Use of Entomogenous Nematode, *Heterorhabditis bacteriophora* in Combination with Fungus, *Metarrhizium anisopliae* and Recommended Pesticides for Management of *Holotrichia consanguinea* Blanch.

होलोट्राइकिया कन्सैंग्यूनिया ब्लैंकाई के प्रबन्धन में कीटजनक सूत्रकृमि, हेटेरोरेबडिटीस बेक्टीरिफोरा का मेटाराइजियम एनीसोपिली, फफूंद एवं अभिस्तापित पीडकनाशकों के संयोजन में प्रयोग

Bhanwar Lal Jat

Thesis

Doctor of Philosophy in Agriculture

उत्तमा वतिस्तु कृषिकमैव

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Department of Agricultural Zoology and Entomology
Rajashan College of Agriculture
Udaipur

Use of Entomogenous Nematode, *Heterorhabditis bacteriophora* in Combination with Fungus, *Metarrhizium anisopliae* and Recommended Pesticides for Management of *Holotrichia consanguinea* Blanch.

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Rajasthan Agricultural University, Bikaner
Rajasthan College of Agriculture, Udaipur

CERTIFICATE - I

Dated - 18/11/1998

This is to certify that **Mr. Bhanwar Lal Jat** has successfully completed the preliminary examination held on 20th may, 1997 as required under the regulation for **Doctor of Philosophy** degree.

18.11.98

(Dr. H.C.L. Gupta)
Head
Department of Agricultural
Zoology and Entomology
Rajasthan College of Agriculture
Udaipur

Rajasthan Agricultural University, Bikaner
Rajasthan College of Agriculture, Udaipur

CERTIFICATE-II

Dated - 18/11/1998

This is to certify that this thesis entitled "**Use of entomogenous nematode, *Heterorhabditis bacteriophora* in combination with fungus, *Metarrhizium anisopliae* and recommended pesticides for management of *Holotrichia consanguinea* Blanch.**", Submitted for the degree of **Doctor of Philosophy in Agriculture** in the subject of **Entomology** embodies bonafide research work carried out by **Mr. Bhanwar Lal Jat** under my guidance and supervision and that no part of this thesis has been submitted for any other degree. The assistance and help received during the course of investigation have been fully acknowledged. The draft of the thesis was also approved by the advisory committee on 27th October, 1998.

18.11.98

(Dr H.C.L. Gupta)
Professor and Head
Department of Agricultural
Zoology and Entomology

18/11/98

(Dr. S.C. Bhardwaj)
Major advisor

19.11.98

(Dr. G.S. Sharma)
Dean
Rajasthan College of Agriculture,
Udaipur (Raj.)

Rajasthan Agricultural University, Bikaner
Rajasthan College of Agriculture, Udaipur

CERTIFICATE -III

Dated: 26/02/1999

This is to certify that this thesis entitled "**Use of entomogenous nematode, *Heterorhabditis bacteriophora* in combination with fungus, *Metarrhizium anisopliae* and recommended pesticides for management of *Holotrichia consanguinea* Blanch.**", Submitted by **Mr. Bhanwar Lal Jat** to the Rajasthan Agricultural University, Bikaner in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** in the subject of **Entomology** was after recommendation by the external examiner defended by the candidate before the following members of the examination committee. The performance of the candidate in the oral examination on his thesis has been found satisfactory, we therefore, recommended that the thesis be approved.

(Dr. H.C.L. Gupta)
Head
Department of Agricultural
Zoology and Entomology

27/2/99
(Dr. S.C. Bhardwaj)
Major Advisor

(Dr. H.C.L. Gupta)
Advisor

(Dr. Hanuman Singh)
Advisor

(Sh. M.S. Bhatnagar)
Advisor

(Dr. B.L. Baser)
Nominee of Dean, PGS

Approved
APPROVED
DEAN
27/2/99
(Dr. R.K. Sharma)
Dean
Post Graduate Studies
Rajasthan Agricultural University,
Bikaner

Post Graduate Studies
Rajasthan Agricultural University
BIKANER (RAJ.)

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1. INTRODUCTION

White grubs are serious pests of agro-horticultural crops and forest plantation, specially, in the arid and semi-arid regions of the country. Several species of this pest cause considerable damage but the most important is *Holotrichia consanguinea* Blanch. which is reported to cause 10 to 100 per cent damage to various crops in different regions in India. (Yadava, 1981). This polyphagous species causes heavy losses mainly in Rajasthan, Gujarat, Uttar Pradesh, Andhra Pradesh, Haryana, Punjab and Bihar. The State of Rajasthan, because of favourable climatic conditions and soil type, is supposed to be suitable for this pest. Both the grubs and beetles cause damage, the former feed on roots whereas the latter defoliate the shrubs and the trees growing nearby cultivated fields. In endemic areas major cause for alarming shrinkage in the area under kharif oilseeds, mainly the groundnut, and pulse crops is the steady rise in the population of this polyphagous pest resulting in drastic yield losses and making cultivation of these crops uneconomical.

Although chemical control recommendations are available for protecting the crops from this abnoxious pest but there are many dis-advantages of their use. Firstly, as the pest is hardy and wide spread, the quantity of toxic chemicals put into the environment and subsequent environmental pollution is quite high. Secondly, the use of insecticide protects the crops in the treated field only and does not curb the pest population in the surrounding areas hence , the recurrent use of chemicals every year becomes necessary. For this reason it is obligatory to search for alternate control measures which may be more eco-friendly and bring down the pest population to a level where the pest can be kept under control without environmental pollution.

Use of biological control agents is one such measure. Nematode as bio-control agent was employed for the first time against Japanese beetles, *Popillia japonica* (Glaser, 1932; Anonymous, 1988) Since, then entomogenous nematodes have been successfully used against several insects in different countries (Kaya and Gaugler, 1993). The Steinernematid and Heterorhabditid nematodes were considered to be

useful as bio-control agent against insect-pests in different agro-ecosystem (Poinar, 1972 and Kaya, 1985). Phytopathogenic fungus, *Metarrhizium anisopliae* was also reported to attack the grubs of *Holotrichia consanguinea* (Avasthy, 1967).

Perusal of literature reveals about the use of microbial control agents against phytophagous scarabs in the country, but information required for proper deployment of these microbs in field and their compatibility with pesticides for white grub management is still wanting. With these facts in mind the present investigations were carried out with the following objectives:

1. To work out relationship between the nematode inoculation dosage/response in the grub and study the relative susceptibility of grubs to the nematode, *Heterorhabditis bacteriophora*.
2. To study the relative susceptibility of grubs to fungus, *Metarrhizium anisopliae*.
3. To find out the compatibility of the nematode with the fungus and nematode and fungus with insecticides recommended for the control of white grub.

2. REVIEW OF LITERATURE

Review of available literature indicate that not much work has been carried out for controlling white grub, *Holotrichia consanguinea* using entomogenous nematodes and entomopathogenic fungi as well as their compatible combinations. The work has been reviewed under following sub-heads.

2.1. Relative susceptibility of white grub, *H. consanguinea* to nematode, *H. bacteriophora*.

2.2 Relative susceptibility of white grub, *H. consanguinea* to fungus, *M. anisopliae*.

2.3 Compatibility of nematode with fungus and nematode and fungus with pesticides.

2.1 RELATIVE SUSCEPTIBILITY OF WHITE GRUB, *H. consanguinea* TO NEMATODE, *H. bacteriophora*

Nematode as bio-control agent was employed for the first time against Japanese beetle, *Popillia japonica* (Glaser, 1932). Since then, entomogenous nematodes have been successfully used against several insects in different countries (Kaya and Gaugler, 1993).

The Stenernematid and Heterorhabditid nematodes were considered to be useful as biological control agent against insect-pests in different agro ecosystems. (Poinar, 1972 and Kaya 1985). These entomogenous nematodes were found to carry specific symbiotic bacteria (Poinar, 1972; 1979). The host finding behaviour of nematodes was studied by Grewal *et al.* (1994). Pionar (1979) described a new species of nematode, *Heterorhabditis megidis* from parasitized third stage larvae of Japanese beetle, *Popillia japonica* from Ohio. *Rhabditis* sp. parasitized the eggs of *Holotrichia serrata* to the extent of 52 to 96 per cent in sugarcane cropping system in South India (David *et al.*, 1986).

Raman and Pionar (1984) conducted green house tests to determine the effectiveness of *Heterorhabditis heliothidis*, *Neoaplectana carpocapsae* and *N. glaseri* for the control of black vine weevil, *Otiorhynchus sulcatus*. They found that *H. heliothidis* applied to the surface of the soil gave better control against early instar larvae than did the other nematodes applied similarly. When placed to the soil surface, all the three species were equally effective against late instar larvae but when larvae were placed 20 cm deep the best control was achieved with *N. glaseri* when injected 5 cm in to the soil. The three species were equally effective against later instar larvae placed at depths of 5, 10 and 20 cm.

Sosa and Beavers (1985) tested *Steinernema glaseri* and *Heterorhabditis heliothidis* against *Ligyrus subropicus* and found significantly higher mortality with third instar *S. glaseri* when applied @ 5000 nematodes per larvae. Mortality of larvae exposed to 200 nematodes per larva did not differ significantly from the larvae with 400 nematodes per larva. The host yielded 139576 nematodes per larva.

Wright *et al.* (1988) tested four species of entomogenous nematodes *Steinernema feltiae, S. glaseri, Heterorhabditis heliothidis* and *Heterorhabditis* sp. against third instar larvae, *Popillia japonica* and, *Rhizotrogus majalis* in potted Japanese Yem, *Taxus cuspidata* and compared with two insecticides (chlorpyrifos and isofenphos). They found that 17-21 days after treatment *Heterorhabditis* sp. at 92 nematodes per cm^2 of soil surface and *H. heliothidis* at 192 nematodes per cm^2 provided more than 90 per cent control of Japanese beetles compared with 71 per cent for chlorpyrifos (9.0 kg ai/ha) and 84 per cent for isofenphos (4.5 kg ai/ha). *S. glaseri* provided 84 per cent and *S. feltiae* 29 per cent control (both at 385 nematodes per cm^2). Control with nematodes, *S. glaseri*, *H. heliothidis* and *Heterorhabditis* sp. 385 nematodes per cm^2 ranged from 46 to 59 per cent.

Further effectiveness of the three nematodes *viz. Steinernema feltiae*, *S. glaseri* and *Heterorhabditis heliothidis* was tested against white grub both in laboratory and field. In the laboratory tests 60-80 per cent grub mortality was achieved within 2 to 4 days while significant reduction was achieved in field with dosages ranging from

0.54 to 10.76 x 10^5 nematodes per m^2 (Kard *et al.*, 1988). Similarly Koizumi *et al.* (1988) found varying degree of root damage in *Chamaecyparis obtusa* with *Steinernema* suspension ranging from 5000 to 10,00000 juveniles per m^2. However, to achieve cent per cent mortality 100000 juveniles per m^2 were required, while 44 and 62 per cent control was obtained with isofenphos and chlorpyrifos, respectively.

Villani and Wright (1988) found 94 per cent control of *Rhizotrogus majalis* larvae with *Heterorhabditis heliothidis* with 19.4 nematodes per cm^2 after 25 days of treatment. Further, *H. heliothidis* (310 per cm^2) provided more than 60 per cent control of mixed population of Japanese beetle and European chafer larvae after 47 days of treatment which was equivalent to the control achieved with insecticides chlorpyrifos and trichlorfan against this combination of white grubs.

The nematode IJs of *Heterorhabditis* sp. H.P. 888 strain when mixed with soil at 50 IJs/cm^3 resulted in 86 per cent control of *Maladera matrida* in lab and green house conditions. Similar mortality was obtained with higher concentration of 160 to 640 IJs/cm^3 when applied on the soil surface. The most susceptible stage was 3.5 week old grubs but the pupae were not affected (Glazer and Allam, 1989). Contrary to this, *Heterorhabditis bacteriophora* (H.P. 88 strain) and *Steinernema carpocapsae* (All and G-13 strain) were found to be pathogenic to larvae and pupae of sweet potato weevil, *Cylas formicarius*. The LD-50 values of the three strains (HP88, All and G-13) for the larvae were 2.6, 2.9 and 3.4 IJs/insect whereas for pupae the values were 4.9, 5.9 and 4.8 IJs/insect, respectively. (Jansoon *et al.*, 1990).

The LC_{50} of *Steinernema carpocapsae*, *S. glaseri* and *Heterorhabditis heliothidis* to the third instar *Phyllophaga hirticula* were 210, 86 and 12 nematodes per grub, respectively (Forschler and Gardner, 1991) However, due to migration actual concentration of nematodes remained less than actually applied.

In a comparative efficacy Karuna Karan (1990) found *Heterorhabditis* sp. more effective than *Steinernema feltiae*. Similarly, *H. bacteriophora* reduced the population of *Polillia japonica* by 60 per cent 34 days after treatment which was increased to 96 per cent before population and 93 to 99 per cent in next generation.

S. carpocapsae provided 51 per cent control after 34 days, 90 per cent 290 days after treatment and zero per cent after 386 days (Klein and Georgis, 1992)

Further entomogenous nematodes, *Steinernema* sp. *S. glaseri*, *S. feltiae*, *Heterorhabditis* sp. and *H. bacteriophora* were found pathogenic to economically important white grub species, (*H. consanguinea*, *A. bengalensis*, *M. insanabilis* and *L. lepidophora*) killing the test larvae within 3 to 10 days depending upon the number of IJs, stage of insect and method of inoculation. The nematodes further multiplied in the dead cadaver and free living infective juveniles emerged after 2 to 4 weeks (Anonymous, 1995).

In the grubs of *L. lepidophora* 33.33 to 66.66 per cent mortality occurred with the dosage of 7000 to 10,000 nemas per grub (*Heterorhabditis* sp.) (Anonymous, 1995). When the first instar grubs were directly exposed to the nematode (500 to 30,000 nematodes/grub). cent per cent mortality was achieved. (Anonymous, 1995). Similar results were obtained when the first instar grubs were inoculated with 2000 nemas/ grub through the food whereas 20 per cent mortality was noticed when the inoculated food and grubs with 2000 nemas were kept at 5 and 10 cm depth in the soil. The mortality in grubs of *H. longipennis* ranged from 30 to 70 per cent in different inoculation dosages (450 to 1950 IJs /grub) of parasite nematode. *S. glaseri* against zero per cent mortality in untreated control in laboratory test. (Anonymous, 1997).

Mathur *et al.* (1995) found that *Heterorhabditis bacteriophora* Poinar and *Heterorhabditis* sp. parasitized the grubs of six species of phytophagous scarabs (*H. consanguinea*, *H. serrata*, *A. bengalensis*, *A. dimidiata*, *Leucopholis lepidophora* and *Maladera insanabilis*). The nematode @ 500 and 1000 IJs/grub by filter paper method and 5000 and 10,000 IJs/grub by soil inoculation method caused 10 to 40 per cent mortality within 2 to 3 days and 70 to 100 per cent after a week of inoculation. From the dead insects 1,500 to 1,50000 IJs/grub were harvested.

Likewise, Shinde *et al.* (1995) evaluated entomophathogenic stenernematid nematodes (*Steinornema glaseri*, *S. feltiae* and *Steinernema* sp.) against third instar

grubs of six species of economically important phytophagous scarabs. They found 10 to 60 per cent grub mortality 2 days after exposure, with filter paper method, while with soil incorporation method it needed one more day to get this level of lethal infection. The number of IJs harvested from each grub ranged from 750 to 1.55,00. Among the six scarabs tested, mean number of IJs harvested was minimum in case of *M. insanabilis* and maximum in *L. lepidophora*.

The first instar grubs of *H. consanguinea* when exposed to infective juveniles on filter paper (5-2000 IJs/grub) and in soil (100-10,000 IJs 100 ml soil/grub) 10 to 90 per cent kill was observed within a week depending on the inoculum dosage (Veer Singh *et al.*, 1995).

Bareth *et al.* (1995) found that insect parasitic nematode, *Steinernema glaseri* (Stein), was very effective against first instar grubs of *Holotrichia consanguinea* Blanch. All the grubs exposed to infected juveniles (IJs) of nematodes on filter paper (10-1000 IJs/grub) died within 2 to 5 days. When grubs were individually confined to soil containing IJs (1000 to 10,000 IJs/100 ml soil) 80 to 100 per cent kill was recorded in a week. The nematodes multiplied inside dead insects. Nematodes (IJs and adults) started emerging from insects within 5 to 11 days of mortality and from a single grub 600 - 2520 nematodes were harvested. The correlation was positive between the inoculum dosage and size of harvest to a certain extent beyond which higher dosages adversely affected the harvest.

2.2 RELATIVE SUSCEPTIBILITY OF WHITE GRUB, *H. consanguinea* TO FUNGUS, *M. anisopliae*

The entomophathogenic fungus, *Metarrhizium anisopliae* is widely distributed naturally but the incidence of this disease among the grubs is usually very low (Hawley and White, 1935). The grubs of *Holotrichia consanguinea* were found to be attacked by fungus *M. anisopliae* (Avasthy, 1967). Further different species of fungi *viz. Metarrhizium anisopliae*, *Beauveria bassiana*, *B. brongniartii* and *Fusarium oxysporous* were found to be pathogenic to economically important phytophagous

scarabs, *H. consanguinea*, *H. lengipennis*, *Anomala dimidiata*, *H. sikkimensis* both in laboratory and field conditions (Anonymous, 1978, 1988, 1989, 1995, 1997).

Ranganathiah *et al.* (1973) were the first to report the efficacy of *Beauveria brongniartii* against white grub, *Holotrichia serrata* from South India. All the stages of white grub were found susceptible to this fungus. High humidity and temperature were considered to be essential for quick growth of the fungus. Two weeks after the treatment with fungus the grubs stopped feeding and by the third week the fungal growth became visible externally. Thereafter, infected grubs became covered with white mass.

Singh (1985) observed 20 per cent mortality of scarabaeid larvae due to fungus *M. anisopliae* in potato field. The fungus, *Verticillium lecanii* (Zimmermann) caused 53 and 58 per cent mortality in first and second instar grubs of *H. consanguinea* respectively by soil inoculation method. (Gour and Dabi, 1988). Similarly 82.5 per cent mortality in *H. consanguinea* grubs was recorded after 30 days of soil inoculation with *M. anisopliae*, whereas only 40.6 per cent mortality was caused by *B. bassiana* as a result of crawling of grubs over fungal culture after 30 days of inoculation (Anonymous, 1978).

When *B. brongniartii* spore dust @ 10^{15} conidia/ha was applied in furrows before sowing of groundnut seeds about 46.0 per cent mortality was recorded in *H. consanguinea* grubs after four weeks of application (Anonymous, 1988). Similarly 16 per cent infected grubs of *H. longipennis* were found (15 days of application) when this fungus at the same rate was applied in standing crop of finger millet in northern hill region. In the third instar grub of *H. serrata* 40 per cent infection of *B. brongniartii* was found (Anonymous 1989).

The green muscardine fungus, *M. anisopliae* and the white muscardine fungus, *B. brongniartii* have been found effective entomopathogenic against white grubs. (Ranganathaiah *et al.*, 1973; Jayaramaiah and Veeresh 1983 b; Patel *et al.*, 1988). Outside also intensive use of *B. brongniartii* has been made against *Melolontha melolontha* in France (Ferron, 1981).

In India, the fungus was first isolated from the grubs of *H. serrata* from Karnataka (Rajanathaiah *et al.* 1973) The fungus, *B. brongniartii* was found pathogenic to all the stages of *H. consanguinea*, the maximum mortality in eggs (52%), grubs (72%), pupae (48%) and adults (72%) was observed at 10^7 spore dosage under laboratory conditions (Vyas,1988).

Verma *et al.* (1988) tested the pathogenicity of *M. anisopliae* and *B. bassiana* against different larval instars of *Leucopholis lepidophora*. They observed that *M. anisopliae* induced 80 and 66 per cent mortality in first and second instar respectively as compared to 100 per cent mortality with *B. bassiana* in both the instars after 55 days of treatments. Similarly 40 per cent grubs of *L. lepidephora* were found infected after 60 days of treatment with *B. brongniartii* in lab test at Kolhapur. (Anonymous 1990). The entomopathogenic fungus, *M. anisopliae* was found to be potential biological control agent of sugarcane white grub, *L. lepidophora* (Samuels *et al.*, 1990).

Milner (1989) emphasised that effective development of a mycoinsecticide for use against soil pest such as scarabaeid larvae is dependent on increased knowledge of ecology of *M. anisopliae* under field conditions. Laboratory data backed up by field observations suggested that temperature, moisture and soil type may either independently or by interaction, affect conidial persistence, invasion of the target host and the production of new conidia.

Rath and Yip (1989) found *M. anisopliae* highly virulent to larvae and adults of red-headed cock-chafer, *Anoryphorus couloni* but noted rapid reduction in fungal level in soil after 12 months. For effective control of cock-chafer, spores of *Metarhizium* were recommended to be placed below soil surface at the dosage of 10^3 to 10^4 spores/cm^3. Further Rath, (1989) found difference in mortality at different temperatures when exposed to *M. anisopliae* ($1x10^4$ spores/g. sand-peat) at 20°C more than 90 per cent mortality was achieved whereas at 10°C it was 70 per cent.

Vyas *et al.* (1990) also found that white muscardine fungus, *B. brongniartii* was infective to grubs, pupae, adults and eggs of all important white grub species like

H. consanguinea, *H. serrata*, *H. fregei* and *Autoseria nathani*. The fungus caused a maximum of 41.5 per cent mortality in the grub of *H. serrata* and 45.5 per cent in the grubs of *H. consanguinea*, when applied at the rate of 10^{15} conidia/ha. Similarly the fungus, *Fusarium oxysporum* was found pathogenic against the third instar grubs of *H. longipennis*. The fungus in dosage of 1 x 10^9 spores/g inflicted 52 per cent mortality and at 1 x 10^7 spores/g the mortality was 40.0 per cent (Anonymous, 1995).

Under All India co-ordinated Research Project on white grub, *M. anisopliae* has been used to control different species of white grubs. At Palampur it was found effective against *H. sikkimensis* (Anonymous, 1990) while at Durgapura it was used against *H. consanguinea*. (Anonymous, 1990). Similarly at Ranichuri it saved the crop of barnyard millet from *Anomala dimidiata* and *H. longipennis* (Anonymous, 1995).

Yadav *et al.* (1998) also found *M. anisopliae* pathogenic to all the three instars of grub of *H. consanguinea* Blanch. The fungus ($1x10^{10}$ spores 100 ml. soil) caused seventy, sixty and fifty per cent mortality in first, second and third instar *H. consanguinea*, respectively under pot condition. Susceptibility decreased from lower to higher instars.

2.3 COMPATIBILITY OF NEMATODE WITH FUNGUS AND NEMATODE AND FUNGUS WITH PESTICIDES

Compatibility trials on the use of nematodes, fungus and pesticides have been carried out mostly outside India. However, recently work on this aspect has been initiated at the all India Co-ordinated Research project on white grub at Durgapura (Jaipur). The notable work includes the studies conducted by:

Kamionek *et al.* (1974 a,b) found period of lethal infection (PLI) of nematode and pathogenic fungus combination shorter than fungus alone against Coleo ptera and lepidoptera. They also observed that only nematodes survived, developed and produced progeny in the insect cadaver.

Tillamans *et al.* (1990) studied the compatibility between *B. brongniartii* and strain of *Heterorhabditis* against black vine weevil (*Otiorhynchus suleatus*) on strawberry and proved that their combined affect was greater. Similar observations were made by Barbercheck and Kaya (1990) who stated that the period of lethal infection (PLI) for last instar *Galleria mellonella* larvae infected with nematodes (*S. feltiae* and *H. heliothidis*) and fungus (*B. bassiana*) simultaneously was shorter than in larvae treated with nematode or fungus alone or sequential dual treatment.

Interestingly neem kernal extract at higher concentration adversely affected Steinernematid and Heterorhabditid nematodes (Rovesti and Deseo, 1989) When infective juveniles of *H. bacteriophora, H. heliothidis, N. carpocapsae, N. glaseri* and *N. bibionis* were used with different concentrations (2% w/vat, 1/2, 1/4, 1/8, 1/16 & 1/32) of neem kernal extract, *Heterorhabditis* sp. was more adversely affected than *Neoaplectana* sp. They suggested that entomophilic nematodes and neem should not be applied together.

On the other hand Rovesti *et al.* (1990) stated that infective juveniles of *H. bacteriophora, H. heliothidis, S. carpocapsae* and *S. feltiae* exposed to large number of pesticides were tolerant to most of the pesticides tested, indicating that there were good possibilities for their use in integrated pest management programmes.

Zimmerman and Cranshaw (1990) tested compatibility of pesticides with nematodes. The fungicides chlorothalonil, benomyl and pentachloronitrobenzene and the herbicide dicamba were found non toxic to *Steinernema feltiae, S. bibionis* and *Heterorhabditis* sp. while carbaryl and bendiocarb insecticides were highly toxic to *Heterorhabditis* sp. but less toxic to *S. feltiae*. However, *S. bibionis* was more sensitive to chlorpyriphos than other nematode species. Further, Diazinon was found significantly toxic only to *Heterorhabditis* sp. while the inorganic mercurial fungicide calor-clor was highly toxic to all species tested. On the whole entomogenous nematode appeared to be compatible with most of the pesticides tested.

Steinernema carpocapsae (Weiser) and *Heterorhabditis heliothidis* (Khan, Brooks and Hirschmann) have been used extensively to control white grub in turf and

pastures. Brain and Wayne (1991) found only 12 per cent grub mortality with nematode concentrations of 0.5 and 1.5 million per m^2 when applied alone or in combination with diazinon (2.25 kg ai/ha.). Comparison of the mean number of grubs recovered from each treatment 2 to 4 week after application showed significant reductions in the grub populations.

Synergistic action was found when combinations of *B. tenella* and organo phosphorus insecticides were tested against *M. melolontha* (Ferron, 1971). It was stated that mortality by mycosis was 55 per cent after 3 months in simultaneous treatment, whereas only 15 per cent in the treatment of fungus alone.

Zimmermann (1975) studied the effect of seven systemic fungicides on *B. tenella* (*B. brongniartii*) and some other entomophathogenic fungi *viz.*, *B. bassiana*, *M. anisopliae*, *Paecilomyces ferinosus* and *P. fumosoroseus*. He reported that sapral and benomyl suppressed germination of almost all the fungal spores. White calaxin inhibited the mycelial growth. Lowest effect on fungi was that of plantvax and milsten while cerobin-M was tolerated by all the fungi tested.

The insecticide, sevidol (0.5%) completely inhibited (100%) the growth of fungus, *B. brongniartii* whereas lindane, carbofuran, quinalphos provided lower inhibition. The fungicide bavistin also inhibited (100%) it in the dosage of 100 ppm, whereas fytolan, thiram, captan, mancozeb at 100 ppm provided inhibition ranging from 12 to 36 per cent (Anonymous, 1988).

Anderson *et al.* (1989) tested the compatibility and efficacy of *B. bassiana* with insecticides (abamection 0.15 EC, triflumuran 4 flowable and carbaryl 50 WP) against Colarado potato beetle and found that insecticides did not inhibit the growth of *B. bassiana* significantly. The combinations of *B. bassiana* with given insecticides were consistently more toxic than *B. bassiana* alone. In field cage tests with *B. bassiana* and insecticides mortality was generally greater than the individual agent.

Compatibility of *B. brongniartii* with commonly used insecticides and fungicides in groundnut was tested by Vyas *et al.* (1990) at the field recommended

and at half and double dosages. The insecticides used were lindane, phorate, sevidol, carbofuran and quinalphos and fungicides were fytolan, thiram, captan. bavistin and mancozeb tested at 100, 500 and 1000 ppm concentrations. The results revealed that phorate did not inhibit the growth and sporulation of the fungus, whereas lindane, sevidol, carbofuran and quinalphos inhibited the growth. In case of fungicides the lowest concentration of fytolan, captan and mancozeb did not show any inhibition, although they exhibited considerable inhibition of fungal growth at higher concentrations. Bavistin inhibited the fungal growth completely at all the three concentrations.

Influence of fungicides and insecticides on entomogenous fungus, *M. anisopliae* was studied by Moorhov e (1992). The 12 fungicides (except chlorothalonil and zineb) and 6 insecticides had no effect on spore germination and mycelial growth of *M. anisopliae* on S.D.A. plates at recommended concentrations. The growth of the fungus was completely prevented by the fungicides etridiazole, triforine and zineb and the insecticides dichlorvos and hoftathion (Triazophos) at 10 times of the recommended rate. Control of larvae (*Otiorhynchus sulcatus*) in pots treated with *M. anisopliae* plus any one of the 12 fungicides and 4 insecticides ranged from 82 to 90 per cent. The insecticide diazinon applied alone reduced larval number completely. Two other insecticides, dichlorvos and cyperpmethrin and the fungicide pyrazophos also reduced weevil population by over 50 per cent.

