

**Assessment of various plant extracts and a synthetic insecticide against lemon butterfly (*Papilio demoleus*) in the citrus (*Citrus aurantium*) nursery**

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### **Abstract**

Research is currently being conducted at the Horticultural Research Farm of The University of Agriculture Peshawar, Pakistan, in the spring of 2021. The experiment was laid down in Randomized Completely Block Design (RCBD) with 3 replications 6 treatments and a control comprising of 1 synthetic pesticide used as a standard against *P. demoleus* and 5 aqueous botanicals extracts were randomly assigned to different plots. At intervals of 14 days, each treatment was applied to the plot it was assigned to. Results revealed that the lemon butterfly (*P. demoleus*) population was lowest in the plot treated with bifenthrin (13.00, 7.00 and 6.00 larvae plant<sup>-1</sup>), neem fruit extract (14.00, 7.22 and 7.55 larvae plant<sup>-1</sup>), ginger rhizome extract (15.00, 8.94 and 8.50 larvae plant<sup>-1</sup>), black pepper fruit extract (16.00, 9.83 and 9.11 larvae plant<sup>-1</sup>), tobacco leaf extract (16.11, 10.27 and 9.83 larvae plant<sup>-1</sup>), garlic bulb extract (17.16, 11.11 and 10.61 larvae plant<sup>-1</sup>) after first, second and third spray respectively. The highest *P. demoleus* population was in the plot control (21.27, 21.33 and 21.38 larvae plant<sup>-1</sup>). The damage assessment percentage of treated plots was also correlated with *P. demoleus*. It was found that damage due to *P. demoleus* ranged from (50.28% to 73.56%) with maximum damage from 15<sup>th</sup> May to 1<sup>st</sup> July whereas, the minimum damage was observed from 1<sup>st</sup> July to 15<sup>th</sup> August but again increased after 15<sup>th</sup> August. Biochemical analysis of healthy vs infested plant leaves was also investigated. It was found that leaves with higher Ca<sup>+2</sup> concentration were more prone to/attack by *P. demoleus*. However, leaves with higher K<sup>+</sup> concentration was attacked in lower numbers/ of *P. demoleus*. Based on the current study among all treatments, bifenthrin was found more effective against both the insect pests of citrus and is therefore recommended for managing these pests effectively. However, among plant extracts, Neem fruit extract proved to be effective against *P. demoleus* and is therefore recommended for environmentally safe management against these pests in nurseries and kitchen gardening.

**Keywords:** botanicals, *Citrus aurantium*, *P. demoleus*, synthetic insecticides

## Introduction

*Citrus* is a genus of blooming trees and bushes belonging to the Rutaceae family. Citrus fruits, like oranges, lemons, grapefruits, pomelos, and limes, are produced by plants of this genus (Wu *et al.*, 2017). Citrus production grew at a steady rate in the latter decades of the 20<sup>th</sup> century, reaching more than 105 million tonnes per year (2000-2004) (Ollitrault and Navarro, 2012). Oranges represent 61% of citrus yield, trailed by mandarins (20%), lemons and limes (14%), and grapefruits (5%). Most of the production is in Brazil (20%), the Mediterranean countries (20%), China (16%), and the United States of America (USA) (11%). These regions account for around a 3<sup>rd</sup> of all citrus production. Citrus fruits are mostly grown in the Mediterranean basin for the fresh fruit market. Spain is the main producer in the region, with a surface area of 305,000 hectares and a production of about 6 million tonnes (FAO, 2006). Pakistan's largest fruit crop is citrus, which is grown on 160,000 hectares and yields 1.5 million metric tonnes annually. Although it is grown throughout Pakistan, Punjab essentially produces the whole yield. A hybrid between a king and a willow leaf produced the citrus variety Kinnow (*C. reticulata*). Its moniker is "kinnow mandarin" (Altaf and Khan, 2008). Pakistan has the tenth-largest kinnow plantation in the world, with an annual output of 2 million tonnes (Syed, 2007). Total citrus output in Khyber Pakhtunkhwa was 1.29% in 2014-2015 (Siddique and Garnevska, 2018) However, the lemon butterfly (*P. demoleus*) is an economically significant harmful insect that severely damages the leaves of the Apiaceae, Anacardiaceae, Fabaceae, Rhamnaceae, Rutaceae, and Sapindaceae families resulting in complete defoliation of young plants. At the point when an invasion occurs at a wide scope of host plants retarded their development and minimize yield amount on the planet however on account of Southeastern Asia, these irritative insects are located at the broad reach and wide dispersal region of the 5<sup>th</sup> caterpillar larval stage causes large infestation on citrus (*C. aurantium*) plantation (Mangrio *et al.*, 2020). The larval stage of the lemon butterfly (*P. demoleus*) shows a serious impact on the developmental stage of host plants which may result in complete defoliation (Pathak and Rizvi, 2002). When fresh foliage and luscious green leaves appear, the lemon butterfly's survival activity peaks (Narayanamma and Savithri, 2002), and their caterpillars regularly find new growing citrus plantations, especially when orchards reach a height of a few feet (Yunus and

