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Research Article

Impact of silicon-based fertilizer and salicylic acid on the population density of *Brevicoryn brassicae* (Hemiptera: Aphididae) and its parasitism by *Diaeretiella rapae* (Hymenoptera: Braconidae)

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Abstract: The cabbage aphid, *Brevicoryne brassicae* (Hemiptera: Aphididae), is an important pest of rapeseed. In this study, the effects of a silicon-based fertilizer, silicate potassium (SP), and salicylic acid (SA) on inducing resistance in rapeseed to the aphid were studied under field conditions. The rapeseed plants were directly sprayed with SP and SA at two application rates, and the population density of alive and parasitized aphids by *Diaeretiella rapae* (Hymenoptera: Braconidae) was recorded every three days. The results showed that the aphid population densities in SP and SA treatments were significantly lower than in control (2.21 and 3.53 folds, respectively). The lowest population density was observed in the SP treatment (1200 g/ ha). Moreover, the parasitized aphid densities in the treatments were significantly increased, and the highest parasitism was recorded in the SP treatment (1200 g/ha). The rapeseed yield in the SP-treated plants was significantly more than control, and the highest yield increase was observed in SP treatment (1200 g/ha). According to the results, SP at 1200 g/ha is recommended for cultural control of *B. brassicae* in the rapeseed fields.

Keywords: Cabbage aphid, plant nutrition, induced resistance, biological control, IPM

Introduction

Rapeseed, *Brassica napus* L., is one of the most important oilseed crops in different parts of the world (Bell, 1984), including Iran (Rameeh, 2016). In addition to direct usage of the plant products as human food or livestock feed, the plant has industrial usages, and its products have been applied as biofuel in recent years (Malça and Freire, 2010).

The cabbage aphid, *Brevicoryne brassicae* L. (Hemiptera: Aphididae), is the most destructive pest of this plant in many world regions. The aphid causes severe damage to the plant at flowering and pod formation stages (Lashkari *et al.*, 2007; Razmjou *et al.*, 2019) by feeding on the plant sap and injecting the saliva into the plant tissues. Photosynthesis reduction due to sooty mold growth and virus transmission indirectly damages by the aphids (Blackman and Eastop 2000; Rajabpour and Yarahmadi, 2012; Rashedi *et al.*, 2019; Kafeshani *et al.*, 2018). Broad-spectrum insecticides, especially pyrethroid and neonicotinoid compounds, have been enormously applied every year (Zhang *et al.*, 2017). However, these chemicals cause hazardous drawbacks such as pesticide resistance, secondary pest outbreaks, adverse effects on non-target organisms, and environmental contaminations (Pedigo, 2002;

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Shahbi and Rajabpour, 2017). Host plant resistance (HPR) is an economical and eco-friendly method in integrated pest management (IPM) programs. This tactic is compatible with other controlling methods, primarily biological control (Pedigo, 2002; Mohammadi et al., 2015; Azadi *et al*., 2018). Insect-host interactions can be significantly influenced by the host plant's nutrition (Barbour *et al*., 1991). Nutrition manipulations through fertilizers are necessary and conventional methods in cultural control. Silicon (Si) is considered a functional plant nutrient that can significantly enhance the HPR of some crops to various pests. Moreover, some Analogues of plant hormones are used for inducing resistance to plant pests and pathogens. Salicylic acid (SA) is a type of phenolic acid considered a plant hormone, which can mediate multiple signaling pathways in the plant's biochemistry of defense (Raskin, 1992; Rodríguez-Álvarez *et al*., 2015; Mahmoudi *et al*., 2021; Ghazalbash *et al*., 2018; Laane *et al.,* 2018).

The HPR can influence the herbivore-natural enemy interactions. Therefore, the abundance and efficacy of the natural enemies (NEs) may differ in resistant and susceptible cultivars (Price *et al*., 1980; van Emden, 1995). The parasitic wasp, *Diaeretiella rapae* McIntoch (Hymenoptera, Braconidae), is an important parasitoid of *B. brassicae* whose efficiency can be affected by variation the host plant nutrition (Karami *et al*., 2018).

