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Efficacy and time mortality of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) by some essential oils through contact and fumigant methods

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Abstract

Tribolium castaneum (Herbst) (Coleoptera: Tenebrionidae) is one of the most damaging stored–product insect pests in Pakistan.Though synthetic insecticides have promising results against *T. castaneum* but use of synthetic insecticides can be hazardous for the environment. Replacing these synthetic insecticides with plant materials to control this pest, however, can be a safe method with low environmental risk especially in stored products. So three important essential oils i.e. neem seed oil, castor seed oil and turpentine oil were evaluated against *T. castaneum* using contact method. Five different doses of each of the oils were prepared. Mortality percentages on different doses with respect to time were not only compared with each other but also with deltamethrin as a standard. Results showed that mortality was directly proportional to dose and time in case of treated oils and deltamethrin. Order of toxicity was deltamethrin>neem seed oil>castor seed oil> turpentine oil. Based on the results obtained by contact toxicity of neem seed oil, castor seed oil and turpentine oil; five different concentrations were made to find out the fumigant toxicity of neem. Highest mortality was recorded at the highest concentration with respect to time in neem seed oil and deltamethrin at 50% and 5ppm doses, respectively.

1 Introduction

Red flour beetle, *Tribolium castaneum* (Herbst) is one of the worldwide insect pests of mills, food warehouses, retail stores, and urban homes (Rees 2004). Scientifically it has been reported that the germ part (embryo portion) of the grain is destroyed by red flour beetle, *T. castaneum*. Their presence in stored grain directly affects both the quantity and quality of the commodity (Okonkwo & Okoye, 1996; Sagheer et al., 2011; Rahman et al., 2011). The population of *Tribolium* spp. was found to be suppressed by the conventional insecticides (Arthur et al. 1990; Mondal 1984, Kamaruzzaman 2000 ; Hasnat 2003), but due to increasing costs of application, pest resurgence, adverse effects on non–target organisms and human health concerns; synthetic insecticides are not used against stored grain pests (Paranagama et al. 2003). It is proven from the research that extracts taken from the plants have great variety of properties which can be insecticidal, growth regulatory, antifungal, antiviral and anti-feedant (Prakash & Rao,1997). Essential oils and especially their important compounds monoterpenoids, offer promising alternatives for classical fumigants (Peterson and Ems–Wilson 2003, Aslan et al. 2004). If used as volatiles, essential oils can act like fumigants thus offering the prospect for use in stored–product protection (Lee et al. 2004). Essential oils are also excellent contact insecticides (Tapondjou et al. 2002, Peterson and Ems–Wilson 2003), anti-feedant or have repellent effects (Kim et al. 2003 a,b,: Park et al. 2003 a,b,: García et al. 2005) and may also affect important biological parameters, such as growth rate, life span and reproduction (Tunç et al. 2000, Kathuria and Kaushik 2005, Rahmat et al. 2006). Bioactivity of essential oils is directly related to its

chemical composition, which can vary dramatically, even within the same species. Sources of compositional variability can include the plant part extracted, the physiological state of the plant, time of year and growth environmental conditions (Angioni et al., 2006)

In the present experiment, 3 essential oils i.e. neem seed oil, castor oil and turpentine oil were screened and compared with a conventional insecticide, deltamethrin. The most effective essential oil was tested after transforming into fumigant.

2 Material and methods

2.1 Insect rearing

Adults of *T. castaneum* uniformed age were used in these experiments. All adults were obtained from Eco–toxicology laboratory in Faculty of Agricultural Sciences and Technology, Bahauddin Zakariya University Multan. Collection of 500 adult beetles (*T. castaneum*) was done from infested grains like wheat, corn, pulses, etc., which were stored in local storage facilities in Multan (+30° 11' 52"N, +71° 28' 11" E). This collection of *T. castaneum* was cultured on whole meal wheat flour with 5% brewer's yeast added to increase fecundity of the parent beetles. The cultures were maintained at room temperature and 40 ± 5% R.H in different jars. The beetles were reared on wheat flour mixed with yeast (10:1 w: w) to increase the population in limited time. The newly pupated larvae retrieved and sexed based on the abdominal characteristics (Halstead, 1962). The uniform aged adult, ten days after they emerged, were used in the experiments. The adults were kept for 24 hours without food before exposing them to the tests.

