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Biodiversity and Pathogenicity Potential of Mycoflora Associated with *Brahmina coriacea* in Potato Fields of North-Western Indian Hills

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ABSTRACT

In the present study, the occurrence and diversity of fungi associated with beetles and white grub, *Brahmina coriacea* (Hope) (Coleoptera: Scarabaeidae) in potato fields of North Western Indian hills was explored for the first time. Sixteen species belonging to 9 genera and two non-sporulating fungi were isolated and identified from white grub cadavers, adult beetles and soil samples in question. Total 1404 out of 14164 (9.91%) beetles were found infected with fungi during year 2007-09 at studied areas. Most abundantly occurring fungi associated with beetles were *Metarhizium anisopliae*, 423 out of 1404 (30.12%); followed by *Aspergillus flavus*, 322 (23.0%); *Fusarium oxysporum*, 262 (18.66%); *Beauveria bassiana*, 143 (10.2%); *Aspergillus clavatus*, 130 (9.25%) and *Fusarium solani*, 120 (8.6%). Whereas, 21 out of 1351 (1.6%) white grubs were found infected with fungi. *A. clavatus* showed high frequency of infection to grubs (33.8%), followed by *A. flavus*, (23.8%) and *Rhizopus oryzae*, (19.04%). Each *Aspergillus niger* and *Alternaria alternata* showed an infection frequency of 9.53% while 4.8% was noticed for *Penicillium griseofulvum*. Total ten fungi were identified from soil samples with high frequency of *R. oryzae* (27.60%) and *Cunninghamella elegans* (24.13%). Preliminary pathogenicity tests were performed on late first instar grubs for all isolated fungi except non-sporulating. *B. bassiana* was found highly pathogenic with 63.70% mortalities followed by 60.37% by *A. flavus* and 50.00% by *M. anisopliae* after 15 days of treatment. *F. oxysporum* and *F. solani* showed mortalities up to 49.63 and 42.59%, respectively.

Key words: Fungal association, *Metarhizium*, *Aspergillus*, *Beauveria*, *Fusarium*, white grub

INTRODUCTION

Entomopathogenic fungi is well distributed and its occurrence in various agricultural soils had been investigated by several workers (Chandler *et al*., 1997; Bidochka *et al*., 1998; Ali-Shtayeh *et al*., 2002; Klingen *et al*., 2002; Keller *et al*., 2003; Meyling and Eilenberg, 2006). Entomopathogenic fungi, *Beauveria*, *Conidiobolus*, *Metarhizium* and *Paecilomyces* are frequently found in soil (Domsch *et al*., 1980). Various other fungal species have also been reported from soil. *Mucor* species have been isolated from the larval cadavers of *Troglophilus neglectus* Krauss (Gunde-Cimerman *et al*., 1998). *F. solani* had been reported from *Tetanops myopaeformis* Roder

and *Hypothenemus hampei* (Ferrari) (Rojas *et al*., 1999; Majumdar *et al*., 2008). *Aspergillus* species were found to be most common among 28 species representing 15 fungal genera, associated with different stages of *Spodoptera littoralis* (Boisduval) (Ismail and Abdel-Sater, 1993). Eleven species of fungi belonging to 5 genera including *Penicillium* have been isolated from mosquito larvae (Da Costa and de Oliveira, 1998; Sur *et al*., 1999). *Aspergillus flavus* and *Fusarium* species were identified from *Chilo partellus* Swinhoe (Atwal *et al*., 1973) and many other fungal species have been reported from diseased soil-inhabiting insects. According to Ali-Shtayeh *et al*. (2002) weak pathogenic fungi can become causal agent of epizootics in predisposed insects. These workers have reported pathogenicity of isolates of the genera *Absidia*, *Aspergillus*, *Fusarium* and *Mucor* against the larvae of *Galleria mellonella* (L.) (Ali-Shtayeh *et al*., 2002). Insects in the soil are always prone to infection by various pathogens. Even avirulent pathogens have been reported to cause various degree of pathogenecity in insects (Thomas *et al*., 2003). Fungal epizootics in soil insect populations are documented by many workers (Samson *et al*., 1988; Keller and Zimmerman, 1989; Klingen and Haukeland, 2006). Lot of work had been done on pathogenicity of *Metarhizium anisopliae* and *Beauveria bassiana* against several insect species (Demirel and Cranshaw, 2006; Khashaveh *et al*., 2008; Abood *et al*., 2010; Cheong *et al*., 2010; Sharififard *et al*., 2011). *B. bassiana*, *M. anisopliae*, *Fusarium* species near *Fusarium solani*, *Cordyceps* species, *Penicillium* species and *Aspergillus* species were isolated from beetles of *Phyllophaga* species, in Southern Quebec, Canada (Poprawski and Yule, 1991). *Aspergillus* species were reported from all stages of white grub in India (Yadav and Mathur, 1987). *Fusarium* species have also been reported from coleopteran insects with weak pathogenicity (Teetor-Barsch and Roberts, 1983). The knowledge of occurrence and diversity of naturally associated fungi can help to explore the potential fungal pathogens other than well known insect pathogenic fungi. It is very useful to get more information on mycoflora associated with different insect pests so that possibilities of bio-control can be explored extensively. Perusals of the literature revealed that no studies have been carried out on the fungal species associated with white grub *Brahmina coriacea* (Hope) (Coleoptera: Scarabaeidae). Therefore, in present study, in order to explore bio-control agents against white grub, a survey was undertaken. Further, to distinguish the relationship between fungi and white grubs, all fungi except non-sporulating species were tested for their pathogenicity.

