Research Article

Pesticide Residue Analysis of three Different Pesticides used against *Helicoverpa Armigera* (Hubner) in Tomato Crop

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Abstract | Pesticide residue analysis experiment of three different pesticides used against *Helicoverpa armigera* was conducted in tomato crop during 2019. The experiment was carried out in (RCBD) Randomized Complete Block Design. There were four treatments i.e., Cypermethrin, Imidacloprid, Lamda cyhalothirn and control. Each treatment was replicated three times. Lowest mean percent fruits infestation (2.84%), highest yield per treatment (9.56 kg) per plot, highest ascorbic acid content (11.06 mg/100g), fresh weight of treated tomato fruits (101.45 g) and lowest content of mean pesticides residue (0.054%) was recorded in plots treated with Imidacloprid. It was followed by Lamda cyhalothrin where 5.97%, 8.02 kg, 8.18 mg/100g, 89.18 g, and 0.071% of mean percent fruits infestation, yield per treatment, ascorbic acid content, fresh weight of treated tomato fruits and content of mean pesticides residue was recorded, respectively. Highest content of pesticide residue (0.088%) was obtained from tomatoes treated with Cypermethrin. Our results further indicated that highest mean percent fruit infestation (8.92%) per plant and fresh weight (92.57g) were recorded from fruits after 3 days of the post treatments, while the lowest mean percent fruit infestation (8.35%) and fresh weigh of tomato fruits (83.46g) were recorded from plants after 7 and 10 days, respectively. Significantly highest ascorbic acid concentration (9.68 mg/100g) was recorded on day 10 while lowest (9.19 mg/100g) was recorded on 3rd day post treatment. Similarly, highest percent concentration of pesticide residue (0.080%) was recorded on day 1 while lowest (0.0624%) was recorded on 10th day of post treatment. Results showed that Imidacloprid is significantly better against tomato fruit worm by reducing the fruit infestation, resulted in lowest percent concentration of pesticide residue on tested tomato fruits as compared to Lamda cyhalothrin and Cypermethrin. Based on the results of the current study Imidacloprid is recommended in the IPM as safe pesticides against tomato fruit worm in tomato crop.

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Introduction

Tomato (*Lycopersicon esculentum* Mill.) is self-pollinated, cosmopolitan crop mainly grown in tropical and sub-tropical regions of the world (Tahir *et al.*, 2012; Kumar *et al.*, 2017). Tomato is a rich source of minerals, vitamins, sugars, essential amino acids, vitamin A, B, C, iron and phosphorus, it also contains Lycopene which is an antioxidant used to decrease the risk neurodegenerative diseases (Mahla *et al.*, 2017).

In Pakistan the total area under tomato cultivation was 0.061 million hectares, while the total production of tomato was 0.580 million tonnes during 2019-20. In Khyber Pakhtunkhwa tomato crop was cultivated on an average area of 0.013 million hectares with a total production of 0.129 million tonnes (Ministry of National Food Security and Research, 2019-2020). Due to soft and tenderness texture of the tomato fruit, it is more prone to insect pests and disease attacked. The major insect pests include aphids, jassids, flea beetle, tobacco caterpillar, spider mite and fruit borer (Kumar *et al*., 2017). Among these insects, the tomato fruit borer, *Helicoverpa armigera* (Hubner) (Lepidoptera; Noctuidae) is considered as one of the most important pests of tomato worldwide (Dhandapani *et al*., 2003). This pest is cosmopolitan, polyphagous, cause severe damages to the fruits and makes these fruits unmarketable, unfit for human consumption (Talekar *et al*., 2006).

The female of tomato fruit borer lay on an average of 412 eggs. The eggs hatch in 3-4 days. After hatching the first and second instar larvae feed on young leaves, buds and flowers for 2-3 days and then bore into the fruits. The larvae pass through six larval instars and become fully mature in 23-25 days. Pupation take place in the soil and lasts for 14-18 days. After pupation, adults emerge. The whole life cycle of tomato fruit borer from egg hatching to emergence of adults is completed in 40-47 days (Herald and Tayde, 2018).

