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Investigation of the induced antibiosis resistance by zinc element in different cultivars of sugar beet to long snout weevil, *Lixus incanescens* (Col: Curculionidae)

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Faramarz HARIRI MOGHADAM[1*], Jafar KHALGHANI[1], Saeid MOHARRAMIPOUR[2], Babak GHARALI[3] and Mehrzad MOSTASHARI MOHASSES[3]

[1]Department of Entomology, Science and Research Branch, Faculty of Agriculture, Islamic Azad University, Tehran, IRAN
[2]Department of Entomology, Faculty of Agriculture, Tarbiat Modares University, Tehran, IRAN
[3]Agriculture and Natural Resources Research and Education Center of Qazvin Province, Qazvin, IRAN
[*]Corresponding author: Fhmoghadam@gmail.com, Tel: +989127852004

***Abstract**.* Sugar beet weevil, *Lixus incanescens* Boh., is one of most dangerous pests in sugar beet. Host plant resistance is known as an efficient strategy for pest management. Thus, this study was conducted to investigate the antibiosis resistance induced by zinc element in different cultivars of sugar beet to long snout weevil, *L. incanescens*. Seeds of eleven commercial monogerm cultivars of sugar beet including Pars, Torbat, Ekbatan, SBSI019, Rizofort, Puma, Dorothea, Brigita, Pecora, Nagano and Jolgeh, were studied. Field was divided to two parts; one part was treated with zinc fertilizer and other part did not treat with fertilizer. Antibiosis parameters were tested. Results clearly did not show that cultivars have antibiosis resistance under non–using fertilizer during 2013 and 2014 years. Rizofort cultivar showed lower susceptibility compared with other cultivars when using zinc fertilizer, because of lower adult weight, female longevity and period length of immature stage during the both years. Thus, Rizofort cultivar was relatively resistant to *L. incanescens* when using zinc fertilizer during the both years, which can be useful in the development of IPM programs for this insect in sugar beet fields

***Keyword**: Lixus incanescens*, antibiosis, sugar beet, zinc fertilizer.

Introduction

Sugar beet is belonging to *Beta vulgaris* L. ssp. *vulgaris* sugar beet group [LANGE *et al.,* 1999]. Sugar beet is extensively cultivated in northern hemisphere in late winter or early spring and harvested after 5–9 months, depending on climatic and soil conditions. Twenty percent of world's supply of sugar is achieved from sugar beet and other part is get from sugar cane which is mainly cultivated in tropical climates in developing countries [FAO, 2009].

The sugar content in sugar beet has different amounts. The by–products of the sugar beet including pulp and molasses increases value of up to 10 % of the value of the sugar. However, sugar beet was protected by herbicides [WINNER, 1993].

Sugar beet is severely sensitive to pests when developing the first 3–4 pairs of leaves [BAZOK *et al.,* 2016]. Sugar beet weevil, *Lixus incanescens* Boh., is one of most dangerous pests in sugar beet which is found in some parts of Iran and other countries. This pest attacks leaf and petiole of sugar beet and larvae can damage up to 75 % root weight loss [ARBABTAFTI *et al.,* 2012; OCETE *et al.,* 1994]. It is well known that the use of insecticides for controlling the insects has caused some problems such as the development of insecticide resistance, health hazards, and the risk of environmental pollution [COX, 2004]. Farmers usually use pesticides for controlling this pest, but often these efforts are not successful, since immature stages are located the petioles [FOSTER *et al.,* 2007], resulting increased resistance of *L. incanescens*. However, the use of insecticides is still efficient strategies for controlling the pest [ROUHANI *et al.,* 2012].

Discovery of some genetic resistances to diseases enhanced sugar yield and lowered reliance on pesticides.
Studies have shown that improvement in hybrid varieties, the pest and disease resistances, breeding assisted by molecular biology, *etc*. caused to produce

hybrid varieties. Host plant resistance is known as an efficient strategy for pest management, because of its importance economic and environment [KARIMI *et al.*, 2012, SAMFIRA et al., 2015].

