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Direct and Indirect Effects of *Aphis gossypii* (Hemi.: Aphididae) and *Lasius brunneus* (Hym.: Formicidae) Mutualism on Cotton Yield in Field Condition

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ABSTRACT

Ant-aphid mutualism may increase or decrease plant yield. This depends on the relative cost of damage by ant-tended aphids versus the relative benefit of ant suppression of other (non-aphid) herbivores and associated yield losses. To evaluate the effect of mutualism between cotton aphid, *Aphis gossypii*, and brown ant, *Lasius brunneus*, on the productivity of cotton plant, a field experiment was conducted in the presence or absence of ants in the Cotton Research Center of Golestan province (Iran), in 2014. During the two-month test, the numbers of *A. gossypii* and visiting ants *L. brunneus* as well as *Coccinella septempunctata* and *Helicoverpa armigera* on each plant were counted in four-day intervals and, finally, the yield of cotton plants was measured. Ant presence significantly increased the abundance of cotton aphid, whereas the ant with suppression in presence of *C. septempunctata* and *H. armigera* significantly reduced their abundance. Regardless of the increase in the abundance of *A. gossypii*, cotton plant yields significantly increased in the presence of *L. brunneus*. This could be due to the effect of ant presence and the reduction of *H. armigera* abundance and the associated yield losses on cotton plant. In general, the results of the current study showed that indirect benefits of ant-aphid mutualism influence cotton plants by suppression of *H. armigera* damage. Since this pest causes serious damage on cotton plant, increasing attention to the role of ant predation is economically useful.

Keyword: Cotton aphid, Brown ant, Cotton bollworm, Plant yield

INTRODUCTION

Mutualism is defined as a beneficial interaction between individuals of two species (Stadler and Dixon, 2008). Perhaps, one of the widely studied mutualistic relationship has been between ants (Hymenoptera: Formicidae), and members of the order Hemiptera, such as aphids, whiteflies, and scale insects (Way, 1963; Buckley, 1987). The benefits of ants stem largely from supply of available carbohydrates in aphids honeydew and, in certain cases, from protein of the aphids themselves, while aphids may benefit in terms of protection from their natural enemies (Holldobler and wilson, 1990; Holway *et al*., 2002), enhancement of survivorship (Morales and Beal, 2006), fecundity and population growth (Flatt and Weisser, 2000). Mutualistic relationship between ants and hemipteran insects may deeply alter the food web, affecting several trophic levels including the host plant, non-honeydew producing herbivores, and entomophagous arthropods (Kaplan and Eubanks, 2002, Styrsky and Eubanks, 2007; 2010). Considering the ubiquity of ant-

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hemipteran mutualism in terrestrial communities, there is growing body of evidence to the effects of ants, mediated by aphids, on host plants, especially with regard to seed production of plants with short life cycles (Canedo-Júnior *et al*., 2017). Some studies have demonstrated that host plants can indirectly benefit from hemipterans as the attracted ants can reduce infestation of other herbivores (Karhu, 1998; Sakata and Hashimoto, 2000). On the other hand, several studies have found no effect e.g. Mahdi and Whittaker, 1993, or negative effect of ant presence on plant performance (Buckley, 1987; Delabie, 2001). However, the final effects of ants on plant yield depends on the ratio of the direct cost of feeding by honeydew-producing hemipteran to indirect benefit of increased ant suppression of other (non-honeydew producing) herbivores (Carroll and Janzen, 1973; Buckley, 1987; Lach, 2003).

One of the economically important pests of cotton plant is cotton aphid, *Aphis gossypii* Glover (Hemi.: Aphididae), which is the most widespread pest in temperate and tropical regions (Ebert and Cartwright, 1997; Afshari *et al*., 2009). This aphid causes damages to the different growth stages of cotton plant and is the vector of several plant viruses that result in greater yield losses (Henneberry *et al*., 2000).

