Plant Archives Volume 20 No. 2, 2020 pp. 3696-3700 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

STUDIES ON EFFECT OF SELECTED FUNGICIDES, BOTANICALS AND ANTAGONISTS AGAINST *TRICHODERMA HARZIANUM* UNDER *IN-VITRO* CONDITION

Saugat Sharma Neupane and Shivam Singh*

Department of Plant Pathology, School of Agriculture, Lovely Professional University, Phagwara (Punjab), India.

Abstract

Present investigation aimed at finding the best methods to bring *Trichoderma harzianum*, causes green mould disease in oyster mushroom (*Pleurotus florida*), under control by evaluating the efficacy of various chemicals, botanicals and antagonists under different concentrations. Chemicals namely Carbandazim and Hexaconzole at 3 different concentrations *viz.*, 100, 150 and 200 ppm were used, in which Carbandazim demonstrated best efficacy against *T. harzianum* mycelium under *in-vitro* condition with maximum average mycelium inhibition of 95.27% with least suppression of *P. florida* mycelium at 33.652% mean inhibition. As for Botanical extracts, neem (*Azadirachta indica*) leaf extract and *Lantana camara* leaf extract were studied *in vitro*, whereby *L. camara* showed higher efficacy against *T. harzianum* mycelium with mean inhibition of 34.02%. Novel concept of utilizing antagonistic properties of *Bacillus subtilis* and *Pseudomonas fluorescence* were tested using dual culture technique. Both were statistically identical in their results with *P. fluorescence* showing relatively better *In-vitro* efficacy with mean mycelium inhibition of *T. harzianum* at 43.545% and much lower mean inhibition against *P. florida* at 7.95%.

Key words: Antagonists; botanicals; chemicals; *Pleurotus florida; Trichoderma harzianum*

Introduction

Pleurotus species usually referred to as the oyster mushroom or dhingri mushroom are grown worldwide among commercially grown mushrooms and contribute about 27 percent of total global production (Royse, 2014). It has the highest protein content and many additional elements, including vitamin B_1, vitamin B_2 and low calorie levels. Besides, they are stated to be low in fat (2-3% by dry weight), a good source of essential amino acids and contain 5-9% fiber (Yang *et al.*, 2001). This mushroom is, unfortunately, subject to many natural vagaries, namely pests and diseases, which adversely affect its production and productivity. Between the various *Pleurotus* spp. competitors and moulds, when growing this fungus, green moulds are stated to be destructive diseases. *Trichoderma viride* and *Trichoderma harzianum* have been described as the principal fungal species producing green mould.

Moulds show rapid growth in these favorable conditions, thereby vying more effectively for space and nutrients than the mushrooms (Chen and Moy, 2004). They are also capable of producing extracellular enzymes, harmful secondary compounds and volatile organic compounds which can lead to a major reduction in production or the wiping out of the entire crop. Pathogenic green moulds may either colonize the substratum or grow on the surface of the developing mushrooms, often heavily spotted and distorted. Besides, in severe outbreaks, no fruiting bodies are produced. Trichoderma spp. produce white mycelia which is not easily distinguishable from mushrooms during spawning, making early infection difficult to detect (Largeteau *et al.*, 2004).

The key symptom of green mould disease is the appearance of greenish mycelium in *P. florida*, bagging layer, or fruiting bodies, 2-5 weeks after the growth cycle has started. Pathogen prevents mushroom growth, and the fruiting bodies aren't developed in extreme outbreaks. It dramatically impacts the mushroom markets, as the green mould epidemic of *Trichoderma* plagues most oyster mushroom farms. Although the first flush of the production can be saved with strict hygiene, *Trichoderma* green mould often decreases second flush yield by 20-30

***Author for correspondence :** E-mail : shivam.23596@lpu.co.in

per cent (Nagy *et al.*, 2012).

Chemicals and fungicides are the frontrunners once the regulation has been achieved for these *Trichoderma*. Some systemic fungicides: Carbendazim, Bitertanol and Hexaconzole, and other fungicides that are non-systemic like Captan and Mancozeb have been producing better results. However, a significant issue is damage to the oyster's fungal mycelium and the hazards of retention of chemicals in the plant, which brings many health and environmental issues. *Bacillus* and *Pseudomonas* antagonism provides a superior alternative to synthetic chemicals. Antagonism is economical, environmentally sustainable and an alternative to chemical fungicides for the control and degradation of oyster mushroom diseases. Bacteria such as *Bacillus* spp. were investigated for their ability to produce antifungal metabolites that protect plants from fungal infection. (Nourozian *et al.*, 2006).

The ultimate goal of this study was to find out the efficacy of synthetic chemicals on the control of *Trichoderma*, compare it with the control provided by *Bacillus* sp. and *Pseudomonas fluorescence* along with the efficacy of different botanicals so that we have an idea to the extent, these methods are victorious against the green mould.

