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Toxicity of botanical and synthetic formulations to the maize weevil, *Sitophilus zeamais* (Coleoptera: Curculionidae)

Toxicidad de las formulaciones botánicas y sintéticas para el gorgojo del maíz, *Sitophilus zeamais* (Coleoptera: Curculionidae)

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Abstract: Maize is attacked by various pest species, including the key-pest of stored maize, *Sitophilus zeamais* (Coleoptera: Curculionidae). This study determined the LC_{50} and LD_{50} of neem-based insecticides (AzaMax® and Natuneem®) in comparison to a synthetic pyrethroid insecticide (Decis® 25 CE) to *S. zeamais*. The bioassays consisted of insects' exposure alone (topical application with microsyringe) and exposure of both insects and seeds treated simultaneously. The final bioassays used four to six concentrations of each formulation per bioassay, diluted in distilled water. The bioassays were run with the use of 10 non-sexed adults of *S. zeamais* per replication and mortality was assessed after 48 h of exposure. The data regarding concentration and dose-mortality were analyzed by probit analysis. Both LC_{50} and LD_{50} were used to calculate LCR and LDR's and their respective confidence interval (CI). After using topical application, the bioassays yielded LD_{50}-values of 51.32, 76.76 and 42.75 µL of AzaMax, Natuneem and Decis/g of insects, respectively. The bioassays with simultaneous exposure of both insects and seeds yielded LC_{50}-values of 4.01, 4.46 and 0.41 µL of AzaMax, Natuneem and Decis/g of seeds, respectively. Regarding the fact that there were no significant differences between the LC_{50}-values of the botanical insecticides, both of them can be used to manage *S. zeamais* infesting stored corn. The cost to treat maize and to obtain effective control of *S. zeamais* is cheapest for Decis<Natuneem<Azamax. The LC_{50}-values found in our study would be equivalent to use 8.02, 8.92 and 0.82 L of AzaMax, Natuneem and Decis/ton of seeds.

Key words: Chemical control, *Zea mays*, *Azadirachta indica*, acute toxicity.

Resumen: Varias especies de plagas atacan al maíz, entre ellas la principal plaga del maíz almacenado, *Sitophilus zeamais* (Coleoptera: Curculionidae). Este estudio determinó las CL_{50} y DL_{50} de insecticidas a base de nim (AzaMax® y Natuneem®) en comparación con un insecticida piretroide sintético (Decis® 25 CE) para *S. zeamais*. Los bioensayos consistieron de exposición de insectos solos (aplicación tópica mediante microjeringa) y de exposición de insectos y semillas tratados simultáneamente. El bioensayo final utilizó de cuatro a seis concentraciones de cada formulación por bioensayo, diluidas en agua destilada. Los bioensayos se hicieron con 10 adultos no sexados de *S. zeamais* por repetición y tras 48h de exposición se evaluó la mortalidad. Los datos de concentración y dosis-mortalidad fueron analizados mediante análisis de probit. Las CL_{50} y DL_{50} fueron utilizadas para calcular la tasa de toxicidad con su respectivo intervalo de confianza. Los bioensayos con aplicación tópica produjeron DL_{50} de 51,32, 76,76 y 42,75 µL de AzaMax, Natuneem y Decis/g de insectos, respectivamente. Los bioensayos con exposición simultánea de insectos y semillas produjeron CL_{50} de 4,01, 4,46 y 0,41 µL de AzaMax, Natuneem y Decis/g de semillas, respectivamente. No se detectaron diferencias significativas entre los valores de CL_{50} de insecticidas botánicos, así que ambos se pueden utilizar para el manejo del maíz almacenado infestado con *S. zeamais*. El costo para tratar el maíz y obtener un control efectivo de *S. zeamais* es menor para Decis <Natuneem <Azamax. Los valores de CL_{50} encontrados serían equivalentes a usar 8,02, 8,92 y 0,82 L de AzaMax, Natuneem y Decis/ton de semillas.