Munir, 1972). One of the naturally occurring chemicals found in plants is referred to as plant extracts. Naturally, occurs insecticides can be utilized as an option in contrast to manufactured formulations, yet they are normally claimed to be more poisoning to people. Probably the deadliest cancer-causing substances, as destructive poisons, grow rapidly and flourish in nature (Sarasan *et al.*, 2011). There are around 2400 plant species in 189 plant families that are believed to be abundant in biologically active plant extracts (Rao *et al.*, 2005). Citrus plants needed an enormous number of leaf macro and micronutrient minerals like ( $\text{Ca}^{++}$ ,  $\text{Mg}^{++}$ , and  $\text{K}^{+}$ ) which play a significant part in their development.  $\text{Ca}^{++}$  is significant for the advancement of roots, cell division, chromosome pressing, and the invulnerable arrangements of plants (Zekri and Obreza, 2003). In recent years, there has been a significant shift in the use of insecticides from synthetic to plant extracts, owing to the harmful effects of synthetic insecticides, which are carcinogens and cause various diseases in animals and humans, as well as the accumulation of different insecticides in the fatty tissue of animals and humans, and the depositions of insecticides in vegetables eaten by animals and returned to humans. The goal of this study was to compare different plant extracts and synthetic insecticides in order to better understand how they work together to control lemon butterfly (*P. demoleus*) infestation and studied the biochemical comparison of healthy and diseased leaves.

### Materials and Methods

Experiment on “assessment of various plant extracts and a synthetic insecticide against lemon butterfly (*Papilio demoleus*) in the citrus (*Citrus aurantium*) nursery” at the Horticultural Research Farm of The University of Agriculture Peshawar, Pakistan during spring 2021. *Citrus aurantium* nursery was transplanted into the field with the essential agronomic operations from February 15<sup>th</sup> to April 1<sup>st</sup> of 2021. The experiment used a Randomized Completely Block Design (RCBD) with 3 replications and 7 treatments including 5 plant extracts and 1 synthetic pesticide (black pepper fruit extract @ 6%, garlic bulb extract@ 3%, ginger rhizome extract@ 3%, neem fruit extract@ 5%, tobacco leaf extract@ 5%, tender 7.5 EC (bifenthrin) and control. Each experimental plot was 5 feet long and 5 feet wide, with a row to row spacing of 2.5 feet and a border area of 6 inches. From each experimental plot, 7 plants were chosen and tagged. A knapsack sprayer was used to apply all treatments, including plant extracts and

synthetic insecticide. The control plot was not treated with any plant extracts or synthetic insecticide.

### **Evaluation of various plant extracts against lemon butterfly (*P. demoleus*)**

In each replication and experimental plot, the population of lemon butterfly (*P. demoleus*) was recorded on 7 randomly selected plants. Each plant was separated into three sections: top, middle, and bottom, with all leaves from each section being picked and tagged. Data was recorded 1 day before the spray. Post-spray data was recorded on 1, 3, 5, 7, and 14 days after the application of treatments.

$$\text{Percentage leaf infestation} = \left\{ \frac{\text{Total number of infested leaves}}{\text{Total number of leaves}} \right\} \times 100$$

### **Sample collection and damage assessment of citrus leaves**

Mustafa *et al.*, (2014) followed for sample collection and damage. From each treatment and control group, 7 plants were chosen. The number of un-infested and infested leaves was counted, and the percentage of damage on each plant was computed. No chemical spray was done during the entire time span of the study from 15<sup>th</sup> February to 1<sup>st</sup> April.

### **Proximate analysis of un-infested versus infested citrus leaves *P. demoleus***

Mustafa *et al.*, (2014) followed this study with leaf samples taken from 3 citrus replications with 6 treatments and control. During the whole research time, no insecticides were utilized. 7 plants were picked and tagged from each experimental plot. 7 un-infested and infested leaves were picked from each plant. The leaves of each experimental plot were individually packaged in a paper bag. Each plant's un-infested and infested leaves were oven-dried at 70°C for 48 hours, and then 1 gram of the sample was digested using 4 ml H<sub>2</sub>SO<sub>4</sub> and 8 ml HCl on a hot plate till the digest becomes clear and colorless. After that, it was filtered and diluted with distilled water at a final concentration of 50 ml. In Shimadzu's (AA-6300) Atomic Absorption Spectrophotometer, (AAS) leaf samples were tested for minerals (Ca<sup>+2</sup>, Mg<sup>+2</sup>, and K<sup>+</sup>) concentrations were approximated using standard curves for these minerals; mineral concentrations were measured in parts per million (ppm).

## Statistical analysis

The data of all collected parameters were analyzed by using Statistical Software "Statistix Version.8.1". In order to compare the effect of mentioned treatments, the Analysis of Variance (ANOVA) technique in Randomized Complete Block Design (RCBD) the Least Significant Difference (LSD) test was applied. Furthermore, to compare the un-infested and infested leaves samples, independent samples T-tests were applied.

