

STUDIES ON THE EFFECTS OF AQUEOUS EXTRACTS OF NEEM BARKS AND LEAVES ON SOIL TERMITES AND EARTHWORMS

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ABSTRACT

Earthworms and termites are two soil macro faunas known to perform important soil forming roles. While earthworms do not have any detrimental effect on crops, termites can attack both annual and perennial crops causing significant yield losses. Though synthetic insecticides are effective in the control of field insect pests like termites, their residues in agricultural soils and water have negative effects on beneficial soil organisms like earthworms. Extracts of different neem part have been reported as eco-friendly alternatives to synthetic insecticides. This study investigated the toxicity of the aqueous extracts of neem barks and leaves to termites in soils treated with varying concentrations i.e. 2%, 5%, 10%, 15%, 30% and 100%. The effects of the 50% lethal concentration (LC₅₀) of both extracts on the burrowing time, weight and mortality of earthworms in treated soils were also determined. Neem bark aqueous extract caused a significantly ($p < 0.01$) higher mean mortality of termites than neem leaf extracts at 1, 3, and 5 days after treatment. Mean percentage mortality of termites increased as the concentration of both extracts increased. In addition, both aqueous extracts reduced the weight of the earthworms exposed to them for 7 and 14 days while also increasing the time they took to burrow. Their effects on the earthworms were however significantly ($p < 0.01$) less detrimental than that of Chlorpyrifos (20EC). Neem based insecticides therefore stand a chance in the control of termites but should be used with discretion since they could exert detrimental effects on the activities of earthworms.

Keywords: Neem bark, Neem leaf, Aqueous extracts, Termites, Earthworms

1.0 INTRODUCTION

As a result of their abundance and soil forming activities, earthworms and termites have been reported to be among the three most important soil macro fauna (Eggleton *et al.*, 2000; FAO, 2008). Termite activities in the ecosystem include the breakdown of wood and other materials made up of cellulose. They also help in the natural recycling of organic matter and nutrients (Ndiaye *et al.*, 2004). Similarly, earthworms help digest organic matter components of the soil like leaf litters. Their excreta known as cast helps improve soil nutrient and makes them readily available for plants. In addition, they help aerate the soil through their burrowing activities (Blakemore, 2006; Nyle and Ray, 2009; Lavelle and Spain, 2011). While earthworms do not cause any damage to crops in agroecosystems, termites infest farmlands causing severe destruction to crops resulting into serious economic losses (Owa *et al.*, 2001; Abe *et al.*, 2014). In the case of termite infestation on crop lands, synthetic insecticides have often been used as a fast and effective way to contain the pest. Unfortunately, the unilateral use of such synthetic pesticides has resulted into a number of health and environmental effects that are all too deleterious. The negative impact of insecticide residues in agricultural soils and water on the biodiversity and ecological roles of earthworms is one of such effects (Hole *et al.*, 2005). In the search for eco-friendly alternatives to conventional insecticides, several plants have been screened and found to have significant mortality effects against a number of insect pests including termites. Neem (*Azadirachta indica*) is one botanical with a wide spectrum of biological activity including termiticidal activities (ICAR, 1993). Neem extract is made up of a mixture of biodegradable natural chemicals that have been reported to be relatively safe to non-target organisms including earthworms. According to Lal *et al.* (2001), chemicals may not result in the mortality of earthworms in soils but may negatively impact important traits, behaviours and activities which influence soil functioning.

This study therefore investigated the effects of aqueous extracts of neem barks and leaves at varying concentrations on termites in treated soils. The effect of the 50% lethal concentration (LC₅₀) of both extracts (from the termite bioassay) on the weight, burrowing time and mortality of test earthworms was also studied.

2.0 MATERIALS AND METHODS

2.1 Soil

Soil was collected from randomly selected farms from within the premises of the study area – University of Ilorin, Ilorin, Nigeria (8.50°N, 4.68°E). Soil samples were collected from 10 maize farms, 9 soybean farms and 13 cassava farms. From each farm, 12 sub samples of soil were collected and homogenized in a polythene bag. Each bag was labelled appropriately and taken to the laboratory where they were air dried for 7 days. The air

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dried soil samples were then sterilized by heating for about 30 minutes. The sterilized soils were thereafter stored in labelled polythene bags and kept in the refrigerator until when needed.

