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Resistance induction in lima bean plants by silicon and ascorbic acid

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Abstract:

Abiotic elicitors of plant defense can induce plant resistance effective against various insect pests. Vegetable crops are currently infested with several lepidoptera caterpillars causing economically devastating crop losses. To date, there is no one treatment or technique has been found to be effective in all cases to control insect pest infestations. In this study the possible effects of silicon and ascorbic acid to induce resistance in lima bean against feeding activity of *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) larvae were evaluated under greenhouse and laboratory conditions. Results indicated that the foliar spray of silicon (1%) and ascorbic acid (0.2%) as individual application on lima bean 30 days after seedlings emergence reduced the feeding activity of the third instar larvae at 5 days after treatment by 29.6 and 19.2%, respectively. The best efficacy was obtained using a combined treatment by soil drench of silicon 1% and foliar spray by a mixture of (silicon 1% and ascorbic acid 0.2%) (1:1 v/v) showed feeding inhibitory activity of 35.7% and larval mortality of 32.7%. This combined treatment exhibited also significant increase in the activities of two enzymes (polyphenol oxidase and peroxidase) involved in plant defense mechanism. Consequently, the use of silicon and ascorbic acid for induced resistance of plant to insects could be contributed in reducing the use of conventional insecticides.

Introduction

Insect herbivores are responsible for about 15- 35 % of crop annual losses in vegetable production in Egypt and worldwide. Because of less agrochemicals being used and less new insecticides coming on the market due to environmental concern, research efforts are now being directed to find acceptable and safer alternatives which are required for economically viable and

environmentally safe crop protection measures. One such possible alternative to synthetic pesticides is host plant resistance by using more resistant varieties and induction of plant resistance by biotic or abiotic elicitors including silicon (Basagli *et al*., 2003; Cherif *et al*., 1994; Eldoksch and El-Sebae, 2009) and ascorbic acid (Felton and Summers, 1993).

Silicon is not considered an essential element for most plants but research findings indicated that absorption of soluble silicon by plants is beneficial to crops via inducing resistance and protection against pest attack (Epstein, 1994 and Massey *et al*., 2006).

The protection in plants by silicon could be due to its accumulation and polymerization in the plant cells, to form a mechanical barrier as silica – cuticle double layers that difficult to be attacked by the insect pests (Massey *et al*., 2006; Ma and Yamaji, 2006 and Teixeira *et al*., 2017). Furthermore, mechanical barriers are not the only defense mechanism against external agents. Investigations with cucumber plants have shown induced resistance of silicon – treated plants to the fungus *Pythium* spp., resulting from the accumulation of phenolic compounds, lignin and phytoalexins (Cherif *et al*., 1994 and Fawe *et al.*,1998). Materials that induce such defense response in plants called elicitors that can trigger the induced resistance process. Freitas *et al*. (2012) evaluated the use of silicon in integrated management of diamondback moth, as a physical barrier, reducing the use of pesticides for cabbage insect control and found that mortality was high in treatment with 12kg/ha of silicon , they concluded that silicon damaged larval jaw, limiting ingestion and causing high mortality.

Defense related proteins and ascorbic acid in higher plants have received also considerable attention as sources of resistance against insect pests. Felton and Summers (1993) indicated that ascorbic acid is essential for both nutritive and antioxidant functions in phytophagous insects. They indicated that the plant enzyme ascorbate oxidase retains activity in the digestive system of the herbivore *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) and high levels of the enzyme are present in several host plants. The enzyme oxidizes L- ascorbic acid to dehydro-L-ascorbic acid, a potentially toxic product. The oxidation of ascorbic acid also produces active oxygen species such as the highly reactive hydroxyl radical, and the nutritional quality of protein for larval feeding was significantly reduced by treatment with ascorbic acid and ascorbate oxidase.

In the present study, using lima bean plants the effect of silicon and ascorbic acid applied alone and in combination as soil or foliar treatment on feeding activity, and mortality of Spodoptera caterpillars were tested. The effect of silicon and ascorbic acid treatment on plant defensive enzymes, peroxidase (POX) and polyphenol oxidase (PPO) was also investigated.