## Results and discussions

### Evaluation of various plant extracts against *P. demoleus* larvae plant<sup>-1</sup>

Table 1 showed the number of *P. demoleus* larvae plant<sup>-1</sup> was recorded both before and after application. The non-significant outcome was found in every one of the treatments before spray application while after spray application data shows that the population of *P. demoleus* larvae plant<sup>-1</sup> was significantly affected by various treatments. One day before application data shows the lowest *P. demoleus* population was recorded in the plot control (18.66 larvae plant<sup>-1</sup>) followed by the plots bifenthrin (20.00 larvae plant<sup>-1</sup>), tobacco leaf extract (20.33 larvae plant<sup>-1</sup>), neem fruit extract (21.00 larvae plant<sup>-1</sup>) and ginger rhizome extract (22.00 larvae plant<sup>-1</sup>), being non-significant with each other. The highest *P. demoleus* population was recorded in the plots black pepper fruit extract (22.66 larvae plant<sup>-1</sup>) and garlic bulb extract (22.66 larvae plant<sup>-1</sup>), being non-significant with each other. One day after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (17.33 larvae plant<sup>-1</sup>) subsequently the plot treated with neem fruit extract (18.33 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with ginger rhizome extract (19.33 larvae plant<sup>-1</sup>), black pepper fruit extract (20.66 larvae plant<sup>-1</sup>) and tobacco leaf extract (21.00 plant<sup>-1</sup>), being non-significant with each other. The plot was treated with garlic bulb extract (21.33 larvae plant<sup>-1</sup>), being significant with other treatments. The highest *P. demoleus* population was recorded in the plot control (23.00 larvae plant<sup>-1</sup>). Three days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (14.00 larvae plant<sup>-1</sup>) subsequently the plots treated with neem fruit extract (15.66 larvae plant<sup>-1</sup>) and ginger rhizome extract (16.33 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with black pepper fruit extract (17.00 larvae plant<sup>-1</sup>), tobacco

leaf extract (18.00 larvae plant<sup>-1</sup>) and garlic bulb extract (18.33 larvae plant<sup>-1</sup>), being non-significant with each other. The highest *P. demoleus* population was recorded in the plot control (20.00 larvae plant<sup>-1</sup>). Five days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (11.33 larvae plant<sup>-1</sup>) subsequently the plots treated with neem fruit extract (12.66 larvae plant<sup>-1</sup>) and ginger rhizome extract (13.66 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with black pepper fruit extract (14.00 larvae plant<sup>-1</sup>), tobacco leaf extract (14.66 larvae plant<sup>-1</sup>) and garlic bulb extract (15.33 larvae plant<sup>-1</sup>), being non-significant with each other. The highest *P. demoleus* population was recorded in the plot control (23.66 larvae plant<sup>-1</sup>). Seven days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (6.00 larvae plant<sup>-1</sup>) subsequently the plot treated with neem fruit extract (7.00 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with ginger rhizome extract (8.00 larvae plant<sup>-1</sup>) and black pepper fruit extract (9.33 larvae plant<sup>-1</sup>), being non-significant with each other. The plots treated with tobacco leaf extract (10.00 larvae plant<sup>-1</sup>) and garlic bulb extract (11.00 larvae plant<sup>-1</sup>), being non-significant with each other. The highest *P. demoleus* population was recorded in the plot control (20.66 larvae plant<sup>-1</sup>). Fourteen days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (9.33 larvae plant<sup>-1</sup>) subsequently the plots treated with neem fruit extract (9.33 larvae plant<sup>-1</sup>), ginger rhizome extract (10.66 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with black pepper fruit extract (12.33 larvae plant<sup>-1</sup>) and tobacco leaf extract (12.66 larvae plant<sup>-1</sup>), being non-significant with each other. The plot was treated with garlic bulb extract (14.33 larvae plant<sup>-1</sup>), being significant with others. The highest *P. demoleus* population was recorded in the plot control (21.66 larvae plant<sup>-1</sup>). The mean column in Table 1 shows that after the 1<sup>st</sup> spray, the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (13.00 larvae plant<sup>-1</sup>) subsequently the plot treated with neem fruit extract (14.00 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest plots treated with ginger rhizome extract (15.00 larvae plant<sup>-1</sup>), black pepper fruit extract (16.00 larvae plant<sup>-1</sup>) and tobacco leaf extract (16.11 larvae plant<sup>-1</sup>), being non-significant with each other. The plot treated with garlic bulb extract (17.16 larvae plant<sup>-1</sup>), being significant with others. The highest *P. demoleus* population was recorded in the plot control (21.27 larvae plant<sup>-1</sup>).



