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Terpinolene Compound: A Bio-preservative for Stored Wheat Grains against R*hyzopertha dominica* Fab. (Coleoptera: Bostrichidae) Infestation

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Abstract
Contact and fumigant bioassay of Terpinolene compound was conducted at five concentration levels of 0.2, 0.4, 0.6, 0.8 and 1.0ml /20 g wheat grains against Rhyzopertha dominica. Parameters assessed were adult mortality at 24, 48, 72 and 96 hours of exposure, adult emergence, percentage inhibition rate and grain weight loss due to infestation by R. dominica. Significantly ($P < 0.05$), direct toxicity was observed on the insects compared to negative control with 100% mortality at 72 and 96 h at 0.8 and 1.0 ml/20 g grains respectively. Percentage mortality was dosage and exposure time dependent. LD_{50} of 3.75 and 4.17ml of terpinolene evoked 50% adult mortality. Adult emergence and percentage weight loss decreased with increased dosages, while percentage inhibition rate increased with increased treatment dosages. It was concluded that, Terpinolene compound proved effective biopreservative for the control of R. dominica infesting stored wheat grains suggesting future evaluation of mode of action for crop protection commercialisation.

Keywords: Biopreservative, Infestation, Inhibition rate, Storability Terpinolene

Introduction
Post-harvest food grains losses remain one of the serious constrictions militating against food security and self-sufficiency in many Tropical and Subtropical countries (Udo, 2011; Adesina *et al.*, 2020). Croser and Andersen (2010) reported that in sub-Saharan Africa, post-harvest losses of grain are appraised to be between 10 - 20% and accounted for about $4 billion of total cereal production. In a related development, Rajashejkar *et al.,* (2012) stated that insect pests destroy roughly one-third of the world's food production, estimated yearly at more than $100 billion from which 43% been the highest losses occurred in the developing world.

Wheat *Triticum aestivum* (L.) with its boundless nutritional value in the diet of developing countries teeming population, when unprotected, damages and losses due to *Rhyzopertha dominica* (Fab.) and other storage insect pests are lost up to 20-60% during storage (Arthur and Throne, 2003; Babu *et al.,* 2003; Gueye *et al.,* 2012). Both the adults and larvae causes' severe damage to stored cereal grains and products and adult beetles are more destructive which destroy healthy grains and reduced them to powdery. They destroy far more than they consume (Arifuzzaman *et al.,* 2014).

Considerable losses associated with the insect during post-harvest storage accounts for importation from neighbouring West African, Asia and other agriculturally developed countries to meet local demand for wheat grains (Adesina and Mobolade-Adesina, 2020).

At present, the control of stored product insect is achieved using some chemical products, for which series of adverse or hazard effects have been reported in humans, animals and environment contributing to the reduction in their usage among farmers (Oni *et al*., 2018). This has necessitated the increased in search for natural products derived from plants that is safe and ecofriendly in nature as replacements to synthetic compounds (Pavela, 2009; Adesina and Mobolade-Adesina, 2020). Bioactive molecules such as R-pinene, eugenol, limonene, terpineol, citronellol, citronellal, camphor, thymol and others derived from plant products have proven to be effective as insecticides, ovicidals, antifeedants, oviposition deterrents and repellent properties for pest control (Yang *et al*., 2004; Park *et al*., 2005; Jaenson *et al*., 2006). In light of the foregoing, the aim of this work was to evaluate the insecticidal properties of terpinolene obtained from plants products against *R. dominica.*

Materials and Methods
Experimental location and condition
The experiment was carried out at the Entomology laboratory of Department of Crop, Soil and Pest Management, Federal University of Technology Akure, Nigeria under ambient conditions of 28±2ºC temperature,

75±5% relative humidity and 12 L: 12 D photoperiodic. Experimental design is a completely randomized design (CRD).

Insect culture

Rhyzopertha dominica used as initial stock were collected from the naturally infested wheat grains from the local market of Oja ugele, Ondo town, Nigeria. Fifty 50 insects were reared in the laboratory at ambient room temperature and relative humidity in 3 litres plastic containers covered with muslin cloth and tightly secured with rubber band to avoid contamination and escape of insect and arranged on laboratory work bench to allow for multiplication and steady supply of insect for the study. Adult insects were 2- 4 days old when used in the bioassays.

Source of terpinolene

Ten (10) ml of Terpinolene was supplied by Prof. M. Isman of Colombia University, Canada and the active compounds was bought from Sigma-Aldrich Chemical Store, Canada. Upon receipt, the active compound was kept in a refrigerator till use. The terpinolene was made into 2% concentration and 0.2, 0.4, 0.6, 0.8 and 1.0 dosages were prepared from it. Diclorovos (DDVP) used as standard check was bought from a local agrochemical store in Akure, Nigeria and made into 0.005% from which 0.1 dosage was made.

