Research Article

Efficacy of Different Pesticides against Mustard Aphid, Lipaphis Erysimi in Selected Mustard Cultivars

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Abstract | Mustard Aphid (*Lipaphis erysimi*) (Homoptera: Aphididae) is a destructive insect pest that causes significant losses in the yield of canola crops. It is essential to find out the management strategies of this pest for higher canola production. Synthetic insecticides and bio-pesticides are used to control *L. erysimi*. The present study evaluated the efficacy of different pesticides against mustard aphids in selected mustard cultivars under field conditions. The experiment was carried out in Split Plot Design with four treatments and three replications. The results showed that Thiamethoxam, Fipronil and Neem oil were effective against mustard aphids. After the first spray, the minimum mean numbers of aphids were recorded (8.49) on a variety of China in a plot treated with Thiamethoxam. In contrast, the maximum mean number of aphids was observed (28.80) in the control plot. In the case of cultivar, Swabi the highest mean numbers of aphids were noted (53.86) in an untreated plot, whereas the lowest mean numbers of aphids were found (19.72) in Thiamethoxam treated plot, respectively. After 2nd spray in cultivar China the lowest aphid was (7.45) in the thiamethoxam treated plot, while the maximum mean numbers of aphids were (29.97) in the check plot, respectively. Compared with cultivar Swabi more numbers of aphids were recorded (47.94) on the control plot, while the least numbers of aphids were recorded (23.80) in a plot treated with thiamethoxam, respectively. In cultivar, China the maximum yield (1064.6) kg/ha was obtained from a thiamethoxam treated plot, while the minimum yield was recorded (562.5) kg ha^{-1} in control plots. Compared with cultivar, Swabi maximum yield (8.16.7) kg/ha was obtained from a thiamethoxam treated plot, and minimum yield (327.1) kg/ha was also recorded in the control plot. Thus overall results indicated that chemical, thiamethoxam along with resistant variety (China) were superior over rest of the all treatments that reduced the aphid and increased the yield productions, whereas regression of the slop of the tested insecticides based IPM strategy is to be applied for aphids control measure in canola crop.

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Introduction

The canola crop (*Brassica napus* L.) is one of the most important oil crops belonging to the family Brassicaceae (Miri, 2007). This crop is sown for various purposes, such as fodder, feed, vegetables, and edible oil. The byproduct of this crop is oilseed cake used as feed for animals. However, the canola crop is more important due to its high oil quality (Chand *et al*., 2017). Pakistan currently produces 70% canola oil, while the remaining oil is imported (Pakistan Bureau of Statistics, 2017-2018). The mandate for edible oil has been increasing gradually in Pakistan. Therefore, to see the requirements of the ever-increasing population, there is a need to improve mustard production by reducing the losses due to biotic and abiotic stresses. Among several conditions, insect pest infestation is the main preventive factor in gaining higher production (Singh *et al*., 2009).

There are many insect pests attacked on canola crops such as mustard aphid (*L. erysimi*), cabbage caterpillar (*Piers brassicae*) and leaf miner (Saljoqi *et al*., 2006). Among these insect pests, *L. erysimi* is a destructive insect pest that causes severe damage to the crop (Mahmoud and Shebl, 2014). Both nymphs and adults, which are louse-like and pale greenish insects, cause damage to flower buds, shoots and pods are seen feeding in huge numbers, often covering the whole surface of flower buds, shoots and pods, (Ahmad *et al*., 2009). According to Talpur and Khuhro (2004) *L. erysimi* occurred on leaves during the 3rd week of January and shifted to the inflorescences during the 2nd week of February and remained active up to harvesting. Peak activities were noted from February 15th to March 5th. Mustard aphid uses two different ways when damaging the plant, first, it sucks the cell sap from leaves, inflorescence, pods and twigs of the plant. Secondly, it releases a juicy honeydew and provides a standard or medium for the growth of the sooty mold fungus, which affects the plant's photosynthetic process. As a result, the plant becomes stunted and fails to maintain its strength and growth (Khan *et al*., 2015). It is as well as a vector for various plant viruses and its honeydew benefits the fungal growth on leaves and thereby disturbing plant photosynthesis (Sato *et al*., 2013, 2014). It can badly affect the canola crop and causes diseases i.e. viruses (TuMV), pathogens, and up to 45 to 60% losses in canola yield (Capinera, 2001). There are different techniques used for the management of mustard aphids (*Lipaphis erysimi*), such as biological control (Jagannath *et al*., 2002), and selection of aphid-resistance soybean strains (Lee *et al*., 2015) still, insecticide applications are at the forefront of management methods owing to rapid reduction of aphid populations (Ullah *et al*., 2019)

