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Towards microbial control of white grubs in Nepal with entomopathogenic fungi

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With an objective to explore the possibility of biocontrol of white grubs using entomopathogenic fungi, an exploratory study was conducted in the Syangja and Parbat districts in Nepal in the winter of 2001/2002. In order to explore the occurrence of indigenous fungal pathogens of white grubs, field and laboratory experiments were carried out and information was collected from all available sources. Upon collection of the white grubs the entomopathogenic fungus *Metarhizium anisopliae* was found to be associated with white grubs in fields with arable crop. Disease prevalence was between 0 and 2% depending on host origin and species. Bioassays revealed that the Nepalese isolates of this fungus species were as pathogenic as a Swiss isolate used for comparison purposes. Therefore, future work will be done exclusively with Nepalese isolates. Analysis of soils from three different regions showed that *M. anisopliae* is common and was present in about 50% of the samples irrespective of their origin. However, the fungus densities were low. Another entomopathogenic fungus, *Beauveria bassiana*, was isolated as well from a few soil samples. Based on these first results, the possibilities to develop mycoinsecticides and to integrate them into existing pest management (IPM) systems are considered as very promising. In the meantime a project funded by Helvetas has been initiated.

Key words: Biological control, entomopathogenic fungi, *Metarhizium anisopliae*, Scarabaeidae, white grubs, Nepal.

INTRODUCTION

Nepal, represented by the Terai (from below 100 m asl) to the highest peak of the world has a wide variety of climatic and agro-ecological zonations, where agriculture is the main source of livelihood. The crops grown are also very diverse, from tropical rice to temperate barley, from mangoes to apples. Agriculture in the past was traditional with few crops per unit land per year, low input in terms of chemicals (fertilisers and pesticides) resulting in low productivity. With increasing population pressure, the need for the adoption of improved technologies has been widely realised at all levels from planners to rural farmers. Adoption of new technologies has increased crop intensities, and has lead to the adoption of high yielding varieties and high input of fertilisers and pesticides. These practices have lead not only to increased production and productivity and greater monetary returns to the farmers but also resulted in severe problems to an extent never faced in the past. Pest problems including insects, diseases, weeds, rodents, and mites have increased greatly in several crops. The National Planning Commission of Nepal has estimated that pests cause a loss in food production of about 15–20% (Baker and Gyawali 1994). Farmers are very vigilant and worried about pest attack in high value crops such as rice and maize and most vegetables and fruits. In such crops they often apply pesticides to reduce the damage to the crops in an excessive way.

Different types of insect pests attack different crops, however, the losses in cereal are one of the major concern for the farmers and technicians as they are major sources of livelihood in Nepal. Crop losses by soil-pest insects have been identified as one of the main causes of low food production (Pandey et al. 1993). Among different insect pests, white grubs (larvae of Coleoptera, Scarabaeidae) are among the destructive insect pests in Nepal. Collaborating Institutions of Sustainable Soil Management Program (SSMP) have reported white grubs as a major limiting factor to soil productivity in several areas. The grubs attack a wide range of crops such as *Arachis hypogaea* (groundnut), *Sorghum bicolor* (sorghum), *Zea mays* (maize), *Saccharum officinarum* (sugarcane), *Glycine max* (soybean), *Vigna mungo* (black gram), *Vigna radiata* (mung bean), *Cajanus cajan* (pigeon pea), cucurbits, *Brassica*, *Solanum melongena* (aubergine), *Lycopersicon esculentum* (tomato), *Ipomoea batatas* (sweet potato), *Cyamopsis tetragonoloba* (clusterbean), *Vigna unguiculata* (cowpea) and *Solanum tuberosum* (potato) (Chen et al. 1997; Nath and Singh 1988; Tian and Hu 1992; Vora & Ramakrishnan 1978; Vyas and Yadav 1993). White grubs, for example, caused an average of about 25% yield loss on groundnut in Baitadi district of Nepal (G. Weber, pers. comm.). In Nepal losses due to white grubs are reported from across the country of the mid to high hills, although quantification of their damage has not been assessed. In Himachal Pradesh, India, potato yield losses of up to 85% have been reported (Misra and Chandla 1989).

