



Contents lists available at ScienceDirect

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journal homepage: www.elsevier.com/locate/indcrop



Toxicity and enzyme inhibition activities of the essential oil and dominant constituents derived from *Artemisia absinthium* L. against adult Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae)



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ARTICLE INFO

Keywords:

Botanical pesticides

Artemisia absinthium

Asian citrus psyllid

Essential oil

Acetylcholinesterase inhibition

ABSTRACT

The extensive use of synthetic chemical pesticides has led to negative effects on the environment and human health. Therefore, scientists are in search of natural products, especially those from plant sources, which are less hazardous, eco-friendly, and effective alternatives to the conventional pesticides. Essential oils are potential alternatives to the synthetic chemical pesticides. However, the toxicity of *Artemisia absinthium* against Asian citrus psyllid *Diaphorina citri* has not been documented. Therefore, here we focused on the *A. absinthium* essential oil (ABEO) extracted from the population grown under the climatic conditions of Pakistan's high plateaus, as a natural pesticide for the control of the citrus psyllid. The results indicated that the oil caused significant acute mortalities in both topical and residual contact applications, with 50% lethal dose (LD_{50}) and 50% lethal concentration (LC_{50}) values of 5.2 $\mu\text{g}/\text{insect}$ and 24.4 mg/mL . Among the constituents tested only carvacrol, (–)- α -bisabolol and chamazulene were toxic, with LD_{50} values of 6.9, 8.2, and 10.3 $\mu\text{g}/\text{insect}$ via topical application and LC_{50} values of 28.5, 33.5, and 39.4 mg/mL via residual contact exposure. Several combinations of dominant constituents were both additive and synergistic when applied topically, especially carvacrol + (–)- α -bisabolol + chamazulene (1:1:1) displayed synergistic effect, with a poison ratio of 1.39. Furthermore, the essential oil and carvacrol inhibit acetylcholinesterase activity in citrus psyllid. It has been concluded that the essential oil and some of the compounds from *A. absinthium* might be developed as eco-friendly approaches for the control of citrus psyllid.

RESEARCH ARTICLE



Development and evaluation of emulsifiable concentrate formulation containing *Sophora alopecuroides* L. extract for the novel management of Asian citrus psyllid

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Received: 30 March 2019 / Accepted: 7 May 2019

© Springer-Verlag GmbH Germany, part of Springer Nature 2019

Abstract

Utilization of non-host plants semiochemicals to mediate insect behavior offers a promising opportunity for novel management of insect pests in field crops and fruits. Therefore, there is still a substantial opportunity for the development of natural prophylactic as an eco-friendly approach in the novel pest management programs. *Sophora alopecuroides* extract has been used as a natural pesticide in the control of agricultural and household pests, but the low persistence effect and rapid biodegradability limit its use on a wider scale in pest management programs. In this study, an emulsifiable concentrate formulation containing *S. alopecuroides* extract (SAE-EC) was developed with a simple procedure and evaluated for its ovicidal, antifeedant, and repellent effects against *Diaphorina citri* under laboratory and semi-field conditions. Our results indicated that SAE-EC at 15, 30, and 50 mg/mL concentrations provide complete protection against psyllids for a period of 96 h after application both under laboratory and semi-field conditions, while the aqueous methanolic extract of *S. alopecuroides* loses its persistence 48 h after application. Furthermore, the emulsifiable concentrate at 20 and 30 mg/mL concentrations, only 15.97% and 31.97% of eggs were able to hatch, and at similar concentrations, 72.86% and 85.5% of honeydew secretion were reduced as compared to the control. SAE-EC at 30 mg/mL concentration has not shown any phytotoxic symptoms on *Murraya paniculata* seedlings. Fourier transform infrared (FTIR) study revealed the presence of alkaloids in emulsifiable concentrate after 3 months of its preparation placed under ambient temperature. Furthermore, the particle size and polydispersity index (PDI) of the emulsifiable concentrate were also confirmed by dynamic light scattering (DLS). Our finding indicated that emulsifiable concentrate formulation prolongs the persistence of *S. alopecuroides* extract and enhances its efficacy both under laboratory and semi-field conditions. It has been concluded that the emulsifiable concentrate formulation containing *S. alopecuroides* extract might be developed as an eco-friendly novel prophylactic against citrus psyllid.

