IJPS INTERNATIONAL JOURNAL OF PLANT SCIENCES
ISSN-0973-1547 Volume **14** | Issue 1 | January, 2019 | 14-18
DOI: 10.15740/HAS/IJPS/14.1/14-18
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RESEARCH ARTICLE

Study the relationship of *Pleurotus florida* with other *Pleurotus* sp. and fungal moulds

■ **R. L. Sharma and M. P. Thakur**

SUMMARY

The present investigation on study the relationship of *Pleurotus florida,* with other *Pleurotus* sp. and fungal moulds was undertaken with the objectives to study the growth, population of substrate mycoflora associated and inhibition of *P. florida*, with other *Pleurotus* sp. and fungal moulds. In all the cases, the test fungus showed less mycelial growth while *P. florida* recorded maximum growth (43.47 mm) in dual culture with *P. ostreatus* followed by combination with *P. flabellatus* (41.54 mm). Regarding the population of mycoflora during different month, it was generally lower from November to March and August to September when the growth of *P. florida* was profuse and abundant due to fovourable prevailing climatic conditions. However, the population of mycoflora was comparatively higher during April to July with rise in temperature, fall in relative humidity and unfavourable conditions for development of mushroom mycelium and fruiting. The results also revealed that all the isolated fungi had relatively faster rate of growth than *P. florida* on potato dextrose agar medium. The rate of growth in *Rhizopus* sp. was more followed by *Aspergillus niger, A. flavus* and *Trichoderma viride*. However, the rate of growth was comparatively less in *Rhizoctonia* sp. Maximum inhibition in radial growth of *P. florida* was recorded with *Rhizopus* sp.(70.45%) followed by *A. niger* (65.90%) and *A. flavus* (63.63%). However, the inhibition of growth of *P. florida* was minimum in *Rhizopus* sp. (29.51%).

Key Words : Relationship of *Pleurotus florida, Pleurotus* sp., Fungal moulds

How to cite this article : Sharma, R.L. and Thakur, M.P. (2019). Study the relationship of *Pleurotus florida* with other *Pleurotus* sp. and fungal moulds. *Internat. J. Plant Sci.*, **14** (1): 14-18, **DOI: 10.15740/HAS/IJPS/14.1/14-18,** Copyright@ 2019: Hind Agri-Horticultural Society.

Article chronicle : Received : 10.10.2018; **Revised :** 04.12.2018; **Accepted :** 13.12.2018

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). 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). But, only a few species have been brought under cultivation on commercial scale. World production of mushroom is around 7.2 million tones

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(Thakur, 2005) 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).

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), more than a dozen are under cultivation in different parts of the country (Balakrishnan and Nair, 1995).

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 (Personal communication). 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. Keeping in view of the above, the present investigation entitled was carried out with the objectives to study the growth, population of substrate mycoflora associated and inhibition of *P. florida,* with other *Pleurotus* sp. and fungal moulds.

MATERIAL 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 per cent 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.).

Under *in vitro* conditions, the radial growth of *P. florida* in relation to *P. sajor-caju, P. columbinus, P. flabellatus, P. sapidus, P. eous* and *P. ostreatus* was studied by dual culture technique. Petridishes containing 20 ml solidified PDA amended with little amount of antibiotic were inoculated with 5 mm disc of *P. florida* in first half of the plate, while second half of the plate was inoculated with a 5mm disc of *Pleurotus* spp. These plates were incubated at 25+2°C and examined frequently to observe the radial growth of *P. florida* in relation to *Pleurotus* spp. When the growth of *P. florida* and *Pleurotus* spp. came in contact to each and started overlapping to one and another, the final observations on radial growth of both the fungi were recorded on eighth day. Four replications were maintained.

Twelve crops of mushroom were taken from one successive year. Frequency of total number of mycoflora associated with paddy straw substrate during this period was recorded. The count of mycoflora was recorded with treated (bavistin 75ppm +formalin 500ppm) and untreated substrate (straw was dipped in plain water) separately.

The population of fungal flora was estimated by serial dilution method (Nallathambi and Marimuthu, 1994). One gram of paddy straw substrate was used for serial dilution. The sample was introduced separately in 250 ml conical flask having 100ml sterilized water. The flask was shaken vigorously for 30 minutes to get homogenous suspension. One ml of this suspension was transferred to a test tube containing 9 ml of sterilized water to get the dilution of 1:10. It was shaken well. One ml of this suspension was again transferred to a test tube containing 9 ml of sterilized water to obtain the final dilution of 1:100. One ml of 1:100 suspensions was poured in each petridishes and then incubated at $25^0 \pm 10^0$ for six days. The observation on CFU/g of straw was recorded on third and sixth day of incubation Pure culture of each fungal colony was obtained in slants having potato dextrose agar medium. The cultures were then examined microscopically and matched with the standard text for identification. Pure cultures of nine fungi observed during *P. florida* cultivation in paddy straw substrate were obtained. The rate of growth of *P. florida* in relation to these fungi were studied under *in vitro* condition to see their relationship, if any. For this purpose, a 5mm -disc of *P. florida* was cut with the help of cork borer and kept in one corner of the petridish having 20 ml potato dextrose agar media, a similar disc of test fungi was cut in the same fashion and placed on opposite side of the petridishes. Four replications were maintained for each treatment. The plates were incubated at 25 ± 2^0 C.

