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Spinosad-induced stress on the maize weevil *Sitophilus zeamais*

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Abstract

Although seldom considered, sublethal insecticide exposure may lead to harmful, neutral, or even beneficial responses that may affect (or not) the behavior and fitness of the exposed insects. Intriguingly, little is known about such effects on stored product insect pests and even less is available regarding the bioinsecticide, spinosad. Thus, we assessed the sublethal effects of spinosad on walking, feeding, drinking and mating behaviors of maize weevils (*Sitophilus zeamais*), also assessing their survival, reproductive output, and grain loss compared with maize weevils exposed to the pyrethroid deltamethrin (as positive control), and water only (negative control). Both spinosad and deltamethrin were able to effectively control the insects, although the latter caused a faster mortality than the former. Behavioral pattern changes were caused by both insecticides, especially deltamethrin, triggering irritability (i.e., avoidance after contact). Different feeding and drinking responses were also detected with significant avoidance to deltamethrin, but not to spinosad. Maize weevil couples sublethally exposed to deltamethrin and spinosad exhibited altered reproductive behavior, a likely consequence of their altered activity, but deltamethrin caused greater behavioral changes. Curiously, higher progeny emergence and grain loss were observed in deltamethrin-exposed insects, suggesting that this pyrethroid insecticide elicits hormesis in maize weevils that may compromise control efficacy by this compound. In contrast, such effect was not detected with spinosad, which did not elicit avoidance allowing the intended weevil exposure and control.

Keywords: biopesticide, hormesis, insecticide avoidance, sublethal exposure, progeny production

Introduction

Insecticides are a familiar class of pest control agents, understandable due to their broad use since the 1940's across many sectors, including stored product protection. Although insecticides are technically defined as "*any substance or mixture of substances intended for preventing, destroying, repelling, or mitigating any insect pest*" (e.g., US Federal Insecticide, Fungicide, and Rodenticide Act), these compounds are popularly defined as substances that kills insects. The blame probably lays with the old Romans and the Latin origin of the suffix *cide* (from *cīda*; = a killer of), which is rather popular and frequent in several nouns of different languages. Regardless, the emphasis of the popular definition of insecticide is on the *killing* of insects, not managing or controlling them, as

advocated by the technical definition. Such fact leads to an important bias on dealing with insecticides – the emphasis and reliance on their mortality effect, while largely neglecting their sublethal consequences (Hardin et al., 1995; Guedes et al., 2016).

Lethal effects of insecticides are certainly important, and the primary intent of most users is indeed to quickly kill the pest species. Importantly, however, sublethal exposures can cause population suppression without necessarily causing death; reproductive impairment, for instance, can be as effective, or even more effective, for pest control. It is critical to understand the issues that contribute to a sublethal exposure. Initial insecticide deposits degrade over time, lowering residue levels, eventually to the sublethal range for a length of time that is a complex function of toxicological and environmental factors. Another contributor is that the lethal concentration applied to target a given pest is potentially sublethal to other species, particularly co-occurring species.

Sublethal insecticide exposure is known to affect arthropod development, longevity, reproduction, and even the genetic make-up of the population (Lee, 2000; Guedes et al., 2016, 2017). Sublethal exposures may lead to shifts in species prevalence (or dominance) (Cordeiro et al., 2014) creating unforeseen pest outbreaks, as well as inadvertent selection(s) for insecticide resistance in non-targeted pest species (Haddi et al., 2015; Guedes et al., 2017). Behavioral changes are also among the potential consequences of sublethal insecticide exposure. These changes may involve general activity, mobility, feeding, mating and egg-laying, among others, all potentially affecting the maintenance and growth of the arthropod pest population (Haynes, 1988; Lee, 2000; Guedes et al., 2016). Therefore, a range of responses may accrue from sublethal insecticide exposure. In fact, novel insecticidal chemistries have increasingly relied on secondary and behavioral effects (Casida and Durkin, 2013; Guedes et al., 2016). Curiously, sublethal insecticide exposure is rarely scrutinized in stored product scenarios, where it is common for pest species co-occur with finite resource (Guedes et al., 2011, 2014).