Keywords Botanical pesticides · Asian citrus psyllid · Emulsifiable concentrate · *Sophora alopecuroides* · Novel prophylactic



Interference mechanism of *Sophora alopecuroides* L. alkaloids extract on host finding and selection of the Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Psyllidae)

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Received: 13 September 2018 / Accepted: 9 November 2018
© Springer-Verlag GmbH Germany, part of Springer Nature 2018

Abstract

Manipulating insect behavior through the deployment of semiochemicals offers a promising opportunity for protecting crops in a sustainable manner. Therefore, there is still a significant opportunity for the development of natural crop protectants as eco-friendly tools in pest management. In this context, the aim of the current investigation is to find a novel prophylactic against the Asian citrus psyllid (ACP) and to gain a better understanding of the host-finding and selection ability of the ACP towards *Murraya paniculata* seedlings treated with *Sophora alopecuroides* alkaloids extract (SAAE). Our results indicate that foliar application of SAAE influences the psyllid host-finding and selection process. The behavioral assay with *M. paniculata* seedlings treated with 15 and 30 mg/mL of SAAE, with masked visual cues, revealed that only 6.6 and 10.4% psyllids were able to locate the host in the vials. The results also indicate that citrus psyllids mainly rely on both visual and olfaction in host-finding and selection. In choice settling experiments, psyllids settled almost completely on control seedlings rather than on seedlings treated with SAAE at a concentration of 30 mg/mL. Chemical analyses of the alkaloids extract revealed the presence of sophocarpine (33.90%), sophoridine (6.23%), anagyrene (2.77%), matrine (2.38%), lupanine (1.68%), aphylline (0.89%), and sophoramine (0.75%). In further behavioral bioassays with the dominant alkaloids sophocarpine and sophoridine, the alkaloids repelled ACP at higher concentrations of 50 and 70 mg/mL as compared to SAAE. Furthermore, the 50 mg/mL (1:1, v/v) combination of sophocarpine and sophoridine displayed a synergistic effect and showed the maximum behavioral effect as compared to the individual alkaloid. Based on our results, SAAE makes *M. paniculata* seedlings unattractive to the psyllids, and therefore, alkaloids could be used in reducing the colonization of citrus plants, subsequently curtailing HLB infection.

Keywords Botanical pesticides · *Diaphorina citri* · *Sophora alopecuroides* · Alkaloids · Host selection · Behavioral effect

CHEMICAL COMPOSITION OF ESSENTIAL OIL OBTAINED FROM (*ARTEMESIA ABSINTHIUM* L.) GROWN UNDER THE CLIMATIC CONDITION OF SKARDU BALTISTAN OF PAKISTAN

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Abstract

The common wormwood *Artemisia absinthium* (L.) is an aromatic perennial herb, which is wildly grown in the Skardu Baltistan. Locally its fresh and dried form is used as both medicinal and insecticidal purposes. This study first time reports the chemical composition of hydro-distilled essential oil obtained from aerial parts of *A. absinthium*. Upon hydro-distillation, it gave dark green colored oil with yield 0.41% (w/w) (based on sheltered dried plant parts). The variation in chemical composition and their relative percentage in essential oil of *A. absinthium* are cited to vary qualitatively and quantitatively according to geographical location and environmental conditions. The GC-MS analysis of *A. absinthium* essential oil obtained from the population collected from Skardu Baltistan also showed variation in chemical composition and their relative percentage. The oil was dominated by Sesquiterpenes (61.01%) and Monoterpenes (17.92%). The major dominating constituents were Guaiol (19.33%), α -Bisabolol (8.83%), Carveol (6.16%), Chamazulene (5.94%), Limonen-6-ol, Pivalate (5.37%) and Geranyl- α -Terpinene (5.63%), which accounts overall 51.62% of total constituents identified.

