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Nematicidal Activity of *Ageratum conyzoides* Leaf Extract Against Root-knot Nematode (*Meloidogyne javanica*) on Eggplant in Jalingo, Nigeria

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Abstract

Abstract*: Devastation of crops by pests is exacerbated by human activities but nature provides remedies that are environmentally friendly. This study was carried out to evaluate the activity of* ***A.*** *conyzoides leaf extract against Meloidogyne javanica on eggplant in Jalingo, Nigeria. Three concentrations (crude extract, 5 ml and 10 ml dilutions) of extract of Ageratum conyzoides as well as control (no application) were applied on juveniles and eggs of M. javanica in petri dishes in the laboratory in 2018 and 10 ml of the same extract was applied into holes at the base of eggplants transplanted into a nematode-infested field in 2018 and 2019. Result of the juvenile mortality test showed that the extract treatments caused the mortality of M. javanica juveniles with crudes extract being highest at 90.3%. Results of the egg hatch inhibition test also showed the extract treatments significantly reduced egg hatch compared to control with crude extract having the highest inhibition of 85.5%. Field experiment in both years indicated that the extract treatments recorded significantly (P<0.05) better result than control in all parameters in both years. There was reduced root galling and nematode reproduction and by extension final nematode population with attendant high yield in extract-treated plants. It is therefore concluded that* ***A. conyzoides*** *has the potential for use as a bio-pesticide against M. javanica.*

Keywords: Inhibition, Extract, ***M. javanica*** , ***A. conyzoides*** , Concentrations

INTRODUCTION

Meloidogyne, the root-knot nematode genus whose members reportedly cause a crop loss yearly estimated to be $157 billion (Deau *et al.*, 2008) and $100 billion (Oka *et al.*, 2010), contains over 80 species (Karssen, 2002). *Meloidogyne incognita*, *M. javanica,* and *M. arenaria* are three species of root-knot nematodes that have been reported to be associated with the root-knot disease of eggplant worldwide (Das *et al.*, 2021). A 50% yield loss and shoot growth reduction occurred when eggplants were inoculated with 4.7 and 3.2 *M. javanica* eggs and juveniles/g soil respectively (Moosavi, 2014).

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Phytotoxicity, environmental pollution and nematode resistance result from the indiscriminate use of synthetic pesticides for controlling nematodes (Yudelman *et al*., 1998; Pandey, 2005; Thligene *et al*., 2019). Compounds that occur naturally are usually inexpensive, easy to apply and do not pollute the environment and have potential to replace synthetic nematicides (Burow *et al*., 2007; Abolusoro *et al*., 2020; Favaz *et al*., 2021; Okechalu *et al*., 2021; Samara, 2022; Mamman *et al*., 2022).

It was in view of the above that this research was carried out in order to explore the potentials of *A. conyzoides* to counter the menace of *M. javanica* on eggplant which is a popular food in Jalingo and Nigeria.

MATERIALS AND METHODS

Experimental sites

The laboratory tests were carried out in the Department of Agronomy Laboratory, Taraba State University, in 2018. The field experiment was conducted at the Teaching and Research Farm of Department of Crop Production Technology, College of Agriculture, Jalingo, Nigeria in 2018 and 2019.

Plant Extract Preparation

The leaves of *Ageratum conyzoides* were removed by hand from the plant within Taraba State University, Jalingo. After washing, they were shade-dried and then ground to powder using mortar and pestle. The powder was stored in plastic bags. Fifty grams of the powder was turned in to a 5 litre plastic bucket with 500 ml distilled water added. The set up was allowed to stand for 48 hours and filtered through Whatman No.1 filter paper. The filtrate obtained was designated as crude extract which was then serially diluted with 5 mL and 10 mL distilled water giving three treatments (PTS1–Crude Extract, PTS2–5 mL dilution, PTS3–10 mL dilution). Also, distilled water was used as control and designated as CT. There were four treatments for each of the egg hatchability and juvenile mortality tests and field experiment.

