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Seed-borne Pathogens Associated with Pumpkin and their *in-vitro* Management

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ABSTRACT: Pumpkin is known by its botanical name *Cucurbita moschata* and belongs to the Cucurbitaceae family. They are crucial in people's daily lives as they contain important vitamins, minerals, and antioxidants. Pumpkins are vulnerable to the attack of various seed-borne pathogens. Seed-borne pathogens including *Aspergillus niger, Aspergillus flavus, Fusarium oxysporum*, etc. were confirmed through pathogenicity tests and cultural and morphological identification from pumpkin. All ten fungicides were tested against the pathogens listed. Maximum percent inhibition (100%) was achieved due to Chlorothalonil in the case of *Aspergillus niger* and *Aspergillus flavus*. While in the case of *Fusarium oxysporum* and *Aspergillus niger*, maximum percent inhibition (100%) was achieved due to Carbendazim. All three bioagents were tested against the pathogens and found significantly effective over control. Maximum percent inhibition was achieved due to *Trichoderma harzianum* in the case of *Aspergillus niger* (64.52%) and *Fusarium oxysporum* (61.91%). While, in the case of *Aspergillus flavus*, maximum percent inhibition (53.74%) was achieved due to *Pseudomonas fluorescens*. All eleven botanicals were tested at 10% concentrations and found significantly effective over control. At 10% concentration, maximum percent inhibition was achieved due to *Curcuma longa* (Turmeric) in the case of *Aspergillus niger* (76.75%) and *Fusarium oxysporum* (46.51%). In the case of *Aspergillus flavus*, maximum percent inhibition (42.24%) was achieved due to *Zingiber officinale* (Ginger).

Keywords: Seed-borne, pathogenicity, fungicides, bioagents, botanicals.

INTRODUCTION

The Cucurbitaceae family includes the main agricultural species of pumpkin (*Cucurbita moschata* Duch), which is grown extensively all year round in India in agriculture. It is thought that North America is where pumpkins first appeared. According to Pranab *et al*. (2006), the crop is widely farmed in South Asia, Africa, Latin America, India, and the United States. The warm-season crop pumpkin does well in sandy, well-drained soil that is rich in organic materials. Pumpkin is primarily cultivated in China, India, Russia, and the USA, with a global production of approximately 27.4 million metric tons (Singh & Kumar 2020). In contrast, India contributed 5.1 million tonnes from an area of 45,000 hectares (FAOSTAT, 2021). Pumpkin is a significant food item; its principal ingredients are lutein and both alpha and beta carotene, the latter of which the body uses to produce vitamin A. Pumpkin has 1.2 g of protein, 0.6 g of dietary fiber, and 7.5 g of carbs in terms of nutrition. Once considered underutilized and wasteful, pumpkin seeds are now gaining recognition in agriculture, medicine, and the food industry due to their health benefits and nutraceutical properties. These seeds are a rich source of edible oil (37.8–45.4%), which contains various fatty acids, including linoleic acid (35.6–60.8%), oleic acid (21.0–46.9%), palmitic acid (9.5–14.5%), and stearic acid (3.1–7.4%), along with smaller amounts of α-linolenic, palmitoleic, arachidic, eicosanoic, lignoceric, and behenic acid (Singh & Kumar 2022). In Kerala, high relative humidity predominates for the majority of the year, causing vegetable seeds, especially cucurbitaceous seeds, to degrade while in storage. Although not thoroughly studied to date in the country, losses due to diseases of seeds and seedlings, in particular rotting caused by *Aspergillus niger*, have been reported in individual plots. The pathogen, *Aspergillus niger*, was found to be predominate with seeds as well as soilborne in nature. Root rot (*Fusarium solani* (Mart.) Sacc.), is one of the most destructive diseases. Some of the common indications of diseases include rot in the main and subsidiary roots, the presence of brown spots on the crown area in contact with the soil surface, wilting and yellowing of lower leaves, which ultimately results in plant mortality (Han *et al*., 2012). However, the use of

