Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2015; 3(5): 84-88

Received: 16-07-2015
Accepted: 18-08-2015

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Laboratory assessment of different botanical extracts and cypermethrin against insect pests

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Abstract
Different botanical extract contains promising pest control substances which are effective against many economically important insects pest of crop and store grain pests. So a comparative study was conducted to assess the contact toxicities of Cypermethrin against important stored products insects, *Sitophilus oryzae*, the rice weevil, and *Tribolium castaneum*, the rust red flour and 4th instar larvae of *Culex pipen*. The rice weevil, *Sitophilus oryzae* were more susceptible than *T. casteneum* and rust red flour beetle, with LC_{50} values of 0.05% and 0.09% respectively. Aqueous extract of *Vitex negundo* showed moderate activity against *T. castaneum* and non significant activity against *S. oryzae*. The extracts of *Nepeta Clarkei* showed activity against the 4th instar larvae of *Culex pipien* with LC_{50} value of (1.5%). Similarly, effects of *Vitex negundo* against *Culex pipien* larvae were not significant. Larvicidal effects of these botanicals were considered to warrant further research into their potential for commercial use.

Keywords: Botanical Extracts, *Tribolium castaneum*, Laboratory assessment.

1. Introduction
Among stored grain beetles *Sitophilus oryzae* is a serious insect pest of various food grains under storage. *Tribolium Castaeum* not only cause a major damage to stored grain, also affect the other commodities like grain, flour, peas, beans, cocoanuts, dried fruits, and spices, but milled grain products such as flour appear to be their preferred food [1].
Wheat suffers heavy losses during storage due to insect pests. According to FAO, the loss done annually by insects and rodent pests is 10 to 25% of world-harvested food [1]. In Pakistan, about 2 - 6% food grain production is lost every year during storage by stored grain insect pests. Similarly, the total post harvest loss of wheat is about 7.37% out of which 3.24% is due to stored grain insect pests. Weight loss of wheat during storage due to insect pests in Multan and Bhawalpur food grain storages on an average ranges between 0.45 and 0.75 % [2].
Moreover, mosquitoes are known as vectors of several disease causing pathogens, which affect millions of people all over the world [3]. Flies and mosquitoes (Dipterans species) constitute two major groups of nuisance species in rural and urban environments and remains a major source of illness and death worldwide. Mosquitoes alone transmit disease to more than 700 million people annually [4]. Flies with their ability for rapid distribution, high rate of reproduction, vast range of host plants, and good ecological adaptability, they are posing a large problem all over the globe and are a big menace in vegetable and fruit production [5].
Synthetic pyrethroids are chemical compounds with an action similar to that of natural pyrethrum. Among pyrethroids Cypermethrin is a synthetic pyrethroid of moderate mammalian toxicity. Cypermethrin acts by interfering with the Na+ and K+ channels in the peripheral and central nervous systems of insects [6]. Various strains of *T. castaneum* had been reported to develop resistance against gamma–HCH, malathion, fumigants, e.g., methyl bromide and phosphine, synthetic pyrethroids, e.g. Cypermethrin, deltamethrin, cyfluthrin, fenvalerate and permethrin and some juvenile hormone analogues [7]. A recent report has shown that *R. dominica* has become so resistant to phosphine that 200 times the recommended dose has to be used [8].
Due to resistant of insect to pesticide and also their persistent in environment and damage to non target organism necessitates the need of developing certain new safe alternatives of these synthetic pesticides, which may have no adverse effect on the environment and non-target animals [9]. Plant based pesticides are component of ecosystem for million years without any ill or adverse affect on ecosystem. Also, some plants have more than one chemical as an active

principle responsible for their biological properties. The chances of developing quick resistance to different chemicals are highly unlikely [10].

2. Materials and Methods

1. Insect rearing

The adult *T. castaneum* and *S. oryzae* were collected from store house of Peshawar. They were reared in plastic container (20 cm height × 7.5 cm diameter) containing wheat and maize for feeding. The temperature was usually between 25 ^{0}C to 30 ^{0}C with relative humidity 70 ±5. All experimental procedures were carried out under the same environmental conditions as the cultures.

1.1 Preparation of chemical for *T. castaneum* and *S. oryzae*

The toxicity of Cypermethrin was used against *T. castaneum* and *S. oryzae* in contact methods. The Cypermethrin was of BASF chemical company with a commercial name Rapicord (10EC) taken from Saleem shop at King Gate Kohat, Khyber pukhtoonkhwa (KPK), Pakistan. The insecticides were dissolved in distilled water and make 1% stock solution. From the stock solution the different concentration of 0.5%, 0.25%, 0.125%, 0.0625%, 0.03125%, 0.015625%, were selected for both species. These doses of Cypermethrin were selected after many preliminary tests.

