Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(4): 304-308

Received: 22-05-2019
Accepted: 24-06-2019

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Insecticidal activity of *Artemisia sieversiana* Ehrh. plant extract against white grub (Scarabaeidae: Coleoptera)

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Abstract
June beetles larva, commonly known as white grub, feed on the roots of many herbaceous and woody plants. White grub is an important agricultural crop pest found in agro-climatic conditions of Gilgit Baltistan. Insecticidal activity of *Artemisia sieversiana* Ehrh. Plant crude extracts prepared in three different solvents (Acetone, Ethanol and P. ether) against white grub was studied in Laboratory. Bioassay for repellency and residual toxicity of crude extracts carried out with three serial doses (1%, 3% and 5%). The results showed that maximum repellency (98.30%) was observed at 5% extract in Ethanol after 3 hours exposure. Maximum mortality (97.00%) in white grub was recorded at 5% extract in acetone after 72 Hr of insect exposure. The results revealed that *A. sieversiana* Ehrh. Has dual properties repellency and residual toxicity and can be used for eco-friendly management of white grub replacing chemical insecticides.

Keywords: Artemisia, insecticidal, white grub, crude extracts, repellency and mortality

Introduction

White grub is a larva of Melolonthidae. The Scarabaeidae is most important family of order Coleoptera. The world fauna of white grub exceeds 30,000 species, and there are about 1300 North American species (Mittal 2000) [15]. White grub is a National pest. Several species of white grubs or scarabs are root feeding pests such as turf grass, forage grass, corn, small grains, sugarcane, strawberry, potato tubers, and young nursery trees (Crocker, 1996) [4].

Potato crop is attacked and damaged by a number of insect pests including wireworms, white grub, aphids, cutworm and others as a result, the yield of the crop is adversely affected (Shakur *et al*, 2007) [17].

They cause damage to roots of commercial crops the damage caused by the White grub up to 70% was recorded (Yadava, 1995) [27]. White grubs are root feeders and their beetles feed on the leaves of host plants. *Holotrichia serrata* as a serious pest recorded in many parts of western Maharashtra, India (Musthak, 2013) [17].

White grub damage is usually most evident in August and September. As damage continues, the dead patches may increase in size, and apparently healthy turf areas may exhibit sudden wilting. The turf may feel spongy as you walk over the infested area (Buss, 2006) [3].

The chemical insecticides have not effective result of the white grub management system and these chemical pesticides have hazardous effects occur on the human being and other organisms. The green plants represent a reservoir of effective and provide valuable natural source of pesticide (Hostettmann *et al*., 1997) [6].

Other eco-friendly insecticides are botanical pesticides, which are synthesized by plants or produced from extracts of plants with insecticidal activity. These biopesticides have considerable advantages over synthetic insecticides in terms of their high selectivity, low mammalian toxicity, rapid degradation, and environmental friendliness. Moreover, botanical pesticides are produced by plants that have undergone long-term co-evolution with pests, and possess diverse insecticidal activities. It is more difficult for pests to develop resistance to these natural products (Isman 2000, Isman and Akhtar, 2007) [7-8]. Therefore, the prospect of using botanical pesticides as alternatives to controversial traditional pesticides has gained increasing interest (White and Leesch, 1995, Nukenine *et al*., 2011, Huang *et al*. 2011) [24-19-6].

Many botanical insecticides derived from commonly available plants effectively meet criteria of affordability and accessibility to small-scale farmers as well as human- and environmental-safety (Isman, 2008) [10]. Botanical insecticides are generally considered to have low toxicity to mammals; with some, like the neem-based azadirachtin, being non-toxic to mammals, fish and pollinators (Naumann and Isman, 1996; Wan *et al*., 1996) [18-23]. Even those botanical insecticides that have some level of toxicity to non-target organisms, their tendency to be rapidly degraded by sunlight renders them non-persistent and as such less likely to have significant impact through residual effect as is often the case with synthetic chemical insecticides (Isman, 2006) [9].

Different species either from Eucalyptus or Artemisia genera have a vast range of insecticidal activities including fumigant, contact and repellent effects (Jemâa, 2014) [12]. Wormwood (*Artemisia sieversiana*) is perennial herb with grey green deeply lobed aromatic leaves. *A. sieversiana* is distributed in Skardu and used as ethnomedicine by local people. It grows abundantly as wild in Gilgit Baltistan along the water channels and uncultivable lands reserved for nonfruit trees and fodders.

The aim of this study was to investigate the insecticidal activity of a locally available herb *Artemisia sieversiana* for eco-friendly management of white grub which is serious Polyphagus insect pests of crops in Skardu, GB, Pakistan.