White grubs are not new pest insects in Nepal, however, in farmers perception their infestation has been increasing every year. A hitherto unknown number of species of white grubs causes damage depending mainly on environmental conditions, however, the damaging species are not identified yet. They are polyphagous and feed on roots, which may lead to the complete destruction of single plants, or of the entire plant cover in a limited area. Their life cycle is species-specific and may last one or more seasons. The adults are considered less damaging but some species are known to feed on leaves, buds and flowers of cultivated plants. Traditional methods to control white grubs are repeated ploughings, preferably soon after the summer rains, which kill them or expose them to predatory animals, removal of host weeds by cleaning nearby areas, the use of well decomposed manure, and an adapted crop rotation. As a control measure, farmers attempt different methods, however, the pests are not amenable to control with the common practices.

In recent years, chemical insecticides including DDT were more and more used to control this pest. In order to avoid detrimental effects of such a practice, the Sustainable Soil Management Programme (SSMP) funded by Swiss development organizations (SDC, Helvetas, Intercooperation) initiated a project with the goal to develop and establish a biological control method using indigenous antagonists. Such methods have been developed and successfully applied in other countries like Switzerland, Austria, New Zealand and Australia (Keller 2000; Strasser 1999; Jackson et al. 1992; Rath et al. 1995). They were either based on the insect pathogenic bacterium *Serratia entomophila* (Jackson et al. 1992) or the insect pathogenic fungi *Metarhizium anisopliae* (Zimmermann 1993) and *Beauveria brongniartii* (Zimmermann 1992).

MATERIALS AND METHODS

Survey of farmer practices for white grub management

During a survey on damages done by white grubs, some of the basic information was recorded which included the awareness of the white grub problems by farmers, their knowledge about white grubs and how they cope with this pest insect.

Observation of natural enemies of white grubs

In order to understand the white grubs intensity in the field, a survey was carried out in white grub prone area of arable (bari) land in two ecological zones of low-mid hills (600–900 m altitude) in Syangja and mid-hills (1500–1800 m altitude) of the Parbat district in Nepal (Fig. 1). Similarly, existing farmer's practices

Fig. 1. Study sites: 1: Durlung and Pang/Parbat; 2: Syangja; 3: Rishing Patan/Tanahun; 4: IAAS and NMRP Rampur/Chitwan. White grubs were collected from 1 and 2; and soil samples were collected from 1, 3 and 4.

for the management of white grubs with chemical pesticides, botanicals and overall crop management practices was also studied during the survey. For this, farmers were interviewed individually and in groups about the prevalent management practices. During field surveys farmers were also consulted in terms of declining or increasing trends of white grub occurrence.

A total of 25 white grubs of the same instar and sizes were collected from each field resulting in 50 and 200 individuals respectively, and a total of 600 individuals (Tab. 1). The larvae were placed in containers in groups of about 5 larvae with soil from the same collection site and transported to the laboratory. While surveying, some of the basic information was recorded. In the laboratory they were reared individually in plastic boxes of different sizes at ambient temperatures until death or adult emergence. They were fed with slices of carrots or potato, checked at regular intervals of 5 days and occurrence of entomopathogenic fungi was recorded and the fungi were isolated (Tab. 1).

Presence of entomopathogenic soil fungi in soils

46 soil samples were taken with a soil borer from fields in Chitwan, Syangja, Tanahun and Parbat districts (Fig. 1). They originated from arable land and from meadows in Chitwan (Rampur, National Maize Research Programme – NMRP), from Syangja (Dhanubanse), from Tanahun (Rishing Patan) and Parbat (Pang, Durlung) and from fruit plantations, arable land and meadows from Chitwan (Rampur, Tribhuvan University, Institute of Agricultural and Animal Sciences – IAAS). All

Tab. 1. Natural occurrence of entomopathogenic fungi on white grubs.