Another conceptual bias that commonly plagues the general public perception of insecticides, and also influences pest research and management, is the deeply-rooted notion that natural compounds are safer than synthetic ones. While this may be valid in certain cases, the notion in based on the false premise that origin is a determinant of toxicity, and safety (Coats, 1994; Isman and Grieneisen, 2014; Guedes et al., 2016). The selection of a "natural" insecticide, including biopesticides, reduced-risk pesticides, biorational compounds, insecticidal proteins, and an increasing variety of neologism, pleonasms, and/or misnomers should be considered based not on semantic qualification, but on the chemistry that dictates toxicity and safety.

Spinosad is an insecticide of natural origin, or bioinsecide, with recent use in stored product protection, not yet subjected to comprehensive sublethal studies. The technical active ingredient (a.i.) is a mixture of spinosyns A and D, fermentation products of the soil actynomycete *Saccharopolyspora spinose* Mertz and Yao (Thompson et al., 2000; Sparks et al., 2001). Earlier launched for field crop use, its market was more recently extended to stored products as a grain protectant efficient against a range of pest species (Toews and Subramanyam, 2003; Huang and Subramanyam, 2007; Athanassiou et al., 2008; Athaniassiou and Kavallieratus, 2014), including the grain weevils (Coleoptera: Curculionidae) (Athaniassiou and Kavallieratus, 2014). In warmer climates, particularly Neotropical America, the maize weevil *Sitophilus zeamais* Motsch is a key pest, and virtually nothing is known about (sublethal) effects of spinosad, despite its potential usefulness for conventional and organic production and storage systems.

We assessed the sublethal effects of spinosad on the overall activity, walking, feeding, drinking and mating behaviors of maize weevils (*Sitophilus zeamais*), and evaluated results relative to conspecifics exposed to the pyrethroid deltamethrin (as positive control), and water only (negative control). Differences in the sublethal effects of the two insecticides were suspected, as the mechanism and modes of neurotoxic action are distinct; deltamethrin is a Na-channel modulator of the axon of neurons (i.e., nerve cells) with quick activity, in contrast with spinosad, which is a disruptor of

nicotinic acetylcholine receptors (nAChRs) in synapses of the insect nervous system with potentially slower activity (Sparks et al., 2001; Casida and Durkin, 2013).

1. Material and Methods

1.1. Insects and insecticides

The maize weevil population used in the study was originally collected in Sete Lagoas county (State of Minas Gerais, Brazil). This population is used as a susceptible standard population in studies of insecticide resistance and has been maintained on maize grains free of insecticide residues under controlled conditions of $27 \pm 2°C$, $70 \pm 10\%$ r.h., and 12:12 h photoperiod (L:D).

The insecticides were used in their respective commercial formulations available in Brazil for stored product protection and at the recommended label rates, as follows: deltamethrin (K-Obiol 25 CE; emulsifiable concentrate at 25 g a.i./L; Bayer, São Paulo, SP, Brazil), and spinosad (Tracer 480 SC; suspension concentrate at 480 g a.i./L, Dow, Mogi-Mirim, SP, Brazil). The insecticides were diluted in distilled and deionized water at the concentrations of 0.25 and 0.50 mf a.i./L of deltamethrin and spinosad, respectively. The insecticide solutions were sprayed in batches of 400 g of maize grains at 0.5 and 1.0 mg a.i./kg grain using an artist air brush (Saguma SW440A, Yamar, São, SP, Brazil) connected to an air compressor (model 131 type 2VC, Primatec, Itu, SP, Brazil) at 3 bar pressure. The maize grains were sprayed within a stainless-steel container coupled to a revolving rotor to homogenize the grain coverage until the residues dried. The air brush and revolving container were cleaned with acetone; distilled and deionized water was used as negative control treatment.

