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Full Length Research Paper

Compared efficiency of a commercial Neem oil (TopBio) and synthetic insecticide (Antouka Super®) in the management of *Dinoderus porcellus* Lesne (Coleoptera: Bostrichidae) infesting yam chips

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Dinoderus porcellus **Lesne is the main pest of stored yam chips in West Africa. Despite their negative impact on the environment and human health, synthetic chemical pesticides are the main control method used by farmers for yam chips protection. This study aims to evaluate the biological effects of different doses (2.5 and 5%) of a commercial Neem oil (TopBio) and the chemical insecticide, Antouka Super® (Permethrin 3 g / kg + pyrimiphos 16 g / kg) against *D. porcellus* fed on yam chips. We evaluated the contact toxicity by topical application, the repellent activity, and ingestion toxicity of both products. Results revealed that *D. porcellus* adults were more sensitive to contact with Neem oil (LC_{50} = 2.17 µl/adult) than with the chemical insecticide (LC_{50} = 644.93 µl/adult) at 72 h post-treatment. The Neem oil (TopBio) also exhibited a strong repulsion (Class V) against *D. porcellus* at all tested concentrations. Antouka Super® at 5% was more effective against *D. porcellus* by ingestion with a corrected mortality percentage of 81.05 ± 11.00% three days post-treatment versus 15.73 ± 11.36% with Neem oil. The lowest weight loss of treated yam chips was obtained with Antouka (0.06 ± 0.02%) comparatively to the Neem oil at 5% (2.12 ± 0.42%). Our study showed that Neem oil (TopBio) is a contact biocide with a highly repulsive potential for integrated management of *D. porcellus* adults in stored yam chips.**

Key words: *Dinoderus porcellus*, yam chips, contact toxicity, repellency, ingestion, bioinsecticide.

INTRODUCTION

Yam (*Dioscorea* ssp.) occupies an important place in the diet of African peoples. Its tubers are an important source of carbohydrates, vitamins and minerals (Olajumoke et al., 2012), and plays an important role in the livelihood

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people in West Africa (Agba et al., 2019). However, despite its nutritional, economic and socio-cultural importance, yams cultivation was subjected to several constraints, including the high perishability of fresh tubers (Ategbo et al., 1997). Post-harvest losses are of the order of 65 to 85% (Ferraro et al., 2016), which leads to an irregularity in the availability of fresh tubers throughout the year (Babadjide et al., 2008). To overcome the highly perishable of tubers, they are traditionally dried and made into chips (Hounhouigan et al., 2003), contributing to food security and alleviate poverty (Oluwole et al., 2013; Olatoye and Arueya 2019).

Unfortunately, these yam chips were severely attacked by insects, which cause important losses (Vernier et al., 2005). Among the diversity of insect pests found in stored yam chips, *Dinoderus porcellus* Lesne is the most abundant and causing the most damage (Loko et al., 2013; Loko et al., 2019a, b). Therefore, alternatives such as resistance varieties, aqueous extracts and powders, essential oils of medicinal plants have been developed for the management of this pest (Loko et al., 2019b). However, shortcomings were still observed in their effective use in farming environments, hence the search for the effectiveness of commercial Neem oil in the integrated fight against *D. porcellus*.

Regarding using of bio-insecticides, pride of place is reserved for products derived from Neem (*Azadirachta indica* A. Juss.), for the most promising results obtained in the fight against pests. Over 400 species of crop and stock insect pests were susceptible to the various effects of Neem (Schmutterer and Singh, 1995; Vernier et al., 2005). Neem oil and its derived products have been shown to be effective against storage insects such as *Rhizopertha dominica* Fabricius (Coleoptera: Bostrichidae) (LMDS, 2010), *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) (Lale and Mustapha, 2000) and *Sitophilus zeamais* Motshulsky (Coleoptera: Curculionidae) (Tamgno and Tinkeu, 2013). Therefore, this study aims to assess the biological effects (contact toxicity, repellent activity, antifeedant activity) of the commercial Neem oil TopBio on *D. porcellus* and compared its bioactivity with the chemical insecticide Antouka Super® recommended for the protection of foodstuffs stored in the Republic of Benin.

