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Influence of Potassium Silicate on the Survival, Development and Reproduction of *Aphis gossypii* Glover (Hemiptera: Aphididae)

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ABSTRACT

Induced plant resistance is an important component of pest control. Therefore, effects of potassium silicate (K_2SiO_3) on the survival, development and reproduction of *Aphis gossypii* Glover at various life stages were tested under laboratory conditions. Efficacy of foliar spraying with K_2SiO_3 compared to lambda-cyhalothrin (LCH) against the field strain of *A. gossypii* third nymphal instar under greenhouse conditions as well as its impacts on the activity of antioxidant enzymes were also evaluated. In laboratory studies, K_2SiO_3 (LC_{50} = 58.21 mg L^{-1}) was less toxic than LCH (LC_{50} = 44.32 mg L^{-1}) against *A. gossypii* first nymphal instar after 48 h treatment. There was no significant difference in the developmental duration of total nymphal stages among the three treatment concentrations (LC_{10}, LC_{25} and LC_{50}) and the control. Treatments with K_2SiO_3 significantly reduced the longevity and fecundity of adult aphids. Reduction percentages of *A. gossypii* field strain after feeding on cotton leaves sprayed with K_2SiO_3 were 47.1 and 55.0 % at 100 and 200 mg L^{-1}, respectively, compared to 67.9 and 77.5% in LCH. The higher levels of catalase and polyphenol oxidase enzymes activities was observed at 24 h with percentages 68.16 and 32.74%, respectively, compared to the control, then declined and reached to minimum value at 96 h. However, peroxidase enzyme activity was increased with percentage 59.72 % at 12 h and decreased to reach minimum value at 48 h. This study could introduce promising plant resistance inductors for use in integrated pest management of *A. gossypii* on cotton plant.

Keywords: *Aphis gossypii*; potassium silicate; induced resistance; biological parameters; antioxidant enzymes.

INTRODUCTION

The cotton aphid, *Aphis gossypii* Glover (Hemiptera: Aphididae), is a devastating polyphagous insect pest worldwide (Blackman and Eastop, 2000). It not only damages to crops by sucking phloem sap and producing honeydew, which promotes the growth of sooty mold fungus, disrupts leaf respiration and photosynthesis but also transmits several plant pathogenic viruses (Campolo *et al*., 2014; Afloukou *et al*., 2021). So, aphids control is heavily reliant on the frequent application of systemic insecticides, which along with short life cycle and rapidly reproductive rate have evolved resistance to a wide spectrum of insecticides in addition to the health and environmental risks (El-Dewy *et al*., 2018; Chen *et al*., 2020; Ma *et al*., 2021).

Plants have evolved varied tactics to defend themselves from herbivore attack. Insect herbivores among biotic stress pose the largest damage to plants, as they have severely depleted crops (War *et al*., 2012). In this respect, there is a pressing need to develop effective and eco-friendly alternative pest control methods. Induced resistance in plants to herbivores may be one of these viable approaches in integrated pest management, where inductors can play a vital role in plant resistance to a variety of biotic and abiotic stresses (Debona *et al*., 2017; Song *et al*., 2021). Most inductors are relatively low in toxicity compared to the synthetic insecticides and consequently limit of frequent uses of insecticides spray, reduce environmental hazardous, delay resistance development, improve crop yield besides prospective compatibility with biological control by natural enemies (Boughton *et al*., 2006). Inductors are applied as foliar

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spray, seed treatment, or soil drench (Gordy *et al.*, 2015).

Studies have shown that using inductors like silicon (Si) enhances plant protection against abiotic and biotic stressors, due to the mechanical barrier created by the silica precipitation in the foliar tissues and in the trichomes, as well as the production of phenolic compounds, chitinases, peroxidases and lignin, which can all interfere with the physiology and development of insect pests (Alcantra *et al.*, 2011; Adss *et al.*, 2021). Si may act directly on insect herbivores by reducing insect growth and reproduction and thus reducing crop damage, or it may act indirectly through lower plant penetration, allowing natural enemies easier access to the crop (Reynolds *et al*., 2009; Züst and Agrawal, 2016).

