

Original Article**Toxicological effects of *Haloxylon recurvum* Bunge ex Boiss (Khar Boti) whole plant extract and novel insecticide chlorantraniliprole against maize weevil, *Sitophilus zeamais* Motschulsky**Zubair Ahmed[#], Muhammad Farhanullah Khan, Habiballah Rana^{*}

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Article history**Received:** August 25, 2016**Revised:** June 16, 2017**Accepted:** July 26, 2017**Authors' Contribution****ZA:** execute experimental work and wrote initial draft, **MFK:** analyzed the data, **HR:** supervise the whole and improved the manuscript**Key words**Plant extract
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*Sitophilus zeamais***Abstract**

A great variety of stored grain pests attack stored commodities at storage facilities. Among these, maize weevil, *Sitophilus zeamais* is one of the most threatening pests. Botanical insecticides derived from plants have increased popularity in stored products protection due to ecological concerns and insect resistance to chemical insecticides. In these regards, a study were carried out to evaluate toxicity of crude methanol extract of whole plant of *Haloxylon recurvum* (Khar boti) and a novel insecticide chlorantraniliprole. A serial concentration of extract i.e., 1.2%, 2.4%, 3.6%, 4.8%, 6.0% and insecticide 0.00224%, 0.00448%, 0.00896%, 0.01792%, 0.02688%, respectively, were topically applied to adult *S. zeamais*. Contact toxicity assayed by topical application showed that 3.6% concentration of *H. recurvum* plant extract and 0.00896% concentration of chlorantraniliprole induced lethal effects in *S. zeamais*. Probit analysis revealed LD₅₀ value for *H. recurvum* plant extract were 16.47 µg/insect and chlorantraniliprole were 0.0358 µg/insect, after 24 hours. The result revealed that *H. recurvum* plant extract had insecticidal potential against the stored grain pest, *S. zeamais*. Current study suggests *H. recurvum* plant extract could be a good alternative against conventional insecticides.

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INTRODUCTION

Protection of crops and stored grain products from insect pest by usage of whole plants, plants parts, plant products and crude plant extracts or botanical insecticides is undoubtedly as old as crop protection itself. For example, in 2000 B.C; poisonous plants in India (Thacker, 2002) and chrysanthemum dried, ground flowers, in ancient China were used against insect pests of crops (Davies *et al.*, 2007). Many recent reported literatures suggested that extracts of locally available indigenous plant were very effective against insect pest, either applied alone or in combination (Isman, 2008; Ahad *et al.*, 2016; Aihetasham *et al.*, 2017; Behera *et al.*, 2017; Benelli *et al.*, 2017). In Pakistan, a large number of biologically affective compounds were extracted from a wide range of indigenous

plants. Many have been reported for their insecticide properties (Anwar *et al.*, 1992; Khan and Ahmed, 2000; Aslam *et al.*, 2002; Ali *et al.*, 2008; Ali *et al.*, 2012; Rana *et al.*, 2014; Saleem *et al.*, 2016; Ali *et al.*, 2017). An increased search interest drove various researchers attention on halophyte's plants (plants those entire biological clock completes in salty environment), reflects recognition of their immense potential as a valuable resource of food, fodder, grass forage, medicinal, oil raw compounds and as well as pest control agent (Lashgari *et al.*, 2016; Shamsutdinov *et al.*, 2017). Total 274 out of 410 halophyte's plant were reported as valuable resources in Pakistan (Dagla and Shekhawat, 2006; Khan and Qaiser, 2006; Abbasi *et al.*, 2011; Qasim *et al.*, 2011).

In the present study, a halophyte, *Haloxylon recurvum* Bunge ex Boiss (Family: Chenopodiaceae) have been focused to

investigate some insecticidal effects on the selected insect pest.

MATERIALS AND METHODS

Collection and rearing of maize weevils

Maize weevils, *Sitophilus zeamais* were reared at temperature 28-31°C with relative humidity of 70-80%, in the laboratory of entomology, M.A.H. Qadri Biological Research Center, University of Karachi. For the rearing purpose, maize weevils were collected from various grain markets and warehouses of Karachi, Pakistan. Weevils were reared on whole maize grain sterilized at 60°C for 90 minutes in sterilized glass jars of 350ml by volume. Each jar was filled with 200g of maize grains and 50 pairs of *S. zeamais* were added to them. The mouth of jars were covered with a piece of muslin cloth tied by means of rubber band to avoid contamination and escape of weevils. Weevils were left for 4 days for egg laying. After 4 days, the weevils were removed with help of aspirator, sieve and camel hairbrush and added to the other sterilized maize grain jars for the continuation of culture. The maize containing eggs were placed again in the same jars. Adult weevils were emerged after 30-35 days and adults were used for experiments.

