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ORIGINAL ARTICLE

Microbial pest control of first larval instars of *Anomala bengalensis* and *Sophrops* sp. (Coleoptera: Scarabaeidae) native to Indian Himalayan region

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ABSTRACT

The present study was carried out to determine the pathogenicity of various entomopathogens against first instars of two notorious and economically important white grub species, (Anomala bengalensis and Sophrops sp.) of Indian Himalayan region. The entomopathogenic bacteria and fungi tested against the white grubs recorded mortality of less than 25% while, two strains of entomopathogenic nematode (Heterorhabditis indica) recorded mortality of more than 70%. The median lethal dose and median lethal time estimation showed LD_{50} value of 1230.27 Infective Juveniles (IJs)/ml and 891.25 IJs/ml against the grubs of A. bengalensis for commercial and native strain of EPN respectively. While, for the grubs of Sophrops sp. LD_{50} value of 1023.29 IJs/ml and 954.99 IJs/ml were obtained for commercial and native strains, respectively. The obtained LT_{50} values were 70.79 hrs and 91.20 hrs for A. bengalensis grubs and 74.13 hrs and 77.62 hrs for Sophrops sp. grubs with commercial and native strains of EPN, respectively. Overall, among all the tested entomopathogens, the H. indica (both commercial and native strain) showed good potential for biological control of grubs of A. bengalensis and Sophrops sp. under NW Himalayan conditions.

Key words: Entomopathogens, *Anomala bengalensis*, *Sophrops* sp., Median lethal values, Microbial pest control, NW Himalayas.

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INTRODUCTION

White grubs (Coleoptera: Scarabaeidae) are polyphagous pests of many agricultural, horticultural and silvicultural crops worldwide [13, 19]. They are widely distributed in all the agro-climatic zones of India and have become pests of national importance [3]. White grub management has always been intricate because of their concealed and hidden feeding nature in soil. A number of control measures have been adopted for the management of white grubs including cultural, mechanical, chemical, biological and integrated methods [17, 22, 26] all over the world. However, due to difficulty in the prediction of damage done by the white grubs, farmers are forced to use chemical control measures to prevent damage to their field crops [28], because many insecticides have proved to be very effective against white grubs [18, 32]. But, they do not provide satisfactory control unless they are used in very high dosages, as white grubs feed under the soil near the root zone of various crop plants, it is difficult to introduce an adequate amount of chemical pesticides into the root zone of an infected plant which is not only uneconomical for farmers but can be dangerous for non-target organisms and also have negative impact on the environment. Also due to the continual development of new and presumably better synthetic insecticides, it has been observed that the various species of pests have developed resistance to a different class of insecticides and successful control of this pest have become very difficult. So, the use of these harmful chemical insecticides against white grubs is not advisable as they infect crop plants and their products or by-products which are used as food. Owing to the negative impacts of hazardous insecticides, there is a

need to limit the application of these chemical insecticides to control pests and develop new control agents with no or low-hazard effects on non-target organisms and the environment to achieve control of pests in an eco-friendly and economically satisfactory manner.

During last several decades, biological control agents have been identified as feasible and ideal alternatives to hazardous chemical insecticides for pest management [4] and they have several advantages over chemical insecticides due to improved performance, cost-effectiveness and increasing resistance of insects to the various chemical insecticides [5]. In addition to this, their specificity to target insect pests and safety to the non-target organisms and environment make them the best biocontrol agents. The entomopathogens (bacteria, fungi and nematodes) play an important role in the suppression of various insect pests including white grubs [29] as like other insects they are susceptible to a variety of diseases caused by entomopathogens. These entomopathogens such as bacteria (*Bacillus cereus*), fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) and nematodes are well-known for their ability to infect white grubs in natural habitats [15, 31].