MATERIAL AND METHODS

Chemical material

The natural insecticide "TopBio" was purchased from the company BioPhyto-Collines located in Allada Togoudo in the Republic of Benin. TopBio is composed of emulsifiable Neem oil (azadirachtin) and trace vegetable essential oils such as citronellal, citronellol, geraniol and nimbin from lemongrass (Toffa et al., 2020). The synthetic insecticide Antouka Super® (SYNGENTA, United Kingdom) is a combined chemical insecticide whose active principles are Pirimiphos-methyl 16% and Permethrin 3% was bought. Antouka Super® is recommended for the protection of stored products against insect pest attacks (Ngoula et al., 2017).

Insect rearing

D. porcellus adults were collected in the infested yam chips from previously rearing in the Laboratory of Applied Entomology (LEnA). The rearing of *D. porcellus* was carried out in plastic boxes (10.5 cm high, 16.8 cm in diameter) containing sterilized yam chips at the laboratory conditions (25 ± 2°C, 70 ± 5% RH, 12L / 12D photoperiod). The progeny were used in all bioassays (Loko et al., 2020).

Contact toxicity

The insecticidal activity of Neem oil (TopBio) and Antouka Super® insecticide against *D. porcellus* adults was evaluated by the contact application method (Mukanga et al., 2010; Loko et al., 2020). Two concentrations (2.5 and 5%) of each insecticides (Neem oil and Antouka) were prepared and applied on the dorsal side of the thorax of *D. porcellus*. Those of Neem oil by dilution in distilled water, and those of Antouka by dilution in alcohol.

Ten pairs of adult *D. porcellus* (3-7 days old) were treated with 1 µl of each dose. After treatment, the insects were transferred to Petri dishes containing 10 g of untreated yam chips. Each treatment was repeated four times. The dishes were then be placed in the dark at 26 ± 2°C and 75 ± 5% relative humidity. Insects were examined daily and the adult mortality rate was recorded after 1 h, 10, 24, 48 and 72 h (Caballero Gallardo et al., 2012). The percentage was calculated according to the formula of Asawalam et al. (2006) (1) and the corrected mortality percentage was calculated using Abbott's formula (2) to eliminate natural mortalities.

$$\text{Percentage of mortality} = \frac{\text{number of } D.\ porcellus \text{ dead}}{\text{number of } D.\ porcellus \text{ introduced}} \times 100 \quad (1)$$

$$\text{Corrected mortality} = \frac{(\%\text{ of death in treadted} - \%\text{ death in control})}{(\mathbf{100} - \%\text{death in control})} \times 100 \quad (2)$$

Repellent activity bioassay

The repellent effect of Neem oil (TopBio) and Antouka Super® insecticides was evaluated using the area preference method on filter paper described by Babarinde et al. (2008) and Loko et al. (2020). Filter paper discs (diameter 9 cm) used for this purpose were cut into two equal parts with 31.8 cm^2 for each half. Two doses (2.5 and 5%) of each insecticide (Neem oil and Antouka) were prepared. Those of Neem oil by dilution in distilled water, and those of Antouka by dilution in alcohol. Then 1 ml of each of the solutions thus prepared was spread evenly over one half of the disc while the other half did not receive treatment. The pieces of treated filter paper were dried to allow evaporation of the solvent during ten minutes (Odeyemi et al., 2008). Treated and untreated halves were fixed using adhesive tape and placed in a Petri dish. Twenty mixed-sex adult of *D. porcellus* were released at the center of each Petri dish. The dishes were then covered and placed in the laboratory at 27 ± 2°C with a relative humidity of 70 ± 5%. Four replications were performed for each dose of insecticides. The number of *D. porcellus* present on the treated and untreated part of filter paper was recorded after 30 min, 2, 4 and 8 h of exposure (Lale and Alaga, 2001). Percentage of repellence (PR) was calculated using the formula of Gusmao et al. (2013):

Table 1. Mortality of adult *D. porcellus* and weight loss (mean ± SE) of yam chips treated with varying concentration of Topbio and the chemical insecticide Antouka in 3 days after infestation.

