RESEARCH NOTE/NOTA DE INVESTIGACIÓN

NEMATICIDAL EFFECT OF SUNN HEMP ROOT AND SHOOT EXTRACTS ON EGGS AND SECOND-STAGE JUVENILES OF *MELOIDOGYNE JAVANICA*

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ABSTRACT

Mayorga, L., D. Jacobs, H. X. Bui, and J. A. Desaeger. 2022. Nematicidal effect of sunn hemp root and shoot extracts on eggs and second-stage juveniles of *Meloidogyne javanica*. Nematropica 52:72-78.

Root-knot nematodes (RKN, *Meloidogyne* spp.) are among the most damaging and widespread nematodes and cause significant yield loss in vegetable production in Florida. Sunn hemp (*Crotalaria juncea*) is a common, fast-growing leguminous summer cover crop that is also known to be a poor or non-host to RKN. In this study, we investigated the nematicidal effect of sunn hemp plant tissue and extracts on egg hatching and second-stage juvenile (J2) survival of *M. javanica* under *in vitro* conditions. Six rates (0, 0.625, 1.25, 2.5, 5, and 10 g/L) of the shoot and root extracts were tested and hatching evaluations were made at 7, 10, 14, 21, and 28 days after treatment. Extract from the shoot was found to have an inhibitory egg hatching effect at 10 g/L, while root extract did not inhibit egg hatch. Second-stage juvenile survival was tested using shoot and root fresh tissue at six rates (0, 1, 5, 10, 15, and 20 g/200 ml soil) under aerobic and anaerobic conditions; survival was evaluated seven days after treatment. Incorporation of shoot and root fresh tissue in soil negatively affected J2 survival in all tested rates under both conditions. Furthermore, J2 survival was more affected by shoot tissue than root tissue. The results suggest that both sunn hemp shoot and roots have nematicidal activity against eggs and J2 of *M. javanica*.

Key words: Egg hatching, J2 survival, M*eloidogyne javanica*, nematicidal activity, sunn hemp extract

RESUMEN

Mayorga, L., D. Jacobs, H. X. Bui, y J. A. Desaeger. 2022. Efecto nematicida de extractos de raíces y brotes de crotalaria en huevos y juveniles en segundo estado de *Meloidogyne javanica*. Nematropica 52:72-78.

Los nematodos formadores de agallas (RKN, *Meloidogyne* spp.) se encuentran entre los nematodos más dañinos y ampliamente distribuidos, y causan pérdidas significativas en el rendimiento de la producción de hortalizas en Florida. La crotalaria es un cultivo de cobertura común para verano, leguminosa, de rápido crecimiento, y que también es conocida por ser un pobre o no hospedero de RKN. En este estudio, investigamos el efecto nematicida del material vegetal y extractos de crotalaria en la eclosión de huevos y sobrevivencia de juveniles de segundo estado (J2) de *M. javanica* en condiciones *in vitro*. Se probaron seis dosis (0, 0.625, 1.25, 2.5, 5 y 10 g/L) de los extractos de brotes y raíces, y se realizaron evaluaciones de la eclosión a los 7, 10, 14, 21 y 28 días después del tratamiento. Se encontró que el extracto de brote tuvo un efecto inhibidor de la eclosión de los huevos a 10 g/L, mientras que los extractos de la raíz no inhibieron la eclosión de los huevos. La sobrevivencia de los J2 se probó utilizando tejido fresco de brotes y raíces a seis cantidades (0, 1, 5, 10, 15, y 20 g/200 ml de suelo) en condiciones aeróbicas

y anaeróbicas. La sobrevivencia de J2 se evaluó siete días después del tratamiento. Los resultados mostraron que la incorporación de tejido fresco de brotes y raíces en el suelo afectó negativamente la sobrevivencia de J2 en todas las cantidades probadas en ambas condiciones. Además, la sobrevivencia de J2 se vio más afectada por el tejido del brote que por el tejido de la raíz. Los resultados sugieren que tanto el brote como la raíz de la crotalaria tienen actividad nematicida contra los huevos y J2 de *M. javanica*.