Globally, various entomopathogens are gaining importance in the management of white grubs as they not only act as a promising and potential alternative to the use of insecticides for white grub control but also are eco-friendly that tend to be long-lasting and sustainable for the environment. However, these entomopathogens have many strains which are species-specific and have little or no cross-infectivity on different species of white grubs. Taking this into consideration; the present study was carried out to evaluate the efficacy of various entomopathogens (commercial as well as native strains) against notorious and economically important white grub species, *Anomala bengalensis* and *Sophrops* sp., so that the effective strains of entomopathogens can be used to develop eco-friendly pest management strategies for sustainable agriculture in NW Himalayan region.

MATERIAL AND METHODS

Investigations were carried out to test the efficacy of different entomopathogens of bacterial, fungal and nematode origin (Table 1) against first larval instars of *A. bengalensis* and *Sophrops* sp. in Entomology Laboratory, Experimental Farm, ICAR-VPKAS, Hawalbagh, Uttarakhand (29.64º N and 79.63º E, 1250 m amsl). These entomopathogens were evaluated at different dosages. The laboratory bioassays were done as per Jackson and Saville [9] with some modifications.

Laboratory rearing and maintenance of the white grubs

The white grub adults of *A. bengalensis* and *Sophrops* sp. were collected either by handpicking or using hand nets at night time (7:00 pm to 8:30 pm) from the second fortnight of May to the second fortnight of July and were bought to the laboratory in a sterile plastic container with food of their preference. Thus, collected beetles were kept in mesh cages with large plastic buckets half filled with autoclaved soil and farm yard manure (FYM) mixture (1:1) and the freshly cut shoots of their preferred hosts *i.e., Ligustrum nepalensis* for *A. bengalensis* and *Carya illinoensis* for *Sophrops* sp., were fed to the adults. The food was replaced daily with freshly cut shoots and the soil moisture was maintained by sprinkling water at regular intervals. The adults were allowed to mate and the eggs laid by females were inspected on daily basis. After eclosion from the egg, the hatched out first instar larvae were maintained in autoclaved sterile soil under laboratory conditions at 27±2^{o}C for 5-7 days and were further used for the bioassay studies.

Bio-efficacy of various entomopathogens against white grubs

The first instar larvae were available from the second fortnight of June to the second fortnight of July. So, the bioassay studies on first instar grubs were conducted from June-July, 2021. Mortality of the white grubs was recorded and was corrected by Abbott's formula [1]. The median lethal concentration and median lethal time were calculated on the probit analysis [6] for only the effective entomopathogens and the most effective among them were recommended to farmers for managing white grubs in fields.

Bio-efficacy of entomopathogenic bacteria against white grubs

Talc-based formulation of *B. cereus* WGPSB- 2, originally isolated by Sushil *et al.* [28] from Uttarakhand was used in the present study. The concentration of commercial products used for bioassay studies was 7x10^{7}spores/gram. The containers (7x6 cm) filled with 100 gram autoclaved soil was set up and the soil was treated with *B. cereus* WGPSB- 2. One grub of first instar was released in each container and the treatment was applied to a set of 10 grubs which was replicated 3 times. In addition to this, an untreated control was also set up. Observations on grub mortality were recorded at 3 days interval up to 15 days. The grubs (if any) were also examined for the symptoms of bacterial infection *i.e.*, the diseased grub appeared shiny white in color while turns muddy brown after death.

Bio-efficacy of entomopathogenic fungi against white grubs

Two commercially available strains of entomopathogenic fungi *viz.*, *B. bassiana* and *M. anisopliae* were evaluated for their efficacy. A mixture of moist autoclaved soil and FYM (1:1) treated with conidial

suspension containing $2x10^8$ spores/ml was used and one grub per container was released. In addition to this, a control treatment (treated with autoclaved double distilled water) was also set up. Observations on grub mortality were recorded at every 3 days interval up to 15 days. The dead larvae were counted, transferred to new petri dishes containing moistened filter paper and examined for growth of fungal hyphae to confirm mycosis.