MATERIALS AND METHODS

Collection of white grubs, adult beetles and soil samples: A survey was undertaken during three years (2007-09) at different locations of North Western Indian hills. All naturally occurring fungi associated with grubs in North Western Indian hills was included. White grubs were collected from potato farms at three different locations, Shimla (31°N -77°E; 2,202 m amsl), Shillaroo (31°N-77°E; 1,820 m amsl) and Kheradhar (30°N-77°E; 1,048 m amsl) of North Western hills of India, during year 2007-08. Sampling was done from root zone of randomly selected potato plants in 30 cm^2 area and minimum of 10 samples from each quadrate of selected location were taken. To collect adults (beetles) of white grubs, two light traps at potato farms (Shimla and Fagu, 32°N-77°E; 2,502 m amsl) were installed during year 2007-09 and beetles were caught weekly. Grubs and beetles were reared and observed for any natural fungal infection. A total of 35 soil samples originating from potato fields of above regions were collected. Only those soil samples were taken in which dead grubs were found. The samples were taken by cylindrical soil core borer to a depth of 20 cm. The samples were placed into plastic bags and stored at 4°C.

Isolation of fungi: Visible conidia from grubs and adult beetles were harvested directly or scraped off on to media plates. Alternatively grub cadavers without external fungal growth were surface sterilized for 2 min each with 70% ethanol, 1% sodium hypochlorite and sterile water (2-3 washes) and were homogenized. Small quantity of homogenate was plated onto the SDAY media (4% Sabouraud dextrose agar media + 2% yeast extract + 0.1% streptomycin) (Papierok and Hajek, 1997). Soil samples were passed through sieve (pore size 2 mm) to remove plant tissues and gravels and mixed thoroughly. Isolation of fungi from soil samples was carried out through soil dilution plate method. One gram soil was mixed in 9 mL (w/v) sterile water. The sample was homogenized on mechanical shaker for 60 min. Aliquots of 100 μL were spread using L-shaped glass rod on above mentioned media. Cultures were incubated at 25°C for one week.

Identification of fungi: All isolated fungi were identified morpho-taxonomically from Maharashtra Association for the Cultivation of Science, Agharkar Research Institute Pune, India.

Preliminary pathogenicity test (Koch's postulates): All fungal isolates with no knowledge about their pathogenicity to the white grubs except non sporulating fungi were bio-assayed. The fungi were grown on SDAY media plate for 10-12 days. Late first instar grubs of *B. coriacea* permitted to walk on the sporulated fungus culture for 5 min (Goettel and Inglis, 1997) and transferred to pots individually filled with sterile soil: Farm Yard Manure (FYM) (1:1) (w/w). Pots were incubated at the temperature 25°C in darkness. Maize plants were grown in the pots so that grubs could feed on roots of maize plants. Thirty laboratory reared grubs were taken for each fungus with three replications of each. Observations on mortality of grubs were recorded at periodic intervals (7, 15, 21 and 30 days after treatment). The cadavers were surface disinfected and fungus was re-isolated.

Data analysis: Mortality in control group was corrected using method suggested by Abbott (1925):

$$\text{Corrected (\%)} = \frac{\text{1-n in T after treatment}}{\text{n in CO after treatment}} \times 100$$

where, n is number of grubs, T is treatment and CO is control.