Key words: *Artemisia absinthium*, Essential oil, GC-MS, Sesquiterpenes, Guaiol.

Introduction

Common wormwood (*Artemisia absinthium* L.) is an aromatic herb, perennial medicinal plant (Tan *et al.*, 1998; Bora & Sharma, 2010). It is native to temperate region of the world and widely distributed in Afghanistan, China, India, Japan, Kazakhstan, Kyrgyzstan, Pakistan, Russia, North Africa, Europe, North America (Bora & Sharma, 2011). Its extract and oil were traditionally used in ethno-pharmacology and ethno-medicine (Abad *et al.*, 2011). From ancient times species of *Artemisia* i.e., *A. absinthium*, *A. annua* and *A. vulgaris* have been used in folk medicine (Hayat *et al.*, 2009; Bora *et al.*, 2011b; Bano *et al.*, 2014; Milena *et al.*, 2016). It is also used as antiseptic, stomachic, cardiac stimulant, antispasmodic, anthelmintic and to reducing liver

In Skardu Baltistan *A. absinthium* grows wildly on roadsides and barren lands and locally it is used as both medicinal and insecticidal purposes, the sun-dried leaf and flowers powder are used for stomach aches, anti-inflammatory, anti-migraine and for high blood pressure and diabetics (Hayat *et al.*, 2009; Bano *et al.*, 2014). Due to ethno-pharmacology, ethno-medicine, insecticidal and antimicrobial properties of *A. absinthium*, much investigation yet has been performed and the previous literature indicated that geographical distribution greatly influences the chemical composition and essential oil content. Nature has blessed the Skardu Baltistan region with a diversity of wild herbs and shrubs of economic importance. The aim of this study is to analyze the chemical composition of *A. absinthium* plant grown under

RESEARCH ARTICLE



Seriphidium brevifolium essential oil: a novel alternative to synthetic insecticides against the dengue vector *Aedes albopictus*

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Received: 6 March 2020 / Accepted: 28 April 2020

© Springer-Verlag GmbH Germany, part of Springer Nature 2020

Abstract

The *Aedes albopictus* mosquito is a vector of several deadly diseases of humans and domesticated animals. Usually, synthetic insecticides are used for mosquito control. The excessive use of synthetic insecticides is hazardous for humans and the environment. Therefore, there is a need to develop environment-friendly and novel mosquito larvicides. In the current study, we evaluated larvicidal and bite protection properties of *Seriphidium brevifolium* essential oil (SBEO) and its active constituents against this mosquito. SBEO and its active constituents, α , β -thujone, and limonene, were toxic to *A. albopictus*, with LC_{50} values of 21.43, 45.99, 47.38, and 49.46 $\mu\text{g/mL}$. The cream formulation of EO at 5 % (w/v) provided complete protection against mosquito bites until 70 min after application. Among the EO constituents tested, α and β -thujone showed considerable protections against mosquito bites but lower as compared with the whole oil. Furthermore, 1:1 combinations of active constituent α -thujone and β -thujone and 1:1:1 combinations of α -thujone, β -thujone, and limonene displayed a synergistic effect against the larvae. Particularly, the EO and its active constituents were safer to *Poecilia reticulata* a mosquito predator, with LC_{50} ranging from 3934.33 to 14,432.11 $\mu\text{g/mL}$. Our current study indicated that SBEO and some of its constituents can be used for the control of *A. albopictus* mosquito, as a novel alternative to hazardous synthetic insecticides and to overcome the problem of increasing insecticides resistance.

