Implantation of *Heterorhabditis indica*-infected *Galleria* cadavers in the soil for biocontrol of white grub infestation in sugarcane fields of western Uttar Pradesh, India

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Over the past 10 years, farmers of Uttaranchal and western Uttar Pradesh, India have been fighting a losing battle against the white grub (Coleoptera: Scarabaeidae) infestation of sugarcane crop. Pesticides have failed to address the problem as evident from the observed infestation levels of 10–24 grubs/m^2. During 2008–2014, the Division of Nematology, Indian Agricultural Research Institute, New Delhi launched a biocontrol project involving treatment of the white grub-afflicted fields with entomopathogenic nematodes (EPNs)-infected Galleria mellonella cadavers. This initiative, spread over the districts of Ghaziabad, Meerut, Amroha, Saharanpur, Gajraula, Bulandshahar and Hapur, was undertaken in collaboration with a non-governmental organization – the Foundation for Resources Management and Environmental Remediation – and local sugar mills, and by enlisting the active participation of the farming community. It was perceived that this technology had a greater possibility of evolving into a long-term, sustainable biocontrol strategy if the EPN-infected Galleria were sourced in each village. Capacity-building programmes were undertaken in the villages with special focus on empowering women, and small and marginalized farmers by educating them on rearing and infecting Galleria. This initiative has resulted in an average reduction of 69.1% in the white grub population and an average increase of 60.49 q/acre in sugarcane yield over untreated control.

Keywords: Biocontrol strategy, *Galleria mellonella* cadavers, *Heterorhabditis indica*, sugarcane, white grubs.

White grub menace

WHITE grubs, the root-feeding larvae of scarab beetles, are soil-borne pests of many agricultural and horticultural plants worldwide[1]. Since the past one decade, farmers in Uttaranchal and western Uttar Pradesh (UP) have been struggling to contain the surge in white grub infestation of sugarcane crop. The grubs feed on the roots and underground stems causing yellowing (chlorosis) of the leaves followed by stunted growth, dense browning, dislodging and eventually death of the plant in heavily infested areas. It is often unviable to harvest heavily infested fields, and at times these fields have to be replanted as ratoon regrowth and productivity is severely hampered amounting to back-breaking losses to the farmers.

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The farmers rely solely on synthetic pesticides (organophosphate and carbamate) to combat the white grub menace, even though many species are reported to have developed resistance to pesticides[2]. Also, in addition to the ecological issues involved, pesticides have limited efficacy in controlling white grubs. Effective management of the larval stages is difficult as the infestation becomes obvious only with the manifestation of the above-ground symptoms. Most white grub species spend a considerable part of their life cycle within the soil as larva. While pesticides have been observed to control the young larvae, only marginal control is seen in the older larvae due to their concealment response: they retreat vertically into the soil to avoid pesticide contact, surviving on their lipid reserves or soil humus, and resurface unharmed once the pesticidal effect wears-off.

EPN potential to control white grubs

The unique attributes of entomopathogenic nematodes (EPNs) make them an ecologically and economically

sound alternative for white grub management. EPNs are soil-dwelling parasites of insects, and they occur widely in natural and agricultural ecosystems across the world. They have been widely accepted as potential biocontrol agents of the larval stages of white grubs[3]. The genera *Heterorhabditis* (family: Heterorhabditidae Poinar 1976) and *Steinernema* (family: Steinernematidae Chitwood and Chitwood, 1937) have generated much interest as potential biocontrol agents as they carry the lethal symbiotic bacteria *Photorhabdus* and *Xenorhabdus* respectively, in their guts. These two nematode–bacteria symbionts exhibit a range of insecticidal activity against several insect pests, including white grubs, causing death within 24–48 h (refs 4–6). *Steinernema* infective juveniles (IJs) enter the host insect through the natural body openings (mouth, anus or spiracles); in addition to these openings, *Heterorhabditis* IJs also enter through the thin intersegmental membrane. IJs release the symbiotic bacteria into the haemocoel of the insect to cause lethal septicemia. These bacteria multiply exponentially by ingesting the host tissue and create a nutrient-rich environment for the nematodes to feed upon and molt. Within 5–10 days (depending upon the nematode species), the nematodes complete up to three generations in the host, after which IJs exit the cadaver to seek out new hosts. Both *Photorhabdus* and *Xenorhabdus*, along with killing the insect, produce an array of antimicrobial factors that suppress the growth of competing microbes in the insect cadaver and prevent it from putrefying[7].

