Indian Journal of Weed Science 41(1&2) : 67-69, 2009

Evaluation of different substrate for mass production and field performance of collor rot fungi strain for the management of Parthenium

Rekha Shukla and A.K. Pandey
Mycological Research Laboratory, Department of Bioscience, RDVV, Jabalpur (Madhya Pradesh)
Email: akpmycol@yahoo.com

ABSTRACT

Mass production of mycoherbicidal agent *Sclerotium rolfsii* (FGCC#02) under solid substrate fermentation was standardized. Mycoherbicidal potential of inoculum, grown on twenty agro wastes, applied on Parthenium at pre-emergence and post emergence stage was determined. Wheat grains and host leaves colonized inocula incited severe infection and was responsible for significant mortality at pre-emergence application while tea-bagasse-wheat bran-wheat straw (TBWW) was highly effective at post emergence stage. Contrary to these, waste cotton failed to cause appreciable mortality at both the stages.

Key words: Solid substrate fermentation, Mycoherbicide, *Sclerotium rolfsii*, Field evaluation

Parthenium hysterophorus L., a deadly weed was probably introduced in India during 1950s and now posing serious health problems , threat to human beings, livestock and animals (Pandey *et al.* 1996). It is also responsible for substantial losses in agriculture and forestry (Knox *et al.* 2006). Conventional methods to control this weed have failed due to several reasons (Hasija *et al*. 1994). Thus, biological control of weed especially with plant pathogenic fungi have attracted the attention of a large group of scientist world over. Several such microbes have been patented and few of them have been commercialized as mycoherbicides. A local strain of *Sclerotium rolfsii (FGCC#02)* incited severe collar rot disease and showed very high pathogenic potential and satisfied most of the parameters required for consideration of any organism as mycoherbicide against Parthenium (Pandey *et al.* 1996a). However, difficulty in mass production of the agent constrained its application as mycoherbicide. Therefore, mass production of the test agent through solid substrate fermentation and its field potential have been determined and discussed in the present communication.

MATERIALS AND METHODS

Recovery of culture and its maintenance

The test strain was obtained from Regional Fungal Germplasm Collection Center, Mycological Research Laboratory, Department of Biological Sciences, R.D. University Jabalpur. It was maintained on PDA (Potato Dextrose Agar) slants and stored in a refrigerator.

Screening of substrates

A total of 20 agro wastes was tried with definite moisture content. All the substrates were initially soaked overnight in distilled water then drained and partially dried to remove all the free water. Plastic bags containing 50 gm of substrates containing 100% moisture were successively autoclaved for 3 days at 121^{0}C and 15 lb pressure for 30 min. Each substrate was seeded with 9 mm^2 mycelial disc obtained from the edge of an actively growing 7 days old colony. All the experiments were performed in triplicates. Fungal biomass, sclerotiarl formation, color, size and number were determined by taking dry weight and visual reading of sclerotiar in all the substrates used (Larena *et al.* 2002, Perez-Gurez *et al.* 2003).

Mycoherbicidal potential

Seedling assay : 0.5 g substrate colonized by the fungus was applied to seedlings of Parthenium raised in pots (10 cm) in greenhouse containing sterilized soil/peat (1:1). Each treatment was replicated thrice. Observations were made after 24 hrs upto 14 days and per cent disease index (PDI) in terms of seedling mortality was determined (Mishra *et al.* 1996).

Field assessment : Mycoherbicidal potential of the test agent at both pre and post emergence stage of the weed were determined through Randomized Block Design (RBD) method. Each treatment was replicated thrice with 50 seedlings in each plot (2 m^2) for post and pre emergent application. The fungal agent was applied just after sowing of seeds for pre emergence treatment, while seedlings at 4-8 leaf stage were treated with the fungi for post emergence treatment. 10g of inoculum (colonized substrate) was applied to each plot. Post emergence application was followed by a second application, which was done after one month of previous application. Percentage mortality and emergence of seedlings was recorded after 2nd day of application for 1 month in post and pre emergent application of field respectively (Knox *et al.* 2006). The

Horsfall-Barratt (1945) rating scale was used to estimate injury to the seedlings (1=91-100%, 2=81-90%, 46.3=71-80%, 4=61-70%, 5=51-60%, 6=41-50%, 7=31-40%, 8=21-30%, 9=11-20%, 47.10=1-10%). Data obtained was subjected to analysis of variance. When significant results were found means were separated by the test of least significant difference (P=0.05).