Extraction of Nematode Eggs and Juvenile

Second stage juveniles (J2) and eggs of *M. javanica* were extracted from pure culture of infested roots of tomato plants. Nematode juveniles were extracted using the modified Baermann method of Whitehead and Hemming (1965). The juveniles were extracted using shallow trays with sieve lined with tissue paper and macerated roots of tomato placed on it. Water was poured in from the side of the tray to a level just submerging the materials on the sieve. This set up was left to stand for 24 hours and the nematode juveniles were collected by decanting into a beaker. Aliquots of 10 mL in syringes were taken and counted under a stereoscopic microscope using a grid counting dish and 1000 juveniles were used for each inoculation of juvenile mortality test. Identification of the nematode (*Meloidogyne javanica*) was done using the head and stylet morphology described by Eisenback *et al.* (1981). Nematode eggs were extracted by agitating tomato roots in 0.05% NaOCl (sodium hypochlorite) for 2 – 3 minutes (Hussey and Barker, 1973). The eggs were collected and rinsed with tap water on nested 150 and 25 um pore sieves (Dong *et al.*, 2007).

Juvenile Mortality Test
Ten ml of the extracts of *T. rhomboidea* in 10 mL syringe was dispensed into petri dishes containing 1000 juveniles of *M. javanica*. There were four treatments as described under preparation of extract above arranged in the completely randomized design (CRD), with 12 petri dishes (Mamman *et al.*, 2022).

Egg Hatchability Test
A 10 mL syringe was used to dispense 10 mL of *A. conyzoides* extracts into 12 petri-dishes containing 1000 eggs of *M. javanica*. There were four treatments arranged in the completely randomized design (CRD) (Mamman *et al.*, 2022).

Field Experiment
The experimental plot, which was naturally infested with *M. javanica*, was ploughed, levelled, demarcated into 12 plots (with each plot measuring 2 m x 2 m (4 m^2), replicated three times (each replication contained four plot) and laid out in a randomized complete block design (RCBD) in 2018 and 2019. Seedlings of a commonly grown local eggplant variety (eggplant cv data) were raised in June (in steam-sterilized loamy soil at 60 ^oC) in 20 cm diameter plastic pots for three weeks and transplanted to the field in July of each of the two years at one plant per stand and nine plant per plot at a spacing of 60 cm x 60 cm. Three-week old seedlings were transplanted to the field into a holes drenched with 10 mL extract of *A. conyzoides* at one seedling per hole. After that, a subsequent application of 10 mL extracts was done weekly for eight weeks. There were three concentrations (crude extract, 5 mL dilution and 10 mL dilution) and control making four treatments in all. Only three plants per plot were sampled for plant height, number of leaves, number of branches, shoot weight, root length, root weight, number of fruits, fruit weight, fruit girth, yield, galling index, nematode population and reproduction factor. All other agronomic practices were applied as required (Mamman *et al.*, 2022).
For soil analysis and initial nematode population, soil was sampled at a depth of 0 – 15 cm with an auger prior to the application of treatments.

Data analysis
At the end of the experiment, all data collected was subjected to analysis of variance (ANOVA) in SAS procedures and means were separated using SEM (Standard Error of the Mean) at $P<0.05$ level of significance.

RESULTS AND DISCUSSION
At the end of the experiment, results of juvenile mortality test showed that juvenile mortality increased with increase in extract concentration. Therefore, the higher the concentration of *A. conyzoides* extract, the higher the percentage death of *M. javanica* juveniles recorded. The 10 mL dilution recorded the lowest mortality rate among the extract dilutions with 60.4%, the 5 mL dilution with 77.7% and the highest result was shown by the crude extract (100% extract) with 90.3%. Control recorded the least juvenile mortality of all the treatments with 3.6% as shown in Figure 1. This goes to show that fewer nematode juveniles died in control as the extract was not applied there. They might have died from some natural causes. *Piper betel*, *Allium sativum* and *Cassia fistula* extracts at concentrations of 5 and 10% showed 76 to 98% mortality and egg hatchability rates 72 h after treatment (Farzana and Faisal, 2022).