Plant species are known to have different suitability for different insect pests when their performance and preference are compared with other plants [RAZMJOU and GOLIZADEH, 2010; RAZMJOU *et al.*, 2012]. Antixenosis, antibiosis, and tolerance are resistance mechanisms known against insects in plants [KOGAN and ORTMAN, 1978; MOTTAGHINIA *et al.*, 2011]. Studies have shown that variations in host plant quality can influence development, reproduction and, hence, population growth of target herbivorous insects [La ROSSA *et al.*, 2013].

Some active ingredients are allowed to control sugar beet weevil, because of implementation of EU pesticide legislation. Zinc can be used to control the pest, since it is known to have antimicrobial activity which can be applied as fungicide [SEVEN *et al.*, 2004; LEVIN *et al.*, 2007].

Zinc acts as antimicrobial agent by generation of reactive oxygen species, oxidative stress, membrane disruption, protein unfolding, and/or inflammation [REDDY *et al.*, 2007; MENG *et al.*, 2009; DONALDSON *et al.*, 2009]. It is also reported the effects of zinc against insects (*Aphis nerii*) [ROUHANI *et al.*, 2012]. Other study has shown impacts of zinc oxide nanoparticle against rice weevil (Coleoptera: Curculionidae) [KERATUM *et al.*, 2015, CAUNII, *et al.*, 2015, BUTNARIU, *et al.*, 2015].

The use of different varieties of sugar beet with diverse quantitative and qualitative characteristics can increase yield and decrease pest losses in the world. So far, based on our knowledge no comprehensive study in Iran is done about induction of antibiosis resistance by zinc in sugar beet varieties to sugar beet long snout weevil. Thus, this study aimed to investigate the induction of antibiosis resistance by zinc in eleven sugar beet varieties to long snout weevil *L. incanescens* in Iran.

Material and methods

Location and Plant materials. The present study was conducted at Pistachio Research Station, Yezbar village Qazvin Province, Iran (36° 2'N, 50° 7'E, 1175 m above sea level). Seeds of eleven commercial monogerm cultivars of sugar beet including Pars, Torbat, Ekbatan, SBSI019, Rizofort, Puma, Dorothea, Brigita, Pecora, Nagano and Jolgeh, were prepared from Seed and Plant Breeding Research Institute, Karaj, Iran.

Preparing land and insect colony. Field testing was achieved in an area of about 0.1 hectare, in the early spring of 2013 and 2014 that was deeply plowed. After tight and weeding, planting seeds of sugar beet varieties were conducted in the second decade of April each year.

Farm irrigation every 7 to 10 days were routine. Weed control was performed by hand removing. Adult's weevils were collected from a sugar beet (*B. vulgaris*) field.

Field preparation for planting and zinc sulphate treatment. Field was divided to two parts; one part was treated with zinc fertilizer and other part did not treat with fertilizer. Each plot was divided to 3 blocks and each block was also divided to 3 plots. Each plot had 11 m length and 5.5 m width and 11 plots were in each row. Plot distance was 1 m and row distance were 11 m.

Antibiosis experiment. After 30 days and to develop 3 to 8 leaves, part of plant in main plot was selected and maintained at cages with dimensions 50·30·30 cm. Insects (3 males and 2 females) with <1 d of age was released into each cage. Insects were weighted and allowed to feeding up to 7 days and were then weighted. This action was in 3 replicates.

Longevity and also larval weight was calculated.

Statistical analysis. The data are shown as mean ± standard deviation (mean ± SD). Prior to analysis, heterogeneity of variance was tested and then all entries were transformed [Log(X+2)]. Numbers of adult weevils deployed at the different times on eleven sugar beet cultivars at two–years were analyzed based on combined analysis of randomized complete block design.

Significance of the differences was examined by analysis of variance (ANOVA) test., followed using Tukey post hoc. The levels of significance were done at α=0.05. Statistical analysis was performed by SAS® 9.1 software (2005).

