RESEARCH ARTICLE

ISOLATION AND CHARACTERIZATION OF VARIOUS FUNGAL STRAINS AS PRIMARY COLONISER FROM WHEAT STRAW AT VARYING NITROGEN CONCENTRATIONS

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Abstract: The present study was undertaken with an aim to search for the fungal strains, which have the potential to efficiently decompose wheat straw with high C:N ratios. Identification and characterization of these microbial species is important to study their decomposition potential for use in soil fertility management. A total 19 strains of fungal primary colonizers were isolated from a sample of wheat straw. Out of these, one belonged to Zygomycota while the remaining 18 belonged to Deuteromycota. *Alternaria, Aspergillus, Cladosporium, Helminthosporium, Stachybotrys, Fusarium* and *Penicillium* were the most frequently isolated genera at low nitrogen concentration. Isolated strains at low nitrogen concentration seem to be the most probable candidates, as initial primary bio inoculants, for hastening the decomposition of wheat straw. The results of this study suggest the possibility of utilizing fungal inoculants as an integrated component of microbe-based strategies for biotechnological management of wheat straw.

Keywords: Wheat Straw, Microorganisms, Isolation, Decomposition, Serial dilution, Fertilizers

INTRODUCTION

Wheat is grown basically for the grains. But the utility of the remaining parts of the wheat plant cannot be ignored. A lot of wheat straw is produced in wheat-growing belts in the world. A significant proportion of wheat straw has been in use as feed for ruminants because of its abundance and low cost (Viola *et al*. 2008; Bals*et al*., 2010; Manriquez *et al*. 2016). However, it is also used for the production of pulp and paper (Zhao *et al.* 2004), strawboards (Deswarte*et al*., 2007), textiles and composites (Avella *et al.,* 1995; Reddy *et al*. 2007), plastics (Avella *et al.,* 1995) and for the removal of metals in wastewater industry (Kumar *et al*., 2000; Doan *et al*., 2008) and hybrid composite materials (Yu *et al.* 2016*).*
Incidentally, a major portion of straw is burnt in the field itself (Gupta *et al*., 2004). This results, on one hand, in a waste of organic sources in soil affecting C:N ratio and biota; and on other hand, leads to global warming and environmental problems (Badrinath *et al*. 2006; Sastre *et al*. 2015). Keeping in mind the harmful effects of burning straw in the field as well as the convenience of the farmer, economical, environment–friendly and low labour strategies should be adopted for effective utilization of the straw.
Primary colonizers are the microorganisms which play an important role in the initiation of decomposition. These organisms span parasitic as well as saprophytic phases. They possess cellulolytic (Sajith *et* al. 2015) and pectolytic activity at low nitrogen level, and are able to grow at faster rate on a comparatively drier resource (Charaya *et al*. 2005, 2006), (Chauhan *et al.,* 2006) and (Rani, 2008) also found that majority of the primary colonizers of plant litter possessed weak parasitic tendency.
Organic matter plays a unique role in soil fertility (Zhao et al. 2016). It acts as a sink as well as a source for nutrient. It prevents environmental pollution and the loss of nutrients. Above all, it helps in maintaining nutrients balance in the soil which is the basic attribute for sustainability (Raman, 2005). In a natural ecosystem, entire biomass of the plants is returned to the soil after the death of the plants through the process of decomposition. However, in agro-ecosystems, a significant proportion of the biomass is removed from the soil. Hence, intensive cultivation of crop requires massive application of synthetic fertilizers to compensate for the loss of nutrients from the soil as a result of their removal by the agricultural crops.
However, the prohibitive cost of chemical fertilizers as also numerous environmental problems associated with their production and use have prompted agricultural scientists to look for better alternatives. It is being gradually realized that organic wastes and biological sources of nutrients are better alternatives and these may serve as substitutes for inorganic fertilizers to a considerable extent, if not absolutely (Shukla and Mathur, 2000). Wheat straw provides one such alternative. The application of biodegraded products of straw into soil has enormous potential to recycle nutrients and maintain soil fertility (Gaind *et al*., 2006; Zhang *et al*. 2015, Rahman *et al*. 2016).
Straw contains approximately 0.5% nitrogen and 40% carbon. Straw, when subjected to colonization by fungi, has only 0.5 units of nitrogen to offer to the fungi which generally require 1.2 to 1.6 units for

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growth; thus, a deficit of 0.7 to 1.1 units of nitrogen appears in the environment (Alexander, 1977). Many workers including Park (1976) pointed out that the fungi which show high degree of cellulolytic ability under laboratory conditions fail to colonize and degrade the plant residues in a correspondingly effective manner.

