ORIGINAL ARTICLE

Assessment of the ancestral use of garlic (*Allium sativum*) and nettle (*Urtica dioica*) as botanical insecticides in the protection of mesquite (*Prosopis laevigata*) seeds against bruchins

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Abstract

Currently, *Prosopis laevigata* (mesquite) has been affected by the Bruchinae coleoptera pest, whic feeds on its seed and causes significant losses in production and grain storage. In the Hñähñu community El Alberto (Ixmiquilpan, Hidalgo, Mexico), the use of aqueous extracts from garlic and nettle as botanical insecticides against different pests in agricultural fields is a known practice. Herein, we assess the efficacy of the method known by locals in the protection of mesquite seeds. Two tests were conducted: 1) Insecticidal effect on adult bruchins, and 2) Seed preservation test from Bruchinae infestation, with a germination test in seeds exposed to the treatments. There are probable insecticidal effects on immature stages of Bruchinae since there were no mortality effects on their adults during the first test. Mortality on adults in the second test was 75.6% with garlic and 50% with nettle. Nettle extract had more efficacy in seed protection with an infestation rate of 4%, whereas 27.5% of the seeds exposed to garlic extract were infested. Seed germination rate was 2.38% with nettle extract, and 1.19% with garlic extract. The method known by local inhabitants requires modifications to increase its efficacy and possible use in Integrated Pest Management in the future.

Keywords: biological control, leguminous, seed storage, weevil

Introduction

Prosopis laevigata (Humb. & Bonpl Ex Wild), commonly known as mesquite, is a tree species of the Fabaceae family, distributed in semi-arid and arid regions of America, where it is characteristic, both ecologically and anthropologically (Sauceda *et al*. 2014).

Its ecological importance is reflected in nitrogen fixation in the soil and formation of soil-root structures. It has been associated to fertility islands in the soil and is considered as a nurse species of several animals and plants (Ruiz *et al*. 2008; Ríos *et al*. 2013; Cuéllar-Rodríguez *et al.* 2017). Mesquite has been associated with medicinal properties. Its seeds are used as food by humans and domestic livestock and its wood is used as a biofuel (Cervantes-Ramírez 2005; Ríos *et al.* 2013; Sauceda *et al*. 2014; Pérez-Serrano *et al.* 2021).

In Mexico, mesquite populations are decreasing because of overlogging, forest fires, land-use changes, the lack of sustainable management plans, the reduction and fragmentation of conserved areas, and the introduction of exotic species which have become potential pests (Cuéllar-Rodriguez *et al.* 2017; Pérez-Serrano *et al.* 2021). As a consequence, arid and semi-arid ecosystems face an increase in soil degradation and ecological imbalance where mesquite is found (Ríos *et al*. 2013).

The coleopteran subfamily Bruchinae has become one of the worst pests that affects not only mesquite populations, but also several species from the Fabaceae family (Romero and Johnson 2004; Ribeiro-Costa and Almeida 2012). Their larvae feed on the seed mesocarp and embryonic structures, leaving a hole with a well-defined contour, which is used by the adult as an exit (Romero-Gómez *et al.* 2014). As a consequence, infestations by bruchins can affect dispersal, production, viability, storage and quality of seeds for later use (Ribeiro-Costa and Almeida 2012).

In America, the genus *Prosopis* L. has been affected by different bruchin species, with infestation rates ranging from 55.9% in *Prosopis juliflora* Swartz in Santa Marta, Colombia (Aguado and Suárez 2006) to 97% in *P. glandulosa* L.D. Benson in Texas, USA (Amdor *et al.* 2006). In Mexico, the infestation rate caused by bruchins on *P. laevigata* has been reported from 44% in Irapuato, Guanajuato (Salas-Araiza *et al.* 2001), to 77% in Durango (Reséndez-Velázquez and González--Castillo 2016). Since *P. laevigata* is widely distributed in the Mezquital Valley, the Hñähñu community El Alberto, in Ixmiquilpan, Hidalgo, also faces this problem.

Some alternatives to use against mesquite pests include botanical insecticides (Pavela 2016). The use of this type of bioinsecticides has advantages due to its low-cost accessibility, and some of them have medicinal or culinary properties (Paul *et al.* 2009; Fatiha *et al.* 2014). In addition, the negative impacts that industrial insecticides have on the environment, and human and animal health, are avoided (Hernández-Carlos and Gamboa-Angulo 2019). Such is the case for calcium hypochlorite, $Ca(ClO)_2$ (CAS: 7778-54-3), a potent biocide which carries risks of acute toxicity through contact or inhalation; as well as a chronic hazards in aquatic environments.

