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RESEARCH ARTICLE

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Bio-active potential of African chewing gum (*Azanza garckeana*) aqueous leaf extract on second stage juvenile mortality of root-knot nematode (*Meloidogyne incognita*)

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ABSTRACT

An experiment was conducted to determine the efficacy Azanza garckeana on root-knot nematode (*Meloidogyne incognita*) juvenile (J2) at the central laboratory, Federal College of Horticulture Dadin Kowa, Gombe State. M. incognita juveniles (J2) were treated with five concentrations (0, 25, 50, 75, and 100% v/v) of *A. garckeana* aqueous leaf extract (ALE) and exposed for 6, 12, 24, 48, and 72 hours. The 5 x 4 factorial experiments was set up in a completely randomized design (CRD) and replicated three times. The results showed that there was a significant difference ($p < 0.05$) among the various concentrations and time of exposure on juvenile mortality of *M. incognita*. Irrespective of the time of exposure, treatment with 100 % (v/v) concentration of *A. garckeana* (ALE) significantly ($p < 0.05$) recorded the highest juvenile mortality. This was followed by treatment with a 75 % (v/v) concentration of *A. garckena* (ALE) while the untreated control had the minimal juvenile mortality of M. incognita. Sensolato, juvenile mortality of *M. incognita* increased with an increase in concentration and time of exposure. The study revealed that Azanza garckena leave extract possessed potential nematicidal properties responsible for juvenile mortality of M. incognita, which was largely influenced by concentration and time of exposure. Therefore, the use of *Azanza garckena* leave extract can serve as an alternative to synthetic nematicides for the management of *M. incognita*.

Keywords: *Azanza garckena*, concentration, exposure, *Meloidogyne incognita*, second stage juvenile

1. INTRODUCTION

Azanza garckeana plant locally called Goron Tula (Hausa, Nigeria); variously it is called

(Afrikaans); chinga, mukole (Bemba); Azanza, tree hibiscus, snot apple, quarters, wild hibiscus, African chewing gum [1]. *Azanza garckeana* is a tree measuring 3-13 m high, with a diameter at breast height of up to 25 cm; it is resistant and can withstand mild frost [1]. In Nigeria, *Azanza garckeana* is found abundantly in Tula, located in Kaltungo Local Government Area, Gombe State. It can also be found around Kankiya, Katsina State, and Daggish Kali hills of Zah district, Michika local government area of Adamawa State [2]. Ripened fruits are consumed as an aphrodisiac and for treatment of infertility in men [3]. Other parts of the plants have been studied with several reports of isolated compounds [4-5]. An Infusion of the roots is dropped into the ear to treat earache or orally as an antiemetic or to treat cough, chest pain, menstruation and in large doses as an abortifacient [6-7]. A tea of the stems and leaves is taken to treat liver problems [7]. A poultice of the pounded fruit is applied to abscesses to sufficiently thin out inflamed tissue encompassing the infected cavity or draw the pus to a head so the abscess may rupture [7-8].

Different plant-parasitic nematodes occurred all over the tropics and subtropics regions, among the PPN which are considered of economic importance is the genus Meloidogney species. The potential for the nematicidal activity of plants and their products against plant-parasitic nematodes has been reported by many researchers, [9-14].

However, there is a paucity of literature on the use of *Azanza garckeana*-leaf extract in the control of plant-parasitic nematodes. The current research, therefore, focuses on the effect of aqueous leaf extract of *Azanza garckeana*, on juvenile mortality of root-knot nematode, Meloidogyne incognita, in the laboratory.

2. METHOD AND MATERIAL

2.1. Experimental Location

The experiment was carried out at Federal College of Horticulture Dadin Kowa central laboratory, Nigeria.

2.2. Extraction and counting of root knot nematodes juveniles

Meloidogyne incognita was extracted from an infected tomato (Roma Variety) using the modified Baermann tray method [15]. The tomato roots were chopped into smaller pieces and placed separately in a plastic sieve lined with a two-ply tissue paper placed in a plastic plate. Tap water was poured carefully into the plastic plate in which the sieve was resting until the tissue became moist. The setup was left for 24hr and then poured separately into beakers. The volume of each suspension was standardized to 50ml. An aliquot of 1ml of each suspension was taken with a pipette into a counting tray and counting was done with the aid of a stereomicroscope. Each suspension was homogenized by blowing air through with a pipette

2.3. Source and Preparation of Plant Aqueous Extracts

The leaves of African chewing gum *Azanza garckena* were sourced from farmers field in Tula town Gombe Nigeria, and were shade dried. The dried leaves were then ground into a fine powder with pestle and mortar, 25 g of African chewing gum leaves were weighed and thoroughly mixed with 75ml distilled water and sieved through a medium-sized plastic sieve. After which the mixtures were left to stand overnight then filtered through a Whatman filter paper. The filtrate contained a 100% stock solution. Serial dilutions were then done to obtain 25, 50, and 75% concentrations, while distilled water only (0% concentration) served as the control.

