POTENCY OF BIOINSECTICIDES AGAINST THE COWPEA BRUCHID, *Callosobruchus maculatus* (F.) (COLEOPTERA: CHRYSOMELIDAE) IN STORAGE

By

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(2009-11-109)

THESIS

Submitted in partial fulfillment of the requirement for the degree of

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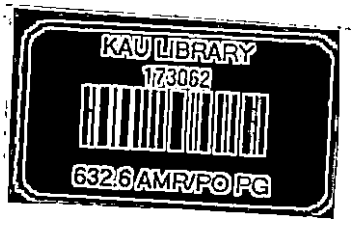

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DECLARATION

I, hereby declare that this thesis entitled **"Potency of bioinsecticides against the cowpea bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) in storage"** is a bonafide record of research work done by me during the course of research and that the thesis has not previously formed the basis for the award of any degree, diploma, fellowship or other similar title, of any other University or Society.

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CONTENTS

Chapter	Title	Page No.
1	INTRODUCTION	1-2
2	REVIEW OF LITERATURE	3-25
3	MATERIALS AND METHODS	26-42
4	RESULTS	43-69
5	DISCUSSION	70-83
6	SUMMARY	84-88
	REFERENCES	i-xvii
	APPENDICES	
	ABSTRACT	

LIST OF TABLES

Table No.	Title	Page No.
1	Mortality of *C. maculatus* treated with *B. bassiana* (direct dipping)	45
2	Mortality of *C. maculatus* treated with *B. bassiana* (residue film)	45
3	Mortality of *C. maculatus* treated with *M. anisopliae* (direct dipping)	47
4	Mortality of *C. maculatus* treated with *M. anisopliae* (residue film)	47
5	Effect of *B. bassiana* and *M. anisopliae* on the cumulative mortality of *C. maculatus* by different bioassay methods	50
6	LC_{50}, LC_{90} values and probit regression parameters for *B. bassiana* and *M. anisopliae* against *C. maculatus* by two bioassay methods	50
7	Effect of spinosad on the mortality of adults of *C. maculatus* (direct dipping)	52
8	Effect of spinosad on the mortality of *C. maculatus* (residue film)	52
9	LC_{50}, LC_{90} values and probit regression parameters for spinosad against *C. maculatus* by two bioassay methods	54
10	Fumigant toxicity of lemongrass oil on *C. maculatus*	54
11	LC_{50}, LC_{90} values and probit regression parameters of lemongrass oil against *C. maculatus*	55
12	LT_{50} and LT_{90} values of entomopathogens against *C. maculatus* by two bioassay techniques	57
13	LT_{50}, LT_{90} and probit regression parameters for spinosad against *C. maculatus* by two bioassay methods	58
14	LT_{50}, LT_{90} and probit regression parameters for lemongrass oil against *C. maculatus*	58
15	Toxicity of bioinsecticides on adults of *C. maculatus* in cowpea storage	60
16	Fecundity and hatchability of *C. maculatus* as influenced by bioagents	63

17	Effect of bioinsecticides on the progeny emergence of *C. maculatus* in cowpea grains	65
18	Effect of bioagents on extent of infestation by *C. maculatus*	67
19	Influence of bioinsecticides on weight loss caused by *C. maculatus*	67
20	Effect of bioagents on germination of cowpea seeds	69
21	Persistent toxicity of bioagents against *C. maculatus*	69

LIST OF FIGURES

Figure No.	Title	Between pages
1	Mortality of *C. maculatus* treated with *B. bassiana* (residue film)	70-71
2	Mortality of *C. maculatus* treated with *M. anisopliae* (residue film)	70-71
3	Cumulative mortality by *B. bassiana* and *M. anisopliae* of *C. maculatus* (two bioassay methods)	71-72
4	Mortality of *C. maculatus* by spinosad (residue film on grains)	71-72
5	Fumigant toxicity of lemongrass oil on *C. maculatus*	73-74
6	LT_{50} values of entomopathogens against *C. maculatus* by two bioassay techniques	73-74
7	Direct toxicity effect of bioinsecticides on adults of *C. maculatus*	77-78
8.	Surface protectant action of bioinsecticides on *C. maculatus*	77-78
9.	Effect of bioagents on extent of infestation by *C. maculatus*	80-81
10.	Effect of bioagents on weight loss caused by *C. maculatus*	80-81
11.	Effect of bioagents on germination of cowpea seeds infested by *C. maculatus*	82-83
12.	Persistent toxicity of bioinsecticides in cowpea storage	82-83

LIST OF PLATES

Plate No.	Title	Between pages
1	Taxonomic characters of *C. maculatus*	26-27
2	*Callosobruchus maculatus* adults	27-28
3.	Rearing set up of *C. maculatus*	27-28
4.	Chracteristics of *B. bassiana* and *M. anisopliae*	28-29
5.	Culturing of *B. bassiana* and *M. anisopliae*	29-30
6.	Fungal infected cadavars of *C. maculatus*	30-31
7.	Fabricated fumigation chamber	35-36
8.	Unhatched and hatched eggs of *C. maculatus*	39-40

LIST OF APPENDICES

Appendix No.	Title
I	Key to *Callosobruchus* species


Introduction

1. INTRODUCTION

Among the various pests of pulses, bruchids, the pulse beetles are of major concern as they infest the grains both in the field and in stores. Several bruchid species attack cereals and pulses in store and cause a loss of 10 to 15 per cent and a germination loss ranging from 50 to 92 per cent (Aduga, 2006). The bruchid beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) is the most destructive pest of cowpea *Vigna unguiculata* (L.) Walp. under storage throughout the tropics and sub tropics. Attack on stored cowpea grains by *C. maculatus* brings about losses in commercial value by reducing quantity, quality and viability of seeds. Development of a single larva in a pulse grain can lead to 8 to 22 per cent weight loss and the bruchids are able to destroy all cowpea grains by causing losses upto 100 per cent within a few months of storage (Cherry *et al.*, 2007). Without specific protection, 80 per cent of the cowpea grains are lost due to bruchid infestation after six months of storage (Aboua *et al.*, 2010).

Arthropod pest management in post harvest storage has been primarily through the prophylactic application of synthetic chemical insecticides and fumigants. The excessive use of conventional chemical insecticides has resulted in an array of serious problems to humans, beneficial insects, other non target organisms and to the environment. The development of new races of insects resistant to synthetic insecticides also resulted in increased losses in storage. The increasing awareness on the deleterious effects of chemical insecticides and the demand for insecticide free food has prompted the development of safer alternative management options.

The use of bioinsecticides offers an alternative management strategy against stored grain pests in post harvest storage of pulses. Bioinsecticides are gaining an increasing importance as an eco-friendly management tool in the current agricultural scenario. Entomopathogenic fungi such as *Beauveria bassiana* (Balsamo) Vuillemin (Ascomycota: Hypocreales) and *Metarhizium anisopliae*

(Metschinkoff) Sorokin (Deuteromycotina: Hyphomycetes) have been reported to have great potential for the control of insect pests of stored grains particularly coleopteran insects in storage (Adane *et al.,* 1996; Lawrence and Khan, 2002; Cherry *et al.,* 2005). New molecules of biological origin with environmental and food safety are also required to be explored as bioinsecticides to afford protection of cowpea seeds from the attack of *C. maculatus* in storage. Spinosad, a newer generation, reduced- risk, bioinsecticide derived from a soil actinomycete, has been suggested as a potential grain protectant against storage pests (Nayak *et al.,* 2005). Plant essential oils exhibiting insecticidal activity offer a viable option against several coleopterans in post harvest storage. Fumigant toxicity effectiveness of essential oil from lemongrass (*Cymbopogon flexuosus*) has been reported against *C. maculatus* (Raja and William, 2008). Not much information is available on the feasibility of utilization of the above mentioned bioinsecticides *viz.*, entomopathogenic fungi, spinosad and lemongrass oil as grain protectants against *C. maculatus* in stored cowpea. In this context, four bioinsecticides comprising of two entomopathogenic fungi- *B. bassiana* and *M. anisopliae,* a chemical molecule derived by fermentation from the naturally occurring soil actinomycete *Saccharopolyspora spinosa*- spinosad and a plant essential oil from the aromatic plant *Cymbopogon flexuosus* (L.) were selected to assess their potency against *C. maculatus* in cowpea and thereby to identify the promising alternative management options in cowpea storage.

The present investigation entitled 'Potency of bioinsecticides against the cowpea bruchid *Callosobruchus maculatus* (F.) in storage' has been carried out with the following objectives.

1. To investigate the potential of bioinsecticides against the cowpea bruchid, *C. maculatus.*

2. To explore the feasibility of utilizing the biologically based grain protectants in post harvest storage of cowpea.

Review of Literature

2. REVIEW OF LITERATURE

Insect pests in stored grains and pulses may cause 10- 40 per cent damage in countries where modern storage technologies have not been introduced. It is generally accepted that 5- 15 per cent of total weight of all the pulses, cereals and oil seeds is lost after harvest (Padin *et al.*, 2002).

Cowpea, *Vigna unguiculata* Walpers, is an important food legume in tropics and subtropics. With the high protein content, cowpea is a natural supplement to cereals. *Callosobruchus maculatus* (F.) is a primary pest of cowpea and other legumes worldwide, both on pods in fields and in stored seeds. It is responsible for losses upto 20- 60 per cent in stored legumes.

C. maculatus belongs to the the super family Chrysomeloidea, family Chrysomelidae and subfamily Bruchinae. Earlier it was included under the family Bruchidae (Lawrence and Britton, 1991)

Attack of stored grains by *C. maculatus* significantly reduces the quantity and quality of seeds destined for both human consumption and sowing purposes (Raja *et al.,* 2001). Many synthetic insecticides are being used for the management of insect pests in food commodities and most of them show adverse effects and have raised a number of ecological and medical problems such as residual toxicity, carcinogenicity, impotency, infertility *etc.* (Dubey *et al.*, 2004). These problems coupled with the demand for insecticide free foods have triggered the efforts to find alternative less obtrusive management options. Bioinsecticides offer an alternative pest management strategy in storage. The important works pertaining to bioinsecticides *viz.,* entomopathogenic fungi- *B. bassiana* and *M. anisopliae*, spinosad and lemongrass oil against storage pests in general and pulse beetle in particular are reviewed in this chapter.

2.1. Entomopathogenic fungi against storage grain pests

Naturally occurring entomopathogens are important regulatory factors in insect populations. Entomopathogenic fungi include those genera of fungi that associate with insects and some other arthropods like spiders and mites. They are characterized by their ability to attach and penetrate host cuticle, multiply within the host and ultimately result in the death of host insect (Easwaramoorthy, 2003). Many species are employed as biological control agents of insect pests in glass house crops, orchards, ornamentals, range, turf and lawn, stored products and forestry and for abatement of pest and vector insects of veterinary and medical importance (Lacey *et al.*, 2001). The capacity of entomopathogenic fungi to control stored grain pests particularly Coleoptera has been investigated in several studies (Adane *et al.*, 1996; Rice and Cogburn, 1999; Kassa *et al.*, 2002). Few studies have evaluated fungal pathogens for the control of *C. maculatus* in cowpea (Vilas-Boas *et al.*, 1996; Lawrence and Khan, 2002).

2.1.1. *Beauveria bassiana* (Balsamo) Vuillemin

The white muscardine fungus *B. bassiana* occurs throughout the world and has the largest host range among the fungi imperfecti. It mainly infects insects belonging to Lepidoptera, Coleoptera, Hemiptera, Diptera, and Hymenoptera (Easwaramoorthy, 2003).

2.1.1.1. *B. bassiana* against pulse beetle, *C. maculatus*

Cherry *et al.* (2007) evaluated eight indigenous and exotic isolates of *B. bassiana* for their virulence and the ability to suppress the populations of *C. maculatus* in stored cowpea in West Africa. They found that the indigenous isolates were more virulent in laboratory bioassays than exotic isolates from other insects. Isolate of *B. bassiana* 0362 was consistently more virulent and LC_{50} values on day six post treatment was 9.1 x 10^4 conidia/ ml. In one kg batch of

cowpea stocked with fifty adults *C. maculatus*, *B. bassiana* 0362 at both 1 x 10^7 and 1 x 10^8 conidia/ gram led to significant adult mortality and reduced F_1 emergence. However, *B. bassiana* 0362 infected and killed *C. maculatus*, it was not suggested that cadavars were sporulating in the stored grain. Without the production of new conidia, persistence was found to be dependent on the survival of the original inoculum.

Murad *et al.* (2007) screened the response of *B. bassiana* isolates in response to *C. maculatus*. They evaluated the insecticidal activity of ten strains of *B. bassiana* against *C. maculatus* and most lethal strains were analysed for proteinaceous secretions and their enzymatic activites. They had suggested that this study could help to establish novel biotechnological tools to use for cowpea bruchid control.

2.1.1.2. *B. bassiana* against other storage pests

Rodrigues and Pratisoli (1990) conducted studies in the laboratory at 25-28°C and 60 per cent relative humidity to evaluate the pathogenicity of an isolate (CA-2) of *Beauveria brongniartii* and another isolate (E-9) of *M. anisopliae* against the curculionid *Sitophilus zeamais* Motch and the bruchid *Acanthoscelides obtectus* (Say). The results showed that both isolates at 1 x 10^8 conidia/ ml were pathogenic to the pests and gave effective protection of treated maize and bean (*Phaseolus vulgaris*) grains stored with the pests for a period of 6 months.

Seventy two isolates of *B. bassiana* and *M. anisopliae* were screened by Moino *et al.* (1998) for the control of *Sitophilus oryzae* (L.), *S. zeamais* and *Rhyzopertha dominica* (F.). *B. bassiana* isolates produced highest mortalities against all the three hosts. Cumulative mortalities after 10 days of exposure and lethal time for 50 per cent of the population (LT_{50}) were calculated and compared.

S. oryzae and *S. zeamais* were less succeptible to *B. bassiana* isolates than *R. dominica* which were completely killed by several isolates.

Adane *et al.* (1996) tested the virulence of ten isolates of *B. bassiana* to maize weevil *S. zeamais* in the laboratory. All isolates tested were capable of infesting *S. zeamais* but their virulence determined by adult mortality and median lethal time varied. A total of five isolates were virulent and one among them 190-520 was selected for further work on dosage-mortality relationships and infectivity of dry conidia against the pest. The lowest dose (1×10^4 conidia/ ml) caused about 88 per cent mortality within eight days. But adult mortality and damage to maize seeds treated with different levels of dry conidia were not significantly different from pirimiphos- methyl treated maize after 14 days of storage.

The effect of different formulations of *B. bassiana* on *S. zeamais* on stored maize was studied by Hidalgo *et al.* (1998). Twenty four hours of direct contact of *S. zeamais* with the fat pellet formulation containing 1×10^{10} conidia/ gram gave 100 per cent mortality after seven days. Oil suspension with *B. bassiana* at a concentration of 1×10^9 conidia/ ml or 200 ml/ kg grain showed the highest level of control in maize grains.

An isolate of *B. bassiana* from rice water weevil, *Lissorhoptrus oryzophilus* (Kusche) (Coleoptera: Curculionidae) as a conidial powder was evaluated against three Coleopteran pests of stored grains: the rice weevil, *S. oryzae*; the lesser grain borer, *R. dominica*; and red flour beetle, *Tribolium castaneum* (Herbst). It was reported that adult mortality ranged from 80 to 100 per cent at higher dosage levels for all insects at 21 days after treatments. Emergence of adult progeny on brown and rough rice was reduced by 83 to 99 per cent at the two highest dosage levels (Rice and Cogburn, 1999).

Lord (2001) reported that *B. bassiana* was most efficacious in high humidity. Interaction between diatomaceous earth and *B. bassiana* enhanced insect control performance. He conducted assays with *B. bassiana* against stored grain beetles at the rate of 11, 33, 100 and 300 mg of conidia/ kg. The assays revealed synergism in the effects on adult *R. dominica* and *Oryzaephilus surinamensis* (L.) at all doses.

The effect of *B. bassiana* on the losses caused to durum wheat and beans by storage insects was investigated by Padin *et al.* (2002). A spore formulation of ground rice and *B. bassiana* was used. Adults of *T. castaneum* or *S. oryzae* were added to treated wheat and *Acanthoscelides obtectus* to bean. The insecticidal effect of *B. bassiana* was tested by measuring the fresh weight and weight loss of grains after four months of storage. Percentage weight loss decreased by 81.5 per cent and was significantly smaller than that of control. Significant differences in weight loss were not found in seeds infested with *T. castaneum* and *A. obtectus*.

Akbar *et al.* (2005) evaluated the impact of plant essential oils, mineral oil and organosilicone carriers on the efficacy of *B. bassiana* conidia against red flour beetle, *T. castaneum* larvae. They reported that for each of the three selected conidial density, the probability of *T. castaneum* larval mortality was significantly greater when conidia were in mineral oil than when they were applied without a carrier. A decrease in median lethal concentration of *B. bassiana* in presence of mineral oil was noticed. They also reported that mineral oil was found to be a more effective carrier of *B. bassiana* for *T. castaneum*.

Cherry *et al.* (2005) evaluated 12 indigenous and exotic isolates of *B. bassiana* and *M. anisopliae* for their virulence and the ability to suppress population of *C. maculatus* in stored cowpea. They reported that LT_{50} values for *B. bassiana* isolates varied from 3.11 to 6.13 days with an average of 4.61 days and that of *M. anisopliae* varied from 3.27 to 5.62 days with an average of 4.60 days. In dose response bioassay by exposure to treated grains gave LC_{50} values of

1.15 x 10^7 and 4.44 x 10^7 conidia/ g grain for *B. bassiana* 0362 and *M. anisopliae* 0351 respectively. In one kg batches of cowpea stocked with 50 adult *C. maculatus*, *B. bassiana* 0362 at both 1 x 10^7 and 1 x 10^8 conidia/ gram grain led to significant adult mortality and reduced F1 emergence. At 1 x 10^8 conidia/ gram, the effect of fungus persisted into F_1 generation.

The insecticidal effect of *B. bassiana* formulations against adults of *R. dominica* and *S. oryzae* on stored wheat was studied in the laboratory by Vassilakos *et al.* (2006). The formulated product of *B. bassiana* Naturalis was applied at three dose rates, 2500, 5000, and 10000 ppm and diatomaceous earth formulations Silico Sec at two dose rates, 0.2 and 0.5 g/ kg of wheat either alone or in combination with each fungal rate. Bioassays were conducted at three temperatures, 22, 26, and 30^{0}C and relative humidity level 55 per cent. Generally the increase of temperature increased the insecticidal effect of diatomaceous earth against *R. dominica* and *S. oryzae,* but *B. bassiana* was more effective at 26^0 C than at 30^0 C, for both species. The simultaneous use of diatomaceous earth in fungal formulation moderated the negative effect. Some combination treatment had an additive effect on fungal efficacy by diatomaceous earth while in others a negative effect was observed.

Athanassiou and Steenberg (2007) studied the insecticidal effect of *B. bassiana* in combination with diatomaceous earth (DE) formulations against adults of granary weevil, *S. granarius*. Bioassays were conducted at three temperatures (20, 25, and 30 ^{0}C) and two relative humidities (55 and 75 per cent). On wheat treated with *B. bassiana* alone, mortality was higher at 55 per cent than at 75 per cent relative humidity and less effective at 20^0 C than at other two temperatures tested. But mortality did not exceed 52 per cent for any condition tested. This study clearly demonstrated that the combination of relatively low doses of DE and *B. bassiana* could provide higher insecticidal effect than the use of *B. bassiana* or diatomaceous earth alone.

The effect of desiccation stress on the efficacy of *B. bassiana* for controlling stored product insects was tested and the results demonstrated that dry stored grain conditions were favourable for *B. bassiana* efficacy (Lord, 2007).

Laboratory bioassays were conducted to evaluate the efficacy of Iranian isolates of *B. bassiana* against adults of *R. dominica* on stored wheat. All the isolates were pathogenic to the beetle although mortality rates were different. *B. bassiana* Iran 187 C showed a LC_{50} value of 9.6×10^5 conidia/ ml. LT_{50} values varied from 6.77 to 9.28 days for the isolates of *B. bassiana* (Mahdneshin *et al.*, 2009).

2.1.2. *Metarhizium anisopliae* (Metchinkoff) Sorokin

The green muscardine fungus *M. anisopliae* is a common and widely distributed fungus with a wide host range. Over 100 species of insects belonging to different insect orders are known to be infected by this fungus.

2.1.2.1. Bioefficacy of *M. anisopliae* against *C. maculatus*

Murad *et al.* (2006) reported that cowpea weevil control could be based on utilization of bacteria and fungi. They evaluated ten *M. anisopliae* isolates according to their virulence, correlating chitinolytic, proteolytic and α-amylolytic activities against *C. maculatus*. They had suggested that this study could help to establish novel biotechnological tools for cowpea bruchid control.

Three indigenous and exotic isolates of *M. anisopliae* were tested for their effect against *C. maculatus* in Benin, Western Africa. The LT_{50} values showed a shortest value (3.27 days) for indigenous isolate and longest value (4.97 days) for the exotic one. *M. anisopliae* isolate 0351 was less virulent than *B. bassiana* 0362 isolate and both were found to be indigenous isolates recovered from *C. maculatus* in Benin (Cherry *et al.*, 2007).

2.1.2.2. Bioefficacy of *M. anisopliae* against other storage pests

The pathogenicity of five isolates of *M. anisopliae* to adult groundnut bruchid, *Caryedon serratus* was evaluated in Nigeria. All isolates tested were virulent to the beetle but pathogeniciy varied among the isolates. Isolate CPD 4 was consistently superior to all other isolates in terms of morality of beetle, protection of groundnut pods from damage, reduction in progeny production and repellency to beetle (Ekesi *et al.*, 2001).

Batta (2004) tested different formulations of *M. anisopliae* conidia with oven ash, chalk powder, charcoal and wheat flour at a ratio of 1:4 (w/w) against *S. oryzae*. Treatment with charcoal and oven ash formulations at the rate of 2.0 per cent or 2.8 mg/ cm^2 of treated area resulted in 73.3- 86.7 per cent mortality of adult *S. oryzae* after seven days. Mortality of F1 adults was 28.9-86.7 per cent. Charcoal or oven ash formulation reduced damage rates to wheat grains to 0.5 per cent and prolonged the development time of *S. oryzae* to 4-8 days.

Batta (2005) studied the effect of *M. anisopliae* formulated in invert emulsions (water in oil formulation) or in wheat flour against *R. dominica* adults infecting *Cicer arietinum* grains. The application rates were 4.1 x 10^5 conidia/ cm^2 of treated area using a concentration of 1.8 x 10^7 conidia/ ml of the invert emulsion and 8.2 x 10^6 conidia / cm^2 of treated area using a concentration of 6.5 x 10^8 conidia/ g of wheat flour formulation. They have reported significant mortality when newly emerged adults were introduced. Residual effectiveness extended to more than two months and infestation rate was also significantly reduced. The infestation with the fungus delayed adult emergence of *R. dominica* by 8-12 days.

Laboratory bioassays were conducted to evaluate the use of *M. anisopliae* against the adults of three stored grain beetle species, *R. dominica*, *S. oryzae*, and *T. confusum* Jacquelin du-Val. Two fungal preparations, a conidial suspension and

a conidial powder were applied to wheat at three dosages 8 x 10^6, 8 x 10^8 and 8 x 10^{10} conidia/ kg of wheat alone or in combination with diatomaceous earth (DE) applied at 0.5 g/ kg of wheat. The mortality of adults after 14 days of exposure to the treated substrate was 100 and 96 per cent for suspension and powder respectively. The respective figures without DE were 94.4 and 74.6 per cent. In contrast, the application of conidial suspension was not effective against *S. oryzae* and *T. confusum* (Kavallieratos *et al.*, 2006).

Insecticidal effect of *M. anisopliae* formulations against the larvae of *T. confusum* on flour and wheat was assessed in the laboratory by Michalaki *et al.* (2006). *M. anisopliae* was applied at three dose rates of 8 x 10^6, 8 x 10^8 and 8 x 10^{10} conidia/ kg and wheat or flour was also treated with diatomaceous earth formulation at two dose rates 0.2 and 0.5 g/ kg either alone or in combination with *M. aniospliae*. They conducted bioassays at three temperatures and two relative humidity levels. For both fungus and diatomaceous earth, the increase of temperature increased their effectiveness. On the other hand, the increase of relative humidity significantly reduced larval mortality for both *M. anisopliae* and diatomaceous earth formulation.