Hence, it is important to take into account the nitrogen level of resources to be degraded in the selection of effective decomposers. Since wheat straw has low nitrogen content, it should be preferable to use those organisms for the purpose of decomposition which can grow, reproduce and carry out polysachharolytic activity at low nitrogen levels. In other words, those microorganisms which can operate the process of decomposition with lesser units of nitrogen would be more suitable as primary colonizers or primary bioinoculants.

However, van Fassen and van Dijk (1979) have demonstrated that hot composting of straw leads to considerable losses of nitrogen in the form of ammonia also, and therefore only relatively small gains in nitrogen appear to take place during decomposition. If it is so, the secondary colonizers also have to face the deficiency of nitrogen. The addition of nitrogen to crop residues has been reported to enhance the rate of decomposition of crop residues but only when a large amount of residues with low nitrogen was decomposing. Additional supply of nitrogen is believed to enhance the growth of decomposers (Fan *et al*., 1981; Yadav, 1987; Mary *et al*. 1996; Hu *et al. 2*015; Wang *et al*. 2015 and Maaroufi et al. 2017).

Primary colonizers are the microorganisms which play an important role in the commencement of decomposition of crop residues. Identification and characterization of these microbial species is important for studying their decomposition potential vis a vis soil fertility management. In the present study an attempt has been made towards isolation and characterization of fungal strains which can survive and flourish at low nitrogen concentrations or higher C/ N ratios.

MATERIAL AND METHOD

Isolation of Fungi

Freshly harvested wheat straw was collected from agricultural fields situated at village Khardoni, Meerut. The samples were collected aseptically in fresh polythene bags and brought to the laboratory for further studies. Serial dilution plate method (Waksman, 1927) was used to isolate the fungi from wheat straw sample. 1 g of the sample was placed in 250 ml of sterile water and stirred for fifteen minutes using a magnetic stirrer to get the stock solution. 10 ml of this solution were immediately transferred to a conical flask containing 90 ml of sterile distilled water to get a suspension of 1:10 dilution. This suspension was used for the preparation of further serial dilutions (1:100, 1:1000). From the suspension of each dilution (1:10, 1:100, 1:1,000), 1 ml aliquots were transferred to each of a set of three Petri dishes followed by the addition of approximately 20 ml of cooled (45°C) and sterilized culture medium. Czapek'sDox Agar medium (Raper and Thom, 1949) with 30 ppm of rose bengal and 30 mg of streptomycin was used for this purpose. This medium served as control and was designated as N. The media with 2/3 (two third), 1/2 (half) and 1/3 (one third) concentrations (i.e. 1.5 g/1, 1.0 g/1 and 0.67 g/1 respectively) of nitrogen (sodium nitrate) as compared to control (2.0 g/1) were also prepared. The media with 2/3, 1/2 and 1/3 concentrations of nitrogen were designated as N/1.5, N/2 and N/3 respectively.

Records of the Fungi Isolated

Isolation and identification of the fungi was done in the microbiology laboratory of the Department of Botany, CCS University, Meerut, India. The Petri dishes were observed from the third day itself when fast-growing fungi started appearing in the Petri dishes. The slow-growing fungi were transferred onto other Petri dishes just after their appearance to prevent them from being overrun by the fast growing fungi. A complete record of the fungal species and their numbers in cfus (cfus: colony forming units) in the Petri dishes was maintained. The identification of the fungal species was done on the basis of their morphology and cultural characteristics following Gilman (1957), Barnett and Hunter (1972), Subramanian (1971), Ellis (1971, 1976), Domsch and Gams (1972), Domsch *et al*. (1980), Nagmani *et al.* (2006), Venkateswarlu et al (2015), Singh et al. (2015).

Purification and Maintenance of Cultures

The fungal strains were purified by hyphal cut method and streak plate method. The pure cultures were maintained on PDA medium (Riker and Riker, 1936) on slants and were stored in a refrigerator.

