" UTILIZATION OF VARIOUS FORMULATIONS OF BIOPESTICIDES AGAINST WHITE GRUB, *Leucopholis lepidophora* (BLANCHARD)"

By

Miss. KUMBHAR RASHMIKA ANANDA
(Reg. No. 15/177)

A Thesis submitted to the

MAHATMA PHULE KRISHI VIDYAPEETH
RAHURI-413 722, DIST. AHMEDNAGAR,
MAHARASHTRA STATE, INDIA

In partial fulfilment of the requirements for the degree

Of

MASTER OF SCIENCE (AGRICULTURE)

In

AGRICULTURAL ENTOMOLOGY

AGRICULTURAL ENTOMOLOGY SECTION
COLLEGE OF AGRICULTURE,
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MAY, 2017

CANDIDATE'S DECLARATION

I hereby declare that this thesis or a part
thereof has not been submitted
by me or any other person
to any other University
or Institute for
a Degree or
Diploma

Place: Kolhapur

Date : / 05/ 2017 (Ms. R.A.KUMBHAR)

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CERTIFICATE

This is to certify that the thesis entitled **UTILIZATION OF VARIOUS FORMULATIONS OF BIOPESTICIDES AGAINST WHITE GRUB, *Leucopholis lespidophora* (BLANCHARD)**, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra State, India, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULTURE)** in **AGRICULTURAL ENTOMOLOGY,** embodies the results of a bona-fide research work carried out by **Ms. KUMBHAR RASHMIKA ANANDA,** under my guidance and supervision and no part of the thesis has been submitted for any other degree or diploma in other form. The assistance and help received during the course of this investigation and sources of literature referred to have been duly acknowledged.

Place: Kolhapur **(Dr. Pandurang B.Mohite)**

Date: / 05/ 017 Chairman and Research Guide

Dr. G. G. Khot
Associate Dean,
College of Agriculture,
Kolhapur – 416 004
Maharashtra State, India

CERTIFICATE

This is to certify that the thesis entitled, **UTILIZATION OF VARIOUS FORMULATIONS OF BIOPESTICIDES AGAINST WHITE GRUB, *Leucopholis lepidophora* (BLANCHARD)**, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar, Maharashtra, India, in partial fulfilment of the requirements for the degree of **MASTER OF SCIENCE (AGRICULURE)** in **AGRICULTURAL ENTOMOLOGY,** embodies the results of a piece of bona-fide research work carried out by **Ms. KUMBHAR RASHMIKA ANANDA** under the guidance and supervision of **Dr. P.B. Mohite,** Professor of Agril. Entomology, College of Agriculture, Kolhapur and that no part of the thesis has been submitted for any other degree or diploma in other form.

Place: Kolhapur **Dr. G. G. Khot**

Date: / 05 /2017 Associate Dean

ABSTRACT

" UTILIZATION OF VARIOUS FORMULATIONS OF BIOPESTICIDES AGAINST WHITE GRUB, *Leucopholis lepidophora* (BLANCHARD)"

By

Ms. Rashmika A. Kumbhar

A candidate for the degree of

MASTER OF SCIENCE (AGRICULTURE)

2017

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Laboratory and pot culture studies were conducted on the "Utilization of various formulations of biopesticides against white grub, *Leucopholis lepidophora* (Blanchard)" during 2016-2017 in the Agriculture Entomology Section, College of Agriculture, Kolhapur. Studies on bioefficacy of various formulations of biopesticides against white grub were conducted at farmers field in the endemic area of white grub in Kolhapur district.

In laboratory bioassay studies, the treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre recorded 81.95 per cent reduction in grub population and it was found to be significantly superior over all the treatments. Entomopathogenic Nematode-Powder with the dose of 5 gm per litre showed 76.93 per cent reduction in grub population at 15

DAT were next in order of efficacy. The treatment with Entomopathogenic Nematode-spong formulation and Entomopathogenic Nematode-Granules found to be equally effective.

In pot culture experiment, treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre was the most promising treatment against *L. lepidophora* recorded 69.41 per cent grub mortality at 30 DAT. Entomopathogenic Nematode-Powder (5 gm/lit), Entomopathogenic Nematode-spong formulation (300 IJs), Entomopathogenic Nematode-Granules (5 gm/lit), *M. anisopliae*-tablet formulation (2 gm/lit) were next in order of efficacy.

Under field conditions, treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre was found to be significantly superior over untreated control and recorded 9.36 per cent clumps mortality at 60 DAT. Treatment with Entomopathogenic Nematode-Powder with the dose of 5 gm per litre showed 11.68 per cent clumps mortality at 60 DAT were next in order of efficacy. Treatment with Entomopathogenic Nematode-spong formulation, Entomopathogenic Nematode Granules were next in order of efficacy.

Pages 1 to 84

1. INTRODUCTION

Sugarcane, *Saccharum officinarum* L. is one of the most important commercial crops of the tropical countries belonging to the family Gramineae and is the main source of sugar in the world; sugarcane contributes nearly 70 per cent of the world's total sugar production. It is considered as a cash crop and plays the key role in the economy of the Maharashtra. Among sugarcane growing countries in the world, India ranks second by producing 34.12 lakh metric tons sugarcane during year 2015. In India sugarcane crop occupies about 4.99 million hectares area with production of 25.5 million metric tons during 2016-2017 (Anonymous, 2016a). In Maharashtra sugarcane is grown on 6.33 lakh hectares with annual production of 49.66 million tones and yields 78.46 tons per hectare during 2016-2017 (Anonymous, 2016b). Sugar industry is the second largest agro-based industry comprising of 453 in India where as 170 in Maharashtra. Major sugarcane growing districts in Maharashtra are Kolhapur, Satara, Sangali, Ahmednagar, Pune, Nashik and Solapur.

Up till now 200 insect pests have been reported causing serious damage to sugarcane crop (David *et al.* 1986). Among them white grub has became the most important polyphagous pest causing serious damage to sugarcane since 1960 (Mohalkar *et al.* 1977). Many species of the white grubs are known pests of a number of crops in India. They belongs to four subfamilies of *viz.*, Dynastinae, Cetoninae, Rutelinae and Melolonthinae (Balasimha and Rajagopal, 2003). About 40 different species under these subfamilies have been recorded major pests of various crops in different parts of the country (Veeresh, 1988). Nearly 20 species of white grubs are reported to attack sugarcane in India. Of these, *Holotrichia* spp, *Anomala varicolor* (Gryll), *A.viridis* (F), *Apogonia destructor* (Bos.), *Cyclocephala parallella* (Casey), *Dermolepidia pica* (Arrow), *Lepidiota stigma, Ligyrous subtropicius* (Blanch), *Leucopholis* spp. (F.), *Phyllophaga helleri* (Brsk), and *Schizonycha* spp. have been reported to assume pest status in sugarcane growing regions (Yubak Dhoj, 2006). In India white grub is one of the five pests of national importance (Yadava and Vijayavergia, 1994). A total of 16 species were recorded under eight genera distributed in three sub families *viz.*, Melolonthinae, Rutelinae, Dynastinae and five tribes in Western Maharashtra (Rathour 2013).

Among the white grubs, *Leucopholis lepidophora* (Blanchard) has recently been reported to thread to sugarcane, paddy and groundnut cultivation in the western Maharashtra especially in Kolhapur region (Patil and Hapse, 1986; Mane, 2010 ; Bharati and Mohite, 2013). In Kolhapur district of India, *Leucopholis lepidophora* (Blanch) is serious phytophagous pest damaging mainly sugarcane crop located along the bank of rivers of Western Maharashtra. Besides *Holotrichia serrata*, *H. fissa,* and *H. karschi* also attained the pest status in field of paddy, jowar, maize, turmeric and tobacco (Bhawane *et al.* 2012). *Phyllognathus dionysius* attending pest status of sugarcane, soyabean and groundnut (Rathour *et al.* 2015).

Several tactics have been adopted for the management of white grubs including cultural, mechanical, biological, chemical and integrated methods suggested by various workers (Srikanth and Singaravelu, 2011). Adult collection and insecticidal application are the major tactics of management followed against all white grub species (Veeresh, 1974 and Raodeo *et al.* 1976). Application of chemical is practically uneconomical, difficult and associated with high cost, environmental pollution and other problems. Chemical pesticides have been primarily means of managing grubs for many years but the control of the grubs is often ineffective because of the difficulty in reaching the pesticides as grubs are in the root zone (Potter *et al.* 1996 and Sankarnarayanan *et al.* 2006) and also there are lot of limitations to use higher dose of insecticides as the infestation is more pronounce on banks of rivers. Hence, there is strong need for the development of alternative strategies for the control of white grubs, which are eco-friendly and economical feasible. The use of bio-pesticides as a vital component in sustainable agriculture assumed greater significance as an important tactic in IPM due to the economic viability, social acceptability and eco friendly in nature (David, 2008 and Mane, 2010).

The use of bio-control agents in general and fungal based myco-insecticides in particular are lacking in country. About 90 genera and 700 species of fungi representing a large group of entomophthroals (*Metarhizium* spp., *Beauveria* spp., and *Verticillium* spp.) which are entomopathogenic have been reported. Among these, *Metarhizium* is of greater importance in management of white grubs. *Metarhizium anisopliae* (Metschnikof) Sorokin can be effectively utilized one of the components in the management of white grubs (Mohi-ud-din *et al.* 2006 and Chroton, 2007). The fungus is eco-friendly, cost effective, highly persistent and also self-perpetuating in nature. The unique character of entomopathogenic fungi as compared to bacteria and virus is that they penetrate through the insect cuticle thus making them as valuable biological control agent of white grub. Moreover the microclimate of sugarcane eco-system is ideal for the fungus to multiply. Further, rainfall, high humidity and soil with high organic content also help the fungus to perpetuate itself in nature.

The entomopathogenic nematodes (EPNs) are potential and most promising biological agents for the control of different insect pest of different crops, those are eco friendly and cost effective (Kaya and Gaugler, 1993 and Ali *et al.* 2005). Entomopathogenic nematodes in the families Steinerematidae and Heterorhabiditidae are potential biocontrol agents (Gaugler, 1988). Most biocontrol agents take days or weeks to kill the pest but entomopathogenic nematodes with their symbiotic bacteria kills the pest within 24-48 hr. Inexpensive mass production, high virulence and broad host range are the important attributes which extends over the other bio-control agent.

However, not much work has been reported on the utilization of various formulations of biopesticides against white grub, *L. lepidophora*. Hence present investigation was therefore undertaken with following objectives,

1. To study the various formulations of biopesticides against white grub under lab and pot culture condition.

2. To study the effect of different formulation of biopesticides against white grub under field conditions.

2. REVIEW OF LITERATURE

The literature pertaining to the study of various formulations of biopesticides against *L. lepidophora* (Blanchard) under different conditions is very meagre, hence related information and on other hosts as per objectives of the present investigations is highlighted here under.

2.1 General Taxonomy

The word fauna of scarabaeidae exceeds 30,000 species (Mittal, 2000) with about 1300 North American species (Borror, *et al.* 1975). The maximum number occurs in the tropical areas of world, particularly in African and Oriental regions. The fauna of Indian sub region is very rich and diverse, but it is yet be fully explored (Richter, 1971; Mishra and Singh, 1999). In 1956, Gupta and Avasthy reported white grub as a pest of sugarcane in India. True white grubs are the larvae of May beetles also called June beetles from the genus *Holotrichia* belonging to the family scarabaeidae and subfamily Melolonthinae. In India, near about 300 species of white grubs are recorded (Raodeo and Deshpande, 1987). Different species of white grubs are found according to the soil type and climatic conditions. Yadava and Sharma (1995) gave the detailed biology, description and management of twelve most economically important species *viz, Holotrichia consnguinea* Blanch., *H. Serrata* (F.), *H. reynaudi* Blanch., *H. longipennis* Blanch., *H. seticollis* Mose, *H. nilgirla* Arrow, *Brahmina (Holotrichia) coriacea* Hope, *Leucopholis lepidophora* Blanch., *L. burmeistri* Blanch., *L. coneophora* Burm., *Anomala dimidiate* Hope, *Maladera insabilis,* which appeared in severe form in different parts of the country. *Leucopholis lepidophora* Blanchard reported as a serious pest of arecanut in Malnad district of Karnataka by Veeresh *et al.* (1982). This pest consider threat to sugarcane cultivation in parts of Kolhapur and Sangali districts (Adsule and Patil, 1990). However, its incidence was also recorded in maize, groundnut, paddy and vegetables (Mane, 2010; Bharati and Mohite, 2013). The *Phyllognathus dionysius* was first time reported causing damage to sugarcane (Rathour *et al.* 2015).

White grub are polyphagous pests having a wide range of hosts, which are damaging both on adult and larval stages; however the larvae are a greater nuisance. The extent of damage caused by white grubs solely depends upon the species involved, the numbers present and host crop. In India, white grub is one of the five pests of national importance (Yadava and Vijayavergia, 1994). In many crops, white grubs cause losses to the extent of 40-80 per cent (Mane, 2010 and Bharati, 2013).

The incidence of *Leucopholis* reduces 40 per cent germination of the cane and causes 40 to 60 per cent reduction in cane yield and 78 units reduction in sugar recovery in heavily infested sugarcane fields. The losses to the extent of 100 per cent were also reported by (Patil and Hapse, 1991 and Mane, 2010).