The larva of tomato fruit borer at young stage mostly feed on leaves, buds and flowers, however at mature stage, bore into fruits making them unmarketable and unfit for human consumption (Meena and Raju, 2014). Tomato fruit borer has many generations with wide host range and high fecundity that migrate from one place to another. and has developed resistance against several insecticides (Ahmed *et al*., 2000). Its losses range from 20-60% (Talekar *et al*., 2006) with global annual crop losses about 5 billion dollars (Sharma, 2001). In Pakistan, 53% losses to tomato fruits occur due to this pest (Inayatullah, 2007). The severity of this pest can be judged from the fact that 80% of the total insecticides are used to control this pest in Pakistan (Shaheen, 2008).

The farmers use different management techniques for managing the fruit borer infestation in tomato crop (Ravi *et al*., 2008) but growers prefer to use chemicals against this pest (Hussain and Bilal, 2007). Chemicals are the prime method of control fruit borer infestation in tomato crop in Pakistan because it is easily available with high return ratio and minimum effort in application (Rasheed *et al*., 2019). However, the indiscriminate use of pesticides has resulted in development of resistance, pest resurgence, harmful pesticide residue in fruits, severe environmental pollution and health problems (Ignacimuthu, 2007). As tomato is a routine-use and perishable commodity, the period between application of pesticide and marketing is hardly observed resulting in contaminated tomato production and supply (Abbas *et al*., 2015).

Pesticides alter food quality by interfering with their biochemical processes (Singh *et al*., 2015) and their biotic stress changes the quantity of phenolics, total ortho-dihydroxy phenols and ascorbic acid resulting in poor uptake of essential micronutrients from soil (Ghosh *et al*., 2010). Unaccepted amounts of pesticides residues in fresh fruits and vegetables pose risk to food safety for importers and a market risk factor for exporters as the shipment can be cancelled and rejected if the pesticide residue exceeds the allowable limits (Shalaby and Gad, 2016).

Due to lack of knowledge of potential risk of using these chemicals, vegetables and fruits growers as well as consumers health are at stake. Residues of these pesticides remain active in the soil and in the edible portions of various crops and fruits (William and George, 2005). Maximum residue limits (MRL) are specific for every food item and used as monitoring tool for these food products. It is used to prevent the illegal and indiscriminate use of pesticides (Dureja *et al*., 2015).

It is necessary to make awareness among common community about potential hazards of using contaminated food (Rahman *et al*., 2015). Therefore, this study was undertaken to analyze the residue status of three different pesticides in tomato fruits with the objectives to find out; mean % field infestation of tomato fruit borer, the effect of different pesticides on the yield of tomato and residues of the selected pesticides on tomato fruit.

Materials and Methods

Seed bed and field preparation

Commercially grown Tomato cultivar namely "Roma" nursery was sown on raised seedbed (15cm high) covered in plastic. The nursery was monitored regularly for germination and watering. When the nursery reaches to two to three leaf stage, the tomato plants were transplanted to the field following two-to-three-day hard days period. Before transplanting into the fields, field was prepared at Agricultural Research Institute Tarnab, under Randomized Complete Block Design (RCBD). Furrows were made (50 cm wide) in the field. The experimental area was divided into three blocks. Each block was then subdivided into four sub-plots (5×2 m^2) consisting of four rows for individual treatments application.

After field preparation, the tomato seedlings were transplanted into each plot with row-to-row distance of 1.5 meter, while plant to plant distance was kept 30 cm. A buffer zone of 1 meter was maintained to isolate sub-plots. All the cultural practices were done at regular intervals. The three insecticides namely cypermethrin @ 100 gm/Lit, imidacloprid @ 250 gm/kg, and lamda cyhalothirn @ 25 gm/Lit were applied separately through knapsack sprayer into each plot, while the control plots received only water spray. All the insecticides were applied at economic threshold level of tomato fruit borer (*Helicoverpa armigera)*.