Materials and Methods

Culture collection

In present investigation, *Trichoderma harzianum* was isolated and identified from Green mold infected bags in the Mushroom Production Room, Department of Plant Pathology, SAGR, LPU, Punjab. *Pseudomonas fluorescence* was isolated and identified from the root zone of rice field whereas *Bacillus subtilis* was acquired from Bio-technology Laboratory, LPU.

In-vitro evaluation

In this study, two systemic chemical fungicides: Carbandazim and Hexaconazole@100, 150 and 200ppm, two botanical extracts *viz*. leaf extracts of neem (*Azadirachta indica*) and *Lantana camara* @5% and 10% concentrations and two antagonists *Bacillus subtilis* and *Pseudomonas fluorescence* were used for estimation of their efficacy against mycelium growth of *T. harzianum* and *P. florida.*

In this experiment, PDA (Potato Dextrose Agar) has been used as the basal medium. Standard methods *viz.*, poisoned food technique and dual culture method, were used for estimation of chemicals, botanicals and antagonists against mycelium of *T. harzianum* and *P. florida*.

The per cent inhibition of growth of the fungus in each treatment was calculated by using the following formula:

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

Where,

I = inhibition of radial mycelial growth

C = radial growth of fungal mycelium in control

T = Radial growth of fungal mycelium in the presence of test chemicals/botanical extracts/antagonists.

Results and Discussion

From the glance at the data, it is clear that both the fungicides were more or less significant in inhibiting the mycelial growth of *T. harzianum* but at varying degree. Among the test chemicals, Carbandazim seemed highly superior at all concentrations. It executed 85.83%, 100% and 100% inhibition at 100,150 and 200 ppm, respectively with the mean inhibition recorded at 95.276%. Similarly, Hexaconazole showed 8.023%, 18.55% and 20.25% mycelial inhibition against pathogen at 100, 150 and 200ppm, respectively with mean inhibition recorded at 15.607% table 1. It was clear that the growth inhibition increases with increasing concentrations. Hence, Carbandazim was found superior with (100%) mycelia inhibition against *T. harzianum* at 150 and 200 ppm

Table 1: *In-vitro* evaluation of selected fungicides against *T. harzianum*:

Sr. No.		Radial growth of mycelium over control (cm)*			Mean radial growth (cm)	Percentage inhibition in mycelia growth over control (%)*			Mean percentage inhibition (%)
	Chemical Fungicides	**100 ppm**	**150 ppm**	**200 ppm**		**100 ppm**	**150 ppm**	**200 ppm**	
1.	Carbandazim	1.275	0	0	0.425	85.83	100	100	95.27667
2.	Hexaconazole	8.278	7.33	7.173	7.59367	8.023	18.55	20.25	15.60767

Source of variation (SOV)	S.Em(±)	C.V	C.D @ 5%
Treatment	1.53	5.553	3.999
Treatment × Concentration	1.014	3.659	3.126

*average of 4 replications

Table 2: *In-vitro* evaluation of selected fungicides against *P.florida.*

Sr. No.		Radial growth of mycelium over control (cm)*			Mean radial growth (cm)	Percentage inhibition in mycelia growth over control (%)*			Mean percentage inhibition (%)
	Chemical Fungicides	100 ppm	150 ppm	200 ppm		100 ppm	150 ppm	200 ppm	
1.	Carbandazim	6.6	5.98	5.31	5.963	26.66	33.356	40.94	33.652
2.	Hexaconazole	2.138	2.0075	1.92	1.92	76.25	77.69	78.64	77.527

Source of variation (SOV)	S.Em(±)	C.V	C.D @ 5%
Treatment	1.168	4.202	3.037
Treatment × Concentration	1.066	3.835	3.288

*average of 4 replications

supported by the study of (Shah *et al.*, 2013) whereby, they reported maximum inhibition (90.8%) exhibited by Carbandazim at 500 ppm.

From the data depicted in table 2, it is evident that both the fungicides were more or less significant in suppressing the mycelial growth of *P. florida* but at varying degree. Among the tested chemical fungicides, Hexaconazole seemed highly inhibitory at all concentrations. It executed 76.25%, 77.69% and 78.64% inhibition at 100,150 and 200 ppm. respectively with the mean inhibition recorded at 77.527%. Similarly, Carbandazim showed 26.66%, 33.356% and 40.94% mycelial inhibition against pathogen at 100 ppm, 150 ppm and 200 ppm respectively with mean inhibition recorded at 33.652%.

