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A survey of the local isolates of entomopathogenic fungi in Uttarakhand region

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Abstract
An extensive survey was conducted in the Kumaun and Garhwal divisions of Uttarakhand. Both insect cadavers and soil samples were collected for the purpose of isolation of local strains of entomopathogenic fungi. *Nomuraea rileyi* was the most predominant entomopathogen isolated from the mycosed larvae followed by *Beauveria bassiana*. *Trichoplusia orichalcea* showed more cases of mycosis. Some *B bassiana* affected coleopteran (beetle) in the soybean field were also collected. Maximum number of mycosed cadavers were collected in the month of September, followed by November and October respectively and none in December. The maximum number of mean diseased insects per meter square was reported at Fatehpur area of Udham Singh Nagar District in the month of September. Isolates were extracted from soil samples also. 52 soil samples were collected for this purpose. *Metarhizium anisopliae* followed by *B bassiana* dominated the soil samples. The collected soil samples upon testing yielded a total of six entomopathogenic fungi among which *M anisopliae* was the predominant one.

Keywords: Entomopathogenic fungi, survey, mycosis, Epizootic

Introduction

Agriculture is regarded as the backbone of our country [1]. India abounds in the number and variety of crops grown throughout its boundaries, owing to the varied agro ecosystems present in the country's domain, but this diversity of the crops as well as the diverse agro ecosystems also add to the assortment of the pest population. This puts up a challenge of protecting our crop efficiently and timely in order to draw the benefits in terms of production and yield. There has always been a need to keep crops free from pests in order to maximize food production. The conventional ways have more or less relied highly on the chemicals for pest management. In the long run the injudicious and excessive use of the chemicals did not bring about the desired control over pests, but added to a series of problems such as environmental degradation, pollution, pest resistance, pest resurgence, secondary pest outbreak and many more.

All these problems and the environmental issues resulted in a higher sense of awareness among the people. Eventually alternate ways to pest control were found. Integrated approaches were made for pest control and integrated pest management with its various tools like cultural, mechanical, physical, biological etc came into being. One of its tools the biological control employs the use of microbes to combat the pest problem and protect the crops. One such microbe employed is the entomopathogenic fungi.

Entomopathogenic fungi are natural enemies of diverse terrestrial arthropods and are important regulators of host populations in terrestrial ecosystems [5]. They naturally occur in insect hosts as infections; they can be collected in the field and grown in the laboratory for the documentation of the fungus. *Beauveria bassiana* have been reported to occur naturally in more than 700 species of hosts [6]. There are more than 700 species of fungi from 9 genera that infect insects [3]. Entomopathogenic hyphomycetes have great potential as biological control agents against insects and as one component within integrated pest management systems. They are being developed worldwide for the control of many pests of agricultural importance [4]. Entomopathogenic fungi are widely distributed in a wide range of habitats including aquatic, forest, agricultural and pasture habitats [9]. However, epizootics caused by entomopathogenic fungi in agricultural habitats are more numerous, particularly in temperate regions, than those in other habitats. Epizootics caused by a range of genera, including *Beauveria*, *Paecilomyces*, *Sporodiniella*, *Stilbella*, *Hirsutella*, *Metarhizium* and *Erynia* have been reported on insect

populations in agricultural habitats [7]. The occurrence of entomopathogenic fungi in India has been reported by many workers [2]. These findings suggest that these fungi act as mortality factors in Indian insect populations. However, the abundance and biodiversity of these fungi is poorly known, especially in Central India. Uttarakhand also holds a lot of fungal biodiversity, much of which can be explored and documented with the help of authentic and accurate techniques, therefore a survey was carried out in various places of Uttarakhand to isolate, identify and document the local fungal isolates.

Materials and Methods

Kumaun and Garhwal are the two administrative divisions of Uttarakhand. Kumaun division comprises of six districts namely U.S Nagar, Nanital, Almora, Pithoragarh, Bageshwar and Champawat, whereas Garhwal comprises of seven districts namely Haridwar, Dehradun, Pauri, Tehri Garhwal, Chamoli, Uttarkashi and Rudraprayag. Intensive and repeated survey for the occurrence of insect mycopathogens was made in various regions of Kumaun and Garhwal. and some forest ecosystems during the cropping season of 2014-2015 from the month of September to December. (Table 1.1 and 1.2) The number of visits to each place varied from one to five depending upon the cropping system and host insect availability.