Corrected mortality data were statistically analyzed by using one-way analysis of variance after Arc sine transformation. Mean difference was tested using Duncan's multiple range test. The difference of two means between treatments exceeding Critical Differences value was taken significant. MSTAT-C software was used for analysis.

RESULTS

Occurrence of insect associated fungi: Total 1404 out of 14164 (9.91%) adult beetles were found infected with fungi during year 2007-09 at Shimla and Fagu. Most frequently occurring fungus was *M. anisopliae*. Out of 1404 infected beetles, 423 (30.12%) were infected by *M. anisopliae* followed by *A. flavus*, 322 (23%) and *F. oxysporum*, 262 (18.66%). Occurrence of infection was comparatively less by *B. bassiana*, 143 (10.2%), *A. clavatus*, 130 (9.25%) and *F. solani* 120 (8.6%). *M. anisopliae* was found with high frequency during three years 2007-09. An infection frequency of 2.96 and 3.29% of *M. anisopliae* out of 9.92 and 9.80% total fungal infection was observed at Shimla and Fagu, respectively. Similar trend was observed with *A. flavus*

(2.28 and 2.12%) and *F. oxysporum* (1.86 and 1.65%), respectively. Well known insect pathogenic fungus *B. bassiana* (1.01 and 1.02%) was significantly less prevalent at both the above mentioned places during all the three years. *A. clavatus* and *F. solani* were also least prevalent with infection frequencies of 0.92 and 0.86% at Shimla and 0.86 and 0.72% at Fagu, respectively (Table 1). Figure 1 shows naturally infected beetles with *A. flavus* and *B. bassiana*.

Table 1: Location wise fungal species found associated with adult beetles of *B. coriacea* during year 2007-09

Studied area	Year	Total No. of beetles collected	No. of beetles infected with fungus	Total percent of infection	Infection by each species: Species	Infection by each species: (%)
Fagu	2007	5971	589	9.86	*Metarhizium anisopliae*	2.95
(L- 32°N -77° E; 2,502 amsl)					*Aspergillus flavus*	2.31
					Fusarium oxysporum	1.80
					Beauveria bassiana	0.99
					Aspergillus clavatus	0.95
					Fusarium solani	0.85
	2008	4442	487	10.96	*M. anisopliae*	3.31
					A. flavus	2.43
					F. oxysporum	2.21
					B. bassiana	1.10
					A. clavatus	0.95
					F. solani	0.92
	2009	2890	203	7.07	*M. anisopliae*	2.01
					A. flavus	1.64
					B. bassiana	1.21
					A. clavatus	0.76
					F. oxysporum	0.69
					F. solani	0.66
Total	2007-09	12889	1279	9.92	*M. anisopliae*	2.96
					A. flavus	2.28
					F. oxysporum	1.86
					B. bassiana	1.01
					A. clavatus	0.92
					F. solani	0.86
Shimla	2007	499	47	9.41	*M. anisopliae*	3.56
(31°N -77°E; 2,202 m amsl)					*A. flavus*	2.00
					F. oxysporum	1.60
					B. bassiana	1.00
					A. clavatus	0.80
					F. solani	0.80
	2008	394	49	12.43	*M. anisopliae*	4.31
					A. flavus	2.79
					F. oxysporum	2.03
					B. bassiana	1.26
					A. clavatus	1.26
					F. solani	0.76
	2009	382	29	7.59	*M. anisopliae*	2.36
					A. flavus	1.57
					F. oxysporum	1.31

Table 1: Continue

Studied area	Year	Total No. of beetles collected	No. of beetles infected with fungus	Total percent of infection	Infection by each species: Species	Infection by each species: (%)
					B. bassiana	0.79
					A. clavatus	0.52
					F. solani	0.52
Total	2007-09	1275	125	9.80	*M. anisopliae*	3.29
					A. flavus	2.12
					F. oxysporum	1.65
					B. bassiana	1.02
					A. clavatus	0.86
					F. solani	0.72
Grand total	2007-09	14164	1404	9.91	*M. anisopliae*	2.89
(Fagu and Shimla)					*A. flavus*	2.27
					F. oxysporum	1.85
					B. bassiana	1.01
					A. clavatus	0.91
					F. solani	0.85
Infection of each fugal species	2007-09	-	1404	-	*M. anisopliae*	30.12
(Fagu and Shimla)					*A. flavus*	23.00
					F. oxysporum	18.66
					B. bassiana	10.20
					A. clavatus	9.25
					F. solani	8.60

