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Natural Compounds with Potential Insecticidal Properties against Banana Weevil *Cosmopolites sordidus*

Elyeza Bakaze [1&2], Julian Kofler [1], Beloved Mensah Dzomeku [3], Jens Wünsche [1]

[1] *Universität Hohenheim, Crop Science/ Crop Physiology of Specialty Crops (340f), Germany.*
[2] *National Agriculture Research Organisation (NARO). National Agriculture Research Laboratories (NARL) - Kawanda, P. O. Box 7065, Kampala, Uganda.*
[3] *CSIR -Crops Research Institute (CRI), Plantain Research Program, P. O. Box 3785, Kumasi, Ghana.*

ABSTRACT:

Introduction: *The banana weevil (Cosmopolites sordidus Germar) causes 30-70% annual yield losses to Musa species in tropical and subtropical regions. Effective control strategies against C. sordidus of low cost, readily available, easy to apply, and safe for homestead growers is sought. The objective was therefore to evaluate natural compounds for potential insecticidal properties against C. sordidus.*

Materials and methods: *Bio-efficacy of extracts from cloves (Syzygium aromaticum), black pepper (Piper guineense), neem (Azadirachta indica) and their synthetic analogs along with Carbofuran were evaluated as botanical control option against Cosmopolites sordidus. They were assessed for egg hatch inhibitory effect, larvicidal toxicity and adult repellency in both laboratory and infested field experiments.*

Results and conclusion: *Results showed that eggs and larvae of C. sordidus were most susceptible to clove extract and its synthetic analogs. Egg hatchability was inhibited to 93% by clove extract whereas to > 50% by its synthetic analogs at 0.5% concentration. Larvae mortality dependent on instar stage but at instar stage 3 it was about 80% for clove extracts and above 60% for all other treatments at 0.5% concentration, respectively. The percentage of adult weevils repelled for between 6 to 48 hours ranged from 80 - 98% with black pepper, 78 - 90% for clove and 63 - 75% for neem. In conclusion, all evaluated extracts and synthetic analogs inhibited egg hatching, caused larvae mortality and repelled adult weevil to an extent that is sufficient to reduce markedly weevil damage and to serve as an alternative to synthetic pesticides, Carbofuran.*

Keywords: *Azadirachta indica, β-Caryophyllene, Eugenol, N-Isobutylamine, Piper guineense, Syzygium aromaticum*

I. INTRODUCTION

Integrated pest management (IPM) has been advocated for since the early 1970s (Knipling, 1972) to ensure that pest populations are suppressed below an economic relevant injury level by following a broad-based approach. It emphasizes the growth of healthy, high quality crops with the least possible disruption to agro-ecosystem and encourages sustainable natural pest control mechanisms. Despite the introduction of regular monitoring practices and mechanical and biological control measures that led to a responsible and targeted use of insecticides, fungicides and acaricides, synthetic pesticides have come under significant scrutiny in the last decade by current or impending legislation being implemented in many countries and due to breaking down of crop resistance to pathogens and pests. Those impediments have spurred the search for crop management strategies that require lower chemical input and make use of effective non-synthetic control options in all food crops (Buss & Brown, 2014; B. I. Murray, 2007; Zeng, Lao, Cen, & Liang, 2010). With particular focus on small farmers in developing countries, Altieri (1993) has described key elements in the design of sustainable pest management systems.

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The banana weevil (*Cosmopolites sordidus Germar*) causes extensive damage to *Musa* species, resulting in 30-70% annual yield losses (Gold et al., 2003; Njau et al., 2011; Speijer et al., 2001) in banana and plantain growing regions of East and West Africa. It is estimated that 90% of the *Musa* production is carried out on small subsistence-oriented family farms (Gold et al., 2001). Therefore, effective pest control strategies against *C. sordidus* should be of low cost, readily available, easy to apply, and safe for homestead growers. IPM strategies have been suggested by Gold et al. (2001) to target successfully different life stages of *C. sordidus*; however, weevil resistance to insecticides like dieldrin (Edge et al., 1975) is widely manifested. Consequently, crop protection strategies based on plant derivatives to control injurious pests have been recognized as a valuable tool (Ian M Scott, Jensen, Philogene, & Arnason, 2008).