Plants are subjected to various stressors, which cause an over accumulation of reactive oxygen species (ROS), including free radicals, resulting in increasing oxidative damage and cell death. The antioxidant enzymes such as catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7) and polyphenol oxidase (PPO; E.C. 1.10.3.2), which protects the cell from oxidative damage, is responsible for maintaining the balance between the detoxification and generation of ROS (Hasanuzzaman *et al.*, 2020; Dumanovic´ *et al.*, 2021). Higher levels of POD and PPO have been linked to reduced insect growth and development in several plants (Zhang *et al.*, 2008; Bhonwong *et al.*, 2009).

The purpose of this study was to test how K_2SiO_3 at sublethal concentrations influenced the survival, development and reproduction of all *A. gossypii* developmental stages. The induction of foliar spraying with K_2SiO_3 on cotton plant resistance against *A. gossypii* field strain under greenhouse conditions as well as the antioxidant enzymes activities were also assessed.

MATERIALS AND METHODS

Test insect

A laboratory strain of *Aphis gossypii* Glover was originally collected from the cotton fields in Abees district, Alexandria, Egypt, and reared on cotton plant leaves, *Gossypium barbadense* Linnaeus var. (Malvaceae) over many generations. It reared in a greenhouse at a temperature of 25±2˚C and relative humidity of 65±5% under natural lighting without exposure to insecticides. A field strain of *A. gossypii* was collected and maintained at the same previous conditions before tested.

Chemicals

Potassium silicate® (K_2SiO_3- contains 22.5 % SiO_2 and 10.25 % K_2O) was purchased from El-Goumhouria Company for trading medicines, chemicals and medical appliances, Cairo, Egypt. Lambda-Cyhalothrin (Lambada® 5% EC) was produced by Dow AgroSciences Co. Other chemicals used for enzyme assays were purchased from Sigma–Aldrich, Germany.

Bioassays

The laboratory experiments were conducted in the Department of Applied Entomology and Zoology, Faculty of Agriculture, Alexandria University, Egypt. The efficacy of K_2SiO_3 against the laboratory strain of *A. gossypii* first instar nymphs was tested using a standard leaf dip method (Moores *et al.*, 1996). Five concentrations of both K_2SiO_3 and LCH (12.5, 25, 50, 100 and 200 mg L^{-1}) were prepared in distilled water. LCH was used as a positive control. Cotton leaf discs (5 cm in diameter) were dipped in each concentration for 20 sec and in distilled water served as a control. Treated and control leaf discs were allowed to dry for one hour and then placed in each Petri dish (9 cm in diameter) containing filter paper. Each treatment plus the control was repeated three times. Twenty of the aphid's nymphs were placed per each replicate. The Petri dishes were kept under laboratory conditions at a temperature of 25±2ºC, relative humidity of 65±5% and natural lighting periods. Recorded the number of aphid's nymphs mortality 48 h after treatment and then calculated the LC_{50} values of K_2SiO_3 and LCH using probit analysis (Finney, 1971).

Biological parameters

The sublethal concentrations at LC_{10}, LC_{25} and LC_{50} values were used for evaluate the effects of K_2SiO_3 on the development and reproduction of 1st, 2nd, 3rd, 4th instar nymphs and adult stage of *A. gossypii*. The method of treatment was similar to the previous method described in ''Bioassays'' section: Cotton leaf discs were dipped in each concentration. One hundred from newly molted 1st, 2nd, 3rd, 4th instar nymphs and adult stage were examined for each treatment. After 48 h, the surviving aphid individuals were transferred to untreated cotton leaf discs until the adult stage. The leaves were changed when necessary. Petri dishes were reserved in the laboratory. During the experiment, the duration of the nymph developmental stage, the longevity of adult aphids, and the daily fecundity of adult viviparous females were recorded.

