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BIO-EFFECTS OF CINNAMON (*Cinnamomum zeylanicum*) BARK EXTRACTS AGAINST *Rhizopertha dominica* (F.) (COLEOPTERA: BOSTRICHIDAE)

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ABSTRACT

Ceylon cinnamon bark extracts were evaluated under constant conditions against *Rhizopertha dominica* adults and the effective lethal concentrations (LC50 and LC95) of each extract were calculated. Effects of LC50 and LC95 on both reduction of progeny (%) and weight loss (%) were inve stigated. Also the persistent effect of LC95 of the tested extracts was done weekly on both adult mortality (%) and grain viability (%). Results showed that percent adult mortality increased with increase of the concentration and exposure time. Chloroform extract was the most effective than other extracts, since its lethal effective concentration (LC50) reached a minimum value of 1.8 ml/Kg, while the acetone extract was the least effective which its LC50 was 3.57 ml/Kg. All tested extracts significantly reduced adult progeny (%) and weight loss (%) compared to control. Tested extracts persisted effective on adult mortality (%) for two months and dropped suddenly thereafter for all extracts. Tested extracts slightly affected germination (%) of wheat treated with LC95 value either initially or at end of the storage compared to control. Cinnamon extracts could be used as wheat protectant for wheat from *R. dominica* attack.

INTRODUCTION

The annual worldwide post harvest losses caused by insect damage, microbial deterioration and other factors are a serious problem. Insect infestation of stored grains and their products is a problem all over the world, since it reduces crop yields, pollutes stored products, damages seed germs and reduces quality of the stored products. Chemical control of insects in storage has been given rise toxic residues, increasing costs of application, environmental pollution, and hazards from handling (Ahmed *et al.*, 1981). These problems created worldwide interest to search for alternative strategies such as botanical insecticides as safe, effective insecticides and easily biodegradable. Some indigenous plants have been used for keeping human beings, food materials and woolens from insect attack (Golob and Webley, 1980; Ahmed *et al.*, 1981; Malik and Naqvi, 1984; Chander and Ahamed, 1987; Patel and Valand, 1994; Tiwari, 1994; Jacob and Sheila 1995; EL-Lakwah *et al.*1997; Al- Moagel and Addel-Baki, 2000; Umotok, 2000, Abdel- Latif, 2004). *Rhizopertha dominica* is a serious primary pest of stored cereal grains, since it causes severe damage by their adult and larval stages. This work evaluates the biological effects of Ceylon Cinnamon, *Cinnamomum zeylanicum* bark extracts against *R. dominica*.

MATERIALS AND METHODS

1. Test insect: The lesser grain borer, *R. dominica* (F.) was the test insect. The adult insects were reared in glass jars on wheat grains (Giza 168 variety) under constant conditions of 28±1 °C and 65± 5% R.H. for several generations at Stored Grain Pests Division, Plant Protection Research Institute, ARC, Egypt. In all tests, adults of 1- 2 weeks old were used. Wheat samples were disinfested by freezing at -18 °C for 3 weeks and then conditioned for other two weeks to equilibrate their moisture content.

2. Source of the cinnamon and the extraction method: Cinnamon, *C. zeylanicum* (Family: Lauraceae) bark was purchased from a local supermarket, grounded into a fine powder by an electrical home mill for ten minutes, sieved for obtaining a very fine powder. About two hundred grams of ground material were placed with 400 ml from one of the three different organic solvents (Petroleum ether, chloroform or acetone) separately in a large glass flask and left for two days with continuous mixing as described by Su (1985) and Afifi (1989). The extract was filtered and concentrated by evaporating the solvent at 30- 40 °C under reduced pressure by a rotary evaporator. The obtained extract was in the form of a crude oily gum and considered of 100% concentration.