2.2 Neem Aqueous Extracts

Neem leaves and barks were collected around 5pm from Tanke, Area of Ilorin, Kwara State. The collected neem barks and leaves were air dried for 24 hours after which they were separately soaked in water for another 24 hours. Muslin cloth was used to filter the extracts and remove any plant debris. The stock solution of neem leaf and bark extracts were diluted with distilled water to lower concentrations of 2%, 5%, 10%, 15% and 30%. These were stored in plastic containers and kept at room temperature as filtrate and until needed.

2.3 Termites

Termite populations used for the toxicity bioassay were obtained from termitaria within the study area. Collected termites were maintained on a mixture of dried litters and termitaria fragments within a ventilated 5 litre plastic container at normal laboratory conditions (25-30°C, 70-75% relative humidity). The soil in the termite culture was occasionally moistened to enhance the activities of the termites. Termites were then identified as belonging to the family termitidae and sub family macrotermitinae using the work of Malaka (1996). Identification of termite castes was done using morphological traits described by Bignell (2010). Only worker and nausate soldiers were used in this study.

2.4 Earthworms

The earthworms used in this study were collected from stream sites adjacent to some agricultural lands in the study area. Earthworms were collected and maintained in a 5 litre plastic containing some of the soil from their habitat mixed with dry grass. Injured or dead earthworms were removed from the culture. The soil in the culture was kept moist by occasionally sprinkling water on it. Earthworms were identified to species level with majority belonging to the genus *Lumbricus*.

2.5 Experimental Procedures

2.5.1 Bioassay on toxicity of neem aqueous extracts to termites

This bioassay determined the toxic effect of neem bark and leaf aqueous extracts on termites. Hundred grams (100g) of sterilized soil was weighed into small plastic containers (350ml capacity). 20mls of the different concentrations of neem leaf aqueous extracts was introduced separately into the small containers using a 20ml syringe. Different syringes were used for different concentrations of each extract type. Each treatment of neem leaf extract was replicated 3 times. Twenty (20) termites (10 workers and 10 soldiers) collected with a pooter were then introduced into each experimental unit of neem leaf extracts. The setups were labelled appropriately and covered with lids bearing minute holes for ventilation. The same procedure was followed with neem bark aqueous extracts. A control experiment with distilled water was also setup. Data on percentage mortality of termites were taken 1, 3 and 5 days after setup.

2.5.2 Bioassay on response of earthworms to LC_{50} of neem aqueous extracts

Five hundred grams (500g) of soil mixed with dry litters was put into a transparent plastic (750ml). The soil was then moistened with 80ml of water to make it wet but not waterlogged. 20mls of 22.0% neem leaf aqueous extracts (which is equivalent to the LC_{50} of leaf extracts obtained during the termite mortality bioassay) was added to the soil. Thereafter 4 active adult earthworms whose initial weights and 90% burrowing time had been measured were introduced into the setup. The 90% burrowing time is the time it takes an earthworm to bury 90% of its body in the moist soil. A similar earthworm bioassay using neem bark aqueous extract was set up. However, 20ml of 10.0% neem bark aqueous extracts (which is equivalent to the LC_{50} of bark extracts obtained during the termite mortality bioassay) was used as treatment. A positive control and negative control experiments which had a Chlorpyrifos (20% EC) and distilled water as treatments respectively were also setup. Each treatment was replicated 3 times. At 7days and 14days after setup, earthworms in each treatment were reweighed and their 90% burrowing time remeasured. Data was taken on the percentage change in weight, percentage change in burrowing time and percentage mortality of earthworms 7 and 14 days after setup

2.6 Statistical Analysis

The experiments for both termites and earthworms were laid out in a Completely Randomized Design. Data from percentage mortality of termites' bioassay were subjected to two way analysis of variance using the Generalized Linear Model. Microsoft Excel 2007 was used to plot a graph of concentration against percentage mortality from which the logarithmic equations (Table 1) used to calculate 50% lethal concentrations were obtained. Data from percentage change in burrowing time of earthworms, percentage change in weight of earthworms and earthworm's percentage mortality rate were subjected to a one way analysis of variance (1-way ANOVA). Where treatment means were significant, separation of means was done using Tukey's Honestly

Significant difference (HSD) at 1% level of significance. All data analysis were done using IBM SPSS statistics version 21.