Observations on radial growth of *P. florida* and test fungus were recorded when they meet each other.

RESULTS AND DISCUSSION

The results obtained from the present investigation as well as relevant discussion have been summarized under following heads :

Growth of *P. florida* in relation to other species of *Pleurotus* :

Radial growth of *P. florida* with other species of *Pleurotus* in dual culture was studied and the data is presented in Table 1. In all the cases, the test fungus showed less mycelial growth while *P. florida* recorded maximum growth (43.47 mm) in dual culture with *P. ostreatus* followed by combination with *P. flabellatus* (41.54 mm). However, with the other species *i.e.*, *P. eous, P. coumbinusi* and *P. sapidus*, the growth was comparatively less (31.66 mm) indicating slower growth compared to other species of *Pleurotus*.

Mycoflora associated with *P. florida* cultivation during different months of the year:

An experiment was conducted to observe the population of mycoflora associated with *P. florida* cultivation during different months of the year. It was carried out with paddy straw as a substrate with and without sterilization and the results are presented in Table 2.

It is evident from the data that the total fungal population (CFU/g of straw) was about 2.5 times higher (1550) on untreated paddy straw substrate compared to that of lower population (612 CFU/g of straw) on treated paddy straw substrate at 10^5 dilution. A sum of nine

Table 1: Studies on radial growth of *Pleurotus florida* in relation to other species of *Pleurotus* spp. in dual culture inoculation

Pleurotus spp.	Radial growth (mm)		Increase over control (%)
	Pleurotus spp.	*Pleurotus florida*	
P. sajor-caju	52.61	37.39	16.91
P. coumbinus	49.57	40.43	10.15
P. flabellatus	48.46	41.54	7.68
P. sapidus	56.24	33.76	24.97
P. eous	58.34	31.66	29.64
P.ostreatus	46.53	43.47	3.4
P. florida (control)	-	45.00	
S.E.±	1.34	1.10	
C.D. (P=0.05)	4.00	3.23	

DAI = Day after inoculation

Table 2: Population of substrate mycoflora associated with *Pleurotus florida* cultivation during different month of the year

Mycoflora (10^5)	April-May		May-June		June-July		July-Aug.		Aug.-Sept.		Sept.-Oct.		Oct.-Nov.		Nov.-Dec.		Dec.-Jan.		Jan.-Feb.		Feb.-Mar.		Mar.-Apr.		Total	
	T	UT	T	UT	T	UT	T	UT	T	UT	T	UT	T	UT	T		T	UT	T	UT	T	UT	T	UT	T	UT
T. viride	2	8	2	11	2	7	1	6	2	5	1	3	1	2	1	2	0	2	0	3	0	4	2	6	14	59
T. harzianum	2	6	1	10	2	5	2	4	1	4	0	2	1	3	1	1	0	2	0	3	0	5	0	5	10	50
A. flavus	4	26	13	127	22	76	6	69	6	31	2	4	0	6	0	3	1	3	3	10	4	21	3	39	64	415
A. niger	5	43	6	48	7	47	2	37	0	24	1	6	0	8	0	5	0	3	2	5	4	20	3	35	30	281
P. citrinum	5	16	10	24	8	18	3	11	1	6	0	0	0	0	0	0	0	1	0	5	2	10	2	12	31	103
Coprinus sp.	3	9	4	12	4	8	3	3	3	3	0	2	0	1	0	2	0	1	0	3	0	3	0	6	17	53
Rhizoctonia	1	8	1	14	0	15	1	12	1	4	0	3	0	2	0	3	0	2	0	2	0	3	1	5	5	73
Rhizopus sp.	42	43	86	89	70	76	50	53	21	26	5	7	4	6	6	8	5	7	7	11	13	18	30	34	339	378
S. rolfsii	15	26	25	31	16	22	10	13	7	7	0	0	2	3	2	2	2	3	4	4	8	11	11	16	102	138
Grand total	79	186	148	366	131	274	78	208	42	110	9	27	8	31	10	26	8	24	16	46	31	95	52	158	612	1550

Population (CFU/g of straw) is the sum of five stages (spawning to third flush)
T-Treated (Bavistin 75 ppm + formalin 500 ppm)
UT-Untreated (Plain water)

species belonging to 7 genera namely *Trichoderma viride, T. harzianum, A. flavus, A. niger, P. citrinum, Coprinus* sp., *Rhizoctonia* sp., *Rhizopus* sp. and *S. rolfsii* were found associated with untreated substrate during different months of *P. florida* cultivation. Among these mycoflora, *A. flavus* (415) *Rhizopus* sp. (378), *A. niger* (281) and *S. rolfsii* (138) were most predominant. On the contrary, the treated paddy straw substrate recorded eight fungi *viz., T. viride, T. harzianum, A. flavus, A. nizer, P. citrinum, Coprinus* sp., *Rhizoctonia* sp., *Rhizopus* sp. and *S. rolfsii* and that too in lower population. Of these, *Rhizopus* sp. (339), S*clerotium rolfsii* (102) and *A. flavus* (64) were most predominant.