Greenhouse Trial

The experiments were carried out in the greenhouse of Cotton Pesticides Evaluation Department, Plant Protection Research Station, El-Sabhia, Alexandria, Egypt. The efficacy of foliar spray application of K_2SiO_3 compared to LCH against the field strain of *A. gossypii* third nymphal instar was evaluated on cotton plants. Cotton seeds (*Gossypium barbadense* Linnaeus var. Giza 92) were cultivated in plastic pots (25-cm diameter and 18-cm height) containing clay mixed with sphagnum peat moss. Thirty days after emergence,

K_2SiO_3 and LCH at 100 and 200 mg L^{-1} were applied by using hand-held sprayer 1 liter capacity until leaves were saturated and left to dry. The untreated plants were sprayed with water only. All treatments plus the control were replicated five times. Each pot was individually infested with twenty third-instar nymphs of *A. gossypii* field strain and enclosed with wooden trusses covered with muslin. Mortality percentages were assessed after 2 h from the infestation and then after 1, 2, 3, 4 and 7 days.

Biochemical assays

- **Homogenate preparation**

Cotton plants were treated with foliar spray application of K_2SiO_3 at LC_{50} concentration. Control plants were sprayed with water only. The leaf samples were randomly collected after 12, 24, 48, 72 and 96 hours from the application and immediately frozen till used. Treated and control plants were replicated 3 times. Leaves (0.5 g) of each replicate were homogenized in 3 ml of ice cold 0.1 M Tris-HCl buffer (pH 7.5) containing 2-mercaptoethanol (5 mM), 1% (w/v) polyvinylpyrolidone (PVP) and 0.5 mM ethylenediamine tetraacetic acid (EDTA). The homogenate was centrifuged at 10,000 rpm at 4°C for 20 min. The obtained supernatant was used for enzyme activities and total protein estimations.

- **Total protein content**

The protein content was determined according to Bradford (1976) by using bovine serum albumin as a standard.

- **Enzyme assessments**

The CAT, POD and PPO activities were measured by using the methods of Aebi, (1984); Kar and Mishra (1976), respectively.

Statistical analysis

Mean values of treatments were compared for significance using analysis of variance (ANOVA) test with $LSD_{0.05}$ (CoStat Statistical Software, 2005). The mortality percentages in the laboratory and greenhouse experiments were corrected according to Abbott equation (Abbott, 1925). LC_{10}, LC_{25}, LC_{50} values and slope of the concentration–mortality regression line values were calculated by using probit analysis (Finney, 1971).

RESULTS AND DISCUSSION

Toxicity of K_2SiO_3 to *A. gossypii* under laboratory conditions

Data presented in Table 1 showed that, K_2SiO_3 (LC_{50} = 58.21 mg L^{-1}) was less toxic than LCH (LC_{50} = 44.32 mg L^{-1}) against the first instar nymphs of *A. gossypii* after 48 h treatment with a leaf dip method. LC_{10} and LC_{25} values were 8.89 and 21.65 mg L^{-1}, respectively for K_2SiO_3 versus to 7.06 and 16.86 mg L^{-1} for LCH.

Effects of K_2SiO_3 on the development and reproduction of *A. gossypii*

The effects of K_2SiO_3 at sublethal concentrations on the developmental duration of nymphs, adult longevity and fecundity of *A. gossypii* at different stages are presented in Table 2. K_2SiO_3 significantly extended the developmental duration of only the second instar nymphs at the LC_{50} concentration (second instar: F = 4.28; df = 3.213; $P < 0.0001$, third instar: F = 5.24, df = 3.213; P = 0.0019, fourth instar: F = 6.18, df = 3.213; P = 0.0085). Statistically, there was no significant difference in the developmental duration of total nymphal stages among the three treatment concentrations and the control (F = 1.96; df = 3.213; P = 0.14). Treatments with K_2SiO_3 significantly reduced the longevity of adult aphids, as the values were 15.45 days (LC_{10}), 14.82 days (LC_{25}), 11.23 days (LC_{50}) and 17.69 days (control) (F = 32.096; df = 3.160; $P < 0.001$). The average number of nymphs per female (fecundity) (F = 73.018, df = 3.160; $P < 0.001$) was 16.28 (LC_{10}), 12.65 (LC_{25}), and 10.09 (LC_{50}) nymph/female compared to 19.50 nymph/female in the control.

