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Research Article

Biological activity of the leaf extract of *Tetrapleura tetraptera* on *Rhyzopertha dominica* (Bostrichidae: Coleoptera) in stored wheat

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Abstract: The bioactivity of the aqueous extracts of the leaf of *Tetrapleura tetraptera* against the wheat storage pest, *Rhyzopertha dominica,* on stored wheat grains was investigated in laboratory conditions. *T. tetraptera* leaf extracts were added to 20.0 g of grains at 25.0 mg/l, 50.0 mg/l, and 100.0 mg/l to assess contact toxicity, damage assessment, reproductive performance, and repellency ability. Results showed that the extracts were toxic to the insects. The leaf extracts applied at 100.0 mg/l significantly caused the highest mortality of 94% after 21 days. It also repelled almost 90% of the insects and inhibited adult emergence up to 98%. The 100.0 mg/l of the leaf extract further offered protection of nearly 99% of the wheat grains against insect damage compared to the control. This study revealed that leaf extracts of *T. tetraptera* can be used to control *R. dominica* in stored grains efficiently, and its incorporation into traditional storage pest management is strongly recommended in developing countries

Keywords: *Rhyzopertha dominica*, grain protection, *Tetrapleura tetraptera*, insect perforation index

Introduction

Wheat is a cereal staple that is grown all over the world (Belderok, 2000). It consumes more land than other food crops (FAO, 2014). Wheat production in 2020 was 761 million tonnes (1.7 trillion pounds), making it the second most-produced cereal after maize (FAO, 2014). Wheat is a good source of carbohydrates (Shewry and Hey, 2015). With a protein content of roughly 13%, it is the world's leading source of vegetable protein in human meals, which is relatively high compared to other major cereals (FAO, 2017). When consumed as a whole grain, wheat contains various nutrients and dietary fiber (Shewry and Hey, 2015).

Although wheat is critical to food security in Africa and Ghana, its storage is jeopardized by various insects (Ileke, 2011). These insect pests are to blame for significant losses in stored wheat. In developing countries, storage loss due to insect infestation has reached up to 70% (Kavita, 2004). These insect pests harm cereals quantitative and qualitatively (Fornal *et al.*, 2007). The lesser grain borer *Rhyzophertha dominica* Fabricius (Bostrichidae: Coleoptera) is one of wheat's most damaging insect pests.

R. dominica is a grain pest that causes havoc, both in larval and an adult stages (Raju, 1984). The adults are strong fliers that move from one warehouse to another, generating new infestations. When the infestation is

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severe, the adults produce a large amount of excreta, contaminating more than just what they eat (Atwal, 1994). The flour created in this manner feeds the baby grubs until they are ready to bore into the grain. It reduces the amount and quality of grain and its products (Atwal, 1994).

Attempts to manage this deleterious wheat bug pest primarily relied on synthesized insecticides. However, these synthesized compounds are not without health and environmental risks (Babarinde *et al*., 2008). Aside from synthesized insecticides' health and environmental hazards, applicators' misuse and overuse have resulted in severe issues such as insecticide resistance, toxic residues on stored products, health risks to handlers, food poisoning, and environmental pollution (Ali, 2009). It has also led to the development of resistance (DeSilva *et al*., 1997), adverse effects on non-target organisms (Hayes and Laws, 1991), prompting a quest for alternate control strategies.

Botanical pesticides have recently been tested to control insect pests and vectors (Khanna *et al.*, 2011). They tend to have minimal environmental persistence and lesser impacts on human health than conventional insecticides (Mohammadpour *et al.*, 2020). However, more concerted attempts have been made to produce environmentally friendly chemicals suitable for use in the field and large-scale pest control operations.

Tetrapleura tetraptera is one such plant. The goal of this study is the biological activities of the plant's leaf extract against *R. dominica* in preserved wheat under laboratory conditions.

Materials and Methods

Insect culture

The wheat for the experiment was purchased at the Winneba market in the Central Region of Ghana. The grain was placed in various jars and covered with muslin cloth before adult *R. dominica* was introduced. The jars were kept at room temperature in the Biology Education Department laboratory at the University of Education, Winneba, so that the insects could breed and multiply at 30 ± 2 °C, 70 ± 5% RH, and a photoperiod of 12:12 h (L: D). The wheat moisture content was adjusted from 12% to 13% (Tapondjou *et al*., 2002). After three weeks of oviposition, the parent insects were sieved out. The grains were later kept in the laboratory for adult emergence while the emerging generation of same-age insects was re-cultured at 30 ± 2 °C, 70 ± 5% RH. The F1 generation was used in the experiments.