RESULTS AND DISCUSSION

Screening of substrates

Data recorded (Table 1) clearly showed significant variation in mycelial coverage and sclerotial production on various agro waste tried. Maximum mycelial coverage was recorded in WG (wheat grains) followed by AB (arhar bran). Other substrates also showed good colonization. In contrast, cotton, maize cob (MC), orange peel (OP) and pomegranate did not support mycelial colonization. Similarly maximum sclerotial production was recorded in gram dal followed by TBWW, WG, TPWS and CC. There was no strict correlation observed between mycelial coverage and sclerotiarl formation. WG, which supported maximum mycelial coverage, produced lesser number of sclerotia. In general it was recorded that substrate supported growth failed to support high sclerotia formation. Poor response of maize cob may be due to hardness, however, similar response with cotton was surprising. Similar variations in substrate colonization have also been recorded by many other workers. Singh *et al.* (2004) obtained fast growth and good sporulation of *Trichoderma harzianum* on combination of wheat straw and wheat bran (WSWB), Sugarcane waste and tea leaves. Addition of wheat bran to the substrate (combinations)

Table 1. Morphological Studies and in vitro mycoherbicidal potential of pathogen on various substrates

Substrates	Mycelia coverage (%)	No. of sclerotiar /50g of substrate	% Seedling mortality	% Inhibition of germination	Seedling mortality
Rice bran (RB)	95.83	1,800	18	73.33	7
Wheat bran (WB)	94.66	500	82	72	7
Tea leaves	53.56	1,000	18	63.33	7
Wheat straw+ wheat bran (WSWB)	96.46	6,000	50	72	5
Rahar bran (RB)	96.90	5,000	50	74.66	6
Gram dal (GD)	95.33	11,800	50	93.33	6
Tea leaves + bagasse(BT)	95.96	670	18	94	7
Wheat straw+ wheat bran+Tea leaves(WWT)	96.75	2500	65	41.33	4
Wheat straw+ wheat bran+ Tea leaves+ bagasse (TBWW)	96.90	10,500	91	80	3
Wheat bran+ bagasse (WBB)	96.00	5,600	40	50.66	6
Tomato pulp+ wheat straw (TPWS)	94.75	6,000	50	20.66	5
Wheat grains (WG)	97.17	6,250	95	100	4
Wheat straw (WS)	95.00	5.,000	0	0	0
Cotton	Nil	40	38	34.66	6
Tomato pulp(dry) (TP)	55.12	4,000	50	100	7
Host leaves (HL)	41.88	5,000	62	33.33	5
Maize cob (MC)	Nil	-	0	75	5
Orange peel (OP)	Nil	-	0	0	0
Pomegranate	Nil	-	0	0	0
Cob cover (CC)	95.00	5,500	0	0	0
LSD (P=0.05)			5.3	1.87	1.33

1. RH = 85-90%, 2. Temperature of cultivation 28±2^{0}C , 3.Age of seedlings: 10 days old, 4. Amount of inoculum = 0.5g for seedling bioassay and 10 g for green house studies, 5. Time of Application (Pre emergence): after sowing seeds and before weeds have emerged 6. Time of application,(post emergence): When the seedlings were at 4-5 leaf stage, 7. Values given in the table are tested by F test and are significant at 5% level of significance

favored the formation of sclerotiar. Regardless of the fact that cotton is a cellulosic material, failed to show significant mycelial growth and sclerotiar production. Maximum mycelial coverage resulted on wheat is in consistent with the results obtained by Harvey (1996) with *Sclerotinia sclerotiorum*.

Mycoherbicidal potential

Seedling assay : Maximum mortality (100%) was observed by fungus, cultivated on wheat grains and TBWW (Table 1). Minimum mycoherbicidal activity observed on cotton waste supported the results obtained during post emergence application of mycoherbicide. Similar observation was also recorded by Harvey (1996) during green house studies with *Sclerotinia sclerotiorum* on cracked wheat.