The obtained results elucidated that K_2SiO_3 at lowest concentration prevented aphids' individuals to reach the adult stage by inhibiting the completion of their life cycle.

Table 1. Toxicity of potassium silicate and lambda-cyhalothrin to the first instar nymphs of *A. gossypii* after 48 h treatment under laboratory conditions

Treatment	LC_{10} (mg L^{-1}) (95% CL)[a]	LC_{25} (mg L^{-1}) (95% CL)	LC_{50} (mg L^{-1}) (95% CL)	Slope ± (SE)[b]	(χ^2)[c]
Potassium silicate	8.89 (4.75 - 13.30)	21.65 (14.78 - 28.38)	58.21 (46.39 - 74.04)	1.57 ± 0.19	0.23
Lambda-cyhalothrin	7.06 (3.66 - 10.77)	16.86 (11.12 - 22.46)	44.32 (35.09 - 55.41)	1.61 ± 0.20	0.25

[a] Confidence limits

[b] Slope of the concentration-mortality regression line ± standard error.

[c] Chi square value.

Table 2. Effects of potassium silicate at sublethal concentrations on the developmental duration of nymphs, adult longevity and fecundity of *A. gossypii* at various life stages

Life stage	Treatment (mean ± SE)			
	Control	LC_{10}	LC_{25}	LC_{50}
First instar (day)	$2.04^{a} \pm 0.10$	$2.15^{a} \pm 0.13$	$2.23^{a} \pm 0.08$	$2.28^{a} \pm 0.07$
Second instar (day)	$2.24^{b} \pm 0.11$	$2.32^{ab} \pm 0.09$	$2.38^{ab} \pm 0.06$	$2.62^{a} \pm 0.11$
Third instar (day)	$2.52^{a} \pm 0.07$	$2.17^{c} \pm 0.09$	$2.55^{a} \pm 0.13$	$2.30^{b} \pm 0.08$
Fourth instar (day)	$2.58^{ab} \pm 0.06$	$2.50^{b} \pm 0.11$	$2.64^{a} \pm 0.07$	$2.68^{a} \pm 0.10$
Total nymph stage (day)	$9.38^{a} \pm 0.17$	$9.14^{a} \pm 0.13$	$9.80^{a} \pm 0.19$	$9.88^{a} \pm 0.21$
Adult longevity (day)	$17.69^{a} \pm 1.12$	$15.45^{b} \pm 1.01$	$14.82^{b} \pm 0.98$	$11.23^{c} \pm 0.92$
Fecundity (individual)	$19.50^{a} \pm 1.47$	$16.28^{b} \pm 1.42$	$12.65^{c} \pm 1.23$	$10.09^{d} \pm 1.38$

Means followed by different letters within the same row denote a significant difference at $P < 0.05$. LC_{10}, LC_{25} and LC_{50} are 8.89, 21.65 and 58.21 mg L^{-1}, respectively

The use of silicon-induced plant resistance as a tactic for protecting plants against insects has been displayed in reducing the feeding time of the aphid, *Schizaphis graminum* (Rondani) in wheat plants (Costa *et al*., 2011), changed in oviposition preference (Peixoto *et al.*, 2011), reduced the palatability of sunflower leaves to attack of caterpillar, *Chlosyne lacinia saundersi* (Assis *et al.*, 2013), decreased fecundity (Alvarenga *et al.*, 2017), on the biology and feeding preference of *Spodoptera frugiperda* (J. E. Smith) (Mondego *et al.*, 2018), reduced growth and the development of pests (Frew *et al.*, 2017; Yang *et al.*, 2018) and inhibited larval performance and their insecticide tolerance (Wang *et al.*, 2020).