Collection and preparation of plant materials

Leaves of *T. tetraptera* were collected from the Agona Swedru area of the Central Region of Ghana. The leaves were rinsed in clean water to remove sand and other impurities before being air-dried at room temperature for 15 days in the laboratory. Using an electric blender, they were pulverized into a fine powder. The powders were then sieved again to pass through perforations of $1mm^2$. To prevent the active ingredients from being lost, the powders were packed in plastic containers with tight lids and stored in the laboratory before use.

Plant material extraction

The extraction was carried out in the Chemistry Education laboratory of the University of Education, Winneba. 400 g of *T. tetraptera* were soaked separately in a 500 ml bottle of absolute n-hexane for three days. The mixture was occasionally stirred with a glass rod, and the extraction was completed after three days. Filtration was performed using a double layer of Whatman No. 1 filter papers, and the solvent was evaporated for 8 hours using a rotary evaporator at 30 to 40 ºC with a rotary speed of 3 to 6 rpm (Udo, 2011). The resulting extracts were air-dried to remove any remaining solvent. The extracts were stored in labeled plastic bottles until they were needed.

Standard stock solution preparation

4 g of crude extracts were dissolved in 1 Litre of water to make standard stock solutions. Different concentrations of 25 mg/l, 50 mg/l,

and 100 mg/l aqueous solutions were prepared from the stock solution and used in the various experiments.

Contact toxicity of leaf extract

The experiment was conducted in a Randomized Complete Block Design (RCBD) with three replications. Forty unsexed *R. dominica* were placed in clean sterilized 250ml plastic containers containing 20.0g of uninfected sterilized wheat at 0.0, 25.0, 50.0, and 100.0 mg/l of *T. tetraptera* leaf extract. At the same time, distilled water was used as the control treatment. An atomizer was used to spray the leaf extract on the wheat grains, then thoroughly shaken to ensure uniform coating. As a ventilated lid, the jars were covered with muslin cloth and secured with rubber bands. The treated grains in the jars were kept for about 21 days, and mortality rate assessments were performed regularly after exposure to *T. tetraptera* leaf extract at 1, 7, 14, and 21 days. Adults were considered dead when probed with blunt objects, and there were no responses (Obeng-Ofori *et al.*, 1997). Adult mortality percentage was corrected using the Abbott (1998) formula thus:

$$\mathrm{PT} = \frac{\mathrm{Po} - \mathrm{Pc}}{100 - \mathrm{Po}} \times 100$$

Where P_T = Corrected mortality (%)
P_o = Observed mortality (%)
P_c = Control mortality (%)

Effect of leaf extract on reproductive performance

The experimental setup was kept inside the laboratory for another 30 days to allow the first filial (F1) generation to emerge. Freshly emerged adult *R. dominica* were counted and documented from the sieved containers. The percentage of adult emergence was calculated using the method of Odeyemi and Daramola (2000).

$$\%\ \text{Adult emergence} = \frac{\text{Total number of adult emergence}}{\text{Total number of eggs laid}} \times 100$$

Damage assessment

The percentage weight loss of the wheat grains was determined by re-weighing after 35 days, and the % loss in weight was determined using the method of Obeng-Ofori *et al*. (1997) as follows:

$$\%\ \text{Weight loss} = \frac{\text{Change in weight}}{\text{TInitial weight}} \times 100$$

After re-weighing, the numbers of damaged wheat grains were evaluated by counting wholesome grains and grains with insect emergent holes. The percentage of grain damaged was calculated using the method of Obeng-Ofori *et al*. (1997) as follows:

$$\%\ \text{Grain damaged} = \frac{\text{Number of grains damaged}}{\text{Total number of grains}} \times 100$$

The Insect Perforation Index (IPI) used by (Fatope *et al*., 1995), quoted by (Ileke, 2015), was adopted for the analysis of the damage. IPI was defined as follows:

$$IPI = \frac{\%\ \text{treated wheat grains perforated}}{\%\ \text{control wheat grains perforate}} \times 100$$

IPI value exceeding 50 was regarded as an enhancement of infestation by the beetle or negative protectability of the plant material tested.