Some of the plants ancestrally used in the El Alberto community for pest control in agricultural fields are: 1) garlic, *Allium sativum* L., (Liliaceae), a cosmopolitan herbaceous plant cultivated for its medicinal, antioxidant and culinary properties (El-Saber Batiha *et al.* 2020) and 2) nettle, *Urtica dioica* L., (Urticaceae), a herbaceous plant that grows along rivers or field banks and is generally considered to be a weed that has antioxidant, antimicrobial and medicinal properties (Gülçin *et al.* 2004; Asgarpanah and Mohajerani 2012). Garlic's insecticidal activity is due to allicin, which is released when crushing garlic bulbs, and in the presence of other organosulfur compounds (Wanyika *et al.* 2011). Nettle has been associated with isolectins involved in the production of chitin and in digestive enzyme docking (Carlini and Grossi-de-Sá 2002; Sadeghi *et al.* 2006).

The ancient use of garlic and nettle as botanical insecticides has passed through generations within this Hñähñu community. However, it is currently not being used in their agricultural practices, which involved crushing garlic bulbs and letting them soak in water for 3 days (personal communication). Regarding nettle, leaves and stem are also soaked in water for 3 days (personal communication). To the best of our knowledge, there are no studies that evaluate scientifically the insecticidal effectiveness of garlic and nettle on local populations of bruchins. Therefore, this study assesses the effects of *Allium sativum* and *Urtica dioica* as botanical insecticides in the form of aqueous extracts, on adult bruchins that attack *Prosopis laevigata* seeds in the El Alberto, Ixmiquilpan, Hidalgo community.

Materials and Methods

Bruchin identification

In order to determine the proportions of bruchins that attack mesquite seeds, the identification of specimens was carried out at the *genus* level, using the two identification keys for coleopterans of the Bruchidae family, in Zimapán, Hidalgo (Hgo), proposed by Godínez-Cortés *et al.* (2013, 2017). The specimens identified were those which emerged in the Seed Conservation Test.

Preparation of aqueous extracts

The aqueous extract of nettle was prepared using stem and leaves (100 g). For garlic extract, small chopped pieces of bulbs (50 g) were used. Both nettle and garlic were placed separately in 1 l of distilled water, obtaining concentrations of 10 and 5%, respectively, and were left to soak for 3 days to obtain the extracts. The positive control was calcium hypochlorite, 0.2 M $Ca(ClO)_2$, due to its potential biocidal activity and usage in the protection and inoculation of seeds in greenhouse installations (Revellin *et al.* 2018). Distilled water was used as the negative control.

Insecticidal effects on adult bruchins

Bruchins, newly emerged from mesquite seeds, were exposed to the aqueous extracts of garlic, nettle and controls. Each treatment was repeated 10 times and consisted of 10 Bruchinae specimens placed in 40-ml Falcon tubes. The exposure was done by spraying for 6 months with four exposure times at 40-day intervals. Seed monitoring was carried out 7 days after each exposure.

Seed Preservation Test from Bruchinae infestation

For this experiment, 20 seeds without superficial infestation symptoms, were placed in 40-ml Falcon tubes, where they were exposed to extracts of garlic, nettle and controls. Ten repetitions were made per treatment. The exposure was carried out by spraying for 6 months with four exposure times at 40-day intervals each. Seed monitoring was carried out 7 days after each exposure.

The insecticidal activity was calculated using the seed infestation rate [*IR* = (Total damaged seeds/Total seeds) × 100] (Aguado and Suárez 2006). The same equation was modified to obtain bruchins' seed emergence, mortality and survival percentages. Mortality and survival percentages do not consider the initial number of eggs laid because the method was designed to be easily reproducible under community greenhouse conditions. Insecticidal efficacy was calculated as follows:

$$\text{Efficacy} = 100 - \left(\frac{\%\ \text{Emergency treatment}}{\%\ \text{Emergency control}} \times 100 \right)$$

A germination test was performed to determine seed viability after the Seed Preservation Test. Twenty-eight seeds of *P. laevigata* were used per treatment, and 14 seeds from the negative control, in semi-closed germinators at 19°C, 60% humidity. The experiment was carried out in triplicate. The composition substrate was vermiculite : soil (3 : 1). The soil used was obtained from rhizospheric soils surrounding *P. laevigata*, from conserved sites in El Alberto, Ixmiquilpan (Hgo, Mexico).

Statistical analysis

Normality was determined with the Shapiro-Wilk Test complemented with Equality of Variances Tests for means (Levene) and medians (Brown-Forsythe). The comparative analysis was performed by Kruskal Wallis among treatments. Subsequently, Dunn's Test was performed, with Bonferroni correction for cases with significant differences. All tests were performed with the NCSS (2020) and STATISTICA 10 software, with significance levels of $\alpha = 0.05$.