Due to complex life cycle and survival of pest in soil, the control of this pest becomes difficult. Indiscriminate use of pesticides to control insect pests of crop has upset natural ecological balance. This has initiated biological control of insect pest with biopesticides is an appealing alternative to the application of synthetic chemicals. They causes lethal infection to their hosts but are harmless for the users as well as for the environment moreover as they are highly host specific. They are not risky for non-target or even to beneficial insects.

2.2 Laboratory bioassay studies

Dhoj and Keller (2005) carried out experiment on *M. anisopliae* for control of white grub in Nepal and tested 70 isolates initially at a concentration of 10^7 conidia ml^{-1}, eight isolates gave over 80 per cent infected grubs, 65 isolates gave over 50-60 per cent infected grubs and rest of isolates had low pathogenicity. The most virulent strains were further assessed with 10^9 spores ml^{-1}, 10^5 spores ml^{-1}, 10^3 spores ml^{-1} and water treatments.

Ibrahim (2005) conducted laboratory experiments on the control of the white grubs, *Pentodon bispinosus* Kust last instar larvae with Heterohabditis and Steinernematid nematodes. In these six nematode strains i.e. three belonging to the genus Steinernema (*S. glaseri, S. carpocapsae,* and *S. riobrave*) and three belonging to Heterrohabditis (*Heterohabditis* spp. HTS-1 and HTS-2) were compared for their capability to control the third instar larvae of *Pentodon bispinosus* a major pest of sugarcane. In that 7 days after treatment at 27^0C, *S. glaseri* recorded the highest mortality rate of 72.7 per cent. Followed by HTS-1 (56%) and HTS-2 (52%) *S. carpocapsae*, *Heterohabditis* sp. and *S. riobrave* recorded lower mortality (20.32% and 28%, respectively) at 38^0C. HTS-2 and HTS-1 showed the highest mortality percentage compared with their original strain while other strain showed no mortality. Contaminating soil surface with nematode was observed to the most effective method to control the white grub larvae.

Ansari *et al.* (2006) compared virulence of the Belgian isolates of *Steinernema glaseri* and *S. scarabaei* to white grub species. The virulence of the entomopathogenic nematodes, *Steinarnema glaseri* Belgian strain and *S. scarabaei* against different development stages of European cockchafer, *Serica brunnea* was compared in the laboratory. The observation combined with those of previous studies on other nematode and white grub species, showed that nematode virulence against white grub development stages varies with white grub and nematode species.

Din *et al.* (2006) conducted experiment on dose optimization of promising entomopathogenic fungi, *B. bassiana* at each level white grub and proved pathogenic at each level of concentration 1 x 10^8, 1 x 10^6, 1 x 10^4, and 1 x 10^2 spores ml^{-1} studied, but mortality in shorter duration was observed with increase in inoculums dose. They recorded that *B. bassiana* @ 1 x 10^8 spores ml^{-1} concentration inflicted initial mortality on 6^{th} day i.e. 46.6 per cent and cent per cent mortality was observed on 12^{th} day. In *B. brongniartii* initial mortality started on 8^{th} day (33.33%) and cent per cent mortality occurred on 20^{th} day, while as in case of *M. anisopliae* mortality was initiated on 8^{th} day (30%) and highest mortality was observed on 20^{th} day (93.91%). They also reported that, all the three screened biocontrol agents caused heavy mortality at the highest concentration of 1 x 10^8 spore ml^{-1}.

In the laboratory experiment Jatt and Choudhary (2006) evaluated bioeffacacy of entomopathogenic nematode, *Heterorhabditis bacteriophora* and fungus *Metarhizium anisopliae* and their coinations against white grub, *H. consanguinae* Blanch. The lethal time required to kill 50 per cent mortality in second and third instar grubs with the treatment of nematode @10,000 IJs/100 ml soil/grub were 3.53 and 5.32 days and with fungus @ 1 x 10^{10} spores /100 ml soil/grubs were 25.76 and 26.42 days. Whereas, with the combination of both were only 3.14 and 4.10 days, respectively. The mortality in second and third instar grubs were 80 and 90 per cent in the treatment of nematode (10,000 IJs) alone and only 10.0 and 6.67 in fungus, in combination 90 and 100 per cent of both, respectively.

The bioefficacy and compatibility of the insect parasitic nematode, *H. bacteriophora* and the insect pathogenic fungus, *M. anisopliae* against *H. consanguinae* was studied by Jat and Choudhary (2006). They reported that combination of nematode with fungus required shorter time to cause lethal infection than either nematode or fungus alone. The median lethal time (LC_{50}) with nematode (2000 IJs) was 4.17 days and with fungus (1 x 10^9 spores) was 14.19 days whereas, with the combination of both was only 2.87 days. The fungus, nematode combination in general gave 10 and 80 per cent more kill than the nematode alone and fungus alone, respectively after 11 days of exposure. The grubs exposed to a suspension of nematode (2000 IJs) and fungus (1 x 10^9 spores) per 100 ml soil per grub gave 96.67 per cent mortality within 11 days of exposure, while the mortality of grubs with nematode alone and fungus alone was 86.67 and 20.0 per cent respectively.

Koppenhofer *et al.* (2006) studied virulence of the entomopathogenic nematode, *Heterorhabditis bacteriophora, H. zealandica* of economic importance in turf grass. The virulence of the nematode species relative to each other differed greatly among white grub species *H. bacteriophora* and *H. zealandica* had similar modest virulence to *P. japonica*, *A. orientatis*, *C. borealis,* and *M. castanea,* but against *R. majalis, H. zealandica* showed low virulence with clear concentration. Whereas *H. bacteriophora* caused only erratic and very low mortality.

Shankarnarayanan *et al*. (2006) evaluated the effectiveness of four EPNs against pupae and adult beetles of *H. serrata* in laboratory. *H. indica* recorded 100 per cent mortality of adult beetles in four days when it is dispended either through soil application or by injection of IJs in to the haemocoel of white grub.

Both laboratory and field studies with environment friendly plant products and entomopathogens on *Leucopholis lepidophora* Blanch. in arecanut to replace present method of chemical control. Rakesh and Prabhu (2007) concluded that among plant products and fungal pathogens, mixture of soapnut and neem oil 5 per cent recorded 50 per cent mortality of third instar grub and found best treatment among entomopathogens, *Metarhizium anisopliae* 2 x 10^8 conidia g^{-1} @ 20 g per palm recorded 26.67 per cent mortality and found superior over other microbial concentration tested.

Ansari *et al.* (2008) evaluated efficacy of entomopathogenic nematodes including *Heterorhabditis bacteriophora* CLO51 strain, *H. megidis* VBM30 strain, *H. indica* , *Steinernema scarabaei, S. glaseri* Belgian and NC strains against larval pupal stages of white grub, *Hoplia philanthus* under laboratory and greenhouse conditions. *Heterorhabditis bacteriophora, H. megidis* and both strains of *S. glaseri* showed highest virulence against third stage larvae and pupae whereas Belgium strain of *S. glaseri* showed high virulence against second stage larvae of *H. philanthus* under laboratory conditions, whereas *H. bacteriophora,* Belgium strains of *S. glaseri* and *S. scarabaei* showed high virulence to third stage than second stage larvae of white grubs under greenhouse conditions.

A series of laboratory and greenhouse experiments evaluated by Kopepenhofer *et al.* (2008) with the entomopathogenic nematodes *Steinernema scarabaei* Stock and Koppenhofer, *Heterohabditis bacteriophora* Poinar, and *H. zealandica* poinar for control of second and third instar cranberry white grub, *Phyllophaga georgiana.* They resulted that *Steinaenema scarabaei* was the most effective species with 76-100% control at the rate of 2.5 x 10^9 IJ/ha in greenhouse experiments.

A series of laboratory tests were conducted by Divya and Sankar (2009) with four EPNs, viz., *H. indica, S. carpocapsae, S. thermophilum* and *S. glaseri* against 25 agricultural important pests. The pathogenicity test conducted by these nematode caused absolutely 100 per cent mortality of these insect pest within 24 to 60 hr of post application. The lethal time of larval mortality by EPNs, *H. indica,* and *S. carpocapsae* on white grub, *H. consanguinea* were 24 and 60 hr, respectively, when applied at 100 IJs/grub.

Kulye and Pokharkar (2009) studied efficacy of *M. anisopliea* and *B. bassiana,* against white grub, *Holotrichia consanguinae* infesting potato crop and estimated LC_{50} of *M. anisopliea* and *B. bassiana* towards third instar larvae of *H.consanguine* were 5.76 x 10^5 conidia ml^{-1}, respectively. The LT_{50} of *M. anisopliae* and *B. bassiana* at the concentration of 8 x 10^5 conidia ml^{-1} against third instar larvae of *H. consanguinea* were 4.88 and 6.73 days. The maximum mycosis was recorded with the use of *M. anisopliae* (44.44%).

Chelvi *et al.* (2010) evaluated laboratory and field efficacy of entomopathogenic to the pest at varying degrees. They reported that under laboratory conditions the spores suspension containing the 3 x 10^9 conidia ml^{-1} all the three evaluated fungi were highly toxic to the grubs.

The efficacy of Six innovative nematode formulations of *H. indica* against cotton bollworm, *H. armigera* were studied by Divya *et al.* (2011) and they reported that the virulence under formulation was leads to cause 85 and 70 per cent pathogenicity on *H. armigera* in hydro gel and saw dust formulation, respectively, when exposed for 48 hr treated with 100 IJs/larvae.

Mane and Mohite (2014) studied the bioefficacy of three species of entomopathogenic fungi *viz*., *Metarhizium anisopliae* (Metsch.) Sorokin, *Beauveria brongniartii* (Sacc.) and *Beauveria* *bassiana* (Balsana) Vuillrmin, against white grub, *L. lepidophora* (Blanchard) infesting sugarcane crop under labortory condition. In this bioassay *M. anisopliae* fungus caused higher rate of grub mortality at an overall concentration range of 4 x 10^5 conidia ml^{-1}.

Bharati and Mohite (2015) tested the two entomopathogenic nematode *viz., H. indica* strain NBAII-104 and *S. carpocapsae* strain NBAII-04 against white grub, *L. lepidophora* (Blanchard) infesting sugarcane under laboratory bioassay studies for first, second and third instar grub. They reported that among this two EPNs, *H. indica* reported to be the most effective and registered LC_{50} value of 106.08 IJs ml^{-1} , 180.09 IJs ml^{-1} and 278.87 IJs ml^{-1} for first, second and third instar grubs, respectively at 5 DAT.

Fedai and Ozgur (2015) studid the Potential of two entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* (Coleoptera: Scarabaeidae), as biological control agents against the June beetle and stated that the highest mortality rates of young and older larvae caused by the *M. anisopliae* product were 74.1 and 67.6% for the GR formulation, 70.2 and 61.8% for the EC formulation, respectively.

Rathour *et al.* (2015) conducted laboratory experiment to study the efficacy of *Steinernema carpocapsae* against white grub, *Phyllognathus dionysius* . They concluded that, the treatments *S. carpocapsea* @100 IJs ml^{-1} for first instar grubs, 150 IJs ml^{-1} for second instar grubs and 250 IJs ml^{-1} for third instar grubs were found most effective in controlling *P. dionysius.*

Kheswa *et al.* (2016) conducted the laboratory bioassay experiments for evaluation of two *Beauveria brongniartii* isolates for pathogenicity against different life stages of white grub species in South African sugarcane and they concluded that both *B. brongniartii* isolates, C17 and HHWG1 have potential as bioinsecticides against adults and larvae, respectively at a concentration of 1 x 10^9 conidia/ml.

2.3 Efficacy of biopesticides in pot culture experiment

Yadav *et al.* (2004) evaluated efficacy of entomogenous fungi, *M. anisopliae* and *B. bassiana* against the eggs, pupa and adult of *H. consanguinea* in pot culture experiment. They revealed that *H. consanguinea* at the egg, pupa and adult stages were susceptible to both entomogenous fungi, with the adult *H. consanguinea* being the most susceptible followed by the egg and the pupa.

In pot culture experiment on vulnerability of potato white grub to entomogenous fungi and nematodes Chandel *et al.* (2005) revealed that *H. indica* caused 100 and 80.46 per cent mortality of second and third instar grubs, respectively.

The field and pot experiments conducted for testing the pathogenicity of EPNs Pillay *et al.* (2009) stated that 100 per cent mortality was achieved with both isoletes *Steinernema* species (EST33D and GINGI3G).

The efficacy of EPNs against different instar of black cutworm (BCW), *A. ipsilon* under laboratory and green house condition was studied by Ebssa and Koppenhofer (2012). They reported that *Heterorhabditis megidis* was the most virulent species, irrespective of larval stage in small arenas, followed most often by *H. bacteriophora.* In pots with grass, *S. carpocapsae* tended to be the most virulent species, followed by *H. bacteriophora, H. megidis* and *S. riobrave*. Fourth and fifth instars were the most susceptible. Furthermore, *H. bacteriophora, H. megidis* and *S. carpocapsae* successfully reproduced in fifth and sixth-instars and pupae.

