

Efficiency of seed disinfection and foliar spray with Imidacloprid and Cypermethrin for control of flea beetles in canola

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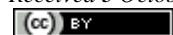
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Abstract

Flea beetles (*Phyllotreta* spp.) are among the most significant pests of canola, causing damage during the early growth stages, particularly in the seedling or cotyledon stage. Chemical control, including seed disinfection and foliar spray, is considered one of the primary strategies for managing this pest. Therefore, this study aimed to evaluate the efficiency of seed disinfection with imidacloprid (Gaucho[®]) on different canola cultivars and foliar spray with imidacloprid (Confidor[®]) and cypermethrin for controlling flea beetles. The experiment was conducted in a factorial arrangement as a randomized complete block design in a canola field in Behshahr, Mazandaran province. The treatments included seed disinfection with Gaucho[®] at a rate of 14 g/kg of seeds for the cultivars Hyola 50, Delgan, and RGS, and the infection coefficient was determined for each treatment. The Henderson-Tilton equation was used to calculate the percentage of insecticide efficiency for imidacloprid and cypermethrin. The results showed that the highest efficiency, with an infection coefficient close to 10%, was observed in the seed-disinfected Delgan cultivar, followed by Hyola cultivars (13.21%) after one week. The evaluation of insecticide efficiency also revealed that cypermethrin had the highest efficiency at approximately 90% after seven and three days of spraying, while the lowest efficiency was observed for Confidor[®] at around 60% three days after spraying. Comparison of the infection coefficients of the sprayed treatments with seed disinfected Hyola cultivars after four weeks indicated that foliar spray with cypermethrin exhibited the best efficiency with an infection coefficient of 15.18%. Based on the results of this study, the Delgan cultivar with seed disinfection and cypermethrin as the most effective insecticide is recommended for controlling flea beetles in canola.

Keywords flea beetles; seed treatment; foliar spraying; Delgan variety; Gaucho[®].

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1 Introduction

Canola, scientifically known as *Brassica napus*, belongs to the Brassicaceae family and is one of the oilseed crops. Historical records indicate that canola cultivation dates back to two thousand years ago in India (Shirani Rad and Dehshiri, 2002; Vafaei Oskoui and Keyhanian, 2021; Barari, 2017). During that time, canola oil was used for lighting and culinary purposes, and the plant itself served as animal feed (ShiraniRad and Dehshiri, 2002; Vafaei Oskoui and Keyhanian, 2021; Barari, 2017). One of the most crucial issues in the field of agriculture worldwide pertains to the presence of pests (Malekzadeh et al., 2023). Flea beetles are among the most important pests of canola, causing damage to canola plants, especially during the early growth stages and in the cotyledon or seedling phase (Kondel, 2017). The active species in canola fields belong to the genera *Phyllotreta* and *Psylliodes*, which are part of the Chrysomelidae family and the subfamily Alticinae. In Mazandaran province, both *Psylliodes Cuprea* and *Phyllotreta Corrugate* are observed simultaneously and cause similar damages (Alavi, 2003). *Phyllotreta* species mainly inflict damage on the leaves and fall-seeded canola cotyledons, while *Psylliodes* species attack the stems and leaves of canola plants in both autumn and spring (Afshari Azad et al., 2016). Management practices used to control this pest worldwide include regular irrigation, early sowing in suitable seedbeds, reducing planting depth, and crop rotation (Kondel, 2017). The most important control methods for flea beetles include the use of resistant varieties, seed disinfection, and the application of granules and sprays (Vafaei Oskoui and Keyhanian, 2021). One of the early-season pest management approaches used in Canada, especially for flea beetles, is seed disinfection with systemic insecticides such as Lindane and systemic insecticides, which provide control of these pests within 7 to 21 days after seedling emergence (Adolphe, 1980). Based on studies conducted in the country, canola seed disinfection with insecticides such as imidacloprid (Gaucho WS70%) at a rate of 12 to 14 g/kg of seed or thiamethoxam (Cruiser FS350) at a rate of 7 to 10 mg/kg of seed has been recommended (Keyhanian et al., 2005; Briar et al., 2018). In addition to seed disinfection, when the population of flea beetles reaches an economic threshold (causing more than 25% damage to the seedlings), foliar spraying is recommended. Studies have shown that after the first application of the insecticide alpha-cypermethrin, the number of flea beetle-like insects in canola seedlings decreased significantly (Hiiesaar et al., 2003). Another strategy used worldwide for controlling this pest is foliar spraying with bio-based insecticides such as neem or pyrethroids, which have shown satisfactory efficiency (Boopathi et al., 2010; Copping and Menn, 2000). Foliar spraying of canola plants with malathion (50% EC) at a rate of one liter per hectare, diazinon (60%EC) at a rate of 1200 milliliters per hectare, imidacloprid (35% SC) at a rate of 400-250 milliliters per hectare, and alpha-cypermethrin at a concentration of 150 g/h has been recommended (Khajezadeh and Keyhanian, 2008; Keyhanian et al., 2021). This study aimed to investigate the impact of seed disinfection using different canola varieties, including Hyola 50, Delgan, and Arjias, with the formulation of imidacloprid insecticide and foliar spraying with imidacloprid and cypermethrin insecticides on the population of canola flea beetles under field conditions.