fungicides as seed treatment has been discouraged due to several drawbacks, including groundwater pollution, residues on food crops, effects on species that are not intended targets, and the development of resistance to chemical fungicides in addition to their high cost (Reddy, 1997). Alternatives to chemicals such as "bio-control agents" may be more environmentally friendly. Baker and Cook (1974) ; Cook and Baker's (1983) reviews of this research are both in-depth. In the past, antagonists such as *Pseudomonas fluorescens*, *Bacillus subtilis*, *Trichoderma* sp., and *Gliocladium virens* have been successfully used as a tool. *Trichoderma* spp. is one of the most important factor used in biological control against pathogens; it has been extensively used and demonstrated to be very successful on some diseases due to its simplicity in obtaining it from the soil, rapid isolation, availability of requirements for its growth and reproduction, and ease in obtaining their growth in temperatures and humidity (Harman, 2000). When compared to fungicides, seed treatment with various biocides (bioagents and plant leaf extracts) has been found to be the safest method.

MATERIAL AND METHODS

Location. The experiment was conducted to study the presence of seed-borne pathogens associated with pumpkins. A study of the efficacy of different fungicides, biocontrol agents, and botanicals to combat the growth of pathogens was carried out in the Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar.

Experimental details. Completely Randomized Design (CRD) was followed to conduct the experiments with three replications. Percent inhibition of test pathogen was calculated by applying the formula given by Vincent (1927). Percent inhibition (I) = (C–T)/C × 100 Where, C = Growth of test fungus (mm) in control plates.

T = Growth of fungus (mm) in treatment plants.

Pathogenicity test. It was conducted using the seed inoculation method, in which the sterilized seeds were rolled over a seven-day-old pure culture petri plate of the test fungi. These inoculated seeds were then placed onto moist blotter paper and incubated at 28±1°C for seven days. Another is by seedling inoculation method in which healthy seedlings that were ten days old were subjected to inoculation at the basal collar region using the conidial/mycelial suspension (10^6 ml) using the pinprick method. The whole seedling was then covered with a polythene to maintain moisture within. All the seedlings were incubated at 23±1°C for 10 days.

***In vitro* evaluation of fungicides against test fungus.** The efficacy of selected common fungicides was compared and evaluated against the test fungi isolated from the seeds of pumpkin by applying a poisoned food technique using PDA as a basal medium. The fungicides used for this purpose are Carbendazim (12%) + Mancozeb (63%), Tebuconazole 25.9% EC, Chlorothalonil 75% WP, Thiophanate Methyl 50% WP, Propiconazole 25% EC, Hexaconazole 5% SC, Difenoconazole 10% WP, Azoxystrobin 25% EC, Carboxin 37.5% + Thiram 37.5%, and Tebuconazole 50% + Trifloxystrobin 25% WG. The poisoned medium was equally distributed in three petri plates depicting three replications. The test fungi was first grown in petri plates. After full growth, the test fungi were cut into 5mm discs from the periphery of the actively growing colony with a sterilized cork borer and transferred to the center of each plate containing the poisoned medium. Control was maintained by placing fungal discs in plates containing untreated medium. All the inoculated petriplates were incubated at 28±2°C in a BOD incubator. The diameter of fungal colonies in treatment was measured when the growth of the fungal colony in the control plate was full. ***In vitro* evaluation of bioagents against test fungus.** In an *in-vitro* study, three different biological control agents, specifically *Trichoderma asperellum*, *Pseudomonas fluorescens*, *and T. harzianum* known for their antagonistic properties, were assessed for their effectiveness against the test fungus. This evaluation was conducted using the dual-culture technique, as outlined by Arora and Upadhay (1978). To carry out the study, small discs (5 mm in diameter) of PDA containing the growth of both the test pathogen and the respective bio-agents were carefully removed using sterilized cork borers. Subsequently, two culture discs, one representing the test fungus and the other the bio-agent, were placed equidistant and directly opposite each other on solidified PDA medium within petri plates under aseptic conditions. These plates were then incubated at a temperature of 27 ± 10°C. Additionally, control PDA plates were prepared with only the culture disc of the test fungus, serving as untreated controls.

