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Effect of Exogenously Field Application of Jojoba Oil and Methomyl on Biochemical Constituents of Broad Bean (*Vicia faba* L.) Against Cotton Leafworm (*Spodoptera littoralis* Boisd.)

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ABSTRACT

Plants respond to a wide range of several synthetic chemicals that can stimulate the production of plant defense compounds that help prevent pest infestation. In this study, we examined the effect of exogenous field application of jojoba oil and methomyl on biochemical constituents of broad bean plant cv "Giza 3", grown in reclaimed agricultural soil, and evaluated its ability to capacity to induce a defense response against *Spodoptera littoralis*. In a plant measurements selection assay, jojoba oil and methomyl had significant increases in mineral elements, carbohydrates and amino acids content of broad bean leaves. Results from bioassays jojoba oil and methomyl showed a significant ($p < 0.05$) decrease in feeding activity and growth rate larvae of cotton leafworm. However, foliar spraying of jojoba oil showed a more significant effect on the treated plant than methomyl. Results revealed that exogenous field application of jojoba oil can play an important role in the protection of broad beans from cotton leafworm.

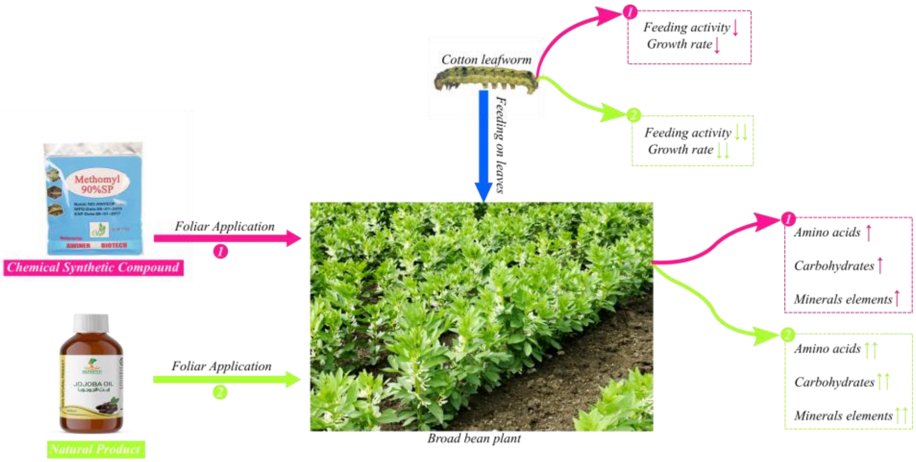

Fig. 1. Effect of exogenous field application of jojoba oil and methomyl on biochemical constituents of a broad bean plant, feeding activity and growth rate of cotton leafworm larvae.

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INTRODUCTION

The cotton leafworm *Spodoptera littoralis* Boisadüval (Lepidoptera: Noctüidae) is the most prevalent one and a serious pest of economic crops worldwide, causing reduced production and quality resulting in huge economic loss to growers (Ismail *et al.*, 2019). Larval stages are the most destructive stages of the insect on cotton and vegetable crops where the larvae can feed on ~90 economically important plant species belonging to 40 families (Ismail *et al.*, 2019). Most applications of insecticides for the control of *S. littoralis* are timed to control the larval instars, which in turn have become highly resistant to most insecticides (Falk *et al.*, 2015). So efforts are being directed for induced host plant resistance as an alternative control tactic to protect themselves from pathogens and herbivores (Inbar *et al.*, 1998).

