Effects of Foliar-Applied Salicylic Acid, Flax and Caraway Oils on Caterpillar Biometrics on Lima Beans

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ABSTRACT

The effects of salicylic acid, flax and caraway oils in either enhancing or inhibiting the lima bean plant's defense against the feeding activity of cotton leafworm *Spodoptera littoralis* were tested. Treatments of salicylic acid as sodium salicylate (NaSA), flax and caraway oils were exogenously applied to lima bean seedlings. Antifeeding activity using cotton leafworm larvae for 48 hrs exposure as well as long-term effect of 12 days exposure were recorded. Plants treated with NaSA had encouraged the feeding ability by *Spodoptera* larvae that showed larger mass after 12 days of feeding with about 49.5% increase in larval growth rate. Flax oil with linolenic acid as the main component and the precursor of jasmonic acid (JA) increased the resistance of the host plants leading to an increased insect antifeeding activity and lower caterpillar growth rate by 39%. Treatment of caraway oil (23.5% carvone, 31.8% limonene) decreased the eating ability of the plant by caterpillars and hence inhibited larval growth rate by about 45% as well as increased average days to pupation with about 1.7 day. The present results indicate the importance of using elicitors of plant resistance in pest control programs that lead to activation of self induced resistance in treated plants. Flax oil and caraway oil have reasonable effects as potential inducers of lima bean plant resistance against cotton leafworm larvae.

Keywords: Induced resistance, Elicitors, Flax and caraway oils, Salicylic acid, Insect antifeedants, Lima beans.

INTRODUCTION

Insect herbivores and plant pathogens are responsible for about 15-30% crop annual losses in Egypt as well as worldwide. Because of less agrochemicals being used and less new insecticides and fungicides coming on the market due to environmental concern, research efforts are now being directed for more acceptable and safe alternatives which are needed for economically viable and environmentally-safe crop protection measures. One such possible alternative to synthetic pesticides is host plant resistance.

Efforts in the area of inducible plant resistance are now being developed concerning the use of induced plant genes for insect herbivory and pathogen resistance and the potential of using induced resistance (IR) (Karban and Baldwin, 1997 and Karban and Chen, 2007), or systemic acquired resistance (SAR) (Agrios, 1997), as environmentally safe methods of insect and disease pest control, respectively. Jasmonic acid (JA) is an endogenous plant growth regulator in plants. Crop plants produce JA in response to injury or application of elicitors which include the expression of defensive compounds (Chen *et al.*, 2006).

The synthesis of JA takes place via the octadecanoid pathway. The precursor of JA is α-linolenic acid (ALA). ALA is converted to JA by lipoxigenation via the octadecanoid pathway. Jasmonic acid then facilitates the induction of plant defensive genes leading to production and accumulation of proteinase inhibitor proteins, polyphenol oxidase, steroid glycoalkaloids as well as release of volatile organic compounds (VOCs) that attract natural enemies of insect herbivores (Greelman and Mullet, 1977; Thaler, 1999; Ryan, 2000 and Chehab *et al.*, 2008). Some secondary metabolites with defensive functions are present constitutively in plants including terpenoids, alkaloids and phenolics. These defensive molecules and insect antifeedant compounds (El-Sebae, 1987; Eldoksch and El-Sebae, 2005) and also lignin are produced via the phenylpropanoid biosynthetic pathway.

Some investigations showed mutual antagonism between jasmonic acid and salicylic acid pathways which increase in pathogen resistance and decrease in insect resistance with the exogenous application of salicylic acid (SA), while others indicated cross protection (Zehnder *et al.*, 1997; Felton *et al.*, 1999; Walling, 2000 and Iverson *et al.*, 2001). In addition, Howe *et al.*(1996) indicated that tomato mutant that was deficient in the ability to induce defense genes, via the octadecanoid pathway, was much more susceptible to damage by the tobacco hornworm (*Manduca sexta*). Thaler (1999) showed that foliar application of JA to tomato plants induced plant resistance to the beet armyworm (*Spodoptera exigua*) damage through the biosynthesis of chemical defenses in tomato plants.

In the present study, using lima bean plants, the effect of foliar applied salicylic acid as sodium salicylate (NaSA) and flax oil (49.7% linolenic acid) as well as their combination (1:1, v/v) on the induction of plant

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resistance against feeding activity by *Spodoptera* caterpillars were tested. The efficacy of caraway oil as a possible inducer of lima bean plant resistance against caterpillar feeding activity of *Spodoptera* larvae was also tested.

