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The potential of entomopathogens in biological control of white grubs

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ABSTRACT

White grubs are highly polyphagous and most destructive soil pests inflicting damage to a wide variety of crops. In India, more than 1000 species of white grubs are known of which over 40 species attack wide range of plants. White grubs are naturally infected by various entomopathogens which include fungi, bacteria and nematodes. Entomopathogenic fungi offer great potential and members of genera *Beauveria* and *Metarhizium* are widely used against white grubs. Several commercial products of entomopathogenic fungi like Bio Green, ORY-X, Grub X 10G, Betel, Biotrol FMA and Meta-Guard have been developed for the control of white grubs. In India, good control of white grubs in paddy, ginger and sugarcane has been achieved with different entomofungi. Among EPNs, *Heterorhabditis bacteriophora* is moderately effective against *Popillia japonica* and *Rhizotrogus majalis*. *H. indica* and *H. bacteriophora* are effective against potato white grubs in India. *Paenibacillus popilliae* cause milky disease in *P. japonica* grubs. The bacterium is pathogenic to *Holotrichia consanguinea*, *H. serrata* and *Leucopholis lepidophora*. In north-western Himalaya, *B. cereus* is highly toxic to the grubs of *H. seticollis* and *Anomala dimidiata*.

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1. Introduction

The superfamily Scarabaeoidea contains a large number of species whose larvae live in the soil and are commonly known as white grubs. As many species of white grubs are root feeders, they are also known as rootgrubs, but not all white grubs are rootgrubs (Gardner 1935).The white grubs are found chiefly in grasslands feeding on roots of various plants. Others grow on decaying organic matter (Misra and Chandel 2003). They live concealed and sudden increase in their population takes up in places having enough food and least soil-disturbance (Chandel and Kashyap 1997). The white grubs cause extensive damage to the roots of grasses, legumes, small fruit plants, shrubs and trees in many parts of the world (Pathania 2014). Larvae of greatest economic importance belong mainly to the tribe Melolonthini (Ritcher 1958). The first known white grub damage to crops in India is that by Stebbing (1902) from Punjab. More than 1000 species of white grubs are known from the Indian sub-continent of which over 40 species attack a wide range of crop plants (Veeresh et al. 1991). First major epidemic of white grubs in India was reported in sugarcane from Bihar during 1956 (Gupta and Avasthy 1956). They have now been included in the category of five national pests (Misra and Chandla 1989), and are reported from every state causing damage to a wide variety of cultivated crops. Yadava and Sharma (1995) reported that *Holotrichia serrata* Fab., *Holotrichia consanguinea* Blanch., *Holotrichia longipennis* Blanch., *Brahmina coriacea* (Hope), *Holotrichia seticollis* Moser, *Anomala dimidiata* (Hope), *Holotrichia reynaudi* Blanch., *Leucopholis lepidophora* Blanch., *Leucopholis coneophora* Brum., *Melolontha* spp and *Lepidiota* spp are the key pest species that attack different plants in different regions of the country.

The current method for the control of white grubs is through the use of chemical pesticides. However, concern about safety, environmental contamination and poor efficacy of recommended insecticides has increased the need to develop IPM approaches for these pests (Kumawat 2001). The naturally occurring entomopathogens (fungi, nematodes, bacteria and viruses) are important components in the soil ecosystem which brings about effective suppression of soil pests in nature. We provide an overview of microbial control of white grubs in different crops with special reference to fungi, nematodes and bacteria and their future prospects in white grub management.

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2. Nature of injury and economic importance of white grubs

All white grubs are polyphagous and they can feed on any root or underground stem (Veeresh 1988). The first stage larvae feed in part on organic matter in the soil, while the second and third instar grubs feed largely on roots or underground stems. The economic importance of chafers is primarily due to feeding activity of the third instar grubs (Chandel et al. 2015). Grubs prefer to feed on fibrous roots for normal growth and the crops with a tap root system suffer more as compared to those with an adventitious root system (Yadava and Vijayvergia 2000). In general, the underground parts of all plants are susceptible to grub feeding. The symptom of the damage is root pruning by grubs thereby showing varying degrees of wilting, yellowing and browning, followed by death of the plant. In crops such as potato and ginger, large holes are inflicted on the tubers or rhizomes which are then rendered unfit for marketing. Mehta et al. (2010) reported that almost all field crops grown during the rainy season, *viz.* potato, vegetables, groundnut, sugarcane, maize, pearl millet, sorghum, cowpea, pigeon pea, green grass, cluster bean, soybean, *rajmash* (kidney beans), upland rice, ginger *etc* are damaged by white grubs.

In India, the grubs of *H. consanguinea* are of major economic importance attacking groundnut and sugarcane in Rajasthan, Bihar, Gujarat, Uttar Pradesh, Haryana and Punjab. In endemic areas, the damage to groundnut ranges from 20% to 100%. The larvae of *H. serrata* are destructive to the roots of vegetables, pulses, oilseeds, cereals, millets, tobacco, sugarcane and sorghum in Karnataka, Maharashtra, Andhra Pradesh, Tamil Nadu and Kerala. In peninsular India, *Leucopholis* larvae injure the roots of coconut palms and also attack cassava, sweet potatoes, yams and colocasia (Nirula et al. 1952). In north western Himalaya, grubs of *B. coriacea* and *H. longipennis* cause wide spread damage to potato and several other crops. Chandel and Chandla (2003) reported that tuber damage in potato often exceeds 50% in the endemic areas of Shimla hills of Himachal Pradesh.

