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An Integrated Approach Towards *In vivo* Control of Mushroom Weeds vis-à-vis Yield

M. R. Chakraborty[1], S. Ojha[1], R. N. Medda[1], and N. C. Chatterjee[2*]

ABSTRACT

Different competitor weed fungi *Rhizopus stolonifer, Penicillium glabrum, Fusarium oxysporum* and *Coprinopsis kimurae* were found to have associated with the fruit beds as constant contaminants of *Pleurotus sajor-caju* and *Lentinula edodes*. Several antagonists, plant extracts and chemicals were tested against the weed fungi to screen the most effective ones and to have an integrated combination for better management of the disease. Among the chemicals used, Baynate and Bavistin at the dose of 0.5% provided highly significant inhibition of all the competitors. Different plant extracts were treated against the weed fungi and it was found that garlic (*Allium sativum*) preparation exhibited the best result followed by *Cycas revoluta* and *Azadirachta indica*. Among the antagonists, *Trichoderma viride* was proved to be the most efficient one. Out of the various compatible combinations, the *T. viride,* garlic extract and Bavistin combination was found to be the most potent integrated combination providing more than 97% growth inhibition of the weed fungi *in vitro*. Under *in vivo* condition, biological efficiency of the combination of *T. viride, T. hamatum*, bavistin and garlic preparation was found to be significantly higher than untreated beds of both edible mushroom species.

Keywords: IDM schedule, *Lentinula edodes,* Plant extracts, *Pleurotus sajor-caju,* Weed fungi.

INTRODUCTION

In India the cultivation of both oyster mushroom *Pleurotus sajor-caju* (Fr.) Singer and shiitake mushroom *Lentinula edodes* (Berk) Singh is being practiced. They are gaining more popularity among the growers due to their health benefits such as diets of low caloric value, availability of most of the essential amino acids and an excellent source of vitamins and minerals. At any stage of growth, an undesirable growth or development of certain molds can occur and can adversely affect the yield of those mushrooms. Many researchers have reported the occurrence of different fungal competitors as weeds in the compost for mushroom cultivation (Sharma and Kumar, 2008; Reyes *et al*., 2009; Singh *et al*., 2010). Weed fungi have been considered as a major factor contributing to low productivity of mushroom in India particularly under seasonal cultivation. *Coprinopsis* sp alone causes 94.4% loss in production (Sharma, 1997). Therefore, a careful production and management system is a pre-requisite for successful cultivation of mushrooms. It has been reported that most of the competitor molds were completely inhibited by fungicides, plant extracts or bio-agents from the cell free extracts of different fungi under *in vitro* and *in vivo* conditions (Wilson *et al*., 1994; Tewari, 2005; Mishra and Singh, 2010; Sangeetha *et al*., 2011). Attempts have been made to control the four obnoxious weed fungi in mushroom cultivation; *Rhizopus stolonifer, Penicillium glabrum, Fusarium oxysporum* and *Coprinopsis*

[1] Department of Botany, Bankura Christian College, Bankura-722101, West Bengal, India.
[2] Department of Botany, UGC Center for Advanced Study, The University of Burdwan, Burdwan – 713 104, West Bengal, India.
[*] Corresponding author; e-mail: nc_chatterjee@rediffmail.com

kimurae by applying antagonists, plant extracts and chemicals *in vitro* and their suitable compatible combinations in an integrated way to control their growth under *in vivo* conditions.

MATERIALS AND METHODS

Mushroom Cultures

The vegetative culture of *Pleurotus sajor-caju* was collected from ITCCC, Indian Agricultural Research Institution, New Delhi (Accession No. ITC-GF-1725) and *Lentinula edodes* was procured from American Type culture collection, USDA, Forest Products Laboratory, Madison, USA (ATCC No. 4086). These individual cultures were subsequently multiplied and maintained in pure line on potato dextrose agar (PDA) slant for further use.

Routine observations were made to study the prevalent occurrence of weed fungi in the mushroom compost. The importance of such contaminants lies in that they may act as competitor inhibitors to edible mushroom to reduce the yields or cause complete damage to the crop. Small pieces or colonies of weed fungi were directly picked up from the compost by sterilized forceps and studied under the microscope for identification. Isolations were made from colonies taken from compost and were placed on sterilized PDA medium in Petri dishes and incubated at 28±1°C for 7 days.