Bharati and Mohite (2013) conducted pot culture experiment for the effectiveness of two entomopathogenic nematodes (EPN) viz., *Heterorhabditis indica* strain NBAII-104 and *Steinernema carpocapsae* strain NBAII-04 against second instar grubs of *L. lepidophora* (Blanchard) and recorded highest mortality (87.60%) at 15 DAT at concentration of 450 IJs ml^{-1}, *H.indica.*

Supekar and Mohite (2015) conducted pot culture experiment for bioefficacy of different entomopathogenic nematodes against second instar grubs of *H. serrata* Fab. infesting sugarcane and the results indicated that *H. indica* with 450 IJs ml^{-1} concentration proved most promising treatment against *H. serrata* recording 72.67 per cent grub mortality at 15 DAT.

Mane and Mohite (2015) studied the pathogenicity of entomopathogenic fungi against white grub, *L. lepidophora* infesting sugarcane under pot culture experiment and resulted that *M. anisopliae* cause highest mortality 34.49-58.62 per cent @ 2×10^8 conidia ml^{-1}.

2.4 Efficacy of biopesticides under field condition

The bioefficacy of *Metarhizium anisopliae* as microbial insecticide was undertaken by Logan *et al.* (2000) under field condition. They tested rice grain based formulations of the isolates FI-1045 and FI-147 against greyback canegrub in north Queensland (Australia) sugarcane. They reported that a commercially viable application rate of 5 gm^{-1} (1 x 10^{10} conidia m^{-1} @33 kg ha^{-1}) of *Metarhizium anisopliae* FI-1045 gave 50-60 per cent control of greyback cane after 6 months.

Field efficacy of talc based conidial formulation of *M. anisopliae* and *B. bassiana* was evaluated against white grub (*Brahmia* spp.) by Bhagat *et al.* (2003) in potato. Treatment comprised, *M. anisopliae* (5 x 10^{13} conidia ml^{-1}), chlorpyriphos (200 to 400 g ha^{-1}) alone or in combination. *M. anisopliae* formulation applied @ 5 x 10^{13} conidia ha^{-1} along with chlorpyriphos 20 EC @ 200 g a.i. h^{-1} was effective in controlling the grub population (56.5%), exhibiting maximum (63.7%),which resulted in higher tuber yield (155 t ha^{-1}).

Field evaluation of *M. anisopliae* (Ma-1) against arecanut root grub (*L. lepidophora*) was reported by Hajeri (2003). He revealed that, mycopathogen @ 2 x 10^{13} conidia ha^{-1} recorded 60.06 per cent reduction in grub population and was next best to chlorpyriphos 20 EC drenching @ 5 lit ha^{-1}.

Singh *et al.* (2003) evaluated efficacy of entomogenous pathogens under field condition and they found that *B. papillae* and *M. anisopliae* cause diseases in the grubs to the extent of 7.70 and 3.85 per cent, respectively.

Cowles *et al.* (2005) reported that the EPNs, *H. bacteriophora* and *S. carpocapsae* suppressed black vine weevil population by 56 to 100 per cent. *S. carpocapsae* was also found to be effective against cranberry girdler, effectiveness ranges from 44 to 92 per cent in field conditions.

The efficacy of four isolates of EPNs against the white grubs in turf grass were evaluated by Hussaini *et al.* (2005). They reported that nematodes were when applied at 5 x 10^9 IJs/ha. *H.indica* and *H.bcteriophora* caused 20-25 per cent mortality at 10 days after nematode application. White grub population was reduced by 42.3 per cent with *H. indica* and 39.6 per cent with *H. bacteriophora*.

Ansari *et al.* (2006) carried out field trials against white grub (*Hoplia philantus*) with a combination of an entomopathogenic nematode and the fungus *M. anisopliae* CLO-53. They examined this interaction in the field and lawn infested in the province of West Flanders. In both trials the combination of *M. anisopliae* with *H. bacteriophora* @ 5 x 10^{12} conidia ha^{-1} + 2.5 x 10^5 IJS ha^{-1} resulted in additive or synergistic effects, causing more than 95 per cent grub mortality when the nematodes was applied 4 weeks after the application of fungus. However, application of nematode, chlorpyriphos or fungus along provided 39-66 per cent, 42-60 per cent,(surface) and 33-76 per cent, 82-100 per cent or 37-65 per cent (subsurface) control of *H.philanthus.*

Field trial conducted by Channakeshava (2006) in arecanut garden near Sagara and Shimoga in Karnataka state, against root grub (*L. lepidophora*) showed that the *M. anisopliae* 2 x 10^8 conidia g^{-1} @ 1.1 kg per acre given 50.97 per cent mortality which is significantly differ with neem cake @ 550 kg per acre which gave only 14.21 per cent mortality.

The field efficacy of *Beauveria bassiana* (bals) vuill. and *M. anisopliae* (Metsch) for the management of white grub in green gram studied by Bhattacharyya *et al.* (2008). They concluded that *B. bassiana* formulation @ 5 x 10^{13} conidia ml^{-1} in combination with imidacloprid 200 SL @ 48 g a.i. ha^{-1} was found to be effective exhibiting lowest plant mortality (1.66%) and lowest grub population (1.60/pit) which resulted in highest yield of 6.83 q ha^{-1} Likewise, *M. anisopliae* when applied @ 5 x 10^{13} conidia ml^{-1} in combination with imidocloprid 200 SL at 48 g a.i. ha^{-1} resulted in lowest plant mortality (2.28%) and grub population (1.12/pit) and highest yield of 6.79 q ha^{-1}

Lozano -Gutierrez and Espana-Luna (2008) studied the virulence of strain Bbz3 and Bbz4 of the entomopathogenic fungus *B.bassiana* against the white grub *Laniifera cyciacles* under field conditions. The result vivid that strain Bbz3 was more virulent with a LT_{50} of 6.4 days while the LT_{50} of Bbz4 was 6 and 7.5 days in field condition.

Kulye and Pokharakr (2009) carried out field experiment for evaluation of entomopathogenic fungi against white grub infesting potato. They revealed that *M. anisopliae* @ 2 x 10^{12} conidia ha^{-1} effective with an average efficacy of 46.47 per cent while, it was 41.32 per cent in phorate 10 G @25 kg ha^{-1}. The maximum mycosis recorded with the use of *M. anisopliae*

(44.44%). The tuber infestation was statistically lowest (6.91%) and yield of marketable tubers highest (27.6 t ha^{-1}) with the use of *M. anisopliae* as against 27.85 per cent and 17.8 t ha^{-1} in untreated plots, respectively.

Sharma *et al.* (2009) studied the efficacy of the entomopathogenic nematodes (EPN) *Steinernema carpocapsae* and *Heterorhabditis indica* against different developmental stages of *Brahmina coriacea* Hope (Coleoptera: Scarabidae) were evaluated under field conditions and resulted that the least number of grubs and damaged tuber were recorded in case of treatment with *S. carpocapsae* @ 6×105 IJ/m^2.

The entomopathogenic fungi, *B. brongniartii, B. bassiana, M. anisopliae* were evaluated by Chelvi *et al.* (2010) against sugarcane white grubs, *Holotrichia serrata.* They revealed that three entomogenous fungi tested were pathogenic to the pest at varying controlled the pest @1 kg per acre of the formulation.

McGraw *et al.* (2010) demonstrated that field application of three species of entomopathogenic nematodes (*S. carpocapsae, S. feltie* and *H. bacteriophora*) @ 2.5 billion nematodes per hectare reduced over 69 per cent population of first generation late instars of the annual bluegrass weevil, *Listronotus maculicollis.*

Shapiro-Ilan *et al.* (2010) tested efficacy of *H. indica* and *S. carpocapsae* against hive beetles and demonstrated that both nematode species when applied through infected cadavers caused up to 78 per cent control in hive beetles.

Chelvi *et al.* (2011) conducted field experiment for efficacy of formulations of microbial insecticide *Metarhizium* *anisopliae* for the control of sugarcane white grub, *Holotrichia serrata* and resulted that the liquid formulation of biopesticide *M. anisopliae* was found to be efficient for control of white grub, *H. serrata.*

Manisegaran *et al.* (2011) concluded that application of *M.anisopliae* against sugarcane white grub, *Holotrichia serrata* (Blanch) at 4 x 10^9 conidia ha^{-1} was found effective next to chlorpyriphos and registered 92% reduction in grub population on 60^{th} DAT under field condition.

Prabhu *et al.* (2011) studied the efficacy of *M. anisopliae* against white grub *L. lepidophora* under field condition and resulted that *M. anisoliae* 2 x 10^8 conidia per g at the rate 20 g per palm recorded the mortality 31.38%.

Shah *et al.* (2011) concluded that application of *H. indica* and *S. carpocapsae* @ 1 billion nematodes per ha can reduce up to 72 and 50 per cent population of alfa alfa weevil, *Hypera postica* grubs, respectively.

Guo *et al.* (2013) evaluated the efficacy of entomopathogenic nematodes (EPNs) against the white grub, *Holotrichia parallela* Motschulsky (Coleoptera: Scarabaeidae) in the peanut (Arachis hypogaea L.) under field condition and showed the benefit of using EPNs for white grub control was estimated to be comparable with that of using chemicals. *S. longicaudum* X-7 and *H. bacteriophora* H06 showed promise for white grub control in peanut fields.

Bhattacharya and Pujari (2014) conducted field experiments on green gram for white grub management using entomopathogenic fungi i.e., *Beauveria brongniartii* and *Metarhizium anisopliae*. Among them *Beauveria brongniartii* @ 5 x 10^{13} conidia/ml was effective for control white grubs.

Visalakshi *et al*. (2015) conducted field experiments on sugarcane for white grub management using two entomopathogenic fungi i.e., *Beauveria bassiana* and *Metarhizium anisopliae*. Among the treatments imposed at the time of planting, *M. anisopliae* @ 5 x 10^{13} spores ha^{-1} mixed with farm yard manure (FYM) was found effective.

3. MATERIALS AND METHODS

Investigation both in laboratory studies and pot culture experiment were carried out to study the efficacy of different formulations of biopesticides against white grub, *Leucopholis lepidophora* . Laboratory evaluations and pot culture experiments were carried out in the laboratory and green house of Entomology Section, College of Agriculture, Kolhapur. Field experiments were conducted at farmer's field in endemic area of pest in Kolhapur region during 2016-2017. The methodology adopted to study these experiments are described in this chapter.

3.1 Field Survey

For conducting various laboratory experiments and pot culture experiments uninterrupted supply of grubs was essential, hence field survey was conducted around Kolhapur region at different locations particularly area nearby river bank to collect grubs of *Leucopholis lepidophora.*

3.1.1 Collection and maintenance of *L.lepidophora* grubs

A grubs of the same instar and same sizes particularly third instar grub stage were collected from infested sugarcane field from river bank area, immediately after the collection of grubs, they were placed in sterile plastic vials (4 cm × 3.5 cm) with soil from the same collection site for transporting them to the laboratory. Only one larva put into each vial. Potato pieces and roots of sugarcane which are disinfected for 10 min. in 0.5% sodium hypochloride solution were added to each vial as a diet and avoid cannibalism. Additional soil was collected from the same field for the pot culture experiment under the natural white grub habitat. The larval culture were maintained at 25±2 ℃ and 65±5% RH were used for laboratory and pot culture experiments (Bharati and Mohite, 2013).

3.2 Bioefficacy of various formulations of biopesticides against *L. lepidophora* in laboratory bioassay studies

3.2.1 Larval dip method

3.2.1.1 Materials required

Different EPNs formulations used in present study were obtained from NBAII, Banglore, the EPF *M.anisopliae* and *Beauveria* available from Entomology section, College of Agriculture, Kolhapur. The other material plastic vials (4 cm x 3.5 cm), conical flask, petriplates, sodium hypochloride, forceps, potatoes and roots of sugarcane were available at Entomology section.

Table 1: Treatment details of laboratory bioassay studies for third instar grubs :

Sr. No.	Insecticide Treatment	Dose/lit
T_1	*Metarhizium anisopliae* - talc based	5 gm
T_2	*Metarhizium anisopliae* – granules	5 gm
T_3	*Metarhizium anisopliae* tablets	2 gm
T_4	Entomopathogenic Nematode - spong formulation	300 IJS
T_5	Entomopathogenic Nematode- Powder	5 gm
T_6	Entomopathogenic Nematode- Granules formulation	5 gm
T_7	*Beauveria* - Oil based	5 ml
T_8	*Metarhizium anisopliae* - Oil based	5 ml
T_9	Untreated Control	-

Table 2 : Treatment details :

Sr. No.	Insecticide Treatment	Formulation	Source
T_1	*Metarhizium anisopliae* - talc based	$1x10^8$ cfu/ml	Entomology section, College of Agriculture, Kolhapur
T_2	*Metarhizium anisopliae* – granules	$1x10^8$ cfu/ml	Shri Biotech, Hyderabad
T_3	*Metarhizium anisopliae* tablets	$1x10^8$ cfu/ml	Criyagen, Bangalore
T_4	Entomopathogenic Nematode - spong formulation	IJS/ml	NBAII, Bangalore
T_5	Entomopathogenic Nematode-Powder	IJS/ml	Shri Biotech, Hyderabad
T_6	Entomopathogenic Nematode-Granules formulation	IJS/ml	Shri Biotech, Hyderabad
T_7	*Beauveria* - Oil based	$1x10^8$ cfu/ml	Nirmal seeds, Pachora (Jalgoan)
T_8	*Metarhizium anisopliae* - Oil based	$1x10^8$ cfu/ml	Nirmal seeds, Pachora (Jalgoan)
T_9	Untreated Control	-	-

3.2.1.2 Method of testing

The suspension of various formulations of EPNs, EPF *M.anisopliae* and *B.bassiana* prepared according to the required quantity as per recommended dose given in Table 1. The dose-response assay included *M.anisopliae*-talc based (1 x 10^8 cfu/ml) 5 gm, *M.anisopliae*-granules (1 x 10^8 cfu/ml) 5 gm, *M.anisopliae*-tablets (1 x 10^8 cfu/ml) 2 gm, EPN sponge formulation (IJs/ml) 300IJs, EPN powder (IJs/ml) 5 gm, EPN granules formulation (IJs/ml) 5 gm, *Beauveria*-oil based (1 x 10^8 cfu/ml) 5 ml, *M.anisopliae*-oil based (1 x 10^8 cfu/ml) 5 ml.

