

Research Article



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Comparative Toxicant Efficacy of Some Novel Insecticides against Metamorphosis of Papilio demoleus (L.) Larvae

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Abstract

The lemon butterfly, *Papilio demoleus* (L.) is a key pest of citrus orchards found throughout the year. Their infestation causes defoliation, retards growth, and yields reduction. The five insecticides namely; Emamectin Benzoate 1.9 EC, Belt 480g/L SC (Flubendiamide), Helmet 40 EC (Chlorpyrifos), Coragen (Chlorantraniliprole 18.5% SC), and Regent 5% SC (Fipronil) were tested on *C. limon* orchard leaves against the larvae population of the lemon butterfly under laboratory conditions during, 2020. The maximum cumulative effect of all treatments was observed in three replications. The mortality % of larvae and efficacy of insecticides were recorded after 24, 48, 72, 120, and 168 hours of post-sprays. The result revealed that Belt pesticide proved with maximum larval mortality % (66.64 ± 8.16) followed by Regent (60.26 ± 0.76), Coragen (59.66 ± 7.72), Helmet (57.14 ± 7.65), Emamectin and (56.18 ± 7.50), respectively. It is concluded that the Belt insecticide provided a better mortality % against the larval population. The one-way ANOVA showed a significant difference among all tested insecticides at (P< 0.05) level. After insecticide application larvae reduced movement, skin became wrinkled, thin, and yellow-brown pigmentation visibly appeared with loose muscle concentration by releasing hyper-salivation and semi-liquid frass. Thus, it is recommended to be applied at a time interval basis to suppress the population density of lemon butterflies.

Keywords: Belt, Coragen, Citrus limon, Emamectin, Helmet, Papilio demoleus, Regent.

Introduction

Citrus, the thorny aromatic orchards that originated first in the Indian Sub-continent has obscure and long domestication history (Rao et al., 2021). Citrus is the largest evergreen prime genus and contains leathery leaves, and stems with sharp spines, the most significant crop in the world with antioxidant values and high content of vitamin C (Gorinstein et al., 2021). China, India, USA, Pakistan, Japan, Korea, and Spain produce massive citrus fruit, but Brazil is the topmost citrus fruitproducing country in the world (Mahato et al., 2019). These fruits are thirst-quenching, and widely popular due to their refreshing fragrance and their nutritional and phytochemical content depending upon maturity, fruit storage, growing conditions, processing, and varieties (Singh et al., 2019). The lemon orchards rapidly grow in large proportion in the summer season, they may be seedy or seedless, spread upright, grow in open habitats, oblong to obovate, and due to their delightful taste, therapeutic and potential healthy citrus fruits are widely preferred (Czech et al., 2021). The Lepidoptera are highly recognized and widely distributed scaly-winged insects with differences in distribution and lifestyles,

more in number than Diptera, Hymenoptera, and Coleoptera (Powell, 2009). They are amazing surrogate herbivore species with marvellous body colors, their life cycle plays a main role in the food supply for spiders, reptiles, predatory insects, and birds and due to the availability of food sources, their habitat is muscularly interlinked (Haroon et al., 2013). Papilio demoleus often called "lime swallowtails" belongs to the family Papilionidae, commonly associated with evergreen forests, dry and humid areas, and human settlements (Smith and Vane-Wright, 2008). This species of pest insect is regularly seen in natural vegetation, gardens, wideopen, and rapid visual power, frequently found in Africa, Madagascar, Cape Verde Islands, Mauritius, India, Bangladesh, Burma, Ceylon, Formosa, Arabia, and all over Pakistan (Rafi et al., 1989). The citrus varieties are mostly harmed by the P. demoleus larvae and their biological development depends on environmental conditions (Narayanamma et al., 2001). From nurseries, up to mature stages of the citrus lemon are vigorously harmed mostly 4th and 5th instar stages of LBF (Mangrio et al., 2020). The Crambidae, Curculionidae, Eriophyidae, Pseudococcidae, Cossidae Drosophilidae, and