3. Bioassay: Primary tests made to determine toxicity of different concentrations of each extract on *R. dominica* adults by direct mixing with wheat grains. Toxicity of each extract was determined by adding one ml of each stock crude extract to 10 ml of the same solvent of its extraction. Grains (10 g) were mixed with each extract at concentrations of (1.0, 2.0, 3.0, 5.0 and 6.0 ml/kg); (1.0, 2.0, 3.0, and 6.0 ml/kg); (1.0, 4.0, 6.0, 7.0 and 8.0 ml/kg) of the three extracts respectively, then shaked in glass vials and left to dry. Twenty five adults of 1- 2 weeks old *R.dominica* was introduced into each vial covered with muslin and held by a rubber band. Each concentration was replicated three times and all replicates were kept at constant conditions of 28± 1°C and 65± 5 R.H. The same number of untreated wheat served as control. Adult mortality counts were recorded after 1, 3, 5, 7, and 14 days and its corresponding percentages were calculated and corrected with Abbott's formula (1925). Mortality (%) was computed three days after treatment according to Finney (1952) for determining the lethal effective concentrations (LC50 and LC95) and toxicity slope for each extract.

4. Weight loss (%) and progeny as affected by extract treatment:

Three replicates (each with10 gm wheat) were treated with LC50 or LC95 of each extract, infested with 25 *R.dominica* adults for two weeks, and placed under the same previous conditions until adult emergence. Two similar groups with untreated wheat, each of three replicates were made: one group left without insect infestation for assessing moisture content and considered as initial dry weight, while the other group was

infested with 25 adults as untreated control and both kept under the same previous conditions. One month later, vials were examined for progeny number, separated and counted at regular intervals of two days until ceased. Reduction (%) of adult emergence was calculated compared to control of the two lethal effective concentrations of the tested extracts as follows:

$$\text{Reduction (\%)} = \frac{\text{Progeny in control - progeny in Treatment}}{\text{Progeny in control}} \times 100$$

Replicates were reweighed again after no adult emergence (and after subtracting its contained moisture content) and considered as final dry weight, to calculate the weight loss (%) on the dry weight basis compared to uninfested sample according to Khare and Johari (1984) as follows:

$$\text{Weight loss(\%)} = \frac{\text{Initial dry weight - final dry weight}}{\text{Initial dry weight}} \times 100$$

4. Persistent effect: Thirty replicates (of ten gm each) were treated with LC95 value of each extract and stored under 28±1 °C and 65±5 % R.H. Other thirty similar untreated replicates were made for comparison. Every week, three replicates were withdrawn from each of the different extracts and control, each replicate was infested with 25 adults of *R.dominica*, newly emerged (1-2 weeks old). Adult mortality (%) was determined three days later and corrected with Abbott's equation (1925). Effects of tested extracts at the maximum lethal concentration (LC95) on the wheat viability (% germination) were also screened as follows: 25 treated grains were planted on a moistened cotton pad within Petri dish of 15.0 cm diameter according to Anonymous, (1965). Four replicates were made for each extract beside untreated ones. Germination count was made one week later and its percentage was determined. Data analyzed statistically using ANOVA and means separated by Duncan multiple range tests (Duncan, 1951) using a computer software.

RESULTS AND DISCUSSION

The following results clarify effects of the different crude extracts (petroleum ether, chloroform and acetone) of cinnamon bark on *R.dominica* adults.

Data of Table 1 indicated that adult mortality (%) values increased with the increase of concentration and time of exposure. After three days from treatment, adult mortality (%) ranged from 30- 95, 25- 90, and 20- 95 for petroleum ether, chloroform and acetone extracts respectively. After five days, the corresponding values were 39- 100, 49- 100 and 38- 100, with complete mortality after 2 weeks at the most concentrations of each extract.

Table 1. Effect of cinnamon bark extracts on adult mortality (%) of *Rhizopertha dominica* (F.)