Natural enemies have fundamental role in the control of the cotton aphid outbreaks and regulation of seasonal population dynamics (Dreistadt and Flint, 1996). Coccinellids (ladybirds) are one of the most important predators of this aphid and are often observed in aphid colonies with mutualistic ants in the fields (Kaneko, 2002). *Coccinella septempunctata* (Linnaeus) is the most common and abundant coccinellid species in nearly all the Iranian cotton fields (Ghahari *et al*., 2009). Ants of the genus *Lasius* are commonly observed in protecting aphids against their natural enemies (Takizawa and Yasuda, 2006; Gavrilyuk and Novogorodova, 2007; Schwartzberg *et al*., 2010). *Lasius brunneus* (Latreille) have been observed attending aphids *Stomaphis quercus* L. on oak trees (Loi *et al*., 2012), and *A. gossypii* on cotton plants, (Mirzamohamadi *et al*., 2015); however, the effects of ant-tending on performance and population growth of aphid partners have not been reported. Cotton boll worm, *Helicoverpa armigera* (Hubner) (Lep.: Noctuidae) is a common insect pest of cotton plants in Iran (Farid, 1986), and is one of the dominant pests on cotton (Mojeni *et al*., 2005). Predators, especially ants, are the most important group of natural enemies of *H. armigera* on cotton plants, but the efficacy of the ant species in suppressing *H. armigera* population is still unknown (Van Den Berg and Cock, 1993 a, b). To date, no work has been done to investigate the effect of mutualism between *L. brunneus* and *A. gossypii* on abundance of other insect communities in cotton field as well as their consequences on the plant yield. Therefore, the objective of present study was to determine the influence of *L. brunneus* presence on the abundance of cotton aphid, cotton bollworm, and seven-spotted ladybird as well as on cotton plant yield.

MATERIALS AND METHODS

The field experiment was conducted during the cotton growing season in a field in Hashem Abad, Golestan province, Iran (36° 53′ N, 54° 20′ E, 134 m altitude), from July to September 2014. This field was selected because the ant *L. brunneus*, which is tending cotton aphid, had been observed there in the previous year (Mirzamohamadi *et al*., 2015). The selected field for this study was planted with *Gossypium hirsutum* seeds (cv. Golestan) on 28th April 2014. Planting was done with 80 cm row spacing and the plant density was estimated at approximately 12-14 plants/m^2 (Bednarz *et al*., 2000). First irrigation was done after sowing, and afterwards the field was irrigated weekly. We applied the fertilizer Urea, $CO(NH3)_2$, as source of nitrogen, and trisodium phosphate, as source of phosphorous at the rates of 50 kg.ha^{-1} and 75 kg.ha^{-1},

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respectively. The fertilizers were applied before planting on 15 May 2014. No insecticide and herbicide were used to control herbivorous insects and weeds in the field. The experimental design included 48 cotton plants organized into six blocks each containing 8 plants. The experiment had two treatments: plants without ants but with aphids' presence (Aphid), and plants with both ants and aphids' presence (Aphid+Ant). Ants were prevented to access the aphid colonies by banding the main stem of the cotton plant with sticky barriers of Tanglefoot® (BIAGRO Company, Valencia) at 75 mm above the soil surface. Samplings were initiated when the plants reached a height of about 30±5 cm (1st of July) and continued until 30th of August 2014, with four-day intervals. During the study period (8 weeks), the plants were visited to examine the number of cotton aphids on the main stem growing from the terminal node and giving rise to each of branches, from 8:00 to 12:00 AM. The number of ants on the same part of each plant was also recorded for five min. Cotton bollworm, *H. armigera* and entomophagous arthropods were counted and recorded on each plant. To quantify plant yield, eight weeks after the final sampling time, matured bolls were collected, and dried at 60 °C in an oven for three days. Thereafter, the bolls were dissected and the seeds were collected and weighted with a microbalance (Sartorius GD503, Germany, sensitivity 1mg) to determine total seed weight. The total number of seeds produced by each plant was also counted.

