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Insecticidal effect of *Piper nigrum* L. (Pipeaceae) and *Prunus cerasus* L. (Rosaceae) Seeds Extract against *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae)

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Abstract **– The study aims was to investigate the insecticidal effect of Petroleum ether extract of *Piper nigrum* and *Prunus cerasus* seeds against *R. dominica*. Maceration method was used to extract *Piper nigrum* and *Prunus cerasus* seeds. Four concentrations (0.25, 0.5, 1 and 2%) were prepared in acetone. The residual film method was used to assess the percentage mortality, while the repellent activity was tested by using the area preference method. The results showed that there was significant difference between petroleum ether extract of pepper and cherry seeds, whereas, the percentage mortality in cherry seeds extract was higher than in pepper. It was 53.33 and 100% within 24 and 48 hours of exposure at 0.25% of cherry seeds extract. On the other hand, in pepper extract, it was 26.66, 36.67 and 56.67% after 24, 48 and 72 hours at 0.25%, respectively, while, the petroleum ether extract of pepper seeds was more repellency than cherry seeds extract. The concentration at 2% of pepper was the strongest repellent effect on *R. dominica* with mean percentage repellency of 76% followed by 1%, it was 42.6%. Finally, it can be concluded that cherry and black pepper seeds extracts possess insecticidal activity and both could be used as insecticides.**

Keywords **– Insecticidal Activity, *Piper nigrum, Prunus cerasus, Rhyzopertha dominica.***

I. INTRODUCTION

Rhyzopertha dominica F. (Coleoptera: Bostrichidae) is thought to originate from India. It has cosmopolitan distribution, and it is a serious pest of the stored products by reducing their dry weight and nutritional value. Synthetic chemical insecticides have been used worldwide to control the pests of stored grain. The EPA in the USA estimated that 270,000 kg of pesticides was used annually between (1987 and 1996) for wheat in storage (https//www.cabi.org/isc/datasheet/47191). Some pesticides have chronic effects including cancers, reproductive problems, birth defects, hormonal disruption and damage to the immune system. Impacts come from direct exposure in use, spray drift, home pesticide storage, pesticide dumps and persistence in the environment. To reduce the use of hazardous pesticides the recommended plant extracts practices are safer, more affordable, and easy to follow. Most of the farm practices, the farmers can do by themselves and the materials that are needed are found in their backyards or in their kitchens or can be purchased in the local agricultural supplier [1]. Botanical insecticides are used as powder, essential oil, solvent extract, repellent agents and fumigants to control the stored insects [2, 3, 4]. *Piper nigrum* has been used as effective insecticide against stored grain pests [5, 6]. Sour cherry is a plant traditionally used as food. Its health care potency has been discovered recently [7]. Moreover, some species of *Prunus* trees have active effect against microorganisms and have medicinal value [8, 9]. This study reports on the insecticidal effect of two botanical seeds extracts (*Piper nigrum* and *Prunus cerasus*) in the control of the lesser grain borer in stored rice.

II. MATERIALS AND METHODS

A. *Experimental Insect*

Adult insects of the lesser grain borer, *Rhyzopertha dominica* F. (Coleoptera: Bostrichidaeas) obtained from already infested rice grains. The insects were reared for about two generations separately in rice grains as growth medium throughout the study. Two hundred adult beetles were introduced into plastic jars containing 300g of rice. These plastic jars were then covered with a muslin cloth with the help of rubber bands to prevent insects escaping and to allow ventilation. The insects were allowed to oviposit for three weeks then they were removed. After emergence the new adults were transfer to other jar containing fresh grains [10]. Unsexed beetles, 2-4 weeks old, were used in bioassays.

