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IN-VITRO STUDIES ON EFFECT OF METHANOL AND WATER EXTRACTS OF *DETARIUM MICROCARPUM* GUILL AND PERR STEM BARK ON ROOT-KNOT NEMATODE (*MELOIGOGYNE JAVANICA*)

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ABSTRACT

Methanol and water soluble extract of *Detarium microcarpum* Guill and Perr effect of Roo-knot nematode (*Meloidogyne javanica)* were studied in Laboratory of the Department of Crop Protection, Modibbo Adama University of Technology, Yola in 2014. Root-knot nematode (*M. javanica*) juvenile mortality in the Laboratory and chemical compound responsible were ascertained. The two extracts (Methanol and water) were run separately, each consisting of six treatments (100%, 80%, 60%, 40% and 20% extract concentration and distilled water as 0%) replicated four times arranged in a Completely Randomised Design placed on Laboratory benches. The dead juveniles of *M. javanica* were counted after 24hrs, 48hrs and 72hrs. Chemical compound extraction and identification were earlier carried out. The results indicated that root-knot nematode (*M. javanica*) juvenile mortality was significant at 100% concentration for both methanol and water extracts. At 100% concentration extract *M. javanica* juvenile mortality was 57.00 while that of water extract gave 61.1 *M. javanica* juvenile mortality. The chemical analysis result indicates that methanol extract contains medium concentration of Tannins (lead acetate) and very high concentration of Terpenoids. Both Tannins and Terpenoids were found in low concentration of the bark.

INTRODUCTION

Crop production worldwide is faced with a lot of constraints that limit or reduce production (Jada *et al.,* 2013). Apart from nutrient and moisture deficiency, pests and diseases are also a major reason for low crop production (Oaya and Jada, 2013). Root-knot nematode (Meloidogyne species) is one of the major plant parasitic nematodes causing major yield loss on a variety of crops worldwide that is estimated at about $100million annually (Oka *et al.,*2000; Javad, *et al*., 2006). Root-knot nematodes alone are known to attack over 2000 plant species including woody plants causing various symptoms that result to stunted growth and finally low yield (Oka *et al.,* 2000).

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Control of plant parasitic nematodes has been a difficult test for most farmers. The use of chemical control has been very successful but it is costly and hazardous to the environment. However, the uses of plant extracts have been reported to be successful in controlling plant parasitic nematodes. Dawar *et al.* (2007) reported 38% *M. juvenile* mortality using *Eucalyptus* bark extract. Earlier on, Jada *et al.* (2014) reported 92.33% *M. javanica* juvenile mortality on exposure to 100% Ethylacetate extract of *D. microcarpun*. This study was performed to determine the nematicidal properties of *D. microcarpun* bark methanol and water extracts on *M. javanica* juveniles. It will also look at possible chemical compounds found in the two extracts.

MATERIALS AND METHODS

Leaves and other plant parts of *D. microcarpun* were removed and brought to the Labrotory of the Department of Forestry

Technology, Adamawa State College of Agriculture, Ganye for proper identification (Reuben and Jada, 2013).

Extraction of *D. microcarpun* Bark Using Methanol and Distilled Water

Using simple hoe, the bark of *D. microcarpun* was removed as practiced by the local herb men. The bark was then left under properly ventilated shade for seven days to dry. The dry bark was then taken to the Laboratory and pulverised after being thoroughly cleaned of any dirt. From the pulverised bark, one thousand grams (1000g) was weighed. This quantity was extensively and sequentially extracted using methanol (MTE) with Soxhelet extractor (WHO, 2000; Geidam *et al.,* 2007). The same procedure above using another one thousand grams (1000g) of *D. microcarpun* pulverised bark for distilled water (AQE) was done.