Likewise, Li and Holdom (1994) found that *M. anisopliae* isolates were more tolerant to the insecticides and herbicides than to the fungicides. Growth of the isolates was unaffected by Furadon (carbofuran), Temik (aldicarb) or Amicide (2, 4-D amine) but significantly reduced by Larsban (chlorpyrifos), Atrazine, Gramoxane (paraquat) and Stomp (pendimethalin) at 0.01 per cent. The fungicides (bavistin & sportak) were particularly toxic to both growth and sporulation of *M. anisopliae* even at a concentration of 0.0001 per cent.

Nicast alone or in combination with mycophatogen formulation each @ 5 per cent produced 100 per cent mortality of the third instar white grub, *H. consanguinea*

within 24 hours ∠very day, fresh grubs were released in the same container in place of dead ones and mortality was noted till 14 days. Whereas in mycophathogen alone as well as in check, no mortality was recorded by this time. However, the grubs released after 14 days in the containers having NICAST + *M. anisopliae* died within 36 days as compared to 60 days needed to cause 100 per cent mortality in *M. anisopliae* alone. Similarly, *B. brongniartii* tested in combination with Nicast took less times (26 days) than *B. brongniartii* alone (>60%). It showed that Nicast enhanced the efficacy of entomofungal pathogens when applied along with them. (Anonymous, 1995).

The insecticides, Azadirachtin (0.3 % EC) was found to be the most compatible with *M. anisopliae* exhibiting no growth inhibition at all up to 1000 ppm and only 20 per cent inhibition at the highest conc. of 2000 ppm. This was closely followed by monocrotophos, chlorpyriphos and quinalphos which did not exhibit more than 50 per cent inhibition at 1000 ppm. The test pathogen was relatively more sensitive to endosulfan showing more than 50 per cent inhibition at higher conc. but was tolerated al lower conc. (20-40 per cent inhibition).

Fungicide, blitox-50 could be tolerated well by *M. anisopliae* even at the highest concentration of 2000 ppm. The growth inhibition at this concentration was only 11.1 per cent. This was closely followed by kavach exhibiting 53.4 per cent inhibition at 2000 ppm ridomil MZ, dithane M-45 and thiram were, however, tolerated at the lower concentration. showing 25.0, 33.3 and 50.0 per cent growth inhibition at 100 ppm respectively. All the test fungicides except bavistin and baynate, allowed the growth of *M. anisopliae* satisfactorily at 10 ppm (Anonymous, 1997).

3. MATERIALS AND METHODS

The materials used and methodology adopted during the present investigation using entomogenous nematode, *Heterorhabditis bacteriophora* in combination with fungus, *Metarrhizium anisopliae* and recommended pesticides for regulating the population of *Holotrichia consanguinea* as envisaged in the plan of work is described below:

3.1 DETAILS OF THE EXPERIMENTS

3.1.1 Site and location of the experiments

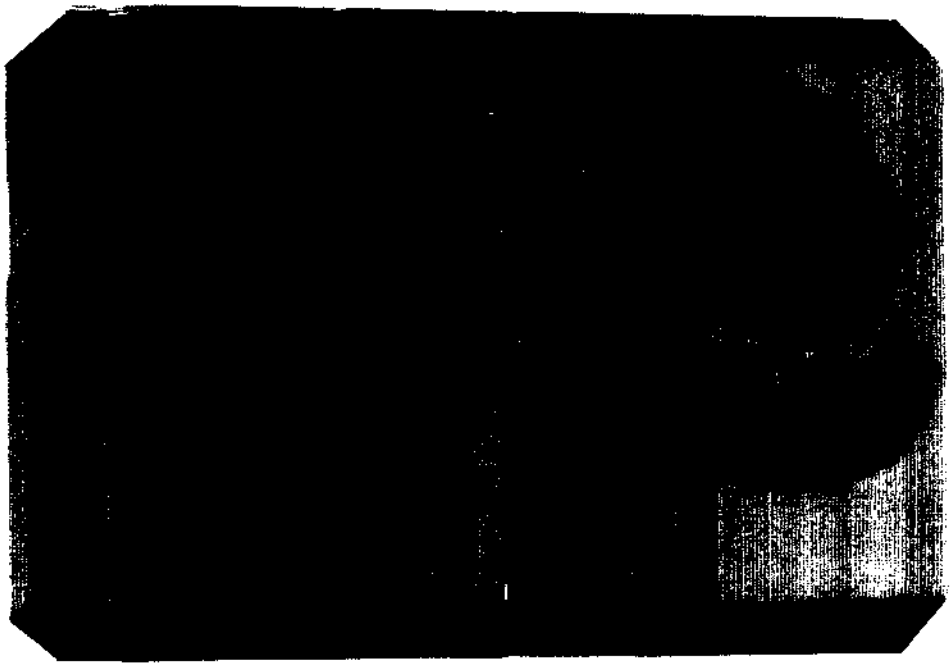
The investigations were conducted under laboratory conditions at Agricultural Research Station, Durgapura-Jaipur. All the facilities for the experiments were available in the laboratory of Project-Co-ordinator, All India Co-ordinated Research Project on White grub.

3.1.2 Rearing of white grub. *H. consanguinea*

The adult beetles of *H. consanguinea* were collected from the host plants, neem, eucalyptus, ber etc. and kept in iron cage (1x1x1 m) filled with 20 cm moist soil. Tender leaves of neem were provided in the cage as food for the beetles. The food was changed as and when required. Every day in the morning the eggs laid in the cage were collected by sieving the soil with 7 mesh sieve and transferred to the small petridishes containing loose moist soil for hatching. On hatching each grub was placed separately in earthen pot containing loose moist soil mixed with organic manure. The pearl millet seedlings were provided in the earthen pots as food for the grubs. The food was changed as and when required.

The grubs selected for tests were first individually cleaned under running tap water using wiremesh containers. The cleaned grubs were then surface sterilized with 1.0 per cent formalin, followed by rinsing with 0.1 per cent formalin and drying on filter paper.

1. Beetles of *Holotrichia consanguinea*



2. Grubs of *Holotrichia consanguinea*

3.1.3 Multiplication of nematodes, *H. bacteriophora*

The nematodes were multiplied in the laboratory on wax moth *Galleria mellonella* larvae as per method described by Dutky *et al.* (1964) partially modified by Poinar (1979). Culture of *G. mellonella* was raised on diet consisting of honey (875 ml), glycerol (100 ml), wheat flour (500 gm), wheat bran (500 gm), milk powder (500 gm) and yeast (250 gm).

For tests third instar infective juveniles (IJs) were obtained from the *in vivo* cultures. Before use the harvested nematodes were washed first with 1.0 per cent and then with 0.1 % formalin and stored for 48 hours at 7.0°C. During this period parasite juveniles and dead adults (if present) could be easily separated by passing the stored nematodes through tissue paper. The active third instar juveniles (IJs) thus obtained were used for tests and counted with nematode counting dish.

3.1.4 Examination of dead grubs with *H. bacteriophora*

To confirm the cause of death, the dead grubs were surface washed, first with 1.0 per cent and then with 0.1 per cent formalin. The washed grubs were kept on filter paper spread on the out surface of watch glass which was then kept in rounded plastic jars with lid containing 0.1 per cent formalin. The nematodes emerging from the cadaver were collected in formalin solution. It was the indication of the death of the grub by nematodes.

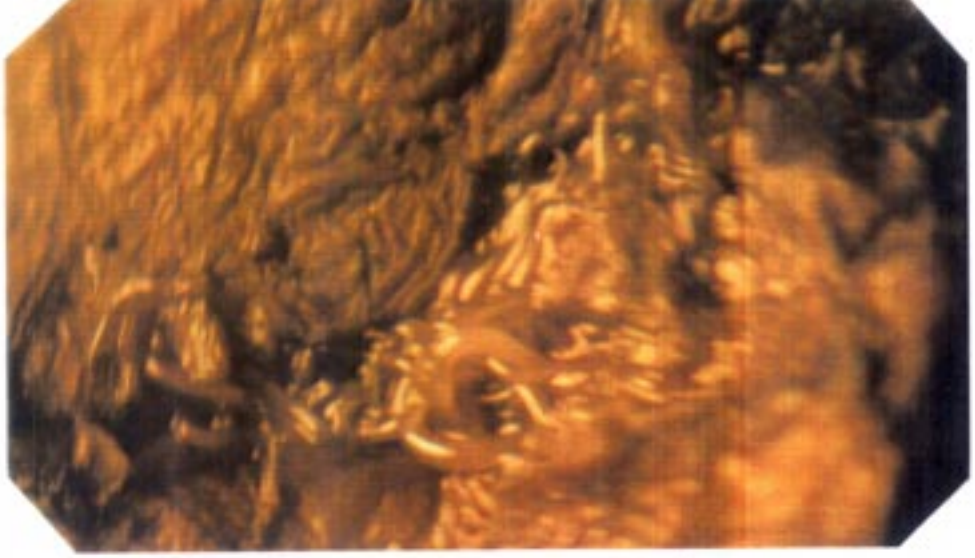
3.1.5 Mass production of fungus, *M. anisopliae*

The sorghum grains were washed and soaked in water for 10 hours. After this period excess water was drained off and the seeds were dried on a cloth. The grains were then placed in 250 ml erlmeyer flasks and sterilized in an autoclave for 20 minutes at 1.5 kq/cm^2 pressure and 121°temperature. After autoclaving the flasks were shaken vigorously for 2-3 minutes to separate the wet grains. To avoid the lumping of the grains $CaCO_3$ was mixed at the rate of 5 gm/kg grains before autoclaving.

3. Culturing of wax moth, *Gallaria mellonella* on artificial diet

4. Nematode, *Heterorhabditis bacteriophora* infected wax moth larvae on trap

5. Chamber for filteration of IJS of nematode



6. IJS of nematode, *Heterorhabditis bacteriophora*

7. Jars containing soil inoculated with nematode and white grub

8. View of observations on nematode multiplication

The autoclaved grains were inoculated with 1.0 ml of spore suspension of *M. anisopliae* (200 spores/ml) and incubated at 25°C for 15 days. After 15 days when spores got fully developed on grains, they were harvested and counted with Haemocytometer.

The spore load per gm (SLPG) was estimated by following formula (Jha, 1995)

$$SLPG = \frac{N \times V \times 10000}{W}$$

Where, N = No. of spores in the central square of Haemocytometer.
V = Volume of mounting fluid to the substrate.
W = Weight of grain.

3.1.6 Examination of dead grubs with *M. anisopliae*

The dead grubs were kept in the petridishes to note the symptoms of green muscardine disease i.e. development of green spores on dead grubs which indicated fungus as cause of the death.

3.1.7 Compatibility of pesticides with bioagents (*H. bacteriophora* and *M. anisopliae*)

Commonly used pesticides were tested for the compatibility with nematode *H. bacteriophora* and fungus, *M. anisopliae*. The insecticides taken for the studies were chloropyriphos and quinalphos and the fungicides were bavistin and thiram. The recommended doses of the pesticides tested were as shown below:

Name of pesticides	Dose	Practice
1. Chlorpyriphos 20 EC	4.0 litre/ha	Applied in standing crop with irrigation
2. Quinalphos 25 EC	4.0 litre/ha	-do-
3. Bavistin 50 WP	160 gm/ha	Used for seed treatment @ 2 gm/kg seed (groundnut)
4. Thiram 50 WP	240 gm/ha	Used for seed treatment @ 3 gm/kg seed (groundnut)

3.1.8 Statistical analysis

The data on percentage mortality (cumulative) were transformed into angular values and subjected to statistical analysis using Complete Randomized Design (CRD). Zero values in mortality were substituted by using 1/4n and 100 per cent mortality was substituted by 100-1/4n equation (Bartlett, 1947).

The mortality data were also subjected to probit analysis (Finney, 1971) to determine LD_{50} and LT_{50} values.

3.2 RELATIVE SUSCEPTIBILITY OF DIFFERENT INSTAR GRUBS OF *H. consanguinea* TO NEMATODE, *H. bacteriophora*

The experiment was conducted with all the three instars (I, II & III) of white grub by soil inoculation method in laboratory. Cylindrical plastic cups of 150 ml capacity were used in this test. Each cup was filled with 100 ml well sterilized air dried sandy loam soil. For each treatment 30 grubs were individually exposed in these containers with three replicationsi.e. 10 grubs per replication. To maintain soil moisture similar to field conditions 10 ml distilled water was mixed in soil before filling in the cups.

The required number of nematode IJs were released on wet soil surface with remaining 6 ml distilled water per cup. In each cup one healthy grub of each instar was released separately and pearl millet seedlings were provided as food. The exposed grub along with container was held at room temperature. The soil moisture in the container was maintained by adding about 2 ml distilled water per cup daily. The grub mortality was recorded daily for ten days. Simultaneously control with soil alone was run with 30 grubs for each instar.

The dead grubs were kept on filter paper traps in plastic containers to observe nematode multiplication in grubs and the nematode IJs were counted.

The mortality data were subjected to probit analysis and LD_{50} (Lethal dosage of nematode IJs required to kill 50 per cent grub) values were determined. The data on percentage mortality were also transformed into angular values and subjected to

statistical analysis using Complete Randomized Design (CRD). The details of the treatments are given below:

A. For tests with first instar grub:

Treatments (Nematode IJs/100 ml soil/grub)

1. 100 IJs
2. 200 IJs
3. 500 IJs
4. 1000 IJs
5. 2000 IJs
6. 5000 IJs
7. Control / check

B. For test with second and third instar grubs:

Treatments (Nematode IJs/100 ml soil/grub)

1. 500 IJs
2. 1000 IJs
3. 2000 IJs
4. 5000 IJs
5. 10,000 IJs
6. 20,000 IJs
7. Control / check

The nematode IJs 10, 20, 50, per 100 ml soil per grub, (as mentioned in synopsis) were also tested but grub mortality was not found hence the dosages were increased.

3.3 RELATIVE SUSCEPTIBILITY OF DIFFERENT INSTAR GRUB OF *H. consanguinea* to FUNGUS, *M. anisopliae*

This experiment was conducted with all the three instars (I, II & III) of white grub by soil inoculation method in laboratory. Cylindrical plastic cups of 150 ml capacity were used in this test. Each cup was filled with 100 ml well sterilized air

dried sandy-loam soil. For each treatment 30 grubs were individually exposed in these containers with three replications i.e. 10 grubs per replication. To maintain soil moisture similar to field conditions distilled water @ 10 ml/100 ml soil was mixed in soil before filling in the cups.

The spore suspension of desired concentration from stock suspension ($1x10^{10}$ spores/m.l.) with remaining 6 ml distilled water was poured in each cup. For getting uniform suspension of fungal spores few drops of tween-80 were added to spore suspension. The treated soil was stirred for uniform mixing of spores in the soil. In each cup one healthy grub of each instar was released separately and pearl millet seedlings were provided as food. The exposed grub along with container was held at room temperature. The soil moisture in the container was maintained by adding distilled water to soil as and when required i.e. about 2 ml per cup per day. The grub mortality for I instar was recorded daily for 16 days and for II and III instar at 5 days interval for 30 days. Separate control with soil alone was run with 30 grubs for each instar.

The dead grubs were put on PDA (Potato Destrose Agar) for sporulation to confirm the cause of mortality. The mortality data were subjected to probit analysis (Finney 1971) and LD_{50} (lethal spores of fungus required to kill 50 per cent grubs) values were determined. The data on percentage mortality were transformed into angular values and subjected to statistical analysis using CRD. The details of the treatments are given below:

A. For tests with first instar grub:

Treatments (Fungus spores/100 ml soil/grub)

1. $1x10^7$ spores
2. $5x10^7$ spores
3. $1x10^8$ spores
4. $5x10^8$ spores
5. $1x10^9$ spores
6. $5x10^9$ spores

7. $1x10^{10}$ spores
8. Control

B. For test with second and third instar grub:

Treatments (Fungus spores/100 ml soil/grub)

1. $1x10^{8}$ spores
2. $5x10^{8}$ spores
3. $1x10^{9}$ spores
4. $5x10^{9}$ spores
5. $1x10^{10}$ spores
6. $5x10^{10}$ spores
7. $1x10^{11}$ spores
8. Control

The fungus spore concentration $1x10^{6}$ and $5x10^{6}$ per 100 ml soil per grub were also tested but the grub mortality was not found, hence, the dosages were increased.

3.4 COMPATIBILITY OF NEMATODE WITH FUNGUS AND NEMATODE AND FUNGUS WITH PESTICIDES

3.4.1. Compatibility of nematode, *H. bacteriophora* with fungus, *M. anisopliae* in relation to different instar grubs of *H. consanguinea*

The experiment was conducted with all the three instars (I, II & III) of white grub by soil inoculation method in laboratory. The methods used to conduct the experiment was similar to the methods used to test the relative susceptibility of white grub with nematode and fungus.

The required number of nematode IJs and fungus spore suspension of desired concentration with 6 ml distilled water was poured in each cup. The grub mortality in I instar was recorded every alternate day for 15 days and for II and III instar for 10 days alternately and after this at 5 days interval up to 30 days.

The dead grubs were kept on filter paper traps in plastic containers to observe nematode multiplication in grubs and sporulation of fungus. The nematode IJs were counted.

The mortality data were subjected to probit analysis and LT_{50} was determined. The statistical analysis was also carried out to compare treatments, the details of the treatments are given below:

A. For test with first instar grubs:

(Treatment dosage/100 ml soil/grub)

1. Nematode (2000 IJs)
2. Nematode (1000 IJs)
3. Fungus ($1x10^9$ spores)
4. Fungus ($5x10^8$ spores)
5. Nematode (2000 IJs) + Fungus ($1x10^9$ spores)
6. Nematode (5,000 IJs) + Fungus ($5x10^8$ spores)
7. Control

B. For test with second instar grub:

(Treatment dosage/100 ml soil/grub)

1. Nematode (10000 IJs)
2. Nematode (5000 IJs)
3. Fungus (1×10^{10} spores)
4. Fungus (5×10^9 spores)
5. Nematode (10000 IJs) + fungus (1×10^{10} spores)
6. Nematode (5000 IJs) + fungus (5×10^9 spores)
7. Control

C. For test with third instar grub:

(Treatment dosage/100 ml soil/grub)

1. Nematode (20000 IJs)
2. Nematode (10000 IJs)

3. Fungus ($5x10^{10}$ spores)
4. Fungus ($1x10^{10}$ spores)
5. Nematode (20000 IJs) + Fungus ($5x10^{10}$ spores)
6. Nematode (10000 IJs) + Fungus ($1x10^{10}$ spores)
7. Control / check

3.4.2. Compatibility of nematode, *H. bacteriophora* with insecticides (chlorpyriphos & quinalphos) in relation to different instar grubs of *H. consanguinea*

The experiment was conducted with all the three instars (I, II & III) of white grub by soil inoculation method in laboratory. Cylindrical plastic cups of 150 ml capacity were used in this test. Each cup was filled with 100 ml well sterilized air dried sandy-loam soil. For each treatment 30 grub were individually exposed in these containers with three replication i.e. 10 grubs per replication. To maintain soil moisture similar to field conditions 10 ml distilled water was mixed in soil before filling in the cups. The required quantity of insecticides i.e. 0.8 mg and 1.0 mg for higher dosage and 0.4 mg and 0.5 mg for lower dosage of chlorpyriphos 20 E C. and quinalphos 25 EC. respectively, per cup was also mixed in soil with 10 ml distilled water per cup. The required number of nematode IJs were released on wet soil surface with remaining 6 ml distilled water per cup. In each cup one healthy grub of each instar was released separately and pearl-millet seedlings were provided as food. The exposed grub along with container was held at room temperature. The soil moisture in the container was maintained by adding distilled water to soil as and when required i.e. about 2 ml per cup per day. The grub mortality was recorded daily for eleven days; separate control with soil alone was run with 30 grubs for each instar.

The dead grubs were kept on filter paper trap in plastic containers to observe nematode multiplication in grubs treated with nematode and insecticides simultaneously and nematode alone. The nematode IJs were harvested and counted.

The mortality data were subjected to probit analysis and LT_{50} values were determined. Statistical analysis was also carried out to test the significance.

The details of the treatments are given below:

A. For test with first instar grub:

Treatment (Dosage/100 ml soil/grub)

1. Nematode 2000 IJs
2. Nematode 1000 IJs
3. Chlorpyriphos 0.8 mg
4. Chlorpyriphos 0.4 mg
5. Quinalphos 1.0 mg
6. Quinalphos 0.5 mg
7. Nematode 2000 IJs + Chlorpyriphos 0.8 mg
8. Nematode 1000 IJs + Chlorpyriphos 0.4 mg
9. Nematode 2000 IJs + Quinalphos 1.0 mg
10. Nematode 1000 IJs + Quinalphos 0.5 mg
11. Control.

B. For test with second instar grub:

Treatment (Dosage/100 ml soil/grub)

1. Nematode 10000 IJs
2. Nematode 5000 IJs
3. Chlorpyriphos 0.8 mg
4. Chlorpyriphos 0.4 mg
5. Quinalphos 1.0 mg
6. Quinalphos 0.5 mg
7. Nematode 10000 IJs + Chlorpyriphos 0.8 mg
8. Nematode 5000 IJs + Chlorpyriphos 0.4 mg
9. Nematode 10000 IJs + Quinalphos 1.0 mg
10. Nematode 5000 IJs + Quinalphos 0.5 mg
11. Control.

C. For test with third instar grub:

Treatment (dosage /100 ml soil/grub)

1. Nematode 20000 IJs

2. Nematode 10000 IJs
3. Chlorpyriphos 0.8 mg
4. Chlorpyriphos 0.4 mg
5. Quinalphos 1.0 mg
6. Quinalphos 0.5 mg
7. Nematode 20000 IJs + Chlorpyriphos 0.8 mg
8. Nematode 10000 IJs + Chlorpyriphos 0.4 mg
9. Nematode 20000 IJs + Quinalphos 1.0 mg
10. Nematode 10000 IJs + Quinalphos 0.5 mg
11. Control.

3.4.3 Compatibility of nematode, *H. bacteriophora* with fungicides (bavistin & thiram) in relation to different instar grubs of *H. consanguinea*

The experiment was conducted with all the three instars (I, II & III) of white grub by soil inoculation method in laboratory. The methodology used to conduct the experiment was similar to methodology used to test the compatibility of *H. bacteriophora* with insecticides.

The required quantity of fungicide i.e. 0.16 mg & 0.24 mg for higher dosage and 0.08 mg and 0.12 mg for lower dosage of bavistin and thiram, respectively per cup was mixed in soil before filling in the cups. The nematode IJs were released on wet soil surface with remaining 6 ml distilled water per cup.

The grub mortality was recorded at one day interval for ten days and the data were subjected for statistical analysis using C.R.D. and also for probit analysis to calculated LT_{50} values. The details of the treatments are given below:

A. For test with first instar grub:

Treatment (Dosage/100 ml soil/grub)

1. Nematode 2000 IJs
2. Nematode 1000 IJs
3. Bavistin 0.16 mg

4. Bavistin 0.08 mg
5. Thiram 0.24 mg
6. Thiram 0.12 mg
7. Nematode 2000 IJs + Bavistin 0.16 mg
8. Nematode 1000 IJs + Bavistin 0.08 mg
9. Nematode 2000 IJs + Thiram 0.24 mg
10. Nematode 1000 IJs + Thiram 0.12 mg
11. Control.

B. For test with second instar grub:

Treatment (Dosage/100 ml soil/grub)

1. Nematode 10000 IJs
2. Nematode 5000 IJs
3. Bavistin 0.16 mg
4. Bavistin 0.08 mg
5. Thiram 0.24 mg
6. Thiram 0.12 mg
7. Nematode 10000 IJs + Bavistin 0.16 mg,
8. Nematode 5000 IJs + Bavistin 0.08 mg,
9. Nematode 10000 IJs + Thiram 0.24 mg,
10. Nematode 5000 IJs + Thiram 0.12 mg,
11. Control.

C. For test with third instar grub:

Treatment (Dosage/100 ml soil/grub)

1. Nematode 20000 IJs
2. Nematode 10000 IJs
3. Bavistin 0.16 mg
4. Bavistin 0.08 mg
5. Thiram 0.24 mg
6. Thiram 0.12 mg
7. Nematode 20000 IJs + Bavistin 0.16 mg

8. Nematode 10000 IJs + Bavistin 0.08 mg
9. Nematode 20000 IJs + Thiram 0.24 mg
10. Nematode 20000 IJs + Thiram 0.12 mg
11. Control.

3.4.4 Compatibility of fungus, *M. anisopliae* with insecticides and fungicides

Insecticides and fungicides commonly used in managing white grub either in field or for seed treatment were tested for the compatibility with *M. anisopliae* by poisoned food technique. The insecticides taken for the studies were chlorpyriphos and quinalphos and the fungicides were bavistin and thiram in food poison tests nutrient glucose medium was used. Three levels *viz.* 100, 500 and 1000 ppm concentration of each were taken and for each level ten flask of 100 ml capacity were maintained. Separate control was maintained without pesticides. After autoclaving the flasks required quantity of pesticides was added to the medium (25 ml) and thoroughly mixed by shaking.

The flask were inoculated with fungal growth (2 mm disc) from 10 days old laboratory culture. The flasks were incubated at 25°C for ten days after which weight of mycelial mat was recorded in each flask. The mean weight of mycelial mat based on 10 flasks was calculated for each concentration and treatment. The per cent growth inhibition of *M. anisopliae* with each pesticides was calculated by the following formula as per Vincent (1947).

$$Percent\ Inhibition = \frac{100 \times (C-T)}{C}$$

Where, C = Average mycelial mat weight in control set.

T = Average mycelial mat weight in experiment set.

4.4.5 Compatibility of nematode, *H. bacteriophora* and fungus, *M. anisopliae* with fungicides and insecticides in relation to first instar grubs of *H. consanguinea*

The experiment was conducted with first instar white grub by soil inoculation method in laboratory. Cylindrical plastic cups of 150 ml capacity were used in this

test. Each cup was filled with 100 ml well sterilized air dried sandy-loam soil for each treatment 30 grub were individually exposed in these containers with three replications i.e. 10 grubs per replicate to maintain soil moisture similar to field conditions 10 ml distilled water was mixed in soil before filling in the cups. The required quantity of insecticides (chlorpyriphos & quinalphos) and fungicides (bavistin & thiram) were also mixed in soil before filling in the cups with 10 ml distilled water. The required number of nematode IJs and fungus spores were released on wet soil surface with remaining 6 ml distilled water per cup and mixed. In each cup one healthy grub of first instar was released separately and pearl-millet seedling were provided as food. The exposed grub along with container was held at room temperature. The soil moisture in the container was maintained by adding distilled water to soil as and when required i.e. about 2 ml per cup per day. The grub mortality was recorded at one day interval for seven days. Separate control with soil alone was run with 30 grubs.

The mortality data were subjected to probit analysis and LT_{50} values were calculated. The statistical analysis were also carried out. The details of the treatments are given below:

Treatment (Dosage/100 ml soil/grub)

1. Nematode (2000 IJs) + Fungus (1 x 10^9 spores) + Chlorpyriphos (0.8 mg)
2. Nematode (2000 IJs) + Fungus (1 x 10^9 spores) + Quinalphos (1.0 mg)
3. Nematode (1000 IJs) + Fungus (5 x 10^8 spores) + Chlorpyriphos (0.4 mg)
4. Nematode (1000 IJs) + Fungus (5 x 10^8 spores) + Quinalphos (0.5 mg)
5. Nematode (2000 IJs) + Fungus (1 x 10^9 spores) + Bavistin (0.16 mg)
6. Nematode (2000 IJs) + Fungus (1 x 10^9 spores) + Thiram (0.24 mg)
7. Nematode (1000 IJs) + Fungus (5 x 10^8 spores) + Bavistin (0.08 mg)
8. Nematode (1000 IJs) + Fungus (5 x 10^8 spores) + Thiram (0.12 mg)
9. Control.

4. RESULTS

4.1 RELATIVE SUSCEPTIBILITY OF DIFFERENT INSTAR GRUBS OF *H. consanguinea* TO NEMATODE, *H. bacteriophora*

Consequent to experiment conducted on the efficacy of *Heterorhabditis bacteriophora* against different instars (I, II & III) of *Holotrichia consanguinea*. Mortality ranging from 0 to 100 per cent depending upon the dosage and period of inoculation was observed. The findings showed that all the instars of white grub were susceptible to nematode, *H. bacteriophora* at the dosages ranging from 100 to 20,000 IJs/100 mlsoil/grub.

4.1.1 Efficacy against first instar grubs of *H. consanguinea*

Relative efficacy of *H. bacteriophora* against first instar *H. consanguinea* was tested with dosage ranging from 100 to 5000 IJs/100 mlsoil/grub. All the treatments were found significantly effective over control, significant difference in efficacy also existed amongst treatments (Table 1.a).

Three days after exposure 6.67 to 63.33 per cent grub mortality was recorded; highest was 63.33 per cent in the treatment of 5000 IJs/100 ml soil/grub though it was at par with the treatment 2000 IJs/100 ml soil/grub (50.0 per cent mortality). The lowest grub mortality (6.67%) was found with 100 IJs/100 ml soil/grub it was at par with the treatment 200 IJs/100 ml soil/grub. The treatment of 500 IJs/100 ml soil/grub registered 16.67 per cent mortality. Statistically the two treatments of 500 IJs and 200 IJs/100 ml/grub were at par. The treatment with 1000 IJs/100 ml soil/grub registered 36.67 per cent mortality and it was at par with the treatment of 2000 IJs/100 ml soil/grub (50% mortality).