To determine the effect of ant presence (or absence) on the abundance of cotton aphid, cotton bollworm and seven-spotted ladybird, a repeated measure one-way ANOVA was used. If the interaction between time intervals and the ant presence (or absence), were significant ($P<0.05$), the Tukey test was employed. A non-pair Student's t-test also was applied to analyze the effect of ant presence (or absence) on the number of seeds and seed weight. The relationships between measured traits and presence (or absence) of ant were tested using multiple Pearson correlation.

Prior to ANOVA analyses, data were log-transformed when necessary to meet assumption of normality and homogeneity of variances. SAS software version 9.2 was used for all statistical analyses (SAS, 2003).

RESULTS

In total, we sampled 4 ant species tending *A. gossypii* including *L. brunneus*, *Plagiolepis taurica* Santschi, *Cardiocondyla* sp., *Tetramorium chefketi* Forel, in which *L. brunneus* was numerically the dominant species while the latter species had sporadic occurrence, and not included in this study.

Across all sampling dates, *A. gossypii* abundance was positively affected by the attending ants presence, showing greater population on the plants with ant than ant-excluded plants; except during the last four sampling times (repeated measures ANOVA: interactive effect of time intervals and the ant presence (or absence): $F_{13, 65} = 7.24$, $P<0.01$; Figure 1). The increase in abundance of *A. gossypii* with increasing the number of attending ants per plant was confirmed by correlation analysis ($r^2 = 0.84$; $P<0.05$). During the first four weeks, the abundance of *A. gossypii* on both plants with and without ant increased, reaching the highest density in the early August, with an average of 146.83 ± 13.9 and 101.45 ± 11.6 (Mean ± SE) individuals per plant, respectively. Thereafter, the population of aphid dropped on both with ant and ant-excluded plants to the end of August.

Helicoverpa armigera abundance was negatively affected by ant presence, showing lower abundance on the plants with ant than that on the ant-excluded plants; except during the last three sampling times (repeated measures ANOVA: interactive effect of time intervals and the ant presence (or absence): $F_{13, 65} = 2.90$, $P<0.01$, Figure 2). Also, there was a negative correlation between the number of cotton bollworm and

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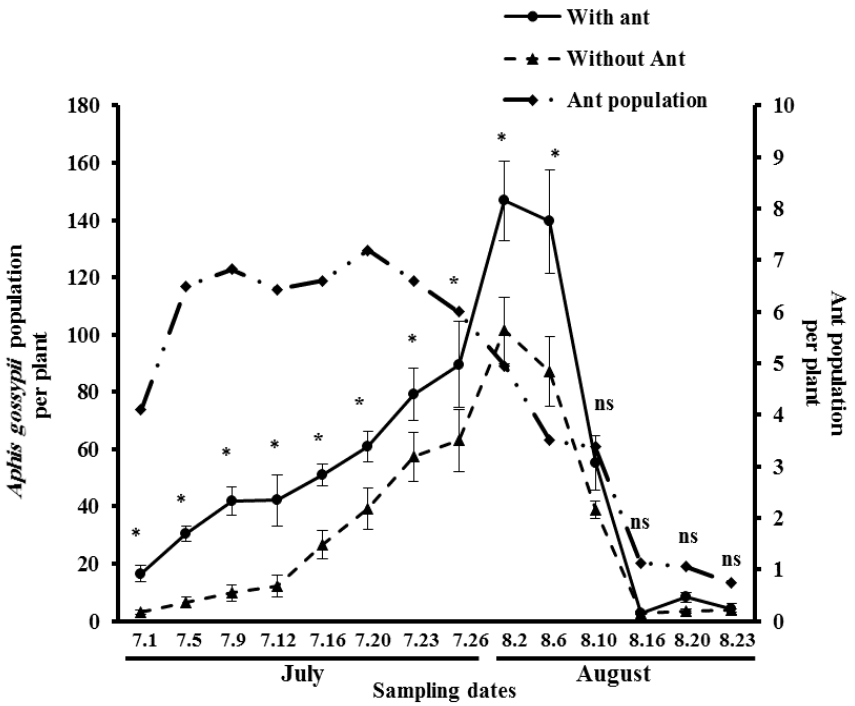

Figure 1.The mean (± SE) number of *Aphis gossypii* per plant in the presence or absence of *Lasius brunneus*. Asterisk indicate significant difference between treatments. Repeated one-way measure ANOVA (P < 0.01).