Bio-pesticides also play an important role in the IPM program as they are naturally available plant extracts materials, which are comparatively not much expensive, less poisonous, less dangerous, environmental, and safe to beneficial organisms (Marghub *et al*., 2010). Therefore, indigenously accessible plants and leaf extracts or oils with insecticidal properties can be used to control mustard aphids. They help maintain the biological diversity of beneficial organisms and reduce environmental contamination human health hazards (Buss and Park, 2002). The Pyrethroids chemicals were also most effective against mustard aphids in canola crops. It effectively controls many insect pests such as aphids, thrips, jassid, mites, wireworms, and a true bug when used as a seed treatment and soil and foliar applications (Sarwar *et al*., 2003). The present study evaluated the efficacy of different pesticides against mustard aphids in selected mustard cultivars under field conditions.

Materials and Methods

The present experiment was carried out at a new developmental research farm, The University of Agriculture Peshawar, to evaluate the efficacy of different pesticides against mustard aphid (*L. erysimi)* in selected mustard cultivars (China and Swabi) during crop growing season 2020-21. The research was conducted in Split Plot Design with three replications and four treatments, i.e. Thiamethoxam 25 WG @100gm/ha, Fipronil @5 SC 1000ml/ha, Neem oil @3% and control in each replication. Two varieties (Cultivar China and Swabi) were selected based on their preliminary test.

Field preparation

The total research area (field) was kept at 30x20 m^2. Each plot size was kept (4 x 4 m^2) considered of seven rows where (plant to plant) and (row to row) distance was kept 50x30 cm, respectively. The buffer zone was left (0.5 m and 0.3 m) between each subplot, while (1 m) buffer zone was left between two blocks on each side. The seeds were sown in rows by hand drill of the mid-October-2020. All agronomic practices were maintained uniform in all plots to raise good crops.

The recommended insecticides Chemicals spray were used before the pest population reached the economic threshold level (ETL) with the help of a knapsack sprayer (Aslam *et al*., 2007).

Data collection

The data were recorded before (incidence of attack) or after 1st, 3rd, 7th and 10 days chemical application of insecticides by counting the numbers of aphid per plant from 10 cm top portion of the terminal shoot on ten plants randomly selected in each treated and un-treated plots, respectively. After threshing, the seed was first weighed and cleaned separately based on yield obtained in each treated and untreated subplot. After that, check the effectiveness of the tested insecticides were assessed based on the percent reduction population of aphids. Percent reduction population was calculated using the following formula.

$$\text{Percent Reduction} = \frac{\text{population before spray} - \text{population after spray}}{\text{population before spray}} \times 100$$

Aphid population per plant

The aphid population/plant numbers were recorded in the field from 10 cm top portion of the terminal shoot with pencil size stick gently beaten with a white paper sheet, randomly selected ten plants from each treated and un-treated plots at days 1, 3, 7 and 10 days after application. Cut these top 10cm terminal portions from randomly selected plants were brought to the Laboratory into polythene bags to count the numbers of aphid population per plant. The aphids were removed from the plant by soft hair camel brush, placed on a white paper sheet and easily counted by the magnifying glass. The numbers of aphid plant^{-1} were converted into percent (%) reduction aphid population over control by given formula (Arif *et al*., 2012).

$$Percent\ Reduction = \frac{population\ before\ spray - population\ after\ spray}{population\ before\ spray} \times 100$$

Biological efficacy of different pesticides against Lipahis erysimi

The biological efficacy of different pesticides tested against the mustard aphid population was calculated by the given formula.

$$B.E\ (\%) = \frac{control\ plot - treated\ plot}{control\ plot} \times 100$$

Numbers of sub-branches per plant

The sub-branches plant^{-1} were counted when the plant was fully matured from top to bottom on 10 randomly selected plants from each subplot after insecticides and botanical extract (Razaq *et al*., 2014).

Numbers of pods per plant

The numbers of pods/plants were observed when the plant is fully matured by counting the number of pods per plant randomly selected 10 plants from each treated and un-treated subplot after application of insecticides and botanical extract (Malik *et al*., 2012).

