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RESEARCH ARTICLE

Solid substrate fermentation of seed borne fungi of *Parthenium hysterophorus*

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Abstract

Use of plant pathogens, especially seed borne fungi have been suggested as one of the best possible means for controlling *Parthenium hysterophorus* as an alternative source of chemical herbicides with ecofriendly properties.

Production of mycoherbicide by solid substrate fermentation using *Drechslera* state of *Cochliobolus spicifer*, a seed borne fungi of the targeted weed as test pathogen was carried out in the present work by using various inexpensive solid wastes as substrate. Mycelial growth was recorded on each of the inoculated substrate but was extensive on rice bran (70%), sugarcane bagasse (70%) followed by wheat straw (65%) and wood shavings (65%), while sporulation was panoptic with wheat straw (7.8 $X10^8$/ml) followed by maize flaps (6.4 $X10^8$/ml) and rice straw (6.2 $X10^8$/ml) respectively. The bioactivity assessment revealed significant mortality against the targeted weed by using wheat straw followed by maize flaps and rice straw, which clearly shows that pathogenicity was in proportion to sporulation of the test pathogen.

Introduction

P. hysterophorus has now become a menace to different parts of the world and has also been reported from many parts of India. It is regarded as one of the most pernicious weed, causes damage in environment and agriculture by spreading disease causing agents in various ways. Particularly its pollen is known to be capable of causing various health hazards to humans and livestock (Kanchan, 1975; Chippendale and Panneta, 1994; Kumar, 1998 and Shukla and Pandey, 2008). Extensive efforts have been tried till date for the management of this dangerous weed by using various mechanical and chemical strategies but have failed due to several harmful reasons as they may cause formidable problems directly or indirectly to human beings, plants and animals. Therefore, biological strategies with ecofriendly properties are gaining more interest nowadays in weed management programs (Zettler and Freeman, 1972; Templeton and Smith, 1977 and McFadyen, 1992). Plant pathogens especially fungi possess great potential as controlling agent against this noxious weed (Pandey *et al.*, 2004 and Chutia *et al.*, 2007).

Mass production by solid substrate fermentation utilizes economically feasible, cost effective and easily available solid wastes as substrates for growth of the test strain which after fermentation become ready for direct application to the field with very little additional processing. Therefore, the present study was conducted to describe economic mass production with significant herbicidal potential of the selected seed borne fungal strain.

Materials and Methods

The mycoherbicidal agent:

The culture of *D.* state of *Cochliobolus spicifer* used during the study was isolated earlier from infected seed samples of *P. hysterophorus* and has been deposited to the Fungal Germplasm Culture Collection (FGCC), R.D. University, Jabalpur (M.P.), India. It appears highly pathogenic against the targeted weed host during preliminary herbicidal assessments, therefore selected for the present work.

Mass production:
For the fermentation process, solid substrates *viz*., arhar bran, bajra semolina, barley bran, barley straw, coconut coir, gram bran, gram semolina, groundnut oilcake, maize cobgrits, maize flaps, mustard oilcake, rice bran, rice straw, sugarcane baggase, soybean oilcake, tea wastes, til oilcake, vegetable wastes, wheat straw and wood shavings were employed.

Solid substrate fermentation:
Each substrate of 250 gm was soaked overnight in water and then kept for drying. These moistened substrates were dispensed in plastic bags and autoclaved under 121^{o}C for 15 min., inoculated with two discs of 0.5 cm diameter from 14 days old actively growing culture of the test fungi in sterilized conditions. These bags were closed with the help of cotton plugs in order to avoid contamination and were incubated in growth chamber for 30 days at 28±2°C in a BOD incubator, as according to Pandey *et al.,* (2001); Siddiqui and Bajwa, (2008) and Singh *et al.,* (2010).

Determination of mycelialcoverage:
Mycelial growth and coverage on each of the substrate was recorded after required period of incubation on the basis of visual observations respectively.

