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Assessment of the attack of *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae) on biomass, seed and oil in fennel intercropped with cotton with colored fibers

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ABSTRACT

The fennel aphids, *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae) attack umbels and may thus, cause to failure of fruit setting and also attack during the phase of seed development. Our research investigated the severity of biomass, seed and essential oil damages related to infestation by the fennel aphid, *H. foeniculi* in fennel (*Foeniculum vulgare* Miller) plots and plots of fennel intercropped with cotton with colored fibers. A randomized complete block design was used with four treatments: (1) two rows of fennel: three rows of cotton (2F: 3C) (non-treated plot), (2) two rows of fennel: three rows of cotton (2F: 3C) (treated plot), (3) fennel (F) (non-treated plot), and (4) fennel (F) (treated-plot), with four replications. The average of the weight of fennel seeds from umbels in the green stage in treated plots (0.19 mg) was higher (21%) than in the non-treated plots (0.15 mg). There was no significant difference in the essential oil extracted from biomass between two cropping systems for both treated and non-treated plots. However, the amount of essential oil extracted from fennel biomass in treated plots was higher in fennel plots than in fennel in non-treated plots. The average of the essential oil of 1000 fennel seeds from fennel–cotton intercropping system (0.17 ml) was higher (18%) than in the fennel cropping system (0.14 mg). The aphid attack reduced 29% of fennel seed weight. On the other hand, the average amount of essential oil extracted of the non-attacked seeds was two folds higher than of the attacked seeds. Therefore, our results showed that the effects of *H. foeniculi* on these yield components were smaller in the plots with fennel–cotton intercropping than in fennel plots. We suggest that seed composition responses to *H. foeniculi* injury should be an important consideration when conducting research to develop IPM systems for this fennel pest.

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1. Introduction

Fennel (*Foeniculum vulgare* Miller) occurs naturally in the North America (Ramalho et al., 2012). It is spreaded in Mediterranean region and Central Europe (Aprotosoaie et al., 2010; He and Huang, 2011) and it is widely cultivated throughout the temperate and tropical regions of the world for its aromatic fruits, widely applied in medicinal preparations (He and Huang, 2011).

It was introduced into Brazil by the first settlers and rapidly spread through the states of Bahia, Sergipe, Paraíba, and Pernambuco (Ferreira and Sousa-Silva, 2004). The fennel has a guaranteed market in northeastern Brazil and is important for family farming in the region (Ramalho et al., 2012).

Fennel is known by the use of its fruits and its essential oil in the manufacture of perfumes, toothpaste, soaps and herbal medicine (Madueno Box, 1973; Silva Júnior, 1997). Herbal drugs and essential oil of fennel have antispasmodic, diuretic, anti-inflammatory, analgesic and antioxidant effects (Parejo et al., 2002; Choi and Hwang, 2004). According to Singh et al. (1988), it has fungicidal activity. The Brazilian cosmetic industry uses fennel oil in many ways (soothing, cleansing, and toning effects on the skin and hairs) (Ramalho et al., 2012).

Factors that impair fennel production and seed quality in Brazil include insect pests, especially the aphid, *Hyadaphis foeniculi* (Passerini) (Hemiptera: Aphididae). *H. foeniculi* is a cosmopolitan

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species and a vector for at least 12 types of virus, including mosaic potyvirus, yellow luteovirus and carlavirus (Ferreira and Sousa-Silva, 2004). Since it continually sucks sap, it causes flowers and fruits to wilt and dry up (Ramalho et al., 2012). It also produces a secretion known as "honeydew" which is favorable to the development of the fungus *Capnodium* spp., leading to the formation of sooty mold (Lazzari and Lazzarotto, 2005) which prevents the plant from transpiring and reduces the photosynthetic area, weakening the plant (Leite et al., 2006). In the state of Paraíba, Brazil, *H. foeniculi* usually reproduces during hot periods, forming colonies inside flowers (Ramalho et al., 2012).