Palabras clave: Control químico, *Zea mays*, *Azadirachta indica*, toxicidad aguda.

Introduction

Maize is infested in the field and during storage by various pest species. During storage the maize weevil, *Sitophilus zeamais* Motschulsky, 1885 (Coleoptera: Curculionidae), is the most important pest causing severe quantitative and qualitative losses. Larvae and adults feed internally in the seeds, causing losses in weight and quality and increasing infection by pathogens, which are harmful for human health, *e.g.*, aflatoxins (Pacheco and Paula 1995; Hell *et al.* 2000; Fontes *et al.* 2003).

Control of this pest is based on use of synthetic insecticide fumigants, *e.g.*, aluminum and magnesium phosphide (inorganic precursor of phosphine) and contact products, e.g., fenitrothion and pirimiphos-methyl (organophosphate), bifenthrin and deltamethrin (pyretroid) and esfenvarelate (pyretroid) + fenitrothion (organophosphate) (Brasil 2016). Because of their non-specific mode of action, acting on the central nervous system and on the energetic metabolism (Brazilian Committee of Action to Prevent Resistance to Insecticides - IRAC-BR 2015), these products can be deadly harmful to non-target organisms.

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Hence, the search for viable alternatives of control to deal with this pest is more than just a reality, but a necessity. Besides, the lack of insecticides with alternative modes of action to be used in rotation for mitigating pest-resistance, this species has exhibited resistance to various synthetic insecticides (Ribeiro *et al.* 2003). Thus, some botanical insecticides are already available including neem-based formulations, *e.g.,* AzaMax®, which is registered in the Brazilian Agriculture, Poultry and Supply Ministry (MAPA) for the control of field pests (Brasil 2016). Additionally, other neem-based formulations, although not registered, are recommended to manage storage pests as is the case of Natuneem®.

Azadirachtins, which make up the active ingredient (a.i.) of neem-based formulations since they are the most active and abundant phytochemical in neem and show action against at least 550 insect species (Gahukar 2014), are considered to be selective to mammals based on their specific mode of action. They act on physiological processes inherent to insects, related to metamorphosis and ecdysis (Mordue and Nisbet 2000). Furthermore, they are of special interest for use in the prevailing condition of the storage environment (darkness), since one of its main components which has known insecticidal activity, e.g. azadirachtin-A (makes up 73 % content of the azadirachtin), is light degraded after 2.47 days while covering foliar surfaces (Johnson *et al.* 2003). To date, there is a lack of information concerning the efficiency of these formulations on control of storage pests, such as *S. zeamais*.

The first step to be taken when selecting alternative products for pest control is to define the discriminating dosages or concentrations (acute toxicity) that are effective in killing the pest. This is generally made through estimation of the LC_{50} and LD_{50}, which are, respectively, the concentration and dosage that kill 50 % of the population. These estimations, when doubled, are expected to harm approximately 100 % of the population. The estimations are made on this range since there is a higher degree of reproducibility and they are more reliable while making assumptions for a population (Costa *et al.* 2008). Thus, this study focused on estimating the LC_{50} and LD_{50} values of neem-based formulations in order to define the discriminating dosages and concentrations effective to control *S. zeamais* and comparing them to a contact synthetic insecticide used against the pest.

Material and methods

Experimental conditions. The insects used were obtained from mass rearing on sweet maize seeds, cv. Doce Cristal from Embrapa. Seeds of the same maize genotype were used in the bioassays. The maize weevil colony was initiated with insects collected from infested maize and was maintained in the laboratory for no more than 20 generations.

Two neem-based formulations were tested, being: AzaMax®, containing 12 g of the active ingredient (a.i.) (azadirachtin A/B) per liter (L) of formulation and registered on the Agriculture, Poultry and Supply Ministry (MAPA) and; 2) Natuneem® with no reference concerning the amount of a.i. per L of formulation and not registered on the MAPA. Distilled water was used as a standard negative control and a synthetic insecticide (Decis® 25 CE – Deltamethrin 25 g a.i./L of formulation) as a standard positive control for comparisons.