The laboratory bioassay studies were under taken as per the method suggested by Rathour *et al.* (2015) with slight modification. The larvae were treated with suspension of various formulations of biopesticides and then treated individual larvae transferred separately into a sterile vial and pieces of sugarcane or potato provided as food for grubs. A set of 10 larvae with three replications of each treatment and a control treated with distilled water was maintained. The sugarcane and potato pieces changed every day. The grub kept at 25±2^0C and 65±5 per cent R.H. till death.

3.2.1.3 Observations

The grub mortality was recorded after the treatment at interval of 7, 10 and 15 days after treatment. The exact time required to kill the test larva was strictly recorded. The cause of larval death was confirmed by body colour change of the cadver.

3.2.2 Pot-Culture experiment

In pot culture experiments various formulations of EPNs and EPFs were evaluated against third instar larvae at dose given in Table 3.

3.2.2.1 Materials required

Different formulations of EPNs and EPF *M.anisopliae* and *Beauveria*, plastic vials (4 x 3.5 cm), conical flasks, petriplates, forceps, sugarcane settlings, FYM, earthen pots (15 cm x 20 cm).

Table 3: Treatment details of various formulations of biopesticides under pot culture experiment :

Sr. No.	Insecticide Treatment	Dose/lit
T_1	*Metarhizium anisopliae* - talc based	5 gm
T_2	*Metarhizium anisopliae* – granules	5 gm
T_3	*Metarhizium anisopliae* tablets	2 gm
T_4	Entomopathogenic Nematode - spong formulation	300 IJS
T_5	Entomopathogenic Nematode-Powder	5 gm
T_6	Entomopathogenic Nematode-Granules formulation	5 gm
T_7	*Beaveria* - Oil based	5 ml
T_8	*Metarhizium anisopliae* - Oil based	5 ml
T_9	Untreated Control	-

3.2.3.2 Method of testing

In pot culture experiments various formulations of EPNs and EPF evaluated against third instar larvae. The methodology for pot culture experiment under taken as per the method suggested by Bharati (2013) with slight modifications. Soil and FYM were mixed at 2:1 proportion. Before the addition of biopesticides suspension, the FYM was solarized. For solarization, the FYM was moistened and then spread into a 10 cm thick layer, which was covered with soil to make leak proof. The solarization was done for 3 weeks. Grubs kept in FYM with sugarcane seedlings in earthen pots. It is placed in green shed to provide 25 ± 2^{o} C temperature and 65 ± 5 per cent Relative humidity by regular watering.

The experiment was carried out in Completely Randomized Block Design with three replications and nine treatments. Eight treatments for grubs in pot were carried out whereas in the ninth treatment, the grub was treated as control. Ten grubs of uniform size were used for each treatment.

3.2.3.3 Observations

The grub mortality was recorded after the treatment at an interval of 7, 15 and 30 days. The exact time required to kill the test larva was strictly recorded. The cause of larval death was confirmed by change in body colour of the cadver.

3.2.4 Statistical analysis

Data on per cent mortality were corrected by Abbott's formula (Abbott, 1925) as follows.

$$\text{Corrected mortality} = \frac{T-C}{100-C} \times 100$$

Where,

T = Per cent mortality in treatment.

C = Per cent mortality in control.

Data on infected grubs in laboratory and pot culture experiments were subjected to arcsine transformations, these transformed data was subjected to analysis of variance (Panse and Sukhatme, 1967).

3.3 Bio efficacy of various formulations of biopesticides against White grub under field condition

3.3.1 Field Layout

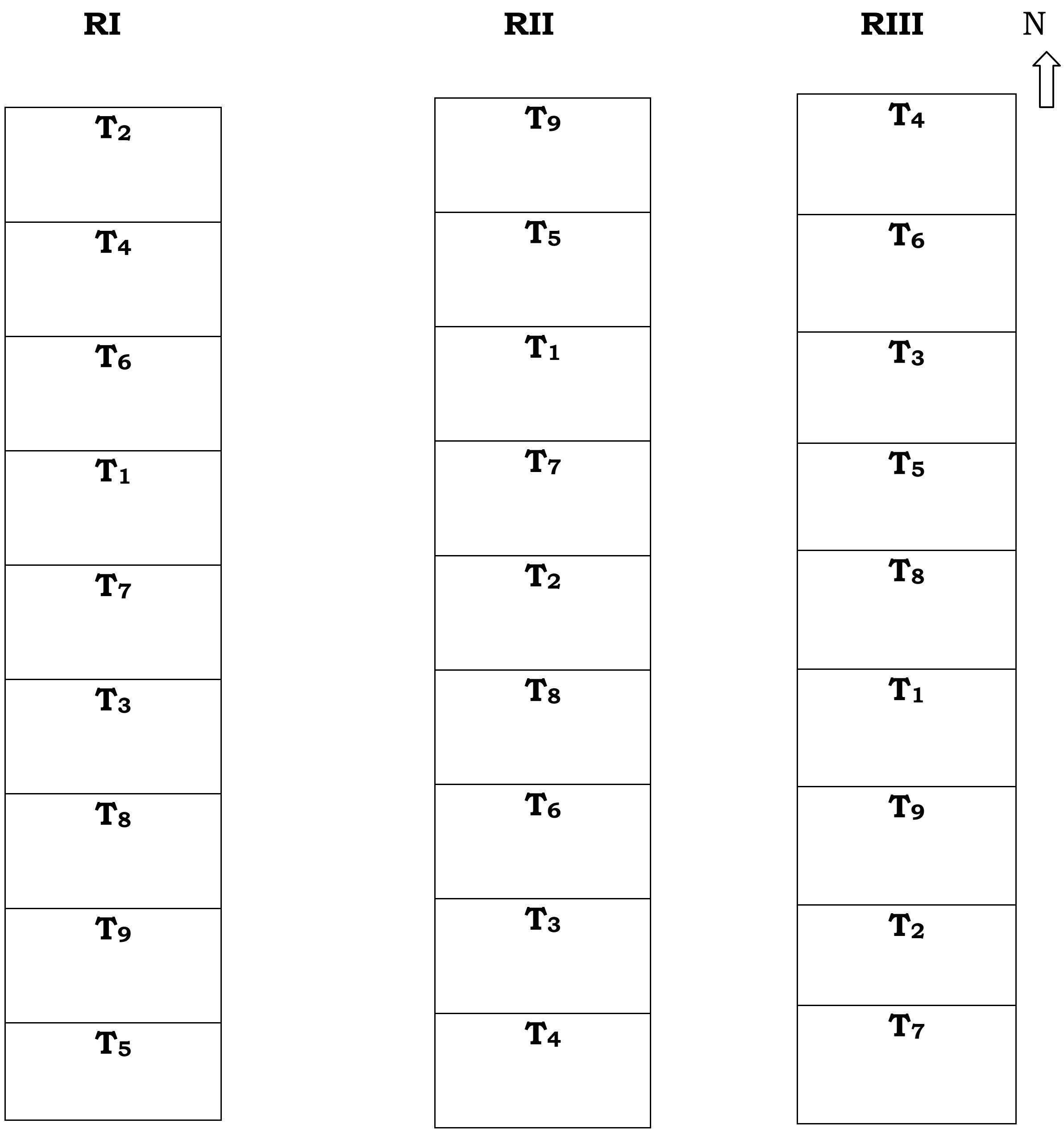
Field trial was conducted at farmer's field in endemic area of pest in Kolhapur region during 2016-2017. Treatment details are as follows initiated at the time of sugarcane planting,

Experimental design	: RBD
Replications	: Three
Treatments	: Nine
Plot Size	: 6 x 5 m^2
Spacing	: 90 cm x 90 cm
Variety of Sugarcane	: CO-86032
Date of planting	: 01 January 2016
Date of drenching	: 15 June 2016

Table 4: Treatment details :

Sr. No.	**Insecticide Treatment**	**Dose/lit**
T_1	*Metarhizium anisopliae* - talc based	5 gm
T_2	*Metarhizium anisopliae* – granules	5 gm
T_3	*Metarhizium anisopliae* tablets	2 gm
T_4	Entomopathogenic Nematode - spong formulation	300 IJS
T_5	Entomopathogenic Nematode-Powder	5 gm
T_6	Entomopathogenic Nematode-Granules formulation	250 gm/plot
T_7	*Beaveria* - Oil based	5 ml
T_8	*Metarhizium anisopliae* - Oil based	5 ml
T_9	Untreated Control	-

Fig 1: Layout of field experiment for efficacy studies of various formulations of biopesticides



3.3.2 Method of various formulations of biopesticides application

The suspension of various formulations of EPNs, EPF *M.anisopliae* and *B.bassiana* prepared according to the required quantity as per recommended dose given in Table 4. The application of various formulations of biopesticides was done by drenching through the hole made by using a crowbar and irrigated the field plots and granules were applied with equal quantity of sand (Manisegaran *et al.* 2011).

3.3.3 Method of recording observations

The observations of field experiments was recorded 5 spots with 1 m^2 area per plot was selected randomly and the number of damaged clumps was counted at 15, 30, 45 and 60 days after treatment (Mane, 2010). The sugarcane clump mortality data recorded and was angularly transformed and subjected to analysis of variance.

3.4 Weather conditions

The data on meteorological parameters viz., temperature, relative humidity and rainfall during the period of field experiment were obtained and presented in Appendix I. The graphical depictions are shown in Fig 2.

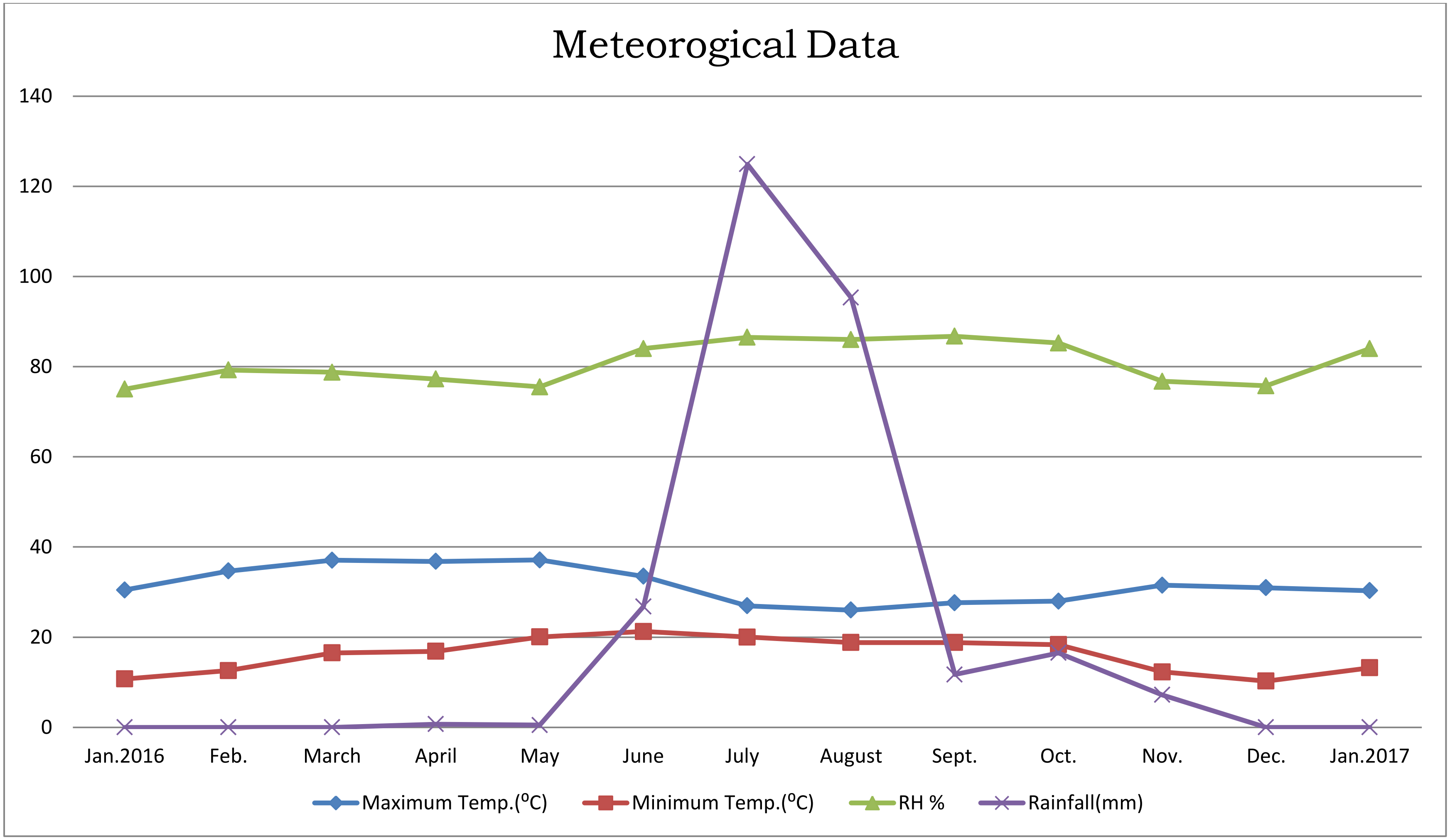


Fig.2. Meteorological data during the period of field experiment

4. RESULTS AND DISCUSSION

Biological control with the help of biopesticides like, entomopathogenic fungi and entomopathogenic nematode is promising alternative to chemical control against the pest infesting annual crop like sugarcane and the search for their host specificity, virulence, safety to natural enemies of target pest against the white grub is a dire need. The various formulations of EPNs and EPFs were assessed for their efficacy against white grub, *L. lepidophora* Blanch. The thought of scientist working with mycopathogens revealed that significance of this biopesticides in management of white grubs may not be as sole control measure but can be effectively incorporated as an important biological weapon in the integrated management.