1.2. Survival bioassay

Time-mortality bioassays were conducted using 3- to 7-days old adults (unsexed). Individual insects were placed inside 30-mL glass vials containing 10 g of treated maize (i.e., deltamethrin, spinosad, or water (control)). Twenty insects were used in each insecticidal treatment and their respective survival was monitored at 30 min (for first two hours) and 1 h intervals for deltamethrin, and at 1 h (first 8 hs) and 6 h-interval afterwards for spinosad and water. The insects were considered as dead if unable to respond when prodded with a fine hair brush.

1.3. Overall group activity

Adult weevils subjected to either 30 min (deltamethrin) or a 10-h exposure (spinosad and water), the duration corresponding to non-observed effect levels as determined in the survival bioassays, were clustered in groups of 10 individuals within Petri dish arenas (9-cm diameter) lined at the bottom with filter paper and coated with Teflon® to prevent insect escape (Guedes et al., 2009b). The overall group activity within each arena was recorded for 15 min and digitally transferred to a computer using an automated video-tracking system equipped with a CCD camera (ViewPoint LifeSciences, Montreal, Canada). Overall activity was digitally recognized as changes in pixels in two successive frames taken every 10^{-2} s from each other representing any change of position and posture of the insects. The bioassays were carried out under the same conditions as previously described and during daytime.

1.4. Walking bioassays

Walking bioassays in half-treated arenas were performed to assess insecticide behavioral avoidance by means of irritability (i.e., with contact with insecticide) and repellence (i.e., without direct contact with insecticide). Unexposed insects were released alone in individual Petri dish arenas lined with filter paper half-treated with either deltamethrin or spinosad and their movement was digitally recorded for 15 min with the tracking system described above (Cordeiro et al., 2010; Morales et al., 2013). Again, 20 replicates were used for each dichotomous bioassay with either deltamethrin or spinosad vs water (control).

1.5. Feeding and drinking preference

A free-choice test modified from Guedes et al. (2009a) was performed using white plastic trays (30 x 18 x 6 cm) with 200 g of water- and insecticide-sprayed grains placed in opposite sides. The inner walls of the trays were covered with Teflon® to prevent insect escape and 25 unsexed adult weevils (1-2 weeks old) were released in the center of the arena. Insect preference was recorded after one hour and the bioassays were replicated five times.

A dichotomous bioassay of water drinking preference was carried out as described by Guedes et al. (2014) providing a choice between 50-µL droplets of water uncontaminated ,or insecticide-contaminated (deltamethrin or spinosad), after maintaining the insects under 30-40% relative humidity for 24 hs. The insecticide contaminations were used at the same rates from previous bioassays, and each droplet was stained with either artificial blue or red dye (Mix Industries, São Bernardo do Campo, SP, Brazil). The choice of water droplets was provided in 9-cm Petri dish arenas and the insects were observed for 5 min. Water intake was confirmed by dissecting the insects and examining evidence of the dye coloration in the insect gut diverticula, what was performed under stereomicroscope (Stemi 2000; Zeiss, Göttingen, Germany).

1.6. Female mate-searching

Virgin weevil females (< one week old) were treated with insecticides (except in the control) as previously described, and transferred to 9-cm Petri dish arenas containing a male weevil caged in its center (Guedes et al. 2017; Cordeiro et al. 2017). The searching activity of the females was recorded again using the ViewPoint tracking system recording search time and velocity for up to 2 hours under the same conditions of the previous experiments.

1.7. Progeny emergence and grain consumption

Three groups of 35 virgin weevil couples (one week old) were treated with insecticide as previously described and subsequently released in 140-mL jars containing 50 g maize free of insecticide residues. The insects were removed after 30 days and progeny production and grain loss were recorded. Progeny production was daily assessed until emergence of the last adult, and grain loss was determined in sequence with eventual correction for humidity change, if necessary.

