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Original scientific paper

Effects of salicylic acid elicitor against aphids on wheat and detection of infestation using infrared thermal imaging technique in Ismailia, Egypt

Mahmoud Farag Mahmoud[1]* and Hatem M. Mahfouz[2]

[1] *Plant Protection Department, Faculty of Agriculture, Suez Canal University, 41522 Ismailia, Egypt*
[2] *Plant Production Department, Faculty of Environmental Agricultural Sciences, Suez Canal University El-Arish, Egypt*
*(*mfaragm@hotmail.com; mfaragm@agr.suez.edu.eg)*

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SUMMARY

Wheat (*Triticum sativum* L.) is one of the most important cereal crops in Egypt. Insect pests, such as aphids, are major threats in terms of yield reduction. Induced resistance in wheat using salicylic acid as a foliar application was tested on the farm of the Faculty of Agriculture, Suez Canal University during 2012/2013 and 2013/2014 seasons. Three wheat cultivars, Gemeza 9, Sakha 93 and Giza 168, were sprayed three times with two concentrations of salicylic acid (SA), 200 mg/l and 100 mg/l, after early detection of aphid infestation by infrared thermal imaging.

The infrared thermal imaging technique is based on significant differences in surface temperature between infested and healthy leaves. Imaging data are digital, and a computer program can be used to detect infestation rapidly. The results showed that aphid infestation raised the temperature of infested leaves, compared to healthy leaves. The range temperature difference between maximum and minimum temperatures (At) was 1.1 ºC in healthy leaves and 3.9 ºC in infected leaves. The results of SA application showed significant differences in the mean number of aphids and in reduction of infestation among treatments and cultivars. The higher of the two SA rates (200 mg/l) gave higher efficacy in the three cultivars than the lower rate (100 mg/l) over the five weeks of trial. The highest efficacy against aphids was reached one week after application (86.28% for Giza, 85.89% for Gemesa and 70.54% for Sakha). Moreover, SA treatment enhanced the wheat yield of all three cultivars, compared with control plants. The three cultivars (Giza, Gemesa and Sakha) produced higher yields than the control when sprayed with 200 mg/l SA. Their grain yield was 2,491.5, 2,455.0, and 2,327.25 kg/feddan (1 fed = 0.42 ha), respectively. In conclusion, infrared thermal imaging can be employed in identification of infected leaves. Also, the application of SA on wheat induced plant resistance to aphids.

Keywords: Aphids; Wheat; Infestation; Salicylic acid; Thermal imaging

INTRODUCTION

Wheat (*Triticum sativum* L.) is one of the oldest and most important cereal crops in Egypt. Although wheat production per unit area has significantly increased in Egypt over the past years, wheat production supplies only 40% of its annual domestic demand. Wheat occupies about 32.6 percent of the total winter land area and is mostly used to make bread, a very important component of the Egyptian diet (Elhamid, 2014).

Several insect pests infest wheat and cause enormous damage during two important growth stages (heading and flowering) (Freier et al., 2007). Major threats regarding yield reduction of wheat come from insect pests. Wheat aphids are devastating insect pests of wheat (Steffey & Grey, 2012). Management of wheat aphids is difficult due to their rapid reproduction and extremely short life cycle. Currently, the use of conventional insecticides is the main approach in controlling aphids (Dogimont et al., 2010). These insecticides have negative impact on the environment, especially on beneficial organisms,. Moreover, plant treatment with insecticides leads to resistance of aphids and other insect pests, which also contributes to management problems (Dogimont et al., 2010).

Induced plant resistance to insect pests has been documented in several crops (Hussein et al., 2014; Mahmoud, 2013). Several investigators have proposed the use of elicitors of plant resistance as a means of controlling insect pests in agriculture (Thaler et al., 1999; Boughton et al., 2006). This new control approach is gaining in topicality because insect pests and diseases are serious constraints in efforts to increase productivity per feddan. Salicylic acid (SA) is a plant phenolic widely distributed throughout the plant kingdom. It is a hormone-like substance which plays an important role in the regulation of many aspects of plant growth and development (Raskin, 1992). However, it is especially famous for its ability to induce systemic acquired resistance in plants (Ryals et al., 1996).

