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Efficacy of Leaf Extract of Piliostigma Thonningii for Control of Root-Knot Nematode (*Meloidogyne javanica*) on Eggplant

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Extracts of *Piliostigma thonningii* were tested for their effectiveness in controlling *Meloidogyne javanica* eggs and juveniles in the laboratory and on eggplant cv 'Yalon Data'. A thousand juveniles were placed in 12 petridishes and extracts of *P. thonningii* (Crude extracts, 5 ml dilution and 10 ml dilution of the crude extracts) were dispensed into the petridishes. One thousand eggs of the *M. javanica* were placed in 12 petridishes and treated with the same extracts used on the juveniles. For the field experiment, 12 plots of size 2m x2m were prepared and planted with nine plants of eggplant cv 'Yalon Data'. Seedlings of eggplants were transplanted after three weeks in the nursery to the field into holes drenched with 10 ml of the extracts used in the laboratory tests. Subsequent applications of extracts were done weekly for eight weeks. The results showed that the crude extract was the most effective against both the eggs (87.43 % hatch inhibition) and juveniles (90.23 % mortality). In the field, eggplants treated with the crude extract recorded the tallest plants ((124.78 cm -2017 and 125.00 cm-2018), highest number of fruits/plant (18.51-2017 and 19.55-2018), highest yield (50.45t/ha-2017 and 53.78t/ha-2018) and the lowest galling indices and final nematode population. It is therefore concluded that the crude extract of *P. thonningii* can be employed for the control of *M. javanica* in the field.

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1. INTRODUCTION

Piliostigma thonningii is a tree 4-15 m in height with a rounded crown and a short but often crooked bole. Twigs are rusty-hairy. The bark is rough and longitudinally fissured, being creamy-brown when fresh and grey-brown later. *P. thonningii* is common in open woodland and wooded grasslands of sub-humid Africa at medium to low altitudes. It is found throughout tropical Africa except in Somalia. It is usually associated with *Annona senegalensis*, *Grewia mollis* and *Combretum* spp [1]. Eggplant (*Solanum melongena* L.) also known as garden egg, is a short-lived perennial herb that belongs to the family Solanaceae [2]. It is one of the most important fruit vegetable crops in West Africa [3, 4]. Four cultivar groups (Gilo, Kumba, Shun and Aculeatum) are recognized within *Solanum* species with the first three being most important for Africa [5]. Eggplant can be planted by direct field seeding or by transplanting and that early varieties mature in 75 – 110 days while the late maturing takes 100 – 200 days. A well-drained sandy loam of pH 5.5 to 6.5 with high organic matter content is ideal for growing eggplant [6].

Eggplant is highly susceptible to plant parasitic nematodes [7]. Six species of nematodes, *Meloidogyne sp., Pratylenchus sp., Rotylenchulus sp., Tylenchorhynchus annulus and Tylenchorhynchus sp.* and *Xiphinema radicola*) are known to damage eggplant [8]. Above-ground symptoms of root-knot nematode infection include wilting, stunted plants, chlorotic or pale green leaves, and reduced yields during periods of moisture stress. The most characteristic symptoms occur on underground plant parts where infected roots swell at the point of infection and form knots or galls. Infected roots are retarded in growth and lack fine feeder roots [9,10]. Plants infected by root-knot nematodes show stunted growth accompanied by symptoms of severe deficiency of some nutritional elements, substantially reduced nutrient and water intake, quantity and quality of yield [11,12].

Root-knot nematodes (*Meloidogyne* spp.) are responsible for a yield loss of up to 16.8 to 85.0% [13]. In eggplant, 50% yield losses and shoot growth reduction occurred when the plants were inoculated with 4.7 and 3.2 *M. javanica* eggs and juveniles/g soil respectively [14]. The use of synthetic nematicides is considered the most effective practical means of combating the menace of plant-parasitic nematodes [15]. On the other hand, indiscriminate use of synthetic pesticides for controlling nematodes will likely lead to phytotoxicity, environmental pollution and nematode resistance [16-19]. Crop productivity can be enhanced with the use of bio-nematicides in place of the environmentally unsafe synthetic nematicides used in the control of plant parasitic nematodes [20]. It is unrealistic to recommend nematicides to the peasant farmer in Nigeria, because of the toxicity to humans, as well as the need for special application equipment that is unaffordable and increase in yield obtained may not even cover the costs involved especially for low value crops. This has encouraged greater focus on plant materials for nematode control [21-24]. Also, synthetic nematicides are costly and beyond the reach of local farmers apart from its hazardous effects on the environment [25].

2. MATERIALS AND METHODS

The experiments were conducted in the laboratory of Department of Agronomy, Taraba State University, Jalingo and the Teaching and Research Farm, Department of Crop Production Technology, College of Agriculture, Jalingo, Nigeria in 2017 and 2018.