Bio-efficacy of entomopathogenic nematodes against white grubs

Two strains of entomopathogenic nematodes (*H. indica*) *i.e.,* commercial and one isolated from the native soil of Uttarakhand were tested against the first instar grubs of *A. bengalensis* and *Sophrops* sp. The infective juveniles (IJs) of both commercial as well as native strain were multiplied in the laboratory *in vivo* on larvae of wax moth, *Galleria mellonella* reared on an artificial diet and the freshly emerged populations of IJs were used for bioassay studies. The concentration of IJs per ml of suspension was determined by counting the nematodes in a counting dish under a stereo-zoom microscope. The average of four counts was taken to estimate the final nematode population/ml. Different graded concentrations ranging from 250-1000 IJs/ml was prepared. A 100 g mixture of moist autoclaved soil and FYM (1:1) was used as substrate. For the treatment of white grubs, 1 ml suspension of different graded concentrations *i.e.,* 250, 500, 750, 1000 IJs, respectively was taken in a dropper and was put into the soil directly in the cups containing grubs while the control was treated with 1 ml of ADDW. Each concentration treatment was applied to a set of 10 grubs which was replicated three times. The grub mortalities were checked at every 24 hours till 7 days of inoculation. The dead grubs (if any) were collected and were kept on White trap for release of IJs to check the pathogenicity. The corrected seven-day per cent mortality was subjected to probit analysis [6] to calculate LD_{50}, LD_{90}, LT_{50} and LT_{90} values.

RESULTS AND DISCUSSION

In present study, laboratory bioassay with *B. cereus* strain WGPSB-2 recorded no grub mortality at 7 days after treatment (DAT) in *A. bengalensis* and only 6.67% grub mortality in *Sophrops* sp. at 7 DAT while 6.67% and 13.33% grub mortality at 15 DAT in *A. bengalensis* and *Sophrops* sp., respectively. Although, *B. cereus* have been identified as one of the most potent biological control agent as it is highly efficient in the management of many white grub species with recorded mortality of 81.3% in *Anomala dimidiata* and 76.3% in *Holotrichia setticollis* @ $1.7x10^{10}$spores/m^2 [28], 92% and 67% mortality in second instar grubs of *A. dimidiata* and *H. setticollis*, respectively [23] and 51.85% at 7 DAT and cent percent mortality at 45 DAT of *Brahmina coriacea* grubs [24] but the results obtained from our studies showed contrasting observations with these findings.

The two commercially available mycopesticides (details in Table 1) were also tested and found to be ineffective causing less than 25% mortality at 15 DAT against first instar larvae of *A. bengalensis* and *Sophrops* sp. The commercial strain Green *Beauveria* recorded no mortality till 5 DAT in both the tested white grub species. After, 7 days of treatment showed 10% and 6.67% grub mortality while at 15 DAT recorded only 13.33% and 16.67% grub mortality in *A. bengalensis* and *Sophrops* sp., respectively. The commercial strain Green Meta recorded 6.67% and 3.33% mortality at 5 DAT, 13.33% and 16.67% mortality at 7 DAT and 23.33% and 20% grub mortality at 15 DAT in *A. bengalensis* and *Sophrops* sp., respectively. Although, the virulence of these mycopathogens against white grubs is well documented [21, 33] but our study does not show similarity with other findings which successfully utilized *M. anisopliae* and *B. bassiana* as potential bioagents for many subterranean insect pests [12, 14]. *M. anisopliae* @ $2x10^{12}$ conidia ha^{-1} was effective against grubs of *Holotrichia consanguinea* with an average efficacy of 46.74% under field conditions [14]. The virulence of an indigenous and a commercial strain of *M. anisopliae* against white grub species, *Chiloloba acuta* under laboratory conditions were evaluated in Nepal and reported 89% to 97.8% grub mortalities at different concentrations ranging from $3.33×10^4$ to $1.04×10^8$ [12]. The relatively low levels of the mortality observed in treatment with *B. cereus* strain WGPSB-2, *M. anisopliae* and *B. bassiana* can be attributed to a number of causes including species specificity of entomopathogens, insufficient dose and insufficient time for mortality to become apparent.

