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Ovicidal effect of *Canavalia ensiformis* seed extract with SiO_2 nanoparticles on *Meloidogyne incognita*

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ABSTRACT

Background/Objective. Seed extracts from *Canavalia ensiformis* have shown both antiparasitic and repellent effects against pests. To evaluate the effectiveness of the extract combined with silicon dioxide nanoparticles (NPs) against *Meloidogyne incognita* eggs.

Materials and Methods. *In vitro* experiments were conducted to assess the effects of *C. ensiformis* seed extracts, alone and combined with silicon dioxide NPs, on *M. incognita* juveniles hatching. 150 eggs were used, and concentrations of 0, 2, 4, 6, 8, and 10 % of the extract were applied. Additionally, concentrations of the extract at 0, 1.5, 2.0, 2.5, and 3.0 %, each combined with NP concentrations at 0.06, 0.08, 0.10, 0.12, and 0.14 %, were evaluated.

Results. None of the treatments prevented more than 30 % of juveniles hatching. It was concluded that modifying the technique for obtaining *C. ensiformis* seed extract could have a complementary ovicidal effect; however, increasing the extract concentrations could serve as a medium for the proliferation of saprophytic fungi and other microorganisms.

Conclusion. The treatments did not show significant ovicidal effects.

Keywords: Nematodes, bioassays, concentrations, hatching.

Introduction

The phytopathogenic nematodes that currently cause large losses in the production of tomato (*Solanum lycopersicum*), cucumber (*Cucumis sativus*), chili

pepper (*Capsicum annuum*), coffee (*Coffea arabica*), and others, are also scarcely studied organisms, resistant to synthetic nematocidal chemical products due to their indiscriminate use and that these, in turn, generate residuality in foods and affect human health (Trujillo-Rugamas *et al*., 2022). Plants have adaptatively and evolutionarily developed defense as a response to biotic and abiotic factors; some of these adaptations are physiological changes, chemical defenses and the production of secondary metabolites (Camacho-Escobar *et al.*, 2020). Therefore, new technologies have been sought in order to mitigate the secondary effects of agrochemicals, and for this reason, phytochemical extracts have been developed that help control nematodes (Cóndor-Golec., 2019). It is important to identify the metabolites in plants and know the methods and solvents to obtain them, since some plants such as Fabaceae, which contain large amounts of secondary metabolites such as flavonoids, phenolic compounds and alkaloids with diverse effects that help control pests (López *et al.*, 2022). Metabolites are distributed in different parts of the plant and can be found in different concentrations, depending on where they are extracted from. Some parts of the plant in which they can be found are leaves, seeds, flowers and stems (Guillén-Andrade *et al.*, 2019). *C. ensiformis* is a Fabacea that is used as food for cattle, although it contains diverse secondary metabolites such as L-canavanine, which, when given to rodents, causes problems in the development of some of their organs (Ruiz-Bedolla and López-Martínez., 2019). The aqueous extracts of *C. ensiformis* seeds were evaluated in phytopathogenic nematodes; the effects of the extract reached an effectiveness of at least 90% in second-stage juveniles (J2) and avoided the ecclosion of juveniles in 80% of *M. incognita* individuals (Rocha *et al.,* 2017). The aim of this study was to evaluate the ovicidal effect og *C. ensiformis*, as well as its potencialization when combined with silicon dioxide NPs.

The study was established in the Universidad Autónoma Agraria Antonio Narro in the Toxciology laboratory of the Agricultural Parasitology department, located in the city of Saltillo Coahuila, Mexico. Five samples of soil and roots infected with the nematodes of the *Meloidogyne* genus were taken from a serrano chili pepper plot in the town of Cristo Rey, in the municipal area of Escuinapa, Sinaloa. The samples were processed and J2 were obtained using the sieving-centrifugation method with sieves with mesh sizes of 50, 100, 400 and 500, followed by centrifugation at 5000 rpm for two minutes. A 45% sucrose solution was then added and centrifuged at 5000 rpm for one minute. The solution was decanted into the 500-mesh sieve and rinsed with sterile distilled water (Cepeda, 1995). The egg masses were obtained using the technique by Mc Clure *et al*. (1973) from roots using a Ninja ® food processor. One hundred grams of roots, previously washed with tap water were used. Distilled water was added until the root was covered. It was then grinded with three 15-second pulsations. Using the 50, 100, 400 and 500 meshed, the remains

of the roots were eliminated and incubated at 28 °C for six days (Cristóbal-Alejo *et al.*, 2018).