families are major pest insect species to citrus (Mal et al., 2014). The female of P. demoleus exclusively lays eggs on twigs continues two to three days, their larvae pass five distinct stages that harm new growing twigs, and leaflets cause complete defoliation to citrus orchards (Mangrio and Sahito, 2022). They are widely dispersed insects with rapid growth rates and for their growth and development feed on soft green parts of the leaves, and behave as potential insect pest species to citrus (Islam et al., 2017). The lead arsenate hydrogen and sodium fluorosilicate were spraved to control the larvae population of LBF and observed the best larvae mortality (Bindra, 1957). In Maharashtra (Sontakay, 1943), used parathion 0.025%, endrin 0.025%, mevinphos malathion 0.05%, 001%. folithion, dichlorodiphenyltrichloroethane, and diazinon against LBF larvae and found best LBF larvae mortality. Sprayed parathion insecticide and recorded 100% mortality in larvae of LBF (Saini and Sharma, 1970). The deltamethrin insecticide was applied against the P. malayanus and P. demoleus species and reported more effective against the LBF larvae population (Riaz et al., 2020). Generally, the wide application of insecticides utmost enhances the resistance power of insect pests, causing loss of natural pollinators, pest predators, bird communities, pollution, and several public health problems (Pimentel, 2005). Therefore, the present work is the first attempt to find out the control against the larvae population of the LBF under laboratory conditions in Sindh-Pakistan.

Materials and Methods

Sample collection site: The new 4th and 5th instar stages of larvae of LBF were collected from each talukas of district Naushahro Feroze, during, 2020. After the collection of the larvae population from the infested *C. limon* leaves were kept in plastic jars and for aeration, plastic jars were covered with a muslin

cloth. The five different insecticide solutions were prepared and tested and also compared with the control group against the larvae of LBF under laboratory examination. The techniques and procedures were adopted to testify to the insecticidal efficacy against the larvae of LBF at $28.5\pm^{\circ}$ C and $70\pm5\%$ RH in the laboratory.

Experimental design: The five treatments were tested against the larvae of LBF and all treatments were replicated three times. The (n=05) petri plates were arranged and (n=40) new green emerging leaves and (n=20) larvae of the LBF (n=200) green lemon were placed individually. For each insecticide examination, a total of (n=100) 4th and 5th instar stage LBF larvae (n=200) insecticide containing green lemon leaves and (n=100) LBF larvae (n=200) without insecticide lush green lemon leaves were used for control. The experiment was done in the Entomology laboratory, new emerged green leaves were dipped in T_1 = Belt (0.62ml), T_2 = Helmet $(2.5ml), T_3 = Coragen (0.62ml), T_4 = Regent (6.25ml)$ and T_5 = Emamectin Benzoate (6.25ml) per litter. For the recommended dosage of each insecticide, the leaves were dipped for two minutes and then kept on blotting paper for dehydration (Table- 1). After that, insecticides containing leaves were placed (6x6) in diameter containing petri plates and placed Whatsman[®] filter paper for moisture absorption. The insecticide efficacy and larvae mortality % were recorded after the 2nd, 3rd, 4th, 6th, and 8th days of insecticide application. For the determination of each insecticide application against LBF larvae (Muhammad et al., 2019) described methodology was used.

Statistical analysis: The analysis of variance and means were significant difference was counted through student package software (8.1) and LBF larvae mortality % was taken through the application of (Abbott, 1925), introduced formula.

-) x 100

Corrected (%)=1--

Insect population in treated plot/lab after treatment

Insect population control plot/lab after treatment

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Brand name	Compound	Name of company	Dose/ litter	Effectiveness
Emamectin 1.9 EC	Benzoate	Sino Crop Science (Pvt). Ltd.	6.25 ml	larvae
Belt 480g/l SC	Flubendiamide	Bayer Crop Science (Pvt). Ltd.	0.62 ml	Larvae/Adults
Regent 5% SC	Fipronil	Bayer Crop Science (Pvt). Ltd.	6.25 ml	Larvae/adults
Coragen	Chlorantraniliprole	FMC (Pvt). Ltd.	0.62	Eggs/larvae
Helmet 40 EC	Chlorpyrifos	Target (Pvt). Ltd.	2.5 ml	Adults/Larvae