Samodra and Ibrahim (2006) evaluated two isolates of *B. bassiana* (BbGc and BbPs) and one strain of *M. anisopliae* (MaPs) as dried conidia against rice weevil, *S. oryzae*. Based on comparative steepness of gradients and supported by EC_{50} and EC_{90} values, isolate BbGc was most infective against *S. oryzae* adults. Fungal formulations in kaolin and talc provided better kill. *B. bassiana* formulated in kaolin showed highest mean per cent mortality and lowest per cent grain weight loss.

Batta (2008) investigated the effect of *B. bassiana* and *M. anisopliae* in two types of diatomaceous earth dusts, against the adults of three species of stored grain insects *S. oryzae*, *R. dominica*, and *T. castaneum*. A synergistic interaction

between the effect of fungal species and the diatomaceous earth dusts was observed in the study.

The virulence of three indigenous Iranian isolates of *M. anisopliae* named DEM 1001, IRAN 715C and IRAN 1018C as well as their ability to suppress populations of granary weevil, *S. granarius* was evaluated by Khashaveh *et al.* (2008). LT_{50} values ranged from 5.54 to 7.9 days and the lowest LT_{50} on day ten was 1/ 4 x 10^{5} conidia/ml for DEM 1001. Cumulative mortality ten days after treatment varied from 9.4 to 88.88 per cent for IRAN 1018C at low and high concentration respectively.

Athanassiou *et al.* (2008a) studied the persistence and efficacy of *M. anisopliae* and diatomaceous earth against *S. oryzae* and *R. dominica* on wheat and maize. Wheat and maize were treated with 8 x 10^{6} and 8 x 10^{8} conidia/kg of the entomopathogenic fungus *M. anisopliae* alone and in combination with 250 ppm diatomaceous earth. Bioassays were conducted after application and monthly for five months using adult *S. oryzae* and *R. dominica*. Mortality of *S. oryzae* on both grains decreased during five month period. Maximum mortality occurred in the combination of the highest fungal rate with the diatomaceous earth. Mortality of *R. dominica* in both grains was generally greater than mortality observed for *S. oryzae*.

2.2. **Spinosad-a naturalyte insecticide**

Spinosad, a biopesticide in the naturalytes family of insecticides, is a promising alternative to other commercially available insecticides for the control of storage insects. It is based on a fermentation product of the bacterium *Saccharopolyspora spinosa* Mertz and Yao, discovered during 1980's (Mertz and Yao, 1990). It has been successfully used for the protection of more than 100 major crops worldwide against insect pests belonging to Lepidoptera, Diptera, Coleoptera, Thysanoptera and Orthoptera. It is attractive as an alternative to

synthetic pesticides because, although it has long term action in storage, it is harmless to mammals and lacks other environmental side effects (Thompson *et al.,* 2000). In addition to replacing synthetic pesticides, spinosad can be used to manage resistance to synthetic pesticides (Huang and Subramanyam, 2004).

Spinosad, a broad spectrum insecticide of low mammalian toxicity, has been registered in many countries against wide range of pests. It was evaluated by many research teams throughout the world as a grain protectant and recently registered for storage purpose in USA (Subramanyam, 2006). It is a mixture of tetracyclic neurotoxins, spinosyn A and D produced during the fermentation of the soil actinomycete *Saccharopolyspora spinosa* .It has a unique mode of action involving post synaptic nicotinic acetyl choline and GABA (Gama aminobutyric acid) receptor (Watson, 2001). This microbial insecticide acts as a stomach and contact poison and degrades rapidly in the environment.

2.2.1. Bioefficacy of spinosad against pulse beetle

Spinosad could be an environmentally safe biopesticide for the control of *C. maculatus* because it has been reported to have fewer hazards for the environment and mammals than many other currently available pesticides (Saunders and Bret, 1997; Thompson *et al.,* 2000).

Sadat and Asghar (2006) studied the effect of post exposure temperature on the toxicity of commercial formulation of spinosad (Tracer 24). It was determined against the adults of *C. maculatus.* The experiment was conducted at four different temperatures under laboratory conditions. A direct relationship between spinosad dosages and post treatment temperature was detected.

Study by Sanon *et al.* (2010) was the first to evaluate this biopesticide against *C. maculatus.* He carried out laboratory and field trials to determine the efficacy of spinosad against cowpea weevil, *C. maculatus.* In laboratory, spinosad

caused high mortality of adult *C. maculatus* and decreased number of eggs. They reported that in on-farm experiments, spinosad was effective in controlling *C. maculatus*. After six months of storage, number of insects emerging from cowpea seeds was reduced by more than 80 per cent by coating seeds with spinosad and less than 20 per cent seeds were found perforated. The on-farm trial confirmed that spinosad could be used for protecting cowpea grains from *C. maculatus* during post harvest storage.

2.2.2. Bioefficacy of spinosad against other storage pests

Spinosad has been found to be effective against many insect pests of stored products (Toews *et al.,* 2003; Huang and Subramanyam, 2004; Flinn *et al.,* 2004). At relatively low dose rates, spinosad was reported to be highly effective against *R. dominica* resistant to traditional pesticides (Arthur, 1996). Spinosad was proved extremely effective at doses as low as 0.1 ppm (Fang *et al.,* 2002a) and for *T. castaneum* 1 ppm of spinosad was required.

Vayias *et al.,* (2009) observed that apart from the species, life stages of the storage pest are also an important factor that affects spinosad efficacy. On the contrary, larvae of rice moth, *Corcyra cephalonica* (Stainton) were very susceptible on maize and sunflower seeds treated with 0.5 ppm spinosad (Huang and Subramanyam, 2004).

Fang *et al.* (2002a) evaluated the performance of spinosad on four classes of wheat against adults of the lesser grain borer, *R. dominica;* rice weevil, *S. oryzae;* saw toothed grain beetle, *O. surinamensis*; red flour beetle, *T. castaneum* and larvae of Indian meal moth, *Plodia interpunctella.* The effects of spinosad on *R. dominica* and *P. interpunctella* were consistent across all wheat classes. Spinosad at 0.1 and one mg a.i./kg of grain reported 100 per cent adult mortality, significant reduction in progeny (84-100%) and kernel damage (66-100%) against *R. domonica.* Spinosad was extremely effective against *P. interpunctella* on all

wheat classes at one mg/kg, based on larval mortality (97.6-99.6%), suppression of egg to adult emergence (93-100%) and kernel damage (95-100%). The type of commodity was also found to have an impact on spinosad efficacy.

The degradation and insecticidal effectiveness of spinosad residues in farm bins holding wheat was studied by Fang *et al.* (2002b). They reported that spinosad residues on wheat were 25 per cent less than the calculated rates. These residues were stable during one year of storage and killed all *R. dominica* adults.

Fang and Subramanyam (2003) evaluated spinosad against adults of *R. dominica* on 12.5 or 14.5 per cent moisture wheat stored at 22, 28 and 34^0 C. Adults of *R. dominica* were exposed for 14 days to untreated wheat and wheat treated with spinosad at 0.1 and one mg a.i. per kg every month for four months. Mortality of adults exposed to untreated wheat ranged from zero to 39 per cent. All *R. dominica* adults exposed to spinosad treated wheat were killed. The activity of spinosad during the four month test period was not affected by three temperatures and two moisture levels tested.

Toews *et al.* (2003) evaluated the contact toxicity of spinosad to adults of eight stored product beetles on four different surfaces. They reported that spinosad has excellent contact toxicity against adults of stored product insects and mortality of insects exposed to concrete was higher than those exposed to floor tile or galvanised steel.

Contact toxicity of spinosad to adults of *R. dominica, S. oryzae* and *T. castaneum* was evaluated in wheat by Toews and Subramanyam (2003). *R. dominica* adults were highly susceptible to spinosad followed by *T. castaneum* and *S. oryzae.*

Flinn *et al.* (2004) conducted field studies for evaluating the effects of controlled aeration and spinosad in suppressing insect populations in stored

wheat. Results suggested that spinosad was very effective in suppressing *R. dominica, Cryptolestes ferrugineus* and *T. castaneum* populations in stored wheat.

Huang and subramanyam (2004) investigated the succeptibility of *Corcyra cephalonica* to pirimiphos-mathyl, spinosad, pyrethrins synergised with piperonyl butoxide and pirimiphos-methyl combined with synergized pyrethrins. *C. cephalonica* was highly succeptible to spinosad at 0.5 and one mg/kg. At both spinosad rates, reduction in larval survival, egg to adult emergence, and seed damage relative to the control treatment was $\geq$ 93 per cent in both corn and sunflower seeds and it appears to be effective against *C. cephalonica* on both commodities at very low rates.

Nayak *et al.* (2005) carried out laboratory experiments on relevant resistant strains of flour beetle and four psocid species, with the aim of determining the potential of spinosad as a new grain protectant. They reported that among the four species tested, spinosad was most effective against *R. dominica*, less effective against *S. oryzae* and least effective against *T. castaneum* and *O. surinamensis*. They also reported that among four psocid species tested, spinosad was most effective against *Liposcelis entomophila* followed by *L. paeta, L. bostrychophila* and *L. decolour*. They investigated the effect of spinosad on progeny reduction of beetles and psocids. Among beetle species tested, spinosad was most effective in reducing the progeny reduction of *R. dominica* at one mg a.i./kg. Spinosad had a significant effect on all the four species of psocids on progeny production.

A laboratory study was undertaken by Daglish and Nayak (2006) to determine the persistence and efficacy of spinosad against *R. dominica* in stored wheat for nine months. Wheat was treated with spinosad at 0.1, 0.5 and one mg/kg grain and sampled after 0, 1.5, 3.0, 4.5, 6, 7.5 and 9 months of storage for bioassays and residue analysis. They reported that spinosad applied at 0.5 or one

mg/ kg was completely effective for nine months, with 100 per cent adult mortality after 14 days of exposure and no F_1 adults produced.

The persistence and insecticidal activity of spinosad was evaluated by Subramanyam *et al.* (2007) on Kansas farm. Laboratory bioassays with monthly grain samples collected from the field showed that spinosad alone or in combination with chlorpyriphos methyl, was effective in killing adults of *R. dominica* irrespective of the sampling month. Single application of spinosad at one mg a.i./kg was effective for managing common stored grain insects, including *R. dominica* for at least six months.

Evaluation of insecticidal efficacy of spinosad against the adults of *R. dominica, S. oryzae,* and *T. confusum* on wheat and *Prostephanus truncatus* on maize indicated that mortality of *R. dominica* and *S. oryzae* was high at 0.01 ppm reaching 100 per cent at 55 per cent relative humidity and 30^0 C after 21 days of exposure. Of the species tested, *R. dominica* and *P. truncatus* were very succeptible to spinosad (Athanassiou *et al.,* 2008b).

Laboratory bioassays were carried out to evaluate the insecticidal effect of spinosad dust against two major stored grain beetle species, *R. dominica* and *S. oryzae* in wheat, barley and maize. Four dose rates- 20, 100, 500, and 1000 ppm of the formulation were tried, corresponding to 0.025, 0.125, 0.625 and 1.25 ppm of a.i. respectively. *R. dominica* adult mortality was 100 per cent at doses $\geq$ 0.125 ppm of a.i. after 14 days of exposure and that for *S. oryzae* was > 95 per cent only at 1.25 ppm of a.i. Accelerated degradation of spinosad was observed in maize and negligible degradation in wheat (Chintzoglou *et al.,* 2008a).

The efficacy of spinosad applied alone or combined with the diatomaceous earth against adult rice weevil, *S. oryzae* and confused flour beetles, *T. confusum* was assessed on wheat and maize at three dosages of spinosad dust formulation corresponding to 0.0625, 0.1875 and 0.625 ppm of a.i. for *S. oryzae*

and 0.1875, 0.625, and 1.25 ppm of a.i. for *T. confusum.* Mortality of *S. oryzae* exposed for 14 days on wheat treated with spinosad ranged between 83 and 100 per cent. For *T. confusum,* mortality on both commodities was lower than for *S. oryzae* and there is a joint action between spinosad and diatomaceous earth for *T. castaneum* exposed on treated maize (Chintzoglou *et al.,* 2008b).

Daglish *et al.* (2008) investigated the potential of spinosad as a grain protectant for lesser grain borer *R. dominica* in a silo-scale trial on wheat. Three hundred tonnes of wheat was treated with spinosad 0.96 mg/ kg and samples were collected at intervals during 7.5 months storage to determine efficacy and residues. Bioassays of all treated wheat samples over 7.5 months resulted in 100 per cent mortality after two weeks of exposure and no live progeny were produced.

Evaluation of spinosad in laboratory bioassays as surface treatment for wheat to control adult *R. dominica, S. oryzae* and three psocid species *Liposcelis paeta, L. bostrychophila,* and *Lepinotus reticulatus* showed that spinosad had some effectiveness as a layer treatment on a column of wheat. Efficacy depended on target species, the depth of treated layer, and the upward and downward mobility of the insect species (Athanassiou *et al.,* 2009).

Hussain *et al.* (2009) tested the effects of LC_{10} and LC_{20} of a commercial formulation of spinosad Tracer 240 SC in the laboratory on ten day old adults of malathion resistant (PAK) and succeptible (FSS-II) strains of red flour beetle, *T. castaneum.* Results showed that spinosad caused inhibition of carboxyl esterase at LC_{20} in PAK strain, while caused elevation of carboxyl esterase activity in FSS II strain with depletion of total protein contents. Glucoamylase activity was inhibited in the adults of both the strains.

In laboratory bioassays to evaluate the insecticidal effect of four spinosad doses, 0.01, 0.1, 0.5 and one ppm against adults of *R. dominica* and *S. oryzae* as

well as adults and larvae of *T. confusum* on four grain commodities, wheat, corn, rice and barley, mortality was observed to be extreamly high for *R. dominica* on all commodities even at lowest concentration. For the other species, mortality was generally increased with the increase in dose rate. Progeny production was highly suppressed in *R. dominica* irrespective of dose rate or commodity, but high progeny production was recorded in *S. oryzae* in all commodities (Vayias *et al.*, 2009).

Results of the study on the effect of short exposures to spinosad treated wheat against the adults of four stored product insect species, *S. oryzae, T. castaneum,* and the psocid *Lepinotus reticulates* indicated that *R. dominica* was very susceptible after short exposures to spinosad treated substrate (Athanassiou *et al.*, 2010).

Kavallieratos *et al.* (2010) carried out laboratory tests to evaluate the efficacy of diatomaceous earth formulations and spinosad against *R. dominica, S. oryzae* and *T. confusum* in three wheat varieties. Spinosad was tried at 0.125, 0.625 and 1.25 ppm a.i./kg. Spinosad was reported to be highly effective against *R. dominica* and *S. oryzae* even at lowest dose.

The insecticidal and residual effect of spinosad in the laboratory against the adults of *S. oryzae. R. dominica, T. castaneum, Cryptolestes ferrugineus* and the larvae of *T. confusum* on wheat, maize and barley were evaluated at three concentrations 0.1, 0.5, and 1 ppm by Vayias *et al.* (2010). It was reported that with exception of *T. confusum* 1 ppm of spinosad was very effective against the reminder of the pest species and provided protection for a period of storage at least four months. Spinosad provided suitable protection for six months against *S. oryzae* and *R. dominica* but it was not suitable for long term protection against *T. confusum* and *C. ferrugineus.*

2.3. Lemongrass oil- a fumigant toxin

Cymbopogon is an important genus of aromatic grasses with about 120 species and of which 27 species occur in India. East Indian lemongrass or *Cymbopogon flexuosus* (L.) is grown commonly in Kerala and adjacent states. The essential oil of *C. flexuosus* has fumigant and insecticidal actions. It is commonly called 'Cochin oil' and the major component (about 80 per cent) of the oil is Citral (Kumar *et al.,* 1993). The toxicity of a number of essential oils has been evaluated against a number of stored product insects. Effectiveness of essential oils extracted from various spices and herbs have shown great promise for the control of storage insects and were found to be active fumigants at low concentration against these insects (Shaaya *et al.,* 1991)

2.3.1. Bioefficacy of lemongrass oil against pulse beetle

Raja *et al.* (2001) studied the effect of plant volatile oils derived from *Cymbopogon nardus, Mentha* spp. in protecting stored cowpea against *C. maculatus* infestation for four months. The oil significantly influenced egg laying, adult mortality, adult emergence and subsequent seed damage.

The toxic and repellent effect of 13 volatile oils, two non edible oils and eight slurries were evaluated against cowpea beetle *C. maculatus*. The volatile oils of *Cymbopogon nardus* and *Cymbopogon shoenanthus* caused majority of the eggs not to develop into adult beetles. Repellent effects were also reported for *C. citrates, C. nardus* and a mixture of *C. nardus, C. flexuosus, Hyptis spicigera,* and *Tagetes minuta.* Non volatile oils were not repellent and had no effect on the number of eggs laid, but the development of eggs was hampered (Boeke *et al.* 2004).

Dubey *et al.* (2004) tested the insecticidal activity of essential oils of *Ocimum* sp., *Lippa alba* and citral (major constituent from essential oil of

C. citratus) against bruchids, *C. chinensis* as well as their ability to influence oviposition on treated stored cowpea. The essential oils affected significantly on mortality of bruchids, oviposition and number of eggs in treated seeds with all oils.

Ketoh *et al.* (2005a) studied the control of the development of *C. maculatus* using a method that combined exposure to essential oil extracted from *Cymbopogon shoenanthus* and the introduction of a pteromalid natural enemy of the bruchid, *Dinarmus basalis.* At the highest concentration tested (33.33 micro l/l), all adults of *C. maculatus* were killed within 24 hours and egg and larvae development also inhibited.

The effect of essential oils of *C. nardus, C. schoenanthus* and *Ocimum basilicum* by using them in fumigation was studied to investigate their absorption by cowpea seeds during treatment and their residual effects on adults of *C. maculatus*, survival, female production and seed germination. During treatment, seeds absorbed essential oil compounds differently. After 72 hour exposure, adult *C. maculatus* mortality was less than 50 per cent in presence of treated and degassed seeds. Treated seeds did not affect reproduction in female *C. maculatus* nor seed germination (Ketoh *et al.,* 2005b).

Ketoh *et al.* (2006) assessed the insecticidal activity of crude essential oil extracted from *C. shoenenthus* and of its main constituent, piperitone, was assessed on different developmental stages of *C. maculatus.* Piperitone was more toxic to adults (LC_{50} value of 1.6 micro litre) than crude extract. Piperitone inhibited the development of newly laid eggs and of neonate larvae, but was less toxic than the crude extract to individuals developing inside the seeds.

Srivastava and Dubey (2007) tested the potency of the essential oil of *Cymbopogon martini* as a botanical pesticide to protect stored wheat and bengal gram (*Cicer arietinum*) from insect infestation. *C. martinii* was a potent fumigant

in stored gram. The oil was an effective repellent against the beetles *Callosobruchus chinensis* and *T. castanium*. *C. martini* oil significantly affected oviposition, adult development and mortality of *C. chinensis* in cowpeas. *C. martini* oil when used as a fumigant did not affect viability, germination and seedling growth of gram.

The impact of essential oils of plants *viz.*, lemongrass (*C. flexuosus*), Citronella (*C. winterianus*), Citrodora (*Eucalyptus citrodora*) and Palmarosa (*C. martinii*) for their insecticidal and ovicidal activities against adults and eggs of *C. maculatus* at five per cent concentration at 96 hours of exposure was assessed by Raja and William (2008). The highest mortality and ovicidal action were recorded in citrodora oil (96 and 88.43% respectively) followed by lemongrass oil (92 and 45.25%) at 96 hours of exposure.

Aziz and Abbass (2010) tested bioefficacy of five plant essential oils including lemongrass (*C. citratus*) as seed protectants at 1.0, 0.5, and 0.25 per cent for *C. maculatus*. All the tested oils elongated the larval and pupal period. Lemongrass oil caused high mortality to larvae and pupae about 92.32 per cent.

2.3.2. Bioefficacy of lemongrass oil against other storage pests

A study was conducted by Parugrug and Roxas (2008) to evaluate the insecticidal action of five locally available plants namely lemongrass (*C. citratus*), neem (*Azadirachta indica*), lantana (*Lantana camara*), basil (*Ocimum basilicum*), and African marigold (*Tagetes erecta*) against maize weevil, *S. zeamais*. Results revealed that all the test materials exhibited repellency action against maize weevil. Powdered leaves of lemongrass was observed to be moderately repellent and showed low mortality. None of them exhibited antiovipositional and growth inhibitory action.

Ojianwuna and Umoru (2010) studied the effects of *C. citratus* and *Ocimum suave* (W.) (wild basil) on *C. maculatus*. Plant mixtures containing *C. citratus* (lemongrass) and *Ocimum suave* powders in the ratios 100:0, 80:20, 60:40, 50:50, 40: 60, 20:80, 0:100 and 0:0 were used to evaluate their effects on oviposition and adult emergence of *C. maculatus*. The mixture of 60: 40 had least mean number of egg counts and least number of F_1 emergence which was significantly reduced.

2.3.3. Bioefficacy of other essential oils against *C. maculatus*

Essential oils from *Tagetes minuta, Hyptis suaveolens, Ocimum* spp., *Piper guineaneense* showed 99 per cent mortality and complete suppression of oviposition against *C. maculatus* (Keita *et al.*, 2000).

Keita *et al.* (2001) studied the effect of essential oils of *Ocimum* spp. as an insecticidal fumigant against *C. maculatus* and reported significant effect on adult mortality, egg hatching and adult emergence for three months.

The fumigant toxicity of essential oil from *Artemisia sieberi* against *C. maculatus* was reported to cause 100 per cent kill (Negahban *et al.*, 2007).

Fumigant action of essential oils extracted from *Eucalyptus leucoxylon* against major stored product pests, *C. maculatus, S. oryzae* and *T. castaneum* was studied by Kambouzia *et al.* (2009).

Insecticidal activity of essential oils from three aromatic plants by fumigation against *C. maculatus* was determined and observed significant reduction in egg laying (Aboua *et al.*, 2010).

2.4. Efficacy of edible oils against pulse beetle

Edible oils can be recommended for the control of *C. maculatus* and other bruchids. A rate of 1.5 to 3 g oil/ kg seeds protected in a warehouse for a period of upto five months. Edible oils could be very useful on the farm level in developing countries and can play an important role in stored grain protection and reduce the need and risk associated with the use of bioinsecticides (Shaaya and Sukprakarn, 1994).

Varma and Pandey (1978) and Pandey *et al.* (1981) showed that groundnut and other oils at 0.3 per cent w/w gave full protection of green gram against *C. maculatus.* Biological activity of the edible oils is attributable to both their physical and chemical properties and also to fatty acid chain length. Among the straight chain fatty acids ranging from C5 to C18, it was found that C9-C11 acids were most effective in protecting oviposition of *C. maculatus.*

The efficacy of vegetable oil in causing inhibition of oviposition and multiplication of bruchids has already been well documented (Singal and Singh, 1990; Ramjan, 1994; Singh, 2003)

Khaire *et al.* (1992) studied the efficacy of vegetable oils *viz.*, sunflower, castor, mustard, safflower, palm, groundnut, sesame, neem, karanj and maize as a grain protectant of pigeon pea against pulse beetle. They reported that oils showed good effects on progeny emergence, loss in grain weight and germination.

Bhatnagar *et al.* (2001) tested six vegetable oils namely groundnut, sesame, soyabean, coconut, mustard and neem for their efficacy as repellent, ovipositional deterrent and ovicidal effect against pulse beetle, *C. maculatus* in cowpea at one, seven, fifteen and thirty days after seed treatment (10 ml/kg seed). Neem oil was found most effective and showed significantly higher repellent; ovipositional deterrent and ovicidal effects against the beetle followed by coconut,

soyabean and mustard oil. All oils except neem oil lost their efficacy at 30 days after treatment.

The efficacy of various plant products *viz.,* some leaf powders, oils of coconut, mustard, groundnut and neem products was assessed against pulse beetle *C. chinensis* on the basis of per cent grain damaged and per cent loss in weight. The mustard and groundnut oils were on par, registered less infestation having 8.86 and 11.35 per cent damage, respectively. The coconut oil provided less infestation *i.e.* 12.40 per cent grain damage. Even though the oils of mustard, groundnut and coconut were not significantly different between them, they were superior to rest of the treatments and control (Umrao and Verma, 2002).

Singh (2003) studied the effect of edible oil *viz.,* coconut, mustard, sunflower and sesame *etc.* at 8 g/ kg of seed proved significantly effective in protecting the seed upto nine months of storage in terms of seed damage and weight loss. The oils prevented egg laying and checked population build up of beetle.

Khalequzzaman *et al.* (2007) evaluated the efficacy of seven edible oils *viz.,* sunflower, mustard, groundnut, sesame, soybean, olive and oil palm against *C. chinensis.* Adult emergence was completely prevented and minimum grain loss was achieved by groundnut oil at one per cent upto 66 days after treatment.

