Biopesticides: Alternatives for management of Callosobruchus maculatus

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ABSTRACT

Among insect pests of stored legumes, *Callosobruchus maculatus* is ranked as the most important with seed loss as high as 60-100% having been reported. Chemical control using synthetic insecticides is usually advocated. These chemicals can have undesirable side effects including human and animal health concerns, development of insecticide resistance and environmental contamination. Biopesticides, especially the use of semiochemicals, botanical extracts and microbial pesticides provide a sustainable and innovative alternative to the sole use of synthetic insecticides. The components of the sex pheromone for *C. maculatus* have been identified as 3-methyleneheptanoic acid, (Z)-3-methyl-3-heptenoic acid, (E)-3-methyl-3-heptenoic acid, (Z)-3-methyl-2-heptenoic acid and (E)-3-methyl-2-heptenoic acid, nonanedioic acid and 2,6-dimethyl-1, 8-octanedioic acid. The use of semiochemicals and pheromones in particular can allow for better monitoring of *C. maculatus* in warehouses and other grain storage facilities. In small scale situations, use of pheromones for mass trapping of *C. maculatus* may be feasible and should be advocated under such circumstances. The use of semiochemicals for enhancement of the effectiveness of biological control agents of *C. maculatus* has been explored. Preliminary research indicates that it may be possible to use semiochemicals to manipulate the behavior and increase the efficacy of natural enemies of storage pests including *C. maculatus*. Various plants and their extracts have been investigated for use in management of all stages of *C. maculatus*. Several of these have repellent, antifeedant, contact toxicity and/or oviposition deterrent properties. Essential oils derived from various plant parts are particularly effective as fumigants due in large measure to their volatile nature. Use of botanical extracts is particularly appealing for resource poor farmers from developing countries as both the plant materials, as well as most of the techniques used for extraction of the active components are readily available to them. Biological control using microbial agents is another viable strategy for management of *C. maculatus*. A number of entomopathogenic fungi in particular have been isolated, tested and their efficacy against the cowpea seed beetle demonstrated. Culture and formulation preparation of these fungi and other microbial agents can also be done at the farm level. The combination of several biopesticidal options offers sustainable and ecologically appropriate alternatives to the sole use of synthetic insecticides for management of *C. maculatus* and these are explored in the current chapter.

Keywords: Biopesticides, semiochemicals, botanical extracts, microbial pesticides

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INTRODUCTION

The cowpea seed beetle, *Callosobruchus maculatus* Fabricius (Coleoptera : Bruchidae) is the most serious insect pest attacking grain legumes in storage throughout the tropics and subtropics. While it has been reported to attack at least 21 species of legumes, its preferred hosts include *Cajanus cajan* (L.) Mill sp., *Glycine max* (L.) Merr., *Phaseolus* spp. and *Vigna unguiculata* (L.) Walp. (CABI 2018). Eggs of *C. maculatus* are oviposited on the surface of leguminous seeds and enclose in 6-7 days. The duration of the larval, pre-pupal and pupal stages is 32, 3.5 and 4 days respectively at 23^0C and 75% R.H. (Moreno *et al.*, 2000). Male and female longevity ranged from 6.6 – 9.7 and 7.2-9.3 days respectively and was related to food source. Females had highest mean oviposition on *V. unguiculata* (80.08 eggs) followed by *Cicer arietinum* L. (70.56 eggs) and *G. max* (58.80 eggs) (Jalpa *et al.,* 2015). *C. maculatus* can cause significant quantitative and qualitative damage to leguminous grain with losses ranging from 60–100% (Pereira, 1983; Bamaiyi *et al.,* 2006; Adebiyi and Tedela, 2012). Management of *C. maculatus* on grain legumes has focused on chemical control using predominantly synthetic insecticides (Al-Mekhlafi *et al.,* 2012; Mahdavi *et al.,* 2012; Vidyashree *et al.,* 2016; Okonkwo *et al.,* 2017). However, the frequent and long-term use of synthetic insecticides required for *C. maculatus* control has led to environmental, human and animal health concerns as well as insecticidal residues on grains destined for human and animal consumption. Development of resistance to frequently and prophylactically used insecticides for its control is yet another disturbing aspect of chemical control of *C. maculatus*. The incidence of resistance of *C. maculatus* to major insecticide groups has increased over time, with reports of resistance to organochlorines (Gouhar *et al.,* 1980; Evans 1985), organophosphates (Evans 1985; Bogamuwa *et al.,* 2002; Srivastava and Singh 2002; Gbaye *et al.,* 2016), carbamates (Gouhar *et al.,* 1980; Bogamuwa *et al.,* 2002) and pyrethroids (Bogamuwa *et al.,* 2002; Srivastava and Singh 2008; Fouad and Abotaleb, 2021). These problems have led to increased public awareness of the deleterious effects of synthetic insecticides and have enhanced the search for alternatives to these compounds. Biopesticides as a group provide one such alternative.

The United States Environmental Protection Agency defines biopesticides as products containing/derived from passive biocontrol agents (EPA 2018). This definition does not include traditional biological control agents of pests such as predators, parasitoids and entomopathogenic nematodes which actively pursue their hosts. The EPA (2018) has categorized biopesticides into three distinctgroups- Biochemical pesticides, Microbial pesticides and Plant-Incorporated-Protectants.

The prominence of biopesticides has increased within the past two decades, with a total of 189 active biopesticide ingredients being registered from 2000 – 2018 alone, compared to 6 in 1960s, 20 in 1970s, 39 in 1980s and 112 in 1990s (EPA 2018). *Bacillus* spp. was the most frequently used microbial pesticides with 49 strains being registered since 1971, twenty-three (23) of which were registered since 2000. Ten entomopathogenic fungi (*Beauveria bassiana*– 5 strains, *Metarhizium* spp. – 2 strains, *Paecilomyces* spp. – 2 strains and *Verticillium* sp. – 1 strain) have been registered for use as biopesticides since 1993 (EPA 2018). Use of biopesticides for management of *C. maculatus* provides distinct advantages over conventional synthetic insecticides including the fact that they are innately less toxic have limited undesirable effects on non-target organisms, effective in low quantities and tend to be feasible at the farm level. The latter is an especially attractive component of biopesticides for developing countries and resource poor farmers.