Sampling and collection

The fields for the purpose of sampling were selected randomly. The fungus infected insect cadavers were counted in 1 m^2 area and Mean no. of diseased insects/m^2 area were calculated.

The cadavers of the insect that appeared to be infected by fungi, and soil samples from moist and damp region of the field were collected during the survey. The infected insects were identified on the basis of visual symptoms by sight inspection. The lethargic insects/dead cadavers adhered to the leaves, any plant part or soil with or without external signs of mycosis were collected with a piece of substrate to which they adhered. The substrate with attached cadavers was placed in a polythene bag air tightly whereas cadavers without substrate were kept in sterilized plastic vials with screw caps. The collected material was transported promptly to the laboratory.

Results and Discussion

During these survey various fungus infected insect cadevars along with soil sample were collected. Among the various entomopathogenic fungi collected (from mycosed larvae) *N. rileyi* was the predominant one followed by *B. bassiana*. Whereas *M. anisopliae* dominated the soil samples followed by *B. bassiana*. Table 1.1 reveals the prevalence of disease caused by *N. rileyi on T. orichalca and S. litura and* that of *B. bassiana* on Beetle in the Soybean growing areas viz Fatehpur, Kalekhera, Haripura, Badigarh and Pantnagar of Udham Singh Nagar District and Baelparao of Nanital district.

It was also observed that the maximum number of mycosed cadavers were collected in the month of September, followed by November and October respectively and none in December. The maximum number of mean diseased insects per meter square to the tune of 0.8 was reported at Fatehpur area of Udham Singh Nagar District in the month of September. An epizootic of the mycopathogen was not reported in the Soybean growing areas of Uttarakhand in the cropping year 2014-2015. Mycosis of *S. litura* to the tune of 0.4 number of mean diseased insects per meter square was observed. It was also observed that *T. orichalcea* showed more cases of mycosis ranging from 0.2 to 0.8 number of mean diseased insects per meter square.

During the survey, occurrence of *B. bassiana* was noticed on some coleopteran beetle in the soybean field with the mean diseased insects per meter square being 0.2.

An extensive soil collection was also done from the localities namely Haripura, Jholia, Shantinagar, Kaelakhera, Fatehpur, Pratappur**,** Lalpur, Jaspur, Bhujjawala, Badigarh, Pratappur, Jaffarpur, Jhagadpuri, Danpur, Lalpur, Kashipur, Konora, Kelakhera, Surajpur, Horticulture Research Centre, Crop Research Centre, Vegetable Research Centre of Udham Singh Nagar district, Ramnagar Forest area, Corbet fall, Baelparao, Golapar, katghariya, Motahaldu of Nanital District, Kinkreig, Wynberg, library of Dehradun District, and Bahadurpur, Habibwala, Mubarakpur of Haridwar District. The soil samples were taken from cropped as well as fallow fields. The samples were collected from damp and shady areas as there are more chances that a damp and shady place would harbor more microflora such as fungus. The soil samples upon testing yielded a total of 6 entomopathogenic fungus, *B. bassiana* was isolated from the samples collected from Afzalgarh and Bahadurpur areas of U.P and Kumaun whereas *M. anisopliae* was isolated from samples collected from Danpur, Lalpur, Kaelakhera and Horticulture Research Centre (HRC) Pantnagar. It was observed that *M. anisopliae* dominated the soil followed by *B. bassiana*.

Isolation of Entomopathogenic fungi from insect has been studied by many researchers. Some of the findings have suggested that *Beauveria bassiana* can easily be isolated from insect cadavers or from soil in forested areas by using media **[13]**, as well as by baiting soil with insects **[14]**. In the current study *B. bassiana* was noticed on some coleopteran beetle in the soybean field. The observation is in line with the studies conducted by Patrick and Fernando in which *B. bassiana* was isolated at high frequency from all the *B. bassiana*-contaminated sap beetles recovered from the overwintering traps [12]. Studies have revealed that many insect species belonging to Lepidoptera including *Spodoptera litura* and some belonging to Coleoptera are susceptible to *N. rileyi* [15]. All these findings are in line with the results obtained in the current studies.