B. *Preparation of Plant Extract*

The active components of *Piper nigrum* L. (black pepper) and *Prunus cerasus* L. (the red sour cherry) seeds were extracted using the cold extraction method (maceration). 100g of seeds were prepared by pulverizing with the help of an electric blender and sieved to obtain fine powder. The fine powder was socked separately in 150 ml of petroleum ether (99%) in sterile conical flask for about 3 weeks at room temperature and was stirred occasionally. Then, the resulting mixture was filtered using filter paper (Whatman No. 1). The cakes of black pepper and red sour cherry after filtration were repacked to the same flask and another 150 ml of petroleum ether (99%) was added. The mixture was socked for 3 weeks with stirring occasionally. After that, the extract was filtrated and then, the collected filtrates were combined and were exposed to air in order to remove the solvent at room temperature. About 13.7% (v/w) of black pepper extract was obtained and 7.8% (v/w) of crud cherry extract. 2 ml of crude extract were dissolved in 48 ml acetone (99.9%) in 50 ml volumetric flask to prepare the stock solution 4% (v/v). four concentrations of *Piper nigrum* and *Prunus cerasus* crude extracts were prepared in acetone (0.25, 0.5, 1 and 2%).

C. *Contact Toxicity (Effect of the Plant Extract on Mortality)*

Contact toxicity was done on Petri dishes (5 cm in diameter) by using the residual film method [11]. 1 ml of each concentration was spread uniformly along the whole surface of the Petri dishes. The solvent was allowed to evaporate for overnight leaving thin film of black pepper and cherry seeds extracts, separately. Ten adults of *R. dominica* were released separately into each Petri dish and covered with a lid. The control was treated with acetone alone. The treatments were replicates three times. Insect mortality was recorded at 24, 48 and 72 hours after exposure of *Piper* and *Prunus* petroleum ether extracts. Insects which did not react or move when probed gently with a needle or when no antennal movements were observed were considered dead. No correction was considered necessary in control because there was not mortality. From the observations of mortality percentages after exposure of *Prunus* extract, it was about 100% at 48 and 72 hours, so it was recorded at 6, 12, 18 and 24 hours after exposure of *Prunus* petroleum ether extract. The percentage mortalities were transformed to arcsine values for repeated measures analysis of variance (ANOVA). If ANOVA results were significant, Fishers Protected Least Significant Differences ($p<0.05$) were used to separate the means. The lethal dose 50 (LD_{50}) values, the confidence upper and lower limits (CL), regression equations, the slope and the standard error (SE) of the slope were calculated according to [12].

D. *Repellent Bioassay*

Repellence activity of petroleum ether extract of black pepper and cherry seeds was evaluated using the area preference method [13] with some modifications. A filter paper disc (Whatman No. 1) was waved and was

divided in half. One half was treated with 0.5 ml of each concentrate separately. While the other half was treated with 0.5 ml of acetone considered as control. After that, both of them were left in air for about 2 hours to remove the solvent completely. The treated and untreated halves were attached without using cello-tape and fixed in Petri dishes. Ten adults of *R. dominica* were released separately into each Petri dish and covered with a lid. The treatments were replicates three times. The number of tested insects on the treated and untreated halves was recorded after 1, 2, 3, 4 and 5 hours. Percentage repellency (PR) was calculated by using the following formula: $PR = (Nc - 50) \times 2$.

Where, Nc = percentage of insects present in the control.

Positive PR values indicate repellence whereas, negative values indicate attraction. For each test dose, the mean PR value was calculated and assigned to repellence classes from 0 to V [14]: class 0 (PR < 0.1%), class I (PR = 0.1-20%), class II (PR = 20.1-40%), class III (PR = 40.1-60%), class IV (PR = 60.1-80%), class V (PR = 80.1-100%). PR values were transformed to arcsine values for repeated measures analysis of variance (ANOVA).