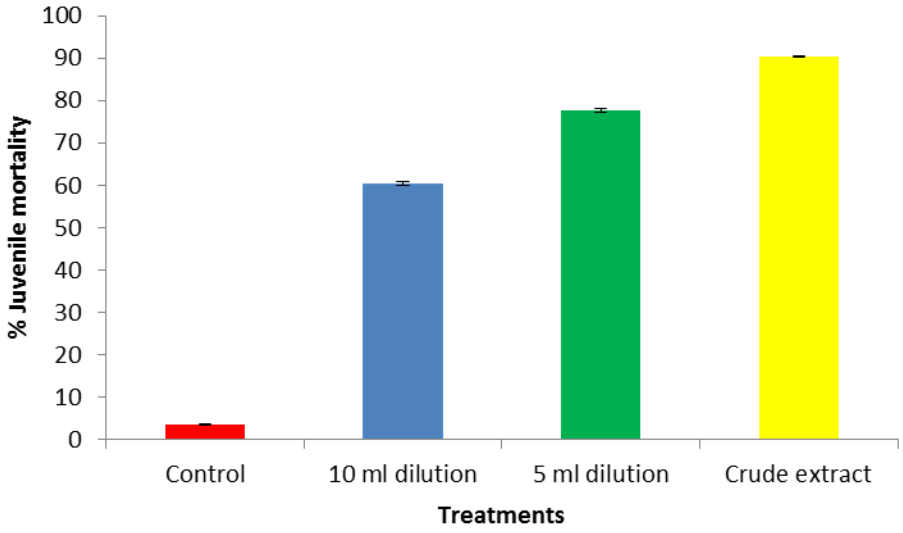

Figure 1: Effect of *A. conyzoides* leaf extract on the mortality of *M. javanica* juveniles

At the end of the egg hatch inhibition test, results showed that egg hatch inhibition increased with increase in concentration of extract. Therefore, the higher the concentration of *A. conyzoides* extract, the higher the percentage of unhatched *M. javanica* eggs recorded. However 10 mL dilution recorded the lowest percentage egg hatch inhibition rate among the extract dilutions with 59.9%, the 5 ml dilution with 79.3% and the highest result was shown by the crude extract (100% extract) with 85.5%. Control recorded the least juvenile mortality of all the treatments with 2.6% as shown on Figure 2. Without any hindrance from the extract, nematode eggs hatched freely in control and the hatch inhibition noticed may have been due to unfavourable environment or natural causes. Jidere and Oluwatayo (2018) observed that at higher concentration of the various botanicals, the greater their effect on egg-hatch inhibition and juvenile mortality. Similar to the results obtained in this study, Izuogu and Oyedunmade, (2008) have reported that the presence of saponins and flavonoids in *Phyllanthus amarus, Morinda lucida* and *Cymbopogon citratus* enhanced the mortality of juveniles, egg hatch inhibition and development of infective J2s of *Meloidogyne incognita* in their study.

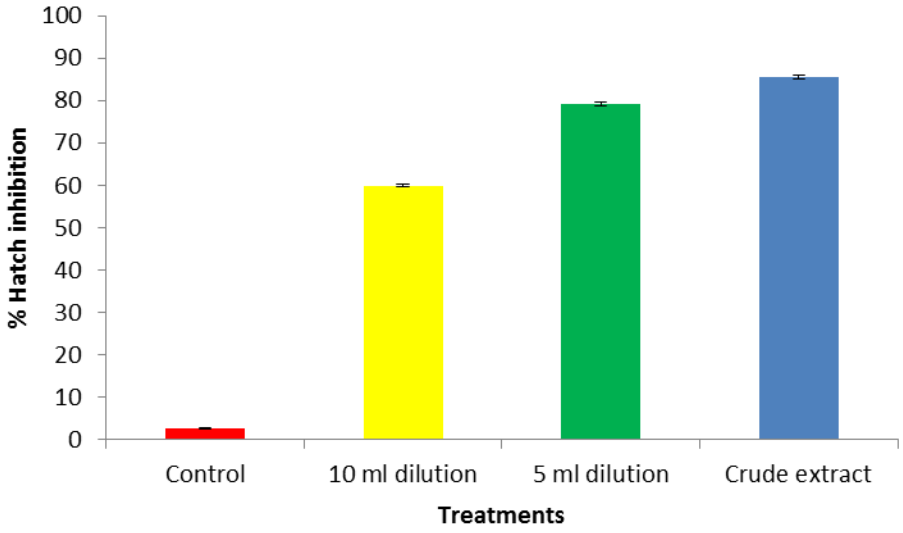

Figure 2: Effect of *A. conyzoides* leaf extract on the hatching of *M. javanica* eggs