1.8. Statistical analyses

Time-mortality data was subjected to survival analyses using Kaplan-Meyer estimators allowing determination of the respective median survival times (LT$_{50}$)(PROC LIFETEST; SAS, SAS Institute, Cary, NC, USA). The curves were compared using Bonferroni's method. Individual and overall group activity, irritability, repellence, and feeding and drinking preferences were subjected to general linear model and contrasted by χ^2 test (PROC GENMOD; SAS). Female searching time, progeny production and grain loss were subjected to analyses of variance and Tukey's HSD test ($P < 0.05$), when appropriate (PROC GLM; SAS).

2. Results

3.1. Survival time

The survival curves of weevils exposed to either insecticide, deltamethrin or spinosad, and the control were significantly different ($\chi^2 = 409.37$, df = 2, $P < 0.001$). Natural (i.e., control) mortality was negligible for up to 15 days, in contrast with insecticide-exposed weevils (Fig. 1). Deltamethrin led to quick mortality among the exposed adult weevils with median survival time of 3.5 hs, while median mortality by spinosad took significantly longer (i.e., 76.5 hs) (Fig. 1).
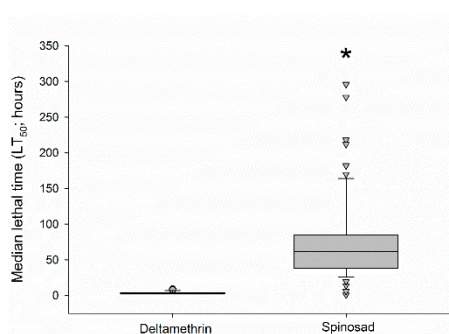
Fig. 1. Median lethal times (TL₅₀) of insecticide-exposed adult maize weevils (*Sitophilus zemais*). The box plots indicate the median and dispersion (lower and upper quartiles, and outliers) of the median lethal times. The asterisk indicate significant difference between insects exposed to the insecticides using Bonferroni's method ($P < 0.05$).
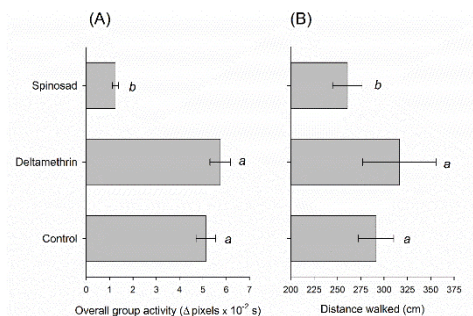
Fig. 2. Overall group activity (A) and distance walked by individual weevils (B) (± SE) subjected to insecticide exposure (except control with unexposed insects). Different low case letters in the bars indicate significant differences by χ^2 test ($P < 0.05$).

3.2. Activity

The overall group activity also significantly differed among treatments with spinosad-exposed adult weevils exhibiting significant less activity than unexposed and deltamethrin-exposed weevils ($\chi^2 = 181.45$, df = 2, $P < 0.001$) (Fig. 2A). When distance walked by individual insects was considered as a proxy of individual activity, a similar trend was observed. Again spinosad compromised activity and led to the lowest distance walked compared with deltamethrin and unexposed insects, which provided similar results ($\chi^2 = 55.68$, df = 2, $P < 0.001$) (Fig. 2B).
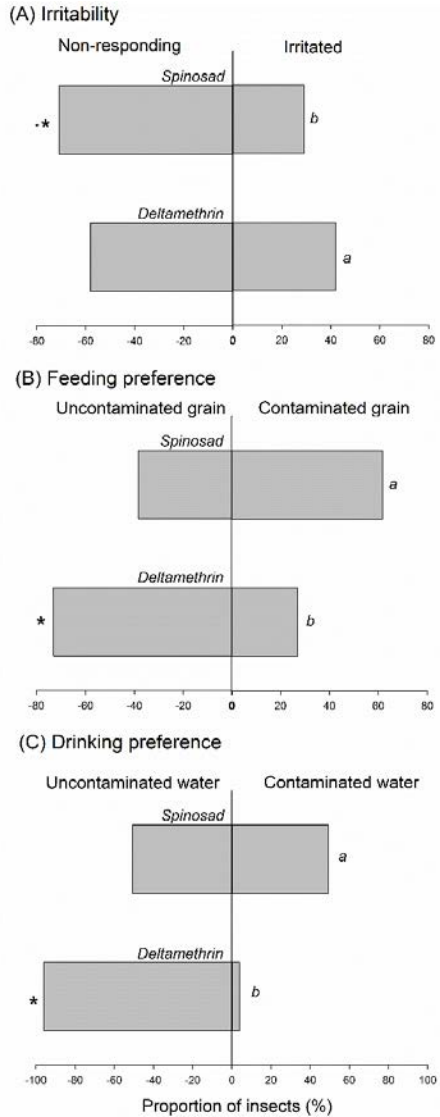
3.3. Feeding and drinking preference