Multiplication of EPNs

The first step in any biocontrol management regime is to ensure adequate availability of the relevant biocontrol agent. EPNs can be efficiently multiplied *in vivo*, on a small scale, on the larvae of the Greater Wax moth, *Galleria mellonella*. It is the conventional host for *in vivo* multiplication of EPNs due to its several attributes, not the least of which is the ease with which it can be reared in the laboratory or at home on diets of wheat/corn flour, wheat/rice bran, wax, yeast, honey and glycerol.

Aim of the study

Our aim was twofold: one, to develop an EPN technology for controlling white grub infestation in the sugarcane fields of western UP; and two, to put in place a mechanism to ensure mass and continuous supply of EPNs in order to reap sustainable benefits. The first was achieved by introducing the technology of implanting *Galleria* cadavers infected by *H. indica* into the soil. The ground was laid for the second by undertaking capacity-building programmes in villages to motivate farmers to take up the rearing and implantation of *Galleria.*

Development of EPN technology to control white grub infestation in sugarcane

The backdrop

Inundative application of EPNs for the control of white grubs was initiated in the 1980s using *Steinernema carpocapsae* and *Heterorhabditis bacteriophora.* A total of 82 field trials were conducted between 1984 and 1988 in USA with *H. bacteriophora* (@ 2.5×10^9 IJs/ha) against the Japanese beetle *Popillia japonica*, which proved to be as effective as standard chemical insecticides. However, the trials of *S. carpocapsae* for white grub control did not yield encouraging results[8]. Over the years, several species of EPNs such as *H. bacteriophora*, *H. zealandica*, *S. scarabaei*, *S. longicaudum* and *Rhabditis* spp. were reported to have successfully infected white grub species in the laboratory and in sugarcane, potato and peanut fields, but the results were variable[9–12].

The Indian initiative – laboratory bioassay

In India, during 2008, preliminary laboratory bioassays of an indigenous population of *H. indica* against different stages of mixed populations of white grubs collected from the sugarcane fields of western UP showed high virulence. The nematodes imparted 100% mortality to all stages of the grubs within 24–72 h (ref. 13). The cadavers turned from a characteristic orange to brick red in colour pointing to full-blown infection (Figure 1 *a*). The nematodes completed their life cycle in 9–10 days, and on an average 135,781.8 IJs (SD ± 27,554.56) were harvested from a fourth instar white grub, indicative of the efficient recycling ability of *H. indica* in the grubs (Figure 1 *b*).

Field trials by inundation with infective juveniles

Based upon the encouraging results of the laboratory bioassays of EPNs against white grubs, field trials were conducted from June to December 2008 in collaboration with a non-governmental organization known as The Foundation for Resources Management and Environmental Remediation (FARMER) and Modi Sugar Mill in Issapur village, Ghaziabad, UP on sugarcane fields heavily infested with white grubs. The soil of the trial sites was analysed as sandy loam (sand : silt : clay = 78 : 4 : 18; pH 8.6, electrical conductivity (EC) 0.12 ds/m). The soil was inundated by releasing *H. indica* via irrigation channels @ 2.5×10^5 IJs/m^2. Post application, a study of IJs indicated a vertical migration of 45 cm and horizontal of 30 cm, suggesting they could spread out to reach and kill the grubs. *H. indica* reduced the population of white grubs by 70% compared to 55% by standard insecticidal application (80 ml/m^2) of chlorpyriphos. A combined dose of nematode and chlorpyriphos had the synergistic effect of killing 85% of the grubs[14].

Figure 1. ***a***, Progression of *Heterorhabditis indica* infection in white grubs. ***b***, Emergence of *H. indica* infective juveniles from white grub cadavers.

Figure 2. ***a***, *Galleria* infected with *H. indica*. ***b***, Implantation of infected *Galleria* cadavers near root zone of sugarcane. ***c***, Dead grubs infected with *H. indica* recovered from cadaver-treated fields.