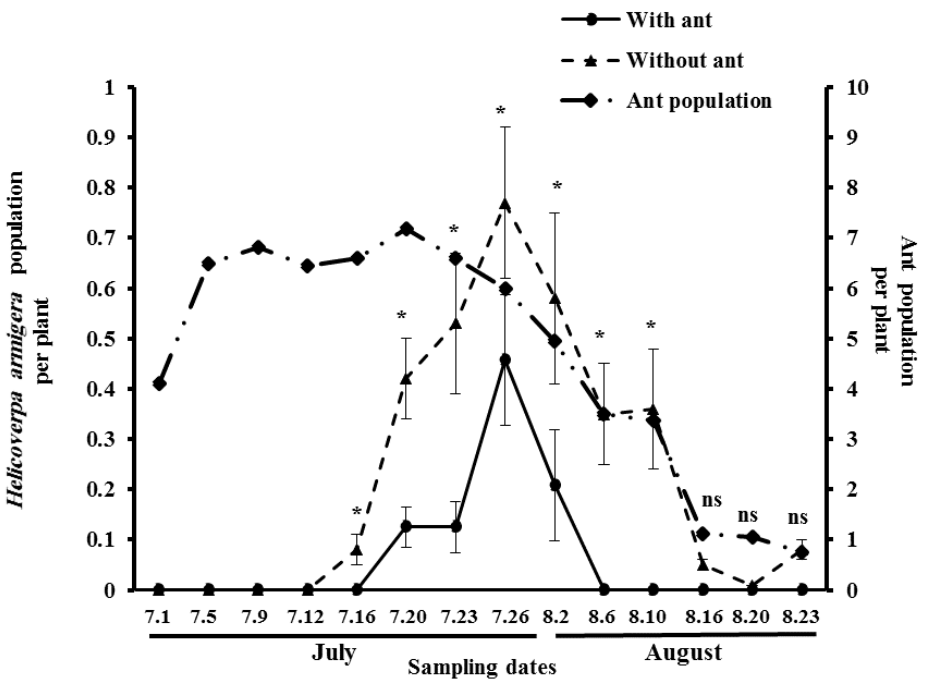

Figure 2. The mean (± SE) number of *Helicoverpa armigera* per plant in the presence or absence of *Lasius brunneus*. Asterisk indicate significant difference between treatments. Repeated one-way measure ANOVA (P < 0.01).

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the number of attending ants per plant (r^2 = -0.94; P<0.01).

From the fourth sampling time, the abundance of *H. armigera* on both plants with and without ant grew up, reaching their peak abundance in the eighth sampling time with an average 0.45 ± 0.11 and 0.75 ± 0.13 (Mean ± SE) per plant, respectively. Then, the population of *H. armigera* on plants with ant lessened to the tenth sampling time, but the population survived three weeks longer on plants without ant.

Seven-spotted ladybird was the most abundant natural enemy attacking *A. gossypii* in cotton field; however, other coccinellid predators including *Hippodamia variegate* Goeze and *Scymnus* sp., and larva of syrphids (*Episyrphus balteatus* DeGeer), chrysopids (*Chrysoperla carnea* Stephens) as well as adults of predatory pirate bugs (*Orius* sp.) were observed in very low numbers.

Averaged over all sampling dates, there were significantly fewer abundance of *C. septempunctata* on the plants with ant than that on the ant-excluded plants (repeated measures ANOVA: interactive effect of time intervals and the ant presence (or absence): $F_{13, 65}$ = 3.61, P<0.05), but this effect varied with sampling times (Figure 3). The abundance of *C. septempunctata* was negatively correlated with the number of attending ants per plant (r^2 = - 0.32; P = 0.32).

