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Short Communication

Mass Production of *Alternaria alternata* Isolates as Potential Bioherbicide Agents for *Rumex dentatus* and *Chenopodium album*

IFFAT SIDDIQUI[1] AND RUKHSANA BAJWA
Institute of Mycology and Plant Pathology, University of the Punjab, Quaid-e- Azam Campus, 54590, Lahore Pakistan
[1]Corresponding author's e-mail: isofpk_73@yahoo.com

ABSTRACT

Laboratory evaluation was carried out of locally available wheat straw, rice straw and bagasse as substrates supplemented with chickpea flour and molasses for mass culturing of *Alternaria alternata* (Fr.) Keissler. isolates, potential bioherbicides for *Rumex dentatus* L. and *Chenopodium album* L. Among three substrates wheat straw with chickpea flour as supplement produced 213 conidia per 10 µL in case of strain R (strain of *A. alternata* isolated from *R. dentatus*) and 203 conidia per 10 µL in strain C (strain of *A. alternata* isolated from *C. album*) after 14 days with 12 h photoperiod at 25-30ºC. The viability test of the *A. alternata* conidia at 10^7 conidia mL^{-1} and 10^9 conidia mL^{-1} for strain R and strain C, respectively mass produced on selected suitable substrate against 4-5 leaf stage of *R. dentatus* and 10-15 leaf stage of *C. album* revealed 100% mortality of the target weeds at 100% humidity for 24 h and at 25-30ºC.

Key Words: Bioherbicides; Wheat and rice straw; Bagasse; Chickpea flour; Molasses; Strain

INTRODUCTION

Rumex dentatus L. and *Chenopodium album* L. are most frequently occurring weeds in wheat fields of Pakistan (Siddiqui & Bajwa, 2001). Mechanical methods are becoming more costly and difficult to control weeds in wheat fields (Khalid, 2003). On the other hand chemical control strategies have produced herbicide resistant weeds during the last 20 years (Friesen *et al*., 2000). Due to these setbacks, in the last few years, there has been increasing interest in integration of biological methods into the control concepts (Amsellem *et al*., 2001).

The genus *Alternaria* contains over 60 species including both parasites on living plants and saprophytes (Rotem, 1994). Some important weed diseases are caused by *Alternaria* species e.g., *A. eichhorinae* has been reported as a fungal pathogen on water hyacinth in Egypt, Sudan, Kenya and many other countries (Evans & Reeder, 2001; Shabana, 2002). Similarly *A. cassiae* has the potential of controlling *Senna obtusifolia* (Pitelli & Amorim, 2003). Many isolates of *A. alternata* have been found to be the bio-control agents of different plants (Masangkay *et al*., 1999; Mohan Babu *et al*., 2003). In Pakistan two isolates of *A. alternata* have also been found to be a promising bio-control agent for *R. dentatus* and *C. album*, especially when formulated in canola oil emulsion. The potential of these isolates for field use have also been demonstrated (Siddiqui & Bajwa, unpublished data).

An important aspect of the initial phase of mycoherbicide is culturing of organism on artificial media followed by optimization of spore production. Spore production on agar media can only provide sufficient inoculum for laboratory and small plot field trials. The success of mycoherbicidal formulations with *A. alternata* demands to stabilize mass production of inoculum on economically feasible substrate. A variety of substrates have been employed by several workers for the better production of conidia (Pfirter *et al.,* 1999; Prasad *et al.,* 2002; Bailey *et al*., 2004). Masangkay *et al*. (2000) have defined sorghum seeds, as the best substrate for mass production of *A. alternata*.

The present studies propose to screen out an economically viable substrate for mass-producing *A. alternata* isolates out of several locally available industrial wastes.

MATERIALS AND METHODS

Substrates for mass production. It is necessary to have mass production of the mycoherbicidal inoculum to be used for this purpose. For this economically feasible and cost effective and easily available substrates are employed. Agricultural residues as wheat, rice straws and sugarcane bagasse were used in mass cultivation.