There were no previous reports on the effects of silicon-based fertilizers or plant hormones, including SA, on induction of HPR in rapeseed. Therefore, the present study aimed to evaluate the fertilizer effects on the population density of *B. brassicae* and its parasitism by *D. rapae* in rapeseed fields for reducing the insecticide applications and protecting the natural enemy. Moreover, the effect of the fertilizers on the rapeseed yield was studied.

Materials and Methods

Experimental design

The study was performed in an experimental rapeseed field, 5000 m^2, in Abid county, Gotvand district, Khuzestan province, southwest Iran (32°29'31.6" N 48°52'20.1" E) during 2018-2019. The field included 20 plots (each plot 200 m^2 with 1 m ridge). Experiments were arranged in a randomized complete block design with four replications (plots) according to the field slope and irrigation (distance to irrigation canal). Seeds of commercial rapeseed cultivar Hyola 401 were cultivated (900000 plants per ha). Agricultural practices were conducted according to the instructions of the Khuzestan province agricultural organization. No chemicals, e.g., insecticide, herbicide, fungicide, other fertilizers, etc., were applied in the experimental field during the trial period.

Treatments are shown in Table 1. The recommended concentrations and half-recommended application rates of the resistance inducers were tested (Table 1). Treatments were applied using a hand-operated knapsack sprayer having a 20-liter capacity fitted with a hollow cone nozzle at the flowering stage. The equipment was set to deliver 1000L/ha, following the growers' usual practice. According to the aphid occurrence on rapeseed in Khuzestan province, the application time was selected at the flowering stage of the plants.

Table 1 Resistance-inducing treatments and their application rates in the rapeseed field.

Fertilizer	Application rate	Trade name	Company
Silicate potassium	600 ml/ha	Silicon Dayan®	Khosheh Parvaran Zist Fanavar company, Iran
Silicate potassium	1200 ml/ha	Silicon Dayan®	Khosheh Parvaran Zist Fanavar company, Iran
Salicylic acid	27.6 g/ha	-	Merck, Germany
Salicylic acid	138 g/ha	-	Merck, Germany
Control	Sprayed with water		

Sampling

Sampling was carried out every three days from December 2018 to the end of the growing season, May 2019. Ten plants were randomly selected at each sampling date by traveling in a zigzag-shaped pattern through each plot, and the numbers of the aphids, nymphs, and adults, and parasitized aphids by *D. rapae* on 2 cm of terminal shoots, were recorded. At the end of the growing season, total yield was measured in each plot separately. For this purpose, the total seed weight of rapeseed plants in each plot was separately recorded. The previous study indicated that *D. rapae* was the active parasitoid wasp on *B. brassicae* in rapeseed fields of Khuzestan province Farsi *et al.*, 2010). The aphid mums parasitized by the parasitoid were identified according to Hafez (1961).

Data analyses

The effect of 5 (resistance inducer treatments) and 16 (times) was studied on the population density of *B. brassicae* and parasitism of *D. rapae* based on a completely randomized block design. The data of this experiment could provide analysis as a factorial, split-plot (resistance inducer treatment as the main factors), or repeated measurement based on the covariance-variance matrix for sampling date residual the best model was selected. Among compound symmetry, Unstructured, Autoregressive, and Vertical component covariance-variance structures, for sampling date residual, the best model was selected based on the minimum value in the criteria such as Akaike information criterion (AIC) and AIC corrected (AICC). All of the analyses were performed by SAS software (version 9.2) (SAS Institute, Inc., Cary, NC). The five levels of resistance inducer factor were SP (600mL/ha), SP 1200 (mL/ha), SA (27.6 g/ha), SA (13.8 g/ha), and control, and the sixteen levels of sampling time factor were performed from 25th February to 22nd April in the year 2019. Duncan's multiple range test was used as an ANOVA post hoc test for means comparisons.