Near infrared reflectance has been proposed for detecting grain insects in wheat (Dowell et al., 1998) and has been applied to tree fruit pests (Hansen et al., 2008), but this technology has not been applied to wheat leaves infested by aphids. Thermal imaging is a technique which converts the radiation emitted by an object into temperature data without establishing contact with the object. It has been successfully used in civil engineering, manufacturing industries, electrical engineering and medicine, but has been rarely applied in agriculture, e.g. for detection of bruises in fruits and vegetables (Varith et al., 2003), detection of foreign substances in food (Meinlschmidt & Maergner, 2003), and detection of insect infestation of stored grain (Manickavasagan et al., 2008).

The objective of the current study was to investigate the efficacy of the elicitor SA in controlling wheat aphids under field conditions, and its impact on wheat yield. Also, the infrared thermal imaging technique was assessed as a new approach in entomological research for early detection of wheat infestation by aphids.

MATERIALS AND METHODS

Experimental design

The experiment was carried out at the Experimental farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt during the growing seasons of wheat (2012/2013 and 2013/2014). Three wheat cultivars (Gemesa 9, Sakha 93 and Giza 168) were selected for this experiment. The cultivars were sawn on December 9, 2012 and 2013. Two rates of salicylic acid and an untreated control were used. The experimental plots were designed as a randomized complete block. Each treatment was replicated 4 times in each block.

Thermal imaging camera

An infrared thermal camera (Fluke Ti32), which is sensitive to 7.5-13 µm wavelengths at 320 X 240 pixels, and a visual mode of camera were used. Thermal calibration ranged from -10.0°C to 600.0°C with 9Hz image speed. A software converts temperatures to images based on a palette. Five temperature features (average, maximum and minimum temperatures of wheat leaves, temperature range difference between maximum and minimum temperatures (At), and standard deviation) were extracted from each thermal image using a Matlab algorithm.

Wheat thermal imaging

Infrared thermal imaging was performed several times on wheat plants before the experiment to adjust surface temperature. Temperature measurements were taken on 5 leaves of each wheat cultivar at different heights. For comparison, infested wheat leaves were also measured.

Treatments with salicylic acid

Plots with the three wheat cultivars were sprayed with salicylic acid (SA) three times after early detection of aphid infestation by the infrared thermal imaging camera. SA was applied at two rates (200 mg/l and 100 mg/l). SA was dissolved in a few drops of ethanol and then dispersed in water to give the required rates. After early detection of aphid infestation, wheat cultivars were sprayed with the two rates of SA at weekly intervals three times using a hydraulic sprayer. Control plots were sprayed only with distilled water and each treatment was replicated 4 times.

Wheat aphid counts

For population counts of wheat aphids, twenty-five plants were randomly selected from each replicate. Aphid counting was done one day before spraying. After SA spraying, aphid population counts were recorded at intervals of one, two, three, four and five weeks. Moreover, in view of these pre-treatment differences, corrected percentage efficacies were calculated according to the following modification of Abbott's formula, as described by Henderson and Tilton (1955):

$$\% \text{ Efficacy} = [1\text{-}(T_a/C_a \times C_b/T_b)] \times 100$$

where T_b is infestation in treated plot prior to application; T_a is infestation in treated plot after application; C_b is infestation in control plot prior to application; C_a is infestation in control plot after application.

Wheat yield

The yields of SA-treated wheat plants were compared with the control. At the end of the experiment random samples were taken to measure the following characters: kernel number, weight of 1000 kernals and grain yield.

Statistical analysis

Data obtained in the present study was subjected to an analysis of variance (ANOVA) with the honestly significant difference value calculated as Tukey's statistic at P ≤ 0.05. (SAS Institute 2004).