2 Materials and Methods

2.1 Location and insecticide

This research was conducted in a canola field located on Behshahr city in Mazandaran province, north of Iran. The experimental treatments consisted of a control group and the insecticide imidacloprid, commercially known as Gaucho® 70%WS. The formulation of this insecticide was a water-soluble powder for seed disinfection of different canola varieties, including Hyola, Delgan, and Arjias, at a rate of 14 g/kg of seed (Khanjani and Pourmirza, 2004).

2.2 Disinfection of seeds

Fifteen hours prior to sowing the canola seeds, seed disinfection was performed based on the recommended insecticide labels. The required quantity of canola seeds for each treatment was weighed and placed in separate containers. Gaucho, 14 g/kg of different canola seeds, was measured separately. Water was added to the container at a ratio of one to one and a half times the amount of insecticide. The weighed Gaucho was gradually added to the water, and after 10 minutes of thorough mixing, the insecticide powder was well dissolved. The resulting mixture was stirred for a few minutes (Kamangar et al., 2009).

Subsequently, while the seeds corresponding to each treatment were being stirred in separate containers, the insecticide was gradually added to create a uniform coverage on the seeds. The disinfected seeds were poured onto paper sheets and placed in the shade for several hours to dry completely. Finally, the disinfected seeds were placed in labeled paper packets and transferred to the experimental field for sowing (Barari, 2014).

2.3 Sampling of disinfection treatments

The sampling for determining coefficient of infestations was conducted on the dates of 17th and 24th of November, 2021 and 1st and 7th of December, 2021. At the time of sampling, the canola plants were at different growth stages, including from germination to cotyledon, cotyledon to first true leaf, two true leaves, and four true leaves. All planting and cultivation operations were carried out according to local practices, but no insecticide spraying was performed in the experimental field. After planting, a weekly assessment was conducted by placing a frame of half a meter by half a meter in the center of each plot. The total number of plants inside the frame, including completely healthy plants, slightly damaged plants, and severely infested plants, was counted and recorded. The coefficient of infestation was then calculated based on the formula (Kazda et al., 2005), where a higher coefficient of infestation indicates more severe plant damage (Addison et al., 2002).

2.4 Experimental treatments

The experimental treatments in this stage consisted of the insecticide Alpha-Cypermethrin (Cypermethrin[®]), at a rate of 150 g/h. Additionally, Imidacloprid (Convidor[®]), was applied at a rate of 0.5 liters per hectare. The control treatment involved spraying water as a solvent (Keyhanian et al., 2021).