Biochemical pesticides

Semiochemicals

Semiochemicals are chemical substances or mixtures that communicate information either between different species (interspecific) or individuals of the same species (intraspecific) and which ultimately modify the behaviour of the receiver. Pheromones are intraspecific semiochemicals released by one organism which elicit a response by another organism of the same species. Allelochemicals however, are interspecific semiochemicals which stimulate a response in a species different from that of the producer. Allelochemicals can further be subdivided into allomones (produce a response which favours the emitter), kairomones (response favours the receiver) and synomones (favours both receiver and emitter). Semiochemicals can play an important role in management of stored product insects in general and *C. maculatus* in particular. It has been suggested that three approaches using semiochemicals – mating disruption by means of pheromones, oviposition disruption and controlling feeding may be utilized for management of *C. maculatus* (Yamamoto 1985; Oksun and Reddy 2021).

Pheromones are by far the most commonly used semiochemicals in pest control and possess tremendous potential as biopesticides in the management of stored product insects. In the majority of cases, sex pheromones are used for monitoring and to a lesser extent, trapping of stored product insects. The contact sex pheromone of female *C. maculatus* was initially identified by Phillips *et al*. (1996) and comprised of five compounds (3-methyleneheptanoic acid, (Z)-3-methyl-3-heptenoic acid, (E)-3-methyl-3-heptenoic acid, (Z)-3-methyl-2-heptenoic acid and (E)-3-methyl-2-heptenoic acid) each of which individually elicited a positive response by males (Figure 1). Combinations of two or more of these compounds evoked synergistic responses in male *C. maculatus.* Two additional components, nonanedioic acid and 2, 6-dimethyl-1, 8-octanedioic acid were later isolated and induced copulation behaviour in male *C. maculatus* (Nojima *et al.*, 2007) (Fig. 1). In most insect species, female sex pheromone production declines with both age and post mating. However, *C. maculatus* appears to be anomalous in this regard as female attractiveness to males was regained one-day post mating (Shu *et al.*, 1996) and this may have important implications in its management using pheromones. While *C. maculatus* is considered a major pest in its own right, in commercial grain storage warehouses several insect pests are frequently encountered simultaneously. The use of pheromone traps baited with a single pheromone capable of attracting several species is an attractive proposition. Ukeh *et al*. (2013) used the multi-attractant pheromonal lure BFL 225 in traps which captured a significant number of stored insect pests including *C. maculatus* compared to the unbaited control traps.

Insects also produce a variety of other pheromones which modify the behavior of conspecifics among which are oviposition marking pheromones. Oviposition marking pheromones are usually deposited at the time of oviposition or shortly thereafter and function to deter conspecifics from ovipositing on the same food resource. This in turn prevents competition for the limiting food resource upon which survival of the developing young depends. The existence of these pheromones has been recognized in a wide array of insects including *C. maculatus.* For example, female *C. maculatus* preferred to oviposit on azuki beans (*Vigna angularis* (Willd.) Ohwi & H. Ohashi (Leguminosae)) which had very few eggs or on beans on which eggs were removed and then washed with ether (Sakai *et al.*, 1986). Oviposition pattern on host legume seeds in *C. maculatus* may thus be as a result of the presence of oviposition marking pheromones (Messina and Dickinson 1993).

Biological control of *C. maculatus* using predators and parasitoids has not been extensively practiced despite reports of high levels of control under natural conditions (Ouedraogo *et al.*, 1996; Iloba *et al.*, 2007; Soundararajan *et al.*, 2012; Ozdemir *et al.*, 2020). The role of semiochemicals in tritrophic interactions in stored product insects is particularly intriguing as their use may enhance natural enemy effectiveness among these pests. Several natural enemies have been recorded for *C. maculatus* including the predaceous mites, *Cheyletus eruditus* Schrank (Cheyletidae) and *Pyemotes tritici* (Lagreze-Fossat & Montagn) (Pyemotidae), two egg parasitoids, *Uscana lariophaga* Steffan (Trichogrammatidae) and *Uscana mukerjii* (Mani) (Trichogrammatidae), seven larval parasitoids, *Anisopteromalus calandrae* Howard (Pteromalidae), *Dinarmus acutus* Thomson (Hymenoptera:Pteromalidae), *Dinarmus basalis* Rondani (Pteromalidae), *Dinarmus vagabundus* Timberlake (Pteromalidae), *Heterospilus prosopidis* Viereck (Braconidae), *Lariophagus distinguendus* (Forster) (Pteromalidae) and *Pteromalus* *cerealellae*(Ashmead) (Pteromalidae) and two larval/pupal parasitoids.