Behavioral avoidance among insecticide-exposed weevils indicated significant irritability ($\chi^2 = 4.73$, df = 2, $P = 0.03$), or avoidance after contact with contaminated surface, but no repellence (i.e., avoidance without contact with contaminated surface). Most insects did not respond to spinosad though, in contrast to deltamethrin against which 40% of the insects exhibited avoidance by irritability (Fig. 3A).

Feeding preference also differed between insecticides when given a choice between uncontaminated and contaminated maize grains. Weevils did not exhibit feeding preference when offered uncontaminated and spinosad-contaminated grains, but the insects significantly avoided deltamethrin-contaminated grains in favor of uncontaminated maize kernels ($\chi^2 = 25.53$, df = 1, $P = 0.0004$) (Fig. 3B). Such a trend was also observed when water was provided for drinking with weevils avoiding deltamethrin-contaminated water ($\chi^2 = 39.32$, df = 1, $P < 0.001$), but no avoidance was detected with spinosad-contaminated water ($\chi^2 = 2.91$, df = 1, $P = 0.10$) (Fig. 3C).

3.4. Female-mate searching, progeny production and grain loss

The female searching for suitable male partner was significantly affected by insecticide exposure ($F_{2,57} = 39.63$, $P < 0.001$). Unexposed females were able to find their mates relatively quicker, while spinosad and particularly deltamethrin significantly extended such searching time (Fig. 4A). Nonetheless, the differences in mate searching time did not significantly affect the total progeny produced by each female weevil ($F_{2,102} = 0.35$, $P = 0.70$) (Fig. 4B), but deltamethrin-exposed weevil led to higher grain loss than those unexposed or exposed to spinosad ($F_{2,102} = 13.93$, $P < 0.001$) (Fig. 4C).

Fig. 3. Proportion of adult weevils showing irritability to insecticide-contaminated surfaces (A), and exhibiting feeding (B) and drinking preferences (C) with choice of uncontaminated and insecticide-contaminated grains and water. Different letters in each bar indicate significant differences between treatments and asterisk indicates significant difference between proportion of uncontaminated and insecticide-contaminated material (i.e., surface, grain, or water). All differences were detected with χ^2 test ($P < 0.05$).
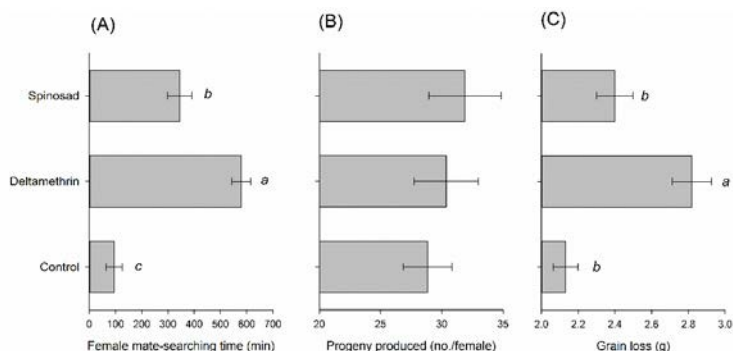
Fig. 4. Female mate-searching time (A), total progeny produced per female (B), and total grain loss (C) (± SE) by weevils subjected to insecticide exposure (except control with unexposed insects). Different lower case letters in the bars indicate significant differences by χ^2 test ($P < 0.05$).