Repellency assessment

The repellency of the plant extract against *R. dominica* was evaluated using the preferential zone on a filter paper method described by (McDonald *et al*., 1970) with some minor modifications. Whatman filter paper (No. 10) was used to line a petri dish. Using an HB pencil, the paper was divided into three equal zones along the diameter of the petri dish. In a clean glass jar, 10 unsexed adult insects were starved for 24 hours. 30.0 g of sterilized wheat grains were placed in the Petri dish's two extreme zones. *T. tetraptera* leaf extract (0.0, 25.0, 50.0, and 100.0 mg/l) was added to one heap of grain in one of the Petri dish's extreme zones. After 10 minutes, the number of insects moving into the two extreme zones was recorded using 10 starved adult wheat beetles placed in the center of the central zone of the divide. The experiment was repeated three times for each dose of plant extracts in CRD. Alzouma (1992) proposed the formula to calculate the percentage repellency.

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$$PR = \frac{\mathrm{NC} - \mathrm{NT}}{\mathrm{NC} + \mathrm{N}} \times 100$$

Where: NC – number of insects in the controlled zone (no plant extract).

NT – number of insects in the treated zone (plant extracts available).

PR – percent repellency. The PR was ranked in six different classes as described by.

(McDonald *et al*., 1970) as shown below (Table 1):

Table 1 Percentage repellency (PR) classes ranked by (McDonald *et al*., 1970).

Class	PR proportion (%)	Description
O	PR < 0.01	Not repellant
I	0.1 < PR ≤ 20	Fair repellant
II	20.1 ≤ PR ≤ 40	Moderate repellant
III	4.01 ≤ PR ≤ 60	Good repellant
IV	60.1 ≤ PR ≤ 80	Very repellant
V	80.1 ≤ PR ≤ 100.0	Perfect repellant

Less than one percent repellency was considered zero (Obeng-Ofori and Akuamoah, 2000). The data from the repellency test was analyzed using the chi-square test to determine the repellency activity of the various plant extract doses and the insect susceptibility. PR_{50} was calculated using the Finny (1971) method, based on the probit regression of mortality as a function of plant extract dose logarithm.

Statistical analysis

ANOVA was used to analyze the data, and treatment means were separated using the new Duncan's Multiple Range Test. The ANOVA was carried out using the SPSS 16.0 software.
Before analysis, egg counts, damaged and undamaged seeds were square root transformed, and percentages were arcsine transformed. The LSD test was used to separate the means of the results ($p \leq 0.05$) (Zettler and Coperus, 1990).

Results

Contact toxicity extracts

The percentage of mortalities of *R. dominica* in wheat treated with various doses of *T. tetraptera* leaf extract is shown in Table 2. All of the doses were effective against the insects. Toxicity increases as *T. tetraptera* leaf extract concentrations increase. However, at all doses, less mortality was found one day after insect exposure to the plant extract. When the insects were treated with the lowest dose (25 mg/l) of the plant extract, there was very little death within 21 days. However, when the insects were exposed to the highest dose of the extract (100 mg/l), there was a higher mortality rate.

The protection ability of the extracts

The level of protection provided by *T. tetraptera* leaf extracts is shown in Table 3. There were significant differences ($P < 0.05$) among the treatments in reducing the damage caused by the stored product pest. The 100 mg/l of *T. tetraptera* leaf extract provided the highest protection (weight loss and seed damage) and prevented the insects' perforation of the wheat seeds. The 25 mg/l of *T. tetraptera* leaf extracts provided the lowest protection and perforation index.

Fecundity of *R. dominica* treated with extracts.

Table 4 shows oviposition and percent progeny development of *R. dominica* after different plant extracts were used as contact insecticides. Various plant extracts greatly inhibited progeny development, with the 100.0 mg/l dose virtually preventing *R. dominica* emergence.

Repellent action of leaf extract

The repellence of *T. tetraptera* leaf extracts to *R. dominica* varied according to the dose. Table 5 shows that the 100 mg/l dose had the highest repellence of 90 percent, while the 25 mg/l dose had the lowest repellence of 63 percent.

Table 2 Percentage mortality of adult *Rhyzopertha dominica* treated with *Tetrapleura tetraptera* leaf extracts.

Dose (g)	Mortality + SE (%)			
	1	7	14	21
Control	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a
25	18.00 ± 2.41b	24.20 ± 3.10b	53.31 ± 4.49b	68.21 ± 2.10b
50	20.04 ± 2.62bc	38.00 ± 2.19bc	67.00 ± 4.34bc	82.14 ± 2.21cd
100	27.7 ± 2.19c	55.20 ± 4.48d	88.24 ± 4.59d	94.42 ± 3.85b

Each value is a mean ± standard error of four replicate means within a column followed by the same letters are not significantly different at (P > 0.05) using New Duncan's Multiple Range Test.