3. Potential entomopathogens for control of white grubs

White grubs are naturally infected by various entomopathogens which are disease causing organisms that kill their hosts or debilitate their future generations (Singh 1991). Important entopathogens which infect white grubs include fungi, bacteria, viruses and nematodes. Entomopathogenic nematodes are not strictly microbes; however, they have been included with entomopathogens, because most of them are associated with insect-pathogenic bacteria (Dhaliwal and Koul 2007). Under certain favourable situations, they cause disease epizootics in the field. Disease is common in dense insect populations and under environmental conditions suitable to the microorganisms and entomopathogenic nematodes. However, at low host density, disease incidence is often low due to lack of contact between the pathogens and their insect hosts (Gullan and Cranston 2005). The infected insects are unable to feed properly, remain stunted, lose their body colour and become paralysed (Singh 1991). Arora et al. (2000) reported that entomopathogens exert a controlling effect on grubs by means of their invasive properties, toxins, enzymes and other substances. Entomopathogenic fungi and nematodes have received more attention than other groups of microbial organisms with potential for use in the management of white grubs. Yadava and Sharma (1995) have reported that several microorganisms such as *Paenibacillus* (=*Bacillus*) *popilliae* (Dutky), *Metarhizium anisopliae* (Metchnikoff) Sorokin, *Beauveria bassiana* (Bals.) Vuill., *B. brongniartii* (Saccardo), *Heterorhabditis bacteriophora* Poinar 1976, *Steinernema glaseri* (Steiner 1929) and *Steinernema feltiae* (Filipjev 1934) are pathogenic to white grubs and are effective in suppressing their population under field conditions. The present review highlights the major developments of last 3-4 decades along with current status as well as comments on the future possibilities.

3.1. Entomopathogenic fungi isolated from white grubs

Entomopathogenic fungi offer great potential in controlling insect-pests, as they have a wide host range, infect at different ages and stages of their hosts and cause spectacular epizootics (Zimmerman 1993). There are about 750 species of entomopathogenic fungi, although only a few dozen naturally infect insect pests (Figure 1) of agricultural importance (Gupta 2004). Fungi are also particularly important for the control of Coleoptera, as viral and bacterial diseases are rare among them (Hajek and St Leger 1994). Members of the genera *Beauveria, Metarhizium, Entomophthora, Verticillium* and *Paecilomyces* have received maximum attention and are widely used against white grubs. *M. anisopliae* has profuse, irregularly branched conidiophores and cylindrical to clavate phialides, tapering abruptly towards the apex (Gupta 2001). In genus *Beauveria,* the conidiogenus cells are swollen, ampulliform or nearly spherical. They form a narrow conidiogenus rachis which elongates sympodially and conidia

range from 2 to 3 × 2.0–2.5 µm in length. In *B. brongniartii*, the conidiogenus rachi are shorter and the ellipsoid conidia are 2–3 × 1.5–2.5 µm long, whereas the conidia are globose to sub globose (2–3 × 2.0–2.5 µm) with conidiogenous structures forming dense clusters in *B. bassiana* (Samson 1981). In *Verticillium*, phialides in whorls are borne on branched verticillate conidiophores. The phialides are typically awl-shaped and form many conidia in heads (Ramarethinam et al. 2005). The primary conidiophores of *Entomophthora* are simple or branched and have zygospores with a hyaline to coloured to very dark outer wall layer. These are never formed within the layers of two gametangia (King and Humber 1981). Bose and Mehta (1953) isolated *Pandora* (=*Entomophthora) brahminae* from adult beetles of *Brahmina* sp and *Anomala rufiventris* Kollar and Redtenbacher in India.

M. anisopliae and *B. bassiana* are perhaps the most heavily researched EPFs because of their high host specificity, non-persistence and non-toxicity to the environment, unique mode of action and an appreciable shelf life (McCoy et al. 1988). Fungal spores that contact and adhere to an insect, germinate and send out hyphae. These penetrate the cuticle, invade the haemocoel and cause death either rapidly, owing to release of toxins, or more slowly, owing to massive hyphal proliferation that disrupts insect body functions (Gullan and Cranston 2005). The genus *Metarhizium* is pathogenic to a large number of insect species, many of whom are agricultural and forest insects (Ferron 1978). *M. anisopliae* was first described in Ukraine from infected larvae of wheat cockchafer *Anisopliae austriaca* in 1879 (Tulloch 1976). *Metarhizium* causes a disease known as 'green muscardine' in insect hosts because of the green colour of its conidial cells (Figures 2 and 3). *B. bassiana* is a fungus that grows naturally in soil and acts as a pathogen on various insect species and causes 'white muscardine' disease in white grubs. The *B. bassiana* or *B. brongniartii* usually appear as loose white cottony growth, at times almost completely enveloping the insect (Figures 4 and 5). Currently *M. anisopliae* consists of four genetic groups (Driver et al. 2000).

Rao and Vijaylakshmi (1959) observed *M. anisopliae* and *B. bassiana* killing large populations of *H. serrata* in south India. Avasthy (1967) reported good control of white grubs in India by *M. anisopliae*. Epizootics of *M. anisopliae* and *B. bassiana* have been reported against several scarab species

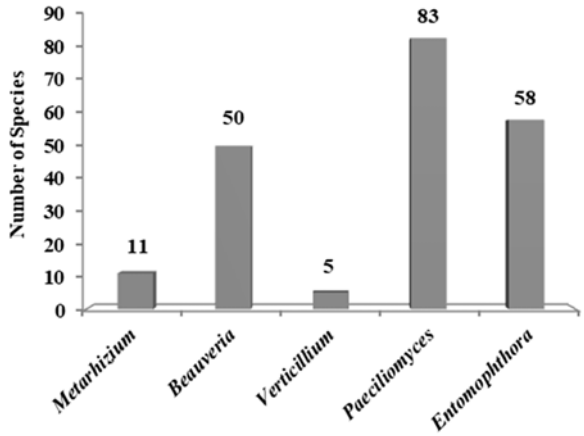

Figure 1. Species diversity in leading genera of entomopathogenic fungi.

Figure 2. *Leucopholis lepidophora* grub infected with *M. anisopliae*.

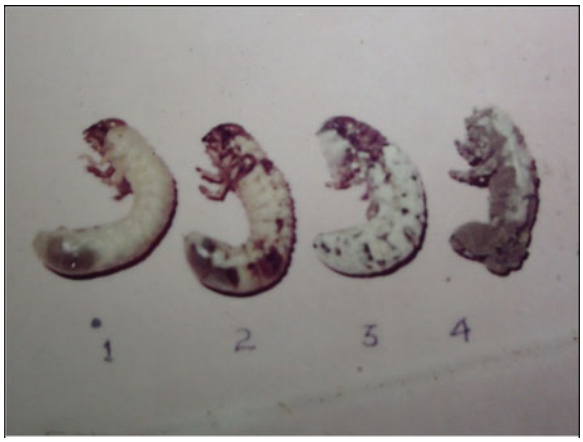

Figure 3. Healthy (1) and *M. anisopliae* infected (2-4) grubs of *Holotrichia consanguinea*.

