International Journal of Zoology and Applied Biosciences **ISSN: 2455-9571**
Volume 9, Issue 6, pp: 1-7, 2024
https://doi.org/10.55126/ijzab.2024.v09.i06.001 **http://www.ijzab.com**

Research Article

EVALUATION OF THE REPELLENT EFFECT OF LEAF AND FRESH KERNEL EXTRACTS OF NEEM (*AZADIRACHTA INDICA* A. JUSS., 1830) ON THE SWEET POTATO WEEVIL (*CYLAS PUNCTICOLLIS* BOHEMAN, 1833) UNDER LABORATORY CONDITIONS IN BURKINA FASO

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Article History: Received 29th September 2024; Accepted 24th October 2024; Published 1st November 2024

ABSTRACT

The sweet potato, *Lpomoea batatas* (L) Lam. is a major subsistance crop in Africa. It plays a major role in the diets of many urban and rural house holds, and is a source of income for farmers. Its production is limited by several constraints, including the weevil *Cylas puncticollis* (Coleoptera:Apionidae). Weevil repellency tests were carried out in the laboratory using aqueous extracts of neem leaves and fresh neem kernels. The experimental design was a Fisher block with four treatments (different extract concentrations and distilled water) and five replicates. The test consisted in accessing the percentage of weevils present in the treated area. Each concentration was applied to one half of Whatman blotting paper placed in a 9-cm diameter Petri dish, on which 10 adult weevils were placed. The results showed that the aqueous extracts of neem leaves and fresh neem kernels caused 100% repulsion of the weevils after 1 hour's exposure, compared with the distilled water control. The tests revealed that aqueous neem leaf extracts had a higher repellent potential than almonds after 30 min of weevil exposure. Neem leaf extracts could be a more environmentally-friendly method of controlling this insect pest, both for human and animal health.

Keywords: Aqueous extracts, Neem, Repellent effect, Sweet potato, *Cylas puncticollis*, Burkina Faso.

INTRODUCTION

Sweet potato is an important food and vegetable crop for its tubers and edible leaves (Oladipo and Adenegan, 2011). It is used in many parts of the world as a lean food during hard times and plays a major dietary role in many households both urban and rural (Somda, 2019). Sweet potatoes are grown for their tubers, which constitute the main edible part (Vernier and Varin, 1994) and are a source of income for farmers (Jones *et al*., 2012). Orange-fleshed sweet potato varieties in particular are an important source of the vitamin A precursor beta-carotene (Vernier and Varin, 1994). These varieties can help combat avitaminosis A in children and pregnant women, who are most at risk of vitamin A deficiency (Andrade *et al*., 2009).

In Burkina Faso, sweet potato production has been estimated at 107,413 tonnes in 2021 (FAOSTAT, 2021). It is the leading tuber crop produced just behind cereals (DGPSA, 2014). Its production is concentrated in the Hauts-Bassins, Centre-South, Centre-West, East, Centre-East and Boucle du Mouhoun regions respectively in the provinces of Kénédougou, Houet, Sissili, Nahouri, Gourma, Kouritenga and Banwa (Some *et al*., 2014). However, sweet potato production is subject to constraints of various kinds. Among the biotic constraints, fungal and

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viral diseases and insect pest splay an important role in sweet potato production in African countries. The weevil, *Cylaspuncti collis* represents the main biotic constraint to sweet potato production in Burkina Faso (Koussoubé *et al*., 2018). Thus, weevils can cause a loss of productivity ranging from 60 to 100% (Stathers *et al*., 2003).

In ordrer to control this insect pest, growers resort to the use of systemic chemical insecticides (Somda, 2016). The use of these chemicals is increasingly criticized for their harmful effects on human and animal health and on the environment. For this reason, the use of biopesticides such as aqueous neem extracts is one of the alternatives to chemical insecticides in plant protection, but also a non-polluting means of control for the environment (Sanon *et al*., 2005). The main objective of the work is to evaluate, in the laboratory, the repellent effect of neem extracts (kernels and fresh leaves) on sweet potato weevils.

MATERIALS AND METHODS

Plant material

The plant material consisted of tubers of the white-fleshed sweet potato variety and extracts of fresh neem leaves and kernels. White-fleshed sweet potato tubers were collected from potato growers in Bama. Fresh neem leaves and kernels were collected from a neem plant located at the study site. Leaf and fresh kernel extracts were prepared in the laboratory.