***In vitro* evaluation of Phytoextracts/Botanicals against test fungus.** Fresh and healthy plant parts of *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), *Allium sativum* (Garlic), *Allium cepa* (Onion), *Azadirachta indica* (Neem), *Pongamia pinnata* (Karanj), *Annona squamosa* (Custard apple), *Eucalyptus* spp. (Eucalyptus), *Lantana camara* (Lantana), *Tagetes* spp. (Marigold) and *Tinospora cordifolia* (Giloy) were collected. Subsequently, 100 g of these plant parts were crushed in 100 ml of acetone. A suitable quantity of each plant extract (at 100% concentration) was thoroughly mixed with autoclaved and cooled PDA medium at 40°C in 250ml conical flasks to achieve the desired concentration of 10 percent. In total, each plant extract and its specific concentration were replicated three times. After the PDA medium solidified, all the treatment and control plates were inoculated under aseptic conditions by placing a 5 mm mycelial disc from a one-week-old actively growing pure culture of test pathogen in the center of each plate. Petri plates filled with regular PDA medium, without any botanical extract, and inoculated with a mycelial disc of the test fungus, were used as the untreated control. Subsequently, all of these plates were placed in an incubator at a temperature of 27 ± 10°C for one week.

Statistical analysis. Data collected from various treatments were subjected to statistical analysis using established methods. When the treatment effects were determined to be statistically significant, the corresponding standard error (S.E.) and critical difference (C.D.) were calculated at a 5 percent significance level.

RESULTS AND DISCUSSION

Pathogenicity test Seed inoculation. In this case, the pumpkin seeds were completely rotten due to which there was no germination of seeds, while the seeds in the control pot were healthy and germinated.

Seedling inoculation. In this case, rotting occurred at the collar region followed by yellowing and wilting of branches of pumpkin seedlings in the pots. While the seedlings in the control pot looked completely healthy and free from infection.

***In-vitro* evaluation of fungicides.** A total of ten fungicides were evaluated *in-vitro* against *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum.* The results obtained are presented in Table 1 and Fig. 1(a) and (b).

Aspergillus niger exhibited no growth in chlorothalonil, carbendazim 12% + mancozeb 63%, and tebuconazole as against 90mm in the untreated control. *Aspergillus flavus* exhibited no growth in chlorothalonil, propiconazole, tebuconazole 50% + trifloxystrobin 25%, thiophanate methyl, and hexaconazole, as against 89.46 mm in the untreated control. *Fusarium oxysporum* exhibited no growth in carbendazim 12% + mancozeb 63% as against 90.00mm in the untreated control.

***In-vitro* evaluation of Bioagents.** A total of three biocontrol agents were evaluated *in- vitro* against *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum.* The results obtained are presented in Table 2 and Fig. 2(a) and (b).

Out of three antagonists tested *Trichoderma harzianum* was found the most effective and recorded significantly least linear mycelial growth of 31.5mm of test pathogen (*Aspergillus niger*) with ultimately the highest percent mycelial inhibition (64.6%).

Out of three antagonists tested *Pseudomonas fluorescens* was found the most effective and recorded significantly least linear mycelial growth of 41.1mm of test pathogen (*Aspergillus flavus*) with ultimately the highest percent mycelial inhibition (53.8%).

Out of three antagonists tested *Trichoderma harzianum* was found the most effective and recorded significantly least linear mycelial growth of 34.28 mm of test pathogen (*Fusarium oxysporum*) with the highest percent mycelial inhibition (61.91%).

***In-vitro* evaluation of botanicals @ 10% concentration.** A total of eleven botanicals @10% conc. were evaluated *in-vitro* against *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum.* The results obtained are presented in Table 3 and Fig. 3(a) and 3(b).