The soils have also been a storehouse of such fungi. The present findings from soil survey and isolation from soil can be compared with a few available reports about the distribution of entomopathogenic fungi**.** The surveys done in nine districts of northern Karnataka revealed that the occurrence of entomopathogenic fungi *N. rileyi* was higher followed by *M. anisopliae* and *B. Bassiana* [10]. Rath also reported that the ability of *M. anisopliae* to persist in cultivated soils is well established, while *B. bassiana* has more likelihood of being present in uncultivated soil [8]. *B. bassiana* was found to be more dominant than *M. anisopliae* in the soil cultivated with wheat and cotton plants [11]. *B. bassiana* and *M. anisopliae* are common everywhere. It is a fact that *B. bassiana* seems to be very sensitive to the disturbance effects of cultivation and thus is restricted to natural habitats. The ability of *M. anisopliae* to persist in cultivated soils is well established. Therefore the first is more frequent in forest, and second in arable soils [16].

As per the current study the maximum Mean no. of diseased insects/m^2 area were observed in the month of September while none in the month of December. This clearly indicates that moderate temperature along with moisture is necessary

for the growth of the fungus. Studies conducted by Huessein also reveal that in general, the highest number of the isolates was recorded during spring and autumn. Whereas during the winter and summer months, the number of isolates were in low detectable level [11].

Thus it can be said that Uttarakhand has a rich storehouse of entomopathogenic fungi. A good inoculum can be recovered from many locations during the time of the year which has favourable conditions for the fungi. These can provide good control over pests and can act as bio control agents.

Table 1: Details of extensive survey conducted for collection of Entomopathogenic fungi affected insect cadavers in Uttarakhand (2014-2015).

Months visited	District	Crops surveyed	Mycosis noticed					
			Locality	Crop	Pest	Causal agent	Total no. of mycosed insects	Mean no. of diseased insects/m^2 area
September	**U.S Nagar** Haripura, Jholia, Shantinagar, Kaelakhera, Fatehpur **Nanital** Ramnagar Forest area, Corbet fall, Baelparao	Soybean,Urd, Arhar, Mung, Paddy Soybean,Urd, Arhar, Mung, Paddy, Tomato	Fatehpur Kaelakhera -	Soybean Soybean -	*T. orichalca* *T. orichalca* -	*Nomuraea spp.* *Nomuraea spp* -	5 2 0	0.8 0.4 0
October	**Nanital** Ramnagar Forest area, Corbet fall, Baelparao **Dehradun Mussoorie** Kinkreig, Wynberg, library **U.S Nagar** Haripura, Jholia, Shantinagar, Kaelakhera, Fatehpur **Rudrapur** Pratappur, Lalpur, **Haldwani** Golapar, katghariya, Motahaldu	Soybean,Urd, Arhar, Mung, Paddy, Tomato Bajra, jhangora, mandua, paddy Soybean,Urd, Arhar, Mung, Paddy Tomato, Urd, Pegion pea Tomato and pulses	Baelparao - Haripura - -	Soybean - Mung bean - -	Beetle - *T. orichalca* - -	*Beauveria spp.* - *Nomuraea spp* - -	1 0 1 0 0	0.2 0 0.2 0 0
November	**U.S Nagar** Jaspur, Bhujjawala, Badigarh, Pratappur, Jaffarpur, Pantnagar, Jhagadpuri, Danpur, Lalpur, Kashipur, Konora, Kelakhera, Surajpur **Pantnagar** Horticulture research centre(HRC), Vegetable research centre (VRC), Crop research centre (CRC) **Haridwar** Bhahadurpur, Habibwala, Mubarakpur,	Soybean, Tomato, Urd, Pegion pea, chickpea S.cane, wheat, barley, Soybean	Badigarh CRC -	Urd Chickpea -	*S. litura* *S. litura* -	*Nomuraea spp* *Nomuraea spp* -	2 2 0	0.2 0.4 0
December	**U.S Nagar** **Pantnagar** **Pantnagar** Horticulture research centre(HRC), Vegetable research centre (VRC), Crop research centre (CRC)	S.cane, wheat	-	-	-	-	0	0