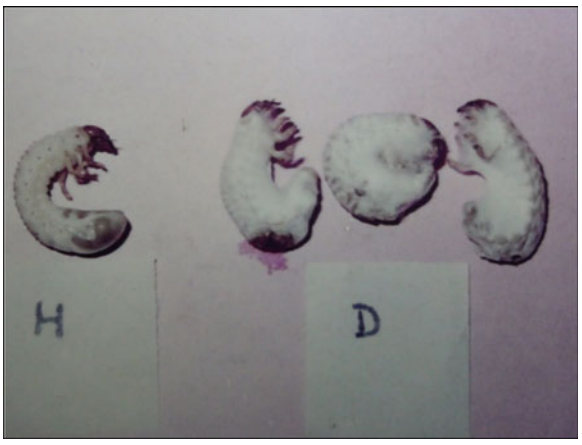

Figure 4. Healthy (H) and *Beauveria bassiana* infected grubs (D) of *H. consanguinea*.

and other soil inhabiting Coleoptera (Fleming 1968; Hurpin and Robert 1977; Young 1974).

Ranganthiah et al. (1973) collected fungus-infected grubs of *H. serrata* from an Areca garden in Mysore and isolated *B. brongniartii* from these grubs. *Oryctes rhinoceros* is a major pest of palm in many Pacific islands and south-east Asian countries. *M. anisopliae* has been used successfully to control *O. rhinoceros* for several years (Gupta 2001).

In Himachal Pradesh, Singh (1978) found about 20% infestation of *M. anisopliae* in potato white grubs. Jongelleen et al. (1979) isolated a number of entomopathogenic fungi from *L. stigma* and *L. rorida* in Indonesia and reported that *M. anisopliae* was the most commonly encountered insect pathogenic fungus. Veeresh (1977) found strong cases of fungal infections in *H. serrata* grubs from coffee estates in Karnataka. Padmanaban et al. (2003) conducted a survey in Karnataka and Kerala, and isolated *Aspergillus flavus*, *Metarhizium* sp and *Fusarium* sp from field collected grubs of *L. brumeisteri*. Yubak Dhoj et al. (2004) conducted exploratory studies for EPFs in Nepal and reported that *M. anisopliae* was associated with white grubs to the tune of about 2% in fields with arable soil. *B. bassiana* was also isolated from a few soil samples.

Milner et al. (1992) reported an unidentified *Hirsutella* sp on scarabs in Burma. In India, *V. lecanii* was isolated from soil which caused 48%–53% grub mortality in *H. consanguinea* after application to soil (Gour and Dabi 1988). Brown and Smith (1957) reported infection of *Paecilomyces fumosoroseus* from *M. melolontha* under synonym '*Spicaria fumoso-rosea*'. Vanderberg et al. (1998) found a strain of *P. farinosus* highly pathogenic to larvae of *Costelytra zealandica* in New Zealand. *Entoderma colletosporium* has been isolated from larvae of *Popillia japonica* by Hanula et al. (1991). In Himachal Pradesh, Chandel (2000) isolated *Entomophthora* sp from full-fed grubs of *B. coriacea* collected from Shimla region. Sharma et al. (2012) isolated 16 species belonging to nine genera from cadavers of white grubs and adult beetles in Himachal Pradesh. Most abundantly occurring fungi associated with beetles/grubs were *M. anisopliae*, *Aspergillus flavus*, *Fusarium oxysporum*, *B. bassiana*, *Aspergillus clavatus*, *Fusarium solani* and *Rhizopus oryzae*. Kalia (2013) isolated *B. brongniartii* from grubs of *B. coriacea*. On the basis of ISSR markers, wide variability exists among isolates of *B. brongniartii* present in Himachal Pradesh. Shillaroo strain has been reported to be highly virulent in nature against potato white grubs.

Figure 5. *Brahmina coriacea* grub infected with *B. brongniartii*.

3.1.1. Use of entomopathogenic fungi for the control of white grubs

Most research on entomopathogenic fungi has been aimed at developing them as inundative biological control agents of white grubs. Most of the commercially produced fungi are species of either *Beauveria* or *Metarhizium* and both are relatively easy to mass produce. Production requirements include reasonable cost, long-term stability, and, most importantly, consistent efficacy under field conditions. The development of a suitable formulation is mandatory in order to enhance spore application and successful utilization in soil. Some important formulations tested against white grubs are listed in Table 1.

Table 1. Commercial products of entomofungi being developed specially for control of white grubs.

Fungi	Product	Target pests	Producer/company
M. anisopliae	Bio Green	Redheaded cockchafer (*Adoryphorus couloni*)	Australia Bio-care Technology
M. anisopliae	ORY-X	*O. rhinoceros*	Malaysian Palm Oil Board, Malaysia
M. flavoviridae	Bio Green	Red headed cockchafer	Australia
B. brongniartii	Engerlingspilz	White grubs	Andermatt, Switzerland
	Schweizer	White grubs	EricSchweizer, Switzerland
	Melocont	White grubs	Kwizda, Austria
	Betel	White grubs	NPP (Valioppe, France)
B. bassiana	Daman	*B. coriacea*	Pancea Biotee, India
M. anisopliae	Grub x 10 G	*B. coriacea*	Pest Control India Ltd., India
B. bassiana	Commercial formulation	White grubs in golf course	Multiplex Agro Technology, Bangalore, India
B. bassiana	ABG 6178	White grubs	Abbott laboratory, USA
B. bassiana	Betel	Scarab beetle	Reunion
M. anisopliae	Biotrol FMA	Scarab larvae	Nutrilite Products, Inc., USA
M. anisopliae	Meta- Gaurd	White grubs and termites	Ajay Biotech (India) Ltd. Pune, India.