At a 10% concentration, the least mycelial growth was recorded by *Curcuma longa* (20.92mm) with the highest percent mycelial inhibition (76.75%) of the test pathogen (*Aspergillus niger*). At a 10% concentration, the least mycelial growth was recorded by *Zingiber officinale* (51.40mm) with the highest percent mycelial inhibition (42.55%) of the test pathogen (*Aspergillus flavus*). At a 10% concentration, the least mycelial growth was recorded by *Curcuma longa* (48.14mm) with the highest percent mycelial inhibition (46.51%) of the test pathogen (*Fusarium oxysporum*).

The results presented were discussed with the findings of the following researchers.

Our reports confirmed with Mohammed and Chala (2014) reported *Aspergillus niger* pathogenicity when seeds were involved, while Mohapatra and Sahoo (2011) reported *Aspergillus niger* pathogenicity through soil inoculation. Prajapati *et al.* (2016) found that among the fungicides they tested, carbendazim, a combination of carbendazim (12%) and mancozeb (63%), a mixture of mancozeb (50%) and carbendazim (25%), difenoconazole (11.4%), trifloxystrobin (25%) in combination with tebuconazole (50%) at concentrations of 500 and 100 ppm, and hexaconazole and propiconazole at concentrations of 1000 and 1500 ppm, all completely inhibited the mycelial growth of *A. niger* compared to the control group. Wani and Kuruchave (2004) reported that carbendazim + mancozeb proved to be the most effective in complete inhibition (100%) of mycelium growth for both *A. niger* and *A. flavus*.

Attri (2016); Sonakshi (2018), both of which reported the highest inhibition of *Fusarium oxysporum* mycelial growth with the use of carbendazim and trifloxystrobin in combination with tebuconazole. Sharma and Saha (2013) and Nathawat and Pratap (2014) also previously documented the effectiveness of *T. harzianum* in reducing the radial growth of *A. niger in vitro*. The effectiveness of *Trichoderma* spp., *P. fluorescens*, and *Bacillus* sp. against *F. oxysporum* has been extensively documented in various studies (Lifshitz *et al*., 1986; Jee and Kim 1987; Moon *et al*., 1988; Cho *et al*., 1989; Leeman *et al*., 1995; Larkin and Fravel 1998; Hamid, 1999; Prasad *et al.*, 2002). Our current findings align with Cho *et al.* (1989); Hamid (1999) research, highlighting that *T. harzianum* is particularly effective against *Fusarium oxysporum* f. sp. *cucumerinum*. These findings are consistent with the results reported by Baig *et al*. (2012); Sudha *et al*. (2013); Spadaro and Gullino (2005), which revealed that the lowest percent inhibition (64.50%) was observed for *Pseudomonas fluorescens* in terms of its effect on the growth of *Aspergillus flavus.* These findings also align with the results reported by Shukla and Dwivedi (2012), who observed an 89.2% inhibition of *Fusarium* sp. growth when using a 15% concentration of turmeric (*Curcuma longa*).

Table 1: *In vitro* evaluation of fungicides against *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum*.

Aspergillus niger				*Aspergillus flavus*		*Fusarium oxysporum*	
Tr. No.	**Treatments**	**Colony diameter (mm)***	**% inhibition**	**Colony diameter (mm)**	**% inhibition**	**Colony diameter (mm)**	**% inhibition**
T1	Chlorothalonil	0.00	100 (90.00)**	0.00	100 (90.00)	6.45	92.83 (74.49)
T2	Propiconazole	5.71	93.65 (75.40)	0.00	100 (90.00)	15.11	83.20 (65.80)
T3	Azoxystrobin	88.8	1.33 (6.62)	59.73	32.80 (34.94)	10.33	88.52 (70.2)
T4	Tebuconazole	0.00	100 (90.00)	7.15	91.95 (73.51)	20.41	77.32 (61.56)
T5	Carbendazim 12% + Mancozeb 63%	0.00	100 (90.00)	5.37	93.95 (75.76)	0.00	100 (90.00)
T6	Tebuconazole 50% + Trifloxystrobin 25%	5.38	94.01 (75.83)	0.00	100 (90.00)	9.61	89.32 (70.92)
T7	Thiophanate methyl	7.83	91.29 (72.83)	0.00	100 (90.00)	12.18	86.46 (68.40)
T8	Carboxin 37.5% + Thiram 37.5%	8.69	90.33 (71.88)	8.6	90.32 (71.87)	12.40	86.21 (68.20)
T9	Difenoconazole	9.43	89.51 (71.10)	10.67	87.98	24.21	73.43 (58.97)
T10	Hexaconazole	6.98	92.23 (73.81)	0.00	100 (90.00)	11.92	86.75 (68.65)
T11	Control	90.00	0.00 (0.00)	88.9	0.00 (0.00)	90.00	0.00 (0.00)
SE(m)		0.53	0.59	0.21	0.24	0.51	0.62
CD 5%		1.57	1.74	0.61	0.69	1.49	1.81
CV		4.58%	1.33%	2.20%	0.5%	4.57%	1.36%