The measured cotton yield showed that plants with ant had significantly more cotton seed numbers (144.5 ± 8.5 vs. 63.1 ± 5.5, Mean ± SE) and weight (24.03 ± 1.22 g / plant vs. 16.07 ± 0.64 g / plant) than those of ant-excluded plants (t-test $_{1, 44}$ = 5.51, P<0.01 and t-test $_{1, 44}$ = 7.89, P<0.01, respectively) (Figure 4). While the numbers of cotton bollworms had negative significant correlations with cotton seed numbers (r^2 = -0.56; P<0.01) and weight (r^2 = -0.41; P <0.01) in the absence of ants, those were not significantly correlated with cotton seed numbers (r^2 = -0.02; P =0.92) and weight (r^2

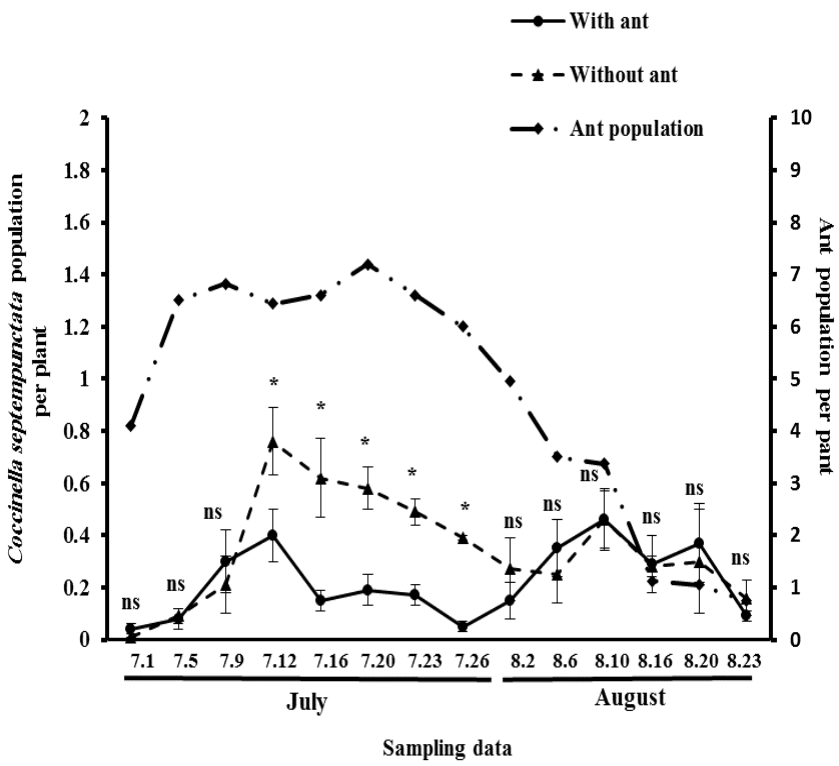

Figure 3. The mean (± SE) number of *Coccinella septempunctata* per plant in the presence or absence of *Lasius brunneus*. Asterisk indicate significant difference between treatments. Repeated one-way measure ANOVA (P < 0.01).

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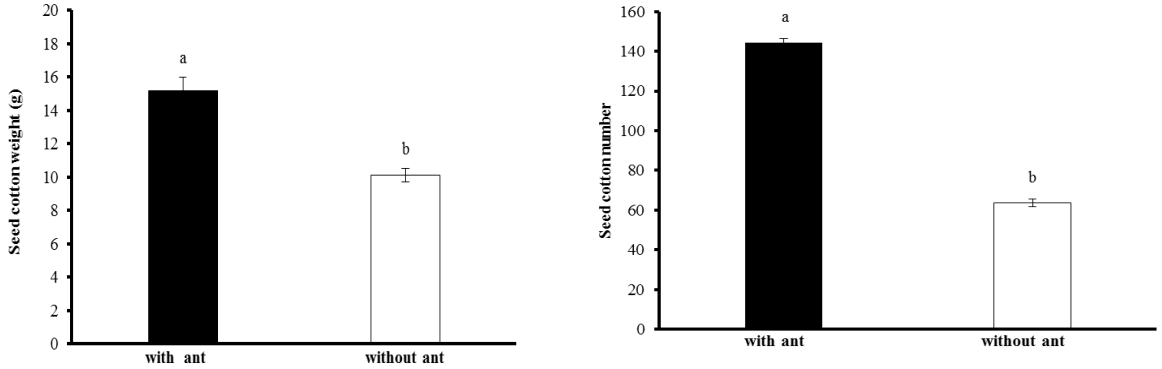