Host plants protect themselves to counteract the effects of herbivore attack through various morphological, biochemical, and molecular mechanisms. The biochemical mechanisms of defense against herbivores are extensive and highly dynamic and are mediated by direct and indirect defenses **(**Arnold *et al.* (2004; Chen *et al.*, 2009; Hendriks *et al.*, 2009). Defense compounds are produced either directly to reduce the performance and preference of herbivores or in response to plant damage, which may negatively affect the nutrition, growth, and survival of herbivores (War *et al.*, 2012; Gordy *et al.*, 2015; Erb and Reymond 2019). In addition, plants also release volatile organic compounds that indirectly attract natural enemies of herbivores (Conboy *et al.*, 2020). These strategies work either independently or in combination with each other (War *et al.*, 2012). In natural systems, because direct and indirect induced defenses are not presented at maximum levels all the time; various stimuli are needed to induce higher levels of resistance (Ismail 2020; Mouden et al. 2020). Elicitors, natural or synthetic chemicals, have a role in stimulating plant responses. Induced plant resistance has been well studied and reported in more than 100 plant species, which included crops such as soybeans, tomatoes (Mouden *et al.*, 2020) and cotton (Ismail 2020). Jasmonic acid and salicylic acid are the best-documented elicitors to induce resistance in herbivores (Heil et al. 2001; Mouden *et al.*, 2020). Plants respond to a wide range of several other synthetic chemicals that have been used as inducers of resistance against herbivores (Boughton *et al.*, 2005) by which plants acquire a variety of systemic immunity; such as Actigard® (Acibenzolar-S-methyl), Regalia® (*Reynoutria sachalinensis*), methyl jasmonate, etc. It acts as a component of integrated pest management for the sustainable production of crops and the reduction of the amounts of insecticides used in pest control. Therefore, it is obvious from the literature that none has shown that foliar application of essential oils or synthetic chemicals may play a primary role in increasing host plant tolerance to infestation by increasing elicitors of induced plant responses. Hence, the main contribution of this study is to alleviate the harmful effects of the cotton leafworm on the broad beans plant by foliar spraying of commercial formulations of jojoba oil as a natural product and methomyl as a chemical synethetic compound by increasing the tolerance of the host plant through affecting on some of its chemical constituents.

MATERIALS AND METHODS

1. Experimental Design and Site

The current experiment was carried out during the year 2020, on broad bean "Giza 3" obtained from Agriculture Researches Center, Ministry of Agriculture and Land Reclamation Egypt, planted at 8.5 m^2 apart in a sandy clay loam soil under surface irrigation in a private farm located at El-Salhia region, Alexandria Governorate; Egypt. The physicochemical analysis of experimental soil was carried out according to method of Page et al. (1982) as follow: pH (7.80), EC (1.12 dS/m), Na^+ (6.67), Ca^{2+} (2.73), Mg2$^+$ (1.84), Cl^- (19.33), HCO_3 (20.19), CO_3^{2-} (3.46) and SO_4^{2-} (20.63). Sowing was done with a plant-to-plant distance of 15 cm and row to row distance of 70 cm. The treatments were arranged in Randomized Complete Block Design (RCBD) with three replicates/treatments. Fertility management and other agricultural practices were similar for all plants.

2. Plant Materials and Treatments

Broad bean seeds were scattered on 20 September during the 2020 season. Plants were allowed to grow for 30 days until fully ripened before exposure to jojoba oil 96% EC (*Simmodsia chinensis*), Egyptian Natural Co., Egypt and methomyl (Lannate® 90% WP), E. I. Du Pont de Nemours USA. Foliar spray treatments were: control (distilled water + 0.5% Triton X-100), jojoba oil (120 mL/feddan), and methomyl (300 g/feddan). Treatments were applied at two different times – 32 days after sowing and 10 days later. Plants were sprayed by a knapsack sprayer equipped with one nozzle spraying (20 L capacity). The wetting agent of Tween (0.5%) was used.

3. Plant Measurements

3.1. Leaf Sampling

Leaf samples of each treatment were randomly collected at two different times – 2 days after the first spray and 12 days after the second spray then transferred directly to a laboratory. 150 g/treatment was dried at 70 °C for 72 hours in a forced-air oven and then ground with an electric grinder. The changes in carbohydrate, free amino acids and minerals content were measured.

3.2. Amino Acids Content

For the determination of free amino acids, 0.5 g of dried leaves were shaken with 20 ml of 5% sulpho salicylic acid for 1 h at room temperature. Then centrifuged at 5.000 rpm for 15 min, after that the clear supernatants were used.

3.3. Carbohydrates Content

The carbohydrate content was extracted using 2 g of dried leaves were boiled with 10 ml of 80% ethanol in a water bath at 50 °C for 10 min. Then centrifuged at 2500 rpm for 5 min, after that the clear supernatants were used.

3.4. Mineral Elements Content

The macro and micronutrient concentrations were determined using the method described by Isacc and Johnson (1975).

4. Insects

Bioassays were conducted using the second-instar larvae of cotton leafworm obtained from a laboratory susceptible culture in the Department of Insect Population Toxicology, Central Agricultural Pesticides Laboratory, Agriculture Research Center, Giza, Egypt. The culture was lab-reared without exposure to any pesticides for several years and kept at 25 ± 1°C, 65 ± 5% relative humidity and a photoperiod of 16:8 (L:D). Larvae were kept in sterilized glass jars and provided soft, fresh, sterilized castor bean leaves (*Ricinus communis* L.) for feeding. To prevent overcrowding and infection from excrement, the developing larvae were transferred daily to clean sterilized glass jars and supplied with fresh sterilized leaves for feeding.