Keywords Bio-pesticides · Mosquito · Toxicity · Larvicidal activity · Eco-friendly · Responsible Editor: Giovanni Benelli



Differences in susceptibility to insecticides among color morphs of the Asian citrus psyllid

Fajun Tian, Syed Arif Hussain Rizvi, Jiali Liu, Xinnian Zeng

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<https://doi.org/10.1016/j.pestbp.2019.11.015>

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Highlights

- *D. citri* occurs in three distinct color morphs.
- Insecticide susceptibility in *D. citri* is associated with color morphs.
- The orange/yellow morph of *D. citri* was the most susceptible to insecticides.
- The activities of P450s, GSTs and ESTs were lowest in the orange/yellow morph.
- The relative expression levels of six detoxification genes were lowest in the orange/yellow morph.

Abstract

Insect color morphs are known to vary in detoxification enzyme activity associated with susceptibility to insecticides. *Diaphorina citri* occurs in three distinct color morphs, and little data are available addressing this question. In this study, susceptibility to nine insecticides was compared among orange/yellow, gray/brown and blue/green morphs of adult *D. citri*. Imidacloprid and lambda-cyhalothrin resulted in higher mortality in the orange/yellow morph than in the blue/green morph. The orange/yellow morph was significantly more susceptible to acetamiprid, bifenthrin and chlorfenapyr than the blue/green and gray/brown morphs. For clothianidin and chlorpyrifos, the orange/yellow morph was significantly more susceptible than the gray/brown morphs. Susceptibility to dinotefuran and thiamethoxam was not significantly different between the three color morphs of *D. citri*. Biochemical enzyme assays revealed that the esterase,



Original Article

Fumigant toxicity and biochemical properties of (α + β) thujone and 1, 8-cineole derived from *Seriphidium brevifolium* volatile oil against the red imported fire ant *Solenopsis invicta* (Hymenoptera: Formicidae)



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ARTICLE INFO

Article history:

Received 8 March 2019

Accepted 13 April 2019

Available online 16 September 2019

Keywords:

Botanical pesticides

Short-leaved wormwood

Fire ants

Fumigant toxicity

Volatile oil

Biochemical activities

ABSTRACT

The excessive use of chemical insecticides has led to negative effects on human health and the environment. Volatile oils are one of the possible potential alternatives to chemical insecticides. Traditionally *Seriphidium brevifolium* (Wall. ex DC.) Ling & Y.R.Ling, Asteraceae, powder from its leaves is used to treat gastric problems and expel intestinal worms by local peoples, but yet there is no literature available regarding its insecticidal activity. In this study fumigant toxicity and enzyme inhibition activities of the *S. brevifolium* volatile oil collected from the highlands of Skardu Baltistan, Pakistan, was evaluated against the red imported fire ant *Solenopsis invicta*. The phytochemical studies indicated that monoterpenes were the most abundant constituents, accounting for 88% of the total oil. The major dominant constituents were 2-bornanone (28.2%), 1,8-cineole (19.9%), α -thujone (7.5%), β -thujone (6.7%) which accounts for 62.3% of total constituents identified, with volatile oil yield of 4.11% (w/w). The fumigation assay indicated that the volatile oil was acutely toxic to fire ants, with an LC_{50} of 16.47 μ l/l. Among the constituents tested, only (α + β) thujone and 1,8-cineole were toxic, with LC_{50} of 17.68 and 30.66 μ l/l after 12 h of exposure. The volatile oil, (α + β) thujone and 1,8-cineole showed strong fumigant activity against the red imported fire ant workers in a time- and dose-dependent manner. The volatile oil caused 100% mortality of the red imported fire ant workers, even at the lowest concentration of 20 μ l/l after 24 h of exposure. In addition, the volatile oil and 1,8-cineole inhibited acetylcholinesterase activity, while (α + β) thujone inhibited carboxylesterase activity in the fire ant workers. It has been concluded that the volatile oil and some of the compounds from *S. brevifolium* might be developed as eco-friendly approaches for the control of red imported fire ants.

