Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; 9(3): 1206-1210
Received: 04-03-2020
Accepted: 08-04-2020

DRS Vineela
Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

SK Beura
Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

A Dhal
AICRP on Groundnut, OUAT, Bhubaneswar, Odisha, India

SK Swain
AICRP on Groundnut, OUAT, Bhubaneswar, Odisha, India

Corresponding Author:
DRS Vineela
Department of Plant Pathology, College of Agriculture, OUAT, Bhubaneswar, Odisha, India

Efficacy of bio-agents, botanicals, organic amendments against groundnut pathogens *Sclerotium rolfsii* and *Aspergillus niger in-vitro*

DRS Vineela, SK Beura, A Dhal and SK Swain

Abstract
Groundnut is an important oilseed crop susceptible to losses incited by soil borne pathogens of which collar rot caused by *A.niger* and stem rot caused by *S.rolfsii* are most important diseases in groundnut that kill the plants and their by direct effects on yields. Considering the limitations of chemical control, biological control is an important approach in this direction and was tested *in-vitro*. In dual culture, of the three Trichoderma species *Trichoderma hamatum* is more efficacious compared to *Trichoderma harzianum* and *Trichoderma viride* in inhibiting growth of test pathogens. In poisoned food technique, *Allium sativum* was proved to be the most effective botanical and recorded 100% reduction in growth of *A. niger* and *S. rolfsii* and of six organic extracts well decomposed FYM and Groundnut cake inhibits mycelial growth of *S. rolfsii* by 100% whereas vermicompost has shown higher growth inhibition of *A. niger* by 71.80%.

Keywords: Botanicals, Organic amendments, Integrated disease management, soil borne diseases

Introduction
The major soil borne diseases of groundnut caused by fungi are collar rot/crown rot/seedling blight (*Aspergillus niger*), stem rot/Sclerotium wilt (*Sclerotium rolfsii Sacc.*). *S. rolfsii* attacks the crop at all the stages causing seed rot, seedling blight, stem rot and pod rot, the most common being stem rot. On the contrary, both *A. niger* and *A. flavus* primarily attack the seedling stage causing collar rot and aflaroot. These two soil borne diseases poses severe threat to groundnut cultivation. Soilborne diseases are especially complicated to manage due to the difficulty of dispersing fungicides through the peanut canopy to the soil profile. The chemical method developed for disease control too has its own limitations such as high capital investment, non- remunerative, poor availability, selectivity, temporary effect, efficacy affected by physico-chemicals and biological factors, development of pest resistance, pollution of food and feeds, health hazards and environmental pollution. Hence, the present investigation was undertaken to exploit the *in-vitro* efficacy of three species of *Trichoderma*, *in-vitro* efficiecy of plant extracts and organic amendments for management of stem rot of groundnut caused by *Sclerotium rolfsii* and collar rot of groundnut caused by *Aspergillus niger* as bioagents, plant extracts and organic amendments can be exploited within framework of integrated disease management.

Materials and Methods
In-vitro evaluation of bioagents
The antagonistic activity of three *Trichoderma* spp. against *Sclerotium rolfsii* and *Aspergillus niger* was determined by dual culture technique. Mycelial discs measuring 5 mm diameter from four days old cultures of both fungal antagonist and the test pathogen were placed at equidistant on sterile petriplate containing PDA medium. The petriplates were then incubated at 28 ± 2°C. Three replications were maintained in each treatment. Suitable controls were kept without antagonist. Growth of antagonists, pathogen and zone of inhibition were measured after recording full growth in control plate and percentage inhibition of mycelial growth of test pathogen was calculated. Antagonistic potential was determined by using parameters *viz.* degree of inhibition or intermingled zone between both the colonies.

1. The percentage inhibition of radial growth was calculated by using equation

Percentage of inhibition I =C-T/C × 100

Where
C= growth of pathogen in control
T = growth of pathogen in treatment

Further, angular transformation values were taken for data and analyzed statistically

2. Efficiency of species was identified by scoring on modified Bell's scale (Bell *et al.*, 1982) [2]. R1=I00% overgrowth; R2=75% overgrowth; R3=55% overgrowth; R4= blocked at point of contact and R5= pathogen overgrows antagonist.
3. The kind and degree of antagonism was determined according to classification by Skidmore and Dickinson (1976) [15]. A=Mutual intermingling growth, Bi=Overgrowth by antagonists, Bii= Intermingling growth in which test fungus under observation has ceased growth and overgrown by another colony, C= Light inhibition, D= Not detected.

