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Bionomics of *Aphis gossypii* (Homoptera: Aphididae) and Its Predators *Coccinella septempunctata* and *Hippodamia variegata* (Coleoptera: Coccinellidae) in Natural Conditions

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ABSTRACT

Bionomics of *Aphis gossypii* Glover and its predators *Coccinella septempunctata* L. and *Hippodamia variegata* Goeze were studied in Varamin (Tehran Province) during 11th May to 5th October 2006 on eggplant crop, with the interaction (density dependence) between the aphid and either of the predators determined. The reliable sample size (number of leaves) with a maximum variation of 6.73% was about 56. The index of dispersion, regression models (Taylor and Iwao), Morisita's index as well as Lloyd's mean crowding to mean were employed to estimate the spatial distribution pattern of the insects. The results indicated that the highest population densities of *A. gossypii*, *C. septempunctata* and *H. variegata* occurred in 8th June (11.62 per leaf), 17th August (0.36 per leaf) and 3rd August (2.11 per leaf), respectively. The index of dispersion, regression models (Taylor and Iwao), Morisita's index and Lloyd's mean crowding to mean showed aggregated distribution for all species. The linear regression model between prey and predators' population densities showed a density independent predation by *C. septempunctata* and *H. variegata* on *A. gossypii*. This study indicated that spatial distribution parameters of the cotton aphid and its natural enemies can be employed to outline a sampling program and to estimate the population densities of these insects for use in integrated pest management programs.

Keywords: *Aphis gossypii, Coccinella septempunctata,* Density dependence interaction, *Hippodamia variegata,* Population density, Spatial distribution.

INTRODUCTION

Cotton aphid, *Aphis gossypii* Glover is a cosmopolitan species widely distributed in tropical, subtropical and in warm temperate regions (Isikber, 2005). It was first recorded as a pest of cucumber by Afshar (1939) in Iran. It can also infest such other economically important crops as cotton, melon, courgette, citrus, coffee, vegetables (eggplant, okra, sweet pepper, etc.) as well as ornamental plants (*Lantana, Hibiscus, Chrysanthemum*) (Razmjou *et al*., 2006). The importance of cotton aphid is not only due to its feeding and resultant honeydew, but also due to a transmission of more than fifty plant viruses (Blackman and Eastop, 2000). Outbreaks of this insect have been attributed to the development of resistance to insecticides: insecticides which are known to have injurious effects on populations of insect's natural enemies as well as changes in nutritional and bioclimatic factors in host plants (Isikber, 2005).

Biological control provides a useful alternative to pesticides in managing various arthropod pests (Opit *et al*., 2005). In order to develop sustainable biological control strategies, it is necessary to understand how habitat structure influences the ability of natural enemies to locate and eliminate

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sparsely distributed prey patches (Stavrinides and Skirvin, 2003). The predaceous coccinellids may play a significant role in reducing aphids and other insect pest populations (Van Emden, 1995); they are linked to biological control more often than any other taxa of predatory organisms (Obrycki and Kring, 1998). Following the successful biological control of the cottony-cushion scale, primarily by vedalia beetle *Rodolia cardinalis* (Mulsant), importation programs focused on Coccinellidae (Nielsen *et al.,* 2002; Arshad and Parez, 2007) with these insects being widely employed in biological control for over a century.

A quantitative knowledge of temporal and spatial distribution patterns of arthropod pests and of their natural enemies is essential to an understanding of their interactions, as well as being a prerequisite for the development of reliable sampling plans for estimating and monitoring the pest and its natural enemy abundance (Onzo *et al.,* 2005). A knowledge of spatial distribution of prey and predator is important in evaluating the system's persistence and the due potential of natural enemy to reduce prey density (Stavrinides and Skirvin, 2003). The spatial distribution of an insect can be employed in investigating population dispersion behavior, establishing a precise sampling scheme and sequential sampling (Margolis *et al.,* 1984), binomial sampling (Binns and Bostanian, 1990), studying of population dynamics (Jarosik *et al.,* 2003), detecting pest levels that justify control measures (Arnaldo and Torres, 2005) and assessing crop loss (Hughes, 1996).