Table 2: Details of extensive survey conducted for collection of soil samples for the incidence of Entomopathogenic fungi in Uttarakhand (2014-2015)

Months visited	District	Crops surveyed	Soil collection		
			Locality	Crop	Entomopathogenic fungi isolated
September	**U.S Nagar** Haripura, Jholia, Shantinagar, Kaelakhera, Fatehpur **Nanital** Ramnagar Forest area, Corbet fall, Baelparao	Soybean,Urd, Arhar, Mung, Paddy Soybean,Urd, Arhar, Mung, Paddy, Tomato	Haripura Jholia Shantinagar Kaelakhera Fatehpur	Soybean Soybean Urd Mung Soybean	Not determined Not determined Not determined Not determined Not determined
October	**Nanital** Ramnagar Forest area, Corbet fall, Baelparao **Dehradun** **Mussoorie** Kinkreig, Wynberg, library **U.S Nagar** Haripura, Jholia, Shantinagar, Kaelakhera, Fatehpur **Rudrapur** Pratappur Lalpur, **Haldwani** Golapar, katghariya, Motahaldu	Soybean,Urd, Arhar, Mung, Paddy, Forest Soybean,Urd, Arhar, Mung, Paddy, S.cane Tomato, Urd, Pegion pea Tomato and pulses	Ramnagar Forest area Corbet fall Baelparao Kinkreig Wynberg library Haripura Jholia Shantinagar Kaelakhera Fatehpur Pratappur Lalpur Golapar, katghariya, Motahaldu	Forest Fallow Soybean Fallow Fallow Fallow Soybean Mung Soybean S. cane Soybean Urd Urd Tomato Soybean Fallow	Not determined Not determined Not determined Not determined Not determined Not determined Not determined Not determined Not determined Not determined Not determined Not determined Not determined Not determined Not determined Not determined
November	**U.S Nagar** Jaspur, Bhujjawala, Badigarh, Pratappur, Jaffarpur, Pantnagar, Jhagadpuri, Danpur, Lalpur, Kashipur, Konora, Kelakhera, Surajpur **Pantnagar** Horticulture research centre(HRC), Vegetable research centre (VRC), Crop research centre (CRC) **Haridwar** Bhahadurpur, Habibwala, Mubarakpur, **Uttar Pradesh** Afzalgarh	Soybean, Tomato, Urd, Pegion pea, chickpea Chickpea, Arhar, Horticultural trees and vegetables S.cane, wheat, barley, Soybean Soybean	Jaspur, Bhujjawala, Badigarh, Pratappur, Jaffarpur, Jhagadpuri, Danpur, Lalpur, Kashipur, Konora, Kelakhera, Surajpur HRC CRC VRC Bhahadurpur Habibwala, Mubarakpur, Afzalgarh	Soybean Urd Arhar Soybean Soybean Fallow Soybean Soybean Fallow Fallow Urd Fallow Fallow Chickpea Fallow Soybean Fallow Fallow Soybean	Not determined Not determined Not determined Not determined Not determined Not determined *Metarhizium spp* *Metarhizium spp* Not determined Not determined *Metarhizium spp* Not determined *Metarhizium spp* Not determined Not determined *Beauveria spp* Not determined Not determined *Beauveria spp*
December	**U.S Nagar** **Pantnagar** **Pantnagar** Horticulture research centre(HRC), Vegetable research centre (VRC), Crop research centre (CRC)	S. cane, wheat	HRC CRC VRC	Fallow Chickpea Fallow	Not determined Not determined Not determined

Conclusion

The diversity and distribution of entomopathogenic fungi occurring in Uttarakhand could play an important role in regulating the forest and agricultural pest populations. The local isolates of entomopathogenic fungi can better manage the existing pest problems owing to its better adaptability in the microenvironment and better host searching ability. The survey reveals that local isolates of entomopathogenic fungi are naturally present in the agro ecosystems of the state. However, in order to obtain concrete results these isolates need to be isolated and used for the management of the pest problems. This is possible when the isolates are available to the farmer in the form of a commercial formulation. Thus we can say that the native isolates play an important rule for the biological control and their significance in managing the pest population is immense.

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