Figure 4. The mean (± SE) number and weight of cotton seeds per plant in the presence or absence of *Lasius brunneus* (P < 0.01).

= -0.22; *P* =0.25) in the presence of ants. Nevertheless, cotton aphid numbers were not significantly correlated with cotton seed numbers and weight in the presence (or absence) of ants.

DISCUSSION

The vast majority of studies on mutualism between ants and honeydew-producing hemipterans have focused historically on the costs and benefits to both partners (Renault *et al*., 2005; Yao, 2014). In contrast, ant-hemipteran mutualism have received comparatively little scrutiny from a broader, community-level perspective, particularly their potential effects on trophic interactions in food webs, arthropod community structure, and host plant fitness (Lach, 2003; Styrsky and Eubanks, 2010, Canedo-Júnior, *et al*., 2017). The results of the current study demonstrated that the aphid-tending ant, *L. brunneus*, positively affected the cotton plants' yield because they reduced the number of non-honeydew producing insect, *H. armigera*, and the associated yield losses. In the field condition, the cotton aphids attracted the *L. brunneus* to the plant, resulting in predation interference of the ants on the *H. armigera* caterpillars and, consequently, suppression of their population. Potential decreased caterpillars' damage to leaves and bolls resulted in yield increase in the cotton plants including the number and weight of cotton seeds. In accordance with our finding, several studies provided some evidences that ants are generally positive, in terms of both reduced plant damage and increased plant growth and reproduction. For instance, Altfeld and Stiling (2009) reported that although the presence of Argentine ant, *Linepithema humile* Mayr, increased abundance of aphid, *Aphis coreopsidis* Thomason *Baccharis halimifolia* (Asteraceae), by over 150%, significantly more of these plants survived relative to plants from which ants were experimentally excluded. This occurred because the aphid-tending ants protected the plants from the stem borer *Oidaematophorus balanotes* Meyrick. Styrsky and Eubanks (2010) also reported a potential effect of a facultative mutualism between the cotton aphid (*A. gossypii*) and the red imported fire ant (*Solenopsis invicta* Buren) on cotton plant. The fire ant, *S. invicta* foraged more on plants with cotton aphids than on plants without cotton aphid, resulting in a significant reduction in *Spodoptera exigua* (Hübner) population and the associated yield losses. However, some studies have found negative effect of ants on plant performance. For example, Rico-Gray and Castro (1996) found that a facultative mutualism between the ant *Camponotus planatus* Roger and an unidentified aphid species hosted by woody vine, *Paullinia fuscenens* (Sapindaceae)

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reduced the proportion of inflorescence with seeds by 7-23% and reduced seed number per inflorescent by 46-60% compared to plants with untended aphid.

The results of this study showed that *L. brunneus* positively influenced the cotton aphid density, suggesting a net positive effect of ant-tending to protect cotton aphid from attack by natural enemies, mainly *C. septempunctata* population (Figures 1 and 3). This agrees with the finding of Kaplan and Eubanks (2002), who found that increased fire ant *S. invicta* presence on the cotton plants was likely to result in enhanced abundance of cotton aphids through interference with aphid predators such as *C. septempunctata* and *Chrysoperla carnea* (Stephen). Renault *et al*. (2005) also suggested that presence of *Camponotus* ant reduced the density of predatory spider on *Bidens pilosa* (Asteraceae), resulting in increased population of *Aphis coreopsidis* (Thomas). Likewise, Powell and Silverman (2010) found that presence of two ant species, namely, *Linepithema humile* (Mayr) and *Tapinoma sessile* (Say), had a positive impact on the *A. gossypii* population, which protected them against *Hippodamia convergence* (Guer) on the cotton plant.

