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# Bioactivity of some plant extracts against the lesser grain borer, *Rhyzopertha dominica* (Fabr.) (Coleoptera: Bostrichidae)

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**Abstract**

**The lesser grain borer, *Rhyzopertha dominica* (Fabr.) is an important insect pest of stored cereals in the world. Plant extracts from *Acacia nilotica*, *Artemisia annua* L. and *Thuja orientalis* were tested for their insecticidal activities against this pest. Contact on surface of a Petri dish and ingestion with wheat grains methods as well as, combinations of mixture of these extracts were used against *R. dominica* adult. Plant extract of *A. annua* had higher mortality of *R. dominica* adult than other tested extracts. The mixtures of *A. annua* with *A. nilotica* or *T. orientalis* are converted to more toxic metabolites against *R. dominica* adult than each plant extracts alone. Understanding the mechanism of mixture of plant extract to enhance activity of them can facilitate the creation of artificial blends that optimize their efficacy against insect pests.**

**Keywords:** plant extract, bioassay, lesser grain borer, mixture, mortality, stored pest.

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## 1. Introduction

The Lesser Grain Borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae) is a pest of economic significance of whole cereal grains in the world (Fields, 2006; Toews et al., 2005; Flinn et al., 2004). Control of this kind of insect relies heavily on the use of synthetic insecticides, particularly organo-phosphates. Organophosphorous (OP) insecticides, including malathion, have been used for post harvest insect control which have led to problems such as environmental pollution, increasing costs of application, pest resistance in many insect species including lesser grain borer, and hazard effects on non-target organisms in addition to direct toxicity to users (Pimentel et al., 2009; Santos et al., 2009; Syed et al., 2005; Ma et al., 2004; Guedes et al., 1996). Plants produce secondary metabolites that many of which have insecticidal properties that can be utilized as an alternative to synthetic insecticides (Potenza et al., 2004). Plant extracts and essential oils have traditionally been used to kill or repel stored product insects (Bandeira et al., 2013; Fouad et al., 2012). Plant extracts of several plants displayed considerable toxic, fumigant and repellent effects on adult of *R. dominica* (Ishtiaq et al. 2016; Arifuzzaman et al. 2014). Acacia (*Acacia nilotica*; Family: Fabaceae), sweet wormwood (*Artemisia annua*; Family: Asteraceae) and Thuja (*Thuja orientalis*; Family: Cupressaceae) are ornamental plants that have been used as botanical insecticides against stored grain insect pests (Taghizadeh & Mohammadkhani, 2017; Forouzan et al. 2016; Lawati et al. 2002). Therefore, the present study was undertaken to investigate the insecticidal activity of *A. nilotica*, *T. orientalis* and *A. annua* extracts against *R. dominica* adult.

## 2. Materials and methods

Experiments were conducted in the Entomology Research Laboratory, Department of Plant Protection, Faculty of Agriculture, Sohag University, El-Kwamel, Sohag, Egypt.

### 2.1 Insect culture

Adults of *R. dominica* were taken from laboratory mass cultures reared in glass jars in an environmentally controlled room at 25 ± 2 °C, 70 ± 10% relative humidity and 12:12 photoperiod. The food media used is whole wheat grains.

### 2.2 Plant materials and extraction

Plants of *A. nilotica*, *A. annua* and *T. orientalis* were obtained from local ornamental farm, Sohag, Egypt. Only leaves were used to obtain the extracts. Leaves from each tested plants were handpicked, chopped in pieces, shade dried and powdered. A weight of 250g of the leaf powder was separately added to conical flask and sequentially extracted with one liter of methanol at room temperature 30 °C for 24 hours and filtered using Whatman No.1 filter paper and again the marc was dissolved in methanol for three days twice. Pooled supernatant was then filtered using filter paper. The extracts were evaporated to dryness and concentrated using a rotary evaporator with the water bath set at 40°C (Anshul et al. 2014; Edeoga et al., 2005). The powdered residue were transferred into vials and stored at 4°C in labeled specimen bottles for bioassays.