Table 3 Protectability of *Tetrapleura tetraptera* leaves extracts on wheat seeds.

Dose (mg/l)	Total number of seeds	Total number of damaged seeds	% seeds damaged	% weight loss	Weevil perforation index
Control	99.0	45.41 ± 3.10b	44.62 ± 4.12b	77.02 ± 2.44b	52.14 ± 1.01c
25	99.5	5.40 ± 1.07a	4.23 ± 0.07a	4.37 ± 1.40a	4.40 ± 0.49b
50	98.5	2.94 ± 0.13a	2.76 ± 0.25a	2.43 ± 0.17a	4.01 ± 1.13b
100	98.5	0.97 ± 0.01a	0.88 ± 0.01a	0.68 ± 0.04a	0.01 ± 0.01a

Each value is a mean ± standard error of four replicates. Means within column followed by the same letters (s) are not significantly different at (P> 0.05) using New Duncan's Multiple Range Test.

Table 4 Fecundity of *Rhyzopertha dominica* treated with *Tetrapleura tetraptera* on wheat seeds.

Dose (g)	Oviposition	% number of progeny development
Control	51.23 ± 5.54c	87.58 ± 6.51c
25	17.04 ± 1.04b	21.00 ± 2.01b
50	9.54 ± 0.24ab	14.24 ± 1.14b
100	4.51 ± 1.91a	0.03 ± 0.02a

Each value is a mean ± standard error of four replicate means within column followed by the same letters are not significantly different at (P> 0.05) using New Duncan's Multiple Range Test.

Table 5 Repellency caused by *Tetrapleura tetraptera* leaf extract against *Rhyzopertha dominica* after 10 min in petri test of preferential zone.

Dose (mg/l)	Mean (± SE) number of insects in controlled zone	Mean (± SE) number of insects in treated zone	%Repelled
Control	7.40 ± 1.50c	7.40 ± 1.50c	0
25	5.41 ± 0.58b	3.41 ± 0.58b	63
50	6.70 ± 0.58ab	2.30 ± 0.58ab	78
100	9.00 ± 1.00a	0.70 ± 0.57a	90

Each value is a mean ± standard error of four replicate means within column followed by the same letters are not significantly different at (P > 0.05) using New Duncan's Multiple Range Test.

Discussion

Botanical pesticides have proven to be indispensable in the control of insect pests. These botanicals are commercially utilized synthetic pesticide alternatives, and many of them have been used against a wide range of stored product insect pests, including those belonging to the Order Coleoptera and lepidoptera. (Nathan *et al*., 2007).

The current investigation found that greater doses of plant extracts caused the highest proportion of mortality. This is consistent with the findings of Alvi *et al*. (2018), who found that

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Rhazya stricta leaf and seed extracts caused substantial mortality in *R. dominica* and *Trogoderma granarium* under laboratory conditions. The extracts induced insect toxicity by blocking over half of the eggs produced and suppressing progeny development.

Additionally, the extract reduced the weight loss of wheat grains treated with this plant material. The efficacy, however, depended on the amount or concentration of T. tetraptera leaf extract and the exposure time. This efficacy could be attributed to more bioactive chemicals in the extract (White, 1995), which has to be investigated further. The substantial mortality effect of the extracts could be attributed to the insects' incapacity to feed on the wheat grains coated with the extract, resulting in hunger. This indicates that the plant possesses antifeedant effects. Insects killed in *T. tetraptera* leaf extract-treated grains also displayed unfurled metathoracic wings and outstretched elytra. According to (Ileke and Olutuah, 2012), the poisoning was caused by the consumption of treated grains and toxicant inhalation. This shows that the plant extract may have interrupted the insects' normal respiratory functions, resulting in asphyxiation and death (Ileke and Olutuah, 2012).