After five days of exposure highest mortality got increased to 80.0 per cent in the treatment 5000 IJs/100 ml soil/grub and the lowest mortality also got increased to 16.67 per cent in the treatment 100 IJs/100 ml soil/grub but statistically it was at par with the treatments of 200 and 500 IJs/100 ml soil/grub where the mortality was

20.0 and 23.33 per cent respectively. The treatments of 2000 and 1000 IJs/100 ml soil/grub registered 50.0 and 40.0 per cent mortality respectively and both were statistically at par.

As the period of exposure increased the mortality also got increased as such seven days after exposure the highest grub mortality (93.33%) was found with the highest number of IJs i.e. 5000/100 ml soil/grub and lowest mortality was 26.67 per cent in the treatment 100 IJs/100 ml soil/grub which was at par with the treatments of 200 and 500 IJs/100 ml soil/grub where the mortality was of 30.0 per cent in both the treatments. In the treatments of 2000 and 1000 IJs/100 ml soil/grub 80.0 and 50.0 per cent grub mortality was observed.

After ten days of exposure mortality ranged from 30 to 100 per cent while cent per cent mortality was found in the treatment of 5000 IJs/100 ml soil/grub. The lowest (30.0%) was found in the treatment 100 IJs/100 ml soil/grub. The treatments of 2000, 1000 and 500 and 200 IJs/100 ml soil/grub registered of 90.0, 60.0 and 53.33 and 40.0 per cent mortality respectively.

The number of IJs harvested from dead grubs varied from 8500 to 24800 per grub depending upon the number of IJs exposed per grub. The highest 24 800 IJs/grub were harvested in the treatment 5000 IJs/100 ml soil/grub and lowest 8500 IJs/grub in the treatment 100 IJs/100 ml soil/grub. In the treatment of 200, 500, 1000 and 2000 IJs/100 ml soil/grub, the number of IJs harvest were 11500, 13200, 16800 and 22500 per grub, respectively (Table 1.a).

The median lethal dosage (LD_{50}) varied from 2320 to 367 IJs/100 ml soil/grub depending upon the period of exposure. The LD_{50} values after 3, 5, 7 and 10 days of exposure were 2320, 1620, 670 and 367 IJs/100 ml soil/grub respectively (Table 2; Fig 1.a).

4.1.3 Efficacy against second instar grubs of *H. consanguinea*

Against II instar *H. consanguinea* 500 to 20,000 nematode IJs were tested and the mortality due to these varied from 0 to 100 per cent (Table 1.b).

Table 1.a **Relative susceptibility of first instar grubs of *Holotrichia consanguinea* to nematode, *Heterorhabditis bacteriophora***

S. No.	Dosage (Nematode IJs/100 ml soil/grub)	Mean per cent mortality Days after exposure				Mean No. of nematode IJs harvested/grub
		3	5	7	10	
1.	100	6.67 (14.97)	16.67 (24.10)	26.67 (31.09)	30.00 (33.21)	8500
2.	200	10.00 (18.43)	20.00 (26.67)	30.00 (33.21)	40.00 (39.23)	11500
3.	500	16.67 (24.10)	23.33 (28.88)	30.00 (33.21)	53.33 (46.91)	13200
4.	1000	36.67 (37.27)	40.00 (39.23)	50.00 (45.00)	60.00 (50.77)	16800
5.	2000	50.00 (45.00)	50.00 (45.00)	80.00 (63.43)	90.00 (71.57)	22500
6.	5000	63.33 (52.72)	80.00 (63.43)	93.33 (75.03)	100.00 (89.01)	24800
7.	Control	0.00 (0.99)	3.33 (10.51)	3.33 (10.51)	3.33 (10.51)	-
	SEm±	2.647	3.284	3.502	2.987	
	CD (P=0.05)	8.030	9.963	10.624	9.060	

Figures in parenthesis are angular transformed values

Three days after exposure grub mortality ranged from 0 to 56.67 per cent and the highest mortality of 56.67 per cent was found in the treatment 20,000 IJs/100 ml soil/grub, but it was at par with the treatments of 10,000 and 5,000 IJs/100 ml soil/grub (50.0 & 40.0% mortality). The treatment of 500 IJs/100 ml soil/grub had not shown any effect against the second instar grubs. The treatments of 1000 and 2000 IJs/100 ml soil/grub registered the mortality of 10.0 and 20.0 per cent respectively which were at par statistically.

After five days of exposure too, all the treatments proved significantly superior over control. The highest grub mortality (73.33%) was found in the treatment 20,000 IJs/100 ml soil/grub which was at par with treatment 10000 IJs/100 ml soil/grub (66.67% mortality). The lowest 6.67 per cent mortality was found in the treatment 500 IJs/100 ml soil/grub. Initially this treatment had shown no effect. In rest of the treatments i.e. 1000, 2000 and 5000 IJs/100 ml soil/grub the mortality was 20.0, 36.67 and 50.0 per cent, respectively.

After seven days of exposure mortality on higher side increased to 96.67 per cent in the treatment 20.000 IJs/100 ml soil/grub which was at par with the treatment 10,000 IJs/100 ml soil/grub (86.67% mortality) and the lowest mortality also increased to 16.7 per cent in treatment 500 IJs/100 ml soil/grub. The treatments of 1000, 2000 and 5000 IJs/100 ml soil/grub registered 33.33, 56.67 and 63.33 per cent mortality respectively. However, the treatments of 2000 and 5000 IJs/100 ml soil/grub were at par statistically.

Similar trend in grub mortality was found after ten days of treatment and all the treatments proved significantly superior over control. The treatments of 10.000 and 20,000 IJs/100 ml soil/grub caused cent per cent mortality in the second instar. The lowest mortality also increased to 30.0 per cent in the treatment 500 IJs/100 ml soil/grub which was at par with the treatment 1000 IJs/100 ml soil/grub (43.33% mortality). The treatments of 2000 and 5000 IJs/100 ml soil/grub also registered higher mortality of 70.0 and 86.67 per cent respectively.

Table 1.b **Relative susceptibility of second instar grubs of *H. consanguinea* to nematode, *Heterorhabditis bacteriophora***

S. No.	Dosage (Nematode IJs/100 ml soil/grub)	Mean per cent mortality Days after exposure				Mean No. of nematode IJs harvested/grub
		3	5	7	10	
1.	500	0.00 (0.99)	6.67 (14.97)	16.67 (24.10)	30.00 (33.21)	18000
2.	1000	10.00 (18.43)	20.00 (26.67)	33.33 (35.26)	43.33 (14.17)	21200
3.	2000	20.00 (26.57)	36.67 (37.27)	56.67 (48.83)	70.00 (56.79)	28000
4.	5000	40.00 (39.23)	50.00 (45.00)	63.33 (52.73)	86.67 (68.59)	32500
5.	10000	50.00 (45.00)	66.67 (54.74)	86.67 (68.59)	100.00 (89.01)	40800
6.	20000	56.67 (48.83)	73.33 (58.91)	96.67 (79.49)	100.00 (89.01)	41200
7.	Control	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	3.33 (10.51)	-
	SEm±	3.212	3.047	3.666	2.884	
	CD (P=0.05)	9.743	9.244	11.120	8.748	

Figures in parenthesis are angular transformed values

The number of IJs harvested also got increased significantly and the number ranged from 18,000 to 41,200 per grub from the treatments of 500 to 20,000 IJs/100 ml soil/grub. The number of IJs harvested from the treatments of 1000, 2000, 5000 and 10,000 IJs/100 ml soil/grub were 21,200, 28,500, 32,500 and 40,800 respectively (Table 1.b).

The median lethal dosage (LD_{50}) varied from 10771 to 1143 IJs/100 ml soil/grub depending upon period of exposure. The LD_{50} values after 3, 5, 7 and 10 days of exposure were 10771, 5017, 2151 and 1143 IJs/100 ml soil/grub respectively(Table 2; Fig. 1.b).

4.1.3 Efficacy against third instar grubs of *H. consanguinea*

Against third instar the dosages tested were same as that of second instar i.e. 500 to 20,000 IJs/100 ml soil/grub (Table 1.c).

Three days after exposure the highest grub mortality (43.33%) was found in the treatment 20000 IJs/100 ml soil/grub and the lowest mortality (10.0%) was found in the treatment of 2000 IJs/100 ml soil/grub but there existed no significant difference between the dosages of 20000 IJs and 10000 IJs and between 5000 IJs and 2000 IJs. The treatment of 500 and 1000 IJs/100 ml soil/grub had not proved lethal at all to this instar within the period of three days.

Five days after exposure mortality increased to 66.67 per cent in the treatment of 20,000 IJs/100 ml soil/grub which was at par with 10,000 IJs/100 ml soil/grub (56.67% mortality). The lowest grub mortality (6.67%) was found in the treatment of 1000 IJs/100 ml soil/grub. The treatments of 2000 and 5000 IJs/100 ml soil/grub resulted in the mortality of 23.33 and 40.0 per cent. However, in the treatment of 500 IJs/100 ml soil/grub, there was no mortality at all.

Similar trend was observed seven and ten days after exposure where all the treatments proved significantly superior over control, but differed significantly with each other. Cent per cent mortality was found in the treatment 20,000 IJs/100 ml soil/grub after ten days of exposure while in the treatment of 500 IJs/100 ml soil/grub

only 6.67 and 13.33 per cent mortality was found after 7 and 10 days of exposure, respectively. However, this treatment was not lethal up to 5 days of exposure against third instar. The treatments of 1000, 2000, 5000 and 10,000 IJs/100 ml soil/grub resulted in the mortality of 26.67, 56.67, 73.33 and 86.67 per cent respectively after ten days of exposure.

The nematode IJs harvested from dead grubs varied from 28,200 to 80,500 per grub. The highest 80,500 IJs were harvested from the treatment 20,000 IJs/100 ml soil/grub whereas lowest 28,200 were recovered from the treatment 500 IJs/100 ml soil/grub. The nematode IJs harvested from the treatments of 1000, 2000, 5000 and 10,000 IJs/100 ml soil/grub were 36,400, 45,000, 48,200 and 62,000 respectively (Table 1.c).

The median lethal dosage (LD_{50}) after 3, 5, 7 and 10 days of exposure were calculated as 19,901, 8293, 4848 and 2143 IJs/100 ml soil/grub respectively (Table 2; Fig. 1.c).

H. consanguinea grubs in all the three instars were found to be susceptible to *H. bacteriophora*. The per cent grub mortality increased as the inoculum dosage of IJs and period of exposure increased. The nematode IJs harvested from dead grub depended on the inoculum dosage and size of grub. Susceptibility decreased with the instars as first instar grubs were highly susceptible.

Table 1.c **Relative susceptibility of third instar grubs of *Holotrichia consanguinea* to nematode, *Heterorhabditis bacteriophora***

S. No.	Dosage (Nematode IJs/100 ml soil/grub)	Mean per cent mortality Days after exposure				Mean No. of nematode IJs harvested/grub
		3	5	7	10	
1.	500	0.00 (0.99)	0.00 (0.99)	6.67 (14.97)	13.33 (21.41)	28200
2.	1000	0.00 (0.99)	6.67 (14.97)	16.67 (24.10)	26.67 (31.09)	36400
3.	2000	10.00 (18.43)	23.33 (28.88)	33.33 (35.26)	56.67 (48.83)	45000
4.	5000	16.67 (24.10)	40.00 (39.23)	50.00 (45.00)	73.33 (58.91)	48200
5.	10000	40.00 (39.23)	56.67 (48.83)	66.67 (54.74)	86.67 (68.59)	62000
6.	20000	43.33 (41.17)	66.67 (54.74)	80.00 (63.43)	100.00 (89.01)	80500
7.	Control	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	-
	SEm±	3.229	2.575	2.651	2.976	
	CD (P=0.05)	9.795	7.811	8.043	9.029	

Figures in parenthesis are angular transformed values

Table 2 **Relative susceptibility of different instar grubs of *Holotrichia consanguinea* to nematode, *Heterorhabditis bacteriophora* based on LD_{50} values**

S.No.	Days after treatment	Time (log) - Kill (probit)	
		Regression equation	LD_{50}
		First instar *Holotrichia consanguinea*	
1.	3	Y = 1.0463 + 1.1748X	2320
2.	5	Y = 1.3557 + 1.1355X	1620
3.	7	Y = 1.3365 + 1.2962X	670
4.	10	Y = 1.3359 + 1.4284X	367
		Second instar *Holotrichia consanguinea*	
1.	3	Y (-) 0.2273 + 1.2964 X	10771
2.	5	Y 0.4001 + 1.2431 X	5017
3.	7	Y (-) 0.3577 + 1.6077 X	2151
4.	10	Y (-) 1.2880 + 2.0562 X	1143
		Third instar *Holotrichia consanguinea*	
1.	3	Y (-) 1.6039 + 1.5362 X	19901
2.	5	Y (-) 1.0029 + 1.5319 X	8293
3.	7	Y (-) 0.1757 + 1.4043 X	4848
4.	10	Y (-) 1.4828 + 1.9463 X	2142

Y = Probit kill
X = log of dosage (Nematode IJs/100 ml soil/grub)
LD_{50} = Lethal dosage calculated to give 50 per cent mortality

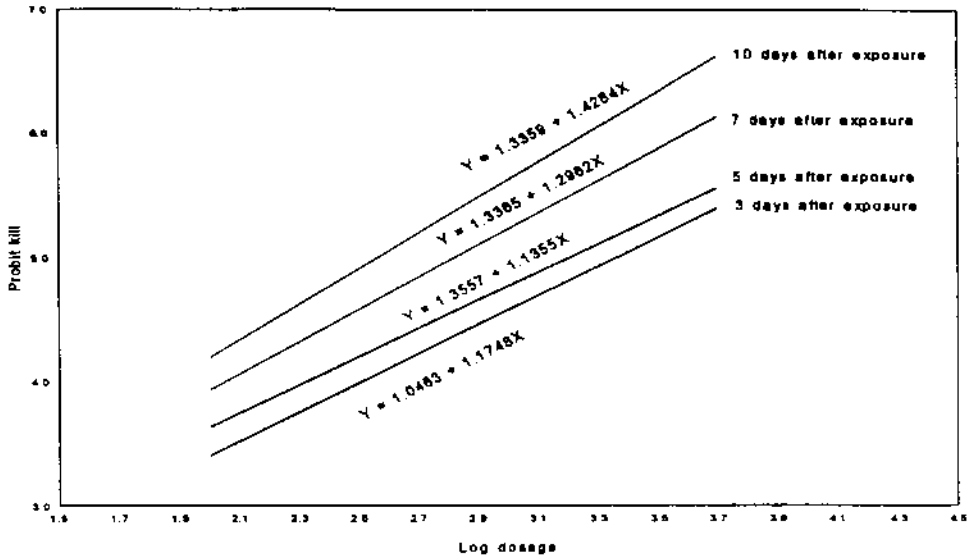


Fig 1.a Log dosage-probit kill line for susceptibility of first instar grubs of *Holotrichia consanguinea* to nematode, *Heterorhabditis bacteriophora*.

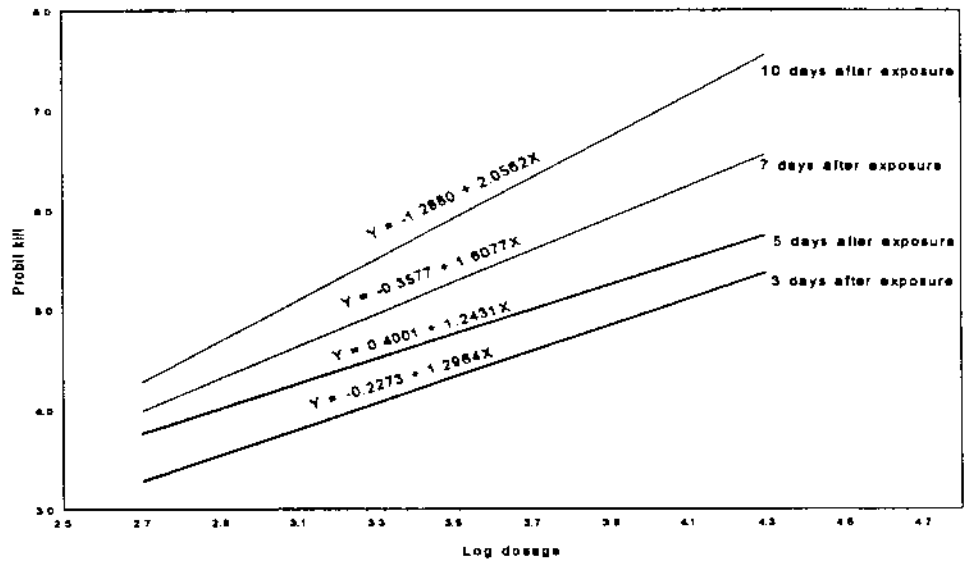


Fig 1.b Log dosage-probit kill line for susceptibility of second instar grubs of *Holotrichia consanguinea* to nematode, *Heterorhabditis bacteriophora*

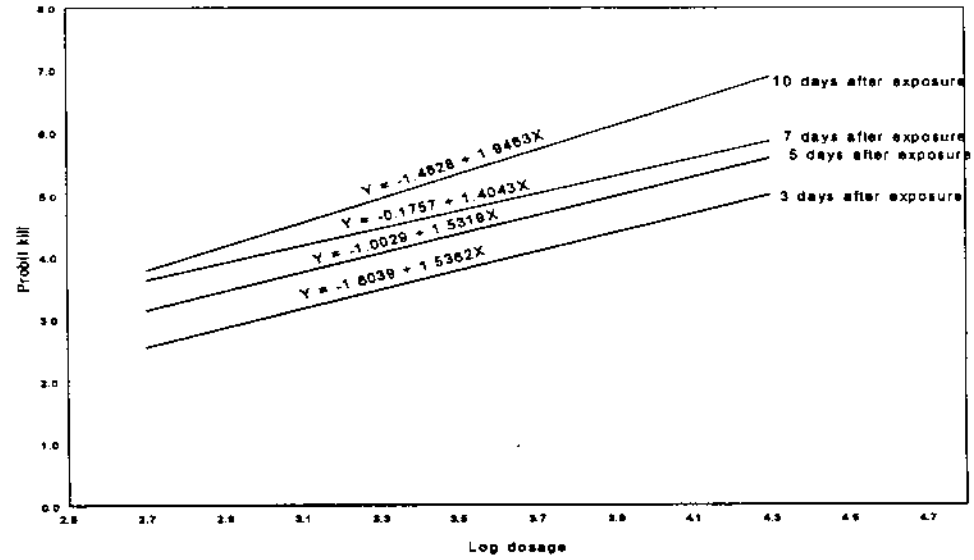


Fig 1.c Log dosage-probit kill line for susceptibility of third instar grubs of *Holotrichia consanguinea* to nematode, *Heterorhabditis bacteriophora*

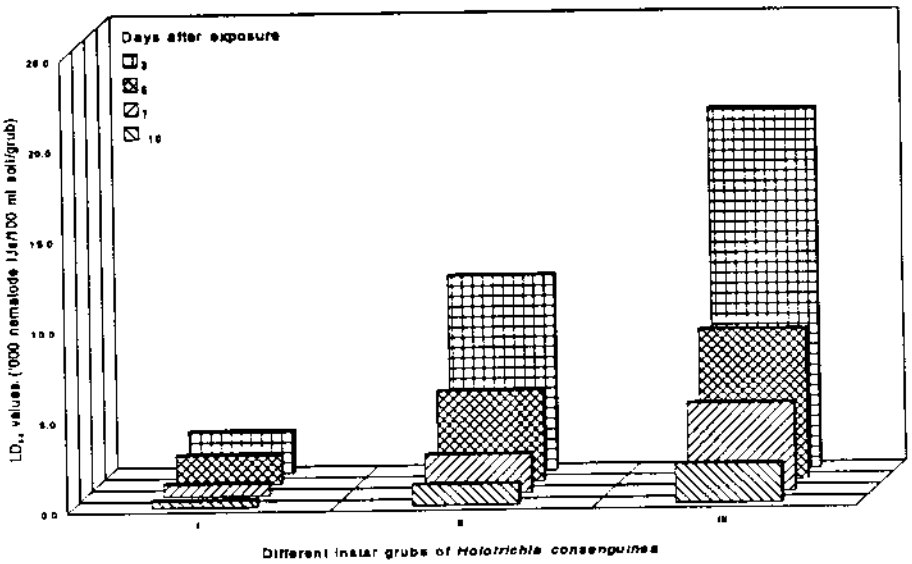


Fig 2. Relative susceptibility of different instar grubs of *Holotrichia consanguinea* to nematode, *Heterorhabditis bacteriophora* based on LD_{50} values

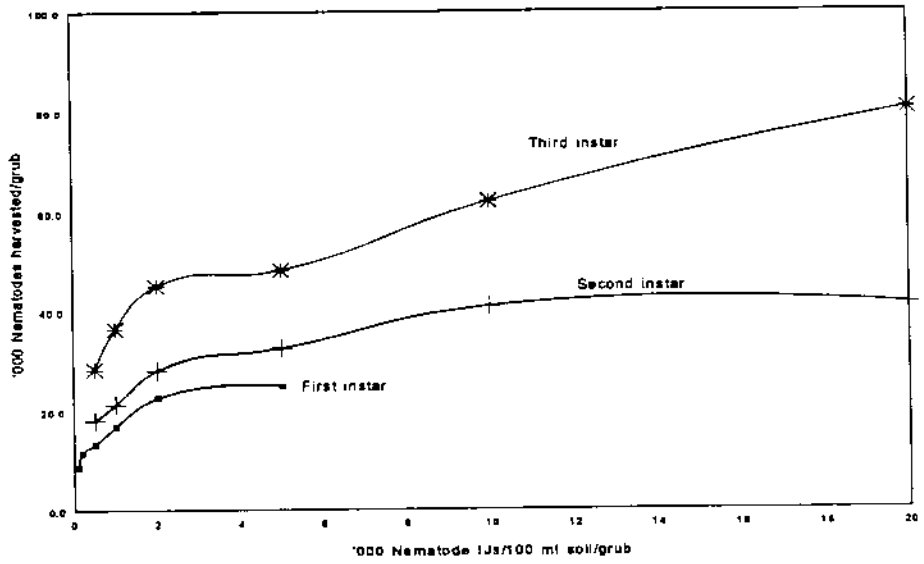
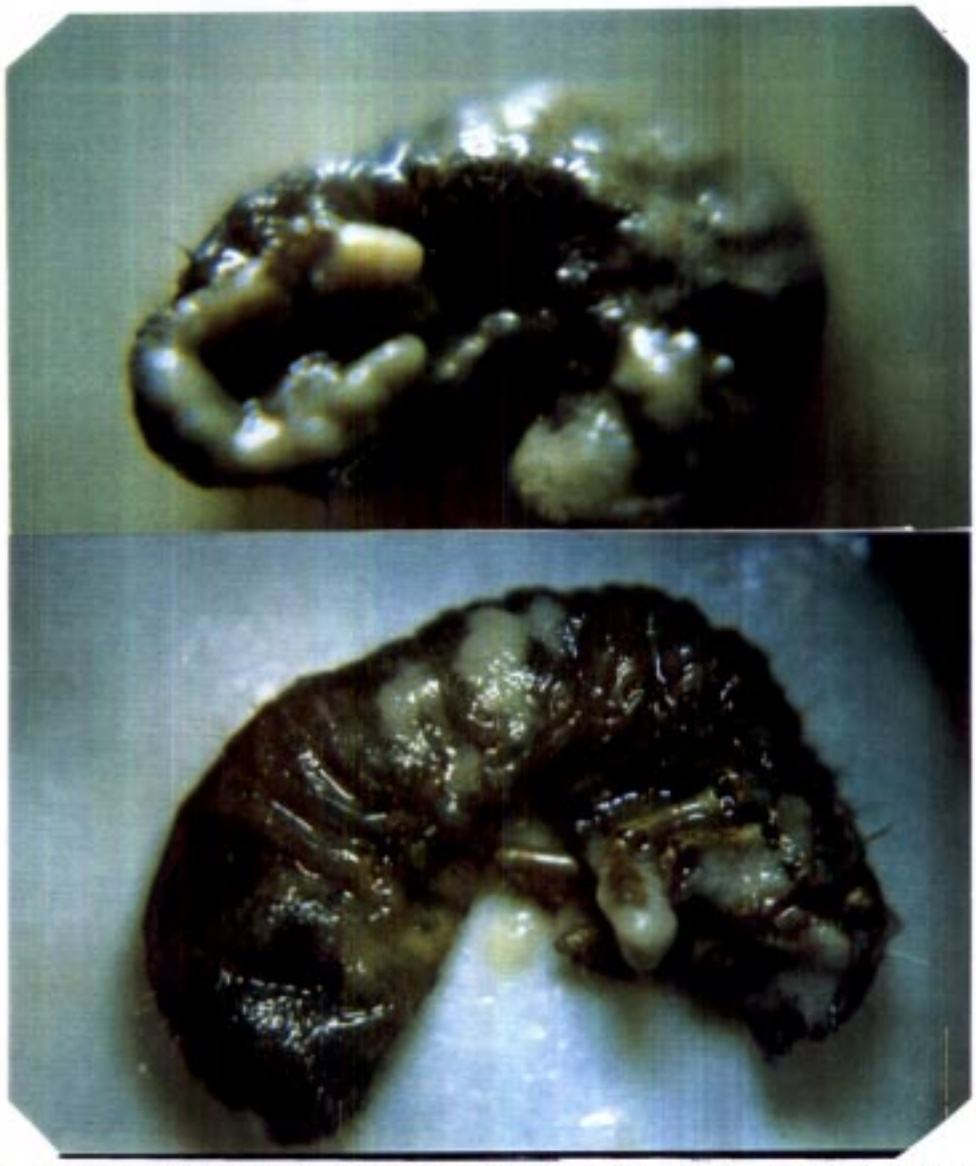
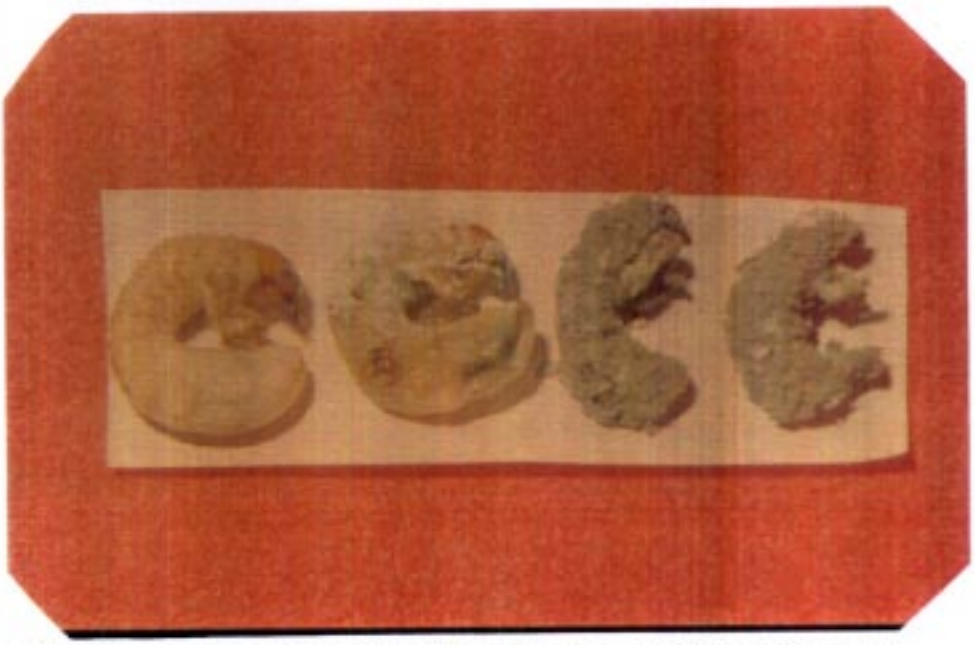


Fig 3. Number of nematode, *Heterorhabditis bacteriophora* harvested from exposed instars of *Holotrichia consanguinea*.



9. Different stages of multiplication of nematode, *Heterorhabditis bacteriophora* on infected grubs



10. Different stages of growth of fungus, *Metarrhizium anisopliae* on infected grubs

4.2 RELATIVE SUSCEPTIBILITY OF DIFFERENT INSTAR GRUBS OF *H. consanguinea* TO FUNGUS, *M. anisopliae*

Efficacy of *Metarrhizium anisopliae* against different instars (I, II & III) of *Holotrichia consanguinea* was tested and mortality upto 70 per cent depending upon the dosage and period of inoculum was observed. The findings showed that all the instars of white grub were susceptible to *M. anisopliae* but required longer lethal time than nematodes. The fungal dosage ranged from 1 x 10^7 to 1 x 10^{11} viable spores/100 ml soil/grub.

4.2.1 Efficacy against first instar grubs of *H. consanguinea*

Relative efficacy of *M. anisopliae* against first instar *H. consanguinea* was tested with dosage ranging from 1 x 10^7 to 1 x 10^{10} viable spores/100 ml soil/grub. It is evident from the data (Table 3.a) that all the concentrations proved significantly superior over control but after fourteen days of exposure only.