Plant height

Plant height was measured in the field from top to bottom with the help of a meter rod randomly selected 10 plants from each subplot after application of insecticides and botanical extract (Ahmed *et al*., 2013).

Yield

The yield was recorded after threshing first the seeds were weighed and cleaned separately based on yield obtained in each treated and un-treated subplot and then converted into kg/ha. Where one hectare is equal to 10000 m^2. The total yield was determined by the given formula (Sarwar, 2013).

$$Total\ yield = \frac{weight\ in\ kg}{per\ plot\ size} \times 10000\ ha$$

Statistical analysis

The data were analyzed through ANOVA by using Statistix software (version 8.1). The significant variances among the treatments, mean for each treatment was separated at least significant difference LSD at (0.05) % level (Steel and Torrie, 1984).

Results and Discussion

Comparative efficacy of different pesticides against mustard aphid population under field condition after the first spray during 2020-21

The results showed a significant difference in the efficacy of various insecticides and Neem oil against the aphid population after the first spray (Table 1). The data indicated that minimum mean numbers of aphid populations in tested cultivar China were recorded (8.49) aphid/plant (10 cm apical shoot) in a plot treated with chemical Thiamethoxam followed by Fipronil and Neem oil (11.41 and 14.45) aphid/ plant, respectively. While the maximum mean numbers of aphid populations were recorded (28.80) aphid/ plant in control plots. In the case of cultivar, Swabi the lowest mean numbers of aphid populations

were (19.72) aphid/plant in a plot treated with Thiamethoxam followed by treatment Fipronil and Neem oil (24.43 and 27.40) aphids/plant, respectively. While the maximum mean numbers of aphid populations were observed (53.68) in the control plot. The mean data regarding time intervals efficacy of the tested pesticides against the aphid population showed a highly significant difference compared to each other (Table 1). The minimum mean numbers of mustard aphid *(L. erysimi)* population were observed (17.41) aphids on Day 10. Whereas the maximum mean numbers of aphid population were recorded (30.11) on Day 1st followed by Day 3rd and Day 7th (25.45 and 21.21) aphids/plant respectively. The Cultivar China maintained minimum numbers of aphid populations compared to the susceptible cultivar Swabi; therefore, insecticides control trend was recorded maximum on Control plot (53.68) aphids/plant followed by Neem oil (27.40) aphids, Fipronil (24.43) aphids and Thiamethoxam (19.72) aphids/ plant, respectively.

Comparative efficacy of different pesticides against mustard aphid population under field condition after the second spray during 2020-21

Table 2 showed a significant difference in the comparative efficacy of different pesticides against the population of mustard aphids after the second spray. The results showed that all the applied treatments were comparatively most effective in reducing the aphid population except control. The effect of different tested insecticides in the case of cultivar China, minimum mean numbers of aphid population were recorded (7.45) aphids/plant in a plot treated with Thiamethoxam followed by Fipronil and neem oil (9.34 and 12.13) aphids/plant respectively. In contrast, the maximum mean number of aphid population was observed (29.97) aphids in an untreated plot. In the case of Cultivar Swabi the lowest mean numbers of aphid population were (23.80) aphids/plant 10 cm apical shoot in a plot treated with Thiamethoxam. While the highest mean number of aphid population was recorded (47.94) aphids/plant in the control plot. The interaction between time intervals on the efficacy of different pesticides against (*L. erysimi)* shown in Table 2. The results showed a highly significant difference among the treatments compared with the first application. The highest aphid population was (27.52) aphids/plant on day 1st followed by day 3rd and day 7th (24.93 and 21.53) aphids/plant. While the lowest mean aphid population was recorded (19.31) aphids/plant 10cm apical shoot on day 10. The resistant cultivar China maintained the minimum numbers of aphid population as compared with susceptible cultivar Swabi, therefore chemical control tendency was calculated highest numbers of aphid population (47.94) aphid/plant followed by neem oil, (29.45) aphids, Fipronil (26.51) aphids and Thiamethoxam (23.80) aphids/plant, respectively.