Materials and Methods

3. MATERIALS AND METHODS

The present investigation entitled 'Potency of bioinsecticides against cowpea bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) in storage' was undertaken in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur during 2010-11. The potency of four bioinsecticides *viz.*, two entomopathogenic fungi- white muscardine fungus, *Beauveria bassiana* and green muscardine fungus, *Metarhizium anisopliae*, spinosad-a broad spectrum insecticide from the naturalytes family with low mammalian toxicity- and lemongrass oil- an essential oil from the aromatic plant *Cymbopogon flexuosus* against *C. maculatus* in storage was assessed by conducting the following studies in the laboratory.

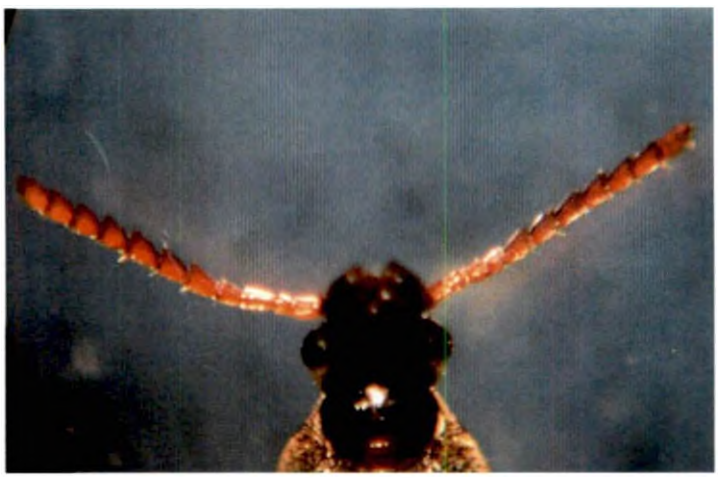
1. Dosage-mortality response
2. Time-mortality effect
3. Biological efficiency in storage
4. Persistent toxicity effect

3.1. DOSAGE- MORTALITY RESPONSE OF BIOAGENTS ON *C. maculatus*

The dosage-mortality response of *B. bassiana, M. anisopliae*, spinosad and lemon grass oil on *C. maculatus* was tested in separate experiments.

3.1.1. Source and identification characters of *C. maculatus*

The initial culture of *C. maculatus* was procured from Krishi Vijnan Kendra, Thrissur. Adult of *C. maculatus* was identified by using the taxonomic key given in Appendix 1 (Haines, 1989). Important taxonomic characters of *C. maculatus* are depicted in Plate 1.



a. Antenna

b. Hind femur (dorsal view)

c. Hind femur (ventral view)

Plate 1 . Taxonomic characters of *C. maculatus*

Sexes were identified according to Beck and Blumer (2010). Male and female beetles are easily distinguished from one another by general appearance. Females are black in colour and males are brown (Plate 2).The most distinguishing characteristic is the colouration of the plate covering the end of the abdomen (pygidium). In female, the pygidium is enlarged and darkly coloured on both sides (Plate 2a) and it is smaller and lacks stripes in males (Plate 2b). In some strains, females are larger in size than males.

3.1.2. Mass rearing and maintenance of culture of C. *maculatus* in the laboratory

Adult beetles of *C. maculatus* were reared on healthy cowpea grains purchased from the local market in Thrissur. Fifty pairs of *C. maculatus* adults were introduced into plastic rearing jars of one litre capacity (9.5cm diameter and 12.5 cm height) containing 200 g cowpea grains. The mouths of rearing jars were covered with pieces of muslin cloth and fastened by rubber bands to prevent contamination and escape of beetles (Plate. 3). The beetles were allowed to oviposit on the grains for a period of seven days and the adults were removed thereafter from the grains. Progeny emergence was checked periodically and the newly emerged adult beetles were used for conducting different experiments. The insect culture was thus maintained continuously in the laboratory and it served as the source for a steady supply of test insects. The culture was maintained in the laboratory under ambient conditions of temperature (23- 31) ^{o} C and relative humidity (62- 84) per cent.

a. *C. maculatus* (Female)

b. *C. maculatus (*Male)

Plate 2. *Callosobruchus maculatus* adults

Plate 3. Rearing set up of *C. maculatus*

3.1.3. Source and identification of the entomopathogens *B. bassiana* and *M. anisopliae* used for experiments

Pure cultures of *B. bassiana* and *M. anisopliae* were obtained from the All India Coordinated Research Project (AICRP) on Biological Control of Crop Pests (BCCP), College of Horticulture, Vellanikkara.

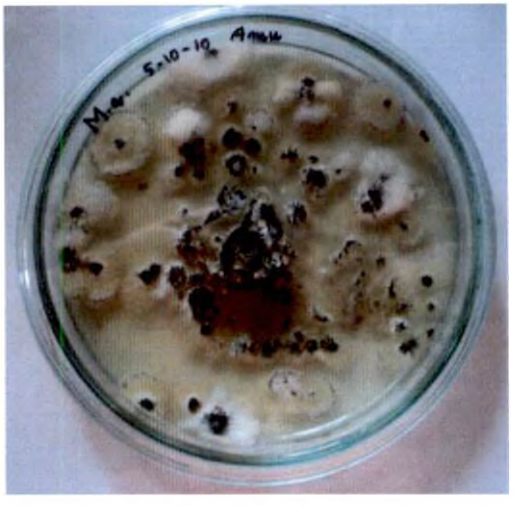
The identity of these pathogens was confirmed under a phase contrast microscope by identifying the following conidial characteristics (Plate 4)

Fungal species	Conidial characteristics	Colour of mycelial mat
B. bassiana	Conidiophores single or branched bearing groups of clustered conidiogenous cells. Conidia smooth and round (Plate 4.c) and white in colour.	Powdery white (Plate 4.a)
M. anisopliae	Conidiophores short, hyaline, simple or branched. Conidia one celled, smooth, long, ovoid to cylindrical shaped (Plate 4.d) with olive green colour.	Olive green (Plate 4.b)

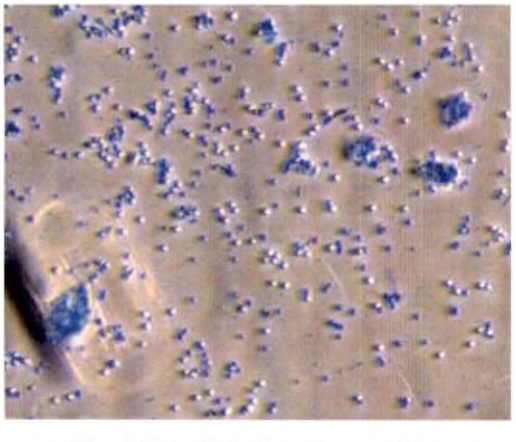
3.1.4. Maintenance and preparation of stock cultures of *B. bassiana* and *M. anisopliae*

The entomopathogenic fungi were cultured on Potato Dextrose Agar (PDA) medium in Petri dishes of diameter 8.5 cm and incubated at room temperature for 10 days for sporulation. The composition of PDA is given below:

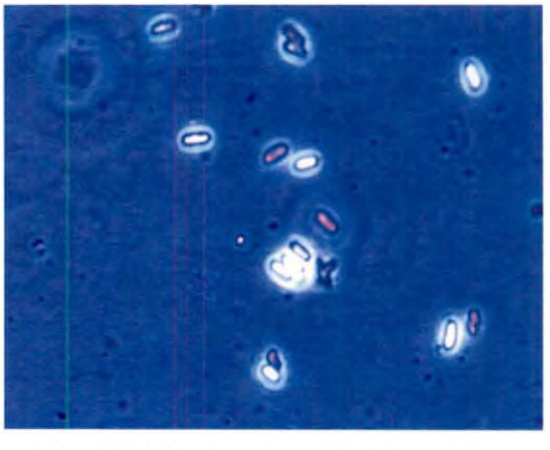
a. *B. bassiana*



b. *M. anisopliae*



c. Conidia of *B. bassiana* (40x)



d. Conidia of *M. anisopliae* (40x)

Plate 4. Characteristics of *B. bassiana* and *M. anisopliae*

Potato : 200 g
Dextrose : 20 g
Agar : 15 g
Distilled water: 1 litre

The fungal isolates were sub cultured periodically and their virulence was maintained by subjecting them to periodic pathogenicity tests.

B. bassiana and *M. anisopliae* were then cultured in two different specific liquid media for getting maximum spore count. *B. bassiana* was cultured in Sabouraud's Maltose Agar enriched with Yeast extract (SMA+Y) liquid medium whose composition is given below:

Maltose : 40 g
Peptone : 10 g
Yeast extract : 2 g
Distilled water: 1 litre

The medium was prepared as per the above composition and poured into glucose bottles of 1 litre capacity at the rate of 300 ml medium per bottle. Then the bottles were plugged with cotton and autoclaved at 15 psi pressure and 121°C for 20 minutes and then allowed to cool. Mycelial disc of 1 cm^2 taken from actively growing colony on PDA medium was transferred to the bottles containing SMA+Y medium and incubated at room temperature for 25 days (Plate 5.a). The bottles were kept in a slanting position for an increased surface area which ensured maximum growth.

Plate 5.a. *B. bassiana*

Plate 5.b. *M. anisopliae*

Plate 5. Culturing of *B. bassiana* and *M. anisopliae*

M. anisopliae was cultured on specific medium, coconut water enriched with magnesium sulphate, in the same method as that of *B. bassiana* (Plate 5.b). The composition of specific medium used for culturing *M. anisopliae* is given below:

Coconut water : 1 litre
Magnesium sulphate : 1.5 g

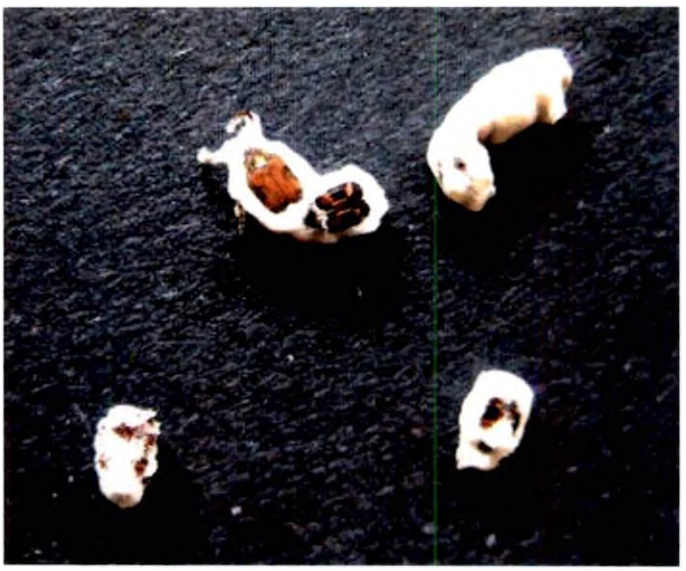
3.1.5. Pathogenicity of entomopathogens to *C. maculatus*

The fungal isolates were subjected to *in vitro* pathogenicity tests. The pathogenicity of *B. bassiana* and *M. anisopliae* was established by dipping one day old adult *C. maculatus* insects in their spore suspensions. Mortality of beetles was observed from second day of inoculation with respective fungal cultures for a period of five days.

3.1.6. Confirmation of mycosis and reisolation of *B. bassiana* and *M. anisopliae*

Dead insects from the pathogenicity tests were taken out, washed with distilled water and placed in a Petri dish lined with a wet filter paper (humid chamber) and observed for the growth of the pathogens. Dead insects on which mycelial growth with characteristic colour developed within two or three days were counted as infected by the pathogens. *B. bassiana* developed white cottony mycelial growth (Plate 6.a) from the cadavars and *M. anisopliae* developed olive green coloured mycelium (Plate 6.b) from the cadavars of *C. maculatus*.

B. bassiana and *M. anisopliae* were then reisolated from the infected insect cadavers on PDA medium to observe the growth of the pathogen. After five days of incubation, fungal mycelia were observed, compared its characters with the original



a. *B. bassiana*

b. *M. anisopliae*

Plate 6. Fungal infected cadavars of *C. maculatus*

cultures and confirmed their identity as respective fungal pathogens by examining under a phase contrast microscope.

3.1.7. Preparation of spore suspensions

The sporulated mycelial mats of *B. bassiana* and *M. anisopliae* along with the culture media were ground separately in an ordinary mixer by adding a drop of Tween 80 solution to disperse the spores and it was made into a liquid spore suspension. It was then filtered through a double layered muslin cloth in order to remove coarse particles. The spore count in the suspension was determined by using a haemocytometer and the spore count was estimated according to the formula given by Lomer and Lomer (1996).

$$\text{Number of spores per ml} = \frac{X \times 400 \times 10 \times 1000 \times D}{Y}$$

Where X - Number of spores counted from 160 small squares

Y - Number of small squares counted

10 - Depth factor

1000 - Conversion factor from mm^3 to cm^3

D - Dilution factor

The spore count in the above prepared suspensions of *B. bassiana* and *M. anisopliae* were adjusted to 1×10^8 spores/ml and it was kept as the stock suspension. Dosage-mortality response of *B. bassiana* and *M. anisopliae* on *C. maculatus* was tested in two different experiments. After preliminary pathogenicity assays, five different concentrations *viz.*, 1×10^8, 1×10^7, 1×10^6, 1×10^5, and 1×10^4 spores/ml were prepared in sterile distilled water containing Tween 80 (0.05% v/v), based on logarithmic series by serial dilution technique from the stock spore suspension of *B. bassiana* and *M. anisopliae.*

3.1.8. Dosage-mortality response of *B. bassiana* and *M. anisopliae* on *C. maculatus*

The prepared concentrations (1 x 10^8, 1 x 10^7, 1 x 10^6, 1 x 10^5, and 1 x 10^4 spores/ml) of *B. bassiana* and *M. anisopliae* were tested in dosage-mortality response experiments by adopting two bioassay techniques-direct dipping and residue film.

3.1.8.a Dosage-mortality response of *B. bassiana* and *M. anisopliae* by direct dipping bioassay

Dosage-mortality response of *B. bassiana* and *M. anisopliae* was studied using the bioassay method of direct dipping of *C. maculatus* adults as described by Cherry *et al.* (2005). Five pairs of one day old adults were collected in glass vials (6 cm x 1.5 cm) from the stock culture and the beetles were made immobilized by keeping in a freezer for three minutes. The immobilized adults were treated by dipping them in the prepared aqueous spore suspensions of five concentrations from 1 x 10^4 to 1 x 10^8 spores per ml for five seconds. The spore concentrations were fixed after preliminary tests. The insects were then transferred to Petri dishes (9.5 cm) lined with a sterile filter paper (9 cm) to absorb excess moisture and covered with upper lid to prevent the escape of beetles. An untreated control treatment by treating the adults with distilled water containing Tween 80 (0.05% v/v) alone was also maintained. Each treatment was replicated thrice. The experiment was maintained for five days to observe the mortality effect. The experiment was conducted separately for *B. bassiana* and *M. anisopliae* in Complete Randomized Design.

Mortality of the beetles was checked daily and counts of dead beetles in treatments and control were recorded at 24 hours interval upto five days after treatment.

3.1.8.b Dosage- mortality response of *B.bassiana* and *M. anisopliae* by residue film bioassay

The mortality response of the same concentrations of *B. bassiana* and *M. anisopliae* on *C. maculatus* as tested above was also determined by another bioassay method- residue film technique. In this method, respective spore concentrations of the fungi were applied on the inner surfaces of both the upper and lower lids of Petri dishes (9.5 cm diameter) at the rate of 1 ml per lid by using a micro pipette. The Petri dishes were gently swirled so that the upper and lower surfaces were fully coated with a thin film of spore suspension and were allowed to dry under a fan. After drying, five pairs of one day old adult beetles were released into each Petri dish. A small piece of cotton soaked with water was placed inside the Petri dish in order to provide sufficient relative humidity for growth and germination of the entomopathogenic fungi. The experiment was conducted in the same design with same number of treatments and replications as described in the preceding section 3.1.7.a. Observations on the mortality of beetles were taken in the same manner as mentioned in 3.1.7.a.

3.1.9. Confirmation of mycosis and reisolation of *B. bassiana* and *M. anisopliae*

After taking the observations on the mortality of *C. maculatus* in different treatments daily upto five days of treatments, the dead insects were taken out, washed with distilled water and placed in a Petri dish lined with a sterilized wet filter paper which served as a humidity chamber for detecting fungal infection. Dead insects,

those developed mycelial growth of characteristic colour of the fungus within two or three days were counted as infected by the pathogens. The fungi were then identified and confirmed by the examination of hyphae and spores under the microscope.

3.1.10. Dosage- mortality response of spinosad on *C. maculatus*

The commercial formulation of spinosad obtained as Spinosad 45 SC from Dow Agro Sciences, Mumbai was used for the study. The toxicity of spinosad to *C. maculatus* was assessed by adopting two methods of bioassay *viz.*, direct immersion of adult beetles and residue deposit on cowpea grains.

3.1.10. a Bioassay by direct immersion of insects

Stock solution and serial dilutions of spinosad were prepared with distilled water. After conducting preliminary tests with different spinosad concentrations, a series of concentrations giving mortality rates between 10 per cent and 95 per cent were selected for bioassay test. The treatments consisted of six doses *viz.*, 1, 3, 5, 7, 9 and 10 ppm of spinosad 45 SC and each treatment was replicated thrice along with an untreated control of distilled water only. For each replication, five pairs of one day old adults were collected from the stock culture in glass vials of 6 cm x 1.5 cm and transferred to five ml of respective concentrations of spinosad. The beetles were allowed to remain in the solution for five seconds and then transferred to Petri dishes (9.5 cm diameter) lined with a filter paper. After covering with the upper lid, the treated insects were incubated at ambient conditions for 24 hours.

Observations on the mortality of adult beetles were recorded 24 h after treatment. While taking observations, beetles which were unable to move after gentle prodding with a thin camel hair brush were recorded as dead.

3.1.10. b Bioassay by residue deposit on cowpea grains

From the prepared stock solution of spinosad, different concentrations were prepared with distilled water by serial dilutions. The concentrations were fixed after preliminary tests. The treatments consisted of six concentrations 10, 20, 30, 40, 50, 60, and 70 ppm (0.225, 0.45, 0.675, 0.9, 1.125, 1.575, 1.8, mg a.i. per kg grain) of spinosad 45 SC. Twenty gram cowpea seeds were taken in Petri dishes (9.5 cm diameter) and the different concentrations of spinosad were applied at the rate of 1 ml per 20 g seeds using a micropipette. The seeds were mixed thoroughly with respective spinosad solutions for proper coverage and allowed to air dry under a fan. After getting air dried, five pairs of one day old *C. maculatus* were released into each Petri dish, covered with upper lid properly for preventing the escape of adult beetles and incubated for 24 hours under ambient conditions. An untreated batch of seeds was also kept as control. Three replications were maintained for each treatment. In the untreated control, the seeds were treated with distilled water only.

The mortality of beetles was observed and recorded from all the treatments after 24 hours of treatment.

3.1.11. Dosage-mortality response of lemongrass (*Cymbopogon flexuous*) oil on *C. maculatus*

Lemon grass oil was obtained from the Aromatic and Medicinal Plants Research Station, Odakkali, Kerala Agricultural University.

Fumigant toxicity of lemongrass oil on *C. maculatus* was studied by dosage-mortality bioassay. The experiment was conducted in plastic jars of one litre capacity (13 cm height and 10 cm diameter) fabricated into airtight fumigation chambers

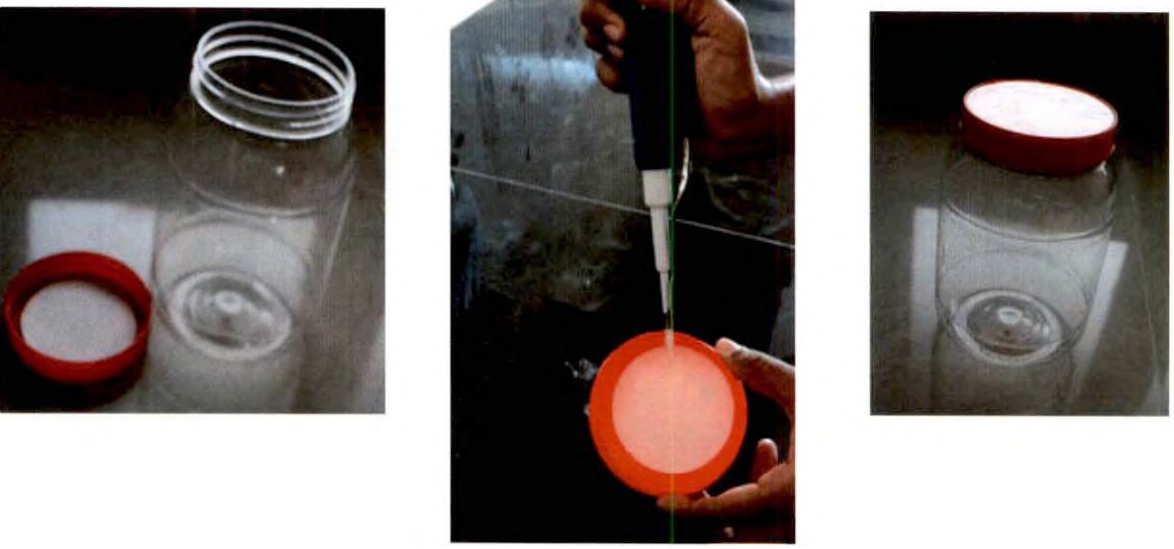


Plate 7. Fabricated fumigation chamber

(Plate 7). Filter paper (Whatman No. 1) discs of four cm diameter were prepared and attached to the lower surface of the jar lid using an adhesive. Twenty gram cowpea seeds were taken in each fumigation chamber and five pairs of newly emerged adults of *C. maculatus* were introduced into the fabricated air tight plastic jars. Lemongrass oil concentrations of 1, 2, 3, 4, 5, 6, 7 and 8 micro litre were prepared after preliminary tests. Filter paper discs attached to lids were impregnated with respective concentration (micro litre per litre of air) of lemongrass oil using a micro pipette and the jars containing insects and grains were then closed. An untreated control without oil was also maintained. Three replications were kept for each treatment. The adult beetles were kept exposed to the fumigant effect of the oil for 24 hours. The dead adults were counted in all the treatments and recorded after 24 hours of exposure.

3.1.12. Statistical analysis of data

Data on mortality counts recorded were converted to per cent mortality and were transformed by square root transformation ($\sqrt{x+0.5}$) to stabilize the variance and the values were subjected to analysis of variance (ANOVA) using the statistical package SPSS (Statistical Package for Social Sciences) in order to determine the difference between treatments. The mean values were compared using DMRT (Duncan's Multiple Range Test) (Duncan, 1951).

Cumulative mortality percentage data obtained from experiments were corrected for control mortality using Abbott's formula (Abbott, 1925). Median lethal concentrations (LC_{50} and LC_{90}) and probit regression parameters were estimated by probit analysis (Finney, 1971)

3.2. TIME-MORTALITY RESPONSE OF BIOINSECTICIDES ON *C. maculatus*

Time-mortality response of *B. bassiana, M. anisopliae,* spinosad and lemongrass oil on *C. maculatus* was studied by the same methods as described in 3.1. Time taken to cause 50 and 90 per cent mortality of *C. maculatus* by *M. anisopliae* and *B. bassiana* at all concentrations was determined. Spinosad and lemongrass oil at their respective LC_{50} values were tested for assessing the LT_{50} and LT_{90} values. Probit analysis was used to estimate median lethal time, LT_{50} and LT_{90} values.

3.3. BIO-EFFICACY OF BIOINSECTICIDES AGAINST *C. maculatus*

The biological efficacy of *B. bassiana, M. anisopliae,* spinosad and lemongrass oil in protecting cowpea grains against *C. maculatus* was assessed in the laboratory for a period of 30 days. The potential of *B. bassiana* and *M. anisopliae* (talc based KAU formulation containing 1×10^{11} spores/g) at 2 per cent, spinosad at LC_{90}, lemongrass oil at LC_{90} and coconut oil at 1 per cent along with an untreated control against *C. maculatus* was evaluated in CRD with three replications. Coconut oil was included as a check in the experiment as it is recommended against *C. maculatus* in the Package of Practices Recommendations of Kerala Agricultural University. Experiments with *B. bassiana* and *M. anisopliae* formulations (2%), spinosad (LC_{90}) and coconut oil (1%) were carried out in 12 Petri dishes (9.5 cm diameter), each containing 20g healthy uninfested cowpea seeds. The seeds were mixed with the required quantities of the above mentioned treatments until compete coverage on the surface of seeds was obtained. An untreated batch of seeds was also maintained as control in the experiment.