2.2 Contact bioassay of essential oils against Tribolium castaneum (Herbst)

Serial dilutions of essential oils were prepared using 90% alcohol as a solvent. Considering azadirachtin as active ingredient five doses of neem seed oil, castor and turpentine oil concentrations i.e. 10%, 5 %, 2.5 %, 1.5 %, 0.63 % were prepared for three oils along with a control having zero concentration of azadirachtin. While in addition to control compared with chemical as five doses of Super delta® (10 % E.C, a.i. deltamethrin) 5 ppm, 2.5 ppm, 1.25 ppm, 0.625 ppm, 0.3125 ppm were prepared to find out the contact toxicity. Five sets of petri–dishes as replications of each concentration were made. Labeling of petri–dishes were done at 1 to 6 (1 to 5; starting from 1 for highest concentration and ending on 5 for lowest concentration of neem seed oil and number 6 for control). Aliquots of 1 ml of the dilutions were applied into each petri–dish (6 cm in diameter) for surface–film bioassay (Busvine 1971). The solvent was allowed to evaporate for 1 hour and six adult insects were released to each petri–dish. Whereas, controls were treated only with alcohol alone. 30 adults of *T. castaneum* were used for each concentration and same quantity was used for control. Five petri–dishes were used for each concentration; each petri–dish with 6 adults. The petri–dishes were kept at room temperature and mortality was observed after 3, 6, 12, 24, 48 and 72 hours of exposure. Mortality percentage was recorded after 3, 6, 12, 24, 48 and 72 hours of exposure. The comparison of essential oils with a synthetic insecticide was done to find out the efficacy of tested oils as replacements of synthetic insecticides.

2.3 Fumigant bioassay of essential oils against Tribolium castaneum (Herbst)

For fumigants bioassay, glass vials (6 cm long, 1.8 cm dia.) capped with polypropylene stoppers was used. Two glass vials were needed to make one unit. Considering azadirachtin as active ingredient five serial dilutions of neem seed oil i.e. 50, 25, 25, 12.5, 6.25 and 3.125% were prepared along with a control. 0.5 ml drop of the dilution was placed into a vial. Then glass vial covered with muslin cloth was secured with adhesive tapes. After the solvent evaporation, other vials which containing the insects were placed by inverting on the first vial which containing the oil fumes to fill the air of other vials containing adult of *T. castaneum*. Five sets as replications of each concentration were made with control. 6 adults were released in each set i.e. replication. Labeling of the vial was done at 1 to 6 (1 to 5, 1 for highest concentration and 5 for lowest concentration of neem seed oil and number 6 for control). Total of 30 vials were made for this experiment. The vials were kept and mortality was recorded after 3, 6, 12, 24, 48and 72 of exposure.

3 Statistical analysis

The mortality data were analyzed using the probit procedures by Statistical Analysis Software (SAS Institute 2002). To compare the toxicity of the same insecticide at different doses against time, as well as the toxicity of

different chemicals with each other, the ratios of the LC_{50} values along with LT_{50} values and their related 95% confidence limits were calculated and compared (Robertson et al., 1992).

4 Results

4.1 Results of contact bioassay of essential oils against Tribolium castaneum (Herbst)

In case of neem seed oil highest mortality, i.e. 43.33±0.79% was observed at 10.00% merely after 3 hours which reached to 50.00±0.00% only after 6 hours. Mortality was found to be 56.67±0.89% after 12 hours which increased to 63.33±1.02% and 70.00±0.10% after 24 hours and 48 hours respectively in case of the highest dose i.e. 10.00%. Highest mortality i.e. 96.33±0.89 was recorded after 72 hours at 50.00% among all the doses. At the lowest dose i.e. 3.125%, mortality percentage reached 56.67±0.89% after 72 hours (Table 1).

Turpentine oil has a sub–lethal effect on *T. castaneum*. In case of turpentine oil highest mortality i.e. 30.00±0.22 was observed in 10.00% acetonic solution of turpentine oil after 3 hours which reached to 36.67±0.89% after 6 hours. Mortality was found to be 43.33±1.02% after 12 hours which increased to 47.70±1.02% and 51.00±0.89% after 24 hours and 48 hours, respectively, in case of the highest dose i.e. 10%. However, at the highest dose (10%) mortality percentage did not exceed 53.33±0.22% even after 72 hours (Table 2).