To identify the *Meloidogyne* species, mounts were prepared in lactophenol with cotton blue using the individuals extracted from the roots and soil. A perineal cut was performed on the females, and these mounts were observed under a compound microscope to examine their internal structures. They were identified using the keys by García *et al.* (2004). The taxonomic keys by Cepeda (2016) were also used and the species of J2 individuals that were confirmed by the Interactive Diagnostic Key to Plant Parasitic, Freeliving and Predaceous Nematodes of the University of Nebraska (Tarjan *et al.*, 1977) were identified. To obtain the *C. ensiformis* seed extract*,* seeds were compared in the Leguminutre® distribution company. The methodology described by Rocha *et al.* (2017) was followed, with some modifications, in which 100 g of seeds were added in a beaker. Two hundred mL of distilled water were added and left in an oscillating shaker for 24 hours at 130 rpm. Subsequently, the seeds in the water were grinded in a Ninja® brand food processor with three 30-second pulsations and the resulting product was left in the oscillator at 150 rpm for 24 hours. With the help of gauzes, the solid and liquid phases were separated and the liquid phase was centrifuged three times at 5000 rpm at 24 °C for 20 minutes each cycle. Finally, the extract was sterilized by filtration using 0.2-micron filters and stored at 4 °C.

The silica dioxide nanoparticles were obtained from the company Culta®. Two experiments were conducted and in both, cell culture plates were used, to which 100 µL of solution containing 150 nematode eggs were added. In all experiments the eggs were exposed to the dose seven days after their extraction and three observations were carried out six days after the applications until 12 days passed since their extractions, since this is the time taken for the juveniles to hatch. At all times the eggs were incubated at 28 °C. In the first experiment, six concentrations were applied (0, 2, 4, 6, 8 and 10% of the *C. ensiformis* seed extracts) with five repetitions per treatments, and these concentrations were determined using previous studies by Rocha *et al.* (2017). In the second experiment, five experiments were implemented with the following combinations: 0.0 1.5, 2.0, 2.5 and 3.0% of the *C. ensiformis* seed extract and each one was combined with 0.0 0.06, 0.08, 0.10, 0.12 and 0.14% of nanoparticles with five repetitions each, which were determined using a biological window. The data obtained from the hatching underwent an analysis of variance (ANOVA) to evaluate the significant differences between the means of the treatments. Later, the Tukey multiple comparisons test was applied with a significance level of 0.05 to identify specifically which groups differed from each other.

None of the *C. ensiformis* seed extract doses avoided the hatching of juveniles. However, none of the doses tested surpassed 30% in the reduction of the hatching.

Table 1 shows the percentages of reduction in hatching obtained after applying the *C. ensiformis* extract. The extract on its own can be seen to have presented a low reduction in the hatching of J2 (lower than 30%), with the 4% concentration being the one which displayed the highest reduction in hatching. The combined doses displayed a better effectiveness in the reduction of hatching in comparison to the non-combined applications. In the case of applications of the extract at 1.5%, the percentage of reduction was obtained in the combination with 0.08%, with 30.6% of eggs not hatched. In the experiment with the treatment at 2.0%, the combination that presented the best results were of 0.06 and 0.08% of nanoparticles, which reduced

Table 1. Means comparison of the effect of the treatments of the extract of *Canavalia ensiformis* seeds and silicon dioxide NPs on *Meloidogyne incognita* eggs.