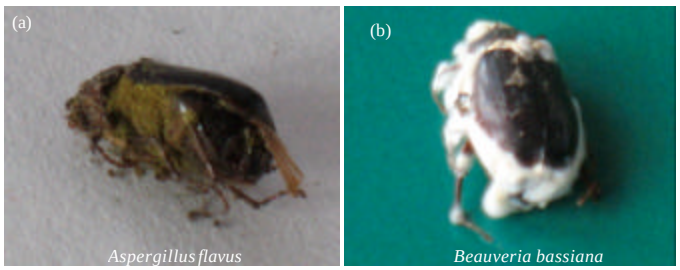

Fig. 1(a-b): (a) Natural infection to *Brahmina coriacea* beetle by *Aspergillus flavus* (b) Natural infection by *Beauveria bassiana*

During the years 2007-08 fungal infection of grubs was very less as compared to adult beetles, at all studied places. Only 21 out of 1351 (1.55%) grubs were found infected. *A. clavatus* was found to cause high frequency of infection among grubs, 7 out of 21 (33.8%), *A. flavus*, 5 out of 21 (23.8%) followed by *Rhizopus oryzae*, 4 out of 21 (19.04%), both *A. niger* and *A. alternata* 2 (9.53%) and *Penicillium griseofulvum,* 1 (4.8%) out of 21 were found infected. In Shimla, 3.19% grub population was found infected with fungi of which *A. clavatus* (1.45%) was dominating followed by *A. flavus*, 0.87 and 0.53% of each of *A. niger*, *R. oryzae* and *P. griseofulvum* during 2007-08. Fungal infection in white grubs was less at Shillaroo (0.60%) with 0.3% each of two species, *A. flavus* and *A. clavatus*. At Kheradhar, 1.73% grubs were found infected with fungi and

Table 2: Fungal species found associated with white grubs of *B. coriacea* during year 2007-08

Location	Year	Total No. of grubs collected	No. of grubs infected with fungi	Percent fungal infection	Infection by each species	
					Species	(%)
Shimla	2007	158	5	3.16	*Aspergillus clavatus*	1.27
					Aspergillus flavus	0.62
					Aspergillus niger	0.62
					Rhizopus oryzae	0.62
	2008	187	6	3.21	*A. clavatus*	1.60
					A. flavus	1.07
					Penicillium griseofulvum	0.53
Total	2007-08	345	11	3.19	*A. clavatus*	1.45
					A. flavus	0.87
					A. niger	0.53
					R. oryzae	0.53
					P. griseofulvum	0.53
Shillaroo	2007	379	2	0.53	*A. clavatus*	0.26
(31°N -77°E 1,820 m amsl)					*A. flavus*	0.26
	2008	280	2	0.71	*A. clavatus*	0.35
					A. flavus	0.35
Total	2007-08	659	4	0.60	*A. clavatus*	0.30
					A. flavus	0.30
Kheradhar	2007	180	1	0.56	*A. niger*	0.56
(30°N -77°E 1.048 m amsl)	2008	167	5	2.99	*Alternaria alternata*	1.20
					R. oryzae	1.80
Total	2007-2008	347	6	1.73	*A. niger*	0.29
					A. alternata	0.58
					R. oryzae	0.86
Grand total	2007-08	1351	21	1.55	*A. clavatus*	0.51
(Shimla, Shillaroo					*A. flavus*	0.37
and Kheradhar)					*A. niger*	0.15
					R. oryzae	0.30
					P. griseofulvum	0.07
					A. alternata	0.15
Percentage of grub infection by	2007-08	-	21	-	*A. clavatus*	33.33
each fungal species					*A. flavus*	23.8
(Shimla, Shillaroo and Kheradhar)					*A.* niger	9.52
					R. oryzae	19.05
					P. griseofulvum	9.53
					A. alternata	4.80

dominating fungal species were *R. oryzae* (0.86%), *A. alternata* (0.58%) and *A. niger* (0.29%) (Table 2). Total fungal infection to white grubs and adult beetles was high during year 2008 than in 2007 and least in 2009 (beetles only) probably due to low rainfall or drought in year 2009, as for optimum growth of fungi high humidity with warm-wet environment are more congenial.