Lemongrass oil was tested for its fumigant effect in plastic jars (one litre capacity) fabricated into air tight fumigation chambers containing 20 g cowpea seeds.

Filter paper discs (4 cm diameter) impregnated with respective concentration of lemongrass oil was fixed on the lower surface of the jar lids using an adhesive as described in 3.1.11.

Five pairs of one day old adults of *C. maculatus* were released in each replication of all treatments. The adult beetles were allowed on the grains for seven days and then removed. The experiment was maintained at room temperature for a period of 30 days and the potency of bioinsecticides was assessed by studying the following biological parameters.

i) Direct adult toxicity/ Insecticidal effect of bioinsecticides
ii) Surface protectant actions by oviposition deterrency, inhibition of hatchability and progeny emergence
iii) Intensity of infestation
iv) Loss in weight
v) Seed viability

3.3.1. Adult toxicity/ Insecticidal effect of bioinsecticides

Mortality counts of all the introduced beetles in all the treatments in the experiment 3.3 were recorded and then removed at 24 hours interval upto seven days. The lethal effect of bioinsecticides was determined by calculating the percentage mortality of adult beetles and mortality data in all treatments were corrected for control mortality by Abbott's formula (Abbott, 1925).

$$\text{Corrected per cent mortality} = \frac{Tm - Cm}{100 - Cm} \times 100$$

Tm - Per cent mortality in treatment

Cm - Per cent mortality in control

3.3.2. Oviposition deterrency and hatching inhibition

Seven days after treatment, all the introduced beetles in all treatments were removed and the grains were observed for oviposition by *C. maculates*. Counts on total number of eggs laid and number of eggs hatched were taken under a microscope. Hatched eggs were distinguished from unhatched eggs (Plate 8). The former were found to be filled with shavings from the seed and turn milky white as the larvae burrow into the seed (Sanon and Ouderaogo, 1998). The oviposition deterrency and hatching inhibition effects of treatments were calculated by the following formulae. Per cent egg hatchability was also worked out.

$$\text{Oviposition deterrency (\%)} = \frac{Ce - Te}{Ce} \times 100$$

Ce - Number of eggs laid in control

Te - Number of eggs laid in treatment

$$\text{Hatching inhibition (\%)} = \frac{Ch - Th}{Ch} \times 100$$

Ch - Number of eggs hatched in control

Th - Number of eggs hatched in treatments

$$\text{Egg hatchability} = \frac{\text{Number of eggs hatched}}{\text{Number of eggs laid}} \times 100$$

a. Unhatched eggs

b. Hatched eggs

Plate 8. Unhatched and hatched eggs of *C. maculatus*

.3.3. Progeny emergence inhibition

Grains in all the treatments were regularly observed for the F_1 progeny mergence for a period of 30 days of storage. Emerged adults were counted daily and ecorded. Progeny emergence inhibition was calculated.

$$\text{Progeny emergence inhibition (\%)} = \frac{Cp - Tp}{Cp} \times 100$$

Cp - Number of progenies emerged in control

Tp - Number of progenies emerged in treatment

The developmental period of *C. maculatus* from egg to adult was also ecorded in all the treatments.

.3.4. Extent of infestation

After recording the progeny emergence upto 30 days of storage, three lots of 5 grains were collected at random from each treatment and counted the number of rains infestsed by observing the holes. The intensity of infestation as influenced by ioinsecticides was calculated.

$$\text{er cent infestation} = \frac{\text{Number of seeds infested}}{\text{Total number of seeds observed}} \times 100$$

$$\text{nfestation reduction (\%)} = \frac{Ci - Ti}{Ci} \times 100$$

Where

Ci - Number of seeds infested in control

Ti - Number of seeds infested in treatment

3.3.5. Weight loss

After 30 days of treatment, grains in each treatment were weighed and recorded in order to assess the weight loss caused by the infestation of *C. maculatus*

$$\text{Per cent loss in weight} = \frac{Cw - Tw}{Cw} \times 100$$

Where

Cw - Final weight of seeds in control

Tw - Final weight of seeds in treatment

3.3.6. Seed viability

In order to assess whether the bioinsecticides have any adverse effect on the seed viability, germination test was conducted at 30 days after treatment. Thirty days after treatment, twenty grains were collected at random from each treatment and subjected to germination test by placing them over a moistened filter paper in a Petri dish. Number of seeds germinated on the third day was counted and per cent germination was worked out.

$$\text{Per cent germination} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

$$\text{Inhibition of germination (\%)} = \frac{Cg - Tg}{Cg} \times 100$$

Where Cg - Per cent germination in control

Tg - Per cent germination in treatment

3.4. PERSISTENT TOXICITY OF BIOINSECTICIDES AGAINST *C. maculatus* IN STORAGE

Cowpea seeds weighing 1 kg each were treated with talc based formulation of *B. bassiana* and *M. anisopliae* at 2 per cent, spinosad and lemongrass oil at LC_{90} concentration and then the seeds were stored in plastic jars (one litre capacity). For lemon grass oil treatment, seeds were subjected to fumigation effect in fabricated fumigation chambers as described in 3.1.11. At fortnightly intervals, 100 g seeds from each of the above treatment were drawn and kept in three plastic jars (250 ml capacity) that served as three replications. Ten newly emerged one day old adults were released in each replication of the treatment. Adult mortality observations were taken 24 hours after release and the experiment was continued for three months storage period. Persistent toxicity/ residual toxicity of the treatments was worked out as PT index (Sarup *et al.*, 1970) where P is the period upto which the toxicity persisted and T is the average toxicity.

$$T = \frac{\text{Sum of percentage mortality}}{\text{Number of observations}} \times 100$$

Results

4. RESULTS

Laboratory studies were undertaken to investigate the dosage- mortality and time- mortality responses of bioinsecticides on *C. maculatus*. Experiments were also carried out to assess the bioefficacy and persistent toxicity of bioinsecticides in protecting cowpea grains against *C. maculatus* in storage. The results of the studies are presented in this chapter.

4.1. DOSAGE-MORTALITY RESPONSE OF BIOINSECTICIDES ON *C. maculatus*

The dosage-mortality response of bioinsecticides *viz.*, *B. bassiana*, *M. anisopliae*, spinosad and lemon grass oil on *C. maculatus* was studied in separate experiments.

4.1.1. Dosage- mortality response of *B. bassiana* on *C. maculatus*

The toxicity of different doses of *B. bassiana* on *C. maculatus* was studied by two bioassay methods *viz.*, direct dipping of adults and residue film technique. The results of the two experiments are presented in Tables 1 & 2.

4.1.1.a. Dosage-mortality response of *B. bassiana* by direct dipping bioassay

Dipping the adults of *C. maculatus* in increasing spore concentrations of *B. bassiana* indicated an increasing mortality with increase in exposure period to *C. maculatus* (Table 1). At one day after treatment, *B. bassiana* at the two lower concentrations (1×10^4 and 1×10^5 spores/ml) caused no mortality while the higher concentrations of 1×10^7 and 1×10^8 spores/ml caused significantly higher mortalities (13.33% and 26.67%). Toxicity of *B. bassiana* increased from 6.67 to 46.67 per cent after two days and further increased from 13.33 to 66.7 per cent at three days after treatment with the different spore concentrations. *B. bassiana* caused 33.33 to 93.33 per cent mortality at four days after treatment. After five days of treatment, *B. bassiana* caused highest mortality of 46.67 to 96.67 per cent

at 1 x 10^4 to 1 x 10^8 spores/ml. *B. bassiana* at the highest spore concentration (1 x 10^8 spores/ml) produced significantly higher mortality upto five days after treatment. However, mortality at a lower concentration of 1 x 10^7 spores/ml was found to be on par with 1 x 10^8 spores/ml at 3, 4, and 5 days after treatment indicating that *B. bassiana* at 1 x 10^7 spores/ml was sufficient to cause significantly higher mortality in *C. maculatus*.

B. bassiana, with five days of exposure on *C. maculatus*, caused a mean mortality of 20 to 66 per cent at 1 x 10^4 to 1 x 10^8 of spores/ml.

4.1.1.b. Dosage-mortality response of *B. bassiana* on *C. maculatus* by residue film bioassay

In the residue film technique also, the trend in mortality of *C. maculatus* was found to increase with an increase in the spore concentration and exposure period of *B. bassiana* (Table 2). *B. bassiana* (1 x 10^4 to 1 x 10^8 spores/ml) caused zero to 93.33 per cent mortality from one to five days after treatment. *B. bassiana* caused very low mortality (0 to 16.67%) at all concentrations upto two days after treatment. Further the mortality showed an increase from 10 to 43.33 per cent at three days after treatment and the maximum mortality (93.33%) was caused at five days after treatment. *B. bassiana* at 1 x 10^7 spores/ml caused 3.33 to 80 per cent mortality while 1 x 10^8 spores/ml, caused 6.67 to 93.33 per cent mortality over a period of five days. However, mortality difference between these two concentrations was not significant. The mean mortality ranged from 15.33 to 49.33 per cent over a period of five days at spore concentrations from 1 x 10^4 to 1 x 10^8 spores/ml

The same trend of increasing mortality with increase in doses and days of exposure was thus observed in both methods of bioassay. In residue film technique, spore concentration of 1 x 10^8 spores/ml caused significantly higher mortality (86.67%) at four days after treatment. By direct dipping, *B. bassiana* caused significantly higher mortality (56.67%) at 1 x 10^7spores/ml at three days after treatment. Significantly higher mortality (46.67%) was achieved

Table 1. Mortality of *C. maculatus* treated with *B. bassiana* (direct dipping)

Spore concentration (spores/ ml)	Per cent mortality at different days after treatment					
	1	2	3	4	5	Mean mortality (%)
1×10^4	0 $(0.71)^c$	6.67 $(2.40)^d$	13.33 $(3.67)^{cd}$	33.33 $(5.72)^c$	46.67 $(6.80)^d$	20.00
1×10^5	0 $(0.71)^c$	6.67 $(1.98)^d$	16.67 $(4.1)^{bc}$	33.33 $(5.8)^c$	56.67 $(7.54)^{bc}$	22.67
1×10^6	6.67 $(1.98)^{bc}$	16.67 $(4.09)^{bc}$	26.67 $(5.19)^b$	53.33 $(7.33)^b$	73.33 $(8.59)^{ab}$	35.33
1×10^7	13.33 $(3.67)^{ab}$	33.33 $(5.8)^{ab}$	56.67 $(7.56)^a$	80.00 $(8.96)^a$	86.67 $(9.32)^a$	54.00
1×10^8	26.67 $(5.19)^a$	46.67 $(6.86)^a$	66.67 $(8.19)^a$	93.33 $(9.68)^a$	96.67 $(9.85)^a$	66.00
Control	0 $(0.71)^c$	3.33 $(1.55)^d$	10.00 $(2.39)^d$	15.00 $(4.1)^d$	26.67 $(5.19)^d$	11.00

Table 2. Mortality of *C. maculatus* treated with *B. bassiana* (residue film)

Spore concentration (spores/ ml)	Per cent mortality at different days after treatment					
	1	2	3	4	5	Mean mortality (%)
1×10^4	0 $(0.71)^b$	0 $(0.71)^b$	10.00 $(2.83)^{cd}$	26.67 $(5.19)^c$	40.00 $(6.42)^c$	15.33
1×10^5	0 $(0.71)^b$	0 $(0.71)^b$	13.33 $(3.67)^{cd}$	30.00 $(5.47)^c$	43.33 $(6.61)^c$	17.33
1×10^6	0 $(0.71)^b$	6.67 $(2.39)^a$	20.00 $(4.43)^{bc}$	56.67 $(7.56)^b$	66.67 $(8.19)^b$	30.00
1×10^7	3.33 $(1.55)^{ab}$	13.33 $(3.67)^a$	33.33 $(5.72)^{ab}$	70.00 $(8.36)^{ab}$	80.00 $(8.19)^{ab}$	34.67
1×10^8	6.67 $(2.39)^a$	16.67 $(3.67)^a$	43.33 $(6.61)^a$	86.67 $(9.33)^a$	93.33 $(9.67)^a$	49.33
Control	0 $(0.71)^b$	0 $(0.71)^b$	10.00 $(2.34)^d$	23.33 $(4.53)^c$	30.00 $(5.52)^c$	12.67

Figures in parentheses are square root ($\sqrt{x} + 0.5$) transformed values

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

two days after treatment with highest concentration of 1 x 10^8spores per ml in direct dipping bioassay.

4.1.2. Dosage-mortality response of *M. anisopliae* on *C. maculatus*

Toxic response of different doses of *M. anisopliae* was tested by two methods of bioassay

4.1.2.a. Dosage-mortality response of *M. anisopliae* on *C. maculatus* by direct dipping bioassay

M. anisopliae caused 12.67 to 50 per cent mean mortality at concentrations ranging from 1 x 10^4 to 1 x 10^8 spores/ml over a period of five days after treatment (Table 3). Mortality rate of *C. maculatus* increased with increase in spore concentration and period of exposure. *M. anisopliae* at 1 x 10^8 spores/ml caused significantly higher mortality (96.67%) at five days after treatment. The lower concentrations caused mortality that ranged from 36.67 to 53.33 per cent at five days after treatment. After one day of treatment with 1 x 10^4 to 1 x 10^8 spores/ml, *M. anisopliae* exhibited very low mortality of zero to 6.67 per cent without any significant difference in the mortality between spore concentrations and control. Mortality increased from 3.33 to 43.33 per cent after three days of treatment with 1 x 10^4 to 1 x 10^8 spores/ml and these concentrations caused maximum lethal effect of 36.67 to 96.67 per cent at five days after treatment. *M. anisopliae* at the two higher concentrations (1 x 10^7 and 1 x 10^8 spores/ml) caused significantly higher mortality of 76.67 and 96.67 per cent. However, the mortality showed no significant difference between these two concentrations.

4.1.2.b. Dosage-mortality response of *M. anisopliae* on *C. maculatus* by residue film bioassay

In residue film bioassay, *M. anisopliae* at 1 x 10^4 to 1 x 10^8 spores/ml caused 20.67 to 61.36 per cent mean mortality to *C. maculatus* (Table 4). Among the different concentrations tested, 1 x 10^8 spores/ml brought about highest

Table 3. Mortality of *C. maculatus* treated with *M. anisopliae* (direct dipping)

Spore concentration (spores/ ml)	Mortality at different days after treatment					
	1	2	3	4	5	Mean mortality (%)
1×10^4	0 (0.71)^a	0 (0.71)^c	3.33 (1.55)^c	23.33 (4.86)^{cd}	36.67 (6.08)^{bc}	12.67
1×10^5	0 (0.71)	3.33 (1.55)^c	13.33 (3.67)^b	26.67 (5.19)^{cd}	43.33 (6.61)^b	17.33
1×10^6	0 (0.71)^a	10.00 (2.83)^{bc}	13.33 (3.67)^b	40.00 (6.33)^{bc}	53.33 (7.29)^b	23.33
1×10^7	3.33 (1.55)^a	20.00 (4.43)^{ab}	36.67 (6.08)^a	63.33 (7.99)^{ab}	76.67 (8.78)^a	40.00
1×10^8	6.67 (1.98)^a	26.67 (5.14)^a	43.33 (6.58)^a	76.67 (8.75)^a	96.67 (9.85)^a	50.00
Control	0 (0.71)^a	0 (0.71)^c	3.33 (1.55)^c	16.67 (3.26)^d	26.67 (4.71)^c	9.33

Table 4. Mortality of *C. maculatus* treated with *M. anisopliae* (residue film)

Spore concentration (spores/ ml)	Mortality at different days after treatment					
	1	2	3	4	5	Mean mortality (%)
1×10^4	0 (0.71)^a	6.67 (2.39)^{bc}	16.67 (4.1)^c	36.67 (6.0)^{cd}	43.33 (6.49)^c	20.67
1×10^5	0 (0.71)^a	13.33 (3.26)^{bc}	20.00 (4.36)^c	50.00 (7.08)^{bc}	56.67 (5.56)^{bc}	28.00
1×10^6	3.33 (1.55)^a	20.00 (4.43)^a	30.00 (5.40)^{bc}	56.67 (7.51)^{bc}	73.33 (8.57)^{ab}	36.67
1×10^7	6.67 (2.39)^a	23.33 (4.79)^a	43.33 (6.61)^{ab}	73.33 (8.57)^{ab}	86.67 (9.33)^a	46.67
1×10^8	10.00 (2.83)^a	46.67 (4.98)^a	66.67 (7.33)^a	90.00 (9.49)^a	93.33 (9.74)^a	61.33
Control	0 (0.71)a	0 (0.71)^c	0 (0.71)^d	26.67 (4.63)^d	30.00 (5.05)^d	11.33

Figures in parentheses are square root ($\sqrt{x} + 0.5$) transformed values

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

mortality of 93.33 per cent followed by 86.87 per cent by 1 x 10^7 spores/ml at five days after treatment. However, there was no significant difference in the mortality caused by 1 x 10^7 and 1 x 10^8 spores/ml at five days after treatment. The increasing doses from 1 x 10^4 to 1 x 10^8 spores/ml of *M. anisopliae* at one day after treatment showed very low mortality (zero to 10%) without any significant difference between the doses and control. Upto three days after treatment, the three higher concentrations *M. anisopliae* showed no significant difference on the mortality of *C. maculatus*. The lethal effect of *M. anisopliae* on *C. maculatus* increased with increased exposure period. Mortality increase varied from 16.67 per cent in 1 x 10^4 spores/ml to 66.67 per cent in 1 x 10^8 spores/ml on third day of exposure. Four days of exposure with *M. anisopliae* caused 36.67 (1 x 10^4 spores/ml) to 90 (1 x 10^8 spores/ml) per cent mortality of *C. maculatus.*

4.1.3. Response of *B. bassiana* and *M. anisopliae* on the cumulative mortality of *C. maculatus* by two methods of bioassay

The cumulative mortality of *C. maculatus* caused by *B. bassiana* and *M. anisopliae* at 1 x 10^4, 1 x 10^5, 1 x 10^6, 1 x 10^7 and 1x 10^8 spores/ml after five days of treatment by two bioassay methods *viz.*, direct dipping the insect and residue film on Petri dish are given in Table 5. *B. bassiana* by direct dipping bioassay indicated higher cumulative mortality (27.27 to 94.93%) than by residue film (14.28 to 90.47%) at 1 x 10^4 to 1x 10^8 spores/ml. But the mortality difference was not significant between the two bioassay methods.

M. anisopliae caused 13.64 to 95.47 per cent cumulative mortality to *C. maculatus* by direct dipping assay whereas in residue film method, the mortality ranged from 20.69 to 90.47 per cent. The bioassay techniques showed no significant difference in mortality to *C. maculatus* by *M. anisopliae* and *B. bassiana*

The highest concentration of *B. bassiana* and *M. anisopliae* (1x10 ^{8}spores/ ml) caused 94.93 and 95.47 per cent cumulative mortality respectively after five days to *C. maculatus* indicating that *B. bassiana* and *M. anisopliae* are equally

effective in causing mortality/pathogenicity to *C. maculatus*. With 1 x 10^7 spores/ ml, mortality ranged from 68.82 to 81.82 per cent and 1 x 10^6 spores/ml caused 40.45 to 63.63 per cent. The mortality was considerably reduced with further lower concentrations wherein the mortality ranged from 19.04 to 40.91 per cent and 13.64 to 27.27 per cent at 1 x 10^5 and 1 x 10^4 spores/ml, respectively.

There was no significant difference between two methods of bioassays on the cumulative mortality of *C. maculatus* by *B. bassiana* and *M. anisopliae*.

4.1.4. Median lethal concentrations of entomopathogenic fungi

Five different concentrations of two fungal pathogens, *M. anisopliae* and *B. bassiana* were tested by two bioassay techniques, direct dipping and residue film against *C. maculatus*. The per cent corrected cumulative mortality at five days after treatment was subjected to Finney's method of probit analysis and the results are presented in Table 6. *M. anisopliae* showed lower LC_{50} values than *B. bassiana* in the two bioassay methods. Lowest LC_{50} value (5.12 x 10^6 spores/ ml) was indicated in direct dipping bioassay of *M. anisopliae*. *B. bassiana* showed highest LC_{50} (7.49 x 10^6 spores/ml) in residue film bioassay. Between the two methods of bioassay, LC_{50} values were found to be higher in residue film technique than in direct immersion bioassay. In direct dipping bioassay, LC_{50} values showed no remarkable difference between B. *bassiana* (5.38 x 10^6 spores/ ml) and *M. anisopliae* (5.12 x 10^6 spores/ml).

Table 5. Effect of *B. bassiana* and *M. anisopliae* on the cumulative mortality of *C. maculatus* by different bioassay methods

Fungal pathogens	Method of treatment	Corrected cumulative mortality percentage at five days after treatment				
		Spore concentrations				
		1 x 10^4	1 x 10^5	1 x 10^6	1 x 10^7	1 x 10^8
B. bassiana	Direct dipping	27.27 $(4.49)^{de}$	40.91 $(6.24)^{bcde}$	63.63 $(8.00)^{abc}$	81.82 $(9.06)^{abc}$	94.93 $(9.78)^{a}$
	Residue film	14.28 $(3.31)^{e}$	19.04 $(3.31)^{e}$	52.39 $(7.09)^{abcd}$	71.43 $(8.11)^{abc}$	90.47 $(9.42)^{abc}$
M. anisopliae	Direct dipping	13.64 $(3.60)^{e}$	22.67 $(3.97)^{de}$	40.45 $(6.11)^{cde}$	68.82 $(8.27)^{abc}$	95.47 $(9.81)^{a}$
	Residue film	20.69 $(3.71)^{e}$	38.10 $(6.18)^{cde}$	61.9 $(7.85)^{abc}$	79.59 $(9.02)^{abc}$	90.47 $(9.53)^{abc}$

Figures in parentheses are square root ($\sqrt{x + 0.5}$) transformed values

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

Table 6. LC_{50}, LC_{90} values and probit regression parameters for *B. bassiana* and *M. anisopliae* against *C. maculatus* by two bioassay methods

Fungal Pathogens	Bioassay method	LC_{50} x 10^6 (Spores/ ml)	LC_{90} x 10^7 (Spores/ ml)	Probit regression parameters			
				Regression equation	Intercept (a) ± SE	Slope (b)±SE	χ^2 value
B. bassiana	Direct dipping	5.38	6.30	Y=0.15±0.0002X	0.15±0.05	0.0002±0.0003	74.10
	Residue film	7.49	7.72	Y=0.0014±0.00017X	0.0014±0.06	0.00017±0.00002	55.11
M. anisopliae	Direct dipping	5.12	5.76	Y=-0.113±0.0002X	0.113±0.058	0.0002±0.0003	43.11
	Residue film	6.7	7.46	Y=0.152±0.00015X	0.152±0.058	0.00015±1.00002	70.23

χ^2- Chi square; LC- Lethal Concentration

4.1.5. Spinosad - A Naturalyte insecticide - against *C. maculatus*

4.1.5.1. Dosage- mortality response of spinosad on *C. maculatus*

Dosage- mortality relationship of spinosad was assessed in the laboratory by two bioassay techniques, direct insect dipping and exposing insects to cowpea grains treated with spinosad to form a residue film on grains. Data on the effect of different concentrations of spinosad on *C. maculatus* by direct dipping bioassay are presented in Table 7. Mortality data showed that spinosad concentrations and mortality of *C. maculatus* were directly proportional. Lowest concentration of one ppm spinosad caused a minimum mortality of 20 per cent and a maximum mortality (100% was observed in the highest concentration of spinosad (10 ppm). Spinosad at 5, 7, 9 and 10 ppm caused significantly higher mortality as they were on par. No significant difference in mortality was observed between 3 and 5 ppm of spinosad.

Data on the toxic effect of different concentrations of spinosad on *C. maculatus* by grain treatment bioassay are given in Table 8. Adult mortality increased significantly from 20.00 to 96.66 per cent with increase in concentration of spinosad (10 ppm to 70 ppm) treatment on grains. Significantly higher lethal effect was caused by spinosad at 70 ppm which was on par with the lower concentration of 60 ppm (80%). However, no significant difference in mortality was observed between 40, 50 and 60 ppm of spinosad. Similarly, 10 to 40 ppm were also on par.

Toxicity of spinosad was found to be higher in direct insect dipping bioassay method than the grain treatment bioassay. Spinosad caused significantly highest mortality (90%) at 9 ppm in dipping bioassay whereas in grain treatment bioassay, highest mortality (96.66%) was obtained at 70 ppm spinosad.