Materials and methods

Plant material

The aerial portion of local herbs *Artemisia sieversiana* harvested at pre-blooming stage and removed wilted parts and pieces of other plants. The plant materials washed thoroughly removed dust and other inert materials then dried in shade. The dried plant materials grinded to form fine powder and stored in air tied jars. Plant specimen was identified by Dr. Amir Sultan, National Herbarium NARC, Islamabad as *Artemisia sieversiana.*

Crude extracts preparation

Artemisia sieversiana plant extract was prepared carried out through Soxhlet extractor in IPEP Laboratory, NARC. Poured 300 ml Ethanol in distillation flask. Filled the thimble with 25gm powder of *A. sieversiana* aerial part then placed the thimble inside the Soxhlet apparatus. The solvent in distillation flask heated adjusting temperature as 40°C. The process of condensed solvent passing through thimble extracting soluble plant materials to distillation flask dropping by siphon repeated for 8 hours. These extracts transferred to Rotary evaporator to evaporate the solvent. Similar process carried out for extraction of Plant materials in Ethanol, Petroleum Ether and Acetone. Recovered crude extracts of *A. sieversiana* stored in glass viols and placed in refrigerator until bioassay.

Bioassay for Repellency

Bioassay for repellence effect of Artemisia extracts in three different solvents on white grub carried out with three serial doses (1%, 3% and 5%). Slices of potato (50 gm) were impregnated with these solutions of serial doses and left overnight to evaporate the solvent. Three treated potato slice were placed in one side of cardboard (20”X12”) and potato slices treated with distilled water were placed in another side of box of each treatments with three replications. Ten white grubs of 3rd instar were placed in center of each box. Number white grub present on treated (NT) and control (NC) sides were recorded after 1 and 3 hours. The percent repellency was calculated according to Hossanah *et al*. [27].

$$\mathrm{PR} = \frac{NC - NT}{NC + NT} \text{ x } 100$$

Residual toxicity Test

A soil based bioassay was carried to determine the efficacy of *A. sieversiana* extract in three different solvent. Three serial doses (1%, 3% and 5% w/v) of each three extracts were prepared in acetone. Slices of potato (50 gm) were impregnated with these solutions of serial doses and left overnight to evaporate the solvent. Three treated potato slice were placed in earthen bowls filled with soil for each treatments with three replications. Potato slices treated with distilled water were placed in bowls of control. Ten white grubs of 2nd instar were allowed feed the treated slice in each bowls. Soil in bowls were kept moist by sprinkling distilled water twice a day. Number of dead insect was recorded after 24 and 72 hours and percent mortality computed using method of (Niber 1994) [28].

$$\%\ \mathrm{Mortality} = \frac{\text{No. of dead insect}}{\text{Total No. of insect}} \times 100$$

Table 1:%Concentration of *A. sieversiana* Ehrh. Crude extract in three different solvent.

S. No	Name of Local Herbs	Treatment/Doses (%w/v)
1	Artemisia Acetone	T1- 1%
		T2- 3%
		T3 5%
2	Artemisia Ethanol	T4- 1%
		T5- 3%
		T6- 5%
3	Artemisia P. Ether	T7- 1%
		T8- 3%
		T9- 5%
4	Control	T10- only acetone

Data Analysis

Data were analyzed for three mean values to test significance among the treated extract, descriptive statistics and LSD values at alpha level 0.05. Data analyses was used by statistical software package Statistics 8.1, USA.

Results

Repellency effect on white grub

A. sieversiana extract in Ethanol was most repellent than two other extracts in Acetone and P. Ether. Maximum average repellency 98.0% was observed at 5% of extract in ethanol after a short time of 1 hour exposure whereas at 1 and 3% extract repellency av. 55 and 56% recorded. Repellency increased to av. 84.00, 87.00 and 98.33% at l, 3 and 5% extract in ethanol after 3 hour of exposure. In acetone extract, av. repellency were 71, 75 and 78% at 1, 3 and 5% concentrations whereas lower repellency 3.0, 13.3 and 30.0% were observed at 1, 3 and 5% extract in P. Ether. At ANOVA Statistic 8.1 Acetone was found more significant as compare to Ethanol and P. Ethanol. Acetone 5% give 61.00 while ethanol 1% is less potential with 3.45 (fig 1).