Percent fruit infestation per plant

Randomly selected four plants from each treatment were visually observed for infested fruits. Data were collected and percent mean infestation was calculated using the following formula.

$$\%\ \text{Fruit infestation} = \frac{\text{Number of infested fruits}}{\text{Total number of fruits}} \times 100$$

Fresh weight and yield of tomato crop

Fresh and dry weight of the treated tomato fruits were calculated by the method of Gabal *et al*. (1984). For yield, mature and healthy fruits were taken from all plants after treatments' application and weighed through electric balance, in Kg/plot and later converted into kg/ha.

Ascorbic acid (mg/100g)

The effect of pesticides on ascorbic acid content of tomato was calculated by proposed method of Giovanelli *et al*. (2002). The tomato fruits collected from treated plots were bring into the lab and were chopped into small pieces and were then frozen at -18 °C. The frozen fresh sub-samples were placed in aluminium foil lined trays and freeze-dried. The frozen tomato samples were then grinded into powder form with the help of mortar and pestle. The powder form was then extracted in 80% acetone in dark for 4 hours at 4 °C following extraction protocol. Finally ascorbic acid was extracted from the dried frozen powder of tomato by Titration method as proposed by Giovanelli *et al*. (2002).

Pesticide residue analysis

Residue analysis was done for tomatoes collected from treated plots in the laboratory at ARI, Tarnab with the steps involved were;

Extraction of residue from tomato: One kg of sample of tomatoes was taken from each replication and each treatment. Ethyl acetate was used as a solvent for the extraction of pesticide residue from tomato fruits. Sample was chopped into small pieces with knife and then 200 grams of chopped tomatoes were transferred to a high-speed mixer for firmly grinding. For the extraction, 100 gm of sample was taken from mixer in 250 ml of Erlenmeyer flask. Ethyl acetate of 75ml along with 5-7gm of anhydrous sodium sulphate was added to Erlenmeyer flask. In a horizontal shaker, sample was shaken for two hours at 80°C. Through a Whatman's filter paper, ethyl acetate was filtered and cleaned.

Cleaning up pesticide residue on Florisil column: To get a clean sample Florisil column adsorption chromatography technique was used for the extracts of pesticide residue.

Analytical techniques used for pesticides residue analyses: In this experiment, High performance liquid chromatography (HPLC) was used for pesticides residue analysis in tomato. Descriptive statistics was used for data analysis.

Statistical analysis

The collected data were analyzed statistically through Statistix 8.1 software, while the means were separated by using Least Significance Difference Test at 5% level (Steel and Torrie 1980).

Results and Discussion

Mean percent fruit infestation per plant

The analysis of variance table data regarding the mean percent fruit infestation per plant of tomato fruit worm showed that the various treatments application significantly affected the (P<0.05) due to (Table 1). The data showed that lowest mean fruit infestation of *H. armigera* per plant (2.84%) was recorded from Imidacloprid treated plants, followed by Lamda cyhalothrin (5.97%) and Cypermethrin (7.37%) per plant, respectively while the highest mean percent fruit infestation (18.74%) per plant was recorde in the control plots.

Table 1: *Effect of three different insecticides on the mean percent fruit infestation per plant against tomato fruit worm in tomato crop during 2019.*

Treatments	Percent Fruit Infestation			Mean
	Day 3	Day 7	Day 10	
Cypermethrin (T_1)	8.92d	6.79e	6.41ef	7.37b
Imidacloprid (T_2)	3.47h	2.69h	2.36h	2.84d
Lamda cyhalothrin (T_3)	7.26e	5.56fg	5.09g	5.97c
Control (T_4)	16.03c	18.38b	21.80a	18.74a
Mean	8.92a	8.35b	8.91ab	

Those means which have different alphabets are statistically different from one another at 5% level of significance using LSD test (P < 0.05); LSD for treatments= 0.6570; LSD for days= 0.5689; LSD for Treatments X Days= 1.1379

The mean values for days in Table 1 further showed significant difference among time intervals. The results revealed that highest mean percent fruit infestation (8.92%) per plant was recorded from plants after 3 days of the post treatments followed by day 10 (8.91%), while the lowest percent fruit infestation (8.35%) was recorded from plants after 7 days post treatment's application.