Table 2 shows the number of *P. demoleus* larvae plant<sup>-1</sup> was recorded both before and after application. The non-significant outcome was found in every one of the treatments before spray application while after spray application data shows that the population of *P. demoleus* larvae plant<sup>-1</sup> was significantly impacted by various treatments. One day before application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (9.33 larvae plant<sup>-1</sup>) subsequently the plots treated with neem fruit extract (9.33 larvae plant<sup>-1</sup>), ginger rhizome extract (10.66 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with black pepper fruit extract (12.33 larvae plant<sup>-1</sup>) and tobacco leaf extract (12.66 larvae plant<sup>-1</sup>), being non-significant with each other. The plot was treated with garlic bulb extract (14.33 larvae plant<sup>-1</sup>), being significant with others. The highest *P. demoleus* population was recorded in the plot control (21.66 larvae plant<sup>-1</sup>). One day after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (7.66 larvae plant<sup>-1</sup>) subsequently the plot treated with neem fruit extract (8.00 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with ginger rhizome extract (10.33 larvae plant<sup>-1</sup>), black pepper fruit extract (10.66 larvae plant<sup>-1</sup>) and tobacco leaf extract (11.66 plant plant<sup>-1</sup>), being non-significant with each other. The plot was treated with garlic bulb extract (13.00 larvae plant<sup>-1</sup>), being significant with others. The highest *P. demoleus* population was recorded in the plot control (22.00 larvae plant<sup>-1</sup>). Three days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (6.66 larvae plant<sup>-1</sup>) subsequently the plots treated with neem fruit extract (7.00 larvae plant<sup>-1</sup>), ginger rhizome extract (8.66 larvae plant<sup>-1</sup>), black pepper fruit extract (9.66 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with tobacco leaf extract (10.00 plant<sup>-1</sup>) and garlic bulb extract (10.33 larvae plant<sup>-1</sup>), being non-significant with each other. The highest *P. demoleus* population was recorded in the plot control (20.66 larvae plant<sup>-1</sup>). Five days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (5.33 larvae plant<sup>-1</sup>) subsequently the plots treated with neem fruit extract (5.33 larvae plant<sup>-1</sup>) and ginger rhizome extract (7.00 larvae plant<sup>-1</sup>), black pepper fruit extract (8.00 larvae plant<sup>-1</sup>) and tobacco leaf extract (8.33 plant<sup>-1</sup>), being non-significant with each other. The plot was treated with garlic bulb extract (8.66 larvae plant<sup>-1</sup>), being significant with others. The highest *P. demoleus* population was recorded in the plot control (21.33 larvae plant<sup>-1</sup>). Seven days after application data shows the lowest *P. demoleus* population was noted in the plot

dealt with bifenthrin (3.00 larvae plant<sup>-1</sup>) subsequently the plot treated with neem fruit extract (3.33 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with ginger rhizome extract (5.00 larvae plant<sup>-1</sup>), black pepper fruit extract (5.66 larvae plant<sup>-1</sup>), tobacco leaf extract (6.00 larvae plant<sup>-1</sup>) and garlic bulb extract (6.66 larvae plant<sup>-1</sup>), being non-significant with each other. The highest *P. demoleus* population was recorded in the plot control (20.33 larvae plant<sup>-1</sup>). Fourteen days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (10.00 larvae plant<sup>-1</sup>) subsequently the plots treated with neem fruit extract (10.33 larvae plant<sup>-1</sup>) and ginger rhizome extract (12.00 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with black pepper fruit extract (12.66 larvae plant<sup>-1</sup>), tobacco leaf extract (13.00 larvae plant<sup>-1</sup>) and garlic bulb extract (12.66 larvae plant<sup>-1</sup>), being non-significant with each other. The highest *P. demoleus* population was recorded in the plot control (22.00 larvae plant<sup>-1</sup>). The mean column in Table 2 shows that after the 2<sup>nd</sup> spray, the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (7.00 larvae plant<sup>-1</sup>) subsequently the plot treated with neem fruit extract (7.22 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with ginger rhizome extract (8.94 larvae plant<sup>-1</sup>), black pepper fruit extract (9.83 larvae plant<sup>-1</sup>) and tobacco leaf extract (10.27 larvae plant<sup>-1</sup>), being non-significant with each other. The plots were treated with garlic bulb extract (11.11 larvae plant<sup>-1</sup>), being significant with others. The highest *P. demoleus* population was recorded in the plot control (21.33 larvae plant<sup>-1</sup>).

Table 3 shows the number of *P. demoleus* larvae plant<sup>-1</sup> was recorded both before and after application. The non-significant outcome was found in every one of the treatments before spray application while after spray application data shows that the population *P. demoleus* larvae plant<sup>-1</sup> was significantly impacted by various treatments. One day before application data shows the lowest *P. demoleus* population was recorded in the plot bifenthrin (10.00 larvae plant<sup>-1</sup>) followed by the plots neem fruit extract (10.33 larvae plant<sup>-1</sup>) and ginger rhizome extract (12.00 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots black pepper fruit extract (12.66 larvae plant<sup>-1</sup>), tobacco leaf extract (13.00 larvae plant<sup>-1</sup>) and garlic bulb extract (12.66 larvae plant<sup>-1</sup>), being non-significant with each other. The highest *P. demoleus* population was recorded in the plot control (22.00 larvae plant<sup>-1</sup>). One day after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (9.00 larvae plant<sup>-1</sup>) subsequently the plots treated with neem fruit extract (9.33 larvae plant<sup>-1</sup>)