The efficacy of *H. indica* (commercial and native strain) against first instar larvae of two predominant and pestiferous species, *A. bengalensis* and *Sophrops* sp. were tested as the interspecific variation in nematodes pathogenicity against the white grub species have been reported *i.e.,* single nematode species with different strains can also vary considerably in their pathogenicity against a given pest species as reported for *H. bacteriophora* in case of *Popillia japonica* and *Cyclocephala borealis* [8]. Both commercial as well as native strain of *H. indica,* has been found to have very high virulence and efficacy with mortality up to 83.33% against the grubs of *A. bengalensis* and *Sophrops* sp. So, to estimate the median lethal dose and median lethal time required to cause grub mortality, the first instar grubs were exposed to different dosages of IJs of *H. indica* (commercial and native strain).

The results obtained from the above study revealed the LD_{50} values 1230.27 IJs/ml at 72 hours after treatment with commercial strain and 891.25 IJs/ml at 96 hours with native strain of *H. indica* against the grubs of *A. bengalensis.* While, against grubs of *Sophrops* sp. LD_{50} values of 1023.29 IJs/ml at 72 hours with commercial strain and 954.99 IJs/ml at 72 hours with native strain of *H. indica* was recorded. The minimum LD_{50} value of 457.09 IJs/ml and 616.60 IJs/ml were obtained against the grubs of *A. bengalensis* and 295.12 IJs/ml, 338.84 IJs/ml against the grubs of *Sophrops* sp. with commercial and native strain of *H. indica*, respectively at 7 DAT**.** The maximum grub mortality in both tested species was recorded at inoculum levels ranging from 500-1000 IJs/ml at 7 DAT which were dependent on period of exposure. Thus, the grub mortality increased with increase in number of IJs of EPN and exposure time. Similar results were also reported by [10], [11], [16].

Moreover, the LT_{50} values ranged from 208.93 hours (8.71 days) at 250 IJs/ml to 70.79 hours (2.95 days) at 1000 IJs/ml after treatment with commercial strain and from 223.87 hours (9.33 days) at 250 IJs/ml to 91.20 hours (3.80 days) at 1000 IJs/ml with native strain of *H. indica* against the grubs of *A. bengalensis.* While, against grubs of *Sophrops* sp. the LT_{50} values ranged from 144.54 hours (6.02 days) at 250 IJs/ml to 74.13 hours (3.09 days) at 1000 IJs/ml for commercial strain and from 154.88 hours (6.45 days) at 250 IJs/ml to 77.62 hours (3.23 days) at 1000 IJs/ml after treatment with native strain of *H. indica*. The median lethal time was dose-dependent. The details of the probit analysis along with LD_{50} and LD_{90} are mentioned in table 2 while LT_{50} and LT_{90} are mentioned in table 3 along with their respective linear equations.

H. indica recorded least LD_{50} values of 44.15 IJs/ml, 97.47 IJs/ml and 150.12 IJs/ml for first, second and third instar grubs of *Phyllognathus dionysius*, respectively under laboratory conditions in Maharashtra [20]. *H. indica* was found to be most effective against *H. serrata* and recorded LD_{50} value of 80.25 IJs/ml, 141.83 IJs/ml and 300.17 IJs/ml for first, second and third instar grubs, respectively for *H. serrata* at 5 DAT under laboratory conditions [27]. In pot culture experiment, *H. indica* at a concentration of 450 IJs/ml recorded mortality of 87.60% at 15 DAT against third instar grubs of *Leucopholis lepidophora* [2]. The bioefficacy of *H. indica* against third instar grubs *H. consanguinea* recorded 30.72% grub mortality at 3 DAT and significantly highest mortality (56.43%) was observed at 4 DAT under controlled laboratory conditions [16]. The grub mortality of 83.33% and 71.66% with *Steinernema glaseri* and *H. indica*, respectively @ 5×10^9 IJs/ha against white grub *Anomala communis* in lab and pot culture [25]. The alone application of native EPN strains of *Steinernema carpocapsae* caused 74.3% and 79.1% mortality and commercial formulation of *H. indica* caused 54.8% and 51.7% reduction in grub population of *L. lepidophora* [7].