Mortality in grubs after ten days exposure was found upto 33.33 per cent, the highest mortality (33.33%) was found in the treatment 1 x 10^{10} spores/100 ml soil/grub, but it was at par with the treatment of 5 x 10^9 spores/100 ml soil/grub (26.67% mortality). The treatments of 1 x 10^7 and 5 x 10^7 spores/100 ml soil/grub had not shown any effect against first instar. However, the treatments of 1 x 10^8, 5 x 10^8 and 1 x 10^9 spores/100 ml soil/grub resulted in 6.67, 10.0 and 13.33 per cent mortality and there existed no significant difference amongst these dosages.

As the period advanced the mortality also got increased. As such twelve days after exposure the highest mortality (50%) was found in the treatment of 1 x 10^{10} spores/100 ml soil/grub which was at par with 1 x 10^9 spores/100 ml soil/grub (40.0% mortality). The treatments of 1 x 10^7 and 5 x 10^7 started showing lethal effect and caused 3.33 and 6.67 per cent mortality. The treatment 5 x 10^7 spores/100 ml soil/grub (6.67% mortality) was at par with the treatment of 1 x 10^8 spores/100 ml soil/grub (13.33% mortality). The treatments of 5 x 10^8 and 1 x 10^9 spores/100 ml soil/grub were also at par and registered mortality of 16.67 and 26.67 per cent. Further the effect was at par with the treatment of 1 x 10^8 spores/100 ml soil/grub.

Fourteen days after exposure all the treatments were found significantly superior over control. The highest grub mortality (66.67%) was found in the highest concentration of 1 x 10^{10} spores/100 ml soil/grub but it was at par with the treatment of 5 x 10^{9} spores/100 ml soil/grub (56.67% mortality). Likewise, the lowest mortality (10.0%) was found in the lowest concentration 1 x 10^{7} spores/100 ml soil/grub, but it was at par with the treatments of 5 x 10^{7} and 1 x 10^{8} spores/100 ml soil/grub (13.33 and 16.67% mortality, respectively). The treatments of 1 x10^{9} and 5 x 10^{8} spores/100 ml soil/grub resulted in 40.0 and 20.0 per cent mortality but the treatment 5 x 10^{8} spores/100 ml soil/grub was at par with the treatments of 1 x 10^{8} and 5 x 10^{7} spores/100 ml soil/grub.

Sixteen days after exposure the grub mortality ranged from 13.33 to 70.0 per cent and the highest mortality (70.0%) was found in the treatment 1 x 10^{10} spores/100 ml soil/grub which was at par with the treatment of 5 x 10^{9} spores/100 ml soil/grub (60.0% mortality). The lowest mortality (13.33%) was found in the treatment 1 x 10^{7} spores/100 ml soil/grub but it was at par with the treatments of 5 x 10^{7} and 1 x 10^{8} spores/100 ml soil/grub which registered 16.67 and 23.33 per cent mortality, respectively. The treatments of 5 x 10^{8} and 1 x 10^{9} spores/100 ml soil/grub resulted in the mortality of 36.67 and 53.33 per cent but result of treatment 1 x 10^{9} spores/100 ml soil/grub was at par with the treatment of 5 x 10^{9} spores/100 ml soil/grub.

The median lethal dosage (LD_{50}) ranged from 3.68^{10} to 1.69^{9} viable spores/100 ml soil/grub depending upon the period of exposure. The LD_{50} values after 10, 12, 14 and 16 days of exposure were 3.68^{10}, 1.20^{10}, 3.49^{9} and 1.69^{9} spores/100 ml soil/grub, respectively (Table 4; Fig. 4.a).

4.2.2 Efficacy against second instar grubs of *H. consanguinea*

Against second instar *H. consanguinea* fungal spore in the dosage of 1 x 10^{8} to 1 x 10^{11}/100 ml soil/grub were tested. The mortality due to these varied from 0 to 66.67 per cent. All the treatments were found significantly effective over control after 30 days of exposure while different dosages differed significantly in efficacy (Table 3.b).

Table 3.a **Relative susceptibility of first instar grubs of *Holotrichia consanguinea* to fungus, *Metarrhizium anisopliae***

S. No.	Dosage (Fungus spores/100 ml soil/grub)	Mean per cent mortality Days after exposure			
		10	12	14	16
1.	1×10^7	0.00 (0.99)	3.33 (10.51)	10.00 (18.43)	13.33 (21.41)
2.	5×10^7	0.00 (0.99)	6.67 (14.67)	13.33 (21.41)	16.67 (24.10)
3.	1×10^8	6.67 (14.97)	13.33 (21.41)	16.67 (24.10)	23.33 (28.88)
4.	5×10^8	10.00 (18.43)	16.67 (24.10)	20.00 (26.57)	36.67 (37.27)
5.	1×10^9	13.33 (21.41)	26.67 (31.09)	40.00 (39.23)	53.33 (46.91)
6.	5×10^9	26.67 (31.09)	40.00 (39.23)	56.67 (48.83)	60.00 (50.77)
7.	1×10^{10}	33.33 (35.26)	50.00 (45.00)	66.67 (54.74)	70.00 (56.79)
8.	Control	0.00 (0.99)	3.33 (10.51)	3.33 (10.51)	3.33 (10.51)
	SEm±	3.657	3.889	2.651	2.765
	CD (P=0.05)	10.965	11.661	7.949	8.289

Figures in parenthesis are angular transformed values

Fifteen days after exposure highest mortality (26.67%) was found in the treatment 1 x 10^{11} spores/100 ml soil/grub but it was at par with the treatments of 5 x 10^{9} and 5 x 10^{10} spores/100 ml soil/grub (16.67% mortality in both). The treatment of 1 x 10^{8} spores/100 ml soil/grub had not shown any effect against this instar. The two treatments of 1 x 10^{9} and 1 x 10^{10} spores resulted in 10.0 per cent mortality only but the effect was statistically at par with the treatment of 5 x 10^{9} and 5 x 10^{10} spores/100 ml soil/grub.

As the period increased, mortality also got increased as such 20 days after exposure highest mortality (46.67%) was found in the treatment of 1 x 10^{11} spores/100 ml soil/grub though statistically it was at par with the treatments of 5 x 10^{10} and 1 x 10^{10} spores/100 ml soil/grub where the mortality was 43.33 and 36.67 per cent, respectively. The treatment of 1 x 10^{8} spores/100 ml soil/grub had not proved lethal at all to this instar up to 20 days of exposure and the treatment of 5 x 10^{8} spores/100 ml soil/grub could produce only 13.33 per cent mortality which was at par with the treatment of 1 x 10^{9} spores/100 ml soil/grub (20% mortality).

After 25 daysof exposure mortality increased to 60.0 per cent in the treatment 1 x 10^{11} spores/100 ml soil/grub but the difference was non significant with 5 x 10^{10}, 1 x 10^{10} and 5 x 10^{9} spores/100 ml soil/grub which resulted in 56.67, 46.67 and 43.33 per cent mortality, respectively. On the lower side also mortality was increased to 16.67 per cent in the treatment of 5 x 10^{8} spores/100 ml soil/grub but the treatment 1 x 10^{8} spores/100 ml soil/grub did not prove lethal to this instar till this time of exposure. The treatment of 1 x 10^{9} spores resulted in the mortality of 33.33 per cent which was statistically at par with the treatments of 5 x 10^{9} and 1 x 10^{10} spores/100 ml soil/grub.

However, after 30 days of exposure all the treatments proved significantly effective over control and the highest mortality increased to 66.67 per cent with the highest concentration of 1 x 10^{11} spores/100 ml soil/grub. There existed non significant difference with the treatment 5 x 10^{10} spores/100 ml soil/grub. The treatment 1 x 10^{8} spores/100 ml soil/grub which was not effective upto 25 days of

Table 3.b **Relative susceptibility of second instar grubs of *Holotrichia consanguinea* to fungus, *Metarrhizium anisopliae***

S. No.	Dosage (Fungus spores/100 ml soil/grub)	Mean per cent mortality Days after exposure			
		15	20	25	30
1.	1×10^8	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	6.67 (14.97)
2.	5×10^8	3.33 (10.51)	13.33 (21.41)	16.67 (16.67)	23.33 (28.88)
3.	1×10^9	10.00 (18.43)	20.00 (26.57)	33.33 (35.26)	40.00 (39.23)
4.	5×10^9	16.67 (24.10)	26.67 (31.09)	43.33 (41.17)	53.33 (46.91)
5.	1×10^{10}	10.00 (18.43)	36.67 (37.27)	46.67 (43.09)	50.00 (45.00)
6.	5×10^{10}	16.67 (24.10)	43.33 (41.17)	56.67 (48.83)	63.33 (52.73)
7.	1×10^{11}	26.67 (31.09)	46.67 (43.09)	60.00 (50.77)	66.67 (54.74)
8.	Control	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	3.33 (10.51)
	SEm±	2.584	2.187	3.356	3.245
	CD (P=0.05)	7.747	6.557	10.061	9.729

Figures in parenthesis are angular transformed values

exposure did show some effect with 6.67 per cent mortality. Similarly the treatments of 5 x 10^8 and 1 x 10^9 spores/100 ml soil/grub caused 23.33 and 40.0 per cent mortality and both the treatments differed significantly from each other. The treatment 1 x 10^9 spores/100 ml soil/grub was at par with the treatments of 5 x 10^9 and 1 x 10^{10} spores/100 ml soil/grub (53.33 and 50.0% mortality respectively).

The median lethal dosage (LD_{50}) for this instar varied from 3.25^{12} to 1.10^{10} spores/100 ml soil/grub depending upon period of exposure. The LD_{50} values after 15, 20, 25 and 30 days of exposure were 3.25^{12}, 8.24^{10}, 2.31^{10} and 1.10^{10} spores/100 ml soil/grub, respectively (Table 4; Fig. 4.b).

4.2.3 Efficacy against third instar grubs of *H. consanguinea*

Dosages against third instar were same as that of second instar i.e. 1 x 10^8 to 1 x 10^{11} viable spores/100 ml soil/grub (Table 3.c). All the treatmentsexcept 1 x 10^8 spores/100 ml soil/grub proved significantly effective over control after 15 days of exposure. Highest grub mortality (23.33%) was found in the treatment of 1 x 10^{11} spores/100 ml soil/grub though it was at par with the treatment 5 x 10^{10} spores/100 ml soil/grub (20.0% mortality). The lowest mortality (10.0%) was found in the treatments of 5 x 10^8 and 5 x 10^9 spores/100 ml soil/grub while the treatments of 1 x 10^9 and 1 x 10^{10} spores/100 ml soil/grub registered mortality of 16.67 and 13.33 per cent, respectively.

After 20 days of exposure highest mortality got increased to 43.33 per cent in the treatment 1 x 10^{11} spores/100 ml soil/grub but the level of significance was at par with the treatment 1 x 10^{10} spores/100 ml soil/grub (36.67% mortality). The treatments of 5 x 10^{10}, 5 x 10^9 and 1 x 10^9 spores/100 ml soil/grub resulted in the mortality of 30.0, 26.67 and 16.67 per cent, respectively out of these treatments 5 x 10^{10} and 5 x 10^9 spores/100 ml soil/grub were at par with treatments of 1 x 10^{10} spores/100 ml soil/grub.

After 25 days of exposure all the treatments proved significantly effective over control and the mortality ranged from 3.33 to 53.33 per cent. The highest mortality

got increased to 53.33 per cent in highest concentration of 1 x 10^{11} spores/100 ml soil/grub but it was at par with treatments of 5 x 10^{10} and 1 x 10^{10} spores/100 ml soil/grub (50% mortality in both). The lowest mortality (3.33%) was found in the treatment 1 x 10^{8} spores/100 ml soil/grub. The treatments of 5 x 10^{8} and 1 x 10^{9} spores/100 ml soil/grub also registered comparatively low mortality of 10.0 and 23.33 per cent respectively while the treatment 5 x 10^{9} spores/100 ml soil/grub resulted in 36.67 per cent mortality which was at par with treatment 1 x 10^{10} and 5 x 10^{10} spores/100 ml soil/grub.

Even after a lapse of 30 days period lowest and highest mortality remained same with a minor change in median dosages where the treatments of 5 x 10^{8}, 1 x 10^{9} and 5 x 10^{9} spores/100 ml soil/grub resulted in 13.33, 33.33 and 43.33 per cent mortality, respectively.

The median lethal dosage (LD_{50}) varied from 2.18^{13} to 2.47^{10} spores/100 ml soil/grub and after 15, 20, 25 and 30 days of exposure the LD_{50} values were found to be 2.18^{13}, 1.47^{11}, 3.33^{10} and 2.47^{10} spores/100 ml soil/grub respectively (Table 4; Fig. 4.c).

The white grub, *H. consanguinea* in all the three instars was found to be susceptible to fungus, *M. anisopliae*. The per cent grub mortality increased as the inoculum dosage and period of exposure increased. But in all *M. anisopliae* required longer time to kill the grub than nematode, *H. bacteriophora*. The median lethal dosage (LD_{50}) increased as the size of grubs (change in instar) increased and the susceptibility of grub decreased with the instar as first instar grubs were highly susceptible as compared to second and third instar grubs.

Table 3.c **Relative susceptibility of third instar grubs of *Holotrichia consanguinea* to fungus, *Metarrhizium anisopliae***

S. No.	Dosage (Fungus spores/100 ml soil/grub)	Mean per cent mortality Days after exposure			
		15	20	25	30
1.	1×10^8	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	3.33 (10.51)
2.	5×10^8	10.00 (18.43)	10.00 (18.43)	10.00 (18.43)	13.33 (21.41)
3.	1×10^9	16.67 (24.10)	16.67 (24.10)	23.33 (28.88)	33.33 (35.26)
4.	5×10^9	10.00 (18.43)	26.67 (31.67)	36.67 (37.67)	43.33 (41.17)
5.	1×10^{10}	13.33 (21.41)	36.67 (37.27)	50.00 (45.00)	50.00 (45.00)
6.	5×10^{10}	20.00 (26.57)	30.00 (33.21)	50.00 (45.00)	53.33 (46.91)
7.	1×10^{11}	23.33 (28.88)	43.33 (41.17)	53.33 (46.91)	53.33 (46.91)
8.	Control	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)
	SEm±	1.566	2.593	2.681	2.653
	CD (P=0.05)	4.690	7.774	8.039	7.953

Figures in parenthesis are angular transformed values

Table 4 **Relative susceptibility of different instar grubs of *Holotrichia consanguinea* to fungus, *Metarrhizium anisopliae* based on LD_{50} values**

S.No.	Days after treatment	Time (log) - Kill (probit)	
		Regression equation	LD_{50}
		First instar *Holotrichia consanguinea*	
1.	10	Y = (-) 2.4964 + 0.7101 X	3.68^{10}
2.	12	Y = (-) 2.1327 + 0.7076 X	1.20^{10}
3.	14	Y = (-) 1.7758 + 0.7108 X	3.49^{9}
4.	16	Y = 0.9528 + 0.6279 X	1.69^{9}
		Second instar *Holotrichia consanguinea*	
1.	15	Y = 1.7684 + 0.4420 X	8.25^{12}
2.	20	Y = 1.5987 + 0.5401 X	8.24^{10}
3.	25	Y = 1.5318 + 0.5837 X	2.31^{10}
4.	30	Y = 1.8712 + 0.5650 X	1.10^{10}
		Third instar *Holotrichia consanguinea*	
1.	15	Y = 2.8964 + 0.3103 X	2.18^{13}
2.	20	Y = 1.7361 + 0.5102 X	1.47^{11}
3.	25	Y = 2.2858 + 0.5783 X	3.33^{10}
4.	30	Y = 1.9949 + 0.5260 X	2.47^{10}

Y = Probit kill
X = log of dosage (Fungus spores/100 ml soil/grub)
LD_{50} = Lethal dosage calculated to give 50 per cent mortality

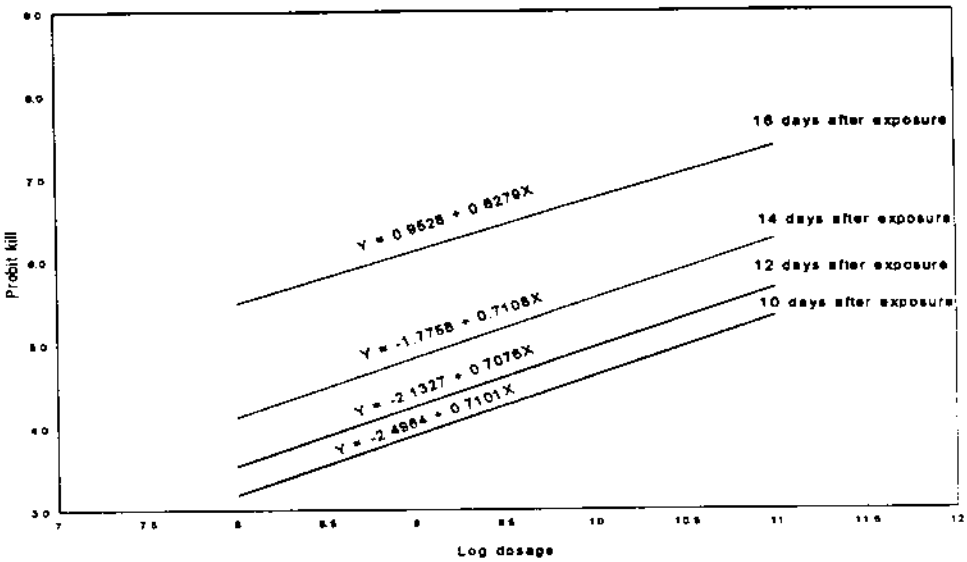


Fig 4.a Log dosage-probit kill line for susceptibility of first instar grubs of *Holotrichia consanguinea* to fungus, *Metarrhizium anisopliae*

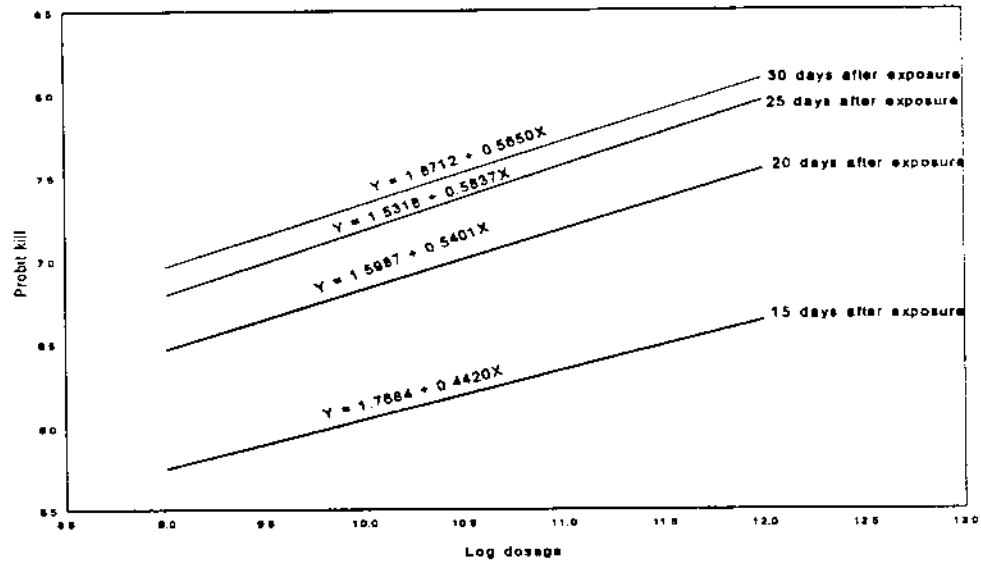


Fig 4.b Log dosage-probit kill line for susceptibility of second instar grubs of *Holotrichia consanguinea* to fungus, *Metarrhizium anisopliae*.

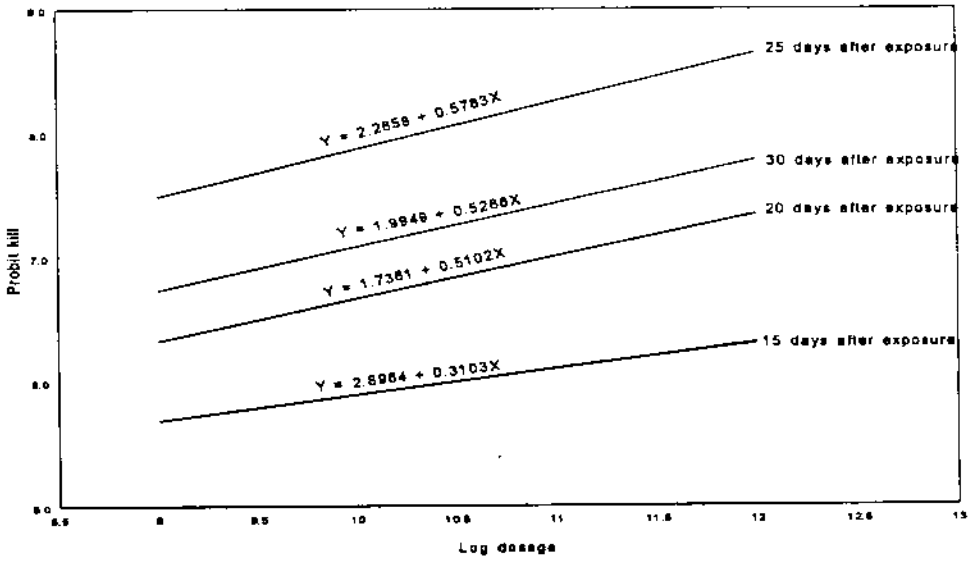


Fig 4.c Log dosage-probit kill line for susceptibility of third instar grubs of *Holotrichia consanguinea* to fungus, *Metarrhizium anisopliae*

4.3 COMPATIBILITY OF NEMATODE, *H. bacteriophora* WITH FUNGUS, *M. anisopliae* IN RELATION TO DIFFERENT INSTARS OF *H. consanguinea*

All the instars of white grubs were found susceptible to nematode and fungus separately as well as in combination of both. With fungal infection the grub mortality was low and longer period was required to get lethal infection as compared to nematode and combinations of the two. The compatibility of nematodes with fungus was assessed based on grub mortality.

4.3.1 Compatibility in relation to first instar grubs of *H. consanguinea*

The nematode, *H. bacteriophora* in two dosages of 2000 and 1000 IJs/100 ml soil /grub and the fungus. *M. anisopliae* also in two dosages of 1 x 10^9 and 5 x 10^8 spores/100 ml soil/grub together with their higher and lower dosage combinations were tested against first instar white grub under laboratory conditions. As such there were six treatments.

The median lethal time (LT_{50}) ranged from 2.87 to 15.93 days. The treatments of nematode 2000 and 1000 IJs/100 ml soil/grub required 4.17 and 6.64 days to cause lethal infection, respectively, whereas fungus in the two above mentioned dosages required comparatively longer time of 14.19 and 15.93 days, respectively. But the combinations of the two required shorter time to cause 50 per cent mortality. The treatments of nematode 2000 IJs + fungus 1 x 10^9 spores/100 ml soil/grub required only 2.87 days in comparison to 4.17 days taken by nematodes alone and 14.19 days by fungus alone. Similarly the lower dosage of the two (nematode 1000 IJs + fungus 5 x 10^8 spores/100 ml soil/grub) required 4.99 days in comparison to 6.64 days taken by nematode alone (1000 IJs) and 15.93 days by fungus alone (5 x 10^8 spores) (Table 5; Fig. 5.a).

All the treatments except fungus alone were found significantly effective over control after three days of exposure. After this period of exposure 36.67 and 27.67 per cent grub mortality was found in the treatment of nematode alone (2000 and 1000 IJs/100 ml soil/grub) while fungus alone did not show any effect, making it clear that

Table 5 **Compatibility of nematode, *Heterorhabditis bacteriophora* with fungus, *Metarrhizium anisopliae* in relation to different instar grubs of *Holotrichia consanguinea* based on LT_{50} values**

S. No.	Treatment (Dosage/100ml soil/grub)	Time (log) - Kill (probit) Regression equation	LT_{50}
	First instar *Holotrichia consanguinea*		
1.	N 2000 IJs	Y = 3.4081 + 2.5651 X	4.17
2.	N 1000 IJs	Y = 3.2547 + 2.1220 X	6.64
3.	F $1x10^9$ spores	Y = 0.1987 + 4.0919 X	14.19
4.	F $5x10^8$ spores	Y = (-)1.0515 + 5.0332 X	15.93
5.	N 2000 IJs + F $1x10^9$ spores	Y = 3.5890 + 3.0826 X	2.87
6.	N 1000 IJs + F $5x10^8$ spores	Y = 3.3348 + 2.3848 X	4.99
	Second instar *Holotrichia consanguinea*		
1.	N 10,000 IJs	Y = 3.3047 + 3.0979 X	3.53
2.	N 5,000 IJs	Y = 3.4351 + 2.004 X	6.04
3.	F 1 x 10^{10} spores	Y = (-) 0.7550 + 4.0787×	25.76
4.	F 5 x 10^9 spores	Y = (-) 1.7591 + 4.7282×	26.89
5.	N 10,000 IJs + F 1 x 10^{10} spores	Y = 3.3856 + 3.2489 X	3.14
6.	N 5,000 IJs + F 5 x 10^9 spores	Y = 3.2058 + 2.6447 X	4.77
	Third instar *Holotrichia consanguinea*		
1.	N 20,000 IJs	Y = 3.0763 + 3.3367 X	3.77
2.	N 10,000 IJs	Y = 3.7897 + 1.6677 X	5.32
3.	F 5 x 10^{10} spores	Y = (-) 1.6336 + 4.7960×	24.16
4.	F 1 x 10^{10} spores	Y = (-) 1.0518 + 4.2563×	26.42
5.	N 20,000 IJs + F $5x10^{10}$ spores	Y = 3.2336 + 3.3956 X	3.31
6.	N 10,000 IJs + F $1x10^{10}$ spores	Y = 3.5686 + 2.3340 X	4.10

N = Nematode F = Fungus
Y = Probit kill X = log of time (days) after exposure
LT_{50} = Period in days calculated to give 50 per cent mortality

fungus enhanced the effect of nematode. The combination of nematode 2000 IJs + fungus 1 x 10^9 spores and 1000 IJs + 5 x 10^8 spores/100 ml soil/grub caused 53.33 and 26.67 per cent mortality in grub, respectively. The combination of higher dosages proved significantly superior over rest of the treatments. Similar trend in grub mortality was noticed after five days of exposure also.

However, after seven days of exposure all the treatments were found significantly effective over control. The combined effect of two microbes resulted in 90 per cent mortality (nematode 2000 IJs + fungus 1 x 10^9 spores/100 ml soil/grub). Fungus also started showing its effect after seven days and the two dosages of 1 x 10^9 and 5 x 10^8 spores/100 ml soil/grub resulted in 13.33 and 3.33 per cent mortality, respectively. The treatments of nematode 2000 IJs/100 ml soil/grub alone and combination of nematode 1000 IJs + fungus 5 x 10^8 spores/100 ml soil/grub resulted in the mortality of 76.67 and 70.0 per cent respectively and they were found statistically at par. The treatment of nematode alone (1000 IJs/100 ml soil/grub) registered 56.67 per cent mortality and was at par with combined treatment of nematode 1000 IJs + fungus 5 x 10^8 spores/100 ml soil/grub. After a lapse of nine days similar trend in grub mortality was observed.

Eleven days after exposure grub mortality reached to its peak. It was 86.67 and 70.0 per cent in the treatment of nematode 2000 IJs and 1000 IJs/100 ml soil/grub, respectively. But the highest mortality (96.67%) was found in the combination dosage of nematode 2000 IJs + fungus 1 x 10^9 spores/100 ml soil/grub. The two treatments of fungus alone produced 20.0 per cent mortality. The results remained same after 13 days of exposure with slight upward change in fungal treatment where the mortality was 36.67 and 33.33 per cent.

After 15 days of exposure increasing trend of grub mortality continued with fungal treatments where the mortality was 60 per cent in 1 x 10^9 spores/100 ml soil/grub treatment and 43.33 per cent with 5 x 10^8 spores/100 ml soil/grub.