*Average of three replications.
**Figures in parentheses are arc sin transformation values.

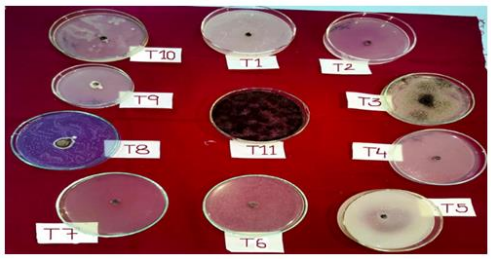

Fig. 1(a). *In vitro* evaluation of fungicides against *Aspergillus niger*.

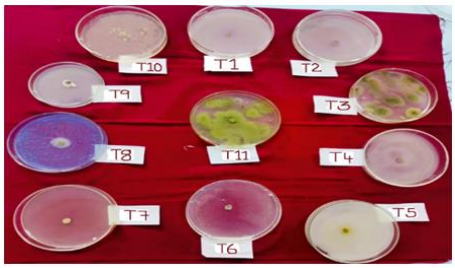

Fig. 1(b). *In vitro* evaluation of fungicides against *Aspergillus flavus*.

Table 2: *In vitro* evaluation of Biocontrol agents against *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum*.

Aspergillus niger				*Aspergillus flavus*		*Fusarium oxysporum*	
Tr. No.	**Treatments**	**Colony diameter (mm)***	**% Inhibition**	**Colony diameter (mm)**	**% Inhibition**	**Colony diameter (mm)**	**% Inhibition**
T1	*Trichoderma asperellum*	56.53	36.47 (37.15)**	42.7	52.01 (46.15)	55.45	38.38 (38.28)
T2	*Pseudomonas fluorescens*	46.3	47.97 (43.83)	41.16	53.74 (47.14)	47.61	47.1 (43.33)
T3	*Trichoderma harzianum*	31.56	64.52 (53.44)	43.73	50.85 (45.48)	34.28	61.91 (51.89)
T4	Control (Untreated)	89.00	0.00 (0.00)	89.00	0.00 (0.00)	90.00	0.00 (0.00)

*Average of three replications.
**Figures in parentheses are arc sin transformation values.

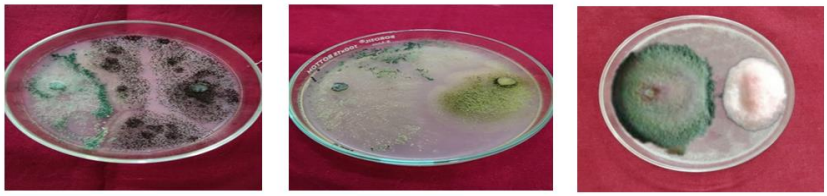

Fig. 2(a). *In vitro* evaluation of *Trichoderma asperellum* against *A. niger*, *A. flavus*, and *Fusarium oxysporum.*

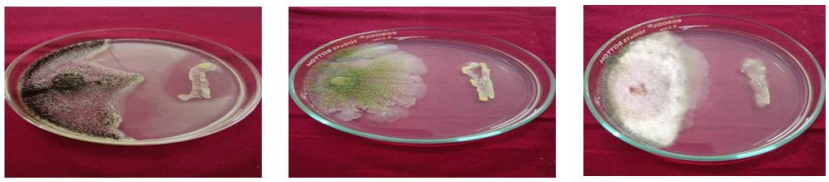

Fig. 2(b). *In vitro* evaluation of *Pseudomonas fluorescens* against *A. niger*, *A. flavus*, and *Fusarium oxysporum*.