The data regarding the combine effect of treatments and time interval (days) in Table 1 also showed significant results. The results showed that significantly lowest percent fruit infestation of *H. armigera* per plant was observed after 10 (2.36%), 7 (2.69%) and 3 (3.47%) days in Imidacloprid treated plants, followed by Lamda cyhalothrin (5.09%) 10 days post treatment application. The control plots resulted in significantly highest percent fruit infestations (21.80, 18.38 and 16.03%) on days 10, 7 and 3 respectively. Among the plots treated with chemicals, highest percent fruit infestation was observed from cypermethrin treated plots after 10 (6.41%) followed by 7 (6.79%) and 3 (8.92%) days post treatment application.

Fresh weight and yield of tomato crop

The analysis of variance for fresh weight of treated tomato fruits showed significant effect (P<0.05) (Table 2). Results revealed that significantly highest fresh weight of treated tomatoes (101.45gm) was recorded from Imidacloprid treated plants, followed by Lamda cyhalothrin (89.18gm) and Cypermethrin (86.76gm), respectively. However, significantly lowest fresh weight of treated tomato fruits (73.09gm) was recorded in control. The mean values for days showed significant difference among time intervals. The results showed that highest fresh weight of tomato fruits (92.57gm) was recorded from plants after 3 days of the post treatments followed by day 7 (86.83gm), while the lowest fresh weight of tomato fruits (83.46gm) was recorded after 10 days post application. Results regarding combine effect of treatments and Time intervals (days) in Table 2 was found non-significant.

Table 2: *Effect of pesticide residue on fresh weight of treated tomato fruits.*

Treatments	Fresh weight of a Tomato Fruit (grams)			Mean
	Day 3	Day 7	Day 10	
Cypermethrin (T_1)	91.24	86.61	82.43	86.76c
Imidacloprid (T_2)	107.27	101.21	95.89	101.45a
Lamda cyhalothrin (T_3)	94.84	88.00	84.71	89.18c
Control (T_4)	76.94	71.51	70.84	73.09d
Mean	92.57a	86.83b	83.46c	

Those means which have different alphabets are statistically different from one another at 5% level of significance using LSD test (P < 0.05); LSD for treatments= 2.1587; LSD for days= 2.0743

Table 3: *Effect of three different insecticides on the yield of tomato crop during 2019.*

Treatments	Yield (Kg) per treatment	Yield(kg/ha)
Cypermehtrin (T_1)	7.39b	7398b
Imidacloprid (T_2)	9.56a	9568a
Lamda cyhalothrin (T_3)	8.02b	8024b
Control (T_4)	5.32c	5326c
LSD	1.0058	1005.8

Those means which have different alphabets are statistically different from one another at 5% level of significance using LSD test (P < 0.05).

The data regarding yield of the tomato fruits in Table 3 showed significant difference (P<0.05) among treatments application and revealed that significantly lowest yield of tomato fruits (5326 kg/ha) was recorded from control while significantly highest yield of tomato fruits (9568 kg/ha) was obtained from Imidacloprid treated plots followed by Lamda cyhalothrin with 8024 kg/ha of tomato fruits.

