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Utilization of some phyto-extracts for control of *Sclerotium rolfsii* during paddy straw mushroom *(Volvariella volvacea)* cultivation - a new approach

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ABSTRACT

***In vitro* and *in vivo* studies were conducted to determine the effect of phyto-extracts of some commonly available plants viz. *Azadirachta indica, Psidium guajava, Lantana camara, Sopindus trifoliat, Rauwolfia serpentina, Lawsonia inermis, Agle marmelos, Cynodon dactylon, Tamarindus indica, Eichhornia crassipes, Adhatoda vasica, Moringa oleifera, Pongamia glabra* and *Tagetes erecta* on the mycelial growth of *Volvariella volvacea* and its common competitor mould, *Sclerotium rolfsii*. Among the phyto-extracts, leaf extract of tamarind *(T. indica)* followed by seed extract of soapnut *(S. trifoliat)* and root extract of drum-stick *(M. oleifera)* appeared promising in suppressing the growth of *S. rolfsii* vis-a-vis *V. volvacea* in laboratory as well as field conditions. Tamarind leaf extract caused highest degree of growth inhibition of *S. rolfsii* while interfering least with the mycelial run of the edible fungus. Paddy straw inoculated with *S. rolfsii* and treated with tamarind leaf extract resulted in the highest sporophore yield of the mushroom followed by soapnut seed extract and drum-stick root extract.**

Key words: Phytoextracts, *Sclerotium rolfsii*, plant protection, *Volvariella volvacea*, mushroom

Paddy straw mushroom *(Volvariella volvacea)* is a popular edible fungus of the tropics and subtropics especially in the South East Asian countries[4]. The mushroom ranks fifth in terms of global production which was 207000 tonnes during 1990[2]. However, the association of a number of undesirable fungi during different stages of crop growth has posed a serious threat to its higher production and productivity in recent times. *Sclerotium rolfsii* is one of the frequently encountered competitors of straw mushroom crop. Primordial abortion/button rot of young fruiting bodies caused by *S. rolfsii* has also been reported[2]. Moreover, the weed fungus also competes for space and nutrition in the cultivation substrate hampering the growth of *V. volvacea*. Fungicidal control of *S. rolfsii* in straw mushroom beds has proved futile because of toxicity of the chemical to both the organisms which are simultaneously present in the cultivation substrate. Increasing awareness about environmental pollution and fear of residual toxicity among consumers also limit the scope of chemical control measures. Phyto-extracts being eco-friendly are being used lately to control different plant pathogens. However, there is no available report regarding utilization of phyto-extracts for control of *S. rolfsii* in *V. volvacea* during its cultivation. In the present study, phyto-extract of some commonly available plants were assayed against *S. rolfsii* and *V. volvacea in vitro* and *in vivo*.

MATERIALS AND METHODS

Sclerotium rolfsii was isolated from a diseased specimen and its pathogenicity was confirmed. Pure culture of *Volvariella volvacea* (ITCC No. 4479) was collected from Indian Type Culture Collection, IARI, New Delhi. Both fungi were maintained on PDA slants at 28° C with regular subculturing at ten days intervals. Phyto-extracts used in the assay included neem leaf *(Azadirachta indica)*, need seed *(A. indica)* guava leaf *(Psidium guajava)*, lantana leaf *(Lantana camara)*, soapnut seed *(Sopindus trifoliat)*, patalgarud leaf *(Rauwolfia serpentina)*, Manjuati root *(Lawsonia inermis)*, bel leaf *(Angle marmelos)*, duba grass *(Cynodon dactylon)*, tamarind leaf *(Tamarindus indica)*, water hyacinth root *(Eichhornia crassipes)*, basang leaf *(Adhatoda vasica)*, drum-stick root *(Moringa oleifera)*, karanja leaf *(Pongamia glabra)* and merigold leaf *(Tagetes erecta)*. These extracts were evaluated against both the fungi in laboratory as well as field conditions.