2.5 Sampling of sprayed treatment

Sampling of the flea beetles' population was conducted one day before spraying, as well as three, seven, and fourteen days after spraying. For sampling within the center of each plot, yellow sticky traps measuring 30 × 30 cm were positioned on a 30 cm high base, approximately at soil level. Additionally, a 25/500 square meter frame located at the center of the plot was utilized. During each sampling event, the number of captured flea beetles on the sticky traps, as well as the number of active flea beetles on a surface measuring half a meter by half a meter at the center of each plot, were counted, and the traps were replaced. The spraying operation was performed using a 20-liter backpack sprayer equipped with a conical nozzle, delivering a calibrated consumption of 300 liters per hectare (Keyhanian, 2019).

To determine the insecticide efficiency percentage, the Henderson-Tilton equation and the counting of live larvae on the plants were employed. This index was calculated for each treatment at each specific time (Henderson and Tilton, 1955).

$$\text{Insecticide efficiency percentage} = \left[1 - \frac{T_a \times C_b}{T_b \times C_a} \right] \times 100$$

The components of the equation are as follows: T_b represents the number of live larvae in the treatment plot before spraying, T_a represents the number of live larvae in the treatment plot after spraying, C_b represents the number of live larvae in the control plot before spraying, and C_a represents the number of live larvae in the control plot after spraying.

2.6 Statistical analysis

This research was conducted in the form of randomized complete block design experiments on fragmented plots during the seed disinfection stage for three varieties, namely Hiyola, Delgan, and Arjias. The coefficient of infestation was assessed in four time intervals. Additionally, during the spray treatment stage, two insecticides, Confidor and Cypermethrin, were applied in three time intervals alongside a control group. This design aimed to determine the coefficient of infestation and efficiency of each insecticide in this comparison. Following the aforementioned regular sampling procedures in the mentioned stages, if necessary, data were transformed, and analysis of variance for the measured factors was performed using SAS software. Means were compared using the Duncan test. The data related to different sampling stages were sorted using Excel software, and the graphs were designed using the same software.

3 Results

3.1 Coefficient of infestation

The results of the analysis of variance for the coefficient of infestation in different sampling weeks demonstrated significant differences in means between various treatments compared to the control (for each treatment, the corresponding control of the same variety was considered, but the results indicated no significant difference in the level of damage caused by this pest among different control varieties; therefore, averages were calculated from all control groups). These differences were statistically significant at a one percent level of probability (Table 1).

Table 1 Variance analysis related to different sampling times

Time	Degrees of Freedom	Mean of Squares	F Value	Pr > F	Grade
1 weeks	3	1731.29	30.91	0.0001	a
2 weeks	3	1131.33	20.20	0.0001	b
3 weeks	3	1232.32	22.05	0.0001	c
4 weeks	3	904.55	16.15	0.0001	d

The results of the comparison of mean infection rates demonstrated that the infection rate increased with time in all sampling instances, reaching its peak in the fourth week (Fig. 1). The lowest infection rate was observed in the "Delgan" cultivar treatment (1.02 ± 10.28), after one week, followed by the "Hyola" cultivar treatment with an infection rate of 2.06 ± 13.12 . No significant differences were observed among different cultivars at weeks two, three, and four, while all treatment groups showed significant differences compared to the control. All three cultivars exhibited the highest infection rates after four weeks, with an approximate 40% infection rate, similar to the damage caused by the pest in the first week in the control treatment (without disinfection). The highest percentage of infection, close to 80%, was also observed in the control treatment after four weeks of cultivation, indicating the effectiveness of this method in controlling the pests.