Table 1. Effect of *Heterorhabditis indica*-infected *Galleria* cadaver implantation on white grub infestation in sugarcane fields in seven districts of western Uttar Pradesh, UP (2010–2014)

District	Acreage	Grub incidence (m^2) ± SD	% Grub reduction over untreated control	% Yield increase (q/acre) over untreated control {probability level of significance of treated versus control}
Hapur	5	10.0 ± 3.87	64.5	64.37 {<0.0001}
Amroha	10	24.0 ± 4.21	72.1	50.33 {<0.0001}
Saharanpur	4	12.5 ± 5.58	74.3	42.08 {<0.0001}
Gajraula	5	10.6 ± 3.46	71.5	74.95 {<0.0001}
Ghaziabad	6	13.7 ± 5.55	65.9	51.96 {<0.0001}
Bulandshahar	4	11.0 ± 4.10	74.5	65.90 {<0.0001}
Meerut	6	12.8 ± 4.09	61.5	73.90 {<0.0001}
Average	5.7	17.8	69.1	60.49 {<0.0001}

{.} denotes probability level of significance for testing equality of yield between treated and control plots obtained using two independent samples (a and b) *t*-test. $a \pm b$ (*a* denotes average over 15 observations and *b* denotes standard deviation of 15 observations).

Field trials by implanting EPN-infected Galleria cadavers in the soil

The application of *H. indica* as an aqueous suspension involves the cumbersome process of collecting, concentrating and applying the nematode suspension. In addition, IJs stored as suspension suffer from certain intrinsic drawbacks: (i) post application, they undergo up to 30% mortality due to exposure to UV radiation; (ii) progressive reduction in infectivity with the use up of lipid reserves, and (iii) poor tolerance to desiccation stress[15].

In view of the above, we customized and adopted the relatively novel EPN-application technology of implanting *H. indica*-infected *Galleria* cadavers in the soil. The application of *Galleria* cadavers has shown encouraging results in the laboratory, greenhouse and field conditions[16,17]. In Brazil, this method has been used for the control of guava weevil in guava plantations[18].

This technology scores over inundation as the emergence of IJs from the insect cadaver straight into the soil ensures that they: (i) are fortified with higher levels of nutritional reserves and thus have greater ability to search for hosts; (ii) exhibit higher infectivity, and (iii) have enhanced ability to survive in an unfavourable environment[19].

Large-scale field trials were carried out between 2010 and 2014 in Amroha, Saharanpur, Gajraula, Ghaziabad, Bulandshahar, Meerut and Hapur, covering 40 acres of cultivated land. The sites were selected on the basis of the incidence of white grub, which ranged from 10 (±3.87) grubs/m^2 in Hapur to 24 (±4.21) grubs/m^2 in Amroha. Under the application regime, fourth instar *Galleria* were infected with *H. indica* IJs (@25 IJs/larva) five days prior to implantation, and the field was irrigated two days in advance to assist in the passive migration of IJs emerging from the cadavers. The white grub management schedule involved two applications of cadavers – one during the first week of June (@3000 cadavers/acre) followed by a second a month later (@2000 cadavers/acre). Cadaver treatment provided overwhelming results by consistently reducing the white grub population from 61.5% in Meerut

Table 2. Relative abundance of white grub species identified by the Division of Entomology, IARI, New Delhi in five districts of western UP

Species	Relative abundance (%)				
	Ghaziabad	Hapur	Amroha	Muzaffarnagar	Saharanpur
Holotrichia consanguinea Blanchard	23.74	15.59	10.62	21.31	65.54
Holotrichia nagpurensis Khan and Ghai	15.43	19.38	31.67	5.37	0.00
Holotrichia serrata (F.)	20.31	11.51	6.21	20.82	14.46
Lepidiota mansueta Burmeister	0.00	0.00	21.77	0.00	0.00
Maladera insanabilis Brenske	1.49	9.25	8.11	9.75	2.50
Schizonycharuficollis (F.)	2.55	8.77	1.22	5.20	1.25
Brahmina sp.	1.50	2.25	0.00	1.75	0.00
Apogonia sp.	0.85	1.50	0.25	2.15	1.10
Anomala dimidiata (Hope)	1.38	7.75	11.12	7.35	1.50
Anomala bengalensis Blanchard	10.36	8.75	2.55	12.55	3.50
Anomala varicolor Gyll.	7.30	2.15	0.75	1.50	0.75
Anomala dorsalis (F).	3.96	5.14	1.35	3.75	0.50
Anomala ruficapilla Burmeister	2.55	1.45	0.00	1.75	1.75
Adoretus flavus Arrow	1.85	1.11	1.00	1.50	0.00
Adoretus duvauceli Blanchard	1.05	2.15	0.55	2.25	1.50
Adoretus versutus Harold	1.01	0.00	0.13	1.25	1.25
Heteronychus sublaevis Fairmaire	1.15	1.50	1.20	1.75	2.20
Pentodon bengalense Arrow	2.17	1.75	1.05	0.00	1.50
Phyllognathus dionysius (F).	1.35	0.00	0.45	0.00	0.70

to 74.5% in Bulandshahar, in comparison to untreated control (Figure 2). An overall increase in sugarcane yield in the cadaver-treated fields was recorded in the 42.08–74.95 q/acre ($P < 0.0001$) range in Saharanpur and Gajraula respectively, over untreated control fields[14,20] (Table 1).

