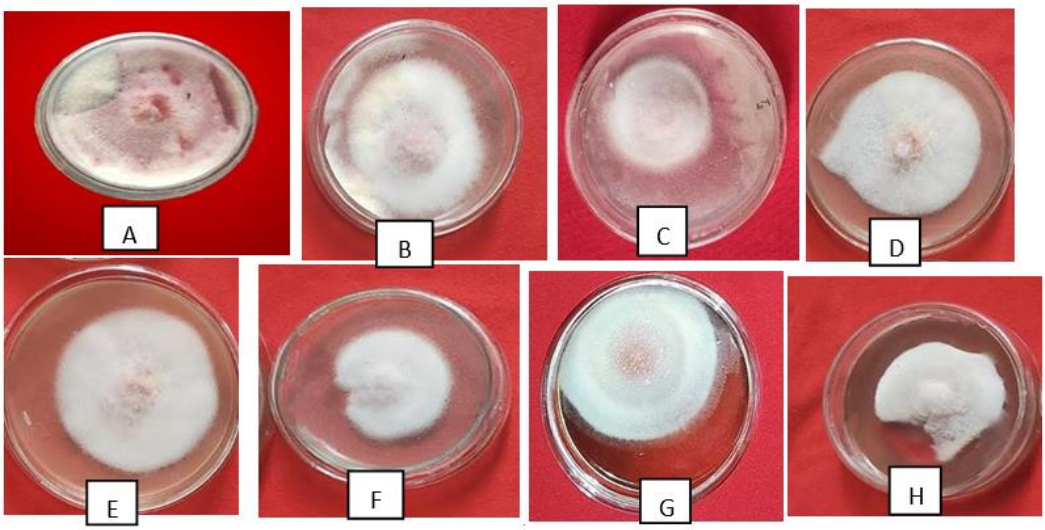

Fig. 3. *In vitro* evaluation of botanicals on radial growth (mm) of *Fusarium equiseti* at 10 % concentration

A- T0 – Control; B= T1–Bidens pilosa ; C- T2 – Artemesia vulgaris ;D- T3 - Stropilanthes crispus; E-T4 -Plantago major; F- T5 -Ageratina adenophora; G- T6 - Centella asiatica; H- T7 -Saurauia napaulensis.

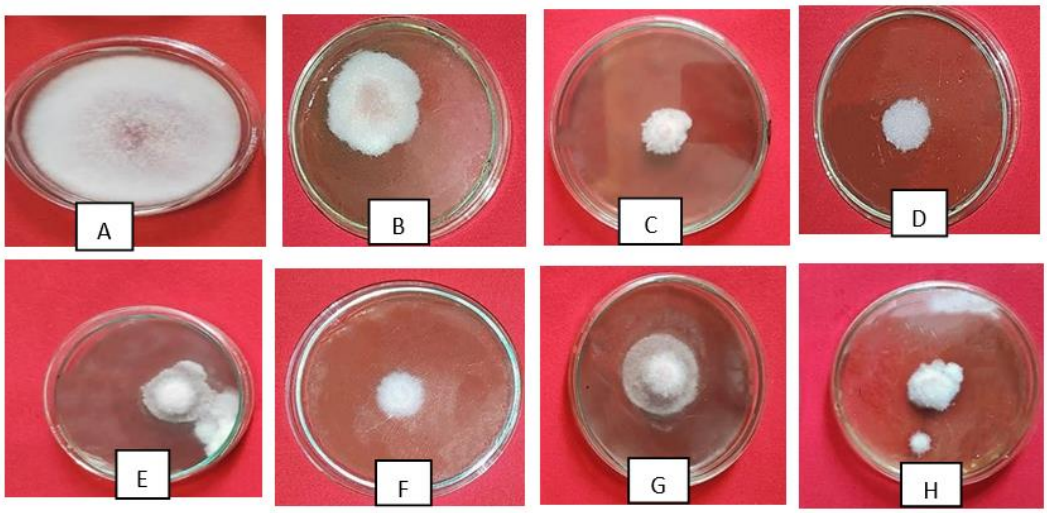

Fig. 4. *In vitro* evaluation of botanicals on radial growth (mm) of *Fusarium equiseti* at 30 % concentration

A- T0 – Control; B= T1–Bidens pilosa ; C- T2 – Artemesia vulgaris ;D- T3 - Stropilanthes crispus; E-T4 -Plantago major; F- T5 -Ageratina adenophora; G- T6 - Centella asiatica; H- T7 -Saurauia napaulensis.

Results data further revealed that irrespective of concentration, among botanicals, mean radial growth inhibition was found highest in T5- *Ageratina adenophora* (78.5%), followed by T2- *Artemesia vulgaris* (75.3%), T7- *Saurauia napaulensis* (72.3%), T3- *Strobilanthes crispus* (69.5%), T4 - *Plantago major* (65.6%), T6- *Centella asiatica* (61.9%), T1 -*Bidens Pilosa* (56.3%) respectively over untreated control. It is also observed that the mean radial growth of the fungus was lowest at a higher respective dose of concentration i.e., at 30% (2.7 cm) and 10% (17.4 cm).

According to earlier studies by Prakash et al. [20] the anti-fungal activity *of Centella asiatica* was tested against three fungal strains using two methods: disc diffusion and broth dilution. The results showed that the crude methanol extract of *C. asiatica* was the most effective in inhibiting fungal activity. Nasrin et al. [21] also found that *C. asiatica* had antifungal properties against

Aspergillus sp. In their study, they used hydrophilic extracts *of C. asiatica* at concentrations of 1% and 5% and found that the bioactive compounds of *C. asiatica* could be a potential source of preservatives that inhibit fungal growth. Seepe et al. [22] observed the *in vitro* antifungal activity of different medicinal plant extracts, either independently or in combination, against four strains of Fusarium. The use of medicinal plant extracts from renewable plant parts, either independently or in combination, is sustainable, affordable, environmentally friendly, and may be more beneficial in the fight against crop pathogenic diseases, particularly in organic farming.

4. CONCLUSION

Based on the results of an in vitro screening test, it is evident that out of the seven botanicals screened, five were found to significantly inhibit the radial growth of *Fusarium culmorum*. *Ageratina adenophora, Artemesia vulgaris*, and *Saurauia napaulensis* were found to be the most effective in comparison to the other botanicals tested. The study also revealed that the efficacy of biomass and radial growth inhibition increased with an increase in extract dose concentration, particularly at 30% and 10% concentrations.The study also found that using eco- friendly bio-fertilizers like Farm yard manure, spent mushroom compost, neem cake, mustard cake, and *Pseudomonas fluorescens* in the field proved to be an effective measure of plant protection. Among the selected treatments, the combination of all treatments (T9: FYM+SMC+NC+MC) significantly reduced the disease intensity of *Fusarium culmorum* at 75, 90, and 105 DAS (3.20%, 10.15%, and 16.35%) respectively, when compared to other treatments. Additionally, the T9 treatment significantly increased plant height (cm), number of leaves, and weight of rhizome at 75, 90, and 120 DAS compared to other treatments.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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