Vegetable powders for the control of *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) in stored maize grains

Pós vegetais no controlo de *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) em milho armazenado

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ABSTRACT

The attack by insects is one of the main causes of losses on maize during storage. The purpose of this research was to investigate the insecticidal potential action of *Urtica urens* L. and *Piper aduncum* L. powders on the maize weevils *Sitophilus zeamais* (Motschulsky) on stored maize. The experiment was carried out in the laboratory, with evaluation of the dry powder of *U. urens* leaves and *P. aduncum* at 3 %, and control without powder, in 80 g of maize grains infested with 0, 8, 16 and 24 maize weevil individuals. A completely randomized design was used on a 4x3 factorial scheme with four replications. The experiment was evaluated after 50 days of storage by: reduction of grain weight, percentage of grain damaged by maize weevils, grain moisture content, number of adult emerged maize weevil and mortality of maize weevil. The application of powders of *P. aduncum* and *U. urens* reduced significantly the grain damaged by *S. zeamais*. *P. aduncum* also caused a significant reduction of grain weight loss, of the number of adult emerged maize weevil and of increased the percentage of dead maize weevil. The powder of *P. aduncum* showed to be promising for the control of *S. zeamais* in stored maize grains.

Key words: integrated pest management; postharvest maize; maize weevil.

RESUMO

Uma das principais causas de perdas na produção de grãos de milho é devido ao ataque de insetos durante seu armazenamento. Objetivou-se nesse trabalho investigar o potencial de ação inseticida de pós de *Urtica urens* L. e *Piper aduncum* L. sobre gorgulhos *Sitophilus zeamais* (Motschulsky) em grãos de milho. O ensaio foi realizado em laboratório, avaliando pós secos de folhas de *U. urens* e de *P. aduncum* a 3 %, em 80 g de grãos de milho infestados com 0, 8, 16 e 24 gorgulhos. Utilizou-se delineamento experimental completamente casualizado em esquema fatorial 4x3 com quatro repetições. O ensaio foi avaliado após 50 dias de armazenamento, por meio de: redução do peso dos grãos, porcentagem de grãos danificados pela ação dos gorgulhos, teor de água dos grãos, número de gorgulhos adultos emergidos e porcentagem de gorgulhos mortos. A aplicação de pós de *P. aduncum* e *U. urens* reduziu significativamente a percentagem de grãos danificados por *S. zeamais*. *P. aduncum* reduziu significativamente a perda de peso de grãos, o número de gorgulhos adultos emergidos e aumentou a mortalidade do gorgulho. Assim, o pó de *P. aduncum* mostrou ser promissor para o controle de *S. zeamais* em grãos de milho armazenados.

Palavras-chave: proteção integrada de pragas; pós-colheita de milho; gorgulho.

INTRODUCTION

Maize is the main cereal produced in Brazil (CONAB, 2020), however, significant productive losses occur after harvest. Those losses, with a reduction in grain quality, are due to inadequate storage conditions. The losses are caused mostly by insect pests.

Sitophilus zeamais (Motschulsky) (Coleoptera: Curculionidae) is the main pest of stored maize in whole grain. This insect perforates the grains (primary pest), adults and larvae feed and develop within the seed's endosperm reducing its weight, quality, and percentage of germination, in addition allows the entry of other insect pests and fungi (Tefera *et al*., 2011; Abdullahi *et al*., 2014; Trematerra *et al*., 2015; Wale and Assegie, 2015). Maize weevils also contaminate the grains with their exuvia and excrement, decreasing the quality of the grains (Caneppele *et al*., 2003), causing devaluation and economic losses.

Maize weevils have high biotic potential due to high fertility and short evolution cycle and show cross-infestation (Lorini *et al*., 2015) when insects infest grains, both in the field and during storage (Lorini, 2008).

In Brazil there are several species of plants with insecticidal potential that could be used in Integrated Pest Management. Compared to synthetic insecticides, botanical extracts or powders are safer for the environment, as they are biodegradable, minimize food residues and are well accepted by farmers (Kim *et al*., 2003; Vendramim and Castiglioni, 2000).

Promising results have been observed in Brazil for the control of *S. zeamais* in stored grains by plants extracts with insecticidal potential, such as *Azadirachta indica* A. Juss (Meliaceae) (Souza and Trovão, 2009), *Capsicum baccatum* (Willd.) Eshb. (Solanaceae) (Guimarães *et al*., 2014) and *Annona mucosa* Jacq. (Annonaceae) (Ribeiro *et al*., 2013). This has also been observed in other regions of the world (Asmanizar and Idris, 2012; Ortiz *et al*., 2012; Karunakaran and Arulnandhy, 2018; Ileke *et al*., 2020).