During the course of present investigation the third instar grubs of *L. lepidophora* Blanch. species were collected from highly infested area nearby river bank in Kolhapur region. The bioefficacy of various formulations of biopesticides were tested under the laboratory bioassay studies and pot culture experiment.

The field experiments were conducted on farmer's field to study the bioefficacy of various formulations of biopesticides against white grub under field condition. The results of the experiments are presented in this chapter.

4.1 Bioefficacy of various formulations of biopesticides in laboratory bioassay studies against grub of *Leucopholis lepidophora*

Eight various formulations of biopesticides were tested for determining their bioefficacy against grubs of *L. lepidophora* (Blanchard). The dead grubs observed at seventh, tenth and fifteenth day interval after the treatment.

4.1.1 Efficacy of various formulations of biopesticides at 7 DAT :

The mortality of the grubs ranged from 13.33 to 64.23 per cent when the observations are taken at 7 DAT. The treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre recorded highest 64.23 per cent mortality and found to be significantly superior over all other treatments. The next best treatments in order of efficacy were Entomopathogenic Nematode-Powder base recorded 61.53 per cent mortality and Entomopathogenic Nematode-spong formulation recorded 56.55 per cent mortality.

4.1.2 Efficacy of various formulations of biopesticides at 10 DAT :

The mortality of the grubs ranged from 14.67 to 74.60 per cent when the observations are taken at 10 DAT. The treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre recorded highest 74.60 per cent mortality and found to be significantly superior over all other treatments. The treatment with Entomopathogenic Nematode-Powder base was next in order of efficacy recorded 67.61 per cent mortality. The next best treatments in order of efficacy were Entomopathogenic Nematode-spong formulation, Entomopathogenic Nematode-granules, *M. anisopliae*-tablet and *M. anisopliae*-granules effective in causing the mortality of the white grub. The treatment with *M. anisopliae*-oil based and Beauveria-oil based are found to be at par with each other.

4.1.3 Efficacy of various formulations of biopesticides at 15 DAT :

The mortality of the grubs ranged from 17.67 to 81.95 per cent when the observations are taken at 15 DAT. The treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre recorded highest 81.95 per cent mortality and found to be significantly superior over all other treatments. The treatment with Entomopathogenic Nematode-Powder base and Entomopathogenic Nematode-spong formulation were found next in order of efficacy recorded 76.93 and 71.64 per cent mortality was recorded.

Entomopathogenic Nematode-Granules form and *M. anisopliae* –tablet were found next best treatment recorded 65.97 and 62.58 per cent grub mortality.

Table 5 Evaluation of various formulations of biopesticides against third instar grub of *L.lepidophora* 7 DAT in laboratory experiment

Sr. No.	Treatment	Dose per litre	Per cent corrected grub mortality			
			R-I	R-II	R-III	Mean
1	*Metarhizium anisopliae* - talc based	5 gm	64.36 (53.35)	64.70 (53.55)	63.63 (52.91)	64.23 (53.27)
2	*Metarhizium anisopliae-granules*	5 gm	51.72 (45.99)	52.94 (46.69)	54.54 (47.60)	53.37 (46.93)
3	*Metarhizium anisopliae* Tablets	2 gm	52.87 (46.65)	52.94 (46.69)	55.68 (48.26)	53.83 (47.20)
4	Entomopathogenic Nematode - spong formulation	300 IJs	56.32 (48.63)	57.64 (49.39)	55.68 (48.26)	56.55 (48.76)
5	Entomopathogenic Nematode-Powder	5 gm	62.06 (51.98)	61.17 (51.45)	61.36 (51.57)	61.53 (51.67)
6	Entomopathogenic Nematode-Granules formulation	5 gm	54.02 (47.31)	52.29 (46.31)	53.40 (46.95)	53.24 (46.86)
7	*Beauveria* - Oil based	5 ml	44.82 (42.03)	43.52 (41.28)	44.31 (41.73)	44.22 (41.68)
8	*Metarhizium anisopliae* - Oil based	5 ml	47.13 (43.35)	48.23 (43.99)	46.59 (43.04)	47.31 (43.46)
9	Untreated Control	_	13.00 (21.13)	15.00 (22.79)	12.00 (20.27)	13.33 (21.41)
	SE±					0.61
	CD at 5%					1.83

Table 6 Evaluation of various formulations of biopesticides against third instar grub of *L.lepidophora* 10 DAT in laboratory experiment

Sr. No.	Treatment	Dose per litre	Per cent corrected grub mortality			
			R-I	R-II	R-III	Mean
1	*Metarhizium anisopliae* - talc based	5 gm	72.94 (58.65)	75.00 (60.00)	75.86 (60.57)	74.60 (59.74)
2	*Metarhizium anisopliae-granules*	5 gm	51.76 (46.01)	53.57 (47.05)	56.32 (48.63)	53.88 (47.23)
3	*Metarhizium anisopliae* Tablets	2 gm	54.11 (47.36)	54.76 (47.73)	56.32 (48.63)	55.06 (47.90)
4	Entomopathogenic Nematode - spong formulation	300 IJs	58.82 (50.08)	64.28 (53.30)	58.62 (49.96)	60.57 (51.10)
5	Entomopathogenic Nematode-Powder	5 gm	67.05 (54.97)	70.28 (56.96)	65.51 (54.04)	67.61 (53.31)
6	Entomopathogenic Nematode-Granules formulation	5 gm	57.64 (49.39)	58.33 (49.80)	55.17 (47.97)	57.05 (49.05)
7	*Beauveria* - Oil based	5 ml	44.70 (41.93)	44.04 (41.58)	44.82 (42.03)	44.52 (41.85)
8	*Metarhizium anisopliae* - Oil based	5 ml	48.23 (43.99)	48.80 (44.31)	47.12 (43.35)	48.05 (43.88)
9	Untreated Control	_	15.00 (22.79)	16.00 (23.58)	13.00 (21.13)	14.67 (22.52)
	S.E±					1.07
	CD at 5%					3.18

Table 7 Evaluation of various formulations of biopesticides against third instar grub of *L.lepidophora* 15 DAT in laboratory experiment

Sr. No.	Treatment	Dose per litre	Per cent corrected grub mortality			
			R-I	R-II	R-III	Mean
1	*Metarhizium anisopliae* - talc based	5 gm	80.48 (63.78)	81.48 (64.51)	83.33 (65.90)	81.95 (64.86)
2	*Metarhizium anisopliae-granules*	5 gm	58.53 (49.91)	59.28 (50.35)	58.33 (49.80)	58.71 (50.02)
3	*Metarhizium anisopliae* Tablets	2 gm	62.89 (52.47)	62.96 (52.51)	61.90 (51.88)	62.58 (52.29)
4	Entomopathogenic Nematode-spong formulation	300 IJs	73.17 (58.80)	69.13 (56.25)	72.61 (58.44)	71.64 (57.82)
5	Entomopathogenic Nematode-Powder	5 gm	76.82 (61.22)	77.77 (61.87)	76.19 (60.79)	76.93 (61.29)
6	Entomopathogenic Nematode-Granules formulation	5 gm	68.29 (55.73)	62.96 (52.51)	66.66 (54.73)	65.97 (54.31)
7	*Beauveria* - Oil based	5 ml	52.43 (46.39)	53.08 (46.77)	53.57 (47.05)	53.03 (46.75)
8	*Metarhizium anisopliae* - Oil based	5 ml	53.58 (47.05)	54.32 (47.48)	54.76 (47.43)	54.22 (47.42)
9	Untreated Control	_	18.00 (25.10)	19.00 (25.84)	16.00 (23.58)	17.67 (24.86)
	S.E±					0.85
	CD at 5%					2.50

4.1.4 Overall performance of various formulations of biopesticides against third instar grub of *L.lepidophora* under laboratory condition

Among eight various formulations of biopesticides were tested for determining their bioefficacy against grubs of *L. lepidophora* Blanch. (Table 7 and Fig.3) The treatment with *M. anisopliae*-talc based was consistently found to be significantly superior over all other treatment where the observations were recorded 7, 10 and 15 DAT. The treatment with Entomopathogenic Nematode-spong formulation, Entomopathogenic Nematode-Powder base formulation, *M. anisopliae*-tablet formulation were next in order of efficacy. The treatment with *M.anisopliae*-oil based formulation found to be superior than the *Beauveria*-oil based formulation and found to be least effective under laboratory condition.

The treatment with *M. anisopliae*-talc based was most effective recording 64.23 per cent grub mortality and it was found to be significantly superior over all other treatments when the observations were recorded at 7 DAT. The treatment with Entomopathogenic Nematode-Powder recorded 61.53 per cent grub mortality and was found next in the order of efficiency. The least of 13.33 per cent grub mortality was observed in treatment with untreated control.

The 74.60 per cent mortality was observed in treatment with *M. anisopliae*-talc based when observations were recorded at 10 DAT which was superior to the rest of the treatment under test. The treatment with Entomopathogenic Nematode-Powder recorded 67.61 per cent grub mortality and was found next in the order of efficiency. The least of 14.67 per cent grub mortality was observed in treatment with untreated control.

The highest grub mortality 81.95 per cent was recorded with the treatment of *M. anisopliae*-talc based when observations were recorded at 15 DAT. However, Entomopathogenic Nematode-Powder were next superior treatment where 76.93 per cent grub mortality observed. The least of 17.67 per cent grub mortality was observed in treatment with untreated control. This may be due to the handling of grubs while recording observations.

The pathogenicity of *M. anisopliae* was first time proved by Hajeri (2003) against *L. lepidophora.*

The present findings are in conformity with Kulye and Pokharkar (2009) and Mane and Mohite (2014) who reported that *M. anisopliae* was more effective than *Beauveria* Against *L. lepidophora.*

The present findings also similar with Bharati and Mohite (2013), Rathour *et al.* (2015), Supekar and Mohite (2015) who reported the efficacy of Entomopathogenic Nematodes against *L. lepidophora.*

Whereas, Fedai and Ozgur (2015) reported that *Beuveria* was more effective than *M. anisopliae* against the larvae of *Polyphylla fullo*. These results are in contrast with present findings. The interaction of *M. anisopliae* and *Beauveria bassiana* may differ species to species.