In the current research, our finding clearly showed that the presence of *L. brunneus* decreased the *H. armigera* population (also see, Figure 2), but the reasons why ant presence caused the lower population of cotton bollworms in the field condition remains unknown. There is evidence that ant species prey on different stages of lepidoptera pest species in many different habitats. For example, van den Berg and Cock (1993 a, b) have shown that, in East Africa, predators, especially ants, are the most important group of natural enemies of *H. armigera* on maize, sorghum, and sunflower. The fire ant, *S. invicta*, also is an active predator of noctuid eggs (Agnew *et al*., 1982; McDaniel and Sterling, 1982) larvae (McDaniel *et al*., 1981; Stewart *et al*., 2001) and pupae (Ruberson *et al*., 1994). Given the potential for *L. brunneus* to suppression *H. armigera* population, future studies are planned to quantify efficiency of ants on *H. armigera* as bio-control agent in cotton field.

In summary, our findings show that the indirect benefit to the cotton plants on suppression of *H. armigera* herbivory by native aphid-tending ants on cotton was greater than any direct cost of infestation by cotton aphids themselves. This is in agreement with the results reported for the positive effects of ant-hemipteran mutualisms involving native ants on the plant growth and reproduction by the suppression of lepidopteran insects' damage (Styrsky and Eubanks, 2010). Consequently, management practices can be developed toward the goal of increasing the role of *L. brunneus* in the biological control of pest insects. To achieve this purpose, it is essential to manage the ants to enhance their density beyond natural levels by limiting use of pesticides, transplanting ant colonies into plantations, and providing supplementary ant feeding during parts of the season.

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اثر مستقیم و غیر مستقیم همیاری شته *Aphis gossypii* و مورچه *Lasius brunneus* بر عملکرد پنبه در شرایط مزرعه ای

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چکیده

همیاری مورچه-شته ممکن است منجر به کاهش یا افزایش عملکرد گیاه شود. این امر به هزینه نسبی خسارت حاصل از گیاهخواری شته در مقابل سود نسبی حاصل از ممانعت مورچه از حضور سایر گیاهخواران (غیرشته) و خسارت وابسته به آن بستگی دارد. به منظور بررسی اثر همیاری شته پنبه *Aphis gossypii* و مورچه قهوه ای *Lasius brunneus* برعملکرد گیاه پنبه در سال ۱۳۹۳، مطالعه مزرعه ای با حضور و عدم حضور مورچه روی پنبه در مرکز تحقیقات پنبه استان گلستان انجام شد. در طول آزمون (۲ ماه) با فواصل زمانی ۴ روز، انبوهی جمعیت های شته و مورچه، کفشدوزک هفت نقطه ای *Coccinella septempunctata* و کرم غوزه پنبه *Helicoverpa armigera* روی هر گیاهو در پایان عملکرد گیاه پنبه اندازه گیری گردید. حضور مورچه باعث افزایش معنی دار جمعیت شته شد، اما با ممانعت از حضور *C. septempunctata* و *H. armigera* بطور معنی داری جمعیت آنها را کاهش داد. در حضور مورچه، عملکرد گیاه پنبه علی رقم افزایش جمعیت شته پنبه، به طور معنی داری افزایش یافت. این نتیجه می تواند به دلیل اثر حضور مورچه در کاهش انبوهی *H. armigera* و خسارت وابسته به آن روی گیاه پنبه باشد. به طور کلی، نتایج مطالعه حاضر بیانگر پیامد اثر غیرمستقیم همیاری مورچه-شته در افزایش عملکرد گیاه با کاهش خسارت *H. armigera* بود. از آنجایی که این آفت خسارت شدیدی روی گیاه پنبه ایجاد می نماید، توجه به شکارگری مورچه از لحاظ اقتصادی سودمند است.

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