2.1 Preparation of Extracts

The leaves of *Piliostigma thonningii* used in this research were collected by removing them from the mother plant within and around Taraba State University, Jalingo. They were washed and shade-dried after which they were ground to powder using mortar and pestle. The powders were stored in plastic containers. Some of the powder was taken to the Biochemistry laboratory of the Modibbo Adama Unibersity of Technology, Yola for phytochemical analysis. Fifty grams of the powder was turned in to a 5 litre plastic bucket with 500ml 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. Serial dilution was carried out 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. This gave a total of four treatments each for both the egg hatchability and juvenile mortality tests and field experiment.

2.2 Extraction of Nematode Eggs and Juveniles

The nematode (*Meloidogyne javanica*) was identified using the head and stylet morphology as described by [26]. Second stage juveniles (J2) and eggs of *M. javanica* extracted from pure culture of infested tomato roots. The extraction of juveniles was done using the modified Baermann method [27]. 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 10ml 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. Nematode eggs were extracted by agitating tomato roots in 0.05% NaOCl (sodium hypochlorite) for 2 – 3 minutes [28]. The eggs were collected and rinsed with tap water on nested 150- and 25-um pore sieves as described by [29].

2.3 Juvenile Mortality Test

10 ml of the extracts of *P. thonningii* in 10 ml syringe was dispensed into petri-dishes containing 1000 juveniles of *M. javanica* in 10ml water. There were four treatments as described under preparation of extracts above arranged in the completely randomized design (CRD), with 12 petri-dishes.

2.4 Egg Hatchability Test

A 10 ml syringe was used to dispense 10 ml of *P. thonningii* extracts into 12 petri-dishes containing 1000 eggs of *M. javanica*. There were four treatments arranged in the completely randomized design (CRD).

2.5 Field Experiment

In 2017 and 2018, the experimental field (which was naturally infested with *M. javanica*) was ploughed, levelled, demarcated into 36 (12 plots per replication) with each plot measuring 2 m x 2 m (4 m^2), replicated three times and laid out in a randomized complete block design (RCBD). Seedlings of a commonly grown local eggplant variety (eggplant cv 'Yalon Data') were raised in June (in steam-sterilized loamy soil) 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. Each three week old seedling was transplanted to the field into a hole drenched with 10 ml extract of *Piliostigma thonningii*. Subsequent application of 10 ml extracts was done weekly for eight weeks. There were three concentration (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 (Gall index was determined using the following rating scale:0 = no galls, 1 = 1 - 2 galls, 2 = 3 – 10 galls, 3 = 11 – 30 galls, 4 = 31 – 100 galls and 5 = >100 galls [30]), nematode population and reproduction factor. All other agronomic practices were applied as required.

Prior to the application of treatments, the soil was sampled with an auger at a depth of 0 – 15 cm for soil analysis and initial nematode population (381 (2017) and 363.2 (2018) per 100cc soil). All Yalon Data collected was subjected to analysis of variance in SAS procedures and means were separated using LSD (Least Significant Difference) at p=0.05 level of significance.

3. RESULTS AND DISCUSSION

The phytochemical analysis of *Piliostigma thonningii* powder showed the presence of saponins, tannins, flavonoids and phenols while glycosides were not detected.

Result of the egg hatchability test showed that extracts of *P. thonningii* produced significantly higher inhibition of *M.* javanica egg hatch than control. The crude extract recorded the highest hatch inhibition of 87.43 % followed by the 5 ml dilution (77.13%), 10 ml dilution (61.20 %) with the least being control with 2.60 % (Fig. 1). The extracts contained the phytochemicals needed to inhibit egg hatch. Also, it was evident from the study that level of toxicity of the extract decreases with increase in dilution [31, 32]. The low level of egg hatch inhibition recorded in control resulted from death of the juveniles in them due to unknown reasons because they had not received any extract treatment.