***In-vitro* evaluation of botanicals**

Ten number of locally available plant parts were taken to test their efficiency against the test pathogens. Fresh plant materials of different plant species were thoroughly cleaned, surface sterilized with 2% sodium hypochlorite and washed well with sterile water. The predetermined plant parts were grounded along with sterile water at the rate of 1:1 w/v using pestle and mortar and the macerate was filtered through a Whatman No.1 filter paper under sterilized condition to get the clear plant extract (100%). The extract of each plant species at two concentrations *viz.,* 10 and 20 per cent were tested against *A. niger* and *S. rolfsii* by poisoned food technique (Nene and Thapliyal, 1971) [10]. Three replications were maintained for each plant extract. The colony diameter was recorded and per cent inhibition of growth of *A. niger and S.rolfsii* over control was estimated. The efficacy of plant extracts or botanicals was expressed as per cent inhibition of radial growth over the control which was calculated by using the formula as described earlier.

Further, corresponding angular transformation were made for data and analysed statistically.

The data obtained *in-vitro* on per cent growth inhibition of test fungus were analysed following completely randomized design (CRD)

***In-vitro* evaluation of organic amendments**

Fifty grams fine powder of six organic amendment were taken into 250 ml conical flasks and 150 ml water was added to each flask and allowed to decompose for 15 days then stained with muslin cloth to obtain the extract and autoclaved at 1.2 kg/cm pressure for 20 min. considered as 100% concentration (standard solution). Fifty ml quantity of standard solution of organic extracts were incorporated separately in melted sterilized PDA medium in conical flasks aseptically at the time of pouring the medium to obtain desired concentrations. After solidification, the Petri plates were inoculated in the centre by placing 4 mm mycelial disc from 7 days old actively growing fungal culture of *S. rolfsii and A.niger* and maintained with 3 replications**.** The efficacy of organic amendments was expressed as per cent of radial growth over the control which was calculated by using the formula as described earlier.

Further, corresponding angular transformations were made for data and analysed statistically.

Results and Discussion

Efficacy of *Trichoderma sp* on growth of *Sclerotium rolfsii* and *Aspergillus niger*

Three *Trichoderma spp viz., Trichoderma viride, Trichoderma harzianum* and *Trichoderma hamatum* were tested to find out their antagonistic potential and type of colony interaction against test pathogens *Sclerotium rolfsi* and *Aspergillus niger*. From the *in-vitro* study it is observed that, antagonist *Trichoderma hamatum* is most efficacious with 72.2% mycelial growth inhibition, 89.02% sclerotial inhibition followed by *Trichoderma harzianum* with 74.7% mycelial growth inhibition, 59.84% sclerotial inhibition and *Trichoderma viride* with 71.9% mycelia growth inhibition, 59.99% sclerotial inhibition .

In-vitro dual culture study of antagonists against *Aspergillus niger* showed that *Trichoderma hamatum* is most efficacious with 56.20% mycelial growth inhibition followed by *Trichoderma viride* with 40.07% mycelia growth inhibition. *Trichoderma hamatum* efficacy is more as compared to *Trichoderma harzianum* and *Trichoderma viride* but *Trichoderma harzianum* is statistically at par with *Trichoderma hamatum.*

Trichoderma hamatum showed better ranking from modified Bells score which decipted ability of antagonist to overgrew and caused lysis of mycelium of test pathogen that is R1(100% growth) in case of *sclerotium rolfsii* and R3 (55%growth) in case of *Aspergillus niger*, whereas *Trichoderma viride* or *Trichoderma hamatum* showed R2 (75%) or R4 (blocked at point of contact) respectively. *Trichoderma* showed Bi type of interaction with *Sclerotium rolfsi* and *Aspergillus niger*(Table.1).