Ascorbic acid concentration in treated tomato fruits

The analysis of variance table data for the ascorbic acid concentration in treated tomato fruits showed significant effect (P<0.05) and revealed that lowest ascorbic acid concentration (4.39) was recorded from cypermethrin treated plants, followed by Lamda cyhalothrin (8.18) and Imidacloprid (11.06), respectively. However, significantly highest ascorbic acid concentration in tomato fruits (14.41) was recorded in control (Table 4).

Table 4: *Effect of pesticide residue on ascorbic acid concentration in treated tomato fruits.*

Treatments	Ascorbic Acid Concentration			Mean
	Day 3	Day 7	Day 10	
Cypermethrin (T_1)	4.08	4.49	4.60	4.39d
Imidacloprid (T_2)	10.87	11.07	11.26	11.06b
Lamda cyhalothrin (T_3)	8.05	8.25	8.26	8.18c
Control (T_4)	13.76	14.87	14.60	14.41a
Mean	9.19a	9.67b	9.68b	

Those means which have different alphabets are statistically different from one another at 5% level of significance using LSD test (P < 0.05); LSD for treatments= 0.3313; LSD for days= 0.2869

The mean values for days showed significant difference among time intervals and indicated that highest ascorbic acid concentration (9.68) was recorded from plants after 10 days of the post treatments followed by day 7 (9.67), while the lowest ascorbic acid concentration (9.19) was recorded from plants after 3 days of post treatment. Results regarding combine effect of treatments and time intervals (days) was found non-significant.

Pesticide residue analysis

The analysis of variance table data for the Percent concentration of pesticide residue on treated tomato fruits in Table 5 showed significant effect (P<0.05). The residue analysis data showed that significantly highest percent concentration of pesticide residue (0.088%) was recorded from cypermethrin treated fruits, followed by lamda cyhalothrin (0.071%) and imidacloprid (0.054).

The mean values for days showed significant difference among time intervals and found that highest percent concentration of pesticide residue on tomato fruits was recorded from plants after 1 day (0.0798%) post treatments, followed by day 3 (0.0930%), while the lowest percent concentration of pesticide residue was recorded from plants after 10 days (0.0624%) post treatments application. Results regarding combine effect of treatments and time intervals (days) was found non-significant.

The mean values for mean Percent Fruits Infestation, yield per treatment, Ascorbic acid (mg/100g) content in treated tomato fruit, fresh weight of treated tomato fruits and pesticide residue content of tomato samples treated with three different pesticides showed significant difference. Lowest percent fruits infestation, highest yield per treatment, highest fresh weight of treated tomato fruits and lowest content of mean pesticides residue was recorded in plots treated with Imidacloprid. These results reveal that Imidacloprid is significantly better than other pesticides used in this experiment. Our results showed that all pesticides used in this experiment reduced the infestation of tomato fruit worm which are in line with Abbas *et al*. (2015) who tested chlorantraniliprole, indoxacarb, flubendamide, spintoram, thiamethaxi, fipronil, emamectin benzoate, chlofenapyr and lufenuron against *H. armigera* and reported that all pesticides increase the percent mortality (55.00 ± 1.35) of tomato fruit worm as compared to control (4.33 ± 0.27). Similar results have also been reported by

Table 5: *Mean pesticide residue analysis for cypermethrin, imidacloprid and lamda cyhalothrin on tomato crop during 2019.*

Treatments	Percent concentration of Pesticide Residue				Mean
	Day1	Day 3	Day 7	Day 10	
Cypermethrin (T_1)	0.096	0.089	0.088	0.079	0.088a
Imidacloprid (T_2)	0.061	0.056	0.053	0.045	0.054c
Lamda cyhalothrin (T_3)	0.081	0.073	0.071	0062	0.071b
Mean	0.0798a	0.0730b	0.0708b	0.0624c	

Those means which have different alphabets are statistically different from one another at 5% level of significance using LSD test (P < 0.05); LSD for treatments= 0.0615; LSD for days= 0.0193.

Ravi *et al*. (2008) who stated that chemical pesticide (indaxacarb) decreased the population of tomato fruit worm from 70.75 to 0.75 after the first application.