There are studies that have described the spatial distribution of *A. gossypii* (Celini and Vaillant, 2004; Kabissa *et al.,* 1996; Tenhumberg and Poehling, 1995), *C. septempunctata* and *H. variegata* (Young and Willson, 1987) using dispersion indices that generally showed aggregation or randomness pattern of these insects' populations (Onzo *et al*., 2005; Bianchi and Warf, 2003; Xia and Sterling, 1987 and Hintze-Poelufal and Thomas, 1997). Although some studies have used chi-square goodness of fit test to compare the observed and expected frequency of negative binomial or Poisson distributions, this statistical procedure is not usually recommended (Ping- Man, 2006).

Interactions between a predator and its prey were expected to be mixed and the variations in observed responses not such a great surprise. A likely characteristic of an efficient specialist predator is its high searching capacity for its preferred food item (Slone and Croft, 2001). Field and laboratory studies of prey- predator systems show marked density fluctuations in either populations (Greco *et al.,* 1999).

Even though aggregation of *A. gossypii* has been widely demonstrated (Celini and Vaiilant, 2004; Kabissa *et al.,* 1996; Tenhumberg and Poehling, 1995), there is little information comparing the aggregation patterns, the population fluctuation of the prey, and its coccinellid predators in specific systems. Accordingly, the objectives of this study were to determine the population fluctuations of *A. gossypii* and its two common coccinellid predators, *C. septempunctata* and *H. variegata*, making the spatial statistical comparison, demonstrating the prey-predator interactions and improving the reliable sampling program on eggplant.

MATERIALS AND METHODS

Sampling Program

Sampling Unit

A leaf of an eggplant bush was selected as a sample unit since this aphid feeds on host plant leaves. Eggplants and the leaves on them were selected randomly in order to get an unbiased estimate of the number of *A. gossypii, C. septempunctata* and *H. variegata* per each selected leaf. Immature as well as adult stages of *A. gossypii* were counted together as numbers of aphid on eggplant. Larval and adult stages of either of the lady beetles were computed. Larval stages of the two coccinellids were differentiated by size and shape (form) of body as well as by shape and color of spot on their bodies. The selected plants were checked and counts mostly done in an eggplant field in Varamin region near Tehran. The field was of an area of 2 hectares. *Solanum melogena* L. was planted on top of furrow banks. No chemical fertilizers or insecticides were applied. Sulfur powder was used only once as a fungicide in 20th June, while dung being used as fertilizer once a month.

Pattern and Timing of Sampling

A basic tenet of any sampling method is that samples are collected randomly so that every sampling item has an equal chance of being picked up (Pedigo and Buntin, 1994). On this basis, eggplant leaves were sampled randomly usually in early morning (8:00-11:00 am and the sampling frequency once a week. The sampling started May 11th and was extended until October 5th 2006.

Sample Size

Differences among primary sampling data are important in sample size determination. To meet the purpose, a primary sampling of 125 samples was adopted. To compare the efficiency of the various sampling methods performed, the relative variation of each sampling method (*RV*) was calculated according to Hillhouse and Pitre (1974):

$$RV = (SE/m)100$$

where *SE* is the standard error of the mean and *m* is the mean of primary sampling data. The reliable sample size was determined using the following equation:

$$N = [ts/dm]^2$$

where *N*= Sample size, *t*= *t*-student, *s*= Standard deviation, *d*= Desired fixed proportion of the mean and *m*= The mean of primary data.

Spatial Distribution

The spatial distribution of *A. gossypii* and its predators was determined through the following five procedural methods: index of dispersion, Taylor's power law, Iwao's patchiness regression, Morisita's coefficient of dispersion and Lloyd's mean crowding.