### 2.3 Residual film method

The contact toxicity of plant extracts and malathion insecticide was tested according to the work described by Fouad and Câmara (2017). Plant extracts of *A. nilotica*, *A. annua* and *T. orientalis* at 100 and 200 µL mL$^{-1}$ concentration and insecticide malathion 57% EC (positive control) at 0.0005 (0.5 ppm) and 0.001 µL mL$^{-1}$ (1 ppm) concentrations were used in this test besides methanol as (control). To study the effect of plant extracts mixture against lesser borer, the extracts were combined in a 1:1 ratio (50 and 100 µL mL$^{-1}$ concentration for each extract) and in 1:1:1 ratio (33.33 and 66.67 µL mL$^{-1}$ concentration for each extract). Using a precision micropipette, 1 mL was applied on the surface of a Petri dish (9 cm diameter, surface area 63.6 cm$^2$). After 15 min, once the solvent had been evaporated, twenty unsexed *R. dominica* adults were placed in each Petri dish. Four replicates were made for each treatment. The dishes were kept in controlled room (25 ± 2°C, 65 ± 5% RH and 12:12 h L: D photoperiod). The mortality (%) was recorded after 24, 48, 72 and 96 h from starting the test.

### 2.4 Toxicity on treated wheat grains

The toxicity of plant extracts of *A. nilotica*, *A. annua*, *T. orientalis* and commercial insecticide malathion 57 EC mixed with wheat whole grains were evaluated by applying 0.5 mL of tested plant extracts at 2.5 and 5 µL g$^{-1}$ concentration and of malathion 57 CE at 0.0001 and 0.0002 µL g$^{-1}$ concentration and the same amount of methanol alone was applied as control using a precision micropipette. Equal amounts of 20 g of wheat whole grains were used in all treatments. To study the effect of plant extracts mixtures against lesser borer feeding on wheat whole grains, the extracts were combined in a 1:1 ratio (1.25 and 2.5 µL g$^{-1}$ concentration for each extract) and in 1:1:1 ratio (0.83 and 1.67 µL g$^{-1}$ concentration for each extract). Jars of 200 mL size were used treatments and solutions or methanol with the wheat whole grains were shaked well for 10 sec. After 15 min, once the solvent had been evaporated, twenty unsexed *R. dominica* adults were separated 24h the rearing culture before the start of experiment. Then insects were left to feed on wheat grains, treated or not treated, in controlled room (25±1 ºC, 70±10% RH and 12:12 h L:D photoperiod). The mortality of beetles was recorded after 24, 48, 72 and 96 h from starting the test (Fouad & Câmara 2017; Tavares et al., 2014).

### 2.5 Statistical analysis

Data from percentage mortality values of different exposure concentrations and times were corrected by Abbot’s formula (Abbot, 1925), then subjected to analysis of variance (one-way ANOVA). Means of treatments were compared at level of 5% probability using the Duncan's Multiple Range Test.

## 3. Results

The efficacy of the tested plant extracts and malathion against *R. dominica* adults by means of mortality in 24, 48, 72 and 96 h are presented in Table 1 and 2 for residual film and ingestion tests. All tested plant extracts were effective to some degree in reducing the number of *R. dominica*. The results of the present study indicated that after 96 h of applications in residual film and ingestion tests, mean percentage of mortality was higher using indoxacrb compared with the other treatments. A mixture of *A. annua*+ *T. orientalis* gave high efficacy of tested plant extracts about 56 % and 60 % of the efficacy of malathion in residual film and ingestion tests, respectively. By considering the mean mortality as a main index, *A. annua* proved to be the most effective of the three tested plants materials against the adult, followed by *A. arabica* and *T. orientalis*. The morality percentages of *R. dominica* were significantly increased with the increase of the exposure times in all tested treatments. In addition, increasing the concentration level of all tested treatments increased the mortality of *R. dominica* adults. The methanol extract of *A. annua* showed insecticide activity with 30.0±3.54 % and 38.25±2.35 % ($F$=35.56; $P$= <0.0001; $F$= 187.95; $P$= <0.0001) mortality of *R. dominica* in contact and ingestion tests at 20 % concentration, respectively (Table 1 and 2).