3.1.2. Field utilization of entomopathogenic fungi against white grubs

Keller (1983) used soil application of *B. brongniartii* against *M. melolontha* by spraying blastospores on adults in Europe. He observed a significant decrease in pest population during the second generation. In Australia, sub-surface application of rice fungus granules of *M. anisopliae* for control of red-headed cockchafer, *Adoryphorus couloni* in pasture and turf, resulted in 60% mortality of third instar grubs (Rath 1992). Li et al. (1992) tested eight varieties of *B. brongniartii* and *B. bassiana* against the grubs of *Blitopertha pallidipennis* on *Larix* (L) seedlings in China. The mortality of larvae infested by AB, LB and YB varieties ranged from 40.3% to 83.3%. The mortality of larvae infected by *M. anisopliae* introduced from Germany was 53.8%.

Cravanzola et al. (1996) found 21.1% grubs of *M. melolontha* to be infected with *B. brongniartii* (58% of them with 1×10^3 CFU/g dry soil and 13% with more than 1×10^4 CFU/g soil) in north-western Italy, with a mean larval density of 9.7grubs/m^2. They did not find any relationship between infestation level of larvae and diffusion of fungus in soil. Keller et al. (1997) conducted large-scale field trials using blastospores of *B. brongniartii* against *M. melolontha* and found reduction in average reproduction rate from 5.09 to 2.19 larvae or adults. In Uttrakhand, good control of *H. longipennis* was achieved with *M. anisopliae* in upland paddy (Gupta 2001). In Sikkim, *M. anisopliae* was applied against grubs of *H. seticollis* attacking ginger. There was 23.5% increase in yield of ginger rhizomes after this treatment (Anonymous 2000).

Chandel and Mehta (2005) tested a Jaipur culture of *B. bassiana* and *M. anisopliae* @ 5×10^{13} conidia/ha against potato white grubs (*B. coriacea*) in Shimla hills, but neither of these fungi were found to be effective. Similarly, Chandel et al. (2005) did not find satisfactory control of *B. coriacea* grubs in potato in Shimla hills with the application of *B. bassiana* and *M. anisopliae* dusts. Contrary to this, *B. bassiana, B. brongniartii* and *M. anisopliae* have been reported to be highly effective in Kashmir against white grubs @ 1×10^8 spores/ml (Mohi-ud din et al. 2006). These cultures produced 100% mortality of grubs after 20–24 days of treatment. Lozano-Gutierrez and Espana-Luna (2008) investigated the virulence of two strains *viz.* BbZ3 and BbZ4 of *B. bassiana* by introducing infected cadavers of *Galleria mel/onella* through the orifices on the stem pads of nopal plant. Both these strains caused 100% mortality in the larvae of *Laniifera cyclades*. Kulye and Pokharkar (2009) studied the efficacy of *M. anisopliae* and *B. bassiana* against *H. consanguinea* infesting potato. Use of *M. anisopliae* @ 2×10^{12} conidia/ha showed average efficacy of 46.74%, with 44.44% mycosis of grubs. The differences in field efficacy of entomopathogenic fungi may be due to variable environmental factors which affect pathogenicity as well as mode of virulence of entomopathogenic fungi. Soil temperature is a major factor which affects the success or failure in the establishment and production of fungal inoculums. Ouedraogo et al. (2004) has demonstrated that stress temperature alters the vegetative growth among isolates of entomopathogenic fungi. Many fungi are also sensitive to pesticides, especially fungicides. In crops like potato, there is heavy use of fungicides having a broad spectrum of activity which adversely affects the efficacy of entomopathogenic fungi, resulting in poor control of white grubs in potato in north western Himalaya. Gupta (2001) reported that *B. bassiana* has a lower temperature profile as compared with *M. anisopliae* and that the combined use of the two ensures better efficacy over a wide range of temperature.

Prasad and Hussain (2011) reported that grubs of *H. serrata, H. consanguinea, H. froges* and *Autoserica nathani* were highly susceptible to *B. brongniartii.* Its application in groundnut fields @ 10.0×10^{14} conidia/ha resulted in 41.5 and 45.5% disease in *H. consanguinea* and *H. serrata* grubs, respectively. Ghosh et al. (2009) observed that two applications of *M. anisopliae* (GRUB X 10% GR) @ 12 kg/ha increased the yield of sugarcane (70t/ha) over its single application @ 8 kg/ha. Srikanth et al. (2010) evaluated *B. brongniartii* against *H. serrata* in sugarcane @ 1×10^{14} and 1×10^{15} spores/ha, applied through fungus-colonized sorghum grains in furrows. Third instar grubs collected from the root zone about a month later, showed equal infection levels at both doses. Application of *M. anisopliae* in sugarcane @ 4×10^9 conidia/ha registered 92% reduction in grub population of *H. serrata* in Tamil Nadu on 60th DAT. The incremental benefit-cost ratio (IBCR) was high with *M. anisopliae* (7.58) as compared with chlorpyriphos (6.09) as observed by Manisegaran et al. (2011). In Assam, Bhattacharya and Pujari (2014) evaluated *B. brongniartii* and *M. anisopliae* alone and in combination with insecticides against white grubs in green gram. Both *B. brongniartii* and *M. anisopliae* in combination with imidacloprid 200 SL were effective in reducing plant mortality caused by white grubs resulting in a significant increase in grain yield.