MATERIALS AND METHODS

Test plant

Lima bean seeds (*Phaseolus lunatus* L.) were sown in sandy loam soil which was placed in 10 cm diameter plastic pots and placed in a greenhouse. Plants were allowed to grow for 30 days before exposure to chemicals or 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.

Application of chemicals and natural oils

Water solutions of 0.05% sodium salicylic acid (NaSA), 0.05% flax oil and 1% caraway oil were prepared for the chemical treatments. Stock solutions of NaSA and flax oil (5%) were prepared with water. Ten ml of each stock solution were then added to 990 ml of deionized water to obtain the desired concentrations of 0.05% for NaSA and flax oil. Five drops of Tween-40 (detergent) were added in each of the prepared solutions 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. Deionized water + Tween-40 was used as a control solution. Caraway oil was obtained by cold pressing extraction of caraway seeds. NaSA and Tween-40 were supplied by El-Nasr Pharmaceutical Chemicals Co., Cairo, Egypt. Commercial flax seed oil was purchased from a local food store. The plants were sprayed by a hand spray bottle twice one day prior and one hour prior to the application of the caterpillar bioassays. Each plant was sprayed with about 2.5-3.0 ml of NaSA, flax oil, caraway oil, control or both NaSA and flax oil solutions.

Chromatographic analysis of flax oil

A sample of flax oil was diluted by diethyl ether and 1.5 µl was injected to a gas chromatography 6000 series GC system controller coupled to flam ionization detector (FID). Authentic linolenic acid was used as a standard material. The GC analytical conditions were as follows: injector temperature, 220°C; program, 140°C (held for 5 min), ramped to 240°C at 4°C/min. Detector temperature was 260°C. Nitrogen gas was used as a carrier gas.

Bioassays

1. Short-term (48 hours) caterpillars bioassay

A laboratory strain of the cotton leafworm (*Spodoptera littoralis*) was continuously reared on castor bean leaves. Five 3rd instar larvae of the cotton leafworm were placed together with a treated and weighed lima bean leaf from exogenously sprayed lima bean plants in a Petri dish (9 cm diameter) and allowed to consume the diet for 48 hrs. Four replicates were made for each treatment including the control. The leaves were removed after 48 hrs exposure and weighed to determine the percentage eaten by the larvae. The average of feeding activity of 20 larvae per each treatment was calculated. The feeding inhibitory activity and acceptability of treated leafy diet was determined using the equation of Wada and Manukata (1968) for feeding ratio determination as follows: Feeding ratio = B/A x 100, where A = amount of diet consumed in control and B = amount of diet consumed in the treated diet.

2. Long-term (12 days) caterpillars boassay

The second instar larvae of *S. littoralis* were selected for assaying the foliar-applied selected natural oils and chemicals on lima bean seedlings. The larvae were allowed to consume foliage for 12 days on the treated 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 weighed and then they were transferred back to the normal leafy diet. The vials were checked daily for pupation. The reduction of larval weight after 12 days of exposure served as the criterion for insect antifeeding activity.

Data of caterpillar weights and lima bean leaf weights were statistically analyzed using analysis of variance (ANOVA) with multiple comparisons tested with the Duncan's method. The P value (0.05) was used for deciding the degree of significance of the treatments.

RERSULTS AND DISCUSSION

Short-term bioassay

The effects of sodium salicylic acid (NaSA), flax oil and their combination (1:1, v/v) as well as caraway oil on caterpillar antifeeding activity during 48 hrs exposure period are presented in Table (1). The data indicated that the treatment of lima bean plants by caraway oil (23.5% carvone, 31.8% limonene) caused the superior antifeeding activity against caterpillars (3rd instar larvae) during 48 hrs feeding period and showed 64.3% reduction in feeding activity compared with the control followed by flax oil (49.7% linolenic acid) by about 52.4% feeding inhibition and then the mixture of flax oil plus NaSA with 33.4% feeding inhibition. NaSA has no antifeeding activity against *Spodoptera* larvae during 48 hrs of exposure and data showed no significant

Table 1. Antifeeding activity of foliar-applied NaSA, flax oil and caraway oil against 3rd instar larvae of *Spodoptera littoralis* (Boisd.)