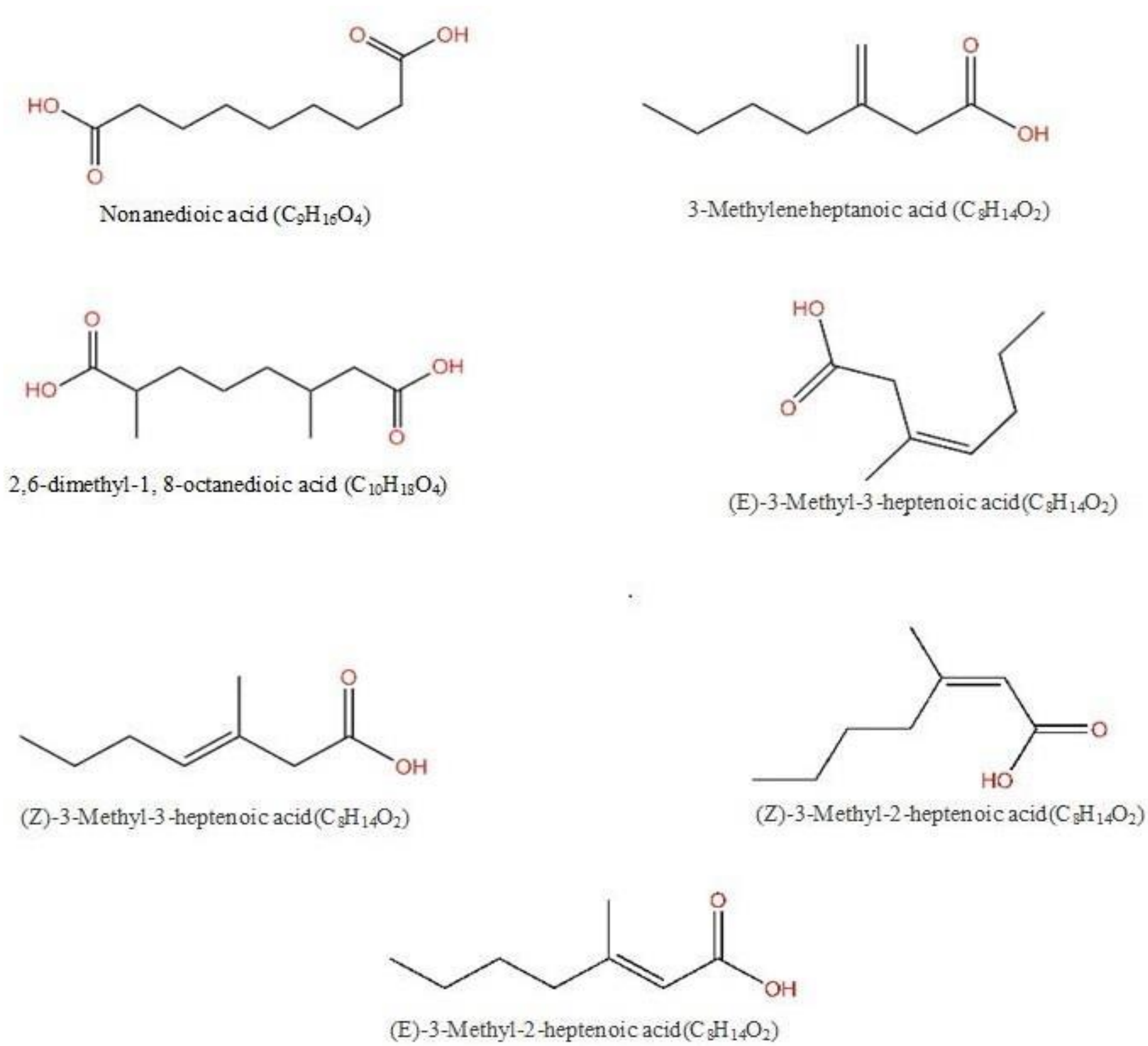

Fig. 1. Components of the contact sex pheromone of *Callosobruchus maculatus* (after Phillips *et al.*, 1996; Nojima *et al.*, 2007)

Eupelmus orientalis (Crawford) (Eupelmidae) and *Eupelmus vuilleti* (Crawford) (Hymenoptera:Eupelmidae) (CABI 2018). Phillips (1997) indicated that it may be possible to use semiochemicals to manipulate the behavior and increase the efficacy of natural enemies of storage pests including *C. maculatus.* Mbata *et al.* (2004) elucidated semiochemical cues used by *P. cerealellae* to locate its host *C. maculatus.* They note that whole body solvent extracts of virgin female *C. maculatus* elicited strong responses from female *P. cerealellae* and that previously exposed *P. cerealellae* had shorter response times to the host compared to unexperienced parasitoids. In the case of the egg parasitoid *U. lariophaga*, cowpea seeds with larger clusters of *C. maculatus* eggs had higher levels of parasitization than those with fewer eggs. This may have been as a result of higher odor concentration around seeds with greater egg densities (Albeek *et al.*, 1997). Chemicals associated with *C. maculatus* egg odour thus have potential for use in increasing levels of parasitization by *U. lariophaga.*

Host preference of *C. maculatus* to various legumes has been fairly extensively demonstrated both between different species and among different cultivars within the same species. This preferential difference has been attributed to physical and/or chemical characteristics of the leguminous seeds (Moreno *et al.*, 2000; Srivastava and Singh 2008; Rosemond and Khan 2013; Jalpa *et al.*, 2015; Sewsaran *et al.*, 2019). Host preference as a result of seed chemical cues has been demonstrated in *C. maculatus*. Comparison of two cultivars of *V. unguiculata* (Ife-Brown and black-eyed cowpea) and *G. max* (soybean) indicated that there were higher levels of 2-ethyl hexanol from Ife-Brown compared to either black-eyed cowpea or soybean and that *C. maculatus* was most attracted to Ife-Brown. Female *C. maculatus* were also highly attracted to synthetic 2-ethyl hexanol (Ajayi *et al.*, 2015). This compound together with the appropriate trap(s) may serve as a potential 'lure and kill' attractant, leading to a reduction of females in a population of cowpea weevil and the concomitant reduced damage to leguminous seed hosts. Kidney bean, *Phaseolus vulgaris* L. has been reported to control and/or inhibit larval of feeding by *C. maculatus*. Yamamoto (1985) and Honda and Ohsawa (1990) noted that an unidentified fraction of *P. vulgaris* with a molecular weight of 48,000 and an isoelectric point of pH 4.6, inhibited *C. maculatus* larval growth and when integrated into artificial beans acted as a growth inhibitor. This fraction was subsequently identified as phaseolin ($C_{20}H_{18}O_4$), which is the main globulin reserve in kidney bean test and has been reported to be unfavorable to the development of *C. maculatus* (Silva *et al.*, 2004). Thus, semiochemicals emanating from *C. maculatus* host leguminous seeds offer another opportunity for management of this pest. *C. maculatus* is primarily a pest of dried seeds in storage but, despite being dried, leguminous seeds do emit volatile organic compounds which can be detected in minute quantities by *C. maculatus.* Five such compounds (1-pentanol, 1-octen-3-ol, (E)-2-octenal, nonanal and 3-carene) were identified from the headspace of dried *Psium sativum* L. seeds. Females were attacted to all five compounds either singly or in various combinations, while males were only attracted to two binary mixtures of aldehydes (Ndomo-Moualeu *et al.*, 2016). A similar suite of 23 volatile compounds was isolated from seeds of *Lathyrus sativus* L. of which 3-octanone, 3-octanol, linalool oxide, 1-octanol and nonanal as a synthetic blend of 448, 390, 1182, 659 and 8114 ng/20µL methylene respectively was most attractive to *C. maculatus* (Adhikary *et al.*, 2015).

Botanical extracts

Botanical extracts have generally been used as plant protectants either as dry (powders, dusts) or wet (essential oils, solvent extracts) formulations. Numerous plant extracts have been utilized as either dry or wet formulations for management of *C. maculatus.* These extracts contain an assortment of bioactive chemical groups which afford stored products protection against *C. maculatus.* These groups include but are not restricted to aldehydes/ketones, alkaloids, phenolics and terpenoids.

Aldehydes and ketones

Aldehydes and ketones are organic compounds with the C=O functional group. In the case of aldehydes, at least one of the other two carbon atom bonds has to be occupied by hydrogen while in ketones neither of the two remaining carbon bonds is occupied by hydrogen. Both aldehydes and ketones in plants and plant components provide initial barriers to insect attack. Combinations of volatile compounds collected from the headspace of dried green pea seeds contained aldehydes and acted as repellents to *C. maculatus* (Ndomo *et al.*, 2016). Extracts from the Japanese spindle, *Euonymus japonicus* Thunb. (Celastraceae) and the golden rain tree *Cassia* *fistula* L. possessed a range of bioactive compounds including aldehydes which were toxic to adult and immature *C. maculatus* and were suggested to be safe substitutes to synthetic insecticides (Ahmed and Gazzy 2011). However, in addition to acting as barriers /repellents to insect attack, some aldehydes and ketones can have attractive properties. Benzaldehyde and octanone were detected in volatiles from Ife-Brown and black-eyed cowpea pods and were attractive to *C. maculatus* (Ajayi *et al.*, 2018). The use of these compounds in traps for *C. maculatus* should be explored.