Wheat grains collection

Wheat grains were procured from the local market in Akure, Ondo State, Nigeria were cleaned thoroughly, sun dried and cooled at 8 ± 2% moisture level, stored in air tight plastic bag and kept in a deep freezer for two weeks to eliminate any hidden infestation. The disinfested wheat grains were air dried under ambient conditions for about two hours, twenty 20 g of wheat grains was weighed into dishes for contact and fumigant toxicity bioassay. (Idoko and Adesina, 2012).

Contact toxicity of terpinolene on adult *R. dominica* survival

Twenty (20) grammes of sterilized wheat grains was treated with 0.2, 0.4, 0.6, 0.8 and 1.0 ml dosages of Terpinolene, the negative control had 1ml of 1% treatment on wheat and positive control had 0.1 ml of 0.005% DDVP. Twenty (20) sexed *R. dominica* adults was introduced into the treated wheat grains dishes and the two controls. Beetle mortality was taken at 24, 48, 72 and 96 hours post-treatment. Insect mortality count in both treated and the control experiment was subjected to correction using Abott's formula (Abbott, 1925) and was subjected to Probit analysis to estimate LD_{50} at 48 hrs. Dead and live insects were removed and recorded on the fourth day. There were five replicates for the experiments.

At day thirty, number of (F1) generation adult insect emergence was taken and inhibition rate (IR) according to Tapondju *et al*. (2002). Percentage weight loss in wheat grains both treated and control setups was calculated by fresh weight basis according to Ileke and Oni, (2011).

Fumigant toxicity of terpinolene on adult *R. dominica* Survival

The fumigant toxicity of terpinolene were tested against *R. dominica* using the method of Zhou *et al.*, (2012) as described by Yang *et al.*, (2014) with little modifications. A cotton wool treated with 0.2, 0.4, 0.6, 0.8 and 1.0 ml of terpinolene at 1% concentration were hung on the cover of the Petri-dishes. Twenty (20) unsexed adult insects were introduced into the treated Petri-dishes, left undisturbed for 4days. The experiment was replicated five times. The observed mortality data and controls was corrected using Abbott (1925) formula. LD_{50} at 48hrs was calculated using Probit analysis (Finney, 1971). Adult emergence, inhibition rate and percentage weight loss were also determined.

Statistical analysis

Data obtained was recorded as mean value ± SE; the statistical analysis was subjected to Analysis of Variance (ANOVA) and means were matched at $P < 0.05$ using Tukey Honestly Significant Test. Mortality data of *R. dominica* was further subjected to probit analysis and log transformation respectively to determine the LD_{50} and LD_{95} (Finney, 1971). All analysis were carried out using SPSS 20.0 software package.

Results

Contact toxicity of terpinolene against adult *R. dominica*

Adult mortality of *R. dominica* in wheat grains treated with *different dosages of* Terpinolene under contact toxicity varied significantly (P>0.05) compared with insects in the controls experiment (Table 1). The bioactive compound showed that adult *R. dominica* mortality increased with increasing dosage rate and hour of exposure. The mean mortality in treated grains ranged from 4 to 100%. Terpinolene was highly effective against *R. dominica* causing highest mortality of 100% after 96 hours of post treatment was recorded at 0.8 and 1.0 ml respectively. However, non-significantly different adult mortality was recorded in wheat grains treated with 0.8 and 1.0 ml Terpinolene and those obtained from negative control irrespective of the duration of exposure. Similar trend was observed in grains treated with 0.6, 0.8 and 1.0 ml respectively at 96 h (Table 1).

Table 1. Contact toxicity of terpinolene against adult *R. dominica*

Treatments	Dosage (ml)	% mortality in hours			
		24	48	72	96
Terpinolene	0.2	8.00±3.74ab	38.00±3.74b	56.00±5.10b	70.00±3.16b
	0.4	24.00±4.00bc	58.00±3.74c	72.00±3.74cd	82.00±3.74bcd
	0.6	32.00±3.74cd	64.00±4.00c	82.00±4.90cde	94.00±4.00de
	0.8	60.00±3.16e	90.00±4.47d	96.00±2.45ef	100.00±0.00e
	1.0	62.00±5.83	90.00±4.47d	98.00±2.00ef	100.00±0.00e
DDVP	0.1	58.00±5.83e	100.00±0.00d	100.00±0.00f	100.00±0.00e
Negative control	0.0	0.00±0.00a	0.00±0.00a	4.00±2.45a	14.00±2.45a

Each value is mean ± standard error of five replicates. Mean followed by the same alphabet are not significantly different (p > 0.05) using Tukey Honestly Significant Test.