In northeastern Brazil, fennel is intercropped with colored-fiber cotton (*Gossypium hirsutum* Linnaeus) (Malaquias et al., 2010; Fernandes et al., 2013; Ramalho et al., 2012). Natural colored cotton is ecologically friendly, as it reduces the dyeing stage in industrial production in which frequently and perhaps incorrectly used chemicals, can be damaging to human health (Horstmann, 1995). Development of cotton with colored fibers intercropping with fennel is an alternative for recuperating agribusiness in cotton cultivation in northeast Brazil. Intercropping fennel with colored-fiber cotton has contributed to a 80% drop in the damage caused by *H. foeniculi* to the fennel crop (Ramalho et al., 2012).

Hyadaphis foeniculi is considered a major insect pest on fennel (Ramalho et al., 2012), mainly attacking flowers, fruits and leaves. By continually sucking the sap, it causes these organs to wilt and dry up, impairing the fennel seed (Ferreira and Sousa-Silva, 2004). On the other hand, essential oils are concentrated in the biomasses and mainly in fruits and provide the unique aroma and taste. The quality of the oil essence of the fennel has great importance and it depends on the stage of maturity of fennel fruits, as well as, insect attack (Maranca, 1985).

We know that fennel aphids reduce the fennel seed yield by 80% in the sole fennel plots compared with 30% for all intercropping systems (Ramalho et al., 2012). However, there is no information on the effect of *H. foeniculi* on the biomass yield, seed weight and oil content of fennel from fennel crop and fennel intercropped with cotton with colored fibers. For this reason, field experiments were carried out in 2009 and 2010 seasons to determine whether fennel aphids cause significant reduction in biomass yield, seed weight and essential oil content of fennel from fennel crop and fennel intercropped with cotton with colored fibers. We have hypothesized that (1) the fennel seeds attacked by *H. foeniculi* are less heavy than the non-attacked seeds; (2) the aphids *H. foeniculi* reduce the amount of essential oil in the fennel seeds; (3) the aphids *H. foeniculi* do not reduce the amount of essential oil in the fennel biomass. Such difference changes depend on the crop systems.

2. Material and methods

2.1. Research location and cotton and fennel cultivars

The study was carried out during 2009 and 2010 seasons at an experimental farm located in the countries of Montadas and Lagoa Seca Paraiba, Brazil, a rural wetland microregion at an elevation of 634 m, S 7°10′5″ W 35°51′13″. A fennel (*F. vulgare*) cultivar (Montadas) and a naturally colored cotton (*G. hirsutum*) cultivar (BRS Safira) were planted under dry land conditions. Field plots were planted between the first and second week of May in 2009 and in the second week of May in 2010. Weed control was done by hoeing.

2.2. Bioassays and experimental design used

A randomized complete block design was used with four treatments: (1) two rows of fennel: three rows of cotton (2F: 3C) (non-treated plot), (2) two rows of fennel: three rows of cotton (2F: 3C) (treated plot), (3) fennel (F) (non-treated plot), and (4) fennel (F) (treated-plot) (Fig. 1), with four replications. The area of each experimental unit was 462 m^2.

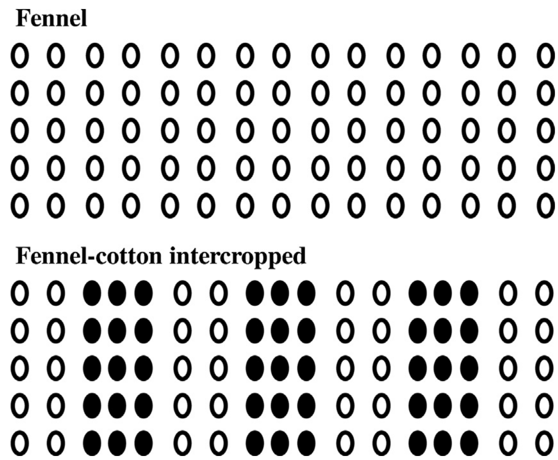

Fig. 1. Layout of experimental units in the fennel–cotton intercropping system (2F: 3C) and fennel (F). Fennel plant (open circles) and cotton plant (closed circles).