To set up the concentrations and dosages that would cause 50 % mortality in the population of *S. zeamais*, we exposed: a) both adults of the insect and food (seeds) simultaneously; and b) only adults treated topically with the aid of a Gilson microsyringe containing the test solutions. All tests were performed in the prevailing laboratory conditions, averaging 27 ± 2 ˚C, 50 ± 20 % R. H. and 12 hours photophase.

Preliminary bioassays were performed to define the range of discriminating dosages and concentrations that would be further tested in the final bioassays and the volume needed to fully impregnate the exposure target (insects and insects + seeds). In such bioassays, we used at least 10 insects per replication and five replications per concentration or dosage, arranged in a completely randomized design.

In the subsequent (final) bioassays run to estimate LC and LD_{50} values the same number of insects per replication (10) was used and a varying number of replications per treatment (concentration or dosage), which was defined depending on species' availability. However, the minimum number of replications used per treatment was five (= minimum number of tested insects per concentration was 50) and, whenever

Table 1. Mortality responses of *Sitophilus zeamais* Motschulsky, 1885 (Coleoptera: Curculionidae) associated to two neem formulations (AzaMax® and Natuneem®) and Deltamethrin (Decis 25 CE®).

Insecticides	DF^a	n^b	Slope ± SEMc	Values (95 % CI)^d		χ^2	P-value
				LC_{50}	LD_{50}		
AzaMax	3	690	2.57 ± 0.26	4.01 (3.05 ± 5.22)	–	6.43	0.0924
AzaMax	2	200	3.38 ± 0.47	–	51.32 (42.47 ± 60.85)	3.34	0.1882
Natuneem	3	690	2.26 ± 0.14	4.46 (3.96 ± 4.99)	–	5.47	0.1403
Natuneem	2	180	3.23 ± 0.64	–	76.76 (15.12 ± 154.84)	4.69	0.0959
Decis	3	750	1.18 ± 0.10	0.41 (0.34 ± 0.49)	–	3.85	0.2784
Decis	1	241	2.88 ± 0.40	–	42.75 (33.66 ± 50.66)	1.61	0.2047

^{a} Degrees of freedom. ^{b} Number of insects treated. ^{c} Slope of the dose-mortality curve and its standard error (SE); ^{d} Lethal concentrations (LC) in µL of formulation/g of seeds and doses (LD) in µL of formulation/g of insects and their respective 95 % confidence intervals (95 % CI).

possible, we added additional replications and this was the reason for the varying number of insects treated (n) as represented in Table 1.

LC_{50} estimation. To estimate the LC_{50}, both insects and seeds were exposed to 2 mL of the test solution using a precision Gilson pipette, followed by homogenization. In order to ensure uniform mixing and coating the vials containing treated seeds and insects were gently shaken for 5 minutes. The exposition method was chosen based on what is done while treating the product (seeds) prior to storage, in order to achieve control, and also based on the poisoning activity of these products which are known to act through cuticular penetration and oral uptake (Mordue and Nisbet 2000; Rehman *et al.* 2014).

The LC_{50} assays were performed within Petri's dishes measuring 9.0 cm diameter and 1.5 cm tall, containing 20 grams of seeds and 10 adults (non-sexed) of *S. zeamais*. The test concentrations were 1.00 %, 2.91 %, 4.76 %, 9.09 %, and 33.33 % for AzaMax; 1.00 %, 3.00 %, 5.00 %, 10.00 %, and 20.00 % for Natuneem; and 0.10 %, 0.30 %, 0.50 %, 1.00 %, and 2.91 % for Decis all provided in volume of the formulation (mL) per volume of distilled water (mL) necessary to obtain the test concentrations. Distilled water (0 %) was used as a control in all assays and as the solvent, to mimic what growers use in the storage to apply these formulations.

