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Occurrence of entomopathogenic fungus, *Beauveria bassiana* (Bals.) on potato whitegrubs in Himachal Pradesh

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Abstract

Survey to isolate and identify the entomopathogenic fungi associated with whitegrubs was conducted in Himachal Pradesh during 2008 and 2009. The whitegrubs were collected from 14 locations and *Beauveria bassiana* (Bals.) was found to be associated with grubs of *Brahmina coriacea* (Hope) in Shillaroo and Kheradhar areas. The fungus infected grubs were observed only in higher hills of Shimla and Sirmour districts. At Shillaroo, 0.98% grubs of *B. coriacea* were observed to have fungal infection, whereas at Kheradhar 20.2% of the grubs showed symptoms of mycosis. When field collected grubs were reared separately location- wise in laboratory, very high percentage of mycosis (55.29%) was recorded. Kheradhar and Shillaroo populations of whitegrubs showed white muscardiane infestation in 8.72 and 5.27% of the grubs, respectively. Conidia of the fungus were globose to subglobose measuring 2.0-3.0×2.0-2.5 µm. Incubation of Shillaroo, Kheradhar, Kharapathar and Solan isolates was done at 20, 22 and 26 °C. After 15 days, the radial growth of Shillaroo isolate of *B. bassiana* was recorded to be 7.66 cm as compared to 7.60, 7.60 and 7.36 cm for Kharapather, Solan and Kheradhar isolates, respectively.

Key words: *Beauveria bassiana*, *Brahmina coriacea*, entomopathogenic fungi, whitegrubs

Microbes are being exploited as alternatives and complimentary to chemical insecticides for the control of insect -pests. Among the various microbes, entomopathogenic fungi are considered as the best agents for whitegrub management (Chandel *et al.* 2005) because of their regular multiplication due to good moisture available in the soil. There is great diversity of fungus-insect interaction and virtually all insect orders are susceptible to fungal diseases. Rai *et al*. (2014) reported that approximately 750 species of fungi from about 90 genera have been documented to be entomopathogenic. However, only a few of these species are currently being developed as pathogens against insect-pests. Fungi are particularly important for control of Coleoptera, because viral and bacterial diseases are rare in beetles (Hajek and Leger, 1994). The unique character of fungi as compared to bacteria and viruses is that they penetrate through the insect cuticle, thus making them as valuable biological control agents of whitegrubs. In many systems, a reduction in feeding in an infected host is one of the first overt changes. This response provides an often-overlooked benefit of fungal pathogens infecting pest insects. The search for and development of commercially viable entomopathogenic fungi entails several steps including isolation from the host insect, followed by studies on ecology and physiology. In past years, attempts to use fungal entomopathogens for inundative releases, similar to use of synthetic chemical insecticides, have frequently been unsuccessful (Chandel and Mehta 2005; Chandel *et al*. 2005). So now it is realized to harness the potential of local entomopathogenic fungi. The main aim of this study was to advance our knowledge of fungal diseases, in general, and use it further in developing control strategies against whitegrubs by means of local entomopathogenic fungi.

Materials and Methods

Survey for entomopathogenic fungi (EPFs): Soil sampling was done in different parts of Himachal Pradesh. A total of 153 samples from 9 districts representing 14 localities (Table 1) were collected. The samples were taken from different habitats *viz*. potato, ginger, pea, maize, cabbage and fruit orchards. Although sampling was done throughout the year,

but most of the samples were taken during July-November. The grubs were collected by digging one cubic feet of soil carefully by shovel and then by searching it by hand for larval, pupal and adult stages. At each site, minimum 5 soil pits were dug. Information regarding location, altitude and total and mycosed grubs was collected for each sample. The infested dead grubs were separated from healthy grubs and brought to the laboratory in individual plastic vials. The healthy grubs were placed in plastic containers in groups of 40-50 with soil from the same collection site.

Isolation and identification of fungi: The diseased grubs showing white mycelial growth on their body were directly used for isolation of fungi. The fugus infected grubs were surface sterilized by immersing them into sodium hypochloride (5%) for two minutes and then rinsed with sterile distilled water thrice under aseptic conditions. Then the sterilized specimen was cut open in a sterile Petri plate and a small portion of infected tissue was streaked on PDA slants; which were incubated at 26 ± 1^0C for further growth. After about 15 days of incubation, the purification of fungal culture was done through streak plate method. A loop full of fungal spores was streaked on PDA in Petri plates under aseptic conditions. These Petri plates were incubated at 26 ± 1^0C for 15-20 days, and after sporulation the fungus was again transferred to PDA slants and maintained in incubator. The identification of fungus was done by preparing the slides of the fungus and the final identification was confirmed at Department of Plant Pathology, CSKHPKV Palampur. The pathogenicity was proved using second instar grubs of *B. coriacea* and Koch's postulates were proved.