The treated plots were sprayed with thiamethoxam insecticide at the rate of 120 g a.i/ha at 1-week intervals beginning with the emergence of the cotton plants to keep the plants aphid-free, i.e. full protection. The spray was made with a hand-operated knapsack sprayer with 200-l water per hectare. The non-treated plots were not sprayed with any insecticide to allow for natural aphid infestation, along with their predators and parasitoids.

The experimental units of the fennel–cotton intercropping system consisted of rows of naturally colored cotton (BRS Safira) between rows of fennel (Montadas cultivar) with a row of 21 m (Fig. 1). Fennel rows were spaced 1.5 m apart, and there was 0.50 m between fennel plants in a row (Fig. 1). In the intercropped plots, cotton rows were spaced 1 m apart, whereas the spacing between the cotton and fennel rows was 1.50 m (Fig. 1).

2.3. Biomass and seed yield and oil content

Samples of biomass and seeds of fennel plants were collected in the plots with 100 (from umbels in green stage) and 150 day-old (from umbels in dry stage). The biomass yield (shoot system plus umbels in green stage) was taken from 10 plants by randomly selecting five plants in each of the second and penultimate rows of all the plots at the umbels green stage. However, at seed ripening (dry umbels) stage only seeds were collected. The extraction of essential oils from fennel biomasses and seeds was achieved by steam-distillation (Bowles, 2003). The biomasses and seeds were distilled for four hours. The oil was stored in a dark glass bottle.

2.4. Data analysis and statistics

The biomass and seed yield and also the oil content from seeds and biomasses were tested for normality (Kolmogorov D normality test) and homogeneity of variance (Bartlett's test). The mean of biomass weight, seed weight, oil content of biomass and seed development were subjected to three-way analysis of variance (ANOVA) [year, crop system, and aphid control (treated and non-treated plots)], using PROC GLM of SAS (SAS Institute, 2006) and the averages were compared using the *F* test or Student–Newman–Keuls test ($P = 0.05$).

Table 1
Summarized model of three-way analysis of variance (ANOVA) for the effects of year, cropping system, and control of the aphids, *Hyadaphis foeniculi* on the fennel biomasss and essential oil for the fennel cultivar Montadas in 2009 and 2010 seasons.

Source	Models	DF	*F* ratio	Prob > *F*
Biomass (kg/plant)	Model	10	0.90	0.5340
	Year (Y)	1	0.09	0.7677
	Cropping system (Cs)	1	0.08	0.7838
	Aphid control (Ac)	1	2.09	0.1824
	Cs × Ac	1	0.07	0.7955
	Y × Cs	1	0.81	0.6898
	Y × Ac	1	0.79	0.6987
	Y × Cs × Ac	1	0.04	0.8867
Essential oil (ml/kg biomass)	Model	6	5.09	0.0152
	Year (Y)	1	0.08	0.7799
	Cropping system (Cs)	1	1.15	0.3106
	Aphid control (Ac)	1	3.41	0.0979
	Cs × Ac	1	9.96	0.0116
	Y × Cs	1	0.78	0.7165
	Y × Ac	1	0.81	0.6709
	Y × Cs × Ac	1	0.05	0.8898

Cropping systems: cotton and fennel intercropped with cotton (2F: 3C). F = fennel and C = cotton. Aphid control: plots treated or not treated with insecticide.

3. Results

From 139 days (2.35 aphids/plant) to 195 days (11 aphids/plant) after transplanting, the fennel plants became infested with aphids. The numbers of aphids per fennel plant in the fennel system and in the intercropped fennel–cotton system were higher at 188 days (36 and 29 aphids, respectively) after transplanting the fennel plants.

With regards to the averages for biomass weight of fennel plants including the umbels in the green stage, it was found that there was no significant interaction among year (Y), cropping system (Cs) and the aphid control (Ac), year (Y) and aphid control (Ac) and year (Y) and cropping system (Cs), cropping system (Cs) and aphid control and also did not differ between aphid control (Ac), cropping system, and years (Table 1). Thus, the analyses were conducted using the pooled data.