Table 1: Mean values ± standard errors of % mortality calculated for contact toxicity of plant extracts and their mixtures against *Rhyzopertha dominica* adults after 24, 48, 72 and 96 h of exposure.

| Treatments | Mortality % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 100 µL mL$^{-1}$ | | | | 200 µL mL$^{-1}$ | | | |
| | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| *Acacia arabica* | 3.25±1.18 c | 5.00±0 cd | 8.25±1.18 cde | 11.50±3.12 cd | 6.50±1.19 d | 8.25±1.18 de | 11.50±3.12 de | 12.50±3.23 d |
| *Artemisia annua* | 9.50±0.5 b | 10.0±0 b | 13.0±2.38 bc | 20.0±0 b | 13.75±2.39 c | 17.50±3.23 c | 20.75±1.49 c | 30.0±3.54 bc |
| *Thuja orientalis* | 1.50±1.19 c | 1.50±1.19 d | 5.0±2.4 e | 5.0±2.04 e | 5.0±2.04 d | 6.50±1.19 e | 9.50±2.10 e | 12.0±3.14 d |
| *A. arabica+A. Annua* | 8.25±1.18 b | 8.75±1.25 bc | 15.25±2.06 b | 20.0±2.04 b | 21.75±4.25 b | 28.25±3.12 b | 34.0±1.35 b | 35.75±2.17 b |
| *A. arabica+T. orientalis* | 8.25±1.18 b | 8.25±1.18 bc | 11.75±2.36 bcd | 13.75±1.25 c | 8.35±1.18 cd | 13.75±2.39 cd | 16.50±2.36 cd | 26.50±3.12 c |
| *A. annua+T. orientalis* | 5.0±0 bc | 8.25±1.18 bc | 10.0±0 bcde | 15.0±2.04 bc | 26.50±2.36 b | 33.25±1.18 b | 35.0±2.04 b | 36.50±2.36 b |
| *A. arabica+A. annua+T. orientalis* | 3.25±1.18 c | 5.0±2.4 cd | 6.50±1.19 ed | 6.50±1.19 ed | 5.0±2.04 d | 6.50±1.19 e | 8.25±1.18 e | 15.0±2.04 d |
| Malathion | 0.0005 µL mL$^{-1}$ | | | | 0.001 µL mL$^{-1}$ | | | |
| | 31.25±3.14 a | 41.25±1.25 a | 53.25±1.18 a | 63.50±2.36 a | 38.25±1.18 a | 48.25±1.18 a | 58.25±1.18 a | 65.0±3.53 a |
| F | 42.29 | 109.17 | 82.45 | 90.24 | 28.21 | 55.20 | 77.07 | 35.56 |
| P | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Means ± Standard errors followed by the same letter per column do not differ by the F test (P = 0.05).

Table 2: Mean values ± Standard errors of % mortality calculated for ingestion toxicity of plant extracts and their mixtures against *Rhyzopertha dominica* adults after 24, 48, 72 and 96 h of exposure.

| Treatments | Mortality % | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | 2.5 µL g$^{-1}$ of grain | | | | 5 µL g$^{-1}$ of grain | | | |
| | 24 h | 48 h | 72 h | 96 h | 24 h | 48 h | 72 h | 96 h |
| *Acacia arabica* | 6.50±1.19 a | 6.50±1.19 cd | 6.50±1.19 e | 8.25±1.18 d | 8.25±1.18 e | 8.25±1.18 d | 9.25±0.48 d | 13.25±1.97 de |
| *Artemisia annua* | 15.0±2.04 bc | 15.0±2.04 c | 21.50±1.19 c | 23.25±1.18 c | 15.0±2.04 d | 18.25±3.12 c | 26.50±2.36 c | 38.25±2.35 c |
| *Thuja orientalis* | 5.0±0 d | 5.0±0 d | 6.50±1.19 e | 8.25±1.18 d | 6.50±1.19 e | 6.50±1.19 d | 8.25±1.18 d | 10.25±0.63 e |
| *A. arabica+A. Annua* | 10.0±0.82 cd | 13.50±2.36 cd | 16.50±4.72 cd | 23.50±2.36 c | 25.0±3.53 c | 33.50±1.18 b | 41.75±1.18 b | 48.75±1.25 b |
| *A. arabica+T. orientalis* | 8.25±1.18 d | 11.75±3.12 cd | 11.75±3.12 de | 13.50±2.36 d | 19.75±1.93 cd | 21.50±3.12 c | 28.25±1.18 c | 34.0±1.35 c |
| *A. annua+T. orientalis* | 20.0±4.08 b | 26.50±6.24 b | 31.25±4.27 b | 41.25±1.25 b | 33.50±1.19 b | 36.50±1.19 b | 43.50±1.19 b | 50.50±2.10 b |
| *A. arabica+A. annua+T. orientalis* | 6.50±1.19 d | 6.50±1.19 cd | 10.0±0 de | 11.75±1.18 d | 6.50±1.19 e | 8.25±1.18 d | 11.75±1.18 d | 17.25±2.50 d |
| Malathion | 0.0005 µL g$^{-1}$ of grain | | | | 0.001 µL g$^{-1}$ of grain | | | |
| | 36.50±3.12 a | 43.50±1.19 a | 56.50±2.36 a | 63.50±2.36 a | 48.25±1.18 a | 51.75±1.18 a | 63.50±2.36 a | 83.50±1.19 a |
| F | 25.24 | 21.77 | 38.18 | 125.06 | 63.81 | 75.63 | 166.99 | 187.95 |
| P | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