Origin of white grubs	White grub species	Number of white grubs observed	Number of white grubs infected with fungus	% fungus infected grubs
Syangja	*Xylotrupes gideon* ("Large grubs")	50	0	0
	Small grubs	200	0	0
	Others	50	0	0
Parbat	*Xylotrupes gideon* ("Large grubs")	50	0	0
	Small grubs	200	4	2
	Others	50	0	0

samples were checked with the *Galleria* bait method (Zimmermann 1986) (Tab. 2). 30 samples, 10 each from Rishing Patan, Pang, and NMRP were analyzed by plating soil suspension on a selective medium (Keller et al. 2000).

Tab. 2. Presence and density of entomopathogenic fungi in soil samples from two sites in Nepal as pointed out with the *Galleria* bait method; EPF = Entomopathogenic fungi. *M.a.* = *Metarhizium anisopliae; B. bass.* = *Beauveria bassiana*; *P.*sp. = *Paecilomyces* sp. NMRP = Samples taken in fields of the National Maize Research Programme.

Origin	Crop	Number of samples	*Soil samples with EPF (%)*	*Species of EPF*
Parbat, Durlung	Arable land	4	3 (75)	3 *M.a.*
	Grassland	4	2 (50)	2 *M.a.*
Parbat, Pang	Arable land	5	3 (60)	3 *M.a.*
	Grassland	5	3 (60)	3 *M.a.* + 1 *B. bass.*
Tanahun, Rishing Patan	Arable land	5	1 (20)	1 *B. bass.*
	Grassland	5	4 (80)	1 *M.a.* + 4 *B. bass.* + 2 *P.* spp.
IAAS, Rampur	Arable land Fruit plantations	4	2 (50)	2 *M.a.*
	Grassland	4	1 (25)	1 *M.a.*
NMRP, Rampur	Arable land	5	2 (40)	2 *M.a.* + 1 *B. bass.*
	Grassland	5	3 (60)	3 *M.a.*
All	Arable land	23	11 (48)	10 *M.a.* + 2 *B. bass.*
	Grassland	23	13 (57)	10 *M.a.* + 5 *B. bass.*

The soil plating method was adapted from Fornallaz (1992). 10 g soil/sample of fresh soil are shaken for 3 h at 140 rpm on a rotary shaker in 250 ml Erlenmeyer flasks with 50 ml tap water containing 1.8 g/l tetra-Sodiumdiphosphate-Decahydrat ($Na_4P_2O_7*10H_2O$) to favour disaggregation of the soil. After 15 seconds of sedimentation 0.1 ml of the suspension is distributed with a Trigalsky spatula on a Petri dish with selective media and intensively rubbed. Three replicates/soil sample were prepared. After 8–10 days at 20°C and darkness the colonies of *M. anisopliae* were counted and a selection of colonies was isolated in tubes with Sabouraud-glucose-agar with 0.6 g/l Streptomycine. The soil suspension was not diluted, even if there were very high fungal densities exceeding 600 colonies/Petri dish, the maximum that could be counted.

The selective medium adapted from Strasser et al. (1997) with the following composition and preparation was used: 10 g peptone from meat pancreatically digested, 20 g glucose, 18 g agar, all dissolved in 1 liter distilled water and autoclaved at 120°C for 20 minutes. At a temperature of 60°C 0.6 g streptomycin, 0.05 g tetracycline and 0.05 g cyclohexamide previously dissolved in distilled, sterile water and 0.1 ml Dodine were added.

The *Galleria* bait method (GBM) was adapted from Zimmermann (1986). About 60 ml of soil/sample were filled in a cylindrical plastic tube with a diameter of 40 mm and a height of 65 mm and 4 larger *Galleria* larvae were added. The samples were kept in darkness at a temperature of 22°C. During the first five days the tubes were turned daily to keep the larvae moving in the soil. After 16–18 days the larvae were examined, fungus infections were recorded and the fungus from infected larvae was isolated.