Adult beetles targeted

Along with killing the white grubs in the soil, from 2012 to 2014 adult beetles were systematically targeted by placing light (160 W mercury bulb) and anisole-loaded pheromone traps in five districts of western UP, viz. Ghaziabad, Hapur, Amroha, Muzaffarnagar and Saharanpur comprising 22 villages covering 196 ha of the sugarcane belt. This exercise was undertaken with the active collaboration of FARMER, and the leading sugar mills of the districts, viz. Simbhaoli Sugar Mills, Hapur; Triveni Engineering and Industries Ltd, Chandanpur Unit in Amroha and Devband Unit in Saharanpur; Modi Sugar Mills in Ghaziabad and Cooperative Sugar Mills, Rohana Unit, Muzaffarnagar, UP. Four lakh adult beetles were trapped and killed. A study of these by the Division of Entomology, Indian Agricultural Research Institute (IARI), New Delhi revealed the presence of 19 species of white grubs lending formidable dimensions to the task of managing the infestation (Table 2).

Production of EPN-infected *Galleria* in villages

As mentioned earlier, a part of our objective was to establish a system for the steady and cheap supply of EPN-infected *Galleria* to the farmers for treating infested fields. The ease and economics (a fourth instar is produced at 50 paise and applied @ 3000 cadavers/acre; hence, it costs Rs 1500 to treat a 1 acre field) of rearing *Galleria* on commonly available ingredients and subsequently implanting it in the fields prompted us to undertake extensive capacity-building programmes in the villages, wherein farmers were trained to rear and implant the infected cadavers.

Steps to control white grub infestation of sugarcane

With a little training, the EPN-infected *Galleria* implantation technology can be easily adopted by the farmers as it comprises three simple steps:

1. Rearing *Galleria*: *Galleria* are reared in plastic boxes or wooden trays on a diet of wheat/corn flour, wheat/rice bran, wax, yeast, honey and glycerol. The seed *Galleria* are provided gratis by the Division of Nematology, IARI, New Delhi and FARMER at Ghaziabad, UP.
2. Infecting *Galleria* with EPN culture: *Galleria* are infected with fresh cultures of EPN provided gratis by the Division of Nematology, IARI, New Delhi and FARMER at Ghaziabad, UP. The infection process involves the release of *Galleria* onto large plastic trays moistened with the nematode suspension. *Galleria* die within 24 h and are ready for release in the fields after four days.
3. Implantation of EPN-infected *Galleria*: Implantation is done @ 3000/acre around sugarcane clumps during June. In cases of acute infestation, reapplication after a month is recommended.

Educative and motivational training programmes

The Division of Nematology and Division of Agricultural Extension, IARI, New Delhi; FARMER, leading sugar

mills of western UP; Krishi Vigyan Kendra (KVK), Ghaziabad, UP, State Department of Agriculture, UP; and Cane Department, UP joined hands in conducting 15 training programmes for farmers, during 2011–2014, on home-rearing and implanting *Galleria*. Farmers were motivated to create self-help groups, and women and small and marginalized farmers were sought to be empowered by encouraging them to adopt *Galleria* rearing as a livelihood-generation scheme.

Success through partnership

The success of this project can be traced to the committed efforts of all stakeholders.

While the Division of Nematology, IARI, New Delhi was the scientific and organizational hub, the wheel could be set in motion only through the active participation of FARMER, local sugar mills and the farming community.

FARMER was associated with the on-ground validation and implementation of the biocontrol project. The Division of Nematology, IARI, New Delhi trained FARMER in the *Galleria* cadaver infection and implantation technology. It provided seed *Galleria* and EPN cultures to FARMER to enable it to set-up a small unit for the production of *Galleria* for gratis supply to farmers.

The support of local sugar mills of western UP was the very backbone of this project. Their day-to-day interactions with farmers, knowledge of the local maladies and remedies of the sugarcane crop, and familiarity with the socio-economic profile of the region proved invaluable in implementing the biocontrol technology and in carrying out capacity-building programmes. R&D departments of the various mills were actively involved in collecting and collating yield information.

The enthusiasm of the farmers and active participation of both men and women in the training programmes energized this project over its long span. The success stories shared with us encourage us to set up new milestones on the path to biocontrol by EPNs.

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