1.2 Method of treatment

The dose of 0.1% with different concentration of Cypermethrin was used. The Petri dishes having a size of medium size 7 cm is used for experiment. The filter paper were impregnated with dose (chemical) at the bottom of glass Petri dishes with the help of micropipette and spread uniformly by rotating the plate then each Petri dish is marked with respective dose and number. After drying, 10 adults of each species with same size and shape were introduced. The control batch of insects was kept under the similar condition without any dose treatment. If mortality more than 10% was investigated in control then the rest of experiments were discarded. When there is no leg or antennal movement the insect were consider dead but take care of fin death was much taken. Then each experiment was repeated five times to get accurate result.

2. Rearing of *Culex pipien*

During preliminary screening the *Culex pipien* was collected from stagnant water near premises of university. They were kept in a plastics jar for rearing in the Entomology/Toxicology lab of Zoology Department, Kohat University of Science and Technology (KUST) Kohat, KPK, Pakistan. The medium containing plant and animal debris is provides as a source of food. The temperature was kept in between 25-30 °C with relative humidity of 70 to 80%. The pupas were collected in separate jar to get adult for identification.

2.1 Method of treatment

The 4th instar larvae were selected for experiments. The larvae were collected with fine brush. Each 10 larvae of *Culex pipien* were taken in each Petri dish (7cm diameter/150mL capacity) and then added 5 ml of water to each of the respective Petri dish. In control batch also, the ten 4th instar larvae with 5% water were taken without adding any chemical (dose). Normally the larvae show wriggling movement when fine brush was touches to their body and when the larvae show no wriggling movement and cannot come to water surface it was consider as dead. Similarly control and check was also run parallel and noted in the same manner. Then each dose was replicated five times to get accurate result. When more than 10% deaths occurred in control and checks then the rest experiment were discarded. The methanolic plant extract of *Nepeta clarkei* and water extract of *Vitex negundo* were obtained with the courtesy of Dr. Javeed Hussain and Izhar-ul-Haq Ali Shah from Department of Chemistry, Kohat University of science and technology (KUST) Kohat, KPK, Pakistan. The dose (*Nepeta clarkei*) of 1%, 2%, 3%, 4%, 5% was used and the results was recorded after 24 hrs and 48 hrs in each Petri dish. Similarly, the biocidal activity of *Vitex negundo* with a dose of 2%, 3%; 4%, 5%, 6% was also recorded after 24 hrs and 48 hrs.

3. Result

The LC_{50} of Cypermethrin against *Sitophilus oryzae* and *Tribolium castaneum* was observed after 24 hrs as 0.05% and 0.09%, which showed that LC_{50} of Cypermethrin against *Sitophilus oryzae* is highest as compared to *Tribolium castaneum*. Similarly, it also means that the *S. oryzae* is more susceptible to Cypermethrin in comparison with *Tribolium castaneum* (Fig 1 and 2). In the percent experimental work, Cypermethrin showed a maximum of 94% and 90% mortality against *S. oryzae* at 0.5% and 0.25% concentrations respectively after 24 hrs of exposure followed by minimum mortality of 40% and 24% at 0.03125%, 0.015625% concentrations respectively (Table1). This shows that increase in dilution of Cypermethrin decrease the mortality rate. Similarly after 48 hrs of treatment Cypermethrin showed regular increase in the mortality of *S. oryzae*. The mortality at high concentrations (0.5% and 0.25%) were 98% and 96% while 82% and 80% deaths were recorded at lowest concentration (0.015625%), as shown in Table 2.

Moreover, the Cypermethrin also showed gradual decline in the adult of *T. castaneum* by decreasing its concentration from 0.5% to at 0.015625% after 24 hrs treatment. The greatest mortality was founded at concentration 0.5%, 0.25% and lowest at 0.03125%, 0.015625% ranging from 94%, 50% 16% and 8 % respectively (Table 3). The mortality also increases to same ratio after 48 hrs treatments and reported that at 0.5 %, 0.25 % mortality reached to 96%, 56% and 26%, 22% at lower concentration 0.03125%, 0.015625% respectively (Table 4). From these it was concluded that after 48 hrs lowering in concentration for *T. castaneum* the mortality was not so much increased as in *S. oryzae.*

LC_{50} of methanol extract of plant *Nepeta clarkei* (Hook) was 1.5% against *Culex pipien* 4th instar larvae. The percent mortality at 1%, 2%, 3%, 4%, 5% concentration were recorded to be 50%, 74%, 84%, 84% and 92% by keeping constant volume of water (Table 5). The *Vitex negundo* resulted in percent mortality after 24hrs were 38%, 12%, 30%, 34%, and 20% at 2%, 3%, 4%, 5%, 6% concentrations (Table 6). These results showed a non-significant activity against *Culex pipien*.