Materials and methods

1. Test plants:

Lima bean seeds (*Phaseolus lanatus* L.) were sown in sandy loam soil in 10 cm diameter plastic pots and grown in greenhouse conditions. Plants could grow for 30 days before exposure to chemical treatment and 35 days before exposure to Spodoptera caterpillars. Plants were placed in rows according to each treatment and six replicate plants were made for each treatment. Plants were watered as needed through the

entire experiment, soil treatment was carried out using 100 ml solution of formulated Mg silicate applied alone with the rate of (1.0%) or in combination with ascorbic acid (0.2%) as 1:1 ratio using drench method.

2. Experimental design:

Chemical treatment in pot experiment consisted of : T1- control (spray with water containing 0.1 % Tween 80), T2- soil treatment with magnesium silicate by drench method (1.0 %), T3- foliar treatment with magnesium silicate, Mg Si (1.0 %) ,T4- foliar treatment with ascorbic acid (0.2%), T5- combined treatment (Soil and foliar treatment) including soil drench with magnesium silicate, 1.0% and (foliar treatment with magnesium silicate, 1.0% + ascorbic acid, (0.2 %) , (1:1, v/v).Tween-80, 0.1% (detergent) was added in each of the prepared solution to allow equal distribution of solution on the lima bean plant leaf surface and to improve the ability of the plant to absorb the solution more readily. Water + tween-80 was used as a control solution. The plants were sprayed by a 500 ml hand spray bottle until run off 5 days prior to the application of the Spodoptera caterpillars bioassays.

3. Bioassays:

3.1. Shorth- term 24 h caterpillars feeding:

A laboratory strain of the cotton leafworm *Spodoptera littoralis* (Boisduval) (Lepidoptera: Noctuidae) was continuously reared on castor bean leaves was used in the bioassays. Five third instar larvae of the caterpillars were placed together with a treated and weighted lima bean leaf from exogenously sprayed lima bean plants in a petri dish (9 cm diameter) or from plants grown in treated soil and allowed to consume the diet for 24 hrs. Six replicates were used for each treatment including the control. The leaves were removed after 24 hrs exposure and weighed to determine the percentage eaten by the larvae. The average of feeding activity of 30 larvae per each treatment was calculated. The feeding inhibitory activity of leafy diet was determine using the equation of Wada and Manukata (1968) for feeding ratio = B/A x 100, Where A = amount of diet consumed in control and B = amount of diet consumed in the treated diet.

3.2. Long – term (12 days) caterpillars feeding:

The second instar larvae of *S. littoralis* were selected for assaying the effect of silicon and ascorbic acid applied alone or in combination and other treatments on lima bean seedlings and their potential resistance to feeding activity of Spodoptera caterpillars. The larvae could consume foliage for 12 days on the different treatments and control leaves present in plastic vials, each vial contains five 2nd instar larvae were placed together on treated lima bean leaf which was changed every two days by fresh one. The vials were covered and maintained at about 26 °C. After 12 days of exposure, the larvae were removed and then they were transferred back to the normal leafy diet. The vials were checked daily for pupation.

3.3. Enzymatic activity determination:

Lima bean leaves were macerated with a mortar and pestle and 10 ml of potassium phosphate buffer (0.1mol/L; pH 6.0) were added to 0.2 g of macerated leaves. The resulting solution was resting for one hour at 4 ºC and the agitation for three times and then the solution was centrifuged at 13000 g for 15 minutes, at 4 ºC for obtaining the supernatants (enzyme extract) which used for enzyme activity determination. The activity of

peroxidase (POX) and polyphenol oxidase (PPO) were measured by spectrophotometry via the increase of optical density (OD), using OD 470 min^{-1} g^{-1} and OD 420 min^{-1} g^{-1}, respectively, following methodology described by Mohammadi and Kazemi (2002) with minor modification . The substrate utilization by peroxidase and polyphenol oxidase were guaiacol / H_2O_2, and catechol, respectively. The enzymatic activity was expressed as units per gram of fresh weight (ug^{-1}). One activity unit was defined as the increment of 0.1 absorbance unit per minute. The result was reported as mean ± SD.