Preparation of plant extract

For extraction Azmir *et al.* (2013) technique followed, fresh *Haloxylon recurvum* whole plant was collected and shade dried at room temperature till complete dryness. After this period, 100g weighed were crushed with the help of pestle and mortar. Crushed material was dipped in 100ml methanol and homogenized by the help of homogenizer. After homogenization, paste was left for 48 hours for extraction of dissolved contents. After 48 hours, contents were carefully filtered (Whatman No. 1 filter paper) and stored in a conical flask, the remaining sediments were again dipped in fresh 100ml methanol, this process was repeated three times to extract all contents. The total 300ml methanolic extract was concentrated under vacuum on a rotary evaporator (EYELA Tokyo Rikakikai Co., Ltd., Japan) at 40°C. The final outcome 3.896g of dry extracted material was redissolved in 38.90ml methanol, making a 10% stock solution that was stored at 4°C for subsequent use.

Formulation of compounds

Haloxylon recurvum plant extract

For toxicity investigation, a wide range of concentrations *i.e.*, 1.2, 2.4, 3.6, 4.8 and 6.0% were formulated from the stock solution using formula ($C_1V_1=C_2V_2$) by dissolving it in methanol for treatment.

Chlorantraniliprole

Chlorantraniliprole formulation coragen® 20 SC was used for experimentation, obtained from the market. Coragen® is a suspension concentrate formulation with 200g/L chlorantraniliprole as active ingredient. For the formulation of different concentrations, 5ml coragen® dissolved in distilled water to a final volume of 100ml solution gave a 1% stock solution of chlorantraniliprole. A wide range of dilutions, 0.00224, 0.00448, 0.00896, 0.01792 and 0.02688% were prepared from stock solution by dissolving it in water for treatment.

Treatment technique

For contact toxicity determination, Liu and Ho (1999) technique were followed with minor modification. Serial concentrations of the *H. recurvum* extract and chlorantraniliprole *i.e.*, 1.2, 2.4, 3.6, 4.8, 6.0% and 0.00224, 0.00448, 0.00896, 0.01792, 0.02688%, respectively were prepared. Ten adult maize weevils of same age and size were selected for each dilution and control. Dilutions were applied (0.5µl/insect) topically to adult maize weevils. A control batch (without any treatment) was kept as reference with each assay to observed environmental factors. Both treated and control insects were then transferred to Petri dishes (90mm x 15mm) separately. For the evaluation of toxic effects the treated and control insects were left for 24 hours, under the control laboratory conditions. Five replicates (60 insect/replicate) were treated.

Statistical analysis

For toxicity test, *Sitophilus zeamais* mortality were expressed as a percentage and corrected by using Abbott's formula (Abbott, 1925). SSPS version 21 (IBM, USA) and Biostat 2009 (Analystsoft Inc. USA) were used for the statistical analysis of the recorded data.

RESULTS

The data (Table I) revealed that *H. recurvum* extract tested against the adult maize weevils produce mortality 12, 32, 46, 68 and 86% under the effect of concentrations 1.20,

2.40, 3.60, 4.80 and 6.00%, respectively. Concentrations 4.80 and 6.00% produced more than 50% mortality as compare to 1.20, 2.40 and 3.60%. As data shown in the Table II, adult maize weevils, *S. zeamais* treated with a series of concentrations of chlorantraniliprole. After 24 hours of exposure it produced mortality 18, 34, 52, 76 and 92% under the effect of concentrations 0.00224, 0.00448, 0.00896, 0.01792 and 0.02688%, respectively. Concentrations 0.00896% produced 52% mortality.

Table I: Contact toxicity of *Haloxylon recurvum* extract applied topically to adult *Sitophilus zeamais*, at 24 hours after treatment.

Concentration of extract (%)	Dosage ($\mu\text{g/insect}$)	Mortality (%Mean \pm SE)
1.20	6.00	1.20 \pm 0.200
2.40	12.00	3.20 \pm 0.374
3.60	18.00	4.60 \pm 0.245
4.80	24.00	6.80 \pm 0.374
6.00	30.00	8.60 \pm 0.245
Control	00.00	0.00 \pm 0.000

Table II: Contact toxicity of Chlorantraniliprole applied topically to adult *Sitophilus zeamais*, at 24 hours after treatment

Concentration (%)	Dosage ($\mu\text{g/insect}$)	Mortality (%Mean \pm SE)
0.00224	0.0112	1.80 \pm 0.200
0.00448	0.0224	3.40 \pm 0.245
0.00896	0.0448	5.20 \pm 0.200
0.01792	0.0896	7.60 \pm 0.245
0.02688	0.1344	9.20 \pm 0.374
Control	0.0000	0.00 \pm 0.000

H. recurvum plant extract and chlorantraniliprole induced mortality in adult weevils in a dose-dependent pattern. By plotting, observed mean percentage mortalities against respective dosages of extract, the LD₅₀ value were 16.47 $\mu\text{g/insect}$, whereas insecticide chlorantraniliprole LD₅₀ value were 0.0358 $\mu\text{g/insect}$, after 24 hours of treatment (Table III). All concentrations tested exhibited insecticidal activity from lower to higher mortality, as dosage increased against the maize weevils.