Fumigant toxicity of terpinolene against adult *R. dominica*

The mortality of *R. dominica* exposed to fumes of different dosages of terpinolene was presented in Table 2. The result shows that, adult insect mortality increased with increase in dosage of the treatments and period of exposure. At 0.2 ml dosage, above 70.0% adult mortality was recorded after 72 h of exposure and same trend was equally observed in 0.8 and 1.0 ml dosage 48 h of exposure (Table 2). There was significant difference (*P*>0.05) in adult *R. dominica* mortality among the grain treated, causing between 2.00 and 92% mortality at all dosage of terpinolene within 24 and 48 h period of exposure. Meanwhile, non-significant difference (*P*<0.05) exists in the mortality recorded at 96 h period of exposure in treated grains irrespective of the dosage rates. Similar results were obtained from grains treated with 0.6, 0.8 and 1.0 ml terpinolene at 72 h exposure respectively. However, the treatment was able to evoked 100% adult mortality 72 and 96 h exposure period with the exception of grains treated with 0.2 and 0.4 ml respectively (Table 2).

From the result presented in Table 2, it is noted that the treatments were not significantly different from the positive control. While, regardless of the dosages used, the effects of the treatments on the adult insect mortality were significantly different from the negative control.

Table 2. Mortality of adult *R. dominica* exposed to fumes of terpinolene

Treatments	Dosage (ml)	% mortality in hours			
		24	48	72	96
Terpinolene	0.2	0.00±0.00a	32.00±3.74b	76.00±10.30b	98.00±0.00b
	0.4	2.00±2.00a	36.00±2.45b	78.00±9.16b	100.00±0.00b
	0.6	24.00±5.10b	56.00±6.78c	100.00±0.00c	100.00±0.00b
	0.8	48.00±4.90cd	76.00±2.45de	100.00±0.00c	100.00±0.00b
	1.0	64.00±4.00de	92.00±3.74f	100.00±0.00c	100.00±0.00b
DDVP	0.1	58.00±5.83cd	100.00±0.00f	100.00±0.00c	100.00±0.00b
Negative control	0.0	0.00±0.00a	0.00±0.00a	4.00±2.45a	14.00±2.45a

Each value is mean ± standard error of five replicates. Mean followed by the same alphabet are not significantly different (p > 0.05) using Tukey Honestly Significant Test.

Lethal dose of terpinolene required to achieve 50 and 95% mortality of *R. dominica* within 24 h

The lethal dosage of terpinolene required to realize 50 and 95% adult mortality of *R. dominica* in both contact and fumigant toxicity was presented in Table 3 and 4 respectively. The result shows that lower dose (3.75 and 4.17 ml) of terpinolene compound was required to achieve 50% adult beetle mortality and substantial amount (16.18 and 8.18 ml) of the compound administered resulted in 95% mortality of the beetle within 24 hours of application respectively for both contact and fumigant toxicity of terpinolene. Thus indicating that lower dosage of terpinolene compound was more in achieving 50% mortality of the insect within 24 hours.

Table 3. Lethal dosage of terpinolene against *R. dominica* at 24 h post treatment

Treatments	Slope ± S.E	Intercept ± S.E	X^2	LD_{50} (FL 95%)	LD_{95} (FL 95%)	Sig.
Terpinolene	2.59±0.13	-1.49±0.07	135.30	3.75(3.31-4.39)	16.18(11.33-28.93)	0.0001

X^2: Chi-square; SE: Standard error; FL: Fiducial limits; LD: Lethal dosage

Table 4. Lethal dosage of terpinolene fumes against *R. dominica* at 24 h post treatment

Treatments	Slope ± S.E	Intercept ± S.E	X^2	LD_{50} (FL 95%)	LD_{95} (FL 95%)	Sig.
Terpinolene	5.63±0.27	-3.50±0.16	97.94	4.17 (3.94-4.46)	8.18 (7.10-10.07)	0.0001

X^2: Chi-square; SE: Standard error; FL: Fiducial limits; LD: Lethal dosage

Adult emergence, percentage inhibition rate of *R. dominica* and weight loss of wheat grains exposed to terpinolene contact and fumigant toxicity

Adult emergence and percentage inhibition rate of *R. dominica* exposed to different dosages of terpinolene as well as the ability of the insect to cause weight loss of treated wheat in both contact and fumigant toxicity were presented in Table 5 and 6. *R. dominica* exhibited dose dependent response to terpinolene application. The adult insect emergence and percentage weight loss of the stored grains decreased with increase in treatment dosages while percentage IR increased with an increase in treatment dosages. In all rate applied, 1.0 ml demonstrated the strongest activities in suppressing adult emergence and weight loss while at the same time exhibited the highest inhibition rate. However, only the positive control was able to completely suppress the adult insect emergence, seed weight loss and 100% inhibition of the insect. Tukey post hoc test showed that significant ($p < 0.05$) differences existed between the various dosages of terpinolene in relation to positive and negative control.