3.4. Statistical analysis:

Data of lima bean leaf weights consumed by *Spodoptera* larvae were statistically analyzed using analysis of variance (ANOVA) with multiple comparison tested with the Duncan's multiple range test method. The *P*-value (0.05) was used for deciding the degree of significance of the different treatments.

Results and discussion

1. Short - term bioassay:

The effects different treatments of soil magnesium silicate (MgSi, 1%), foliar MgSi 1% and foliar ascorbic acid (AA, 0.2%) as well as the combined treatment of soil MgSi and foliar (MgSi + AA) (1:1, v/v) on caterpillar anti-feeding activity during 24 hrs exposure period are presented in Table (1). The data indicated that the combined treatment of soil drench with Mg silicate and foliar application with the mixture of ascorbic acid and Mg Si (1:1) gave the highest feeding inhibitory activity against 3rd instar larvae of spodoptera caterpillars for 24 h feeding period with 35.7 % feeding inhibition followed by foliar Mg silicate, foliar ascorbic acid and then soil drench using Mg silicate with 29.6, 19.2 and 17.4 % reduction in feeding activity respectively compared with the control.

Table (1) : Anti-feeding activity of soil and foliar - applied magnesium silicate (MgSi) and ascorbic acid (AA) against 3rd instar larvae of *Spodoptera* caterpillars.

Treatment	Concentr-ations %	Avg. wt of died consumed per 5 larvae during 24h (mg ±SD)	Feeding ratio (and of control)	Feeding inhibition %
T1: control	-	115±12d	100	0.0
T2: soil MgSi	1.0	95±15c	82.6	17.4
T3: foliar MgSi	1.0	81±8b	70.4	29.6
T4: foliar AA	0.2	93±11c	80.8	19.2
T5: Soil MgSi and Foliar MgSi + AA (1:1)	1.0 + (1.0 + 0.2)	74±7a	64.3	35.7

Mean values followed by different letters differ significantly at 5% level using Duncan's multiple range test

2. Long- term bioassay:

Results after 12 days feeding period of 2nd instar larvae of spodoptera caterpillar are presented in Table (2). The data showed that the effect of the combined treatment of soil drench by Mg silicate (1%) and foliar spray by (Mg silicate (1%) + ascorbic acid (0.2%) (1:1, v/v) exhibited the highest larval mortality (32.7%) followed in a descending order by foliar spray with Mg silicate (24.4%) then, foliar spray treatment with ascorbic acid and then soil drench with Mg silicate with larval mortality of 19.4 and 17.7 %

respectively. Larval mortality was based on % of larvae that did not pupate.

The data of long-term bioassay of caterpillars feeding activity indicated that foliar spray by silicon or ascorbic acid alone made lima bean plants more resistance to spodoptera larvae damage compared with the control. Also, treatment of soil with silicon alone or in combination with ascorbic acid made the plant leaves more resistance to the consumption by *Spodoptera* larvae.

Treatments of soil with silicon alone, foliar spray with silicon alone as well as foliar spray with ascorbic acid alone caused reduction in pupation compared with the control with percent pupation 82.3, 75.6 and 80 6% respectively. Ma and Yamaji (2006) indicated that following silicon uptake by the roots, silicic acid is rapidly translocated to the shoot and leaves, and with increasing Si concentration in the plant sap silicon is polymerized to form amorphous silica and silicon increases the resistance of plants to the green aphid *schizaphis graminum* (Rond.). The conducted results agree with conclusion of many investigators. Shalata *et al.* (2001) reported that the antioxidant and pro-oxidant properties of ascorbic acid are becoming increasingly appreciated for induction of plant resistance against insect pests. Rice cultivar with low tissue silicon is associated with increased susceptibility to insect pests as well as increased problems with crop lodging. Basagli *et al.* (2003) and Keeping and Kvedaras (2008) reported that silicon proved to be as a plant defense against insect herbivory and the application of sodium silicate is deposited on cell wall material forming silica – cuticle double layers and silica cellulose double layer in the leaves and stems of the treated plants that affect the performance of insect pests.