III. Results and Discussion

A. *Contact Toxicity*

The contact toxicity of petroleum ether extract of black pepper and red sour cherry seeds as insecticide against the lesser grain borer (*R. dominica*) was evaluated by using the residual film method. The average mortality percentages of *R. dominica* which exposed to four concentrations 0.25, 0.5, 1 and 2% of tested petroleum ether extracts and the control groups after 24, 48 and 72 hours of exposure period were showed in Table (1). There was significant difference of the mean mortality between petroleum ether extract of black pepper and red sour cherry seeds. Whereas, the percentage mortality in cherry seeds extract was higher than in black pepper. Also, there were significant differences among tested concentrations. Moreover, the mortality percentages were increased with increasing of the time and the exposure time. However, it was evident that, 53.33 and 100% mortality was observed within 24 and 48 hours of exposure at 0.25% of cherry seeds extract. Furthermore, 100% mortality was observed in all concentrations. On the other hand, the percentage mortality in black pepper extract was 26.66, 36.67 and 56.67% after 24, 48 and 72 hours at 0.25%, respectively. The percentage mortality was about 43, 63 and 73.33% at 0.5% after 24, 48 and 72 hours, respectively. Noticeably, the percentage mortality at 1 and 2% was 76.67 and 96.67% after 24 hours of exposure, respectively, and 100% after 48 and 72 hours at the same concentrations. The result in Table (2) showed the percentage mortality in cherry seeds extract against *R. dominica* at four times, 6, 12, 18 and 24 hours, whereas, there were significant differences among tested concentrations and the times of exposure. The percentage mortality was 36.66, 60, 70 and 100% at 0.5% after 6, 12, 18 and 24 hours, respectively, while, it was 90, 96.67 and 100% at 1% 0.5% after 6, 12 and 18 hours, respectively. LD_{50} values were showed in Table (3), where, it was 0.53, 0.37 and 0.05% of *R. dominica* petroleum ether extract of *Piper nigrum* for three times 24, 48 and 72 hours, respectively. Finally, it can be concluded that cherry seeds extract was more toxic than black pepper and both of them could be used as insecticides. Piperaceae are that Piper extracts offer a unique and useful source of biopesticide material for controlling small-scale insect out-breaks and reducing the likelihood of resistance development when applied as a synergist with other botanical insecticides such as pyrethrum Scott *et al.*, 2008 [15]. The present results

support the finding of Ahmad *et al.,* 2016 [16], who showed that *P. nigrum* was observed effective against *Rhyzopertha dominicam,* whereas, the mortality percentage was 55.66% in case of acetone extract of *P. nigrum* when applied with 20% concentration. While, Khani *et al.,* 2011 [17] revealed that the major extracted components of *P. nigrum* were piperine (74.34%) and caryophyllene (18.53%), and the mortality of *S. oryzae* adults increased with increasing concentration of extracts from 2 to 10 µl/g of rice and exposure time from 24 to 72 h. The petroleum ether (LC_{50} = 1.61 µl/g) of *P. nigrum* showed higher mortality rate compared to petroleum ether extracts (LC_{50} = 6.82 µl/g) of *J. curcas,* respectively. In addition to, piperine a clinically established pesticide was investigated in the seeds, fruit and leaves of *Piper nigrum.* The results showed that mortality rate on *S. zeamais* and *C. maculatus* are directly proportional to each equivalent concentration of piperine in the extracts. Pesticidal potency on *S. zeamais* was significantly higher ($P<0.05$) relative to *C. maculatus* within 9000 s of exposure period Awoyinka *et al.,* 2006 [18]. Moreover, three extracted fractions from black pepper fruits, *Piper nigrum* L, in four concentrations were revealed for their toxicity effects against stored grain pests *T. castaneum* and their stages, and *S. oryzae* L. They revealed that the etheric fraction caused higher mortality than that of two other solvent fractions, towards all target pests. The adult rice weevil was the most susceptible, followed by the larvae, adult > eggs > pupae of red flour beetle. The mortality percentage reached to 100% at a concentration 2.5% (w/w) after 21 day of treatment of adult rice weevil, while was 100% at 5% (w/w) in same period in case of adult red flour beetle, and the LC_{50} between both adult pests were 2.43 and 3.52, respectively. By using GC/MS analysis was found a functional groups and compounds in the oil, were determined and identified the main constituents which may cause the mortality, as well as their structures, formulae and percentages. The main components were monoterpenes, Sesquiterpenes, fatty acids and alkaloids, and some of effective constituents were α-pinene, Linalool, α-Copaene, Linoleic acid and Piperin Hussein *et al.*, 2017 [5]. In addition to, Tamara *et al.,* 2016 [7] investigated the antimicrobial activity of sour cherry towards different pathogens by micro dilution method. Results showed that juice and extract exhibit antibacterial activity, but have no antifungal and antialgal activity against tested pathogens. In terms of break point, better results were obtained against Gram positive bacteria. *Rhodococcus equi* was the most susceptible specie to both juice and extract. Juice showed better results to: *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Sallmonela Typhymurium* and *Acinetobacter lwoffi*, but for all other investigated species extract showed superior activity. Moreover, Berroukche *et al.,* 2018 [19] showed that the antimicrobial activity of *Prunus cerasus* leaves was greater than that of *Chamaemelum nobile* stalks aqueous extract with a broader antimicrobial spectrum at lower concentrations. Hassanshahi *et al.,* 2016 [20] showed that *Amygdalus scoparia* and *Prunus dulcis* var *amara* seed extracts were effective against *Agonoscena. pistaciae*. The results showed that *A. scoparia* with LC_{50} value of 1494.00 ml/L was more toxic than *P. dulcis* var *amara* (1968.78 ml/L) on fifth instars nymphs of *A. pistaciae*.