Table III: Probit analysis–toxicity of *Haloxylon recurvum* extract and Chlorantraniliprole insecticide applied topically to adult *Sitophilus zeamais*, at 24 hours after treatment

Tested Materials	LD ₅₀ ($\mu\text{g/insect}$)	Chi-square	Df	p-value
<i>H. recurvum</i> extract	16.47	1.6215	3	0.6545
Chlorantraniliprole	0.0358	0.6474	3	0.8855

Probit analysis, using Finney method [Lognormal Distribution]

DISCUSSION

Boxall (2001) reported that insect directly or indirectly damages the stored grain commodities after harvest. Direct damages to grain commodities, pest feeding on them and indirectly, due to infestation, commodities lost their economic value from higher to lower, leading not fit for human consumption and rebuffed. Currently, Plant extract have significant role in the modern insect pest management strategies (Gerwick, 2014). Bourgaud *et al.* (2001) and Wink (2010) reported that plant extracts contain a varieties of biochemical components *i.e.*, alcohols, aldehydes, coumarins, esters, fatty acids, flavonoids, ketones, phenols, terpenes and waxes *etc.* This encourages many researchers in discovering hiding potential in plants. Presently, the extract of *H. recurvum* whole plant showed toxicity against adult maize weevils, *S. zeamais* with LD₅₀ value 16.47 $\mu\text{g/insect}$. When compared with the famous botanical insecticide, pyrethrum extract, the *H. recurvum* extract showed 3.84 folds less toxicity against the adult maize weevils, *S. zeamais* because pyrethrum extract exhibited a LD₅₀ value 4.29 $\mu\text{g/insect}$ (Liu *et al.*, 2010). Present result, when compared with other plant extracts in the literature, the extract of *H. recurvum* possessed stronger contact toxicity against adult maize weevils, *S. zeamais i.e.*, 1.5 folds more toxic than extract of *Hyssopus cuspidatus* (Li *et al.*, 2013), 1.7 folds more toxic than extract of *Illicium fragesii* (Wang *et al.*, 2011a), 2 folds more toxic than extract of *Cayratia japonica* (Liu *et al.*, 2012), 3 folds more toxic than extract of *Artemisia vestita* (Chu *et al.*, 2010), 3.9 folds more toxic than extract of *Lonicera japonica* (Zhou *et al.*, 2012), 2.5 and

4.6 folds more toxic than extract of *Artemisia giraldii* and *Artemisia subdigitata*, respectively (Chu *et al.*, 2012).

On the basis of attributes required in chemicals for controlling insects pest that feed on stored grains *i.e.*, adulticide, pupicide, ovicide, larvicide and reduction in fecundity and fertility, family chenopodiaceae showed significant potential under these criteria (McGregor, 1944, Dinan *et al.*, 1998, Tapondjou *et al.*, 2002, Denloye *et al.*, 2010). The potential of *H. recurvum* is in respect to activity limits assessed in the current investigation. Present findings agreed with reports of Hasan *et al.* (2006) investigated the toxic effect of *H. recurvum* and deltamethrin against *Trogoderma granarium*. Ahmed *et al.* (2005) have studied the extract efficacy of *H. recurvum*, *Azadiracta indica* and some conventional insecticides against the ground termites and reported *H. recurvum* was toxic to termites. Present findings showed *S. zeamais* adults were highly susceptible to *H. recurvum* plant extract and this finding was in accordance with responses it showed against extract of *Ricinodendron heudelotii*, organic solvents (methanolic, ethanolic and petroleum ether) extracts of *Illicium verum* extracts of *Acorus gramineus* and *Croton tiglium* (Udo and Epedi, 2009, Yao *et al.*, 2004, Wei *et al.*, 2014). Wang *et al.* (2011b) investigated the toxic effect of *Zanthoxylum schinifolium* fresh fruits extract against the adult maize weevils *S. zeamais* and reported the similar results. The lethal effect exhibited by plant extract at minimum concentrations against insect pests was due to the presence of such constituents which are neurotoxicant to target insects and induced mortality. Furthermore, plant constituents are their armor which protect them from insect pest, herbivory and diseases by acting in multiple directions like contact toxicant, antifeedant, repellent and sterilant (Isman *et al.*, 2011). Traditionally, *H. recurvum* was known to possess natural medicinal properties and used to treat diarrhea, dysentery, healing of wound and kidney problems (Ahmed *et al.*, 2014). *H. recurvum* also exhibited anti-microbial and anti-fungal activities (Wahab *et al.*, 2008). Abid *et al.* (1997) investigated nematocidal properties of nearly 60 plant species including *H. recurvum* against second stage juveniles of *Meloidogyne javanica*. The ethanolic extract of *H. recurvum* induced mortality in more than 50 % juveniles. The *H. recurvum* plant extract insecticidal properties are undoubtedly linked to the presence of some active