Castor seed oil was less effective than neem seed oil. In case of castor oil highest mortality i.e. 30.00±0.22 was observed at 10.00% acetonic solution of castor oil merely after 3 hours which reached to 50% only after 6 hours. Mortality was found to be 51.00±0.22% after 12 hours which increased to 63.33±0.89% and 70.00±0.10% after 24 hours and 48 hours respectively in case of the highest dose i.e. 10%. Highest mortality i.e. 93.33±1.02 was recorded after 72 hours at 10 % acetonic solution of castor oil. (Table 3).

In case of standard, synthetic insecticide i.e. Super delta (10 % E.C, a. i. deltamethrin) gave more than 33.33±0.22% mortality at a shortest time period of 3 hours at 5 ppm dose which increased to 58.00±0.22% after 6 hours. Mortality was found to be 79.33±0.79% after 12 hours which increased to 90.00±0.89% and eventually 100.00±0.00% after 24 hours and 48 hours, respectively, in case of the highest dose i.e. 5 ppm. (Table 4). The order of toxicity was deltamethrin>neem seed oil>castor seed oil> turpentine oil.

4.2 Results of fumigant bioassay of essential oils against Tribolium castaneum (Herbst)

Fumigant bioassay of neem seed oil showed that mortality increased with increase in time and dose. Highest mortality i.e. 13.33±0.89% was observed at 50.00% merely after 3 hours which reached to 26.67±0.79% only after 6 hours. Mortality was found to be 36.67±0.61% after 12 hours which increased to 50.00±0.22% and 76.67±0.22% after 24 hours and 48 hours, respectively, in case of the highest dose i.e. 50.00%. Highest mortality i.e. 86.67±0.71% was recorded after 72 hours at 50% among all the doses. At the lowest dose i.e. 6.25%, mortality percentage was found to be 43.33±0.22% after 72 hours (Table 5).

The LC_{50} values of these oils like Neem seed, Turpentine and Castor seed oils were recorded after 3, 6, 12, 24, 48 and 72 hours were recorded that was more toxic in deltamethrin after 72 hours (Table 6).

The LC_{50} values in fumigation of neem seed oil was compared in different hours so after 72 hours i.e. 10.182% in which 50% mortality was achieved (Table 7).

Table 1. Percent mortality of *T. castaneum* against five different concentrations of neem seed oil

Neem oil	Total No.	Mortality % after					
	Populations	3 hours	6 hours	12 hours	24 hours	48 hours	72 hours
10 %	30.00	43.33±0.79f	50.00±0.00e	56.67±0.89d	63.33±1.02c	70.00±0.10b	96.33±0.89a
5 %	30.00	33.33±0.31f	40.00±0.22e	53.33±0.79d	56.67±0.89c	66.67±0.89b	86.67±0.79a
2.5 %	30.00	20.00±0.22e	23.33±0.31d	43.33±0.79c	43.33±0.79c	60.00±0.10b	76.67±1.02a
1.50 %	30.00	13.33±0.31f	20.00±0.10e	23.33±0.31d	33.33±0.31c	53.33±0.79b	66.67±1.21a
0.63 %	30.00	6.67±0.13f	13.33±0.22e	16.67±0.22d	20.00±0.10c	43.33±0.79b	56.67±0.89a
Control	30.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Mean values followed by a common letter do not differ statistically in rows (P≤0.05)

Table 2. Percent mortality of *T. castaneum* against five different concentrations of turpentine oil

Terpene	Total No.	Mortality % after					
Oil	Populations	3 hours	6 hours	12 hours	24 hours	48 hours	72 hours
10%	30.00	30.00±0.22f	36.67±0.89e	43.33±1.02d	47.70±1.02c	51.00±0.89b	53.33±0.22a
5%	30.00	23.33±0.31e	20.00±0.22d	20.00±0.22d	26.67±0.89c	30.00±0.22b	36.67±0.89a
2.50%	30.00	10.00±0.31d	10.00±0.31d	16.67±0.79c	23.33±0.79b	23.33±0.79b	26.67±0.89a
1.50%	30.00	6.67±0.22c	6.67±0.22c	10.00±0.31b	10.00±0.31b	16.67±0.22a	16.67±0.22a
0.63%	30.00	0.00±0.00c	0.00±0.00c	3.33±0.10b	3.33±0.10b	6.67±0.22a	6.67±0.22a
Control	30.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Mean values followed by a common letter do not differ statistically in rows (P≤0.05)