Urticaceae family is found in Neotropical regions and includes 50 genera and 1200 species (Sousa and Lorenzi, 2008). The leaves in some species of the *Urtica* genus are rich in flavonoids, polyphenols and tannins and used in folk medicine with anti-inflammatory, antioxidant, anxiolytic, analgesic effects, treatment of benign prostatic hyperplasia and have chemoprotective properties against cancer agents (Koch & Biber, 1994; Doukkali *et al*., 2015; Mzid *et al*., 2017).

Piper aduncum L., popularly known as spiked pepper, belongs to the Piperaceae family, occurring throughout the tropical region. Approximately 260 species occur in Brazil and 140, specifically, in the Brazilian Amazon (Cronquist, 1981). Plants of *P. aduncum* are rich in dilapiol, phenyl ether, with fungicidal, insecticidal, molluscicidal, acaricidal, bactericidal and larvicidal properties with the advantage of being a biodegradable product (Duarte *et al*., 2014; Almeida *et al*., 2009). Scopel *et al.* (2018) studied the *Piper nigrum* L. (Piperaceae) extract on adults of *S. zeamais* and verified 49 % of mortality and a repellent effect.

In this context, the present work was conducted with the objective of studying the insecticidal potential of *Urtica urens* L. (Urticaceae) and *P. aduncum* powder on *S. zeamais*.

MATERIAL AND METHODS

The experiments were conducted at the Phytotechnics Laboratory of the Institute of Education Agriculture and Environment (IEAA), Federal University of Amazonas (UFAM), in the municipality of Humaitá, southern of the Amazonas State, Brazil.

Adults of *S. zeamais* were collected in samples of commercial maize grains in Humaitá. These insects were then reared in the laboratory using glass jars, with 3 L capacity, and opening sealed with a thin tissue (*voil*) at 27 °C and 85 % relative humidity, approximately.

Leaves of *P. aduncum* and *U. urens* plants were collected in areas with natural growth of these plants, in the municipality of Humaitá, Amazonas, Brazil. The plant dried powders were obtained by drying the leaves in oven with air circulation at 40 °C for 72 hours. Dried leaves were crushed in a blender and subsequently stored in hermetically sealed and identified glass containers. The powders were kept at 25 ± 2 °C.

To measure the injuries caused by *S. zeamais*, 80 g of maize grains cultivar BR 25 with 12.3 % moisture content, were exposed to: 0, 8, 16, and 24 non-sexed insects. During 24 hours preceding the infestation, the insects were counted, isolated and not fed. The grains were weighed and placed in a glass container, with a capacity of 300 ml and opening sealed with a thin tissue (*voil*), and then the infestation was carried out. Subsequently, a dose of 3 % (w/w) of *U. urens* and *P. aduncum* powder was applied, separately. Grains without application of plant extract were used as a control treatment.

The experiment was conducted in a completely randomized design with the treatments arranged in a 4x3 factorial scheme (maize weevil infestation level and type of treatment), with four replications.

The grains were evaluated after 50 days of storage in laboratory conditions at approximately 27 ± 3 °C.

The parameters analysed were: (a) percentage of damaged grain: all the grains with and without injury caused by maize weevil were counted, separately, and the percentage of damaged grains was calculated; (b) weight loss of grain: grains were weighed before and after the exposure to maize weevils; (c) moisture content of grain: four samples of 30 g of maize grains of each treatment were placed in an oven at 105 ± 3 °C, for a period of 24 h and after it was carried out the weighing and calculated the moisture content in humid base (MAPA, 2009); (d) number of adult emerged maize weevil after infestation: total number of maize weevil minus number of maize weevil used in the artificial infestation; and (e) maize weevil mortality, in percentage, in the final population: dead maize weevils present in the grains was counted and the percentage was calculated (maize weevil dead/total maize weevil x 100).

The normality of residuals and homogeneity of variances of data were tested using Shapiro-Wilk and Bartlett tests, respectively. The number of adult maize weevils emerged after infestation was transformed by √(x+1). These were analysed using a standard variance analysis. When the differences were significant at the 5 % probability level by F test, the averages were compared with a post hoc Tukey test for the treatments. A regression analysis was done to study the maize weevil infestation level. Statistical analyses were performed with statistical software SISVAR (Ferreira, 2014).

