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ENVIRONMENT FRIENDLY PEST CONTROL AGENTS: USE OF BIO 1020 TO CONTROL WHITE GRUB DAMAGE ON TEA

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This experiment was conducted with a view to determine the efficacy of the biological control agent BIO 1020 against white grub *Holotrichia disparilis* damage of roots of young tea plants. Bio 1020 is a Commercial formulation of the fungus. *Metarhizium anisopliae* which is an effective bio control agent of many ornamental crop pest species.

Experiment was conducted in field no. 9 and 10 of the St. Coombs Estate which are the white grub prone areas. The results revealed that the formulation is effective against the white grub damage and can be used as a control measure. The required application rate per plant is 0.5 - 1g and method of application should ensure contactability of the target organism. Further from the results it can be concluded that the BIO 1020 can be used to control the grub damage effectively when other conventional methods are not effective on the target pest.

INTRODUCTION

Biological control is one of the oldest methods of insect pest control which uses one biological system to control another in an ecosystem. Biological control is of two types namely Classical and Natural. In classical biological control man introduces the biological agent to control another organism in the environment while natural biological control exists by itself in the environment. Biological control agents include bacteria, viruses and fungi which can be parasites, predators or pathogens hence called as entomopathogens. Entomopathogens enter the body of a particular host by various means and develop inside the host producing toxic substances causing various disorders in the host tissue leading to mortality of the target pest Sweetman, (1936).

Past studies on the subject indicated that there are over 700 species of entomopathogenic fungi which are grouped approximately into 100 genera. From these only 4 genera are registered as biological control agents in the world (Sweetman, 1936). Namely *Beauveria bassiana, Metarhizium anisopliae* which are having broad spectrum of activity and *Verticillium lecanii and Hirsutella thompsanii* of specific in action.

It is necessary to produce these pathogens in large numbers for mass release to control given pest organism. Since these agents are very sensitive to environment, cause difficulties in multiplication in an artificial environment which needs optimal conditions.

Metarhizium anisopliae

Metarhizium anisopliae is a facultative pathogen which can be easily grown on artificial media when provided with suitable conditions. Some farmers have grown the spores of this pathogen in semisolid media on boiled corn or rice in plastic bags which has been used for small scale cropping systems. (Aquino et al. 1975). the fungus is known as the causal agent of the green muscardine disease (Barnett 1955-1960).

However these processes are laborious and time consuming for large scale productions hence attempts have been made to formulate these microbial agents into easily applicable forms and are referred to as microbial insecticides. Only a few microbial insecticides have been produced and one of them is *Metarhizium anisopliae* (Metschnikoff) "BIO 1020" which has a natural host range of more than 200 species Veen, (1968). In the past history even as a crude formulation *Metarhizium* has given good results in controlling many other crop pests in the past Friederich, 1913; Zimmerman, 1981; 1984, Gottwald and Tedders 1983).

History of Metarhizium fungus

The fungus *Metarhizium anisopliae* was first isolated from a diseased beetle by a Russian scientist in 1878. The fungus belongs to the Class fungi imperfecti and to order Moniliales. The spores, olive green in colour; cylindrical and of two varieties which differ in pathogenicity, and produced apically in chains. (Refer Plate 5). Fungus can be divided into two varieties depending on the shape of the spore, the long spore variety known as the M. *anisopliae* var. major, and the short spore variety is M. *anisopliae var. anisopliae.* The two isolates differ in there activity. *M. anisopliae* is highly virulent on the palm pest *Oryctes rhinoceros*, whereas the latter has hardly any virulence on the same pest (Zacharuk, 1970).

Mechanism of infection

The conidiospores of the fungus will adhere to the insect cuticle by hydrophobic action and then germinate in the insect body (Zacharuk, 1970; Dillon and Charnley, 1990). The germ tube forms an appresorium which is the structure for anchoring itself to the insect body and penetrates through the cuticle by means of a penetration tube into the interior of the insect. (Zacharuk, 1970 ; St. Leger, 1990). Huber (1958) demonstrated that entomopathogenic fungi can hydrolyse chitin of the insect integument and gives rise to infection hyphae or the mycelium forming blastospores which spread through the haemolymph into the insect and set in a systemic infection resulting the death of the host. At the time of death the blastospores produce hyphae completely colonising the insect cadaver and surface mycelium can be formed within few days. These spores lie dormant in the soil until they contact a new host. After infection of a particular host, fungus, will appear only after 3 to 5 days on the host appearing as yellow or brown colour spots.

