INT. J. BIOL. BIOTECH., 12 (4): 639-642, 2015.

TOXICOLOGICAL EVALUATION OF INSECTICIDES AND PLANT EXTRACTS AGAINST C*ALLOSOBRUCHUS CHINENSIS* L. (COLEOPTERA: BRUCHIDAE) WITH EFFECTS ON FECUNDITY

Seema Tahir[1] and Tahir Anwar[2]

[1]*Pakistan Agricultural Research Council (PARC), Karachi University Campus, Karachi, 75270, Pakistan*
[2]*Pest Management Research Institute, Southern zone Agricultural Research Center (SARC), Pakistan Agricultural Research Council (PARC) University Campus, Karachi*

ABSTRACT

Two synthetic insecticides i.e. cypermethrin and imidacloprid and botanicals neem oil, *Aloe vera* and chilli extracts were bioassayed under laboratory conditions for their ability to protect stored grain from damage by pulse beetle, *Callosobruchus chinensis* L. at different concentrations. Both insecticides caused significant adult mortality even on low doses whereas the neem oil, *Aloe vera* and chilli extracts had no toxic effects but they repelled and deterred the insects with effect on fecundity which also enhanced their residual effects on grain seeds. *Aloe vera* and chilli extracts significantly reduced the oviposition potentials of adults and thus can be used as a safe grain protestant.

Key-words: Insecticides, plant extracts, pulse beetle, *Callosobruchus chinensis*.

INTRODUCTION

During storage several stored product pests cause economic damage because infected grain lose their quality, weight loss and poor nutritional value. The Food and Agriculture Organization (FAO) estimates that 5-10% of grain is lost between harvest and consumption. Several bruchids species attack cereal & pulses in the store and cause a loss of 10-15% with germination loss ranging from 50-92% (Adugna, 2006). About 100% loss of cereals and pulses was found due to infestation by the pulse beetle. *Callosobruchus chinensis* (L.) and *C. maculatus* (F.) are the major pests of cow-pea in the tropics (Raja *et al.*, 2007).

Fumigants and insecticides are widely used against insect pests (Mamun *et al.*, 2009, Chaubey *et al.*, 2012, and Anwar *et al.*, 2012). Some grain protectors particularly organophosphates have high mammalian toxicity and the residues of these insecticides left in water, fruits and vegetables (Anwar *et al.*, 2011 and Tahir *et al.*, 2001) may cause health concerns because they are conventional neurotoxins that effect the human nervous system, so due to their persistency in food and effects in the environment (Tahir *et al.*, 2001) their negative impact is being felt. There is a need for searching alternative effective safe pest control methods.

The pulse beetle can reproduce both in field and in storage. Synthetic pesticides have been used as an effective grain protector for many years but due to their environmental hazards, there have been a growing concern in recent years against them like toxicity, destruction of beneficial fauna, pest upset, pest resurgence and residues. So many compounds have been phased out due to danger to the user safety, high cost, development of resistant strains and toxic residues. To avoid such hazards bioactive compounds of plant origin having insecticidal, ovicidal properties are being used as grain protector against beetles in storage (Raja *et al.*, 2007). Historical usage of nicotine & pyrethrum has encouraged the researchers to focus their attention on alkaloids, flavonoids, terpenoids, and others compounds for use against insect pests. In recent years attention has been focused on the use of plant materials for the insect pest control.

The use of grain protector is a common preventive measure to protect stored products from insect damage many of these are effective at relatively low doses and can provide long term protection range from 6-12 months. Plant extracts and botanicals are also used to control stored product pests (Stefanazzi, *et al.*, 2011; Mahfuz and Khaliquzzan, 2007; Aziz and Abbass, 2011; and Rajapakse and Ratnasekera, 2008). The fecundity in *C. maculatus* has been reported to be reduced by botanicals (Abdullahi, 2011; Rawat and Srivastava, 2012). Plant extracts specially of neem tree with low mammalian toxicity reported to control many insects and also gave ideal protection for stored products with excellent residual contents (Siddiqui, *et al.*, 2009; Stefanazzi *et al.*, 2011). The toxicity of synthetic pyrethroids & botanicals against stored products is reported but little work is done on their residual toxicity and fecundity deterrents.