For preparation of phyto extracts, fresh leaves/ seeds/roots of the selected plants were thoroughly washed in water and processed in a grinder mixer with sterile water (1:1 w/v). The homogenate was filtered through double layer muslin cloth and finally through Whatman No. 1 filter paper. The cold aqueous extracts were boiled for 10-15 minutes prior to use.

For *in vitro* study, phyto-extracts at concentrations of 2,5, 10 and 20 per cents were added to the molten PDA in petridishes following poison food technique. The petriplates were inoculated separately with mycelial blocks (5mm) of test fungi taken from seven days old pure mycelial cultures. Petriplates devoid of any phyto extract served as control. The inoculated petriplates were incubated at 30±2°C till mycelial growth in control reached the maximum. Per cent growth inhibition of test fungi was determined as per Vincent[12]. Growth inhibition ratio (GIR) of a phyto-extract was computed as the ratio between the mean per cent growth inhibitions of *S. rolfsii* and *V. volvacea* induced by the particular phyto-extract. The GIR would indicate the ability of the phyto-extract to cause comparatively higher degree of growth inhibition of the competitor while interfering least with the mycelial growth of the edible fungus.

For *in* › study well dried paddy straw (CR-1014) was made into bundles and soaked in water overnight. Excess water was then drained off. The wet bundles were pasteurized with steam above 80°C for 1 hour[6]. These bundles were steeped in selected phyto-extracts at 5,19,20 and 50 percent concentrations for 2 hours. A moisture content of 60-70 percent was retained in the substrate at the time of bed preparation. Mycelial suspension of *S. rolfsii* derived from seven days old pure mycelial culture was mixed with the straw @ 10ml/kg on dry weight basis. Square size beds (2ft x 2ft) were prepared for straw mushroom cultivation using one month old wheat grain spawn supplemented with 2% calcium carbonate[5]. For each bed, 350g spawn and 250g. chickpea power were used. The wet bundles with their butt ends on one side were closely laid on a bamboo platform. Some bundles were placed over them in opposite direction. This constituted the first layer. One-fourth of spawn bits (thumb-size) were placed at 3" apart leaving 4" from the periphery. One-fourth of gram power was sprinkled over the spawn bits. The second layer was put in similar manner but at right angle to the previous layer. It was spawned and supplemented like the first layer. The third layer which was put opposite to the second layer was spawned and supplemented on the entire surface with remaining amount of spawn and chickepea powder, respectively. A thin layer of straw was covered at right angle to the third layer. Thickness of individual layer was 6 inches except the top layer which was 2 inches thick. After preparation, the heap was slightly pressed from the top for better contact of spawn with adjacent layers as well as to create compactness. The entire bed was covered with a transparent polythene sheet till initiation of fruiting bodies. The beds were regularly sprayed with water to keep them moist except during fruiting. Mushrooms were harvested from three flushes and the fresh weights were immediately recorded. The data pertaining to yield were subjected to statistical analysis.

RESULTS AND DISCUSSION

The degree of inhibitions of mycelial growth of both the fungi by different phyto extracts varied widely (Table 1). The phyto extracts were more effective against *S. rolfsii* than *V. volvacea*. At lower concentration, the phyto-extracts failed to produce any noticeable growth inhibition of *V. volvacea* while causing comparatively higher growth inhibition of its competitor. There was a positive correlation

Table-1: Effect of different phyto-extracts on the mycelial growth of *Volvariella volvacea* and *Sclerotium rolfsii in vitro.*