<sup>1</sup>) and ginger rhizome extract (11.00 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with black pepper fruit extract (11.66 larvae plant<sup>-1</sup>), tobacco leaf extract (12.00 larvae plant<sup>-1</sup>) and garlic bulb extract (12.66 larvae plant<sup>-1</sup>), being non-significant with each other. The highest *P. demoleus* population was recorded in the plot control (22.00 larvae plant<sup>-1</sup>). Three days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (7.00 larvae plant<sup>-1</sup>) subsequently the plot treated with neem fruit extract (7.66 larvae plant<sup>-1</sup>), ginger rhizome extract (8.00 larvae plant<sup>-1</sup>), black pepper fruit extract (8.33 larvae plant<sup>-1</sup>) and tobacco leaf extract (9.33 plant<sup>-1</sup>), being non-significant with each other. The plot was treated with garlic bulb extract (10.66 larvae plant<sup>-1</sup>), being significant with others. The highest *P. demoleus* population was recorded in the plot control (20.66 larvae plant<sup>-1</sup>). Five days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (3.00 larvae plant<sup>-1</sup>), being significant with the plots treated with neem fruit extract (5.66 larvae plant<sup>-1</sup>), ginger rhizome extract (6.33 larvae plant<sup>-1</sup>), black pepper fruit extract (7.00 larvae plant<sup>-1</sup>) and tobacco leaf extract (7.66 larvae plant<sup>-1</sup>), being non-significant with each other. The plot treated with garlic bulb extract (8.33 larvae plant<sup>-1</sup>), being significant with others. The highest *P. demoleus* population was recorded in the plot control (21.33 larvae plant<sup>-1</sup>). Seven days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (0.00 larvae plant<sup>-1</sup>), being significant with the plots treated with neem fruit extract (1.66 larvae plant<sup>-1</sup>), ginger rhizome extract (2.33 larvae plant<sup>-1</sup>), black pepper fruit extract (3.00 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with tobacco leaf extract (4.00 larvae plant<sup>-1</sup>) and garlic bulb extract (4.66 larvae plant<sup>-1</sup>), being non-significant with each other. The highest *P. demoleus* population was recorded in the plot control (20.33 larvae plant<sup>-1</sup>). Fourteen days after application data shows the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (7.00 larvae plant<sup>-1</sup>), being significant with the plots treated with neem fruit extract (10.66 larvae plant<sup>-1</sup>), ginger rhizome extract (11.33 larvae plant<sup>-1</sup>), black pepper fruit extract (12.00 larvae plant<sup>-1</sup>) and tobacco leaf extract (13.00 larvae plant<sup>-1</sup>), being non-significant with each other. The plot treated with garlic bulb extract (13.66 larvae plant<sup>-1</sup>), being significant with others. The highest *P. demoleus* population was recorded in the plot control (22.00 larvae plant<sup>-1</sup>). The mean column in Table 3 shows that after the 3<sup>rd</sup> spray, the lowest *P. demoleus* population was noted in the plot dealt with bifenthrin (6.00 larvae plant<sup>-1</sup>),

being significant with the plots treated with neem fruit extract (7.55 larvae plant<sup>-1</sup>) and ginger rhizome extract (8.50 larvae plant<sup>-1</sup>), being non-significant with each other, while the rest of the plots treated with black pepper fruit extract (9.11 larvae plant<sup>-1</sup>) and tobacco leaf extract (9.83 larvae plant<sup>-1</sup>), being non-significant with each other. The plot treated with garlic bulb extract (13.55 larvae plant<sup>-1</sup>), being significant with others. The highest *P. demoleus* population was recorded in the plot control (21.38 larvae plant<sup>-1</sup>). Similar results were found by Hussein *et al.*, (2017), they found that the black pepper fruit extract suppresses the *P. citrella* population in *C. aurantium*. Peng *et al.*, (2010) who utilized extracts from ginger rhizome, tobacco leaf, and garlic bulb on populations of *P. citrella* and discovered that they were all successful treatments for these insect pests. Our findings are consistent with those of Fiaz *et al.*, (2012), who found that *P. citrella* mortality is significantly increased by neem fruit extract at 5%. Our findings on garlic bulb extract also yield similar results to that of Khanzada and Khanzada *et al.*, (2018), who found garlic bulb extract as an effective plant extract against *P. demoleus* on *C. aurantium*. In our experiment bifenthrin gave best results against *P. citrella*. The results can be compared with that of Manigano *et al.*, (2008), who reported that high rates of bifenthrin have worked well against *P. citrella*.

**Table 1: Effects of different plant extracts and a synthetic insecticide on *P. demoleus* population before and after 1<sup>st</sup> spray application in the *C. aurantium* nursery during spring 2021.**

Treatments	1 DBS	1 DAS	3 DAS	5 DAS	7 DAS	14 DAS	Mean
Black pepper fruit extract	22.66	20.66 bc	17.00 bc	14.00 bc	9.33 bc	12.33 cd	16.00 bc
Garlic bulb extract	22.66	21.33 ab	18.33 ab	15.33 b	11.00 b	14.33 b	17.16 b
Ginger rhizome extract	22.00	19.33 cd	16.33 bcd	13.66 bcd	8.00 cd	10.66 de	15.00 cd
Neem fruit extract	21.00	18.33 de	15.66 cd	12.66 cd	7.00 de	9.33 e	14.00 de
Tobacco leaf extract	20.33	21.00 bc	18.00 abc	14.66 bc	10.00 b	12.66 bc	16.11 bc
Bifenthrin	20.00	17.33 e	14.00 d	11.33 d	6.00 e	9.33 e	13.00 e
Control	18.66	23.00 a	20.00 a	23.66 a	20.66 a	21.66 a	21.27 a
LSD (0.05)	3.57	1.99	2.38	2.48	1.87	1.8	1.24

Figures in columns followed by the same letters are non-significant from each other at P (0.05%), ns (non-significant)

**Table 2: Effects of different plant extracts and a synthetic insecticide on *P. demoleus* population before and after 2<sup>nd</sup> spray application in the *C. aurantium* nursery during spring 2021.**