	T	N	M±DS	A
Extract *C. ensiformis*	2	5	23.6±1.517	B
	4	5	30.6±2.191	A
	6	5	27.2±1.924	A B
	8	5	25±0.080	B
	10	5	25±0.080	B
Extract at 1.5 % and NP´s	0.06	5	29±1.414	A B
	0.08	5	30.6±2.191	A
	0.10	5	30.4±1.140	A B
	0.12	5	27.4±1.673	B
	0.14	5	30.2±1.789	A B
Extract at 2.0 % and NP´s	0.06	5	33.2±1.304	A
	0.08	5	33±2.350	A B
	0.10	5	31.8±1.304	A B
	0.12	5	30.2±1.483	B
	0.14	5	30.4±1.140	A B
Extract at 2.5 % and NP´s	0.06	5	34.8±2.168	A B
	0.08	5	37.2±1.924	A
	0.10	5	33.4±1.517	A B
	0.12	5	31.2±1.304	A B
	0.14	5	31±0.707	B
Extract at 3.0 % and NP´s	0.06	5	34±1.225	A
	0.08	5	32.8±1.643	A
	0.10	5	29.6±0.894	B
	0.12	5	29.4±0.894	B
	0.14	5	29.8±1.095	B

T= Treatments, N= Repetitions, M=Means, DS= Standard Deviation, A= Statistical differences.

hatching by 33.2%. In the case of 2.5 and 3.0% extract combined with 0.08% of nanoparticles, hatching decrease by 34.8 and 32.8%, respectively. Table 1 shows the statistically significant differences between treatments, that is, the clusters show that there are significant differences between them. The analysis of variance showed that the *C. ensiformis* alone showed no differences at the concentration of 6%. The analysis indicates that the combined applications showed significant differences with 1.5% extract and 0.08 and 0.12% nanoparticles, whereas other combinations did not. The combination of 2.0% with 0.06 and 0.12% of nanoparticles showed significant differences in hatching. For the 2.5% extract with 0.08 and 0.14% of nanoparticles, differences between applications were recorded regarding hatching, whereas for the 3.0% extract and its combinations, significant differences were observed. On the other hand, some effects were observed on the chorion (shell) of the eggs, where vacuoles formed inside them. These formations were noted six days after the inoculations of the treatments (Figures 1B and C). In addition, in the image of one of the controls, the formation of a juveline *M. incognita* was observed (Figure 1A). Likewise, the formation of a juvenile was observed in eggs inoculated with the extract, where the fully developed body of the juvenile was visible; however, this juvenile did not hatch (Figure 1D).

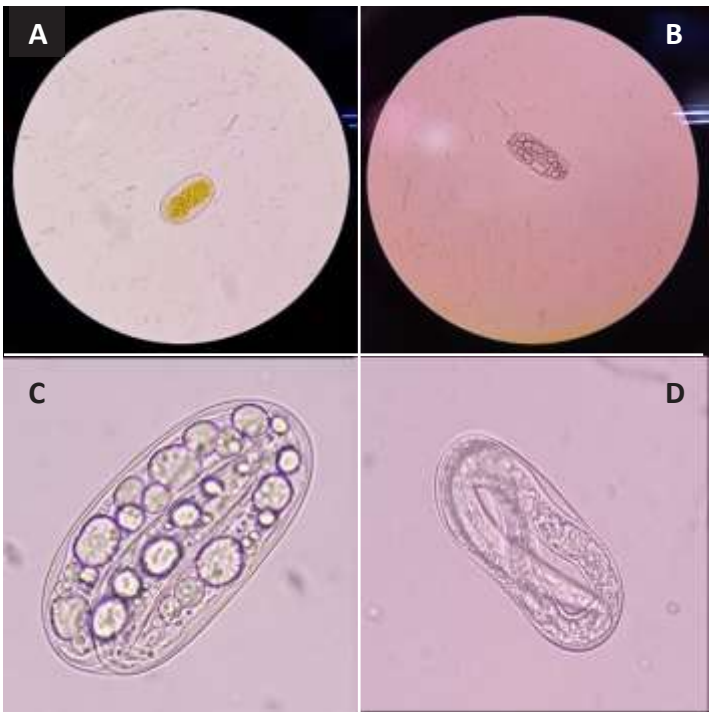

Figure 1. A: egg corresponding to the non-inoculated control and fixed with cotton blue (100).-B: egg inoculated with *Canavalia ensiformi*s extract and NPs (100X). C: amplified image of egg inoculated with *C. ensiformis* and NPs (100X). D: egg inoculated with *C. ensiformis* extract (100X).