When grubs were exposed to combined infection of nematode and fungus, there were no visible external symptoms of fungal infection but nematode

Table 6.a **Compatibility of nematode, *Heterorhabditis bacteriophora* with fungus, *Metarrhizium anisopliae* in relation to first instar grubs of *Holotrichia consanguinea***

S.No.	Treatment (Dosage/100 ml soil/grub)	Mean per cent mortality Days after exposure								Mean No. of NIJs harvested/grub
		1	3	5	7	9	11	13	15	
1.	N 2000 IJs	0.00 (0.99)	36.67 (37.27)	73.33 (58.91)	76.67 (61.12)	76.67 (61.12)	86.67 (68.59)	86.67 (68.59)	86.67 (68.59)	
2.	N 1000 IJs	0.00 (0.99)	26.67 (31.09)	46.67 (43.09)	56.67 (48.85)	56.67 (48.85)	70.00 (56.79)	70.00 (56.79)	70.00 (56.79)	
3.	F $1x10^9$ spores	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	13.33 (21.41)	16.67 (24.10)	20.00 (26.57)	36.67 (37.27)	60.00 (50.77)	
4.	F $5x10^8$ spores	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	13.33 (21.41)	20.00 (26.57)	33.33 (35.26)	43.33 (41.17)	
5.	N 2000 IJs + F $1x10^9$ spores	6.67 (14.97)	53.33 (46.91)	76.67 (61.12)	90.00 (71.57)	96.67 (79.49)	96.67 (79.49)	96.67 (79.49)	96.67 (79.49)	20,800
6.	N 1000 IJs + F 5 x 10^8 spores	3.33 (10.51)	26.67 (31.09)	56.67 (48.33)	70.00 (56.79)	73.33 (58.91)	76.67 (61.12)	80.00 (63.43)	86.67 (63.43)	16,500
7.	Control	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	3.33 (10.51)	3.33 (10.51)	
	SEm±	3.108	1.582	2.699	2.660	2.977	3.377	3.445	3.234	
	CD (p = 0.05)	9.427	4.800	8.187	8.007	9.029	10.245	10.451	10.026	

N = Nematode
F = Fungus
Figures in parenthesis are angular transformed values

multiplication was observed in these. There was no significant difference in the nematode size, when the grubs were subjected to either nematode infection alone or to a combined infection of fungus and nematode both. The nematode IJs harvested per grub from higher dosage combination of 2000 IJs + 1 x 10^9 spores and lower dosage combination of 1000 IJs + 5 x 10^8 spores/100 ml soil/grub were 20,800 and 16,500, respectively (Table 6.a).

4.3.2 Compatibility in relation to second instar grubs of *H. consanguinea*

Similar to first instar the compatibility was based on grub mortality and assessment was done by testing the nematode *H. bacteriophora* in two dosages of 10000 and 5000 IJs and the fungus, *M. anisopliae* also in two dosages of 1 x 10^{10} and 5 x 10^9 spores/100 ml soil/grub together with their higher and lower dosage combination in the laboratory against second instar white grub by soil inoculation method. There were six treatments.

The median lethal time (LT_{50}) ranged from 3.14 to 26.89 days as compared to 2.87 and 15.93 days in first instar. The treatments of nematode 10,000 and 5000 IJs/100 ml soil/grub required 3.53 and 6.04 days to cause lethal infection respectively whereas fungus in two dosages (1 x 10^{10} & 5 x 10^9 spores/100 ml soil/grub) required comparatively longer time of 25.76 and 26.89 days respectively. But the combinations of the two required shorter time to cause 50 per cent mortality. The higher dosage combination of nematode 10,000 + fungus 1 x 10^{10} spores/100 ml soil/grub required only 3.14 days in comparison to 3.53 days taken by nematode alone (10000 IJs) and 25.76 days by fungus (1 x 10^{10} spores). Similarly the lower dosage of the two (5000 IJs + 5 x 10^9 spores) required 4.77 days in comparison to 6.04 day taken by nematode alone (5000 IJs) and 26.89 days by fungus alone (5 x 10^9 spores) (Table 5; Fig. 5.b).

All the treatments except fungus alone were found significantly effective over control after three days of exposure. Nematode alone (10,000 and 5000 IJs) 100 ml soil/grub) caused 40.0 and 26.67 per cent mortality respectively, only after three days to exposure. While combination of higher dosages (nematode 10,000 IJs + fungus

Table 6.b **Compatibility of nematode, *Heterorhabditis bacteriophora* with fungus, *Metarrhizium anisopliae* in relation to second instar white grubs of *Holotrichia consanguinea***

S.No.	Treatment (Dosage/100 ml soil/grub)	Mean per cent mortality Days after exposure									Mean No. of NIJs harvested/grub
		1	3	5	7	10	15	20	25	30	
1.	N 10000 IJs	6.67 (14.97)	40.00 (39.23)	56.67 (48.83)	80.00 (63.43)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	
2.	N 5000 IJs	3.33 (10.51)	26.67 (31.09)	43.33 (41.17)	53.33 (46.91)	83.33 (65.90)	83.33 (65.90)	83.33 (65.90)	83.33 (65.90)	83.33 (65.90)	
3.	F 1 x 10^{10} spores	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	10.00 (18.45)	16.67 (24.10)	33.33 (35.26)	50.00 (45.00)	56.67 (48.83)	
4.	F 5 x 10^{9} spores	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	13.33 (21.41)	30.00 (33.21)	46.67 (43.09)	53.33 (46.91)	
5.	N 10000 IJs + F 1 x 10^{10} spores	10.00 (18.43)	43.33 (41.17)	63.33 (52.73)	86.67 (68.59)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	41,200
6	N 5000 IJs + F 5 x 10^{9} spores	3.33 (10.51)	33.33 (35.26)	50.00 (45.00)	60.00 (50.77)	90.00 (71.57)	93.33 (75.03)	96.67 (79.49)	96.67 (79.49)	96.67 (79.49)	28,500
7.	Control	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
	SEm±	3.882	1.345	1.794	2.417	2.425	2.746	2.892	2.827	2.632	
	CD (0.05)	11.776	4.080	5.441	7.333	7.356	8.330	8.773	8.576	7.986	

N = Nematode
F = Fungus
Figures in parenthesis are angular transformed values

5 x 10^9 spores) and lower dosages (5000 IJs + 5x10^9 spores) caused 43.33 and 33.33 per cent mortality respectively The effect of higher dosage combination was significantly superior over rest of the treatments except higher dosage of nematode (10,000 IJs). Similar trend in grub mortality was noticed after 5 and 7 days of exposure also except that fungus did not show any lethal effect even after seven days.

After ten days of exposure all the grubs tested died and the mortality reached to its peak in the treatments of higher dosages combinations and nematode alone. The mortality was to the extent of 83.33 per cent in lower dosage nematode alone. After a period of 10 days fungus started showing its effect and the two dosages of 1 x 10^{10} and 5 x 10^9 spores/100 ml soil/grub resulted in 10.0 and 3.33 per cent mortality respectively. However, in lower dosages combination mortality also reached to its peak (96.67%) after 20 days of exposure. The increasing trend of grub mortality continued with fungal treatments also where the mortality was 56.67 and 53.33 per cent in the fungal treatments of 1 x 10^{10} and 5 x 10^9 spores/100 ml soil/grub respectively after 30 days of exposure. Though the fungus alone required longer period to cause lethal infection but in combination it enhanced the effect of nematodes. The IJs harvested per grub from higher and lower dosages combination were 41,200 and 28,500 respectively (Table 6.b).

4.3.3 Compatibility in relation to third instar grubs of *H. consanguinea*

The dosages of microbes were increased (nematode 20,000 and 10,000 IJs/100 ml soil/grub and fungus 5 x 10^{10} and 1 x 10^{10} spores/100 ml soil/grub) to test compatibility in relation to third instar white grub.

The median lethal time for third instar (LT_{50}) ranged from 3.31 to 3.77 days. The treatments of nematode alone (20,000 and 10000 IJs/100 ml soil/grub) required 3.77 and 5.32 days, respectively to cause lethal infection whereas fungus in the two above mentioned dosages required comparatively longer time of 24.16 and 26.42 days, respectively. However, the combinations of the two required shorter time to cause 50 per cent mortality.

The treatments of higher dosages combination (20,000 IJs + 5 x 10^{10} spores) required only 3.31 days in comparison to 3.77 days taken by nematode alone (20,000 IJs) and 24.16 days by fungus (5 x 10^{10} spores). Similarly the lower dosages of the two (10,000 IJs + 1 x 10^{10} spores) required 4.10 days while the nematode (10,000 IJs) and fungus (1 x 10^{10} spores) alone required 5.32 and 26.42 days respectively to cause 50 per cent mortality (Table 5; Fig. 5.c).

Similar to first and second instars all the treatments except fungus alone also were found significantly effective over control after three days exposure. The fungus did not show any effect even after 7 days of exposure.

Three days after exposure 40.0 and 36.67 per cent grub mortality was observed in the treatments of nematodes alone (20,00 and 10,000 IJs/100 ml soil/grub) whereas the combination of higher (nematode 20,000 IJs + fungus 5x10^{10} spores) and lower (nematode 10,000 IJs + fungus 1x10^{10} spores) dosages registered 43.33 and 40.0 per cent mortality, respectively. The mortality (40%) was equal in the treatments of lower dosages combinations and higher dosages nematode (20,000 IJs) alone (Table 6.c).

After ten days of exposure cent per cent mortality was found in the treatments of higher dosages combination and higher dosages nematode alone. The mortality reached to its peak (80%) in lower dosage of nematode alone. The treatment of lower dosages combination resulted in 90 per cent mortality, which touched its peak (93.33%) after 20 days exposure. The fungus started showing its effect after 10 days and the two dosages of 5 x 10^{10} and 1 x 10^{10} spores/100 ml soil/grub resulted in 6.67 and 3.33 per cent mortality, respectively. The increasing trend of grub mortality continued with fungal treatments where the mortality was 56.67 per cent in 5 x 10^{10} spores/100 ml soil/grub and 50.0 per cent with 1 x 10^{10} fungal spores/100 ml soil/grub after 30 days of treatment.

The nematode IJs harvested per grub from higher dosage combination of (20,000 IJs + 5 x 10^{10} spores/100 ml soil/grub) and lower dosage combination (10,000 IJs + 1 x 10^{10} spores/100 ml soil/grub) were 60,500 and 54,200 respectively (Table 6.c).

Table 6.c **Compatibility of nematode, *Heterorhabditis bacteriophora* with fungus, *Metarrhizium anisopliae* in relation to third instar grubs of *Holotrichia consanguinea***

S.No.	Treatment (Dosage/100 ml soil/grub)	Mean per cent mortality Days after exposure									Mean No. of NIJs harvested/ grub
		1	3	5	7	10	15	20	25	30	
1.	N 20,000 IJs	3.33 (10.51)	40.00 (39.23)	60.00 (50.77)	73.33 (58.91)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	
2.	N 10,000 IJs	0.00 (0.99)	36.67 (37.27)	50.00 (45.00)	63.33 (52.73)	80.00 (63.43)	80.00 (63.43)	80.00 (63.43)	80.00 (63.43)	80.00 (63.43)	
3.	F 5 x 10^{10} spores	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	20.00 (26.57)	46.67 (43.09)	53.33 (46.91)	56.67 (48.85)	
4.	F 1 x 10^{10} spores	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	6.67 (14.97)	16.67 (24.10)	40.00 (39.23)	46.67 (43.09)	50.00 (45.00)	
5.	N 20,000 IJs + F 5 x 10^{10} spores	6.67 (14.97)	43.33 (41.17)	63.33 (52.73)	83.33 (65.90)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	100.00 (89.01)	60,500
6.	N 10,000 IJs + F 1 x 10^{10} spores	3.33 (10.51)	40.00 (39.23)	56.67 (48.85)	73.33 (58.91)	90.00 (71.57)	90.00 (71.57)	93.33 (75.03)	93.33 (75.03)	93.33 (75.03)	54,200
7.	Control	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
	SEm ±	3.882	1.661	1.475	2.207	3.108	1.913	2.314	2.426	2.314	
	CD (0.05)	11.776	5.038	4.475	6.694	9.427	5.803	7.021	7.358	7.021	

N = Nematode
F = Fungus
Figures in parenthesis are angular transformed values

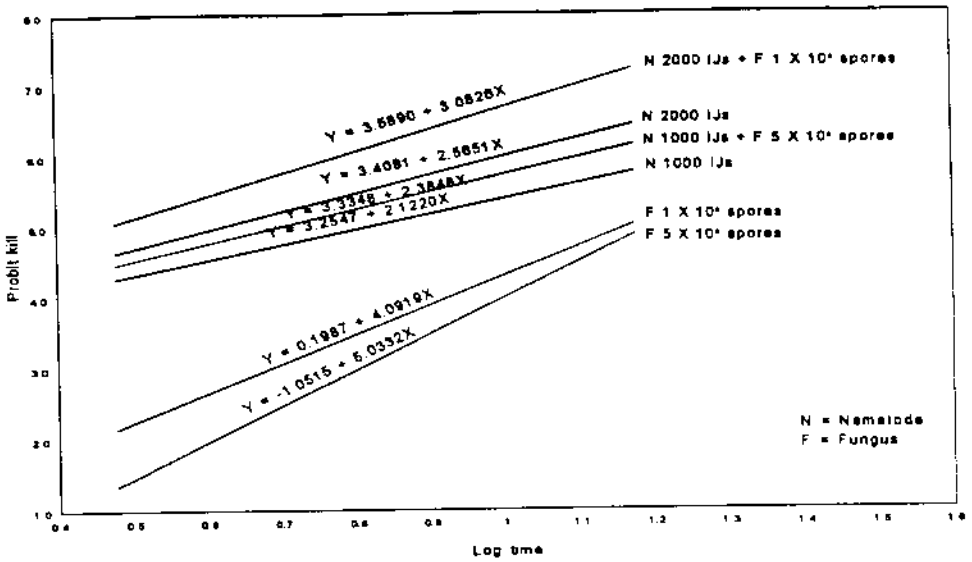


Fig 5.a Log time-probit kill line of compatibility of nematode, *Heterorhabditis bacteriophora with* fungus, *Metarrhizium anisopliae* in relation to first instar *Holotrichia consanguinea*

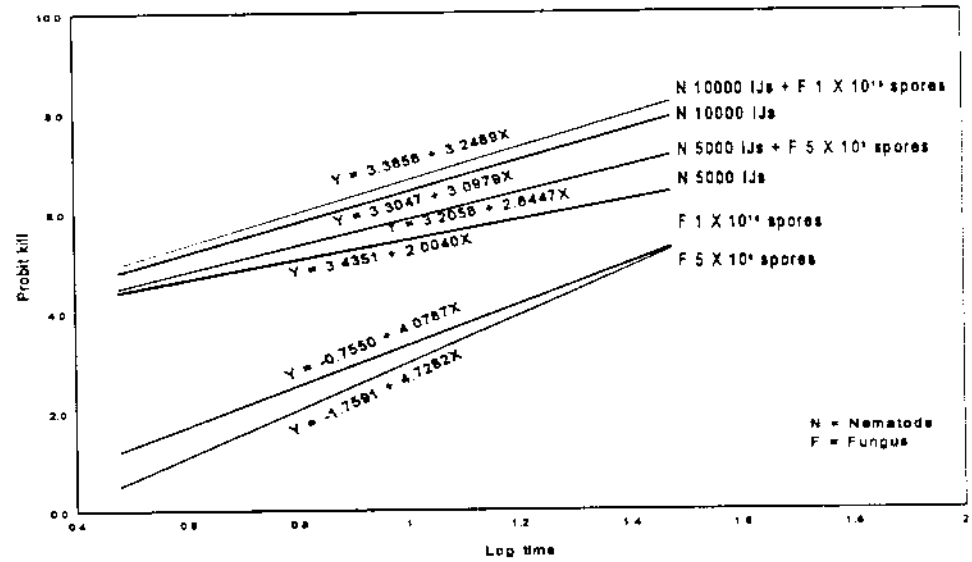


Fig 5.b Log time-probit kill line of compatibility of nematode, *Heterorhabditis bacteriophora with* fungus, *Metarrhizium anisopliae* in relation to second instar *Holotrichia consanguinea*

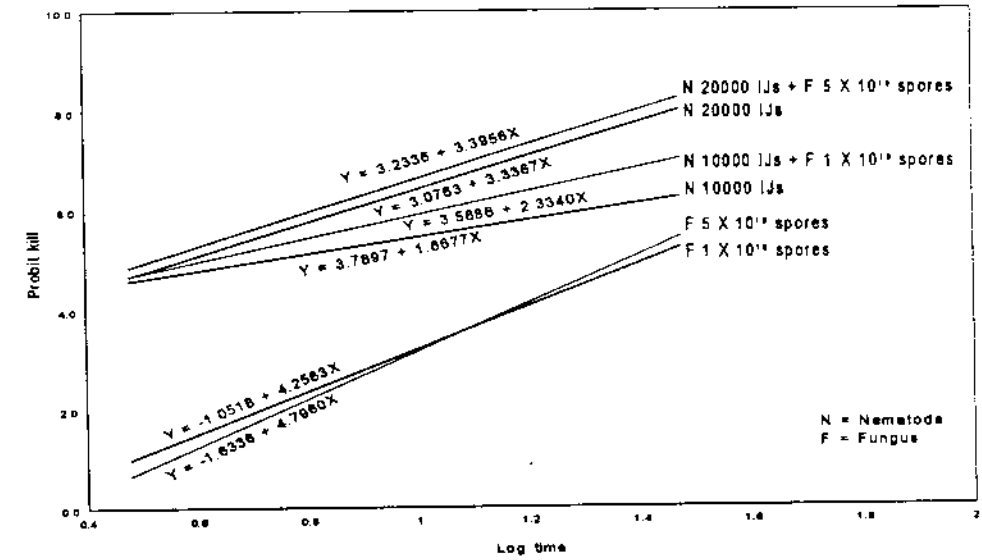


Fig 5.c Log time-probit kill line of compatibility of nematode, *Heterorhabditis bacteriophora with* fungus, *Metarrhizium anisopliae* in relation to third instar *Holotrichia consanguinea*

4.4 COMPATIBILITY OF NEMATODE, *H. bacteriophora* WITH INSECTICIDES (chlorpyriphos and quinalphos)

All the instars of white grubs (*H. consanguinea*) were found susceptible to nematode and insecticides separately as well as to combinations of the two. The compatibility of nematode with insecticides was assessed based on per cent grub mortality and lethal time required to kill 50 per cent grubs.

4.4.1 Compatibility in relation to first instar grubs of *H. consanguinea*

The compatibility of nematode with chlorpyriphos and quinalphos was assessed on the basis of per cent grub mortality. Two dosage each of nematode, *H. bacteriophora* (2000 and 1000 IJs/100 ml soil/grub), chlorpyriphos (0.8 and 0.4 mg/100 ml soil/grub) and quinalphos (1.0 and 0.5 mg/100 ml soil/grub) were tested individually as well as in combination in relation to first instar in laboratory. In all there were ten treatments.

The median lethal time in different treatments (LT_{50}) ranged from 1.30 to 6.64 days. The treatments of nematode (2000 and 1000 IJs/100 ml soil/grub) required 4.17 and 6.64 days to cause lethal infection, respectively. Whereas chlorpyriphos (0.8 and 0.4 mg/100 ml soil/grub.) required comparatively shorter time of 1.44 and 2.06 days and quinalphos (1.0 and 0.5 mg/100 ml soil/grub) 2.02 and 3.76 days respectively. The combination of the two also required shorter time than nematode and insecticide individually to cause 50 per cent mortality. The treatment of nematode 2000 IJs + chlorpyriphos 0.8 mg/100 ml soil/grub and nematode 2000 IJs + quinalphos 1.0 mg/100 ml soil/grub required only 1.30 and 1.60 days respectively in comparison to 4.17 days taken by nematode alone and 1.44 days by chlorpyriphos and 2.02 days by qunalphos. Similarly the lower dosage of the two, nematode 1000 IJs + chlorpyriphos 0.4 mg/100 mg soil/grub and nematode 1000 IJs + quinalphos 0.5 mg/100 ml soil/grub required 2.24 and 2.46 days respectively in comparison to 6.64 days by nematode alone (1000 IJs) and 2.06 days by chlorpyriphos (0.4 mg) and 3.76 days by qunalphos (0.5 mg) alone to cause 50 per cent mortality (Table 7; Fig. 6.a).

Table 7 **Compatibility of Nematode, *Heterorhabditis bacteriophora* with insecticides in relation to different instar grubs of *Holotrichia consanguinea* based on LT_{50} values**

S. No.	Treatment (Dosage/100 ml soil/grub)	Time (log) - Kill (probit)	
		Regression equation	LT_{50}
	First instar of *Holotrichia consanguinea*		
1.	N 2000 IJs	Y = 3.4081 + 2.5651 X	4.17
2.	N 1000 IJs	Y = 3.2547 + 2.1220 X	6.64
3.	Ch 0.8 mg	Y = 4.4415 + 3.5157 X	1.44
4.	Ch 0.4 mg	Y = 3.5800 + 3.1130 X	2.06
5.	Qu 1.0 mg	Y = 4.0677 + 3.0581 X	2.02
6.	Qu 0.5 mg	Y = 3.7552 + 2.1660 X	3.76
7.	N 2000 IJs + ch 0.8 mg	Y = 4.5026 + 4.3679 X	1.30
8.	N 1000 IJs + Ch 0.4 mg	Y = 3.6714 + 3.7878 X	2.24
9.	N 2000 IJs + Qu 1.0 mg	Y = 4.2883 + 3.4756 X	1.60
10.	N 1000 IJs + Qu 0.5 mg	Y = 4.0886 + 2.3352 X	2.46
	Second instar of *Holotrichia consanguinea*		
1.	N 10,000 IJs	Y = 3.3047 + 3.0979 X	3.53
2.	N 5,000 IJs	Y = 3.4351 + 2.004 X	6.04
3.	Ch 0.8 mg	Y = 4.1383 + 3.5202 X	1.76
4.	ch 0.4 mg	Y = 3.6268 + 2.9792 X	2.90
5.	Qu 1.0 mg	Y = 3.8873 + 3.3789 X	2.13
6.	Qu 0.5 mg	Y = 3.5432 + 2.4459 X	3.94
7.	N 10,000 IJs + ch 0.8 mg	Y = 4.2392 + 4.0623 X	1.54
8.	N 5000 IJs + ch 0.4 mg	Y = 3.4266 + 3.6082 X	2.73
9.	N 10,000 IJs + Qu 1.0 mg	Y = 4.1413 + 3.1537 X	1.87
10.	N 5000 IJs + Qu 0.5 mg	Y = 3.4041 + 3.3348 X	3.01

S. No.	Treatment (Dosage/100 ml soil/grub)	Time (log) - Kill (probit)	
		Regression equation	LT_{50}
	Third instar of *Holotrichia consanguinea*		
1.	N 20,000 IJs	Y = 3.0763 + 3.3367 X	3.77
2.	N 10,000 IJs	Y = 3.7897 + 1.6677 X	5.32
3.	Ch 0.8 mg	Y = 4.1226 + 3.1689 X	1.89
4.	Ch 0.4 mg	Y = 3.7367 + 3.1689 X	3.57
5.	Qu 1.0 mg	Y = 3.9913 + 2.5622 X	2.48
6.	Qu 0.5 mg	Y = 3.7533 + 1.9734 X	4.28
7.	N 20,000 IJs + Ch 0.8 mg	Y = 4.3123 + 3.3050 X	1.61
8.	N 10,000 IJs + Ch 0.4 mg	Y = 3.5838 + 3.1458 X	2.82
9.	N 20,000 IJs + Qu 1.0 mg	Y = 4.1485 + 2.8914 X	1.97
10.	N 10,000 IJs + Qu 0.5 mg	Y = 3.5371 + 2.8556 X	3.25

N = Nematode
Qu = Quinalphos
Ch = Chlorpyriphos
Y = Probit kill
X = Log of time (days) after exposure
LT_{50} = Period in days calculated to give 50 per cent mortality

Grubs started dying after one day of exposure to insecticidal and combination treatments. All the treatments except nematode alone were found significantly effective over control. The higher dosage of insecticides alone and combination with nematodes caused 30 to 40 per cent mortality in grubs in comparison to 10.0 to 16.67 per cent in lower dosages alone and combination of the two.

After three days of exposure mortality increased and all the treatments proved significantly superior over control. Treatment of nematode 2000 IJs + chlorpyriphos 0.8 mg/100 ml soil/grub. caused cent per cent mortality whereas higher dosages of chlorpyriphos (0.8 mg) caused 70 per cent mortality and higher dosage of nematode (2000 IJs) proved lethal to only 36.67 test insects. The treatment of nematode (2000 IJs) + quinalphos (1.0 mg) resulted in 80.0 per cent mortality in comparison to 36.67 and 60.0 per cent in nematode (2000 IJs) and qunalphos (1.0 mg) alone (Table 8.a).

The lower dosage combination of nematode (1000 IJs) with chlorpyriphos (0.4 mg) and quinalphos (0.5 mg) resulted in 66.67 and 56.67 per cent mortality respectively whereas individual treatment of nematode, chlorpyriphos and qunalphos caused only 26.67, 50.0 and 46.67 per cent mortality respectively.

With advancement of exposure period cent per cent mortality was observed in test insects; it was after five days in case of chlorpyriphos (0.8 mg) and nematode (2000 IJs) + qunalphos (1.0 mg) and after seven days in case of nematode (1000 IJs) + chlorpyriphos (0.4 mg) and qunalphos (1.0 mg). Increasing trend in grub mortality continued and after ten days of exposure it was 100 per cent in all the treatments except nematode alone (both higher and lower dosages) and qunalphos (0.5 mg) alone which resulted in 86.67, 70.0 and 80 per cent kill respectively.

Combination of nematodes with insecticides resulted in higher mortality than individual use of nematode and insecticides. Therefore, it may be inferred that nematodes are compatible with the two insecticides tested.

The harvested IJs from dead grubs exposed to nematodes (2000 IJs) with chlorpyriphos (0.8 mg) and qunalphos (1.0 mg) were 16,500 and 13,400 per grubs respectively whereas in lower dosage combinations of nematode (1000 IJs) with

Table 8.a **Compatibility of Nematode *Heterorhabditis bacteriophora* with insecticides in relation to first instar grubs of *Holotrichia consanguinea***

S.No.	Treatment (Dosage/100 ml soil/grub)	Mean per cent mortality Days after exposure					Mean No. of N IJS harvested/grub
		1	3	5	7	10	
1.	N 2000 IJs	0.00 (0.99)	36.67 (37.27)	73.33 (58.91)	76.67 (61.12)	86.67 (68.59)	22,500
2.	N 1000 IJs	0.00 (0.99)	26.67 (31.09)	46.67 (43.09)	56.67 (48.83)	70.00 (56.79)	16,800
3.	Ch 0.8 mg	36.67 (37.27)	70.00 (56.79)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	-
4.	Ch 0.4 mg	10.00 (18.43)	50.00 (45.0)	80.0 (63.43)	90.00 (71.57)	100.00 (88.19)	-
5.	Qu 1.0 mg	30.0 (33.21)	60.00 (50.77)	83.33 (65.90)	100.00 (88.19)	100.00 (88.19)	-
6.	Qu 0.5 mg	10.0 (18.43)	46.67 (43.09)	60.00 (50.77)	70.0 (56.79)	80.00 (63.43)	-
7.	N 2000 IJs + ch 0.8 mg	40.00 (39.23)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	16,500
8.	N 1000 IJs + ch 0.4 mg	13.33 (21.41)	66.67 (54.74)	80.00 (63.43)	100.00 (88.19)	100.00 (88.19)	12,400
9.	N 2000 IJs + Qu 1.0 mg	30.00 (33.21)	80.00 (63.43)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	13,400
10.	N 1000 IJs + Qu 0.5 mg	16.67 (24.10)	56.67 (48.83)	70.00 (56.79)	80.00 (63.43)	100.00 (88.19)	9,200
11.	Control.	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	-
	SEm ±	1.993	1.691	1.204	2.368	1.526	
	CD (P=0.05)	6.484	5.501	3.917	7.702	4.965	

N = Nematode
Ch = Chlorpyriphos
Qu = Quinalphos
Figures in parenthesis are angular transformed values

chlorpyriphos (0.4 mg) and quinalphos (0.5 mg) the number was 12,400 and 9,200. The IJs harvested from nematode alone (both higher and lower dosages) were 22,500 and 16,800 per grub respectively (Table 8.a).

4.4.2 Compatibility in relation to second instar grubs of *H. consanguinea*

In relation to second instar grubs the dosages of nematode IJs were increased to 10,000 and 5000 while insecticidal dosages were the same and in all there were ten treatments.

The median lethal time (LT_{50}) for second instar ranged from 1.54 to 6.04 days. The treatments of nematode (10000 and 5000 IJs) required 3.53 and 6.04 days to cause lethal infection, respectively whereas chlorpyriphos (0.8 and 0.4 mg) required only 1.76 and 2.89 days and quinalphos (1.0 and 0.5 mg) 3.13 and 3.94 days respectively. The higher dosages combinations of the two i.e. nematode (10000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) required only 1.54 and 1.87 days respectively while lower dosages combinations of nematode (5000 IJs) with chlorpyriphos (0.4 mg) and quinalphos (0.5 mg) required 2.73 and 3.01 days respectively making it clear that combinations of nematodes with the insecticides required shorter time in comparison to nematode and insecticides alone (Table 7, Fig.6.b). Lethal infection could be noticed after one day of exposure and all the treatments were significantly effective over control. The per cent kill was higher in higher dosages combination of nematode (10,000 IJs) with chlorpyriphos (33.33%) and quinalphos (26.67%). It was more or less similar in case of higher dosages of chlorpyriphos (30.0%) and Quinalphos (26.67%).

After three days of exposure 88.33 and 70.0 per cent grub mortality was found in the combinations of higher dosages of nematodes with chlorpyriphos and quinalphos, respectively. The treatments of higher dosages of nematode, chlorpyriphos and quinalphos alone resulted in 40.0, 70.0 and 60.0 per cent mortality respectively.