With regards to the averages for essential oil extracted from fennel biomass, there were no interactions among the year (Y), cropping system (Cs), and aphid control (Ac); year (Y) and aphid control (Ac) or year (Y) and cropping system (Cs) (Table 1). However, there was a significant interaction between cropping system (Cs) and aphid control (Ac) (Table 1). This means that the effect of the crop system on the production of essential oil depends of the aphid control. There was no significant difference in the essential oil extracted from biomass between two cropping systems for both treated (Fig. 2A) and non-treated (Fig. 2B) plots. However, the amount of essential oil extracted from fennel biomass in treated plots was significantly higher in fennel plots than in fennel plots in non-treated plots.

There were no interactions among the year (Y), cropping system (Cs) and aphid control (Ac), year (Y) and aphid control (Ac), or year (Y) and crop system (Cs) (Table 2) for fennel seed weight from umbels in the green stage. However, there were effects of cropping system (Cs) and aphid control (Ac) (Table 2) for fennel seed weight. The average of the weight of the fennel seed from umbels in the green stage in treated plots (0.19 mg) was significantly higher (21%) than in the non-treated plots (0.15 mg) ($F_{Ac\,(1,21)} = 10.23$, $P = 0.0100$) (Fig. 3A). On the other hand, the average of the weight of the fennel seed in fennel–cotton intercropping system (0.16 mg) was significantly higher (13%) than in the fennel cropping system (0.14 mg) ($F_{Cs\,(1,21)} = 4.52$, $P = 0.0425$) (Fig. 3B).

There were no significant interactions among year (Y), cropping system (Cs) and aphid control (Ac) (Table 2), and between year (Y) and aphid control (Ac), year (Y) and cropping system (Cs) and

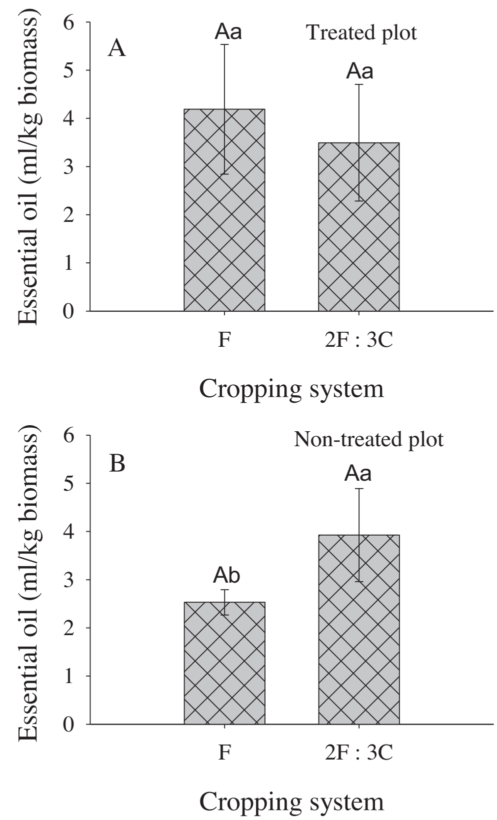

Fig. 2. Essential oil (ml/kg biomass) (mean ± SE) of fennel biomass, cultivar Montadas in treated plots (A) and non-treated plots (B) of fennel and fennel intercropped with cotton with colored fibers ($F_{Cs \times Ac\,(1,21)} = 9.96$, $P < 0.0116$). Mean with a common lowercase letter between the two groups (treated and non-treated plots) of bars within the same cropping system or with a common uppercase letter within each group (treated or non-treated plot) of bars are not significantly different based on the Student–Newman–Keuls test ($P = 0.05$).

cropping system and aphid control (Ac) (Table 2) for amount of essential oil per 1000 seeds from umbels in the green stage. However, there were effects of aphid control (Ac) (Table 2) and cropping system (Cs) (Table 2) on the amount of essential oil per 1000 seeds. The average of the essential oil extracted in 1000 fennel seeds

Table 2
Summarized model of three-way analysis of variance (ANOVA) for the effects of year, cropping system, and control of the aphids, *Hyadaphis foeniculi* on the weight and essential oil of fennel seeds from umbels in the green stage for the fennel cultivar Montadas in 2009 and 2010 seasons.