Bioassay of Chemicals *In vitro*

Effects of different chemicals, Baynate (a.i methyl thiophanate 500 g kg^{-1}), Bavistin (a.i carbendazim 500 g kg^{-1}), Captan (a.i captan 800 g kg^{-1}), Mancozeb (a.i mancozeb 800 g kg^{-1}), and Formalin (a.i formaldehyde 40%) were studied following 'Food poisoned technique' (Mondal *et al*., 1995). PDA broth at the amount of 15 ml at pH 6.5 was sterilized, cooled at room temperature and mixed thoroughly with 1 ml of chemicals baynate, bavistin, captan and mancozeb at the concentration of 0.5% and formalin at 1.5%. The amended medium was inoculated with a 5mm disc of the tested pathogens and incubated at 28±2°C for 7 days. Identical control sets were also maintained.

The colony diameter was measured after five days of incubation and compared with the control set. The percentage of growth inhibition was calculated by the following formula

$$\text{Percentage inhibition} = \frac{\text{control - treatment}}{\text{control}} \times 100$$

Preparation of Plant Extracts

Fresh healthy and young parts (5 ml) of different plants were used: leaves of neem (*Azadirachta indica* Adr. Juss.), bulb of garlic (*Allium sativum* L.), leafy twigs of *Cycas revoluta* Thunb., leafy sheath of lemon grass (*Cymbopogon citratus* (DC. Ex Nees) Stapf.), leafy twigs and fruits of *Datura metel* auct. Non L., roots of *Rauwolfia* sp, leafy twigs of *Eucalyptus globosus* Labill., and *Lantana camara* L. Parts of plants were cut into pieces of 1-2 cm, plunged separately in boiling ethyl alcohol (80%) at a rate of 5- 10 ml of alcohol per gram of tissue and boiled for 5-10 minutes on a steam bath or hot water bath under the hood. They were cooled to room temperature and crushed thoroughly in a mortar with a pestle for 5-10 minutes and filtered through two layers of cheese cloth. The residue was again extracted with 2-3 ml of alcohol for three minutes using the same procedure. Both extracts were mixed and the mixture was filtered through Whatman No.1 filter paper. Alcohol from the extract was evaporated till the volume was reduced to 80-90%. The extracts of each plant were passed through a sterilized sintered glass filter (G-5) under UV-Chamber and a known volume of plant extract was mixed with an equal volume of ethyl alcohol 80% (40-60°C) at room temperature (30°C). The filtration through sintered glass filter was

repeated and alcoholic fractions were combined and evaporated to complete dryness in a rotary film evaporator at 45°C. The residue was dissolved in 10 ml distilled water and stored at –15°C until use (Mishra and Singh, 2006).

Bioassay of Plant Extracts *In vitro*

In vitro effect of different plant extracts on the vegetative growth of weed fungi was studied following 'food poisoning technique' (Mondal *et al*., 1995) Alcoholic extracts of different plant materials, 5ml each were added separately to 15 ml of sterilized PDA medium at pH 6.5 in Petri dishes and mixed thoroughly to have a homogeneous mixture, cooled at room temperature and inoculated with 5 mm disc inoculum of the test pathogen. The plates were incubated at 28±2°C for 7 days and radial growth of the fungi was measured against the control set at the end of the incubation period.

Preparation of Antagonistic Metabolites

Effective antagonists: *Trichoderma viride*, *T hamatum, T. harzianum, Penicillium citrinum* and *Aspergillus niger* were chosen through random screening and employed for growth inhibition of different weed fungi.

To obtain the growth metabolites of different antagonists, Czapek's synthetic medium was taken as the basal medium. In each of the 250 ml flasks, 100 ml of the medium was taken, plugged and sterilized at 15 lbs psi pressure for 15 minutes. The medium was cooled and inoculated separately with 5 mm inoculum discs cut from the margin of actively growing cultures of *T. viride*, *T. hamatum*, *T. harzianum*, *P citrinum* and *A. niger*. The flasks were then incubated at 28±2°C for 15 days. After the incubation period, the mycelial mats from the liquid culture were removed and the remainder was filtered through Whatman filter paper No. l and passed again through a sterilized sintered glass filter (G-5) in order to remove the mycelia and spores of the antagonists. The filtrate obtained served as the crude metabolite of the respective antagonists (Chakraborty *et al*., 2004).