MATERIALS AND METHODS

Insects

The test insects *Callosobruchus chinensis* L. were used in this study. The culture strain was obtained from public sector godowns in Karachi and was reared in the laboratory in sterilized jars covered with muslin cloth tied with rubber bands. Whole 500 gram chickpea seeds were used as culture medium. The culture was maintained in the laboratory at 30 ± 5^{o}C temperature and 65 ± 5 relative humidity. Each jar was set up with 40 pairs of adult insects. After 15-20 days eggs were observed and the newly emerged adults after 40-45 days were utilized for toxicological studies.

Insecticides

Commercial grade insecticides, Cyperkil, 10 EC (Cypermethrin, pyrethroid) from R.B Avari Enterprises (Pvt) Ltd. and Imidacloprid 20% SL (Acetmiprid) from Capricor Associates were purchased from the local market. The Neem (*Azadirachta indica*) fruits were collected from the university campus and oil extracted from the local market. Different concentrations of insecticides and neem oil ie.1.0%, 0.5%, 0.25%, 0.125%, 0.0625% and 0.03125% were prepared by dissolving the 1% stock solution of each in acetone. Fresh leaves of *Aloe vera* were collected from the university campus and juice (100 %) was obtained by removing green parts. 100g of chilli stem was cut and soaked in 500 mL acetone into 1000 mL conical flask and left for 24, filtered and evaporated to 30 mL.

Insect Bioassay

Different dilutions of insecticides and neem oil were used in bioassay. Whatman filter paper (6 cm diameter) impregnated with one mL of different concentrations of tested compounds were placed in 6 cm diameter Petri plates. The control plates had no compound and check plates had only acetone. Three replicates of each dose were run. Ten adults of same size and weight were kept in each Petri plate. The mortalities were recorded at 24, 48 and 96 HAT (hours after treatment). The experimental data were analyzed statistically.

For fecundity trials three doses i.e. 0.5 mL, 1 mL and 2 mL of *Aloe vera* and chilli extracts were mixed in 100g seeds with a control. The experiment had three replicates. Five pairs of insects in each bottle and kept for egg laying. After 10 days, number of eggs laid was counted and after 30–40 days newly emerged adults were collected with aspirator.

RESULTS AND DISCUSSION

The LC_{50} of the tested compounds Commercial grade insecticides Cyperkil, 10 EC (Cypermethrin, pyrethroid), R.B Avari enterprises (Pvt) Ltd. and Imidacloprid 20% SL (Acetmiprid), Capricor Associate and Neem oil (*Azadirachta indica*) against pulse beetle are given in Table 1.

The effects of different concentrations of insecticides and neem oil i.e. 1.0%, 0.5%, 0.25%, 0.125%, 0.0625%, and 0.03125% at 24, 48 and 72 HAT (hours after treatment) indicated that cypermethrin possessed the highest toxic effects at conc. ranging (0.03-1%) i.e. 15-94% , 18-92% and 21-95% mortality percentages were recorded at 24, 48 and 96 HAT respectively .whereas Imidacloprid at conc. ranging (0.03-1%) i.e. 10-80% , 14-93% and 18-93% mortality percentages were recorded at 24, 48 and 96 HAT respectively and neem oil with lowest toxic effects. at conc. ranging (0.03-1%) i.e. 5-37%, 7-46% and10-50% mortality percentages were recorded at 24, 48 and 96 HAT, respectively.

Plant extracts i.e. *Aloe vera* and chili significantly reduced the oviposition i.e. 75, 154 and 138 eggs at 2 mL, 1 mL and 0.5 mL whereas, 59, 29 and 42 eggs at 2 mL, 1 mL and 0.5 mL, respectively. The viability, survival of immature stages as well as adult and emergence were highly reduced in all treatments. However, the reduction in adult emergences was found to be higher on seed treated with chili extract as no emergence was recorded in any treatments as compared to 20 adults emerged in control batch whereas at 0.5 mL, 1 mL and 2 mL *Aloe vera* extract 93, 115 and 88 adults were emerged, respectively as compared to 179 in control (Table 2).