Similar findings also have been reported by Basumatary *et al*., (2015), where maximum percentage of inhibition on growth of *Sclerotium rolfsii* was observed with *Trichoderma harzianum* (77.39%) followed by *Trichoderma viride* (76.54%). Biswas *et.al*., (2000) reported that in groundnut among the 11 isolates of *T. harzianum*, three *viz*., T8, T10 and T2 were found to be effective against *S. rolfsii* and they overgrew the pathogen by 92, 85 and 79% respectively. *Trichoderma harzianum* (Sardarkrushi Nagar) showed significantly maximum growth inhibition (77.7%) and grew over *S. rolfsii* colonies followed by *T. viride* (Sardarkrushi Nagar) as 68.5% (Senjaliya, 2015) [13].

Nathawat (2014) [9] reported similar results where *Trichoderma harzianum* (Navsari) showed significantly maximum growth inhibition (81.4%) followed by *T. viride* (S. K. Nagar) as 77.4% growth inhibition of collar rot fungus. Sharma and Saha (2013) demonstrated that *T. harzianum* inhibited the growth of 2 virulent isolates of *A. niger* by 63 and 58%, respectively. *Trichoderma* (Local,) and *T. harzianum* were found to be effective against *A. niger* which is responsible for causing collar rot disease of groundnut. However, the degree of inhibition varied with the species of *Trichoderma*. It ranged from 31.11 to 57.0%. *Trichoderma* (LocaI1) and *T.harzianum* inhibited the pathogen to the extent of 57.77 and 55.55% respectively in comparison to *T. viride* (37.0%), *Trichoderma* Local2 (33.33%) and *T. hamatum* (31.11%). (Shlvraj S. Patale *et al*., 2009) [14].

However, the present investigation includes *Trichoderma hamatum* which performed better in both inhibiting mycelial growth of *Sclerotium rolsii* and *Aspergillus niger* as well as suppressing the formation of sclerotial bodies of *Sclerotium rolfsii*.

Proposed mechanisms of antagonism include mycoparasitism by the action of cell-wall degrading enzymes, antibiosis by the production of antibiotics, competition for space and nutrients through rhizosphere competence, facilitation of seed germination and growth of the plants via releasing important minerals and trace elements from soil and induction of the defense responses in plants (Herrera and Chet, 2003; Howell, 2003) [5].

Table 1: Efficacy of *Trichoderma* spp. on growth of *Sclerotium rolfsii* and *Aspergillus niger*

	treatment	*Sclerotium rolfsii*				*Aspergillus niger*		
		Radial growth(mm)	%inhibitoin	% inhibition of sclerotial bodies	Score (Bell scale)	Radial growth (mm)	%inhibition	Score (Bell scale)
T1	*Trichoderma viride*	25.00	71.9 (57.99)*	84.07 (66.96)	R2	53.75	40.07 (39.24)	R4
T2	*Trichoderma harzianum*	22.50	74.7 (59.84)	79.57 (63.52)	R2	65.50	26.82 (31.18)	R4
T3	*Trichoderma hamatum*	22.00	75.2 (60.19)	9.02 (71.130)	R1	39.25	56.20 (48.54)	R3
T4	Control	89.25	0.00 (0.00)	0.00 (0.00)		89.75	0.00 (0.00)	
	CD 5%	0.297	2.126	8.373		2.514	1.636	
	SE(m)±	0.095	0.682	2.688		0.807	0.525	
	CV%	4.811	3.066	10.664		2.6	3.531	

* Figures in the parenthesis are arc sin transformed value

Bio-efficacy of phyto-extracts on growth of *Sclerotium rolfsii* and *Aspergillus niger in vitro*

Bioefficacy of plant extracts on growth of *A. niger* and *S. rolfsii* were tested *in-vitro* at two concentrations such as 10% and 20%. All the plant extracts at both the concentrations were significantly effective in reducing growth of *A. niger* and *S. rolfsii* as compared to control. The inhibition of growth of *S. rolfsii* ranged from 0.0cm to 88.66cm against 90cm in control and of *A. niger* ranged from 33.3cm to 63.30cm against 90cm in control (Table.2).

Maximum reduction of mycelium was observed at 20% concentration which was significantly superior over 10% concentration. The interaction between plant extracts and concentrations was found significant. All plant extracts were increasingly effective in reducing mycelial growth with increase in concentration.