Among 35 soil samples, 26 (74.28%) soil samples were found positive for the presence of fungi, out of which 10 species were identified. 14 out of 29 isolates were found at Shimla, showed more diversity of fungal species as compared to Fagu (7) and Shillaroo (5). Only 3 fungal species were found in soil samples collected from Kheradhar. Out of 29 isolates, *R. oryzae* were 8 (27.6%),

Table 3: Fungi isolated from soil samples in potato fields

		Locations				
Fungi isolated	Total isolates	Shimla	Fagu	Shillaroo	Kheradhar	Total percent of fungal occurrence
Rhizopus oryzae	8	4	1	2	1	27.60
Cunninghamella elegans	7	2	3	1	1	24.13
Curvularia prasadii	3	2	-	1	-	10.34
Curvularia pallescens	2	1	-	-	1	6.89
Alternaria raphani	2	-	1	1	-	6.89
Aspergillus niger	2	1	1	-	-	6.89
Fusarium equiseti	2	1	1	-	-	6.89
Aspergillus terreus	1	1	-	-	-	3.44
Non-sporulating hyaline	1	1	-	-	-	3.44
Non-sporulating	1	1	-	-	-	3.44

Table 4: Mortality among late first instar grubs of *B. coriacea*

	Corrected percent mortality			
Fungal isolates tested	(7 Dat)	(15 Dat)	(21 Dat)	(30 Dat)
Metarhizium anisopliae	41.48 (40.11)	50.00 (45.03)	71.48 (57.83)	85.93 (68.20)
Beauveria bassiana	51.48 (45.97)	63.70 (53.30)	81.85 (65.14)	92.59 (77.06)
Aspergillus flavus	44.81 (42.04)	60.37 (51.09)	78.52 (62.43)	89.26 (70.91)
Fusarium oxysporum	37.78 (37.85)	49.63 (44.77)	75.19 (60.21)	85.93 (68.20)
Fusarium solani	27.41 (31.53)	42.59 (40.71)	64.44 (53.44)	81.85 (65.14)
Aspergillus clavatus	17.04 (24.21)	28.15 (31.85)	42.96 (40.97)	53.70 (47.15)
Penicillium griseofulvum	13.70 (21.50)	24.81 (29.84)	38.89 (38.53)	49.63 (44.82)
Alternaria alternata	10.00 (15.01)	21.11 (26.95)	31.85 (34.23)	42.59 (40.71)
Aspergillus niger	3.33 (6.15)	10.74 (19.14)	14.07 (21.85)	24.81 (29.84)
Fusarium equiseti	6.67 (12.30)	10.74 (19.14)	17.78 (24.73)	21.11 (26.95)
Rhizopus oryzae	6.67 (12.30)	7.04 (12.64)	17.78 (24.73)	21.11 (27.62)
Curvularia prasadii	3.33 (6.15)	6.67 (8.86)	14.07 (21.85)	21.11 (27.62)
Curvularia pallescens	3.33 (6.15)	7.037 (12.64)	14.07 (21.85)	21.11 (27.62)
Alternaria raphani	3.33 (6.15)	7.037 (12.64)	17.78 (24.73)	21.11 (27.62)
Aspergillus terreus	3.33 (6.15)	7.037 (12.64)	28.52 (32.22)	35.19 (36.15)
Cunninghamella elegans	0.00 (0.00)	7.04 (12.64)	6.67 (8.81)	10.37 (15.35)
CD (5%)	10.55 (11.76)	10.50 (11.40)	9.40 (8.11)	10.22 (9.09)

Corrected % mortality was calculated by Abbots formula, Dat: Day after treatment, Values in brackets are transformed value, Results are based on isolated fungal species recorded in their preliminary tests, CD: Critical difference

Cunninghamella elegans 7 (24.13%), *Curvularia prasadii* 3 (10.34%), each *C. pallescens, A. raphani* and *A. niger* were 2 (6.89%) and 1 (3.44%) of each *F. equiseti* and *A. terreus* and 1 (3.44%) each of non-sporulating hyaline form and non-sporulating form (Table 3).