Table 1. The average percentage mortality of *R. dominica* exposed to four concentrations of petroleum ether extract of *Piper nigrum* at three times, 24, 48 and 72 hours.

Plant Extract	Conc. (%)	% Mortality (Mean± S.E)				
		24 hrs.	48 hrs.	72 hrs.	Mean	Mean
Piper nigrum	0.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	00.00a	56.89A
	0.25	26.66 ± 0.19	36.67 ± 0.19	56.67 ± 0.19	40.00b	

Plant Extract	Conc. (%)	% Mortality (Mean± S.E)				
		24 hrs.	48 hrs.	72 hrs.	Mean	Mean
	0.5	43 ± 0.5	63.0 ± 1.73	73.33 ± 0.2	53.33c	
	1	76.67 ± 0.2	100 ± 0.00	100 ± 0.00	92.22e	
	2	96.67 ± 0.2	100 ± 0.00	100 ± 0.00	98.89f	
Prunus cerasus	**0.0**	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	00.00a	
	0.25	53.33 ± 8.1	100 ± 0.00	100 ± 0.00	84.44d	
	0.5	100 ± 0.00	100 ± 0.00	100 ± 0.00	100g	76.89B
	1	100 ± 0.00	100 ± 0.00	100 ± 0.00	100g	
	2	100 ± 0.00	100 ± 0.00	100 ± 0.00	100g	

Values followed by the same letter are not significantly different according to the Fisher's LSD test (p≤0.05) L.S. D0.05 between *Piper nigrum* and *Prunus cerasus* extract = 0.017 L.S.D0.05 among 0, 0.25, 0.5, 1 and 2% = 1.9.

Table 2. The average percentage mortality of *R. dominica* exposed to four concentrations of petroleum ether extract of *Prunus cerasus* at four times, 6, 12, 18 and 24 hours.

Conc. (%)	% Mortality (Mean± S.E)				
	6hrs.	12 hrs.	18 hrs.	24 hrs.	Mean
0.0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	00.00a
0.25	3.33 ± 3.33	20.0 ± 5.78	33.33 ± 3.3	53.33 ± 8.1	22.00b
0.5	36.66 ± 3.33	60.0 ± 5.77	70.0 ± 11.5	100 ± 0.00	66.67c
1	90.0 ± 5.77	96.67 ± 3.33	100 ± 0.00	100 ± 0.00	96.67d
2	100 ± 0.00	100 ± 0.00	100 ± 0.00	100 ± 0.00	100d
Mean	45.99A	55.33B	60.67C	70.67D	

Values followed by the same letter are not significantly different according to the Fisher's LSD test (p≤0.05) L.S.D. 0.05 among 0, 0.25, 0.5, 1 and 2% = 6.52 L.S.D0.05 among 6, 12, 18 and 24 hours = 4.59.

Table 3. Probit analysis of mortality for *R. dominica* exposed to four concentration of petroleum ether extract of *Piper nigrum* for three times 24, 48 and 72 hours.

Plant Extract	Time (hour)	LD_{50} %95 Confidence Limit	Regression Equation	Slope ± SE
Piper nigrum	24	0.53 (0.4-0.84)	Y = 1.126 X + 2.36	1.126 ± 0.27
	48	0.37 (0.28-0.41)	Y = 4.403X + 1.66	4.403 ± 0.98
	72	0.05 (0.07-0.09)	Y = 1.21X + 3.94	1.21 ± 1.17