The objective of this study was therefore to evaluate natural compounds for potential insecticidal properties against *C. sordidus*. Three plant extracts have been selected that have been previously shown to control various pest; however, they have not yet been evaluated as a biological control option against *C. sordidus*. The first pest control agent, neem (*Azadirachta indica*) and other Meliaceae species, have been studied for pesticidal properties on different agricultural pests (Mulla & Su, 1999). It was, for example, shown that neem derivatives were effectively reducing the egg hatchability of Okra fruit borer (Thara et al., 2009) and had larvicidal and ovideterrent properties against *Aedes albopictus*, also known as Asian tiger mosquito (Benelli et al., 2015). The second pest control agent, pepper (Piper) extracts and in particular various secondary compounds such as amides (N-Isobutylamine), have shown promising results for controlling various crop pest (I M Scott et al., 2007). Scott et al. (2008) reviewed the insecticidal activity of piperamides and concluded that piper extracts offer a unique and useful source of biopesticide in combination with other botanical insecticides such as pyrethrum. Earlier results of Scott et al. (2003) suggest that Piper extracts from two Piperaceae species, *Piper nigrum* L. and *P. tuberculatum* Jacq. could be used effectively as contact insect control agents to protect potato plants from developing *L. decemlineata* larvae at concentrations less than 0.1%. Moreover, *Piper nigrum* extract might be useful for the control of sawflies and tent caterpillars, two common Canadian forest pest insects (Scott et al., 2007). The third pest control agent, clove (*Syzygium aromaticum*) and other species of the Myrtaceae family, have been traditionally used as insecticides against many plant pests and pathogens (I. B. Murray, 2000). Main oil compounds from clove have some potential insecticidal activity against several grain storage pests (Zeng et al., 2010). Moreover, the bioactivity of eugenol, a natural oil of clove, was evaluated against four Coleoptera species (Obeng-Ofori and Reichmuth, 1997). Eugenol was highly repellent (80-100%) to all four beetle species tested and its effectiveness in terms of beetle mortality was dosage-dependent and reduced with increased length of grain storage after application. Powdered seeds of clove and *P. guineense* elicited 60-80% repellence of *C. sordidus* adults in laboratory studies (Inyang and Emosairue, 2005).

In this study, the hypothesis was tested that plant derivatives from *A. indica*, *P. guineense* and *S. aromaticum* as well as the synthetic analogs eugenol, eugenyl acetate, β-Caryophyllene, and N-Isobutylamine possess effective insecticidal properties against *C. sordidus*, thus can be used as biological control options.

II. MATERIALS AND METHODS

Study site. Field experiments were conducted at the Council for Scientific and Industrial Research (CSIR), Crops Research Institute (CRI) in Kumasi (latitude 6° 41' 0" North, longitude 1° 37' 0" West), Ghana, between August and December 2015. The area is within the semi-deciduous forest region of Ghana, characterized by prevailing hot and dry air masses from the Sahara during the dry season (December to February) and a bimodal rainfall season with up to 1300 mm precipitation and tropical, south-westerlies from the southern Atlantic Ocean between March and November (Oppong-Anane 2006). The annual mean temperature is 27°C (Oppong-Anane, 2006).

In addition, laboratory experiments were carried out at the National Agricultural Research Laboratories (NARL), Kawanda, Uganda (latitude 0° 24' 30" North, longitude 32° 32' 9" East) between September and December 2016.

Substances with insecticidal properties. For identifying natural compounds with potential insecticidal properties against *C. sordidus*, the following plant species were selected: seeds from neem (*A.zadirachta indica*), fruit from Ashanti pepper (*Piper guineense*) and flower buds from clove (*Syzygium aromaticum*). Pepper and cloves were bought from local markets, whereas neem seeds were collected from farmers' backyards in Kumasi. These materials were sundried and milled to a fine powder using a locally made motorized mill. Extracts were prepared according to the method described by Musabyimana et al. (2001). Percentage concentration of crude extracts (w/v) at 0.2, 0.4, 0.6 and 0.8 % were prepared by soaking 20, 40, 60 and 80 g of the powder in 1 L of distilled water, respectively, for 24h and filtering the solution through a muslin cloth.

Various compounds of these plant species with reported insecticidal properties were included as synthetic analogs in the study: eugenol ($C_{10}H_{12}O_2$), eugenyl acetate ($C_{12}H_{14}O_3$) and β-caryophyllene ($C_{15}H_{24}$) from clove (Nazrul, Bhuiyan, Begum, Nandi, & Akter, 2010; Razafimamonjison et al., 2013) and N-isobutylamine ($C_4H_{11}N$) from pepper (Parmar et al., 1997; PARK et al., 2002; Scott et al., 2008). These chemicals (Merck; Munich, Germany) were used at 98% purity. Although eugenol occurs in both clove buds and pepper, it is more abundant in *S. aromaticum* (Meghwal & Goswami, 2013; Ian M Scott et al., 2008).