Bioassays

The susceptibility of white grubs to some isolates of *M. anisopliae* and *B. brongniartii* was tested with the dipping method. The fungus isolates (Tab. 3) were grown on selective medium (Strasser et al. 1997) and the spores washed off with 0.1% Tween 80. The spore suspension was adjusted to 10^7 spores/ml and the white grubs dipped for 5 seconds in the suspension. After the excess liquid had dropped off, the grubs were placed individually in rearing containers with a capacity of 500 ml. They were checked weekly, occurring fungal infections were recorded and the fungi identified.

RESULTS

Farmer practices for white grubs management

The majority of the farmers were unaware of the increasing problem of white grubs, their nature and stages of damage and appropriate control measures. In the surveyed area, farmers ignored crop management such as cultural practices, use of resistant varieties, fertilizer and irrigation management. It turned out that the farmers had no knowledge about the use of decomposed farmyard manure, mixed cropping, crop rotation and other adequate practices.

Use of chemical pesticides

In recent years, pesticide use in Nepal has increased significantly, and this trend is likely to continue and even accelerate in the near future due to missing

Tab. 3. Presence and density of entomopathogenic fungi in soil samples from two sites in Nepal. EPF = Entomopathogenic fungi. SM: Soil suspension spread on selective medium. CFU = Colony Forming Units. *M.a.* = *Metarhizium anisopliae*.

Origin	Crop	Number of samples	Soil samples with EPF (%)	SM CFU/g fresh soil	Species of EPF
Parbat Pang	Arable land	5	1 (20)	275	*M.a.*
	Grassland	5	1 (20)	35	*M.a.*
Tanahun Rishing Patan	Arable land	5	0		
	Grassland	5	1 (20)	15	
Rampur NMRP	Arable land	5	0		
	Grassland	5	2 (40)	265*	*M.a.*
all		30	5 (17)		

* Data from a single sample only, the other sample was partially overgrown by saprophytic fungi.

alternatives. The area along the open boarder with India where agro-chemical production is growing without much quality control is particularly vulnerable. However, in the surveyed area, 25–30% of the farmers used different types of pesticides such as DDT, benzene-hexachloride (BHC), aldrin, dieldrin, heptachlor and chlorpyrifos to control white grubs, however, the percentage is much bigger in case of off-season vegetable crops. During survey, it was also learnt that few farmers also used soil acting fungicides such as Ridomil because of wrong perception.

The most alarming aspect is that the majority of the farmers cannot read the labels on the pesticide and often rely on verbal instructions of the retailer to use pesticide. There is still great misconception among some farmers as they think pesticides are less dangerous chemical compounds. Because of this, there is high level of negligence and misuse while handling chemical pesticides. Moreover, most farmers are not aware of the hazards related to chemicals and do not have adequate knowledge of safety measures.

Presence of entomopathogenic fungi among white grubs and in soil

The white grubs belonged to different and not completely identified scarabaeid species. They were collected at 24 locations in two districts and separated into three groups: "large larvae", later on identified as *Xylotrupes gideon*, "small larvae" and "other larvae". The disease prevalence varied between 0% and 2% infected larvae. Infections were found only in the group of "small larvae" (Tab. 1). All entomopathogenic fungi were identified as *Metarhizium anisopliae*.

Two methods were used to study the presence of entomopathogenic fungi in soils: the *Galleria* bait method (GBM) and spreading soil suspensions on selective media. Using the GBM 52% of the soil samples proved to contain entomopathogenic fungi. The difference between soils from arable land (48% positive samples) and soils from grassland (57% positive samples) were only small and statistically not significant ($p = 0.73$). *Metarhizium anisopliae* was found at all locations and in all types of crop except in arable land at Tanahun. *Beauveria bassiana* was found in grassland at Parbat (Pang), Tanahun (Rishing Patan), and in arable land at Tanahun (Rishing Patan), and Chitwan (NMRP, Rampur) (Tab. 2). Two fungus specimen attributed to *Paecilomyces* spp. were found in grassland at Tanahun.