2.4. Application of treatments

Juvenile standardization per unit volume was done so that 1ml suspension contained approximately 20 Meloidogyne incognita juveniles thereafter introduced into two millimeters (2 ml) of aqueous extract of African chewing gum leaves, (25, 50, 75, and 100% concentrations) in the Petri dish. Juveniles introduced into distilled water only served as control. The setups were kept on laboratory benches at room temperatures.

2.5. Experimental design

The experiment was laid in a completely randomized design (CRD) with 5x4 treatments, replicated 3 times.

2.6. Data collected

The number of dead juveniles (mortality) and their nature was observed and recorded at 6, 12, 24 48, and 72 hours after the application of treatments. Juveniles were considered dead when they were found to have lost their body content and are inactive.

2.7. Data analysis

Data collected were analyzed using the Genstat statistical soft ware. Means separation was done using Duncan's multiple range.

3. RESULT AND DISCUSSION

3.1. Effect of botanical concentration of African chewing gum on juvenile mortality

There was a significant difference ($p< 0.05$) among the various concentrations on juvenile mortality (Table 1). One hundred percent (v/v) concentration of African chewing gum recorded significant ($p<0.05$) juvenile mortality of 50%. At 25% v/v the least percent mortality of 6.67% was recorded. The untreated controlled had 90% number of juveniles alive. However, 25 and 50 % v/v concentration at 24hours after exposure, were not significantly different ($P>0.05$) from each other but differ significantly ($P<0.05$) with the other treatments (75, 100%v/v). A similar trend was observed at 25, 50, 75, and 100 % v/v concentrations. As the concentration of botanical extract increases juvenile mortality also increases in the order of mortality thus, 25% <, 50<, 70 %<, 100 %v/v. Similarly, 75 and 100% v/v at 72 hours after exposure do not differ significantly ($p>0.05$) with each other but were significantly different to 25, 50, and 0% v/v concentrations respectively. At 100 %v/v juvenile mortality of

Table 1. Effect of African chewing gum aqueous extract on root-knot nematode juvenile mortality

Concentration % (v/v)	Time of Exposure (Hours)				
	6 SEM	12 SEM	24 SEM	48 SEM	72 SEM
25	6.67^{e}±1.67	16.67^{e}±1.67	33.33^{d}±4.41	45.00^{e}±2.89	60.00^{d}±2.89
50	18.33^{d}±3.33	30.00^{d}±2.88	43.33^{d}±1.67	61.67^{d}±4.41	75.00^{c}±2.89
75	38.33^{c}±1.67	41.67^{c}±1.67	55.00^{c}±2.89	80.00^{c}±2.89	96.67^{b}±1.67
100	50.00^{b}±2.89	60.00^{b}±2.89	78.33^{b}±4.40	93.33^{b}±1.67	100.00^{b}±0.00
Control(J2 alive)	0.00^{a}±0.00	0.00^{a}±0.00	0.10^{a}±0.00	0.00^{a}±0.00	0.00^{a}±0.00

J2= Juvenile. Figures followed by the same letter in the column are not significantly different at P < 0.05 using Duncan's Multiple Range Tests

Table 2. Effect of time on Juvenile Mortality at 72 hours

Time of Exposure (hours)	%Juvenile Mortality	SEM
6	42.67^{c}	8.70
12	49.33^{c}	6.20
24	61.00^{bc}	4.98
48	74.00^{ab}	3.99
72	82.67^{a}	3.32

SEM=Standard error of means. Figures followed by the same letter in the column are not significantly different at P< 0.05 using Duncan's Multiple Range Tests)

up to 100% were recorded at this concentration of the botanical extract. The untreated control recorded the least juvenile mortality of <2%. Figure (1-5).

3.2. Effect of time of exposure on juvenile mortality

Table 2 reveals that the varying exposure times to juvenile mortality resulted in statistically significant variations (p0.05). Significant mortality (p<0.05) was seen 72 hours after botanical extract exposure, with about 83 percent mortality recorded, closely followed by 48 hours, with 60 percent juvenile death recorded. Similarly, at 12 and 24 hours after exposure, the death rates were 49 and 61 percent, respectively. The youngster with the lowest fatality rate, on the other hand, was discovered 6 hours after being found.