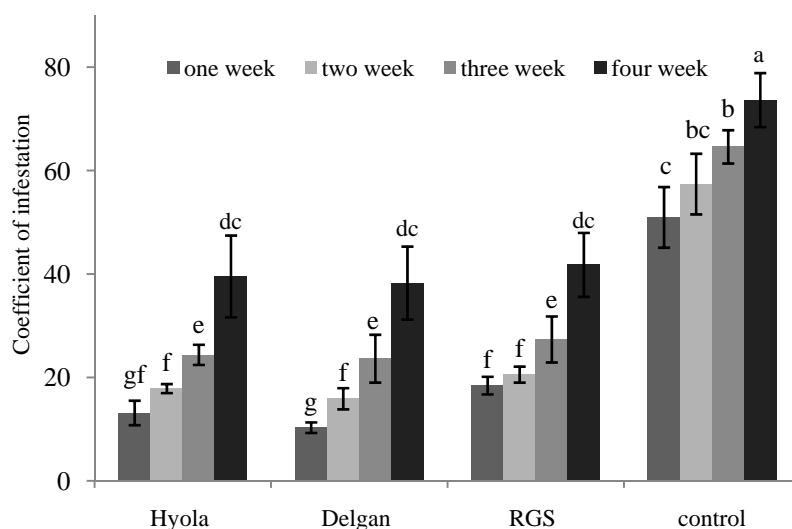


Fig. 1 Comparison of the average percentage of coefficient of infestation of different treatments during four weeks after cultivation.

The analysis of variance table pertaining to different time intervals also demonstrates that the time factor significantly contributes to the variations in the percentage of infection among the conducted treatments. Therefore, the interaction effect of time and treatments was also evaluated.

Table 2 Variance analysis related to the interaction of two factors, treatment and time.

Source	Degrees of Freedom	Mean of Squares	F Value	Pr > F
Time	3	1166.02	20.82	0.0001**
Cultivar	3	4907.05	78.61	0.0001**
Time × Cultivar	9	30.81	0.55	0.8265 n.s.
Error	32	56.01		
Total	47			

The results indicate that both time and treatment had significant effects, as shown in Table 2. However, their interaction did not have a significant effect on each other. Specifically, the treatments exhibited similar performances at all-time points, except for the first week where the Delgan variety showed the lowest percentage of infection rate distinctly. The findings of this study revealed that there was no significant difference among the control treatments with different varieties. However, there was a significant difference between all treatments and their respective controls.

3.2 Assessment of insecticide efficiency

Considering that seed disinfection has partially reduced the damage caused by this pest, farmers across the country also use spraying in addition to seed disinfection. In this project, the efficiency of two insecticides with the highest consumption rate in the region was evaluated, and the results are presented in the graph.

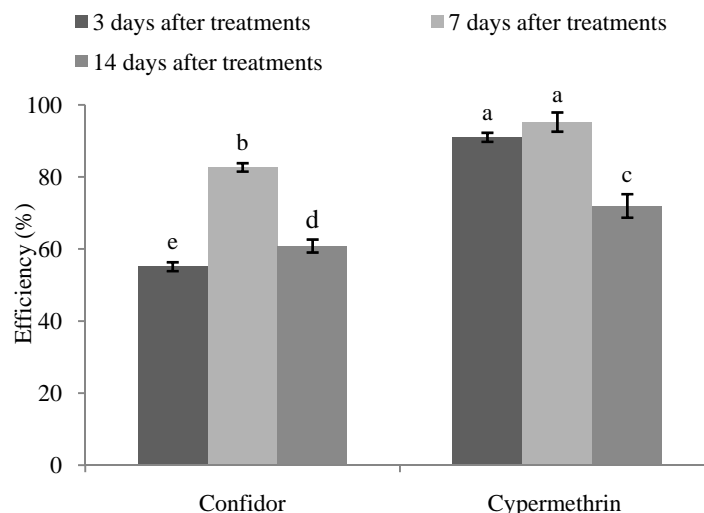


Fig. 2 Comparison of the average efficiency of different insecticides at different sampling times.

As observed in Fig. 2, the highest efficiency is attributed to the insecticide Cypermethrin, which, in both the seven-day and three-day intervals after spraying, achieved over 90% efficiency in minimizing pest damage. On the other hand, the treatment applied three days after spraying with the insecticide Confidor exhibited the lowest efficiency, measuring less than 60%.