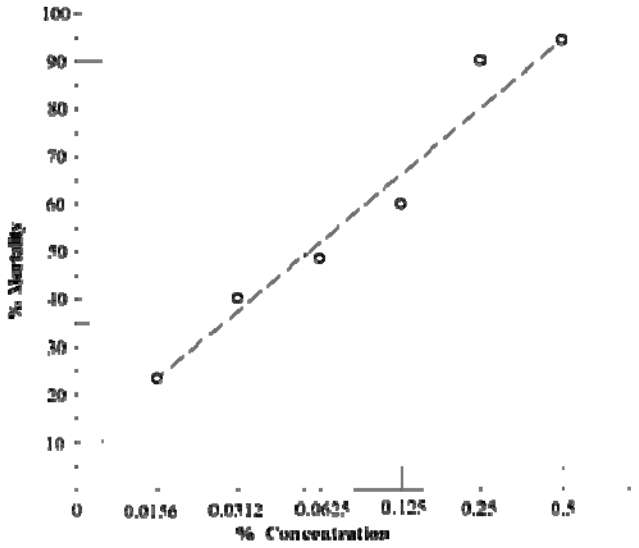

Fig 1: Graph showing LC_{50} of cypermethrin against *T. castaneum*

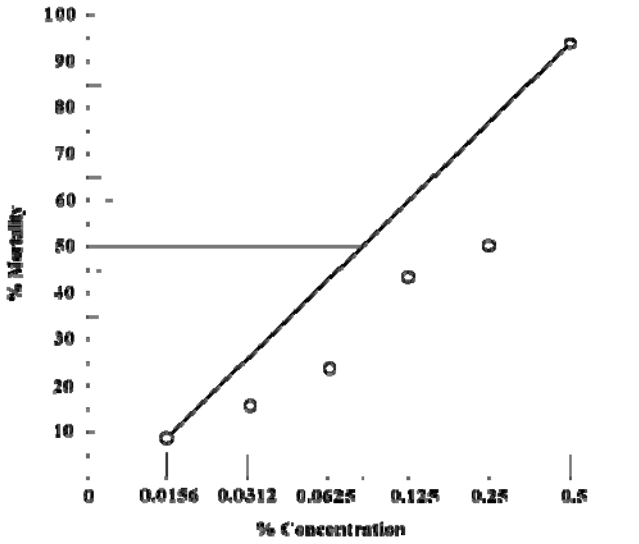

Fig 2: Graph showing LC_{50} of cypermethrin against *S. oryzae.*

Table 1: Percent mortality of *Sitopilus oryzae* after 24 hour of treatment with different concentration of cypermethrin.

S. No	%Concentration	Mean % mortality
1	0.5	94
2	0.25	90
3	0.125	60
4	0.0625	48
5	0.03125	40
6	0.015625	24
7	Control	0

Table2: Percent mortality of *Sitophilus oryzea* after 48 hour of treatment with different concentrations of cypermethrin.

S. No	%Concentration	Mean %Mortality
1	0.5	98
2	0.25	96
3	0.125	86
4	0.0625	86
5	0.03125	82
6	0.015625	80
7	Control	2

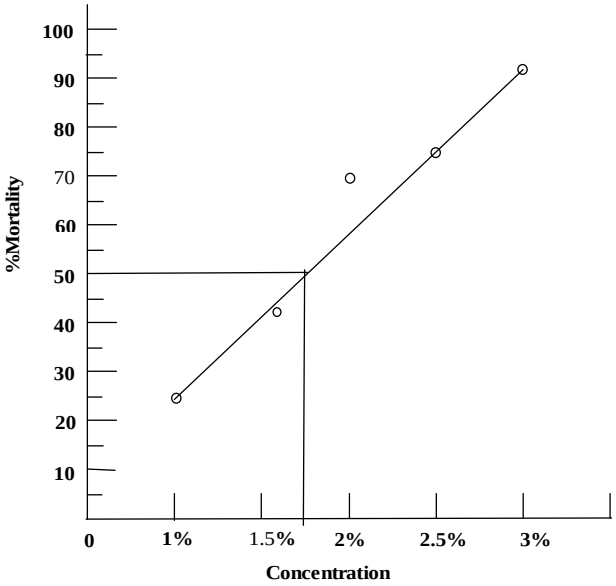

Fig 3: LC_{50} diagram showing % mortality of *Culex pipien*.