A. sieversiana extract in Acetone was most effective than two other extracts in Ethanol and Petroleum Ether. Maximum mortality (97%) was observed at 5% at acetone after 24 hr. *A.*

sieversiana extract in ethanol at 5% gave second highest mortality (90.30%). Lower mortality 21, 36.0 and 40.0% was recorded at 1, 3 and 5% extract in P. Ether. Extract concentration after 72 hr%mortality increased to 72.0, 83 and 91. at l, 3 and 5% extract in Acetone after 72 hours of exposure. In Ethanol extract,%mortality was 81, 83 and 90 at 1. 3 and 5% concentrations whereas lower%mortality of grub observed 21, 36 and 40 were observed at 1, 3 and 5% extract in P. Ether after 72 hours of exposure whereas 0.0% mortality was observed in control. At 5% of Ethanol extract% mortality is significant than all other treatments at LSD (P<0.05) while at 1, 3 and 5% P. Ether extract is no significant at LSD (P<0.05) (fig 2).

At analysis of ANOVA Statistic 8.1 Acetone was highly significant to P. Ether and Ethanol. Mortality of ethanol at 5% is very effective with 91.33 while ethanol 5% give mortality only 20.45 (fig 2).

Table 2:%Repellency effect of *A. sieversiana* plant extract in three different solvent on white grub

Concentration		After 1 hr	After 3 hrs	ANOVA Result
Acetone	*1%*	55.02%	*71.00%*	44.000 b
	3%	56.00%	*75.01%*	54.000 c
	5%	66.40%	78.04%	61.333 e
Ethanol	*1%*	50.01%	*84.00%*	3.450k
	3%	53.00%	*87.20%*	7.333j
	5%	98.00%	98.03%	11.333i
P. Ether	*1%*	13.03%	*20.00%*	33.000d
	3%	30.00	*34.00%*	39.030f
	5%	36.00%	*38.08%*	42.652g
LSD (P<0.05)		--------		0.0000

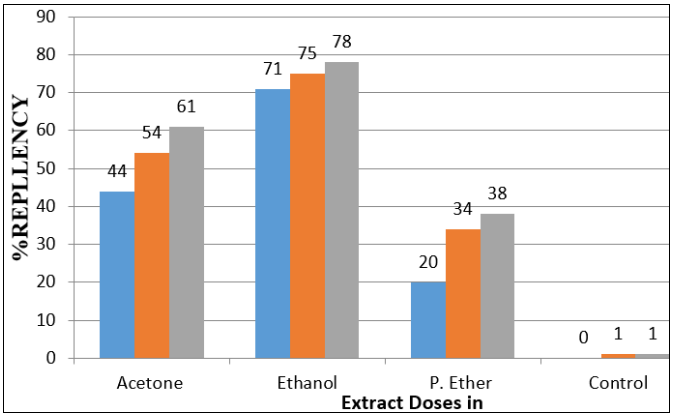

Fig 1:%Repellency of white grubs caused by *A. sieversiana* plant extracts three different solvent *in vitro* after 3 hr.

%Mortality in White grub

A. sieversiana extract in Acetone was most effective than two other extracts in Ethanol and Petroleum Ether. Maximum mortality (97%) was observed at 5% at acetone after 24 hr. *A. sieversiana* extract in ethanol at 5% gave second highest mortality (90.30%). Lower mortality 21, 36.0 and 40.0% was recorded at 1, 3 and 5% extract in P. Ether. Extract concentration after 72 hr%mortality increased to 72.0, 83 and 91. At l, 3 and 5% extract in Acetone after 72 hours of exposure. In Ethanol extract,%mortality was 81, 83 and 90 at 1. 3 and 5% concentrations whereas lower%mortality of grub observed 21, 36 and 40 were observed at 1, 3 and 5% extract in P. Ether after 72 hours of exposure whereas 0.0% mortality was observed in control. At 5% of Ethanol extract% mortality is significant than all other treatments at LSD (P<0.05) while at 1, 3 and 5% P. Ether extract is no significant at LSD (P<0.05) (Fig 2).

At analysis of ANOVA Statistic 8.1 Acetone was highly significant to P. Ether and Ethanol. Mortality of ethanol at 5% is very effective with 91.33 while ethanol 5% give mortality only 20.45 (Fig 2).

