Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(5): 2911-2915
Received: 16-07-2018
Accepted: 18-08-2018

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Evaluation of different fungicides and biopesticides under *in vitro* and *in vivo* conditions of oyster mushroom (*Pleurotus florida* Eger) in Chhattisgarh

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Abstract
The present investigation entitled "Evaluation of different fungicides and biopesticides under *in vitro* and *in vivo* conditions of oyster mushroom (*Pleurotus florida* Eger) in Chhattisgarh" was undertaken with the objectives to find out the effect of fungicides and biopesticides under *in vitro* and *in vivo* conditions on radial growth, inhibition per cent, growth and yield of *P. florida*. The radial growth of *P. florida* varied significantly with respect to different fungicides and their concentrations used. Bavistin, subeej and sultaf at 50–75 ppm were highly effective fungicides under *in vitro* conditions as they did not much inhibit the growth of *P. florida.* Straw treatment with rilon + formalin (75ppm+500ppm), bavistin+ formalin (75ppm+500ppm) and subeej+ formalin (75ppm+500ppm) were effective under *in vivo* conditions. The radial growth of *Pleurotus florida* differed significantly with respect to different biopesticides and their recommended concentration. Minzyme, neem gold and raze were found to be effective under *in vitro* as well as *in vivo* conditions.

Keywords: Systemic fungicides, Biopesticides, radial growth, inhibition, yield, *Pleurotus florida*

Introduction
Mushrooms are reproductive structures of edible fungi and considered as delicacy of food. They have been in existence for millions of year and were known to us even before the origin of man (Kohli, 1990) [13]. Mushroom occurs seasonally all over the world in various habitats varying from sandy plains to thick forests or green meadows to roadside pathways. There are over 10,000 kinds of fleshy fungi, of which over 100 are widely consumed and over 50 are traded internationally (Kohli, 1990) [13]. But, only a few species have been brought under cultivation on commercial scale. World production of mushroom is around 7.2 million tones (Thakur, 2005) [18] with an average annual growth of 7.5 per cent and the production is mainly concentrated in Asia (77.4%), Europe (16.3%) and North America (7%). During 1990, oyster mushroom was estimated to be 24.1 per cent of the total world production of commercial mushrooms (Bahl, 1995) [2].

Oyster mushrooms (*Pleurotus* spp.) are a group of edible fleshy fungi belonging to division basidiomycotina and family Tricholomataceae. It now ranks third among the important cultivated mushrooms of the world. Out of 28 species of *Pleurotus* reported from India (Verma, 1996) [19], more than a dozen are under cultivation in different parts of the country (Balakrishnan and Nair, 1995) [3].

India produced 40,000 tonnes of cultivated mushrooms during 1996-97 (Dhar, 1997), which has further been increased to 55,000 tonnes during 2004-05 of which, 1000-1200 tonnes of mushroom were estimated to be produced from Chhattisgarh. The important species of *Pleurotus* grown in India are *P. eryngii, P. eous, P. florida, P. fossulatus, P. squarrosulus, P. cornucopiae, P. platypus, P. columbinus, P. sajor-caju, P. ostreatus, P. tubereginum, P. flabellatus, P. membranaceus, P. petaloides,*. of these, *P. florida* is very much liked by the people of Chhattisgarh and grown in a widespread area.

In India, majority of the people are vegetarian and mushroom became an important source of nutrition in the cereal- based diet. The nutritive value of mushroom varies with the genotype, maturity, substrate, cultivation technology, post-harvest care and processing (Chadha and Sharma, 1995) [2, 3, 6]. It is considered in between fruits and vegetables. It contained 20-30 percent protein with all essential amino acids (Leucine, lysine and tryptophan) which is deficient in most of the staple cereals and vegetables. Digestibility of mushroom protein is about 60 to 70 percent with digestive coefficient of about 87 per cent. It is an excellent source of folic acid and contains a good amount of vitamin C and vitamin B complex group

(thiamine, riboflavin, niacin) with minerals like calcium, sodium, potassium, iron, copper, zinc, manganese and magnesium). It contains low fat and sugar, which makes them a choice diet for those suffering from diabetic, obesity and hypertension.

The chemical composition of different *Pleurotus* spp. thus differed with the substrate, time of harvesting, moisture content of the substrate and method of assessing the biochemical constituent and hence, it was attempted to study the biochemical constituents as influenced by locally available substrates.