Table 3: Percent mortality of *Tribolium casteneum* after 24 hour treatment with cypermethrin.

S. No	%Concentration	Mean % mortality
1	0.5	94
2	0.25	50
3	0.125	44
4	0.0625	24
5	0.03125	16
6	0.015625	8
7	Control	4

Table 4: Percent mortality of *Tribolium castaneum* after 48 hrs of treatment with different cypermethrin.

S. No	%Concentration	Mean %mortality
1	0.5	96
2	0.25	56
3	0.125	52
4	0.0625	30
5	0.03125	26
6	0.015625	22
7	Control	0

Table 5: Efficacy of Methanol extract of plant concentration of *Nepeta clarkei* Hook F (Labiatae) against *Culex pipien* after 24 hrs of treatment.

S. No	Concentration	Mean% mortality
1	1%	50
2	2%	74
3	3%	84
4	4%	84
5	5%	92
6	Control	0
7	Check	6

Table 6: Efficacy of water extracts of plant *Vitex negundo* (verbenaceae) against *Culex pipien* after 24 hrs of treatment.

S. No	Concentration	Mean % mortality
1	2%	38
2	3%	12
3	4%	30
4	5%	34
5	6%	20
6	Control	0

Table 7: The mortality of *T. castaneum* and *S. oryzae* caused by *Vitex negundo* after 24 treatment.

S. No	Concentration	*T.castaneum* mean% Mortality	*S.oryzae* Mortality mean %
1	Control	00	00
2	2%	00	00
3	3%	3	00
4	4%	11	00
5	5%	12	00
7	6%	14	19

Remarks: Aqueous fraction of *Vitex negundo* showed moderate activity against *Tribolium castaneum* and non - significant activity against *Sitophilus oryzae.*

4. Discussion

The current research presents investigation of the comparative analysis of Cyprmethrin and different plants extracts against agricultural and medical pests and is compatable with the previously published reports of Hasan *et al* [2]. Hasan *et al* [2] reported the comparative efficacy of two plant extracts, *Amaranthus viridis* and *Salsola baryosma* along with Cypermethrin against *Trogoderma granarium*, they reported that Cypermethrin was more effective and give high mortality (37.64%) as compared to plant extract of A. *viridis* (19.58%) and *S. baryosma* (22.08%). These comparative analysis show that plant extract give less mortality as compared to pyrethroids (synthetic product), this may be because of the dilution of crude extract of plants which contain very little actual active ingredients as compared to the pure synthetic compounds. The differences in biocidal activities of plant extracts and synthetic compounds (cypermethrin and pyrethroids) may be due to merely immediate neurotoxic affect of synthetic products on insect's pests as reported previously [11].

Similarly, among pyrethroids, cypermethrin is more effective and show toxicity to wide range of insect's pests belonging to different orders [12]. Rani *et al* [12] compared the toxicity of pyrethroids among which cypermethrin was most effective against *Drosophila melanogaster*, the LC_{50} was found to be 16ppm for deltamrthrin, 4 ppm for coopex and just 0.36 ppm for cypermethrin. The similar results have been observed by Naqvi *et al* [13], who determined the toxic and teratogenic effects of Coopex (25 E.C), and neem extract (N-7) against 3rd instar larvae of *Musca domestica*. The present study has proved the efficacy of plant extract against insect (pests), furthermore, suggests that if the pure compounds obtained from plant, will significantly reduce the use of synthetic products.

Moreover, another study of Elbanna [14] has observed the efficacy of *Eucalyptus globulus* plant against *Culex pipiens*, Elbanna [14] used the seed as well as leaf extracts at 1000 ppm and reported the 100% and 80% mortality rates of *Culex pipiens* larvae. The result of Elbanna [14] is similar to present research work because the mortality rate of the plants extracts was dose and time dependent. Furthermore, the study of Cetin *et al* [15] has also reported the efficacy of five species of plants belonging to Labiatae (Lamiaceae) famil, against *Culex pipien*. In support of our results, Sharma *et al* [16] have also reported the significant biocidal activities of acetone extract of *Nerium indicum* and *Thuja orientalis* against third instar larvae of *A. stephensi* species of mosquito.