Alkaloids

Alkaloids are primarily produced by higher plants especially from the families Apocyanaceae, Papaveraceae, Ranunculaceae, Rubiaceae, Rutaceae and Solanaceae. However, a few alkaloids are derived from certain bacteria (eg. *Pseudomonas aeruginosa* and *Serratia marcescens*), fungi (eg. ergot alkaloids from *Penicillum* spp. and *Aspergillus* spp.), insects (some ants and wasps) and the skin of frogs especially from the family Dendrobatidae. Alkaloids are nitrogen containing cyclic organic compounds that are physiologically active in a range of organisms including insects, where they act as neurotoxins and antifeedants. Glutathione S-transferases from *C. maculatus* have been suggested as a potential target for alkaloid plant extracts and that the competitive binding inhibition on glutathione S-transferases may contribute to the efficacy of alkaloid plant extracts on this pest (Kolawole *et al.*, 2009).

A range of plant species containing varying levels of alkaloids has been examined as potential biopesticides for use against *C. maculatus.* Methanolic and aqueous extracts of the stem and root of *Periploca hydaspidis* Falc. (Apocynaceae) exhibited insecticidal activity against *C. maculatus.* The extracts contained several bioactive groups including flavonoids and alkaloids (Ullah *et al.,* 2018). Methanolic extract of *Ficus thonningii* leaves also caused high mortality in *C. maculatus* adults 3-8 days post exposure. Phytochemical analyses indicated that the extract contained alkaloids, flavonoids and polyphenols which may have contributed to the insecticidal activity observed (Diouf *et al.,* 2014). Powder extracts from different plants and their component parts have exhibited antifeedant and mortality effects in *C. maculatus.* High mortality (85%) of *C. maculatus* exposed to the dry powder of *Aframomum melegueta* K. Schum. (Zingiberaceae) on cowpea seeds was attributed to the presence of alkaloids. *C. maculatus* mortality gradually increased with both dose and exposure time (Adesina *et al*, 2015). Ekeh *et al*. (2013) also examined the effectiveness of powders from *A. melegueta* seed as well as that of several other plants including *Allium sativum* L. (Amaryllidaceae), *Capsicum nigrum* L. (Solanaceae), *Zingiber officinale* Roscoe (Zingiberaceae), *Azadirachta indica* A. Juss. (Meliaceae) and*Ocimum gratissimum* L. (Lamiaceae) against *C. maculatus* on three host legumes *V. unguiculata, Vigna subterranean* (L.) Verdc. (Leguminosae) and *C. cajan.* All powders contained high concentrations of alkaloids along with steroids and glycosides and concluded that *O. gratissimum* powder was the most effective of the six botanicals examined. Hexane and acetone extracts of another species of basil, *Ocimum basilicum*L. (Lamiaceae) similarly contained high levels of alkaloids and cardiac glycosides as caused 75-80% mortality in *C. maculatus* (Olotu *et al.*, 2013). Ethanolic extracts of *O. gratissimum* leaves have also exhibited dose dependent repellent effects against *C. maculatus* with the highest dose (50%) being classified as a Class IV (60 -80% repellency) repellent (Koubala *et al.*, 2013). Root bark powder of Senegal prickly-ash, *Zanthoxylum zanthoxyloides* (Lam.) Zepern. &Timler (Rutaceae) reduced *C. maculatus* oviposition by as much as 63%, adult eclosion by 50% and seed attack by 69-93%. This reduction may have been as a result of the presence of alkaloids, terpenoids and phenolics in the root bark powder (Ogunwolu *et al.*, 1998).

Protection of host legume by seed surface treatment is another option against attack by *C.*

maculatus. Surface treatment of *V. unguiculata* seeds with 3000 ppm of any of the alkaloidal amines, wisanine((2E,4E)-5-(6-methoxy-1,3-benzodioxol-5-yl)-1-piperidin-1-ylpenta-2,4-dien-1-one); delta, alpha, beta-dihydrowisanine ((E)-1-[5-(6-methoxy-1,3-benzodioxol-5-yl)-1-oxo-2-pentenylpiperidine) and piperine ((E,E)-1-[5-(1,3-benzodioxol-5-yl)-1-oxo-2,4-pentadienyl) piperidine) from *Piper guineense* Schumach. (Piperaceae) protected the seeds from attack by *C. maculatus* by acting as antifeedants (Su and Sondengam 1980).

In addition to plant extracts with alkaloids contributing toward the neurotoxic and antifeedant activity of *C. maculatus*, the seeds of several species of legumes which are hosts of this bruchid also possess alkaloid compounds which confer, at least in part, resistance to this pest. Seed coat extracts of both *P. vulgaris* and *V. unguiculata* contain alkaloid and other functional groups which reduce oviposition and disrupt larval development (Hudaib *et al.*, 2017; Pumnuan *et al.*, 2021). However, it should be noted that complete protection of the seeds from oviposition and larval development was not achieved in this study.

Phenolics

Phenolic compounds are based on an aromatic benzene ring with one or more hydroxyl groups. Plant phenolics is a term broadly used to describe secondary metabolites produced by a plant which confer a variety of benefits to the plant against abiotic and biotic stresses including defence against herbivores. Plant extracts containing high concentrations of phenolic compounds may be used to protect grain legume seeds from attack by *C. maculatus*. The presence of polyphenols in ethanolic extracts of *Tithonia diversifolia* (Hemsl.) A. Gray (Asteraceae), *Cyperus rotundus* L. (Cyperaceae) and *Hyptis suaveloensis* (L.) Poit. (Lamiaceae) has been reported to contribute to the insecticidal activity of these plants to *C. maculatus* (Kolawole *et al.*, 2011). Root bark powder of *Z. zanthoxyloides* which contained several phenolic compounds, reduced *C. maculatus* oviposition, adult eclosion and seed attack by as much as 63%, 50% and 93% respectively (Ogunwolu *et al.*, 1998).

While plant extracts with phenols are toxic to *C. maculatus*, the seed coat of legumes themselves possesses a range of chemical compounds including phenolics which provide some level of protection against herbivores. Analysis of seed coat extracts of *P. vulgaris* and *V. unguiculata* indicated that they contained phenolic and alkaloid compounds. Coating of artificial host beans with these extracts resulted in reduction in the number of eggs oviposited as well as disruption of larval development by *C. maculatus*, which was partially attributed to the presence of these compounds (Hudaib *et al.*, 2017).