Assessment of sporulation:
All the inoculated bags were opened and 10 g of material from each of them was suspended in 1000 mL of distilled water seperately. The mixture was shaken and allowed to stand for 15 min., in order to facilitate the loosening of conidia. The resulting suspension was filtered through muslin cloth to remove large mycelial masses and the remnants of substrates. Spores Load Per Gram (SPLG) was estimated according to Bharadwaj and Trivedi, (1996) by using following formula:

$$SPLG = \frac{N \times V \times 1000}{W}$$

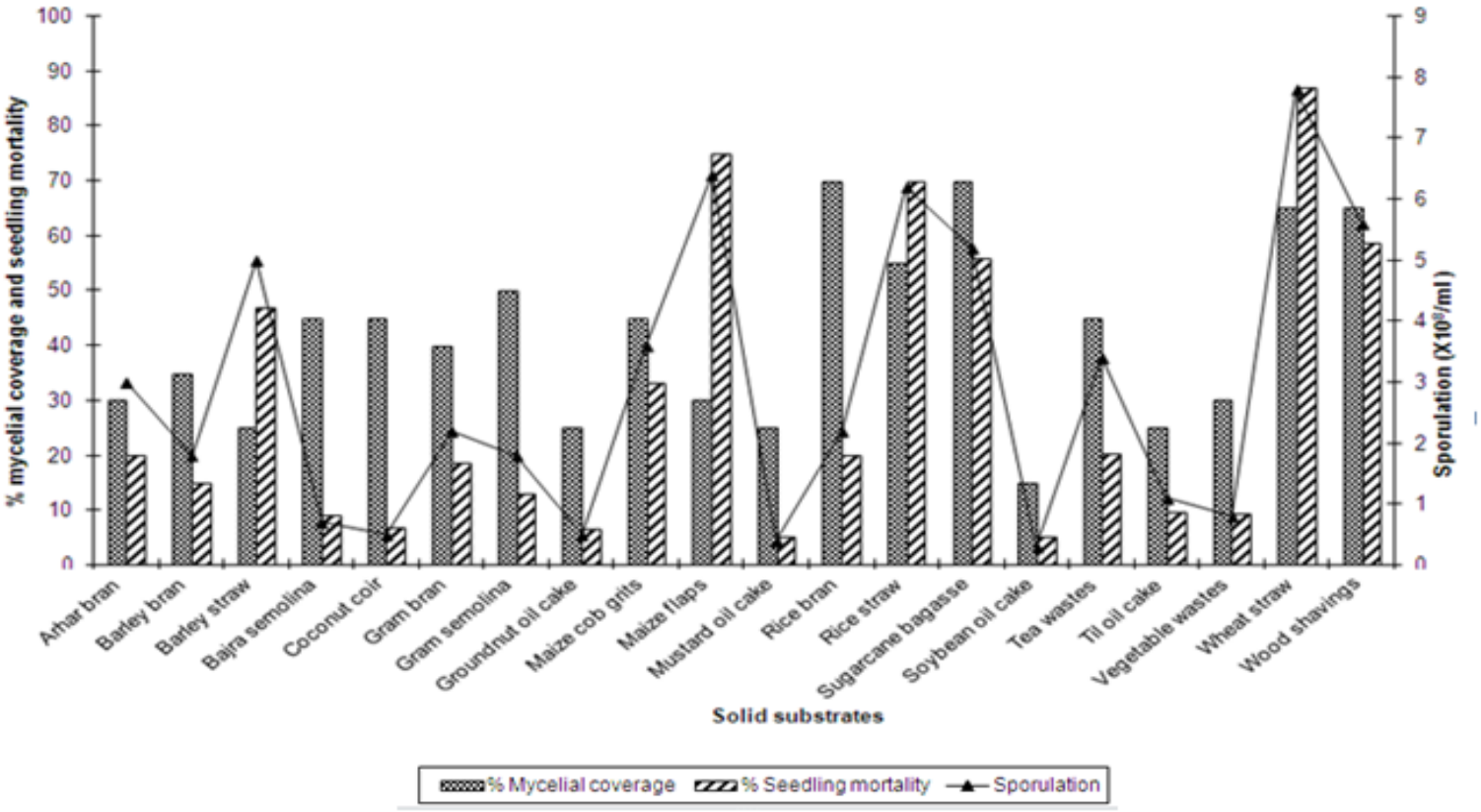
Where:
N = Number of spores in centre square of haemocytometer
V = Volume of mounting fluid added to the substrate
W= Weight of the substrate

Bioactivity assessment:
The viability of the mass produced conidia was assessed under green house conditions. The seedlings of targeted weed were raised in plastic pots of 7 cm diameter and 10 cm deep, filled with sterilized soil, sand and peat in the ratio of 1:1:1, sprayed with each of the prepared mycoherbicide and also 1 ml. of Tween 80 was added as an adjuvant. The pots were then incubated in growth chamber under defined conditions (100% humidity for 24 hrs. at 25ºC) for establishment and development of disease as in Fig. 1.