Pathogenicity tests: All fungal species, except non-sporulating were separately tested for their pathogenicity on late first instar white grubs. Corrected percent mortality data of fungi against late first instar grubs up to 30 days of treatment is presented in Table 4. *B. bassiana* was found highly pathogenic with 51.48% mortalities followed by 44.81% due to *A. flavus* and 41.48% by *M. anisopliae* after 7 days of treatments, whereas *F. oxysporum*, *F. solani* and *A. clavatus* had shown mortalities in range 17.04-37.78%. After 15 days of treatment, *B. bassiana*

showed significantly higher mortalities of 63.70% followed by 60.37% by *A. flavus* and 50.00% by *M. anisopliae*. *F. oxysporum* and *F. solani* showed mortalities up to 49.63 and 42.59%, respectively. Significantly low mortalities were caused by *A. clavatus* (28.15%), *P. griseofulvum* (24.81%) and *A. alternata* (21.11%). The rest of tested fungal species viz., *A. terreus*, *A. niger*, *F. equiseti*, *R. oryzae*, *C. prasadii*, *C. pallescens* and *A. raphani* showed between 3.57-10.74% mortalities. *B. bassiana* caused highest mortalities 92.59% after 30 days of treatment followed by 89.26% by *A. flavus*, 85.93% by both, *M. anisopliae* and *F. oxysporum*, respectively and 53.70% by *A. clavatus*. Rest all isolates caused mortalities in the range 10.37-49.63%. *A. flavus*, *F. solani and F. oxysporum* were found significantly pathogenic in preliminary studies besides well known pathogenic fungi.

DISCUSSION

The present study is the first detailed investigation of fungi associated with white grub and adult beetles of *B. coriacea*. *Aspergillus*, *Fusarium*, *Penicillium*, *Alternaria* and *Rhizopus* species besides well known entomopathogenic species like *Metarhizium* and *Beauveria* were found associated with white grubs. Insect associated mycoflora have been reported earlier on several insects, including bees (Shoreit and Bagy, 1995), termites (Zoberi and Grace, 1990), lepidopterous (Ismail and Abdel-Sater, 1993), mosquitoes (Da Costa and de Oliveira, 1998), cockroaches (Moraes *et al*., 2001a), triatomines (Moraes *et al*., 1998) and beetles (Poprawski and Yule, 1991). In all of the above studies, *Aspergillus*, *Fusarium* and *Penicillium* have been found the predominating genera. *B. bassiana*, *M. anisopliae*, *F. solani*, *Penicillium* and *Aspergillus* species have been reported from white grubs *Phyllophaga* species earlier (Poprawski and Yule, 1991). *A. flavus* had been isolated from *Opisina arenosella*, *Stephanitis typica* and *Proutista moesta* (St Leger *et al*., 2000; Gupta and Gopal, 2002). In a survey of the fungal microbiota associated with adults of *Triatoma infestans* Klug, *Penicillium*, *Aspergillus* and *Alternaria* were the predominating genera (Marti *et al*., 2007). *A. flavus*, one *Aspergillus* and *Fusarium* species associated with one white grub species and *Leucopholis burmeisteri* Brenske have also been reported earlier (Yadav and Mathur, 1987; Padmanaban *et al*., 2003). The wide distributions and high occurrences of *M. anisopliae* and *B. bassiana* in coleopteran and other insects have been reported by many workers (Milner, 1992; Bidochka *et al*., 1998; Khashaveh *et al*., 2008; Abood *et al*., 2010). In present study, *M. anisopliae* was also found most frequently occurring in adult beetles. Earlier, *M. anisopliae* had been reported from *Phytoscaphus* species (Verma *et al*., 1988). The present study revealed that *A. flavus* and *F. oxysporum* were predominant species than *B. bassiana*. *A. flavus* and *A. clavatus* were found associated with both white grub and imago beetle in all locations. *A. flavus* had been found associated with all stages of *S. littoralis*, *C. partellus* and *T. neglectus* earlier (Atwal *et al*., 1973; Ismail and Abdel-Sater, 1993; Gunde-Cimerman *et al*., 1998). Recently, *A. clavatus* has been isolated from *Oedaleus senegalensis* Krauss (Seye *et al*., 2009). *F. oxysporum*, *F. solani* and *A. flavus* have also been reported from larvae and adults of *Hypolixus haerens* Boheman, *Dendroctonus frontalis* Zimmermann, *T. myopaeformis* and *H. hampei* (Moore, 1971, 1973; Rojas *et al*., 1999; Blodgett *et al*., 2004; Carrion and Bonet, 2004; Majumdar *et al*., 2008). Although in the present study fungal infection in grubs was less, which may be due to their aggressive behavior or conidia detachment from the host cuticle (Yaginuma *et al*., 2006). The most frequently occurring fungi in grubs were *A. clavatus* followed by *A. flavus*. Many of the fungi isolated in present study had never been reported from grubs elsewhere in earlier studies. Further, in present study not even a single grub out of 21 infected grubs was found infected with well