RESULTS

Reflectance values of infrared thermal images indicated that the leaves infected by aphids reflected higher in the visible region of the electromagnetic spectrum, rather than in the infrared region, compared to healthy leaves (Figure1). The average surface temperature of 20 healthy wheat leaves at 70 pixels was 28.6°C ± 0.23 whereas the

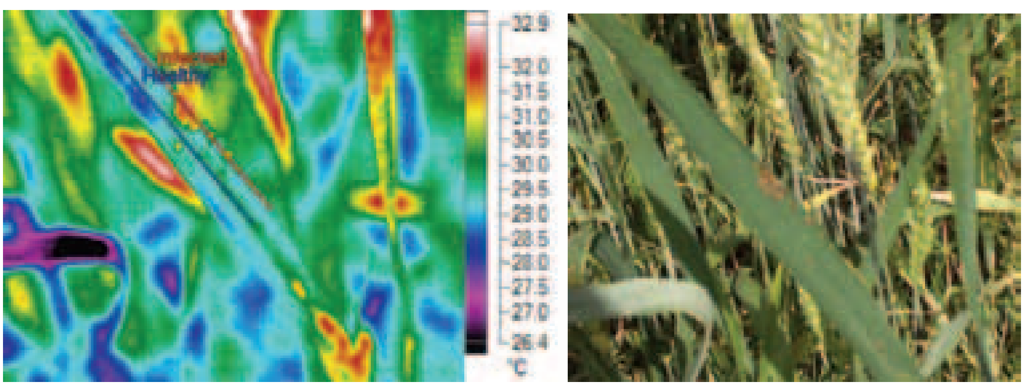

Figure 1. Thermal image of infected and healthy leaves

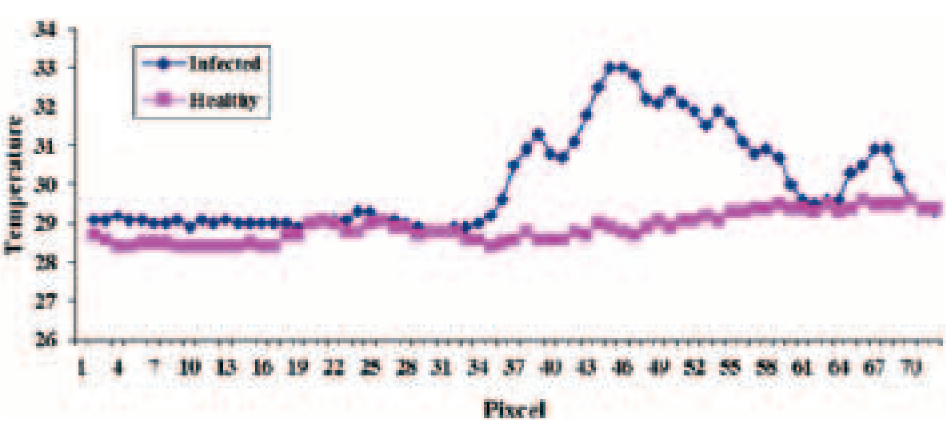

Figure 2. Variation of temperature between healthy and infected leaves at 70 pixels.

average surface temperature of wheat leaves infested by aphids was 30.1°C ± 1.04. The maximum surface temperature of infected and healthy leaves was 32.2°C ± 1.07 and 29.3°C ± 0.35, respectively. In addition, the minimum temperature of infected and healthy leaves was 28.8°C ± 0.91 and 28.2°C ± 0.2, respectively. The background temperature (30.1°C) and emissivity (0.97) were the same for both the infected and healthy leaves of wheat. The (At) range was higher in infected than in healthy leaves. It was 3.9 and 1.1, respectively (Table 1). Thermal images of the healthy and infected leaves are shown in Figure 2.

The average maximum and minimum surface temperatures of healthy leaves were 29.3°C ± 0.35, and 28.2°C ± 0.21, respectively. The average maximum and minimum surface temperature of infested leaves were 32.7°C ± 1.07, and 28.8°C ± 0.91, respectively. Our classification of healthy and infested leaves using thermal imaging was based on that variation in leaf temperatures.