Table (2) : Effect of magnesium silicate (MgSi) and ascorbic acid (AA) applied alone and in combination on some biological aspects of *Spodoptera* carerpillars.

Treatment %	Method of application	Larval mortaility	Avg. days to pupation	Pupation %
T1: control	-	5.9	19.8±0.4c	94.1
T2: MgSi (1.0)	Soil dernsh	17.7	16.9±0.7b	82.3
T3: MgSi (1.0)	Foliar spray	24.4	14.8±0.8a	75.6
T4: AA (0.2)	Foliar spray	19.4	17.3±0.4b	80.6
T5: MgSi & MgSi / AA (1:1)	Soil dernsh + Foliar spray	32.7	14.1±0.1a	67.3

-Different letters indicate significantly difference results at 5% level using Duncan's multiple range test.

3. Enzymatic activity determination:

Results of enzymatic activity determination are presented in Table (3). The data indicated that the greatest PPO activity occurred in the combined treatment of soil Mg silicate and foliar Mg silicate mixed with ascorbic acid (1:1) with (420 ug^{-1} fresh weight), followed by the treatment of foliar Mg silicate alone and foliar ascorbic acid alone (374 and 306 ug^{-1} fresh weight respectively) and then the treatment of soil Mg silicate which showed the least PPO activity (261 ug^{-1} fresh weight). All treatments showed higher activity than the control (144 ug^{-1} fresh weight) (Table, 3).

Table (3) : Polyphenol oxidase (PPO) and peroxidase (POX) activities (mean±SD) in lima bean plants.

Treatment	Enzyme activity of Polyphenol oxidase (PPO) ug^{-1} fresh weight	Enzyme activity of peroxidase (POX) ug^{-1} fresh weight
T1 control	144±11d	152±18d
T2 soil MgSi	261±21c	270±31c
T3 foliar MgSi	374±16b	390±17b
T4 foliar AA	306±28b	345±15b
T5 soil MgSi + Foliar MgSi + AA (1:1)	420±18a	511±15a

-Different letters indicate significantly different results at 5% level using Duncan's multiple range test.

The combined treatment of soil Mg silicate and foliar Mg silicate + ascorbic acid (1:1) (511 ug^{-1} fresh weight) showed the higher POX activity than the treatments with foliar Mg silicate and foliar ascorbic acid which had an intermediate activity (390 and 345 ug^{-1} fresh weight, respectively), treatment of soil Mg silicate (270 ug^{-1} fresh weight) showed the least POX activity but all of these treatments showed higher activity than that of the control (152 ug^{-1} fresh weight) (Table, 3). Khattab (2007) indicated that polyphenol oxidase (PPO) and peroxidase (POX) play an important role in the defense mechanism of cabbage plants (*Brassica olereacea* var. capitata) against phloem sucking aphid (*Brevicoryne brassicae* L.) by increasing enzymatic activity. Peroxidases play an important role in defense against other insects (Dowd and lagrimini, 1997) and their activity has reported to increase as a response to herbivory or wounding. In addition, POX can contribute to insect resistance by quinone oxidation which can bind to protein to reduce digestibility in insects. Polyphenol oxidase is the major anti-nutritive enzyme induced also in response to wounding and insect herbivory. This enzyme oxidizes phenolic compounds to quinone reactive molecule which can interact with other biological molecules (Dowd and Lagrimini, 1997).

The application of silicon compounds and ascorbic acid in crop management may provide a viable component of integrated management of insect pests because they leaves no pesticide residues in food or the environment and can be easily integrated with other pest management practices (Liang *et al.*, 2003 and Massey *et al.*, 2006). Consequently, the use of silicon and ascorbic acid for induced resistance of plant to insects would be contributed in reducing the use of conventional insecticides.