RESULT AND DISCUSSION

The current practice of using media having low C:N ratio for the isolation of microflora colonizing the resources with much higher C:N level in most biological laboratories is not appropriate as it might not present a true picture of the colonizer microorganisms. There are greater chances that the microbes present on the resource would be favored by the medium having higher nitrogen levels, thus distorting the picture completely by tilting the balance against the active microorganisms not capable of growing fast enough on the medium with high nitrogen and in favor of those microbes which are not actually active on the litter but grow better on the medium employed. Therefore, it would be worthwhile to use media with the C:N ratio comparable to that in the resource under study. The dominance of Deuteromycota observed in the present study is in full agreement with the earlier reports (Hudson, 1968; Dickinson and Pugh, 1974; Hayes and Lim, 1979; Charaya, 2006; Tiwari and Charaya, 2006; Sen and Charaya, 2010), Guleri *et al.* (2016). Only 1 members belonging to the order Mucorales were isolated in the present study thus supporting the findings of Galloway (1935), Singh and Charaya (1975), Dube *et al.* (1980), Singh (2004), Charaya (2006) and Tiwari (2010) which indicated the paucity of Mucorales in the tropical regions of India.

A total of 19 strains of fungal primary colonizers were isolated from the wheat straw through serial dilution plate method using media containing different levels of nitrogen. 19 fungal strains were isolated as: *Aspergillus flavus, A. fumigatus , A. luchuensis, A. oryzae, A. niger, A. sydowi, A. ustus, A. wenti, Alternaria alternata, Alternaria citri, Alternaria longipus, Alternaria palandui, Chaetomiumflavum, Dreschleraavenacae, Cladosporium herbarum, Fusarium* sp., *Helminthosporium microsorum, Penicillium frequentans, P. funiculosum, P. implicatum, P. paxilli, P. spinulosum, Rhizopus* sp., and *Stachybotrys atra.* Out of these, only one belonged to Zygomycota while the remaining 18 belonged to Deuteromycota.

Among the Deuteromycota, the Hyphomycetes constituted the major component. 13 species belonging to Moniliaceae and 5 species belonging to Dematiaceae were obtained. Among the Moniliaceae, 6 species belonged to the genus *Aspergillus* while only 5 belonged to the genus *Penicillium*. It is widely believed that Aspergilli are more common in warmer regions of the world while the Penicillia are more abundant in the colder regions

Three species were isolated and identified as *Alternaria alternata, Aspergillus niger and Stachybotrys atra* at all concentration of media used in this study. Among these isolated fungal strains, *A. alternate* was obtained exclusively. 12 Isolates of this fungal sp. with 66.66 frequencies were obtained at N media while at N/1.5, N/2 and N/3 media total 8, 10 and 10 isolates were obtained with 33.33, 55.55 and 44.44 frequency respectively.

Total 33 fungal isolates belonging to 11 species were identified as *A. alternate (12), A. longipes* (1), *A. luchuensis* (2), *A. niger* (1), *A. ustus* (2), *Fusarium sp. (2), C. herbarum* (3) *and P. funiculosum* (1), *P. paxilli* (1) *Rhizopus* sp (3) *Stachybotrys atra* (5). *A. Citri, A. palandui, A. flavus, A. fumigates, A. oryzae, H. microsorum* and *P. frequentans* were note obtained at media N. Total 26 fungal isolates belonging to 6 species were obtained from N/1.5 media and identified as *A. alternate* (8), *A.Citri* (1), *A. niger* (5), *A. oryzae* (7), *P. spinulasum* (1) and *Stachybotrys atra* (4). *A. longipes, A. palandui, Aspergillus flavus, A. fumigates, A. luchuensis, A. ustus, Cladosporium herbarum, Fusarium sp. H. microsorum, Penicillium frequentans, P. funiculosum, P. implicatum, P. paxilli and Rhizopus* species were absent at N/1.5 media. Results of this study have been summarized in table 1 and represented by figure 1.

Table 1. A comparison of the Total Isolate (TI) and Frequency (F), Frequency Class (FC) of fungal species isolated using media of different nitrogen levels (N, N/1.5, N/2, N/3) from wheat straw.