Treatment (%)	Aver. wt. of diet [2] consumed per 5 larvae during 48 hrs (mg±SD)	Feeding ratio (% of control)	Feeding inhibition (%)
NaSA [1] (0.05)	78 ± 11 [b]	92.8	7.2
Flax oil (0.05)	40 ± 7 [a]	47.6	52.4
NaSA + flax oil (0.05+0.05)	56 ± 5 [a]	66.6	33.4
Caraway oil (1.0)	30 ± 7 [a]	35.7	64.3
Control	84 ± 10 [b]	100	0.0

[1]NaSA = sodium salicylic acid

[2]Different letters indicate significantly different results (P<0.05) using Duncan's multiple range test.

differences between leafy diet consumed in control and those treated by NaSA.

Long-term bioassay

Results after 12 days caterpillars feeding period are presented in Table (2). The data showed significant effects of exogenously applied NaSA, flax oil as well as caraway oil on the defense of lima bean plants against insect herbivory. Caterpillars (2nd instar larvae) of *S. littoralis* after 12 days of exposure on NaSA-treated lima bean leaves grew 49.5% larger than those on control and 145% larger than those grown on flax oil-treated plant leaves. Caterpillars fed on flax oil-treated leaves showed 39% reduction in growth rate compared to the control, whereas those fed on plant leaves treated by flax oil + NaSA exhibited 26% reduction in growth rate.

These data of long-term bioassay of caterpillars weight indicated that foliar application of NaSA made lima bean plants more susceptible to *Spodoptera* larvae damage and that flax oil (49.7% linolenic acid) made lima bean plants less susceptible to *Spodoptera* larvae damage. Reduced resistance in plants treated by NaSA may be due to the well-known phenomena where SA blocks the production of allen oxide synthase (AOS), a necessary enzyme to produce jasmonic acid through the octadecanoid pathway (Fig. 1). As a result, the plant becomes unable to produce the defensive compounds such as polyphenol oxidase (PPO) and/or proteinase inhibitors (PIs) and, subsequently, becomes more palatable to the herbivore (Thaler *et al.*, 1999 and Fidantsef *et al.*, 1999). In addition, Ryan (2000) indicated that the major insecticidal gene products in induced tomato plants are proteinase inhibitors and polyphenol oxidase, both of which are interfering with insect digestion and then nutrient uptake. On the other hand, plants treated by flax oil containing 49.7% alpha linolenic acid, which is the precursor of jasmonic acid, showed significant reduction in caterpillar feeding activity with a reduced weight gain than those feeding on control leaves as well as showed increase in average days to pupation (Table 2).

Table 2. Effect of NaSA, flax oil and caraway oil on cotton leafworm *S. littoralis* weights and days to pupation

Treatment (%)	Mean weight of [1,2] caterpillars (mg±SD)	Caterpillar weight (% of control)	Average[3] days to pupation
NaSA (0.05)	142 ± 18 [c]	149.5	23.5 ± 0.5
Flax oil (0.05)	58 ± 7 [a]	61.0	25.0 ± 0.2
NaSA + flax oil (0.05+0.05)	70 ± 11 [ab]	73.0	24.2 ± 0.5
Caraway oil (1.0)	52 ± 8 [a]	55.0	25.5 ± 1.1
Control	95 ± 13 [b]	100.0	23.8 ± 0.8

[1]Caterpillars consumption (2nd instar larvae) on treated lima bean leaves was allowed to proceed for 12 days.

[2]Different letters indicate significantly different results (P<0.05) using Duncan's multiple range test.

[3]Averages based on 20 larvae tested in 4 replicates of 5 larvae each. The larvae were transferred back to normal leafy diet after 12 days.

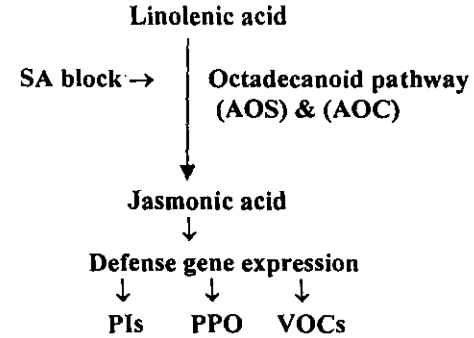

Fig. 1. Pathway of jasmonic acid (JA) synthesis in plants, also showing the point of inhibition by salicylic acid (SA) in the pathway