References

Basagli, M.A.B.; Moraes, J.C.; Carvalho, G.A. *et al.* (2003) : Effects of sodium silicate application on the resistance of wheat plants to the green aphid *Schizaphis graminum* (Rond.) (Hemiptera: Aphididae). Neotropical Entomology, 32 (4): 459-663.

Cherif, M. ; Asselin, A. and Belanger, R.R. (1994) : Defense responses induced by soluble silicon in cucumber roots infected by Pythium spp. Phytopathology, 84 : 236-242.

Dowd, P.F. and Lagrimini, L.M. (1997) : The role of peroxidase in host insect defense. In: N. Carozzi and M. Koziel (eds): Advances in insect control. Taylor and Francis Ltd., London, England., Pp. 195-223.

Eldoksch, H. and EL-Sebae, A.H. (2009) : Resistance induction in crop plants against insect herbivores and pathogens by elicitors. J. Pest Cont. and Environ. Sci., 17(1/2): 1-18.

Epstein, E. (1994) : The anatomy of silicon in plant biology . Proceedings of the National of Academy of Science of the USA , 91 : 11-17.

Fawe, A.; Abou-Zaid, M.; Menzies, J. and Bélanger, R. (1998): Silicon-mediated accumulation of flavonoid phytoalexins in cucumber. Phytopathology, 88 : 396–401.

Felton, G.W. and Summers, **C.B. (1993) :** Potential role of ascorbate oxidase as a plant defense protein against insect herbivory, Journal of Chemical Ecology , 19 (7): 1553-1568

Freitas, L.M.; Junqueira, A.M. *et al*. (2012) : Potential use of silicon in the integrated management of diamondback moth, *Plutella zylostella* , on cabbage plants. Revesta Coatinga, 25(1): 8-13 .

Keeping, M.G. and Kvedaras, O.L. (2008) : Silicon as a plant defense against insect herbivory. Journal of Animal Ecology, 77: 631-633.

Khattab, H. (2007) : The defense mechanism of cabbage plant against phloem sucking aphid (Brevicoryne brassicae L.) , Aust. J. Basic Appl. Sci., 1(1):56-62 .

Liang, Y.; Chen, Q. *et al*. (2003) : Exogenous silicon (Si) increases antioxidant enzyme activity and reduces lipid peroxidation in roots of salt- stressed barley (Hordeum vulgare L.) Journal of Plant Physiology, 160 :1157-1164.

Ma, J.F. and Yamaji, N (2006) : Silicon uptake and accumulation in higher plants. Trends in Plant Science ,11: 392-397.

Massey, F.P.; Ennos, R.A. and Hartley, S.E. (2006) : Silica in grasses as a defense against herbivores: contrasting effects on folivores and phloem feeder. Journal of Animal Ecology , 75 : 595- 603.

Mohammadi, M. and Kazemi , H. (2002) : Changes in peroxidase and polyphenol oxidase activities in susceptible and resistant wheat heads inoculated with Fusarium graminearum and induced resistance. Plant Science, 162 : 491-498.

Shalata, A.; Peter, M. and Neumann, E. (2001) : Exogenous ascorbic acid (vitamin C) increases resistance of salt stress and reduces lipid peroxidation. Journal of Experimental Botany , 52 (364) : 2207-2211.

Teixeira, N.C.; Oliveira, J. *et al.* (2017). Silicon -mediated resistance against specialist insects in sap- sucking and leaf- chewing guilds in the Si non- accumulator collard. Entomologia Experimentalis et Applicata , 165 (1): 94- 108.

Wada, K. and Manukata, K. (1968) : Naturally occurring insect control chemicals . Isobolidin, a feeding inhibitor and coccoloidine, an insecticide in the leaves of *Cocculus trilobus* D.C. J. Agric. Food Chem., 16:471-474.