Collection and maintenance of weevils. Adult weevils were trapped from plantations of the CRI and maintained in the laboratory in agreement with protocols described by Ogenga-Latigo and Bakyalire (1993) and Night et al. (2010). Weevils were kept on weekly supplied fresh plantain rhizomes inside 10 L plastic buckets, closed with perforated lids for aeration, and maintained at room temperature. This procedure ensured an adequate supply of eggs, larvae and adult weevils for the experiments.

Egg inhibition assays. Two experiments, each repeated twice, were conducted to evaluate the potential efficacy of plant extracts and some of their specific synthetic analogs on the inhibitory effect of *C. sordidus* egg hatching. For the first experiment at the CRI in Ghana, extracts of clove buds, neem seed and pepper fruit at 0 (control), 0.2, 0.4, 0.6 and 0.8 % (w/v) were evaluated using 26 brown spotted eggs (4-days-old) for each extract and concentration, respectively. The second experiment at NARL in Uganda constituted of nine treatments, each applied at concentrations of 0, 0.1, 0.25, 0.5% (w/v): eugenol, β-caryophyllene, eugenyl acetate, N-isobutylamine, a combination of the clove synthetic analogs eugenol, β-caryophyllene and eugenyl acetate, a combination of the pepper synthetic analogs eugenol and N-isobutylamine as well as clove buds, pepper fruit and a mixture of clove and pepper. Each treatment at a given concentration was tested on 32 brown spotted eggs. Eggs were first soaked in a respective solution for 20 min and thereafter placed on moistened filter paper to be incubated at ambient temperature. The percentage of inhibited eggs (PR) per treatment concentration was calculated in accordance with the equation of Zeng et al. (2010) after 5 days of incubation:

where NC is the total number of hatched eggs in the control treatment and NT is the total number of hatched eggs in the treatment.

$$PR = 100 \times \left[\frac{NC - NT}{NC + NT} \right]$$

Larval toxicity assays. In the experiment at the CRI, extracts of clove buds, neem seeds and pepper fruit at 0.8% (w/v) were each tested twice for insecticidal effects on 20 *C. sordidus* larvae at instar stage 1 to 5, respectively. The experiment at NARL in Uganda included the same nine treatments as in the egg inhibition assays, each applied at concentrations of 0, 0.1, 0.25, 0.5% (w/v). Each treatment at each concentration was tested on 20 larvae of instar stage three (5-day-old).

Larvae in each treatment received a diet that was based on published recipes and approximated growth requirements (Shimoji and Yamagishi, 2004). The diet was a composite of different ingredients (Table I), including banana rhizomes, used from weevil susceptible maiden sucker, that were sliced, solar dried and milled with a cutting mill (Fritsch, Pulverisette 15, Fritsch, Idar-Oberstein, Germany) to a fine powder. The diet was autoclaved at 121°C and

1034.21 hPa for 15 min before antibiotics (Table I) was added at 55°C prior to dispensing. For the bioassay, 0.2 ml of each treatment concentration and sterile water for the control were randomly pipetted into 24 well plates (BD Bioscience, MA, USA). Thereafter, approximately 1.8 ml diet at 55°C was added to each well and gently mixed before setting. Plates were allowed to cool overnight when two larvae were introduced per well. Ten wells were used for each treatment concentration and instar stage. The plates were incubated for 8 days at ambient temperature in the darkroom and the experiments were replicated two times. Percentage mortality was calculated as per Zeng et al. (2010). The larvae were recorded dead if its body was not moving when mechanically prodded.

Weevil repellent assay. Adult weevil repellent assays were conducted at NARL with 15 unsexed adult weevils for each extract (clove buds, neem seeds and pepper fruits), each at a concentration of 0.8% (w/v), and the substances eugenol, β-caryophyllene, eugenyl acetate and N-isobutylamine, each applied at 0.5% (w/v). A cup bioassay technique was used as described by Kumar et al. (2004). Freshly pared rhizomes from plantain sucker were soaked in 1 L of each treatment solution or in water (control) for 30 min, respectively. Treated rhizome pieces were each placed in plastic buckets (37 cm width, 16 cm height), perforated with 8 holes (each of 5-6 mm diameter) at mid-position and equally distributed around the circumference.