Table 8 Evaluation of various formulations of biopesticides against third instar grub of *L. lepidophora* in laboratory experiment

Sr. No.	Treatment	Dose per litre	Mean per cent mortality of grub		
			7 DAT	10 DAT	15 DAT
1	*Metarhizium anisopliae* - talc based	5 gm	64.23 (53.27)	74.60 (59.74)	81.95 (64.86)
2	*Metarhizium anisopliae-granules*	5 gm	53.37 (46.93)	53.88 (47.23)	58.71 (50.02)
3	*Metarhizium anisopliae* Tablets	2 gm	53.83 (47.20)	55.06 (47.90)	62.58 (52.29)
4	Entomopathogenic Nematode - spong formulation	300 IJs	56.55 (48.76)	60.57 (51.10)	71.64 (57.82)
5	Entomopathogenic Nematode-Powder	5 gm	61.53 (51.67)	67.61 (55.31)	76.93 (61.29)
6	Entomopathogenic Nematode-Granules formulation	5 gm	53.24 (46.86)	57.05 (49.05)	65.97 (54.31)
7	*Beauveria* - Oil based	5 ml	44.22 (41.68)	44.52 (41.85)	53.03 (46.75)
8	*Metarhizium anisopliae* - Oil based	5 ml	47.31 (43.46)	48.05 (43.88)	54.22 (47.42)
9	Untreated Control	_	13.33 (21.41)	14.67 (22.52)	17.67 (24.86)
	SE±		0.61	1.07	0.85
	CD at 5%		1.83	3.18	2.50

DAT- Days After Treatment
(Figures in the parenthesis are arc sin transformation)

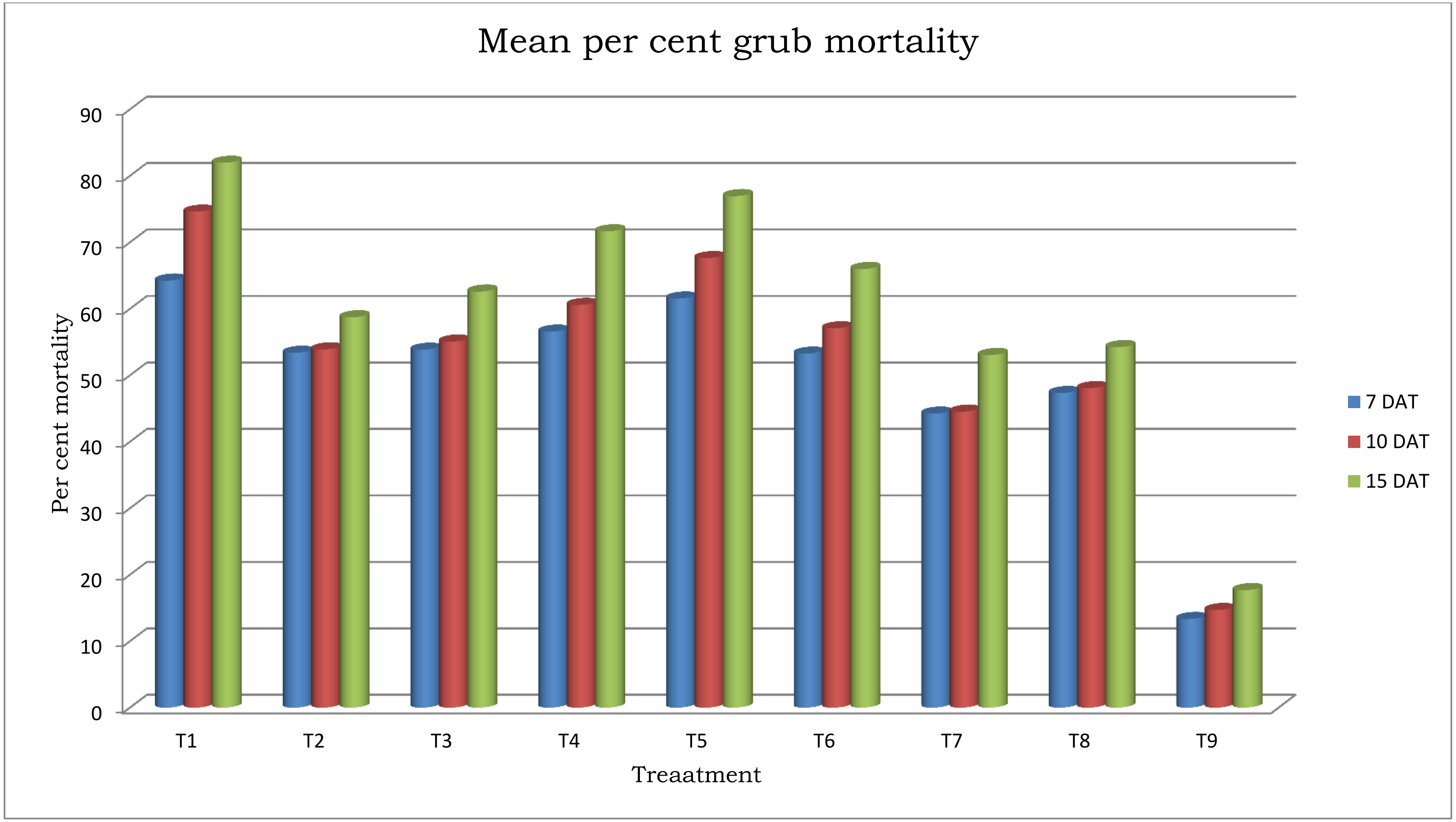


Fig.3. Bioefficacy of various formulations of biopesticides under laboratory bioassay studies against White grub, *L.lepidophora*

4.2 Bioefficacy of various formulations of biopesticides against third instar grub of *L.lepidophora* in pot culture experiment

Response of eight various formulations of biopesticides were tested for determining their bioefficacy against grubs of *L. lepidophora* (Blanchard) under pot culture experiment. The dead grubs observed at seventh, fifteenth and thirtieth day interval after the treatment.

4.2.1 Efficacy of various formulations of biopesticides at 7 DAT :

The mortality of the grubs ranged from 0.00 to 36.33 per cent when the observations are taken at 7 DAT. The treatment with *M.anisopliae*-talc based at the dose of 5 gm per litre recorded highest 36.33 per cent mortality of grub under pot culture experiment which is significantly superior over all other treatments. However, this treatment was found to be on par with Entomopathogenic Nematode-Powder base, Entomopathogenic Nematode-spong base where 33.00 and 32.33 per cent grub mortality was registered. The treatment with *Beuveria*-oil based found to be least effective treatment.

4.2.2 Efficacy of various formulations of biopesticides at 15 DAT :

The mortality of the grubs ranged from 10.33 to 58.36 per cent when the observations are taken at 15 DAT. The treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre recorded highest 58.36 per cent mortality of grub under pot culture experiment which is significantly superior over all other treatments.

However, the treatment with Entomopathogenic Nematode-Powder and Entomopathogenic Nematode-spong formulation were found next best treatment where 54.30 and 53.89 per cent mortality was observed. In untreated control 10.33 per cent grub mortality was observed. The significant differences did not existed among rest of the treatment.

4.2.3 Efficacy of various formulations of biopesticides at 30 DAT :

The mortality of the grubs ranged from 11.67 to 69.41 per cent when the observations are taken at 30 DAT. The treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre recorded highest 69.41 per cent mortality of grub under pot culture experiment which is significantly superior over all other treatments. However, it was on par with the treatment with Entomopathogenic Nematode-Powder where 67.95 per cent mortality was recorded.

The treatment with Entomopathogenic Nematode-spong formulation, Entomopathogenic Nematode-Granules and *M. anisopliae*-tablet were found to be on par with each other and next in order of efficacy where 62.64, 59.99 and 58.06 per cent mortality was registered. *M. anisopliae* and *Beauveria*-oil based formulation found to be less effective in causing the mortality of the grub under pot culture condition.

Table 9 Evaluation of various formulations of biopesticides against third instar grub of *L.lepidophora* 7 DAT under pot culture experiment

Sr. No.	Treatment	Dose per litre	Per cent corrected grub mortality			
			R-I	R-II	R-III	Mean
1	*Metarhizium anisopliae* - talc based	5 gm	35.00 (36.27)	36.00 (36.87)	38.00 (38.06)	36.33 (37.07)
2	*Metarhizium anisopliae-granules*	5 gm	27.00 (31.31)	25.00 (30.00)	26.00 (30.66)	26.00 (30.66)
3	*Metarhizium anisopliae* Tablets	2 gm	29.00 (32.58)	31.00 (33.83)	30.00 (33.21)	30.00 (33.21)
4	Entomopathogenic Nematode - spong formulation	300 IJs	31.00 (33.83)	32.00 (34.45)	34.00 (35.67)	32.33 (34.65)
5	Entomopathogenic Nematode-Powder	5 gm	32.00 (34.45)	33.00 (35.06)	34.00 (35.67)	33.00 (35.06)
6	Entomopathogenic Nematode-Granules formulation	5 gm	28.00 (31.95)	29.00 (32.58)	30.00 (33.21)	29.00 (32.58)
7	*Beauveria* - Oil based	5 ml	21.00 (27.27)	24.00 (29.33)	22.00 (27.97)	22.33 (28.20)
8	*Metarhizium anisopliae* - Oil based	5 ml	22.00 (27.97)	24.00 (29.33)	26.00 (30.66)	24.00 (29.33)
9	Untreated Control	_	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	SE±					0.74
	CD at 5%					2.21

Table 10 Evaluation of various formulations of biopesticides against third instar grub of *L.lepidophora* 15 DAT under pot culture experiment

Sr. No.	Treatment	Dose per litre	Per cent corrected grub mortality			
			R-I	R-II	R-III	Mean
1	*Metarhizium anisopliae* - talc based	5 gm	60.00 (50.71)	56.57 (48.78)	58.42 (49.85)	58.36 (49.81)
2	*Metarhizium anisopliae-granules*	5 gm	43.33 (41.17)	45.56 (42.45)	44.94 (42.10)	44.61 (41.91)
3	*Metarhizium anisopliae* Tablets	2 gm	52.22 (46.27)	51.11 (45.64)	49.43 (44.67)	50.92 (45.53)
4	Entomopathogenic Nematode - spong formulation	300 IJs	55.56 (48.19)	54.44 (47.55)	51.68 (45.96)	53.89 (47.23)
5	Entomopathogenic Nematode-Powder	5 gm	54.54 (47.60)	55.56 (48.19)	52.80 (46.61)	54.30 (47.47)
6	Entomopathogenic Nematode-Granules formulation	5 gm	51.11 (45.64)	53.33 (46.91)	50.56 (45.32)	51.67 (45.96)
7	*Beauveria* - Oil based	5 ml	44.44 (41.81)	42.22 (40.12)	42.69 (40.80)	43.12 (41.05)
8	*Metarhizium anisopliae* - Oil based	5 ml	43.33 (41.17)	44.55 (41.87)	43.82 (41.15)	43.87 (41.48)
9	Untreated Control	_	10.00 (18.43)	10.00 (18.43)	11.00 (19.37)	10.33 (18.75)
	SE±					0.77
	CD at 5%					2.29

Table 11 Evaluation of various formulations of biopesticides against third instar grub of *L.lepidophora* 30 DAT under pot culture experiment

Sr. No.	Treatment	Dose per litre	Per cent corrected grub mortality			
			R-I	R-II	R-III	Mean
1	*Metarhizium anisopliae* - talc based	5 gm	69.31 (56.36)	71.11 (57.49)	67.81 (55.43)	69.41 (56.42)
2	*Metarhizium anisopliae*-*granules*	5 gm	47.72 (43.69)	47.77 (43.72)	48.27 (44.01)	47.92 (43.81)
3	*Metarhizium anisopliae* Tablets	2 gm	57.95 (49.57)	60.00 (50.77)	58.62 (49.96)	58.86 (50.01)
4	Entomopathogenic Nematode-spong formulation	300 IJs	62.50 (52.24)	62.22 (52.07)	63.21 (52.66)	62.64 (52.32)
5	Entomopathogenic Nematode-Powder	5 gm	68.18 (55.66)	65.56 (54.07)	70.11 (56.86)	67.95 (55.52)
6	Entomopathogenic Nematode-Granules formulation	5 gm	56.81 (48.91)	61.11 (51.42)	62.06 (51.98)	59.99 (50.76)
7	*Beauveria* - Oil based	5 ml	37.50 (37.76)	37.78 (37.93)	39.08 (38.69)	38.12 (38.13)
8	*Metarhizium anisopliae* - Oil based	5 ml	42.04 (40.42)	41.11 (39.88)	42.52 (40.70)	41.89 (40.33)
9	Untreated Control	_	12.00 (20.27)	10.00 (18.43)	13.00 (21.13)	11.67 (19.98)
	SE±					0.80
	CD at 5%					2.60

4.2.4 Overall performance of various formulations of biopesticides against third instar grub of *L.lepidophora* under pot culture experiment

The overall performance of eight formulations indicated that the treatment with *M. anisopliae*-talc based formulation consistently superior over all other treatments in causing the mortality of white grubs under pot culture experiment when the observations were recorded 7, 15 and 30 DAT. The treatment with Entomopathogenic nematode-Powder formulation was found next best treatment in causing the mortality and was on par with the earlier treatment. The treatment with Entomopathogenic Nematode-spong formulation, Entomopathogenic Nematode-Granules and *M. anisopliae*-tablet were found to be next in order of efficacy. The treatment with oil formulation of *M. anisopliae* and *Beauveria* were found least effective as compared to all other treatments.

The data recorded at 7 DAT revealed that the mortality of grubs varies from 0.00 to 36.33 per cent. The treatment with of *M. anisopliae*-talc based at the dose of 5 gm per litre was significantly superior over the other treatments and recorded maximum of 36.33 per cent grub mortality. While, the treatments with untreated control recorded no grub mortality.

The treatment with *M. anisopliae*-talc based was most effective recording 58.36 per cent grub mortality and it was found to be significantly superior over all other treatments when the observations were recorded at 15 DAT. The treatment with Entomopathogenic Nematode-Powder recorded 54.30 per cent grub mortality and was found next in the order of efficiency. The least of 10.33 per cent grub mortality was observed in treatment with untreated control.

The treatment with *M.anisopliae*-talc based was most effective recording 69.41 per cent grub mortality and it was found to be significantly superior over all other treatments when the observations were recorded at 30 DAT. The treatment with Entomopathogenic Nematode-Powder recorded 67.95 per cent grub mortality and was found next in the order of efficiency. The least of 11.67 per cent grub mortality was observed in treatment with untreated control. This may be due to the handling of grubs while recording observations.

The present findings are in agreement with Mane and Mohite (2014) who reported that *M. anisopliae* was more effective than *Beauveria* against *L. lepidophora*.

Bharati and Mohite (2013.), Rathour (2014) and Supekar and Mohite (2015) reported the efficacy of Entomopathogenic Nematodes against white grub which is similar to the present findings.

Due to the paucity of the information the present results could not be compared.