Biological efficacy of various pesticides against the population density of mustard aphid (L. Erysimi) in canola crop during 2020-21

Table 3 showed the percent biological efficacy of different pesticides against mustard aphids (*Lipaphis erysimi*). The results revealed that the minimum mean biological efficacy in cultivar China was recorded (50.81%) in a plot treated with neem oil followed by Fipronil (64.73%). While the maximum

Table 1: *Comparative efficacy of different pesticides against mustard aphid population under field condition after first spray during 2020-21.*

Varieties	Before spray	Treatments	Aphid population/ plant				
			Time intervals				
			Day 1st	Day 3rd	Day 7th	Day 10th	Mean
China	27.23	Thiamethoxam	15.16p	10.33r	6.17t	2.31v	8.49h
	26.23	Fipronil	18.41m	13.56p	9.51rs	4.17u	11.41g
	25.35	Neem oil	21.21L	17.33mn	12.17q	7.12t	14.45f
	29.16	Control	27.33j	29.31hi	28.44i	30.15gh	28.80b
Swabi	45.33	Thiamethoxam	33.17f	21.45L	15.76m	8.53s	19.72e
	43.21	Fipronil	36.31e	27.17j	20.91 L	13.33p	24.43d
	41.32	Neem oil	38.11d	31.23g	23.15k	17.13n	27.40c
	46.76	Control	51.23c	53.27b	53.63b	56.59a	53.68a
Means			30.11a	25.45b	21.21c	17.41d	

Mean followed by different letters significantly different from each other (p<0.05). Lsd Values for Treatments and Varieties= 0.7856. Lsd Values for Time Intervals (T.I)= 0.3086; Lsd Values for V x T x T.I = 0.8729.

Table 2: *Comparative efficacy of different pesticides against mustard aphid Population under field condition after second spray during 2020-21.*

Varieties	Treatments	Aphid population/plant				
		Time intervals				
		Day 1st	Day 3rd	Day 7th	Day 10th	Mean
China	Thiamethoxam	13.33w	9.17y	4.97b	2.35d	7.45h
	Fipronil	15.67 t	11.41x	6.81z	3.47c	9.34g
	Neem oil	18.31r	14.46v	9.13y	6.62z	12.13f
	Control	27.12n	29.33j	30.45i	33.00g	29.97b
Swabi	Thiamethoxam	31.12h	27.64 l	21.33q	15.12u	23.80e
	Fipronil	33.32f	29.13k	24.17 o	19.43q	26.51d
	Neem oil	36.17e	31.17h	27.31m	23.15p	29.45c
	Control	45.12d	47.19c	48.13b	51.33a	47.94a
Means		27.52a	24.93b	21.53c	19.31d	

Mean followed by different letter (s) significantly different from each other (p<0.05). Lsd Values for Treatments x Varieties= 0.1020; Lsd Values for Time Interval (T.I)= 0.0599; Lsd Values for V x T x T.I = 0.1786

Table 3: *Biological efficacy of various pesticides against the population density of mustard aphid in canola crop during 2020-21.*

Varieties	Treatments	Treatments means	Biological efficacy
China	Thiamethoxam	7.97	72.87%
	Fipronil	10.37	64.73%
	Neem oil	13.29	50.81%
	Control	29.38	
Swabi	Thiamethoxam	21.76	57.17%
	Fipronil	25.47	49.87%
	Neem oil	28.42	44.06 %
	Control	50.81	

mean biological efficacy was recorded (72.89%) in a Thiamethoxam treated plot, and it was found comparatively most effective among all the treatments. Compared with cultivar Swabi the mean maximum biological efficacy was observed (57.17%) against mustard aphid in a plot treated Thiamethoxam followed by Fipronil (49.87%). The minimum mean biological effectiveness was recorded (44.06%) against mustard aphid in the Neem oil plot. Thus the cultivar China proved with different treatments i.e., Thiamethoxam, Fipronil and Neem oil against mustard aphid *(L. erysimi)* based on the high percentage of biological efficacy, which is a strong indicator of resistance as compared with cultivar Swabi.