Bioassay of Antagonistic Metabolites *In vitro*

In order to study the effect of antibiosis of the antagonists, 'Food poisoning technique' (Mondal *et al*., 1995) was followed. For designing the 'Food poisoning technique', cultures on PDA medium in petri dishes were maintained to assess the differential antagonistic activities of *T. viride*, *T. hamatum, T. harzianum, A. niger* and *P. citrinum* against weed fungi *viz. R. stolonifer, P. glabrum, F. oxysporum* and and *C. kimurae* that remained constantly associated with the mushroom beds. In this technique 15 ml of the PDA medium at pH 6.5 was poured in each of the sterilized Petri dishes and allowed to solidify. Just before solidification, 6 ml of each of the crude antagonistic metabolites was added separately and mixed homogeneously. The Petridishes were inoculated with active mycelial discs of respective weed fungi and incubated at 28±2°C for 7 days. At the end of the incubation period, the radial growth of the weed fungi were measured against control sets and the percentage of growth inhibition was calculated.

Bioassay of Integrated Approach *In vitro*

An integrated approach towards *in vitro* management of different weed fungi was taken with the antagonistic metabolites, the effective fungicide, Bavistin and plant extracts, garlic in all compatible combinations. Different compatible combinations of fungicide + antagonistic metabolites: *T. viride*+*T. hamatum*+Bavistin, *T. viride*+*A. niger*+Bavistin, *T. hamatum*+*A. niger*+Bavistin, combinations of plant extracts+antagonistic metabolites like *T.*

viride+*T. hamatum*+ garlic, *T. viride*+*A. niger*+garlic, *T. hamatum*+*A. niger*+garlic, and the combinations of antagonistic metabolites+plant extracts+fungicide like *T. viride*+garlic+Bavistin, *T. hamatum*+garlic+Bavistin, *A. niger*+garlic+Bavistin, were made at 2:2:1 ratio, respectively. The concentration of Bavistin remained fixed at 0.001%. The concentration of plant extracts remained fixed at 5%. Potato dextrose broth (15 ml) was put in each 100 ml conical flask, sterilized and cooled. Different combinations were prepared and mixed thoroughly in the medium, inoculated with the respective weed fungi and incubated at 28±2°C for 7 days. After the incubation, the mycelial mats of weed fungi were harvested, washed and oven dried to a constant weight. The dry weight of the mycelial mat was recorded and compared with the control sets. The inhibition percentage of growth of the respective weed fungi was calculated.

Bioassay of Integrated Approach *In vivo*

In vivo effects of different plant extracts, chemicals and the antagonistic metabolites of biocontrol agents were tested against the different weed fungi in the mushroom beds both individually and in an integrated way and the associated yields of *P. sajor-caju* and *L. edodes* were studied following the method of Pani and Patra, (1997).

Cumulative combined form of compost fibreless jute stick (dust and sugarcane bagasse, 1:2 ratio) supplemented with different N-rich organic and inorganic materials previously standardized as the basal substrates for the mushroom was taken in polypropylene bags (1 kg bag^{-1}) and pasteurized with steam above 80°C for one hour (Patil and Jadhav, 1989). The pasteurized bags were seeped in different selected solutions of chemicals (Bavistin and Formalin at 0.5 and 1.5%), plant extracts (garlic and *Cycas* sp at 5%) and in different antagonistic metabolites (*Trihoderma viride*, *T. hamatum* and *A. niger*) at 5% doses. Bags were also steeped in an integrated form of the components like *Trihoderma viride*+*T. hamatum*+ Bavistin+garlic each at 0.001% concentration in equal proportions. Inoculums in the form of suspensions of respective weed fungi derived from seven day old pure cultures were mixed with the prepared compost (10 ml suspension kg^{-1} of dry compost). A cubical design of compost having perforations on all sides was used.

Mushrooms were harvested regularly and the average yields with the biological efficiency were recorded. This indicated the ability of the plant extracts, antagonistic metabolites and chemicals to cause comparatively higher degrees of growth inhibition of the weed fungi than negative interference with the mycelial growth of the edible mushrooms. Biological Efficiency (BE) was calculated as follows (Jongman *et al*., 2010):

$$\text{Biological efficiency} = \frac{\text{Fresh weight of mushroom harvested}}{\text{Dry weight of substrate used}} \times 100$$

Statistical Analysis

There were five replications for each treatment. The results were analyzed statistically using one-sample Student's t-test at $P< 0.05$ and the data for a particular treatment were considered significant if the calculated value of t was greater than the tabulated value. Duncan's multiple range tests were performed for the comparison of means of different treatments. All statistical analyses were carried out using a statistical software package (SPSS 10.0, SPSS Inc.).