Each of wheat and rice straw and bagasse were taken with two supplements, molasses and chickpea flour, for each substrate, respectively. The supplements were used to compensate the deficiency of carbon and nitrogen,

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important sources of energy for the growth of pathogen. The treatments were Wheat straw; Wheat Straw + Chickpea flour + Inoculum; Wheat Straw + Molasses + Inoculum; Rice Straw; Rice Straw + Chickpea flour + Inoculum; Rice Straw + Molasses + Inoculum; Bagasse; Bagasse + Chickpea flour + Inoculum; Bagasse + Molasses + Inoculum. Each treatment was replicated thrice and experiment was repeated twice. These substrates with their supplements were inoculated with both the *A. alternata* strains of the test weeds.

Preparation of bags. Each substrate of 250 g was soaked in water overnight and next day excess water was removed by pressing the substrate with hand. Moistened substrates and chickpea flour were dispensed in the plastic bags and then autoclaved at a pressure of 15 lb and 121°C for 15 min. After that, the bags were transferred to the laminar air flow cabinet where they were opened and each substrate was provided with 2 g of chickpea flour or 2.5 mL of molasses. In case of both the strains of *A. alternata,* isolated from the test weeds, two discs of 0.5 cm diameter were cut with a sterilized cork borer from 8 days old culture and were transferred to the bags of the respective setup of treatments. These bags were closed with the help of a rubber band in order to avoid contamination. The prepared bags were incubated in growth chamber for 14 days at 12 h photoperiod and 25-30ºC.

Assessment of fungal growth and sporulation on substrates. After 14 days, bags were opened and 2 g of material was suspended in 20 mL of distilled and sterilized water. 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. The concentration of the conidia was measured with the help of haemacytometer and expressed as number of conidia per 10 µL of water.

Viability of mass produced inoculum. The viability of the conidia, mass produced on selected suitable substrate (wheat straw+chickpea flour), trials against target weeds with respective *A. alternata* strains were carried out under controlled conditions. The target weeds, *R. dentatus* at 4-5 leaf stage and *C. album* at 10-15 leaf stage were sprayed with these mycoherbicidal formulations in plastic pots. Plastic pots of 7 cm diameter and 10 cm deep were filled with clay loam soil collected from a cultivated field of Punjab University, Lahore. Plants of *C. album* at 10-12 leaf stage were transplanted from field into the pots @ two plants per pot. In case of *R. dentatus,* plastic pots of 12 cm diameter and 5 cm deep were similarly filled with clay loam soil and planted with 3-4 leaf stage transplants (2 plants per pot). The conidial concentrations of *A. alternata* strain R at 10^7 conidia mL^{-1} for *R. dentatus* and 10^9 conidia mL^{-1} of *A. alternata* strain C for *C. album* in 20% canola oil emulsion were prepared. The pots in triplicate were incubated in growth chambers under defined conditions (100% humidity for 24 h at 25ºC) for the establishment and development of disease. The viability tests were repeated thrice. Data on inoculum density was subjected for analysis of variance, ANOVA (Steel & Torrie, 1980).