Table 3. Percent mortality of *T. castaneum* against five different concentrations of castor oil

Castor	Total No.	Mortality % after					
Oil	Populations	3 hours	6 hours	12 hours	24 hours	48 hours	72 hours
10%	30.00	30.00±0.22f	46.67±0.79e	51.00±0.22d	63.33±0.89c	80.00±0.79b	93.33±1.02a
5%	30.00	23.33±0.31f	30.00±00.89e	36.67±1.02d	50.00±0.00c	66.67±1.02b	73.33±0.89a
2.50%	30.00	13.33±0.22f	20.00±0.22e	30.00±0.22d	40.00±0.31c	46.67±0.89b	60.00±1.21a
1.50%	30.00	10.00±0.31f	13.33±0.89e	20.00±0.22d	30.00±0.22c	36.67±0.22b	46.67±0.79a
0.63%	30.00	3.33±0.10f	6.67±0.22e	13.33±0.31d	20.00±0.22c	30.00±0.22b	26.67±0.22a
Control	30.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Mean values followed by a common letter do not differ statistically in rows (P≤0.05)

Table 4. Percent mortality of *T. castaneum* against five different concentrations of deltamethrin

Deltamethrin	Total No.	Mortality % after					
	Populations	3 hours	6 hours	12 hours	24 hours	48 hours	72 hours
5 ppm	30.00	50.00±0.22e	80.00±0.22d	83.33±0.79c	90.00±0.89b	100.00±0.00a	100.00±0.00a
2.5 ppm	30.00	40.00±0.79f	53.33±0.31e	73.33±0.22d	66.67±0.79c	80.00±0.89b	93.33±1.02a
1.25 ppm	30.00	43.33±0.31f	26.67±0.89e	66.67±1.02d	56.67±0.22c	63.33±0.31b	73.33±0.22a
0.63 ppm	30.00	30.00±0.10f	10.00±0.31e	26.67±0.89d	46.67±1.02c	53.33±0.89b	63.33±0.79a
0.32 ppm	30.00	26.67±0.22f	8.00±0.31e	20.67±1.02d	40.00±0.22c	46.67±1.02b	53.33±0.22a
Control	30.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Mean values followed by a common letter do not differ statistically in rows (P≤0.05)

Table 5. Fumigant percent mortality of *T. castaneum* against five different concentrations of neem seed oil

Fumigation	Total No.	Mortality % after					
Neem oil	**Populations**	**3 hours**	**6 hours**	**12 hours**	**24 hours**	**48 hours**	**72 hours**
50.00%	30.00	13.33±0.89f	26.67±0.79e	36.67±0.61d	50.00±0.22c	76.67±0.22b	86.67±0.71a
25.00%	30.00	6.67±0.22f	20.00±0.22e	30.00±0.89d	43.33±0.89c	60.00±1.02b	73.33±0.89a
12.50%	30.00	3.33±0.10f	10.00±0.31e	20.00±0.79d	36.67±0.31c	50.00±1.21b	63.33±0.22a
6.25%	30.00	0.00±0.00d	0.00±0.00d	0.00±0.00d	23.33±0.79c	43.33±0.89b	53.33±1.02a
3.125%	30.00	0.00±0.00d	0.00±0.00d	0.00±0.00d	13.33±0.22c	30.00±0.79b	43.33±0.22a
Control	30.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Mean values followed by a common letter do not differ statistically in rows (P≤0.05

Table 6. Toxicity of neem seed oil, castor oil, turpentine oil and deltamethrin against *T. castaneum* after 3, 6, 12, 24, 48 and 72 hours by contact method