3.2. Biocontrol potential of entomopathogenic nematodes in controlling white grubs

The nematodes belonging to the families Steinernematidae and Heterorhabditidae are

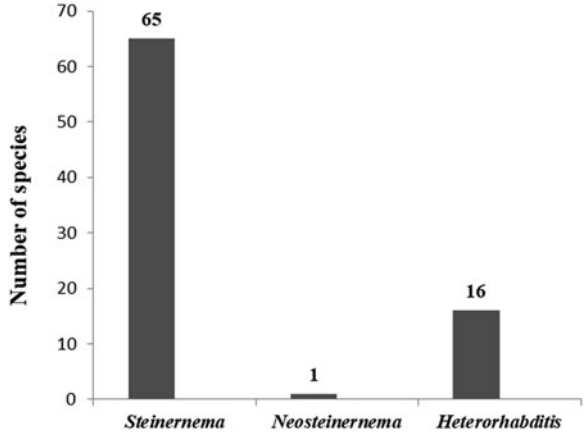

Figure 6. Species diversity in predominant genera of entomopathogenic nematodes.

commonly termed as entomopathogenic nematodes (Ganguly et al. 2011). A highly desirable attribute of entomopathogenic nematodes in control programmes is rapid host mortality which prevents the degree of insect damage to crops (Kaya 1985). They have host-searching ability and are highly virulent, have a high reproductive potential and have the potential to recycle themselves in environment. There are two genera under Steinernematidae - *Steinernema* with 65 species and *Neosteinernema* with single species. In Heterorhabditidae, there is only one genus - *Heterorhabditis* with 16 identified species as shown in Figure 6 (Kepenckci 2014).

These nematodes have symbiotic relationships with bacteria that are species specific. The *Xenorhabdus* spp are associated with *Steinernema*, while *Photorhabdus* spp are associated with *Heterorhabditis*. On locating a host insect, they enter through the natural openings (Steinernematids), and additionally by rupturing the insect cuticle (*Heterorhabditis*) to finally reach the haemocoel as their ultimate destination (Gaur and Mohan 2005). Insects infected by EPNs may often be recognized by their appearance. Infected insects are often flaccid, and change colour to orange, yellow or brown (Steinernematids, Figure 7) or a brownish-red to brick red that shows faint luminescence in the dark (*Heterorhabditis*, Figure 8). Internal tissues are disintegrated to a mass of gummy consistency. The EPNs have a potential in inundative and inoculative releases and have insignificant effects on non-target organisms (Bathon 1996). They are mobile in soil and can persist for years. Chandel et al. (2009) reported that *Heterorhabditis* are encountered frequently in sandy loam soil that has high organic matter content. There are several reports where natural infection of nematodes has been recorded in white grubs as summarized in Table 2.

3.2.1. Field application of entomopthogenic nematodes in biological control of white grubs

Entomopathogenic nematodes (EPNs) have been applied successfully against soil-inhabiting insects (soil application) as well as above-ground insects (foliar spray) in cryptic habitats (Arthers et al. 2004). Howerver, EPNs are better able in controlling soil pests as compared with foliage-feeding insects (Sharma et al. 2011). The major reason for lack of success of foliar application is the intolerance of juveniles to extremes of desiccation (Lello et al. 1996), temperature (Grewal et al. 1994) and ultraviolet radiations (Gaugler et al. 1992). In India, the EPNs were first used when DD-136 (exotic strain of *S. carpocapsae*) was employed against insect pests of rice, sugarcane and apple in 1966 (Hasan et al. 2009).

The first attempt to control white grubs with nematodes was made in the 1930's by using *S. glaseri* against *P. japonica*. Initial results were encouraging with high beetle mortality (Glaser 1932). Subsequent applications were not as encouraging against this insect (Glaser and Farrell 1935; Glaser et al. 1940) or on grass grubs (Hoy 1955). Later, Kain et al. (1982) conducted a trial using nematodes cultured with associated bacteria and found 66% reduction in the population of grass grubs. Kaya and Gaugler (1993) reported that failure of EPNs against *P. japonica* results from the use of unsuitable nematode species or strains. The instances of poor field efficacy despite excellent laboratory results may often be due to use of a nematode poorly adapted to the target insect. Potter and Held (2002) reported that grubs of Japanese beetle possess defense mechanisms against entomopathogenic nematodes. These include grooming with legs, mouth parts and raster when nematodes are present on the cuticle (Wang et al. 1995). Mannian et al. (2001) evaluated *H. bacteriophora* and *H. marelatus* and observed poor to moderate control of *P. japonica* @ 5×10^9 IJs/ha. Wright et al. (1988) investigated the use of various nematode species applied to potted Japanese yew a few days after inoculation with *P. japonica* and *Rhizotrogus majalis*. Control of *P. japonica* grubs with *H. heliothidis* ranged from 60–90% and 0–58% with *S. glaseri*. However, against *R. majalis*, the control with two nematode species ranged between 0 and 86%. Mannian et al. (2001) and Neilson and Cowles (1998) reported poor results with *H. bacteriophora* against *R. majalis*, *P. japonica* and *A. orientalis* in potted cotoneaster. Inability of the nematodes to persist or survive may have been the reason for the unsuccessful control. Temperature also affects the efficacy of entomopathogenic nematodes, and the grub mortality increases at higher temperatures (Wu et al. 2014). Georgis et al. (2006)

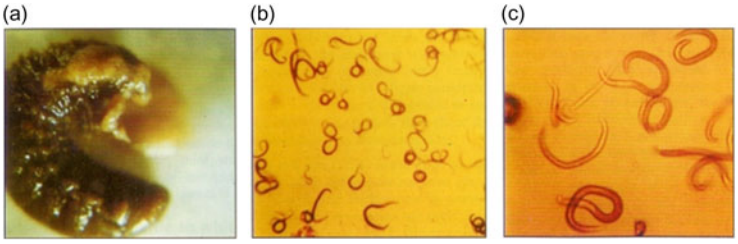

Figure 7. (a) *H. consanguinea* grub infected with *Steinernema glaseri;* (b) Infective juveniles; (c) Adults and parasitic juveniles.

Figure 8. *H. longipennis* grub infected with *H. indica*.

reported that application timing is critical for successful use of EPNs against white grubs in nurseries and greenhouses. In Germany, epizootics have been observed in grub populations infested with *Heterorhabditis* sp achieving 71% control in a sugarcane field (Akhurst et al. 1992), and 80% reduction in the population of the garden chafer, *Phyllopertha horticola* (Peters 1996). An increased infestation of EPNs in white grubs (56%), following one time release of *H. bacteriophora* was reported during second year in New Zealand (Jackson and Wouts 1987). In Germany, *H. bacteriophora* is used inundatively to control *P. horticola* grubs in turf. On many plots treated with nematodes, the grub population was below the damage threshold in following years (Ehlers and Peters 1998).