Ten soil samples from three localities each were analyzed for the density of *M. anisopliae* (Tab. 3). This fungus was found in two samples from Parbat (Pang), in one sample from Tanahun and in two samples from Rampur (NMRP). The highest density found was 275 CFU/g fresh soil (Table 3). However, these data are not representative since all three replicates of 15 out of 30 samples were overgrown with fast growing fungi and the presence of *M. anisopliae* could no more be checked.

Susceptibility of white grubs

The bioassays with the three isolates showed for the Swiss isolates (546 and 617) that the small larvae were less susceptible than the large ones (*Xylotrupes gideon*). *B. brongniartii* 546 and *M. anisopliae* 617 caused about the same mortality in the comparable larval group. Bioassays with small larvae resulted in a mortality of 24% and 48% when infected with *M. anisopliae* 617 and the indigenous isolate *M. anisopliae* Nepal 1 (isolated from an unidentified white grub) respectively (Tab. 4).

DISCUSSION

Increasing damages in many kinds of farm crops due to larvae of Scarabaeidae (white grubs) have drawn attention to this group of root feeding pest insects. First surveys revealed crop losses of 25% and white grubs pointed out to be among the most important pest insects in Nepal. However, damages result not only in crop loss, the feeding on grass roots may cause instability and collapses of terraces and loss of arable land or of the possibility to irrigate the crops. In many regions the farmers recently became aware of the white grub problem, but no traditional pest management practice turned out to be effective. As a consequence, more and more synthetic insecticides including the persistent organochlorines are applied although they are banned for agricultural use. Some of them have no effects on white grubs, but have well documented impacts on the environment. This development alarmed agricultural authorities and lead to the initiation of a project for the biological control of white grubs.

Several workers have reported a number of biological control agents, which attack white grubs in different parts of the world. The most important ones belong to the fungi. The development of commercial products based on entomopathogenic fungi for the use in integrated pest management programmes needs several steps. Fungal species and isolates must first be obtained from diseased insects or from the environment, and be identified. The most promising candidates are evaluated under laboratory conditions and then produced in large scale as mycopesticides. However, none of the steps have been initiated in Nepal. Keeping these views in mind, Tribhuvan University, Institute of Agriculture and Animal Sciences (IAAS), and Sustainable Soil Management Project, (SSMP/Helvetas) initiated a preliminary work in this direction in the winter of 2001. During this study *Metarhizium anisopliae* was found for the first time in Nepal.

The species of Scarabaeidae causing damages in Nepal are not yet identified. Research in this direction is initiated together with a survey on the presence of entomopathogenic fungi attacking white grubs. A first survey started in summer 2002 and revealed low natural prevalence of fungus diseases. Depending on species and origin, a maximum on 2% of the grubs was infected with *M. anisopliae* (Tab. 1). Analysis of soil samples taken in four regions showed, that entomopathogenic fungi, especially *M. anisopliae*, are common in all places. Using the GBM 52% of the soil samples proved to contain entomopathogenic fungi (mainly *M. anisopliae*), while with the isolation on selective media, only 17% of the soil samples showed the presence of *M. anisopliae*. *B. brongniartii*, an important pathogen of white grubs (*Melolontha* spp.) in Europe was neither found on white grubs nor in soil samples.