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Review Article | Open Access

Volume 2020 | Article ID 9375619 | <https://doi.org/10.1155/2020/9375619>

[Show citation](#)

Review of Compounds and Pharmacological Effects of *Delphinium*

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Academic Editor: Pedro M. Mancini

Received	Revised	Accepted	Published
15 Jun 2020	21 Aug 2020	02 Sep 2020	21 Sep 2020

Abstract

Plants of *Delphinium* are herbal medicine used in the Tibet region with whole grass as a drug, which have the effects of analgesic, antibacterial, antipyretic, and anticancer. The main bioactive compounds are alkaloids, flavonoids, and sterols. This review summarized the compounds and pharmacological effects of *Delphinium* and provides a reference for further research on *Delphinium*.



OPEN

Detection and biochemical characterization of insecticide resistance in field populations of Asian citrus psyllid in Guangdong of China

Received: 25 September 2017

Accepted: 30 July 2018

Published online: 22 August 2018

Fajun Tian, Xiufang Mo, Syed Arif Hussain Rizvi, Chaofeng Li & Xinnian Zeng

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is one of the most damaging pests of citrus-producing regions throughout the world. The use of insecticides is the main strategy for controlling psyllid and has increased year by year. In this study, four field populations of *D. citri* were evaluated for resistance to nine different insecticides using the leaf-dip method. The results showed that the highest level of resistance for *D. citri* was found in imidacloprid with a resistance ratio of 15.12 in the Zengcheng population compared with the laboratory susceptible population. This was followed by chlorpyrifos (6.47), dinotefuran (6.16), thiamethoxam (6.04), lambda-cyhalothrin (4.78), and bifenthrin (4.16). Piperonyl butoxide (PBO) and triphenyl phosphate (TPP) showed significant synergism on imidacloprid effects in the Zengcheng population (3.84- and 2.46-fold, respectively). Nevertheless, diethyl maleate (DEM) had no synergism on imidacloprid. Biochemical enzyme assays suggested that general esterase, glutathione *S*-transferase and cytochrome P450 monooxygenase activities were higher in the field-collected populations than in the laboratory susceptible population. However, glutathione *S*-transferase may play a minor role in the resistance of adult *D. citri* to insecticides. At the molecular level, resistance of *D. citri* to imidacloprid is mainly related to the increased expression of *CYP4C68* and *CYP4G70* (>5-fold).

Article

Larvicidal, Ovicidal, Synergistic, and Repellent Activities of *Sophora alopecuroides* and Its Dominant Constituents Against *Aedes albopictus*

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Received: 20 February 2020; Accepted: 9 April 2020; Published: 15 April 2020



Abstract: In the current study, to combat insecticide resistance, we explored larvicidal, ovicidal, synergistic, and repellent activities of *Sophora alopecuroides* extract and its dominant constituents against *Aedes albopictus*. The results of the toxicity bioassays demonstrated that the extract of *S. alopecuroides* exerted significant larvicidal activity (16.66–86.66%) against the third-instar larvae of *Ae. albopictus* at different concentrations (5–50 µg/mL) and low hatchability of eggs (2.32–75%) at 5–50 µg/mL. The constituents of *S. alopecuroides* showed a synergistic effect when applied as a mixture (LC₃₀ + LC₃₀) against larvae, while no synergistic effect was observed against the eggs of *Ae. albopictus*. *S. alopecuroides* extract provided 93.11% repellency in the first 90 min and gradually decreased to 53.14% after 240 min, while the positive control DEET (N,N-diethyl-3-methylbenzamide) showed 94.18% in the first 90 min and 55.33% after 240 min. All of the results exhibited a concentration-dependent effect. To the best of our knowledge, this is the first time that a study has identified a highly effective



RESEARCH PAPER

Morphology and function of ovipositorial and tarsal sensilla of female Asian citrus psyllid

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Received 26 November 2017;
accepted 8 November 2018.