Results and Discussion

Bruch in identification

A total of 128 specimens was obtained from *Prosopis laevigata* seeds, and classified into the subfamily Bruchinae, belonging to the genera *Algarobius* Bridwell and *Amblycerus* Thunberg; which represent, respectively, 72% and 28% of the bruchin pests affecting mesquite seeds under laboratory conditions.

Insecticidal effects on adult bruchins

Regarding mortality on the bruchin pest's adult stage, aqueous extracts of garlic (*A. sativum*) and nettle (*U. dioica*) obtained no effects, under laboratory conditions. Neither positive nor negative controls caused mortality. Aqueous extracts of garlic and nettle have not been used against the genera *Algarobius* or *Amblycerus*. However, Regnault-Roger and Hamraoui (1993) also found no effect on mortality in *Acanthoscelides obtectus* Say when using freshly cut garlic bulbs. For its part, nettle did not show significant activity on the mortality in the homopterans *Hyalopterus pruni* Geoffroy and *Cryptomyzus ribis* Linnaeus (Bozsik 1996). The efficacy of both botanical insecticides on adults may be due to the different concentrations used in this study than those of Regnault-Roger and Hamraoui (1993) and Bozsik (1996), who used 1 g of freshly cut cloves and 500 g of nettle in 5 l of water, respectively. Different effects depending on concentrations will be discussed later.

Seed Preservation Test from Bruchinae infestation

The infestation rate (*IR*) was obtained with the relation between damaged seeds and the total number of seeds used per treatment. The infestation rate was lower using nettle extract (4%) with a total of 8 adults emerging during the entire test ($n = 10$). Comparative analysis showed significant differences between nettle extract and distilled water ($p = 0.000596$). The positive control, $Ca(ClO)_2$, and nettle extract had similar issues as they didn't show significant differences ($p = 0.1976$), though a minor infestation rate was observed with nettle extract (Table 1). Studies relating aqueous nettle extract to seed protection have not been carried out. The results provided in this paper give a positive indicator about nettle properties for seed protection under laboratory conditions, by bruchin infestation in El Alberto. The *IR* using garlic extract was 27.5%, without significant differences with distilled water ($p = 1$), or $Ca(ClO)_2$ ($p = 0.657$) with 41 adults emerged during the entire test. However, a reduction in the number of damaged seeds was observed using garlic against *Callosobruchus maculatus* Fabricius (Tiroesele *et al.* 2015), and *Zabrotes subfasciatus* Boheman (Mulungu *et al.* 2007). Due to the high standard deviation in garlic, it was not possible to determine significant differences between garlic and water; therefore, its effect on seed protection cannot be ruled out (Fig. 1). The significance value between distilled water and $Ca(ClO)_2$ was $p = 0.00524$.

The emergence percentage was higher with garlic extract and lower with nettle extract, corresponding to an efficacy of 47 and 89%, respectively, in seed protection (Table 1). $Ca(ClO)_2$ had the lowest emergence percentage, reflecting an efficacy of 96%. Comparing nettle extract and distilled water yielded a significance of $p = 0.0076$, and a significance of $p = 0.353$ against $Ca(ClO)_2$. No significant differences were obtained between garlic extract and distilled water ($p = 1.38$). Aqueous extracts from garlic and nettle have not been

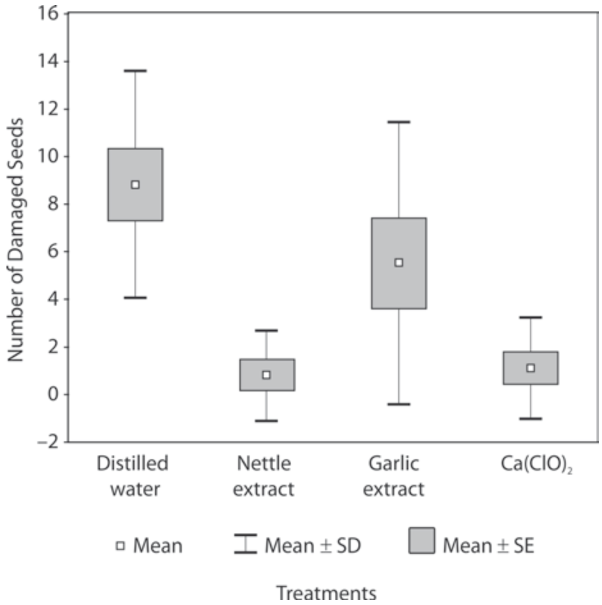

Fig. 1. Damaged seeds during the Seed Preservation Test

Table 1. Effect of the extracts on adult Bruchinae during the Seed Preservation Test