Pre-emergence application : Maximum mycoherbicidal potential of *S. rolfsii* (FGCC#02) was observed when cultivated on WG (100%) and HL, BT, GD and TBWW also showed significantly high potential as they revealed good colonization. Rest of the substrates showed moderate potential. WT and cotton were found unsuitable for inoculum buildup, perhaps due to the improper nutrient supply. Results obtained by Mishra (1994) is in agreement with the current findings.

Post-emergence application : TBWW exhibited maximum mycoherbicidal potential as it provided the suitable environment (substrate) form which organism can derive the energy required for infection and mortality (Table 1). Contrary to this, WG induced the most severe symptoms but failed to cause significant mortality. Amongst 20 agro waste tested as substrate to achieve maximum sclerotiar formation, pulses bran was found to be most suitable for mass multiplication probably due to the nutrients present in the substrate. Similar results have also been recorded by Singh (2004) with *Colletotrichum dematium* against *Parthenium hysteroporus*.

ACKNOWLEDGEMENT

Authors are thankful to the Head, Department of Biological Sciences, R.D. University, Jabalpur for providing necessary laboratory facilities. Financial assistance provided by MPCST, Bhopal is also thankfully acknowledged.

REFERENCES

Hasija SK, Rajak RC and Pandey AK. 1994. Microbes in the management of obnoxious weed. In: *Vistas in seed Biology* Singh, T and Trivedi, P.C.(Eds), Print Well, Jaipur Vol. I : 82–104.

Harvey CI. 1996. *Sclerotinia sclerotiorum*- Prospects as a mycoherbicide in pastures. In : *Pastures and Forage Crop Pathology* : 621-642.

Horsfall JG and Barratt RW. 1945. An improved grading system for measuring plant disease. *Phytopathology Abstracts* **35**, 655.

Larena I, Melgarejo P and Cal A De. 2002. Production acts, survival and evaluation of solid substrate inocula of *Penicillium oxalicum*, a biocontrol agent against *Fusarium* wilt of Tomato. *Biological Control* **92**(8)**:** 863-869.

Knox Jai, Dass A, Thomas M and Paul MS. 2006. Management of *Parthenium hysterophorus* L. through Atrazine with *Cassia uniflora* extract. *Annals of Plants Protection Science* **14** (2): 459-461.

Mishra J, Pandey AK and Hasija SK. 1996a. Mycoherbicidal potential of *Sclerotium rolfsii* Sacc. against *Parthenium*: Factors affecting *in vitro* growth and sclerotiarl formation. *Journal of Pathology Research* **9**(1): 19 - 24.

Mishra J, Pandey AK and Hasija SK. 1996b. Mycoherbicidal potential of *Sclerotium rolfsii* Sacc. against *Parthenium hysterophorus L.*:histopathological studies.*Indian Journal of Applied and Pure Biol*ogy **11:**73-77.

Perez-Gurez N, Torrado-Agrasar A, Lopez-Macias C and Pastrana L. 2003. Main characteristics and applications of solid substrate fermentation. Electronic *Journal of Environmental Agricultural Food Chem*istry **2**(3): 343-350.

Pandey AK, Mishra J, Rajak RC and Hasija SK. 1996. Potential of indigenous strains of *Sclerotium rolfsii* Sacc. for the management of *Parthenium hysterophorus* L In : *A serious threat to biodiversity in India*. *Herbal medicines, Biodiversity and Conservation Strategies*. Rajak RC and MK Rai (Eds) International Book distributors, Dehradun : 104-138

Singh SR, Singh HV and Singh Y. 2004. Efficacy of *Trichoderma* sp. against *Rhizoctonia solani* and reduction of substrates for their mass production. *In: Proceeding of Phytopathology Society, Golden Jubilee* **1**: 393-394.

Singh J. 2002. *Management of Parthenium by developed mycoherbicide from Colletotrichum* sp. Ph.D Thesis, R.D.V.V, Jabalpur.