doi: 10.1111/1748-5967.12337

Abstract

The Asian citrus psyllid (ACP), *Diuraphis citri* Kuwayama (Hemiptera:Psyllidae), is one of the most important pests of citrus plants worldwide, due to be the dominant vector of the devastating citrus disease Huanglongbing (HLB). Selection of suitable oviposition site is a crucial event of phytophagous insect life. Usually, the chemical and physical characteristics of a host plant were recognized by sensilla on the ovipositor and tarsi of a phytophagous insect after settling on a plant surface. In this study, the morphology, number, and distribution pattern of the ovipositorial and tarsal sensilla of adult female psyllids, were observed by scanning electron microscopy (SEM) techniques. The effect of these sensilla on oviposition was investigated by sensilla deactivation using hydrochloric acid (HCl). The results showed that sensilla trichoid (ST), sensilla chaetica (ChS) and sensilla basiconica (SB) were distributed on ovipositor, while sensilla trichoid (ST) were distributed on the external genitalia. Sensilla chaetica (ChS) and basiconica (SB) were mainly distributed on the end of the ovipositor back plate, and on the base of the sternum. On tarsus, sensilla trichoid (ST), sensilla chaetica (ChS), sensilla triangular (TeS) and sensilla ear ball (SE) were observed. However, whole tarsal segments were covered with Böhm's mane, which was arranged in a circular line. Oviposition assay revealed that a significantly lower number of eggs were laid on the *Murraya paniculata* seedlings confined by the psyllids with deactivated ovipositorial sensilla followed by protarsal sensilla, mesotarsal sensilla and hindtarsal sensilla. These results suggested that the ovipositorial and tarsal sensilla of citrus psyllid have an important role in the oviposition.

Key words: *Diuraphis citri*; morphology; ovipositorial sensilla; oviposition; tarsal sensilla.

Evaluating the repellent effect of four botanicals against two *Bactrocera* species on mangoes

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ABSTRACT

Background: *Bactrocera dorsalis* and *B. correcta* are economically important fruit fly pests of crops, vegetables, fruits, and nuts worldwide, especially in China. Nowadays in China, *B. correcta* is a second notorious pest of many fruits after *B. dorsalis*. Different botanicals have been tested against the *B. dorsalis* but in the case of *B. correcta*, no records were published.

Methodology: This study evaluated the repellency of four botanicals (*Seriphidium brevifolium*, *Piper nigrum*, *Azadirachta indica* and quercetin) in acetone dilutions (5%, 2.5% and 1%) against the *B. dorsalis* and *B. correcta* at the laboratory conditions (25 ± 2 °C, $60 \pm 5\%$ relative humidity, and a photoperiod of L:D 14:10 h).

Results: The number of visits after 24–48 h, oviposition punctures, and pupae made by both species were lower on the treated mangoes in comparison to untreated mangoes. *S. brevifolium*, *P. nigrum*, *A. indica* and quercetin have significantly reduced the visits, ovipositional punctures, and pupae of both species. Among botanicals, the *P. nigrum* was the most effective repellent against *B. correcta* and as well as *B. dorsalis*. However, the harmful effects of these botanicals against natural enemies are still unknown.

Submitted 5 July 2019

Accepted 9 January 2020

Published 4 March 2020

Corresponding authors

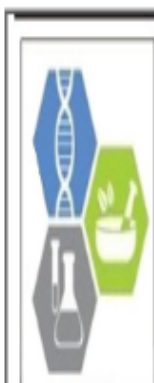
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Academic editor

Najat Saliba

Additional Information and
Declarations can be found on



Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com



E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2017; 5(1): 317-321
© 2017 JEZS
Received: 18-11-2016
Accepted: 19-12-2016

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Standard IPM measures against an invasive pest Mealy bug, *Drosicha Sp.* (Homoptera: Coccoidea) on Willow tree (*Salix Wilhelmsiana*) in Skardu, Pakistan