4.1. Larvae Treatment

In order to evaluate the short- and long-term effects of the foliar spray treatments, broad bean leaf samples collected 2 days and 12 days after foliar spray were weighed using an electronic balance. Larvae were kept and divided two groups into 16 equal clean sterilized glass jars. The above larvae were precisely weighed on the first day using an electronic balance. Eight larvae ($n = 64$) were placed in each jar. These jars were kept under the same laboratory conditions as stated earlier, larvae were considered dead if they did not move with gentle prodding. All the experiments were replicated thrice.

In Group_1: larvae were provided broad bean leaf samples collected 2 days after the first foliar spray and let to feed for 48 h, after that leaves were removed and weighed. The average feeding activity of 32 larvae per treatment was calculated. The feeding inhibitory activity and acceptability of treated leaf diet were determined using the equation of Wada and Manukata (1968) for feeding ratio determination as follows:

$$FA = \left(\frac{TD}{CD}\right) x\ 100$$

Where that FA; Feeding ratio (%), TD; Amount of diet consumed in treatment, CD; Amount of diet consumed in control.

In Group_2: larvae were provided broad bean leaf samples collected 12 days after the second foliar spray for feeding. The larvae feed on these leaves for 48 h that were changed daily with a fresh one, moistened cotton pad was placed in each jar to sustain humidity. After that, all larvae were removed and weighed. Surviving larvae were shifted to sterilized jars, provided with fresh sterilized castor bean leaves for feeding and observed daily till the pupation. Each test was replicated three times to calculate larval weight and pupation. Additionally, the growth larval index was calculated according to the following equation (Itoyama *et al.*, 1999):

Larval growth index = Pupation/Larval period (days)

5. Statistical Analysis

All data were expressed as a mean of replicates ± standard error (SE). Parameters were compared with ANOVA followed by Tukey's multiple range test to determine differences between means of treatment.

RESULTS

1. Chemical Composition

The changes of carbohydrates content in response to foliar application of jojoba oil and methomyl treatments are shown in **Table 1.** The maximum increase in carbohydrates content is recorded in leaves treated with jojoba oil after 42 days of sowing compared with those after 32 days. On the contrary, a slight increase in carbohydrate content was recorded in leaves treated with methomyl after 32 days compared to those after 42 days. In sum, leaves of broad bean, treated with jojoba oil retained a higher carbohydrates content than leaves treated with methomyl.

Table 1. Changes in the carbohydrates of broad bean leaves (%) treated with jojoba oil and methomyl in field conditions.

Treatment	Percentage of sugars (glucose) in the leaves	
	32 days[a]	42 days[b]
Jojoba oil	0.175[c] ± 0.002	0.188[b] ± 0.004
Methomyl	0.169[ab] ± 0.007	0.167[a] ± 0.003
Control	0.165[a] ± 0.003	0.166[a] ± 0.005

[a,b]Foliar spraying 32 days and 42 days after sowing; Data represent mean of three replications; Different letters indicate a significant difference between treatments at $p < 0.05$ according to Tukey's test.

In Table 2, results for jojoba oil and methomyl on amino acids content in broad bean leaves are presented. In both compounds, amino acid values were varied were between first and second foliar sprayings. However, results showed that in both foliar sprayings, jojoba oil and methomyl treatments have no significant effects on amino acids.

Table 2. Changes in the amino acids content of broad bean leaves (mg/100gm dry matter) treated with jojoba oil and methomyl in field conditions.

Amino acids	**32 days[a]**			**42 days[b]**		
	Control	**Jojoba oil**	**Methomyl**	**Control**	**Jojoba oil**	**Methomyl**
Aspartic acid	700.18	704.82	700.33	469.20	474.91	469.21
Threonine	239.97	239.97	239.99	239.97	239.97	239.98
Serine	187.71	187.71	187.79	187.71	187.71	187.72
Glutamic acid	61.21	61.95	61.2	207.91	208.66	207.92
Proline	160.13	160.64	160.19	160.13	160.04	160.14
Glycine	126.06	130.35	126.11	104.64	104.99	104.66
Alanine	78.27	79.15	78.29	64.93	65.11	64.94
Valine	86.85	86.66	86.87	39.56	409.00	39.57
Methionine	189.17	189.73	189.17	189.17	189.78	189.18
Isoleucine	480.29	480.78	480.34	265.05	265.86	265.07
Leucine	315.64	315.64	315.64	170.66	170.05	170.70
Tyrosine	143.21	143.89	143.26	140.24	140.94	140.29
Phenylalanine	613.47	613.30	613.50	385.02	385.29	385.03
Histidine	326.88	326.06	326.91	255.21	255.64	255.23
Lysine	09.24	309.13	309.29	33.69	233.89	233.71
Arginine	433.82	433.97	433.88	483.59	484.22	484.62