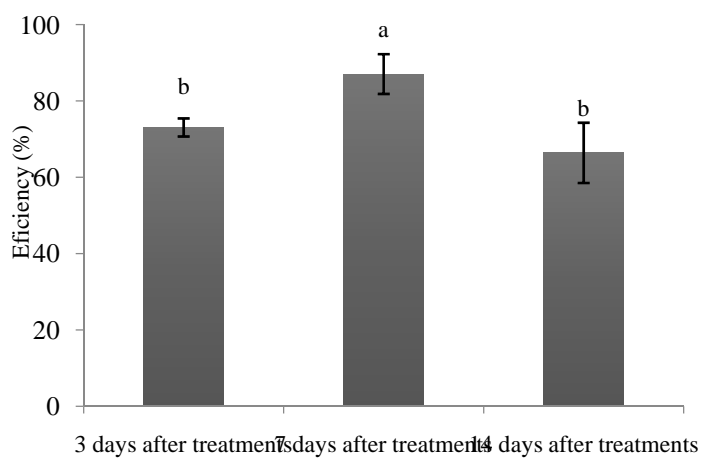


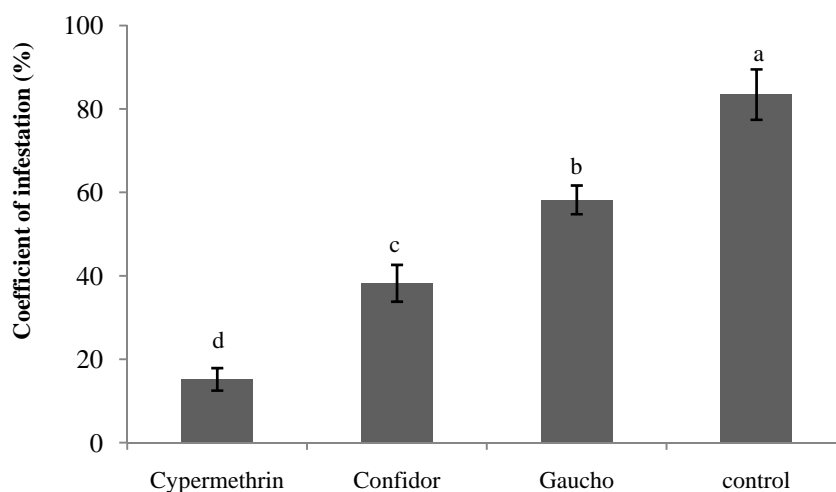
Fig. 3 Comparison of the average efficiency based on time.

Investigation of different time intervals also reveals that the highest average efficiency for both insecticides was observed at the seven-day interval after spraying, with an average of 87.04%. The two other time intervals, namely three days (73.05%) and fourteen days (66.04%) after spraying, showed statistically similar results and did not exhibit a significant difference from each other in the statistical grouping (Fig. 3).

Table 2 Variance analysis related to the interaction of two factors, treatment and time.

Source	Degree of Freedom	Mean of Square	F Value	Pr > F
Block	2	154.68	16.40	0.0118*
Treatment	1	122.21	12.96	0.0001**
Block × Treatment	2	101.49	10.76	0.0246*
Time	2	314.35	33.33	0.0034**
Treatment × Time	2	641.10	67.97	0.0216*
Block × Time	4	28.69	3.04	0.8143 ^{n.s.}

As observed in Fig. 2, the highest efficiency is attributed to the insecticide Cypermethrin, which, in both the seven-day and three-day intervals after spraying, achieved over 90% efficiency in minimizing pest damage. Conversely, the treatment applied three days after spraying with the insecticide Confidor exhibited the lowest efficiency, measuring less than 60%.