The findings of these studies were similar to these of Raja *et al.*, (2007) where the bags of pulses stored for 6 months without any damage of bruchids when treated with aqueous extract from leaves of *Melia azedarach*. The extract coated seeds may have the contact poison effect and during oviposition it can penetrate the body and possibly interfering with the normal development of insects by suppressing the hormonal and biochemical processes. Earlier report have shown that product of plant materials have been used in the control of stored product pests as they may impair the respiration of the insects (Hall and Harmann, 1991) as it blocks the spiracles leading to suffocation. The findings of this study were comparable with that of Abdullahi (2011) where viability, survival of immature stages and adult emergence were highly reduced in all treatments. From the above results it is clear that all the tested compounds may be more or less effective for controlling pulse beetle but cypermethrin was found most effective one. The present study revealed the reduction of pest population by using neem oil which is in line with the previous findings of Anwar *et al.*, (1993) and Siddiqui *et al*., 2009, and this also suggest that the use of plant products may be of value of combating the growing threat of insecticides resistance (Mamun *et al.*, 2009). These findings, considered together, suggest that neem oil shows potential to be developed as natural insecticides/fumigant for control of stored product pests. However, for the practical application of the neem oil and two pesticides as novel insecticides/fumigants further studies on the safety of the neem oil and two tested compounds towards human and on the development of formulations are necessary to improve the efficacy and stability, and to reduce cost. The insecticidal constituents of many plant extracts and oil are mainly terpenoids and are volatile. They can easily penetrate into insects body and interfere their physiological functions thus by controlling the fecundity and control the pests. *Aloe vera* gel and chili extract possessed the ovicidal activity against the pulse beetle which is in favour of previous findings, where the bio-effects of five plant essential oils are known to reduce the population by reducing the oviposition in pulse beetle (Aziz and Abbass, 2010). Similarly, the insects treated with tested compounds reduce the fecundity and thus can be used as efficient insecticidal tool against pulse beetle as fumigants and can replace the conventional comparatively toxic fumigant methyl bromide. However, before using on commercial scale further investigations are needed to confirm the results. Due to their volatility they have fumigant and gaseous action and might be of importance for stored product insects and could be used as a biodegradable natural bio protector for controlling stored product pest.

Table1. Lethal concentrations of synthetic insecticides and neem oil against C*allosobruchus chinensis* L. (Coleoptera: Bruchidae) after 24, 48 and 96 hours of treatments.

S. No	Insecticide HAT	LC_{50} (%)	LC_{90} (%)	Regression model	R^2
1	Cypermethrin				
	24hours	0.32	0.83	Y=77.59X + 25.28	0.86
	48 hours	0.19	0.77	Y=68.77X + 37.20	0.69
	96 hours	0.00	0.68	Y=58.90X + 49.99	0.56
2	Imidacloprid				
	24hours	0.41	1.03	Y=64.03X + 23.87	0.82
	48 hours	0.29	0.85	Y=71.25X + 29.39	0.84
	96 hours	0.19	0.72	Y=75.36X + 35.60	0.77
3	Neem oil				
	24hours	1.33	2.68	Y=29.78 X +10.24	0.85
	48 hours	0.97	2.07	Y=36.51X + 14.46	0.73
	96 hours	0.87	1.99	Y=35.63X + 19.19	0.59

HAT stands for hours after treatment; LC_{Lethal} concentrations in percentage (%) ; Y = % mortality; X = % concentration.

Table 2. Effect of *Aloe vera* gel on the fecundity and emergence of C*allosobruchus chinensis* L. (Coleoptera: Bruchidae) after 10 days of treatment.

Effects	Extract	Control	2 ml	1 ml	0.5 m
Fecundity	*Aloe vera*	367.0 ± 188.37	75.4 ± 48.1	154.0 ± 97.3	138.0 ± 7.6
	Chili	46.6 ± 22.5	59.7 ± 30.2	29.0 ± 14.8	42.0 ± 20.5
Emergence	*Aloe vera*	179.6 ± 92.5	88.0 ± 73.4	115.3 ± 65.6	93.0 ± 41.2
	Chili	20.6 ± 12.0	0	0	0

Mean values ± S.D are the average of three replicates

Acknowledgements

We are thankful to the evaluation team for selection and providing funds under the "Dean Faculty of Science Research projects". University of Karachi, Karachi.