Results and discussion

Mean comparison of treatments in 2013 year (Table 1) showed that when using zinc fertilizer, the lowest insect weight was observed in Rizofort cultivar and the highest insect weight was observed in Brigita cultivar (P<0.05).

However, when non–using zinc fertilizer, the highest insect weight was observed in Pars and Puma and the lowest were considered in Nagano cultivar (P<0.05) in 2013 year (Table 2).

Considering males longevity, Jolgeh cultivar had highest male longevity and puma showed the lowest male longevity (Table 1), while when non–using zinc fertilizer, Pars showed highest male longevity (Table 2).

Table 1.

Means comparison of antibiosis test in different cultivars when using zinc fertilizer in 2013 year (Means ± SE)

Cultivars	Adult weight (mg)	Male longevity (d)	Female longevity (d)	Period length of immature stages (d)	Larval weight (mg)
Pars	0.011±0.001bcd	6.66±0.33cd	11.00±0.57a	28.00±0.57a	0.015±0.000a
Torbat	0.012±0.001bcd	7.66±0.33bc	10.00±0.57ab	26.66±0.88a	0.013±0.000a
Ekbatan	0.009±0.00cde	8.33±0.33abc	9.66±0.33ab	29.66±0.88a	0.012±0.001a
SBSI019	0.013±0.001abc	7.66±0.33bc	8.66±0.33ab	27.33±0.88a	0.012±0.000a
Rizofort	0.006±0.000e	7.33±0.33bcd	7.66±0.33b	28.33±0.33a	0.015±0.000a
Puma	0.008±0.000de	5.66±0.33d	9.00±0.00ab	26.33±1.20a	0.015±0.000a
Drothea	0.012±0.001bcd	8.00±0.00abc	8.66±0.33ab	27.00±1.15a	0.012±0.001a
Brigita	0.016±0.000a	7.66±0.33bc	10.66±0.33ab	30.33±1.20a	0.012±0.000a
Pecora	0.015±0.000ab	9.00±0.00ab	10.66±0.33ab	30.33±1.20a	0.012±0.001a
Nagano	0.014±0.000ab	7.66±0.33bc	9.00±0.00ab	29.33±1.85a	0.015±0.000a
Jolgeh	0.014±0.000ab	9.66±0.33a	10.33±1.20ab	29.00±1.15a	0.011±0.000a

Mean values in a column followed by different lowercase letters (a–e) are significantly different on the basis of ANOVA with Tukey's test (p<0.05)

Considering female longevity, when using zinc fertilizer, Pars cultivar had the highest longevity, but it was not observed significant differences when non–using zinc sulphate fertilizer.

There was no significant difference among groups for period length of immature stage during two years (P>0.05).

When using zinc fertilizer, it was not found significant differences among groups for larval weight, however, Rizofort cultivar showed the highest for when non–using zinc fertilizer.

Table 2.

Means comparison of antibiosis test in different cultivars when non–using zinc fertilizer in 2013 year (Means ± SE)

Cultivars	Adult weight (mg)	Male longevity (d)	Female longevity (d)	Period length of immature stages (d)	Larval weight (mg)
Pars	0.024±0.001a	8.33±0.33a	9.00±0.57a	25.00±0.57a	0.016±0.001ab
Torbat	0.015±0.000bc	7.33±0.33ab	8.66±0.33a	26.33±0.88a	0.016±0.000ab
Ekbatan	0.020±0.001ab	6.66±0.33ab	8.66±0.88a	26.00±0.57a	0.018±0.001ab
SBSI019	0.016±0.000bc	6.00±0.00b	8.33±0.33a	24.66±0.66a	0.017±0.000ab
Rizofort	0.016±0.001bc	6.66±0.33ab	8.66±0.33a	25.33±0.88a	0.021±0.001a
Puma	0.025±0.000a	6.33±0.33b	9.33±0.33a	25.00±0.00a	0.015±0.001b
Drothea	0.018±0.001bc	6.00±0.00b	8.00±0.57a	25.66±1.15a	0.017±0.000ab
Brigita	0.016±0.000bc	7.33±0.33ab	9.00±0.57a	24.00±57a	0.015±0.000ab
Pecora	0.017±0.001bc	7.00±0.57ab	7.66±0.33a	25.00±0.57a	0.018±0.000ab
Nagano	0.014±0.001c	6.33±0.33b	9.00±0.00a	26.33±0.66a	0.015±0.000b
Jolgeh	0.016±0.000bc	6.33±0.33b	7.66±0.33a	27.00±1.00a	0.016±0.001ab