Bio 1020 Formulation

"Bio 1020" is an insoluble granulate of hyphal cells of a wild-type strain of the fungus : *Metarhizium anisopliae* (Metschn.) Sorok. 1883 var. *anisopliae* (Domsch et al., 1980), which has been formulated into a commercially viable plant protection agent. This is

suitable only for soil treatment (Reinecke, et al., 1990). The granules of 0.5-1.0 mm in diameter are viable even after a year in storage. The fungal cells burst into new life when they come in contact with soil moisture and an intensive condition take place on the granules.

Mycopathogens in the soil are affected by abiotic factors, mainly temperature, relative humidity or soil water content, agro-chemicals and composition of soil types. has been reported that infection of the fungal conidia takes place at soil temperatures of 15°C and above and is largely independent of soil moisture and substrate type (Ferron, 1970 & 1971 ; Zimmermann, 1982; Hartwig and Oehmig, 1992). Even though soil water is important, soil moisture for plant growth is adequate for activation of M. *anisopliae* in soil.

Target Organism

Current study was conducted with a view to find out a suitable method to control the target organism white grub the larval stage of the beetle *Holotrichia disparilis* Arrow (Coleoptera: Scarabidae) which is considered as the primary pest of the white grubs or Cockchafers attacking the tea.

Damage

It causes death of the tea plants of 2-3 years of age in new clearings. *Holotrichia disparilis* is the most abundant species in up country districts above 4000 a.m.s.l., particularly in Maturata, Hewaheta, Udapussellawa and Dimbula planting districts of Sri Lanka.

Currently existing control Methods

H.disparilis has proved resistant to chemical control (Cranham, 1966). Pattern of life cycle of white grub (see fig. -1) shows that the period of larval development extends over several months which require a persistant chemical in the soil that long. Conventional chemical insecticides do not meet these requirements.

Prophylactic control can be achieved with "Carbosulfan CR" which is a controlled - release formulation and are granules of 2 mm in having 1.5 - 2 year release rate. To achieve a better control these granules should be incorporated in to the planting hole ensuring coverage of the rhizosphere or the root zone of the young plant. (TRI Advisory Circular 1-9). However if an outbreak occur in an untreated area alternative control agents should be used as post planting treatments.

MATERIALS AND METHODS

2.1 Experimental site - Field No. 9 and 10 of St. Coombs Estate which are prone to white grub damage.

Experiment 1

Experiment 1 was conducted in a block of tea planted in June 1991 was selected as the experimental area. White grub damage was encountered after 4 months of planting thus in October 1991. After detecting the while grub damage this block was treated with 3 different concentrations of BIO 1020 formulation as follows.

Treatments/Dosages of BIO 1020	*Amount per plant*
T1	0.5g
T2	1.0g
T3	2.0g
T4	Control (untreated)

These amounts were applied in 3 holes made down to a depth of 15cm, at a distance of 15cm away from the plant and equally distributed among the rhizosphere of the plant.

After 6 months of the application of the insecticides sampling was commenced. 3 soil cores were taken 15 cm away from the treated plant by means of a soil auger. Soil cores were placed in polythene bags and brought in to the lab for processing. Soil samples were seived through metal mesh sieves to collect various stages of the white grub. Number of dead and live specimens were recorded. Dead white grubs and soil were cultured on plain agar media, in the lab for presence of the *Metarhizium anisopliae* fungus. Viability of the BIO 1020 granules present in the soil was tested by culturing them in plain agar plates in the lab.

Experiment 2

This experiment was conducted to compare the activity of the BIO 1020 with Carbosulfan CR called as suscon fore which is the insecticide used to control white grub damage on tea.

A block of tea at field No. 10 was selected as the experimental area for experiment 2, where the tea was planted in June 1992. Similarly plants were treated with the insecticide when grubs were encountered in October 1992. treatments were given as follows.