A large number of substrates viz., wheat straw, paddy straw, cotton stalks and various other agro and industrial wastes were evaluated for cultivating different *Pleurotus* spp. by several workers all over the country (Jandaik, 1974; Bano *et al.*, 1987; Khandar *et al.*, 1991; Mehetre, 1996; Biswas, 1992 and Ram, 1995) [11, 4, 12, 14, 5, 17]. But, cereal straw gave consistently good yields. Similarly, various substrates for spawn preparation have been tried but wheat grain spawn was found to be most popular in our country (Chadha and Sharma, 1995) [2, 3, 6]. An attempt was further made to evaluate locally and cheaply available substrates for production of spawn as well as crop of *P. florida* in Chhattisgarh.

Oyster mushroom cultivation is normally practiced on paddy straw and wheat straw as a substrate. These straw substrates were contaminated by fungal, bacterial, viral and nematodes problems resulting in partial or total failure of the mushroom crop. Attempts were made by different workers to manage the weed fungi/competitor moulds and increase the yield (Chakravarty *et al* 1982 and Vijay and Sohi, 1987) [7, 20]. In the present study, attempts were therefore made to study some bio-pesticides / systemic fungicides, which may take care of the contaminants and enhance the crop yield without being adversely affecting the host fungus. Keeping in view of the above, the present investigation was carried out with the objectives to evaluate the effect of different fungicides and biopesticides under *in vitro* and *in vivo* conditions on radial growth, inhibition per cent, growth and yield of *P. florida*.

Materials and methods

The present research experiments were conducted in the Mushroom Research Laboratory, Department of Plant Pathology, College of Agriculture, Indira Gandhi Krishi Vishwavidyalaya, Raipur and College of Agriculture and Research Station, IGKV, Jagdalpur (C.G.). Completely Randomized Design was employed for all the statistical analysis work. The critical difference (C.D.) or least significant difference (L. S. D) was calculated at 5% probability level. The pure cultures of *Pleurotus spp*. used during present experiment were procured from Mushroom Research Laboratory, Department of Plant Pathology, IGKV, Raipur (C.G.). The straw substrates used were paddy, wheat, til, maize, moong, gram, arhar, linseed, safflower, mustard, sugarcane baggasse, lucerne, pea, kodo, kutki, ragi soybean, sesamum, sunflower, groundnut pods and ramtil. These were obtained from the Instructional Farm, College of Agriculture, IGKV, Raipur. The empty glucose bottles, polyethylene bags, cereal grains (wheat, rice, sorghum, maize, bajra, kodo and kutki), supplements (rice bran, rice flour, gram, dal powder, wheat bran, soybean meal), chemicals and other things used during the study were made available in the Department of Plant Pathology, College of Agriculture, IGKV, Raipur.

Eleven fungicides viz., bavistin, foltaf, rilon, subeej, sultaf, topaz, topsin – M, antracol, contaf, copper hydroxide and sporgon each with three concentrations (50, 75, 100 ppm) were incorporated in the medium using food poisoned technique (Nene and Thapliyal, 1987). A 5 mm disc cut from young growing culture of *Pleurotus florida* was placed in the centre of each petridish aseptically. A suitable control was kept where no fungicide was incorporated. Four replications were maintained. The plates were incubated at 25°C $\pm$ 2°C till the mycelial growth in any of the treatments reached to 90 mm. The radial growth of *P. florida* at each concentration was recorded and inhibition percentage was calculated using the standard formula.

The above fungicides were also used for substrate treatment under *in vivo* conditions. They were used in combination with formalin 500 ppm constituting 12 treatments. In control, substrate was immersed in plain water. The experiment was conducted in the month of January- February, 2003. Four replications were maintained. The treatments were, Bavistin + Formalin (75+500ppm), Foltaf + Formalin (75+500ppm), Rilon + Formalin (75+500ppm), Subeej + Formalin (75+500ppm), Sultaf + Formalin (75+500ppm), Topaz + Formalin (75+500ppm), Topsin M + Formalin (75+500ppm), Antracol + Formalin (75+500ppm), Contaf + Formalin (75+500ppm), Copper hydroxide + Formalin (75+500ppm), Sporogan + Formalin (75+500ppm) and Plain water (Control).

These chemicals in combination with formalin 500 ppm were dissolved in 20 litres of water and then the substrate (2 kg) was dipped in it for 18 hours as per the method described by Vijay and Sohi (1987) [20]. The observations on soawn run, yield and BE of *P. florida* was recorded.