Cent per cent mortality was observed in the treatment of nematode (10,000 IJs) + chlorpyriphos (0.8 mg) after five days of exposure whereas with the treatments

Table 8.b **Compatibility of Nematode, *Heterorhabditis bacteriophora* with insecticides in relation to second instar grubs of *Holotrichia consanguinea***

S.No.	Treatment (Dosage/100 ml soil/grub)	Mean per cent mortality Days after exposure					Mean No. of N IJS harvested/grub
		1	3	5	7	10	
1.	N 10,000 IJs	6.67 (14.97)	40.00 (39.23)	56.67 (48.83)	80.00 (63.43)	100.00 (88.19)	40,800
2.	N 5,000 IJs	3.33 (10.51)	26.67 (31.09)	43.33 (41.67)	53.33 (46.91)	83.33 (65.90)	32,500
3.	Ch 0.8 mg	30.00 (33.21)	70.00 (56.79)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	-
4.	Ch 0.4 mg	10.00 (18.43)	50.00 (45.0)	70.0 (56.79)	90.00 (71.57)	100.00 (88.19)	-
5.	Qu 1.0 mg	20.00 (26.57)	60.00 (50.77)	80.00 (63.43)	100.00 (88.19)	100.00 (88.19)	-
6.	Qu 0.5 mg	3.33 (10.51)	36.67 (37.27)	63.33 (52.73)	80.00 (63.43)	80.00 (63.43)	-
7.	N 10,000 IJs + ch 0.8 mg	33.33 (35.26)	83.33 (65.90)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	24,200
8.	N 5,000 IJs + ch 0.4 mg	10.00 (18.43)	53.33 (46.91)	83.33 (65.90)	93.33 (75.03)	100.00 (88.19)	18,500
9.	N 10000 IJs + Qu 1.0 mg	26.67 (31.09)	70.00 (56.79)	86.67 (68.59)	100.00 (88.19)	100.00 (88.19)	21,500
10.	N 5,000 IJs + Qu 0.5 mg	10.00 (18.43)	40.00 (39.23)	70.00 (56.79)	90.00 (71.57)	100.00 (88.19)	15,500
11.	Control.	0.00 (0.99)	0.00 (0.99)	0.00 0.99	0.00 (0.99)	0.00 (0.99)	-
	SEm ±	3.166	1.348	1.542	2.208	0.819	
	CD (P=0.05)	10.300	4.384	5.014	7.181	2.662	

N = Nematode
Ch = Chlorpyriphos
Qu = Quinalphos
Figures in parenthesis are angular transformed values

of chlorpyriphos (0.8 mg) quinalphos (1.0 mg) alone and nematode (10000 IJs) + quinalphos (1.0 mg) same results same could be achieved after seven days of exposure. After 10 days, mortality increased to 100 per cent in all the treatments except lower dosages of nematode (5000 IJs) and quinalphos (0.5 mg) which resulted 83.33 and 80.0 per cent mortality respectively.

The nematode IJs harvested from dead grubs exposed to higher dosages combination of nematode (10,000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (0.8 mg) were 24,200 and 21,500 per grub respectively whereas in lower dosages combinations of nematode (5000 IJs) with chlorpyriphos (0.4 mg) and quinalphos (0.5 mg) the number was 18,500 and 15,500 per grub, respectively. The nematode IJs harvested from higher and lower dosages (10,000 & 5,000 IJs) of nematode were 40,800 and 32,500 per grub, respectively (Table 8.b).

4.4.3. Compatibility in relation to third instar grubs of *H. consanguinea*

As mentioned in materials and methods the increased dosages of nematodes tested against third instar grubs were 20,000 and 10,000 IJs/100 ml soil/grub. together with chlorpyriphos (0.8 and 0.4 mg) and quinalphos (1.0 and 0.5 mg).

The median lethal time (LT_{50}) for third instar was found to range from 1.61 to 5.32 days. The treatments of nematode (20,000 and 10,000 IJs) required 3.77 and 5.32 days respectively to cause lethal infection in 50 per cent of tested population whereas chlorpyriphos (0.8 and 0.4 mg) took shorter time i.e. 1.89 and 3.57 days in comparison 2.48 and 4.28 days taken by quinalphos (1.0 and 0.5 mg). The combination of nematode (20,000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) separately required shorter time of 1.61 and 1.97 days in comparison to 2.82 and 3.25 days taken with the lower doses combination of nematode (1000 IJs) with chlorpyriphos (0.4 mg) and quinalphos (0.5 mg) respectively. The LT_{50} for nematode (10,000 IJs) alone was 5.32, chlorpyriphos (0.4 mg) 3.57 and quinalphos (0.5 mg) 4.28 days (Table 7; Fig. 6.c).

The mortality after a day of exposure ranged from 0 to 33.33 per cent. It was 33.33 in case of higher dosage combination of nematode with chlorpyriphos. The

treatment of chlorpyriphos (0.8 mg) alone as well as nematode (1000 IJs) and quinalphos (1.0 mg) resulted in 30 per cent grub mortality.

After three days of exposure 70 per cent mortality was found in the treatment of nematode (20,000 IJs) + chlorpyriphos (0.8 mg) followed by 60 per cent in the treatments of chlorpyriphos (0.8 mg) alone and nematode (20,000 IJs) + quinalphos (1.0 mg). The higher mortality (70%) was observed in the treatment of nematodes (20000 IJs) in combination with chlorpyriphos (0.8 ml) in comparison to 40 per cent achieved with higher dosages of nematode as well as chlorpyriphos separately. Similarly 60 per cent mortality was found in higher dosage combination of nematode with quinalphos.

After five days cent per cent mortality was achieved with higher dosage combination of nematode and chlorpyriphos whereas nematode with quinalphos and chlorpyriphos separately provided similar results after seven days of exposure while after ten days it so happened with all other treatments except nematode (10,000 IJs), chlorpyriphos (0.4 mg) and quinalphos (0.5 mg) which resulted in 80.0, 83.33 and 80.0 per cent mortality respectively.

The nematode IJs harvested with dead grubs from higher dosages combination of nematode (20,000 IJs) with chlorpyriphos (0.8 m.) and quinalphos (1.0 m.) were 35 800 and 31,600 in comparison to 80500 IJs harvested from nematode (20000 IJs). In lower dosage of nematode (10,000 IJs) with chlorpyriphos (0.4 mg) and quinalphos (1.0 mg) the counts were 32,500 and 24,200 respectively whereas in the treatment of nematode (10,000 IJs) alone the IJs counts were 62000 (Table 8.c).

It may be concluded that nematodes in combinations with chlorpyriphos and quinalphos proved more effective and caused higher mortality than nematode alone as well as insecticidal treatment. The time required for lethal infection was also shorter. The multiplication of nematode in the dead grub treated with combinations of nematode and insecticides was also recorded which proved that insecticides did not effect nematode multiplication adversely but the IJs harvested from the treatment of nematode-insecticidal combination was less than nematode alone.

Table 8.c **Compatibility of Nematode *Heterorhabditis bacteriophora* with insecticides in relation to third instar grubs of *Holotrichia consanguinea***

S.No.	Treatment (Dosage/100 ml soil/grub)	Mean per cent mortality Days after exposure					Mean No. of N IJS harvested/grub
		1	3	5	7	10	
1.	N 20000 IJs	3.33 (10.51)	40.00 (39.23)	60.00 (50.77)	73.33 (58.91)	100.00 (88.19)	80,500
2.	N 10000 IJs	0.00 (0.99)	36.67 (37.27)	50.00 (45.00)	63.33 (52.73)	80.00 (63.43)	62,000
3.	Ch 0.8 mg	30.00 (33.21)	60.00 (50.77)	90.00 (71.57)	100.00 (88.19)	100.00 (88.19)	-
4.	Ch 0.4 mg	6.67 (14.97)	40.00 (39.23)	63.33 (52.73)	80.00 (63.43)	83.33 (65.90)	-
5.	Qu 1.0 mg	20.00 (26.57)	46.67 (43.09)	83.33 (65.90)	86.67 (68.59)	100.00 (88.19)	-
6.	Qu 0.5 mg	3.33 (10.51)	36.67 (37.27)	60.00 (50.77)	70.00 (56.79)	80.0 (63.43)	-
7.	N 20,000 IJs + ch 0.8 mg	33.33 (35.26)	70.00 (56.79)	100.00 (88.19)	100.00 (88.19)	100.00 (88.19)	35,800
8.	N 10,000 IJs + ch 0.4 mg	10.00 (18.43)	53.33 (46.91)	70.00 (56.79)	90.00 (71.57)	100.00 (88.19)	32,500
9.	N 20,000 IJs + Qu 1.0 mg	30.00 (33.21)	60.00 (50.77)	83.33 (65.90)	100.00 (88.19)	100.00 (88.19)	31,600
10.	N 10,000 IJs + Qu 0.5 mg	10.00 (18.43)	50.00 (45.00)	63.33 (52.73)	76.67 (61.12)	100.00 (88.19)	24,200
11.	Control.	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	-
	SEm ±	3.096	1.185	2.626	1.388	2.344	
	CD (P=0.05)	10.069	3.855	8.542	4.515	7.626	

N = Nematode
Ch = Chlorpyriphos
Qu = Quinalphos
Figures in parenthesis are angular transformed values

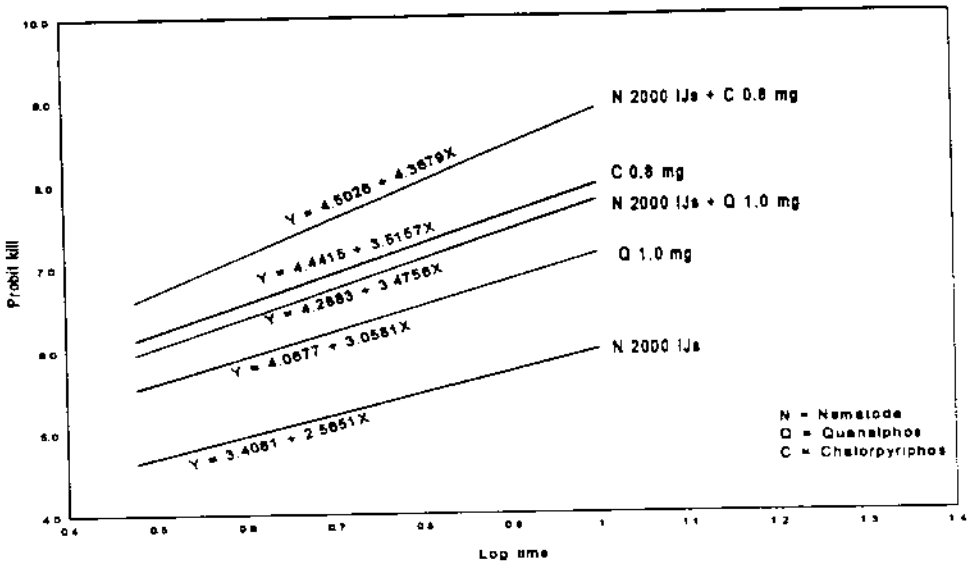


Fig 6.a Log time-probit kill line of compatibility of nematode, *Heterorhabditis bacteriophora* with insecticides in relation to first instar *Holotrichia consanguinea*

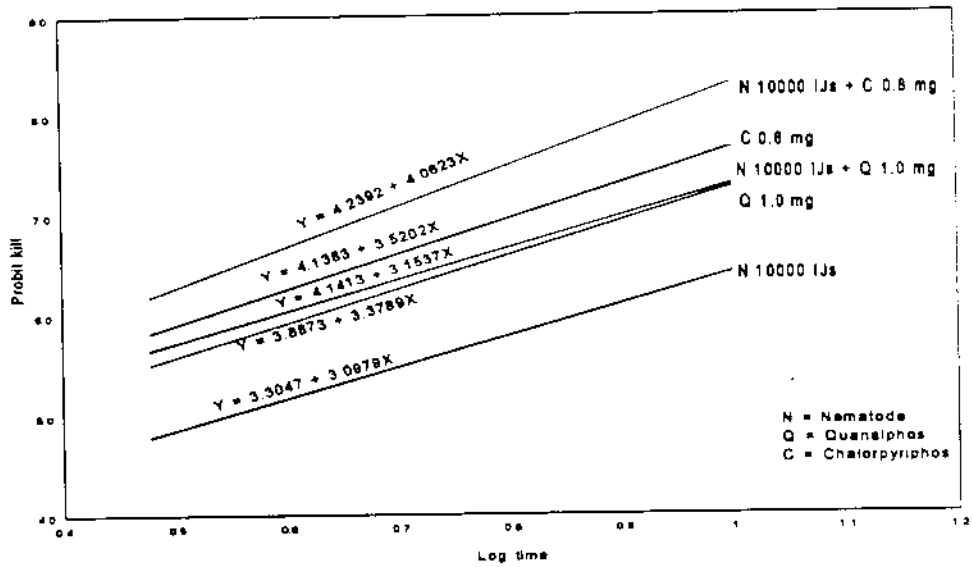


Fig 6.b Log time-probit kill line of compatibility of nematode, *Heterorhabditis bacteriophora* with insecticides in relation to second instar *Holotrichia consanguinea*

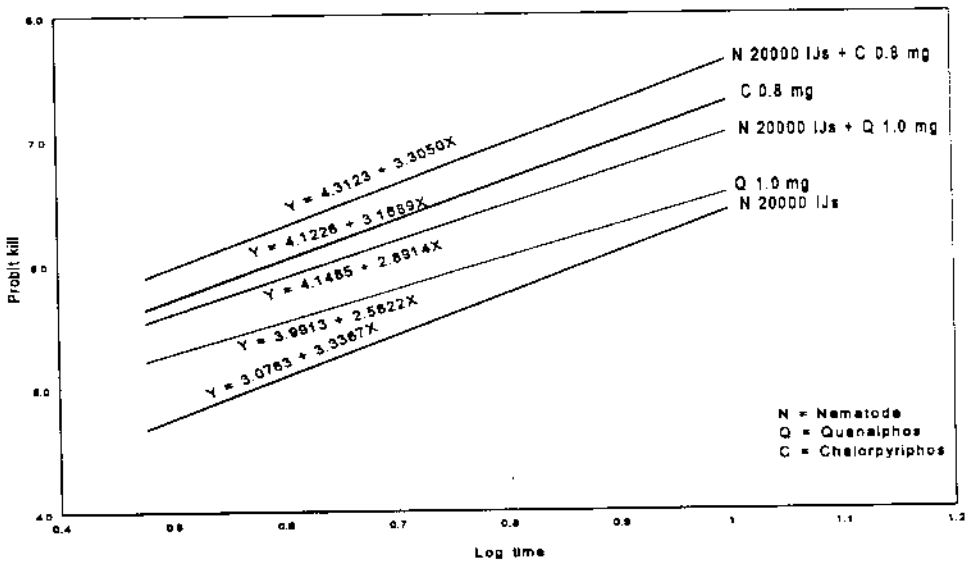


Fig 6.c Log time-probit kill line of compatibility of nematode, *Heterorhabditis bacteriophora* with insecticides in relation to third instar *Holotrichia consanguinea*

4.5. COMPATIBILITY OF NEMATODE, *H. bacteriophora* WITH FUNGICIDES (bavistin and thiram)

All the instars of white grub were found susceptible to nematode even when nematodes were used in combination with fungicides. The compatibility of the two was assessed on the basis of per cent mortality and lethal time required for 50 per cent tested population.

4.5.1 Compatibility in relation to first instar grubs of *H. consanguinea*

The nematode *H. bacteriophora* in two dosages of 2000 and 1000 IJs/100 ml soil/grub and fungicides also in two dosages i.e. bavistin (0.16 and 0.08 mg) and thiram (0.24 and 0.12 mg) together with their higher and lower dosages combination were tested against first instar white grub under laboratory conditions. As such there were ten treatments. The median lethal time (LT_{50}) ranged from 3.22 to 5.89 days. The treatments of nematode 2000 and 1000 IJs/100 ml soil/grub required 3.91 and 5.89 days, respectively to cause lethal infection where as slightly less time was required to cause lethal infection in combinations with fungicides. The treatments of nematode (2000 IJs) + bavistin (0.16 mg) and nematode (1000 IJs) + bavistin (0.08 mg) required 3.22 and 5.07 days respectively while nematode (2000 IJs) + thiram (0.24 mg) and nematode (1000 IJs) + thiram (0.12 mg) took 4.09 and 5.31 days, respectively (Table 9; Fig. 7.a).

All the treatments except fungicides alone were found significantly superior over control after two days of exposure. The grub mortality (30.0 and 20.0%) in the combinations of nematode with bavistin and thiram was found slightly higher than nematode alone (20%) though both were statistically at par. Similar trend in grub mortality was also found in lower dosages combination of nematode with bavistin and thiram where the mortality was 20 and 16.67 per cent respectively. After six days of exposure mortality got increased to 70.0 and 53.33 per cent in the treatment of nematode alone (2000 and 1000 IJs) but in the combinations of nematode with bavistin both at higher and lower dosages (70 and 60 %) and thiram (66.67 and 56.67%) it was slightly higher than nematode alone. In higher dosage (0.24 mg) of thiram alone also grub mortality was noticed it was 6.67 per cent only. Increasing

Table 9 **Compatibility of Nematode, *Heterorhabditis bacteriophora* with fungicides in relation to different instar grubs of *Holotrichia consanguinea* based on LT_{50} values**

S. No.	Treatment (Dosage/100 ml soil/grub)	Time (log) - Kill (probit)	
		Regression equation	LT_{50}
	First instar *Holotrichia consanguinea*		
1.	N 2000 IJs	Y = 3.1760 + 3.0787 X	3.91
2.	N 1000 IJs	Y = 2.7613 + 2.9058 X	5.89
3.	Bavistin 0.16 mg	Low mortality hence, LT_{50} not calculated	
4.	Bavistin 0.08 mg	-do-	
5.	Thiram 0.24 mg	-do-	
6.	Thiram 0.12 mg	-do-	
7.	N 2000 IJs + Bavistin 0.16 mg	Y = 3.3976 + 3.1576 X	3.22
8.	N 1000 IJs + Bavistin 0.08 mg	Y = 3.5571 + 2.0470 X	5.07
9.	N 2000 IJs + Thiram 0.24 mg	Y = 3.5290 + 2.3252 X	4.09
10.	N 2000 IJs + Thiram 0.12 mg	Y = 3.2290 + 2.4431 X	5.31
	Second instar *Holotrichia consanguinea*		
1.	N 10,000 IJs	Y = 3.2483 + 2.8468 X	4.12
2.	N 5,000 IJs	Y = 3.1345 + 2.4076 X	5.95
3.	Bavistin 0.16 mg	Low mortality hence, LT_{50} not calculated	
4.	Bavistin 0.08 mg	-do-	
5.	Thiram 0.24 mg	-do-	
6.	Thiram 0.12 mg	-do-	
7.	N 10,000 IJs + Bavistin 0.16 mg	Y = 3.4914 + 2.5528 X	3.90
8.	N 5,000 IJs + Bavistin 0.08 mg	Y = 2.9024 + 2.8268 X	5.52
9.	N 10,000 IJs + Thiram 0.24 mg	Y = 3.4497 + 2.6561 X	3.83
10.	N 5,000 IJs + Thiram 0.12 mg	Y = 3.2622 + 2.3269 X	5.58

S. No.	Treatment (Dosage/100 ml soil/grub)	Time (log) - Kill (probit)	
		Regression equation	LT_{50}
	Third instar of *Holotrichia consanguinea*		
1.	N 20,000 IJs	Y = 3.7297 + 2.4024 X	3.38
2.	N 10,000 IJs	Y = 3.5383 + 1.8967 X	5.90
3.	Bavistin 0.16 mg	Low mortality hence, LT_{50} not calculated	
4.	Bavistin 0.08 mg	-do-	
5.	Thiram 0.24 mg	-do-	
6.	Thiram 0.12 mg	-do-	
7.	N 20,000 IJs + Bavistin 0.16 mg	Y = 3.9756 + 1.9820 X	3.29
8.	N 10,000 IJs + Bavistin 0.08 mg	Y = 3.9538 + 1.5800 X	4.59
9.	N 20,000 IJs + Thiram 0.24 mg	Y = 4.1461 + 2.4024 X	2.85
10.	N 10,000 IJs + Thiram 0.12 mg	Y = 3.4820 + 2.2031 X	4.89

N = Nematode
Y = Probit kill
X = Log of time (days) after exposure
LT_{50} = Period in days calculated to give 50 per cent mortality.

Table 10.a **Compatibility of nematode, *Heterorhabditis bacteriophora* with fungicides in relation to first instar grubs of *Holotrichia consanguinea***

S. No.	Treatment (Dosage/100ml-soil/grub)	Mean per cent mortality Days after exposure.			Mean No. of NIJs harvested/grub
		2	6	10	
1.	Nematode 2000 IJs	20.00 (26.57)	70.00 (56.79)	93.33 (75.03)	22500
2.	Nematode 1000 IJs	10.00 (18.43)	53.33 (46.91)	70.00 (56.57)	16800
3.	Bavistin 0.16 mg	0.00 (0.99)	0.00 (0.99)	6.67 (14.97)	
4.	Bavistin 0.08 mg	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
5.	Thiram 0.24 mg	0.00 (0.99)	6.67 (14.97)	10.00 (18.43)	
6.	Thiram 0.12 mg	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
7.	Nematode 2000 IJs + Bavistin 0.16 mg	30.00 (33.21)	70.00 (56.79)	100.00 (88.19)	21,500
8.	Nematode 1000 IJs + Bavistin 0.08 mg	20.00 (18.43)	60.00 (50.77)	80.00 (63.43)	15,800
9.	Nematode 2000 IJs + Thiram 0.24 mg	20.00 (26.57)	66.67 (54.77)	90.00 (71.51)	22,100
10.	Nematode 1000 IJs + Thiram 0.12 mg	16.67 (24.10)	56.67 (48.83)	73.33 (58.91)	15,200
11.	Control.	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
	SEm ±	1.997	2.526	2.743	
	CD (P=0.05)	6.497	8.216	8.922	

N = Nematode

Figures in parenthesis are angular transformed values.

trend of grub mortality continued even after ten days of exposure. The treatments of nematodes alone (2000 and 1000 IJs) registered 93.33 and 70.00 per cent mortality whereas , combination treatments with bavistin (0.16 & 0.08 mg) showed 100 and 80 per cent and thiram (0.24 and 0.12 mg) 90.00 and 73.33 per cent mortality respectively. In higher dosages bavistin (0.16 mg) and thiram (0.24 mg) also 6.67 and 10.0 per cent mortality was recorded (Table 10.a).

The nematode IJs harvested from dead grubs varied from 15800 to 22500 per grub. There was no significant difference in the treatment of nematode alone as well as with the combination of fungicides on this account. The IJs harvested from higher dosages nematode alone and combinations with bavistin and thiram were 22500, 21500 and 22,100 per grubs whereas with lower dosages the number was 16800, 15,800 and 15200 per grub respectively (Table 10.a).

4.5.2 Compatibility in relation to second instar grubs of *H. consanguinea*

In relation to second instar the dosages of nematode, *H. bacteriophora* were increased to 10,000 and 5000 IJs/100 ml soil/grub while those of fungicides were kept same. The compatibility was assessed on the basis of grub mortality and median lethal time (LT_{50}) here the median lethal time (LT_{50}) ranged from 3.90 to 5.95 days. The treatments of nematode (10,000 and 5000 IJs) required 4.12 and 5.95 days to cause lethal infection whereas when combined with fungicides the LT_{50} was slightly low. The treatments of nematode (both at higher and lower dosages) with bavistin required 3.90 and 5.52 day and with thiram 3.83 and 5.58 days to cause 50 per cent mortality respectively (Table 9, Fig. 7.b).

All the treatments (except fungicides alone) were found significantly effective over control after two days of exposure. The grub mortality in the two dosages of nematode was 20 and 10 per cent and with higher dosage combination with bavistin also it was 20 per cent however, with of thiram it was slightly higher (26.67 and 20.0%). After six days of exposure mortality increased to 63.33, 66.67 and 60.0 per cent in the treatments of higher dosage nematode alone and in combination with bavistin and thiram respectively. The treatment of bavistin (0.16 mg) and thiram

Table 10.b Compatibility of nematode, *Heterorhabditis bacteriophora* with fungicides in relation to second instar grubs of *Holotrichia consanguinea*

S. No	Treatment (Dosage/100ml-soil/grub)	Mean per cent mortality Days after exposure			Mean No. of N IJS harvested/grub
		2	6	10	
1.	Nematode 10,000 IJs	20.00 (26.57)	63.33 (52.73)	90.00 (71.57)	40,800
2.	Nematode 5,000 IJs	10.00 (18.43)	50.00 (45.0)	70.00 (56.79)	32,500
3.	Bavistin 0.16 mg	0.00 (0.99)	3.33 (10.51)	3.33 (10.51)	
4.	Bavistin 0.08 mg	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
5.	Thiram 0.24 mg	0.00 (0.99)	6.67 (14.97)	6.67 (14.97)	
6.	Thiram 0.12 mg	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
7.	Nematode 10,000 IJs + Bavistin 0.16 mg	20.00 (26.57)	66.67 (54.74)	90.00 (71.57)	39,000
8.	Nematode 5000 IJs + Bavistin 0.08 mg	10.00 (18.43)	50.00 (45.0)	76.67 (61.12)	31,200
9.	Nematode 10,000 IJs + Thiram 0.24 mg	26.67 (31.09)	60.00 (50.77)	93.33 (75.03)	40,100
10.	Nematode 5000 IJs + Thiram 0.12 mg	20.00 (26.67)	46.67 (43.09)	80.00 (63.43)	30,800
11.	Control.	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
	SEm ±	1.451	2.686	3.769	
	CD (P=0.05)	4.721	8.736	12.260	

N = Nematode

Figures in parenthesis are angular transformed values.

(0.24 mg) also proved lethal to 3.33 and 6.67 per cent grubs. Mortality continued to increase and after ten days of exposure it was 90.0 and 70.0 per cent in the two dosages of nematode. The higher dosage of nematode in combination with bavistin (0.16 mg) and thiram (0.24 mg) registered 90.0 and 93.33 per cent mortality whereas in lower dosage combination it was 76.67 and 80.0 per cent. The treatments of nematode higher dosage alone and combinations with higher dosages fungicides were at par statistically but differed significantly with lower dosages,

The difference between the harvested IJs from dead grub treated with nematode alone and in combination with fungicides was non significant and the number was 40,800, 39000 and 40100 per grub with higher dosage of nematode (10000 IJs), bavistin (0.16 mg) thiram (0.24 mg) while with lower dosages it was 32,500, 31200 and 30,800 per grub respectively (Table 10.b).

4.5.3 Compatibility in relation to third instar grubs of *H. consanguinea*

The dosages of nematodes were increased to 20,000 and 10,000 IJs with no change in the dosages of fungicides and number of treatments.

The median lethal time against third instar ranged from 2.85 to 5.90 days. The treatments of nematode at 20,000 and 10,000 IJs/100 ml soil/grub took 3.38 and 5.90 days respectively to cause 50 per cent grub mortality whereas combination with fungicides took slightly less time. The nematodes both at higher and lower dosages combination with bavistin required 3.29 and 4.59 days while with thiram the LT_{50} values were 2.85 and 4.89 days respectively (Table 9; Fig. 7.c).

Significant effect of treatment was noticed after two days over the check except the treatment of fungicides alone. Ten days after exposure grub mortality in the treatment of nematode alone (20,000 IJs) was to the extent of 93.33 per cent which was statistically at par with the higher dosages combinations of bavistin (96.67%) and thiram (93.33%). The treatment with nematode (10,000 IJs) resulted in 66.67 per cent grub mortality which was at par with the lower dosages combination of bavistin (73.33 %) and thiram (73.33 %). The effect of higher and lower dosage

Table 10.c Compatibility of nematode, *Heterorhabditis bacteriophora* with fungicides in relation to third instar grubs of *Holotrichia consanguinea*

S.No	Treatment (Dosage/100ml-soil/grub)	Mean per cent mortality Days after exposure.			Mean No. of N IJS harvested/grub
		2	6	10	
1.	Nematode 20,000 IJs	33.33 (35.26)	66.67 (54.74)	93.33 (75.03)	62,000
2.	Nematode 10,000 IJs	20.00 (26.57)	53.33 (46.91)	66.67 (54.74)	48,000
3.	Bavistin 0.16 mg	0.00 (0.99)	0.00 (0.99)	3.33 (10.51)	
4.	Bavistin 0.08 mg	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
5.	Thiram 0.24 mg	0.00 (0.99)	3.33 (10.51)	6.67 (14.97)	
6.	Thiram 0.12 mg	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
7.	Nematode 20000 IJs + Bavistin 0.16 mg	40.00 (39.23)	63.33 (52.73)	96.67 (79.49)	60,800
8.	Nematode 10000 IJs + Bavistin 0.08 mg	33.33 (35.26)	50.00 (45.00)	73.33 (58.91)	51,000
9.	Nematode 20,000 IJs + Thiram 0.24 mg	40.00 (39.23)	70.00 (56.79)	93.33 (75.03)	60,300
10.	Nematode 10,000 IJs + Thiram 0.12 mg	20.00 (26.57)	60.00 (50.77)	73.33 (58.91)	46,500
11.	Control.	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	
	SEm ±	1.337	2.314	3.695	
	CD (P=0.05)	4.348	7.528	12.017	

N = Nematode

Figures in parenthesis are angular transformed values.