Table 3: %Mortality of white grub caused by *A. sieversiana* plant extract in three different solvent

Concentration		After 24 hr	After 72 hr	Ancova Result
Acetone	*1%*	78.00%	*82.00%*	13.000 C
	3%	62.01%	*89.00%*	16.333 BA
	5%	63.20%	97.00%	20.450 ABC
Ethanol	*1%*	71.07%	*81.00%*	72.000 AC
	3%	82.30%	*83.04%*	83.000 AB
	5%	93.03%	90.30%	91.333 BCD
P. Ether	*1%*	3.03%	*21.00%*	40.000 B
	3%	3.00	*36.00%*	41.030 BA
	5%	9.00%	*40.00%*	45.652 CD
Control		--------		0.1000 D
LSD (P<0.05)		--------		0.0000

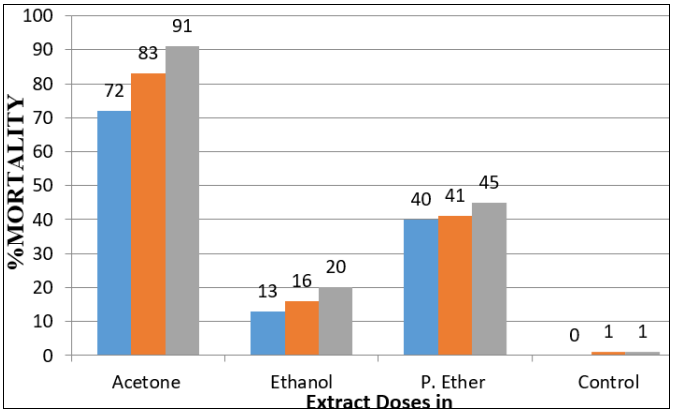

Fig 2:% Mortality of white grubs caused by plant extracts *Artemisia sieversiana* three different solvent *in vitro* after 72 hours.

Discussions

White Grub is an important agricultural root feeding pest widely found in different agro-climatic conditions of Pakistan and throughout the world. White grubs attack one of the four major staples which have a significant contribution to national domestic consumption and food needs. The damaging stage of the life cycle is the larvae or white grub (Buss, 2006) [3].

Biopesticide of *Thevetia peruviana* (Pers) is acts as excellent broad spectrum control of white grub adults on commercial growing or economical crops as well as medical important host plants (Theurkar *et al*., 2014) [22].

Present study revealed that *A. sieversiana* extract in Ethanol was most repellent than two other extracts in acetone and Petroleum Ether. Maximum repellency (98.0%) was observed at 5% of extract in ethanol after 2 hours. At 5% acetone extract highest% mortality was recorded as 97.00 after 72 hr insect exposure.

Various extracts of *A. sieversiana* have been found to exert strong insecticidal activity against *Callosobruchus chinensis*, maize weevil *Sitophilus zeamais* Motsch. *A. sieversiana* also contains essential oil in its aerial parts and various volatile chemical components have been identified by gas chromatography mass spectrometry. These components include eucalyptol (9.2%), geranyl butyrate (9.1%), borneol (7.9%), cam-phor (7.9%), germacrene D (5.5%) and Caryophyllene (5.3%) Yuan *et al* (2007) [25] and Liu *et al* (2010) [14].

Ethanolic crude seed extracts of sweetsop (*A. squamosa*), soursop (*A. muricata*) and biriba (*R. mucosa*) had repellent or feeding deterrent effects on the Asian subterranean termite *C. gestroi*. Soil treated with 5-10% crude extract of the three Annona species investigated prevented tunneling and penetration of *C. gestroi*. Repellents are harmless to ecosystem because they protect the treated materials from insect pests without killing them Acda [1]. Repellents are safe in pest control operations as they minimize residues, ensure safety of food, environment and wild life keeping away insect pests from treated materials [23,13].

Medicinal plants could use as repellent of *P. Interpunctella*, especially *Anethum graveolens*, *Thymus vulgaris* and *Rosmarinus officinalis.* The Strongest repellency showed in *Anethum graveolens* (100%), *Thymus vulgaris* (100%) and *Rosmarinus officinalis* (93.33%) and the weakest repellency in *Hyossopus officinalis* (7.69%) and *Petroselinum sativum* (9.48%). Essential oils these plants can use for protecting stored products from injury of Indian meal moth. They have good potential to replace with chemical repellents for this pest and they are safe for human and had no residue on stored products [13].

Plant extracts from citrus: *Citrus rinenris* cocoa: *Theobroma cacoa*, sunflower: *Tithonia diversifolia* and cashew: *Anacardium occidented* caused 80 – 100% mean insect mortality in termite 10 hr after insect exposure on field and laboratory condition [21].

Conclusion

Results of this study revealed that *A. sieversiana* extract has potential insecticidal activity and can be used as alternative of chemical insecticides for eco-friendly management of white grub. Further studies on fractionation of active compound, formulation and field application are needed to develop a botanical insecticide for control of white grub.

Acknowledgement

This investigation was supported by financial grant received from Pakistan Agricultural Research Council (PARC) under Agricultural Linkages Program (ALP) which is gratefully acknowledged. Authors are also thankful to IPEP Lab. and Insectary, NARC, Islamabad, Pakistan for assistance in preparation of plant crude extracts

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