Means ± Standard errors followed by the same letter per column do not differ by the F test (P = 0.05).

Maximum mortality was recorded in residual film test with 20% concentration after 96 h of treatment 36.50±2.36 % and 35.75±2.17 % for *A. annua* and *A. arabica* + *A. annua*, respectively, while the minimum mortality was 12.50±3.23 % and 12.0±3.14 % for *A. arabica* and *T. orientalis*, respectively (Table 1). In ingestion test, it is evident that the total mean % mortality of *R. dominica* adults for treatment *A. annua+T. orientalis* was 41.25±1.25 at concentration of 10% , and 50.50±2.10 at concentration of 20% after 96 h (Table 2).

## 4. Discussion

Results of the present investigation indicated that botanical derivatives might be useful as store insect control agents for commercial use. The efficiency of the plant extracts on mortality of *R. dominica* adults shown in Table 1 and 2 for the different time periods. Mortality was noted with different individual plant extracts, mixtures of extracts and exposure times. The tested plant extracts showed slight toxicity against *R. dominica* adults. The most effective mortality was observed in extract of *A. annua,* and *T. orientalis* extract was the least effective against of *R. dominica* (Table 1 and 2). The binary mixtures of *A. annua* with *A. Arabica* or *T. orientalis* synergized the toxicity against *R. dominica* adult. The toxicity of these plant extracts against *R. dominica* could depend on several factors among which are the chemical composition of the plant extract and insect susceptibility. Lawati et al., (2002) reported the extract of *A. nilotica* in methanol was toxic causing high mortality to *Callosobruchus chinensis*. Negahban et al. (2007) reported that the essential oil of *A. sieberi* demonstrated fumigant toxicity to *Callosobruchus maculatus* (L.), *Sitophilus oryzae* (L.) and *Tribolium castaneum* (Herbst). Artemisinin in the leaves of *A. annua*, the natural compound that offered by the plant, have natural bitterness that gives protection for stored products against storage pests (Harnisch, 1980). Essential oil of *A. annua* was found to inhibit the fecundity and fertility of the Indian meal moth *Plodia interpunctella* adults (Maggi et al., 2005). Tripathi et al. (2000) reported that there was a significant effect of *A. annua* oil on mortality of *T. castaneum* and *C. maculatus*. Extract from *T. orientalis* had significantly more antifeedant activity against *T. castaneum* (Taghizadeh & Mohammadkhani, 2017). Foliar application of *T. orientalis* extract on maize was very effective against *Chilo partellus* (Anju and Sharma 1999). Application of essential oil of *Thuja occidentalis* lead to 95% mortality of females and 100% of males of *C. maculatus* after 6 h exposure (Kéïta et al. 2001). In the present study mixtures of *A. annua* with *A. arabica* or *T. orientalis* are more toxic metabolites against *R. dominica* adult than these extracts alone. Synergists are among the most straightforward strategy for overcoming metabolic resistance because they can directly inhibit the resistance mechanism itself. Efficacy of plant extract or oil is affected by proportion of chemical constituents and synergism or antagonism among them (Sampson et al., 2005; Hummelbrunner & Isman, 2001). Synergistic effect of complex mixtures is very important in plant defense against herbivores. It is often observed that the mixtures of plant extracts or essential oils are more efficient than the plant extract, essential oil or pure compounds alone (Chaubey, 2012; Ho et al., 1997; Don-Pedro, 1996). A study to improve the effectiveness of botanical derivatives as insecticides will benefit the agricultural sectors of developing countries as these substance are not only of low cost, but also have less environmental impact in terms of insecticidal hazard. The mixture of plant extracts together is more effective due to the fact that the insecticidal spectrum of some binary mixtures is increased. More studies on a larger scale are needed to develop binary mixtures more effective to control insect pests in order to be utilized in an IPM system.

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