REFERENCES

Abdullahi, N. (2011). Studies on the efficacy of leaf extract of *Balanites aegyptiaca* on the oviposition and survival of immature stages of *Callosobruchus maculatus* (F.) on treated cowpea seed. *Bajopas*,.1(4): 40-43.

Adugna, H. (2006). On-farm storages studies in Eritrea. *African J. Biotechnol*, *5* (17): 1537-1544.

Anwar, T., S. Tahir., R. Ali, I. Ahmad and J. Naseem (2012). Field evaluation and residual toxicity of insecticide against fruit fly (*Corpomyia vesuviana)* in ber Z*iziphus jujube*) at SARC- experimental field university campus, Karachi. *Pak. J. Entomol*. Karachi, 27(1): 27-32

Anwar. T., I. Ahmad and S. Tahir (2011). Determination of pesticide residues in fruits of Nawabshah District, Sindh. *Pak. J. Bot*., 43(2): 1133-1139.

Anwar. T., S. Tahir and A. Jabbar (1993). Effect of neem oil on the fecundity and longevity of chickpea pod borer. *Pak. J. Agric. Res*., 14(4): 340-343.

Aziz, E. E., and M. H. Abbass (2010). Chemical composition and efficacy of five essential oils against the pulse beetle *Callosobruchus maculates on Vigna radiata* seeds. *American-Eurasian J. Agric. and Environ. Sci*., 6(4): 411-499.

Chaubey. M. K.. (2012). Acute, Lethal and Synergistic effects of some terpenes against *Tribolium castaneum. Ecologia Balkanica*, 4(1): 53-62.

Hall, J.S. and G. E. Harman (1991). Protection of stored legume seeds against attack by storage fungi and weevils: mechanism of oil seed treatment. *Crop Prot*., 10: 375-80.

Mahfuz, I. and M. Khaliquzzan (2007).Contact and fumigant toxicity of essential oils against *Callosobruchus maculatus*.. *J. Zoology*. *Rajshahi University*, 26: 63-66.

Mamun. M. S. A., Shahjahan and M. Ahmed (2009). Laboratory evaluation of some indigenous plant extracts as toxicants against red flour beetle. *J. Bangladesh Agril. Univ. 7*(1): 1-5.

Raja, M., S. J. William and M. Jayakumar (2007). Repellent activity of plant extract against pulse beetle *Callosobruchus maculatus. Hexapoda.* 14(2): 142-145.

Rajapakse, R. H. S. and D. Ratnasekera (2008). Pesticidal potential of some selected tropical plant extracts against *Callosobruchus maculatus* (F.) and *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae). *Tropical Agricultural Research & Extension*, 11: 69-71.

Rawat, S., and M. Srivastava (2012). Evaluation of efficacy of formulations of plant *Prosopis juliflora* against *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae). *Global J. Science Frontier Research*, 4(12): 29-31.

Siddiqui, B. S., S.K. Ali, S. N. H. Naqvi and R. M. Tariq (2009). Variation of major liminoids in *Azadirachta indica* fruits at different ripening stages and toxicity against *Aedes aegypti*. *Nat. pro. Comm.*, 4(4): 473-476.

Stefanazzi. N., T. Stadler and A. Ferrero (2011). Composition and toxic repellent and feeding deterrent activity of essential oils against the stored-product pests Tribolium castaneum (Coleoptera: Tenebrionidae) and Sitophilus oryzae (Coleoptera: Curculionidae). *Pest Manag. Sci.* 67: 639-646.

Tahir. S., T. Anwar. I. Ahmad. S. Aziz. M. Ashiq and K. Ahad (2001). Determination of pesticide residues in fruits and vegetables in Islamabad market. *J. Enviorn. Biol.*, 22(1): 71-74.

(*Accepted for publication October 2015*)