This result demonstrates that the GBM is much more efficient and appropriate to analyze soils for the presence of entomopathogenic fungi. In addition, this method is simple and allows to find other species of entomopathogenic fungi. Therefore, it is recommended to use it in future investigations, either in surveys for entomopathogenic soil fungi or in monitoring the fungus distribution in control trials. The poor results of the isolation with selective medium can not be attributed to the method itself. It is attributed to the characteristics of the soils that contain high densities of fungi, which rapidly overgrew the medium. In the experiment all three replicates of 15 out of 30 samples could not be analyzed due to heavy contaminations. The problem is also known from Swiss soils, where an average of 5–10% of the Petri plates can not be analyzed. However, it is very rare that all replicates of a sample are that heavily contaminated. In Switzerland comparable results are obtained with either method (Keller et al. 2003).

The percentage of soil samples containing entomopathogenic fungi as well as the densities found are low as compared to data from Switzerland, but are in the range of other European countries. In Switzerland, 96% of the fields analyzed were found to contain *M. anisopliae* with a mean density of about 1000 CFU/g dry soil (Keller et al. 2003), in Germany and Norway 60% and 7.5%, respectively, of the soil samples contained entomopathogenic fungi dominated by *M. anisopliae* (Kleespies et al. 1989; Klingen 2000).

Bioassays with Swiss isolates showed that *B. brongniartii* and *M. anisopliae* resulted in comparable mortalities of two different host species. The higher mortality of the large grubs in comparison to the small grubs is probably not a result of

Tab. 4. Mortalities of white grubs obtained with different isolates of entomopathogenic fungi at a concentration of 107 conidia/ml. No fungus induced mortality was found in control treatments.

Isolate	Host	Number of larvae tested	% fungus induced mortality after	
			42 days	68 days
B. brongniartii 546	Small larvae	25		40
B. brongniartii 546	*Xylotrupes gideon*	10		90
M. anisopliae 617	Small larvae	25	24	52
M. anisopliae 617	*Xylotrupes gideon*	10		80
M. anisopliae "Nepal 1"	Small larvae	25	48	

a higher sensitivity of this species, but a result of the larger surface, which picked up more conidia. A bioassay with a Swiss and a Nepalesian isolate of *M. anisopliae* showed, that the latter caused a higher mortality (Tab. 4). This result is a very useful indication that indigenous virulent isolates exist which allows to base all future activities in microbial control of white grubs and possibly also of other pest insects on local isolates of entomopathogenic fungi.

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ZUSAMMENFASSUNG

Um die Möglichkeiten der biologischen Bekämpfung von Scarabaeiden-Larven (Engerlingen) in Nepal zu untersuchen, wurden in den Distrikten Syangja und Parbat im Winter 2001/2002 erste Abklärungen durchgeführt. Dabei wurden Engerlinge gesammelt und auf die Anwesenheit von pilzlichen Pathogenen untersucht sowie Umfragen bei Bauern und Beratern durchgeführt. Auf Engerlingen konnte dabei erstmals der Pilz *Metarhizium anisopliae* nachgewiesen werden. Die natürlichen Infektionsraten waren sehr gering und lagen je nach Herkunft und Wirtsart zwischen 0 und 2%. Anschliessende Untersuchungen zeigten, dass nepalesische Pilzisolate mindestens so virulent waren wie schweizerische. Weitere Arbeiten werden deshalb ausschliesslich mit nepalesischen Isolaten durchgeführt. Analysen von Bodenproben zeigten, dass *M. anisopliae* weit verbreitet ist und in rund 50% der Proben nachgewiesen werden konnte, unabhängig davon, ob die Proben aus Wiesland oder Ackerland stammten. Allerdings erwiesen sich die Dichten als sehr gering. Aus einigen Bodenproben konnte *Beauveria bassiana* als weiterer entompathogener Pilz isoliert werden. Die Möglichkeiten für die Entwicklung eines Mycoinsektizides und sein Einsatz in der integrierten Schädlingsbekämpfung sind auf Grund der Ergebnisse als sehr aussichtsreich zu beurteilen. In der Zwischenzeit ist ein von Helvetas finanziertes Projekt zur mikrobiellen Engerlingsbekämpfung initiiert worden.

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