Phyto-extracts	Per cent inhibition of mycelial growth over control										Growth Inhibition Ratio (GIR)
	S. rolfsii					*V. volvacea*					
	2%	5%	10%	20%	Mean	2%	5%	10%	20%	Mean	
1. Neem leaf *(Azadirachta indica)*	7.8	17.1	28.8	41.5	23.8	0.9	4.4	10.6	30.1	11.5	2.0
2 Neem seed *(A. indica)*	10.3	18.6	30.6	44.6	26.0	1.5	5.9	17.6	34.5	14.8	1.7
3 Gauva leaf *(Psidium guajava)*	18.6	20.5	49.8	59.6	37.1	0.0	3.0	10.8	19.2	8.2	4.5
4 Lantana leaf *(Lantana camara)*	29.8	27.8	59.9	70.0	46.8	0.5	5.4	14.6	31.8	13.0	3.6
5 Soapnut seed *(Sopindus trifoliat)*	49.7	70.1	95.5	100.0	78.8	0.0	0.9	10.0	29.8	10.1	7.8
6 Patalgarud leaf *(Rauwlfia sarpentina)*	10.0	14.8	21.4	40.2	21.6	0.2	3.1	4.9	13.6	5.4	4.0
7 Manjuati root *(Lawsonia inermis)*	14.8	20.0	32.8	39.8	26.8	0.0	0.9	5.9	19.8	6.6	4.0
8 Bel leaf *(Agle marmelos)*	11.8	29.3	41.9	71.6	38.6	0.3	5.1	10.5	18.8	8.6	4.4
9 Duba grass *(Cynadon dactylon)*	20.8	28.3	39.6	61.9	37.6	3.1	21.7	37.6	40.5	25.7	1.4
10 Tamarind leaf *(Tamarindus indica)*	31.8	40.8	96.4	100.0	67.2	0.0	1.6	9.8	14.1	6.3	10.6
11 Water hyacinth root *(Eichhornia crassipls)*	2.9	3.9	5.8	10.6	5.8	0.0	2.5	3.8	8.4	3.6	1.6
11 Water hyacinth root *(Euchhornia crassipes)*	2.9	3.9	5.8	10.6	5.8	0.0	2.5	3.8	8.4	3.6	1.6
12 Basung leaf *(Adhatoda vasica)*	5.9	14.8	20.3	30.6	17.9	0.0	0.9	7.6	9.7	4.5	3.9
13 Drumstick root *(Moringa oleifera)*	40.3	54.8	88.7	100.0	70.9	0.6	5.1	18.1	31.9	13.9	5.1
14 Karanj leaf *(Pongamia glabra)*	13.8	29.8	36.6	51.9	33.0	0.0	2.3	9.8	20.6	8.1	4.0
15 Merigold leaf *(Tagetes erecta)*	5.1	10.6	17.8	28.6	15.5	0.0	3.1	12.9	16.9	8.2	1.8
Mean	18.2	26.7	44.3	56.7		0.4	4.3	12.3	22.5		
SE(m)±	0.22	0.31	2.07	3.92		0.1	0.12	0.48	0.22		
CD at 5%	0.63	0.89	5.99	11.35		0.31	0.34	1.39	0.63		

Each observation is the average of three determinations

between the concentration of different phyto extracts and per cent growth inhibition of test fungi. Highest degree of growth inhibition (78.8%) of *S. rolfsii* was recorded against drumstick root extract followed by extracts of soapnut seed (70.9%) and tamarind leaf (67.2%). The mycelial growth of the weed fungus was completely checked by all these phyto-extracts at higher concentrations. Mycelial growth of *V. volvacea* was inhibited to the maximum (25.7%) by duba grass extract followed by drumstick root extract (13.9%) and soapnut seed extract (13.0%). Inhibitions in mycelial growth of both the fungi in response to root extract of water hyacinth were least pronounced as compared to other phyto-extracts. The growth inhibition ratio (GIR) was taken into consideration for selecting some phyto-extracts for their assay *in vivo*. Significantly higher GIR (10.4) was recorded with tamarind leaf extract followed by sopanut seed extract (7.8) and drumstick root extract (5.1). Phyto-extract of duba grass (GIR 1.4) was almost equally effective in inhibiting the mycelial growth of *V. volvacea* and *S. rolfsii*.