Table 1: Fifty percent lethal concentration LC_{50} of neem bark and neem leaves aqueous extracts

Extracts	Logarithmic equation	R-Square Value (R^2)	LC_{50} Value
Neem bark aqueous extract	$Y=13.57\ln(x)+81.734$	0.9222	9.65%
Neem leaf aqueous extract	$Y=5.7785\ln(x)+32.134$	0.823	22.01%

3.0 RESULTS AND DISCUSSION

The mean percentage mortalities (46.19, 68.33 and 78.10) of termites exposed to the bark extracts were significantly ($p<0.01$) higher than the percentage mortalities (17.38, 45.00 and 60.71) observed with the leaf extracts (Table 2). Worker termites were also observed to be more susceptible to the extracts than soldier termites.

Table 2: Main effect of neem bark and neem leaf aqueous extract, on the percentage mortality of termites

Test Materials	Mean percentage mortality of termites (n=20)			Mean percentage mortality of worker termites (n=10)			Mean percentage mortality of nausate termites (n=10)		
	1das	3das	5das	1das	3das	5das	1das	3das	5das
Neem Bark	46.19 ^a	68.33 ^a	78.10 ^a	56.0 ^a	73.33 ^a	80.48 ^a	36.67 ^a	64.29 ^a	76.67 ^a
Neem Leaf	17.38 ^b	45.00 ^b	60.71 ^b	21.91 ^b	49.05 ^b	62.00 ^b	12.86 ^b	40.00 ^b	59.05 ^b
SEM									

Means value in a column followed by the same letter(s) were not significantly different at $p=0.01$. das: days after setup

Generally, mean percentage mortality was observed to increase as extract concentration increased from 0% to 100 % (Table 3). There was significant difference ($p<0.01$) in the mean percentage mortality of termites caused by 0% and 100% extract concentrations at each day of observation. Other extract concentrations had intermediate percentage mortality values that were significantly different ($p<0.01$) from those of the control and 100% concentration. Percentage mortality values of the 30% extract concentration was however not significantly different ($p>0.01$) from 100% extract concentration at each period of observation.

Table 3: Main effect of concentration of neem bark and neem leaf aqueous extracts on the percentage mortality of termites.

Concentration of Neem extracts	Mean percentage mortality of termites (n=20)			Mean percentage mortality of worker termites (n=10)			Mean percentage mortality of nausate termites (n=10)		
	1das	3das	5das	1das	3das	5das	1das	3das	5das
0%	0.00 ^c	0.00 ^d	0.00 ^d	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^c	0.00 ^d	0.00 ^e
2%	23.35 ^d	55.00 ^b	71.67 ^b	35.00 ^b	60.00 ^b	75.00 ^b	11.67 ^{de}	50.00 ^b	68.33 ^d
5%	22.50 ^d	54.17 ^b	73.33 ^b	33.33 ^b	63.33 ^b	80.00 ^b	13.33 ^{cde}	48.33 ^b	70.00 ^{cd}
10%	33.33 ^{cd}	61.67 ^{bc}	77.50 ^b	40.00 ^b	70.00 ^{ab}	80.00 ^b	26.67 ^{bcd}	53.33 ^{bc}	75.00 ^{cd}
15%	39.17 ^{bc}	67.50 ^{abc}	80.83 ^{bc}	46.67 ^{ab}	70.00 ^{ab}	81.67 ^{ab}	31.67 ^{abc}	65.00 ^{abc}	80.00 ^{bc}
30%	46.67 ^{ab}	75.00 ^{ab}	89.17 ^{ab}	53.33 ^{ab}	78.33 ^{ab}	88.33 ^{ab}	40.00 ^{ab}	71.67 ^{ab}	90.00 ^{ab}
100%	57.50 ^a	83.33 ^a	93.33 ^a	66.67 ^a	86.67 ^a	95.00 ^a	48.33 ^a	80.00 ^a	91.67 ^a
SEM	2.44	3.10	1.92	3.88	4.18	3.78	3.62	3.62	1.98

Means value in a column followed by the same letter(s) were not significantly different at $p = 0.01$. das: days after setup.

Significant interaction effects were observed between neem parts and extract concentrations at each day (Table 4). Each concentration of neem bark aqueous extracts had a significantly higher ($p<0.01$) mean percentage mortality of termites than corresponding concentrations of neem leaf aqueous extracts. All treatment combinations of neem leaf aqueous extracts were however significantly different ($p<0.01$) from the control experiment.