Results

Population density of *Brevicoryne brassicae*

The autoregressive covariance matrix was selected due to smaller fitted statistics for all user information criteria (Table 2). The treatments, SP and SA, and sampling date significantly affected the population density of *B. brassicae* (Table 3).

The aphid density at the beginning of sampling (end-February) was low. However, the density dramatically increased ad peaked in mid-March when it was significantly higher than in other sampling dates. Afterward, the density gradually decreased (Figure 1).

Table 2 Autoregressive covariance matrix parameters for a population density of *Brevicoryn brassicae*.

Information Criteria	Compound Symmetry	Autoregressive Lag 1	Vertical component, Diagonal
-2 Res Log Likelihood	1251.5	1125.6	1280.3
AIC (smaller is better)	1255.5	1129.6	1282.3
AICC (smaller is better)	1255.6	1129.6	1282.3
BIC (smaller is better)	1257.5	1131.6	1285.8

Table 3 Parameters of repeated measure analyses of variance for the population density of *Brevicoryne brassicae* based on Autoregressive variance-covariance matrix.

Source of variation	df	Den df	F	P-value
Replication	3	33.1	0.56	0.6421
Resistance inducers (RI)[1]	4	30.7	69.48	< 0.0001
Sampling date	15	215.1	19.43	< 0.0001
RI × Date	60	203.1	5.62	< 0.0001

[1] Resistance inducers (silicate potassium and salicylic acid).

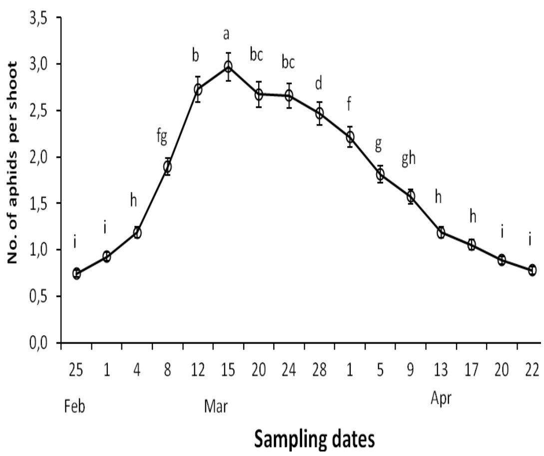

Figure 1 Mean (± SE) population density of *Brevicoryne brassicae* in 2019. Means with the same letters are not significantly different (Duncan's test, P < 0.05).

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The population density of the aphid in the treatments was significantly lower than control. The lowest aphid density was observed in SP (1200 g/ha) (Fig. 2), in which the aphid density (0.99 aphids per shoot) was 3.53 folds lower than control (3.5 aphides per shoot). Aphid density reduction in the SA (27.5 g/ha) treatment was relatively lower than other SP and SA treatments. The aphid density in SA (27.5 g/ha) treatment was 2.21-fold lower than control. The induced resistance (IR) in SP treatments was relatively higher than SA treatments. Moreover, the IR level was enhanced by increasing the application rates of the fertilizers.

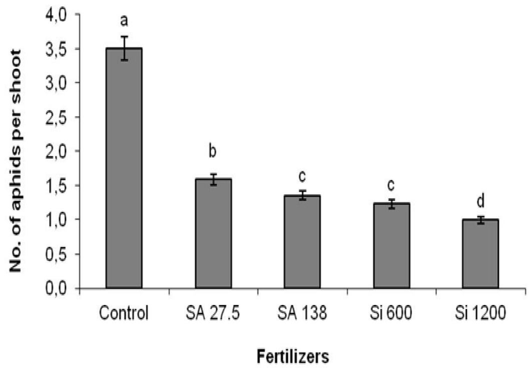

Figure 2 Mean (± SE) population density of *Brevicoryne brassicae* in various treatments, Si 600: Silicate potassium (600mL/ha); Si 1200: Silicate potassium (1200 mL/ha); SA 27.5: Salicylic acid (27.5 g/ha); and SA 138: Salicylic acid (138 g/ha). Means with the same letters are not significantly different (Duncan's test, P < 0.05).