Preparation of Liquid Extract from the Methanol Extract

From the powder of *D. microcarpun*, methanol extract obtained above five grams (5g) was weighed using sensitive weighing balance and added to 1000 litres of chlorine free tap water. This was left for 24hours in the Laboratory and used for *M. javanica* juvenile mortality test. The initial concentration served as 100% concentration of the extract. Twenty millilitres (20mil) of water was added to eighty millilitres (80mil) of the extract above to obtain 80% concentration. The same method was used to obtain 60, 40 and 20% concentrations while chlorine free pipe water was only used as 0% to serve as the control. From the Acquese extract of *D. microcarpun* bark powder five grams (5g) was weighed and added 1000 litres of distilled water and left 24hours. This serves as 100% AQE for the first treatment. Eighty millilitres is taken using pipette and to it 20mil of chlorine free tap water is added to obtain 80% AQE as the second treatment. Similar procedure was used to obtain 60, 40 and 20% concentrations of the AQE. Chlorine free tap water was used as 0% concentration to serve as the control.

***Meloidogyne javanica* Juvenile Mortality Test**

Extracts from methanol and water (AQE) were used as two separate experiments to test the *M. javanica* juvenile mortality.

Use of Methanol Extract of *D. microcarpun* to Test *M. javanica* Juvenile Mortality

From Root-knot nematodes (*Meloidogyne javanica*) tomato culture established at the Screen House of the Department of Crop Protection, Modibbo Adama University of Technology, Yola. One plant was uprooted from the infested roots and about 500-600 egg masses handpicked under the microscope (X100) using needle and forceps and then dropped in 200mil of water in 250mil beaker. They were left to stay for 24hours to allow hatching of *M. javanica* J_2 juveniles. The water in the beaker with J_2 of *M. javanica* was scanted gently to pour off the water and allow the *M. javanica* J_2 in small quantity of water. This juvenile suspension of *M. javanica* was made to a level of one drop drawn using a pipette contained 100 J_2 juveniles when counted. One drop of *M. javanica* J_2 was then drawn and dropped at the back of 250µ sieve.

From the 100% methanol extract concentration, 10mil was drawn and used to wash the J_2 juvenile on the sieve into a petridish. This was repeated two times to get three replicates. This was done for each methanol extract concentration level of 80, 60, 40 and 20 and 0%. They were laid on the Laboratoty bench in a Completely Randomised Design (CRD) form. The dead *M. javanica* juveniles were counted under a binocular microscope X100 magnification. The dead *M. javanica* were identified by pushing them at least three times using a needle and if they cannot move remaining straight, they are counted as dead ones. The counting of dead *M. javanica* J_2 was done at 24hours, 48hours and 72hours and the experiment was terminated at 72hours.

Use of Water Extract (AQE) of *D. microcarpun* Bark to Test *M. javanica* J_2 Juvenile Mortality

This experiment was started after terminating the methanol extract of *D. microcarpun* bark test on *M. javanica* J_2. The same procedure was used as described above and data were taken at 24hours, 48hours and 72hours. This experiment was also terminated after 72hours. All the data above were subjected to statistical analysis and Least Significant Difference (LSD) was used to separate the means.

RESULTS

Phytochemical analysis for both methanol and water extracts of *D. microcarpum* Guill and Perr bark are presented in Table 1. The methanol extracted substances is brown shiny powder, weighing 208.83g obtained from the 1000g of the powder. While the substances obtained from the water extract was dark brown shiny crystal, weighing 62.80g out of 1000g of the original powder. Looking at Table1, the methanol extract contains high concentration of temperature, moderate lead acetate and no saponins. The water extract contains low lead acetate and moderate tannins.

Table 2 shows the mean *M. javanica* juvenile mortality at different concentration levels of methanol and water extracts of *D. microcarpum* Guill and Perr, where all the concentrations showed significant difference (≤ 0.05) for the two extracts. In methanol extracts, the highest mean mortality *M javanica* juvenile mortality of 57.60 ±4.88% was obtained in 100% methanol extract concentration. At 20% methanol extract concentration only a mean of 30.60±3.67% *M. javanica* juvenile mortality was obtained. In the case of water extract of *D. microcarpum* Guill and Perr, it showed 61.11±4.63% mean juvenile mortality at 100% concentration of the extract. This juvenile mortality was significantly ($P \leq 0.05$) higher than what was obtained in all other % concentrations of the water extract of *D. microcarpum* Guill and Perr.