Treatments	Concentration (%)	Mortality rate (%) after different periods of exposures (hours)					Weight loss (mean ± SE) of yam chips
		1 h	10 h	24 h	48 h	72 h	
Neem oil (Topbio)	2.5	62.5 ± 27.23[a]	65.00 ± 24.83[a]	67.10 ± 21.54[a]	69.78 ± 18.39[a]	71.37 ± 15.97[a]	1.52 ± 0.43[a]
	5	83.15 ± 4.78[a]	85.76 ± 2.93[a]	86.89 ± 2.67[a]	90.47 ± 6.73[a]	90.47 ± 6.73[a]	1.55 ± 0.12[a]
Antouka	2.5	0.00 ± 0.00[b]	2.50 ± 2.88[b]	7.50 ± 6.45[b]	8.75 ± 8.53c	4.69 ± 13.67[b]	3.50 ± 4.46[a]
	5	1.25 ± 2.50[b]	3.75 ± 2.50[b]	11.25 ± 4.78[b]	12.63 ± 6.39[b]	7.63 ± 9.62[b]	3.42 ± 4.54[a]
Df		15	15	15	15	15	15
F		47.796	40.326	33.870	30.746	27.712	0.265
Probability		0.000	0.000	0.000	0.000	0.000	0.849

Means within the same rows followed by the same letter are not significantly different (p< 0.05).

$$\text{Percentage of repellence} = [(\text{Nc} - \text{Nt}) / (\text{Nc} + \text{Nt})] \times 100 \quad (3)$$

where Nc was the number of insects on the untreated area after the exposure interval; Nt was the number of insects on the treated area after the exposure interval. The average repulsion value of each insecticide was assigned to repulsion classes from 0 to V: class 0 (PR ≤ 0.1%), class I (PR = 0.1–20%), class II (PR = 20.1–40%), Class III (40.1–60%), Class IV (60.1– 80%) and Class V (80.1–100%).

Antifeedant activity bioassay

The ingestion toxicity of Neem oil (TopBio) and Antouka Super® insecticides was performed according to the method described by Hamdani (2012) and Loko et al. (2020a, b). Different concentrations (2.5 and 5%) of Neem oil were applied on 10 g of yam chips placed in plastic boxes (7 cm in diameter × 3.7 cm in height). Yam chips were treated with chemical insecticide Antouka Super® at the recommended dose (50 g / 100 Kg) (Taghizadeh et al., 2014). The application volume of insecticides is 1 µl per inoculation. Twenty adults (2-3 day old) of *D. porcellus* starved for 3 h were introduced into treated yam chips. Control treatment consisted in 20 *D. porcellus* adults in boxes containing 10 g of untreated yam chips. Each treatment was repeated 4 times. The dead insects were counted 1, 10, 24, 48 and 72 h after exposure. Percentage weight loss was calculated using equation (Chijindu and Boateng, 2008):

$$\text{Weight loss } \% = \frac{\text{initial weight} - \text{final weight}}{\text{initial weight}} \times 100 \quad (4)$$

Statistical analysis

Mortality values were corrected with Abbott's formula to eliminate natural mortality of control (Abbott, 1925). Data on percentage mortality and repellency were arcsine transformed, in order to homogenize their variance before being subjected to one-way ANOVA using IBM SPSS Statistic Software Version 23.0. Significant differences between means were separated using Newman-Keuls test at 5% probability (Addinsoft, 2019). Probit analysis was used to calculate the median repellent dose RD_{50} (dose that repelled 50% of the exposed insects). The relationship between the TopBio concentration applied and percentage mortality was determined using probit regression analysis of transformed data to estimate lethal concentration that kills 50% (LC_{50}) of test insects.