Fungal Species	**N**			**N/1.5**			**N/2**			**N/3**		
	TI	**F**	**FC**	**TI**	**F**	**FC**	**TI**	**F**	**FC**	**TI**	**F**	**FC**
Alternaria alternata	**12**	**66.66**	**VI**	**8**	**33.33**	**II**	**10**	**55.55**	**III**	**10**	**44.44**	**III**
A. citri	–	–	–	1	11.11	I	–	–	–	1	11.11	I
A. longipes	1	11.11	I	–	–	–	–	–	–	4	22.22	II
A. palandui	–	–	–	–	–	–	1	11.11	I	1	11.11	I
Aspergillus flavus	–	–	–	–	–	–	5	22.22	II	1	11.11	I
A. fumigates	–	–	–	–	–	–	5	22.22	II	–	–	–
A. luchuensis	2	11.11	I	–	–	–	–	–	–	–	–	–
A. Niger	1	11.11	I	5	33.33	II	3	33.33	II	1	11.11	I
A. oryzae	–	–	–	7	11.11	I	–	–	–	–	–	–
A. ustus	2	22.22	II	–	–		3	22.22	II	–	–	–
Cladosporium herbarum	3	22.22	II	–	–	–	3	22.22	II	–	–	–
Fusarium sp.	2	22.22	II	–	–	–	–	–	–	1	11.11	I
Helminth. microsorum	–	–	–	–	–	–	1	11.11	I	1	11.11	I
Penicillium frequentans	–	–	–	–	–	–	–	–	–	1	11.11	I
P. funiculosum	1	22.22	II	–	–	–	–	–	–	–	–	–
P. implicatum	–	–	–	–	–	–	–	–	–	1	11.11	I
P. paxilli	1	11.11	I	–	–	–	–	–	–	–	–	–

P. spinulosum	–	–	–	1	11.11	I	–	–	–	–	–	–
Rhizopus sp.	3	22.22	II	–	–	–	1	11.11	I	–	–	–
Stachybotrys atra	5	33.33	II	4	33.33	II	3	22.22	II	1	11.11	I
Total Isolates	**33**			**26**			**35**			**23**		
Total Species	**11**			**6**			**8**			**11**		