RESULTS AND DISCUSSION

The results clearly reveal that Bavistin at the dose of 0.5% happens to be the most efficient one among all fungicidal treatments, as it provides 98.45% growth inhibition of the weed fungi tested (Table 1). On the other hand, Baynate at the dose of 0.5% concentration was also found to be effective against the growth of the weed fungi as it exhibited up to 97.40% growth

Table 1. Growth inhibition of different weed fungi by chemicals *in vitro*.

Fungicidal chemicals	Dose (%)	Weed fungi[a]							
		Rhizopus stolonifer		*Penicillium glabrum*		*Fusarium oxysporum*		*Coprinopsis kimurae*	
		Radial growth	Growth inhibition (%)	Radial growth	Growth inhibition (%)	Radial growth	Growth inhibition (%)	Radial growth	Growth inhibition (%)
Bavistin	0.5	1.8	80.75 b	0.2	98.45 a	0.7	92.45 a	0.8	91.11 a
Baynate	0.5	0.3	97.40 a	0.4	95.00 ab	0.8	90.85 a	0.9	90.00 a
Mancozeb	0.5	3.5	61.45 c	2.7	69.25 c	3.6	60.75 c	3.7	57.77 c
Captan	0.5	3.8	57.00 d	3.0	66.65 cd	3.8	56.82 cd	3.2	63.00 b
Formalin	1.5	0.8	90.60 ab	0.3	96.80 a	1.1	88.25 b	0.7	92.22 a
Control		9.0	00.00	9.0	00.00	9.0	00.00	9.0	00.00

[a] Data are the mean values of five replicates. Means in a column with the same letter are not significantly different according to Duncan's multiple range test at $P< 0.05$.

inhibition while formalin at the dose of 1.5% concentration showed growth inhibition of the weed fungi from 88.25 to 96.80%.

Excellent performance of Baynate, a methyl thiophanate compound may be the due to its easy transformation into methyl benzimidazole carbamate (MBC), which is effective against a wide range of pathogenic fungi. MBC is well known for its adverse effects on DNA, RNA, protein synthesis and respiration of fungi. Moreover, a secondary action can also be attributed to butyl isocyanate, a co-product of MBC, which aids in the hydrolysis of the systemic fungicides (Mehrotra and Aggarwal, 2010). A good response towards the growth inhibition of the weed fungi by Bavistin, another systemic fungicide, is due to its capacity of having both prophylactic and curative actions. According to Sharma and Kumar (2008) Bavistin is the best fungicide for the control of *Sepedonium* yellow mold.

The results in Table 2 clearly indicate the efficacy of various plant extracts as biocides against the *in vitro* growth of different weed fungi. However the percentage of growth inhibition varies significantly where garlic extract shows maximum inhibition of all the weed fungi 94.44% in case of *Penicillium glabrum*, 90% in *Coprinopsis kimurae*, 70.11% in *Rhizopus stolonifer* and 68.45% in *Fusarium oxysporum*. This was followed in the rank of efficacies by *Cycas* leaf extract, neem extract, lemongrass extract respectively.

Such a good response of garlic extract against the weed pathogens may be attributed to the fact that the cell free extract contains some antifungal volatile metabolites such as allicin and ajoene, which are responsible for damaging the fungal cell walls (Singh and Divedi, 1999). Similarly the antifungal activity of neem extract might be due to the presence of active chemicals such as azadirachtin, nimbidin, nimbinin, nimbolidin, nimbin etc. (Amadioha, 2002; Brahmachari, 2004). Sangeetha *et al*. (2011) reported that *Coprinopsis kimurae*

population in mushroom beds was controlled by spraying neem oil before spawning.

It is clear from the results in Table 3 that different antagonists play significant roles in the inhibition of growth of weed fungi. Metabolites of *T. viride* and *T. hamatum* exhibit highly significant inhibitory effects on the growth of all weed fungi. Interaction between the antagonists and weed fungi leading to the development of all modes of antagonistic action such as competition, mycoparasitism and antibiosis has been reported by earlier researchers (Cliquet and Scheffer, 1996; Chakraborty and Chatterjee, 2008). The process of antibiosis is due to the production of some fungitoxic metabolites in the medium where they are grown and these toxic metabolites or the antibiotics may aid in the inhibition of fungal growth and/or germination of fungal spores.