The effect of the extracts revealed in this investigation on decreasing *R. dominica* offspring development and oviposition can be related to the insects' toxicity and mortality, interfering with the physiological processes of egg formation. This finding is consistent with the findings of Oigiangbe *et al*. (2007), who concluded that extracts of *Alstonia boonei* leaves harmed the survival and growth of *Sesamia calamistis*. Upadhyay and Jaiswal (2007) discovered that botanical pesticides greatly inhibited the development of *Tribolium castaneum* offspring. According to Chaubey (2011), *Piper nigrum* oil inhibited the development of *Callosobruchus chinensis* offspring. The extracts presented in this study may contain compounds responsible for adult insects' inability to emerge, as they have been shown to disrupt growth, reduce larvae survival, and disrupt the insect life cycle. Mukanga *et al*. (2010) previously discovered that *T. vogelli* extracts inhibited the development of the larger grain borer progeny (*Prostephanus truncatus*). The inability of these insects to emerge could be attributed to the mortality of the insect's larvae, which could be caused by the larvae's inability to entirely cast off their exoskeleton, which remained attached to the posterior section of their abdomen. This is consistent with the findings of Oigiangbe *et al.* (2010), who investigated the insecticidal activities of an alkaloid from *Alstonia boonei*. The growth suppression could be due to the plant extracts' toxicity or feeding deterrent characteristics. The insecticidal activity of various plant extracts in inhibiting offspring growth of stored insect pests has been reported by Akhtar and Isman (2004), Erturk (2006), and Suleiman *et al*. (2018).

The toxicity of the extract on the test organism indicates the relative importance of the extracts in preventing *R. dominica* damage to wheat grains. This is consistent with the findings of Adeniyi *et al.* (2010), who discovered that plant extracts from *Vernonia amygdalina*, *Sida acuta*, *Osmium gratissimum*, and *Telfaria occidentalis* were effective against beans weevil. This discovery contributes to knowledge about the efficiency of plant extracts as biopesticides for preserved food.

R. dominica was considerably repelled by *T. tetraptera* leaf extract. The observed repellent action could be attributed in part to the presence of volatile compounds such as terpenoids in *T. tetraptera* leaves (Steentoft, 1988), which are well-known phytophagous insect repellents that act on olfactory receptors in the vapour form (Sintim *et al.*, 2019). Several repellent components in *T. tetraptera* leaves, such as tannins, flavonoids, and starch, could explain their enhanced repellent properties(Steentoft, 1988).

Conclusion

The findings of this study demonstrated that a *T. tetraptera* leaf extract could go a long way toward providing an alternative to the use of chemical insecticides in the storage of wheat grain. More research is needed to investigate the insecticidal potential of bark and root extracts to incorporate them into integrated pest management strategies in developing countries because they have a broad spectrum of action, are locally available, may be less expensive for traditional farmers, and are less harmful to human health and the environment.

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فعالیت بیولوژیکی عصاره برگ *Tetrapleura tetraptera* روی *Rhyzopertha dominica* (Bostrichidae: Coleoptera) در گندم‌های انباری

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چکیده: فعالیت زیستی عصاره آبی برگ *Tetrapleura tetraptera* در برابر آفت انباری *Rhyzopertha dominica* روی دانه‌های گندم انباری در شرایط آزمایشگاهی بررسی شد. عصاره برگ *T. tetraptera* در غلظت‌های ۲۵، ۵۰ و ۱۰۰ میلی‌گرم در لیتر به ۲۰ گرم دانه اضافه شد و سمّیت تماسی، ارزیابی آسیب، عملکرد تولیدمثلی و توانایی دورکنندگی آن مورد بررسی قرار گرفت. نتایج نشان داد که عصاره‌ها برای حشرات سمّی بودند. عصاره برگ با غلظت ۱۰۰ میلی‌گرم در لیتر به‌طور معنی‌داری با ۹۴ درصد بیشترین تلفات را پس از ۲۱ روز ایجاد کرد. همچنین تقریباً ۹۰ درصد از حشرات را دفع کرد و تا ۹۸ درصد از ظهور حشرات کامل جلوگیری کرد. ۱۰۰ میلی‌گرم در لیتر عصاره برگ بیشتر از ۹۹ درصد از دانه‌های گندم در برابر آسیب حشرات در مقایسه با شاهد محافظت شدند. این مطالعه نشان داد که عصاره برگ *T. tetraptera* را می‌توان برای کنترل مؤثر *R. dominica* در انبار استفاده کرد و کاربرد آن در مدیریت تلفیقی آفات در کشورهای در حال توسعه به‌شدت توصیه می‌شود.

واژگان کلیدی: *Rhyzopertha dominica*، *Tetrapleura tetraptera*، حفاظت غلات، ارزیابی خسارت

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