[a,b]Foliar spraying 32 days and 42 days after sowing.

Foliar application of jojoba oil and methamyl had significant differences in the micro and macronutrient content of broad bean leaves at almost both application times compared to the control group (**Table 3**). In the vast majority of experimental, no significant effect of the foliar application on the content of Magnesium, Copper and Iron in the leaves was found. While a significant increase in the content of Calcium was observed with a significant reduction in Manganese content in broad bean leaves of treatment compared with the control.

Table 3. Changes in the essential macro and microelements content of broad bean leaves (ppm) treated with jojoba oil and methomyl in field conditions.

Treatment	**32 days[a]**					**42 days[b]**				
	Ca	**Mg**	**Fe**	**Cu**	**Mn**	**Ca**	**Mg**	**Fe**	**Cu**	**Mn**
Jojoba oil	65.80[a]	30.71[a]	118.88[a]	8.63[ab]	50.80[c]	67.16[b]	33.25[b]	125.20[ab]	10.23[b]	56.07[ac]
Methomyl	65.62[a]	30.65[a]	118.80[a]	8.60[ab]	50.47[b]	66.99[ab]	33.20[b]	125.17[a]	10.16[a]	55.77[b]
Control	65.51[a]	30.73[a]	118.75[a]	8.56[a]	51.00[a]	66.80[a]	33.31[a]	125.16[a]	10.15[a]	56.26[a]

[a,b]Foliar spraying 32 days and 42 days after sowing; Data represent mean of three replications; Different letters indicate a significant difference between treatments at $p < 0.05$ according to Tukey's test.

2. Feeding Behavior

The jojoba oil and methomyl caused feeding inhibition in *S. littoralis* larvae compared to the control. Based on the percentage reduction of feeding values presented in **Table 4,** a significant decrease in the feeding activity of larvae with jojoba oil (61.1%) was recorded, while a slight decrease in the feeding activity with methomyl (17.4%) was observed.

Table 4. Feeding behavior larvae of *Spodoptera littoralis* caused by jojoba oil and methomyl applied on broad bean leaves in field conditions.

Treatment	Average weight of consumed leaves/8 larvae during 48 h (mg)	Feeding ratio (%)	Feeding inhibition (%)
Jojoba oil	33.2[c] ± 1.15	38.9	61.1[b] ± 1.4
Methomyl	76.5[b] ± 0.45	82.6	17.4[a] ± 1.3
Control	87.2[a] ± 0.59	100.0	

Data represent mean ± SE of three replications; Different letters indicate significant difference between treatments at $p < 0.05$ according to Tukey's test.

3. Larval Growth

Feeding 2nd instars larvae on leaves treated with jojoba oil and methomyl caused loss in larval weight and achieve larval periods more than control as illustrated in **Table 5.** So, jojoba oil treatment caused significant body weight loss with longer larval periods than the control. Whereas**,** methomyl did not affect the larval weight and larval periods significantly. On the other hand, the highest reduction in larval growth and pupation by jojoba oil treatment.

Table 5. Larval weight, pupation and larval growth rate of *Spodoptera littoralis* caused by jojoba oil and methomyl applied on broad bean leaves in field conditions.

Treatment	Larval weight (mg)	Pupation (%)	Larval growth index
Jojoba oil	50.6 ± 1.41c	26.4 ± 0.82b	1.80
Methomyl	72.6 ± 1.91b	24.9 ± 1.36a	2.24
Control	97.8 ± 1.34a	24.5 ± 1.96a	2.53

Data represent mean ± SE of three replications; Different letters indicate significant difference between treatments at $p < 0.05$ according to Tukey's test.