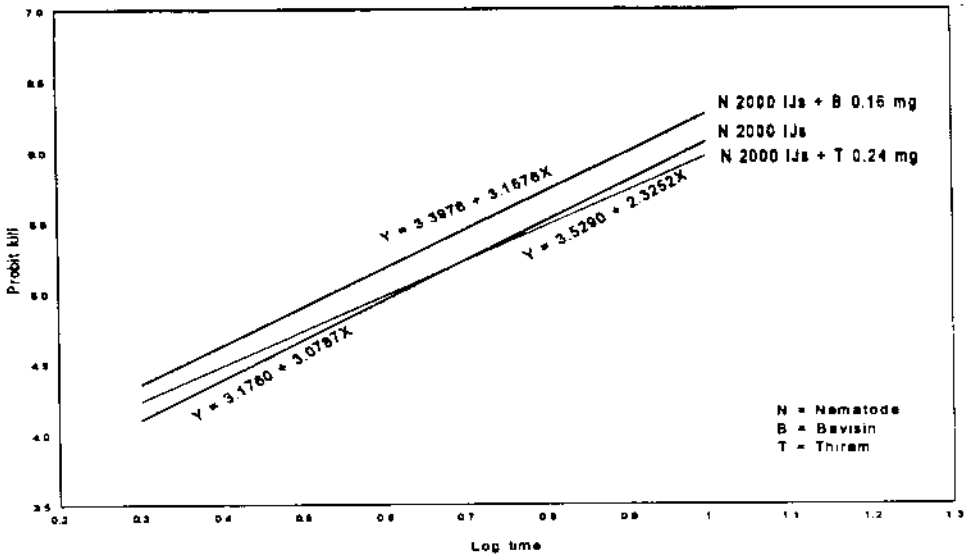


Fig 7.a Log time-probit kill line of compatibility of nematode, *Heterorhabditis bacteriophora* with fungicides in relation to first instar *Holotrichia consanguinea*

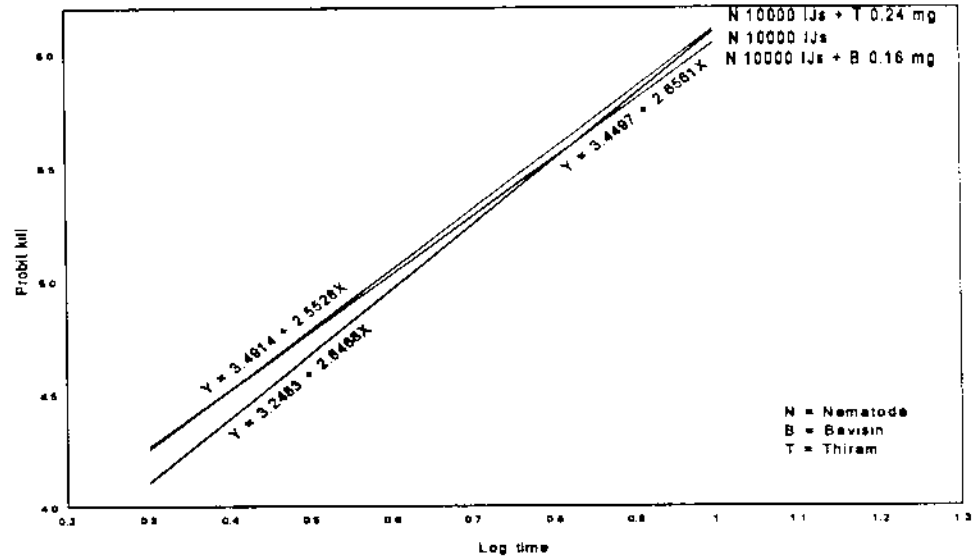


Fig 7.b Log time-probit kill line of compatibility of nematode, *Heterorhabditis bacteriophora* with fungicides in relation to second instar *Holotrichia consanguinea*

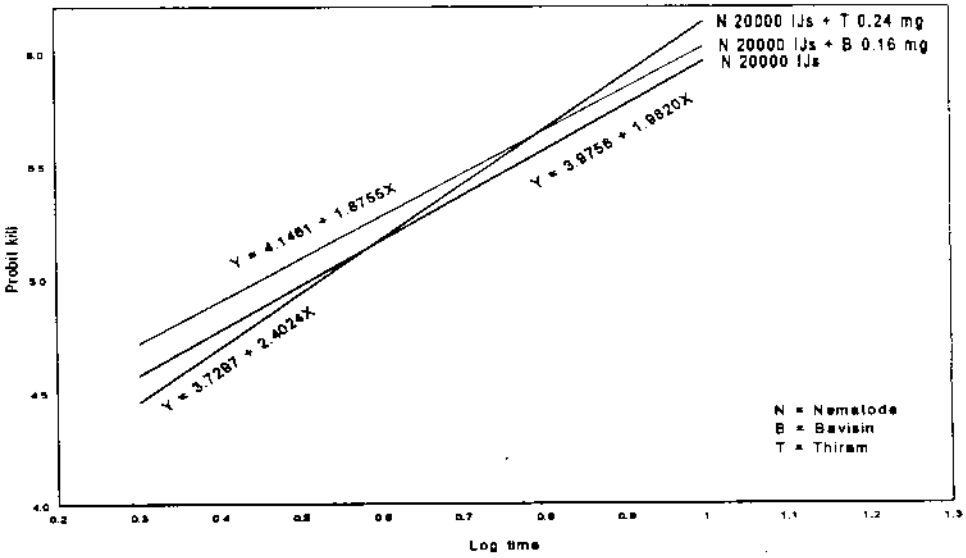


Fig 7.c Log time-probit kill line of compatibility of nematode, *Heterorhabditis bacteriophora* with fungicides in relation to third instar *Holotrichia consanguinea*

differed significantly. The treatment of bavistin and thiram at higher dosages also proved lethal causing 3.33 and 6.67 per cent mortality of grubs respectively.

The number of nematode IJs harvested depended on the size of the grub and number increased with size. The number ranged from 46,500 to 62,000 per grub. The nematode IJs harvested from the treatment of nematode alone (20,000 IJs) and with the combinations of higher dosages bavistin (0.16 mg) and thiram (0.24 mg) were 62,000, 60,800 and 60,300 per grub, respectively. Similarly with lower dosages nematode alone (10,000 IJs) and combinations with bavistin (0.08 mg) and thiram (0.24 mg) the number was 48,000, 51000 and 46,500 per grub respectively.

On the basis of mortality data it may be inferred that nematode was compatible with fungicides as per cent mortality slightly increased in combination treatments (Table 10.c).

4.6 COMPATIBILITY OF FUNGUS, *M. anisopliae* WITH INSECTICIDES AND FUNGICIDES

The compatibility of *M. anisopliae* was tested with insecticides *viz.*, chlorpyriphos and quinalphos and fungicides *viz.*, bavistin and thiram at 100, 500 and 1000 ppm concentration. Among the tested insecticides and fungicides cent per cent inhibition of mycelial growth was found in the treatment of bavistin at all concentrations, other treatments were found to be moderately compatible at 100 ppm concentration (less than 50% inhibition). The inhibition by chlorpyriphos, quinalphos and thiram at 100 ppm concentration was 40.98, 34.43 and 44.26 per cent respectively. At higher concentration (1000 ppm) none of the insecticide and fungicide tested was found compatible with *M. anisopliae*. The inhibition at this concentration was more than 50 per cent i.e. 70.05, 67.21 and 77.05 per cent with chlorpyriphos, quinalphos and thiram respectively. At 500 ppm concentration quinalphos (42.62 % inhibition) was found to be slightly compatible. The inhibition at this concentration by chlorpyriphos and thiram was 54.10 and 60.60 per cent, respectively. The inhibition of mycelial growth by fungicides was found to be higher than insecticides (Table 11; Fig. 8).

Table 11 Compatibility of fungus, *M. anisopliae* with insecticides and fungicides

S.No.	Insecticides/ Fungicides	% growth inhibition at different conc. (ppm)		
		100	500	1000
1	Chlorpyriphos	40.98 (180)	54.10 (140)	70.05 (90)
2	Quinalphos	34.43 (200)	42.62 (175)	67.21 (100)
3	Bavistin	100.00 (0)	100.00 (0)	100.00 (0)
4	Thiram	44.26 (170)	60.66 (120)	77.05 (70)
5	Control	- (305)	- (305)	- (305)

Figures in the parenthesis are average dry mycelial mat in mg.

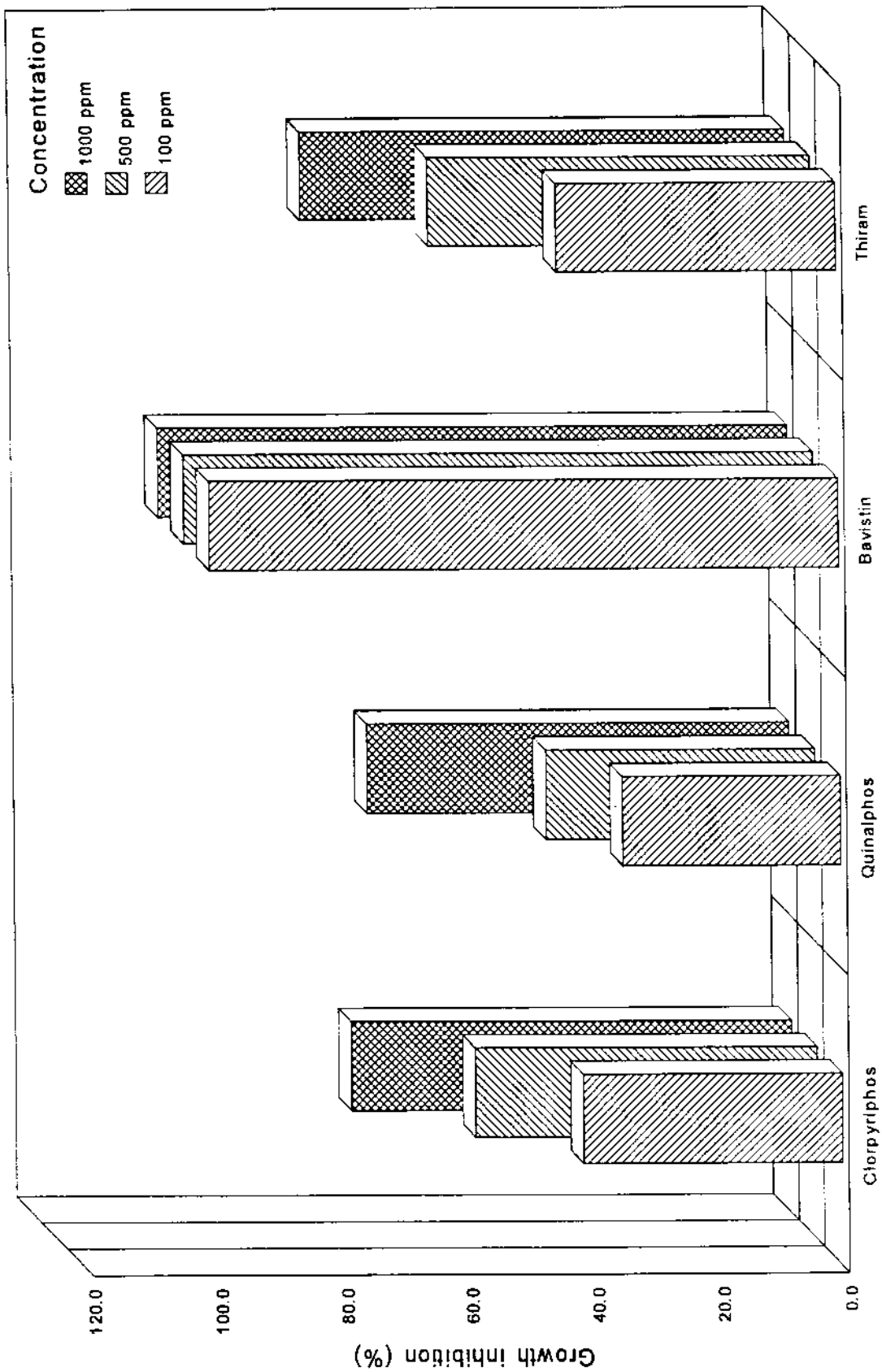


Fig 8. Compatibility of fungus *Metarrhizium anisopliae* with insecticides and fungicides

4.7 COMPATIBILITY OF NEMATODE, *H. bacteriophora* AND FUNGUS, *M. anisopliae* WITH FUNGICIDES AND INSECTICIDES IN RELATION TO FIRST INSTAR GRUBS OF *H. consanguinea*

Compatibility of nematode and fungus with insecticides and fungicides in relation to first instar, *H. consanguinea* was assessed based on per cent grub mortality and lethal time required to kill 50 per cent grubs. The two dosages of nematodes (2000 and 1000 IJs), fungus (1 x 10^9 and 5 x 10^8 spores), chlorpyriphos (0.8 and 0.4 ml), quinalphos (1.0 and 0.5 ml), bavistin (0.16 and 0.08 mg) and thiram (0.24 and 0.12 mg) with their higher and lower dosages combinations of nematode and fungus with insecticides and fungicides were tested. In all there were eight treatments.

The median lethal time (LT_{50}) in different treatments ranged from 1.49 to 4.91 days. The treatments of higher combinations of nematode 2000 IJs + fungus 1 x 10^9 spores with chlorpyriphos 0.8 ml quinalphos 1.0 ml per 100 ml soil/grub required 1.49 and 2.06 days to cause lethal infection and lower dosages combination required 2.74 and 3.54 days. respectively. Similarly higher dosages combinations of nematode and fungus with bavistin (0.16 mg) and thiram (0.24 mg) required 3.29 and 4.02 days to cause lethal infection and lower dosages combinations required 4.24 and 4.91 days, respectively. The nematode and fungus with the combinations of insecticides required less time to kill 50 per cent test grubs as compared to nematode and fungus combination with fungicides. The treatments of higher dosages nematode and fungus combinations with chlorpyriphos and quinalphos required only 1.49 and 2.06 days to cause lethal infections whereas nematode and fungus combinations with bavistin and thiram required 3.29 and 4.02 days. Similar trend of mortality was also found in lower dosages combinations (Table 12).

One day after exposure all the treatments except lower dosages combinations of nematode (100 IJs) and fungus (5 x 10^8 spores) with bavistin and thiram (3.33 %) were found significantly effective over control. The highest grub mortality (33.33%) was found in the treatment of higher dosages nematode (2000 IJs) and fungus (1 x 10^9 spores) with chlorpyriphos (0.8 ml) followed by quinalphos (1.0 ml) which resulted

Table 12 **Compatibility of nematode, *Heterorhabditis bacteriophora* and fungus, *Metarrhizium anisopliae* with fungicides and insecticides in relation to first instar grubs of *Holotrichia consanguinea* based on LT_{50} values**

S. No.	Treatment (Dosage/100 ml soil/grub)	Time (log) - Kill (probit)	
		Regression equation	LT_{50}
1.	N 2000 IJs + F 1 x 10^9 spores + Chlorpyriphos 0.8 mg	Y = 4.3591 + 3.7292 X	1.49
2.	N 2000 IJs + F 1 x 10^9 spores + Quinalphos 1.0 mg	Y = 3.9350 + 3.3939 X	2.06
3.	N 1000 IJs + F 5 x 10^8 spores + Chlorpyriphos 0.4 mg	Y = 3.8055 + 2.7264 X	2.74
4.	N 1000 IJs + F 5 x 10^8 spores + Quinalphos 0.5 mg	Y = 3.6476 + 2.4642 X	3.54
5.	N 2000 IJs + F 1 x 10^9 spores + Bavistin 0.16 mg	Y = 3.6511 + 2.6116 X	3.29
6.	N 2000 IJs + F 1 x 10^9 spores + Thiram 0.24 mg	Y = 3.2207 + 2.9461 X	4.02
7.	N 1000 IJs + F 5 x 10^8 spores + Bavistin 0.08 mg	Y = 3.2156 + 2.8930 X	4.14
8.	N 1000 IJs + F 5 x 10^8 spores + Thiram 0.12 mg	Y = 3.2924 + 2.4719 X	4.91

N = Nematode F = Fungus Y = Probit kill
X = Log of time (days) after exposure
LT_{50} = Period in days calculated to give 50 per cent mortality.

in 16.67 per cent mortality, whereas only 6.67 per cent mortality was found in the higher dosages combination of nematode and fungus with bavistin and thiram.

Three days after exposure all the treatments were found significantly superior over control. The highest grub mortality (73.33%) was found in the treatment of higher dosages combinations of nematode and fungus with chlorpyriphos followed by quinalphos which resulted in 63.33 per cent mortality and both were at par statistically. Similarly, 53.33 and 43.33 per cent grub mortality was found in the treatment of lower dosages combinations of nematodes and fungus with chlorpyriphos and quinalphos. The results were statistically at par with treatment of higher dosages combinations of nematodes and fungus with bavistin (0.16 mg) which resulted 46.67 per cent mortality. The lowest grub mortality (26.67 %) was found in the treatment of lower dosages combinations of nematode and fungus with thiram. It was statistically at par with the lower dosages combinations of nematode and fungus with bavistin (36.67 %) and higher dosages combinations of nematode and fungus with thiram (36.67 %). Higher mortality was found in the combinations of nematode with insecticides than with fungicides. Similar trend of grub mortality was found after five days of exposure. Cent per cent mortality was found in the treatment of higher dosage combination of nematode and fungus with chlorpyriphos (Table 13).

Ten days after exposure, cent per cent mortality was also found in the higher dosages combinations of nematode and fungus with quinalphos. The lower dosages combinations of nematode and fungus with chlorpyriphos and quinalphos resulted in 93.33 and 83.33 per cent grub mortality, respectively and were found to differ statistically from each other. Higher grub mortality was found in the combination of nematode and fungus with chlorpyriphos than with quinalphos. The higher dosages combination of nematode and fungus with bavistin and thiram registered 83.33 and 80.0 per cent mortality respectively and were statistically at par. Similarly 70.0 and 63.33 per cent mortality was observed with lower dosages combinations. Further higher grub mortality was observed in the combinations of nematode and fungus with bavistin as compared with thiram.

Table 13 **Compatibility of nematode, *Heterorhabditis bacteriophora* and fungus *Metarrhizium anisopliae* with fungicides and insecticides in relation to first instar grubs of *Holotrichia consanguinea***

S. No.	Treatment (Dosage/100 ml soil/grub)	Mean per cent mortality Days after exposure			
		1	3	5	7
1.	N 2000 IJs + F 1 x 10^9 spores + Chlorpyriphos 0.8 mg	33.33 (35.26)	73.33 (58.91)	100.00 (88.19)	100.00 (88.19)
2.	N 2000 IJs + F 1 x 10^9 spores + Quinalphos 1.0 mg	16.67 (24.10)	63.33 (52.73)	86.67 (68.59)	100.00 (88.19)
3.	N 1000 IJs + F 5 x 10^8 spores + Chlorpyriphos 0.4 mg	10.00 (18.43)	53.33 (46.91)	73.33 (58.91)	93.33 (75.03)
4.	N 1000 IJs + F 5 x 10^8 spores + Quinalphos 0.5 mg	6.67 (14.97)	43.33 (41.17)	60.00 (50.77)	83.33 (65.90)
5.	N 2000 IJs + F 1 x 10^9 spores + Bavistin 0.16 mg	6.67 (14.97)	46.67 (43.09)	63.33 (52.73)	83.33 (65.90)
6.	N 2000 IJs + F 1 x 10^9 spores + Thiram 0.24 mg	6.67 (14.97)	36.67 (37.27)	60.00 (50.77)	80.00 (63.43)
7.	N 1000 IJs + F 5 x 10^8 spores + Bavistin 0.08 mg	3.33 (10.51)	36.67 (37.27)	60.00 (50.77)	70.00 (56.79)
8.	N 1000 IJs + F 5 x 10^8 spores + Thiram 0.12 mg	3.33 (10.51)	26.67 (31.09)	50.00 (45.00)	63.33 (52.73)
9.	Control	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)	0.00 (0.99)
	SEm ±	3.656	2.152	1.347	2.344
	CD (p=0.05)	12.978	7.638	4.780	8.322

N = Nematode F = Fungus

Figure in parenthesis are angular transformed values

5. DISCUSSION

Exclusive dependence on chemical pesticides is unlikely to provide sustained solutions to the existing pest problems. Their frequent massive mis and overuse has led to problems like resurgence of secondary pests, development of resistance, elimination of natural enemies of pests and toxicity hazards (Doutt, and Smith, 1971; Vanden Bosh, 1978). Biopesticides based on the pathogenic microorganisms such as fungi, bacteria, viruses and nematodes can be effectively exploited under the ambit of integrated pest management.

The present investigations were aimed to explore the possibilities of using the two bio-agents (nematodes and fungus) against the grubs of *Holotrichia consanguinea*. The following criteria were taken in to consideration.

1. To workout the relationship between the nematode inoculation dosage/response in the grubs and study the relative susceptibility of grubs to the nematode *Heterorhabditis bacteriophora*.
2. To study the relative susceptibility of grubs to fungus *Metarrhizium anisopliae*.
3. To find out the compatibility of the nematode with the fungus and nematode and fungus with insecticides recommended for the control of white grub.

5.1. RELATIVE SUSCEPTIBILITY OF DIFFERENT INSTAR GRUBS OF *H. consanguinea* TO NEMATODE, *H. bacteriophora*

White grub *Holotrichia consanguinea* in all the three instars was found susceptible to nematode *Heterorhabditis bacteriophora*. Depending upon the dosage (100 to 20,000 IJs/100 ml soil/grub) and period of exposure (0 to 10 days) mortality up to 100 per cent was observed. Earlier finding of Kaya and Gaugler (1993) had shown successful use of entomogenous nematode in different countries for the pest management. The Stenernematid and Heterorhabditid nematodes were considered useful bio-control agents against pests in different agroecosystem (Poinar, 1972 and Kaya, 1985).

The infective juveniles (IJs) of the nematode either get entry through ingestion or through integument and anus. In the haemocoel nematode releases the bacterium (*Xenorhabdus luminercens* and *X. nematophilus*) which is pathogenic and multiplies quickly in blood to cause septicemia. Several generations may occurs to produce nematodes enormously within the dead preserved hosts body. The dead grub is slightly discoloured to become reddish and remains unputrified for more than three weeks because of bacteria which produces an antibiotic that inhibits the growth of other microorganisms. Before death the grubs stopped feeding. The body wall of the dead grubs changed to transparent in which nematode IJs were clearly visible through stereoscopic microscope at 25 x (Plate 9). Such types of observations were also made by Burges and Hussey (1971), Veeresh (1980), S .rivastava (1993), Lewis and Raun (1978) and Morris (1985). Nematodes of the genera *Steinernema* and *Heterorhabditis* are regarded as excellent bio-control agents as they kill the insect pests by infecting them with symbiont entomopathogenic bacteria which they carry. The relatively rapid death of the insect host (24 to 48 hours), safety to plants and vertebrates and the wide host range of these nematodes have generated great interest in their use as biological control agents in IPM system Gaugler (1981); Kara and Gaugler (1993). Nematodes produce a toxin that destroys the inducible enzymatic defense response of the insects (Gotz *et al.*, 1981).

Nematode IJs ranging from 100 to 5000 per 100 ml soil per grub were tested against first instar grubs of *H. consanguinea* which resulted 6.67 to 63.33 and 30.0 to 100 per cent mortality after 3 and 10 days of exposure. Highest mortality of 63.33 and 100 per cent was observed in the treatment of 5000 IJs/100 ml soil/grub after 3 and 10 day of exposure, respectively followed by 50.0 and 90.0 per cent mortality in the treatment of 2000 IJs/100 ml soil/grub. The per cent grub mortality increased with the increase in inoculum dosage and period of exposure.

Kard *et al.* (1988) also achieved 60 to 80 per cent grub mortality within 2 to 4 days by entomogenous nematodes (*S. feltiae, S. glaseri* and *H. Heliothidis*). Similarly, Mathur *et al.* (1995) found 10 to 40 per cent grub mortality (*H. consanguinea*) by nematode (*H. bacteriophora*) @ 5000 and 10,000 IJs/100 ml

soil/grub through soil inoculation within 2 to 3 days and inoculation. Galzer and Allam (1989) reported that nematode IJs (*Heterorhabditis* sp.) @ 50 to 640 IJs/cm^3 resulted in 86.0 per cent control of *Maladera matrida* in laboratory and green house conditions. Hence the results achieved in the present studies are well within the limits earlier reported.

Against second and third instar grubs of *H. consanguinea* nematode at the dosages ranging from 500 to 20,000 IJs/100 ml soil/grub were tested which resulted in the mortality upto 56.67 per cent in 2nd instar and 43.33 per cent in 3rd instar after 3 days of exposure. The mortality in different dosages got increased to 73.33 per cent in II instar and upto 66.67 per cent in III instar after 5 days of exposure. Cent per cent mortality was found in the treatments of 10,000 and 20,000 IJs/100 ml soil/grub in II instar grubs and with the treatments of 20,000 IJs/100 ml soil/grub in III instar grubs after 10 days of exposure.

The grub mortality increased with the period of exposure as well as number of IJs. The lowest mortality of 30.0 and 13.33 per cent was found in the lower dosage of treatment i.e. 500 IJs per 100 ml soil per grub in II and III instar grubs after 10 days of exposure. The treatment of 20,000 IJs/100 ml soil/grub resulted in 56.67 and 100 per cent grub mortality in II instar and 43.33 and 100 per cent in III instar after 3 and 10 days of exposure followed by treatment of 10,000 IJs/100 ml soil/grub which registered 50 and 100 per cent mortality in II instars and 40 and 86.67 in III instar grubs. Whereas the lowest dosage (500 IJs) which had no effect after 3 days resulted in 30 per cent mortality after 10 days in II instar. Similarly exposure of III instar for 10 days could produce only 13.33 per cent mortality.

Earlier too, similar results were obtained by Villani and Wright (1988), who found 94.0 per cent control of *Rhizotrogus majalis* larvae with *H. heliothidis* @ 19.4 nematodes per cm^2 after 25 day of treatment. Like wise, Shinde *et al.* (1995) found 10 to 60 per cent grub mortality in III instar of scarabs by entomopathogenic nematodes (*S. glaseri* and *S. feltiae*) after 3 day of exposure by soil inoculation method. Karuna karan (1990) observed that *H. bacteriophora* reduced the population

of *Popillia japonica* by 60 per cent, 34 days after exposure which was increased to 96 per cent before population. Similarly more than 90 per cent mortality was observed by Wright *et al.* (1988) in third instar *Popillia japonica* and *Rhizotrogus majalis* by entomogenous nematodes (*Heterorhabditis* sp. and *H. heliothidis*) after 17 to 21 days of exposure. The present results in this regard are confirmatory to earlier findings on different species of scarabs.

The susceptibility was also assessed based on median lethal dosage (LD_{50}) obviously the LD_{50} value was low for first instar grubs in comparison to second and third instar. Further the susceptibility decreased with the instars. The LD_{50} values also decreased with the period of exposure. Three days after exposure the LD_{50} values for I, II and III instar grubs were 2320, 10771 and 19901 IJs/100 ml soil/grub respectively whereas only 367, 1143 and 2142 IJs/100 ml soil/grub were required to kill 50 per cent grubs after 10 days of exposure. Similarly, Forschler and Gardner (1991) calculated the LC_{50} of *S. carpocapsae*, *S. glaseri* and *H. heliothidis* to the third instar *Phyllophaga hirticula* as 210, 86 and 12 nematodes per grub, respectively.

To confirm the cause of grub mortality nematode IJs multiplied in the dead grub cadaver were harvested and counted which ranged from 8500 to 24,800, 18,000 to 41200 and 28,200 to 80500 per grub in I, II and III instar *H. consanguinea* respectively. The number of IJs harvested increased with the increase in size of grub, i.e. average nematode harvested from first instar grub were low as compared to second and third instar grubs. The number of IJs harvested also increased with the increase in inoculum dosage. The highest number of IJs (80500) harvested was in the treatment of 20,000 IJs/100 ml soil/grub with third instar grub whereas lowest (8500) was in the treatment of 100 IJs/100 ml soil/grub with first instar grub.

Similar studies in this regard were made by Sosa *et al.* (1985) who reported that dead larvae of *Ligyrus subropicus* yielded 139576 nematodes (*H. heliothidis*) per larva when exposed to 5000 IJs per larva. Likewise, Bareth *et al.* (1995) found that when first instar grub of *H. consanguinea* was exposed to nematode *S. glaseri* dosages ranging from 1000 to 10,000 IJs/100 ml soil yielded 600 to 2520 nematodes

per dead grub. Mathur *et al.* (1995) also reported that dead grubs of *H. consanguinea* yielded 1500 to 150000 IJs/grub when exposed to *H. bacteriophora* @ 5000 and 10,000 IJs/grub. Nematode IJs ranging from 750 to 155000 per grub were harvested from third instar *H. consanguinea* when exposed to *Steinernema glaseri* (Shinde *et al.*, 1995).