Palabras clave: Eclosión de huevos, sobrevivencia de J2, *Meloidogyne javanica*, actividad nematicida extracto de crotalaria

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Root-knot nematodes (RKN, *Meloidogyne* spp.) are among the most damaging and widespread nematodes worldwide (Jones *et al.*, 2013; Janati *et al.*, 2018). Root-knot nematodes thrive in Florida due to the humid warm climate, sandy soils, and almost year-round crop production (Crow, 2020; Bui *et al*., 2022). *Meloidogyne javanica*, *M. incognita*, *M. arenaria*, *M. haplanaria*, *M. hapla*, and *M. enterolobii* are the most common RKN species causing significant yield loss in vegetable production in Florida (Brito *et al.*, 2008; Bui *et al.*, 2022). Root exudates play a key role in egg hatching and second-stage juvenile (J2) mobility and mortality of *Meloidogyne* spp. in the rhizosphere. Several studies have shown that root exudates either inhibit or stimulate egg hatching depending on the resistance or susceptibility of the host plant to *Meloidogyne* spp. (Yang *et al.*, 2016). Similarly, root exudates attribute to the attraction or repellence of nematodes to the roots, and mobility inhibition or mortality of *Meloidogyne* spp. J2 (Dutta *et al.*, 2012).

Cover crops have been used since the early days of agriculture in Florida, both to restore soil fertility and to help manage RKN. However, they were largely abandoned when synthetic fertilizers and fumigants became the standard practice after the 1950s. If cover crops are used for RKN management, they have to be non- or poor hosts to the target *Meloidogyne* species. In addition, cover crops can also release nematicidal compounds against plant-parasitic nematodes, which is the case for marigold (*Tagetes* spp.) (Hooks *et al.*, 2010) and many plants belonging to the Brassica family (Kruger *et al.*, 2013).

Sunn hemp (*Crotalaria juncea*) is a leguminous plant favorable to growing in tropic and subtropic areas and one of the most popular cover crops in Florida (Gill and McSorley, 2011; Wang *et al.*, 2022). It is well known to be a poor host to many different RKN species (Crow *et al.*, 2001; Wang *et al.*, 2002; Gill and McSorley, 2011; Bui and Desaeger, 2021). Also, some studies have shown that sunn hemp also contains nematicidal compounds (Wang *et al.*, 2002; Ji *et al.*, 2005; Colegate *et al.*, 2012; Mansoor *et al.*, 2015). Amulu *et al.* (2021) showed that sunn hemp leaf extract reduced the number of egg masses, density, and gall index of *M. incognita* in *Amaranthus cruentus* at 40 g/L. A reduction in gall rating, reproduction factor, and density of *M. incognita* was observed in a study of tomato and sunn hemp intercropping system under greenhouse conditions (Tesleem *et al.*, 2014). Also, the incorporation of sunn hemp in soil (75 g/kg soil) decreased *M. incognita* population density by 62% in eggplant (*Solanum melongena*) and promoted plant growth parameters such as root and shoot length, and total biomass (Patel and Dhillon, 2017) under greenhouse conditions. Similarly, a 94% reduction of *M. incognita* was recorded in field trials where sunn hemp was incorporated into the soil at 6 kg/ha (Kankam *et al.*, 2015). Another study demonstrated that the growth and incorporation of sunn hemp before taro planting reduced *M. javanica* density below the economic threshold level and increased taro yield (Sipes and Arakaki, 1997).

In Florida, sunn hemp is one of the most popular and commonly planted summer cover crops. While sunn hemp is well known to be a poor host to RKN (Bui and Desaeger, 2021), less is known about how sunn hemp biomass contributes to nematode suppression. Our study aimed to investigate the nematicidal effect of sunn hemp shoot and root extracts on RKN egg hatching and tissue on J2 survival in *in vitro* experiments.