In India, EPNs have been tested against white grubs by many workers. In Tamil Nadu, Sundrababu et al. (1984) conducted preliminary tests with *S. carpocapsae* (DD-136) against potato chafer grub and found this to be pathogenic to *Anomala* sp. Poinar et al. (1992) isolated *H. indicus* from sugarcane top borer, *Scirpophaga excerptalis* and found this species pathogenic to *H. serrata.* In Himachal Pradesh, Gupta et al. (1992) reported that young grubs of *B. coriacea* are more sensitive than the older ones, and *H. bacteriophora* was more effective than *Neoaplectana bibionis*. Inoculum level of 40 dauer larvae/cm^2 produced 60 and 50% mortality in second instar larvae of *B. coriacea* with *H. bacteriophora* and *N. bibionis,* respectively. Against third instar larvae, 200 dauer larvae/cm^2 resulted in maximum kill of 54.5 and 45.5% with *H. bacteriophora* and *N. bibionis* after three weeks of treatment. When these species were tested against third instar grubs of *Maladera insanabilis*, *H. bacteriophora* recorded maximum virulence. When third instar grubs were exposed to *H. bacteriophora* infective juveniles (IJs) in soil, lower inoculation doses were required to kill the host (LD_{50} 14090 IJs/100 g soil/grub), the IJs invaded the host at a faster rate (LT_{50}: 18.38 h), host mortality occurred earlier (LT_{50} 5–65 days) and more IJs were produced per cadaver of infected host (69840/grub; 607.30 IJ/mg host body weight) as compared with other tested nematodes (Bhatnagar et al. 2004). Shinde et al. (1995) evaluated *S. glaseri*, *S. feltiae* and *Steinernema* sp (Ecomax strain) against grubs of *H. consanguinea*, *H. serrata, Anomala bengalensis, A. dimidiata*, *M. insanabilis* and *L. lepidophora*. They reported that all these species of white grubs were susceptible to the tested EPNs. Mathur et al. (1995) reported the susceptibility of all these white grub species to *H. bacteriophora* and *Heterorhabditis* sp (Ecomax strain). In *H. consanguinea*, first instar grubs were more vulnerable to the attack of *S. glaseri* as compared with second instar, third instar, pupae and adults. Bhatnagar (2001) evaluated six EPNs against *M. insanabilis* and found *H. bacteriophora* to be highly virulent with maximum susceptibility to first instar grubs as compared with other stages of this insect. In general, the eggs of white grubs are not susceptible to infection by EPNs (Bareth 2001; Bhatnagar 2001; Gupta et al. 1992). Chandel et al. (2005) reported that *B. coriacea* grubs were highly susceptible to *H. indica*. In laboratory assays, *H. indica* caused 100 and 80.46% mortality of second and third instar grubs, respectively. The applications of *H. indica* in field reduced the damage by grubs to potato tubers. Fewer number of *B. coriacea* grubs were observed in nematode-treated potato fields and white grub infestation was 8.13% as compared with 11.28% in untreated fields.

In Srinagar, the infestation of *H. longipennis* is wide spread in golf courses. Hussaini et al. (2005) applied talc-based formulations of *H. indica* PDBC EN 13.3, *H. bacteriophora*, *S. carpocapsae* PDBC EN 11 and *S. abbasi* PDBC EN 3.1 @ 5×10^9 IJs/ha in a heavily infested golf course with 40–50 grubs/m^2. *S. carpocapsae* and *S. abbasi* caused 30–40% mortality, whereas for *H. indica* and *H. bacteriophora*, it was

Table 2. Natural infection of EPNs in field populations of white grubs.

Species	Habitat	Host	Country	Reference(s)
S khoisanae, Steinernema sp, *H. bacteriophora*	Fruit orchards	White grubs	South Africa	Hatting et al. (2009)
S. feltiae, H. bacteriophora, H. downesi	Oak, deciduous forests, new plantation and fruit orchards	*M. melolontha*	Hungary	Toth (2006)
H. bacteriophora	Maize	White grubs	Mexico	Ruiz-Vega and Aquino-Bolanos (2002)
S. glaseri, H. bacteriophora	Turfgrass	*Adoretus tenuimaculatus*	Korea	Lee et al. (2002)
S. glaseri	Turfgrass	*Anomala* sp	Japan	Yamanaka et al. (1995)
S. carpocapsae	Vegetable fields	*Anomala* sp	India	Rajeswari et al. (1984)
H. megidis	–	*P. japonica*	USA	Poinar et al. (1987)
H. zealandica	–	*Heteronychus arator*	New Zealand	Poinar (1990)
S. glaseri	–	*P. japonica*	USA	Wouts et al. (1982)
S. kushidai	–	*Anomala cuprea*	Hamikita	Mamiya (1988)

20–25%, 10 days after nematode application. The overall reduction in grub population was to the tune of 55.7% with *S. carpocapsae* and 53.1% with *S. abbasi*. The grub population decreased by 42.3 and 39.6% with *H. indica* and *H. bacteriophora*, respectively. Singh and Gupta (2006) isolated *H. bacteriophora* and *S. feltiae* from fruit orchards of Himachal Pradesh and tested them against third instar grubs of *B. coriacea* and *Holotrichia* sp in sterilized soil in glass jars. There was 100% mortality in *B. coriacea* and 58.6% mortality in *Holotrichia* sp after 21 days of inoculation. The *S. feltiae* showed 59.2% mortality in *B. coriacea* grubs. In Shimla hills of Himachal Pradesh, *S. carpocapsae* and *H. indica* used @ 1, 3 and 6×10^5 IJs/m^2 were effective in reducing the population of *B. coriacea* grubs and resultant tuber damage in potato. *H. indica* reduced grub population by 66%–80%, while a 83% reduction in grub population was reported with *S. carpocapsae*. There was more than 60% reduction in tuber damage (Sharma et al. 2009).