Mean values in a column followed by different lowercase letters (a–c) are significantly different on the basis of ANOVA with Tukey's test (p<0.05)

The mean for antibiosis tests for different cultivars in 2014 year are shown in Table 3. When application of zinc fertilizer, Pecora and Jolgeh showed the highest insect weight and Puma showed lowest, however, Nagano showed highest insect weight when non–application the insect.

Table 3.

Means comparison of antibiosis test in different cultivars when using zinc fertilizer in 2014 year (Means ± SE)

Cultivars	Adult weight (mg)	Male longevity (d)	Female longevity (d)	Period length of immature stages (d)	Larval weight (mg)
Pars	0.015±0.001bcd	7.33±0.33bcd	10.00±0.00abc	29.00±0.57cd	0.012±0.001a
Torbat	0.016±0.000ab	8.00±0.00abc	11.00±0.57ab	30.33±0.88bcd	0.014±0.000a
Ekbatan	0.013±0.00bc	8.66±0.33abc	10.33±0.66abc	31.66±0.88abc	0.012±0.000a
SBSI019	0.012±0.000c	7.33±0.33bcd	8.33±0.33cde	26.66±0.88de	0.013±0.000a
Rizofort	0.012±0.000c	7.00±0.57cd	7.00±0.00e	24.00±0.57e	0.016±0.000a
Puma	0.008±0.000d	5.66±0.33d	9.00±0.00bcde	29.33±0.88bcd	0.014±0.000a
Drothea	0.012±0.000c	8.66±0.33abc	7.66±0.33de	32.33±0.33abc	0.012±0.000a
Brigita	0.013±0.000bc	9.00±0.00ab	9.66±0.33abcd	27.66±0.33de	0.013±0.001a
Pecora	0.017±0.000a	7.66±0.33abc	10.66±0.33ab	33.00±0.57ab	0.014±0.001a
Nagano	0.016±0.000ab	7.33±0.33bcd	11.33±0.66a	35.33±0.881a	0.012±0.001a
Jolgeh	0.017±0.000a	9.33±0.33a	9.66±0.33abcd	27.66±0.88de	0.011±0.000a

Mean values in a column followed by different lowercase letters (a–e) are significantly different on the basis of ANOVA with Tukey's test ($p<0.05$)

With regards to males longevity, Puma cultivar showed the lowest male longevity (Table 3), while when non–using zinc fertilizer, while Drothea showed lowest male longevity (Table 4). Considering female longevity, when using zinc fertilizer, Rizofort cultivar had the lowest longevity, while when non–using zinc sulphate fertilizer, Pecora cultivar showed the lowest significant differences. It was not found significant differences among groups for larval weight ($P>0.05$).

Table 4.