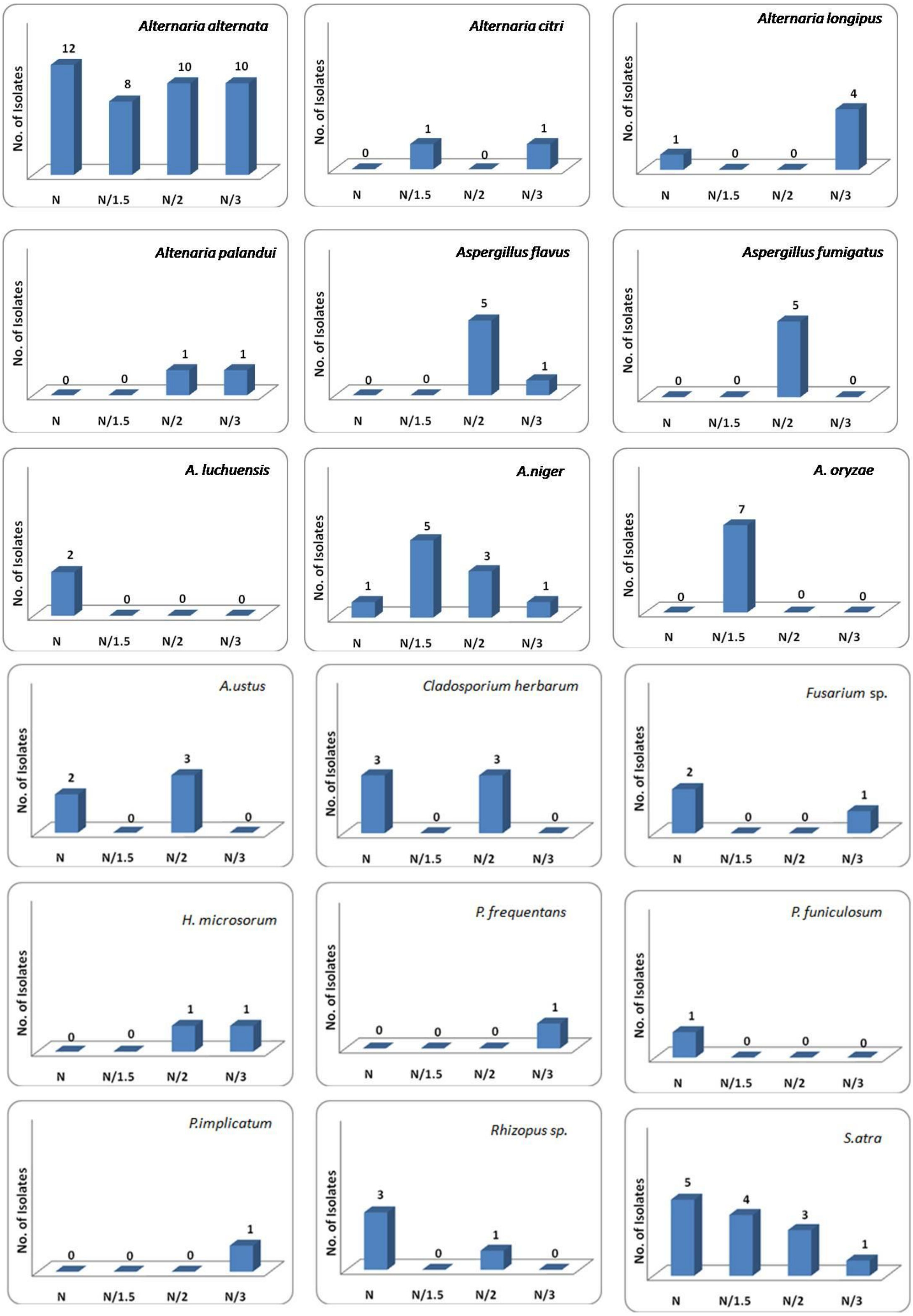

Fig 1: A comparison of the Total Isolate at different nitrogen levels

Total 35 isolates at N/2 media belonging to 8 fungal species were identified as *A. alternate* (10), *A. palandui* (1), *A. flavus* (5), *A. fumigates* (5), *A. niger* (3), *A. ustus* (3), *C. herbarum* (3) *and H. microsorum* (1). A. citri, A. *longipes, A. luchuensis, A. oryzae, Fusarium sp. P. frequentans, P. funiculosum, P. paxilli and P. spinulosum* were not obtained at this nitrogen concentration.

Total 23 fungal isolates belonging to 11 species were obtained at N/3 media and identified as *A. alternata* (10), *A. citri* (1), *A. longipes* (4), *A. palandui* (1), *Aspergillus flavus* (1), *A. niger* (1), *Fusarium* sp. (1*), H. microsurum* (1), *Penicillium frequentans* (1), *P. implicatum* (1) and *Stachybotrys atra* (1). *A. fumigates, A. luchuensis, A. oryzae, A. ustus, C. herbarum, P. funiculosum, P. paxilli, P. spinulosum and Rhizopus* sp. were not btained at N/3 media. It was apparent from the results that *Alternaria alternata* was abundant at all nitrogen concentrations used in this study (table 1 and and fig. 1).

CONCLUSION

The present study describes isolation and identification of fungal colonizers growing actively at low nitrogen concentrations, having potential decomposing activity and their enzyme make-up. Attempt has also been made to identify the fungal strains which respond favourably to supplementation of the resource with additional nitrogen. The results suggest the possibility of utilizing fungal inoculants as an integrated component of microbe based strategies for biotechnological management of agricultural wastes. Czapek's media with different concentrations of nitrogen were prepared to the study the effect of different nitrogen levels on radial growth of selected fungal species Eighteen fungal species *viz. Alternaria alternata, A. citri, A. longipes, A. palandui, Aspergillus flavus, A. fumigatus, A. luchuensis, A. niger, A. oryzae, A. ustus, Cladosporium herbarum, Fusarium* sp., *Helminthosporium microsorum, Penicillium frequentans, P. funiculosum, P. implicatum, P. paxilli, P. spinulosum* and *Stachybotrys atra* were taken for studying effect of nitrogen and results of this study would be communicated in a subsequent paper.

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