It is clear that the growth inhibition increases with increasing concentrations. So, it is understood that all the chemicals more or less suppresses the growth of *P. florida*. Hence, Carbandazim was found least inhibitory with (26.66%) mycelia inhibition against *P. florida* at 100 ppm supported by the study of (Shah *et al.*, 2011) whereby, they reported minimum inhibition (18.1%) exhibited by Carbandazim at much lower concentration of 25 ppm.

From table 3, it is comprehensible that both the botanicals were more or less significant in inhibiting the mycelia growth of *T. harzianum* at both concentrations (5% and 10%). Among the tested botanicals, *Lantana camara* leaf extract deemed slightly superior at both concentrations. It executed 48.72% and 52.34% inhibition at 5% and 10% concentrations, respectively with the mean inhibition recorded at 50.53%. Similarly, Neem leaf extract (*Azadirachta indica*) showed 31.58% and 36.45% mycelial inhibition against pathogen at 5% and 10% concentrations respectively with mean inhibition recorded at 34.015%.

Hence, it was clear that the growth inhibition increases with increasing concentrations. Hence, *L. camara* leaf extract was found superior with (36.45%) mycelia inhibition against *T. harzianum* at 10% concentrations supported by the study of (Pervez et al., 2009) whereby, they reported maximum inhibition (52.3%) exhibited by *L. camara* ethanol extract at 10% concentrations.

Among the tested botanicals, for their suppression against mycelia growth of *P. florida*, *L. camara* leaf extract appeared to be less inhibitory at all concentrations. It executed 23.51% and 28.94% inhibition at 5% and 10% concentration respectively with the mean inhibition of 26.225% table 4. Similarly, Neem leaf extract (*A. indica*) showed 24.96% and 32.45% mycelia growth inhibition against *P. florida* at 5% and 10% concentrations, respectively with mean inhibition of 33.652%.

So, it is understood that both the botanical extracts more or less suppresses the growth of *P. florida*. Hence, *Lantana camara* was found least inhibitory with

Table 3: *In-vitro* evaluation of selected botanicals against *T. harzianum.*

Sr. No.		Radial growth of mycelium over control (cm)*		Mean radial growth (cm)	Percentage inhibition in mycelia growth over control (%)*		Mean percentage inhibition (%)
	Botanicals	@ 5%	@ 10%		@ 5%	@ 10%	
1.	Neem leaf extract (*Azadirachta*)	6.148	5.71	5.929	31.58	36.45	34.015
2.	*Lantana camara* leaf extract	4.482	4.288	4.385	48.72	52.34	50.53

Source of variation (SOV)	S.Em(±)	C.V	C.D @ 5%
Treatment	1.74	8.217	5.527
Treatment × Concentration	0.85	4.046	2.960

*average of 4 replications

Table 4: *In-vitro* evaluation of selected botanicals against *P. florida*.

Sr. No.	Botanicals	Radial growth of mycelium over control (cm)*		Mean radial growth (cm)	Percentage inhibition in mycelia growth over control (%)*		Mean percentage inhibition (%)
		@ 5%	@ 10%		@ 5%	@ 10%	
1.	Neem leaf extract (*Azadirachta*)	6.984	6.075	6.529	24.96	32.45	28.705
2.	*Lantana camara* leaf extract	6.184	6.339	6.689	23.51	28.94	26.225
Source of variation (SOV)		S.Em(±)		C.V		C.D @ 5%	
Treatment		0.79		5.850		2.511	
Treatment × Concentration		1.31		6.482		3.194	

*average of 4 replications

(23.51%) mycelia inhibition against *P. florida* at 5% concentration supported by the study of (Pervez et al., 2009) whereby, they too reported minimum inhibition (23.5%) exhibited by exhibited by *Lantana camara* ethanol extract at 5% concentrations.

From table 5, it is evident that both the antagonists had statistically identical significance in inhibiting the mycelia growth of *T. harzianum*. Among the tested strains, *Pseudomonas fluorescence* seemed slightly superior in inhibiting the pathogen. It executed 43.545% inhibition whereas *Bacillus subtilis* showed 42.215% mycelial inhibition. The study was further bolstered by the similar finding from the research by (Shah & Nasreen, 2011) whereby they reported *P. fluorescence* had maximum mycelia growth inhibition of 44.6% using dual culture technique as well.

Table 5: *In-vitro* evaluation of selected antagonists on growth of *T. harzianum.*

Sr. No.	Antagonistic Bacteria		Colony dia-meter (cm)*	Percentage inhibition in mycelia growth (%)*
1.	*Bacillus subtillis*		5.2	42.215
2.	Pseudomonas fluorescence		5.07325	43.545
Source of variation (SOV)		S.Em(±)	C.V	C.D @ 5%
Treatment		6.03	10.618	2.36

* mean of 4 replications

From table 6, it is intelligible that both the antagonists were more or less significant in inhibiting the mycelial growth of *P. florida*. Among the tested strains, *P. fluorescence* seemed less inhibitory against the mycelium of *P. florida*. It executed 7.95% inhibition whereas *B. subtilis* showed 21.84% mycelial inhibition. The study was further bolstered by the similar finding from the research by (Shah & Nasreen, 2011)whereby they reported *P. fluorescence* had maximum mycelia growth inhibition of 6.23% using dual culture technique as well.