While the mean juvenile mortality of *M. javanica* juveniles were considered for the two extracts (methanol and water) at different times of exposure, there were significant difference at $P \leq 0.05$. In methanol extract, 47.72±5.56% mean juvenile mortality was obtained at 72hours. In the case of water extract at 72hours, 50.56±5.96% juvenile mortality of *M. javanica* was obtained and it was significantly higher than what was obtained at 24 and 48hours.

Table 1. Phytochemical Analysis of Methanol and Water Extracts of *D. microcarpum* Guill and Perr Bark for Bioactive Constituents

S/N	Bioactive constituents and Test	Level			
MTE	AQE				
1.	Alkaloids				
i.	Dragendroff's Test	-		-	
ii.	Mayor's Test	-		-	
2.	Carbohydrate Test				
i.	General Test (Molisch's Test)	+		-	
ii.	Monosaccharide (Barford's Test)	-		-	
iii.	Fehling Test (Free reducing sugar)	++		++	
iv.	Combine reducing sugar	++		+	
v.	Test for ketoses (Salivanoff's Test)	+		-	
vi.	Test for Pentoses	+		-	
3.	Test for soluble starch		+		-
4.	Test for Tannins		++		++
i.	Ferric Chloride Test ($Fecl_3$)	++		+-	
ii.	Lead Acetate Test	++		+	
5.	Test for Phlobatannins		+		+
6.	Test for Cardiac Glycosides				
i.	Salkowski Test	++		+	
ii.	Liebarman-Barchard Test	+		-	
7.	Test for Terpenoids		+++		+

Key:
MTE= Methanol Extract
AQE= Aqueous Extract
+++= Highly Present
++ = Moderately Present
+ = Low Present
- = Nil

Table 2. Effect of Methanol and Water Extract of *D. microcarpum* Guill and Purr Stem Bark on *M. javanica* juveniles

Concentration (%)	Percentage Mortality	
	Methanol	Water
100	57.00a ± 4.88	61.11a ± 4.63
80	50.11b ± 3.99	54.56b ± 3.30
60	44.11c ± 3.19	49.37c ± 3.69
40	37.44d ± 3.45	40.78d ±3.06
20	30.00e ± 3.67	35.00e ± 3.80
0	0.89f ± 3.67	0.56f ± 0.24
LSD	2.32	2.06
Sig.	*	
	Duration (Hours)	
72	47.72a ± 5.56	50.56a ± 5.96
48	36.28b ± 4.44	39.56b ± 4.71
24	25.78c ± 3.32	29.56c ± 3.85
LSD	1.64	1.46
Sig.	*	

Means with the same letter along the same column are not significantly different (Fisher's LSD P≤0.05)
LSD: Least Significant Difference
*: Significant

Table 3. Effect of Methanol and Water Extracts of *D. microcarpum* Guill and Perr Extracts on Mortality of *M. javanica* Juveniles on Exposure for 24, 48 and 72hours

Concentration (%)	Hours of Exposure (Hours)					
	Methanol			Water		
	24	48	72	24	28	72
0	0.00	1.00	1.67	0.33	0.33	1.00
20	18.00	29.33	42.67	19.33	34.67	45.00
40	26.67	35.67	50.00	30.33	41.00	50.00
60	33.00	44.67	54.67	37.33	48.33	62.33
80	36.67	50.00	63.67	44.33	52.67	66.67
100	40.33	57.00	73.67	45.67	60.33	77.33
LSD	1.90	2.31	2.53	1.95	2.33	2.58

Table 3 gave a more detailed *M. javanica* juvenile mortality for the various treatments at 24, 48 and 72 hours for both methanol and water extracts of *D. microcarpum* Guill and Perr bark.