Technical equipment

A number of items of equipment were used in the laboratory

- A graduated cylinder, plastic tubs, 5 mm muslin cloth, glass jars, a 1000 ml graduated beaker and distilled water were used to prepare the various solutions
- A mortar was used to crush the neem extracts (fresh kernels and fresh leaves)
- A chisel was used to cut the Whatman blotting papers
- Petri dishes and Whatmanpaper were used for the repulsion test
- A micropipette for pipetting the various 1000 µl solutions
- A Radwag AS 110.R1 precision electronic balance was used to weigh the fresh material and almonds.

Animal material

The animal material consisted of weevil adults. The adults were mass-reared in the laboratory in plastic containers under ambient conditions.

Weevil rearing

The weevils were reared in the entomology laboratory in Bobo-Dioulasso at a temperature of 37±1°C. Tubers containing *C.puncticollis* damage collected in Bama were placed in plastic containers. Insect rearing was carried out on a variety of white-fleshed sweet potato showing damage by *Cylas puncticollis* and one showing holes for insecte mergence. Old potatoes no longer containing insect larvae were removed from the tubs and replaced. The 10-l bins were covered with muslin cloth to ensure good aeration and prevent the emergent insects from escaping.

Preparation of different concentrations

Fresh neem leaves

The fresh neem leaves collected were weighed and 0.5 kg of them were ground using a mortar. Two and a half liters of distilled water were added to the crushed leaves. The mixture was covered with 5mm mesh muslin cloth and kept in the laboratory for 12 hours. After 12 hours, the resulting macerate was filtered through a 5mm mesh muslin cloth, and the yield was calculated using the ratio: volume of fresh neem leaf solution/volume of distilled water. 2.3l of fresh neem leaf solution was obtained, corresponding to a yield of : 2.3l/2.5l= 92%. Four concentrations were prepared from this filtrate: 10 cl/l; 20 cl/l; 30 cl/l and 40 cl/l. These concentrations were obtained with 5 cl, 10 cl, 15 cl and 20 cl of fresh neem leaf solution respectively, which were then made up to 50 cl with distilled water.

Fresh neem kernels

To obtain the fresh neem kernel solution, 0.5 kg of neem kernels picked directly from the neem plant were crushed and soaked in 2.5 l of distilled water for 12 hours. After 12 h, a filtrate of 2.51 l of neem kernel solution was obtained, giving a yield of 2.51 l of fresh neem kernel solution/2.5 l of distilled water = 100%. With this filtrate obtained, concentrations of 10 cl/l; 20 cl/l; 30 cl/l and 40 cl/l were prepared from 5 cl; 10 cl; 15 cl and 20 cl of neem kernel solution and 50 cl of distilled water.

Neem extract repellency and mortality test

The various tests were carried out using a micropipette. 110 mm Whatman paper was used to cover the bottom of 9 cm diameter Petri dishes. The Whatman paper was cut into two equal pieces and placed on the bottom of the Petri dish, 1 cm apart. 1 ml of each of the prepared solutions was spread evenly over one of the two parts of the Whatman paper, called the treated area, while the other part received nothing and is called the untreated area. A batch of 10 adult weevils was then placed in the center of the Whatmanpaper treated with the neem extract contained in the Petri dish.

Under the same conditions, for the control, the Whatman paper was cut into two equal parts and placed at the bottom of the Petri dish, one centimetre (1 cm) apart. 1ml of distilled water was spread evenly over one of the two parts of the Whatman paper, called the treated area, while the other part received nothing and is called the untreated area. Ten insects were also placed in the center of the Whatman paper treated with the neem extract contained in the Petri dish. Five replicates were carried out for each concentration of the different neem extract solutions (0.5 kg fresh leaves and 0.5 kg fresh kernels). The number of weevils present on the treated part of the Whatman paper and those present on the untreated part was recorded after 30 minutes and one (1) hour. The experimental set-up was a completely randomized Fisher block with five treatments and five replicates.

The Microsoft Office 2019 Excel spread sheet was used to enter and process the data collected and to produce the various graphs. R software version 3.6.2 was used for analyses of variance. When the data distribution did not follow the normal distribution, the non-parametric Kruskal-Wallis test was used to detect differences between treatments. Where there was a significant difference between treatments, pair wise comparison of means was performed using the pair wise t-test at the 5% threshold. The analyses concerned the following parameters: - Repulsion rate (RR) was calculated using the following formula: TR= (Nc - Nt/ Nc + Nt) × 100, withNc: number of weevils in the untreated area and Nt: number of weevils in the treated area; - The average repulsion rate for fines and leaves was calculated and assigned to one of several repellent classes ranging from 0 to V, according to the classification of Mc Donald *et al*. (1970). According to these later authors, class 0 (TR < 0.1%), class I (TR = 0.1 - 20%), class II (TR= 20.1 - 40%), class III (TR = 40.1 - 60%), class IV (TR = 60.1 - 80%) and class V (TR = 80.1 - 100%).