RESULTS AND DISCUSSION

Significant interactions were observed between maize weevil infestation and types of grain treatment relatively to adult emerged maize weevil, percentage of mortality and weight loss of maize grains (Table 1). Infestation level and type of treatment showed a significant effect on the percentage of damaged grains, and treatment effect was significant from water content of the grains.

Table 1 - Analysis of variance of number of weevils emerged (WE), weevil mortality (%) (DW), maize grain weight loss (GW), damaged grains (%) (DG) and moisture content of maize grains (WC) infested with *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) and submitted to no treatment (control) or treatment with plant powders (*Urtica urens* L. (Urticaceae) or *Piper aduncum* L. (Piperaceae))

SV	DF	Mean Squares				
		WE	DW	GW	DG	WC
Infestation (I)	3	27.79**	256.88*	21.76^{ns}	275.93**	5.247^{ns}
Powder (P)	2	222.31**	4332.98**	518.54**	9050.25**	20.175**
I x P	6	6.50*	273.91**	25.86*	74.20^{ns}	1.624^{ns}
Residual	36	2.42	69.53	10.90	41.75	2.878
C.V. (%)	-	-	66.3	28.5	12.6	15.2

*,**Significant at 5% and 1% probability, respectively; ^{ns}Not significant; SV: source of variation; DF: degrees of freedom; C.V.: coefficient of variation.

The increase of maize weevils emerged during the test was slower when the *U. urens* powder was applied compared to the control treatment (Figure 1A). This shows that the *U. urens* powder can affect the maize weevil's fertility because it did not increase the mortality of these insects (Figure 1B).

Similarly, Asawalam *et al.* (2006) found a reduction in the adult emergence of *S. zeamais* in maize grains treated with powder of *Piper guineense*

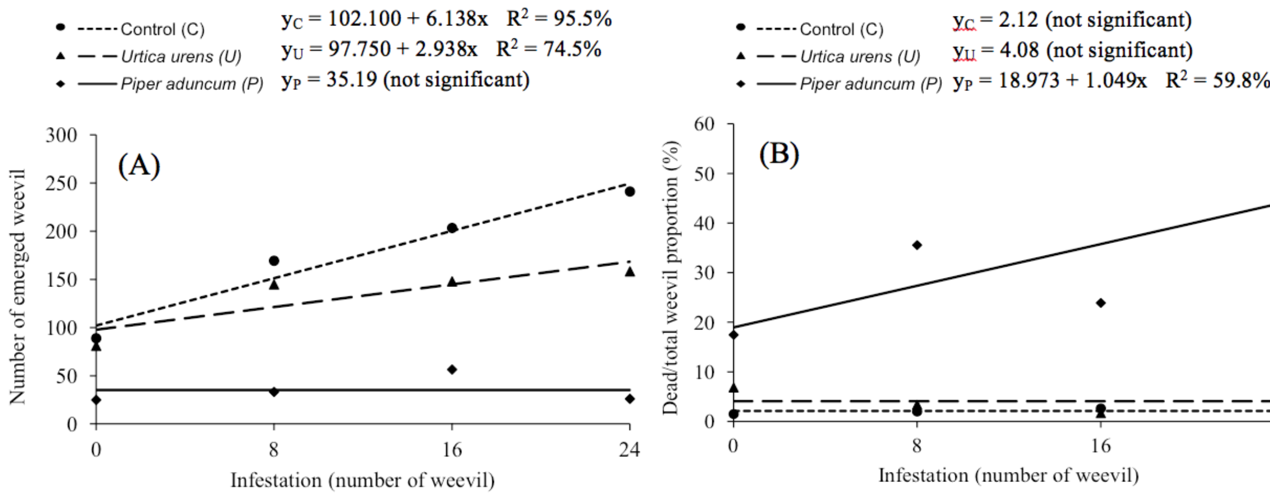

Figure 1 - Number of adult emerged weevils after infestation (A) and weevil mortality (%) (B) on maize grains infested with different densities of *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) and submitted to no treatment (control) or treatment with plant powders (*Urtica uren*s L. (Urticaceae) or *Piper aduncu*m L. (Piperaceae)).

(Shum. and Thonn.). Nukenine *et al.* (2007) also observed a reduction in the progeny production of *S. zeamais* by the application of vegetable powders of *Plectranthus glandulosus* Hook. (Lamiaceae) and *Steganotaenia araliacea* Hochst. (Apiaceae).