Table 1. Mean temperature values (± SD) of healthy and infested wheat leaves

Temperatures	Healthy leaves	Infected leaves
Average temperature	28.6°C ± 0.23	30.1°C ± 1.04
Maximum temperature	29.3°C ± 0.35	32.7°C ± 1.07
Minimum temperature	28.2°C ± 0.21	28.8°C ± 0.91
Background temperature	30.1°C	30.1°C
Emissivity	0.97	0.97
Range (At)	1.1	3.9
Standard deviation	0.25	1.04

The results in Table 2 show the mean number of aphids on three wheat cultivars. Their population decreased after SA application and then increased again in the following counts, particularly when sprayed with the high rate of 200 mg/l. Aphid counts were consistently lower on the plots sprayed with SA than on the control plots. There was a significant difference in the mean number of aphids on SA-treated and control plots. After a week, it was 8 for Gemesa (F= 227.239; p≤ 0.0000), 10 for Giza (187.948; p≤ 0.0000) and 24 for Sakha (F= 104.564; p≤ 0.0000). After two weeks, it was 23 for Gemesa (F= 110.873; p≤ 0.0000), 27 Giza (F= 121.510; p≤ 0.0000) and 36 for Sakha (F= 42.879; p≤ 0.0000). After three weeks, it was 41 for Gemesa (F= 60.931; p≤ 0.0000), 58 for Giza (F= 54.071; p≤ 0.0000) and 78 for Sakha (F= 11.815; p≤ 0.0030). After four weeks, it was 83 for Gemesa (F= 94.659; p≤ 0.0000), 79 for Giza (F= 24.930; p≤ 0.0002) and 100 for Sakha (F= 2.242; p≤ 0.1621). After five weeks, it was 141 for Gemesa (F= 14.333; p≤ 0.0016), 186 for Giza (F= 25.723; p≤ 0.0002) and 211 for Sakha (F= 0.607; p≤ 0.5657).

Table 2. Mean number of wheat aphids on three wheat cultivars

Treatments	Mean number of aphids/25 plants		
	Gemesa 9	Sakha 93	Giza 168
	Before treatment		
SA1	36 a	63 a	41 a
SA2	39 a	49 a	35 a
Control	40 a	58 a	36 a
	1 week after treatment		
SA1	8 b	24 c	10 c
SA2	15 b	35 b	22 b
Control	63 a	75 a	64 a
	2 weeks after treatment		
SA1	23 c	36 c	27 c
SA2	37 b	49 b	43 b
Control	88 a	69 a	87 a
	3 weeks after treatment		
SA1	41 c	78 b	58 c
SA2	57 b	97 a	83 b
Control	93 a	105 a	121 a
	4 weeks after treatment		
SA1	83 c	100 a	79 b
SA2	109 b	115 a	100 b
Control	147 a	117 a	133 a
	5 weeks after treatment		
SA1	141 b	211 a	186 b
SA2	177 a	220 a	227 a
Control	183 a	223 a	246 a

SA1 = 200 mg/l, SA2 = 100 mg/l
Means followed by the same letter (columnwise) are not significantly different (Tukey' HSD; P≤0.05)

Data in Table 3 show that the higher rate of SA (200 mg/l) gave higher efficacy in the three cultivars than the lower rate (100 mg/l) over the five-week period. The highest efficacy against aphids was found one week after application (86.28% for Giza 168, 85.89% for Gemesa 9 and 70.54% for Sakha 93, respectively). The efficacy of the two SA rates decreased gradually and reached 33.61% and 5.09% for Giza 168, 14.39% and 0.8% for Gemesa

9, and 12.89% and 0.0% for Sakha 93, respectively. SA achieved a satisfactory reduction in aphid population within the five-week period, especially on the cultivars Gemesa 9 and Giza 168.