5.2 RELATIVE SUSCEPTIBILITY OF DIFFERENT INSTAR GRUBS OF *H. consanguinea* TO FUNGUS, *M. anisopliae*

White grub, *Holotrichia consanguinea* in all the three instars was found susceptible to fungus, *Metarrhizium anisopliae* but required longer period to cause lethal infection than nematodes. Depending upon the dosages (1×10^7 to 1×10^{11} spores/100 ml soil/grub) and period of exposure (0 to 30 days) mortality up to 70.0 per cent was observed. Earlier findings of Patel *et al.* (1988) had proved that *M. anisopliae* was an effective entomopathogenic fungus against white grub. Similarly another fungus, *B. brongniartii* was reported infecting white grub in India (Ranganathaiah *et al.*, 1973; Jayaramiah and Veeresh, 1983 a, b). Avarthy (1967) also found that grubs of *H. consanguinea* were attached by fungus, *M. anisopliae*.

The conidium of fungus, *M. anisopliae* got entrance in the grub body through cuticle and spiracles and pores of the sense organs. In moist conditions conidium germinates into a short germ tube which gives out small swellings called appressoria. The appressorium in cuticle sends out infection pegs which provide firm attachment. The hyphae then penetrate the layers of the integument by enzymatic dissolution of chitin and protein, ramify first in the cuticle and then reach the haemocoel and internal organs. Conidiophores are then produced which erupt through the cuticle and produce spores outside of body. Death of the grubs takes place by obliteration (choking) of the tissues and also by the toxins (destruxin A and B) produced by the fungus (Burges and Hussey, 1971; Srivastava, 1993).

The diseased grubs stopped feeding and lost appetite and showed restlessness, emerged on surface of the soil only to die. General sluggishness, weakness and decreased irritability were also visible. Due to green colour of the spores the grub

also looked green and the fungus caused green muscardine disease (Plate 10). Similar type of fungal actions and symptoms of grubs were reported by Veeresh (1980).

The fungal spores ranging from 1 x 10^7 to 1 x 10^{10} spores/100 ml soil/grub were tested against first instar grubs of *H. consanguinea* which resulted upto 33.33 and 70.0 per cent mortality after 10 and 16 days of exposure, respectively. Highest mortality was 70.0 per cent in the treatment of 1 x 10^{10} spores/100 ml soil/grub followed by mortality of 60.0 per cent in the treatment of 5 x 10^9 spores/100 ml soil/grub. The mortality increased with the increased inoculum dosage and period of exposure. Similarly Vyas (1988) found that fungus *B. brongniartii* was pathogenic to all the stages of *H. consanguinea*. The maximum mortality in eggs (52%), grubs (72%), pupae (48%) and adults (72%) was observed at 1 x 10^7 spores dosage under laboratory conditions.

Against second and third instars grubs dosages ranging from 1 x 10^8 to 1 x 10^{11} spores/100 ml soil/grub resulted in 26.67 and 23.33 per cent mortality, respectively after 15 days of exposure. The mortality in II and III instars got increased to 46.67 and 43.33 per cent, respectively after 20 days of exposure and after a lapse of 30 days it was enhanced to 6.67 to 66.67 in II instar and 3.33 to 53.33 per cent in III instar. The grub mortality increased with the period of exposure and inoculum dosage. The mortality was only 6.67 and 3.33 per cent with the treatments of 1 x 10^8 spores/100 ml soil/grub in II and III instar grubs. Whereas it was 66.67 and 53.33 per cent in the treatment of 1 x 10^{11} spores/100 ml soil/grub.

Similar results were earlier reported by Yadav *et al.* (1998) with the fungus, *M. anisopliae* at 1 x 10^{10} spores/100 ml soil/grub who found 70, 60 and 50 per cent mortality in I, II and III instar grubs of *H. consanguinea*. The fungus @ 10^{15} conidia/ha. caused a maximum of 41.5 per cent mortality in the grubs of *Holotrichia serrata* and 45.5 per cent in *H. consanguinea* (Vyas *et al.* 1990).

On the basis calculated LD_{50} values it may inferred that susceptibility decreased with instars. The LD_{50} value also decreased with the period of exposure. The LD_{50} values for first instar grub after 10, 12, 14 and 16 days of exposure were

3.68^{10}, 1.20^{10}, 3.49^{9} and 1.69^{9} spores/100 ml soil/grub, respectively. Fifteen days after exposure the LD_{50} values for II and III instar grubs were 3.25^{12} and 2.18^{13} spores/100 ml soil/grub, respectively whereas values were only 1.10^{10} and 2.47^{10} after 30 days of exposure.

5.3 COMPATIBILITY OF NEMATODE, *H. bacteriophora* WITH FUNGUS, *M. anisopliae* IN RELATION TO DIFFERENT INSTAR GRUBS OF *H. consanguinea*

The nematode, *Heterorhabditis bacteriophora* and fungus, *Metarrhizium anisopliae* were found pathogenic to all the three instars of white grub, *Holotrichia consanguinea*. The nematodes required shorter time to kill the grubs as compared to fungus. When entomogenous nematode, *H. baeteriophora* were used in combination of fungus, *M. anisopliae* against white grub, *H. consanguinea* in laboratory, the LT_{50} was shorter that is to say that higher per cent grub mortality was observed than when only either nematode or fungus was used. It was also observed that only nematodes survived, developed and produced progeny in the grub cadaver and there was no visible external symptoms of fungal infection.

The median lethal time (LT_{50}) for first instar grubs of *H. consanguinea* with nematode (2000 IJs) was 4.17 days and with fungus ($1x10^{9}$ spores) 14.19 days whereas with the combination of both it was only 2.87 days. With the second instar grubs nematode (10,000 IJs) and fungus ($1x10^{10}$ spores) required 3.53 and 25.76 days, respectively whereas combination of both required comparatively shorter time of only 3.14 days to kill 50 per cent grubs. Similar trend was also found in third instar grubs. The nematode (20,000 IJs) and fungus ($5x10^{10}$ spores) required 3.77 and 24.16 days respectively whereas, combination of both required only 3.31 day to kill 50 percent grubs of third instar.

The relevant work of Kamionek *et al.* (1974 a,b) indicated that period of lethal infection (PLI) of nematode and fungus combination was shorter than fungus alone against Coleoptera and lepidoptera. Barbercheck and Kaya (1990) stated that the period of lethal infection for last instar, *Galleria mellonella* larvae infected with

nematode (*S. feltiae* and *H. heliothidis*) and fungus (*B. bassiana*) simultaneously was shorter than in larvae treated with nematode or fungus alone or sequential dual treatment.

The per cent grub mortality was found comparatively higher in combination of nematode and fungus than when applied individually against *H. consanguinea*. The highest grub mortality (96.67%) of first instar was found in the treatment of nematode (2000 IJs) + fungus ($1x10^9$ spores) whereas, 86.67 per cent was in nematode alone and only 20.0 per cent in fungus after 11 days of exposure. It was indicated that in initial stage fungus only enhanced grub mortality due to green muscardine disease by which grubs stopped feeding.

In second instar grubs cent per cent mortality was found in the treatment of nematode (10,000 IJs) alone and combination with fungus ($1x10^{10}$ spores) whereas, only 10.0 per cent mortality was observed with fungus alone after 10 days of exposure. The fungus alone required longer period to kill the grubs. Similar trend of grub mortality was also observed in the third instar where 90.0 per cent mortality was found in the combination of nematode (10,000 IJs) + fungus ($1x10^{10}$ spores) whereas, 80.0 per cent by nematode alone and only 6.67 per cent by fungus. These results are in agreement with the results of Tillemans *et al.* (1990) who studied the compatibility between *B. brongiartii* and strain of *Heterorhabditis* against Black vine weevil (*Otiorhynchus suleatus*) on strawberry and proved that their combined affect was greater. Further, Barbercheck and Kaya (1990) found that inundative release of entomopathogenic nematodes where *B. bassiana* occurred provided greater control of soil borne insect-pests than application of nematode alone.

When grubs were exposed to combined infection of nematode and fungus, there were no visible external symptoms of fungal infection but nematode multiplication was observed in these. The nematode IJs harvested per grub with the treatment of nematode (2000 IJs) + fungus ($1x10^9$ spores) from first instar was 20,800. With the increased size of grub and inoculum dosage harvest was also increased. The IJs harvested from second and third instar grubs were 41,200 and

54,200 per grub, respectively in the treatment of nematode (10,000 IJs) + fungus ($1x10^{10}$ spores) and in third instar grub the number was 60,500 in the treatment of nematode (20,000 IJs) + fungus ($5x10^{10}$ spores).

These results are in agreement with the results of Komionck *et. al.* (1974 a,b) who observed that only the nematode survived, developed and produced progeny in the insect cadaver.

5.4 COMPATIBILITY OF NEMATODE, *H. bacteriophora* WITH INSECTICIDES IN RELATION TO DIFFERENT INSTAR GRUBS OF *H. consanguinea*

A perusal of results indicate that combination of nematode and insecticides enhanced the effectiveness of the either components tested individually. The probable reason may be that nematodes survived in the insecticide treated soil at recommended dosage so the activity of nematodes to kill the grubs was not adversely affected. Secondly the lethal dosage of both insecticides and nematodes were doubled than when applied individually. The combinations of nematode (2000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) required 1.30 and 1.60 day to kill 50 per cent grubs of first instar *H. consanguinea* whereas, nematode, chlorpyriphos and quinalphos individually required 4.17, 1.44 and 2.02 days, respectively which was slightly higher than combinations.

Similarly, in relation to second instar grubs combination of nematode (10,000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) required slightly shorter period i.e. 1.54 and 1.87 day to cause lethal infection as compared to when applied individually which required 3.53, 1.76 and 3.13 days respectively. Similar trend of grub mortality was observed in third instar also. The results thus revealed that nematodes were compatible with both the insecticides used.

In relation to per cent grub mortality the combinations of nematode (2000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) registered 100 and 80 per cent mortality whereas, individually these caused only 36.67, 70.0 and 60.0 per cent

mortality respectively after 3 days of exposure. The lower dosages combinations of nematode (1000 IJs) with chlorpyriphos (0.4 mg) and quinalphos (0.5 mg) resulted in 66.67 and 56.67 per cent mortality which were equivalent to higher dosages insecticidal treatments. These results thus revealed that lower concentration of insecticides were more compatible to nematodes than higher concentration. Similar trend of grub mortality was also observed in second and third instars. On the basis of per cent grub mortality and lethal time required to kill 50 per cent grubs it may be concluded that nematodes were compatible with both the tested insecticides. The per cent grub mortality in combination of nematode with chlorpyriphos was higher than combination of nematode with quinalphos.

These results are in agreement with the results of Rovesti *et al.* (1990) who stated that IJs of *H. bacteriophora*, *H. heliothidis*, *S. carpocapsae* and *S. feltiae* were tolerant to pesticides indicating that there were good possibilities for their use in IPM programmes. Zimmerman and Cranshaw (1990) also reported that entomogenous nematodes appeared to be compatible with pesticides. Brain and Wayne (1991) found only 12 per cent grub mortality with nematode concentrations of 0.5 and 1.5 million per m^2 when applied alone or in combination with diazinon (2.5 kg ai/ha). Comparison of the mean number of grubs recovered from each treatment 2 to 4 week after application showed significant reduction in the grub population.

To conform the compatibility of nematodes with tested insecticides number of nematode IJs harvested from dead grubs treated with nematode in combination with insecticides was recorded. The number of nematode IJs from treatments of nematode with combinations of insecticides were comparatively less than nematode alone. The number of nematode IJs harvested from first instar dead grubs exposed to nematode (2000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) were 16,500 and 13,400 per grub, respectively whereas, with nematode alone it was 22,500. Similar trend was also observed in second and third instar grubs. The number increased with the increased size of grubs and inoculum dosages.

5.5 COMPATIBILITY OF NEMATODE, *H. bacteriophora* WITH FUNGICIDES IN RELATION TO DIFFERENT INSTAR GRUBS OF *H. consanguinea*

The combined effect of *H. bacteriophora* with insecticides (chlorpyriphos and quinalphos) was manifested in enhanced mortality of pest but fungicides neither enhanced the mortality nor decreased it. Fungicides when used for seed treatment at recommended rate did not adversely affect the nematode activity. The per cent grub mortality in the treatment of nematode alone and combinations with fungicides were more or less equal, hence, the two may be referred as compatible.

The LT_{50} values of nematode (2000 IJs) with bavistin (0.16 mg) and thiram (0.24 mg) were 3.22 and 4.09 days, respectively for the first instar grubs of *H. consanguinea* in comparison to 3.91 days by nematode. For second instar nematode alone (10,000 IJs) took 4.12 days in comparison to 3.90 and 3.83 days in combination with bavistin (0.16 mg) and thiram (0.24 mg), respectively. Similar trend was also observed in third instar.

The per cent grub mortality in combination of nematode (2000 IJs) with bavistin (0.16 mg) and thiram (0.24 mg) was 100.0 and 90.0 in comparison to 93.33 per cent by the nematode alone after 10 days of exposure. The mortality recorded in the treatment of fungicides alone was below 10.0 per cent with more or less similar effect on second and third instar grubs.

Previous tests on chemical compatibility have shown that *N. carpocapsae* was compatible with a wide range of pesticides (Hara and Kaya, 1982) and the fungicides (chlorothalonil, benomyl and pentachloronitrobenzene) were found non toxic to *Steinernema feltiae*, *S. bibionis* and *Heterorhabditis* sp. (Zimmerman and Cranshaw, 1990).

Number of nematode IJs harvested from dead grub exposed to nematode in combinations with fungicides were more or less equal to nematode alone. The nematode IJs harvested from first instar dead grubs exposed to nematode (2000 IJs) with bavistin (0.16 mg) and thiram (0.24 mg) was 21,500 and 22,100 per cent grub,

respectively and with nematode alone it was 22,500. Similar trend was observed in second and third instar grubs also.

5.6 COMPATIBILITY OF FUNGUS, *M. anisopliae* WITH INSECTICIDES AND FUNGICIDES

The compatibility of *Metarrhizium anisopliae* with insecticides (chlorpyriphos and quinalphos) and fungicides (bavistin and thiram) was tested at 100, 500 and 1000 ppm concentrations. The results revealed that bavistin inhibited cent per cent mycelial growth at all the tested concentrations. Other insecticides and fungicides were found to be compatible with *M. anisopliae* at lower concentration (100 ppm) only. The inhibition of mycelial growth by fungicides was found to be higher than insecticides. At 500 ppm chlorpyriphos, quinalphos and thiram inhibited 54.10, 42.62 and 60.6 per cent growth of mycelium on nutrient glucose medium respectively.

Similar observations were made by Vyas *et al.* (1990) who reported that phorate did not inhibit the growth and sporulation of the fungus, whereas lindane, sevidol, carbofuran and quinalphos inhibited the growth. In case of fungicides, the lowest concentration of fytolan, captan and mancozeb did not show any inhibition, although they exhibited considerable inhibition of fungal growth at higher concentrations. Bavistin inhibited the fungal growth completely at all concentrations(100, 500 and 1000 ppm) tested. Zimmerman (1975) reported that saprol and benomyl suppressed the germination of fungal spores of *M. anisopliae*, *B. brongniartii* and *B. bassiana*. Calaxin inhibited the mycelial growth but the effect of plantvax and milstem was very mild and cerobin-M was tolerated by the tested fungi.

5.7 COMPATIBILITY OF NEMATODE, *H. bacteriophora* AND FUNGUS, *M. anisopliae* WITH FUNGICIDES AND INSECTICIDES IN RELATION TO FIRST INSTAR GRUBS OF *H. consanguinea*

When nematode, *H. bacteriophora* and fungus, *M. anisopliae* were simultaneously mixed with either insecticides (chlorpyriphos and quinalphos) or fungicides (bavistin and thiram) positive results regarding control of white grub were obtained. On the basis of LT_{50} values and per cent grub mortality it may be concluded

that these were compatible. Lower dosages combinations of nematode and fungus with insecticides and fungicides too showed positive results with first instar grubs of *H. consanguinea*. The combinations of nematode (2000 IJs) + fungus ($1x10^9$ spores) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) required only 1.49 and 2.06 days, respectively to cause lethal infection, similarly nematode (2000 IJs) + fungus ($1x10^9$ spores) with bavistin (0.16 mg) and thiram (0.24 mg) required 3.29 and 4.14 days respectively.

Not much work on *in vitro* compatibility of nematode and fungus simultaneously with insecticides and fungicides has been carried out. However, Anderson *et al.* (1989) found greater mortality in Colarado potato beetle when *B. bassiana* was used with insecticides than the individual agents. Zimmerman and Cranshaw (1990) also found that chlorothalonil, benomyl and pentachloronitrobenzene were non toxic to entomogenous nematodes. Likewise, Rovesti *et al.* (1990) stated that entomogenous nematodes were tolerant to most of the pesticides. Vyas *et al.* (1990) reported that bavistin inhibited the fungal growth completely while quinalphos did it moderately.

6. SUMMARY

Investigations on the "Use of entomogenous nematode, *Heterorhabditis bacteriophora* in combination with fungus, *Metarrhizium anisopliae* and recommended pesticides for management of *Holotrichia consanguinea* Blanch." were carried out at ARS Durgapura (Jaipur) during the year 1995-96. The white grub, *H. consanguinea* in all the three instars (I, II and III) was found susceptible to nematode, *H. bacteriophora*. Depending upon the dosages (100 to 20,000 IJs/100 ml soil/grub), instar of grub and period of exposure (0 to 10 days) mortality up to 100 per cent was observed. The treatment of 5000 IJs/100 ml soil/grub registered 100.0, 86.67 and 73.33 per cent mortality in I, II and III instar grubs, respectively after 10 days of exposure. Cent per cent mortality was also observed with 1000 IJs/100 ml soil/grub in second instar and 2000 IJs/100 ml soil/grub in third instar after 10 days of exposure. The LD_{50} values for I, II and III instar grubs after 3 days of exposure were 2320, 10771 and 19901 IJs/100 ml soil/grub, respectively whereas, the values were 367, 1143 and 2142 IJs after 10 days of exposure. The susceptibility decreased as the instar advanced. The number of nematode harvested from dead grubs ranged from 8500 to 24,800, 18,000 to 41200 and 28,200 to 80,500 per grub in I, II and III instar respectively. The harvest increased with increase in size of grub and inoculum dosages.

The grubs of *H. consanguinea* in all the three instars was also found susceptible to fungus, *M. anisopliae* but required longer period to cause lethal infection. Depending upon dosages ($1x10^7$ to $1x10^{11}$ spores/100 ml soil/grub), instar of grub and period of exposure (0 to 30 days) mortality up to 70.0 per cent was observed. The treatment of $1x10^{10}$ spores/100 ml soil/grub registered 70.0 per cent mortality in first instar after 16 days of exposure and 50.0 per cent in second and third instar after a lapse of 30 days. The LD_{50} value for first instar was 1.69^9 spores/100 ml soil/grub after 16 days of exposure and for second and third instars the values were 1.10^{10} and 2.47^{10} spores after 30 days of exposure.

Both the bio-agents i.e. *H. bacteriophora* and *M. anisopliae* were found compatible with each other in relation to all the three instars of *H. consanguinea*. When used in combination against white grub, the mortality got increased and did take shorter period to cause lethal infection than when only either nematode or fungus was used. The median lethal time (LT_{50}) for first instar with nematode (2000 IJs/100 ml soil/grub) was 4.17 days and with fungus ($1x10^9$ spores/100 ml soil/grub) 14.19 days whereas, with the combination of both it was only 2.87 days. The grub mortality in first instar in the treatment of nematode (2000 IJs) + fungus ($1x10^9$ spores) was registered as 96.67 per cent whereas, it was 86.67 per cent in nematode alone and only 20 per cent in fungus after 11 days of exposure. The nematode IJs harvested per grub were 20800. The IJs harvest increased with the increase in size of grub and inoculum dosage. Similar trend was observed in second and third instar grubs also with regard to mortality and LT_{50}.

The nematode, *H. bacteriophora* was also found compatible with insecticides (chlorpyriphos and quinalphos) and fungicides (bavistin and thiram) in relation to different instars of white grub. The combination of nematode with insecticides and fungicides tested enhanced the effectiveness of the either of the components. The LT_{50} values of nematode (2000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) were 1.30 and 1.60 days for first instar in comparison to 4.17, 1.44 and 2.02 days with nematode, chlorpyriphos and quinalphos individually. The per cent grub mortality caused in combinations of nematode (2000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) was 100.0 and 80.0 while individually the three caused 36.67, 70.0 and 60.0 per cent mortality, respectively only after 3 days of exposure. The number of nematode IJs harvested from dead grubs exposed to nematode (2000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) were 16,500 and 13,400 per grub, respectively whereas, with nematode alone it was 22,500. The combination of nematode (2000 IJs) with bavistin (0.16 mg) and thiram (0.24 mg) required 3.22 and 4.09 days, respectively to cause lethal infection in first instar grubs of *H. consanguinea* while nematode alone required 3.19 days.

The fungus, *M. anisopliae* was not found compatible with bavistin even at low concentration (100 ppm) whereas, thiram at this concentration was found compatible. The insecticides (chlorpyriphos and quinalphos) were also found compatible at lower concentration (100 ppm). At 500 ppm chlorpyriphos, quinalphos, bavistin and thiram inhibited 54.10, 42.62, 100.0 and 60.60 per cent mycelial growth on nutrient glucose medium, respectively.

When nematode and fungus were used simultaneously in combination with insecticides and fungicides positive results were obtained. The combinations of nematode (2000 IJs) + fungus ($1x10^9$ spores) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) required only 1.49 and 2.06 days,respectively to cause lethal infection similarly, nematode (2000 IJs) + fungus ($1x10^9$) with bavistin (0.16 mg) and thiram (0.24 mg) required 3.29 and 4.14 days, respectively.

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Use of Entomogenous Nematode, *Heterorhabditis bacteriophora* in Combination with Fungus, *metarrhizium anisopliae* and Recommended Pesticides for Management of *Holotrichia consanguinea* Blanch.

B.L. Jat
Research Scholar

Dr. S.C. Bhardwaj
Major Advisor

ABSTRACT

The White grub, *Holotrichia consanguinea* Blanch. in all the three instars was found susceptible to nematode, *Heterorhabditis bacteriophora* and fungus, *Metarrhizium anisopliae*. The grub mortality up to 100 per cent was found by nematode after 10 days period of exposure and up to 70 per cent by fungus after 30 days of exposure. The LD_{50} values of nematodes for I, II and III instar grubs after 3 days of exposure were 2320, 10771 and 19901 IJs/100 ml soil/grub,respectively whereas, the values were 367, 1143 and 2142 after 10 days of exposure. The number of nematode harvested from dead grubs ranged from 8500 to 80500 per grub. The harvest increased with increase in size of grub and inoculum dosage. The LD_{50} value of fungus for I instar was 1.69^{9} spores/100 ml soil/grub after 16 days exposure and for II and III instars the values were 1.10^{10} and 2.47^{10} spores after 30 days of exposure.

The nematode and fungus were found compatible with each other. When used in combination against white grub, the mortality got increased and did take shorter period to cause lethal infection than when only either nematode or fungus was used. The LT_{50} value and per cent grub mortality for first instar with nematode (2000 IJs/100 ml soil/grub) were 4.17 days and 86.67 per cent and with fungus ($1x10^{9}$

spores/100 ml soil/grub) 14.19 days and 20.0 per cent whereas, with the combination of both it was 2.87 days and 96.67 per cent. The nematode was also found compatible with insecticides (chlorpyriphos and quinalphos) and fungicides (bavistin and thiram). The LT_{50} value of nematode (2000 IJs) with chlorpyriphos (0.8 mg) and quinalphos (1.0 mg) were 1.30 and 1.60 days for first instar while individually the values of the three were 4.17, 1.44 and 2.02 days respectively, the number of nematodes harvested from first instar dead grubs exposed to nematode with chlorpyriphos and quinalphos were 16,500 and 13,400 per grub, respectively whereas, with nematode alone it was 22,500. The LT_{50} values and nematode harvest were more or less equal in combination of nematode with fungicides and nematode alone.

The fungus was not found compatible with bavistin but compatible with thiram at low concentration (100 ppm). The chlorpyriphos and quinalphos were also found compatible at lower concentration (100 ppm). At 500 ppm chlorpyriphos, quinalphos, bavistin and thiram inhibited 54.10, 42.62, 100.0 and 60.6 per cent mycelial growth,respectively. When nematode and fungus were used simultaneously in combination with either insecticides or fungicides positive results were obtained.

होलोट्राइकिया कन्सैंग्यूनिया ब्लैंकार्ड के प्रबन्धन में कीटजनक सूत्रकृमि, *हेटेरोरेबडिटीस बेक्टीरिफोरा* का *मेटाराइजियम एनीसोपिली*, फफूंद एवं अभिस्तावित पीड़कनाशकों के संयोजन में प्रयोग

अनुक्षेपण

बी.एल. जाट
शोधकर्त्ता

डॉ. एस.सी. भारद्वाज
मुख्य सलाहकार

सफेदलट, होलोट्राइकिया कन्स्सैंग्यूनिया ब्लैकार्ड के तीनों इंस्टार सूत्रकृमि, हेटेरोरेबडिटीस बैक्टीरिफोरा एवं फफूंद, मेटाराइजियम एनीसोपिली के प्रति सुग्राही पाये गये। लट की मृत्युदर सूत्रकृमि उद्‌भासन के 10 दिन बाद 100 प्रतिशत एवं फफूंद उद्‌भासन के 30 दिन बाद 70 प्रतिशत पायी गई। सूत्रकृमि की घातक मात्रा मान I, II एवं III इंस्टार के लिये उद्‌भासन के 3 दिन वाद क्रमशः 2320, 1077। एवं 1990। आई.जे.एस. प्रति 100 मिली. मृदा प्रति लट पायी गई। जबकि, उद्‌भासन के 10 दिन वाद 367, 1143 एवं 2142। पायी गई। मृतलट से 8500 से 80500 सूत्रकृमि प्रति लट संग्रह किये गये जो लट के आकार एवं संरोप मात्रा के बढ़ने पर ज्यादा पाये गये। फफूंद की घातक मात्रा मान प्रथम इंस्टार लट के लिये उद्‌भासन के 16 दिन बाद $1x69^{9}$ स्पोरस प्रति100 मिली. मृदा प्रति लट तथा II एवं III इंस्टार लटों के लिये उद्‌भासन के 30 दिन बाद क्रमशः $1x10^{10}$ एवं $2x47^{10}$ स्पोरस पाये गये।

सूत्रकृमि एवं फफूंद में आपस में संगतता पायी गयी। सूत्रकृमि एवं फफूंद के अलग-अलग प्रयोग करने की बजाय संयोजन में प्रयोग करने से लट की मृत्युदर कम समय में अधिक पायी गई। प्रथम इंस्टार लट के लिये घातक समय मान एवं मृत्युदर प्रतिशत सूत्रकृमि (2000 आई.जे.एस. प्रति 100 मिली. मृदा प्रति लट) के साथ 4.17 दिन (86.67%) तथा फफूंद ($1x10^{9}$ स्पोरस प्रत 100 प्रति मिली. मृदा प्रति लट) 14.19 दिन (22.0%) जबकि दोनों के संयोजन में प्रयोग से 2.87 दिन (96.67%) पाये गये। सूत्रकृमि की कीटनाशी (क्लोरपायरीफॉस एवं क्यूनालफॉस) तथा फफूंदनाशी (बावस्टिन एवं थायरम) के साथ भी संगतता पायी गयी। प्रथम इंस्टार लट के लिये सूत्रकृमि (2000 आई.जे.एस.) का क्लोरपायरीफॉस (0.8 मिग्रा.) एवं क्यूनालफॉस (1.0 मिग्रा.) के साथ घातक समय मान क्रमशः 1.30 एवं 1.60 दिन पाया गया जबकि अलग अलग के लिये क्रमशः 4.17, 1.44 एवं 2.02 दिन पाया गया। प्रथम इंस्टार मृत लट

के सूत्रकृमि के क्लोरपायरीफॉस एवं क्यूनालफॉस के साथ प्रयोग करने से क्रमश:16500 एवं 13400 सूत्रकृमि प्रति लट संग्रह किये गये जबकि 22500 केवल सूत्रकृमि के प्रयोग से किये। सूत्रकृमि का फफूंदनाशी के साथ तथा अकेले प्रयोग पर घातक समय मान एवं मृत लट से सूत्रकृमि संग्रह लगभग बराबर पाये गये।

फफूंद की बावस्टिन के साथ संगतता नहीं पायी गई जबकि थायरम के साथ कम सांद्रता (100 पी.पी एम.) पर संगतता पायी गई। क्लोरपायरीफॉस एवं क्यूनालफॉस की भी कम सांद्रता (100 पी.पी.एम.) पर संगतता पायी गयी। क्लोरपायीफॉस, क्यूनालफॉस, बावस्टिन एवं थायरम 500 पी.पी.एम. पर क्रमश: 54.10, 42.62, 100.00 एवं 60.60 प्रतिशत कवकजाल वृद्धि को निरूद्ध करते है। जब सूत्रकृमि एवं फफूंद एक साथ कीटनाशी या फफूंदनाशी के संयोजन मे प्रयोग किये गये तो सकारात्मक परिणाम पाये।

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