Effect of different pesticides on Physiological characteristics of canola crop during 2020-21

In Table 4 the results showed that physiological characteristics of plants were recorded after insecticides application on canola varieties. The data indicated that significantly affects on mean numbers of sub-branches plant^{-1}. In cultivar, China the maximum mean numbers of sub-branches plant^{-1} were recorded (28.81) in a plot treated with Thiamethoxam followed by Fipronil and neem oil (28.95 and 27.86) branches per plant, respectively. While the mean minimum branches/plant were recorded (26.13) in the control plot. The numbers of sub-branches per plant in treated and untreated plots were found significantly different from each other. The highest mean numbers of pod plant^{-1} were observed (120.29) from the Thiamethoxam treated plot followed by Fipronil and neem oil (115.40 and 109.60), respectively. While the lowest mean number of pods were recorded (103.48) in an untreated plot. The maximum plant height (87.40 cm) was observed in Thiamethoxam treated plots followed by Fipronil and neem oil (82.40 cm and 77.42 cm) respectively. In contrast, the control plot recorded the minimum mean plant height (71.19 cm).

In the tested cultivar Swabi, the highest mean number of sub-branches/plants were recorded (15.40) in the Thiamethoxam treated plot followed by Fipronil (9.49). While the lowest mean number of sub-branches/plant were recorded (3.22) in an untreated plot followed by Neem oil (6.35). Canola plants in a plot treated with Thiamethoxam (97.45 /plant) produced maximum mean the number of pods followed by Fipronil and Neem oil (92.33 and 88.70), respectively. The minimum mean number of pods/plant (83.37) is produced in control plots. The highest plant height (80.11 cm) was observed in the

Thiamethoxam treated plot followed by Fipronil and neem oil (75.23 cm and 69.12 cm), respectively. While minimum plant height was recorded in control.

Table 4: *Effect of various chemical on Physiological characteristics of canola crop during 2020-21.*

Varieties	Treatments	Sub branches (no.)	Pods/ Plant (no.)	Plant height (cm)
China	Thiamethoxam	28.81ab	120.29a	88.63a
	Fipronil	28.95a	115.40b	79.19c
	Neem oil	27.86b	109.60c	74.74d
	Control	26.13c	103.48d	68.00f
Swabi	Thiamethoxam	26.23c	97.45e	83.51b
	Fipronil	25.50cd	92.33f	71.30e
	Neem oil	25.11d	88.70g	63.03g
	Control	24.12e	83.37h	58.29h
LSD (0.05)		0.9754	0.1795	3.0558

Mean followed by different letter significantly different from each other's ($p<0.05$).

Table 5: *Efficacy of different insecticides and neem oil on yield per treatment in canola crop during 2020-21.*

Varieties	Treatments	Mean yield (kg/ Plot)	Mean yield (kg/ ha)
China	Thiamethoxam	1.70a	1064.6a
	Fipronil	1.54b	964.6b
	Neem oil	1.40c	877.1c
	Control	0.90f	562.5g
Swabi	Thiamethoxam	1.30c	816.7d
	Fipronil	1.19d	747.9e
	Neem oil	1.09e	681.3f
	Control	0.52g	327.1h
LSD (0.05)		0.0877	54.83

Mean followed by different letters statistically different from each other ($p<0.05$).

Yield of canola crop

In Table 5 the results showed that maximum mean yield was obtained (1064.6) kg/ha from the treated plots in the case of tested cultivar China with Thiamethoxam followed by Fipronil and Neem oil (964.6 and 877.1) kg/ha, respectively. While the minimum mean yield was recorded (562.5) kg ha^{-1} in the control plot. In the case of cultivar Swabi significantly maximum yield was obtained (816.7) kg/ha from Thiamethoxam treated plots followed by Fipronil and neem oil (747.9 and 681.3) kg ha^{-1} respectively. Whereas the lowest yield was recorded (327.1) kg/ ha in the control plot. The results revealed that all the applied treatments were statistically different, as shown in Table 5. Whereas in tested cultivar China significantly more yield was obtained (1.70 kg) per plot in Thiamethoxam treated plot followed by Fipronil and Neem oil (1.54 and 1.40) kg/ plot compared to untreated plot in which significantly less yield was obtained (0.90) kg/plot. In the cultivar, Swabi the maximum mean yield was recorded (1.30) kg per plot in a Thiamethoxam treated plot followed by Fipronil and Neem oil (1.19 and 1.09) kg $^{-1}$ plot. While the mean minimum yield was obtained (0.52) kg/ plot in an untreated plot.