Table 7. Effect of spinosad on the mortality of adults of *C. maculatus* (direct dipping)

Spinosad concentration (ppm)	Mean mortality at 24 hours after treatment (per cent)	
1	20.00	$(4.43)^{c}$
3	43.33	$(6.57)^{b}$
5	63.33	$(7.98)^{ab}$
7	82.12	$(8.75)^{a}$
9	90.00	$(9.51)^{a}$
10	100.00	$(10.02)^{a}$
Control	10.00	$(2.82)^{d}$

Figures in parentheses are square root ($\sqrt{x} + 0.5$) transformed values

Table 8. Effect of spinosad on the mortality of *C. maculatus* (residue film)

Spinosad concentration (ppm)	Mean mortality at 24 hours after treatment (per cent)	
10	20.00	$(4.50)^{d}$
20	33.33	$(5.47)^{d}$
30	40.00	$(6.02)^{cd}$
40	46.60	$(6.51)^{bcd}$
50	66.60	$(8.02)^{bc}$
60	80.00	$(8.87)^{ab}$
70	96.66	$(9.85)^{a}$
Control	3.33	$(3.24)^{d}$

Figures in parentheses are square root ($\sqrt{x} + 0.5$) transformed values

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

4.1.5.2. Median Lethal Concentration, LC_{50} of spinosad against *C. maculatus*

Different concentrations of spinosad were bioassayed against *C. maculatus* in two different methods. The per cent corrected mortality was subjected to Finney's method of probit analysis and the results are given in Table 9.

Spinosad caused 50 per cent mortality to *C. maculatus* at 4.02 ppm by direct dipping bioassay where as a higher LC_{50} value (36.39 ppm) was observed with residue film on grain bioassay. It showed nine fold increase in LC_{50} value as compared to dipping bioassay.

LC_{90} of spinosad varied from 8.33 ppm (direct dipping) to 68.80 ppm (by residue film on grains).

4.1.6. Lemongrass oil – a fumigant toxin – against *C. maculatus*

4.1.6.1. Dosage- mortality response of lemongrass oil on *C. maculatus*

Fumigant action and dosage-mortality relationship of lemongrass oil was studied in the laboratory by using a simple fabricated fumigation chamber. Eight different concentrations, 1, 2, 3, 4, 5, 6, 7 and 8 micro litre/litre of air in the fumigation chamber were employed for bioassay after preliminary experiments. Data on mortality recorded 24 hours after treatment are given in Table 10. Maximum mortality (96.67%) was observed in the highest concentration 8 micro litre per litre and minimum mortality (16.67%) in lowest concentration 1 micro litre. The concentrations 6, 7 and 8 ppm showed no significant difference in mortality.

4.1.6.2. Median Lethal Concentration, LC_{50} of lemongrass oil

Data on per cent mortality obtained from bioassay were subjected to probit analysis and the results are given in Table 11. Lemongrass oil caused 50 and 90 per cent mortalities at 3.93 and 7.51 ppm.

Table 9. LC $_{50}$, LC $_{90}$ values and probit regression parameters for spinosad against *C. maculatus* by two bioassay methods

Method of treatment	LC_{50} in ppm (FL)	LC_{90} in ppm (FL)	Probit parameters			
			Regression equation	Intercept (a) ± SE	Slope (b)±SE	χ^2 value
Direct dipping	4.02 (3.29-4.72)	8.33 (7.36-9.73)	Y=-1.19±0.30X	-1.19±0.18	-0.30± 0.030	2.228
Residue film	36.39 (31.72-41.17)	68.80 (61.29-80.04)	Y=-1.44±0.039X	-1.44±0.19	0.039±0.005	4.821

χ^2 - Chi square; LC- Lethal Concentration; FL- Fiducial limit

Table 10. Fumigant toxicity of lemongrass oil on *C. maculatus*

Lemongrass oil concentration (Micro litre/ litre of air)	Mean mortality of *C. maculatus* (per cent)	
1	16.67	$(4.09)^{f}$
2	26.67	$(5.19)^{ef}$
3	40.00	$(6.33)^{de}$
4	46.67	$(6.84)^{cd}$
5	63.33	$(7.95)^{bc}$
6	73.33	$(8.56)^{ab}$
7	86.67	$(8.94)^{ab}$
8	96.67	$(9.85)^{a}$
Control	0	$(0.71)^{h}$

Figures in parentheses are square root ($\sqrt{x}$ + 0.5) transformed values

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

Table 11. LC_{50}, LC_{90} values and probit regression parameters of lemongrass oil against *C. maculatus*

LC_{50} (FL) ppm	LC_{90} (FL) ppm	Probit regression parameters			
		Regression equation	Intercept (a) $\pm$ SE	Slope (b)$\pm$SE	χ^2 value
3.93 (3.40-4.43)	7.51 (6.73-8.70)	Y=-1.41$\pm$0.36X	-1.41$\pm$0.21	0.36$\pm$ 0.044	1.353

χ^2 - Chisquare; LC- Lethal Concentration; FL- Fiducial limit

4.2. TIME-MORTALITY EFFECT OF BIOINSECTICIDES

4.2.1. Time-mortality effect of *B. bassiana* and *M. anisopliae* against *C. maculatus*

The time taken by *B. bassiana* and *M. anisopliae* (at all tested concentrations) to cause 50 per cent mortality in *C. maculatus* was estimated and data are given in Table 12. LT_{50} values consistently increased as the conidia concentration decreased, but they differed between fungal isolates. LT_{50} values for *B. bassiana* at 1 x 10^8 spores/ml (direct dipping) was found to be lowest (49.26 hours or 2.1 days). But in residue film bioassay, *B. bassiana* took 76.67 hours or 3.02 days for causing 50 per cent mortality. For *M. anisopliae,* the LT_{50} values were 71.69 or 2.99 days (dipping assay) and 57.64 hours or 2.40 days (residue film).

B. bassiana (1x 10^8 spores/ml) required 92.77 (3.9 days) to 109.31 (4.6 days) hours whereas *M anisopliae* had taken 111.67 (4.6 days) to 101.28 (4.2 days) hours to bring about 90 per cent mortality to *C. maculatus* by direct dipping and residue film bioassay techniques respectively.

4.2.2. Time-mortality effect of spinosad against *C. maculatus*

Time taken by spinosad at LC_{50} concentration for 50 per cent mortality was estimated by Finney's method of probit analysis. LT_{50} of spinosad at its LC_{50} concentration was found out by two methods of bioassay (Table 13). Spinosad caused 50 per cent mortality at 20.51 hours and 90 per cent mortality in 62.99 hours (2.62 days) by direct dipping. In residue film on grains bioassay the LT_{50} value was 33.09 hours (1.38 days) and LT_{90} was achieved in 80.59 hours (3.4 days). Residue film bioassay indicated 1.3 fold increase in LT_{90} value of spinosad than by direct dipping bioassay.

Table 12. LT $_{50}$ and LT $_{90}$ values of entomopathogens against *C. maculatus* by two bioassay techniques

Fungal pathogens	Method of bioassay	Concentration (spores/ ml)	LT_{50} value (hours)	95 % fiducial limits (hours)		LT_{90} value (hours)	95 % fiducial limits (hours)	
				Upper	Lower		Upper	Lower
B. bassiana	Direct dip	$1x10^8$	49.26	30.48	76.73	92.77	69.11	136.4
		$1x10^7$	62.66	58.05	105.33	105.33	97.17	116.7
		$1x10^6$	82.05	55.62	125.48	125.48	92.29	224.5
		$1x10^5$	93.49	84.86	125.69	125.69	113.5	148.4
		$1x10^4$	101.24	91.06	138.99	138.99	122.6	174.3
	Residue film	$1x10^8$	76.67	62.36	105.31	109.31	96.44	132.4
		$1x10^7$	85.30	80.79	129.44	129.44	121.6	139.7
		$1x10^6$	98.97	86.83	142.08	142.08	122.4	189.2
		$1x10^5$	123.92	119.14	175.18	175.18	166.9	185.6
		$1x10^4$	135.15	129.64	193.19	193.19	182.6	206.9
M. anisopliae	Direct dip	$1x10^8$	71.69	67.52	111.67	111.67	105.2	119.9
		$1x10^7$	86.12	81.07	137.48	137.48	127.8	150.5
		$1x10^6$	110.99	97.28	163.57	163.57	137.3	231.2
		$1x10^5$	120.98	115.78	169.89	169.89	159.9	183.2
		$1x10^4$	145.20	134.41	204.07	204.07	183.7	239.6
	Residue film	$1x10^8$	57.64	40.87	101.28	101.28	85.31	135.7
		$1x10^7$	76.82	72.102	124.39	124.39	116.4	134.9
		$1x10^6$	91.32	85.88	145.92	145.92	134.9	161.2
		$1x10^5$	108.66	95.59	169.81	169.81	146.9	215.9
		$1x10^4$	126.62	114.67	119.72	119.72	177.1	238.6

LT- Lethal Time

Table 13. LT $_{50}$, LT $_{90}$ and probit regression parameters for spinosad against *C. maculatus* by two bioassay methods

Method of treatment	LT $_{50}$ (FL) hours	LT$_{90}$ (FL) hours	Probit regression parameters			
			Regression equation	Intercept (a) ± SE	Slope (b)±SE	χ^2 value
Direct dipping	20.51 (14.68-25.30)	62.99 (55.98-73.05)	Y=-0.62±.030X	-0.62±0.13	0.030±0.003	0.499
Residue film	33.09 (27.87-38.03)	80.59 (71.55-93.91)	Y=-0.9±0.027X	-0.9±0.13	0.027±.00295	3.336

χ^2- Chi square; LT- Lethal Time; FL- Fiducial limit

Table 14: LT $_{50}$, LT $_{90}$ and probit regression parameters for lemongrass oil against *C. maculatus*

LT $_{50}$ (FL) hours	LT $_{90}$ (FL) Hours	Probit regression parameters			
		Regression equation	Intercept (a) ± SE	Slope (b) ±SE	χ^2 value
23.80 (18.89-28.08)	62.55 (55.99-71.75)	Y=-0.8±0.03X	-0.8±0.13	0.03±0.003	3.123

χ^2- Chi square; LC- Lethal Concentration

4.2.3. Time-mortality effect of lemongrass oil against *C. maculatus*

A single concentration bioassay (LC_{50} value of lemongrass oil) was carried out to estimate LT_{50}. Lemongrass oil took 23.8 hours (18.89- 28.08) to produce 50 per cent mortality to *C. maculatus* (Table 14). Ninety per cent mortality occurred in 62.55 hours (2.6 days).

4.4. BIOLOGICAL EFFICIENCY OF BIOINSECTICIDES AGAINST *C. maculatus*

The bioefficacy of *B. bassiana, M. anisopliae,* spinosad and lemongrass oil against *C. maculatus* was assessed by studying their effect on adult toxicity, fecundity, egg hatchability, progeny emergence, developmental period, extent of infestation, grain weight loss and seed viability.

4.4.1. Adult toxicity

Results on direct toxicity effect of bioinsecticides manifested in the form of adult mortality of *C. maculatus* are given in Table 15. One day after treatment, lemongrass oil caused highest adult mortality (87.07%) followed by spinosad (75.87%). Coconut oil treatment resulted in 28.45 per cent adult mortality. *B. bassiana* and *M. anisopliae* caused very low adult mortality (9.91 to 12.36%). At three days after treatment, spinosad and lemongrass oil brought about 100 per cent mortality while coconut oil caused 100 per cent mortality at five days after treatment. After seven days of treatment, *B. bassiana* and *M. anisopliae* caused 100 and 66.67 per cent mortality.

Spinosad increased adult mortality from 75.87 at one day after treatment to 100 per cent at three days after treatment. Lemongrass oil caused a higher mortality of 87.07 to 100 per cent within three days of treatment. Coconut oil (recommended check) caused a lower mortality of 28.45 per cent at one day after

Table 15. Toxicity of bioinsecticides on adults of *C. maculatus* in cowpea storage

Treatments	Mortality at days after treatment (%)						
	1	2	3	4	5	6	7
B. bassiana (2%)	12.5 (9.91)	20.56 (11.73)	30.00 (16.00)	50.00 (34.78)	60.00 (25.01)	73.33 (27.27)	100.0 (100.0)
M. anisopliae (2%)	15.28 (12.36)	15.56 (6.18)	33.33 (30)	53.66 (39.56)	66.67 (37.51)	80.00 (45.45)	93.33 (66.67)
Spinosad (LC_{90})	76.67 (75.87)	95.5 (95)	100.0 (100.00)	-	-	-	-
Lemongrass oil (LC_{90})	87.50 (87.07)	97.5 (97.22)	100.0 (100.00)	-	-	-	-
Coconut oil (1%)	30.83 (28.45)	51.74 (46.38)	80.00 (78.00)	93.33 (70.00)	100.0 (100.00)	-	-
Control	3.33	10.00	16.67	23.33	46.66	63.33	80.00

Figures in parentheses are corrected mortality by Abbott's formula

treatment and subsequently the mortality rate was increased to 100 per cent at five days after treatment. *B. bassiana* and *M. anisopliae* caused higher mortalities at seven days after treatment only. Among all the treatments, the lowest mortality (66.67%) was indicated by *M. anisopliae* at seven days after treatment.

4.4.2. Surface protectant action

The surface protectant action of bioinsecticides was assessed by studying their effect on oviposition deterrency, egg hatchability, progeny emergence and developmental period of *C. maculatus.*

4.4.2.1. Oviposition deterrency

Oviposition deterrency of bioinsecticides to *C. maculatus* was studied by counting the number of eggs laid on treated and untreated cowpea grains and the results are given in Table 16.

All bioinsecticides except entomopathogenic fungi were significantly effective in deterring the oviposition. *C. maculatus* laid significantly lower number of eggs (34.25) on grains treated with spinosad followed by lemongrass oil (48.25). Spinosad showed 81.65 per cent deterrency of oviposition and lemongrass oil inhibited oviposition by 74.16 per cent. However, there was no significant difference in the number of eggs laid between the treatments of spinosad and lemongrass oil. *M. anisopliae* and *B. bassiana* indicated 14.86 and 17.00 per cent oviposition deterrency and they were on par. Fecundity on grains treated with entomopathogenic fungi showed no significant difference with that of untreated control. Spinosad and lemongrass oil were significantly more effective than coconut oil in deterring the oviposition of *C. maculatus.* But coconut oil treatment significantly reduced fecundity (87) as compared to control. It caused 53.41 per cent inhibition of oviposition over the untreated control. Spinosad, lemongrass oil and coconut oil were significantly effective in reducing the fecundity by causing 53.41 to 81.65 per cent oviposition deterrency. However, spinosad and lemongrass oil showed significantly higher inhibition of oviposition

than coconut oil. Both spinosad and lemongrass oil were equally effective in deterring the oviposition.

4.4.2.2. Egg hatchability

No eggs hatched out in coconut oil treatment indicating cent per cent inhibition of hatching (Table 16). Spinosad and lemongrass oil significantly reduced the number of eggs hatched as compared to untreated control but egg hatchability in *B. bassiana* and *M. anisopliae* was on par with untreated control. Per cent egg hatchability in spinosad was significantly lower (67.63) than that of lemongrass oil (93.66). *B. bassiana and M. anisopliae* recorded a higher hatchability (88.55 and 83.72%). Spinosad caused higher inhibition of egg hatching (82.85 per cent) followed by lemongrass oil with 68.36 per cent. However, both were found to be equally effective in inhibiting egg hatching as they were on par. *B. bassiana and M. anisopliae* recorded significantly lower hatching inhibition (2.47-7.97%) as compared to untreated check.

Coconut oil ranked third in terms of oviposition deterrency but proved to be the best one in inhibiting the hatching of eggs. Spinosad and lemongrass oil were found to be superior to other bioagents in terms of number of eggs laid. Spinosad recorded lowest number of eggs, lowest number of eggs hatched, lowest per cent hatchability, higher oviposition deterrence and highest hatching inhibition. Lemongrass oil was also equally effective in causing lower fecundity, higher oviposition deterrency and higher hatching inhibition over control.

Table16.Fecundity and hatchability of *C. maculatus* as influenced by bioagents

Treatments	Number of eggs laid	Number of eggs hatched	Egg hatchability (Per cent)	Oviposition deterrency over control (Per cent)	Hatching inhibition over control (Per cent)
B. bassiana (0.2 %)	155 (12.43)^{c}	138 (11.77)^{b}	88.55^{bc}	17.00^{c}	2.47 (1.72)^{b}
M. anisopliae (0.2 %)	159 (12.56)^{c}	130.25 (11.43)^{b}	83.72^{bc}	14.86^{c}	7.97 (2.91)^{b}
Spinosad (LC_{90})	34.25 (5.84)^{a}	24.25 (4.97)^{a}	67.63^{a}	81.65^{a}	82.85 (9.13)^{a}
Lemongrass oil (LC_{90})	48.25 (6.92)^{a}	44.75 (6.73)^{a}	93.66^{c}	74.16^{a}	68.36 (8.29)^{a}
Coconut oil (1 %)	87 (9.35)^{b}	0	0	53.41^{b}	100
Control	187 (187)^{c}	141.5 (11.92)^{b}	76.11^{ab}	-	-

All cell values are the mean of four replications

Figures in parentheses are square root ($\sqrt{x} + 0.5$) transformed values

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

4.4.2.3. F_1 progeny emergence

No progenies emerged in coconut oil treatment as there was no hatching of eggs and hence it indicated cent per cent inhibition of progeny emergence (Table 17). Spinosad and lemongrass oil treatment of cowpea grains resulted in significantly lower number of progenies (12.25 and 29.50) of *C. maculatus.* However, progeny emergence in spinosad and lemongrass oil showed no significant difference between them. *B. bassiana* and *M. anisopliae* were not effective in inhibiting the progeny production. Higher number of progenies emerged in *B. bassiana* (75.75) and *M. anisopliae* (101.25) and they did not indicate any significant difference with the untreated control (104).

Spinosad and lemongrass oil caused 88.22 and 72.12 per cent inhibition of progeny emergence over control while coconut oil caused cent per cent progeny emergence inhibition. Next to coconut oil, spinosad and lemongrass oil were equally effective for inhibiting progeny emergence of *C. maculatus.* Entomopathogenic fungi, *B. bassiana* and *M. anisopliae* inhibited only 20.68 and 27.16 per cent progeny emergence.

4.4.3. Developmental period

The bioinsecticides showed no significant effect on the developmental period of *C. maculatus* (Table 17). The mean developmental period ranged from 22.12 to 22.93 days in all treatments including control.

4.4.4. Extent of infestation

Extent of infestation is a measure of damage caused by *C. maculatus* on cowpea grains and it was assessed based on the number of grains infested. No infestation was recorded in treatment with coconut oil in which 100 per cent inhibition of infestation was observed (Table 18). Least infestation was found in spinosad (12.25) followed by lemongrass oil (13) and both were on par. Extent of infestation was very high in treatments with fungal pathogens and was found to

Table 17. Effect of bioagents on the progeny emergence of *C. maculatus*

Treatments	Mean number of adults emerged at different days after treatment									Inhibition on progeny emergence (%)	Mean developmental period (days)
	20	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**Total**		
B. bassiana (2%)	6.3	19.3	20.8	13.3	8.5	4.5	2.25	1.0	75.75[b]	27.16[b]	22.93
M. anisopliae (2%)	4.5	16.7	27.0	16.8	16.0	11.5	7.0	1.75	101.25[b]	20.68[b]	22.34
Spinosad (LC_{90})	0.5	5.5	2.3	1.8	1.0	0.75	0.5	0	12.25[a]	88.22[a]	22.12
Lemongrass oil (LC_{90})	1.0	8.0	4.8	7.0	4.0	3.25	1.5	0	29.50[a]	72.12[a]	22.70
Coconut oil * (1%)	0	0	0	0	0	0	0	0	0	100.00	*
Control	14.3	23.3	21.3	16.3	10.0	8.5	7.0	3.5	104.00[b]	-	22.11

* No adults emerged & most effective, not included for analysis

have no significant difference as compared to control. The per cent seed damage on bioinsecticide treatments ranged from 49 to 87 per cent as compared to 91 per cent in untreated control.

Treatment with lemongrass oil reduced infestation by 37.35 per cent and spinosad caused 40.96 per cent reduction of infestation over control and they were found to be on par. Both *B. bassiana* and *M. anisopliae* were significantly inferior to other treatments.

4.4.5. Grain weight loss

Results on the effect of bioinsecticide treatments on weight loss incurred after 30 days of storage are given in Table 19. Coconut oil recorded no loss in seed weight and observed to be most effective treatment. Spinosad significantly reduced weight loss (1.9 per cent) and it caused 94.48 per cent reduction in weight loss over control. Lemongrass oil treatment resulted in 20.89 per cent loss in weight and indicated 39.29 per cent reduction in weight loss over control. *B. bassiana* and *M. anisopliae* treatments resulted in 26.5 and 29.8 per cent loss in weight of cowpea grains and they showed no significant difference with the untreated grains.

Based on the effect on weight loss, the bioinsecticides can be ranked as

Coconut oil > Spinosad > Lemongrass oil > *M. anisopliae* > *B. bassiana*

Table 18. Effect of bioagents on extent of infestation by *C. maculatus*

Treatments	Number of seeds infested (Mean)	Seed damage (Per cent)	Reduction of infestation over control (Per cent)
B. bassiana (2%)	20.75^{b}	83.00^{b}	9.63 $(3.18)^{b}$
M. anisopliae (2%)	21.75^{b}	87.00^{b}	4.82 $(2.31)^{b}$
Spinosad (LC_{90})	12.25^{a}	49.00^{a}	40.96 $(6.44)^{a}$
Lemongrass oil (LC_{90})	13.00^{a}	52.00^{a}	37.35 $(6.15)^{a}$
Coconut oil (1%)	0	0	100
Control	22.75^{b}	91.00^{b}	-

Table 19. Influence of bioinsecticides on weight loss caused by *C. maculatus*

Treatments	Seed weight after one month of storage (g)	Seed weight loss (Per cent)	Reduction of weight loss over control (Per cent)
B. bassiana (2%)	13.12^{c}	29.84^{bc}	13.28^{c}
M. anisopliae (2%)	14.93^{b}	26.48^{bc}	23.05^{bc}
Spinosad (LC_{90})	19.62^{a}	1.90^{a}	94.48^{a}
Lemongrass oil (LC_{90})	15.82^{b}	20.89^{b}	39.29^{b}
Coconut oil (1%)	20.00	0	100.00
Control	12.84^{c}	34.41^{c}	-

In a column, means superscripted by a common letter are not significantly different by DMRT (P=0.05)

Figures in parentheses are square root ($\sqrt{x} + 0.5$) transformed values

4.4.6. Seed viability

The most accurate test of seed viability is the germination test. Germination test was carried out at 30 days after treating the grains with respective bioinsecticides along with untreated control and the results are given in Table 20. The tested bioagents caused no inhibition of germination. All the bioinsecticides indicated a higher germination that ranged from 55 per cent in *B. bassiana* to 95 per cent in spinosad as compared to the lowest germination (50%) in untreated control. Seed germination in spinosad treatment showed 90 per cent increase over control followed by lemongrass oil (80%) and coconut oil (60%). *B. bassiana* and *M. anisopliae* showed lowest germination rates with 10 to 50 per cent increase over control. Least germination was reported in entomopathogen, *B. bassiana* with 55 per cent at 30 days after treatment respectively.