Extract	Concentration ml/kg	Adult mortality (%) after the indicated days				
		1	3	5	7	14
Petroleum Ether	1.0	0.0	30.0±2.6	39.0±2.1	45.0±0.0	72.0±4.0
	2.0	0.0	40.0±0.0	52.0±5.3	59.0±2.1	79.0±0.0
	3.0	0.0	50.0±2.5	57.0±0.0	71.0±3.8	100.0
	5.0	27.0±3.5	80.0±1.5	98.0±1.2	100.0	100.0
	6.0	32.0±4.2	95.0±2.9	100.0	100.0	100.0
Chloroform	1.0	0.0	25.0±1.5	49.0±3.1	73.0±4.2	89.0±0.6
	2.0	8.0±0.0	53.0±4.6	71.0±1.0	89.0±0.0	96.0±2.0
	3.0	14.0±1.0	75.0±0.0	98.0±1.2	100.0	100.0
	6.0	19.0±0.6	90.0±0.0	100.0	100.0	100.0
Acetone	1.0	0.0±	20.0±0.5	38.0±0.0	47.0±2.0	78.0±0.0
	4.0	0.0	40.0±1.5	57.0±4.6	65.0±0.0	98.0±0.0
	6.0	0.0	50.0±3.5	63.0±3.5	89.0±1.7	100.0
	7.0	17.0±1.5	75.0±2.5	92.0±0.0	98.0±2.0	100.0
	8.0	21.0±0.0	95.0±0.0	100.0	100.0	100.0

Results of Table 2 indicated the calculated values of LC50, LC90, LC95 and relative potency (RP) of the tested extracts. It is clear from this table that, chloroform extract was the most effective because its lethal concentration (LC50) was of a minimum value (1.8 ml/kg), while the acetone extract was the least effective(LC50=3.57 ml/kg) after three days of treatment. Values of the lethal concentration (LC95) ranged from 8.0-19.0 ml/kg for chloroform and acetone extracts respectively. Relative potency based on LC50 of the least effective extract (acetone) showed that chloroform extract has a double effect than the acetone one (Table2).

Table 2: Calculated LC50, LC90 and LC95 values and relative potency (RP) of the cinnamon bark extracts against *R. dominica* adults.

Extract	LC50 (ml/Kg)	LC90 (ml/Kg)	LC95 (ml/kg)	Slope value	Relative potency (RP)*
Chloroform	1.80	5.64	8.00	2.53	1.98
Petroleum ether	2.17	7.56	8.90	2.61	1.65
Acetone	3.57	16.2	19.00	1.95	1.0

*Relative potency (RP) was based on LC50 of the acetone extract.

These results showed that *R. dominica* adults were more sensitive to chloroform extract compared to other two extracts. Data of Table 3 indicated the outcome biological effects of the two calculated concentrations (LC50 and LC95) of the tested extracts on both adult progeny of *R. dominica* emerged and the resultant weight loss(%). Significant differences were found among the extracts. Results of LC95 completely prevented adult emergence compared to control. The results showed significant differences between (petroleum ether and acetone) and chloroform extracts on the adult emergence. Tested extracts could be arranged ascendingly according to effect of its LC50 value on adult

progeny reduction as follows: acetone (75.0 %), petroleum ether (76.4 %), and chloroform (92.3%) compared to control (0.0).

The effects of LC50 and LC95 values on the dry weight loss (%) and its reduction (%) showed significant variations which acetone and pet. ether extracts were the least effective while the chloroform extract was the most effective in reducing weight loss (%). LC50 value resulted in 2.31, 6.88 and 12.8 of the dry weight loss (%) in treatments with chloroform, acetone and pet. ether extracts respectively compared to control (31.4 %). Treatment with LC95 value was more effective and ascertained that chloroform extract was the most effective and produced the lowest weight loss (1.69 %) while pet.ether extract was the least effective (2.7 %). Chloroform extract was the most effective in reducing weight loss (%) to 92.6 %.

Table 3: Adult progeny of *R. dominica* and weight loss (%) as affected by cinnamon bark extracts at LC50 and LC95 level.