DISCUSSION

In this study, exogenous field application of jojoba oil and methomyl led to an increase in carbohydrates, amino acids and mineral elements content of broad bean plants compared to untreated plants. It also negatively affected the feeding activity and growth rate of cotton leafworm larvae. However, significant differences were detected between the broad bean plants treated with jojoba oil and methomyl. Foliar application of some compounds can improve induced resistance for a host plant to counteract the effects of the herbivorous insect through the production of toxic secondary metabolites, changes of biochemistry, physiology, or morphology in the host plant (Mazid *et al.,* 2011; Pokhare *et al.,* 2012; Thakur and Sohal 2014; Thakur *et al.,* 2016). The results of the current study are in line with previous findings of Inbar *et al.* (1998) who reported that exogenous field applications of several abiotic elicitors of defensive systems in tomato (*Lycopersicon esculentum*), reduced leaf mineral elements and whitefly populations. Similar results are reported by Boughton *et al.* (2006) who observed a significant decrease in the populations of green peach aphid (*Myzus persicae*) with the exogenous application of chemicals on tomato leaves in the greenhouse. Gao and Zhang (2013) showed that exogenous application of salicylic acid on pear leaf induced resistance to pear ring rot. Recently, Nouri-Ganbalani *et al.* (2018) indicated that foliar application by of chemical compounds on oilseed rape plants negative effect on *Plutella xylostella* L. fitness. Morimoto (2019) observed a high level of resistance in plants against *Heterotheca subaxillaris* due to the applying three orobanchaceae species as a foliar spray.

Conclusion

Exogenous field application of jojoba oil and methomyl led to an increase in carbohydrates, amino acids and mineral elements content of broad bean plants compared to untreated plants. It also negatively affected the feeding activity and growth rate of cotton leafworm larvae. However, foliar spraying of jojoba oil showed a more significant effect on the treated plant than methomyl. Hence, the current study provided evidence that exogenous field application of jojoba oil can play an important role in the protection of broad beans from cotton leafworm.

REFERENCES

Arnold T, Appel H, Patel V, Stocum E, Kavalier A, Schultz J. 2004. Carbohydrate translocation determines the phenolic content of *Populus* foliage: a test of the sink source model of plant defense. *New Phytologist*,164 :157–164.

Boughton AJ, Hoover K, Felton GW. 2005. Methyl jasmonate application induces increased densities of glandular trichomes on tomato, *Lycopersicon esculentum*. *Journal of Chemical Ecology,* 31:2211–2216. https://doi.10.1007/s10886-005-6228-7.

Boughton AJ, Hoover K, Felton GW. 2006. Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, *Myzus persicae*. *Entomologia Experimentalis et Applicata*, 120:175-188. https://doi.org/ 10. 1111/j.1570-7458.2006.00443.x.

Chen Y, Ni X, Buntin GD. 2009. Physiological, nutritional, and biochemical bases of corn resistance to foliage feeding fall armyworm. *Journal of Chemical Ecology*, 31:2211–2216. 35:297-306. https://doi.10.1007/s10886-009-9600-1.

Conboy NJA, Daniel T Mc, George D, Ormerod A, Edwards M, Donohoe P, Gatehouse AMR, Tosh CR. 2020. Volatile organic compounds as insect repellents and plant elicitors: an integrated pest management (IPM) strategy for glasshouse whitefly (*Trialeurodes vaporariorum*). *Journal of Chemical Ecology,* 46:1090-1104. https://doi.10.1007/s10886-020-01229-8.

Erb M, Reymond P. 2019. Molecular interactions between plants and insect herbivores. *Annual Review of Plant Biology* ,70:527–557.

Falk MG, O'Leary R, Nayak M, et al. 2015. A Bayesian hurdle model for analysis of an insect resistance monitoring database. *Environmental and Ecological Statistics,* 22:207–226. https://doi. org/10.1007/s10651-014-0294-3

Gao L, Zhang Y. 2013. Effect of salicylic acid on pear leaf induced resistance to pear ring rot. *World Applied Sciences Journal*, 22:1534-1539.https://doi.10.5829/ idosi. wasj.2013.22.11. 1732.

Gordy JW, Leonard BR, Blouin D, Davis JA, Stout MJ. 2015. Comparative effectiveness of potential elicitors of plant resistance against *Spodoptera frugiperda* (JE Smith) (Lepidoptera: Noctuidae) in four crop plants. *Plos One*, 10:me0136689. https://doi. 10.1371/journal.pone.0136689.

Heil M, Koch T, Hilpert A, Fiala B, Boland W, Linsenmair KE. 2001. Extrafloral nectarie production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proceeding of the National Academy of Sciences of the United States of America* 98:1083-1088. https://doi.org/10.1073/ pnas.98.3.1083.