Weevils were starved for 12 h before released into each bucket, which was subsequently closed with a lid. Each bucket was placed into a larger plastic bucket and covered with a perforated lid for aeration. A weevil was considered repelled by the treatment if found in the outer bucket after 6, 12, 24 and 48 h for extracts, and 0.5, 3, 6, 12 and 24 h for synthetic analogs after release. Repellence was measured as the percentage number of adult weevils repelled out of the bucket at each observation time. The experiment was repeated twice at ambient room temperature.

Field evaluation. Field evaluations were conducted twice in a weevil-infested mature plantation in Ghana. Forty plantain plants of cv. Apantu, spaced apart at 3 m, were selected and assigned in a randomized complete block design to five replicated blocks of eight treatments. Twenty-six border plants surrounded the experimental field. Weevils were trapped for one week using a method described by Ogenga-Latigo and Bakyalire (1993) to estimate field weevil population. Ten unsexed weevils were marked with pedicure polish (Drahokoupilová and Tuf, 2012) per replicate, using treatment specific colour labels, and released to each plantain mat a day prior to the first of three treatment applications.

Within each block, one plantain mat was subjected to one of the following treatments at three-week intervals: extracts (0.8% w/v) and powder (80 g) of clove, neem and pepper, respectively, Carbofuran (60 g) and water control. The selected rate of Carbofuran is commonly used by farmers to control *C. sordidus* (Gold et al., 1999; Musabyimana et al., 2000). Treatments were incorporated into soils around the respective plant mats. At the end of each treatment interval, the weevil population for each mat and treatment was monitored using the Lincoln-Peterson Index of population size (capture-mark-recapture) method (Bellemain, Swenson, Tallmon, Brunberg, & Taberlet, 2005). The number of marked and unmarked weevils were recorded for each plant mat, with unmarked weevils being marked treatment specific and marked weevils that crossed to different treatments placed back to their respective treatment mats.

Data analyses. Data were analysed using a generalized linear mixed model (GLIMMIX) procedure of SAS 9.4 (SAS Institute Inc., Cary, NC, USA) to evaluate for treatment effects on response variables such as percentage egg hatching inhibition, larvicidal toxicity, adult repellency and weevil population reduction. The GLIMMIX procedure assumed equal variance and was specified with a binomial distribution and logit link function for the dependent variables (Piepho W et al., 2006; Kiernan et al., 2011). Data were graphically displayed with SigmaPlot (Systat Software Inc., San Jose, CA, USA).

III. RESULTS AND DISCUSSION

Egg inhibition assay. The inhibitory effect of botanical extracts on weevil egg hatching is presented in Table II. In general, the percentage of egg inhibition increased with increasing extract concentration. Among the treatments, egg hatchability was suppressed by 86-93 % for clove, 41-52 % for neem and 39-42 % for pepper. Clove and pepper extracts repressed egg hatching equal or even better than their synthetic analogs (Fig. I). However, clove extracts and eugenol had the most potential to inhibit egg hatching, particularly at the higher concentrations, indicating that eugenol is a key ingredient for repressing *C. sordidus* egg hatchability. In contrast, neem extracts are versatile to a wide range of insect species and target different developmental stages. While, for example, neem extracts repressed 52 % weevil egg hatchability in the current study, 79 % of Okra fruit borer eggs did not hatch when exposed to neem oil (Thara et al., 2009).

Larvae toxicity assay. All extracts at 0.8% concentration were significantly lethal to all instar larval stages (Fig. II) when compared to the untreated control where larvae mortality did not occur. The effectivity of the evaluated control measures against *C. sordidus* depended on developmental stage and treatment concentration. This is in agreement with reports on age-dependent susceptibility to plant extracts (Thara et al., 2009) and dose-dependent insecticidal effects of eugenol against pests like ants, American and German cockroaches (Enan, 2001). Specifically, larvae mortality across instar stages was between 78-100 % for clove, 13-85 % for pepper and 18-74 % for neem. Musabyimana et al., (2001) reported 40 to 60 % *C. sordidus* larvae mortality due to neem extract, a range that is comparable to 19-74% instar larvae dependent mortality reported in this study. While clove extracts tended to increase larvae mortality with larvae development, the toxicity of pepper and neem extracts decreased with increasing instar larvae stages. Since neem extracts induced only less than 50% larvae mortality, except instar stage 1, its synthetic analogs were not considered for further evaluation.