Treatments / Dosages of insecticide	*Amount per plant*
BIO 1020	1g
Carbosulfan	2g
Control	Non treated

Carbosulfan was applied in a 2.5 cm furrow at 15cm away from the base of the plant and Bio 1020, was applied as in the Experiment 1.

Please refer Tables 1,2 and Figures 1,2,3,4 for detailed description of the results.

TABLE 1 – *Duration of the experiment - 3. September 1991-14. November 1991*

Treated	*No. of weeks from treatment*					
Whitegrubs found	4th	5th	6th	7th	8th	9th
Dead	2	1	1	2	1	0
	0	0	0	0	0	0
Untreated						
Dead	0	0	0	0	0	0
Live	5	3	4	1	3	1

TABLE 2 – *Live white grub counts encountered in the period of 1992 November to 1993 October.*

	Monthly assessment numbers											
T	1	2	3	4	5	6	7	8	9	10	11	12
T1	1	0	0	0	0	0	2	5	1	1	0	0
T2	2	0	0	0	0	0	0	3	0	0	0	0
T3	3	1	0	0	0	0	0	1	1	1	0	0

T1 = Treated with BIO 1020 or *Metarhizium anisopliae*

T2 = Treated with Carbosulfan or Suscon formulation

T3 = Untreated control.