Treatments	1 DBS	1 DAS	3 DAS	5 DAS	7 DAS	14 DAS	Mean
Black pepper fruit extract	12.33	10.66 c	9.66 bcd	8.00 bc	5.66 b	12.66 bc	9.83 bc
Garlic bulb extract	14.33	13.00 b	10.33 b	8.66 b	6.66 b	13.66 b	11.11 b
Ginger rhizome extract	10.66	10.33 c	8.66 bcd	7.00 bc	5.00 bc	12.00 bcd	8.94 c
Neem fruit extract	9.33	8.00 d	7.00 cd	5.33 c	3.33 cd	10.33 cd	7.22 d
Tobacco leaf extract	12.66	11.66 bc	10.00 bc	8.33 bc	6.00 b	13.00 b	10.27 bc
Bifenthrin	9.33	7.66 d	6.66 d	5.33 c	3.00 d	10.00 d	7.00 d
Control	21.66	22.00 a	20.66 a	21.33 a	20.33 a	22.00 a	21.33 a
LSD (0.05)	1.8	2.14	3.05	3.01	1.94	2.37	1.6

Figures in columns followed by the same letters are non-significant from each other at P (0.05%), ns (non-significant)

**Table 3: Effects of different plant extracts and a synthetic insecticide on *P. demoleus* population before and after 3<sup>rd</sup> spray application in the *C. aurantium* nursery during spring 2021.**

Treatments	1 DBS	1 DAS	3 DAS	5 DAS	7 DAS	14 DAS	Mean
Black pepper fruit extract	12.66	11.66 b	8.33 bc	7.00 bc	3.00 cd	12.00 bc	9.11 cd
Garlic bulb extract	13.66	12.66 b	10.66 b	8.33 b	4.66 b	13.66 b	10.61 b
Ginger rhizome extract	12.00	11.00 bc	8.00 bc	6.33 bc	2.33 d	11.33 bc	8.50 de
Neem fruit extract	10.33	9.33 c	7.66 bc	5.66 c	1.66 d	10.66 c	7.55 e
Tobacco leaf extract	13.00	12.00 b	9.33 bc	7.66 bc	4.00 bc	13.00 bc	9.83 bc
Bifenthrin	10.00	9.00 c	7.00 c	3.00 d	0.00 e	7.00 d	6.00 f
Control	22.00	22.00 a	20.66 a	21.33 a	20.33 a	22.00 a	21.38 a
LSD (0.05)	2.37	2.19	3.16	2.47	1.63	2.37	1.26

Figures in columns followed by the same letters are non-significant from each other at P (0.05%), ns (non-significant)

### Damage assessment percentage of *C. aurantium* leaves

The damage assessment percentage of *C. aurantium* leaves was calculated in all the treated plots by the following procedure mentioned by Mustafa *et al.*, (2014). As mentioned in Table 4 the damage assessment percentage of *C. aurantium* leaves done by *P. demoleus* in black pepper fruit extract was (53%-36%), garlic extract was (57%-23%), ginger rhizome extract was (61%-53%), neem fruit extract was (54%-76%), tobacco leaf extract was (66%-59%), bifenthrin was (50%-28%) and control was (73%-56%). Table 4 there was a decrease in the rate of percentage damage from 1<sup>st</sup> July to 15<sup>th</sup> August, but it gets increased on 15<sup>th</sup> August. For all the treatments, highest damage was observed on 15<sup>th</sup> May and 1<sup>st</sup> July and the maximum value was registered for control (73%-56%) and minimum damage was observed on 1<sup>st</sup> July and 15<sup>th</sup> August the lowest value was registered for treatment bifenthrin (50%-28%). Damage done by *demoleus* in *C. aurantium* leaves ranged between (50.28%-73.56%). Numerous different scientists, for example, Lara *et al.*, (1998), detailed a 12%-85% leaf damage assessment. Zeb *et al.*, (2011), announced 2%-55% complete leaf damage assessment. *P. demoleus* caused the most harm, which was most evident between December and April. Legaspi *et al.* reported comparative findings (2001). From January to March, a decline in the percentage damage assessment rate was seen. It agrees with Pena *et al.*, (1996), who noted that January and the winter months had the lowest population density.



**Table 4: Damage assessment percentage of *C. aurnatium* leaves in the *C. aurantium* nursery during spring 2021.**

Treatments	Damage assessment (%)
Black pepper fruit extract	53.36 d
Garlic bulb extract	57.23 cd
Ginger rhizome extract	61.53 bc
Neem fruit extract	54.76 cd
Tobacco leaf extract	66.59 ab
Bifenthrin	50.28 d
Control	73.56 a
LSD (0.05)	7.43

Figures in columns followed by the same letters are non-significant from each other at P (0.05%), ns (non-significant)