3. Discussion

The lack of information regarding the sublethal effects of the bioinsecticide spinosad led us to evaluate its impact on walking, feeding, drinking and mating behaviors of maize weevils (*Sitophilus zeamais*), also assessing their survival and reproductive output compared with maize weevils exposed to the pyrethroid deltamethrin (as positive control), and water only (negative control). Interference of both neurotoxic compounds with weevil activity were expected with potential consequences for mating, reproduction and grain (weight) loss. Indeed behavioral changes were induced by both insecticides although the effects of deltamethrin were stronger, but unexpectedly enhancing grain loss instead of reducing it.

Both deltamethrin and spinosad were effective against adult maize weevils exposed to contaminated grains at their respective recommended label rates as grain protectants, although deltamethrin exhibits faster activity, as previously been shown (Athanassiou et al., 2008; Athaniassiou and Kavallieratus, 2014). Nonetheless, the impact of both compounds go beyond mortality. Sublethal effects of deltamethrin and spinosad have been reported in other species (Elliott et al., 1978; Huang and Subramanyam, 2007; Amakware et al., 2014; Velki et al., 2014). Overall group activity and walking activity were both significantly reduced by spinosad, a likely consequence of its mode of action modulating nicotinic acetylcholine receptors (nAChR) and interfering with receptors of γ-aminobutiric acid (GABA) at synapses of the nervous system (Sparks et al., 2001; Casida and Durkin, 2013).

Among the two different types of insecticide avoidance behavior reported among insects, repellence (i.e., avoidance with little or no contact) and irritability (i.e., avoidance after contact), only the latter was observed in weevils and sole with deltamethrin, not with spinosad. Weevils exhibited significant irritability to deltamethrin and avoided feeding on deltamethrin-contaminated grains and water, which may potentially reduce exposure to, and targeted-efficacy of this compound. Irritability and associated behavioral responses toward pyrethroids, such as deltamethrin, were already reported among other arthropod species (Quisenberry et al., 1984; Vatandoost, 2001; Pekar and Hadda, 2005; Guedes et al., 2009ab, 2014). In contrast, spinosad avoidance was reported only in a couple of species of predatory stink bugs (Castro et al., 2013).

Insecticides may also interfere with insect communication (Guedes et al., 2016, 2017), which may potentially disrupt mating and reproduction (Lürling and Scheffer, 2007; Guedes et al., 2016). Indeed, spinosad and deltamethrin extended the mate searching time in exposed female weevils, but without significant effect on progeny production. In fact, the trend was of increased progeny production with insecticide exposure, which was reinforced by the higher grain loss obtained, particularly for deltamethrin. With sublethal insecticide exposure, we expected a decrease in progeny production and an extension of the time spent searching for a mate. However, the opposite

was observed in our study, suggesting that the longer searching time may have favored the selection of better quality male partners, potentially leading to higher (and/or better quality) progeny production and more feeding leading to heavier grain losses. The lack of significant difference in the total progeny produced is a likely reflex of not accounting for the time of 1st reproduction in our assessment, and not assessing progeny quality. The former condition shortens generation time, leading to higher population growth and progeny numbers with time, conditions that were beyond the scope fo this investigation. The consequence of higher population growth is more feeding, and ultimately higher grain loss, as was observed to a greater extent for deltamethrin, relative to spinosad.

Higher grain losses resulting from sublethal insecticide exposure is a counter-intuitive outcome. However, this outcome takes place when insecticide-induced hormesis is present. Hormesis is a biphasic dose-response phenomenon that takes place when a stimulatory effect is observed from the low dose of a compound demonstrated to be toxic at higher doses (Guedes and Cutler, 2014; Guedes et al., 2016, 2017). Deltamethrin-induced hormesis has already been reported in the maize weevil (Guedes et al., 2010), and the same likely occurred in our study. The pyrethroid effective against the exposed parental population may have induced higher reproductive output of the better-quality (surviving) parents, leading to higher and/or better-quality progeny. Although the final progeny population was not significantly higher with deltamethrin, the grain loss observed provides at least partial support for this contention. Hormesis, in this case, was probably the result of a trade-off where energy resources for the parent self-maintenance are diverted to offspring production (Guedes and Cutler, 2014), which are of better quality and/or in higher numbers leading to higher grain loss.