Ganguly et al. (2011) evaluated the bio-efficacy of liquid formulations of *S. thermophilum* and *S. glaseri* against grubs of *H. consanguinea* in groundnut at Jaipur. The nematodes @ 10000 IJs were applied around the rhizosphere of groundnut plants infested with white grubs. There was 65.71 and 42.85% reduction in plant mortality with *S. thermophilum* and *S. glaseri*, respectively. Prasad et al. (2012) recorded high variability in mortality of *H. consanguinea* grubs by *H. indica* in Uttar Pradesh. They reported that white grubs have strong mandibles that crush the nematodes entering through the mouth, perform frequent defecation that prevents nematode entry through anal route, have the presence of tuft-like hairs on the spiracles that pose an obstacle in nematode entry and possess a strong immune system which encapsulates the invaders before they can kill the grub. The EPNs are compatible with chemical insecticides, fungicides, acaricides, and other entomopathogens (Ishibashi 1993; Wu et al. 2014), and therefore can be applied with other pesticides as IPM tool. Some pesticides, such as imidacloprid (Koppenhofer et al. 2000), tefluthrin (Nishimatsu and Jackson 1998) and *Bacillus thuringiensis* (Koppenhofer and Kaya 1997) are synergistic with EPNs. *P. popilliae* acts as a stressor and boosts susceptibility of white grubs to nematode infection (Thurston et al. 1994). *S. glaseri* and *H. bacteriophora* interact synergistically with imidacloprid against *P. japonica* grubs (Koppenhofer et al. 2000). There is a reduction in grooming and evasive behaviour in response to nematode attack in treated grubs. The sluggishness of imidacloprid-treated white grubs facilitates host attachment and subsequent penetration of infective juvenile nematodes. Wu et al. (2014) have observed additive or synergistic interactions between *Heterorhabditis megidis* and *B. bassiana*, and between *H. bacteriophora* and *M. anisopliae* or *B. bassiana* against third instar of *Cyclocephala lurida*. The combination of nematodes and fungi may achieve an effect comparable or superior to insecticides for curative control of white grubs.

3.3. Biological control potential of entomopathogenic bacteria against white grubs

Using entomopathogenic bacteria as biological control agent offers considerable potential. Only a few strains of bacteria, such as *Paenibacillus* (=*Bacillus*) *popilliae* and *Serratia* spp have been tested and used against white grubs (Bourner et al. 1996). *P. popilliae* and *P. lentimorbus* are the causal organisms of types A and B milky disease, a lethal infection of *P. japonica* grubs and other species of white grubs. Grubs ingest spores along with soil and roots. These spores then germinate in the gut, and vegetative cells invade the haemocoel, leading to depletion of fat bodies (Sharpe and Detroy 1979). Proliferation of spores and parasporal bodies during the final stage of infection gives the haemolymph a milky white colour (Potter and Held 2002) termed as milky disease. These bacterial infections can be seen as apparent epizootics in insect populations (Kaya et al. 1993) or can weaken insects leading to their

Figure 9. Healthy and bacterial infected white grubs collected in north western Himalaya.

death either directly or indirectly (Thurston et al. 1994). *P. popilliae* has been used as a biopesticide since 1937 when Dutky artificially added diseased larvae to field plots (Dutky 1940). Klein and Kaya (1995) reported that *P. popilliae* was the first microbial agent registered in the United States in 1948. Matsuki et al. (1997) isolated a new strain of *P. popilliae* that caused natural incidence of milky disease for the first time in *P. japonica* larvae in Japan. Small-scale use of *P. popilliae* dusts did not increase milky disease incidence nor did it reduce localized grub infestations in turf (Redmond and Potter 1995). Lack of methods for *in vitro* sporulation has limited the commercial production of *P. popilliae* (Klein 1986). The sporulation of this bacterium occurs easily in insect body and the *in vivo* production technology makes the product highly expensive, thus limiting its commercialization. In USA, M/S Fairfax Biological Laboratory marketed *P. popilliae* under the trade name "Doom" for the first time during the 1980s (Mathur 2001). The application of "Doom" was recommended @ 11.0 kg/ha as talc formulation containing 10^8 spores/g to the soil surface against white grubs (Gupta 2001). *P. popilliae* was tested against different species of white grubs in India and in Gujarat, 20–25% grubs of *Holotrichia* spp showed infection of *P. popilliae* in the subsequent years following the application of Doom. The pathogenicity of this bacterium has been reported by Shinde and Sharma (1971) against *Lachnosterna* (=*Holotrichia*) *consanguinea* from Rajasthan. SubbaRao and Veeresh (1988) tested *P. popilliae* against *H. serrata* in Karnataka. Veeresh et al. (1982) conducted studies on the management of *L. lepidophora* with *P. popilliae* and observed its infection in grubs present only upto a depth of 15 cm, whereas grubs of *L. lepidophora* are distributed up to 60 cm. Birds are indiscriminate feeders and they pick up both healthy and diseased grubs at the time of ploughing. The birds like *Acridotheres tristis* (Linn), *Corvus splendens* (Vieillot), *Corvus macrorynchos* Wagler and *Bubulcus ibis* (Linn.) have shown to reduce 45%–60% grub population during three subsequent ploughings in Gujarat in groundnut fields (Parasharya et al. 1994). The digestive juices of birds, such as house sparrows, have no effect on *P. popilliae* spores. These predatory birds can be useful dispersal agents of the milky disease organisms (Vyas et al. 1988). The birds being long-distance fliers, indirectly help in the dispersal of this pathogen and create natural epizootic control (Parasharya et al. 1994). *P. popilliae* was totally ineffective against grubs of *B. coriacea* in Himachal Pradesh. A few similar reports are available which indicate that the use of *P. popilliae* does not provide adequate control of grubs (Knodel et al. 2012). This may be due to lack of persistence of the bacterium in the soil and/or loss of virulence. *P. popilliae* formulated as wettable powder or dusts loses its virulence under wet conditions, and its application also becomes difficult because of lumping and caking of carrier (Mathur 2001).