The chemical insecticides were comparatively most effective than botanical extract. The result showed that minimum mean numbers of aphid population were recorded in plot treated with chemical Thiamethoxam 25 WG followed by Fipronil 5 SC and neem oil @5%, while maximum mean numbers of aphid populations were observed in control plots. The present investigation conformed to Rohilla *et al.* (2004), who evaluated the efficacy of different pesticides against mustard aphids. Among these Thiamethoxam 25 WG, Fipronil 5 SC and botanical extract (neem oil) @5% proved the most effective insecticides. These findings were also similar with Singh and Lal (2009) reported that neem oil @ 5% is comparatively effective in reducing the population density of mustard aphid. The results indicated that a plot treated with Thiamethoxam significantly decreased the mean aphid population (4.13) aphids/ plant followed by Fipronil (5.42) aphids/10 cm apical shoot. Our findings were in line with Maurya *et al.* (2018) concluded that Thiamethoxam was recorded most effective chemical in suppressing the population density of mustard aphid. Similar results were supported by Patel *et al.* (2017) who studied the comparative efficacy of different synthetic insecticides under field conditions against *(L. erysimi)* population of which Thiamethoxam showed comparatively better efficacy among all the treatments except control.

In tested cultivars, the results showed that minimum mean numbers of aphid population were observed in cultivar China considered as resistant, while maximum mean numbers of aphid population were observed in cultivar Swabi which was considered as susceptible. The present investigation was similar to Ali *et al.* (2018), who reported that the least numbers of aphid population were recorded on resistant cultivar and maximum aphid populations were recorded on susceptible cultivar Swabi. Therefore,

the tested cultivar Swabi is recommended to control substantial aphid abundance from starting the seasons. Similar results have also been reported by Singh *et al*. (2012) who reported the highest density of aphids were counted in cultivar Swabi and the least numbers of aphids were observed in cultivar China. The variations in aphid abundances might be causing the combined effects of antibiotics, canola plants' tolerant capacities, and consuming similarity of aphid behavior. The highest aphid abundances beneath the current investigation were observed during the crops' inflorescences stage. The mean number of mustard aphids (*L. erysimi*) showed significant variation among the applied treatments.

All the tested treatments gave significantly maximum yield (kg/ha) over control. The effect of different treatments on crop yield was recorded maximum in Thiamethoxam as compared with other tested treatments. The results showed that all the chemicals had better yield comparisons in botanical. The data indicated that a plot treated with Thiamethoxam gives maximum yield (kg/ha) followed by Fipronil and neem the oil, while mean minimum yield was recorded in the control plot. These findings were similar to Sinha and Sharma (2008) and Rohilla *et al*. (2004), who reported that Thiamethoxam and Fipronil were found lethal to canola aphid under higher field conditions yields of canola crop. Our result is also in a line with Ullah *et al*. (2020) who reported that the low lethal (LC15) and sublethal (LC5) concentrations of thiamethoxam significantly reduced the longevity and fecundity of the directly exposed aphids. Our crop yield results were in line with Maurya *et al*. (2018) who evaluated the efficacy of synthetic insecticides against mustard aphids. Maximum yield was obtained from a plot treated with Thiamethoxam, and the aphid population's mortality was less mortality in a Fipronil treated plot. The maximum yields were recorded in the thiamethoxam treated plot compared with the control plot. The present investigation was also following the findings of Sharma *et al*. (2012), who reported and evaluated that the highest yield kg/ha was recorded in thiamethoxam 25 WG treated plot followed by fipronil 5SC and neem oil @5% treated plots, respectively.

Conclusions and Recommendations

It is concluded that cultivar China showed comparatively better performance than susceptible Swabi varieties based on the numbers of aphid populations per plant. In tested insecticides, the chemical Thiamethoxam showed better effectiveness against mustard aphid *(Lipahis erysimi)* by suppressing the pest populations and obtaining higher canola crop yields. It is recommended for farmers to use resistant cultivar China and chemical Thiamethoxam 25 WG @ 100 g/ha to control (*L. erysimi*) and achieve higher yield in canola crop.

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Novelty Statement

The Novelty of this research is to study the effects of different pesticides, in which Thiamethoxam gives a good result on the new mustard china cultivar. Which was very effective, and the china cultivars showed resistance to Mustard aphids.

Author's Contribution

Misbah Ud Din: Conducted the experiment.
Shah Alam Khan: Design the experiment.
Said Hussain Shah: wrote the Manuscript
Najeeb Ullah: Technical help

Conflict of interest

The authors have declared no conflict of interest.

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