Thirteen biopesticides viz., Vanish (1000ppm), Achook (1000ppm), Neemazol T. (1000ppm), Multiplex (1500ppm), Dipel-8L (1000ppm), Neem gold (1500ppm), Halt (1000ppm), Raze (1000ppm), Neemol (2000ppm), Econeem (1000ppm), Minzynic (1000ppm) along with two controls, Bavistin (75 ppm-standard control –I and Plain water Control-II) were incorporated in the medium using food poisoned technique (Nene and Thapliyal, 1987). A 5 mm disc cut from young growing cultures of *P. florida* was placed in the center of each petridish aseptically. Suitable controls were maintained. The plates were incubated at 25 $\pm$ 2^0 C till the mycelial growth in any of the treatment reached to 90 mm. The observations on radial growth of different *Pleurotus* spp. were recorded and per cent inhibition was calculated using the standard formula.

The above biopesticides were also used for paddy straw substrate treatment under *in vivo* conditions constituting sum of 13 treatments including control. Four replications were maintained. The above biopesticides in combinations with formalin (500 ppm) were dissolved in 25 litres of water and then the substrate (2 kg) was immersed in it for 18 hour as per the method described by Vijay and Sohi (1987) [20]. Substrate was spawned and filled in polyethylene bags (500 g dry substrate/bag). The spawned bags were transferred to cropping room. The observations on period of spawn run, days to pinning and yield were recorded.

Results

Evaluation of different fungicides on growth and yield:

a. Effect of fungicides on radial growth (mm) and Inhibition (%)

The effect of systemic fungicides on growth and inhibition of *P. florida* at various concentrations was studied under *in vitro* conditions and the results are presented in Table 1.

The radial growth of *P. florida* varied significantly with respect to different fungicides and their concentrations used.

At 50 ppm, the radial growth of *P. florida* was less than control in all the treatments amended with fungicides. Among fungicides the radial growth of *P. florida* was significantly higher when it was amended with bavistin (81.20 mm) followed by subeej (80.34 mm), rilon (80.23 mm), sultaf (80.20 mm) and copper hydroxide (79.65 mm). However, the radial growth was significantly less when the media was amended with contaf (25.64 mm). Similarly, the radial growth at 75 ppm was higher in media amended with bavistin (77.25 mm) followed by sultaf (76.32 mm) and subeej (76.25 mm). When the fungicidal concentration was further increased the radial growth was significantly higher only in case of bavistin (71.25 mm) whereas, it was significantly less in topaz (14.34 mm) followed by contaf (15.74 mm).

The percent inhibition in radial growth of *P. florida* was minimum and varied from 9.7 to 11.5 per cent at 50 ppm of bavisitin (9.7%) followed by 14.06-14.16 percent inhibition at 75 ppm of bavistin, sultaf and subeej.

At 100 ppm, the percent inhibition in radial growth *P. florida* was minimum (20.27%) compared to that of 84.06% inhibition in case of topaz. Thus, it appears that the fungicides, bavisitin, subeej and sultaf can only be use for treatment of straw substrates as they reduced the radial growth only from 9.7 to 14.16 per cent at 50 to 75 ppm concentration. At higher concentrations, the inhibition in radial growth was higher which indicates that the fungicides at higher concentrations may suppress the growth of test fungus and should not be used.

Table 1: Effect of fungicides on growth and inhibition of *Pleurotus florida*

Fungicides	Radial growth (mm)			Inhibition (%)		
	50	75	100	50	75	100
Bavistin	81.20	77.25	71.25	9.7	14.16	20.27
Foltaf	39.48	30.50	28.25	56.13	66.11	68.61
Rilon	80.23	69.31	60.74	10.85	22.98	32.51
Subeej	80.34	76.25	63.75	10.73	14.16	29.16
Sultaf	80.20	76.32	63.41	10.88	14.08	29.54
Topaz	39.14	26.16	14.34	56.51	70.93	84.06
Topsin M	70.00	69.72	64.31	22.22	22.53	28.54
Antracol	36.50	22.75	20.65	59.44	74.72	77.05
Contaf	25.64	18.62	15.75	64.36	79.31	82.5
Copper hydroxide	79.65	74.35	68.43	11.5	17.38	23.96
Sporogon	46.35	44.00	40.29	48.5	51.11	55.23
Control	90.00	90.0	90.0	0.0	0.0	0.0
SEm±	0.99	0.90	0.86			
CD (0.05%)	2.84	2.57	2.47			

b. Efficacy of promising fungicides on growth and yield

Effect of promising fungicides on spawn run, yield and B.E. of *P. florida* was studied and the results are presented in Table 2.