Table 4: Interaction effect of neem bark and neem leaf aqueous extracts on percentage mortality of termites.

Test materials	Concentration	Mean percentage mortality of termites (n=20)			Mean percentage mortality of termites caste					
					Workers (n=10)			Soldiers (n=10)		
					Days after treatment					
		1das	3das	5das	1das	3das	5das	1das	3das	5das
Control	0%	0.00 ^g	0.00 ^g	0.00 ^f	0.00 ^f	0.00 ^e	0.00 ^f	0.00 ^e	0.00 ^e	0.00 ^f
Barks	2%	36.67 ^{cde}	70.00 ^{bcd}	83.33 ^{bcd}	53.33 ^{bcd}	80.00 ^{abc}	90.00 ^{abc}	20.00 ^{cde}	60.00 ^{bcd}	76.67 ^{cde}
	5%	35.00 ^{cde}	70.00 ^{bcd}	85.00 ^{abc}	53.33 ^{bcd}	80.00 ^{abc}	93.33 ^{ab}	20.00 ^{cde}	66.67 ^{bc}	83.33 ^{bcd}
	10%	46.67 ^{bcd}	73.33 ^{abcd}	86.67 ^{ab}	56.67 ^{bcd}	83.33 ^{ab}	86.67 ^{abcd}	36.67 ^{bcd}	63.33 ^{bc}	86.67 ^{ab}
	15%	55.00 ^{bc}	78.33 ^{abc}	91.67 ^{ab}	63.33 ^{abc}	80.00 ^{abc}	93.33 ^{ab}	46.67 ^{abc}	76.67 ^{ab}	90.00 ^{ab}
	30%	63.33 ^b	86.67 ^{ab}	100.00 ^a	73.33 ^{ab}	90.00 ^{ab}	100.00 ^a	53.33 ^{bc}	83.33 ^{ab}	100.00 ^a
	100%	86.67 ^a	100.00 ^a	100.00 ^a	96.67 ^a	100.00 ^a	100.00 ^a	76.67 ^a	100.00 ^a	100.00 ^a
Leaves	2%	10.00 ^{fg}	40.00 ^{ef}	60.00 ^e	16.67 ^{ef}	40.00 ^e	60.00 ^e	3.33 ^e	40.00 ^{cd}	60.00 ^e
	5%	10.00 ^{fg}	38.33 ^f	61.67 ^e	13.33 ^{ef}	46.67 ^{cd}	66.67 ^{de}	6.67 ^{de}	30.00 ^{de}	56.67 ^e
	10%	20.00 ^{efg}	50.00 ^{def}	68.33 ^{de}	23.33 ^{def}	56.67 ^{bcd}	73.33 ^{bcd}	16.67 ^{cde}	43.33 ^{cd}	63.33 ^{de}
	15%	23.33 ^{ef}	56.67 ^{cdef}	70.00 ^{cde}	30.00 ^{cdef}	60.00 ^{bcd}	70.00 ^{cde}	16.67 ^{cde}	53.33 ^{bcd}	70.00 ^{cde}
	30%	30.00 ^{def}	63.33 ^{bcd}	78.33 ^{bcd}	33.33 ^{cdef}	66.67 ^{abcd}	76.67 ^{bcd}	26.67 ^{bcd}	60.00 ^{bcd}	80.00 ^{bcd}
	100%	28.33 ^{def}	66.67 ^{bcd}	86.67 ^{ab}	36.67 ^{cde}	73.33 ^{abcd}	90.00 ^{abc}	20.00 ^{cde}	60.00 ^{bcd}	83.33 ^{bcd}
SEM		3.45	4.39	2.71	5.49	5.91	3.78	5.12	5.12	2.67

Means value in a column followed by the same letter(s) were not significantly different at $p = 0.01$. das: days after setup