Table 5. Adult emergence of *R. dominica*, percentage inhibition and seed weight loss by contact toxicity test

Treatments	Dosage (ml)	Adult emergence	% IR	% weight loss
Terpinolene	0.2	42.00±1.64e	38.60±2.40b	15.36±0.42e
	0.4	35.20±0.92e	48.54±1.34c	11.08±0.98d
	0.6	27.40±2.18cd	59.94±3.19d	7.21±0.58c
	0.8	14.20±1.50b	79.24±2.19e	4.91±0.70bc
	1.0	5.80±1.16a	91.52±1.69f	2.67±1.04ab
DDVP	0.1	0.00±0.00a	100.00±0.00f	0.00±0.00a
Negative control	0.0	68.00±2.65f	0.00±0.00a	41.70±1.23f

Each value is mean ± standard error of five replicates. Mean followed by the same alphabet are not significantly different ($p > 0.05$) using Tukey Honestly Significant Test.

Table 6. Adult emergence of *R. dominica*, percentage inhibition and seed weight loss caused by the insect in fumigant toxicity test.

Treatments	Dosage (ml)	Adult emergence	% IR	% weight loss
Terpinolene	0.2	45.80±0.80f	33.04±1.17b	23.96±1.30h
	0.4	38.20±1.07e	44.15±1.56cd	19.25±0.98fg
	0.6	29.20±1.39c	57.31±2.04ef	15.25±0.61ef
	0.8	26.40±0.50c	61.40±0.75ef	9.18±1.46cd
	1.0	17.60±1.63b	74.27±2.38g	5.20±0.40bc
DDVP	0.1	0.00±0.00a	100.00±0.00h	0.00±0.00a
Negative control	0.0	68.00±2.65g	0.00±0.00a	41.70±1.23i

Each value is mean ± standard error of five replicates. Mean followed by the same alphabet are not significantly different ($p > 0.05$) using Tukey Honestly Significant Test.

Discussion

Stored food grains represent a major percentage of agricultural produce but its storability has been hampered by numerous insect species under storage condition; causing colossal economic and nutritional damages. Utilization of plant derived bioactive compounds with insecticidal properties is becoming an attractive option in contrast to the costly and hazardous synthetic insecticides and fumigants.

In the present exploration, terpinolene compound exhibited as grains preservative in the protection of stored wheat against insect infestation by exerting significant adult mortality and suppressing adult emergence, weight loss and enhanced inhibition rate.

The survivability of the adult insect in both contact and fumigant toxicity has been negatively impaired with the application of terpinolene. From the outcome, it is apparent that the insect survival percentage decreased with increased concentration of terpinolene and with exposure period. Thus indicating that, the insect mortality was directly connected to the exposure time and concentration of terpinolene.

The result of the contact toxicity did not follow the same trend as observed in the fumigant toxicity. In contact toxicity, 100% mortality was achieved at 96h exposure time using 0.8 and 1.0ml dosage. While, 100% mortality was recorded at 96 and 72h in insect exposed to the fumes of 0.4, 0.6, 0.8 and 1.0ml dosages respectively.

The disparity observed between the contact and fumigant toxicity might be due to the fact that the adult insect had no escape route from the fumigation chamber and the insects were constrained to absorb the dosage of the toxic compound into their body system via their spiracles causing respiratory impairment due to hindrance in normal supply of oxygen to the trachea. This agrees with opinion of Babarinde *et al*., (2019). The result obtained from contact toxicity can be ascribed to the possibility of the bioactive compound been absorbed by the grains; hence, could not be available for the envisaged bioactivity. This result generally supports the findings of Babarinde *et al*., (2019) who infers that the bioactivity of *Jatropha curcas* seed oil against *Sitophilus zeamais* relatively depends on the bioassay type and methods of application.

Probit analysis showed that all the dosage tested would be more or less effective for controlling lesser grain borer. The lowest LD_{50} values of the compound specified that the highest toxic effects against the insect subdue their infestation and damage in treated wheat grains. This is in tandem with similar observation by Ileke and Oni (2011) and Adesina *et al.,* (2020).

Conclusion and Recommendation

This study has shown that all treated grains irrespective of dosage, recorded considerable significant reduction in terms of percentage weight loss caused by the insects than the untreated control. This reduction could be attributed to low adult insect survival, adult emergence, high inhibition rate in treated grains and compared to high adult insect survival, adult weevil emergence and low inhibition rate in the negative control. Attractive potentials of terpinolene as plant derived insecticides against *R. dominica* was demonstrated. Thus alluding to the fact that botanicals is worth developing as protectants of stored food grains against insect pest infestation and consequently can serve as an inexpensive alternative for synthetic insecticides.

This study therefore suggests characterization and identification of plant bioactive compounds present in other plant materials with insecticidal properties for their development as plant derived insecticides for the management of field and store produce pests.

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