Table 12 Evaluation of various formulations of biopesticides against third instar grub of *L.lepidophora* under pot culture experiment

Tr. No.	Treatment	Dose per litre	Mean per cent grub mortality		
			7 DAT	15 DAT	30 DAT
1	*Metarhizium anisopliae* - talc based	5 gm	36.33 (37.07)	58.36 (49.81)	69.41 (56.42)
2	*Metarhizium anisopliae-granules*	5 gm	26.00 (30.66)	44.61 (41.91)	47.92 (43.81)
3	*Metarhizium anisopliae* Tablets	2 gm	30.00 (33.21)	50.92 (45.53)	58.06 (50.01)
4	Entomopathogenic Nematode-spong formulation	300 IJs	32.33 (34.65)	53.89 (47.23)	62.64 (52.32)
5	Entomopathogenic Nematode-Powder	5 gm	33.00 (35.06)	54.30 (47.47)	67.95 (55.52)
6	Entomopathogenic Nematode-Granules formulation	5 gm	29.00 (32.58)	51.67 (45.96)	59.99 (50.76)
7	*Beauveria* - Oil based	5 ml	22.33 (28.20)	43.12 (41.05)	38.12 (38.13)
8	*Metarhizium anisopliae* - Oil based	5 ml	24.00 (29.33)	43.87 (41.48)	41.89 (40.33)
9	Untreated Control	_	0.00 (0.00)	10.33 (18.75)	11.67 (19.98)
	SE±		0.74	0.77	0.80
	CD at 5%		2.21	2.29	2.60

DAT- Days After Treatment
(Figures in the parenthesis are arc sin transformation)

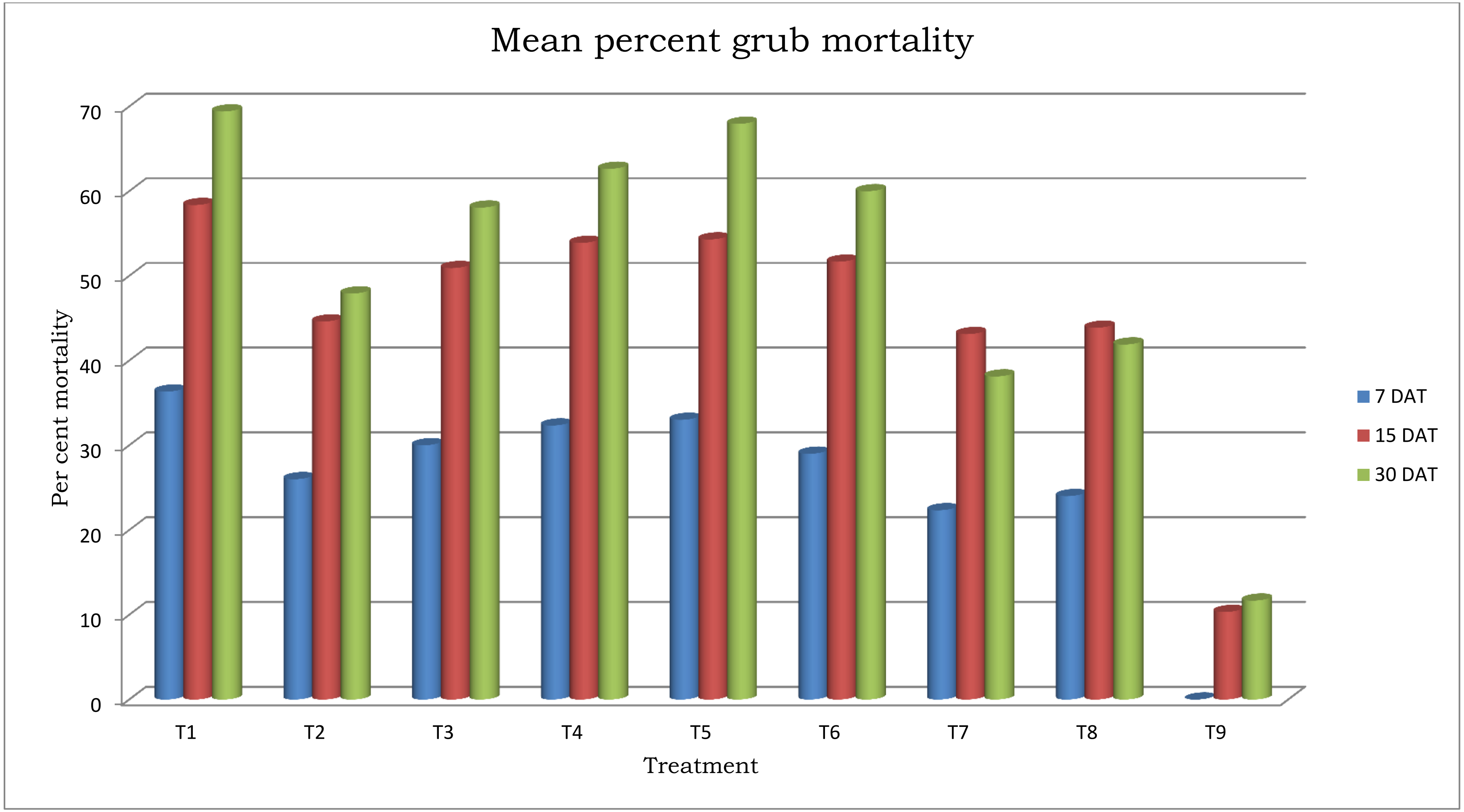


Fig.4. Bioefficacy of various formulations of biopesticides against White grub, *L.lepidophora* under pot culture experiment

4.3 Bioefficacy of various formulations of biopesticides against third instar grub of *L.lepidophora* under field condition

Response of eight various formulations of biopesticides were tested for determining their bioefficacy against grubs of *L. lepidophora* (Blanchard) under field condition. The clumps mortality observed at fifteenth, thirtieth, forty-fifth and sixtieth day interval after the treatment.

4.3.1 Efficacy of various formulations of biopesticides at 15 DAT :

The mortality of clumps ranged from 6.49 to 31.16 per cent as compared to 35.93 per cent in untreated control when the observations are taken at 15 DAT. The treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre was significantly superior over all other treatments (6.49%) in reducing mortality of clumps. The treatment with Entomopathogenic Nematode-Powder was found next best treatment (8.49%) in reducing mortality of clumps. The next best treatments were Entomopathogenic Nematode-spong formulation and Entomopathogenic Nematode-Granules formulation where 10.81 and 15.58 per cent mortality of clumps was recorded. The significant differences did not exist among the rest of the treatment.

4.3.2 Efficacy of various formulations of biopesticides at 30 DAT :

The mortality of clumps ranged from 7.36 to 32.45 per cent as compared to 37.52 per cent in untreated control when the observations are taken at 30 DAT. The treatment with *M.*

anisopliae-talc based at the dose of 5 gm per litre was significantly superior over all other treatments (7.36%) in reducing mortality of clumps. The treatment with Entomopathogenic Nematode-Powder was found next best treatment (10.06%) in reducing mortality of clumps. The next best treatment was Entomopathogenic Nematode-spong formulation where 12.55 per cent mortality of clumps was recorded. The treatment with Entomopathogenic Nematode-Granules, *M.anisopliae*-tablet and *M.anisopliae*-Granules next in order of efficacy. Significant differences did not exist among the rest of the treatment. *M.anisopliae*-oil based and *Beauveria*-oil based formulations registered high clumps mortality (32.45 and 27.27 per cent).

4.3.3 Efficacy of various formulations of biopesticides at 45 DAT :

The mortality of clumps ranged from 9.20 to 33.76 per cent as compared to 38.53 per cent in untreated control when the observations are taken at 45 DAT. The treatment with *M.anisopliae*-talc based at the dose of 5 gm per litre was significantly superior over all other treatments (9.20%) in reducing mortality of clumps. The treatment with Entomopathogenic Nematode-Powder was found next best treatment (10.65%) in reducing mortality of clumps, However Entomopathogenic Nematode-spong formulation, Entomopathogenic Nematode-Granules formulation and *M.anisopliae*-tablet where 13.85, 18.18 and 23.80 per cent mortality of clumps was recorded.

4.3.4 Efficacy of various formulations of biopesticides at 60 DAT :

The mortality of clumps ranged from 9.36 to 35.06 per cent as compared to 40.25 per cent in untreated control when the observations are taken at 60 DAT. The treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre was significantly superior over all other treatments (9.36%) in reducing mortality of clumps. The treatment with Entomopathogenic Nematode-Powder and Entomopathogenic Nematode-spong formulation were found next best treatment recorded 11.68 per cent and 15.15 per cent mortality.

The Entomopathogenic Nematode-Granules formulation was found to be superior than remaining treatments where 19.91 per cent clumps mortality was recorded.

Table 13 Efficacy of various formulations of biopesticides against third instar grub *L.lepidophora* 15 DAT under field condition

Sr. No.	Treatment	Dose per litre	Per cent clumps mortality			
			R-I	R-II	R-III	Mean
1	*Metarhizium anisopliae* - talc based	5 gm	5.19 (13.17)	7.79 (16.21)	6.49 (14.76)	6.49 (14.76)
2	*Metarhizium anisopliae-granules*	5 gm	20.77 (27.11)	23.37 (28.91)	24.67 (29.78)	22.94 (28.62)
3	*Metarhizium anisopliae* Tablets	2 gm	19.48 (26.19)	20.77 (27.11)	22.07 (28.02)	20.77 (27.11)
4	Entomopathogenic Nematode - spong formulation	300 IJs	10.38 (18.19)	11.68 (19.98)	10.38 (18.79)	10.81 (19.20)
5	Entomopathogenic Nematode-Powder	5 gm	7.79 (16.21)	9.90 (18.34)	7.79 (16.21)	8.49 (16.94)
6	Entomopathogenic Nematode-Granules formulation	250 gm/plot	14.28 (22.20)	15.58 (23.25)	16.88 (24.26)	15.58 (23.25)
7	*Beauveria* - Oil based	5 ml	24.67 (29.78)	25.97 (30.64)	27.27 (31.48)	25.97 (30.64)
8	*Metarhizium anisopliae* - Oil based	5 ml	29.87 (33.13)	31.16 (33.93)	32.46 (34.73)	31.16 (33.93)
9	Untreated Control	_	35.06 (36.31)	36.36 (37.08)	36.36 (37.08)	35.93 (36.83)
	S.E±					0.47
	CD at 5%					1.42

Table 14 Efficacy of various formulations of biopesticides against third instar grub *L.lepidophora* 30 DAT under field condition

Sr. No.	Treatment	Dose per litre	Per cent clumps mortality			
			R-I	R-II	R-III	Mean
1	*Metarhizium anisopliae* - talc based	5 gm	6.49 (14.76)	7.79 (16.21)	7.79 (16.21)	7.36 (15.74)
2	*Metarhizium anisopliae- granules*	5 gm	20.77 (27.11)	22.07 (28.02)	23.37 (28.91)	22.07 (28.02)
3	*Metarhizium anisopliae* Tablets	2 gm	22.07 (28.02)	24.67 (29.78)	25.97 (30.64)	24.24 (29.49)
4	Entomopathogenic Nematode - spong formulation	300 IJs	11.68 (19.98)	12.98 (21.12)	12.98 (21.12)	12.55 (20.75)
5	Entomopathogenic Nematode- Powder	5 gm	9.90 (18.34)	10.38 (18.79)	9.90 (18.34)	10.06 (18.49)
6	Entomopathogenic Nematode- Granules formulation	250 gm/plot	16.88 (24.26)	16.88 (24.26)	18.18 (25.24)	17.31 (24.59)
7	*Beauveria* - Oil based	5 ml	25.97 (30.64)	27.27 (31.48)	28.57 (32.31)	27.27 (31.48)
8	*Metarhizium anisopliae* - Oil based	5 ml	31.16 (33.93)	32.46 (34.73)	33.73 (35.51)	32.45 (34.73)
9	Untreated Control	_	36.36 (37.08)	37.66 (37.86)	37.66 (37.86)	37.52 (37.77)
	SE±					0.30
	CD at 5%					1.15

Table 15 Efficacy of various formulations of biopesticides against third instar grub *L.lepidophora* 45 DAT under field condition

Sr. No.	Treatment	Dose per litre	Per cent clumps mortality			
			R-I	R-II	R-III	Mean
1	*Metarhizium anisopliae* - talc based	5 gm	7.79 (16.21)	9.90 (18.34)	9.90 (18.34)	9.20 (17.66)
2	*Metarhizium anisopliae-granules*	5 gm	24.67 (29.78)	25.97 (30.64)	25.97 (30.64)	25.54 (30.36)
3	*Metarhizium anisopliae* Tablets	2 gm	23.37 (28.91)	23.37 (28.91)	24.67 (14.28)	23.80 (29.20)
4	Entomopathogenic Nematode - spong formulation	300 IJs	12.98 (21.12)	14.28 (22.20)	14.28 (22.20)	13.85 (21.85)
5	Entomopathogenic Nematode-Powder	5 gm	10.38 (18.17)	11.68 (19.98)	9.90 (18.34)	10.65 (19.05)
6	Entomopathogenic Nematode-Granules formulation	250 gm/plot	18.18 (25.24)	18.18 (25.24)	18.18 (25.24)	18.18 (24.24)
7	*Beauveria* - Oil based	5 ml	27.27 (31.48)	28.57 (32.31)	29.87 (33.13)	28.57 (32.81)
8	*Metarhizium anisopliae* - Oil based	5 ml	32.46 (34.73)	33.76 (35.52)	35.06 (36.31)	33.76 (35.52)
9	Untreated Control	_	37.66 (37.86)	38.96 (38.62)	38.96 (38.62)	38.53 (38.37)
	SE±					0.37
	CD at 5%					1.12