Antunes *et al.* (2011), evaluating physical damage in maize grains, cultivar AS-32, caused by *S. zeamais* within three storage periods (25 ± 5 °C and 60 ± 10 % relative humidity - RH), concluded that longer exposure time of the grains to maize weevils was related to a higher final population and more observed damages.

There was no significant increase in the number of adult emerged maize weevils due to the infestation level when the grains were treated with powder of *P. aduncum* leaves, which kept the maize weevil population at significantly lower levels when compared to the other treatments.

The percentage of dead maize weevils showed no significant increase in control treatment and *U. urens* powder, so these treatments did not affect the insect mortality rate (Figure 1B). For treatments with *P. aduncum* powder there was a significantly higher mortality of maize weevil in all treatments. The larger initial infestation with maize weevil, the higher mortality, which explains the absence of an increase in population of living insects verified for *P. aduncum* powder, even with the increased population of introduced maize weevils. Silva *et al.* (2016) found that the powder of *P. aduncum* applied on bean grains reduced infestation by adults of *Zabrotes subfasciatus* Bohemann (Coleoptera: Chrysomelidae, Bruchinae).

The essential oil of *P. aduncum* has insecticidal effect on *S. zeamais* and it depends on the exposure methods and concentration of the applied oil, being more effective by fumigation and by contact with topical application than by contact on a contaminated surface (Estrela *et al.*, 2006). It also has an insecticidal effect by contact (filter paper) on *Cerotoma tingomarianus* Bechyné (Coleoptera: Chrysomelidae) at 0.04 % concentration, causing physiological disturbances by topical application in concentrations greater than 2.5 % (Fazolin *et al.*, 2005).

The *P. aduncum* powder was efficient for the control of *S. zeamais* for 50 days, evaluated in this experiment. Pereira *et al.* (2009) found that *P. aduncum* had a high residual effect and was effective in protecting cowpea grains from *Callosobruchus maculatus* Fabr. (Coleoptera: Chrysomelidae, Bruchinae) during 120 days of storage. Additional studies are needed to verify the residual effect of *P. aduncum* powder against *S. zeamais*.

The weight reduction on maize grains did not differ significantly according to the different

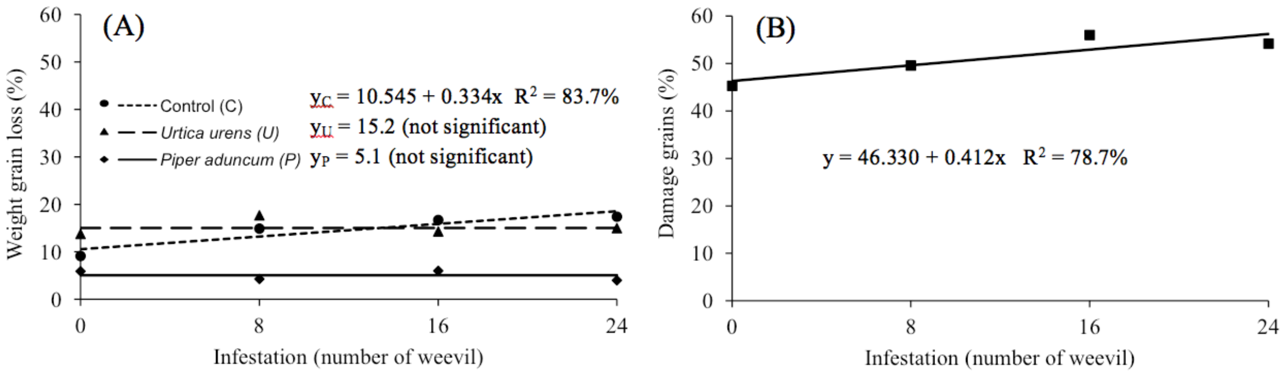

Figure 2 - Percentage of maize grain weight loss (A) and percentage of damaged maize grains (B) infested with different densities of *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae) and submitted to no treatment (control) or treatment with plant powders (*Urtica urens* L. (Urticaceae) or *Piper aduncum* L. (Piperaceae)).

maize weevil infestation level when the treatment with *U. urens* was used (Figure 2A). The number of emerged maize weevils was higher with the increase of maize weevil infestation level in the treatment with *U. urens* (Figure 1) but probably there was an inverse relationship between the number of insects and the percentage of food consumed per insect in this treatment.

There was a significant increase in the weight loss on maize grains at the control treatment when the maize weevil infestation level increased. However, weight loss in the control treatment and *U. urens* powder were not different. This indicate that the limited resources can also explain grain weight loss around 15 % in both treatments even with a high level of initial infestation.