RESULTS

Contact toxicity

Our results showed that the *D. porcellus* mortality varied significantly as a function of the different concentrations of the two insecticides tested at 1 h (F = 47.796; df = 15; p = 0.000), 10 h (F = 40.326; df = 15; p = 0.000), 24 h (F = 33.870; df = 15; p = 0.000), 48 h (F = 30.746; df = 15; p = 0.000), and 72 h (F = 27.712; df = 15; p = 0.000) post-exposition (Table 1). However, death rates increased over time of exposure regardless of the Neem oil (TopBio) dose used. The highest mortality rate (90.47 ± 6.73%) of *D. porcellus* was observed with Neem oil at 5% concentration after 48 h of exposure.

Unlike Neem oil, Antouka Super® was less toxic to *D. porcellus* adults. The highest mortality rate of *D. porcellus* obtained with Antouka insecticide at 5% after 48 h of exposure was only 12.63 ± 6.39% (Table 1). On the other hand, the application of the different insecticides on *D.*

Table 2. LC_{50} value after contact application of neem oil (Topbio) and the chemical insecticide Antouka on adults of *D. porcellus.*

Insecticides	Exposure time (hours)	$CL_{50}{}^{a}$ (µL/adult)	95% Confidence Interval (lower-upper)	Slope ± ES	Intercept
Neem oil (Topbio)	1	1.79	0.95-3.36	2.21 ± 0.13	4.439
	10	1.70	0.87-3.29	2.10 ± 0.14	4.516
	24	1.85	0.90-3.72	1.95 ± 0.14	4.476
	48	2.13	1.03-4.39	1.82 ± 0.16	4.400
	72	2.17	0.93-5.03	1.55 ± 0.18	4.477
Antouka	1	-	-	-	-
	10	72.73	12.10-437.24	1.53 ± 0.39	2.149
	24	186.34	19.45-1785.01	1.04 ± 0.50	2.623
	48	374.67	27.29-5142.39	0.81 ± 0.58	2.888
	72	644.93	34.71-11982.20	0.68 ± 0.64	3.084

[a]CL_{50}: lethal concentration 50; [b]dl: degrees of freedom.

Table 3. Percent repellence (mean ± SE) of adult *D. porcellus* and repellent class of Antouka and Topbio to varying exposure time and concentrations in a choice bioassay.

Treatments	Concentrations (µl/ml)	Repellence rate (%) after treatments				Mean repellency	Repellency class	Classification
		30 min	2 h	4 h	8 h			
Neem oil (Topbio)	2.5	77.50 ± 17.05^{ab}	77.50 ±15.00^{bc}	70.00 ± 14.14b	75.00 ±17.32b	75.00 ±14.60^{c}	V	Repulsive
	5	90.00 ± 11.54^{b}	95.00 ± 5.77^{c}	95.00 ± 5.77^{c}	97.50 ± 5.00^{c}	94.40 ± 7.25^{d}	V	Repulsive
Antouka	2.5	45.00 ± 20.81^{a}	27.50 ± 9.51a	15.00 ± 19.14a	17.50 ± 15.00a	26.25 ±19.27^{a}	II	Repulsive
	5	50.00 ± 14.14^{a}	60.00 ± 18.25^{b}	62.50 ± 15.00^{b}	65.00 ± 5.77^{b}	59.38 ±13.88^{b}	III	Repulsive
df		15	15	15	15	63	-	-
F		5.822	12.884	16.752	17.164	28.927	-	-
Probability		0.011	0.000	0.000	0.000	0.000	-	-

Means within the same rows followed by the same letter are not significantly different (p< 0.05).

porcellus did not significantly ($F = 0.265$; $df = 15$; $p = 0.849$) reduce the yam chips weight loss. The lowest LC_{50} values were obtained with TopBio and are 1.70; 1.85; 2.13 and 2.17 µl/adult respectively at 10, 24, 48 and 72 h post-exposure (Table 2).