JA then enhances the production of secondary metabolites and defensive compounds via the octadecanoid pathway. The data also indicated that treatment of caraway oil increased plant resistance to eating by caterpillars with 45% reduction in larval weight as well as increased average days to pupation by 1.7 day compared to the control. This promising insect antifeeding activity of caraway oil may be due to its main monoterpene components, carvone (23.5%) and limonene (31.8%) (Eldoksch and Hassanein, 2007). Iverson *et al.*(2001) indicated that JA, endogenously or exogenously applied on plants is necessary for defense against insect herbivory and SA disrupts JA biosynthesis leading to reduced resistance to the feeding insects. The phenomenon of reduced insect resistance in lima bean plants treated by NaSA in the present work is potentially attributed to the increased SA levels or inhibition of JA biosynthesis. Increased antifeeding activity in lima bean plants treated by flax oil with 49.7% α-linolenic acid may be due to the induction of JA biosynthesis that takes place via the octadecanoid pathway. Thaler (1999) reported that foliar JA application on tomato plants increased levels of polyphenol oxidase an oxidative enzyme implicated in resistance against several insect herbivores. Furthermore, Omer *et al.*(2000) demonstrated that foliar jasmonic acid application on grapevines induced resistance in plants against the Pacific spider mite, *Tetranychus pacificus* McGregor as well as the root-feeding grape Phylloxera, *daktulosphaira vitifoliae* (Fitch).

It can be concluded that the present results indicate the importance of using elicitors of plant resistance in pest control that leading to activation in plants of induced resistance in a vaccine form. Flax oil (49.7% linolenic acid) and caraway oil (23.5% carvone, 31.8% limonene) increased the defensive mechanisms and insect antifeeding activity of the plant. They are potential inducers of lima bean plant resistance that significantly inhibited feeding activity and growth rate of caterpillars.

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الملخص العربي

دراسة تأثير الرش الورقى لنباتات اللوبيا بحمض الساليسليك وزيوت الكتان والكراوية على القياسات البيولوجية للطور اليرقى لحشرة دودة ورق القطن

حمدى على الدكش، عبدالخالق حامد السباعى

تم دراسة تأثير حمض الساليسليك وزيوت كل من الكتان والكراوية فى تنشيط أو تثبيط قدرة المقاومة الذاتية لنباتات اللوبيا ضد نشاط التغذية ليرقات حشرة دودة ورق القطن. تم رش بادرات اللوبيا عمر ٣٠ يوم بحمض الساليسليك على صورة صوديوم ساليسيلات وأيضاً زيت الكتان وزيت الكراوية ومخلوط حمض الساليسليك وزيت الكتان بنسبة (١:١). تم تسجيل نشاط التغذية ليرقات دودة ورق القطن على أوراق اللوبيا المعاملة وذلك لمدة ٤٨ ساعة وأيضاً على مدى أطول لمدة ١٢يوم. تبين من النتائج أن النباتات المعاملة بساليسيلات الصوديوم شجعت قدرة التغذية لليرقات الحشرية واكتسبت اليرقات زيادة فى الوزن بعد ١٢ يوم من التغذية مع نسبة ٤٩,٥% زيادة فى معدل النمو اليرقى. المعاملة بزيت الكتان المحتوى على ٤٩,٧% ألفا-حمض اللينولينيك والذى يعتبر المصدر الأساسى للتخليق الحيوى لحمض الجاسمونيك أدى إلى زيادة مقاومة نباتات اللوبيا وزيادة التأثير المضاد لتغذية الحشرات مما أدى إلى إنخفاض فى معدل النمو اليرقى بنسبة ٣٩%.

أيضا أوضحت النتائج أن المعاملة بزيت الكراوية والمحتوى على ٢٣,٥% كارفون، ٣١,٨% ليمونين قد خفض من نشاط التغذية لليرقات الحشرية مما أدى إلى أنخفاض فى معدل النمو اليرقى بنسبة ٤٥% مع زيادة فى متوسط عدد الأيام اللازمة للوصول إلى طور العذراء بحوالى ١,٧ يوم.

لقد تبين من النتائج أهمية استخدام المواد المحفزة للمقاومة الذاتية فى النباتات ضد الحشرات فى برنامج مكافحة الآفات والذى يؤدى إلى تنشيط المقاومة الذاتية للنبات العائل. إن معاملة نباتات اللوبيا بكل من زيت الكتان وزيت الكراوية أعطى تأثيرات جيدة وواعدة كمواد محفزة محتملة لتنشيط المقاومة الذاتية فى النباتات ضد هجوم يرقات دودة ورق القطن.