LD_{50} estimation. Topical application of the tested solutions to adult weevils was used to estimate LD_{50}. Before performing the final LD_{50} bioassays, we sampled and weighed five replicates of 10 adult weevils, to obtain an average weight, and this was used in the calculations of LD_{50} unit.

To prevent insects from moving and allow precise topical application of the solutions, 10 adults of *S. zeamais* were paralyzed by keeping them inside Petri's dishes of 9.0 cm in diameter and 1.5 cm tall within a freezer for two minutes (time estimated in preliminary tests). After that, the group of 10 insects was treated by applying 20 µL of the test solution to adults using a Gilson microsyringe. The insects were left in the prevailing laboratory conditions until the solution dried out and then untreated maize (20 grams) seeds were added as a food source.

The dosages used to estimate the LD_{50} for *S. zeamais* were 2.00 %, 5.00 %, 10.00 %, and 20.00 % for AzaMax; 5.00 %, 10.00 %, 20.00 %, and 40.00 % for Natuneem, and 5.00 %, 10.00 % and 20.00 % for Decis, all provided in volume of the formulation (µL) per volume of distilled water (µL) necessary to obtain the test concentration. Distilled water (0 %) was used as a control in all bioassays.

Variables measured and statistical analysis. Mortality was recorded 48 hours after treatment. Adults of *S. zeamais* were confirmed dead when they failed to move any part of the body while touched with a sharp tweezer at the abdomen (Kemabonta and Falodu 2013). Observed mortality in the treatments was corrected to the mortality that occurred in the control and it has reached a maximum of 10 %, in only one bioassay.

Data were subjected to a Probit analysis using the SAS software (Sas 2002) to estimate the lethal concentrations or doses desired. Lethal dose and concentration ratio (LDR and LCR) of the botanical insecticides compared with deltamethrin and with each other were calculated by dividing the higher value by the lower value, following what was done in Biddinger *et al.* (2013). An LCR and LDR provide a means to test whether two LC's or LD's are significantly different (i.e., when the 95 % CI for the LCR or LDR did not include the value 1.0 (Robertson *et al.* 2007).

Results and discussion

The LC_{50} -values for AzaMax® and Natuneem® were around 4 µL of formulation/g of grains while the LC_{50} -value for Decis was 0.41 µL of formulation/g of grains (Table 1). This also means that these compounds were almost 10-fold (9.67 and 10.74) less toxic than deltamethrin (Table 2).

Topical application of neem-based formulations yielded LD_{50} values of 51.32 and 76.76 µL of formulation/g of insects for AzaMax and Natuneem, respectively (Table 1). The LD_{50} value for Decis was 42.75 µL of formulation/g of *S. zeamais* (Table 1), which was the lowest value observed in this study. Hence, it takes a dosage around 20 % and 80 % higher of AzaMax and Natuneem to cause the same mortality rate in *S. zeamais* while exposed to Decis (Table 2).

The differences found between the botanical and synthetic formulations were expected and agree with other findings (Brito *et al.* 2006; Dadang and Prijono 2009; Olaitan and Abiodun 2011). They are attributed to the diversity in the nature of the products tested, i.e., neem-based products which are botanical insecticides versus deltamethrin which is a synthetic insecticide. The a.i. of synthetic insecticides such as delthametrin is more environmentally stable than any known botanical insecticide (Olaitan and Abiodun 2011). Hence, the response of *S. zeamais* to higher concentrations of the botanical formulations can be associated with the ability of the effective concentration to withstand photo-decomposition (Johnson *et al.* 2003), allowing for the remaining residues to show acute toxicity against the weevil. This pattern was maintained for both botanical formulations tested, strengthening this hypothesis.