The results presented in Table 2 indicates that there was significant difference in period of spawn run and yield of *P. florida* among different fungicidal treatments. Treatment of paddy straw with rilon + formalin (75+500 ppm) took significantly less time (14 days) followed by bavistin + formalin (15 days), subeej + formalin (15 days) and sporogon + formalin (15days).The period required for pinning of *P. florida* in case of rilon + formalin was significantly less (21days) followed by bavistin + formalin (22 days) subeej+ formalin (23 days) and sporogon + formalin (22 days) as against maximum days in control. The fresh yield and B.E. of *P. florida* was significantly higher when the straw was treated with rilon + formalin (407g and 81.4% B.E.) followed by subeej + formalin (398 + 79.6% B.E.) and bavistin + formalin (397g and 79.4% B.E.). Minimum yield and B.E. was recorded in case of control (117.5g and 23.0% B.E.).

Thus, it can be said that rilon, subeej and bavistin in combination with formalin was highly effective in increasing the yield without being adversely affecting the mycelial growth of *P. florida.*

Table 2: Efficacy of selected fungicides and vegetative growth and yield of *Pleurotus florida*

Fungicides	Spawn run (days)	Pin head stage (days)	Yield (g/500 g dry substrate)	Biological efficiency (%)
Bavistin 75 ppm	15	22	397.0	79.4
Foltaf 75 ppm	21	29	258.5	51.7
Rilon 75 ppm	14	21	407.0	81.4
Subeej 75 ppm	15	23	398.0	79.6
Sultaf 75 ppm	18	24	371.0	74.2
Topaz 75 ppm	19	26	328.5	65.7
Topsin M 75 ppm	17	25	347.0	69.4
Antracal 75 ppm	20	27	267.5	53.5
Contaf 75 ppm	19	26	322.0	64.4
Copper hydroxide 75 ppm	19	27	282.5	56.5
Sporogan 75 ppm	15	23	367.5	73.5
Control	23	31	117.5	23.5
SEm±	0.74	0.91	6.13	
CD (0.05%)	2.10	2.61	18.52	

Effect of biopesticides on radial growth and inhibition:

a. Effect of biopesticides on *Pleurotus florida* under *in vitro*

The effect of biopesticides on growth and inhibition of *Pleurotus florida* at various concentrations was studied under *in vitro* conditions (Table 3). The radial growth of *Pleurotus florida* differed significantly with respect to different biopesticides and their recommended concentration. The radial growth of *Pleurotus florida* in case of minzyme (a growth promoter) was 88.40 mm, after 7 to 8 days of incubation as against 84 mm in control-II (No biopesticide). The incorporation of minzyme thus promoted the growth of *Pleurotus florida.* In medium amended with neem gold (77.77mm), neemazol-T (76.77 mm), Achook (76.34mm), neemol(76.34mm) the radial growth was though less than control but was statistically at par among themselves and was less affected. However, the radial growth in Dipel-8L was severely affected in comparison to control-II.

There was no inhibition in radial growth of *Pleurotus florida* at 1000 ppm of minzyme rather increase in radial growth was recorded. The average inhibition in mycelial growth of *Pleurotus florida* was less in neem gold (7.41%), neemazol-T (8.90%), achook and neemol (9.11) amended medium. However, the per cent inhibition was more in case of dipel-8L (18.55%) followed by econeem (13.75%) as against 19.1% in control-I (bavistin).

Table 3: Effect of biopesticides on the growth and inhibition of *Pleurotus florida*

Biopesticides	Concentrations (ppm)	*P. florida*	
		Growth (mm)	Inhibition (%)
Vanish	1000	74.50	11.30
Achook	1000	76.34	9.11
Neemazal-T	1000	76.52	8.90
Multiplex	1500	73.48	12.52
Dipel-8L	1000	68.41	18.55
Neemgold	1500	77.77	7.41
Halt	1000	73.98	11.92
Raze	2000	75.00	10.71
Neemol	2000	76.34	9.11
Econeem	1000	72.45	13.75
Ecosteem	1000	73.68	12.28
Minzyme	1000	88.40	+5.23
Neemactin	1500	73.62	12.35
Bavistin (Control-I)	75	72.46	13.73
Control-II (without pesticides)		84.00	
SEm ±		0.85	
CD (0.05%)		2.43	

* Average of four replications.

b. Effect of biopesticides on *Pleurotus florida* under *in vivo* condition

Influence of biopesticides on spawn run and yield of *Pleurotus florida* was studied and the results are presented in Table 4.