The effect of clove and pepper and their synthetic analogs at varying concentrations on instar larvae stage 3 is shown in Figure III. All treatments followed a similar pattern with larvae mortality steadily increasing at higher concentrations. Larvae exposure to 0.5 % induced around 80 % larvae mortality in all treatments, except for pepper extract and its constituent N-Isobutylamine with a larvicidal effect of only 60-65 %. Treatment mixtures of synthetic analogs had no synergetic effect on larvae mortality when compared to single product assays. N-Isobutylamine may be antagonistic to eugenol since the observed eugenol toxicity in a separate assay was not effective when blended with N-Isobutylamine (data not shown).

The observed 100 % mortality at instar larvae stage 5 prior to pupation might have been a result from increased dietary intake of clove bioactive compounds with insecticidal properties that inhibit the gut proteinases serine or cysteine in phytophagous insects (Macedo & Freire, 2011). Through such mode of action clove metabolites such as eugenol, eugenyl acetate and Caryophyllene (Razafimamonjison et al., 2013) are likely responsible for restraining *C. sordidus* performance. Similar observations were reported for grain storage pest (Sitophilus zeamais and Tribolium castaneum) (Obeng-Ofori & Reichmuth, 1997; Yan, Shuit-Hung, Hsien-Chieh, & Yen-Ling, 2002) and Culex mosquito (Culex pipiens) (Chaieb et al., 2007) when clove chemical derivatives inhibited egg hatchability and caused larvae and adult mortality. Clove extract effects on *C. sordidus* were comparable to those of their synthetic analogs which; however, exhibited a shorter efficacy. A loss of eugenol activity within 24 h of the application was also reported by Obeng-Ofori and Reichmuth (1997), results that may indicate a need for improved formulations to prolong insecticidal activity.

Weevil repellent assay. The repellency effect of the three plant extracts, each applied at 0.8 % concentration, to *C. sordidus* is summarized in Figure IV. Pepper extract effectively repelled most adult weevils, ranging between 80 to 98 %, followed by clove extract with 78 to 90 % repellency and neem extract that was least efficient with only repelling 63 to 75 % of the weevils. Moreover, neem extracts repellency of adult weevils is in good agreement with earlier studies, reporting 89 % (Musabyimana et al., 2000, 2001) and 65 to 73% repellency (Inyang and Emosairue, 2005). It is suggested, that the high efficacy of neem products in controlling *C. sordidus* is due to its key secondary metabolites azadirachtin and nimolinone. Azadirachtin works by demobilizing the ecdysteroid molting hormone (Dorn et al., 1986), preventing the larvae from developing into adults. In addition, dipping plantain or banana suckers in 20 % neem extract prior to planting provided protection from weevil attack through repellency that discouraged egg oviposition (C. S. Gold & Messiaen, 2000).

The potential of various synthetic analogs of clove and pepper metabolites, each applied at 0.5 % concentration, on repellency of *C. sordidus* is shown in Figure V. Repellency activity was greatest after 3 to 6 hours of exposure to all synthetic analogs, followed by a gradual decline over the observation period of 24 hours. The repellency activity of eugenol and β-caryophyllene was still around 60 % after 24 hours; however, that of eugenyl acetate and N-isobutylamine was reduced to below 40 %. Nonetheless, the demonstrated insecticidal properties of pepper to *C. sordidus* is likely attributable to a complex plant ingredient matrix since, for example, total extracts had more repellent effect than the synthetic analog of its ingredient N-Isobutylamine. These observations are similar to reports of Samuel et al. (2016), showing that piperine, a chemical derivative of *P. guineense*, had less toxicity to Anopheles larvae than what was inducible by total plant extracts. Therefore, the limited efficacy of N-Isobutylamine on weevil egg inhibition, larviciding and repellency properties indicates that it may not be the key pepper ingredient for controlling *C. sordidus*.

Plant extracts and synthetic analogs of their key ingredients restrained to varying degrees the vitality of *C. sordidus* based on the evaluated parameters egg hatching, larval development and adult repellency during laboratory and field studies. Although the efficacy of plant extracts to control insect pests may vary with species, many species are susceptible to similar active compounds (Mundi, Adamu, Ajayi, Bamayi L.J., & Egwurube, 2012; Musabyimana et al., 2001). For example, the observed larviciding effect of pepper against *C. sordidus* was also reported on *Anopheles gambia* species complex (Samuel, Oliver, Coetzee, & Brooke, 2016).