Extract	Concentration ml/kg	Progeny number	Red. of adult Progeny (%)	Dry weight Loss(%)	Red. of weight loss (%)
		a.LC50 effects			
Chloroform	LC50 (1.8)	7.0±1.0c	92.3±1.4a	2.3±0.5c	92.6±1.6a
Petroleum Ether	LC50 (2.17)	17.0±3.1b	76.4±4.2b	12.8±1.2b	59.3±3.9b
Acetone	LC50 (3.57)	18.0±1.2b	75.0±1.6b	6.9±1.0c	78.1±3.6ab
Control	0.0	72.0±4.4a	0.0c	31.4±2.6a	0.0c
		b.LC95 effects			
Chloroform	LC95 (8.0)	0.0b	100.0a	1.7±0.2b	94.6±0.7Aa
Petroleum Ether	LC95 (8.9)	0.0b	100.0a	2.7±0.2b	91.3±0.7a
Acetone	LC95 (19.0)	0.0b	100.0a	2.6±0.03b	91.8±0.7a
Control	0.0	72.0±4.4a	0.0b	31.4±2.6a	0.0b

* Data analyzed statistically by ANOVA test and means separated by Duncan's multiple test for L50 and LC95 separately, ** Vertical means with same letters are not significantly different.

The residual effect of tested extracts was bio-assayed weekly against *R. dominica* adults exposed to wheat grains treated with LC95 value of the different extracts separately was shown in Table 4. Extracts lasted effective until 8 weeks, which gave more 90 % adult mortality and then dropped suddenly from 96.0 to 59.0 % (Pet. Ether), 97.0- 64.0 % (chloroform) and from 95.0- 58.0 % (acetone) after ten weeks.

Data of Table 5, showed effect of LC95 on percent germination initially after treatment and at end of storage period. Initially after treatment germination differed significantly and ranged from 80.0-88.0 % for acetone and chloroform extracts respectively compared to control (95.0 %). With end of storage period after ten weeks, percent germination ranged from 74.0- 82.0 % for acetone and chloroform extracts respectively. Significant reduction (%) of germination was slightly observed with the end of storage period. Thus, treatment of wheat grains showed a little effect on viability of grains especially with the end of storage.

Table 4: Adult mortality (%) of *R .dominica* exposed to wheat grains treated with cinnamon bark extracts (at LC95) after different storage periods

Period after treatment (weeks)	Petroleum Ether Extract	Chloroform extract	Acetone extract
Initial	96.0±2.3	97.0±1.2	95.0±1.7
1	97.0±0.0	95.0±0.0	95.0±0.0
2	95.0±0.5	95.0±0.6	96.0±0.6
3	96.0±0.6	96.0±0.0	94.0±0.6
4 (One month)	95.0±1.0	95.0±2.5	96.0±2.0
5	94.0±1.0	94.0±1.0	95.0±1.0
6	95.0±2.5	95.0±0.6	95.0±0.0
7	95.0±0.4	94.0±2.5	93.0±1.2
8 (Two months)	94.3±0.9	92.0±1.5	90.0±1.0
9	75.0±2.5	83.0±4.0	71.0±2.5
10	59.3±2.6	64.0±0.0	58.0±3.1

Table 5: Germination (%) of wheat grains treated with LC95 of cinnamon bark extracts initially and after ten weeks of storage.

Extracts	Concentration ml/kg	Initial period		End of storage period (Ten weeks)	
		Germination (%)	Reduction (%)	Germination (%)	Reduction (%)
Petroleum Ether	8.9	82.0±3.1c	13.7±1.3a	79.0±1.5b	17.7±1.4b
Chloroform	8.0	88.0±2.3b	7.4±1.9b	82.0±0.6b	14.6±0.2b
Acetone	19.0	80.0±2.3c	15.7±0.4a	74.0±2.1c	22.9±2.2a
Control	0.0	95.0±1.2a	0.0c	96.0±0.6a	0.0c

*Data were statistically analyzed by ANOVA test and means separated by Duncan's multiple range test, ** Vertical means with same letter are not significantly different.