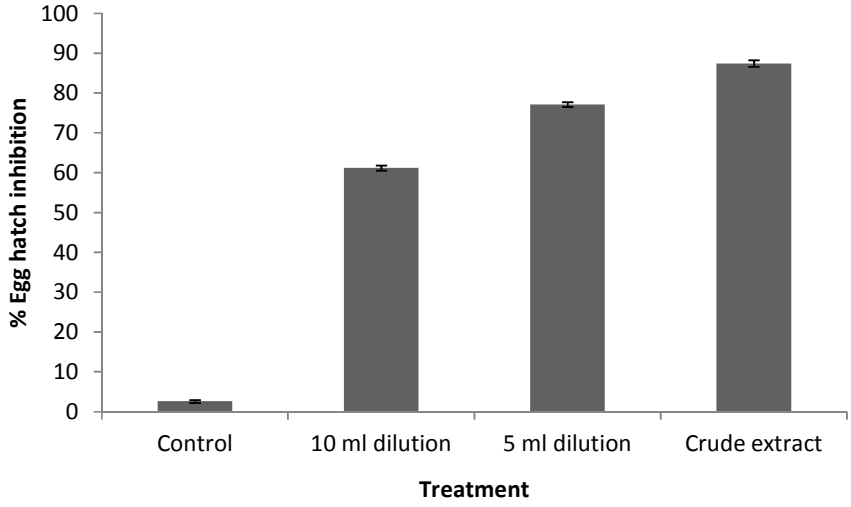

Fig. 1. Effect of extracts of *Piliostigma thonningii* on inhibition of *M. javanica* egg hatch

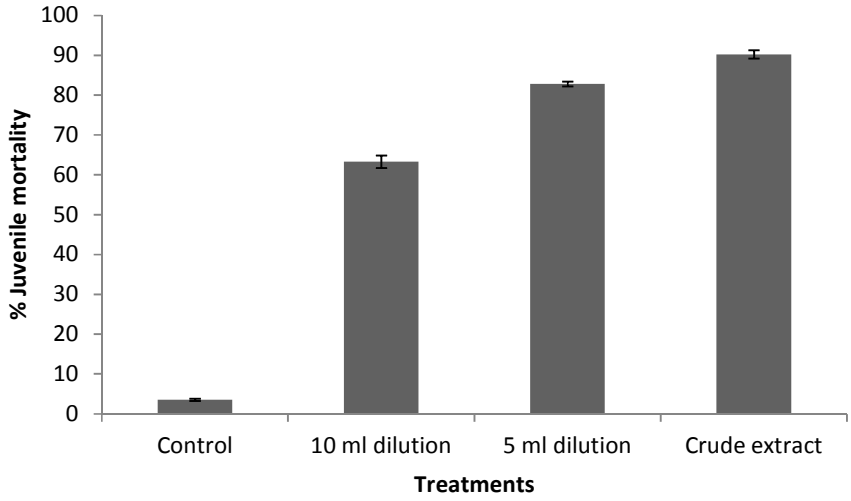

Fig. 2. Effect of extracts of *Piliostigma thonningii* on mortality of *M. javanica* juveniles

The result of the juvenile mortality test indicated that extracts of *P. thonningii* caused significantly higher mortality of *M. javanica* juveniles than control. The crude extract had the highest mortality of the juveniles with 90.23 %, followed by the 5 ml dilution (82.80 %), 10 ml dilution (63.30 %) with the least being control with 3.53 % (Fig. 2). At higher concentration of the various botanicals, the greater its effect on egg-hatch inhibition and juvenile mortality [33]. The nematodes juveniles that died in control may have died of natural causes since they were not treated with the extracts. Several other authors have shown the potential of controlling root-knot and other plant-parasitic nematodes using plant extracts [34-37].

Result of the field experiment showed that eggplant cv 'Yalon Data' plants that were treated with extracts of *P. thonningii* performed better in most parameters than control. The crude extract produced better result than the two dilutions in both years for all parameters such as plant height (124.78 cm -2017 and 125.00 cm-2018), number of leaves (131.88-2017 and 132.44-2018) (Table 1), number of fruits/plant (18.51-

Table 1. Effect of extract of *Piliostigma thonningii* on *M. javanica* on eggplant in 2017 and 2018

	2017					2018				
	PH (cm)	**NL**	**NB**	**SW (g)**	**RL (cm)**	**PH (cm)**	**NL**	**NB**	**SW (g)**	**RL (cm)**
Crude extract	124.78[a]	131.88[a]	16.99[a]	1508.03[a]	65.33[a]	125.00[a]	132.44[a]	17.88[a]	1474.77[a]	65.96[a]
5 ml	119.33[a]	124.99[ab]	15.33[ab]	1289.60[b]	61.66[a]	118.88[a]	125.55[ab]	15.88[ab]	1307.53[a]	62.50[a]
10 ml	106.67[a]	112.22[b]	12.77[b]	859.10[c]	45.33[b]	106.88[a]	112.55[b]	13.33[b]	930.73[b]	45.43[b]
Control	58.05[b]	59.33[c]	8.49[c]	129.70[d]	37.00[c]	57.22[b]	59.55[c]	8.99[c]	129.87[c]	37.16[c]
Mean	102.20	107.10	13.39	946.60	52.33	101.99	107.52	14.02	960.72	52.76
LSD (0.05)	29.05	18.77	3.63	128.83	6.86	28.58	18.66	3.77	180.39	7.44
SE(±)	8.59	8.91	1.10	160.62	3.70	8.66	8..97	1.14	156.62	3.73