In the same way as it observed to *U. urens* powder, the increase of maize weevil infestation level did not affect grain weight loss when *P. aduncum* powder was used. However, *P. aduncum* powder showed a smallest grain weight loss. This may be explained by the maintenance of a lower number of emerged maize weevils and mortality of maize weevil when *P. aduncum* powder was applied (Figure 1).

Higher initial maize weevil populations caused an increase in the maize grain weight loss when grains were not treated with vegetable powders, as a consequence also of the increase in the number of live maize weevils found in these samples, as already presented.

The percentage of damaged grains increased linearly with the increase in the initial number of maize weevils (Figure 2B).

The application of vegetable powders significantly reduced the incidence of damaged grains, in relation to the control treatment, with emphasis on the treatment with *P. aduncum*, which presented the lowest percentage of damaged grains (Table 2). This probably occurred due to lower number of living insects feeding on the grains observed in this treatment. The powders may have caused the interruption of feeding as observed by Fazolin *et al.* (2005) when studying the effects of the application of *P. aduncum* on *C. tingomarianus*.

The water content of the grains was significantly lower in the treatment with *P. aduncum* powder when compared to the control and *U. urens* powder (Table 2). The lower water content observed in the treatment with *P. aduncum* can be explained by the smaller population of live maize weevils, and consequently less water release by the respiratory activity of insects. Silva *et al.* (2003) also observed an increase in the water content of maize grains with an increase in the population of *S. zeamais* during storage, according to Pinto *et al.* (2002) this increase is due to the greater metabolic activity of insects.

Moisture content below 14 % is ideal for maize grains storage in tropical regions (Suleiman *et al.*, 2013). The treatments tested, control without powders, *U. urens* and *P. aduncum* powder, showed adequate water contents for maize storage.

Table 2 - Means and standard error of the percentage of damaged grains by *Sitophilus zeamais* (Motschulsky) (Coleoptera: Curculionidae), and the moisture content of maize grains submitted to no treatment (control) or treatment with plant powders (*Urtica urens* L. (Urticaceae) or *Piper aduncum* L. (Piperaceae))

Treatment	Damaged grains (%)*	Moisture content (%)*
Control	70.2 ± 2.3 a	11.8 ± 0.3 a
Urtica urens	59.1 ± 1.4 b	11.8 ± 0.6 a
Piper aduncum	24.6 ± 2.1 c	9.9 ± 0.3 b

* Means followed by the same letter, are not statistically different by Tukey test ($P \leq 0.05$).

Dilapiol, a phenylpropanoid present in plants of *P. aduncum* from the Amazon (Almeida *et al.*, 2009), interacts with Cytochrome P450, inhibiting the activity of monooxygenases responsible for the metabolism of excretion of toxins in insects (detoxification) (Bernard *et al.*, 1995), resulting in the death of the insects. It has a similar action to piperonyl butoxide, a synthetic benzodioxol derivative that may increase the action of insecticides through the inhibitory action on the Cytochrome P450 enzyme system (Hodgson and Levi, 1998) accounting for insect resistance to xenobiotic toxins (Berenbaum and Johnson, 2015). For this reason, it has been studied for use in synergy with other compounds and synthetic insecticides to enhance their potential insecticide power (Belzile *et al.*, 2000).

In addition, powders, extracts and essential oils of *P. aduncum*, from the Amazon, which are rich in dillapiol, have caused repellency to *Lasioderma serricorne* Fabricius (Coleoptera: Anobiidae) (Pérez *et al.*, 2012), food paralysis in *C. tingomarianus* (Coleoptera: Chrysomelidae) (Fazolin *et al.*, 2005) and mortality of *Euschistus heros* Fabricius (Hemiptera: Pentatomidae) and *Anticarsia gemmatalis* Hübner (Lepidoptera, Erebidae) (Krinski *et al.*, 2018).

Thus, it is believed that dillapiol is the main insecticidal active compound of *P. aduncum* powder used in this experiment. To complement this exploratory work, phytochemical analysis, tests on fractions, study of penetration pathways, doses and residual power of *P. aduncum* powder would be necessary in the future.

CONCLUSION

The application of powder of *Piper aduncum* and *Urtica urens* reduced the damaged grains percentage by *Sitophilus zeamais*.

Piper aduncum also significantly reduced grains weight loss and the number of adult emerged maize weevils and caused a significant increase on mortality of maize weevil.

The powder of *P. aduncum* seems to be a promising tool for the control of *S. zeamais* in stored maize grains.

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