Effect of temperature on growth of *B. bassiana*: Four strains of *B. bassiana* isolated from Shillaroo, Kheradhar, Kharapathar and Solan were taken for their growth studies at different temperatures. The growth and development of these four isolates were recorded at 20, 22 and 26 ^{0}C. The PDA medium from the flask was poured into Petri plates and allowed to solidify. Small fungal discs (5 mm diameter) of different isolates were placed in the center of each Petri plate (5 plates for each isolate) and the plates were incubated at 20, 22 and 26 ^{0}C in BOD incubator. Data on radial growth of fungus were recorded at an interval of 5, 10 and 15 days. The statistical analysis was done in Completely Randomized Design using computer based CPCS software.

Results and Discussion

The whitegrubs were collected from 14 different locations of Himachal Pradesh. *B. coriacea* was found to be the predominant species. The fungus infected grubs were collected only in high altitude areas. However, per cent infestation was recorded to be very low. At Shillaroo, population of whitegrubs varied from 6 to 18 grubs/feet^3, however, only 0.98% of the grubs showed mycosis (Table 1). Kheradhar area in Sirmour district was another endemic pocket of whitegrubs and *B. coriacea* was by far the most prevalent whitegrub species encountered in soil sampling from potato fields. At Kheradhar, a total of 321 grubs were collected, out of which 20.2% were found to be infected with the fungus. The dead grubs showed clear cut symptoms of fungal infestations and their body was completely covered with white growth of fungus (Fig. 1). At most other locations none of the collected grubs showed visible symptoms of fungal infestation irrespective of altitude of the area and species of whitegrubs. Farmers resort to frequent application of pesticides like chlorpyriphos, phorate, Ridomil and Diathane M-45 to control various insect-pests and diseases and all these have antagonistic effects against entomopathogenic fungi which seems to be the possible reason for low natural infestation of entomopathogenic fungi in soil arthropods like whitegrubs. When the field collected grubs were reared in laboratory at Palampur, many of the grubs died due to fungal infestation. Among Shillaroo culture, 5.27% of the grubs showed mycosis during rearing in laboratory. Similarly, from Kheradhar 8.72% mycosis was observed under laboratory rearing. In Kharapathar population, 55.29% of the grubs were found dead due to infection. In laboratory, proper soil moisture was maintained through regular watering which provided ideal conditions for growth and conidia production of fungus. Cisneros and Vera (2000) also reported that mycelial growth reactivates in fungus infested specimens when they are exposed to proper moisture conditions.

The data in Table 1 clearly showed that the fungus occurs only in high hill temperate wet zone of Himachal Pradesh. According to Muller-Kogler (1965), the epizootics of fungi are usually associated with period of high humidity particularly, rainy periods. Germination of spores is seriously affected, since most fungi germinate only at very high RH usually 90% or higher. Arthurs and Thomas (2001) reported that the efficacy of fungal entomopathogens is highly dependent on suitable climatic conditions, in particular the availability of a high level of environmental moisture. In case of *B. brongniartii*, high soil moisture (50% FC) favours the development of mycelia outside the infected insect's body, whereas low moisture (25% FC) favours the production of conidia.

Isolation and identification of EPFs from whitegrubs

The fungus was isolated on PDA from field infected

a b

Fig 1. (a) Healthy whitegrubs, and (b) fungal infected whitegrub

Table 1. Natural infection of entomopathogenic fungi in white grubs

Location	Altitude (m amsl)	Sampling field	Predominant species	Total number of grubs collected	Mycosed grubs observed in		Mycosis (%)	
					Field	Lab	Field	Lab
Shillaroo	2450	Potato	*B. coriacea*	815	8	43	0.98	5.27
Kharapathar	2580	Potato	*B. coriacea*, *Holotrichia* sp.	85	0	47	0	55.29
Kufri	2500	Potato	*B. coriacea*	28	0	0	0	0
Fagu	2650	Potato	*B. coriacea*	105	0	0	0	0
Kheradhar	1950	Potato	*B. coriacea*	321	65	28	20.2	8.72
Kheri	300	Maize	*Lepidiota stigma*, *A. dimidiata*	73	0	0	0	0
Sangrah	1800	Ginger	*H. longipennis*	23	0	0	0	0
Janjehli	2200	Potato, Peas	*B. flavosericea*	55	0	0	0	0
Baragaon	1835	Potato, Cabbage	*Melolontha* sp.	107	0	2	0	1.86
Seobagh	2100	Fruit orchards	*B. coriacea*, *B. crinicollis*	61	0	0	0	0
Sangla	2580	Pea	*Melolontha* sp.	12	0	0	0	0
Palampur	1110	Potato, Cabbage	*H. longipennis*	45	0	0	0	0
Phulladhar	2250	Potato	-	0	-	-	-	-
Kamrah	2400	Potato	-	0	-	-	-	-