Examination of 150 genotypes of *Vigna mungo* (L.) Hepper seeds for susceptibility against *C. maculatus* indicated that egg eclosion, survival and number of F1 *C. maculatus* adults were much higher in susceptible genotypes. Phenolic content of the seed coats was significantly higher in resistant compared to that in susceptible genotypes (Haque *et al.*, 1992).Higher levels of total phenols, tannins and flavonoids in resistant varieties of *V. radiata* were positively correlated with increased resistance to *C. maculatus* in these varieties. Incubation period, larval-pupal duration and total developmental time were all significantly longer in resistant compared to susceptible *V. radiata* varieties examined (Lazar *et al.*, 2014). Similarly, Venugopal *et al*. (2000) evaluated the seeds of ten wild (*Cajanus albicans* (Wight &Arn.) (Leguminosae), *Canavalia virosa*(Roxb.) (Leguminosae), *Dolichos tribolus* L. (Leguminosae), *Dunberia ferruginea* Wight & Arn. (Leguminosae), *Lablab purpureus* (brown) (L.) (Leguminosae), *Lablab purpureus* (black) (L.) (Leguminosae), *Neonotonia wightii* (Wight & Arn.) (Leguminosae), *Rhynchosia cana* (Willd.) DC. (Leguminosae), *Rhynchosia rufescens*(Willd.) DC. (Leguminosae) and *Vigna bourneae*Gamble (Leguminosae)) and ten cultivated (*Cajanus cajan* ICP14770, *C. cajan* ICP14990, *Cicer arietinum* COG29, *C. arietinum* CO3, *Vigna unguiculata* VCP8, *V. unguiculata* CO4, *V. mungo* CO5, *V.*

mungo VBN 1, *V. radiata* K851 and *V. radiata* UGG 4) grain legumes. They concluded that non-protein anti-metabolites including total phenols, ortho-dihydroxy phenols and tannins occurred in significantly higher concentrations in the wild compared to cultivated legume varieties. Wild varieties were also less attacked by *C. maculatus* than cultivated varieties of legumes examined. They attributed the higher anti-metabolite levels which were predominantly phenolic in origin, for providing resistance in the seeds of the wild legumes examined.

Terpenoids

Terpenoids or isoprenoids are the largest group of naturally occurring plant secondary metabolites. They are usually classified based on the number of isoprene units in the terpenoid molecule with each isoprene unit generally arranged head-to-tail and consisting of five carbons with two unsaturated bonds and a branched chain. Hemiterpenoids (C_5H_8) thus possess one isoprene unit, monoterpenoids ($C_{10}H_{16}$) have two, sesquiterpenes ($C_{15}H_{24}$) have three, diterpenoids ($C_{20}H_{32}$) have four, sesterpenoids ($C_{25}H_{40}$) have five, triterpenoids ($C_{30}H_{48}$) have six and tetraterpenoids ($C_{40}H_{64}$) have eight isoprene units. Terpenoids are known to exhibit strong bioactivity against numerous insects and usually act as neurotoxins, feeding deterrents and/or hormonal disruptors in insects including *C. maculates* (Dele and Oyeteju 2021). Application of 500 – 1000 ppm of the crystalline sesquiterpene, dehydrococtus lactone, isolated from the root of *Saussurea lappa* Clarke (Compositae) prevented eclosion of *C. maculatus* adults (Kailey *et al.*, 1979). Another terpenoid lactone, costunolide, also isolated from *S. lappa* reduced total number of *C. maculatus* eggs by 89% compared to the control, while egg eclosion and adult emergence were also significantly reduced (Singh, 1998). Mono- and sesquiterpenoids in particular, are volatile and thus especially suited as fumigants against insects. Cardamom, *Elettaria cardamomum* (L.) Maton (Zingiberaceae) essential oil, containing the major constituents 1,8-cineol, α-terpinylacteate, terpinene and fenchyl alcohol was used as a fumigant against *C. maculatus.* Fifty percent mortality was achieved after 12h exposure. The oil also exhibited good oviposition deterrence activity (Abbasipour *et al.*, 2011).

Terpenoids also act as repellents against *C. maculatus.* Islam (2009) found that eugenol and neem oil both achieved >80% repellency (Class V repellent) against *C. maculatus* adults 5-60min post treatment with a dose of 1μL. The effectiveness of the monoterpenoids geraniol, geranial, (+) citronellal, citronellol and eugenol as repellents and fumigants was determined against several stored grain pests including *C. maculatus.* Eugenol emerged as the most efficient fumigant and repellent against this pest (Reis *et al.,* 2016). The essential oil of *Teucrium polium* L. (Lamiaceae) is rich in sesquiterpenoids, the major constituents of which have been identified as: α-cadinol (46.2%), caryophyllene oxide (25.9%), α muurolol epi (8.1%), cadalene (3.7%) and longiverbenone (2.9%). This essential oil was tested as both a repellent and fumigant against *C. maculatus* and displayed 52% repellency while the fumigant LC_{50} was 148.9μL/L air (Khani and Heydarian 2014).

Botanicals -Antifeedants

Insect antifeedants or feeding deterrents prevent or retard insects from feeding. They do not appear to affect the insect's appetite or feeding receptors or repel them from their food source. This lack of feeding by the insects consequently results in their death from starvation. Several plant-derived extracts have been tested as antifeedants against *C. maculatus.* Essential oils extracted from leaves of both *Chenopodium ambrosioides* L. (Chenopodiaceae) and *Clausena pentaphylla* (Roxb.) (Rutaceae) were potent antifeedants and exhibited 100% reduction in feeding by *C. maculatus* (Pandey *et al.*, 2011).