Table 16 Efficacy of various formulations of biopesticides against third instar grub *L.lepidophora* 60 DAT under field condition

Sr. No.	Treatment	Dose per litre	Per cent clumps mortality			
			R-I	R-II	R-III	Mean
1	*Metarhizium anisopliae* - talc based	5 gm	9.90 (18.34)	10.38 (18.79)	7.79 (16.21)	9.36 (17.81)
2	*Metarhizium anisopliae- granules*	5 gm	24.67 (29.78)	27.27 (31.48)	28.51 (32.27)	26.82 (31.19)
3	*Metarhizium anisopliae* Tablets	2 gm	23.37 (28.91)	24.67 (29.78)	28.57 (32.31)	25.54 (30.36)
4	Entomopathogenic Nematode - spong formulation	300 IJs	14.28 (22.20)	15.58 (23.25)	15.58 (23.25)	15.15 (22.91)
5	Entomopathogenic Nematode- Powder	5 gm	11.68 (19.98)	12.98 (21.12)	10.38 (18.79)	11.68 (19.98)
6	Entomopathogenic Nematode- Granules formulation	250 gm/plot	19.48 (26.19)	19.48 (26.19)	20.77 (21.11)	19.91 (26.50)
7	*Beauveria* - Oil based	5 ml	28.57 (32.31)	28.57 (32.31)	31.16 (33.93)	29.43 (32.85)
8	*Metarhizium anisopliae* - Oil based	5 ml	33.76 (36.31)	35.06 (36.31)	36.36 (37.08)	35.06 (36.31)
9	Untreated Control	_	38.96 (38.62)	41.55 (40.14)	40.25 (39.35)	40.25 (39.38)
	SE±					0.79
	CD at 5%					2.37

4.3.5 Overall performance of various formulations of biopesticides against third instar grub of *L.lepidophora* under field condition

The overall performance of the various formulations of the biopesticides against the white grub under field condition revealed that the treatment with *M.anisopliae*-talc based formulation registered significantly less 6.49, 7.36, 9.20 and 9.36 per cent clumps mortality. The treatment with Entomopathogenic Nematode-Powder base, Entomopathogenic Nematode-spong formulation, *M. anisopliae*-tablet and Entomopathogenic Nematode-Granules formulation were next in order of efficacy. The oil based formulations of *M. anisopliae* and *Beauveria* was found to be least effective in reducing the clumps mortality.

The data recorded at 15 DAT revealed that the mortality of clumps varies from 4.49 to 35.93 per cent. The treatment with of *M. anisopliae*-talc based at the dose of 5 gm per litre was significantly superior over the other treatments (4.49%) in reducing the mortality of clumps. The treatment with Entomopathogenic Nematode-Powder (8.49%) was found next in the efficacy of reducing mortality of clumps.

The treatment with *M. anisopliae*-talc based (7.36%) was most effective in reducing mortality of clumps and it was found to be significantly superior over all other treatments when the observations were recorded at 30 DAT. The treatment with Entomopathogenic Nematode-Powder recorded 10.06 per cent clumps mortality and was found next in the order of efficiency.

The treatment with *M. anisopliae*-talc based was most effective in reducing mortality of clumps (9.20%) and it was found to be significantly superior over all other treatments when the observations were recorded at 45 DAT. The treatment with Entomopathogenic Nematode-Powder recorded 10.65 per cent clumps mortality.

The treatment with *M. anisopliae*-talc based was most effective in reducing mortality of clumps (9.36%) and it was found to be significantly superior over all other treatments when the observations were recorded at 60 DAT. The treatment with Entomopathogenic Nematode-Powder recorded 11.68 per cent clumps mortality and was found next in the order of efficiency.

The present findings are in conformity with Kuley and Pokharkar (2009) and Visalakshi *et al.* (2015) who reported that *M. anisopliae* was effective than *Beauveria* under field condition for controlling white grub.

Hussaini (2005) reported the efficacy of four EPNs against white grub when applied at 5 x 10^9 IJs/ha which is similar to present findings.

Bhattacharya and Pujari (2014) reported that *Beauveria* 5 x 10^{13} conidia/ml was more effective than *M. anisopliae* against The white grub.

Thamarai *et al.* (2011) revealed that *M. anisopliae* liquid formulation was more effective than other formulations for the control of *Holotrichia serrata.* This results are in contrast with present findings.

Table 17 Efficacy of various formulations of biopesticides against third instar grub of *L.lepidophora* under field condition

Sr. No.	Treatment	Dose per litre	Per cent clumps mortality			
			15 DAT	30 DAT	45 DAT	60 DAT
1	*Metarhizium anisopliae* - talc based	5 gm	6.49 (14.76)	7.36 (15.74)	9.20 (17.66)	9.36 (17.81)
2	*Metarhizium anisopliae- granules*	5 gm	22.94 (28.62)	24.24 (29.49)	25.54 (30.36)	26.82 (31.19)
3	*Metarhizium anisopliae* Tablets	2 gm	20.77 (27.11)	22.07 (28.02)	23.80 (29.20)	25.54 (30.36)
4	Entomopathogenic Nematode - spong formulation	300 IJs	10.81 (19.20)	12.55 (20.75)	13.85 (21.85)	15.15 (22.91)
5	Entomopathogenic Nematode- Powder	5 gm	8.49 (16.94)	10.06 (18.49)	10.65 (19.05)	11.68 (19.98)
6	Entomopathogenic Nematode- Granules formulation	250 gm/plot	15.58 (23.25)	17.31 (24.59)	18.18 (24.24)	19.91 (26.50)
7	*Beauveria* - Oil based	5 ml	25.97 (30.64)	27.27 (31.48)	28.57 (32.81)	29.43 (32.85)
8	*Metarhizium anisopliae* - Oil based	5 ml	31.16 (33.93)	32.45 (34.73)	33.76 (35.52)	35.06 (36.31)
9	Untreated Control	_	35.93 (36.83)	37.52 (37.77)	38.53 (38.37)	40.25 (39.38)
	SE±		0.47	0.30	0.37	0.79
	CD at 5%		1.42	1.15	1.12	2.37

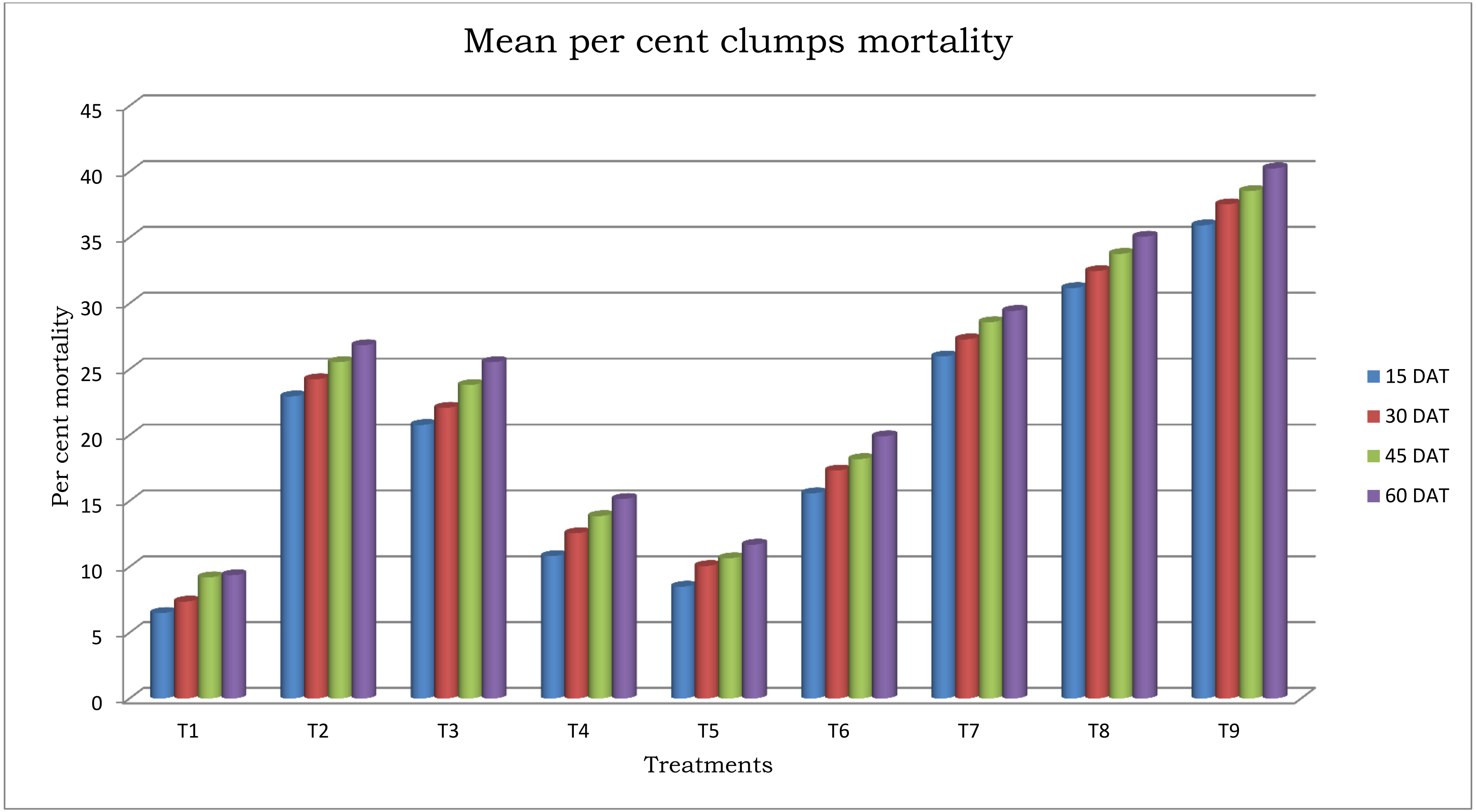


Fig.5. Bioefficacy of various formulations of biopesticides against White grub, *L.lepidophora* under field condition

5. SUMMARY AND CONCLUSIONS

The present investigations were undertaken to find out bioefficacy of various formulations of biopesticides against third instar grubs of *L.lepidophora* under laboratory, pot and field conditions.

The various formulations of biopesticides were tested against third instar grubs proved pathogenic to *L. lepidophora* of white grub in laboratory bioassay studies, pot culture experiment and field experiment. Among these various formulations of biopesticides *M. anisopliae*-talc based was found to be the most effective at the dose of 5 gm per litre.

5.1 Efficacy of various formulations of Biopesticides against third instar grubs of *L.lepidophora* in larval dip method

The reduction of grub population at 15 DAT indicated that all the treatments were significantly superior to untreated control in reduction of *L. lepidophora* grub population. The treatment with *M. anisopliae*-talc based at the dose of 5 gm per litre recorded 81.95 per cent reduction in grub population and it was found to be significantly superior over all the treatments. Entomopathogenic Nematode-Powder with the dose of 5 gm per litre showed 76.93 per cent reduction in grub population at 15 DAT were next in order of efficacy. The treatment with Entomopathogenic Nematode-spong formulation, Entomopathogenic Nematode-Granules were next in order of efficacy.

5.2 Efficacy of various formulations of biopesticides against third instar grubs of *L.lepidophora* in pot culture experiment

Results indicated that *M. anisopliae*-talc based at the dose of 5 gm per litre was the most promising treatment against *L. lepidophora* recorded 69.41 per cent grub mortality at 30 DAT. Entomopathogenic Nematode-Powder, Entomopathogenic Nematode-spong formulation, Entomopathogenic Nematode-Granules, *M. anisopliae*-tablet formulation were next in order of efficacy.

5.3 Efficacy of various formulations of biopesticides against third instar grubs of *L.lepidophora* under field condition

Field evaluation of various biopesticides against white grub in sugarcane revealed that application of *M. anisopliae*-talc based at the dose of 5 gm per litre was found to be significantly superior over untreated control and recorded 9.36 per cent clumps mortality at 60 DAT. Treatment with Entomopathogenic Nematode-Powder with the dose of 5 gm per litre showed 11.68 per cent clumps mortality at 60 DAT were next in order of efficacy. Treatment with Entomopathogenic Nematode-spong formulation, Entomopathogenic Nematode Granules were next in order of efficacy.

5.4 Conclusion

1. Among the various formulations of biopesticides *M.anisopliae*-talc based at the dose of 5 gm per litre was promising in laboratory bioassay studies.
2. In pot culture experiment *M.anisopliae*-talc based at the dose 5 gm per litre was promising among other formulations.
3. In field studies among various formulations of biopesticides *M.anisopliae* - talc at the dose 5 gm per litre showed higher efficacy in reducing clumps mortality. Entomopathogenic Nematode-Powder were next in order of efficacy.

FUTURE LINE OF WORK

1. Efficacy of various formulations of different entomopathogenic nematodes needs to be tested in sugarcane as well as other cropping system.
2. Evaluation of other bioagents like bacteria alone and in combination with EPF and insecticides needs to be tested under pot culture and field condition.
3. Studeis on synergistic interaction of EPNs and EPFs with chemicals like insecticides, fungicides and herbicides is need to be tested under pot culture and field conditions.
4. Studies on bio-ecology and life table of sugarcane white grub is necessary to find out weakest link and time of application.

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