It is evident from the results in Table 4 that management of mushroom weeds in the cultivation beds can be achieved through the integrated way of applying antagonists along with a compatible form of fungicide and/or plant extract. Comparative efficacy of different plant extracts chemicals and metabolic products of different antagonists individually as well as in the form of an integrated way clearly indicates the superiority of using integrated management for the control of weed fungi over any other individual treatments. The average yield along with biological efficiency of both mushrooms was recorded to be significantly higher when the mushroom beds were under integrated treatments than under the individual treatments. Out of the different integrated combinations, *T. viride*+*T. hamatum*+Bavistin, *T. viride*+garlic+Bavistin and *A. niger*+garlic+Bavistin were found to produce significant inhibition of almost total biomass production of the weed fungi (Table4).

Many weed fungi, predominantly *Coprinopsis kimurae* and *Rhizopus stolonifer,* which remain constantly associated with the mushroom compost, interfere with the growth and development of the experimental mushrooms. Therefore, sterilization of the substrates for mushroom cultivation is indispensable for obtaining improved yield. However, pasteurization of substrates by conventional hot water treatment or normal steaming is often costly and cumbersome for the production of the crop. In the absence of proper pasteurization facilities, the growers are left with no other choice but to use unsterilized substrates for mushroom cultivation. Some growers resort to chemical pasteurization with Bavistin and formalin as reported by Vijoy and Sohi, (1987). The present study indicates that chemicals like Bavistin and formalin could inhibit the growth of weed fungi in both mushroom beds but when yield is concerned, the compost beds treated with plant extracts or metabolites of different antagonists show more or less higher yield than the chemically treated beds.

It is advisable to manage the diseases in mushrooms through hygienic measures because of several factors. There are a limited number of pesticides registered for use on mushrooms because they are grown indoors and have a very short shelf-life. Mushrooms themselves are fungi and most of the weed fungi are also fungi thereby making the choice of fungicides very difficult. Moreover, because of short cropping cycle, residual toxicity of different chemicals is of great concern and this must be below the tolerance limit. Besides, mushrooms are very sensitive to fumes, toxic gases and several chemicals. This also limits the frequent use of chemicals in mushroom industry. An equally important factor which limits the use of fungicides for the management of diseases in mushrooms is the problem of acquired resistance as repeated and regular applications of any chemical greatly increase the chance of resistance developing.

In this context, the present study summarizes that the application of plant extracts as botanicals along with minimal dosages of chemicals assumes to be the best integrated management strategy for fungal weeds in mushroom cultivation.

Table 2. Growth inhibition of different weed fungi by botanicals *in vitro*.

Biocides	Plant parts[b]	Weed fungi[a]							
		Rhizopus stolonifer		*Penicillium glabrum*		*Fusarium oxysporum*		*Coprinopsis kimurae*	
		Radial growth	Growth inhibition (%)	Radial growth	Growth inhibition (%)	Radial growth	Growth inhibition (%)	Radial growth	Growth inhibition (%)
Datura metel	Leafy twigs + fruits	4.8	45.55 c	4.6	50.00 e	6.7	25.55 e	6.8	35.45 f
Allium sativum	Bulb	2.8	70.11 a	0.5	94.44 a	2.8	68.45 a	0.8	90.00 a
Azadirachta sp	Leafy twigs	3.3	63.00 b	2.3	75.55 bc	3.2	60.45 b	3.7	58.89 c
Rauwolfia sp	Roots	5.9	36.00 d	7.8	15.20 f	7.1	20.44 ef	5.6	38.88 f
Cycas revoluta	Leafy twigs	4.5	51.11 bc	1.7	80.00 b	4.3	52.00 cd	1.00	88.88 a
Cymbopogon sp	Leaf	3.9	56.66 c	2.8	68.85 cd	4.1	55.55 c	3.3	65.55 b
Eucalyptus sp	Leaf	6.6	26.66 e	3.0	66.00 d	4.2	54.00 c	4.8	46.66 e
Lantana sp	Leaf	4.3	52.25 bc	4.5	50.11 e	4.0	55.55 c	4.4	51.88 d

[a] Data are the mean values of five replicates. Means in a column with the same letter are not significantly different according to Duncan's multiple range test at $P< 0.05$, [b] In each case quantity of biocide is 5 ml.

Table 3. Growth inhibition of different weed fungi by antagonistic metabolites *in vitro*.