Efficacy of K_2SiO_3 against *A. gossypii* under greenhouse conditions

Results on Table 3 revealed that, the mean mortality percentages of the field strain of *A. gossypii* third nymphal instar after feeding on cotton plants leaves sprayed with K_2SiO_3 were 47.1 and 55.0 % at 100 and 200 mg L^{-1}, respectively, compared to 67.9 and 77.5 % in LCH. The mortality percentages of all treatments decreased over 7 days from the infestation. Using of silicon has been reported as an inducer of plant resistance to pest attack (Reynolds *et al*., 2009; Teixeira *et al*., 2017; Yang *et al*., 2017). Effectiveness of silicon applications on plant resistance induction and protect crop losses from chewing insects e.g., fall armyworm (*S. frugiperda*) was tested by Gordy *et al*., 2015; Nascimento *et al.*, 2018; Acevedo *et al.*, 2021; Nagaratna *et al*., 2021 and sucking insects e.g., green peach aphid, *Myzus persicae* (Sulzer) (Boughton *et al*., 2006; Ranger *et al*., 2009; Hong *et al.*, 2019) and whitefly (*Bemisia tabaci* Gennadius) (Ferreira *et al*., 2011; Abassi *et al*., 2020). When also compared to soil application alone on sunflower plants, combining applications of silicon with the soil and foliar spray together reduced the number of sunflower caterpillar, *Chlosyne lacinia saundersii* almost 10 % (Assis *et al*., 2013).

Activity of antioxidant enzymes on cotton plant

The catalase (CAT), peroxidase (POD) and polyphenol oxidase (PPO) activities were significantly increased after 12, 24, 48, 72 and 96 h from the spraying with K_2SiO_3 at LC_{50} concentration on cotton plant compared to the control. Where, the CAT activity was highly increased at 24 h from the application (29. 36 µmol min^{-1} mg^{-1} protein) compared to 17.46 µmol min^{-1} mg^{-1} protein in the control, then decreased and reached to the minimum value at 96 h compared to the control (Fig. 1).

Table 3. Efficacy of potassium silicate and lambda-cyhalothrin against the field strain of *A. gossypii* third nymphal instar under greenhouse conditions

Treatment	Conc. (mg L^{-1})	Corrected mortality (%) after the indicated periods from the infestation						General mean ± SE
		2 h	1-day	2-days	3-days	4-days	7-days	
Potassium silicate	100	95.0	77.5	60.0	32.5	15.0	2.5	$47.1^{d} \pm 1.4$
	200	100.0	85.0	67.5	45.0	22.5	10.0	$55.0^{c} \pm 1.6$
Lambda-cyhalothrin	100	100.0	95.0	80.0	62.5	45.0	25.0	$67.9^{b} \pm 1.3$
	200	100.0	100.0	90.0	75.0	60.0	40.0	$77.5^{a} \pm 1.2$

Means followed by different lowercase letters within the same column denote significant difference at $P < 0.05$. Each treatment in each period from the infestation was replicated five times with twenty nymphs.