Table 3. Efficacy of salicylic acid in reducing aphid infestation on three wheat cultivars

Treatments	Efficacy %		
	Gemesa 9	Sakha 93	Giza 168
	1 week after treatment		
SA1	85.89	70.54	86.28
SA2	75.58	44.76	64.64
	2 weeks after treatment		
SA1	70.96	51.97	72.75
SA2	56.88	15.49	49.16
	3 weeks after treatment		
SA1	51.02	31.61	57.91
SA2	37.14	0	31.40
	4 weeks after treatment		
SA1	37.26	21.31	47.85
SA2	23.95	0	22.66
	5 weeks after treatment		
SA1	14.39	12.89	33.61
SA2	0.80	0	5.09

SA1 = 200 mg/l, SA2 = 100 mg /l
Means followed by the same letter (columnwise) are not significantly different (Tukey' HSD; P≤0.05)

A comparison of yields of the three wheat cultivars (Gemesa 9, Sakha 93 and Giza 168) revealed significant differences in kernal numbers/ear, weight of 1000 kernals (g), and grain yield (kg/feddan) between the untreated control and the SA treatments. There were significant differences among the cultivars regarding kernal numbers/ear (F=19.0027; p≤0.0009 for Gemesa 9, F=19.529; p≤0.0000 for Sakha 93 and F=19.529; p≤0.0000 for Giza 168), weight of 1000 kernals (g) (F=7.142; p≤0.0032 for Gemesa 9, F=32.255; p≤0.0000 for Sakha 93 and F=32.255; p≤0.0000 for Giza 168), and grain yield (kg/fed.) (F=13.143; p≤0.0021 for Gemesa 9, F=16.002; p≤0.0011 for Sakha 93 and F=22.228; p≤0.0003 for Giza 168).

The three cultivars (Giza 168, Gemesa 9 and Sakha 93) sprayed with 200 mg/l SA had higher yields than the control, i.e. 2,491.5, 2,455.0, and 2,327.25 kg/fed, respectively. Control yield was 2,189.0, 2,157.0 and 2,227.5. Also, yield data revealed that the lower rate of salicylic treatments enhanced yield relative to control. A significant difference was detected between treated and control cultivars (Table 4).

Table 4. Effects of salicylic acid on kernal number/ear, weight of 1000 kernals (g) and grain yield (kg/fed.)

Salicylic treatment	Kernal number/ear	Weight of 1000 kernals (g)	Grain yield (kg/fed.)
(Gemesa 9) SA1	61.4 a	40.1 a	2455.0 a
(Gemesa 9) SA2	60.7 a	39.4 ab	2335.0 ab
Control	56.3 b	38.2 b	2227.5 b
(Sakha 93) SA1	60.0 a	39.5 a	2327.25 a
(Sakha 93) SA2	58.6 a	38.8 a	2255.0 a
Control	55.7 b	36.5 b	2157.0 b
(Giza 168) SA1	62.6 a	42.0 a	2491.5 a
(Giza 168) SA2	60.8 b	40.9 a	2347.0 b
Control	58.6 c	38.9 b	2189.0 c

SA1 = 200 mg/l, SA2 = 100 mg/l
Means followed by the same letter (columnwise) are not significantly different (Tukey' HSD; P≤0.05)

DISCUSSION

Early detection of wheat infested by aphids using infrared thermal imaging can help us prevent increase in aphid numbers and their management at the beginning of infestation. It can improve the yield and quality of crop, and reduce the use of pesticides.

Aphid numbers were lower in the treated than in untreated plots, which may be attributed to salicylic acid application that deterred aphids from wheat and increased foraging by parasitoids and predators attacking herbivorous insects of wheat crop. Plants treated with SA produce a volatile of methyl salicylate, which normally repels polyphagous herbivores and may attract specialist herbivores and their natural enemies which use a volatile as a host location cue. This study was similar to that reported by Pickett and Poppy (2001), who indicated that methyl salicylate prevented aphids from colonizing plants and populations of natural enemies of herbivorous pests. Salicylic acid acted as a signal in some induced responses to pathogens, as well as insect pests (Pickett & Poppy, 2001). Over 40 insect species from five separate orders have been identified as having olfactory receptors for the methylated form, methyl salicylate (Chamberlain et al., 2000; Pickett et al., 2003). The cereal aphids *R. padi*, *S. avenae* and *Metopolophium dirhodum* have a specific olfactory neuron on the sixth antennal segment to detect methyl salicylate (Pickett et al., 2006).