whitegrubs. After purification, the fungus was identified as *Beauveria bassiana* (Bals.). To test the pathogenicity of collected isolates of *B. bassiana*, the grubs were dipped in a spore suspension to facilitate better fungus-insect contact. After about 4 weeks, half of the treated whitegrubs died due to mycosis. There was no visible growth of fungus in some dead grubs, especially where early mortality was recorded. This may be due to the reason that fungi secrete a wide array of compounds that exhibit biological activity against insects (Vey *et al.* 2001). *B. brongniartii* produces oosporein which affects enzymes functioning by redox reactions and is effective against cockchafer larvae (Vey *et al*. 2001). Growth pattern on insect body was characterized by formation of loose or tough mycelial mat with cushions or areas of conidial structures. The conidia were formed solitarily on a laterally proliferating conidiogenous cell often showing a geniculate or zig-zag type of elongation. However, after 5-6 weeks, most of the grubs were covered with white mat of mycelium. The globose shape and size (2.0-3.3x 2.0-2.5 µm) of conidia and shape and structure of conidiogenous cells resembled the description given by Samson (1981).

Establishing the pathogenicity of *B. bassiana* against whitegrubs

The pathogenicity of all four strains of *B. bassiana* isolated from Shillaroo, Kheradhar, Kharapathar and Solan was tested against second instar grubs of *B. coriacea* under laboratory conditions at Palampur. The dose of each strain was standardized to $401x10^4$ conidia/ml. The grubs were given dip treatment in the conidial suspension for about 10 seconds. There was no mortality of grubs up to 7 days, however, after about 2 weeks, 20% of mortality of whitegrubs was recorded with Shillaroo strain of *B. bassiana*. After about 4 weeks, considerable mortality (77%) was recorded with all the strains. Prior to death, the whitegrubs stopped feeding, turned sluggish and the growth was arrested. After about 6 weeks, there was development of white mycelial growth on the body of grubs. When the fungal infested grubs were dissected, their haemocoel was completely filled with whitish fungal growth. Koch's postulates were proved to confirm the pathogenicity of the isolated fungus (*B. bassiana*).

Effect of temperature on growth and development of *B. bassiana*

Growth and sporulation of 4 isolates of *B. bassiana* collected from Shillaroo, Kheradhar, Kharapather and Solan was observed at 20 to 26 ^oC. There was gradual increase in radial growth of fungus with increase in temperature up to 26^oC as shown in Table 2. After 15 days of incubation, the diameter of Shillaroo isolate was recorded to be 7.66 cm.

B. bassiana strain obtained from Solan exhibited radial growth of less than 6.0 cm at 20 ^oC. However, at 26 ^oC, both Solan and Shillaroo isolates were statistically at par with each other. The Kharapather isolate showed growth at par with Solan isolate both at 20 and 22 ^oC. At 26 ^oC, the Kharapather and Solan isolates produced equal growth (7.60 cm) and were at par with Shillaroo isolate. There was lesser growth of Kheradhar isolate at all the temperatures as compared to all other isolates. The optimum temperature for growth and sporulation of *B. bassiana* has been reported to be ranging between 20-30 ^oC (Kuberappa and Jayaramaiah 1987). Sharma *et al.* (1998) demonstrated that temperature of 28 ^oC is most favourable for growth and sporulation of *B. bassiana* and *M. anisopliae*. They observed gradual increase in growth of *B. bassiana* with temperature. The results in the present study are totally in accordance with their findings. Among different isolates, Shillaroo isolate showed significantly higher multiplication in terms of mycelium development. This higher multiplication of Shillaroo isolate may be related to the virulence of the isolate and can cause early mortality in whitegrubs in high hills where average soil temperature fluctuates between 20 to 25 ^oC during cropping period.

Table 2. Effect of different temperatures on growth of *B. bassiana*

Strain	Radial growth (cm) of fungus after 15 days of incubation			
	20 ^0C	22 ^0C	26 ^0C	Mean
Shillaroo	6.19	6.90	7.66	6.92
Kharapather	5.80	6.10	7.60	6.50
Solan	5.93	6.19	7.60	6.57
Kheradhar	5.50	5.80	7.36	6.22
Mean	5.85	6.25	7.55	
LSD (P=0.05)	Strain	Temperature	Strain x Temperature	
	0.13	0.11	0.23	

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