**Fig. 4** Comparison of the average percentage of coefficient of infestation between spraying and seed treatments.

As observed in Fig. 4, the results indicate that the highest percentage of infection rate is associated with the treatment of Hyola seed disinfection using the insecticide Gaucho, reaching nearly 60%. Following that, spraying with the insecticide Confidor resulted in approximately 40% pest damage in this treatment. Ultimately, the best efficiency and lowest infection rate were achieved in the treatment involving the insecticide Cypermethrin, with an infection rate of less than 20%. The findings of this study, considering all spraying treatments and comparing infection rates with seed disinfection treatments, demonstrate that the spraying treatment with the insecticide Cypermethrin exhibited the highest efficiency. Cypermethrin, belonging to the pyrethroid family of synthetic insecticides, is commonly used for controlling insects on various agricultural crops.

4 Discussion

Given the importance of developing oilseed crops in the world and the strategic need for providing feed for livestock and poultry, increasing the cultivation area of these plants, including canola, has gained significant importance. Therefore, any pests and diseases that affect the performance of this plant should be promptly investigated, and necessary measures for their control should be taken (Antwi and Reddy, 2016). Flea beetles of canola are one of the primary pests in the growth stages of canola plants, and if left uncontrolled, they can cause irreparable damage to farmers. Extensive and diverse research has been conducted worldwide on the use of seed treatment against canola stem weevils, and in all of these studies, this method has proven to be one of the most effective approaches, similar to the present study, where significant differences have been observed between treated and untreated treatments (Reddy et al., 2014; Antwi and Reddy, 2016; Hladik et al., 2018).

In comparison to the application of insecticide sprays, seed treatment can prevent direct contact between insecticides and natural enemies, and it can also provide the capacity for insects to modify their feeding regime and consume fewer bait containing insecticides (Chatterjee et al., 2009). Therefore, this method has become an important component in integrated pest management strategies (Ding et al., 2018). Gaucho is a neonicotinoid insecticide with excellent systemic properties that is absorbed through the roots and translocated to the aerial parts of the plant. The residue of this insecticide in the soil is minimal. Studies conducted on seeds treated with neonicotinoid insecticides have shown that it not only does not lead to a reduction in germination and growth of various crops but also affects the growth processes of these crops (Tsvetkov et al., 2017; Woodcock et al., 2017). The impact of Gaucho against canola stem weevils during germination and early growth stages of canola has been reported in various sources (Addison et al., 2002; Kazda et al., 2005). Gaucho is the most widely used insecticide as a seed disinfectant worldwide and has been recommended for seed disinfection in more than 15 crops to combat approximately 45 pest species, including canola stem weevils and aphids (Zaharia et al., 2023). Based on research conducted in the Rudbar region of Gilan, canola seeds treated with 70% Gaucho at a rate of 14 g/kg of seed showed better control efficiency against canola stem weevils compared to 12 g of Gaucho per kg of seed (Keyhanian, 2004).

Other studies have investigated the effects of seed treatments with Gaucho and Cruiser on the cabbage moth (*Argaerosae* L.). The researchers concluded that Cruiser treatments at doses of 7.5, 10, and 12.5, as well as Gaucho treatment at a rate of 12.5 gr/kg of canola seeds, had the greatest impact on reducing the population of larvae of this pest (Kamangar, 2012; Kamangar et al., 2009). The researchers reported that seed disinfection with the insecticides imidacloprid (Gaucho) at a rate of 14 mg/kg and thiamethoxam (Cruiser) at a rate of 10 ml/kg of canola seeds significantly reduced pest damage compared to the control (Barari, 2014), which is consistent with previous research findings.

The lowest infestation level was observed one week after sowing, and infestation levels increased over time, leading to a significant decrease in the efficiency of the insecticides in all treatments. This decrease is attributed to the degradation of the insecticides within the plant. According to the conducted studies, the residual amount of insecticide in the leaves and other vegetative parts of canola plants decreased to less than one-fourth of the initial applied amount after 7 days (Sekulic and Rempel, 2016).