Antagonists[b]	Weed fungi[a]							
	Rhizopus stolonifer		*Penicillium glabrum*		*Fusarium oxysporum*		*Coprinopsis kimurae*	
	Radial growth	Growth inhibition (%)	Radial growth	Growth inhibition (%)	Radial growth	Growth inhibition (%)	Radial growth	Growth inhibition (%)
Trichoderma viride	1.07	88.09 a	0.40	25.55 d	1.90	98.88 a	0.90	90.00 a
Trichoderma harzianum	1.90	78.88 b	0.60	93.00 b	1.20	86.66 b	1.70	81.05 b
Trichoderma hamatum	1.70	81.00 ab	0.10	98.88 a	2.60	71.11 c	1.50	83.33 b
Aspergillus niger	6.40	28.88 c	4.90	45.35 c	1.00	88.88 b	1.10	87.00 ab
Penicillium niger	7.00	22.45 d	8.2	8.88 e	1.10	87.77 b	8.00	11.11 c
Control	9.00	0.00	9.00	0.00	9.00	0.00	9.00	0.00

[a] Data are the mean values of five replicates. Means in a column with the same letter are not significantly different according to Duncan's multiple range test at $P< 0.05$, [b] In each case quantity of growth metabolites of the antagonists is 6 ml.

Table 4. Integrated approach towards *in vitro* biomass production of different weed fungi

Treatments[b]	Ratio	Weed fungi[a]							
		Rhizopus stolonifer		*Penicillium glabrum*		*Fusarium oxysporum*		*Coprinopsis kimurae*	
		Dry mycelial wt.(mg)[c]	Inhibition (%)	Dry mycelial wt.(mg)	Inhibition (%)	Dry mycelial wt. (mg)	Inhibition (%)	Dry mycelial wt.(mg)	Inhibition (%)
TV+TL+Bavistin	2 : 2 : 1	0.72	99.45 a	0.00	100.00 a	6.31	96.85 b	0.00	100.00 a
TV+AN+Bavistin	2 : 2 : 1	23.67	82.15 c	5.84	95.14 b	5.57	97.22 b	6.78	95.50 b
TH+AN+Bavistin	2 : 2 : 1	22.41	83.10 c	1.02	99.15 a	3.11	78.45 e	22.30	85.20 d
TV+TH+garlic	2 : 2 : 1	9.75	92.65 b	5.82	95.15 b	22.76	88.64 d	11.53	92.35 c
TV+AN+garlic	2 : 2 : 1	21.69	73.65 d	8.93	92.56 ab	19.80	90.15 c	8.06	94.65 b
TH+AN+garlic	2 : 2 : 1	21.42	83.85 c	8.19	93.18 ab	17.23	91.40 c	15.07	90.00 cd
TV+garlic+Bavistin	2 : 2 : 1	0.00	100.00 a	1.19	99.01 a	5.51	97.25 b	0.00	100.00 a
TV+garlic+Bavistin	2 : 2 : 1	0.87	99.35 a	0.00	100.00 a	21.14	89.45 cd	14.69	90.25 cd
TH+garlic+Bavistin	2 : 2 : 1	9.62	92.75 b	17.22	85.65 c	0.00	100.00 a	29.61	80.35 e
Control		132.60	0.00	120.00	0.00	200.30	0.00	150.65	0.00

[a] Data are the mean values of five replicates. Means in a column with the same letter are not significantly different according to Duncan's multiple range test at $P< 0.05$, [b] TV= *Trichoderma viride*; TH= *Trichoderma hamatum*, AN= *Aspergillus niger*. [c] weight.

Table 5. Biological efficacy (BE) of different antifungal substances towards control of weed fungi on *Pleurotus sajor-caju* and *Lentinula edodes*.