Effect of biopesticides on spawn run, pinhead and yield was found to vary significantly with respect to different biopesticides studied. The time required for spawn run was significantly less in neemazal T, raze (11 days) followed by 12 days in multiplex and neemactin compared to that of 13 days in control. Similarly, period required for pinning was significantly less (20 day) in case of neemazal T, raze followed by 21 days in case of multiplex, neemol, halt and 22 days in case of Achook and econeem. However, the period required for pinning was significantly more in case of bavistin and control (26 days). The fresh yield and biological efficiency was significantly higher in neem gold (350g) followed raze (348.10 g) and neemactin (207.4 g). On the other hand, fresh yield was significantly less in case of vanish. Thus, neem gold and raze were found to be most effective biopesticides in minimizing the period of spawn run and increasing the yield.

Table 4: Efficacy of selected biopesticides on spawn run, pinning yield and biological efficiency of *Pleurotus florida*

Biopesticides	Concentrations (ppm)	Spawn run(days)	Pin head stage (days)	Yield (g/500g drysubstrates)	B. E. (%)
Vanish	1000	16	23	193.6	38.72
Achook	1000	15	22	215.80	43.16
Neemazal-T	1000	11	20	219.20	43.84
Multiplex	1500	12	21	263.35	52.67
Dipel-8L	1000	14	24	167.50	33.50
Neemgold	1500	13	23	350.00	70.0
Halt	1000	12	21	168.15	33.63
Raze	2000	11	20	348.10	69.6
Neemol	2000	13	21	213.05	42.61
Econeem	1000	13	22	281.80	56.36
Ecosteem	1000	14	25	217.70	43.54
Minzyme	1000	0.0	0.0	0.0	0.0
Neemactin	1500	12	24	207.40	41.48
Bavistin+Formalin (Control-I)	75+500	13	26	344.85	68.97
Control-II		-	-	-	-
SEm±		0.60	0.79	6.87	0.91
CD (0.05%)		1.70	2.25	19.55	2.58

No spawn run

Discussion

Bavistin, subeej, rilon, sultaf and copper hydroxide at 50 ppm, bavistin, sultaf and subeej and 75 ppm, and Bavistin alone at 100 ppm were highly effective fungicides at they exhibited good mycelial growth of *P. florida.* But, as the concentration was increased to 100 ppm, the radial growth of *P. florida* in other fungicides was adversely affected Chakravarty *et al.*, (1982) [7] and Doshi and Singh (1985) [9] reported less or no inhibition in growth of *Pleurous* sp. by carbendazim under *in vitro* conditions. Further, Ram (1995) [17] found bavistin and rilon to be good fungicides for the vegetative growth of *P. florida* which again confirms the present results. The inhibition of growth of *P. florida* by antracol was reported by Rajput (1996).

Under *in vivo* study, rilon + formalin (75+500 ppm), bavistin+formalin (75+500 ppm) and subjeej+formalin (75+500ppm) were found to completely inhibit the growth of competitor moulds without being inhibiting the growth and increasing the yield of *P. florida.* Similar results on rilon + formalin (75+500 ppm) against *P. florida* were reported by Ram (1995) [17]. Bavistin + formalin (75+500 ppm) was reported to be a known and effective combination for treatment of straw substrates (Vijay and Sohi, 1987) [20].

Under *in vitro* conditions, minzyme, neemgold, neemazal -T, neemol and achook proved to be better biopesticides as they exhibited good radial growth of *Pleurotus florida.* Different neem based biopesticides viz. neemshield, neemax and ecosulf were also reported to be extremely good as they inhibited only 2.5 to 13.4 per cent growth of *P. sajor-caju* (Anonymous, 1997-98) [1]. Gupta (1999) [10] reported minzyme, neemazal-T, neemactin, neem gold and halt to be effective biope sticides as there was no inhibition in radial growth of *Pleurotus* spp.

The time required for spawn run and pinning was significantly less in neemazal-T, raze, multiplex and neemactin but the fresh yield and biological efficiency was significantly higher in neem gold, followed by raze and neemactin. Thus, neem gold and raze were found to be most effective biopesticides in minimizing the period of spawn run and increasing the yield. Straw treatment with biopesticides resulted in complete suppression of fungal contaminants. The biological efficiency of *P. florida* was higher when the straw was treated with biopesticides like neemazal and neem gold (Anonymous (1997-98) [1]. Gupta (1999) [10] also reported that straw treated with multiplex and minzyme gave higher yield as against lower yield in bavistin + formalin.

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