Field evaluation. The number of recaptured weevils was highly variable, depending on treatment and observation time (Table III). Carbofuran had the most consistent toxic effect of all treatments, reducing the weevil population by 45-80% compared to the control. This effect was closely matched by the 0.8 % neem extract treatment, with 40-70% lower weevil numbers than in the control. The clove extract had 40-50% fewer weevils than in the control; however, there was less consistent efficiency throughout the experimental period. In addition, pepper extract field activity against *C. sordidus* decreased with exposure time (Table III), an effect that was also reported for *P. nigrum* on Colorado potato beetles (Scott et al., 2003). This might be explained with the loss of volatility of pepper extracts and thus a time-dependent declining repellent effect on weevils. All other treatments were too variable or less efficient in controlling the weevil population.

IV. CONCLUSION

This study demonstrates the potential of using botanical pesticides such as extracts of neem, clove and pepper for controlling *C. sordidus* at varying stages throughout the lifecycle. Therefore, it can be concluded that effective alternatives to synthetic insecticides are water extractable plant metabolites that target octopaminergic neurons in invertebrates (Enan, 2001; I. B. Murray, 2000). It is recommended to use all plant extracts as a repellent, although their effectivity to repel weevils is reduced with time, possibly because of evaporation. In addition clove extract has also potential as a toxicant and egg inhibiting agent against *C. sordidus*. These natural low-cost products constitute an alternative pest management strategy for smallholder farmers and may help to reduce the occurrence of weevil resistance to synthetic insecticides. In contrast, all evaluated synthetic analogs proved to be useful repellents, but eugenol and eugenyl acetate were also effective as toxicants and egg hatch inhibitors. Proceeding studies should provide a toxicological understanding of how these botanical extracts and their synthetic analogs penetrate into insect cuticle and its metabolic target to give an insight into their specific mode of action.

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Table I. Composition of *C. sordidus* artificial diet.

Table II. Treatment effects on the mean percentage of inhibited hatching of *C. sordidus* eggs.

Table III. Mean number of recaptured weevils per treatment mat at three-week intervals over a period of 9 weeks.

Figure I. Treatment effects on mean percentage of inhibited hatching of *C. sordidus* eggs. Each point is the mean of two experimental repeats, each with n = 32. Eu_EuAc_Ca is a mixture of clove synthetic analogs eugenol, eugenyl acetate and β-caryophyllene; Eu_N-iso is a mixture of pepper synthetic analogs eugenol and N-isobutylamine. $LSD_{0.05}$ for each concentration is represented with a vertical bar.

Figure II. Treatment effects (0.8 % extract) on the mean percentage of *C. sordidus* larvae mortality at instar larvae stages 1 to 5. Each bar is the mean of two experimental repeats, each with n = 20. Vertical bars indicate $LSD_{0.05}$ with ***, **, * referring to significance at *P* value ≤ 0.001 and ≤ 0.01, ≤ 0.05, respectively, for each instar larvae stage.

Figure III. The effect of varying treatment concentrations on the mean percentage of *C. sordidus* larvae mortality at instar larvae stages 3. Each point represents a mean of two experimental repeats, each with n = 20. Eu_EuAc_Ca is a mixture of clove synthetic analogs eugenol, eugenyl acetate and β-caryophyllene; Eu_N-iso is a mixture of pepper synthetic analogs eugenol and N-isobutylamine. $LSD_{0.05}$ for each concentration is represented with a vertical bar.

Figure IV. The effect of *C. sordidus* exposure to various plant extracts, each applied at 0.8 % (w/v) concentration, on mean percentage of repelled adult weevils over 48 hours. Each point is the mean of two experimental repeats, each with n = 15. $LSD_{0.05}$ for time (hours) is represented with a bar.

Figure V. The effect of *C. sordidus* exposure to various synthetic analogs of plant metabolites, each applied at 0.5 % (w/v) concentration, on mean percentage of repelled adult weevils over 24 hours. Each point is the mean of two experimental repeats, each with n = 15. $LSD_{0.05}$ for time (hours) is represented with a bar.