Also, the differences in the mode of action may partially explain the differences found between the botanical and synthetic insecticides. Neem-based products are known to act mainly on growth and molting, also interfering on reproduction and cellular processes. These effects can increase mortality related to disruption of endocrine system controlling the described processes. Neem-based formulations also cause antifedant effects which are, however, a lot less pronounced in Coleoptera than in Lepidoptera (Mordue and Nisbet 2000; Okweche *et al.* 2013). Kavallieratos *et al.* (2007) tested two azadirachtin formulations against adults of *Sitophilus oryzae* (Linné, 1763) (Coleoptera: Curculionidae) and found that further dosage increases and longer exposure times were needed to guarantee high rates of mortality. Deltamethrin based products, for instance, has a rapidly disabling effect on feeding insects and their death seems to be due to irreversible damage to the nervous system occurring when poisoning lasts more than a few hours (Rehman *et al.* 2014).

The LC_{50} of crude neem seed oil determined for *S. zeamais* infesting maize was found to be much lower than the values found herein, i.e., 1.46 mL of formulation/kg of seeds which is equivalent to 1.46 µL/gram of seeds (Nukenine *et al.* 2011). However, because it was not a formulation but, instead, a crude extract or seed oil, such differences are again acceptable and confirm the potential of the tested formulations. Crude

Table 2. Lethal dose (LDR) and concentration ratios (LCR) and 95 % Confidence Interval (CI) of botanical insecticides (AzaMax and Natuneem) compared with a synthetic insecticide (Decis) and with each other to *Sitophilus zeamais* Motschulsky, 1885 (Coleoptera: Curculionidae).

Insecticides	**Decis**		**AzaMax**	
	LCR[a] 95 % CI[b]	**LDR[a] 95 % CI**	**LCR 95 % CI**	**LDR 95 % CI**
AzaMax	9.67 6.63-14,11	1.20 0.96-1.50	1.00 –	1.00 –
Natuneem	10.74 8.61-13.39	1.80 1.48-2.17	1.11 0.81-1.527	1.50 1.16-1.94
Decis	1.00	1.00	–	–

[a] LCR and LDR is the higher LC and LD divided by the lower LC and LD. [b] If the 95 % CI of the LCR and LDR includes the value 1.0, then the LC's and LD's are not significantly different.

seed oil is not emulsified and hence may face an increase in individual contribution of toxic components in the final applied solution, what can act increasing toxicity. Some emulsified concentrate contains only 32 % of neem seed oil (Pandiyan 2011) which is far lower than the content of crude oil (~100 %).

Concerning the differences in the LC_{50} and LD_{50} between the two botanical insecticides, AzaMax and Natuneem, the former was 1.11 and 1.50 more toxic than the latter, although the only significant differences found according to the 95 % CI was between the LD_{50} values of AzaMax and Natuneem (Table 2). This result agrees with that found by Brito *et al.* (2006) who described that Natuneem was less toxic to adult females of *Tetranychus urticae* Koch, 1836 (Acari: Tetranychidae) when topically applied than all the other tested formulations (Neemseto, Callneem and the extract of seeds powder).

Considering that AzaMax has a known amount of a.i. (azadirachtin A/B) in the commercial product while in Natuneem that is not specified, the differences in toxicity due to topical application might be caused by the differences in the concentration of the a.i. between the two formulations. In that matter, Gahukar (2014) mentions in his revision that the LC_{50} values of five neem-based formulations tested against three major tea pests (thrips, tea mosquito bug and leaf hoppers) decreased as the azadirachtin content in the formulations increased. Also, the toxicity may vary according with the components of the formulation or used in the extraction (Mansour *et al.* 1987) and with the part of the plant used in the formulation. For instance, Mansour *et al.* (1993) have tested three neem formulations against phytophagous mites and have found that the only truly acaricidal formulation among the three tested was the one having the lowest content of azadirachtin. The authors attributed such effect to the extraction of neem seed kernels with lipophilic solvents resulting in much higher oil content in the final formulation of this product. Therefore, final products possessing higher oil content act as a contact poison particularly against mites and soft bodied insects once in such formulations the variation on bioactivity can be driven from a great variation in azadirachtin content (Gahukar 2014). Since both tested formulations were extracted from the seeds/kernels and then were oily, that might also account for the differences found in acute toxicity of topically applied formulations, although *S. zeamais* is not a soft bodied insect.