The culture of *M. javanica* was maintained on RKN susceptible tomato cv. HM1823 for two months in a greenhouse at the University of Florida Gulf Coast Research and Education Center (UF/GCREC), Wimauma, FL. Eggs were extracted from infected roots by the sodium hypochlorite method (Hussey and Barker, 1973). For the *in vitro*

assay, eggs were collected immediately and counted to adjust to a final concentration of approximately 200 eggs/ml. For the J2 survival experiment, eggs were kept in hatching boxes and *M. javanica* J2 were collected every 2 days for 2 wk in a chamber maintained at 27-29ºC. The collected *M. javanica* J2 were stored at 10°C prior to the start of the experiment.

Sunn hemp cv. Crescent Sunn AMS 535 was grown at the research farm of UF/GCREC. The plants were harvested 90 days after planting when the first flowers appeared. Roots and shoots of sunn hemp were separately chopped into 1-cm pieces, then blended with distilled water at a ratio of 20 g/L. The flask was kept on the shaker (ELMI-3.02.20L, NewBury Park, CA) at 1,000 rpm for 72 hr at room temperature and then the extracts were filtered through a 75-µm sieve. In each well of a 48-well plate, 500 µl of the extract was mixed with 500 µl of nematode egg solution (the final concentration was 100 eggs per cell). Tested rates of the shoot and root extracts were 0.625, 1.25, 2.5, 5, and 10 g/L, and distilled water was used as the control. Egg hatching was evaluated at 7, 10, 14, 21, and 28 days after treatment (DAT). Each treatment was replicated five times in a randomized complete block design (RCBD), and the experiment was performed twice to confirm the result. The hatching percentage was calculated as (Sikandar *et al.*, 2020): % hatching = (hatched J2/ total eggs) x 100.

The soil used in this experiment was collected from the research farm at UF/GCREC. It was characterized as Myakka fine sand (96% sand, 3% silt and 1% clay, pH 7.6 and 0.8% organic matter). The soil was pasteurized twice at 80°C for 2 hr using the SST-15 96-L 120 v Soil Sterilizer (Durable Greenhouse & Nursery Equipment LLC, Phoenix, AZ) and then kept in plastic bins for 2 wk before use. Plastic boxes (0.5 L) were filled with 200 ml of pasteurized soil, then shoot or root pieces (approximately 1 cm) were mixed with the soil at the following rates: 0, 1, 5, 10, 15, and 20 g of fresh tissue per 200 ml soil. Additionally, *M. javanica* J2 survival was tested under anaerobic and aerobic conditions by leaving the box with the lid closed or open, respectively. Each box was inoculated with 2,000 *M. javanica* J2 in the first trial and with 2,300 *M. javanica* J2 in the second trial. The boxes were watered with 50 ml of distilled water at the beginning of the experiment, and boxes that were left open were watered daily with 30 ml of distilled water to prevent drying of the soil. The boxes were left for seven days at room temperature (23ºC, 52% RH). At the end of the experiment, the soil from each box was extracted using a modified Baermann method for 48 hr at room temperature. *Meloidogyne javanica* J2 were collected on a 25-µm sieve, transferred in water into vials, and stored at 4°C for counting (Hooper and Evans, 1993; Forge and Kimpinski, 2007; Saikai *et al.*, 2021). Each treatment was replicated five times and arranged in a RCBD. The experiment was performed twice in the spring and fall of 2021, respectively. *Meloidogyne javanica* J2 were counted under an inverted compound microscope (Zeiss AxioVert A1, Carl Zeiss Microscopy, Jena, Germany). The survival percentage was calculated as: % survival = (extracted J2s in the treatment/extracted J2 in the control) * 100.

The second (repeat) experiments followed similar trends as experiment one. Data for the *M. javanica* egg hatching experiment were combined and analyzed by repeated measure ANOVA with $P \leq 0.05$ with trials as a random effect. Data for the *M. javanica* J2 survival experiment were combined and analyzed using a generalized linear mixed model (PROC GLIMMIX) for three-way ANOVA with $P \leq 0.05$, trials as a random effect and data were fit to a lognormal distribution. The mean comparison was done by Tukey-Kramer's posthoc test. The analyses were performed in SAS Studios (SAS Institute Inc., Cary, NC, USA).