Results of the field experiment are presented on Tables 1, 2 and 3. These Tables show the effect of *M. javanica* infestation on eggplant cv data and the mitigating effect of application of *Ageratum conyzoides* leaf extract. For all parameters measured, there was significant ($P<0.05$) difference between all three extract treatments and control. For plant height in 2018, the crude extract recorded the tallest plants with 109.88 cm followed by 5 mL dilution with 102.55 cm and 10 mL dilution with 89.99 cm. control produced the shortest plants with a mean height of 42.10 cm. In 2019, the result followed same trend with the crude extract having the tallest plants at 110.33 cm followed by 5 ml dilution-treated plants (103.10 cm) and least was control with 42.99 cm. Eggplants treated with the crude extract recorded the longest roots in both 2018 and 2019. In 2018, root length for plants treated with the crude extract were longest at 82.76 cm followed by 5 mL dilution (75.63 cm) and least was control with 37.12 cm whereas in 2019, crude extract-treated eggplants still recorded the longest roots with 81.50 cm and least was still control with 37.16 cm (Table 1). The study showed that the application of *A. conyzoides* extract on eggplant resulted in a significant increase in plant height and an increase in the mean root length of the plants compared to the control group. The crude extract treatment had the most pronounced effect in both years of the study, leading to the tallest plants and the longest plant roots. These findings are consistent with previous studies that have demonstrated the potential of *A. conyzoides* as a natural pesticide and growth enhancer for various crops. Dada *et al.* (2020) found that the application of *A. conyzoides* extract significantly ($P<0.05$) reduced root-knot nematode infection in tomato plants while also promoting plant growth and fruit yield. Similarly, Ekeh *et al.* (2019) reported that *A. conyzoides* extract improved the growth and yield of okra plants while also reducing the incidence of pest and disease attacks. Also, statistical analysis showed that various aqueous extract treatments of moringa and bitter leaf significantly increased plant height and fresh tuber weight ($p< 0.05$) of carrot as compared to the untreated control (Okechalu *et al*. 2021).

In both 2018 and 2019, eggplant treated with extracts of *A. conyzoides* recorded significantly ($P<0.05$) lower root weight and higher mean number of fruits per plant that control. In 2018,

eggplants that received the crude extract (100%) of *A. conyzoides* had the lowest root weight of 238.63 g which is lower than plants treated with 5 mL dilution of the extract with 247.85 g, and the 10 mL dilution-treated plants had root weight of 275.98 g; the highest root weight was recorded by control at 359.52 g. In 2019, the eggplants treated with crude extract still recorded the lowest root weight of 238.40 g lower than the 5 mL dilution with 246.76 g and least was control (360.33 g). Eggplants treated with extracts of *A. conyzoides* produced significantly ($P<0.05$) more fruits per plant than control. The crude extract-treated plants had the highest number with 30.66 fruits per plant followed by the 10 mL dilution-treated plants with 28.05 fruits/plant and 10 mL dilution with 21.77 fruits while control was least with 12.55 fruits per plant in 2018. In 2019, the crude extract produced the highest number of fruits per plant with 31.77 fruits followed by 5 mL dilution (28.33 fruits) and 10 mL dilution (22.33fruits) while control was still the least with 12.77 fruits per plant (Table 2). The result suggests that treating eggplant with extracts of *Ageratum conyzoides* could result in a significant maintenance of healthy root weight seen in treated plants as against increase in root weight due to the presence of much more galls seen in control and an increase in the mean number of fruits per plant compared to the control group in both 2018 and 2019. The crude extract showed the most significant effect in both years, resulting in the lowest root weight and highest number of fruits per plant. This finding agrees with previous research that has reported the potential of *A. conyzoides* extracts as a natural alternative for controlling plant-parasitic nematodes. According to Opara *et al.* (2018), the active compounds in *A. conyzoides* have been shown to possess anthelmintic and pesticidal properties, which may explain their effectiveness against nematodes. Furthermore, increased fruit production in treated eggplants recorded in this study could be attributed to the reduced damage caused by the nematodes. Nematode infestation can lead to stunted growth, wilting, and decreased yields in crops (Sikora *et al.*, 2018).

Eggplants that received crude extract treatment recorded the lowest galling index of 1.66 followed by 5 mL dilution (3.00), 10 mL dilution (3.33) with control having significantly ($P<0.05$) the highest galling with 4.33 in 2018. In 2019, the crude extract treatment still recorded the lowest galling index with 1.70 followed by 5 mL dilution treatment (2.36) and highest was control with 4.40. For final nematode population in 2018, control recorded the highest number with 923.00 nematodes followed by the 10 mL dilution treatment with 283.66 nematodes, 10 mL dilution (195.33 nematodes) and least was crude extract treatment (176.00). In 2019, control recorded the highest number of nematodes with 912.46 followed by 10 mL dilution (269.86) and least was crude extract treatment with 168.53 nematodes. The reproduction factor was highest in control (2.42) followed by 10 mL dilution (0.74), 5 mL dilution (0.51) and least in the crude extract treatment with 0.46 in 2018. In 2019, the reproduction factor was still highest in control with 2.39 followed by 10 mL dilution treatment (0.71), 5 mL dilution (0.48) and least was the crude extract (0.44) (Table 3). The study showed that the lower galling index was an indication that extract treatments of *A. conyzoides* reduced activities of *M. javanica* on eggplant leading to the lowering of gall formation on the roots. Also, lower reproduction factor showed the inhibitory effect of the extracts on nematode reproduction leading to the lower final nematode population recorded. Shukla *et al.*, (2016) demonstrated that extract of *Ageratum conyzoides* has nematocidal activity against several species of plant-parasitic nematodes, including Meloidogyne spp. Furthermore, *A. conyzoides* extract showed significant nematicidal activity against *Meloidogyne incognita* in tomato plants (El-Shahawy *et al.,* 2019). Therefore, the potential