In conclusion, *A. bengalensis* and *Sophrops* sp. population of the Indian Himalayas are highly susceptible to *H. indica* (both commercial and native strain). So, they can be used and recommended to farmers as an environmentally safe and IPM-compatible alternative to chemical insecticides for the management of white grub species. But, the susceptibility of grubs to EPNs differs with the species. So, further species-specific studies are needed to understand the bio-efficacy of EPNs against white grub species. In addition to this, the combination of novel insecticides with various entomopathogens against various white grub species needs to be investigated as biological–chemical synergisms are one of the important tactics that must be exploited for reduced risk. White grub management through the strategic combination of bio-control agents with reduced rates of synthetic insecticide may represent a valuable control tactic for the suppression of the white grub population in the NW Himalayan region.

Table 1: Details of entomopathogens used for bioassay studies

Entomopathogen used	Scientific name	Strain	Formulation used	Source
Bacteria	*Bacillus cereus* WGPSB- 2	Native strain	Talc	Isolated by Sushil *et al.* [28] from diseased white grub collected from Almora (29.64º N and 79.63º E, 1250 m amsl), Uttarakhand
Fungus	*Beauveria bassiana*	Commercial strain	Conidial suspension	Greenlife Biotech Laboratory, Coimbatore, Tamilnadu
	Metarhizium anisopliae	Commercial strain	Conidial suspension	Greenlife Biotech Laboratory, Coimbatore, Tamilnadu
Nematode	*Heterorhabditis indica*	Commercial strain	Infective juveniles in ADDW	Anshul Agro Chemicals, Karnataka
	Heterorhabditis indica	Native strain	Infective juveniles in ADDW	Isolated form native soil of Almora (29.64º N and 79.63º E, 1250 m amsl), Uttarakhand

Table 2: Lethal dose of entomopathogenic nematodes against first larval instars of white grubs at Experimental farm, ICAR-VPKAS, Hawalbagh, Almora, Uttarakhand.

White grub species	Entomopathogenic nematode	Linear equation (Y= ax+b)	Slope±SE	χ^2	LD_{50} (IJs)	LD_{90} (IJs)
Anomala bengalensis	*H. indica* (Commercial strain)	Y=1.95x-1.02	1.95±0.2	0.91	1230.27	5495.41
	H. indica (Native strain)	Y=2.69x-2.93	2.69±0.06	0.99	891.25	2630.27
Sophrops sp.	*H. indica* (Commercial strain)	Y=1.67x-0.02	1.67±0.11	0.96	1023.29	5888.44
	H. indica (Native strain)	Y=2.09x-1.23	2.09±0.12	0.97	954.99	3890.45

Table 3: Lethal time of entomopathogenic nematodes against first larval instars of white grubs at Experimental farm, ICAR-VPKAS, Hawalbagh, Almora, Uttarakhand.

White grub species	Entomopathogenic nematode	Linear equation (Y= ax+b)	Slope±SE	χ^2	LT_{50} (in hrs)	LT_{90} (in hrs)
Anomala bengalensis	*H. indica* (Commercial strain)	Y=2.71x-0.02	2.71±0.08	0.99	70.79	208.93
	H. indica (Native strain)	Y=2.54x+0.01	2.54±0.22	0.93	91.20	295.12
Sophrops sp.	*H. indica* (Commercial strain)	Y=2.64x+0.06	2.64±0.11	0.98	74.13	229.09
	H. indica (Native strain)	Y=3.26x-1.16	3.26±0.2	0.97	77.62	190.55

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