Data presented in Table-2 indicate that there was not much difference in time periods for primordia initiation among different phyto-extracts at various concentrations. In general, the fungus took 8-10 days for initiation of fruiting bodies. Highest sporophore yield was recorded in response to treatment of substrate with tamarind leaf extract followed by soapnut seed extract and drumstick root extract. Increase in sporophore yield was to the tune of 41.4 to 67.64 per cent compared to control. Mushroom yield was enhanced by 11.7 to 35.2 per cent and 2.94 to 41.1 per cent when extracts of soapnut seed and drumstick root were mixed with the inoculated substrate, respectively. Phyto-extracts at 5-10 per cent concentration sustained higher sporophore yield of *V. volvacea* compared to higher concentrations. There was no significant difference in yields between 5 and 10 per cent concentrations of the selected phyto-extracts in supporting the mushroom yield. The number and weight of sporophores were positively co-related. The average weight of sporophore was highest (15.1g) in the substrate treated with drumstick root extract at 5 per cent concentration.

Many weed fungi, predominantly *S. rolfsii* are associated with the undecomposed substrate, interfering with the growth and development of paddy straw mushroom. Therefore, sterilization of

Table 2: Effect of phyto-extracts treated substrate but inoculated with *Sclerotium rolfsii*, on the sphorophore yield of *Volvariella volvacea*

Phyto extracts	Concentration (%)	Number of sporophore (g)	Weight of sporophore (%)	Biological efficiency
Tamarind leaf *Tamarindus indica)*	5	98	1400 (64.7)	14.0
	10	102	1425 (67.6)	14.2
	20	92	1350 (58.8)	13.5
	50	89	1200 (41.1)	12.0
Mean			1343.7	
Soapnut seed *(Sopindus trifoliat)*	5	85	1200 (29.4)	11.0
	10	89	1150 (35.2)	11.5
	20	77	975 (14.7)	9.7
	50	71	950 (11.7)	9.5
Mean			1043.7	
Drum-stick root *(Moringa oleifera)*	5	79	1200 (41.7)	12.0
	10	75	1050 (73.5)	10.5
	20	70	925 (8.8)	9.2
	50	70	875 (2.9)	8.7
Mean			1012.5	
Control (Paddy straw inoculated with *S. rolfsii)*		69	850	8.5
SE(m)t			14.24	4.3
CD at 5%			43.15	

Each observation is the average of three determinations.

Data in parentheses indicate per cent increase in yield over control.

cultivation substrate is indispensable for obtaining improved sporophore yield. However, pasteurization of paddy straw by conventional hot water treatment or normal steaming is often costly and cumbersome for production of the crop. In the absence of proper pasteurization facilities, the growers are left with no choice but to use unsterilized substrate for mushroom cultivation. Some growers resort to chemical pasteurization with bavistin and formalin as reported by Vijay and Sohi[11]. However, higher concentration of these chemicals have proved to be toxic to the growth of mushroom mycelia which ultimately hamper the spawn run and yield[3,9]. In this context, the results of the present investigation assume immense importance. Tamarind leaf extract at lower concentration was proved to be nontoxic to the growth of mushroom mycelia while causing appreciable growth inhibition of the competitor thereby sustaining higher sporophore yield of *V. volvacea*. This finding substantiates earlier reports of some scientists [8,10] who obtained higher yield of paddy straw mushroom by supplementing paddy straw with tamarind leaves. However, the use of tamarind leaf extract for suppressing growth of *S. rolfsii* during straw mushroom cultivation is reported for the first time. Moreover, extracts of soapnut seed and drumstick root could be effectively used against *S. rolfsii* vis-a-vis *V. volvacea* which have not been reported previously. Therefore, the present investigation opens up a new approach for developing some botanical fungicides derived from these phyto-extracts which will be cheaper and selective in action compared to present substrate-sterilization methods for improved sporophore yield of paddy straw mushroom.

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