Syed Arif Hussain Rizvi, Waqar Jaleel, Walter Maldonado Jr, Zahid
Mahmood Sarwar, Saleem Jaffar and Muhammad Ayub

Abstract

The *Drosicha* sp (mealy bug) is an invasive and polyphagous pest in Baltistan, Pakistan. This pest was recorded in 2005, as primary pest of willow tree (*Salix wilhelmsiana*). The secondary hosts of *Drosicha* sp was recorded in Skardu region are apricot, apple, cherry, and mulberry. Our study was designed to find out Integrated Pest Management strategies against the *Drosicha* mealy bug. The Gunny bag wrappings along with mud paste was showed best cultural practice to stop the crawlers as in June only 6.0 ± 0.92 mealy bug was recorded per plant as compared to control (12.000 ± 1.03). The dispersal behavior (altitude and water availability) mainly affects the infestation of mealy bug in Skardu region. The mealy bug infestation at Chumik was statistically found maximum as compared to Halqa two and Hassan colony. The *Sumnius renardi* was observed as a predator of mealy bug in the field. The population dynamics of mealy bug was showed positive correlation with temperature. The temperature greatly affects the population and reproduction of mealy bug in Skardu. The maximum percent mortality was caused by Movento® 240SC (Spirotetramat +Imidacloprid) 47.13% after 72 hours of application.

Keywords: Mealy bug, insecticides, *Salix wilhelmsiana*, population dynamics, gunny bag, *Sumnius renardi*



Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

J
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Journal of
Entomology
and
Zoology Studies

E-ISSN: 2320-7078

P-ISSN: 2349-6800

JEZS 2015; 3(3): 50-52

© 2015 JEZS

Received: 25-06-2015

Accepted: 29-07-2015

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Efficacy of some selected chemical insecticides and bio-pesticides against tomato fruit worm, (*Helicoverpa armigera*) under the agro climatic condition of Gilgit Baltistan, Pakistan

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Abstract

A Field Studies were carried out for the control of Tomato fruit borer (*Helicoverpa armigera*) during July 2014 at District Skardu Baltistan Pakistan. Variety of tomato grown was Shalkot when fruit setting starts in Skardu region we applied chemical insecticide and bio-pesticide at their recommended doses. The aim of investigation is to promote use of environment friendly bio-pesticides. Data were taken as number of fruit borer larvae plant⁻¹ of tomato. Data was recorded at weekly interval. The data after first week of application of pesticides and bio-pesticides revealed that lowest number (0.93) of *H. armigera* larvae plant⁻¹ recorded in the plot treated with Volium Flexy 300 EC followed by Delegate 25 EC where (1.19) numbers of *H. armigera* larvae plant⁻¹ while on the other hand Bio-pesticides also shown control of fruit borer at some extant which was recorded as 1.24 and 1.26 numbers of *H. armigera* larvae plant⁻¹ by Sea-buckthorns and Populus extracts while in the check plot highest number(1.56) of fruit borer larvae plant⁻¹ was recorded. Overall performance of synthetic insecticides and bio-pesticides against Tomato fruit borer *H. armigera* the data showed that Volium Flexy 300 EC lowest (0.64) numbers of *H. armigera* larvae plant⁻¹ followed by Delegate 25 EC, Sea-buckthorns and Populus Extracts (0.98, 1.02, 1.03) while highest number of (1.38) *H. armigera* larvae plant⁻¹ was recorded from the check plot. From the results of present investigation we concluded that although percent mortalities showed by Populus and Sea-buckthorns plant extracts was low as compared to the synthetic insecticides but theses bio-pesticides were helpful to some extant for control of tomato fruit borer because of organic in nature the bio-pesticides have less environmental hazards while the synthetic insecticides have lots of ill effects on environment.

Keywords: Tomato, Bio-pesticides, Synthetic insecticides, *Populus euphratica* (Populus), *Hippophae rhamnoides* (Sea-buckthorns)