Similar results regarding efficacy of pesticides and yield have also been reported by Kumar *et al*. (2017) who stated that pesticides reduced the larval population and decreased the percent fruit damage as compared to control as well as highest yield (13.21 t/ha) was also reported from plot treated with profenophos as compared to control (8.14 t/ha). Similar results regarding yield have also been reported by Rasheed *et al*. (2019) who stated that plots treated with chemical pesticides gives highest yield (9450 kg/ha) as compared to control (5607 kg/ha). Our results are further in line with the work of Hussain and Bilal (2007) who reported a yield of 28000 kg/ha from plots treated with imidacloprid. Results regarding the yield on cypermethrin treated plot are also in line with Singh *et al*. (2015) who reported that yield of 25.72 t/ha was recorded from cypermethrin treated plots.

Our findings regarding the residue analysis of three pesticides showed that highest percentage concentration of residue was recorded from cypermethrin which are in conformity with the findings of Rahman *et al*. (2015) who reported that cypermethrin residue was recorded from eggplant fruits after 5 days of the application and the quantity of residue was above maximum residue limit (MRL) up to 3 days (0.762 ppm) post application as food and agriculture organization (FAO) has setup 0.5 ppm as the maximum residue limit (MRL) for insecticides used in vegetables (FAO, 1993). Home processing of some vegetables also reduces the amount of pesticides from vegetables (Nath and Srivastava, 1990).

As we have reported in our research that the lowest percentage of pesticide residue on fruit was recorded from Imidacloprid as compared to other pesticides, these results are in line with Kapoor *et al*. (2013) who tested 10 samples of tomato fruit and all samples have shown residue of imidacloprid lower than maximum residue limit (MRL) while residue was detected in only two samples. So, it can be concluded from these findings that lifetime consumption of vegetables sprayed with imidacloprid pose no hazard to human health as the residue was below the maximum residue limit (Darko and Akoto, 2008). Our results regarding residue concentration of imidacloprid are in conformity with Sharma *et al*. (2018) who stated higher amount of residue (0.018%) in tomato crop which is below the maximum residue limit (MRL). However, Suganthy *et al*. (2010) reported that safe waiting period of 1.2-4.5 days are required for imidacloprid in chilli fruit, while in tomato fruit, 3 days safe period is required (Dharmurajan and Dikshit, 2010).

Conclusions and Recommendations

It was concluded that imidacloprid is significantly better as compared to lamda cyhalothrin and cypermethrin against tomato fruit worm by reducing the fruit infestation. Further, this insecticide results significantly highest yield per treatment, ascorbic acid content and fresh weight of tomato fruit. Further, imidacloprid also results in lowest percent concentration of pesticide residue on tested tomato fruits as compared to lamda cyhalothrin and cypermethrin. Moreover, all pesticides used in this experiment decreased the pest population as compared to control. Imidacloprid is recommended as comparatively safe pesticides against tomato fruit worm in tomato crop and can also be used in the IPM as it results in highest fresh weight of fruits with lowest residue content and can be used for the control of tomato fruit worm in tomato crop.

Novelty Statement

This study highlights the efficacy and persistency of new chemistry insecticides viz Imidacloprid, Lamda cyhalothrin and Cypermethrin against *Helicoverpa armigera* in tomato crop. The study suggests that Imidacloprid was found significantly effective with less fruit infestation and lowest percent concentration of pesticide residue on tested tomato fruits as compared to the other treatments and hence can be used in the IPM of *H. armigera* in tomato crop.

Author's Contribution

Sumayya Amin: Conducted the research trial, compiled the data.
Taufiq Nawaz: Helped in data compilation.
Muhammad Salim: Wrote the article.
Ahmad Ur Rahman Saljoqi and Farida Anjum: supervised the whole research.

Conflict of interest

The authors have declared no conflict of interest.

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