Extracts from leaves and seed oil of neem, *Azadirachta indica* A. Juss. (Meliaceae) are perhaps one of the best known biopesticides with potent antifeedant activity against a range of insect pests. The extracts contain a diversity of compounds including terpenoids, steroids, saponins and phenolics, which may contribute towards its antifeedant properties. However, addition of neem with other plant extracts has been demonstrated to have additive antifeedant effects against *C. maculatus.* Combination of Malabar catmint, *Anisomeles malabarica* (L.) (Lamiaceae) leaf extract and *A. indica* seed kernel extract increased the antifeedant effect against *C. maculatus* compared to either extract individually (Murugan 2010). Neem seed oil together with moringa (*Moringa oleifera* Lam. (Moringaceae)) oil resulted in 73-94% uninfested cowpea seeds after 6 months under storage (Ilesanmi and Gungula 2010). Saeidi (2014) compared the effectiveness of two species of eucalyptus, *Eucalyptus globules* Labill. (Myrtaceae) and *Eucalyptus camaldulensis* Dehnh. (Myrtaceae) as potential antifeedants and concluded that, while both species exhibited feeding deterrence against *C. maculatus*, *E. globulus* exhibited greater antifeedant activity. Aqueous extracts from the aril of ackee (*Blighia sapida* Koenig (Sapindaceae)) caused significant mortality as well as served as a potent antifeedant to *C. maculatus* adults and could be used at the farm level for seed protection against this pest (Khan *et al.,* 2002). Seed extracts from the leguminous plant, monkey's earring, *Pithecellobium dumosum* Benth. (Leguminosae), produced a trypsin inhibitor (PdKI) which, in addition to possessing insecticidal properties, also demonstrated potent antifeedant activity against several insect pests including *C. maculatus* (Oliveira *et al.*, 2007; Jamal *et al.*, 2019).

Oviposition deterrents

Use of techniques to either prevent or reduce the number of eggs laid by insects is an interesting concept in pest management since reduced insect egg load ultimately leads to decreased damage on the crop or plant part by pest larvae. Numerous botanical extracts have demonstrated varying levels of oviposition deterrence in *C. maculatus* (Bavarsad *et al.*, 2020; Estekhdami *et al.*, 2020). Crude extracts of the bark of three species of Meliaceae, *Khaya grandifoliola* (C. DC.), *Khaya nyasica*Stapf ex Baker f. and *Khaya senegalensis* (Desr.) A. Juss.) were evaluated for *C. maculatus* oviposition deterrent activity. Extracts between 250 – 1000ppm from all three species significantly reduced oviposition, with *K. grandifoliola* treated cowpea seeds having the least *C. maculatus* eggs (Babarinde and Ewete 2008). Pigeon pea seeds treated with green inflorescence extracts from breadfruit, *Artocarpus altilis* (Parkinson) Fosberg (Moraceae), at 1000ppm reduced *C. maculatus* oviposition significantly (Eccles *et al.,* 2019), while methanolic leaf extracts from *Clusia palmicida* Rich. ex Planch. & Triana (Clusiaceae) was reported to be an effective oviposition deterrent for *C. maculatus* on pigeon pea treated seeds at 1000ppm (Powder-George *et al.*, 2018).

Various essential oils, especially those with terpenoids and phenolics, have been observed to be oviposition deterrents to *C. maculatus.* Monoterpenoids may inhibit egg laying by *C. maculatus* by interfering with oviposition reflexes (Mbata and Payton, 2013; Bavarsad *et al.*, 2020). Essential oils from *Citrus* spp. have terpenoids (eg limonene, cineole) comprising a major group of their constituents. Short-term oviposition deterrent capability of *Citrus* spp. extracts against *C. maculatus* has been demonstrated. *C. maculatus* oviposition was inhibited on cowpea seeds treated with 0.8% lime (*Citrus aurantifolia* (Christm.) Swingle (Rutaceae)) peel extracts for 7 days. However, maximum oviposition deterrence was obtained with a 2:1 mixture of grapefruit (*Citrus paradisi* Macfad. (Rutaceae)) and lime peel extract (Musa and Sulyman, 2014). Sour orange, *Citrus aurantium* L. (Rutaceae), peel extract on cowpea treated seeds also exhibited high (82%) oviposition deterrence against *C. maculatus* at 10% but also displayed at least 50% deterrence at 1.25% (Jayakumar, 2010).

Essential oils from aromatic herbs generally possess numerous terpenoids (eg. α and β pinene, camphene, sabinene) as their major constituents with most of these terpenoids having been confirmed as oviposition deterrents in various insects including *C. maculates* (Estekhdami *et al.*, 2020). Shahkarami *et al.* (2010) investigated the oviposition deterrent capability of essential oils from four aromatic herbs, summer savory, (*Satureja hortensis* L. (Lamiaceae)), pennyroyal mint (*Mentha pulegium* L. (Lamiaceae)), ostokhodus (*Nepeta menthoides* Boiss. & Buhse (Lamiaceae)) and rosemary (*Rosmarinus officinalis* L. (Lamiaceae)) against *C. maculatus.* At the highest concentration of oils tested (190µL/L air) *M. pulegium, S. hortensis, R. officinalis* and *N. menthoides* gave 92.45%, 83.22%, 73.24% and 62.34% oviposition deterrence against *C. maculatus.* Similar results were also obtained with the essential oils from other aromatic herbs. Comparison of sage (*Salvia officinalis* L. (Lamiaceae)), rosemary and coriander (*Coriandrum sativum* L. (Apiaceae)) essential oils against *C. maculatus* indicated that at the lowest concentration tested (6.25 µL/mL) all oils reduced oviposition significantly. Rosemary essential oil exhibited the highest degree of oviposition deterrent ability against *C. maculatus* which lasted for 120h (5days) (Dayaram and Khan 2016). Likewise, garlic essential oil in combination with vegetable oils completely inhibited oviposition by *C. maculatus* on cowpea seeds (Abd El-Salam 2005).

Sublethal doses of essential oils may adversely affect the fecundity of *C. maculatus* and consequently lead to a reduction in the number of eggs produced. While this may not be considered as deterrence of oviposition, it does ultimately result in decreased egg load on stored seed hosts. Exposure of *C. maculatus* to sublethal (LC_{20}) doses of essential oils from *E.camaldulensis* and *Heracleum persicum* Desf. ex Fisch. (Apiaceae) resulted in a significant decrease in both the total and daily number of eggs produced compared to the unexposed (control) adults (Izakmehri *et al.*, 2013).

Apart from the use of chemicals to deter oviposition, the use of physical barriers such as vegetable oils and powdersto protect the seeds should also be considered. A single application of groundnut or palm oil significantly reduced *C. maculatus* oviposition of cowpea seeds (Uvah and Ishaya 1992). Use of corn, groundnut, sunflower or sesame oil to cowpea seeds at a rate of 10mL/kg or *Cymbopogon citratus (DC.) Stapf. (Poaceae), Cinnamomum camphora* (L.) J. Presl. (Lauraceae), *Derris inudata* (Leguminosae), *Monodoramyristica* (Gaertn.) *Dunal* (Annonaceae), *Zingiber s pectabile* Griff. (Zingiberaceae) or *Alpinia zerumbet* (Pers.) B.L. Burtt and R.M. Smith (Zingiberaceae) powder significantly reduced oviposition on the seeds (Rajapakse and Van Emden, 1997). Powdered grapefruit (*C. paradisi*) and lime peel at 20g / 200g cowpea seeds significantly reduced *C. maculatus* oviposition (Onu and Sulyman 1997). Application of cashew (*Anacardium occidentale*L. (Anacardiaceae)) gum exudate to cowpea seeds prevented oviposition by *C. maculatus* possibly because it acted as a physical barrier preventing oviposition on the seeds (Marques *et al.*, 1992). Similarly, cowpea seeds treated with the latex from *Euphorbia tirucalli* L. (Euphorbiaceae), *Calotropis procera* (Aiton) W.T. Aiton (Apocynaceae) and *Plumeria rubra* L. (Apocynaceae) deterred oviposition by *C. maculatus* (Ramos *et al.*, 2011). However, the authors suggest that the presence of proteins and volatiles in the latex may not be responsible for the repellent activity demonstrated.