The effect of LC_{50} values of neem bark and neem leaf aqueous extracts on percentage change in weight, burrowing time and mortality of earthworms presented in tables 5 to 7. Generally, there was significant ($p < 0.01$) reduction in weight and an increase in burrowing time of earthworms exposed to the different extracts and control after 7 and 14 days. Percentage change in weight and burrowing time at 7das was lowest in the control (0.60g and 2.53secs) followed by neem leaf extracts (4.75g and 8.96secs) and then neem bark extracts (6.83g and 10.58secs) respectively. Similarly, at 14das, percentage change in weight and burrowing time was lowest in the control (2.94g and 10.57secs) followed by neem leaf extracts (22.98g and 96.61secs) and highest in neem bark extracts (34.18g, 100.00secs) respectively. While no mortality was observed in the negative check, 100% mortality of earthworms was observed in the positive check in less than 7das. The aqueous extracts had intermediate percentage mortality values and were significantly ($p < 0.01$) different from both the positive and negative checks at 14 days after setup. Neem leaf and bark extracts showed significant insecticidal activity against both worker and soldier termites in this study. In addition, the mortality of termites increased with an increase in the concentration of both extracts. These observations agree with Achio *et al.*, (2012) who reported the insecticidal effects of several neem preparations against some insects of agricultural and public health importance. Furthermore, it was observed in this study that neem bark aqueous extracts caused higher mortality of termites than neem leaf aqueous extract. In contrast, Achio *et al.*, (2012) and Aliero (2003) reported that neem leaf aqueous extract caused higher mortality of some insects such as *Anopheles* mosquito, weevils, termites, cockroaches than neem bark aqueous extract. The difference in observation may be attributed to the concentration of the bioactive components of the neem parts as at the time of harvest. Azadirachtin is the major constituent of neem and has been reported to be present in higher proportions in neem leaves than in the neem bark. On the other hand, nimbin and salannin are bioactive components that occur more abundantly in neem bark than in neem leaves (Kudom *et al.*, 2011 and Mitchell *et al.*, 2002). Neem bark and neem leaves aqueous extracts reduced the weight of the earthworms exposed to them. In addition, they negatively impacted the natural and beneficial burrowing activities of the earthworms by increasing their burrowing time. Their effects on the earthworms were however significantly less detrimental than those of synthetic insecticides.

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Table 5: Effect of neem bark and neem leaf aqueous extracts on percentage change in weight of earthworms

Neem parts	Percentage change in weight of earthworms (n=4)	
	7 das	14 das
Control	(-)0.60 ^c	(-)2.94 ^c
Bark	(-)6.83 ^a	(-)34.18 ^a
Leaf	(-)4.75 ^b	(-)22.98 ^b
Chlorpyrifos (20EC)		0.00
SEM	0.00	

Mean values followed by the same letter are not significantly different at $p=0.01$. das: days after setup. * Earthworms in the chlorpyrifos (20EC) had all died. Negative sign (-) represents a decrease in burrowing time

Table 6: Percentage change in burrowing time of earthworms exposed to neem bark and leaf aqueous extracts

Neem parts	Percentage change in burrowing time (n=4)	
	7 das	14 das
Control	(+)2.53 ^b	(+)10.57 ^b
Bark	(+)10.58 ^a	(+)100.00 ^a
Leaf	(+)8.96 ^a	(+)96.61 ^a
Chlorpyrifos (20EC)		0.00
SEM	0.00	

Mean values followed by the same letter are not significantly different at $p=0.01$. Das: days after setup. Positive sign (+) represents an increase in burrowing time

Table 7: Effect of LC₅₀ of neem bark and neem leaf aqueous extract on the mean percentage mortality of earthworms

Neem parts	Percentage change in mortality of earthworms (n=4)	
	7 das	14 das
Control	0.00±0.00 ^c	0.00±0.00 ^c
Bark	0.00±0.00 ^c	58.33±8.33 ^b
Leaf	25.00±0.00 ^b	58.33±8.33 ^b
Chlorpyrifos (20EC)	100.00±0.00 ^a	100.00±0.00 ^a
SEM	0.00	0.00

Mean values followed by the same letter are not significantly different at $p=0.01$. das: days after setup. * Earthworms in the chlorpyrifos (20EC) had all died

4.0 Conclusion

In conclusion, this study investigated the effect of different concentrations of neem bark and neem leaf aqueous extracts on termites and earthworms. The study revealed that neem bark and neem leaf aqueous extracts are toxic to the castes of termites and are capable of causing loss in weight, increased burrowing time and even mortality at 50% lethal concentrations of earthworms if exposure period is prolonged. The extracts may thus be used as eco-friendly insecticides for the management of termites in agricultural soils. However, their use should be with discretion since they may exert non-toxic but detrimental effects on the activities of earthworms at low concentrations.

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