### Biochemical estimation percentage of healthy and damaged leaves of *C. aurantium*

It was estimated that among all the tested leaves %  $\text{Ca}^{+2}$ , %  $\text{Mg}^{+2}$  and %  $\text{K}^{+}$  were found in un-treated and treated leaves, whereas in Table 5 the biochemical estimation percentage of un-treated and treated leaves of *C. aurantium* was calculated by the following procedure mentioned by Mustafa *et al.*, (2014). In healthy leaves, the range and mean values of the studied elements  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$  and  $\text{K}^{+}$  in the *C. aurantium* leaves samples, expressed on dry matter basis in a total of 1029 leaves sample size. Element  $\text{Ca}^{+2}$  ( $\text{g } 100 \text{ g}^{-1}$ ), range (0.89-6.77), mean (2.09) and standard deviation ( $\text{SD}^{\text{a}}$ ) (0.61), element  $\text{Mg}^{+2}$  ( $\text{g } 100 \text{ g}^{-1}$ ), range (0.14-0.80), mean (0.41) and standard deviation ( $\text{SD}^{\text{a}}$ ) (0.03), element  $\text{K}^{+}$  ( $\text{g } 100 \text{ g}^{-1}$ ), range (0.42-1.92), mean (1.38) and standard deviation ( $\text{SD}^{\text{a}}$ ) (0.38) was observed in healthy leaves. In infested leaves, the range and mean values of the studied elements  $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$  and  $\text{K}^{+}$  in the *C. aurantium* leaves samples, expressed on dry matter basis in a total of 1029 leaves sample size. Element  $\text{Ca}^{+2}$  ( $\text{g } 100 \text{ g}^{-1}$ ), range (0.89-6.77), mean (2.35) and

standard deviation ( $SD^a$ ) (0.53), element  $Mg^{+2}$  ( $g\ 100\ g^{-1}$ ), range (0.14-0.80), mean (0.39) and standard deviation ( $SD^a$ ) (0.03), element  $K^+$  ( $g\ 100\ g^{-1}$ ), range (0.42-1.92), mean (0.90) and standard deviation ( $SD^a$ ) (0.28) was observed in infested leaves. A negative relationship between  $Ca^{+2}$  was observed with  $Mg^{+2}$ . Comparative outcomes were accounted for by Fudge and Fehmerling, (1940). They announced the repressing impact of  $Ca^{+2}$  on  $Mg^{+2}$  when  $Ca^{+2}$  is available in maximum concentration it suppresses the absorption of  $Mg^{+2}$ . This discrepancy in correlation values may be caused by the makeup of the soil's macro- and micronutrients. In replications number one, number two, and number three, the plots were present in the same areas. The plot area may likewise impact the correlation.  $Ca^{+2}$  showed a significant strong positive correlation with  $K^+$  in un-infested leaves and a weak negative correlation in infested leaves. Smith and Reuther *et al.*, (1950), also reported negative correlation between  $Ca^{+2}$  and  $K^+$ . So, they also observed that maximum  $K^+$  depresses the concentration of  $Ca^{+2}$  in *C. aurantium* leaves.

**Table 5: Biochemical estimation percentage of ( $Ca^{+2}$ ,  $Mg^{+2}$  and  $K^+$ ) in un-infested and infested leaves in the *C. aurantium* nursery during spring 2021.**

Elements	Healthy leaves			Infested leaves	
	Range	Mean	$SD^a$	Mean	$SD^a$
$Ca^{+2}$ $g\ 100\ g^{-1}$	0.89-6.77	2.09	0.61	2.35	0.53
$Mg^{+2}$ $g\ 100\ g^{-1}$	0.14-0.80	0.41	0.03	0.39	0.03
$K^+$ $g\ 100\ g^{-1}$	0.42-1.92	1.38	0.38	0.90	0.28

## Conclusions and Recommendations

From the current study we conclude that for controlling the one main insect pests of the *C. aurantium* nursery, *P. demoleus*, all the tested treatments were shown to be superior to the control. Similarly, bifenthrin was the best management tool in managing both the pests while among plant extracts *P. demoleus* were effectively controlled by neem fruit extract. It was observed that *P. demoleus* has a weak correlation with leaf minerals ( $\text{Ca}^{+2}$ ,  $\text{Mg}^{+2}$  and  $\text{K}^{+}$ ) and it was influenced by soil composition, abundance of *P. demoleus*, environmental factors such as temperature and place of *C. aurantium*. Based on the above conclusion, it is recommended to use Bifenthrin for effective management of *P. demoleus* on *C. aurantium* in nurseries grown for commercial purposes. However, neem fruit extract can be a useful alternate against *P. demoleus* on *C. aurantium* for environmentally safe management of these pests in citrus nurseries or home-based nurseries and kitchen gardens.

## References

- Altaf, N., and Khan, A. R. 2008. Variation within kinnow (*Citrus reticulata*) and rough lemon (*Citrus jambheri*). Pak. J. Bot. 40(2): 589-598.
- Fiaz, M., Hameed, A., ul Hasan, M., and Wakil, W. 2012. Efficacy of plant extracts on some cotton (*Gossypium hirsutum*) pests: *Amrasca bigutulla bigutulla* Ishida and *Thrips tabaci* Lindeman. Pakistan Journal of Zoology. 44(1).
- Fudge, B. R., and Fehmerling, G. B. 1940. Edition. Some effects of soils and fertilizers on fruit composition. *Some effects of soils and fertilizers on fruit composition*.
- Hussein, A. E., Abd Elhaseeb, H., Mohamed, R. A., Abdel-Mogib, M., and Abou Elnaga, Z. 2017. Toxicity of three chemical extracts of black pepper fruits against two stored grain insect pests. International Journal of Pharmaceutical Science Invention. 6(10): 20-29.
- Khanzada, K. K., and Khanzada, B. 2018. Using plant extracts to control the sucking insect pests of Brinjal. International Journal of Zoological Studies. 3(4): 58-62.