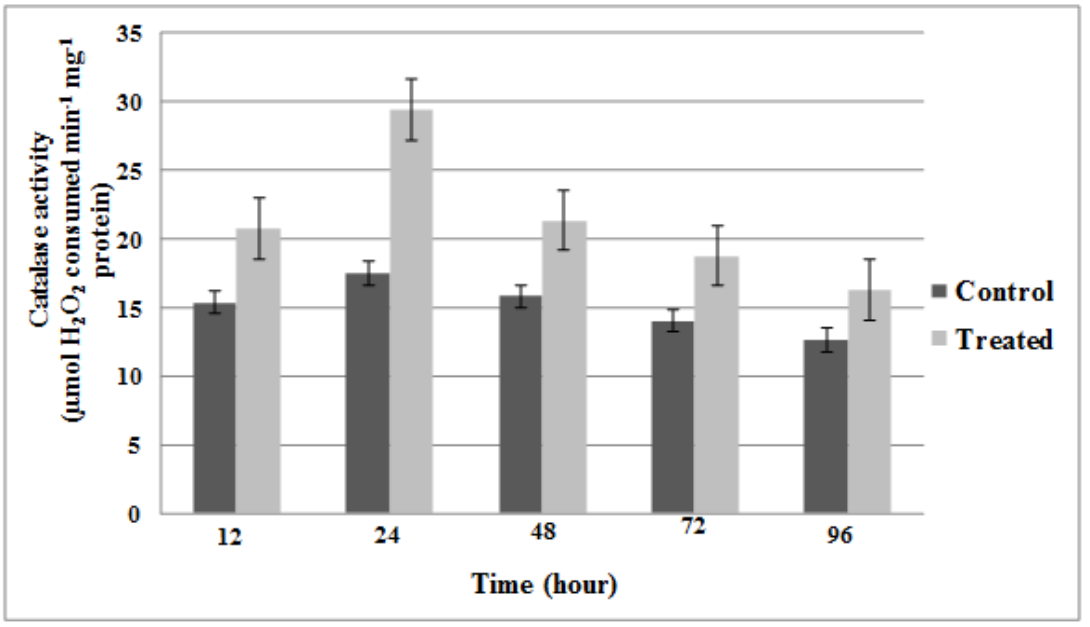

Figure 1. Induction of catalase (CAT) activity on cotton plants after different intervals from the application with potassium silicate at LC_{50} concentration

The POD activity was increased at maximum value at 12 h (14.62 µmol min^{-1} mg^{-1} protein) compared to 9.76 µmol min^{-1} mg^{-1} protein in the control, then gradually decreased and reached to the minimum value at 48 h. POD activity was increased again at 96 h (Fig. 2). However, the activity of PPO was gradually increased after 24 h from the application (11.19 µmol min^{-1} mg^{-1} protein) compared to 8.43 µmol min^{-1} mg^{-1} protein in the control, and then declined to reach the minimum value at 96 h compared to the control (Fig. 3).