The studied of aqueous plant extract of *A. garckeana*, an African chewing gum, was beneficial against *M. incognita* second stage juvenile mortality. This is in consistent with the findings of Suzan *et al.* (2015), Lashein (2002) and Elnagdi and mansour (2003) who found that several plant extracts induced increases in juvenile mortality in the laboratory [4, 16-17]. Mamman *et al.* (2021) found out that the leaf extract of *Piliostigma thonningii* caused 90.23 percent juvenile death [18]. Haseeb *et al.* (2017) observed that A. indica seed powder was effective against M. incognita mortality

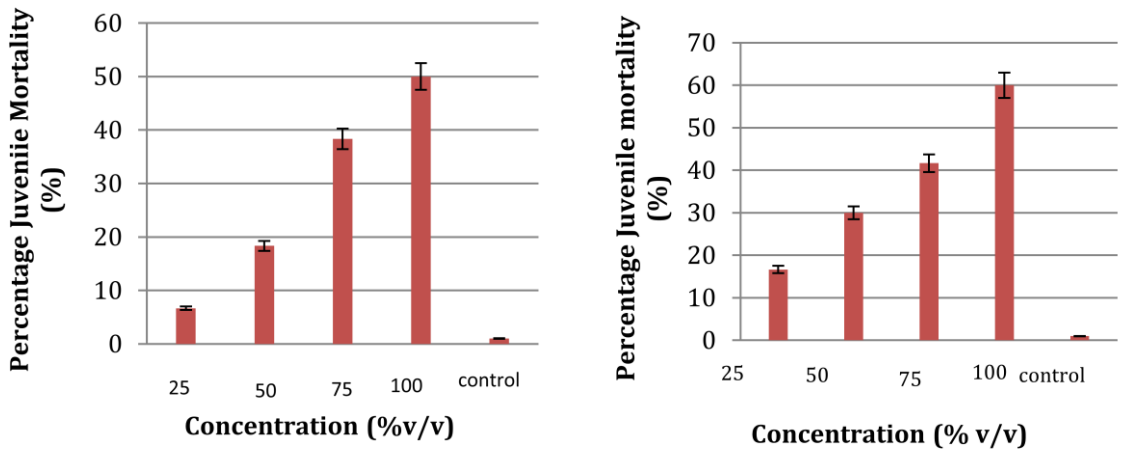

Figure 1a. (Left) Effect of concentration on Juvenile Mortality at 6 hours
Figure 1b. (Right) Effect of concentration on Juvenile Mortality at 12 hours

with more than 50% mortality achieved [19]. It is that leaf and root extract of lead tree (*Leucaena leucocephala*) and Quick stick (*Gliricidiasepium*) were efficient against root-knot nematode resulting in significant mortality. Nidhi and Trivedi (2002) discovered that leaf extract of *Calotropis procera* and *R. communis* were particularly efficient against root-knot nematode juvenile mortality with over 60% mortality recorded [20]. Neem (*Azadirachta indica*) Dharek (*Melia aadirach*) and Castor (*R. communis*) have 100%, 94.48% and 91.47 percent juvenile mortality respectively [6]. The juveniles of *M. incognita* and *Radopholus similis* died within three days of being exposed to a 100 percent concentration of Bixaorellana root bark extract. Jidere and Oluwatayo (2019) also reported that *R, communis, M. oleifera* and *J. curcas* leaf and seed extract were effective against juvenile mortality, resulting in 80-100% mortality [21]. The presence of specific components such as flavonoid, alkaloid, tannins, and saponins in the examined extract may be ascribed to its nematicidal impact; these accords with the findings of Dikko *et al*, (2016) who mentioned the existence of such compounds in the leaves and pulp of *A. garckeana* extract [3]. Plant extract action methods may include protein denaturing and degradation, enzymes inhibition, and interfering with electron flow in the respiratory chain or ADP phosphorylation as detailed by Konstantopoulou *et al*. (1994) [22].

4. CONCLUSION

This study found that African chewing gum leaf extract had nematicidal effects and was efficient in increasing juvenile mortality at various doses and times of exposure. Because its impact against the second-stage juvenile of M. incognita was not immediate, it was discovered that the nematicidal characteristics in this leaf extract work as contact systemic rather than contact alone. The time and concentration of the botanical extract also have an important effect since as the time and

concentration of the botanical extract increases, so does juvenile mortality.

5. ACKNOWLEDGEMENT

NA

6. CONFLICT OF INTEREST

The authors have declared that there is no conflict of interest.

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