Sunn hemp root extract did not inhibit *M. javanica* egg hatching at any of the tested concentrations (0.625, 1.25, 2.5, 5, and 10 g/L) (Fig. 1A). Similarly, sunn hemp shoot extract did not have an inhibitory effect on egg hatching, except at the highest rate of 10 g/L, which showed a significant inhibitory effect with 50% egg hatching inhibition compared to the control (Fig. 1B). This confirms a study conducted in Hawaii under *in vitro* conditions, which demonstrated that sunn hemp leaf extracts had a greater effect on *M. incognita* J2 mortality and egg hatching inhibition than flower and root extracts (Zasada *et al.*, 2012). Several studies have shown that *Crotalaria* spp. produce a large number of pyrridozine alkaloids and other secondary metabolites that have nematicidal activity (Wang *et al.*, 2002; Mansoor *et al.*, 2015). A study using sunn hemp cv. Tropic Sun showed that the total concentration of pyrridozyne alkaloids was higher in the stem compared to roots, leaves, and seeds (Colegate *et*

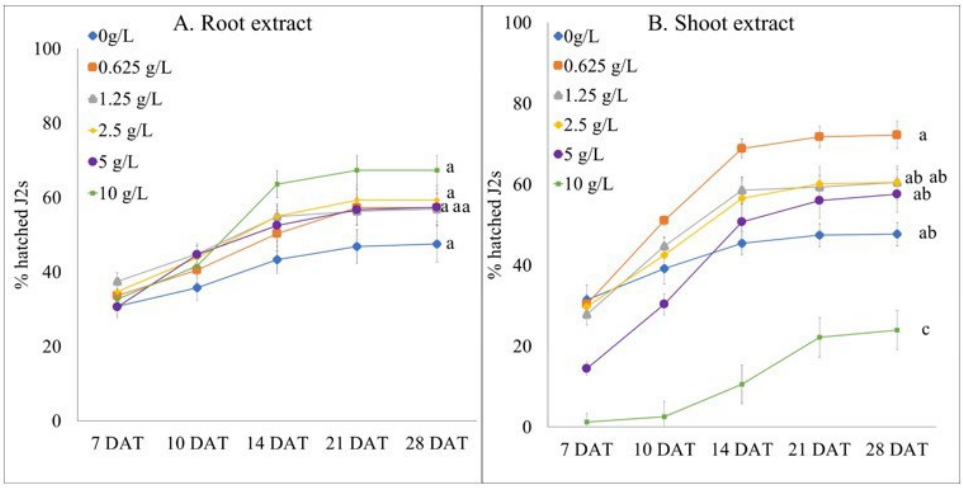

Figure 1. The effect of sunn hemp root (A) and shoot (B) extracts (0, 0.625, 1.25, 2.5, 5, and 10 g/L) on *Meloidogyne javanica* egg hatch. Treatment means with different letters indicate statistical significance based on the Tukey-Kramer test ($P \leq 0.05$). Error bars = standard error mean. DAT: days after treatment.

al., 2012). The most common pyrridozyne alkaloids are junceine, trichodemine, hemijunceine, and isohemijunceine, and their relative quantities likely vary among sunn hemp cultivars (Ji *et al.*, 2005; Colegate *et al.*, 2012).

Significant interactions were observed between sunn hemp rates, tissue type, and testing conditions (Table 1). Sunn hemp tissue rates significantly affected *M. javanica* J2 survival and a negative correlation was observed between sunn hemp rates and *M. javanica* J2 survival for both root and shoot tissue (Fig. 2). *Meloidogyne javanica* J2 survival averaged 42.5% at the 1g rate as compared to 6.6% on average for the 20 g rate (Table 1).

Meloidogyne javanica J2 survival in the

Table 1. Survival of *Meloidogyne javanica* second-stage juveniles after exposure to sunn hemp rates, sunn hemp tissues, and testing conditions.