Fumigants

Fumigants for use in stored products perform best where grains are stored in hermetically sealed environments. This allows the fumigant to achieve high concentrations for a prolonged period and thus increases its efficacy. A wide array of essential oils has been examined for their fumigant action against stored product pests. The fumigant activity of these oils is dependent in large measure on their volatility at normal temperatures. These essential oils contain numerous terpenoids which account for their volatility.

Terpenoids accounted for approximately 66% of the active compounds in *Thymus carmanicus* Jalas (Lamiaceae) and included carvacrol (41.14%), p-cymene (12.09%), thymol (6.35%) and γ-terpinene (6.21%) as the major constituents. The highest fumigant concentration (65.62 µL/L air) caused 84% mortality to *C. maculatus* after 5h exposure

(Jarrahi *et al.,* 2012). *Teucrium polium* subsp. *Capitatum* L. (Lamiaceae) also had a predominance of terpenoids as the major components, α-cadinol (46.2 caryophyllene oxide (25.9%), α muurolol epi (8.1%), cadalene (3.7%) and longiverbenone (2.9%) and *C. maculatus* was highly susceptible to the extracted essential oil (Khani and Heydarian, 2014).

When used as a fumigant, the essential oils of *S. hortensis, M. pulegium, N. menthoides, R. officinalis* at a concentration of 560µL/L air caused 96%, 88%, 57% and 65% mortality in *C. maculatus* respectively. While the corresponding fumigant LC_{50} values for *S. hortensis, M. pulegium, N. menthoides, R. officinalis* were 69, 106, 322 and 205 µL/L respectively on adult *C. maculatus* (Shahkarami *et al.,* 2010). The fumigant mortality of six essential oils from chilli pepper (*C. annuum*), garlic (*A. sativum*), tea tree (*Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae)), peppermint (*Mentha pulegium* L. (Lamiaceae)), cardamom (*E. cardamomum*) and camphor (*E. globulus*) was assessed against *C. maculatus.* All six oils caused mortality at 0.8mL (w/v) as a result of their fumigant action, however, *C. annuum, M.pulegium, M. alternifolia* and *E. globulus* had the highest mortality at 96.2%, 85.1%, 85.1% and 77.7% respectively. It was concluded that the fumigant action of the oils was both dose and time dependent (Abdel-Fattah and Dooa, 2017). Similar dose and time dependent fumigant mortality of essential oils against *C. maculatus* was also reported by Nikooei and Moharramipour (2011); Dayaram and Khan (2016); Bavarsad *et al.* (2020) and Estekhdami *et al.* (2020).

Combinations of essential oils have been reported to exhibit synergistic fumigant action. Erler*et al.* (2009) reported that anise (*Pimpinella anisum* L. (Apiaceae)), thyme (*T. vulgaris*) and rosemary (*R. officinalis*) essential oils at a dose of 120µL/L air in binary (1:1) and tertiary (1:1:1) mixtures caused higher fumigant mortality in *C. maculatus* than single oils. Tertiary combination of all oils provided the highest mortality.

Repellents

The majority of insect repellents are volatile and consequently their effectiveness is sort-lived. However, the persistence of these repellents may be extended by using the appropriate formulation. Combination of repellents with less volatile compounds (eg clays, diatomaceous earth) is one technique to protract the period of their usefulness. Most insect repellents are of plant origin, the more notable being Asteraceae, Cupressaceae, Labiatae, Lamiaceae, Lauraceae, Meliaceaeand the essential oils from several of these have been tested for their repellent activity against stored product pests (Reis *et al.,* 2016; Kosini *et al.,* 2021).

A number of plant families have repellent activity against *C. maculatus,* however the most notable is the Meliaceae of which *A. indica* has been extensively studied. The repellent action of neem oil at concentrations of 1, 5 and 10mL/kg cowpea seeds was highly evident. Application of neem oil at 5 or 10mL/kg of cowpea seeds repelled *C. maculatus* adults and protected seeds for up to 6 months (Daniel and Smith, 1990). The use of an 5% emulsifiable concentrate of azadirachtin (Neemazal-F®) on cowpea seeds caused 10.6 - 18.7% repellency of *C. maculatus* 7-days post treatment (El-Lakwah *et al.,* 1994). Other members of the Meliaceae have also been studied as repellents against *C. maculatus* including the chinaberry tree, *Melia azedarach.* The fruit powder, petroleum ether and acetone extracts *M. azedarach* were tested against *C. maculatus* adults. Over a 21-day period the repellency ranged from ranged from 9.5-28%, 7.3-22 3% and 12-26% for the powder and petroleum ether and acetone extracts respectively (El-Lakwah *et al.,* 1994).

Extracts from members of the plant family Piperaceae have also demonstrated repellency against a range of stored product insects (Satongrod *et al.,* 2021). Cowpea treated with hexane extract from *Piper cubeba* L.f. (Piperaceae) was highly repellent to *C. maculatus* adults (Su, 1990). Similarly, when the essential oil from *Piper hispidinervum* (C. DC. (Piperaceae)) was used to treat cowpea seeds, *C. maculatus* was significantly more repelled than in untreated seeds (Oliveira *et al.,* 2017). High repellent activity using essential oils against *C. maculatus* has also been reported from other plant families including Chenopodiaceae (*C. ambrosioides*) and Lamiaceae (*Adhatodavasica* (L.) Nees) (Pandey *et al.,* 2014). Freeze dried powdered leaves of tamarind, *Tamaridus indica* L. (Leguminosae) mixed with cowpea seeds at 5% (w/w) was an effective repellent against *C. maculatus* adults (Dharmasena *et al.,* 1998). At a dose of 1µL all of the three compounds, eugenol, zimtaldehyde and neem oil exhibited more than 80% repellency (Class V repellent) toward *C. maculatus* after 5-60 minutes. The highest repellent action was shown by zimtaldehyde (87.4% after 10 minutes exposure), while eugenol had the lowest repellency (79.5% after 25 minutes exposure) (Islam, 2009). The repellent activity of the monoterpenoids geranial, geraniol, citronellal, citronellol and eugenol was evaluated against *C. maculatus.* Repellent activity ranged from 50 – 88% indicating that all compounds exhibited relatively strong repellency against *C. maculatus* (Reis *et al*., 2016)