Parasitism of *B. brassicae* by *D. rapae*

Based on Table 4, compound Symmetry and Autoregressive variance-covariance matrix did not have any superiority over variance component matrix based on all of the Information Criteria. Therefore, a factorial model was used for data analysis. There were significant effects of the resistance inducer treatments and sampling dates on parasitism of *B. brassicae* by *D. rapae* (Table 5).

The aphid parasitism by *D. rapae* was low at the beginning (February and early March) and at the end of sampling (March). The highest parasitized aphid density was observed in Late-March (Figure 3).

Table 4 Autoregressive covariance matrix parameters for parasitism of *Brevicoryn brassicae* by *Diaretiella rapae.*

Information criteria	Compound symmetry	Autoregressive Lag 1	variance component, Diagonal
-2 Res Log Likelihood	-207.8	-207.1	-207
AIC (smaller is better)	-203.8	-203.1	-205
AICC (smaller is better)	-203.7	-203	-205
BIC (smaller is better)	-201.8	-201.1	-201.5

Table 5 GLM parameters of main effects and interactions for resistance inducer treatments and sampling date on parasitism of *Brevicoryne brassicae* by *Diaeretiella rapae.*

Source	df	MS	F	P-value
Replication	3	0.00067	0.14	0.9379
Resistance inducers (RI)[1]	4	0.05731	11.79	< 0.0001
Sampling date	15	0.07328	15.07	< 0.0001
RI × Date	79	0.00709	1.46	0.0255
Error	237	0.00486		

[1] Resistance inducers (silicate potassium and salicylic acid).

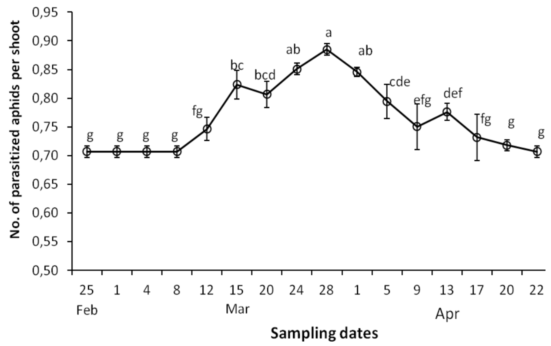

Figure 3 Mean (± SE) population density of parasitized aphids, *Brevicoryne brassicae*, by *Diaeretiella rapae* in 2019. Means with the same letters are not significantly different (Duncan's test, P < 0.05).

The percentage of the parasitized aphids in all SP and SA treatments was significantly higher than control (Figure 4). The highest percentage of the parasitized aphids was observed in SP (1200 g/ ha) treatment which was about 10% more than the control. Moreover, the percent of the parasitized aphid density increased significantly when a higher fertilizer rate was applied.

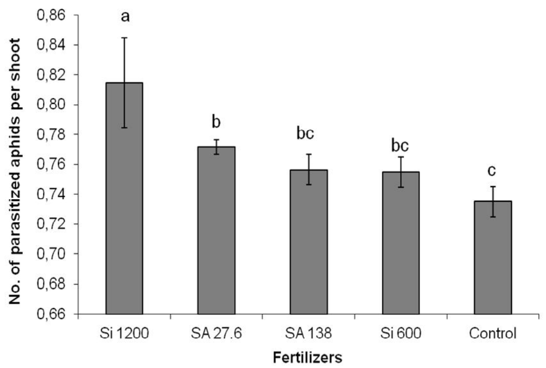

Figure 4 Mean (± SE) population density of parasitized aphids, *Brevicoryne brassicae*, by *Diaeretiella rapae* in various treatments, Si 600: Silicate potassium (600mL/ha); Si 1200: Silicate potassium (1200 mL/ha); SA 27.5: Salicylic acid (27.5 g/ha); and SA 138: Salicylic acid (138 g/ha). Means with the same letters are not significantly different (Duncan's test, P < 0.05).