None of the treatments reached an effectiveness of 50% regarding hatching, although significant differences were observed between treatments. The *C. ensiformis* seed extract on its own displayed a low reduction in the hatching of juveniles, despite the fact that, if there is a reduction in hatching, it was not in a significant percentage, probably due to the extract not containing stabilizing contributor, since it is an aqueous extract. If we take this into account, it can be compared to other extracts obtained using water solvents, ethanol, dichloromethane and hexane obtained from *Embelia schimperi*; in those experiments, the aqueous extracts did not surpass 50% in the reduction of the hatching, whereas the extracts obtained with solvents were greater than 80%. L-canavanine is a metabolite that hydrolyzes at a temperature equal to or greater than 40 °C, therefore its extraction must be performed cold (Rocha *et al.,* 2017). The technique used in this work was modified in order to reduce costs; it was developed at room temperature (26 °C +/- 1 °C) and incubation was carried out with a constant temperature of 26 °C. It is important to stress that, due to the nature of the L-canavanine, they cannot undergo concentration processes that involve high temperatures, which is why Rocha *et al.* (2017) placed the extract under a lyophilization process to preserve its properties. In this study, as a part of the modification of the technique, this step was omitted. This point may be the main differentiating factor in hatching, as when compared to other aqueous extracts where a rotary evaporator was used, the results achieved hatching inhibitions of over 70%. Metabolites can be obtained from different parts of plants and through various methods; their effectiveness depends on several factors such as mobility and availability in the soil, temperature and relative humidity, as well as pH and soil type (Shanmuga *et al*., 2019). In the complementary part of this study, the silica dioxide nanoparticles were used to innovate in seeking a ptentializing effect of the *C. ensiformis* seeds. Data anlysis showed that the use of nanoparticles enhanced the *C. ensiformis* extract, reducing the concentrations required to reduce hatching. To date, there is no information on the use of silica dioxide nanoparticles for nematode control. Nevertheless, studies have formulated plant extracts with nanoparticles, such as those from *Syzygium aromaticum*, *Lantana camara* and *Conyza dioscoridis*. These studies have achieved favorable results in which egg hatching has been reduced by 100%, which displayed greater effectiveness in comparison to aqueous extracts of the same plants (El-Habashy, 2022). The aforementioned results differ from those obtained with *C. ensiformis*, due probably to the fact that, despite being penetration of the extracts and the nanoparticles, these do not penetrate the secondary layers composed of proteins and lipids. It is in this part of the egg structure that vacuoles form (Perry *et al.*, 1982). The combination of *C. ensiformis* with the nanoparticles prevented juvenile hatching compared to treatments without the combination and required lower amounts of the extract. A potentializing effect was observed as a result of

combining silica dioxide nanoparticles with *C. ensiformis* extract, which may be attributed to small agglomerations of the extract since gelatinous formations were formed during the experiments.It is concluded that the application of the extract caused no significant ovicidal effects, inhibiting juvenile hatching by less than 30%. The combination of the silica dioxide nanoparticles with the extract yielded better results, inhibiting 37% of hatching at a concentration of 2.5 and 0.08% of nanoparticles. This suggests a 10% enhancement in the reduction of hatching, in addition to requiring significantly lower concentrations. The modification of the technique implemented at room temperature, combined with the nanoparticles displayed ovicidal effects. Molecular and morphometric studies are needed to confirm and support the identification of the nematode species.

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