- Lara-Guerra, J., Quiroz-Martinez, H., Sanchez, J. A., Badii, M. H., and Rodriguez-Castro, V. A. 1998. Citrus leaf miner (*Phyllocnistis citrella*) (Stainton), incidence, damage and natural enemies in Montemorelos, Nuevo Leon, Mexico. *The Southwestern entomologist* (USA). 23(1): 93-94.
- Legaspi, J. C., French, J. V., Zuniga, A. G., and Legaspi Jr, B. C. 2001. Population dynamics of the citrus leaf miner, (*Phyllocnistis citrella*) (Lepidoptera: Gracillariidae), and its natural enemies in Texas and Mexico. *Biological Control*. 21(1): 84-90.
- Mangrio, W. M., Sahito, H. A., Chandio, N. H., Kousar, T., Shah, Z. H., Khaskheli, N. A., and Jatoi, F. A. 2020. 35. Food and feed consumption of lemon butterfly, (*Papilio demoleus*) under laboratory conditions. *Pure and Applied Biology (PAB)*. 9(1): 340-351.
- Manigano, P., D. Severtson. 2008. Edition. 'Pest Fax Issue Number 01.' Department of Agriculture and Food, Western Australian Government.
- Mustafa, I., Aslam, M., Arshad, M., Muhammad, S., Ullah, I., Mustaqeem, M., and Haroon, A. 2014. Association of citrus leaf miner (*Phyllocnistis citrella*) (Lepidoptera: Gracillariidae: Phyllocnistinae) with leaf biochemical factors ( $\text{Ca}^{+2}$   $\text{K}^{+}$  and  $\text{Mg}^{+2}$ ) in Kinnow leaves of district Sargodha Punjab Pakistan. *Pakistan Journal of Zoology*. 46(4): 953-958.
- Narayanamma, V. L., and Savithri, P. 2002. Seasonal abundance of the citrus butterfly, (*P. demoleus*) (Linn.). On sathgudy sweet orange and Tenali acid lime. *J of App Zoo Rese*. 13(1): 54-56.
- Ollitrault, P., and Navarro, L. 2012. Edition. Citrus. In *Fruit breeding*. Springer, Boston, MA. 623-662.
- Pathak, M., and Rizvi, P. Q. 2002. Age specific life tables of *Papilio demoleus* on different hosts. *Annals of plant protection sciences*. 10(2): 375-376.
- Pena, J. E., Duncan, R., and Browning, H. 1996. Seasonal abundance of *Phyllocnistis citrella* (Lepidoptera: Gracillariidae) and its parasitoids South Florida citrus. *Environmental Entomology*. 25(3): 698-702.

- Peng, Y., Shao, X. L., Hose, G. C., Liu, F. X., and Chen, J. 2010. Dimethoate, fenvalerate and their mixture affects *Hylyphantes graminicola* (Araneae: Linyphiidae) adults and their unexposed offspring. *Agricultural and Forest Entomology*. 12(4): 343-351.
- Rao, N. V., Maheswari, T. U., and Manjula, K. 2005. Edition. Review on botanical pesticides as tools of pest management. *Green pesticides for insect pest management*. Ignacimuthu, S. and S. Jayaraj. Pp 1-16.
- Sarasan, V., Kite, G. C., Sileshi, G. W., and Stevenson, P. C. 2011. Applications of phytochemical and in vitro techniques for reducing over-harvesting of medicinal and pesticidal plants and generating income for the rural poor. *Plant cell reports*. 30(7): 1163-1172.
- Siddique, M. I., and Garnevska, E. 2018. Citrus value chain (s): A survey of Pakistan citrus industry. *Agric. Value Chain*. Pp 37.
- Smith, P. F., and Reuther, W. 1950. Seasonal changes in Valencia orange trees. I. Changes in leaf dry weight, ash, and macro-nutrient elements. In *Proceedings. American Society for Horticultural Science*. 55: 61-72.
- Syed, R. 2007. Searching new avenues to enhance citrus fruit export. *Daily Times Pakistan*.
- World Health Organization. 1991. Joint FAO/WHO expert committee on food additives. *Evaluation of certain food additives and contaminants. 37<sup>th</sup> report*. Geneva: World Health Organization.
- Wu, J., Zhao, Y., Qi, H., Zhao, X., Yang, T., Du, Y., and Wei, Z. 2017. Identifying the key factors that affect the formation of humic substance during different materials composting. *Bio resource technology*. 24(4): 1193-1196.
- Yunus, M., and Munir, M. 1972. Host plants and host preference of lemon butterfly, (*Papilio demoleus*) (Linn.). *Caterpillars. Pakistan Journal of Zoology*. 4(2): 231-232.
- Zeb, Q., Khan, I., Inayatullah, M., Hayat, Y., Khan, M. A., Saljoqi, A. R., and Khan, M. A. 2011. Population dynamics of citrus whiteflies, aphids, citrus psylla leaf miner and their biocontrol agents in Khyber Pakhtunkhwa. *Sarhad J. Agric*, 27(3): 451-457.

Zekri, M., and Obreza, T. A. 2003. Edition. *Plant nutrients for citrus trees*. University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences. Pp 1-5.