Means comparison of antibiosis test in different cultivars when non–using zinc fertilizer in 2014 year (Means ± SE)

Cultivars	Adult weight (mg)	Male longevity (d)	Female longevity (d)	Period length of immature stages (d)	Larval weight (mg)
Pars	0.027±0.001ab	7.00±0.57ab	8.66±0.33a	24.66±0.88a	0.016±0.000a
Torbat	0.019±0.004bc	6.66±0.66ab	7.66±0.33ab	26.33±0.33a	0.015±0.000a
Ekbatan	0.021±0.000bc	7.00±0.57ab	8.66±0.33a	25.00±0.57a	0.018±0.001a
SBSI019	0.014±0.000c	6.33±0.66ab	8.00±0.00ab	25.33±1.45a	0.017±0.001a
Rizofort	0.024±0.002bc	6.00±0.00ab	8.33±0.66ab	26.00±0.57a	0.021±0.000a
Puma	0.021±0.001bc	5.66±0.33ab	9.00±0.57a	25.66±0.33a	0.015±0.002a
Drothea	0.019±0.000bc	5.33±0.66b	9.00±0.57a	25.33±0.88a	0.016±0.001a
Brigita	0.023±0.001bc	6.00±0.00ab	9.00±0.00a	24.66±88a	0.015±0.000a
Pecora	0.019±0.001bc	7.66±0.33a	6.00±0.57b	25.33±0.33a	0.017±0.001a
Nagano	0.035±0.003a	6.66±0.33ab	7.66±0.66ab	25.66±0.88a	0.017±0.000a
Jolgeh	0.017±0.002bc	6.33±0.33ab	7.66±0.33ab	28.33±0.33a	0.019±0.000a

Mean values in a column followed by different lowercase letters (a–c) are significantly different on the basis of ANOVA with Tukey's test ($p<0.05$)

The results of data analysis for the mechanism of antibiosis test in the year 2013 year (Table 5) and 2014 year (Table 6) show significant differences between experimental for cultivars tested.

Table 5.

Combined analysis of antibiosis test when using zinc fertilizer in different sugar beet cultivars during 2013 and 2014 years

S.O.V.	Df	Adult weight (mg)	Male longevity (d)	Female longevity (d)	Period length of immature stages (d)	Larval weight (mg)
Year	1	0.000064**	0.060 ^{NS}	0.060 ^{NS}	29.333 **	0.0000000 ^{ns}
Block (year)	4	0.000000 ^{NS}	0.424 ^{NS}	0.530 ^{NS}	2.424 ^{NS}	0000000 ^{NS}
Cultivar	10	0.000044 **	6.003 **	7.469 **	21.442 **	0.00001 **
Year × Cultivar	10	0.000010 **	0.760 *	1.627 ^{NS}	16.133 **	0.000003 ^{NS}
Error	40	0.000001	0.340	0.880	2.624	0.0000028
C.V.	–	10.348	7.497	9.829	5.574	12.720

NS: non–significant, *: $p<0.05$ and **: $p<0.01$

There was significant interaction between year and cultivar for insect weight (P<0.05).

The sugar beet is known to be one of the most important productions in farming fields in Qazvin province, Iran.

This study investigated antibiosis responses of eleven sugar beet cultivars against *L. incanescens*. The results of present study showed a wide range of host plant responses to sugar beet long snout weevil between sugar beet cultivars when using and non–using zinc fertilizer.

The results of this study can help to use the best cultivar in selection of suitable cultivar, regarding to longevity and period length of immature stages.

Antibiosis test can be used as efficient in preventing their populations from reaching economic damage levels [KENNEDY *et al.*, 1987, IANCULOV, *et al.*, 2004, BUTU, *et al.*, 2014]. To evaluate the resistance by population growth parameters of an insect is beneficial tools in showing degree of plant resistance to insects [TSAI and WANG, 2001, RASHED and BUTNARIU, 2014]. In 2013 year, Rizofort cultivar showed lower insect weight, female longevity and period length of immature stage. However, it did not show significant difference with those which had male longevity for male longevity traits when using zinc fertilizer. It can be stated that Rizofort cultivar has lower susceptibility compared with other cultivars when using zinc fertilizer.

Table 6.