Results

Toxicant efficacy of Belt insecticide against LBF larvae on *C. limon* **leaves :** To examine the efficacy of the five novel insecticides namely; Belt, Helmet, Coragen, Regent, Emamectin, and control were applied against the 4th and 5th instar stages of LBF larvae under laboratory conditions, during, 2020. The larvae mean population was recorded before the application of the insecticides and recorded at (20.00 ± 1.41) and after the application of Belt insecticide the maximum to minimum mortality % was recorded during 3rd spray (87.95±9.38) (2nd day), followed by (87.50±9.35) (3rd day), (72.22±8.50) (4th

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day), (60.00 ± 7.75) (6th day) (36.36±6.03) (8th days) with overall mortality % (68.81±8.30), respectively. In the 2nd spray, the mortality % recorded (96.00±9.80) (24^h), (73.68±8.58) (48^h), (62.50±7.91) (72^h), (53.85±7.34) (120^h), (41.67±6.46) (168^h) with overall (65.54±8.07) of post-spray. The mortality % counted (90.91±9.53) (2nd day), (81.82±9.04) (3rd day), (66.67±8.17) (4th day), (50.00±7.07) (6th day), (38.46±6.20) (8th day) with overall (65.57±8.10) mortality % in 1st spray, respectively. Against the LBF larvae, the Belt insecticide proved with the best effect after 24^h, 48^h, and 72^h post-spray. The analysis of variance found (DF= 2; F= 0.66; P= 0.55) with non-significant mortality % difference in three treatments and (DF= 4; F= 47.35; P= 0.02) significant difference in post-spray days at (P< 0.05) as given in (Fig- 1).



Toxicant efficacy of Regent insecticide against LBF larvae on C. limon leaves: The maximum to minimum mortality % of the LBF larvae recorded (83.33 ± 9.13) $(24^{\rm h})$, (78.26 ± 8.85) $(48^{\rm h})$, (66.67 ± 8.17) $(72^{\rm h})$, (53.33 ± 7.30) $(120^{\rm h})$, (27.27 ± 5.22) $(168^{\rm h})$, with overall mortality % (61.77±7.86) in 3rd spray. In 1st spray (86.38±9.29) (2nd day), (77.27±8.79) (3rd day), (60.00±7.75) (4th day), (50.00±7.07) (6th day), (30.77 ± 5.55) (8th day), with overall (60.88±7.80) mortality % was observed. While as; mortality % was counted at (92.00±9.59) (2nd day), (78.95±8.89) (3rd day), (56.27±7.50) (4th day), (38.46±6.20) (6th day), (25.00 ± 5.00) (8th day), with overall mortality % (58.13±7.62), after the application of regent insecticide under laboratory conditions. This insecticide seemed with more efficacies in the early days (24^h, 48^h, 72^h) of post-treatment. The analysis of variance found a non-significant difference in treatments (DF= 2; F= 0.73; P= 0.51), and (DF= 4; F= 68.89; P= 0.01) significant difference in postdays, at (P<0.05) among the homogenous groups in days and treatments as justified in (Fig- 2).

Toxicant efficacy of Coragen insecticide against LBF larvae on C. limon leaves: The maximum to

minimum mortality % of LBF larvae was recorded at (79.17 ± 8.90) (2nd day), (73.91\pm8.60) (3rd day), (72.22 ± 8.50) (4th day), (60.00±7.75) (6th day), (18.18±4.26) (8th day) with overall mortality % (60.70±7.79) in 3rd spray. In the 1st spray the LBF larvae mortality % (81.82±9.05) (24^h), (65.00±8.06) $(48^{\rm h})$, (60.00 ± 7.75) $(72^{\rm h})$, (46.15 ± 6.79) $(120^{\rm h})$, (42.86 ± 6.55) (168^{h}) , with overall (59.17 ± 7.69) mortality % was recorded. The larvae mortality % remained at (80.00 ± 8.94) (2nd day), (73.68±8.58) (3rd day), (62.50±7.91) (4th day), (46.15±6.79) (6th day), (33.33 ± 5.77) (8th day), with overall mortality % (59.13 ± 7.64) in 2nd spray, after the application of Coragen insecticide under laboratory conditions. The coragen insecticide was found with maximum effectiveness in the earlier days of the post-spry against the larvae of LBF. When data was statistically analysed found non-significant at (DF= F= 12.34; P= 0.94) in treatments and (DF= 4; F= 12.34; P= 0.01) significant difference in post-spray days. The least significant difference T-test was recorded at (P<0.05), as shown in (Fig- 3).