RESULTS AND DISCUSSION

After 30 min of exposure (Table 1), a pest presence rate of 0.00±0.00% was observed in the 40 cl/l treated zone, compared with 100.00 ± 0.00% in the untreated zone. This difference was significant (p = 0.0081). Similarly, with a concentration of 30 cl/l, 7.51±0.78% of *C. puncticollis* were recorded in the treated zone versus 95.00±2.88% in the untreated zone (p=0.018). Finally, a significant difference was revealed between the two zones for 20 and 30 cl/l, i.e. 2.5±0.20% of *C. puncticollis* in the treated zone versus 97.5±2.50% in the untreated zone.

Table 1. Average repellency rates of *C. puncticollis* according to area (treated or untreated) after 30 min exposure of *C. puncticollis* to macerated extracts of fresh neem leaves, Burkina Faso.

Time of exposure	Aqueous extracts	Concentration	Treatments		Probabilit y
			Treated	Non treated	
30 mn	Fresh leaves	40 cl/l	0.00±0.00a	100.00±0.00b	0.0081
30 mn	Fresh leaves	30 cl/l	7.51±0.78a	95.00±2.88b	0.018
30 mn	Fresh leaves	20cl/l	2.5±0.20a	97.5±2.50b	0.015
30 mn	Fresh leaves	10 cl/l	2.5±0.20a	97.5±2.50b	0.015
30 mn	Fresh leaves	00 cl/l	-	10.00±7.05	-

In each column, values followed by the same letter are not significantly different from each other at the 5% threshold according to the pairwise-test.

After 1 hour's exposure of *C. puncticollis* (Table 2), 0.00% of weevils were observed in the treated zone, compared with 100.00% in the untreated zone, for all the different concentrations. These differences in pest presence rates were significant (p = 0.0081).

Table 2. Average repellency rates of *C. puncticollis* according to area (treated or untreated) after 1 h exposure to macerated extracts of fresh neem leaves, Burkina Faso.

Time of exposure	Aqueous extracts	Concentration	Treatments		Probability
			Treated	Non treated	
1h	Fresh leaves	40cl/l	0.00±0.00a	100.00±0.00b	0.0081
1h	Fresh leaves	30cl/l	0.00±0.00a	100.00±0.00b	0.0081
1h	Fresh leaves	20cl/l	0.00±0.00a	100.00±0.00b	0.0081
1h	Fresh leaves	10cl/l	0,00±0,00a	100.00±0.00b	0.0081
1h	Fresh leaves	00cl/l	-	12.5±7.5	-

In each column, values followed by the same letter are not significantly different from each other at the 5% threshold according to the pairwise-test.

After 30 min of exposure, (Table 3) an average rate of 0.00±0.00% of weevils was recorded in treated areas versus 100.00±0.00% in untreated areas for concentrations of 40 and 30 cl/l with a significant difference between treatments (p = 0.008). An average weevil presence rate of 5.00±2.88% was noted in the treated zone, compared with 95.00±2.88 in the untreated zone at 20 cl/l. For 10 cl/l, the average rate was 12.50±4.78% in the treated zone versus 87.50±4.78% in the untreated zone. These rates were significantly different from one another (p=0.019).

Table 3. Average repellency rates of *C. puncticollis* according to area (treated or untreated) with macerated extracts of fresh neem kernels, Burkina Faso

Time of exposure	Aqueous extracts	Concentration	Treatments		Probablilty
			Treated	Non treated	
30 mn	Fresh kernels	40 cl/l	0.00±0.00a	100.00±0.00b	0.008
30 mn	Fresh kernels	30 cl/l	0.00±0.00a	100.00±0.00b	0.008
30 mn	Fresh kernels	20 cl/l	5.00±2.88a	95.00±2.88b	0.017
30 mn	Fresh kernels	10 cl/l	12.50±4.78a	87.50±4.78b	0.019
30 mn	Fresh kernels	00 cl/l	-	0.00±0.00	-

In each column, values followed by the same letter are not significantly different from each other at the 5% threshold according to the pair wise-test.

After 1 hour's exposure (Table 4), 0.00±0.00% of weevils were noted in treated areas versus 100.00±0.00% in untreated areas for all concentrations. These values were significantly different from each other (p=0.008)

Table 4. Average repellency rates of *C. puncticollis* according to area (treated or untreated) with macerated extracts of fresh neem kernels, Burkina Faso.