Table 3: *In vitro* evaluation of Botanicals @10% against *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium oxysporum*.

		Aspergillus niger		*Aspergillus flavus*		*Fusarium oxysporum*	
Tr. No.	**Treatments**	**Colony diameter (mm)***	**% inhibition**	**Colony diameter (mm)**	**% inhibition**	**Colony diameter (mm)**	**% inhibition**
T1	*Curcuma longa* (Turmeric)	20.92	76.75 (61.17)**	53.49	39.89 (39.16)	48.14	46.51 (42.99)
T2	*Allium cepa* (Onion)	86.60	3.77 (11.19)	77.29	13.15 (21.26)	55.12	38.75 (38.49)
T3	*Pongamia pinnata* (Karanj)	84.86	5.71 (13.82)	68.52	23.00 (28.65)	79.07	12.14 (20.39)
T4	*Tagetes* sp. (Marigold)	75.49	16.12 (23.67)	85.66	3.74 (11.15)	79.89	11.23 (19.57)
T5	*Annona squamosa* (Custard apple)	62.24	30.84 (33.73)	85.81	3.57 (10.89)	74.72	16.97 (24.32)
T6	*Azadirachta indica* (Neem)	78.21	13.1 (21.22)	61.56	30.83 (33.72)	61.37	31.81 (34.33
T7	*Allium sativum* (Garlic)	87.77	2.47 (9.04)	87.93	1.12 (6.07)	70.49	21.67 (27.74)
T8	*Lantana camara* (Lantana)	51.01	43.32 (41.16)	86.45	2.86 (9.73)	52.87	41.25 (39.96)
T9	*Zingiber officinale* (Ginger)	63.19	29.78 (33.07)	51.40	42.24 (40.53)	77.35	14.05 (22.01)
T10	*Tinospora cordifolia* (Giloy)	68.10	24.33 (29.55)	78.52	11.77 (20.06)	81.85	9.05 (17.50)
T11	*Eucalyptus* sp. (Eucalyptus)	41.76	53.6 (47.06)	80.68	9.68 (18.12)	81.38	9.57 (18.02)
T12	Control (Untreated)	90.00	0.00 (0.00)	89.00	0.00 (0.00)	90.00	0.00 (0.00)
SE(m)		0.50	0.55	0.57	0.65	0.54	0.60
CD 5%		1.45	1.61	1.69	1.91	1.58	1.76
CV		1.27%	3.83%	1.32%	7.48%	1.33%	4.95%

*Average of three replications; **Figures in parentheses are arc sin transformation values.