Toxicant efficacy of Helmet insecticide against LBF larvae on C. limon leaves: The maximum and minimum mortality % of LBF larvae were recorded (73.33±8.56) at (24^h), (70.00 ± 8.37) $(48^{h}),$ (72^h), (50.00 ± 7.07) (65.00 ± 8.06) $(120^{h}),$ (38.46 ± 6.20) (168^h), with overall (59.36±7.70) mortality % in 1st spray. In 3rd spray (77.78±8.82) (2nd day), (73.91) (3rd day), (70.83±8.42) (4th day), (53.33 ± 7.30) (6th day), (09.09±3.01) (8th day) with overall (56.99±7.55) mortality % was recorded. While as; the mortality % remained (68.75 ± 8.29) overall (55.08±7.42) mortality % after (3rd spray), after the application of Helmet insecticide under laboratory conditions. Helmet insecticide was observed with maximum mortality % of the larvae during the (2nd day) of the post-spray followed by other days of the post-spray, respectively. The analysis of variance was found with a non-significant difference in treatments at (DF= 2; F= 0.32; P= 0.07) and a significant difference (DF= 4; F= 14.58; P= 0.03) in post-spray days. The significance was counted through the T-test at (P<0.05) as shown in (Fig- 4).



Toxicant efficacy of Emamectin insecticide against LBF larvae on C. limon leaves: The overall mortality % of LBF larvae on post-spray days on insecticide-containing leaves was recorded at (68.75 ± 8.29) (2nd day), (68.42\pm8.27) (3rd day), (68.00 ± 8.25) (4th day), (53.85 ±7.34) (6th day), (33.33 ± 5.77) (8th day), with overall mortality % (58.47 ± 7.65) in 2nd spray. In 1st spray the mortality % was recorded (73.33±8.56) (24^h), (70.00±8.73) (48^h), (72^h), (68.18±8.26) (42.86 ± 6.55) $(120^{h}),$ (23.08 ± 4.80) (168^h), with overall (55.49±7.45) mortality % in the larvae of LBF. The mortality % recorded (66.67±8.17) (2nd day), (65.22±8.08) (3rd

day), (69.57±8.34) (4th day), (53.33±7.30) (6th day), (18.18±4.26) (8th day), with overall (54.49±7.38) mortality % in 3rd spray, after the application of Emamectin insecticides under laboratory conditions. Emamectin insecticide against the larvae of LBF was found to best control during earlier days as compared to the later days of the post-spray. ANOVA was found with a non-significant difference in treatments at (DF= 2; F= 0.9; P= 0.44) and a significant difference (DF= 4; F= 49.66; P= 0.02), in post-spray days. T-test was found at (P<0.05), further validation is given in (Fig- 5).



Overall toxicity of five novel insecticides against LBF larvae: The toxicant effect of the abovementioned insecticides against the LBF larvae with the overall mean in pre-treatment of all insecticides under laboratory was recorded at (20.00 ± 1.41) . Belt insecticide was observed maximum to minimum mortality % at (91.47 ± 9.56) (2nd day), (81.15 ± 9.01) (3rd day), (67.13\pm8.19) (4th day), (54.62\pm7.39) (6th) day), (38.83 ± 6.23) (8th day), with overall (66.64±8.16) mortality % of LBF larvae. The efficacy of Regent insecticide was recorded (87.24±9.34) (2nd day), (78.16±8.84) (3rd day), (60.97±7.81) (4th day), (47.26±6.87) (6th day), (27.68±6.25) (8th day), with overall mortality % (60.26±7.76) against the larvae. The larvae mortality % counted (80.33±8.96) (2nd day), (70.86±8.42) (3rd