Time of exposure	Aqueous extracts	Concentration	Treatments		Probablilty
			Treated	Non treated	
1 h	Fresh kernels	40 cl/l	0.00±0.00a	100.00±0.00b	0.008
1 h	Fresh kernels	30 cl/l	0.00±0.00a	100.00±0.00b	0.008
1 h	Fresh kernels	20 cl/l	0.00±0.00a	100.00±0.00b	0.008
1 h	Fresh kernels	10 cl/l	0.00±0.00a	100.00±0.00b	0.008
1 h	Fresh kernels	00 cl/l	-	0.00±0.00	-

In each column, values followed by the same letter are not significantly different from each other at the 5% threshold according to the pair wise-test.

Table 5 shows that after 30 min of insect exposure, a low rate of repellency was observed in areas treated with fresh kernels and fresh leaves respectively (23.09±2.00% and 5.77±0.50%; 10.00±1.20% and 5.00±0.50%) with 40 and 20 cl/l. However, no repellency was observed in areas treated with fresh kernels and fresh leaves. But no repulsion was recorded with 30; 10 and 00 cl/l respectively with the same extracts. No repulsion was recorded in untreated areas after 30 min of exposure.

Table 5. Comparison of average repellency rates of *C. puncticollis* according to area (treated or untreated) with macerated extracts of fresh neem leaves and kernels, Burkina Faso

Time of exposure	Concentrations	Treatments			
		Fresh kernels		Freshleaves	
		Non treated	Treated	Non treated	Treated
30 mn	40 cl/l	0.00±0.00	23.09±2.00b	0.00±0.00	0.00±0.00a
30 mn	30 cl/l	0.00±0.00	0.00±0.00a	0.00±0.00	10.00±1.02c
30 mn	20 cl/l	0.00±0.00	5.77±0.50b	0.00±0.00	0.00±0.00a
30 mn	10 cl/l	0.00±0.00	0.00±0.00a	0.00±0.00	5.00±0.50b
30 mn	00 cl/l	0.00±0.00	0.00±0.00a	0.00±0.00	0.00±0.00a
Probabilité	0,02				

In each column, values followed by the same letter are not significantly different from each other at the 5% threshold according to the pairwise-test.

Table 6 shows that after 1h of exposure of *C. puncticollis*, an average repellency rate of 100% of the insect (p=6.810-12) was observed in areas treated respectively with fresh neem kernels and fresh neem leaves with 40, 30, 20, 10 and 00cl/l. No repellency of the insect was observed in areas treated with fresh neem kernels and fresh neem leaves with 40, 30, 20, 10 and 00cl/l respectively. No insect repellence was recorded in untreated areas after 1h of exposure with the 2 types of extracts.

Table 6. Comparison of average repellency rates of *C. puncticollis* according to area (treated or untreated) with macerated extracts of leaves and fresh kernels of neem, Burkina Faso.

Time of exposure	Concentrations	Treatments			
		Fresh Kernels		Freshleaves	
		Non treated	Treated	Non treated	Treated
1 h	40 cl/l	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
1 h	30 cl/l	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
1 h	20 cl/l	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
1 h	10 cl/l	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
1 h	00 cl/l	0.00±0.00	100.00±0.00	0.00±0.00	100.00±0.00
Probability	6.810^{-12}				

Average repellency rates of *Cylas puncticollis* according to different concentrations of neem extracts are summarized in Table 7.

The different concentrations of leaf extracts (00; 10; 20; 30; 40 cL/L) caused 0.00; 50.99; 50.99; 51.25; 51.25 and 50% average repellency rates to *Cylas puncticollis* respectively. On the other hand almonds (00; 10; 20; 30 and 40 c/l) caused 00; 50; 50; 50 and 53.45% average insect repellency respectively. The highest repellency class for almond extracts was class III, with an average repellency rate of 53.45%,compared with 51.25% for leaves.

Table 7. Mc Donald *et al*. (1970) classification of average repellency rates of *Cylas puncticollis* as a function of neem extract type and concentration. Burkina Faso.