Table I

Ingredients	Quantity (g)
Agar	40.0
Banana rhizome powder	80.0
Dextrose	40.0
Yeast extract	9.0
Cellulose	14.4
Casein	21.6
Vitamin B	0.045
Salt mixture	2.7
Ascorbic acid	1.8
Chlorine chloride	0.45
Methyl 4-hydroxybenzoate (Nipagine)	0.67
Inositol	0.36
Stigmasterol	0.72
Potassium sorbate	0.67
Tetracycline	0.2
Ethanol	10.0[1]
Distilled water	1000.0[1]

Table II

Species	Extract concentration (%)					Statistics	
	0	0.2	0.4	0.6	0.8	*F-Value*	$LSD_{0.05}$
Clove	6	86	86	93	93	267.02	9.0
Neem	6	41	46	52	47	8.72	24.5
Pepper	6	39	42	39	42	60.36	7.8
F-Value		95.18	79.13	57.32	33.79	10.30	
P-Value		0.010	0.012	0.017	0.029		
$LSD_{0.05}$		16.8	16.8	22.4	29.6		11.6

Each treatment was repeated twice, each with n = 26. Least significant difference ($P \leq 0.05$).

Table III

Treatment	Mean number of weevils per mat at week			
	3	6	9	Average
Control	20 [1]	10	27	19.0
Carbofuran	11	2	6	6.3
Clove extract	10	12	10	10.5
Clove powder	15	7	17	13.1
Neem extract	8	6	8	7.3
Neem powder	18	16	11	14.8
Pepper powder	9	6	29	14.7
Pepper extract	14	6	29	16.2
F-Value	1.03	1.55	1.68	1.21
P-Value	0.43	0.19	0.15	0.27
$LSD_{0.05}$	12.69	9.83	22.08	15.77

[1] Each number is the mean of two experimental repeats and five replicates per treatment, each with n = 10 per mat.