4.5. PERSISTENT TOXICITY OF BIOAGENTS IN COWPEA STORAGE

The results of the study on the persistent toxicity of bioagents for 90 days on cowpea grains are given in Table 21. Spinosad showed highest persistence with a PT value of 5699.7. All other bioagents showed less persistence. The fungal pathogens *B. bassiana* and *M. anisopliae* showed almost similar PT values of 83.34 and 83.31. Spinosad caused 56.66 per cent mortality even at 90 days after treatment. The lowest PT value (16.5) was shown by lemongrass oil showing its least persistence. Lemongrass oil indicated the lowest average toxicity (1.1) and period (15 days) of toxicity persistence. The persistent toxicity of bioinsecticides based on their PT index values can be ranked as

Spinosad > *B. bassiana* > *M. anisopliae* > Lemongrass oil

(5699.7) (83.34) (83.31) (16.5)

Table 20. Effect of bioagents on the germination of cowpea seeds

Treatments	Germination at 30 DAT (Per cent)	Increase in germination over control (Per cent)
B. bassiana (2%)	55	10
M. anisopliae (2%)	75	50
Spinosad (LC_{90})	95	90
Lemongrass oil (LC_{90})	90	80
Coconut oil (1%)	80	60
Control	50	-

DAT - Days after treatment

Table 21. Persistent toxicity of bioagents against *C. maculatus*

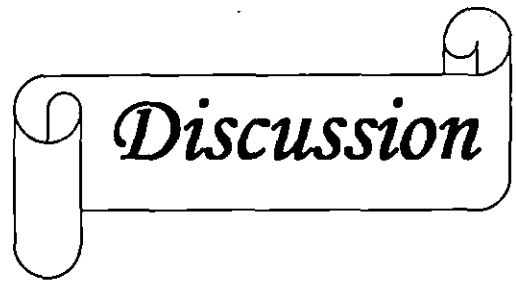
Treatments (Dose)	Percentage mortality at different days after treatment						P	T	PT	ORE
	15	30	45	60	75	90				
B. bassiana (2%)	10	6.67	0	0	0	0	30.00	2.78	83.34	2
M.anisopliae (2%)	13.33	3.33	0	0	0	0	30.00	2.77	83.31	3
Spinosad (LC_{90})	76.66	70.00	66.66	60.00	50.00	56.66	90.00	63.00	5699.7	1
Lemongrass oil (LC_{90})	6.60	0	0	0	0	0	15.00	1.10	16.50	4

PT - Persistent toxicity Index

P - Period upto which the toxicity is persisted (days)

T - Average Toxicity

ORE - Order of relative efficacy


Discussion

5. DISCUSSION

The potential of four bioinsecticides *viz.*, two entomopathogenic fungi- *Beauveria bassiana* and *Metarhizium anisopliae*, a new generation insecticide molecule (derived from a soil actinomycete)-spinosad and an essential oil from lemongrass (*Cymbopogon flexuosus*) were studied for their protectant actions against *C. maculatus*. The results obtained from the studies on dosage- mortality response, time-mortality effect, bioefficacy and persistent toxicity of bioinsecticides against *Callosobruchus maculatus* in storage are discussed hereunder to elucidate the observations and findings.

5.1. DOSAGE- MORTALITY RESPONSE OF BIOINSECTICIDES

5.1.a. *B. bassiana* and *M. anisopliae*

Two entomopathogenic fungi- *B. bassiana* and *M. anisopliae* were tested at five concentrations from 1 x 10^4 to 1 x 10^8 spores/ ml on *C. maculatus* by two methods of bioassay *viz.* direct dipping and residue film. Results on the mortality of *C. maculatus* treated with *B. bassiana* indicated an increase in mortality rate with increase in dosage and period of exposure (Table 1 & 2). *B. bassiana* (1 x 10^4 to 1 x 10^8 spores/ ml) caused very low mortality (zero to 6.67 %) at one day after treatment. But at five days after treatment, the mortality was increased from 40.00 to 93.33 per cent (Fig.1) and the mortality caused by the two higher concentrations (1 x 10^7 and 1 x 10^8 spores/ ml) were found to be on par . It is thus indicated that *B. bassiana* at 1 x 10^7 spores/ ml is sufficient to cause higher mortality (80 to 93.33 %) to *C. maculatus*.

Results on the dosage- mortality response of *M. anisopliae* also revealed the same trend of increasing mortality rate with increase in dosage and exposure period (Table 3 & 4). In the case of *M. anisopliae* also 1 x 10^7 and 1 x 10^8 spores/ ml caused significantly higher mortality (86.67 and 93.33%) at five days after treatment (Fig. 2) and they were on par.

Fig. 1. Mortality of *C. maculatus* treated with *B. bassiana* (residue film)

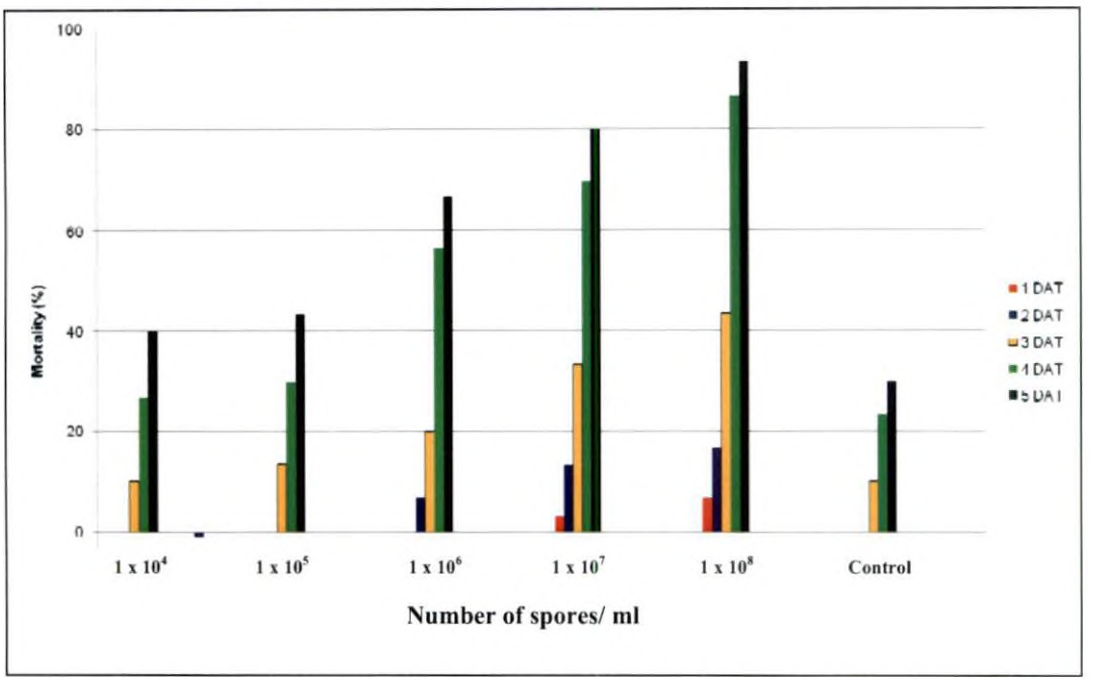
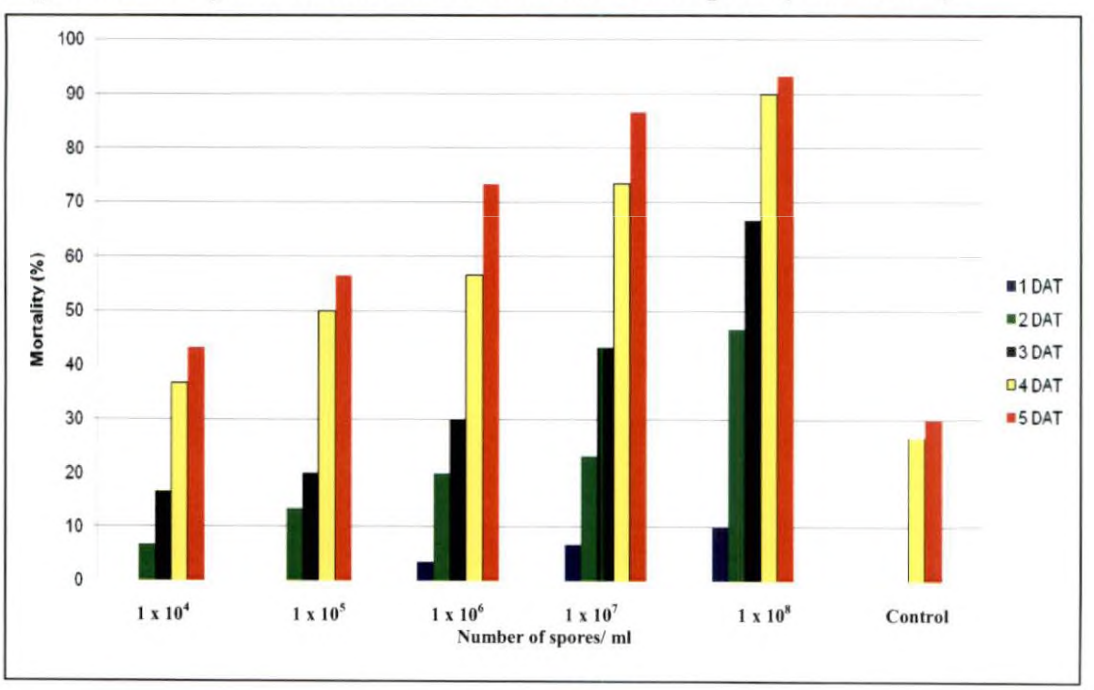


Fig. 2. Mortality of *C. maculatus* treated with *M. anisopliae* (residue film)



For *M. anisopliae* also, a concentration of 1 x 10^7 spores/ml was found to be effective in causing a higher mortality. The overall results on the mortality of *C. maculatus* treated with five dosages (1 x 10^4 to 1 x 10^8 spores/ml) of *B. bassiana* and *M. anisopliae* for a period five days thus revealed that both *B. bassiana* and *M. anisopliae* were pathogenic and worked similarly in causing mortality to *C. maculatus*. Both *B. bassiana* and *M. anisopliae* at 1 x 10^7 spores/ml caused significantly higher mortality (76.67- 86.67%) to *C. maculatus* over five days of exposure. This finding is in conformity with Bello *et al.* (2001) who reported that mortality of storage pests increased with time of exposure with *B. bassiana.*

Cumulative mortality data of *C. maculatus* by *B. bassiana* and *M. anisopliae* at five days after treatment with five concentrations revealed no significant difference between the two entomopathogens (Table 5, Fig. 3). The two bioassay methods, direct dipping and residue film used in the study also showed no significant difference in cumulative mortality thus indicating equal effectiveness of the two techniques for dosage- mortality response study. The cumulative mortality of *B. bassiana* and *M. anisopliae* at 1 x 10^7 spores/ml ranged from 68.8 to 81.82 per cent. At the highest concentration (1 x 10^8 spores/ml), *B. bassiana* and *M. anisopliae* caused 90.47 to 95.47 per cent cumulative mortality to *C. maculatus*. This finding corroborates with Adane *et al.* (1996) who reported a cumulative mortality of 93.5 to 100 per cent at seven days after treatment in *Sitophilus zeamais* by the treatment with *B. bassiana* isolates. Kavallieratos (2006) also reported a significant increase in mortality of *Rhizopertha dominica* with seven days of exposure with *B. bassiana.*

Several studies have documented that entomopathogenic fungi, *B. bassiana* and *M. anisopliae* can be used with success against stored product insects (Ekesi *et al.,* 2001; Ferron *et al.,* 1991). The present finding is also in consonance with Cherry *et al.* (2005) who had demonstrated that different isolates of *B. bassiana* and *M. anisopliae* can provide good control over *C. maculatus* by

Fig. 3. Cumulative mortality by *B. bassiana* and *M. anisopliae* of *C. maculatus* (two bioassay methods)

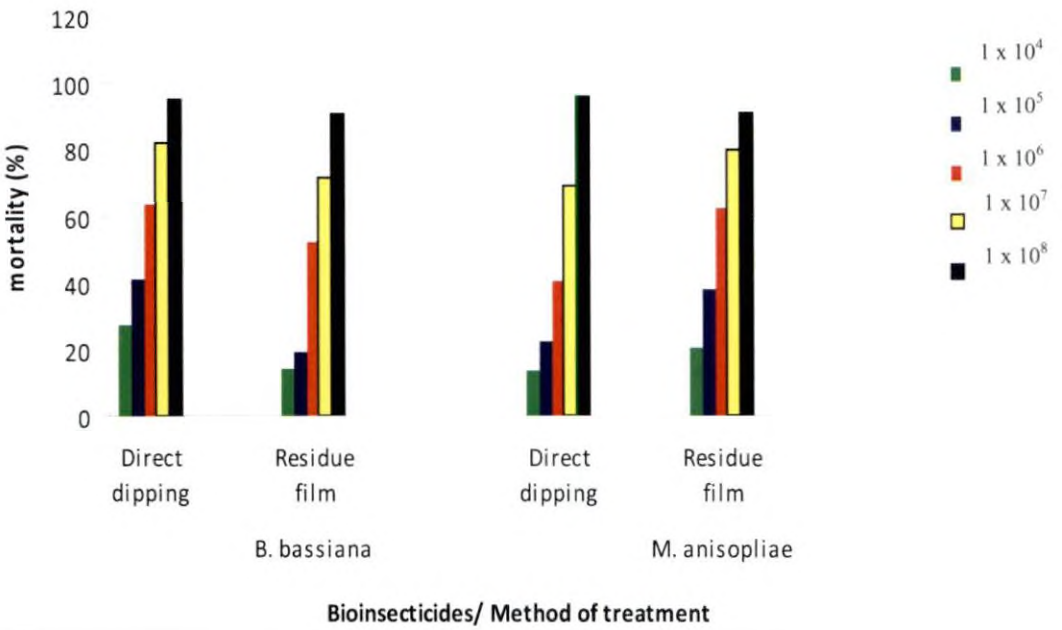
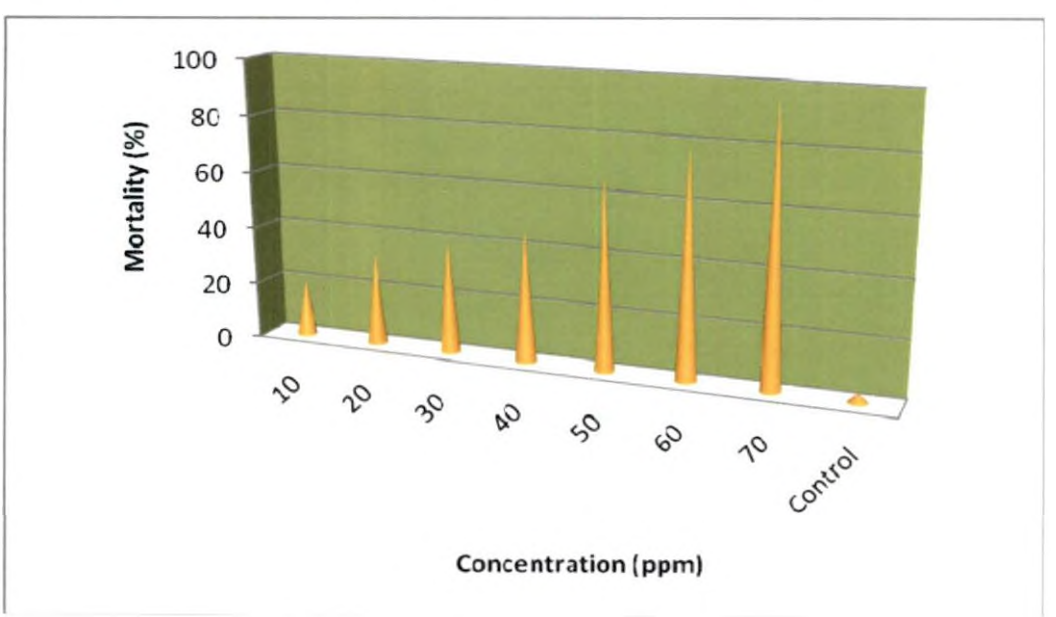


Fig. 4. Mortality of *C. maculatus* by spinosad (residue film on grains)



immersion bioassay. They reported 100 per cent mortality of *C. maculatus* with *B. bassiana* at six days after treatment by adult immersion bioassay technique.

5.1.b. Spinosad

Spinosad was tested at 1 to 10 ppm and it was found to cause 20 to 100 per cent mortality to *C. maculatus* by direct dipping bioassay (Table 7). In residue film bioassay method of treating the cowpea grains, higher concentrations of spinosad (10 to 70 ppm) were required to cause 20-96.6 per cent mortality in *C. maculatus* (Fig. 4). Spinosad at 70 ppm showed significantly higher mortality (96.67 %) but the next lower concentration of 60 ppm also showed equal effectiveness (80 % mortality) as both these concentrations was observed to be on par. Other lower concentrations of spinosad (10 to 40 ppm) caused no significant mortality (20-46.66 %) to *C. maculatus.* The insecticidal effect of spinosad to *R. dominica* and *Tribolium* sp. was earlier documented by Toews *et al.* (2003) and Nayak *et al.* (2005). However, Sanon *et al.* (2010) were the first to evaluate this bioinsecticide, spinosad against *C. maculatus.* They reported that spinosad significantly increased the mortality of adult *C. maculatus* that were exposed to cowpea seeds immediately after the seeds had been coated and adult mortality increased significantly with increased quantities of spinosad applied.

5.1.c. Lemongrass oil

Lemongrass oil at eight concentrations (1 to 8 µl/l of air) were tested to study its fumigant toxicity against *C. maculatus.* Results revealed that all concentrations of lemongrass oil were significantly effective than control in causing higher mortality to *C. maculatus* (Table 10, Fig. 5). The mortality ranged from 16.67 to 96.67 per cent with 1 to 8 ppm lemongrass oil. The three higher concentrations of 6, 7 and 8 ppm lemongrass oil showed no significant difference in mortality between them and thus indicated that they were equally effective in

causing higher mortality (73 to 97%) to *C. maculatus* within 24 hours. Aromatic spices and herbs contain volatile essential oils which possess insecticidal activity. The toxicity of a large number of essential oils has been evaluated against a number of storage insects (Deshpande *et al.,* 1974). Varma and Dubey (1998) found that essential oil of *Cymbopogon citratus* showed its *in vitro* fumigant activity in the management of storage insects of some cereals without exhibiting mammalian toxicity. The present finding is in consonance with Srinivas (2008) who reported that lemongrass oil acted as an effective fumigant against *C. chinensis.*

5.1.1. Median lethal concentration of bioinsecticides

Probit analysis of cumulative mortality revealed that *M. anisopliae* had lower LC_{50} values (5.12 x 10^6 and 6.7 x 10^6 spores/ml) than *B. bassiana* (5.38 x 10^6 and 7.49 x 10^6 spores/ml) by direct dipping and residue film bioassay indicating higher toxicity of *M. anisopliae* (Table 6) to *C. maculatus.* Cherry *et al.* (2005) obtained LC_{50} values of 9.1 x 10^4 and 7.1 x 10^5 spores/ml for *B. bassiana* 0362 and *M. anisopliae* 0351 by immersion bioassay of *C. maculatus.* They reported that bioassay with treated cowpea grains gave LC_{50} values of 1.15 x 10^7 and 4.44 x 10^7 spores/g grain for *B. bassiana* 0362 and *M. anisopliae* 0351. The difference in LC_{50} values of the present study might be due to difference in fungal strains and experimental conditions. Samodra *et al.* (2006) reported LC_{50} values of 9.49 x 10^6 and 1.38 x 10^7 spores/ml for two strains of *B. bassiana* and 1.12 x 10^7 spores/ml for *M. anisopliae.* Lower LC_{50} values were reported for indigenous isolates of *B. bassiana* than *M. anisopliae* isolate against *R. dominica* (Mahdneshin *et al.* 2009).

LC_{50} of spinosad was found to be 4.02 ppm in direct dipping method, where as a higher LC_{50} value (36.39 ppm) was observed by residue film bioassay (Table 9). Vayias *et al.* (2009) observed extreamly high mortality of *R. dominica* even with lowest dose of spinosad.

Fig. 5. Fumigant toxicity of lemongrass oil on *C. maculatus*

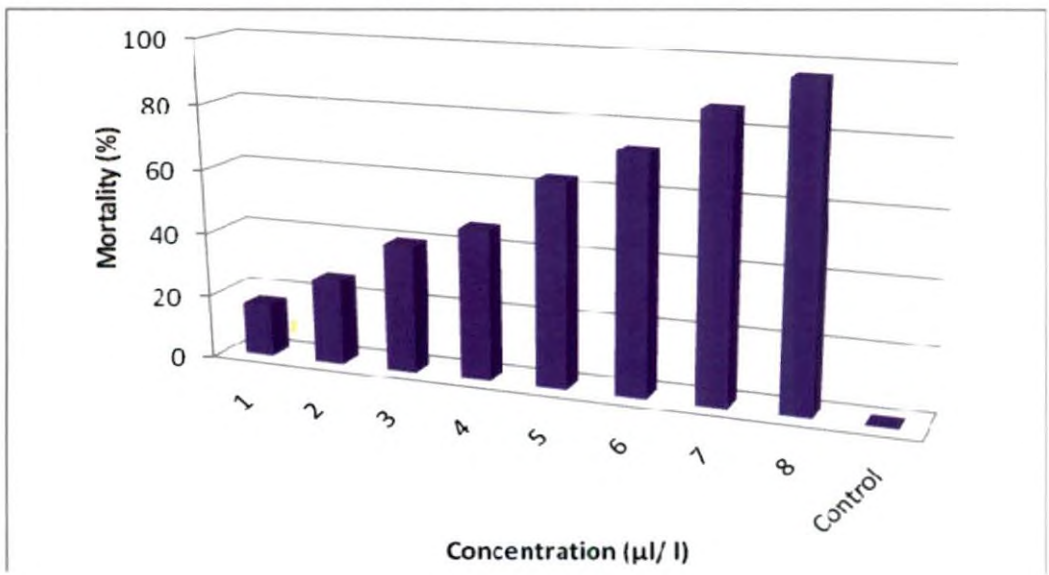
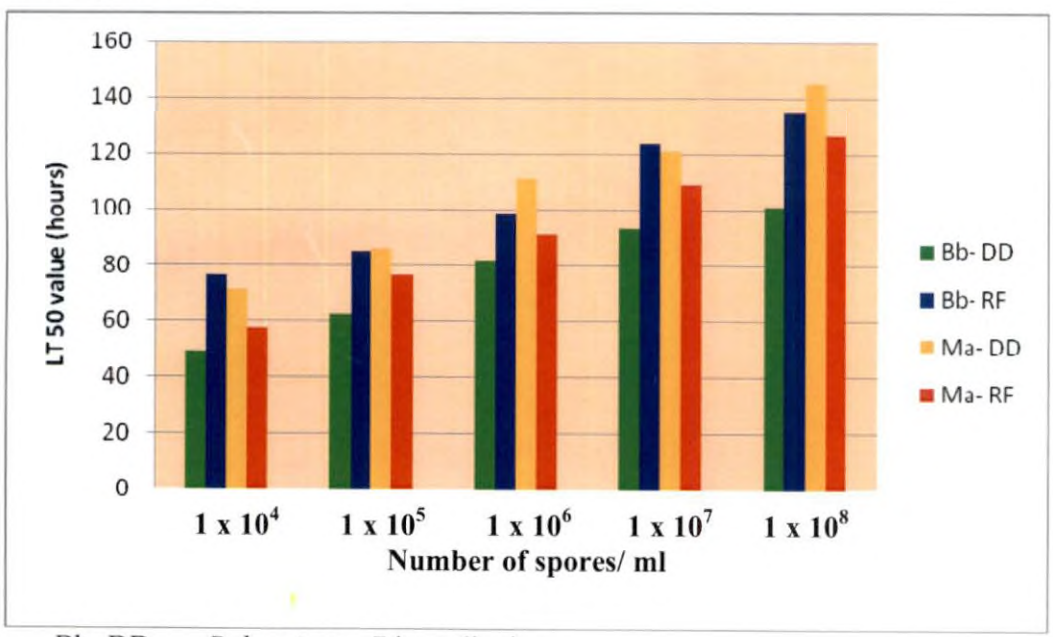


Fig. 6. LT $_{50}$ values of entomopathogens against *C. maculatus* by two bioassay techniques



Bb- DD - *B. bassiana* - Direct dipping

Bb- RF - *B. bassiana* - Residue film

Ma- DD - *M. anisopliae* - Direct dipping

Ma- RF - *M. anisopliae*- Residue film

Lemongrass oil caused 50 per cent mortality at 3.93 ppm and 7.51 ppm was required to cause 90 per cent mortality (Table 11). The toxicity of a number of essential oils has been evaluated against stored product insects. Klingauf *et al.* (1983) studied fumigant toxicity of 16 essential oils against *Acanthoscelides obtectus.*

5.2. TIME MORTALITY EFFECT OF BIOINSECTICIDES

LT_{50} values for *B. bassiana* and *M. anisopliae* at all the tested concentrations were estimated by probit analysis. Results showed LT_{50} values of the entomopathogens consistently increased as the conidia concentration decreased (Table 12). LT_{50} values for *B. bassiana* (1×10^8 to 1×10^4 spores/ml) varied from 49.26 to 101.24 hours (direct dipping) and 76.67 to 135.15 hours by residue film bioassay (Fig. 6). *B. bassiana* at 1×10^8 spores/ml caused 90 per cent mortality in 92.77- 138.99 hours by direct dipping bioassay. *M. anisopliae* caused 50 per cent mortality in 71.69 hours (direct dipping) and 57.64 hours (residue film) at 1×10^8 spores/ml. Out of the two entomopathogenic fungi, *M. anisopliae* (1×10^8 spores/ml) recorded shorter LT_{90} (4.2 days) than *B. bassiana* (4.5 days). However, the difference in LT_{50} between the two entomopathogens was not significant as both *B. bassiana* and *M. anisopliae* caused 90 per cent mortality within four to five days. Mahdneshin *et al.* (2009) reported that LT_{50} of *B. bassiana* isolates varied from 6.77 to 9.28 days and those for *M. anisopliae* varied from 7.48 to 8.25 days against *R. dominica.* The median lethal time value of *B. bassiana* against *S. zeamais* was between 2.74 to 8.75 days (Adane *et al.,* 1996). Different isolates of *M. anisopliae* and *B. bassiana* provided good control of *C. maculatus* by immersion bioassay and LT_{50} values for *B. bassiana* varied from 3.11 to 6.13 days, with an average of 4.60 days and those of *M. anisopliae* isolates values varied from 3.27 to 5.62 days, with an average of 4.60 days (Cherry *et al.,* 2005).