PATTERN OF DEVELOPMENT OF LIFE STAGES OF WHITE GRUBS AND ITS SIGNIFICANCE ON CONTROL ASPECTS

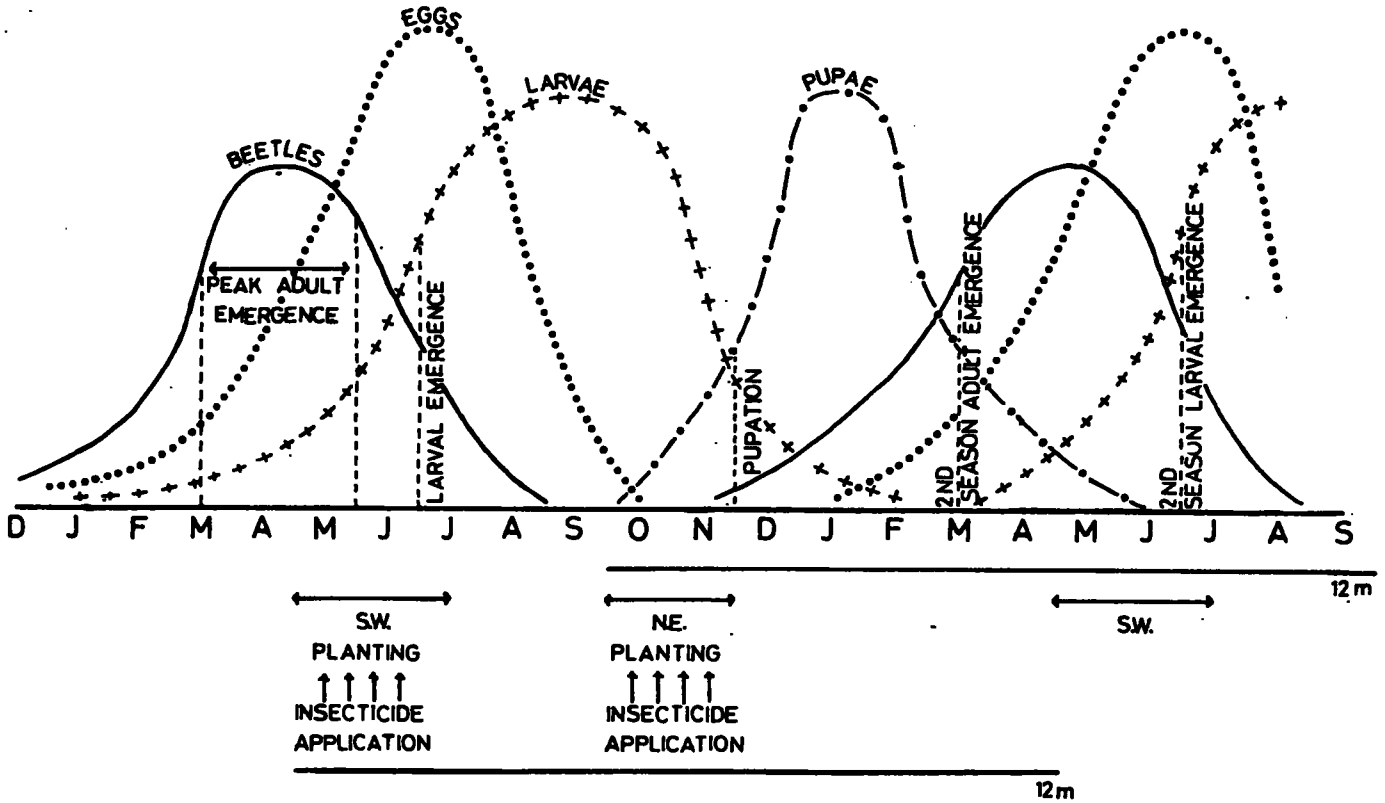

Fig-01

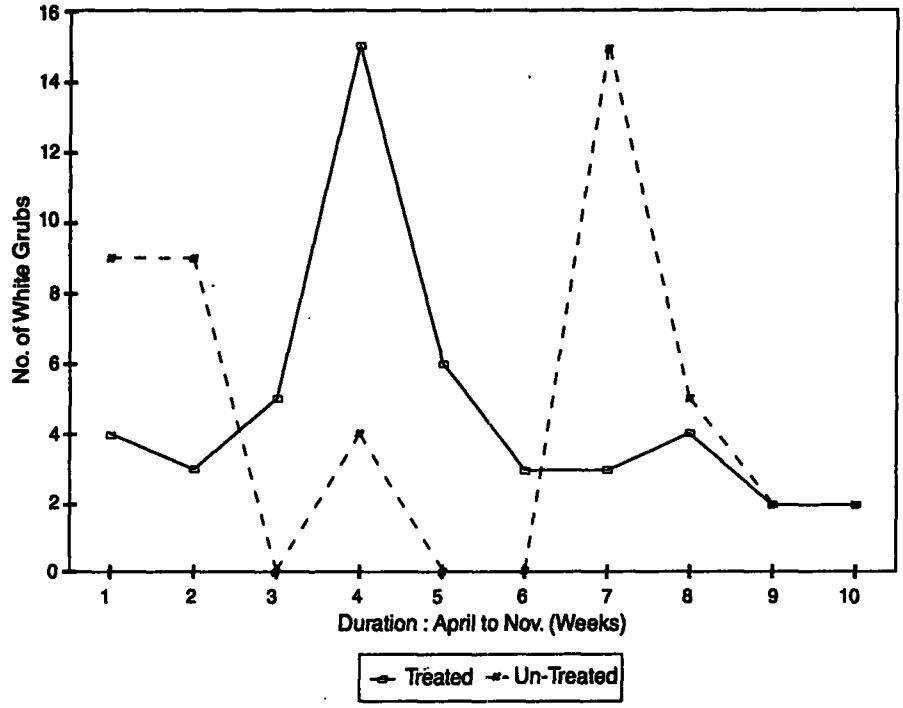

Fig. 2 – White Grub counts in 1992

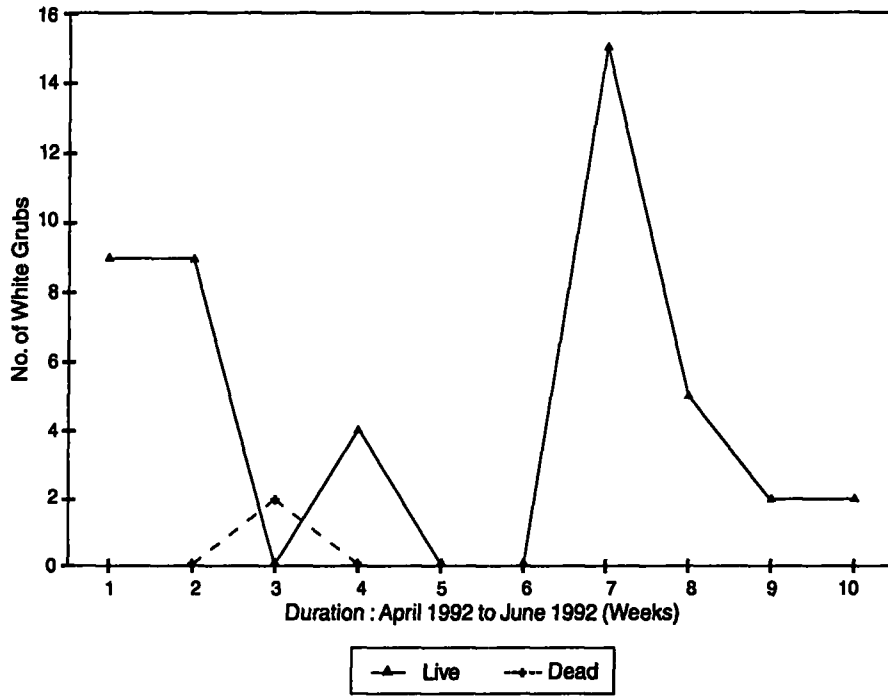

Fig. 3 – White Grub counts in un-treated plots

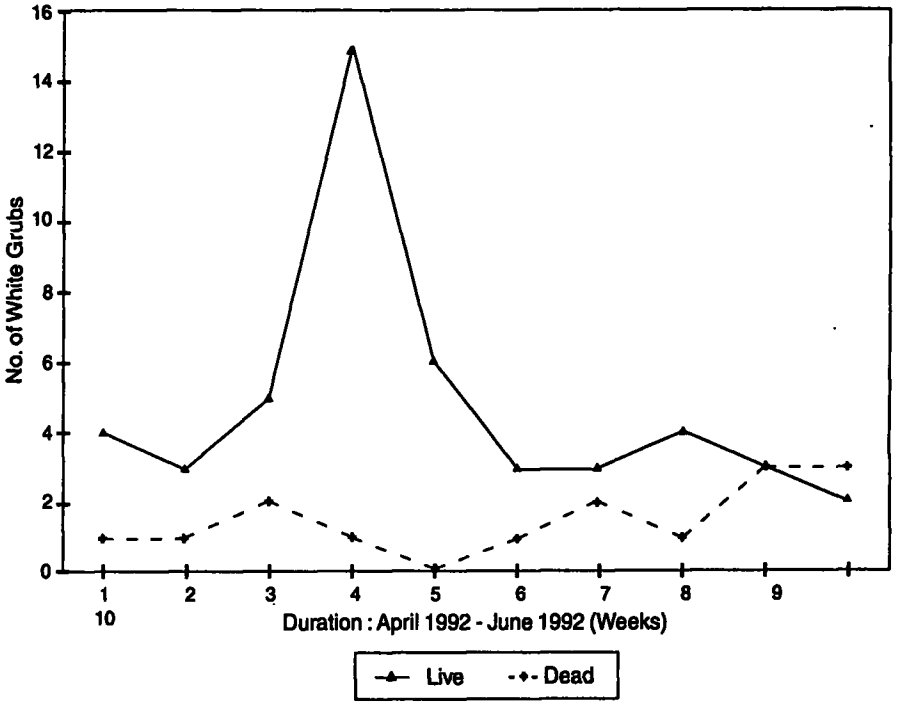

Fig. 4 – White Grub counts in treated plots

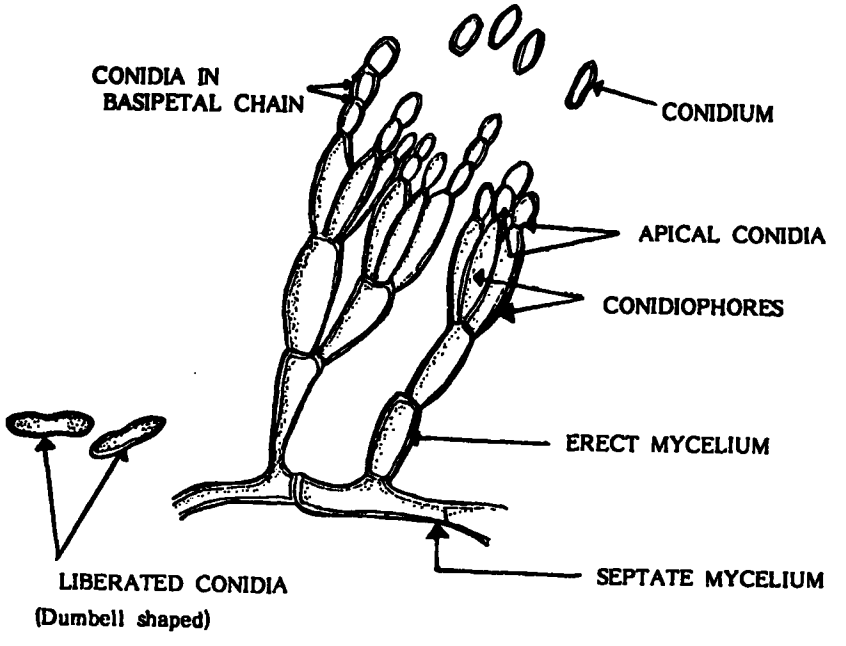

Fig. 5 – Morphological features of the fungus

Metarhizium anisopliae (Fungi Imperfecti : Moniliales)

RESULTS AND DISCUSSION

In Experiment 1 it could be seen in the treated plots the white grub population increased until April 1992 and then decreased abruptly in May. In this period No. of dead white grubs were increased (Refer fig. 1).

In the untreated plots the live population counts continued to remain high and population counts of the dead white grubs were negligible. (Refer Fig 2). Viable granules or infected body fluids produced muscardine spores of *Metarhizium anisopliae* on agar.

The deaths of the white grubs were due to the infection of the biological agent thus the *Metarhizium anisopliae* fungus, which can be confirmed with the development of the fungus from the dead grubs body fluids in culture plates. The cultures produced the fungal mycelia of the *Metarhizium anisopliae* fungus. Therefore it can be concluded that the grubs were infected by the fungul agent BIO 1020. It could be observed that the BIO 1020 remained viable upto the next white grub season of the following year too.