5. Conclusion

In the present study Cypermethrin reported sufficient control of different insect at lower concentration as compared to plant extract so it can be used in large scale to keep insect population below threshold level. Keeping in mind the ecological health hazards and increasing resistant of different insect to synthetic products, the plant based pesticides may be preferred. The eco-friendly, safe and local availability of natural products necessitates further researches in order to discover new plant origin pesticides against different insect pest.

6. References

1. Sahaf BZ, Moharramipour S, Meshkatalsadat MH. Chemical constituents and fumigant toxicity of essential oil from *Carum copticum* against two stored product beetle. Insecect Science 2007; 14:213-218
2. Hasan M, Siddique MA, Sagheer M, Aleem M. Comparative efficacy of ethanol leaf extracts of *Amaranthus viridis* and *Salsola baryosma* (schultes) and cypermethrin against *Trogoderma granarium* (Everts). Pak. J Agri Sci. 2005; 42:3-4.
3. Rahuman AA, Gopalakrishnan G, Venkatesan P, Geetha K, Bagavan A. Mosquito Larvicidal activity of isolated compounds from the Rhizome of Zingiber officinale. Phytother. Res. 2008; 22**:**1035-1039.
4. Taubes G. Vaccines. Searching for a parasite's weak spot, *Science* 2000; 290(5491):434-437.
5. Bonnefoy X, Kampen H, Sweeney K. *Public Health Significance of Urban Pests*, pp. 565. World Health Organization Regional Office for Europe, Copenhagen, 2008, 209-238
6. Saleem MA, Shakoori AR, Mantle D. Macromolecular and enzymatic abnormalities induced by a synthetic pyrethroid, Ripcord (Cypermethrin), in adult beetles of a stored grain pest, *Tribolium castaneum* (Herbst.) (Coleoptera: Tenebrionidae) Arch. Ins. Biochem. Phy 1998; 39:144-154
7. Hussain R, Ashfaq M, Saleem MA, Ahmed S. Toxicity of some insecticides with novel modes of action against Malathion resistant and organophosphate susceptible strains of *Tribolium castaneum* Larvae. Int. J Agri Biol. 2005, 7(5).
8. Wu H, Zhang G, Zeng S, Lin. Extraction of allyl isothiocyanate from horseradish (Armoracia rusticana) and its fumigant insecticidal activity on four stored-product pests of paddy. *Pest* Manag sci. 2009; 65(9):1003-8.
9. Ngamo TSL, Ngatanko, Ngassoum MB, Mapongmestsem PM, Hance T. Persistence of insecticidal activities of crude essential oils of three aromatic plants towards four major stored product insect pests. Afr. J Agric Res. 2007; 2(4):173-177.
10. Saxena J, Mathela CS. Antifungal activity of new compounds from Nepeta leucophylla and Nepeta clarkei. Appl. Environ. Microbiol 1996; 62(2):702-704.
11. Dyck PJ, Masatake S, Gerald PS, Alfred CL, Karen FO, Margaret FS. The evaluation of a new synthetic pyrethroid Pesticide (Permethrin) for neurotoxicity. j environ pathol Toxicol oncol 1985; 5:109-118.
12. Rani S, Tabassum R, Naqvi SNH, Ahmed I, Sultan B, Khan MF. Comparative toxicity of different formulation of cypermethrin, delmethrin and permethrin against *Drosophila melanogaster* (K.U strain). J of Environ boil. 1997; 18(4):343-349.
13. Naqvi SNH, Jahan M, Tabassum R, Qamar SJ, Ahmad I. Toxicity and teratogeny caused by Coopex (25 E.C), and a neem extract (N-7) against 3rd instar larvae of Musca domestica L. Pak. J Zool. 1995; 27(1):27-31.

14. Elbanna SM. Larvaecidal effects of *Eucalyptus* extract on the Larvae of *Culex pipiens* Mosquito. Int. J Agri Biol. 2006, 8(6).
15. Cetin H, Cinbilgel I, Yanikoglu A, Gokceoglu M. Larvicidal activity of some Labiatae (Lamiaceae) plant extracts from Turkey. Phytother. Res 2006; 20:1088-1090.
16. Sharma P, Mohan L, Srivastava CN. Larvicidal potential of *Nerium indicum* and *Thuja oriertelis* extracts against malaria and Japanese encephalitis vector. J Environ Biol. 2005; 26(4):657-660.