Regarding the population of mycoflora during different month, it was generally lower from November to March and August to September when the growth of *P. florida* was profuse and abundant due to fovourable prevailing climatic conditions. However, the population of mycoflora was comparatively higher during April to July with rise in temperature, fall in relative humidity and unfavourable conditions for development of mushroom mycelium and fruiting.

Mycelial growth of *P. florida* in relation to isolated mycoflora :

Nine different mycoflora isolated from paddy straw substrate during *P. florida* cultivation were tested for their growth performance in relation to mushroom fungus and the results are presented in Table 3.

The results revealed that all the isolated fungi had relatively faster rate of growth than *P. florida* on potato dextrose agar medium. The rate of growth in *Rhizopus* sp. was more followed by *Aspergillus niger, A. flavus* and *Trichoderma viride.* However, the rate of growth was comparatively less in *Rhizoctonia* sp. Maximum inhibition in radial growth of *P. florida* was recorded with *Rhizopus* sp.(70.45%) followed by *A. niger* (65.90%) and *A. flavus* (63.63%). However, the inhibition of growth of *P. florida* was minimum in *Rhizopus* sp. (29.51%). Thus, it can be said that *Rhizopus* sp., *A. niger* and *A. flavus* did allow only the partial growth of *P. florida* in their presence.

In all the cases, *Pleurotus florida* showed less mycelial growth when compared to other species. However, the growth was maximum (43.47 mm) in dual culture with *P. ostreatus* followed by combination with *P. flabellatus* (41.54 mm). However, with the other species *i.e., P. eous, P. columbinus* and *P sapidus*, the growth was comparatively less. Solanki (1999) studied the radial growth of *P. columbinus* in relation to other species of *Pleurotus.* He found slow growth of *P. columbinus* with other *Pleurotus* species except *P. flabellatus.* Namdev (2000) studied the radial growth of *P. flabellatus* in relation to other species of *Pleurotus* and found slow growth of *P. flabellatus* than *P. florida*, while faster growth was observed with other species of *Pleurotus.* It was surprising to note that the mycelial growth of *P. florida* was less compared to other species but the spawn prepared from *P. florida* produced better yield than rest of the species under Chhattisgarh conditions.

Mycoflora associated with paddy straw substrate revealed 2.5 fold higher population (1550 CFU/g of straw) on untreated paddy straw substrate compared to

Table 3: Inhibition of growth of *Pleurotus florida* by different species of fungal moulds

Other mycoflora	Radial growth		Per cent inhibition over control
	Isolated mycoflora	*Pleurotus florida*	
Trichoderma viride	73	17	61.36
Trichoderma harzianum	72	18	59.09
Aspergillus flavus	74	16	63.63
Aspergillus niger	75	15	65.90
Penicillium citrinum	66	24	45.45
Coprinus sp.	61	29	34.09
Rhizoctonia sp.	51	31	29.54
Rhizopus sp.	77	13	70.45
Sclerotium rolfsii	68	22	50.0
Control	-	44	
S.E.±	0.85	0.93	
C.D. (P=0.05)	2.43	2.65	

that of lower population (612 CFU/g of straw) on treated paddy straw substrate at 10^5 dilution. However, there was no much difference in the type of mycoflora associated with untreated and treated paddy straw substrate. Population of mycoflora during different months was generally lower in November to March and August to September and higher during April to July which may probably be due to rise in temperature and fall in relative humidity. Thakur *et al.* (2001) also reported about three fold higher (1324 CFU/g of straw) mycoflora on untreated paddy straw substrate during *P. florida* cultivation as compared to only 496 CFU /g of straw population in chemically treated paddy straw substrate. The population of mycoflora was maximum (144 and 3490 on treated and untreated straw substrate) during 1st May to 15th July whereas, it was minimum (10 and 16) during 1st January to 17th March. Association of 11 fungal species with untreated paddy straw substrate was also reported by several workers (Chakravarty *et al.*, 1982; Pandey and Tiwari, 1988 and Nallathambi and Marimuthu, 1994).

It was observed that all the nine fungal flora isolated did grow at a faster rate than the mushroom fungus on potato dextrose agar medium. The rate of growth of *Rhizopus* sp. was faster than *A. niger, A. flavus* and *T. viride.* Maximum inhibition in radial growth of *P. florida* was recorded by *Rhizopus* sp. followed by *A. niger* and *A. flavus*. Several workers noticed faster rate of growth in weed fungi compared to mushroom mycelium (Pandey and Tiwari, 1988; Doshi and Singh, 1983). Similarly, Rai *et al*. (1993) observed *Trichoderma* sp. as a serious competitor of *Pleurotus* sp.

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