Rapeseed yields

Yields of rapeseed in various treatments were significantly different (Table 6). The yield in the resistance inducer treatments was significantly higher than control. The highest rapeseed yield was recorded in SP (1200 g/ha) and was 11% more than control.

Table 6 Effects of silicon and salicylic acid on rapeseed yield.

Treatments	Rate (g/ha)	Yield ± SE (Kg per plot)[1]
Silicon	600.0	246.6 ± 0.4b
Silicon	1200.0	265.1 ± 1.6d
Salicylic acid	27.5	244.9 ± 1.4b
Salicylic acid	138.0	259.7 ± 2.2c
Control	0	238.8 ± 2.2a
F (df = 4, 19)		38.3
P-value		< 0.0001

[1] Means with the same letters are not significantly different (Duncan's test, P < 0.05).

Discussion

All of the resistance inducer treatments caused a significant reduction of *B. brassicae* density in rapeseed. Therefore, the silicon-based fertilizer (SP) and SA stimulate IR of rapeseed to the aphid. Si may be deposited in host plant tissues and provides a mechanical barrier to the herbivore insect feeding. For instance, it is demonstrated that silicon IR is associated with callose deposition in sieve tubes that blocks the sap intake by hemipteran pests (Alhousari and Greger, 2018). Moreover, the element has a crucial role in the physiological process of defensive products of host plants, including tannins and phenolic compounds (Reynolds *et al*., 2009). The compounds play important role in HPR (Zandi-Sohani *et al.*, 2018; Rajabpour *et al.*, 2019). The HPR, especially IR, can also be manipulated by using chemical elicitors of the defensive plant chemicals (War *et al.*, 2012). In this case, the Si may have acted as the elicitor. A previous study showed that this element, as an elicitor, triggers some defensive enzymes, peroxidase, polyphenol oxidase, and phenylalanine ammonia-lyase. The lignification and suberization process was catalyzed using the peroxidase enzyme (Goodman *et al*., 1986). Researchers indicated that polyphenol oxidase and phenylalanine ammonia-lyase enzymes oxidated some important defensive phytochemicals, including phenolic compounds and quinines. The phytochemicals reduce the host plant's nutritional quality and inhibit plant protein digestion (Felton *et al*., 1994; Bi and Felton, 1995; Safaei *et al*., 2016).

Si-mediated resistance of some plants to hemipteran pests has been previously documented. For instance, it is reported that silicon-based fertilizers increased resistance of: rice to brown planthopper *Nilaparvata lugens* Stål (Hemiptera, Delphacidae) (He *et al*., 2015), wheat to the grain aphid *Sitobion avenae* F. (Dias *et al*., 2014), and the green wheat aphid *Schizaphis graminum* (Rond.) (Hemiptera, Aphididae) (Basagli *et al*., 2003), the greenhouse whitefly, poinsettia to *Trialeurodes vaporariorum* Westwood (Hemiptera, Aleyrodidae) (Hogendorp

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et al., 2010), cucumber to the silver whitefly, *Bemisia tabaci* Gennadius biotype B (Hemiptera, Aleyrodidae) (Correa *et al*., 2005), corn to the corn leaf aphid *Rhopalosiphum maidis* Fitch (Boer *et al*., 2019; Moraes *et al*., 2005) and increased resistance of common zinnia to green peach aphid *Myzus persicae* Sulzer (Hemiptera, Aphididae) (Ranger *et al*., 2009).

The SA and jasmonic acid are considered two phytohormones that play important roles in regulating plant defense against insect pests, especially sap feeders (Moran and Thompson, 2001). There was a significant correlation between Si absorption and the concentration of the phytohormones in the host plants (Alhousari and Greger, 2018).

Similarly, inductions of resistance of some host plants, including, barely to *S graminum* (Chaman *et al*., 2003) and tomato to *Macrosiphum euphorbiae* Thomas (Hemiptera, Aphididae) (Li *et al*., 2006) and *B. tabaci* (Rodríguez-Álvarez *et al*., 2015) were previously documented.