of *A. conyzoides* extracts to suppress nematodes could have significant implications for sustainable agriculture.

Table 1: Effect of Extract of *Ageratum conyzoides* on *M. javanica* on Eggplant in 2018 and 2019

	2018					2019				
	PH (cm)	**NL**	**NB**	**SW (g)**	**RL (cm)**	**PH (cm)**	**NL**	**NB**	**SW (g)**	**RL (cm)**
Crude extract	109.88	118.11	21.05	1265.6	82.76	110.33	118.77	21.44	1266.67	81.50
5 mL	102.55	111.11	17.55	1056.87	75.63	103.10	111.55	18.22	1057.53	76.13
10 mL	89.99	103.15	13.5	943.77	49.36	90.44	103.55	13.99	943.77	49.83
Control	42.10	50.66	7.66	144.4	37.12	42.99	51.33	8.55	143.43	37.16
Mean	86.13	95.75	14.94	852.65	61.22	86.71	96.30	15.55	852.85	61.15
SEM (±)	15.24	15.33	2.87	245.31	10.77	15.14	15.30	2.78	245.74	10.57

PH-Plant height, NL-Number of leaves, NB-Number of branches, SW-Shoot weight, RL-Root length, SE-Standard error

Table 2: Effect of Extract of *Ageratum conyzoides* on *M. javanica* on Eggplant in 2018 and 2019

	2018				2019			
	RW (g)	**NF**	**FW (g)**	**FG (cm)**	**RW (g)**	**NF**	**FW (g)**	**FG (cm)**
Crude extract	238.63	30.66	71.97	4.66	238.40	31.77	73.33	4.62
5 mL	247.85	28.05	66.08	4.25	246.76	28.33	66.21	4.32
10 mL	275.98	21.77	44.51	4.19	278.00	22.33	44.32	4.14
Control	359.52	12.55	30.07	3.06	360.33	12.77	30.1	3.31
Mean	280.49	23.25	53.15	4.04	280.87	23.8	53.49	4.09
SEM(±)	27.51	4.02	9.69	0.34	27.82	4.16	9.94	0.28

RW-Root weight, NF-Number of fruits, FW-Fruit weight, Fruit girth, SEM-Standard error of the Mean

Table 3: Effect of Extract of *Ageratum conyzoides* on *M. javanica* on Eggplant in 2018 and 2019

	2018				2019			
	Yield (tons/ha)	**GI**	**FNP**	**RF**	**Yield (tons/ha)**	**GI**	**FNP**	**RF**
Crude extract	50.07	1.66	176.00	0.46	52.69	1.70	168.53	0.44
5 mL	41.52	3.00	195.33	0.51	42.09	2.36	183.73	0.48
10 mL	21.65	3.33	283.66	0.74	22.17	3.40	269.86	0.71
Control	8.54	4.33	923.00	2.42	8.72	4.40	912.46	2.39
Mean	30.44	3.08	394.49	1.03	31.41	2.96	383.64	1.01
SE(±)	9.42	0.55	177.71	0.466	9.86	0.59	177.67	0.46

GI-Galling index, FNP-Final nematode population, RF-Reproductive Factor, SE-Standard error

CONCLUSION

The study found that extract of *A. conyzoides* particularly the crude extract reduced the damaging effect of *Meloidogyne javanica* on eggplant in Jalingo. In Nigeria, eggplant is a popular food crop but many plant-parasitic nematodes especially the *root knot* nematodes tend to reduce its yield significantly. Chemical nematicides are almost out of reach of farmers just as other agro-chemicals in Nigeria. The plant *A. conyzoides* is common weed across the country and with the result obtained from this research, it is concluded that it is a potentially good bio-

pesticide that can be used to curb the threat posed by *M. javanica* and increase the yield of eggplant in Jalingo.

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