Factor	Treatment	% Survival
Rate (g/200ml soil)	1g	42.5 a[z]
	5g	20.6 ab
	10g	13.5 bc
	15g	11.1 cd
	20g	6.6 d
Sunn hemp tissue	Root	26.9 A
	Shoot	10.7 B
Testing conditions	Anaerobic	20.1 β
	Aerobic	17.3 β
P-value	Rate	<0.0001
	Sunn hemp tissue	<0.0001
	Testing conditions	0.12
	Rate*Sunn hemp tissue	<0.0001
	Rate*Testing conditions	0.004
	Testing conditions*Sunn hemp tissue	0.004
	Testing conditions*Sunn hemp tissue* Rate	0.001

[z]Treatment means in each factor with different letters indicate statistical significance based on the Tukey-Kramer test ($P \leq 0.05$).

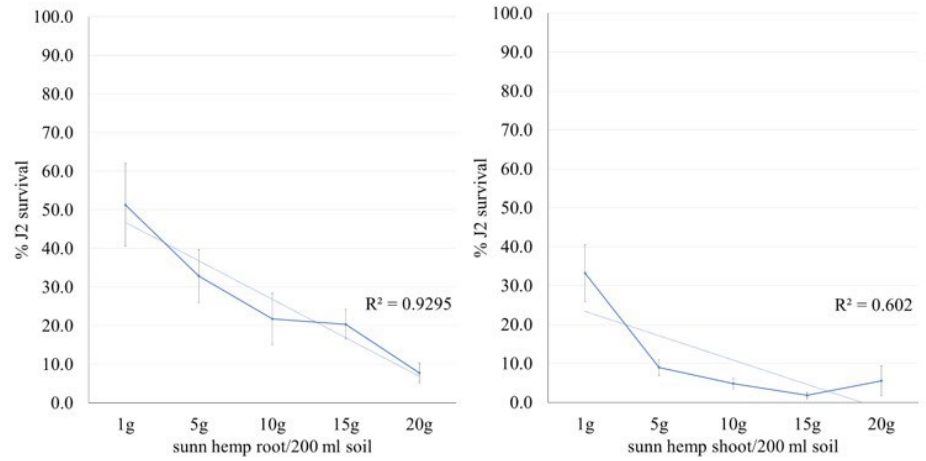

Figure 2. The linear regression represents the nematicidal effects of sunn hemp root and shoot (1, 5, 10, 15, and 20 g/200 ml soil) on *Meloidogyne javanica* second-stage juvenile (J2) survival. The linear regression model was performed by PROC REG in SAS Studio with a lack of fit test (*P*-value = 0.41 for root and *P*-value = 0.09 for shoot). Root and shoot data were SQRT-transformed and LOG-transformed to satisfy the normality. Error bars = standard error mean.

control (non-amended) soil was somewhat lower in under anaerobic condition as compared to the aerobic condition (respectively 391 and 279 *M. javanica* J2 were recovered from control boxes, data not provided), but testing conditions (aerobic or anaerobic) did not significantly impact the effect of tissue incorporation on *M. javanica* J2 (Table 1).

Meloidogyne javanica J2 survival was significantly lower for incorporated sunn hemp shoot (10.7% on average) as compared to sunn hemp root tissue (26.9% on average) (Table 1). Several studies have shown that leaf extracts were more toxic to *M. incognita* J2 than root extracts (Zasada *et al.*, 2012; Colegate *et al.*, 2012). This has also been shown to be the case for other plant species like *Bidens pilosa* and *Tagetes erecta* (Taba *et al.*, 2008; Natarajan *et al.*, 2006). Also, other nematodes, such as soybean cyst nematode (*Heterodera glycines*), were suppressed by incorporating *Crotalaria spectabilis* aboveground biomass (Nguyen *et al.*, 2022).

In summary, inhibitory effects of sunn hemp extracts on nematode egg hatching were limited, but nematicidal effects of fresh shoot material on *M. javanica* J2 survival in soil were more pronounced. Our results suggest that the suppressive effect of sunn hemp as a cover crop on *M. javanica* is not only due to its non-host status to root-knot, but also because of nematicidal effects of its tissues, in particular, shoot tissue.

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