Spinosad brought about 50 per cent mortality in 20.51 to 33.09 hours (1.38 days) and 90 per cent mortality was effected in 2.6 to 3.3 days (Table 13).

Lemongrass oil caused 50 per cent mortality to *C. maculatus* in one day (23.8 hours) and 90 per cent mortality occurred in 3.4 days.

A summary on the time-mortality effect indicated that among four bioinsecticides, both spinosad and lemongrass oil recorded lower LT_{50} and LT_{90} values than *B. bassiana* and *M. anisopliae.* Spinosad and lemongrass oil caused 50 per cent mortality in one day while 90 per cent mortality was achieved in three days. The entomopathogenic fungi, *B. bassiana* and *M. anisopliae* brought about 50 and 90 per cent mortality in 2-3 and 4-5 days.

5.3. BIOLOGICAL EFFICIENCY OF BIOINSECTICIDES

The bioefficacy of *B. bassiana, M. anisopliae,* spinosad and lemongrass oil against *C. maculatus* was assessed by studying their effect on adult mortality, fecundity, egg hatchability, progeny emergence, developmental period, extent of infestation, grain weight loss and seed viability.

5.3.1. Adult mortality

Results on the adult mortality of *C. maculatus* by bioinsecticides indicated that mortality showed a positive relation with exposure time. Spinosad and lemongrass oil caused highest mortality of the introduced adults of *C. maculatus* (Table 15, Fig. 7). Spinosad caused 75.87 to 100 per cent mortality within 1-3 days of treatment. Similar results were reported by Sanon *et al.* (2010) who observed increased mortality of adults of *C. maculatus* that were exposed to cowpea seeds immediately after the seeds had been treated with spinosad. As reported for other insecticides, the efficacy of spinosad depends on the duration of insect exposure. Spinosad's suitability as a stored grain protectant has been

progressively highlighted in a series of publications from 1999 (Subramanyam *et al.*, 1999; Subramanyam *et al.*, 2002 and Mutambuki *et al.*, 2002)

Lemongrass oil with its fumigant effect caused 87.5 per cent mortality at 24 hours and 100 per cent mortality at three days after treatment. The fumigant toxicity effect of a number of plant essential oils has been evaluated against *C. chinensis* by Deshpande and Tipinis (1979). Effectiveness of essential oils had shown great promise for the control of major stored product insects and they were found to be active fumigants at low concentrations against these insects (Shaaya *et al.*, 1991). Volatile oils mostly affect adult beetles. The vapours have a repellent effect causing the beetles to fly away. A major advantage of volatile oils is that they need not be mixed with seeds and no physical contact is required between seeds and protectant as the effect is through fumigation (Rajapakse, 2006). The fumigant toxicity of lemongrass oil against *C. maculatus* has been reported by Raja and William (2008) who observed a lower adult mortality of 92 per cent at 96 hours after treatment with lemongrass oil. Parugrug and Roxas (2008) reported that lemongrass oil was found effective in deterring a wide variety of insects. The volatile oil of *Cymbopogon citratus* was found effective as repellent against cowpea beetle, *C. maculatus* Fab. *C. citratus* gave a higher adult mortality compared to the others. Fumigant efficacy of essential oils were also reported by Srinivas (2008) who found that oils of 11 species of plants acted as effective fumigants against *C. chinensis.*

Coconut oil caused direct toxicity effect by causing adult mortality that ranged from 28.45 (one day after treatment) to 100 per cent at five day after treatment. Singh (2003) proved coconut oil as a protectant of pigeon pea seeds against *C. chinensis*. Several authors have reported the use of plant oils against *Callosobruchus* adults. Varma and Pandey (1978) showed groundnut and other oils gave complete protection against *C. maculatus.*

The entomopathogenic fungi, *B. bassiana* and *M. anisopliae* as compared to other treatments were found to cause lower mortality of introduced adults. *B. bassiana* caused 9.91 to 27.27 per cent while *M. anisopliae* brought about 12.36 to 45.45 per cent mortality from one to six days after treatment.

Based on direct toxicity effect on adult mortality, the decreasing order of effectiveness of bioinsecticides is

Lemongrass oil > spinosad > Coconut oil > *M. anisopliae* > *B. bassiana*

5.3.2. Surface protectant action of bioinsecticides against *C. maculatus*

5.3.2.1. Oviposition deterrency and egg hatching inhibition

Among the four bioinsecticides, spinosad was most effective as an inhibitor of oviposition and egg hatchability of *C. maculatus* (Table 16; Fig. 8). Spinosad caused highest oviposition deterrency (81.65 per cent) as indicated by the lowest fecundity (34.25) recorded. It was also found to be an effective inhibitor of egg hatching as evident from the higher rate of hatching inhibition (82.85 %) and lowest number of hatched eggs (67.63). The death of introduced adult bruchids caused by spinosad led to a decrease in number of eggs laid by the females. The present findings are in accordance with Sanon *et al.* (2010) who reported that spinosad significantly reduced viability of eggs of *C. maculatus.*

Next to spinosad, lemongrass oil was found to be more effective as it brought about 74.16 per cent deterrency in oviposition and 68.36 per cent egg hatching inhibition. This finding is in consonance with Raja and William (2008) who also revealed significant ovicidal action of lemongrass oil against *C. maculatus.* Risha *et al.* (1990) reported that oil vapour possess a strong toxic effect to eggs of *C. phaseoli.* Singh (1995) suggested that the toxic principle present in the volatile oils could block the micropyle region of egg chorion

Fig. 7. Direct toxicity effect of bioinsecticides on adults of *C. maculatus*

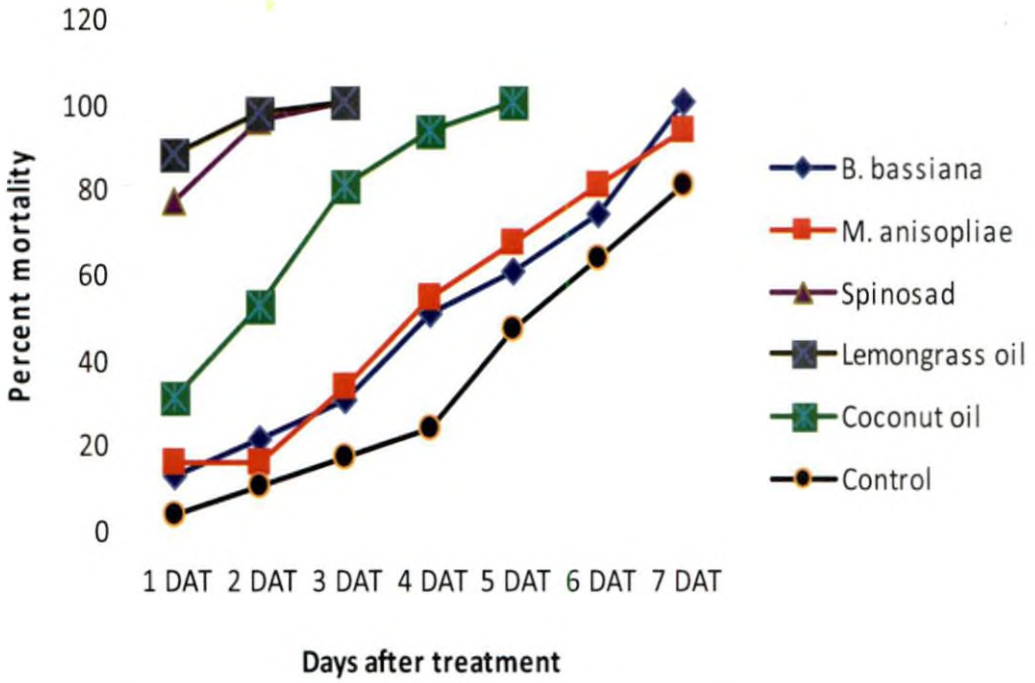
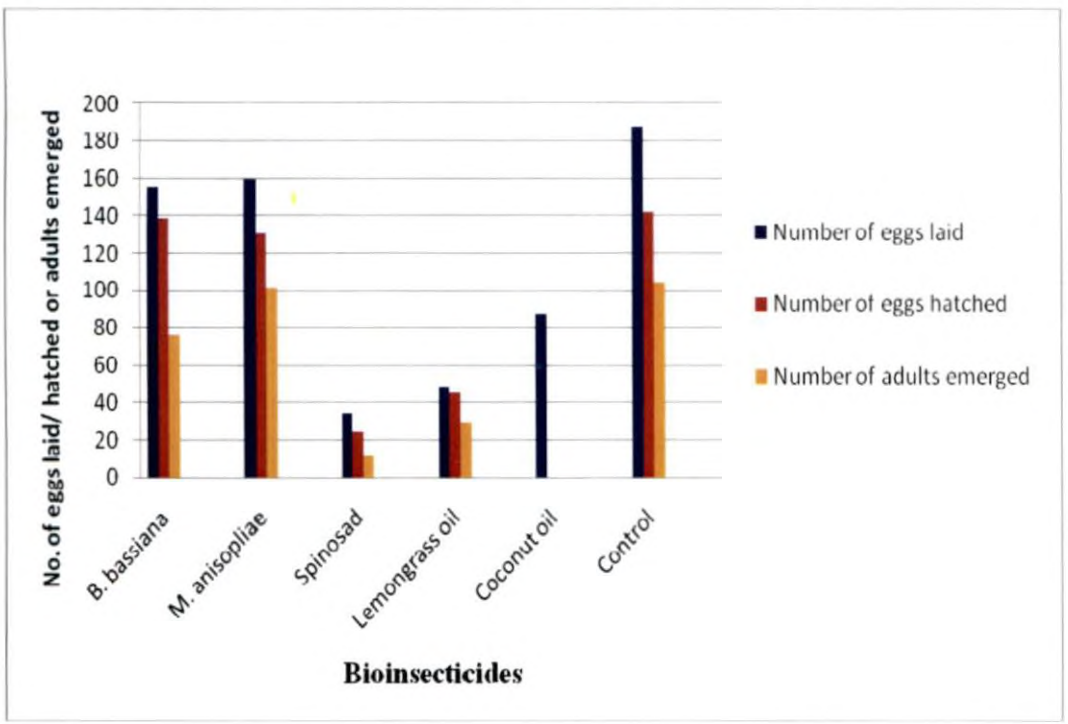


Fig. 8. Surface protectant action of bioinsecticides on *C. maculatus*



leading to the death of embryo due to depletion of oxygen for respiration.

Though coconut oil caused only 53.41 per cent deterrency for oviposition but it acted as the most efficient inhibitor of egg hatching (100 %) as it showed no hatching of eggs. Singh *et al.* (1978) proposed that the toxicity of vegetable oil was primarily to the eggs of *C. maculatus* and that the effect was both physical and chemical. This view was supported by Van schoonhoven (1978) who showed that vegetable oils of different purity varied in toxicity to eggs. There have been various reports of oils causing reduced oviposition and higher egg and adult mortality, but all not agreed (VanHuis, 1991) and indicated great discrepancy about the extent of protection offered by the oils against stored products insects (Pereira, 1983; Doharey *et al.*, 1988). Kachare *et al.* (1994) observed that coconut oil and other vegetable oils had repellent action for egg laying and effective in suppressing egg hatching. Coconut oil has been reported to cause inhibition of oviposition of bruchids (Sharma *et al.,* 1999). Rajapakse (2006) observed reduced oviposition of *C. maculatus.* Tripatti *et al.* (2007) observed significant oviposition reduction in bruchids by plant oils.

B. bassiana and *M. anisopliae* exhibited significantly lower effect on oviposition and hatching as evident from lower values for oviposition and hatching inhibition. *B. bassiana* and *M. anisopliae* caused 17 and 14.86 per cent deterrency of oviposition. Egg hatching inhibitory effect of *B. bassiana* and *M. anisopliae* were 2.47 and 7.97 per cent.

5.3.2.2. Progeny emergence

No progeny production was observed in the treatment of coconut oil and thus it indicated 100 per cent inhibitory effect on progeny emergence (Fig. 8). This is due to its cent per cent inhibitory effect on egg hatching. Similar reports were made by Bhatnagar *et al.* (2001) and Singh *et al.* (1978) with coconut oil inhibiting progeny emergence of *C. maculatus.*

Spinosad was also effective as a progeny inhibitor by causing 88.2 per cent inhibition of progeny emergence and lemongrass oil brought about 72 per cent inhibition of progeny production. This finding is in accordance with Sanon *et al.* (2010) who also observed a reduction in emergence of adults of *C. maculatus* from spinosad treated cowpea seeds. Progeny production of *R. dominica* was highly suppressed in wheat, corn, rice, and barley by spinosad and indicated that spinosad could be used with success against stored pests by taking into account the target species and the commodity (Vayias *et al.*, 2009).

B. bassiana and *M. anisopliae* were observed to be not effective in inhibiting progeny emergence of *C. maculatus* in cowpea as indicated by higher progeny emergence. These entomopathogenic fungi caused higher fecundity and egg hatchability of *C. maculatus* in the experiment. Similar findings were reported from Project Directorate of Biological Control and Punjab Agricultural University (PDBC, 2006) where in it was found that progeny emergence of *Callosobruchus* spp. during storage of green gram after treatment with three isolates of *B. bassiana* and *M. anisopliae* was on par with that of control. The present finding is contrary to Ekesi *et al.* (2001) and Cherry *et al.* (2005) who observed reduced progeny emergence of *C. maculatus* in cowpea seeds treated with indigenous isolates of *B. bassiana* and *M. anisopliae.* The difference in the present finding might be due to the difference in the virulence of strains of entomopathogenic fungi used in the present study.

Even though the different bioinsecticides indicated significant effect on progeny emergence, the mean developmental period of *C. maculatus* was found to be on par with that of untreated control. The developmental period from egg to adult ranged from 22-23 days in all the treatments thus indicating no effect of bioinsecticides on the developmental period of *C. maculatus.*

5.3.3. Extent of infestation

Extent of infestation is a measure of damage caused by a pest. Data on the effect of bioagents on the extent of infestation by *C. maculatus* revealed that coconut oil was the most effective treatment as it caused zero infestation (Table 18; Fig. 9). Spinosad and lemongrass oils were the next effective ones with 49- 52 per cent infestation. Both were equally effective in reducing the infestation by 37.35 to 40.96 per cent over control. Entomopathogens were found to be not effective in reducing the infestation as they were on par with control. *B. bassiana* and *M. anisopliae* treatments resulted in 83 and 87 per cent damage in cowpea seeds that was on par with untreated seeds (91 per cent). This is in consonance with the report of PDBC (PDBC, 2006) where in 96.8- 99.2 per cent damage was observed in green gram seeds treated with *B. bassiana* and *M. anisopliae* as against 99.6 per cent damage in untreated control. Similar results were reported from PAU (PDBC, 2006) also where in 100 per cent grain damage was observed in all the isolates of *B. bassiana* and *M. anisopliae* treated seeds except that of one per cent groundnut oil. Sanon *et al.* (2010) observed less than 20 per cent infestation by *C. maculatus* after five months of storage of cowpea seeds treated with spinosad. It was found to be more effective in controlling *C. maculatus* than deltamethrin.

5.3.4. Weight loss

Results on the weight loss caused by *C. maculatus* in cowpea grains after one month of treatment with bioinsecticides revealed that coconut oil was the most effective treatment wherein no loss in weight occurred as compared to highest weight loss (34.4 per cent) in control (Table 19; Fig. 10). Varma and Pandey (1978) reported coconut oil to be more protective than groundnut and sesamum oil against *C. maculatus*. Spinosad also showed significant effect in protecting cowpea grains as evident from the lowest weight loss of 1.9 per cent and 94.45 per cent reduction of loss in weight as compared to untreated control.

Fig. 9. Effect of bioagents on extent of infestation by *C . maculatus*

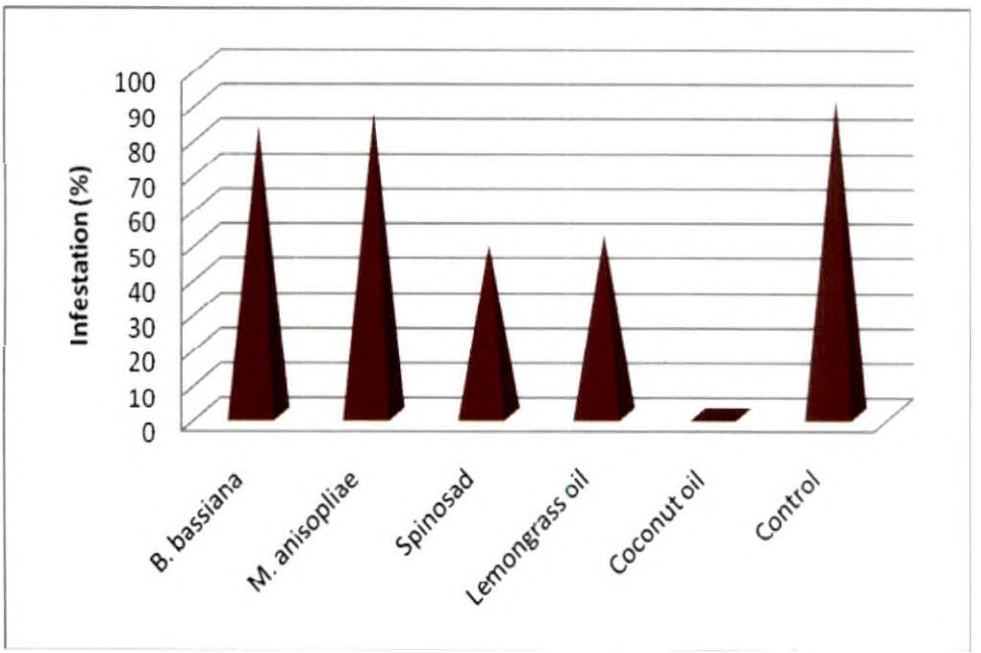
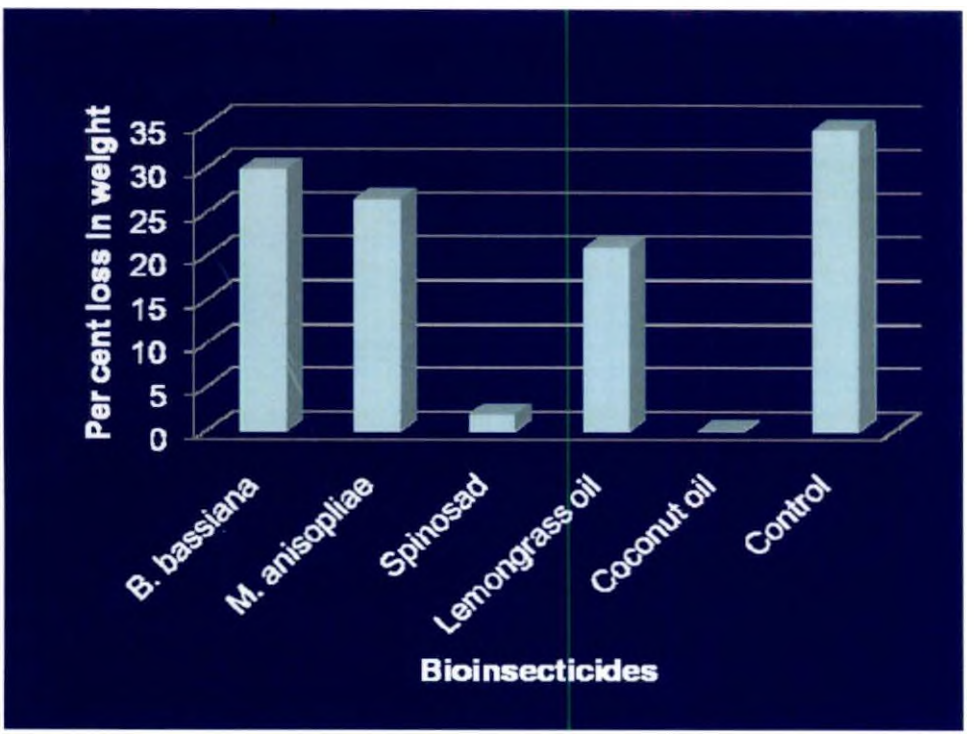


Fig. 10. Effect of bioagents on weight loss caused by *C. maculatus*



Similar findings were reported by Sanon *et al.* (2010) wherein spinosad controlled *C. maculatus* for six months of cowpea storage. Singal (1995) observed that vegetable oils proved to be effective against *C. maculatus* upto eight months of storage in terms of seed damage and weight loss.

Next to spinosad, lemongrass oil proved to be effective against weight loss in cowpea by *C. maculatus* in storage. It caused 20.89 per cent weight loss and 39.29 per cent reduction of weight loss as compared to control (34.4%). Treatment with *B. bassiana* and *M. anisopliae* resulted in minimum reduction (13-23%) of seed weight loss and weight loss was on par with control and hence proved to be not effective grain protectants. This finding is in conformity with the report by PDBC (PDBC, 2006) wherein it was reported that cowpea grain damage in *B. bassiana* and *M. anisopliae* isolate treated seeds were on par with untreated control.

5.3.5. Germination

Untreated cowpea seeds with *C. maculatus* infestation, after 30 days of storage indicated lowest germination (50%). Treatments with bioinsecticides caused no adverse effect on germination of cowpea seeds as evident from a higher germination than control (Table 20; Fig. 11). Spinosad showed highest germination (95%) as against the lowest germination (50 per cent) in untreated control. This might be due to the highest protective potential of spinosad and the highest insect damage in untreated seeds. The results are consistent with Sanon *et al.* (2010) who observed higher germination rates for cowpea seeds treated with spinosad. Though coconut oil treatment resulted in cent per cent reduction of infestation , it produced only 60 per cent increase in germination over control thus indicating certain adverse effects on germination.

This corroborates Shaaya and Sukprakarn (1994) who reported that edible oils had detrimental effects on seed germination which make them unsuitable for insect control in grains meant for seed purpose and thus can be used in seeds meant for consumption.

Next to spinoasd, lemongrass oil treatment resulted in 80 per cent more germination as compared to untreated control. *B. bassiana* and *M. anisopliae* treatments showed lower germination of cowpea seeds.

5.4. PERSISTENT TOXICITY OF BIOINSECTICIDES IN COWPEA STORAGE

Among the bioinsecticides, spinosad showed highest persistent toxicity (5699.7) and lemongrass oil indicated lowest PT index (Table 21; Fig. 12). Toxicity of spinosad persisted upto 90 days while lemongrass oil showed lower persistence only upto 15 days. Both *B. bassiana* and *M. anisopliae* showed lower persistent toxicity effects on the protection of cowpea grains against *C. maculatus*. The lack of light during storage of grains probably might have contributed to the persistence of spinosad as it degrades only slowly in dark (Saldago, 1998). The present finding on persistent toxicity of spinosad is in conformity with Sanon *et al.* (2010).

The results of the present study are consistent with those of many earlier studies reporting that spinosad is effective against several insect pests (Liang *et al.*, 2002; Toews *et al.*, 2003; Flinn *et al.*, 2004; Sanon *et al.*, 2010).