Repellent activity

The results showed that both insecticides act as repellent against D. porcellus even at low concentration (Table 3). Contrary to Antouka Super®, all concentrations of TopBio had a strong repellent effect on adults of D. porcellus. Indeed, there is a significant difference between the different insecticides at various concentrations ($F = 63$; $df = 28.927$; $p = 0.000$). In addition, all

Table 4. RD_{50} value after contact application of neem oil (Topbio) and the chemical insecticide Antouka on adults of *D. porcellus.*

Insecticides	Exposure time (hours)	RD_{50} (µl/insect)	95% Confidence Interval (lower-upper)	Slope ± ES	Intercept
Neem oil (Topbio)	30 min	0.32	0.04-2.53	0.66 ± 0.46	5.333
	2 h	0.55	0.13-0.23	1.03 ± 0.31	5.265
	4 h	1.17	0.45-3.00	1.48 ± 0.20	4.899
	8 h	0.83	0.28-2.46	1.33 ± 0.24	5.106
Antouka	30 min	174.99	3.02-10.52	0.33 ± 0.89	4.259
	2 h	7.25	4.22-12.44	2.71 ± 0.12	2.719
	4 h	6.42	4.31-9.56	3.95 ± 0.08	1.808
	8 h	5.83	4.34-7.83	5.72 ± 0.06	0.613

ES : standard error.

Table 5. Average corrected mortality rate of *D. porcellus* after ingestion of treated yam chips with neem (Topbio) and Antouka oil and weight loss (mean ± SE) of yam chips 3 days after treatment.

Insecticides	Concentrations	Mortality rate (%) after treatments					Weight loss (mean ± SE) of yam chips
		1 h	10 h	24 h	48 h	72 h	
Neem oil (Topbio)	2.5%	1.25 ± 2.50[a]	4.74 ± 4.50[a]	8.88 ± 6.33[a]	16.51 ± 8.39[a]	25.78 ± 10.40[a]	2.37 ± 0.62[a]
	5%	2.50 ± 2.88[a]	3.75 ± 4.78[a]	3.75 ± 4.78[a]	6.51 ± 6.64[a]	15.73 ± 11.36[a]	2.12 ± 0.42[a]
Antouka	50 g/100 kg	0.00 ± 0.00[a]	11.25 ± 4.78[a]	66.71 ± 25.85[b]	78.34 ± 11.53[b]	81.05 ± 11.00[b]	0.06 ± 0.02[b]
df		11	11	11	11	11	19
F		1.286	2.432	16.668	37.344	30.522	5.411
Probability		0.323	0.143	0.001	0.000	0.000	0.009
Control	0	0.00 ± 0.00	0.13 ± 0.35	1.87 ± 2.58	3.75 ± 3.53	4.37 ± 3.20	2.71 ± 3.49[a]

Means within the same rows followed by the same letter are not significantly different ($p < 0.05$).

tested concentrations (2.5% and 5%) of TopBio exhibited a strong repellency class (class V) while Antouka Super® at the same concentration exhibited repellency class II and III, respectively. The repellency was dose-dependent with Antouka Super®. However, the values of RD50 indicate that TopBio at 30 min post-treatment was more repellent than Antouka Super® (Table 4).

Ingestion toxicity

After consumption of yam chips treated with Neem oil (TopBio) and Antouka Super® insecticides by *D. porcellus* adults, we observed a gradual increase of mortality rate over hours of observation ($F = 30.522$; $df = 11$; $p = 0.000$) (Table 5). At 24 h, Antouka Super® killed more than 66% of *D. porcellus* adults. This rate reached 81%, 72 h post exposition (Table 5). Whereas, after treatment, the concentrations of 2.5 and 5%

of Neem caused 25 and 15% mortality of Dinoderus, respectively. However, the lowest weight loss obtained for yam chips treated with Antouka Super® against *D. porcellus* was significantly different (df = 19; F = 5.4; p = 0.009) of those caused by TopBio (Table 5).