The lower population density of *B. brassicae* may be due to increased aphid parasitism by *D. rapae,* its dominant parasitoid. Our data indicated that the density of parasitized aphids in rapeseed plants treated by silicon-based fertilizers was significantly increased. Increased efficiency of the biological control can serve as indirect IR of the silicon-treated rapeseed plants to the aphid. Ode and Crompton (2013) showed that HPR affected the foraging behavior of *Aphidius colemani* Viereck (Hymenoptera, Braconidae), an important parasitoid of *Aphis glycines* (Homoptera, Aphididae), in soybean fields. On resistant soybean cultivars, the parasitoid attack rate was higher than on the susceptible ones. Moreover, the female wasps showed lower handling time for finding the aphid in the soybean-resistant cultivar than susceptible cultivar (Ode and Crompton, 2013). Usually, the defensive characteristics of resistant host plants influence herbivore size and behavior. The aphid feeding on the resistant plants may exhibit lower defensive behavior against the female attacks (Ode and Crompton, 2013).

Moreover, Si stimulates the jasmonic acid pathway in host plants which modulates volatile plant synomones. The synomones increase the biocontrol of the plant pests by attracting their NEs, including parasitoids (Alhousari and Greger, 2018).

Conclusion

Our results indicated that the SP and SA treatments significantly increased rapeseed resistance to *B. brassicae*. Moreover, biological control of the aphid by *D. rapae* was significantly increased by the treatments. The resistance level, biocontrol efficacy, and rapeseed yield in SP treatment (at 1200 g/ha) were significantly higher than the other treatments. Results of this study can be used for cultural control of the aphid, reduction of insecticide application, and biocontrol efficiency in rapeseed fields.

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تأثیر کود سیلیس پایه و اسید سالیسیلیک روی تراکم جمعیت شته *Brevicoryne brassicae* (Hemiptera: Aphididae) و پارازیتیسم آن توسط *Diaeretiella rapae* (Hymenoptera: Braconidae)

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چکیده: شته مومی کلم *Brevicoryne brassicae* (Hemiptera: Aphididae) یکی از آفات مهم کلزا می‌باشد. در این مطالعه، اثرات یک کود سیلیس پایه (سیلیکات پتاسیم SP) و اسید سالیسیلیک (SA) روی القای مقاومت به این شته در شرایط مزرعه‌ای مورد مطالعه قرار گرفت. گیاهان کلزا به‌صورت مستقیم توسط SA و SP در دو غلظت محلول‌پاشی شدند و تراکم جمعیت شته‌های زنده و پارازیته شده توسط زنبور پارازیتوئید *Diaretiella rapae* (Hymenoptera: Braconidae) هر سه روز یک‌بار به‌صورت جداگانه ثبت شد. نتایج نشان داد که تراکم جمعیت این شته در تیمارهای SP با ۲/۲۱ برابر و SA با ۳/۵۳ برابر به‌صورت معنی‌داری کم‌تر از تیمار شاهد بود. کم‌ترین تراکم جمعیت در تیمار SP (غلظت ۱۲۰۰ گرم در هکتار) مشاهده شد. هم‌چنین، تراکم شته‌های پارازیته شده در این تیمارها به‌صورت معنی‌داری افزایش یافت. بیش‌ترین پارازیتیسم در تیمار SP (غلظت ۱۲۰۰ گرم در هکتار) دیده شد. عملکرد کلزا در گیاهان تیمار شده با SP به‌صورت معنی‌داری بیشتر از تیمار شاهد بود. بیش‌ترین افزایش عملکرد در تیمار SP (غلظت ۱۲۰۰ گرم در هکتار) مشاهده شد. بنابراین کود SP با غلظت ۱۲۰۰ گرم در هکتار برای کنترل زراعی شته مومی کلم در مزارع کلزا می‌تواند توصیه شود.

واژگان کلیدی: شته کلم، تغذیه گیاهی، مقاومت القایی، کنترل بیولوژیک، مدیریت تلفیقی آفات

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