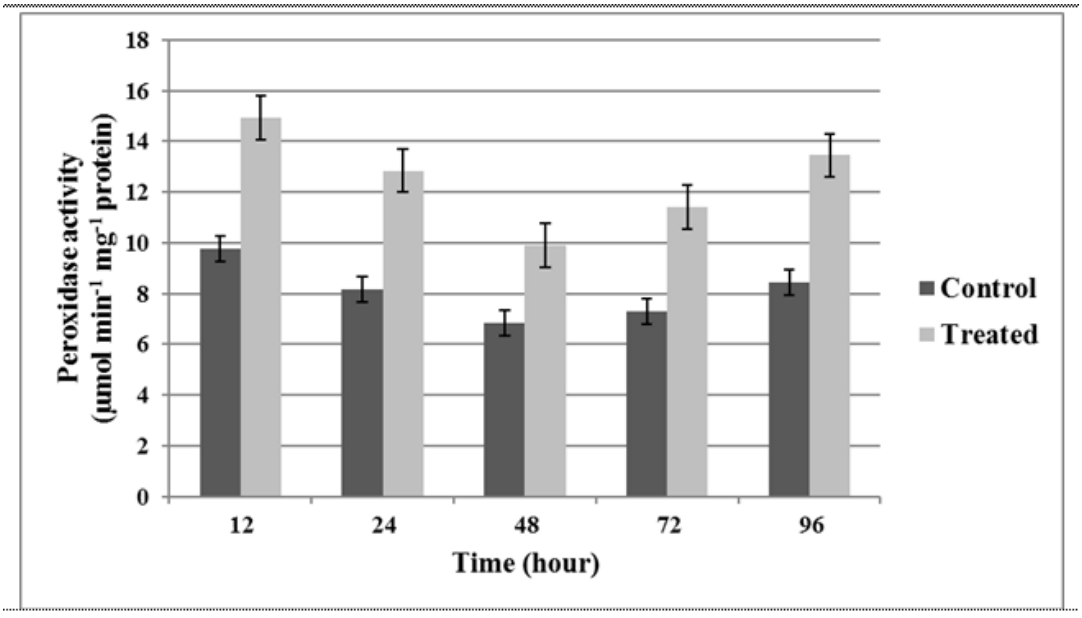

Figure 2. Induction of peroxidase (POD) activity on cotton plants after different intervals from the application with potassium silicate at LC_{50} concentration

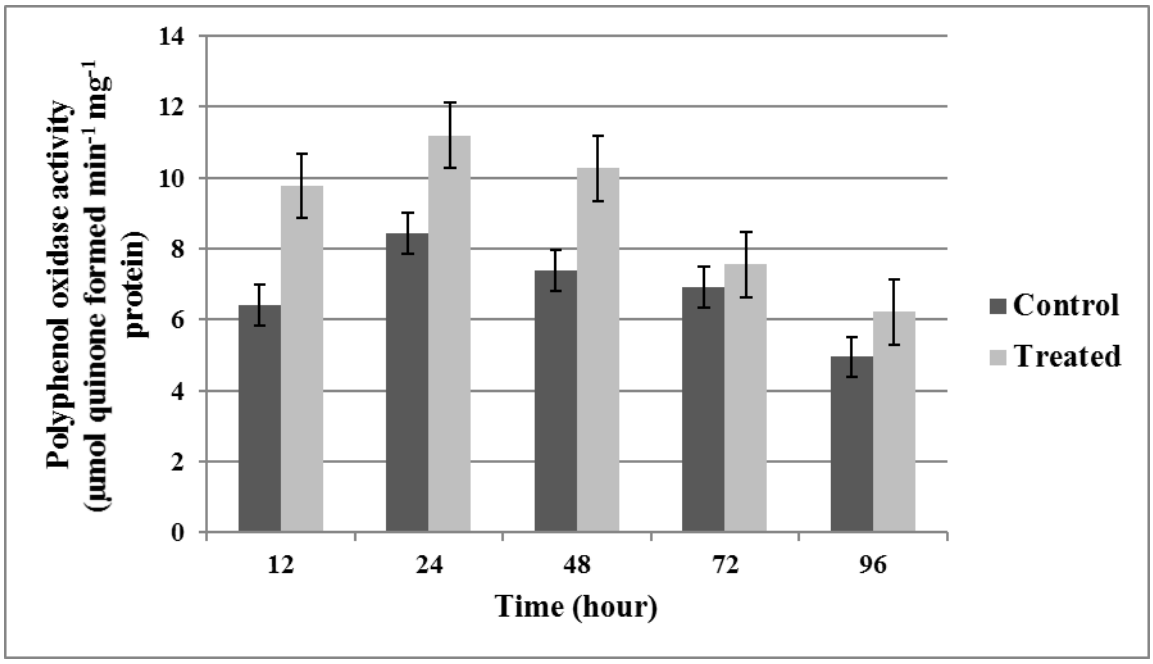

Figure 3. Induction of polyphenol oxidase (PPO) activity on cotton plants after different intervals from the application with potassium silicate at LC_{50} concentration