Source	Models	DF	*F* ratio	Prob > *F*
Seed weight (mg)	Model	10	2.93	0.0719
	Year (Y)	1	0.15	0.8343
	Cropping system (Cs)	1	4.52	0.0425
	Aphid control (Ac)	1	10.23	0.0100
	Cs × Ac	1	0.16	0.8132
	Y × Cs	1	0.98	0.5144
	Y × Ac	1	0.84	0.6332
	Y × Cs × Ac	1	0.08	0.9108
Essential oil (ml/1000 seeds)	Model	10	3.87	0.0611
	Year (Y)	1	0.10	0.7799
	Cropping system (Cs)	1	12.78	0.0012
	Aphid control (Ac)	1	5.15	0.0428
	Cs × Ac	1	0.96	0.1454
	Y × Cs	1	0.86	0.1712
	Y × Ac	1	0.91	0.1511
	Y × Cs × Ac	1	0.12	0.7565

Cropping systems: cotton and fennel intercropped with cotton (2F: 3C). F = fennel and C = cotton. Aphid control: plots treated or not treated with insecticide.

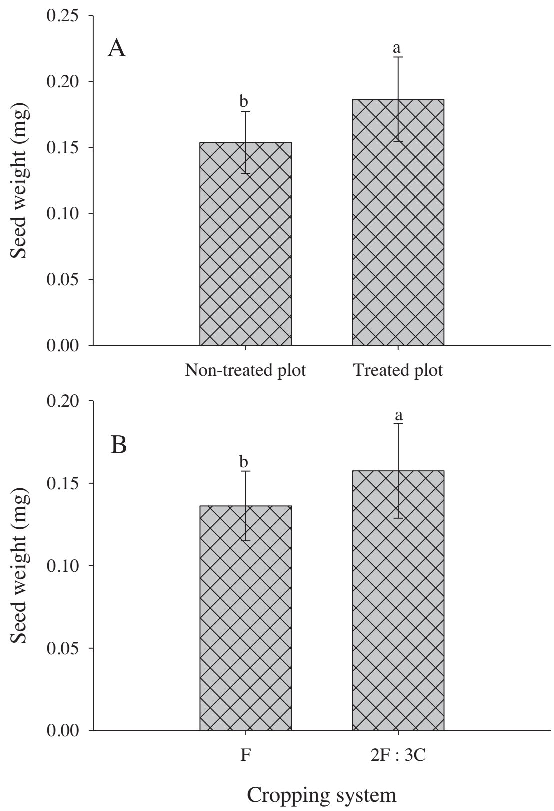

Fig. 3. Weight of fennel seeds (mean ± SE) from umbels in green stage, cultivar Montadas in treated plots and non-treated plots ($F_{Ac(1,21)}$ = 10.23, P = 0.0100) of fennel (A) and fennel and fennel intercropped with cotton with colored fibers ($F_{Cs\,(1,21)}$ = 4.52, P = 0.0425) (B). Different lowercase letters indicate a significant difference between mean within each variable.