According to the results it can be concluded that several overlapping generations of white grubs inhabits in tea lands in Sri Lanka which can feed over tea roots for an extended period of about 6-8 months leading destructions to young plants. The peak levels of beetle population occurred during March to May. The eggs laid by them hatch out and considerable numbers of grubs are encountered in tea soils from about October and November.

In experiment 2 the population levels of white grubs remained very low throughout 1993 in all plots.

The initial increase of the white grub population upto the end of April 1992 in the second grub season indicated that the grubs were infected with BIO 1020, a few days after emergence from the eggs confirmed that the susceptible stage is the larve or the white grub. However in contrast Stenzel (1992) reported that eggs and other development stages of black wine weevil (*Otiorhynchus sulcatus*) are susceptible to *Metarhizium fungus.*

In ornamental crops, pest control was achieved by incorporating 1g of BIO 1020 per litre of potting mixture (Reinecke et al., 1990). However it is important that BIO 1020 is mixed properly with the entire soil to ensure the contact of the target pest organism present in the soil. Nevertheless it could be seen in this experiment even at localised application of lower dosages such as 0.5 g per plant on surface soil has given confirmative results comparable to 1.0 and 2.0 g per plant treatment.

The reason for this is the mobility of the target pest *Holotrichia disparilis* in the soil substrate and also due to its behaviour of surfacing at dawn and at dusk in the soil has made it contactable by the control agent.

These observations help us to minimize the utilisation of labour for applications at field scale. However application to the soil in a furrow was insisted to prevent the losses due to washing away with rainwater.

From the results it indicated that the fungus was able to survive in the soil during a period of year until the next grub season arises in the following year (From April - October 1992).

CONCLUSION

From the results it could be seen that BIO 1020 is an effective biological, control agent against white grub *Holotrichia disparilis*. The required rate of application is 0.5-2.0 g per plant. Application in a shallow furrow at the base of the plant of 15cm depth would be most effective since the granules remain viable for more than 12 months in the soil. In situations where conventional insecticides are not effective or where there is organic farming in practice usage of BIO 1020 to control white grub damage is advisable.

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