Irrespective of concentration, *Allium sativum* was proved to be effective botanical and recorded maximum reduction of growth of both *A. niger* and *S. rolfsii* by 100% which was significantly superior to all other plant extracts. The next best treatment against *S. rolfsii* was *Curcuma longa*(55.50%) followed by *Lantana camara* (53.26%), *Allium cepa*(52.16%), *Pongamia pinata (40.33%), Annona squamosa(32.20%), Eucalyptus* (32.90%), *Zingiber officinale(32.16%)* and *Azadirachta indica* (*25.13%)* at 20% concentration. *Chrysanthemum* at both the concentration was least effective in reducing fungal growth.

In case of *A. niger*, best extract after *Allium sativum* was *Annona squamosa (55.13%)* followed by *Lantana camara (43.47%), Allium cepa(40.70%), Zingiber officinale (27.73%), Azadirachta indica(20.70%)* and *Curcuma longa z(18.46%)* at 20% concentration. *Pongamia pinata* at both concentration was least effective in reducing fungal growth.

Garcia and Padilla (1996) [3] reported that extracts from garlic were found to be the most effective against *S. rolfsii* to inhibit the mycelial growth. Different plant extracts *viz*., onion (*Allium cepa*), carrot grass (*Parthenium hysterophorus)*, ashok (*Polyalthia longifolia*), neem (*Azadirachta indica*), batri (*Clerodendron spp*.) and datura (*Datura stremonium*) have also been reported to limit the growth of *S. rolfsii in-vitro*(Pariya and Chakravarthi, 1977; Annapurna *et al*., 1983; Humanthegowda and Adiver, 2001 and Meena and Muthuswamy, 2002) [11, 1, 6, 8]. The growth of *S. rolfsii* was inhibited by all the botanicals at both concentrations(25% and 50%) tested in comparison to control. Maximum inhibition was obtained by neem leaves followed by turmeric and garlic clove extracts. (Gour.,2010) [4].

Evaluation of different botanicals by poisoned food technique showed that all plant extracts tested *in-vitro* were found significantly effective in reducing the percentage mycelial growth of *Aspergillus niger* over untreated control. However, plant extract @ 5, 10 and 15% of garlic, recorded lowest mean colony diameter (1.57 cm) and highest mean mycelial growth inhibition (81.51%) followed by *Polyalthia longifolia, Annona squamosa and Pongamia glabra* which recorded the mycelial growth of 3.38 cm, 3.75 cm, 3.87 cm and the mean mycelial growth inhibition of 60.18%, 55.87% and 54.44% respectively. Among the concentrations and plant extracts, *A. sativum* (@10 and 15%) was most effective in reducing the growth of *A. niger* (100%) followed by *Polyalthia longifolia* @15% (77.73%), *Annona squamosa* @ 15% (74.61%) and *Pongamia glabra* @ 15% (70.33%). (Rajasekharam *et al*.,2013) [12]. The phytoextracts screened *in-vitro* by poisoned food technique against *A. niger* revealed that neem leaves showed maximum growth inhibition of the fungus followed by parthenium, neem seed, barmasi and ardusi leaves. (Nathawat, 2014) [9].

However the present study revealed that plant extract of *Allium sativum* is most effective in inhitbiting mycelia growth of *Sclerotium rolfsii* and *Aspergillus niger* at both 10% and 20% concentration.

Table 2: Bio-efficacy of phyto-extracts on growth of *Sclerotium rolfsii* and *Aspergillus niger*

	Treatment	*Sclerotium rolfsii*				*Aspergillus niger*			
		Concentration 10%		Concentration 20%		Concentration 10%		Concentration 20%	
		Radial growth(mm)	%inhibitoin	Radial growth (mm)	% inhibition	Radial growth(cm)	%inhibition	Radial growth(mm)	%inhibition
T1	*Curcuma longa*	50.33	44.03 (41.53)*	40.00	55.50 (48.14)*	77.33	14.03 (21.92)*	73.33	18.46 (25.35)*
T2	*Zinziber officinale*	80.33	10.70 (18.97)	61.00	32.16 (34.52)	81.33	9.60 (17.99)	65.00	27.73 (31.75)
T3	*Allium sativum*	0.00	100.00 (90.00)	0.00	100.00 (90.00)	33.00	63.30 (52.70)	0.00	100.00 (90.00)