The present study showed that the chloroform extract of cinnamon bark was the most effective than the other extracts since it affects adult mortality (%) as well as it showed a higher reduction at its LC50 and LC95 on both adult emergence and weight loss (%). Also, the persistent effect of chloroform extract lasted and remained effective tested for more than two months. These results agree with those mentioned by EL-Lakwah *et.al.*, (2004) which mentioned that toxicity of cinnamon extracts might due to its chemical constituents of terpenoids, glycosides or similar substances which posses many interactive effects, that are finally lethal to insects. Cinnamon bark powder is used for flavoring food products and with medicinal properties as well as preferred as hot public drink all over the world (Su, 1985), so its use for wheat protection is considered safe but regards about its cost is unknown especially under the large scale application. Such extracts can be used to protect small stocks of wheat from *R.dominica* infestation at the peasant level.

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التاثيرات الحيوية لمستخلصات القرفة ضد حشرة ثاقبة الحبوب الصغري

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في الدراسة الحالية تم تقييم التأثيرات البيولوجية لمستخلصات الأسيتون والكلوروفورم وايثير البترول لقلف القرفة Ceylon cinnamon, *Cinnamomum zeylanicum* bark (N.) ضد حشرة ثاقبة الحبوب الصغري(وهي حشرة أولية خطيرة تصيب الحبوب النجيلية السليمة كالقمح) وذلك بالمعاملة السطحية لحبوب القمح، وتقدير تركيزات السمية الفعالة القاتلة ل٥٠% و٩٥%. وكذلك تم دراسة تأثير المعاملة بهذه التركيزات علي الخلفة الناتجة ونسبة الفاقد في الوزن ونسبة حيوية البذور عند إستخدامها كتفاوي، كذلك تم تقييم الأثر الباقي لهذه المستخلصات الثلاثة ضد الحشرات الكاملة أسبوعيا لمعرفة مدة بقاء فاعليتها، وتحديد نسبة موت الحشرات الكاملة بعد أسبوع من العدوي بالحشرات.

أظهرت النتائج أن نسبة الموت للحشرات الكاملة تزداد مع زيادة كل من التركيز وفترة التعريض لكل مستخلص ، حيث وصلت نسبة الموت ١٠٠% بعد أسبوع من المعاملة بالتركيزات العالية، وكان مستخلص الكلوروفورم أفضليا فاعلية حيث وصل تركيزه القاتل ل٥٠% إلي ١,٨ مللي/كجم قمح بينما كان الاسيتون اقلها فاعلية (٣,٥٧ مللي/كجم). وجد عدم خروج خلفة وذرية عند المعاملة بالتركيز القاتل ل ٩٥% ، كما أحدثت إنخفاضا كبيرا في نسبة الفقد في الوزن مقارنة بالكونترول ، وكان مستخلص الكلوروفورم أفضليا تأثيرا.

وعند دراسة الأثر الفعال الباقي للمستخلصات الثلاثة أسبوعيا ضد الحشرات الكاملة، وجد أن كل المستخلصات المختبرة أستمرت فعالة حتى الأسبوع الثامن حيث وصلت نسبة الموت ٩٠% فاكثر، ولم تظهر إختلافات معنوية بين الثلاث مستخلصات ، ثم إنخفضت نسبة الموت لتتراوح بين ٥٨%- ٦٤% بعد الأسبوع العاشر، وكذلك وجد إنخفاض طفيف في نسبة إنبات الحبوب المعاملة بالجرعة LC95 من المستخلصات الثلاثة بعد عشرة أسابيع من المعاملة مقارنة بالكونترول. توصي الدراسة الحالية بإستخدام مستخلص قلف القرفة في الكلوروفورم كمادة واقية لحبوب القمح من الإصابة بثاقبة الحبوب الصغري لانه أكثر المستخلصات فاعلية حيث أدي إلي منع ظهور ذرية من الحشرات المعرضة لقمح معامل بتركيز ٩٥% لمدة تزيد عن شهرين، كما أن قلف هذا النبات أمن الإستخدام لأنه ذو إستخدامات غذائية وطبية عديدة لغالبية الناس علي مستوي العالم.