The broad spectrum activity of spinosad may be explained by its unusual mode of action. Spinosad is a nervous poison, acting on the nicotinic acetyl choline and gamma amino butyric acid (GABA) receptor sites of the insect nervous system (Saldago, 1998). The bioinsecticide spinosad is attractive as an alternative to the synthetic insecticides because, although it has long term action

Fig. 11. Effect of bioagents on germination of cowpea seeds infested by *C. maculatus*

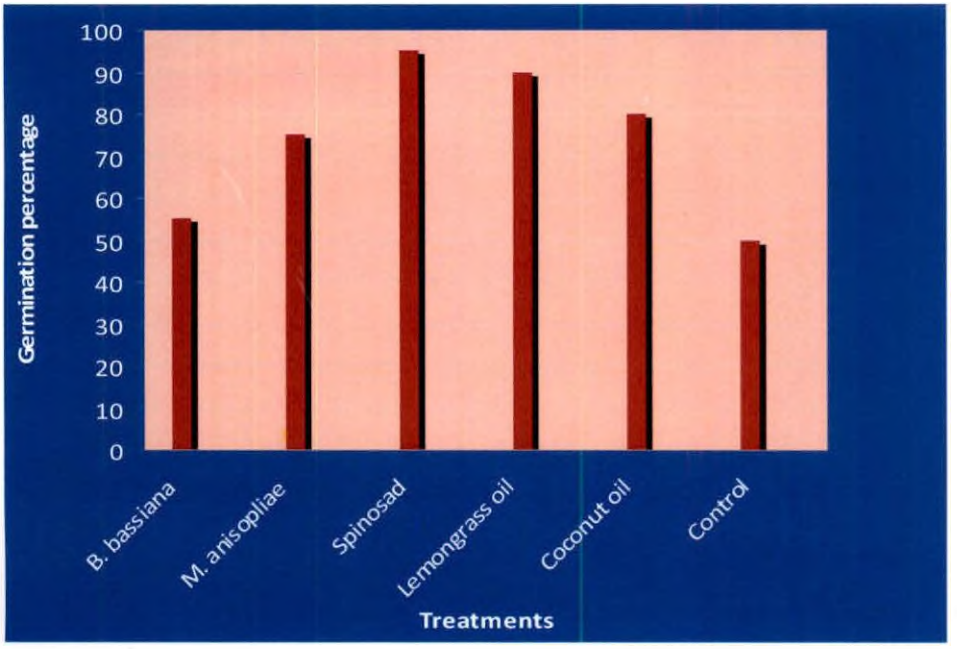
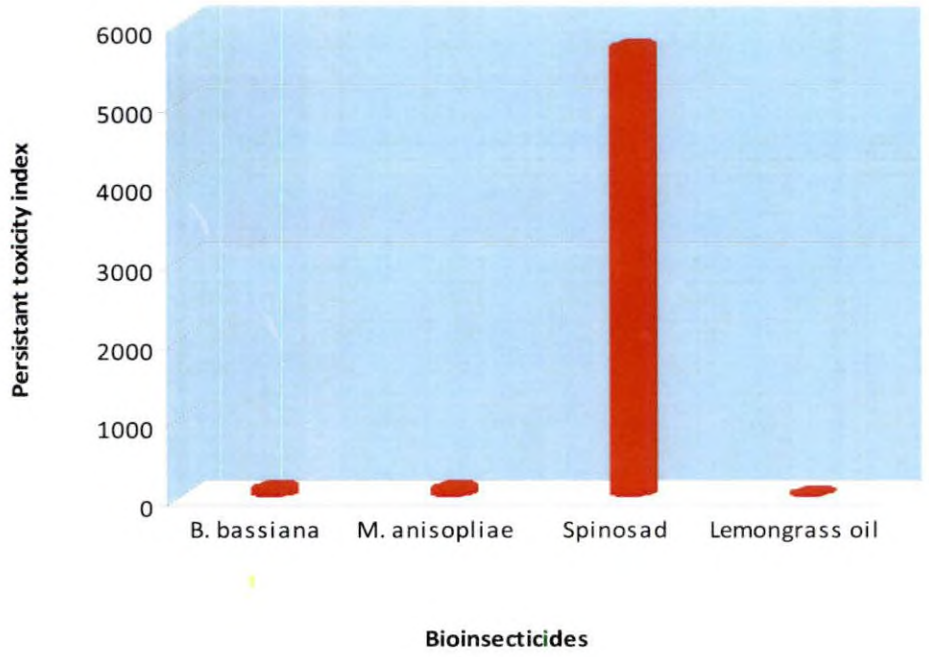


Fig. 12. Persistent toxicity of bioinsecticides in cowpea storage



in storage, it is harmless to mammals and lacks other environmental side effects (Thompson *et al.,* 2000). In addition to replace synthetic insecticides, spinosad can be used to manage resistance to synthetic insecticides (Huang and Subramanyam, 2004). Spinosad is considered as a natural product and thus approved for use in organic agriculture by numerous national and international certification bodies (Cleveland, 2007).

It can be concluded that spinosad could be considered as a new bioagent for protecting cowpea grains from *C. maculatus* during post harvest storage. The combination of high efficacy, broad spectrum, low mammalian toxicity, persistence and sound environmental profile will be unique among existing grain protectants and can positively impact global food security (Hertlein *et al.*, 2011).


Summary

6. SUMMARY

The study entitled 'Potency of bioinsecticides against cowpea bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) in storage' was carried out in the Department of Agricultural Entomology, College of Horticulture, Vellanikkara, Thrissur during 2010-11. Four bioinsecticides *viz.*, two entomopathogenic fungi-*B. bassiana* and *M. anisopliae;* an actinomycete based insecticide of naturalyte family-spinosad, and a plant derived essential oil-lemongrass (*Cymbopogon flexuosus*) oil were evaluated for their dosage-mortality response, time-mortality effect, bioefficacy and persistent toxicity in protecting cowpea grains against *C. maculatus* in storage. The salient findings of the present investigation are summarized below.

The dosage-mortality response of the entomopathogenic fungi-*B. bassiana* and *M. anisopliae* were tested at five concentrations from 1 x 10^4 to 1 x 10^8 spores/ml on *C. maculatus* by two methods of bioassay *viz.* direct dipping and residue film. The mortality of *C. maculatus* treated with *B. bassiana* and *M. anisopliae* indicated an increase in mortality rate with increase in dosage and period of exposure for a period of five days. Both *B. bassiana* and *M. anisopliae* were pathogenic and caused mortality to *C. maculatus.* At the highest concentration (1 x 10^8 spores/ml) both *B. bassiana* and *M. anisopliae* caused more than 90 per cent mortality on the fifth day of exposure. A lower concentration of 1 x 10^7 spores/ml was also found to be effective in causing a higher mortality at five days after treatment by the two bioassay methods. Cumulative mortality data of *C. maculatus* by *B. bassiana* and *M. anisopliae* at the five tested concentrations after five days of treatment revealed no significant difference in mortality by *B. bassiana* and *M. anisopliae* and the two bioassay methods thus indicating an equal effectiveness of the two entomopathogens and the two bioassay techniques for dosage-mortality response study. The cumulative

mortality of *B. bassiana* and *M. anisopliae* at 1 x 10^7 spores/ml ranged from 68.8 to 81.82 per cent which was on par with that of 1 x 10^8 spores/ml.

Spinosad 45 SC was tested at 1 to 10 ppm and the adult mortality increased significantly with increased dose. It caused 20 to 100 per cent mortality to *C. maculatus* by direct dipping bioassay. In residue film bioassay method of treating the cowpea grains, higher concentrations of spinosad (10 to 70 ppm) were required to cause 20- 96.6 per cent mortality in *C. maculatus*. Significantly higher mortality was shown by 60 ppm (80% mortality) and 70 ppm (96.67% mortality) of spinosad and both these concentrations were on par.

Lemongrass oil at eight concentrations (1 to 8 µl/l of air) was tested to study its fumigant toxicity action against *C. maculatus*. Results revealed that all concentrations of lemongrass oil were significantly effective in causing higher mortality to *C. maculatus*. The mortality ranged from 16.67 to 96.67 per cent with 1 to 8 ppm lemongrass oil.

Median lethal concentration value (LC_{50}) was lower for *M. anisopliae* (5.12 x 10^6 and 6.7 x 10^6 spores/ml) than *B. bassiana* (5.38 x 10^6 and 7.49 x 10^6 spores/ml) by direct dipping and residue film bioassay techniques indicating a slightly higher toxicity of *M. anisopliae* to *C. maculatus*. LC_{50} of spinosad was 4.02 ppm in direct dipping bioassay where as a higher LC_{50} value (36.39 ppm) was observed by residue film bioassay. Lemongrass oil caused 50 per cent mortality at 3.93 ppm and 7.51 ppm was required to cause 90 per cent mortality.

LT_{50} values for *B. bassiana* and *M. anisopliae* at all the tested concentrations were estimated by probit analysis and the results showed that LT_{50} values of the entomopathogenic fungi consistently increased as the conidial concentration decreased. LT_{50} values for *B. bassiana* (1 x 10^8 to 1 x 10^4 spores/ml) varied from

49.26 to 101.24 hours (direct dipping) and 76.67 to 135.15 hours by residue film bioassay. *M. anisopliae* caused 50 per cent mortality in 71.69 hours (direct dipping) and 57.64 hours (residue film) at 1 x 10^8 spores/ml. Out of the two entomopathogenic fungi, *M. anisopliae* (1 x 10^8 spores/ml) recorded shorter LT_{90} (4.2 days) than *B. bassiana* (4.5 days). Spinosad brought about 50 per cent mortality in 20.51 to 33.09 hours (1.38 days) and 90 per cent mortality was effected in 2.6 to 3.3 days. Lemongrass oil caused 50 per cent mortality to *C. maculatus* in one day (23.8 hours) and 90 per cent mortality occurred in 3.4 days

The biological effectiveness of bioinsecticides *viz., B. bassiana, M. anisopliae,* spinosad and lemongrass oil against *C. maculatus* was assessed along with coconut oil by studying their effects on adult mortality, fecundity, egg hatchability, progeny emergence, developmental period, extent of infestation, grain weight loss and seed viability.

Results on the adult mortality of *C. maculatus* by bioinsecticides indicated that mortality showed a positive relation with exposure time. Spinosad and lemongrass oil caused cent per cent mortality to the introduced adults of *C. maculatus* within three days of exposure and coconut oil reported cent per cent mortality within five days of exposure. Seven days were required for *B. bassiana* and *M. anisopliae* to bring about adult mortality.

Among the four bioinsecticides, spinosad was the most effective one as an inhibitor of oviposition and egg hatchability of *C. maculatus.* Next best oviposition inhibitor was lemongrass oil. Though coconut oil caused only 53.41 per cent deterrency for oviposition but it was found to be the most efficient inhibitor of egg hatching. *B. bassiana* and *M. anisopliae* exhibited significantly lower effects on oviposition and hatching of eggs of *C. maculatus.*

On comparing the progeny emergence of *C. maculatus* in the bioinsecticide treated cowpea grains, it was observed that no progeny was produced in the grains treated with coconut oil. Spinosad was also very effective as a progeny inhibitor as it caused 88.2 per cent inhibition of progeny emergence and lemongrass oil brought about 72 per cent inhibition of progeny production. *B. bassiana* and *M. anisopliae* were not effective in inhibiting the progeny emergence of progenies of *C. maculatus*.

Data on the effect of bioagents on the extent of infestation by *C. maculatus* in stored cowpea revealed that coconut oil was the most effective treatment as it caused zero infestation. Spinosad and lemongrass oil were the next effective ones. *B. bassiana* and *M. anisopliae* were found to be not effective in reducing seed damage.

Results on the weight loss caused by *C. maculatus* in cowpea grains after one month of treatment with bioinsecticides revealed that coconut oil was the most effective treatment wherein no loss in weight occurred. Spinosad also showed significant effect in protecting cowpea grains as evident from the lowest weight loss of 1.9 per cent as compared to 34.41 per cent weight loss in untreated control. It could reduce the weight loss by 94.5 per cent over control. Lemongrass oil was the next effective one as it brought about 39.3 per cent reduction of weight loss. *B. bassiana* and *M. anisopliae* were not effective as they could reduce the weight loss by only 26.5 to 30 per cent.

Treatment of cowpea seeds with the tested bioinsecticides viz., *B. bassiana* *M. anisopliae,* spinosad, lemongrass oil and coconut oil caused no adverse effect on the seed viability. Spinosad showed highest germination (95%) as against the lowest germination (50 per cent) in untreated control. This might be due to the highest protective potential of spinosad and the highest bruchus damage in untreated seeds. Lemongrass oil treatment resulted in 80 per cent more germination as compared with that of control. *B. bassiana* and *M. anisopliae* treated grains showed lower

germination of 55 and 75 per cent as compared to 50 per cent germination in the untreated seeds of cowpea after a period of 30 days of treatment.

In the study on the persistent toxicity of bioagents for 90 days on cowpea seeds, spinosad showed highest persistence with a highest PT value of 5699.7. All other bioagents showed lesser persistence. Lemongrass oil exhibited least persistence with the lowest PT index (16.5). *B. bassiana* and *M. anisopliae* showed lower persistence with PT values of 83.34 and 83.31. Higher persistence of spinosad was evident even at 90 days after treatment as it caused 56.66 per cent mortality.

From the study it can be concluded that spinosad is the most effective bioinsecticide against *C. maculatus* in storage. Spinosad has good direct toxicity effect and surface protectant action against *C. maculatus* in cowpea storage. Its persistent action was more than 90 days and hence we can recommend it as a safe alternative to synthetic chemicals. Lemongrass oil can also be considered as an alternative grain protectant with a limitation of short persistent action. Entomopathogens-*B. bassiana* and *M. anisopliae* are not at all effective in cowpea storage because of its delayed effect on adult mortality of *C. maculatus,* least surface protectant action and reduced persistence.

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Appendices

APPENDIX- 1

Key to *Callosobruchus* species

(1) Inner tooth of hind femur conspicuously longer than the short blunt outer tooth. Body cuticle black, with a pattern of grey, black and brassy setae on the dorsum. Length 2.75-3.0 mm. .....***C. udemptus*** (Sharp)

- Inner tooth of hind femur usually only as long as, or shorter than, outer tooth; if inner tooth is longer than outer, then colour is not as above. Body cuticle and setae not as above; if cuticle is black, then setal pattern on dorsum is indistinct, inner tooth is only slightly longer than outer tooth, and length is more than 4 mm.....2

(2) Body cuticle uniformly black or very dark brown, occasionally with dark reddish highlights on legs and antennae; setae grey or brown, never forming a distinct pattern on the elytra, but usually with a vague pattern of whitish setae on the elytra of females. Length 4.0-5.5 mm. (Mainly restricted to West Africa, also recorded occasionally in parts of the Caribbean and South America, rarely intercepted elsewhere; usually infesting Bambarra groundnuts *(Vignu (= Voandzeia) subterrunea)* but able to breed on some other pulses. . . . . .***C. subinnotutus*** (Pit)

- Body colour never as above: cuticle usually with pale marks (red, yellowish-red, or brown); these and the setae on the elytra of mature specimens forming a distinct and contrasting colour pattern. Length usually less than 4 cm.....3

(3) Inner tooth of hind femur less than half as long as outer tooth, sometimes very small or absent. Pronotum with uniformly reddish-brown cuticle and with sparse golden setae everywhere, except on basal median gibbosities which have the usual

(though sparse) white setae. (Mainly restricted to South and South-East Asia; very rarely reported elsewhereand, except for a confirmed record in Tanzania, most non-Asian records are misidentifications; mainly infesting grams and cowpeas, but also recorded from several otherpulses)..... Normal form of ***C. unulis*** (Fabricius)

- Inner tooth of hind femur more than half as long as outer tooth, often equal in length, inner tooth sometimes longer than outer. Pronotum usually with a distinct pattern (in addition to the usual white patches of setae on the basal median gibbosities.....4

(4) Lateral margins of abdominal sternites 2 to 5 with dense patches of coarse white setae: these setae are much coarser and whiter than the other setae on the sternites.....5

- Lateral margins of abdominal sternites without dense patches of coarse white setae: instead with the same fine whitish or yellowish setae as elsewhere on the sternites.....7

(5) Eyes very prominent in dorsal view: ratio of dorsal width of one eye to minimum distance between eyes 5-6: 1 in males, 2-3: 1 in females. Body colour light and dark brown, mainly with whitish or silvery-cream setae but with patches of dark brown setae; elytra with a complex pattern of coloration and without any reddish colour. Occasionally, females may have black cuticle . Male genitalia (median lobe and parameres) very elongate; parameres spatulate at apex; median lobe with two sclerotized plates at about its mid-point. (Uncommon; mainly known from India and Sri Lanka on pigeon peas and other pulses, both in the field and in store, but also found in Indonesia.).....***C. theobromae*** (Linnaeus)

- Eyes not so prominent: ratio of dorsal width of one eye to minimum distance between eyes 2.5-3: 1 in males, 1.5: 1 in females. Body colour not as above: elytral pattern relatively simple; cuticle black and reddish-brown; setae whitish, yellowish and dark brown or black, following pattern of cuticle. Male genitalia not as above: if parameres are very elongate, then they are not spatulate and the median lobe is not elongate; if the median lobe has two sclerotized plates, they are basal.....6

(6) Male antennae pectinate, segments 4-10 conspicuously expanded antero-laterally; female antennae serrate; antennae of both sexes usually with segments 4-l 1 dark brown (rarely yellow-brown). Pygidium of female (and male) covered with white or silver setae. Inner tooth of hind femur with sides more or less parallel, converging near apex. Male genitalia: median lobe more elongate, apex with exophallic valve spearhead-shaped, and base with two sclerotized plates; parameres normal and rather broadly spatulate. (Major pest, especially of cowpeas and grams; especially common in the oriental region, but found throughout the tropics and subtropics.) .....
.....***C. chinensis*** (Linnaeus)

- Male and female antennae serrate; antennae yellow-brown (rarely darker). Pygidium of female mainly with yellowish or pale brown setae, but with a median longitudinal line of white setae; pygidium of male with white setae (as in above species). Inner tooth of hind femur narrowly triangular, acute. Male genitalia: median lobe less elongate, apex with exophallic valve triangular, and base with six sclerotized plates; parameres elongate, very thin, longer than median lobe, narrow and truncate at apex. (Infesting cowpeas and grams; mainly restricted to southern Africa, but is common in some parts of Africa and occurs occasionally in West Africa.....
.....***C. rhodesianus*** (Pit)

(7) Body yellowish-brown to brown, with grey and dark brown setae. Pronotum typically yellowish-brown or mid-brown with a pair of dark brown longitudinal marks either side of the midline; these lines sometimes reduced to paired dark brown spots. Inner tooth of hind femur as long as, or slightly longer than, outer tooth. Male genitalia: median lobe without sclerotized denticulate areas near the middle; parameres broadly spatulate. (Widespread in tropics, but not very common; often found infesting dolichos beans *(Lablab purpureus),* but also found on other pulses.).....C. ***phaseok*** (Gyllenhal)

- Colour not as above. Pronotum black or reddish-brown, never with dark brown marks on a lighter background. Inner tooth of hind femur as long as outer tooth, or shorter. Male genitalia not as above: either median lobe with two sclerotized areas near the middle, or parameres rather slender and only narrowly spatulate.....8

(8) Inner carina of hind femur smooth; inner tooth typically as long as, or very slightly longer than, outer tooth. Pronotum of mature specimens with black cuticle, and with golden setae, except on the basal median gibbosities, which extend well beyond the posterior margin and are covered with white scale-like setae. Eyes very deeply emarginate, prominent and bulbous. Male genitalia distinctive: median lobe with two longitudinal sclerotized denticulate areas near its middle; parameres rather stout and broadly spatulate. (Major pest of several pulses, but especially cowpeas and grams, throughout the tropics and subtropics.....
.....***C. maculatus*** (Fabricius)

- Inner carina of hind femur with numerous irregularly-spaced small denticles along its proximal two-thirds; inner tooth rather shorter than, or as long as, outer tooth.

Pronotum with uniformly reddish-brown cuticle, and with sparse golden setae, except on the basal median gibbosities, which extend only slightly beyond the posterior margin and have sparse white setae. Eyes less deeply emarginate, rather flattened and less prominent. Male genitalia: median lobe without sclerotized areas near its middle; parameres rather slender and only narrowly spatulate. (At present, known only from Indonesia, infesting green gram and white..... soya.)......"Long toothed" form of ***C. analis*** (Fabricius).

POTENCY OF BIOINSECTICIDES AGAINST THE COWPEA BRUCHID, *Callosobruchus maculatus* (F.) (COLEOPTERA: CHRYSOMELIDAE) IN STORAGE

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ABSTRACT OF THE THESIS

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ABSTRACT

The present study entitled 'Potency of bioinsecticides against cowpea bruchid, *Callosobruchus maculatus* (F.) (Coleoptera: Chrysomelidae) in storage' was undertaken to understand the feasibility of utilizing few biologically based grain protectants in post harvest storage of cowpea by investigating their biological efficacy and persistent/ residual toxicity.

The bioinsecticides under study were two entomopathogenic fungi *Beauveria bassiana* and *Metarhizium anisopliae*; spinosad, an actinomycete based broad spectrum insecticide with low mammalian toxicity and lemongrass oil- an essential oil from the aromatic plant, *Cymbopogon flexuosus*.

Laboratory bioassays were carried out to investigate the dosage-mortality response of the bioinsecticides against *C. maculatus*. Toxicity of entomopathogens was studied by two bioassay techniques-direct dipping and residue film. *B. bassiana* and *M. anisopliae* were tested at five different concentrations 1 x 10^4, 1 x 10^5, 1 x 10^6, 1 x 10^7 and 1 x 10^8 spores/ml. Observations on mortality were taken at 24 hours interval upto five days. Results on the mortality of *C. maculatus* indicated an increase in mortality with increase in dosage and period of exposure.

Cumulative mortality data of *C. maculatus* by *B. bassiana* and *M. anisopliae* at five days after treatment with five concentrations revealed no significant difference in mortality between the two entomopathogens and the two bioassay methods.

Probit analysis of dosage-mortality response of *B. bassiana* and *M. anisopliae* indicated lower LC_{50} value for *M. anisopliae* than *B. bassiana* (5.12 x 10^6 and 6.7 x 10^6 spores/ml) than *B. bassiana* (5.38 x 10^6 and 7.49 x 10^6 spores/ml). However the difference was not remarkable.

Time-mortality effect of *B. bassiana* and *M. anisopliae* indicated an increase in LT_{50} values as the spore concentration decreased.

Toxicity of spinosad to *C. maculatus* was also investigated by direct dipping and residue film bioassay methods. LC_{50} of spinosad was 4.02 ppm by direct dipping where as a higher LC_{50} value (36.39 ppm) was observed by residue film bioassay. Spinosad brought about 50 per cent mortality in 20.51 to 33.09 hours and 90 per cent mortality in 2.6 to 3.3 days.

Lemongrass oil, at eight concentrations (1 to 8 µl/l of air), was tested to study the fumigant toxicity action against *C. maculatus* and the mortality ranged from 16.67 to 96.67 per cent. Lemongrass oil caused 50 per cent mortality at 3.93 ppm in one day and 90 per cent mortality at 7.51 ppm in 2.6 days.

Bio-efficacy of the four bioinsecticides along with coconut oil as a recommended check was assessed for a period of 30 days in the storage by studying their effects on adult mortality, fecundity, egg hatchability, progeny emergence, developmental period, extent of infestation, grain weight loss and seed viability.

Regarding adult toxicity, lemongrass oil caused highest adult mortality (87.1 to 100%) followed by spinosad (75.87 to 100%) at three days after treatment. Spinosad was most effective as an inhibitor of oviposition and egg hatchability. On comparing the progeny emergence in different treatments, no progenies were produced in the cowpea grains treated with coconut oil. Spinosad and lemongrass oil also showed higher inhibition of progeny emergence. Data on the effect of bioagents on the extent of infestation by *C. maculatus* revealed that coconut oil was the most effective treatment as it caused zero infestation. Spinosad and lemongrass oil were the next effective ones as they resulted in 49 and 52 per cent reduction of seed damage. Entomopathogens were found to be ineffective in reducing bruchus damage to seeds. Effects on weight loss also

recorded the same trend. Results on the impact of bioagents on seed viability indicated that spinosad treated grains showed the highest germination followed by lemongrass oil and coconut oil.

Results of the persistent toxicity action of the tested bioinsecticides revealed that spinosad had highest persistence with a PT value of 5699.7. All other bioagents showed less persistence. Lemongrass oil showed the least persistent toxicity against in storage.

It can be concluded from the present investigation that among the four bioagents, spinosad is the most effective one in terms of bioefficacy as well as persistent action in cowpea seeds. It can be recommended as an alternative option for bruchid management in storage. The existing recommendation of coconut oil is also proved to be effective against *C. maculatus* in storage of cowpea seeds. Lemongrass oil is also a very effective bioagent as a fumigant for protecting cowpea grains from *C. maculatus* with a limitation of short persistence that warrants frequent applications in storage. *B. bassiana and M. anisopliae* are not effective against *C. maculatus* in cowpea storage.