T4	*Allium cepa*	87.33	2.77 (7.80)	42.66	52.56 (46.45)	72.33	19.60 (26.22)	53.33	40.70 (39.61)
T5	*Azadirachta indica*	87.33	2.93 (5.75)	67.33	25.13 (30.05)	84.33	6.26 (13.84)	71.33	20.70 (26.95)
T6	*Pongsmia pinnata*	80.00	11.06 (19.28)	53.66	40.33 (39.41)	88.67	1.46 (4.03)	77.67	13.67 (21.62)
T7	*Annona squamosa*	67.33	25.13 (30.06)	61.00	32.20 (34.55)	85.33	5.13 (12.27)	40.33	55.13 (47.93)
T8	*Eucalyptus*	72.66	19.23 (25.94)	60.33	32.93 (34.99)	85.00	5.50 (13.37)	74.67	17.00 (24.29)
T9	Lantana camara	51.00	43.30 (41.12)	42.00	53.26 (46.85)	82.00	8.83 (17.08)	37.33	43.47 (40.31)
T10	*Chrysanthemum*	87.66	2.56 (5.3)	88.66	1.46 (4.03)				
T11	*CONTROL*	90.00	0.00 (0.00)	90.00	0.00 (0.00)	90.00	0.00 (0.00)	90.00	0.00 (0.00)
	CD 5%	5.348	8.485	3.868	4.347	4.57	6.592	4.581	8.682
	SE(m)±	1.812	2.874	1.31	1.473	1.538	2.219	1.542	2.923
	CV%	4.578	19.158	4.115	6.859	3.419	21.414	4.581	14.54

*Figures in parenthesis are arc sin transformed value

Evaluation of organic extracts on growth of *Sclerotium rolfsii* and *Aspergillus niger in vitro*.

Organic extracts were tested *in-vitro* against *S. rolfsii* and *A. niger* at 10% concentration. Results showed that well decomposed FYM and Groundnut cake inhibited mycelial growth of *S. rolfsii* by 100% followed by Mustard oil cake(88.8%), Neem cake(60.70%), Karanja(60.7%) and Vermicompost(59.96%) which were significantly higher than control. However Vermicompost has shown higher growth inhibition of *A. niger* by 71.8% followed by Mustard cake and Neem cake(70%), FYM and Groundnut cake (68.1%) which were statistically at par with each other. Least inhibition was recorded by Karanja cake (50%) (Table.3)

Similar findings have been reported by Senjaliya, (2015) [13] that among 6 different organic extracts (mustard, groundnut, neem, castor, cotton cakes & FYM) evaluated and results revealed that all the extracts significantly inhibited the growth of *Sclerotium rolfsii in-vitro* except FYM. Among all the organic extracts, castor cake recorded significantly 100% inhibition of growth and sclerotial production of *S. rolfsii* at both the concentrations (10 & 20%). Also cent per cent inhibition of growth and sclerotial production was recorded in neem, groundnut and cotton cakes at 20% concentration. However, present study showed that 100%inhibition by well decomposed FYM. Management of stem rot in groundnut has also been demonstrated with different doses of amendments such as cakes and FYM by Jonson *et al*. (2003) [7].

Table 3: Evaluation of organic extracts on growth of *Sclerotium rolfsii* and *Aspergillus niger in vitro*

	treatment	**dosage**	***Sclerotium rolfsii***		***Aspergillus niger***	
			Radial growth(mm)	**%inhibitoin**	**Radial growth(cm)**	**%inhibition**
T1	Mustard	10%	10.00*	88.83* (70.51)**	26.66*	70.30* (57.05)**
T2	Groundnut	10%	0.00	100.00 (90.00)	28.66	68.10 (55.60)
T3	Karanja	10%	35.33	60.70 (51.16)	45.00	50.00 (42.240
T4	Neem	10%	27.67	69.20 (56.29)	27.00	70.00 (56.74)
T5	Vermicompost	10%	36.00	59.96 (50.74)	25.33	71.80 (57.92)
T6	FYM	10%	0.00	100.00 (90.00)	28.66	68.10 (54.86)
T7	Control		90.00	0.00 (0.00)	90.00	0.00 (0.00)
	CD 5%		4.314	3.061	7.571	3.822
	SE(m)±		1.409	1	2.472	1.248
	CV%		8.582	2.965	11.046	4.664