Figure I

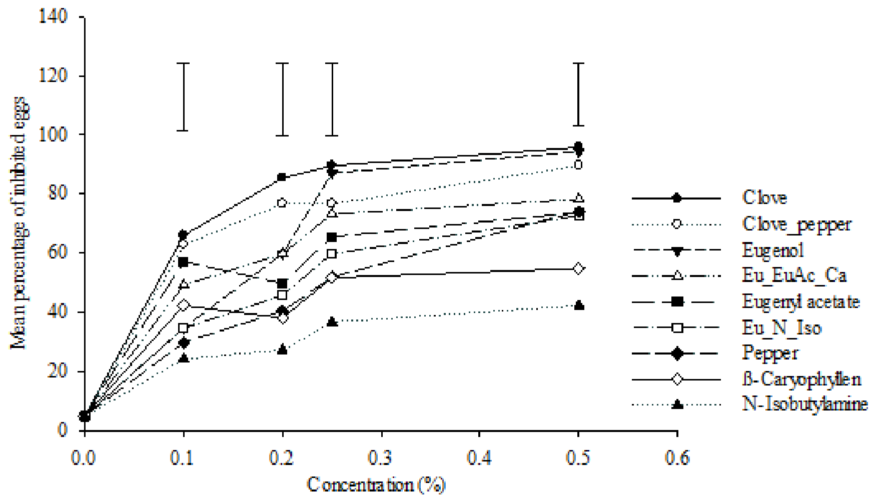


Figure II

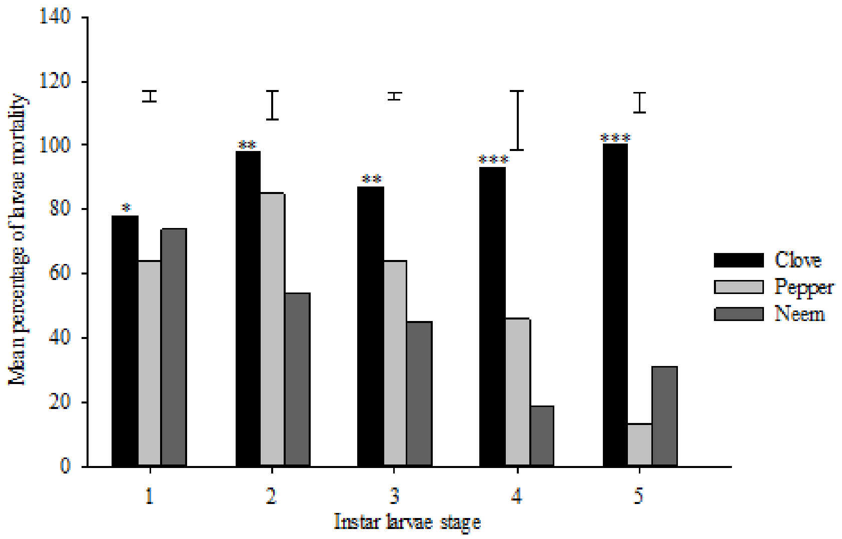


Figure III

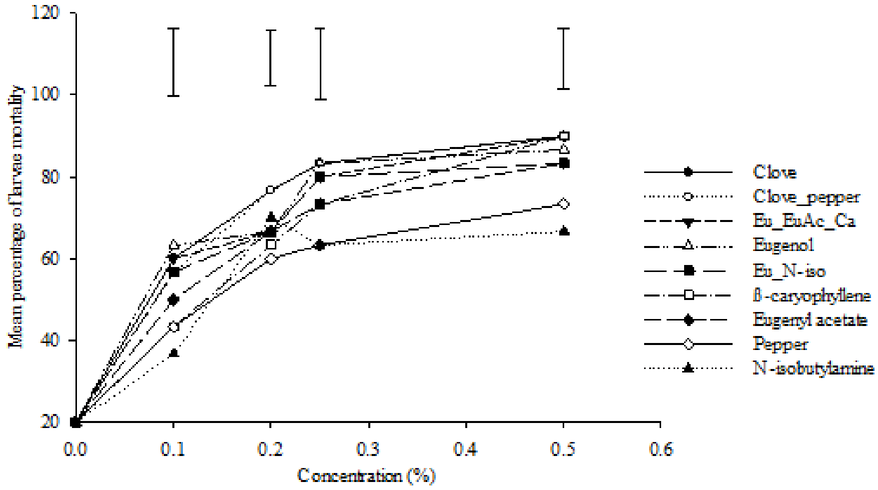


Figure IV

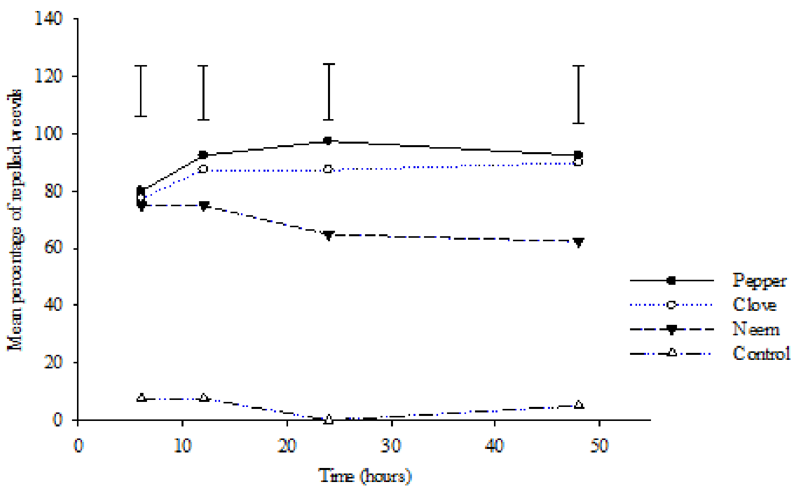
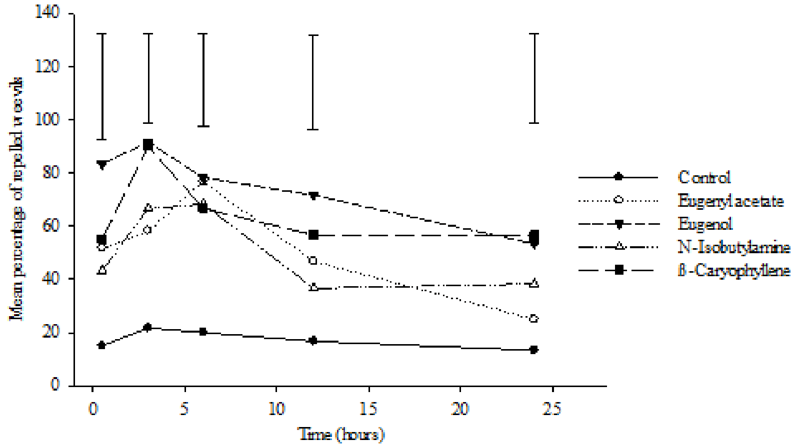


Figure V


Mean percentage of repelled weevils
140
120
100
80
60
40
20
0
0
5
10
15
20
25
Time (hours)
Control
Eugenyl acetate
Eugenol
N-Isobutylamine
ß-Caryophyllene