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day), (64.91 ± 8.06) (4th day), (49.67±7.01) (6th day), (32.55±5.71) (8th day), with overall (59.66±7.72) mortality % after the application of Coragen insecticide. The efficacy power of the Helmet was calculated as (73.29±8.56) (2nd day), (69.02±8.31) (3rd day), (66.61±8.16) (4th day), (49.83±7.06) (6th day), (26.96±5.19) (8th day), with overall (57.14±7.56) mortality % against the larvae of LBF. Similarly, the larvae mortality % was recorded as (70.55±8.40) (2nd day), (67.88±8.24) (3rd day), (67.62±8.22) (4th day), (50.01±7.07) (6th day), (24.86±4.99) (8th day), with overall (56.18±7.50) mortality % after post-treatment of Emamectin. All the novel insecticides were observed with more effective initial days as followed by the post-spray days. When all data have statistically analysed the analysis of variance found a non-significant difference in post-treatments at (DF= 4; F= 2.40; P= 0.09), and (DF= 4; F= 56.18; P= 0.01), significant difference days replication. The least significant difference and T-test were found at (P<0.05) among the homogenous groups as shown in (Fig- 6).



Overall efficacy of five different insecticides against larvae of LBF on different days under laboratory conditions: All five insecticides proved with the most efficacy power against the 4th and 5th larvae stages of LBF under laboratory conditions. Belt insecticides proved to have a maximum mortality % as compared to Emamectin, Regent, Helmet, and Coragen against the pest population. The overall maximum to minimum mortality % against the larvae after the application of all five insecticides recorded (78.65±8.86) (2nd day), (73.41±8.65) (3rd day), (67.37±8.20) (4th day), (50.28 \pm 7.09) (6th day), (30.18 \pm 5.49) (8th day), respectively. Above all tested insecticides were found with maximum mortality % (2nd day) compared with other post-spray days. The ANOVA was found with a significant difference at (DF= 4; F= 56.18; P= 0.02), among the post-treatment. The LSD during the post-spray days was found through (SWX) software (8.1), USA at (P<0.05). The Origin 2017 64Bit graphing and analysis software was used for the minimum to maximum mortality % of larvae on different days after post-treatment as given in (Fig-7).



Discussion

Belt insecticide was found with maximum LBF larvae mortality % in 3rd spray. The same findings were reported (Pawandeep and Anita, 2020); they used quinalphos 25% EC and chlorpyriphos 20% EC with the best LBF larvae mortality % on the citrus, nursery leaves. The mortality % in 1st and 2nd sprays with the work agreement of (Singh et al. 1983), discussed diofenolan as the best insecticide for the control of the citrus butterfly's population also observed pyrethroid and synthetic phoxim was found to be the best mortality % against the Achaea janata larvae. The maximum mortality % of the LBF larvae counted in 3rd spray by the application of Regent is more comparable with the work of (Imen et al. 2021), who applied acrinathrin insecticide and reported (26%) mortality of Thysanoptera on the leaves of Citrus sinensis in Tunisia. The mortality % in 1st and 2nd sprays is similar work of (Patil and Rajashekaragouda, 1985), who mentioned the efficacy of 18 liters of water with 35 ml of endosulfan against the larvae of LBF.