Microbial pesticides

Microbial pesticides have as their active ingredients either bacteria, fungi, nematodes, protozoans or viruses. These pesticides are used to control a variety of pests including insects, plant pathogens and weeds and usually display some degree of host specificity. This trait, together with the increase in resistance of pests to conventional synthetic pesticides and their undesirable environmental and health concerns make microbial pesticides an attractive alternative. The use of microbial pesticides for management of stored product insects appears to be gaining importance. This may be due to several factors including the fact that they are relatively non-toxic to non-target organisms, exhibit host specificity and can self perpetuate once established in a pest population. Two categories of microbial agents have been studied for use in management of *C. maculatus* – entomopathogenic bacteria and entomopathogenic fungi.

Entomopathogenic bacteria

Despite the fact that bacterial biopesticides are the most commonly utilized microbial formulations, few studies have been conducted on the use and efficacy of entomopathogenic bacteria for management of *C. maculatus.* Malaikozhundan and Vinodhini (2018) investigated the effectiveness of the entomopathogenic bacterium, *Bacillus thuringiensis* on *C. maculatus.* They concluded that application of $4x10^8$ cells/mL of *B. thuringiensis* caused delay in the developmental time for immature stages as well as causing 100% mortality to *C. maculatus* adults when applied at this concentration. They also noted reduction in the activity of several gut enzymes including mid-gut α-amylase, cysteine protease, α and β-glucosidases, lipase, glutathione S-transferase and lactate dehydrogenase. Most biologically based pesticides possess low stability and environmental persistence however, the use of nanotechnology and nanomaterials may significantly mitigate these undesirable effects. Increased stability and persistence of *B. thuringiensis* under grain storage conditions could enhance mortality of *C. maculatus* thus leading to better control. Malaikozhundan *et al.* (2017) synthesized and evaluated *B. thuringiensis* coated zinc oxide nanoparticles against *C. maculatus.* The particles were moderately stable under normal environmental conditions and caused 50% mortality (LC_{50}) at 10.71ug/mL. The authors concluded that *B. thuringiensis* zinc oxide nanoparticles were effective against *C. maculatus* and recommended its use in stored grain insect management.

Entomopathogenic fungi

Entomopathogenic fungi are parasitic fungi on insects which eventually kill them during their normal course of development. The mode of action of these fungi usually follows a similar pattern and includes conidial attachment to the host insect followed by germination, hyphal penetration of the host insect's cuticle and epidermis, multiplication of hyphae in the insect's haemolymph during which death of the insect occurs as a result of fungal growth and finally production of conidiophores which disperse, causing further infection of other host insects. Entomopathogenic fungi occur in a several divisions including: the sac fungi, Ascomycota (eg. *Beauveria* spp., *Cordyceps* spp., *Metarhizium* spp.), Entomophthoromycota (eg. *Conidiobolus* spp., *Neozygites* spp.), Microsporidia (eg. *Nosema* spp.) and the club fungi, Basidiomycota (eg. *Septobasidium* spp.).

Entomopathogenic fungi have been isolated from a variety of sources including soil and insect hosts themselves. Wakil *et al*. (2014) isolated 24 different fungal species from various stored grain insects in Pakistan including *C. maculatus.* Entomopathogenic fungi isolated included *Beauveria bassiana* sensu lato, *Metarhizium anisopliae* sensulato, *Purpureocillium lilacinum* (Thom) (Ophiocordyciptaceae) and *Lecanicillium attenuatum* Zare & W. Gams. (Cordycipitaceae). The most frequently tested entomopathogenic fungi against *C. maculatus* are *B. bassiana* and *M. anisopliae.* Comparison of the pathogenicity of strains of *B. bassiana, M. anisopliae* and *Paecilomyces fumoso-roseus* Wize (Clavicipitaceae)) against *C. maculatus* indicated that, while the LD_{50} of *M. anisopliae* was the lowest among the three entomopathogenic fungal species examined, *B. bassiana* had the lowest LT_{50} (4.14 days) compared to either *M. anisopliae* or *P. fumoso-roseus* and caused *C. maculatus* mortality fastest (Lawrence and Khan, 2002). Vilas Boas *et al*. (1996) also concluded that strains of *B. bassiana* had shorter LT_{50}s compared to *M. anisopliae* when tested against *C. maculatus.*

The efficacy entomopathogenic fungi against *C. maculatus* partially depends upon both their pathogenicity and virulence. Comparison of two isolates of *B. bassiana* (ARSEF-1186 and IMI-351833) against *C. maculatus* adults at 20^0C, 25^0C and 30^0C indicated that isolate ARSEF-1186 exhibited highest virulence at 30^0C (Lawrence and Khan 2009). Pathogenicity of *M. anisopliae* against *C. maculatus* has similarly been demonstrated. Mortality of *C. maculatus* adults to 10^8 conidia/mL of *M. anisopliae* var. *anisopliae* (URM3349) and *M. anisopliae* var. *acridum* (URM4412) was 74.5% and 58.3% respectively while the LC_{50} of *M. anisopliae* var. *anisopliae* was 9.2×10^3 conidia/mL (Rodrigues Guaraná *et al.*, 2012).

Apart from using entomopathogenic fungi only, they have been combined with various other agents to increase their effectiveness. Combination of diatomaceous earth (DE) with either *M. anisopliae* or *B. bassiana* resulted in increased efficacy in terms of time to 50% mortality (LT_{50}) when used to control *C. maculatus*. The time to 50% mortality was significantly shorter in both *B. bassiana*+ DE (LT_{50} = 122.2h) and *M. anisolpiae*+ DE (LT_{50} = 128.2h) compared to sole use of either *B. bassiana, M. anisolpiae*or DE (Nabaei *et al.*, 2012). Essential oils have also been combined with entomopathogenic fungi. *B. bassiana* was combined with the essential oil of *C. ambrosioides* and the effectiveness of the combination examined against *C. maculatus. C. ambrosioides* essential oil enhanced the potency of *B. bassiana* resulting in a reduction of the LC_{50} value when compared with either agent alone. Additionally, *C. ambrosioides + B. bassiana* combination completely inhibited *C. maculatus* oviposition on cowpea seeds in storage for as much as 60 days (Radha *et al.*, 2014).

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