Nature of treatments	Treatments	Dose (%)	Weed fungi[a]															
			Rhizopus stolonifer				*Penicillium glabrum*				*Fusarium oxysporum*				*Coprinopsis kimurae*			
			Av. yield (gm kg^{-1} com[c])		BE (%)		Av. yield (gm kg^{-1} com)		BE[b] (%)		Av. yield (gm kg^{-1} com)		BE (%)		Av. yield (gm kg^{-1} com)		BE (%)	
			PS[d]	LE[e]	PS	LE	PS	LE	PS	LE	PS	LE	PS	LE	PS	LE	PS	LE
Chemicals	Bavistin	0.50	875bc	320e	87.5b	32.0c	800e	350de	80d	35d	780b	290e	78ab	29.0d	700f	300g	70e	30.0f
	Formalin	1.50	890b	290e	89.0a	29.0d	900c	360d	90c	36d	790b	300d	79ab	30.0d	750e	320f	75d	32.0e
Botanicals	Garlic bulb	5.00	850c	360d	85.0c	36.0c	1000b	480ab	100b	48a	800b	330c	80a	33.0b	900b	395b	90b	39.5a
	Cycas leaf	5.00	500e	350de	50.0e	35.0c	800e	450b	80e	45b	540f	340c	54de	34.0a	850c	390b	85c	39.0a
Antagonistic metabolites	TV[f]	5.00	850c	380c	85.0c	38.0bc	850d	380cd	85cd	38c	690d	345b	69c	34.5a	890b	380c	89b	38.0b
	TH[g]	5.00	800d	400b	80.0d	40.0b	800e	400c	80d	40c	600e	335c	60d	33.5b	800d	370d	80cd	37.0c
	AN[h]	5.00	450e	300f	45.0f	30.0d	650f	330e	65f	33e	750c	750a	75b	32.0c	860c	350e	86c	35.0d
Integrated approach	TV+TH+ Garlic+ Bavistin (equal proportion)	0.01 (each)	900a	450a	90.0a	45.0a	1100a	500a	110a	50a	850a	350b	85a	35.0a	950a	400a	95a	40.0a
Control			300	200	30.0	20.0	400	300	40	30	250	100	25	10.0	200	150	20	15.0

[a] Data are the mean values of five replicates. Means in a column with the same letter are not significantly different according to Duncan's multiple range test at $P< 0.05$; [b] Biological Efficiency; [c] Dry compost; [d] *Pleurotus sajur-caju*; [e] *Lentinula edodes*; [f] *Trichoderma viride*; [g] *Trichoderma hamatum*, [h] *Aspergillus niger*.

REFERENCES

1. Amadioha, A. C. 2002. Fungitoxic Effect of Extracts of *Azadirachta indica* against *Cochliobolus miyabeanus* Causing Brown Spot Disease of Rich. *Arch. Phytopatn. Pflanz.*, **35**: 37-42.
2. Brahmachari, G. 2004. Neem, an Omnipotent Plant: A Retrospection. *Chem. Biochem.,* **5**: 408-421.
3. Chakraborty, M. R., Dutta, S., Ojha, S. and Chatterjee, N. C. 2004. Antagonistic Potential of Biocontrol Agents Against *Botryodiplodia theobrome* Causing Die-back of Bottle Brush (*Callistemon citrinus*). *Acta Botanica Hungarica*, **46(3-4):** 279-286.
4. Chakraborty, M. R. and Chatterjee, N. C. 2008. Control of Fusariam Wilt of *Solanum melongena* by *Trichoderma* spp. *Biologia Plantarum*, **52(3)**: 582-586.
5. Cliquet, S. and Scheffer, R. J. 1996. Biological Control of Damping off Caused by *Phytrium ultimum* and *Rhizoctonia solani* using *Trichoderma* spp. Applied as Industrial Film Coating on Seeds. *Europ. J. Pl. Pathol*., **102(3)**: 247-255.
6. Jongman, M., Khonga, M., Khare, K. B. and Musyana, T. 2010. Effect of Seasonal Variation and Supplementation on Yield of Oyster Mushroom Cultivated on Indigenous Grasses in Botswana. *Mushroom Res.,* **19(2):** 54-61.
7. Mehrotra, R. S. and Aggarwal, A. 2010. Management of Plant Diseases. In: "*Plant Pathology*". 11th Reprint, Tata McGraw Hill Education Private Ltd., New Delhi, PP. 228-290.
8. Mishra, S. K. and Singh, R. P. 2006. Management of Dry Bubble Disease of Button Mushroom Using Botanicals and Bacterial Biocides. *Indian J. Mush*., **24(1):** 31-39.
9. Mishra, S. K. and Singh, R. P. 2010. Interaction of Bioformulations and Pathogenic Fungi over Case Run and Yield of *Agaricus bisporus* (Lange) Imbach. *Mushroom Res.,* **19 (1)**: 16-21.
10. Mondal, G., Srivastava, K. D. and Aggarwal, R. 1995. Antagonistic Effect of *Trichoderma* spp. On *Ustilago segetum* var. *tritici* and Their Compatibilities with Fungicides and Biocides. *Indian Phytopath*., **48(4):** 466-470.
11. Pani, B. K. and Patra, A. K. 1997. Utilization of Some Phytoextracts for Control of *Sclerotium rolfsii* during Paddy Straw Mushroom Cultivation: A New Approach. *Mushroom Res.,* **6(1):** 37-42.
12. Patil, P. L. and Jadhav, S. W. 1994. Studies on Substrate Supplementation for Higher Yield of Oyster Mushroom. *Mushroom Res.,* **3(1):** 54.
13. Reyes, R. G., Lopez, L. L. M. A., Kalaw, S., Kikukawa, T. and Eguchi, F. 2009. *Coprinus comatus*, a Newly Domesticated Wild Nutricentical Mushroom in the Philippines. *J. Agric. Technol.,* **5(2),** 299-316.
14. Sangeetha, A., Kumar, P. S., Eswaramurthy, S. and Neelamegam, R. 2011. Effect of Different Leaf Extracts for Controlling Microflora Associated with *Pleurotus citrinopileatus* Cultivation. *Mushroom Res.,* **20(1):** 51-54.
15. Sharma, S. R. 1997. Plant Protection Operations in Mushroom Production. In: "*Advances in Mushroom Biology and Production*", (Eds.): Rai R. D., Dhar, B. L. and Verma R. N.. Mushroom Society of India, Solan, PP. 209-223.
16. Sharma, V. P. and Kumar, R. 2008. Management of *Sepedonium* Yellow Mould through Chemicals. *Mushroom Res.,* **17(1):** 19-23.
17. Singh, R. K. and Divedi, R. S. 1990. Fungicidal Properties of Neem and Blue Green against *Sclerotium rolfsii* Sacc. A Foot Rot Pathogen of Barley. *Acta Bot. Indica*. **18:** 26-262.
18. Singh, A., Sharma, V. P., Kumar, S., Varshney, A and Singh, R. 2010. Prevalence of Competitor and Parasitic Moulds during Milky and White Button Mushroom Cultivation in Haryana. *Mushroom Res.,* **19(1):** 45-49.
19. Tewari, A. K. 2005. Pretreatment of Compost and Casing with Fungicides for the Management of *Agaricus bisporus* Moulds. *Mushroom Res.,* **14(2):** 72-75.
20. Vijoy, B. and Sohi, H.S. 1989. Studies on Fungal Competitors of *Pleurotus sajor-caju* (Fr.) Sig. *Mushroom J. Tropics*, **9**: 29-35.
21. Wilson, C. L., Chalutz, A., Droby, E., Stevens, S., John, C. and Khan, Y. K. 1994. Potential of Induced Resistant to Control, Post Harvest Diseases of Fruit and Vegetables. *Plant Dis.*, **78**: 837-844.