Insecticide behavioral avoidance and hormesis are two management concerns for pest species in general, and the maize weevil in particular. The former potentially minimizes exposure to the insecticide, while the latter favors population growth with exposure. Evidence for both phenomena was observed in our study with the maize weevil, but only with the insecticide deltamethrin, not spinosad. Therefore, deltamethrin use deserves particular attention and spinosad, although not as quick in leading to adult mortality, is also a very effective insecticide against the maize weevil and without apparent risk of minimizing exposure or leading to hormesis, at least at the label rate conditions used in our study. Thus, spinosad is an attractive alternative for weevil management, not due to its natural origin, but due to its insecticidal activity. The natural origin of spinosad however, makes it an enticing alternative for organic production and storage systems, a condition in which the origin, rather than chemistry, receives emphasis.

Acknowledgement

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Effects of *Hemizygia welwitschii* leaf extract fractions on postharvest infestation of maize by *Sitophilus zeamais* Motsculsky (Coleoptera: Curculionidae)

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Abstract

As part of on-going efforts to use eco-friendly alternatives to chemical pesticides, leaf powder of *Hemizygia welwitschii* was sequentially extracted in hexane, acetone and methanol. Bioassays were carried out to establish the most active fraction(s) against *Sitophilus zeamais* in maize. Maize grains (50 g) were treated with concentrations within the range 2, 4, 6, and 10 g/kg of extract and *Azadirachta indica* seed oil (positive control) in the laboratory. The total number of progeny emerging from grains infested separately with *S. zeamais* eggs, larvae and pupae were recorded. Adult mortality counts were carried out 1, 3, 7 and 14 d post-exposure. Acetone extract was more toxic to the eggs, larvae and pupae than the other extracts, inhibiting progeny production by 90.90%, 88.10% and 100%, respectively, at the concentration 10 g/kg. For the same concentration, *A. indica* seed oil reduced progeny production by 100% for eggs, 96.08% for larvae and 70.93% for pupae. Hexane extract was more potent to the adult weevil than the other extracts, recording 100% mortality for the concentration 10 g/kg within 14 d. LC_{50} values were 0.78 (Hexane), 5.52 (acetone) and 1.69 g/kg (methanol). Extracts of *H. welwitschii* leaves had sufficient efficacy to be a component of storage pest management package for *S. zeamais*.

Key words: Leaf powder, Mortality, Grain damage, Pest management

1. Introduction

Maize (Zea mays L.) is a staple food for a large proportion of the world with significant economic importance. It is currently the third most-cultivated and traded cereal after wheat and rice (FAO, 2006). The highest amounts of maize consumed as food are found in Southern Africa at 85 kg/capita/year as compared to 27% in East Africa and 25% in West and Central Africa (Smale *et al.,* 2011). The crop is characterized by the diversity of its consumption forms: fresh, boiled, roasted, and "foufou" (Ndjouenkeu *et al.,* 2010). A world challenge is to increase the global maize production to feed nine billion people by 2050 (Godfray *et al.,* 2010).

The production and storage of maize have faced many constraints throughout developing countries such as scarcity of rain, diseases and lack of inputs (Brisibe *et al.,* 2011), and most important constraint being the field-to- store infestations of maize weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) (Akob and Ewete, 2007). This insect inflicts severe damages leading to weight loss and reduction of the economic value, grain viability and nutritive value of maize (Akunne *et al.,* 2013). According to Obeng-Ofori and Amiteye, (2005) and Yuya *et al.,* (2009), about 20 to 40% of