Table 6: *In-vitro* evaluation of selected antagonists on growth of *P. florida.*

Sr. No.	Antagonistic Bacteria		Colony dia-meter (cm)*	Percentage inhibition in mycelia growth (%)*
1.	*Bacillus subtillis*		7.115	21.94
2.	Pseudomonas fluorescence		8.3112	7.95
Source of variation (SOV)		S.Em(±)	C.V	C.D @ 5%
Treatment		1.179	15.763	4.076

* mean of 4 replications

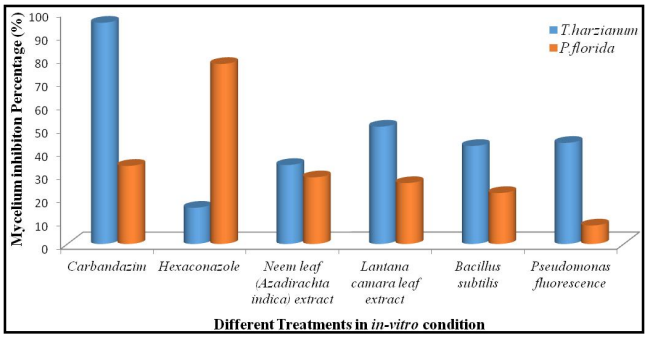

Fig. 1: Percentage of inhibition by selected chemical fungicides, botanicals and antagonists on *T. harzianum* and *P. florida* mycelia growth.

Conclusion

In a nutshell, the present investigation fulfilled its aim to compare and contrast the efficacy of various treatments, suggest the best chemical fungicide, provide an alternative with botanical extracts as well as proving a hypothesis for utilization of antagonism of bacteria as biological control measure which are economically viable, environmentally sound, easily available to remote areas, better farmer access and lower drift hazard.

References

Chen, A.W. and M. Moy (2004). Mushroom cultivation: building mold contamination. *Proceedings of 16th ISMS International Congress, Miami, USA.*

Largeteau, M.L., G. Mata and J.M. Savoie (2004). *Verticillium*

fungicola var. fungicola affects *Agaricus bisporus* cultivation in Mexico. *FEMS Microbiology Letters*, **236(2):** 191–196. https://doi.org/10.1016/j.femsle.2004.05.032.

Nagy, A., L. Manczinger, D. Tombácz, L. Hatvani and J.G. Ê (2012). Biological control of oyster mushroom green mould disease by antagonistic *Bacillus* species. *Biological Control of Fungal and Bacterial Plant Pathogens*, **78:** (May 2014), 289-293.

Nourozian, J., H.R. Etebarian and G. Khodakaramian (2006). Biological control of *Fusarium graminearum* on wheat by antagonistic bacteria. *Songklanakarin Journal of Science and Technology*, **28(1):** 29-38.

Pervez, Z., M. Islam and S. Islam (2009). Evaluation of some plant extracts in controlling green mold (*Trichoderma harzianum*) associated with substrate of oyster mushroom. *Bangladesh Res. Pub. J.*, **7(33):** 194-200. http://www.bdresearchpublications.com/admin/journal/upload/09333/09333.pdf.

Royse, D.J. (2014). A Global Perspective on the High Five/: Agaricus, Pleurotus. *International Conference on Mushroom Biology and Mushroom Products, Usitc 2010*, 2010-2015.

Shah, S. and S. Nasreen (2011). Evaluation of Bioagents against the Infection of Green Mould (Trichoderma spp.) in *Pleurotus sajor-caju* Cultivation. *International Journal of Plant Pathology*, **2:** 81-88. https://doi.org/10.3923/ijpp.2011.81.88.

Shah, S., S. Nasreen and S. Kousar (2013). Efficacy of fungicides against Trichoderma spp. Causing Green mold disease of Oyster mushroom (*Pleurotus sajor-caju*). In *Research Journal of Microbiology*, **8(1):** pp. 13-24. https://doi.org/10.3923/jm.2013.13.24.

Yang, W., Y.H. Hong, X.Q. Shen, C. Frankowski, H.S. Camp and T. Leff (2001). Regulation of transcription by AMP-activated protein kinase. Phosphorylation of p300 blocks its interaction with nuclear receptors. *Journal of Biological Chemistry*, **276(42):** 38341-38344. https://doi.org/10.1074/jbc.C100316200.