Treatment	Emergence[1] [%]	Mortality[2] [%]	Infestation rate
Distilled water	38.0 a	5.263	44 .0 a
$Ca(ClO)_2$	1.5 b	100.00	5.5 b
Nettle extract	4.0 b	50.00	4.0 b
Garlic extract	20.5 a	75.609	27.5 a

Values bearing the same letter a OA_04_JPPR_61_2_0952_Kurniadie.pdf re not significantly different

[1, 2]Emergence and mortality percentages refer to the bruchids emerged from seeds, without considering the number of eggs laid

used against the genera *Algarobius* nor *Amblycerus*. Nevertheless, Oparaeke (2005) observed less damage to the pods and a population reduction in *Maruca vitrata* Fabricius (Lepidoptera) and *Clavigralla tomentosicollis* Stål (Hemiptera) using an aqueous extract of garlic at concentrations of 10 and 20%. The efficient concentrations used by Oparaeke (2005) were two to four times higher than the concentration of 5% in garlic extract used in this present study. Additionally, Denloye (2010) observed a LC_{50} of 0.110 using aqueous garlic extract obtained from garlic powder on mortality in *C. maculatus*. Meriga *et al.* (2012) reported a larvicidal activity of 64% using aqueous extract against *Spodoptera litura* Fabricius (Lepidoptera). Since allicin is only liberated from crushed garlic bulbs, powdered garlic must have a greater efficacy effect than the solvent used. An aqueous extract, obtained from garlic powder and the use of higher concentrations, may boost its efficacy against the immature stages of the insect as well as the adult.

Regarding nettle effects on emergence, only isolated UDA lectins were used by Sadeghi *et al.* (2006) against *C. maculatus*, and Machuka (1999) against *M. vitrata*, with contrasting results: 30 ± 5% and 80 ± 5%, respectively. On the other hand, Jovanović *et al.* (2007) observed that the ethanolic extract of nettle obtained total mortality on *A. obtectus* when using a concentration of 100%, and such activity was considerably reduced when they used a concentration of 30%. Differences in aqueous nettle extract efficacy could depend on the plant part which has a higher lectin concentration as well as an increase in concentration during the extract preparation.

The highest percentage of Bruchinae adult mortality was obtained with garlic extract, followed by nettle extract (Table 1). However, in the comparative analyses, no significant differences were obtained between treatments or controls, which contrast with the results observed during the insecticidal effect on the adult bruchin test. Effects on adult mortality could occur as a result of the exposure of the organisms during embryonic development or other immature stages. Considering these results, War *et al.* (2012) reported that UDA lectins have antinutritive effects in the digestive tract, cause tissue deterioration, and alter the immune and hormonal status, threatening the growth and development of the insect. For its part, ASAI lectins, present in garlic, interact with various proteins involved in the insect life cycle, causing physiological disorders in development, growth retardation and premature death (Upadhyay and Singh 2012). In addition, insecticidal effects of the aqueous extract of garlic have been reported on the larvae of *M. vitrata* (Oparaeke 2005). It caused a reduction in the fecundity and fertility of females, as well as in oviposition and hatching in bruchins (Douiri *et al.* 2013; Chaubey 2014; Tiroesele *et al.* 2015).

The germination test allowed us to define the germination capacity of the treated seeds. The germination percentage in seeds treated with controls positive and negative was 0%, nettle extract 2.38%, and with garlic extract 1.19%. This outcome could be due to the fact that *P. laevigata* seeds present germination problems as a result of physical dormancy during prolonged storage (Sobrevilla-Solís *et al.* 2013). Post-treatment tests are proposed to determine viability in the seeds.

Conclusions

The artisanal use of *Allium sativum* and *Urtica dioica* as botanical insecticides showed that both have toxic effects on the developmental stages of *Amblycerus* and *Algarobius*. Aqueous nettle extract proved to be more effective than garlic extract in the protection of *Prosopis laevigata* seeds.

An increase in the concentration of aqueous extracts, the use of aerial and underground parts of nettle, the crushing of garlic bulbs into powder (associated with their incorporation in boiling water), as well as the combined use of both extracts and other native plants in El Alberto, Ixmiquilpan (Hidalgo, Mexico) could increase the efficacy of artisanal use of botanical insecticides.

Finally, studies should be carried out on the treatment of these pests. These studies must be focused on traditional knowledge, history of the use of botanical insecticides in the region, and in the collaboration of scientific groups and indigenous communities to promote the application of Comprehensive Management Plans in agroforestry.

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