یک رویکرد یکپارچه برای کنترل *in vivo* قارچهای هرز در برابر عملکرد

م. ر. چاکرابورتی، س. اوجها، ر. ن. مدا و ن. س. چاترجی

چکیده

مشخص گردید که قارچ‌های هرز رقیب *Rhizopus stolonifer*، *Penicillium glabrum*، *Fusarium oxysporum* و *Coprinopsis kimurae* در بستر میوه قارچ‌های *Pleurotus sajor-caju* و *Lentinula edodes* به صورت آلاینده های دائمی وجود دارند. چندین آنتاگونیست، عصاره گیاهی و مواد شیمیایی بر علیه قارچ‌های هرز مورد استفاده قرار گرفتند تا موثرترین آنها شناسایی شده و ترکیب یکپارچه ای برای مدیریت بهتر بیماری به دست آید. در بین مواد شیمیایی مختلف، باینیت و باویستین در دوز ۰/۵٪ به طور معنی داری موجب جلوگیری از رشد همه رقبا شدند. عصاره‌های گیاهی مختلف در برابر قارچ‌های هرز استفاده شدند و از آن میان عصاره سیر (*Allium sativum*) بهترین نتیجه را داد و بعد از آن *Cycas revoluta* و *Azadirachta indica* قرار گرفتند. در بین آنتاگونیست ها، *Trichoderma viride* بهترین بود. در بین ترکیبات مختلف سازگار، ترکیب *T. viride*، عصاره سیر و باویستین بهترین ترکیب شناسایی گردید و موجب ۹۷٪ کاهش در رشد قارچهای هرز در شرایط in vitro گردید. در شرایط in vivo، کارایی بیولوژیک ترکیب *T. viride*، *T. hamatum*، باویستین و عصاره سیر بیش از بسترهای تیمارنشده هر دو گونه قارچ خوراکی بود.