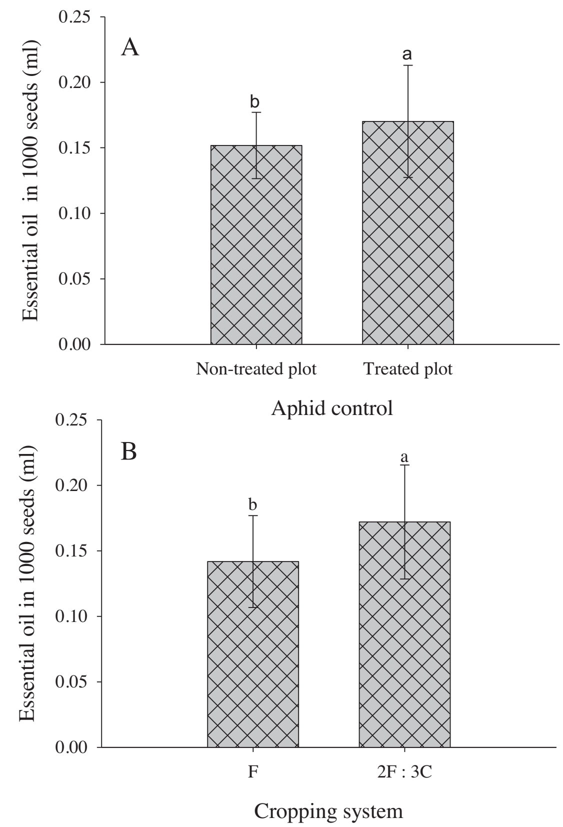

Fig. 4. Essential oil of fennel seeds (mean ± SE) from umbels in green stage, cultivar Montadas in treated plots and non-treated plots ($F_{Ac(1,21)}$ = 5.15, P = 0.0321) of fennel (A) and fennel and fennel intercropped with cotton with colored fibers ($F_{Cs\,(1,21)}$ = 12.78, P = 0.0012) (B). Mean with different lowercase letters within each group (aphid control or cropping system) of bars are significantly different.

from treated plots (0.17 ml) was significantly higher (12%) than in 1000 fennel seeds from non-treated plots (0.15 ml) ($F_{Ac\,(1,21)}$ = 5.15, P = 0.0428) (Fig. 4A). The average of the essential oil of 1000 fennel seeds from fennel–cotton intercropping system (0.17 ml) was significantly higher (18%) than in the fennel cropping system (0.14 mg) ($F_{Cs\,(1,21)}$ = 12.78, P = 0.0012) (Fig. 4B).

There was effect of aphid attack in the weight of fennel seeds ($F_{(1,9)}$ = 76.36, P < 0.0018) (Fig. 5A) and amount of essential oil ($F_{(1,9)}$ = 295.31, P < 0.0001) (Fig. 4B) extracted of seeds from umbels in dry stage. The average weight of non-attacked seed by *H. foeniculi* was higher than attacked seeds (Fig. 5A). The aphid attack reduced 29% of fennel seed weight (Fig. 5A). On the other hand, the average amount of essential oil extracted of the non-attacked seeds was two folds higher than of the attacked seeds (Fig. 5B).

4. Discussion

Insect damage to fruit and seed crops causes heavy losses of seed every year (Schimidt, 1988). Destruction of seed crops is caused by insect directly infecting the reproductive structures (flowers, fruits and seeds) (Schimidt, 1988). The fennel aphids attack umbels and may thus cause to failure of fruit setting and they also attack during the phase of seed development (Ramalho et al., 2012).

Our research investigated the severity of biomass, seed and essential oil damages related to infestation by the fennel aphid, *H. foeniculi* in fennel plots and plots of fennel intercropped with cotton with colored fibers. Data on biomass, seed and essential oil yield of fennel clearly showed that the aphid *H. foeniculi* had a negative effect on these yield components. The results showed that the effects of *H. foeniculi* on these yield components were smaller in the plots with fennel–cotton intercropping than in fennel plots.

High aphid densities can have a negative impact on fennel seed yield and can result in economic losses (Ramalho et al., 2012). According to Ramalho et al. (2012), fennel aphids reduced the fennel seed yield by 80% in the fennel plots compared with 26, 29, 28, and 28% in the intercropping systems consisting of two rows of fennel with three rows of cotton, one row of fennel with two rows of cotton, three rows of fennel with one row of cotton, and two rows of fennel with one row of cotton, respectively. Ramalho et al. (2012) showed that the resource concentration and natural enemies probably can account for reduced fennel aphid loads in fennel intercropped with cotton. According to Root (1973), both resource concentration and natural enemies work together in regulating phytophagous insect populations.