known insect pathogenic fungi *M. anisopliae* and *B. bassiana*. However, adult beetles had shown higher infection of *M. anisopliae*. A few reports about *A. alternata*, *P. griseofulvum*, *A. niger* and *R. oryzae* associated with other insects have already been published (Christias *et al*., 2001; Moraes *et al*., 2001a-d; Greif and Currah, 2007; Sun and Liu, 2008) but there are no such reports on white grubs. In present study, soil sample containing dead grubs had shown predominance of *R. oryzae* and *C. elegans*. Distribution of these species within agricultural field soils had been investigated in earlier studies (Ali-Shtayeh *et al*., 2002; Meyling and Eilenberg, 2006). In preliminary pathogenecity test, *A. flavus, F. oxysporum* and *F. solani* resulted in the significant mortality of grubs besides well-known pathogenic species. *M. anisopliae* and *B. bassiana* had earlier been reported pathogenic to various insect species including white grubs (Shahid *et al*., 2003; Mohi-ud-din *et al*., 2007; Khan *et al*., 2008; Mahdneshin *et al*., 2009; Dal Pogetto *et al*., 2011). *A. flavus* and *Fusarium* species have also been reported pathogenic to white grub *L. burmeister* (Padmanaban *et al*., 2003). According to earlier studies, chitinase producers can be considered as potential pathogens (Sur *et al*., 1999; Kumar *et al*., 2004). High chitinolytic activities were observed with *A. flavus* on *S. littoralis, Bombyx mori* (L.) and termites (Domsch *et al*., 1980; Gunde-Cimerman *et al*., 1998). *F. oxysporum* and *F. solani* are proven entomo-pathogenic species for several insects groups including Coleoptera (Miller *et al*., 1985; Hajek *et al*., 1993; Ali-Shtayeh *et al*., 2002). *A. flavus*, *F. oxysporum* and *F. solani* had also been reported to cause mortalities of *G. mellonella* to the tune of 30.8, 80.0 and 66.7%, respectively in preliminary tests (Sun and Liu, 2008). *F. oxysporum* had caused 100% mortality of *Nilaparvata lugens* (Stal) (Kuruvilla and Jacob, 1979, 1980). *F. solani* had been found pathogenic to *Scolytus scolytus* F., *T. myopaeformis* and lobster (Barson, 1976; Claydon *et al*., 1977; Majumdar *et al*., 2008). Low levels of moralities by *A. clavatus, P. griseofulvum* and *A. alternata* to grubs were also observed in the present study. *A. clavatus* had already been reported highly pathogenic to the larvae of *Aedes aegypti* (L.), *Anopheles gambiae* Giles and *Culex quinquefasciatus* (Say) (Seye *et al*., 2009). 100% mortality by *A. alternata* had been reported to the aphids (Christias *et al*., 2001). *P. griseofulvum* had been reported pathogenic to termites earlier (Moraes *et al*., 2001a, 2001b).

CONCLUSION

Present work is a first report of listing 16 species belonging to 9 genera with two non-sporulating fungi associated with *B. coriacea* white grubs, adult beetles and soil containing dead grubs. This study showed the diversity and spectrum of the fungal species associated with white grubs and beetles. More over many fungal species tested for their pathogenicity against first instar grubs had shown pathogenicity with more or less virulence. Fungi which caused significant mortalities were *A. flavus, F. oxysporum* and *F. solani* besides well known pathogenic fungi. These species could be further developed as a potential biological control agent against white grubs in their initial life cycle stage. However, present studies conducted are able to provide framework for further investigation and generating hypothesis to conduct preliminary pathogenicity required to test in different developmental stages of white grubs. Further studies are needed for fungal strains optimization and development of better substrates for mass production and practical uses. Characterization and application of toxins on different stages of grubs including eggs and beetles are also needed to understand their killing effects. Side effects and safety tests with beneficial insects, mammals and human cells need to be conducted. Further, it reveals that opportunistic pathogens, largely been overlooked till date can be tested to prove their pathogenicity.

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