Time of exposure : 30 mn			
Extracts	Concentrations	Average repulsion rates (%)	Classes
Fresh leaves	40 cl/l	50.00	III
	30 cl/l	51.25	III
	20 c/l	50.99	III
	10 cl/l	50.99	III
	00 cl/l	0.00	0
Fresh kernels	40 cl/l	53.45	III
	30 cl/l	50.00	III
	20 cl/l	50.00	III
	10 cl/l	50.00	III
	00 cl/l	0.00	0

Average repellency rates for different concentrations of neem extracts are shown in Table 8. The different doses of leaf extracts (00; 10; 20; 30; 40 cl/l) caused 0.00; 53.45; 53.45; 53.45 and 53.45% repellence to weevils respectively. By contrast, almonds (00; 10; 20; 30 and 40 cl/l) caused 00; 53.45; 53.45; 53.45 and 53.45% repellence respectively. The highest repellency class for leaf and almond extracts was class III with an average repellency of 53.45%.

Table 8. Average rates and classes of repulsion of *Cylas puncticollis* by neem extracts (kernels and leaves), Burkina Faso.

1 h exposure			
Extracts	Concentrations	Average repulsion rates (%)	Classes
Fresh leaves	40 cl/l	53.45	III
	30 cl/l	53.45	III
	20 cl/l	53.45	III
	10 cl/l	53.45	III
	00 cl/l	0.00	0
Fresh kernels	40 cl/l	53.45	III
	30 cl/l	53.45	III
	20 cl/l	53.45	III
	10 cl/l	53.45	III
	00 cl/l	0.00	0

Repellency is defined by Regnault-Roger *et al*. (2012) as the ability of an applied insect repellent substance to reduce the normal contact time of arthropods with the treated surface. The results recorded revealed that the average repellency rate of sweet potato weevils increased with the concentration of neem extracts, whatever the type (fresh leaves or kernels). All the concentrations tested proved relatively effective, with an average repellency rate of 100% for the 40 cl/l concentration. These results may be explained by the fact that neem leaf extract contains azadirachtin, an alkaloid from the non-mutagenic and biodegradable terpen group, which can paralyze the insect's digestive tract, causing the insects to stop feeding, according to Simmonds *et al*. (1996).This repellency of neem extracts is attributable to the presence of azadirachtin, the main active ingredient found in neem.

These results are comparable to those of Faye (2010), who reported that the high rate of insect pest repellency in the different neem treatments compared to the control was due to the presence of azadirachtin. Boeke *et al*. (2004) showed that all parts of neem have pesticidal properties that are responsible for weevil repellence. According to Simmonds and Blaney (1996), azadirachtin binds to the taste receptor, causing insects to reject any treated food. The average rate of weevil repellence increased in proportion to the duration of insect exposure. After 1 hour's exposure, no insects were present in treated areas, even with the lowest concentrations of neem extract, whether leaf or kernel. Weevil repellence appears to be a function of exposure time. Tests carried out with neem extracts showed that neem extracts had an insect repellent effect on sweet potato weevil adults. Fresh leaf extracts produced a higher average rate of insect repellence than other types of plant extracts. This difference in repellency observed between the different neem-derived products could be explained by a higher concentration of azadirachtin in fresh leaf extracts than in fresh kernels extracts.

After 30 min exposure to neem extracts, the classification of McDonald *et al*. (1970) shows an increase in the repulsion rate with both fresh leaf extracts and fresh kernels. This result clearly shows that the rate of repulsion increases with the concentration of extracts, whatever their nature. From these results, we can conclude that neem extracts have an insect repellent effect on the sweet potato weevil *Cylas puncticollis*. After 1 h of exposure to neem extracts, the average repellency rate of sweet potato weevils was the same for both fresh leaf extracts and fresh kernels extracts. In fact, macerated leaf and fresh kernels extracts produced 53.45% repulsion of adult insects at neem concentrations of 10, 20, 30 and 40 cl/l. The different repellency classes show, however, that macerated leaf extracts (Class 0 to III) were as repellent as those from seed kernels (Class 0 to III). These results could be explained by the presence of a substance with insect repellent potential, azadirachtin, with a remarkably higher concentration in the leaves. Our results differ from those of Douan *et al* (2022), who showed that macerated extracts of fresh leaves (class II to V) are more repellent than those of kernels (class I to III).

CONCLUSION

Tests carried out to determine the repellent and lethal potential of neem extracts yielded interesting results: all the neem extracts used effectively repelled the sweet potato weevil; the rate of weevil repellence increased with exposure time. However, these studies could be continued in the field to confirm the results obtained in the laboratory.

ACKNOWLEDGMENT

The authors express sincere thanks to the head of the Institut de Recherches en Sciences Appliquées et Technologies, Direction Régionale en Sciences Appliquées et Technologies 01 BP 2393 Bobo-Dioulasso, Burkina Faso for the facilities provided to carry out this research work.

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