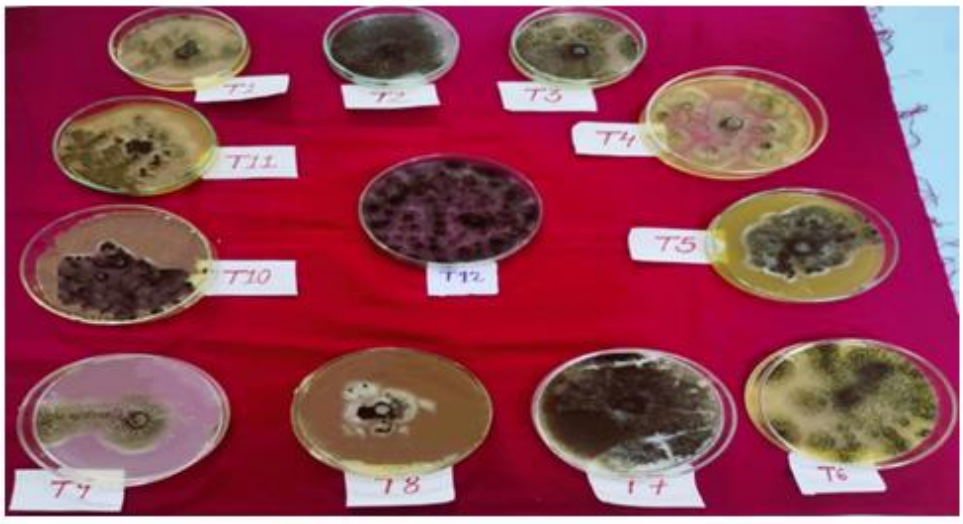

Fig. 3(a). *In vitro* evaluation of botanicals @10% concentration against *Aspergillus niger*.

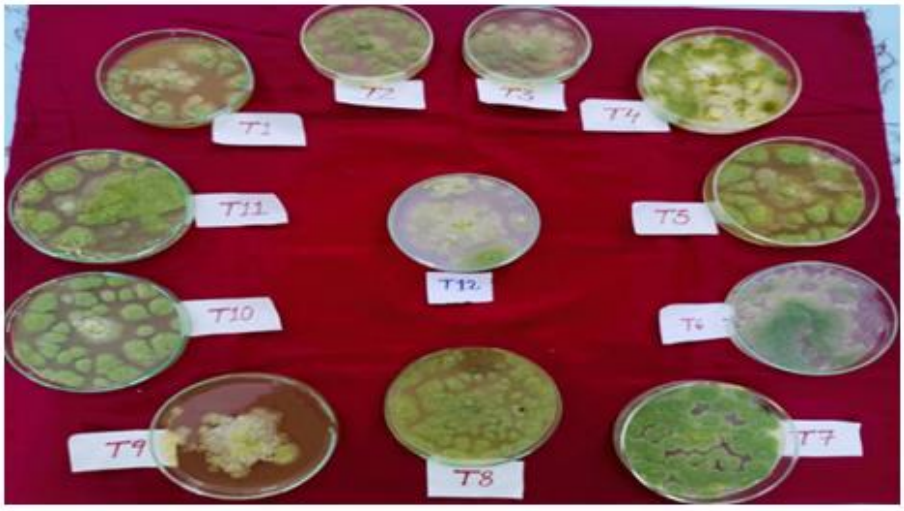

Fig. 3(b). *In vitro* evaluation of botanicals @10% concentration against *Aspergillus flavus*.

CONCLUSIONS

In the majority of agricultural crop development initiatives, a significant emphasis is placed on enhancing the quality of seeds. This involves increasing their potential for high yields, ensuring their purity, and viability, and promoting strong germination capabilities. The presence of microorganisms can severely impact many of these attributes.

All three test fungi's highest mycelial growth inhibition was recorded in the fungicide Carbendazim 12%+ Mancozeb 63% (100%) and Chlorothalonil (100%). However, *Trichoderma harzianum* was recorded to be the most effective against *Aspergillus niger* (64.52%) and *Fusarium oxysporum* (61.91%) with the least linear mycelial growth and the highest percent of mycelial growth inhibition. *Pseudomonas fluorescens* was recorded to be the most effective, having the least linear mycelial growth with the highest percent of mycelial inhibition of *Aspergillus flavus* (53.74%). The highest average mycelial growth inhibition recorded with botanicals @10%, *Curcuma longa* showed 76.75% inhibition of *Aspergillus niger*. The highest average mycelial growth inhibition was recorded in *Zingiber officinale* at 10% concentration revealed 42.24% inhibition of *Aspergillus flavus*. The highest average mycelial growth inhibition was recorded in *Curcuma longa* at 10% concentration which exhibited 46.51% inhibition of *Fusarium oxysporum*.

FUTURE SCOPE

The future of disease management will likely be shaped by the need for more sustainable, eco-friendly solutions, where biocontrol agents, botanicals, and fungicides all have roles to play. However, the conflict of interest between the chemical industries, regulatory bodies, and environmental groups will continue to influence the pace and direction of these advancements. Balancing innovation with sustainability and overcoming these conflicts will be key to the future of crop protection.

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