Salicylic acid caused a reduction in aphid population within five weeks after application. Pettersson et al. (1994) reported that cereal crops treated with a slow-release formulation of methyl salicylate had been avoided by many insects. Thus, in spring field trials, methyl salicylate applied to wheat significantly reduced (by 30-40%) the overall number of aphids colonizing the crop.

The experiments showed that salicylic acid affected the yield of the three wheat cultivars. The significant increase in wheat yield may have been due to the SA treatments that reduced wheat aphid damage. This result is consistent with Ibrahim et al. (2014), who reported that SA applied to the wheat cultivar Sakha 93 had increased the yield of crop and its components.

CONCLUSION

The infrared thermal imaging technique can be used to identify aphid-infected leaves of wheat. Also, the results indicate that SA application after such early detection could be helpful in aphid management strategies to increase wheat yield and reduce the use of chemical insecticides.

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Delovanje salicilne kiseline kao elicitora na biljne vaši u pšenici i otkrivanje zaraženosti infracrvenom termovizijskom kamerom, Ismailia, Egipat

REZIME

Pšenica (*Triticum sativum* L.) je jedna od najvažnijih žitarica u Egiptu. Štetni insekti, kao što su biljne vaši, predstavljaju pretnju u pogledu smanjenja prinosa. Otpornost indukovana kod pšenice folijarnom primenom salicilne kiseline ispitivana je u polju Poljoprivrednog fakulteta, Univerziteta Suez Canal u Egiptu tokom sezona 2012/2013 i 2013/2014. Tri sorte pšenice: Gemesa 9, Sakha 93 i Giza 168 prskane su tri puta koncentracijama salicilne kiseline (SA) od 200 mg/l i 100 mg/l nakon otkrivanja zaraze biljnim vašima korišćenjem infracrvene termovizijske kamere.

Tehnika infracrvene termovizije zasniva se na značajnim razlikama u temperaturi površine zaraženih i zdravih listova. Dobijaju se digitalne slike, a računarski program omogućava brzo otkrivanje zaraze. Rezultati su pokazali da zaraza biljnim vašima povećava temperaturu zaraženih listova, u poređenju sa zdravim listovima. Opseg razlike između maksimalne i minimalne temperature (At) bio je 1.1 ºC kod zdravih listova i 3.9 ºC kod zaraženih listova. Rezultati primene SA pokazali su značajne razlike u srednjim vrednostima za broj biljnih vaši i smanjenje zaraženosti među tretmanima i sortama. Viša od dve ispitivane koncentracije SA (200 mg/l) postigla je višu efikasnost kod sve tri sorte nego niža koncentracija (100 mg/l) tokom ogleda koji je trajao pet nedelja. Najviša efikasnost u suzbijanju biljnih vaši postignuta je jednu nedelju nakon primene (86,28% za sortu Giza, 85,89% za Gemesa i 70,54% za Sakha, respektivno). Pored toga, primena SA dovela je do povećanja prinosa sve tri sorte u odnosu na kontrolu. Sve tri sorte (Giza, Gemesa i Sakha) imale su više prinose nego kontrola nakon primene 200 mg/l SA. Uporedni prinos zrna je bio 2.491,5, 2.455,0 i 2.327,25 kg/fed. Može se zaključiti da se tehnika infracrvene termovizije može koristiti za otkrivanje zaraženih listova. Takođe, primena SA u pšenici indukuje otpornost na biljne vaši.

Ključne reči: Biljne vaši; Pšenica; Zaraženost; Salicilna kiselina; Termovizija