In methanol extracts at 24hours, the highest *M. javanica* juvenile mortality of 40.33% (P≤0.05) was observed in the treatment of 100% extract concentration. The juvenile mortality at this level was significantly higher at P≤0.05 than

what was obtained in other treatments. At 48 and 72hours there were also significant differences (P≤0.05) between the juvenile mortality in all the concentration levels. In water extract of *D. microcarpum* bark, the *M. javanica* juvenile mortality showed significant difference (P≤0.05) between the concentration levels at all times (24, 48 and 72 hours) of time exposure. At 24 hours of exposure, *M. javanica* juvenile mortality of 19.33% was observed in 20% *D. microcarpum* , however, it reached maximum at 100% with 45.67% J_2 mortality. The highest juvenile mortality of 77.33% was obtained in 100% concentration.

DISCUSSION

The juvenile mortality of *M. javanica* increased with increase in concentration of *D. microcarpum* Guill and Perr bark extracted with methanol from 30.00±3.67 dead juveniles at 20% to 57.60±4.88 at 100% extract concentration. This juvenile mortality increased with increase in methanol bark extract concentration even within the same time of exposure (24, 48 and 72hours). The mortality recorded is an indication that *D. microcarpum* bark extracted with methanol has nematicidal properties. Elbadria, *et al.* (2008) earlier reported that methanol extracts of *Solenostemma argel, Aristolorchia bracteolate* and *Ziziphus spinachristi* leaves and that of seeds of *Areginome mexicana, Datura steamanium* and *Azadirachta indica* caused 80-90% juvenile mortality of *M. incognita* juveniles tested. The methanol extract of *D. microcarpum* bark efficacy also increase with increase in time of exposure. At 100% concentration of the extract in 24 hours caused 40.33% *M. javanica* juvenile mortality which increased to 73.67% mortality at 72hours of exposure. Similar results were reported by Azhagumurugan and Rajan (2014) in an acetone leaf extracts of *Gmelina asiatica* that recorded 16.34 juvenile of *M. incognita* at 24hours and increased to 22.34 juvenile mortality at 48 hours in 15ppm of the acetone extracts. Extracts of various plant leaves using ethanol were tested at 24hours exposure time, Clove caused 98.00±2.30, Tobacco caused 94.00±3.0 and Betlevine caused 83.0±1.20 *M. incognita* juvenile mortality (Taniwiryono *et al.,* 2009).

The *D. microcarpum* bark water extract also causes *M. javanica* juvenile mortality indicating nematicidal properties. At 24hours exposure time, the *M. javanica* juvenile mortality in 20% concentration was 19.33% and significantly (P≤0.05) increased to 45.67% juvenile mortality at 100% concentration of the extract. However, at 48hours of exposure, *M. javanica* juvenile mortality in 80% concentration treatment reached more than 50% juveniles. While at 72hours, more than 50% juvenile mortality was observed in 40% water extract concentration. This indicates that, *M. javanica* juvenile mortality was not only increasing with increase in concentration but also increases with increase in exposure time. Chedekal (2013) reported *M. incognita* juvenile mortality of 90.17%, 81.33% and 74.00% water extracts of *A. indica, Clerodendrium inerune* and *Lantana camara* respectively at 72hours time of exposure. From the chemical analysis, flavonoids are moderately present in both methanol and water extracts which could be responsible for the juvenile mortality. The high presence and low presence of terpenoids in methanol and water extracts respectively could also be another chemical compound responsible for juvenile mortality. Earlier, Knoblock, *et al. (*1989) and Konstantopoulou, *et al. (*1994) observed that the mechanism of plant extracts action may include denaturing and degrading of proteins, inhibitory of enzymes and interfering with electron flow in respiration or with ADP phosphorylation. Saponins, flavonoids and glycosides are known to have lipophytic properties that enable them dissolve the cytoplasmic membrane of the nematode cells and their functional groups interfering with enzyme protein structures of nematodes (Trifonovo and Atanasov, 2009).

Conclusion

It could be concluded from this work that, both methanol and water extracts of *D. microcarpum* Guill and Perr bark contain nematicidal properties. The extracts can be used in controlling root-knot nematode (*Meloidogyne* species) by the local farmers to reduce the cost of production. Further research should be carried out to further identify the specific chemical compound responsible and also find out its effect on other soil organisms.

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