Chemicals	Hours	Total numbers (n)	LC_{50} and 95 % confidence limit	Slope ± SE	Chi-square	Order of toxicity	Df
Neem seed oil	3	180	67.494(34.325-141.321)	1.507±0.337	1.715	4	3
Castor oil	3	180	30.239(11.597-1431.228)	1.220±0.325	0.15	3	3
Turpentine oil	3	180	20.530(10.462-148.035)	1.382±0.378	1.215	2	3
Deltamethrin	3	180	5.222(5.433-211.660)	1.103±0.327	1.396	1	3
Neem seed oil	6	180	52.131(27.582-309.072)	0.9480.270	0.395	4	3
Castor oil	6	180	12.830(7.011-59.826)	1.1470.296	0.123	2	3
Turpentine oil	6	180	16.657(9.473-70.226)	1.5430.388	0.934	3	3
Deltamethrin	6	180	2.222(1.716-3.061)	2.1100.324	1.142	1	3
Neem seed oil	12	180	25.701(15.835-63.533)	1.0120.260	1.095	4	3
Castor oil	12	180	6.811(4.267-17.169)	1.1540.272	1.120	2	3
Turpentine oil	12	180	16.140(8.575-85.643)	1.2540.324	0.942	3	3
Deltamethrin	12	180	0.404(0.067-0.752)	0.8520.261	0.065	1	3
Neem seed oil	24	180	19.263(11.757-41.239)	0.9770.256	0.254	4	3
Castor oil	24	180	4.624(2.801-11.511)	0.9610.257	0.036	2	3
Turpentine oil	24	180	10.733(6.619-30.101)	1.3940.316	0.914	3	3
Deltamethrin	24	180	0.700(0.349-1.081)	1.123±0.266	2.263	1	3
Neem seed oil	48	180	5.094(0.039-11.520)	0.5780.247	0.133	4	3
Castor oil	48	180	2.502(1.700-3.683)	1.3730.268	0.245	2	3
Turpentine oil	48	180	10.110(5.963-33.467)	1.1980.290	0.805	3	3
Deltamethrin	48	180	0.499(0.267-0.724)	1.4650.300	4.800	1	3
Neem seed oil	72	180	2.623(0.683-4.587)	1.2200.303	0.725	3	3
Castor oil	72	180	1.582(1.048-2.182)	1.5740.285	1.084	2	3
Turpentine oil	72	180	8.630(5.330-23.647)	1.2350.288	0.211	4	3
Deltamethrin	72	180	0.356(0.174-0.523)	1.6650.345	2.970	1	3

Table7. Toxicity of neem seed oil against *Tribolium castaneum* after 3, 6, 12, 24, 48 and 72 hours by fumigation method

Chemicals	Hours	Total numbers (n)	LC_{50} and 95 % confidence limit	Slope ± SE	Chi-square	Order of toxicity	Df
Neem	3	150	462.902(163.963-8094365.678)	1.5990.670	0.473	6	3
Fumigation	6	150	191.525(107.105-1053.098)	1.711(0.471	2.111	5	3
	12	150	121.138(78.524-309.778)	1.7510.399	4.895	4	3
	24	150	81.881(44.732-422.594)	0.9000.263	0.541	3	3
	48	150	21.914(11.740-37.675)	0.9630.254	0.435	2	3
	72	150	10.182(3.530-16.883)	1.0060.263	0.326	1	3

5 Discussion

The population of *T. castaneum* in the present experiment was significantly reduced by the pyrethroid insecticides especially deltamethrin. This result was similar to those of Mondal (1987), Kamaruzzaman (2000) and Amin (2000) mainly due to the fact that the pyrithroids are very fast killers of the target insect pests because of their mode of action and low resistance in target pests (Saeed et al., 2012) . The results in relation to time and dose were similar to Pugazhvendan el al., (2009) as they used some plant parts having insecticidal and repellent activities and they showed the effectiveness, as the doses were increased and for longer period.

Some insecticides are developed with the biological activities like nicotine and azadiractin, resulting in slow resistance to the insects which are attacking the commodities. These secondary chemical compounds are very effective alternative strategy of controlling the insect pests (Talukdar 2006).

Neem seed oil was found more effective contact poison and fumigant against the adults of *T. castaneum* among the three essential oils. Azadirachtin in the neem seed oil is a proven chemical for its insecticidal properties (Xie et al. 1995; Rahila 2006). Lowest toxicity of Terpentine oil was due to its only sub–lethal effect on adults of *T. castaneum* even at high dose (Chaubey 2012).

The synthetic insecticides are very fast killers of the target insect pests and can give excellent control when they are treated. However, in stored grains these insecticides have some lethal effects or have toxic residues, which can harm the consumers. Therefore, this study was conducted to give the idea of alternative use of these botanicals for replacement of these insecticides in the storage and stored grains to minimize the toxic effect insecticides. So neem, tobacco and castor oils, which are less toxic for human consumption and can give excellent control of the insect pests particularly *T. castaneum*. Testing these in two different methods for their efficacy was to check the best possible way to use them in the stored grains for pests control as neem oil was found best for the pest control so was tested as fumigant as well.

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