The cultivars used in this study were Hyola, Delgan, and Argis. Although no significant differences were observed among them at time points beyond two weeks, the best efficiency, indicated by the lowest coefficient of infestation, was attributed to the Delgan cultivar at the first sampling date, which was one week after planting. The conducted research also demonstrated that this cultivar exhibited desirable performance in terms of yield in the northern region of the country compared to RGS and Hyola cultivars (Pyeghamzadeh et al., 2022). In another similar study that evaluated different canola cultivars in terms of performance, the Delgan cultivar showed superior efficiency compared to other cultivars, especially the Argis cultivar as the control

(Fanaei et al., 2019).

The results of the present study, in all cases of foliar application and comparing coefficient of infestations with seed disinfection treatments, demonstrated that the best efficiency was associated with foliar spraying of the insecticide cypermethrin. Cypermethrin, which belongs to the pyrethroid family of synthetic insecticides, is used to control insects on various agricultural crops (Rimoldi et al., 2012). It exhibits minimal residual effect for more than 10 days under favorable conditions. Generally, pyrethroids, including cypermethrin, have a similar mode of action, resembling that of DDT, by targeting sodium channels in the nerve cell membranes. Cypermethrin primarily acts on the nervous system of arthropods and is a contact and stomach poison (Ware and Whitacre, 2011; Bastan et al., 2020). In recent years, 11 insecticides were investigated for controlling the large white cabbage butterfly. The results of the experiments were as follows: fenvalerate, deltamethrin, cypermethrin, malathion, and fenitrothion controlled the pest by 100%, 97.3%, 96.8%, 96.8%, and 93.3%, respectively, with the highest impact attributed to fenvalerate. In this study, fenvalerate and cypermethrin had no adverse effects on non-target species and were deemed safe. However, the toxicity of malathion against natural enemies was evident within 24 hours. Therefore, synthetic pyrethroids, along with the use of parasitoid wasps, are suitable for controlling the large white cabbage butterfly (Thakur and Deka, 2012, Rafiei et al. 2019). In another study evaluating the insecticidal effect, a new insecticide called alpha-cypermethrin WG (15%) was assessed against cabbage aphids in canola fields, compared to malathion EC (57%) at a rate of 600 milliliters per hectare, thiacloprid OD (24%) at a rate of 300 milliliters per hectare, and lambda-cyhalothrin (CS10%) (Karate Zeon) at a rate of 75 milliliters per hectare. The results demonstrated that all tested insecticide treatments showed significant differences at a 1% probability level compared to the control treatment (no spraying). The alpha-cypermethrin treatment at rates of 300 and 150 g/h, in Mazandaran and Golestan provinces, respectively, exhibited efficacies of 87.17 ± 3.29 , 84.19 ± 2.59 , 81.66 ± 3.78 , and 76.66 ± 0.33 in Group A. Alpha-cypermethrin, at rates of 150 and 300 g/ha, can be used as a new insecticide for controlling cabbage aphids. Therefore, alpha-cypermethrin insecticide, with minimal adverse effects on natural enemies and significantly higher efficiency compared to imidacloprid, can be a suitable alternative for controlling aphids in canola fields. Additionally, considering that this insecticide belongs to a different chemical family, the pyrethroids, it reduces the likelihood of resistance development. By activating non-similar detoxification systems in aphids, it greatly enhances mortality rates compared to the widely used imidacloprid insecticide.

The conclusion of this section of the research also showed that both insecticides were capable of controlling this pest compared to the control treatment. However, alpha-cypermethrin insecticide demonstrated a higher efficiency of over 90% at three and seven days after application, making it a more suitable option for controlling this pest compared to the confidor insecticide. The examination of coefficient of infestations four weeks after planting, with a fourteen-day interval after spraying, due to the similar growth stage of the canola plants (variety Hyola), revealed that the lowest coefficient of infestation was still associated with the treatment using alpha-cypermethrin insecticide. Therefore, it can be concluded that one of the best measures for controlling aphids in canola fields in Mazandaran province is the initial disinfection with Gauchoinsecticide, and ultimately, if damage from this pest continues, the next step could be spraying with alpha-cypermethrin insecticide, with field monitoring.

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