metabolites. Previously reported that *H. recurvum* plant extract possessed toxic metabolic derivatives *i.e.*, halosterols, leukotrienes (a neurotoxic compound), recurvoside (a steroidal glucoside), octacosanoic acid, octadecanoic acid, steroids, β -sitosterol, β -sitosterol 3-O- β -D-glucopyranoside, 1-triacontanol, triacontanoic acid, ursolic acid reported by Ahmed *et al.* (2004), Sharif *et al.* (2006) and Hussain *et al.* (2006). Ahmed *et al.* (2007) reported *H. recurvum* also exhibited enzyme (Chymotrypsin) inhibitory component *i.e.*, haloxylase (a triglyceride), saturated fatty acid and unsaturated fatty acid methyl ester. The maximum biological activity against adult maize weevils of the *H. recurvum* extract, is due to the presence of above mention metabolic derivatives which induced mortality in adult maize weevils and showed their potential insecticidal activities against insects' pest.

Novel insecticides have been assessed for their ability to protect grains against stored grain pest yield promising results (Saglam *et al.*, 2013). In present investigation, newer insecticide chlorantraniliprole shown promising result against adult *S. zeamais*. Kavallieratos *et al.* (2013) reported the toxicological effects of two chlorantraniliprole formulations (WG and SC) against *Ephestia kuehniella* larvae, *Liposcelis bostrychophila* adults, *Rhyzopertha dominica* adults, *Sitophilus oryzae* adults and *Tribolium confusum* larvae and adults. Furthermore, present findings in accordance with Saglam *et al.* (2013) reported that chlorantraniliprole was effective against stored grains pest *Tribolium confusum*. Chlorantraniliprole was effective against insect pest that have developed resistance to most senior generation of insecticides (Hannig *et al.*, 2009). Chlorantraniliprole insecticide target insect ryanodine receptor (RyR), releasing stored calcium from the sarcoplasmic reticulum of muscle cells and endoplasmic reticulum of non-muscle cells (Lahm *et al.*, 2007). Lahm *et al.* (2009) reported RyR is a protein channel tetramer complex, name was derived from the plant product "ryanodine", which binds at the channel pore and changed the conductance of the channel. Chlorantraniliprole insecticide bind to a site at RyR complex and activated insect RyR. Activation of receptors induced uncontrolled released of stored calcium from sarcoplasmic reticulum of muscle cells or endoplasmic reticulum of non-muscle cells. In insect cells, calcium influx occur through

sarcoplasmic or endoplasmic receptors and voltage dependent ion channels. The gradual depletion of intracellular calcium contributes elevation on cytosolic calcium leads to indirect sensitization of membrane targets through activation of kinases and phosphatases enzyme. Based on cytosolic activities in target insect resulted in induction of muscles paralysis which finally lead to the death of insect. In addition, anthranilic diamide exhibited binding site selectivity and specificity against insect RyR over mammalian RyR, on the basis of these traits, chlorantraniliprole possess distinct position in latest pest control programme (Cordova *et al.*, 2007, Aparie-Marchais *et al.*, 2016). Present finding of chlorantraniliprole insecticide toxicity against the adult maize weevils, *S. zeamais* in accordance with the Seo *et al.* (2007) reported toxicity of novel insecticide flubendiamide (a phthalic diamide). Current findings lower LD₅₀ value (0.0358 µg/insect) against the adult maize weevils, *S. zeamais*, revealed aqueous formulation of chlorantraniliprole toxicity, a calcium-dependent novel mechanism of action on *S. zeamais* neuronal receptors and channels. Novel chlorantraniliprole was more toxic than *H. recurvum* whole plant extract at low dosage. This study revealed that the *H. recurvum* whole plant extract had toxicity against the adult maize weevil, *S. zeamais*. This suggested that plant extract posses insecticidal potential and also shown as a parallel resource along with synthetic insecticides chlorantraniliprole.

In conclusion, obtained findings of the present study suggested that researchers should give major concentration towards isolation and identification of further hidden metabolic derivatives in *H. recurvum* plant which possess diverse bio-activities. Their discovery is necessary, in developing natural or plant based commercial insecticide against *Sitophilus zeamais* and other stored grain pest.

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