* Figures in the parenthesis are arc sin transformed value

Conclusion

The results of present investigation revealed that *Trichoderma hamatum* is more efficacious compared to *Trichoderma harzianum* and *Trichoderma viride* but *Trichoderma harzianum* is statistically at par with *Trichoderma hamatum.* Bioefficacy of plant extracts on growth of *A. niger* and *S. rolfsii* were tested *in-vitro* at two concentration, 10% and 20%. Irrespective of concentration, *Allium sativum* was proved to be the most effective botanical and recorded highest reduction of growth of both *A. niger* and *S. rolfsii* by 100% which was significantly superior to all other plant extracts under study. well decomposed FYM and Groundnut cake inhibits mycelial growth of *S. rolfsii* by 100% followed by Mustard oil cake(88.8%), whereas Vermicompost has shown higher growth inhibition of *A. niger* by 71.80% followed by Mustard cake(70.30%) and Neem cake(70.00%).

Reference

1. Annapurna M, Mitra S, Lyengan DS, Rao SN, Bholerao UT. Antimicrobial activity of leaf extracts of *Polyalthia longifolia*, Phytopathologgische Zeischrif. 1983; 106:183-185.
2. Bell DK, Wells HD, Markham CR. *In vitro* antagonism of *Trichodrema* species against six fungal plant pathogens. Phytopathology. 1982; 72:379-382.
3. Garcia RP, Padilla CL. Evaluation of potential plant materials against some seed borne fungal pathogens of corn, Philippine Journal of Biotechnology. 1996; 5:159.
4. Gour HN, Pankaj Sharma, Kausha R. Efficacy of Phytoextracts and Bioagents against Root Rot of Groundnut Induced by *Sclerotium rolfsii*. Indian journal of plant protection. 2010; 38(1):73-79.
5. Herrera, Estrella A, Chet I. The biological control agent, Trichoderma from fundamentals to applications. In: Arora, D.K., Bridge, P.D. and Bhatnagar, D. (eds),Fungal biotechnology in agricultural, food and environmental applications, Marcel Dekker, New York, 2003, 147-156.
6. Humanthe Gowda B, Adiver SS. Effect of plant extracts, bioagents, rotational crops and their root exudates on *S. rolfsii* causing stem rot of groundnut, Journal of Oilseeds Research. 2001; 18:240-243.
7. Johnson M, Subramanyam K, Balaguvaih D, John Sudheer M. Management of stem rot in groundnut through soil amendments. Annals of Plant Protection Sciences. 2003; 11:83-85
8. Meena B, Muthusamy M. Fungitoxic properties of plant extracts against *Sclerotium rolfsii* in Jasmine. Journal of Ornamental Horticulture. 2002; 5:82-83.
9. Nathawat BDS, Mahendra Partap. Evaluation of fungicides, botanical and *Trichoderma* spp. against collar rot of Groundnut (*Arachis hypogaea* L.) caused by *Aspergillus niger* van Tiegham. Annals of Plant Protection Science. 2014; 22(2):382-385.
10. Nene YL, Thapliyal PN. Fungicides in plant disease control (3rd Ed.,). Oxford and IBH Publishing Company, New Delhi, 1993.
11. Pariya S, Chakravarthi DK. Antifungal activity of some Indian medicinal plant extracts on phytopathogenic fungi. Phytopathologia Mediterranea. 1977; 16:23-24.
12. Rajasekharam T, Subba Reddy C. Evaluation of antagonists and natural plant extracts in controlling collar rot of groundnut caused by *Aspergillus niger*. Plant Disease Research. 2013; 29(1):26-32.
13. Senjaliya BD, Nathawat BDS. Evaluation of bioagents, phyto-extracts and organic amendment on stem rot of Groundnut, Annals of Plant Protection Science. 2015; 23(1):98-101.
14. Shlvraj S, Patale, Vasant P Mall. Efficacy of *Trichoderma* in management of collar rot of groundnut. Bioinfolet. 2009; 6(2):154-156.
15. Skidmore AM, Dickinson CH. Colony interaction and hypheal interference between *Septoria nodorum* and Phylloplane fungi. Transactions of the British Mycological Society. 1976; 66(1):57-64.