Referring to the CAT, POD and PPO activities of cotton plant, the elevated levels of these antioxidant enzymes could indicate that play an important role in plant defense following herbivores infestation. Where, they represent the majority components in the scavenging mechanism of reactive oxygen species. Previous studies have reported the induction of antioxidant enzymes activities in various plants to combat oxidative stress caused by insects (El-Sherbeni *et al.,* 2018; Yildiztekin *et al.,* 2019; El-Zahi *et al.,* 2021). The increases in activity of many of these enzymes are contributed to plant resistance by lowering the nutritional value of host plants (Chen *et al.,* 2009). The increased levels of CAT seem to be linked with enhancing the cell wall resistance, averting plant cell damage and acting as signals for the induction of defense genes (Rani and Jyothsna, 2010). When POD interacts with phenols, it produces phenoxy and other oxidative radicals that act as feeding deterrents and/or produce toxins that diminish the plant tissue digestibility, affecting insect growth and development dramatically (Zhang *et al.,* 2008). Whereas, the role of PPOs in reducing the nutritional quality, digestibility and palatability of plant tissues to insects as well as the formation of quinones by stimulating phenolic oxidation is a highly appreciating defensive response against herbivores (Mahanil *et al.,* 2008; Bhonwong *et al.,* 2009). Overall, these findings demonstrate that K_2SiO_3 could be used successfully to inhibit the build-up of cotton aphid's population at low rate of application. The elevated levels of CAT, POD and PPO enzyme activities may be one action mode of cotton plants to counteract *A. gossypii* attack.

CONCLUSIONS

Induced plant resistance can be conferred by using K_2SiO_3, which inhibits the survival, development and reproduction of *A. gossypii* on various life stages. Effects of sublethal concentrations on biological traits are very important because offspring can then be reduced and as a consequence, the insect population can be maintained below a level of economic loss and minimize the environmental hazardous. This approach may potentially serve as an alternative technique that can be integrated into the management of *A. gossypii* on cotton plants.

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الملخص العربي

تأثير سيليكات البوتاسيوم على بقاء وتطور وتكاثر منّ القطن*Aphis gossypii* Glover

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تعد مقاومة النبات المستحثة عنصرًا مهمًا في مكافحة الآفات. ولذلك، تم أختبار تأثير سيليكات البوتاسيوم على بقاء وتطور وتكاثر منّ القطن *Aphis gossypii* Glover في مراحل النمو المختلفة تحت الظروف المعملية. تم أيضا تقييم فعالية الرش الورقي لسيليكات البوتاسيوم ضد حوريات الطور الثالث لمنّ القطن في ظروف الصوبة الزجاجية بالأضافة لتأثيرة على أنشطة الإنزيمات المضادة للأكسدة. في الدراسات المعملية، كانت سيليكات البوتاسيوم أقل سمية من اللمدا– سيهالوثرين ضد حوريات العمر الأول لمنّ القطن بعد ٤٨ ساعة من المعاملة. لم يكن هناك فروق معنوية واضحة بين المعاملات الثلاثة في الفترة الكلية لتطور الحوريات مقارنة بالكنترول. أدت المعاملة بسيليكات البوتاسيوم إلى إنخفاض كبير في طول العمر وخصوبة حشرات المنّ البالغة. كانت نسب الموت في السلالة الحقلية لمنّ القطن بعد تغذيتها على أوراق القطن المعامل بسيليكات البوتاسيوم ٤٧,١ و٥٥,٠ ٪ عند ١٠٠ و٢٠٠ ملجم/ لتر على التوالي، مقارنة بـ ٦٧,٩ و٧٧,٥ ٪ في اللمدا – سيهالوثرين. كما لوحظ ارتفاع مستويات أنشطة إنزيمات الكاتلاز والبولى فينول اكسيديز عند ٢٤ ساعة بنسب ٦٨,١٦ و٣٢,٧٤ ٪ على التوالي مقارنة بالكنترول، ثم تراجعت ووصلت إلى أدنى قيمة عند ٩٦ ساعة. وزاد نشاط إنزيم البيروكسيديز بنسبة ٥٩,٧٢ ٪ عند ١٢ ساعة وانخفض ليصل إلى أدنى قيمة عند ٤٨ ساعة. يمكن أن تقدم هذه الدراسة محاثات واعدة لمقاومة النبات لإستخدامها في المكافحة المتكاملة لمنّ القطن.

الكلمات المفتاحية: منّ القطن، سيليكات البوتاسيوم، المقاومة المستحثة، الجوانب البيولوجية، الإنزيمات المضادة للأكسدة.