B. *Repellent Bioassay*

Percentage repellency values of *R. dominica* exposed to petroleum ether extract of black pepper and the red sour cherry seeds were showed in Table (4). Generally, the percentage repellency values were fluctuated at all concentrations and all time of exposure. There was no significant difference affected by increasing of time of exposure. Positive PR values indicate repellence whereas, negative values indicate attraction. There were significant differences between petroleum ether extract of black pepper and the red sour cherry seeds. Whereas, the petroleum ether extract of pepper seeds was more repellency than the red sour cherry seeds extract. Noticeably, the concentration at 2% of black pepper was the strongest repellent effect on *R. dominica* with mean percentage repellency of 76% (class 4) followed by 1%, the mean repellency was 42.6% (class 3). The percentage repellency was the highest values at 2% of pepper extract after 3 hours it was 86.67 followed by 4 hours and 1 hour, 1t was 80 and 73%, respectively. Then it decreased to 60% after 5 hours of exposure. Moreover, the percentage repellency at 1% was 33% after 1 hour, and then decreased to 20% after 2 hours, after that, it increased to 40 and 66.6% after 3 and 4 hours, respectively, and then decreased again to 53% after 5 hours. While the percentage repellency at 0.5% was 47% after 1 hour, and decreased to 13.3 and then, it increased more significantly. It was 20 and 46.67 % after 3 and 4 hours, respectively, after that, it decreased again to 26% after 5 hours of exposure. Meanwhile, the percentage repellency at the concentration of 0.25% decreased statically, it was 40, 33 and 0% after 1, 2 and 3 hours, respectively. After that, it increased to 53% then decreased to 40% after 4 and 5 hours of exposure, respectively. For petroleum ether extract of *Prunus* seeds, there was both of repellent and attractive effect, whereas, the highest repellent value was 66.6% at concentrations of 0.5 and 0.25% after 1 and 2 hours of exposure, respectively. It decreased to 53% at concentrations of (0.25% after 1 and 5 hours) and (0.5% after 3 hours). In addition to, there was attractive affect at concentration of 0.25% after 4 hours, it was -6.67%. While at concentration of 2%, it was 46.6 and 40% after 4 and 1 hour of exposure, respectively. After that, it decreased to 33, 20 and 13% after 2, 5 and 3 hours of exposure the same concentrate. On the other hand, the concentrate at 1% of petroleum ether extract of *Prunus* seeds was the lowest repellent effect on *R. dominica*, it was 17 % (class 1). Khani *et al.*, 2011 [17] revealed that The *P. nigrum* extracts (92.0%) were significantly repelled *S. oryzae* compared to *J. curcas* extracts (69.6%). And Ahmad *et al.*, 2016 [16] showed that the repellency percentage was observed greater in treatment where acetone extract of *P. nigrum* applied. While, Chaubey, 2017 [21] found that *C. cyminum* and *P. nigrum* essential oils reduced AChE activity in *S. zeamais* adults, where *P. nigrum* significantly reduced AChE activety to 53.04 and 39.06 % of control.

Table 4. Percentage repellency of *R. dominica* treated with different concentrations of petroleum extract of *Piper nigrum* and *Prunus cerasus* seeds for 1, 2, 3, 4 and 5 hours.

Plant Extract	Conc. (%)	Time of Exposure (hours)					Mean	Mean	Repellent Class
		1	2	3	4	5			
Piper nigrum	0.25	40	33.33	0	53.33	40	33.33	45.5A	II
	0.5	47	13.3	20	46.67	26.67	30		II
	1	33.33	20	40	66.67	53	42.67		III
	2	73	80	86.67	80	60	76		IV

Prunus cerasus	0.25	53.33	66.67	0	-6.67	53	33.33	30.3B	II
	0.5	66.67	46.67	53.33	6.67	26.67	40		II
	1	13.33	26.67	6.67	13.33	26.67	17.33		I
	2	40	13.33	33.33	46.67	20	30.67		II

Values followed by the same letter are not significantly different according to the Fisher's LSD test (*p≤0.05) $L.S.D_{0.05}$ between *Piper nigrum* and *Prunus cerasus* = 10.99.

IV. Conclusion

This study reports that black pepper and red sour sherry have insecticidal activity against stored grains pest, *Rhyzopertha dominica. Prunus cerasus* seeds extract was the highest toxicity and *Piper nigrum* was the highest repellency. Hence, black pepper and red sour sherry extracts can be used as alternative pesticides against *Rhyzopertha dominica* and has a promising future in plant protection and IPM programs.

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