Hendriks RJJ, Luijten L, Van Groenendael JM. 2009. Context-dependent defence in terrestrial plants: the effects of light and nutrient availability on plant resistance against herbivory. *Entomologia Experimentalis et Applicata*, l131:233-242. https://doi.10.1111/j. 14869-8137.2004.01157.x.

https://doi.10.1146/annurev-arplant-050718-095910.

Inbar M, Doostdar H, Sonoda RM, Leibee GL, Mayer RT. 1998. Elicitors of plant defense systems reduce insect densities and disease incidence. *Journal of Chemical Ecology,* 24:135-149.

Isacc RA, Johnson WC. 1975. Collaborative study of the wet and dry ashing techniques for the elemental analysis of plant tissue by atomic absorption spectrophotometry. .*Journal of AOAC,* 58:436-440.

Ismail SM, Abdel-Galil FA, Hafez SSh, Abu El-Ghiet UM. 2019. Influence of someinsecticides on the incidence of common Lepidopterous insect-pests in cotton field. *Egyptian Academic Journal of Biological Scinces*, 2:23-30. https://doi. org/10.21608/ eajbsf. 2020. 73377.

Ismail SM. 2020. Gossypol as a natural insecticide in cotton plants against cotton thrips and pink bollworm. *Progress in Chemical and Biochemical Research*, 4:68-79. https://doi.10.22034/ pcbr.2021.120448.

Itoyama K, Kawahira Y, Murata M, Tojo S. 1999. Fluctuations of some characteristics in the common cutworm, *Spodoptera litura* (Lepidoptera: Noctuidae) reared under different diets. *Applied Entomologyy and Zoology,* 34:315-321.

Mazid M, Khan TA, Mohammad F. 2011. Role of secondary metabolites in defense mechanisms of plants. *Biology and Medicine,* 3:232-249.

Morimoto M. 2019. Chemical defense against insects in *Heterotheca subaxillaris* and three orobanchaceae species using exudates from trichomes. *Pest Management*

Mouden S, Kappers IF, Klinkhamer PG, Leiss KA. 2020. Cultivar variation in tomato seed coat permeability is an important determinant of jasmonic acid elicited defenses against western flower thrips. . *Frontiers in Plant Science,* 11:576505 https:// doi. 10.3389/ fpls.2020.576505.

Nouri-Ganbalani G, Ehsan Borzoui E, Shahnavazi M, Alireza Nouri A. 2018. Induction of resistance against *Plutella xylostella* (L.) (Lep.: Plutellidae) by jasmonic acid and mealy cabbage aphid feeding in *Brassica napus* L. *Frontiers in Physiology*, 9:859. https://doi.org/10.3389/fphys.2018.00859.

Page AI, Miller RH, Keeney-eds DR. 1982. Methods of soil analysis. *Part 1, 2 American Society of Agronomy.*

Pokhare S, Pankaj S, Shakil NA, Kumar J, Singh K. 2012. Foliar application of chemical elicitors induces biochemical changes in wheat against the cereal cyst nematode, *Heterodera avenae*. *Nematologia Mediterranea*, 40:181-187.
Science, 75:2474-2481. https://doi.10.1002/ps.5395.

Thakur M, Sohal BS. 2014. Effect of elicitors on physiomorphological and biochemical parameters of Indian mustard (*Brassica juncea*) and rapeseed (*B. napus*). *Journal of Applied and Natural Science*, 6:41-46.

Thakur T, Sangha MK, Arora R, Javed M. 2016. Effect of foliar spray of elicitors on status of defense proteins in relation to mustard aphid infestation in crop brassica cultivars. *Journal of Applied and Natural Science*, 8:2242- 2248. https://doi.org/ 10.31018/jans.v8i4.1119.

Wada K, Manukata K. 1968. Naturally occurring insect control chemicals. isobolidine, a feeding inhibitor and cocculolidine, an insecticide in the leaves of *Cocculus trilobus* DC. *Journal of Agricultural and Food Chemistry*, 16:471-474. https://doi. org/ 10.1021/jf60157a034.

War AR, Paulraj MG, Ahmad T, Buhroo AA, Hussain B, Ignacimuthu S, Sharma HC. 2012. Mechanisms of plant defense against insect herbivores. *Plant Signaling and Behavio,* 7:1306-1320. https://doi.org/10.4161/psb.21663.