Combined analysis of antibiosis test when non–using zinc fertilizer in different sugar beet cultivars during 2013 and 2014 years

S.O.V.	Df	Adult weight (mg)	Male longevity (d)	Female longevity (d)	Period length of immature stages (d)	Larval weight (mg)
Year	1	0.000232 **	1.833 ^{NS}	2.560 ^{NS}	0.545 ^{NS}	0.0000000 ^{NS}
Block (year)	4	0.000021 ^{NS}	0.757 ^{NS}	0.242 ^{NS}	0.803 ^{NS}	0000002 ^{NS}
Cultivar	10	0.000068 **	2.078 **	2.581 **	4.678 *	0.000016 **
Year × Cultivar	10	0.000067 **	0.733 ^{NS}	0.793 ^{NS}	0.745 ^{NS}	0.000001 ^{NS}
Error	40	0.000008	0.474	0.692	1.703	0.0000040
C.V.	–	14.494	10.448	9.967	5.102	11.762

NS: non–significant, *: p<0.05 and **: p<0.01

At same year, Brigita had the highest insect weight and period length of immature stage. It seems that Brigita cultivar has higher sensivity when using zinc fertilizer. Insects reared on Rizofort had lowest female longevity and period length of immature stages [STOLERU et al., 2018].

Thus, it decreases reproduction. However, Nagano cultivar showed lower insect weight and male longevity and Pars cultivar showed the highest insect weight and male longevity when non–using.

Thus, to treat with zinc can influence resistance in different cultivars. The decreased insect weight can implicit on antibiosis mechanism [HORTON *et al.*, 1997].

In 2014 Fathi and Abedi showed that Period length of immature stages were different among sugar beet cultivars [FATHI and ABEDI, 2014]. In the study published by Golizadeh and colleagues on five sugar beet cultivars (Doroti, Perimer, Pershia, Rozier and 006) found relative high levels of susceptibility in Pershia cultivar to *Aphis fabae*; however, the cultivars Rozier and Perimer were relatively less susceptible [GOLIZADEH *et al.*, 2016, PETRACHE, *et al.*, 2014, BUTNARIU, *et al.*, 2016].

On the basis our findings, under application and non–application of zinc fertilizer, the different cultivars showed the different antibiosis resistance. It seems that different cultivars have various interactions with zinc. It is shown that heavy metals such zinc in insects have a clear effect on growth [WARRINGTON, 1987], mortality [MITTERBOCK and FUHRER, 1988], and physiology [ILIJIN *et al.*, 2009, BUTU, *et al.*, 2014, RODINO, *et al.*, 2014, BUTNARIU and SAMFIRA, 2012].

Thus, zinc can help to antibiosis resistance in different cultivars. At 2014 year, Rizofort cultivar again showed lower susceptibility compared with other cultivars when using zinc fertilizer, because it had lower insect weight, female longevity and period length of immature stage.

During 2013 and 2014 years, this cultivar showed better efficiency for antibiosis test. Thus, Rizofort cultivar and the use of zinc can be suggested as resistance cultivar.

It was studied the effects of four cultivars of sugar beet on biology and demographic parameters of *A. fabae* and showed that the use of Shirin cultivar could upgrade the natural biological control of *A. fabae* by *Aphidius matricariae* [TAHRIRI ADABI *et al.*, 2010].

Our findings also indicated that the antibiosis parameters of *L. incanescens* were differentially affected by the sugar when non–using zinc fertilizer.

The data about the quality of sugar beet cultivars and their interactions with fertilizers can affect the antibiosis parameters for *L. incanescens* and it also can help us understand the population dynamics and may help in the development of better management programs for this pest.

However, Rizofort cultivar can beneficial when using zinc fertilizer. Our observations showed that the Rizofort cultivar was relatively resistant to *L. incanescens* when using zinc fertilizer, which can be useful in the development of IPM programs for this insect in sugar beet fields.

Conclusions

This study was conducted to investigate the antibiosis resistance induced by zinc element in different cultivars of sugar beet to long snout weevil. Our findings clearly did not show that cultivars have antibiosis resistance under non–using fertilizer.

However, Rizofort cultivar was relatively resistant to *L. incanescens* when using zinc fertilizer, which can be useful in the development of IPM programs for this insect in sugar beet fields.

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