A novel isolate of *B. thuringiensis japonensis* strain (Btj) isolated from Japanese soils is highly effective against grubs of *P. japonica* (Suzuki et al. 1992). In Uttrakhand, a region of north western Himalaya, upto 20% population of the white grubs exhibits symptoms of bacterial infection. White grubs killed by bacteria rapidly darken in colour (Figure 9) and are often very soft. The internal tissue and organs are rapidly broken down into a viscid consistency, accompanied sometimes by a putrefied odour. The cadaver shrivels, dries and hardens. Sushil et al. (2008) isolated *B. cereus* from white grubs that showed symptoms of bacterial infection at Almora. Of the 27 bacterial isolates tested against *A. dimidiata*, WGPSB-2 was found to be highly toxic. The first instar grubs *of A. dimidiata* and *H. seticollis* were more susceptible than second instars. In outdoor microplots, a dose of 1.7×10^{10} spores/m^2 provided good control of white grubs. In Himachal Pradesh, *B. cereus* (WGPSB-2) was tested against grubs of *B. coriacea* by mixing in soil and through oral feeding by treating the tubers. The results were not very encouraging and maximum mortality of 37.77% was recorded when the dust containing 1×10^{10} spores/g was mixed @ 12 g/kg soil. Similarly, under field conditions, *B. cereus* failed to control the infestation of white grubs in potato in Shimla hills. There was 47.80% tuber infestation in potato after seed treatment with *B. cereus* dust (1×10^{10} spores/g) @ 5g/kg seed. When this dust was applied in furrows (12 g/plot) at the time of sowing, the tuber infestation was recorded to be 45.02% as compared with 60.75% in control (Anonymous 2009). In Himachal Pradesh, Sharma et al. (2013) isolated 10 bacteria belonging to genera *Bacillus*, *Psychrobacter*, *Paracoccus*,

Paenibacillus, *Mycobacterium*, *Staphylococcus* and *Novosphingobium* from infected grubs of *B. coriacea*. Bioassay studies revealed 100% mortality with *B. cereus* and 88.89% with *P. pulmonis* after 30 days of treatment. In Karnataka, extensive surveys have been conducted to identify and select the strains of *B. thuringiensis* that are highly toxic to *H. serrata* for use as bio control agents. Manju et al. (2009) identified 34 putative *B. thuringiensis* isolates that are active against white grubs. Activity tests of these cultures against grubs of *H. serrata* revealed that the proportion of *B. thuringiensis* active against white grubs was not correlated to the total load of CFU bacilli per g ($r = 0.273$), while there was significant correlation between the members of *B. thuringiensis* and the proportion infective to *H. serrata* ($r = 0.616$). Yu et al. (2006) indicated that the cultures of *B. thuringiensis* active against one species would potentially be useful in tackling other white grubs.

4. Future perspectives and conclusion

The key factor for successful microbial control of pests is the availability of a highly virulent strain or isolate of pathogen that can be economically mass produced *in vitro*, be compatible with the prevailing environmental conditions and with commonly used agro chemicals. Ecological studies on natural occurrence and distribution of entomofungi in different soil types and in different geographical regions for specific target species of white grubs are needed. Most often, fungal epizootics do not contain insect populations to economic threshold levels. To harness epizootics, we must understand which interactions are critical determinants of pathogenicity and epizootic development. Inoculation of the white grub endemic fields with fungal entomopathogens has provided limited control in some situations, but the inundative use of formulated products has proven to be a more effective method for decimating insect populations. Only naturally occurring fungal strains indigenous to limited geographic sites have been tried successfully, while many strains remain to be examined against white grubs. In many cases, the success or failure of a myco-insecticide usage is determined by the temperature and RH that occur after inoculative applications. Hence, there is an urgent need for improved formulation of technologies to preserve spore viability during storage and to allow spore germination at sub-optimal RH. The knowledge of EPF ecology such as tolerance to environmental stress can contribute to a better understanding of the effect of optimum factors on the survival and distribution of EPFs in field. This in turn can enable prediction and application time, and/or promote habitats that encourage amplification of natural inoculums and the induction of epizootics. Molecular biology provides exciting opportunities for improving fungi for pest control. There can be many features that may benefit from genetic improvement. Further investigations on new technologies are needed, especially genetic manipulation and hybridization, to induce new biotypes for improved performance.

The opportunities for using entomopathogenic nematodes against white grubs in the soil are excellent. The challenges for researchers include isolation of more native nematode species, their characterization and matching them against the target species of white grubs. Field efficacy is one of the required components for commercialization. Efficacy data which are essential to support any claim are lacking for many of the economically important species of white grubs. The conditions under which the nematodes are or are not effective need to be delineated. This type of data will require extensive field testing as laboratory data do not necessarily predict field efficacy. The selection or development of nematode strains that can undergo anhydrobiosis will be a great challenge. Apparently, Steinernematids and *Heterorhabditis* do not have this capability; however, selection for this trait has not been attempted seriously. A more promising future for nematodes in white grub management may lie in developing alternative approaches to their use as bio-pesticides. The alternative approaches include conservation and even better manipulation of the widespread natural nematode populations in soils that could be used to buffer white grub outbreaks. In addition, although the nematodes may be produced and formulated, they must still be made easily available to the farmers who must be then properly trained so that the nematodes are used effectively against the target pests. Bacteria, especially *P. popilliae*, may also play a role if their virulence can be genetically enhanced and *in vitro* production methods are developed. The isolation of white grub active strains of *B. cereus* in north-western Himalaya and *B. thuringiensis* in peninsular India, offers more scope for bacterial products in the management of white grubs. There exists immense variability in activity of different bacterial isolates and their testing against different species of white grubs can help in selection of effective and selective isolates of bacteria.

Making entomopathogens as a component of an integrated approach can provide significant and selective insect control. In the near future, we expect to see synergistic combinations of different microbial control agents with other technologies such as semio-chemicals, soft chemicals, other natural enemies, ecological engineering that will enhance

the effectiveness and sustainability of integrated control strategies against white grubs. A truly integrated approach is required in which all good agricultural practices, including other control options, should be considered to obtain maximum effect from a given intervention or practice without interfering with the effectiveness of other interventions. Further, the IPM, which combines the best of all pest control methods, holds the key to sustainable agriculture through the effective and successful containment of white grubs which are extremely destructive and highly polyphagous soil pests.

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