Results and discussion

Growth and mycelial coverage of the test pathogen was recorded high on rice bran (70%), sugarcane bagasse (70%) followed by wheat straw (65%) and wood shavings (65%), average on rice straw (55%), gram semolina (50%), bajra semolina (45%), coconut coir (45%), maize cobgrits (45%), tea wastes (45%), gram bran (40%). While minimum growth was found on barley bran (35%), arhar bran (30%), maize flaps (30%), vegetable wastes (30%) barley straw (25%), groundnut oilcake (25%), mustard oilcake (25%), til oilcake (25%) and soybean oilcake (15%) respectively. The bioactivity assessment results showed that pathogenicity of the test pathogen was in proportion to sporulation i.e., both sporulation and seedling mortality was maximum with wheat straw (7.8 X10^{8}/ml and 87.33%) followed by maize flaps (6.4 X10^{8} and 75.26%) and rice straw (6.2 X10^{8}/ml and 70.13%) respectively as in Fig.1. Therefore, wheat straw was considered to be the suitable substrate for mycoherbicide production, whereas average sporulation as well as pathogenicity occurred with wood shavings (5.6 X10^{8}/ml and 58.53%), sugarcane bagasse (5.2 X10^{8}/ml and 56.26%) and barley straw (5.0 X10^{8}/ml and 47.19%). Maize cob grits (3.6 X10^{8}/ml and 33.13%), tea wastes (3.4 X10^{8}/ml and 20.4%), arhar bran (3.0 X10^{8}/ml and 20.1%), gram bran (2.2 X10^{8}/ml and 18.16%), rice bran (2.2 X10^{8}/ml and 20.33%), barley bran (1.8 X10^{8}/ml and 15.13%), gram semolina (1.8 X10^{8}/ml and 13.26%), til oilcake (1.1 X10^{8}/ml and 9.06%), vegetable wastes (0.8 X10^{8}/ml and 9.13%), bajra semolina (0.7 X10^{8}/ml and 9.04%), coconut coir (0.5 X10^{8}/ml and 6.73%), groundnut oilcake (0.5 X10^{8}/ml and 6.6%), mustard oil cake (0.4 X10^{8}/ml and 5.33%) and soybean oil cake (0.3 X10^{8}/ml and 5.03%) sporulate to a lesser extent hence caused comparatively very less pathogenicity.

Fig. 1: Effect of various solid substrates on growth, sporulation and seedling mortality of *D.* state of *Cochliobolus spicifer*



Discussion

During solid substrate fermentations, growth and sporulation of mycoherbicide was affected by the nature of substrates. Rice bran and sugarcane bagasse followed by wheat straw and wood shavings showed successful colonization of fungal mycelia. Whereas, wheat straw followed by maize flaps and rice straw were found to be good substrates for spore production and rice bran, groundnut oilcake, coconut coir, mustard oilcake, soybean oilcake and vegetable wastes did not supported much sporulation as in Fig.: 1. Pathogenicity was recognized on the basis of symptoms of infection appeared *viz*., wilting, chlorosis, severe necrosis and ultimately death of the seedlings. During the present study, it was also found that seedling mortality of the targeted weed is directly proportional to the sporulation of test strain.

Several low cost media have also been used earlier by many other workers. Morin *et al.*, (1990) reported significant variations in growth and sporulation by solid substrate fermentation of *Phomopsis convolvulus* for field bindweed, also Siddiqui and Bajwa, (2008) used various agrowastes as substrates for the mass production of *Alternaria alternata* isolates effective against *Rumex dentatus* and *Chenopodium album*. And Zambare, (2010) used various agricultural residues for the solid substrate fermentation of *Aspergillus oryzae*.

Conclusion

The present work is the preliminary study of the screening of various agrowastes as substrates for production of cost effective mycoherbicide. And it was found that wheat straw provided an ideal substrate for moderate sporulation of the test pathogen which has not been established by the other selected substrates. The bioactivity test of the mass produced mycoherbicide against the respective weed revealed that the herbicidal efficacy of the test seed borne pathogen was not affected during mass culturing and causes significant mortality against the weed host.

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