According to Marotti et al. (1993), the oils distilled from whole fennel plant biomass were characterized by lower contents of *trans*-anethole, fenchone and methyl chavicol and higher amounts of limonene in comparison to those extracted from fennel seeds. However, the essence quality of the essential oil extracted from fennel seeds has great importance and it depends on the stage of maturation of its fruits, as well as, of damage caused by insects (Maranca, 1985).

There is no published information available that describes the effects of fennel aphids on fennel biomass yield, seed weight, and oil content. However, studies with soybean showed that aphid injuries in soybean does not lead to a significant loss in total soybean (*Glycine max* (Linnaeus) Merrill) seed yield; however, aphid-induced reductions in oil concentration in the seeds is high (Riedell and Catangui, 2008). Therefore, Riedell and Catangui

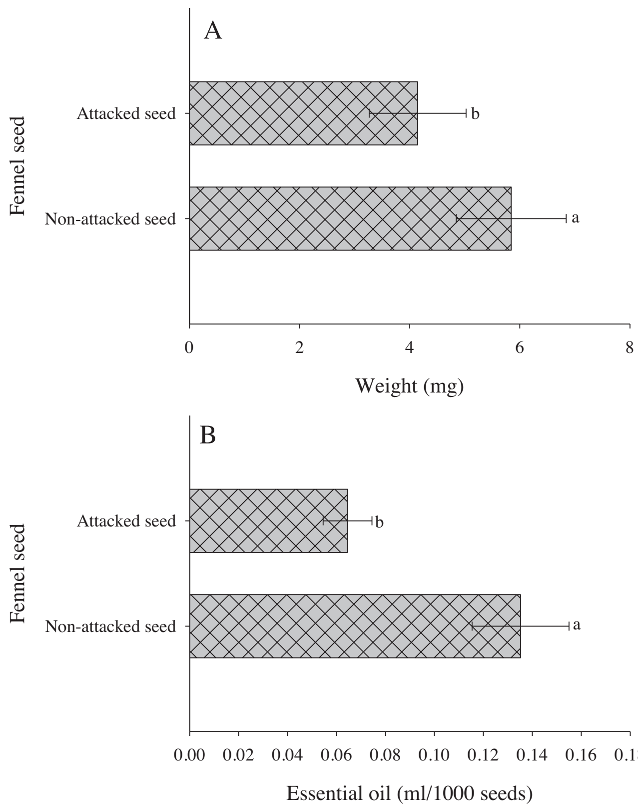

Fig. 5. Weight (mean ± SE) (mg) ($F_{(1, 9)} = 76.36$, $P < 0.0018$) (A) and essential oil (ml/1000 dry seeds) ($F_{(1, 9)} = 295.31$, $P < 0.0001$) (B) of attacked and non-attacked seed of fennel by *Hyadaphis foeniculi* from umbels in dry stage, cultivar Montadas. Means with different lowercase letters within each group (weight or essential oil) of bars are significantly different *F* test.

(2008) suggested that seed composition responses to aphid injury should be an important consideration when conducting research to develop IPM systems for this soybean pest. According to Corrêa-Ferreira and Azevedo (2002), soybean plants attacked by stink bugs can have seeds with low vigor and lower oil content. Oilseed content of canola species is significantly reduced by insect damage although oil quality, indicated by fatty acid profile, is not affected by insect attack (Brown et al., 1999).

The oils distilled from whole fennel plant biomass were characterized by lower contents of *trans*-anethole, fenchone and methyl chavicol and higher amounts of limonene in comparison to those extracted from fennel seeds (Marotti et al., 1993). However, the essence quality of the essential oil extracted from fennel seeds has great importance and it depends on the stage of maturation of its fruits, as well as, of damages caused by insects (Maranca, 1985).

We believe that seed composition responses to fennel aphid injury should be an important consideration when conducting research to develop IPM systems for this fennel pest.

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