The higher values of the LD_{50} compared to the LC_{50} found in our study, while testing the same botanical formulations, can be explained by the increased exposition of *S. zeamais* in the tests performed to estimate the LC_{50} (contact and ingestion) in comparison to the contact exposure alone (topical application). Similar differences were found by Scoz *et al.* (2004) in tests with fruit flies. Furthermore, the mode of action of neem-based products is diversified and incremented by ingestion (exposure of both insects and food) when compared to contact exposure alone (topical exposition) (Mordue and Nisbet 2000) and this also contributes to explain the differences found concerning the LC_{50}- and LD_{50}-values. In addition, according to Gahukar (2014) azadirachtin-based products are mostly stomach poisons and when applied at higher doses, exhibit contact toxicity what also account for the differences found.

In spite of the numerical differences in the LC and LD's values among the insecticides, the only significant differences found, according to the 95 % CI of the LCR and LDR, are those seen between the LC_{50} values of AzaMax and Decis and Natuneem and Decis; the LD_{50} values of Natuneem and Decis and the LD_{50} values of AzaMax and Natuneem (Table 2). However, higher numerical values have some other implications, including economic issues.

Taking into account only the cost to treat maize and to obtain effective control of the pest, it would be much cheaper to control *S. zeamais* using a synthetic formulation of deltamethrin. This is the case because the effective concentration to manage *S. zeamais* with deltamethrin was approximately 10 times lower and the formulation is also cheaper (~US$ 25.98 per liter) compared to the doses required and prices paid for a neem-based formulation. However, other non-economical issues should be taken into consideration while selecting an insecticide and these are related to the lasting residues (Gahukar 2012) and relative selectivity to some non-target organisms (Ziaee 2014). This approach is especially important when seeking alternatives concerning the few options available to manage storage pests and when the known alternatives we are aware of, to manage insecticide resistance, seem to have no effect on this pest (Oliveira *et al.* 2005). The neem-based formulations can thus be considered as alternative options. Concerning the two botanical formulations tested, it would be cheaper to manage *S. zeamais* with Natuneem, since despite the fact that the effective concentrations and doses to kill *S. zeamais* are lower for AzaMax, the latter is more expensive (~R$ 82.44 per liter) than the former (~US$ 22.56 per liter). However, AzaMax is already registered on the MAPA while Natuneem is not, which may restrict availability of this formulation on the market.

Mortality as an estimation of the acute toxicity may only be a partial measure of the deleterious effects of these products. The dynamics of botanical insecticides in the environment that they are used, mainly with respect to rapid degradation (Johnson *et al.* 2003), also indicates sublethal effects in the physiology and behavior of pests; this is the reason why a lower concentration, even without the ability to cause any mortality, can harm a population (Jafarbeigi *et al.* 2014) by causing deterrent effects, diminishing food intake and frass production, retarding larval and pupal development, pupal weight and adult emergence (Li *et al.* 2003). Hence, such effects should be considered in future works for those concentrations that do not cause mortality.

Conclusions

S. zeamais can be managed with both neem-based formulations by using a doubled LC$_{50}$ and LD$_{50}$ which result in the following values: 8.02 and 8.92 µL of formulation/ g of seeds and 102.64 and 153.52 µL of formulation/g of insects for AzaMax and Natuneem, respectively. Since there were no significant differences between the LC$_{50}$-values of the botanical insecticides, both of them can be used to manage *S. zeamais* infesting stored corn. However, it would cost less to manage the pest with Natuneem. The LC$_{50}$- and LD$_{50}$-values of the synthetic insecticide (Decis® 25 CE) against *S. zeamais* are lower than those found for neem-based formulations and in order to manage the pest with this formulation (cause mortalities rates close to 100 %) it takes 0.82 µL of formulation/g of seeds and 85.5 µL of formulation/g of insects. Such LC$_{50}$ values found in our study would be equivalent to use 8.02, 8.92 and 0.82 L of AzaMax, Natuneem and Decis/ton of seeds.

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