All means in the same column bearing the same letters are not significantly different at P=0.05; PH-Plant height, NL-Number of leaves, NB-Number of branches, SW-Shoot weight, RL-Root length, LSD-Least significant difference, SE-Standard error

Table 2. Effect of extract of *Piliostigma thonningii* on *M. javanica* on eggplant in 2017 and 2018

	2017				2018			
	RW (g)	**NF**	**FW (g)**	**FG (cm)**	**RW (g)**	**NF**	**FW (g)**	**FG (cm)**
Crude extract	137.02[c]	18.51[a]	121.11[a]	7.01[a]	136.10[c]	19.55[a]	122.21[a]	7.10[a]
5 ml	142.77[c]	17.33[a]	120.48[a]	6.97[a]	142.46[c]	17.55[b]	120.44[a]	6.99[a]
10 ml	169.23[b]	15.33[b]	114.13[b]	6.53[a]	170.46[b]	15.99[c]	114.33[b]	6.77[a]
Control	255.76[a]	9.66[c]	48.07[c]	3.76[b]	257.00[a]	10.44[d]	49.10[c]	3.92[b]
Mean	176.19	15.20	100.95	6.07	176.50	15.88	101.52	6.19
LSD (0.05)	10.82	1.58	4.09	0.88	10.46	1.37	4.22	1.19
SE(±)	14.39	1.05	9.25	0.41	14.60	1.03	9.17	0.41

All means in the same column bearing the same letters are not significantly different at P=0.05; RW-Root weight, NF-Number of fruits, FW-Fruit weight, Fruit weight, LSD-Least significant difference, SE-Standard error

Table 3. Effect of extract of *Piliostigma thonningii* on *M. javanica* on eggplant in 2017 and 2018

	2017				2018			
	Yield (tons/ha)	**GI**	**FNP**	**RF**	**Yield (tons/ha)**	**GI**	**FNP**	**RF**
Crude extract	50.45[a]	2.66[b]	172.33[c]	0.45[c]	53.78[a]	2.70[b]	160.43[c]	0.42[c]
5 ml	46.96[b]	2.66[b]	189.00[c]	0.49[c]	47.57[b]	3.00[b]	169.77[c]	0.44[c]
10 ml	39.36[c]	3.00[ab]	261.67[b]	0.68[b]	41.15[c]	3.03[b]	252.63[b]	0.66[b]
Control	10.45[d]	4.33[a]	892.33[a]	2.34[a]	11.55[d]	4.43[a]	876.27[a]	2.30[a]
Mean	36.80	3.16	378.83	0.99	38.51	3.29	364.77	0.95
LSD (0.05)	3.41	1.59	26.18	0.072	4.38	1.02	25.12	0.068
SE(±)	4.77	0.27	90.01	0.23	4.90	0.24	89.73	0.23

All means in the same column bearing the same letters are not significantly different at P=0.05;GI-Galling index, FNP-Final nematode population, RF-Reproductive Factor, LSD-Least significant difference, SE-Standard error

2017 and 19.55-2018) (Table 2), yield (50.45t/ha-2017 and 53.78t/ha-2018) (Table3). The 5ml dilution treatment was second to the crude extract in performance. Final nematode population was much higher in control (892.33-2017 and 876.27-2018) than in other treatments. Extracts of neem have been found to be effective in controlling root-knot nematode (*M. javanica*) on sweet gourd [38]. At the end of the experiment, it was also found that most of the nematodes extracted from the soil were juveniles. This indicates that the extracts were effective in preventing nematode egg hatch and that the eggs only started to hatch after the application of extract was stopped after the eight week. It was observed that 100% concentration of root extracts of Siam weed and Neem exhibited 100% inhibition of egg hatch and larval mortality of root-knot nematode (*Meloidogyne* spp) of soybean (*Glycine max*) [39]. Also, a remarkable improvement in eggplant growth parameters including fresh shoot and root lengths, and shoot and root weights with single application of Abamectin or BioNematon followed by plant extracts or vermicompost which resulted in a significant ($P<0.05$) suppression in nematode population in soil and root as well as the number of galls and egg masses was observed [40]. The poor result obtained from control eggplants could have been due to the higher population of nematodes extracted from the soil at the end of the experiment.

4. CONCLUSION

The results of the study show that extracts of *P. thonningii* are effective in keeping down the population of *M. javanica* in the field and causing high juvenile mortality and preventing egg hatch in the laboratory. This allowed eggplants to grow and give economic yield. The crude extract gave the best performance and can be used in areas with similar levels of infestation.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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