Coragen pesticide was found with more efficacy in 3rd spray against the pest population as discussed (Prakash, 2012), who applied different insecticides such as; copper sulfate, endosulfan, lauryl alcohol ethoxylate, dimethoate, fenthion, chlorpyrifos, Parathion methyl, buprofezin, cuprous oxide and lime sulfur against larvae of citrus insect pests and recorded with best mortality %. The larvae mortality % in 1st and 2nd sprays were found more or less comparable with the work of (Lewis et al. 2011), they recorded 100% LBF larvae mortality % when they were given treated leaves of methionine human nutrient amino acid and observed the positive effect on the larvae amino acid modulated system. Against the larvae of LBF the Helmet insecticide in proved more effective in 3rd spray as described (Wen *et al*. 2021), reported 50% and 80% mortality of Diaphorina citri at LC50 and LC80 dosages of flupyradifurone insecticide. The larvae mortality % in 1st and 2nd sprays are comparable with the result of (Butani, 1979), sprayed with monocrotophos 0.04 % and chlorpyriphos 0.05 % and at the rate of twenty litters per tree applied 0.04% phosphamidon and observed effects. The maximum toxicant efficacy of the Emamectin was found in 2nd spray with work similarity of (Radosevich and Cloyd, 2021), applied acetamiprid insecticide and counted (50%) mortality citrus mealybug on horticultural plants. The larvae mortality in 1st spray as (Batra and Bindra, 1982), documented that Malathion, formothion, and oxydemotonmethyl insecticides are less effective compared with endosulfan but more effective compared with untreated control plants against the larvae of LBF. In 3rd spray mortality % in the pest population counted which is comparable with the work of (Singh and Rao, 1978), applied seventeen insecticides were of which they proved methyl parathion 0.05%, methamidophos 0.05% and leptophos 0.06% with more effective against larvae of LBF.

The overall maximum toxicity recorded Belt and Regent (Ghosh et al. 2015), they administrated carbaryl against the 4th instar of LBF larvae through foliar spray 0.25, 0.50, 1.00 mg/l and proposed that the above appropriate doses of the insecticide are recommended and fitting range against the biomonitoring and malformation of LBF larvae. The mortality % of LBF by the application of Coragen, Helmet, and Emamectin is with the work agreement of (Ghosh et al. 2015), they administrated quinallphas 2ml, B. thuringiensis 0.05%, fenitrothion 1ml, carbaryl, dimethoate 1.5ml contact and systematic insecticides against the mortality % of LBF larvae and also found adults with wrinkled and paralytic flight. By the application of all insecticides maximum, mortality % counted 2nd the day of the spray (Afzal et al. 2018), documented mortality

73.57% against Drosicha mangiferae with the application of methidathion, 59.96% bifenthrin, 58.07% profenopfos, and 59.81% carbosulfan. The pest population on the 3rd day of post-spray with the same findings (Bindra, 1957), administrated DDT 40 Ib, parathion 0.44 Ib, benzene- hexachloride 40 Ib, folithion 0.4 Ib, and diazinon 1.0 Ib, along with 100 gallons of water and observed all mentioned insecticides effective against the larvae population of LBF. On the 4th day as described (Delano et al. 2011), they recorded 100 % fourth instar stage mortality by the application of methionine. On the 6th day with work similarity of (Pawandeep and Anita, 2020), who counted 20.03 mean mortality in the control plot and larvae mortality on the 8th day with the agreement of (Resham et al. 1986), they applied quinalphos 0.04% and juvenile hormone against the mortality % LBF larvae.

Conclusion and Recommendations

It was concluded that within the comparison of five novel insecticides the Belt proved more effective followed by the Emamectin, Regent, Helmet, and Coragen against the LBF larvae hence it is recommended. It is recommended that on the residual effect of toxic insecticides more work should be carried out. Typically, for insect pest control and quality improvement of the citrus several insecticides have been administrated in many parts of the world but along with insect pest control, different insecticides are the causative agent in health hazards, soil contamination, environmental pollution, and wildlife demolition it should be wisely used. This study will be an authentic and more informative tool because previously no such type of scientific documentation has been reported from Sindh. Pakistan.

Author's Contribution

W.M. Mangrio is the main author of this research paper, who conducted the experiments in laboratory conditions and wrote the article whereas; H.A. Sahito, reviewed the research article and supervised the experiments.

Impact Statement

The lemon orchards are widely grown in the district of Naushahro Feroze with massive fruit production. It is the main economic commodity of this area but is vigorously harmed by larvae of LBF. It is necessary to bring awareness and apply certain control measures through IPM strategies to secure the citrus from potential citrus insect pests.

Declaration and Competing Interest

There is no personal relationship or financial interest regarding the authors to persuade work which is reported in this research article.

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larvae of *P. demoleus* and proved the toxicity of novel insecticides.

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Availability of data and materials

Due to certain restrictions and ethical privacy the findings and data of this research work are available at the request of the corresponding author. **Consent for Publication**

The author accepts responsibility for releasing this scientific documentary.

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