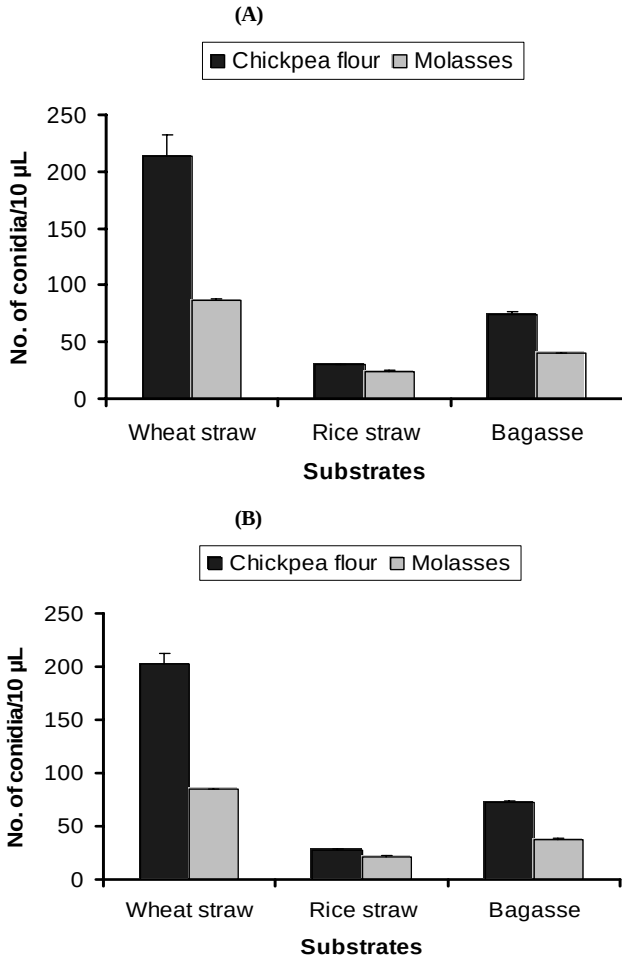
RESULTS AND DISCUSSION

Assessments revealed that among all the substrates wheat straw, with chickpeas flour as N-supplement, was the best substrate for mass culturing of both *A. alternata* strains. It was biologically most active source and supported healthy growth of the fungus, evidenced in terms of conidial productivity. It helped to raise 213 conidia/10 µL in the case of strain R for *R. dentatus* and 203/10 µL conidia in strain C of *C.album* (Fig. 1). This was followed by the combination of substrate, wheat straw supplemented with molasses, which induced production of 87 conidia/10 µL and 85 conidia/10 µL of strains R and C, respectively.

Bagasse with Chickpea flours N-source led to produce 74 conidia per 10 µL in the case of strain R and 72 conidia per 10 µL of strain C, while the same substrate with molasses

Fig. 1. Mass production of *Alternaria alternata* strains isolated from (A) *Rumex dentatus* and (B) *Chenopodium album*

Vertical bars show standard errors of means of three replicates.



was found to be comparatively less effective substrate. It only supported 40 conidia per 10 µL for strain R and 38 conidia per 10 µL for strain C. Bagasse apparently was a relatively better substrate as compared to rice straw. Rice straw was found to be least active biological source with chickpeas flour supplement the conidial productivity of the fungal strains was substantially declined. The production was restricted to 30 conidia per 10 µL for strain R and 27 conidia per 10 µL of strain C. Rate of conidia production on rice straw supplemented with molasses was further depressed and was found to be the lowest (24- 21 conidia per 10 µL).

Among these three substrates, wheat straw with chickpea flour as supplement was found to be most suitable for both the pathogenic strains of *A. alternate* (Fig. 1). Wheat straw is a lignocellulosic material containing about 35-40% cellulose, 30-35% hemicellulose, 10-15% lignin, 5-10% mineral and small amount of other compounds (Dunford *et al*., 2006). Found to be more effective than rice straw. Rice straw has protein content 2-7% and very high silica content (8-14%) (Drake *et al*., 2002). Bagasse has α-Cellulose 30-39%, Pentosans 24-30% and Lignin 1-22% (Dinu, 2006). Bagasse produces better results than rice straw but less so than wheat straw. It is plausible that silica may be inhibitory to the growth of conidia present in the rice straw.

A comparison of chickpea flour and molasses revealed that molasses is entirely deficient in nitrogen and highly rich in Carbon with 34.1%, sucrose 16.5%, reducing sugars 16.3%, acids and ash 11.3% (Curtin, 1983; Schaffler, 1997) as compared to chickpea flour, which contains 13-33% proteins and 40-55% carbohydrates (Berrada *et al*., 1999).

CONCLUSION

Conidia of *A. alternata* strains require more nitrogen than carbon further growth; the reason chickpea flour has advantage over molasses. However, chickpea flour and wheat straw provided an ideal environment for the growth of fungus, which might not have been established by bagasse and chickpea flour. The viability tests of conidia, mass produced on selected suitable substrate (wheat straw+chickpea flour), against respective target weeds revealed (visual) that the efficacy of inocula (strains R & C) was not affected during mass culturing. Both strains caused 100% mortality of their respective weed hosts.

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