DISCUSSION

The results showed that the Neem oil (TopBio) and Antouka Super® are toxic to *D. porcellus* adults. The dose-dependent toxicity of Neem oil and the resulting high contact mortality rates of *D. porcellus* are not surprising. Indeed, Islam (2010) showed that the dose-dependent insecticidal activity of Neem oil formulations is due to the high concentration of insecticidal compounds. The insecticidal effect of TopBio on *D. porcellus* could be due to its active components, which certainly induced the blockage of the respiratory stigmata of the insect and created its asphyxia or its observed death. In fact, the active components such as azadirachtin, citronellal, citronellol, geraniol and nimbin present in Neem oil affect the biochemical and physiological processes of the insect system and cancel out the insect's detoxification mechanism, thus preventing the pest from developing resistance (Goektepe et al., 2004; da Costa et al., 2014). These potentially bioactive compounds are effective in controlling stored grain and tubers pests (Kumar et al., 2003). Coitinho and Oliveira (2006) also showed the acute toxicity by contact of Neem oil in *S. zeamais* adults. The good inhibitory effects of TopBio on the *D. porcellus* survival suggest its use as yam chips protectants. However, more research is needed to evaluate the mechanism of action of TopBio on *D. porcellus* to better plan an efficient control program. TopBio was significantly more repellent for *D. porcellus* adults than Antouka Super®. Loko et al. (2017) also reported the low repulsive activity of Antouka Super®.

However, these authors showed that Antouka Super® was more repellent in respect of *D. porcellus* than leaves powders of *Bridelia ferruginea*, *Blighia sapida*, and *Khaya senegalensis*. The strong repulsive activity (Class V) of TopBio could be due to the slightly pungent odour of Neem oil (Forim et al., 2013). Moreover, the persistence of the repellent activity of TopBio over the time could be explained by the low volatility of azadirachtin, which would cause a rapid increase in repellency activity over time (Morgan, 2009; Forim et al., 2013). Similar results were found by Bidiga (2014), which showed that Neem oil induced a strong repulsion against *Aphtona* spp.

Nevertheless, Loko et al. (2021) observed a low repellency class against *D. porcellus* with the essential oils of *Petroselinum crispum* and *Pimenta racemosa*. Therefore, TopBio may be considered as a new repellent against *D. porcellus*. However, the combination of Topbio with other biological control methods should be explored and could contribute to effective management of *D. porcellus*.

The insecticide Antouka Super® demonstrated higher insecticidal activity against *D. porcellus* by ingestion of treated yam chips and considerably reduced weight loss compared to TopBio. These results are consistent with those of Loko et al. (2017), which showed that Antouka caused a strong anti-appetite activity against *D. porcellus* but a low mortality by contact. Toxic effects from ingestion of the synthetic insecticide Antouka may be due to the entry of active compounds into the insect's body through the digestive system (Ngoula et al., 2017).

The low ingestion toxicity of TopBio against *D. porcellus* is surprising because some authors showed that Neem-derived products can significantly cause mortality of several insect storage pests such as *Prostephanus truncatus* Horn (Mukanga et al., 2010), *Sitophilus oryzae* (L.) (Ivbijaro, 1983; Nukenine et al., 2011; Tamgno and Tinkeu, 2018). In fact, a wide range of biological activities of Topbio affecting the feeding behavior of insects has also been proven by Abdullah and Subramanian (2008). In addition, Tamgno and Tinkeu (2013) have shown that azadirachtin concentrated and processed by ingestion induced a high mortality rate on *S. zeamais*. Other authors confirmed that Neem-derived products can protect stored foodstuffs over several months (Taponjou et al., 2002; Isman, 2006). Therefore, further investigation is necessary to evaluate the exposure time, doses and application formulations of Topbio in the management of *D. porcellus*.

Conclusion

The results revealed the good potential of the commercial Neem oil, TopBio as contact insecticide and repellent agent against *D. porcellus*. Likewise, Topbio reduced the weight loss of yam chips caused by the pest. It follows that it can be used as an alternative to synthetic insecticides. However, further studies are needed to assess the anti-appetite activity of Neem oil (Topbio) against adults of *D. porcellus* on yam chips. Investigations must then continue on the exposure time, doses and application formulations of Topbio to improve its effectiveness as bioinsecticide in the sustainable management of *D. porcellus* adults.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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