Index of Dispersion

The variance (S^2) to mean (m) ratio recalls that the mean and variance would be equal in a randomly distributed population. Dispersion of a population can be classified by evaluation of the variance to mean ratio as follows:

$S^2/m>1$ Aggregated
$S^2/m=1$ Random
$S^2/m<1$ Regular

Departure from a random distribution can be tested by calculating the index of dispersion, I_D, where *n* is the number of samples:

$$I_D = (n-1)S^2/m$$

In the next stage, *Z* coefficient was

$$Z = \sqrt{2I_D} - \sqrt{(2\nu - 1)}$$

calculated for testing the goodness-of-fit:

where ν is the number of degrees of freedom (n-1).

If $1.96 \geq Z \geq -1.96$ the spatial distribution would be random but if $z>1.96$, $z<-1.96$ it would be either aggregative or uniform, respectively (Patil and Stiteler, 1974).

Taylor's Power Law and Iwao's Patchiness Regression

Taylor's power law states that the variance (S^2) of a population is proportional to a fractional power of the arithmetic mean (m):

$$S^2 = am^b \text{ or } \log S^2 = \log a + b \log m$$

where the parameter *a* is a scaling factor related to sample size and the slope *b* is an index of aggregation which indicates a uniform (b< 1), random (b= 1) and

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aggregated (b> 1) dispersion of a population (Taylor, 1961).

Iwao's patchiness regression method quantifies the relationship between mean crowding index (m*) and mean (m) using the following equation:

$$m^* = \alpha + \beta m$$

where α indicates the tendency to crowding (positive) or dispersion (negative) and β reflects the distribution of a population in space and is interpreted in the same manner as *b* of Taylor's power law (Iwao, 1968). Student *t*-test can be used to determine if the colonies are randomly dispersed.

Test $b = 1$ $t = (b-1)/s_b$ and Test $\beta = 1$ $t = (\beta - 1)/s_\beta$

where s_b and s_β are the standard error of the slope for the mean crowding regression. Calculated values are compared with tabulated *t*-values with *n-2* degrees of freedom.

Morisita's Coefficient of Dispersion

Morisita (1962) reported a hypothesis for testing the uneven distribution coefficient of I_δ calculated through the following equation:

$$I_\delta = \frac{n\sum x_i(x_i - 1)}{N(N-1)}$$

where *n*= The number of sample units, x_i= The number of individuals in each sample unit and *N* = Total number of individuals in *n* samples.

To determine if the sampled population significantly differs from random, the following large sample test of significance can be applied:

$$z = \frac{(I_\delta - 1)}{\left(\frac{2}{nm^2}\right)^{\frac{1}{2}}}$$

If 1.96≥ z≥ -1.96 the spatial distribution would be random but if z< -1.96, z> 1.96 it would be either regular or aggregated, respectively (Pedigo and Buntin, 1994).

Lloyd's Mean Crowding

Mean crowding (m^*) was proposed by Lloyd to indicate the possible effect of mutual interference or competition among individuals. Theoretically mean crowding is the mean number of other individuals per individual in the same quadrate:

$$m^* = m + \frac{s^2}{m} - 1$$

As an index, mean crowding is highly dependent upon both the degree of clumping and population density. To remove the effect of changes in density, Lloyd introduced the index of patchiness, expressed as the ratio of mean crowding to the mean. As with the variance-to-mean ratio, the index of patchiness is dependent upon quadrate size m^*/m= 1 random <1 regular and >1 aggregated (Lloyd, 1967).

Sample Size Model

Taylor's *a* and *b* coefficients, taken from Taylor's power law, describe the relationship between variance and mean ($s^2 = am^b$) for individuals distributed in a natural population. The mean and variance of sampled insects was determined for each weekly sampling date. Taylor's *a* and *b* coefficients were calculated by log-log linear transformation of the mean-variance data, where *b* is the slope of the transformed data and *a* the antilog of transformed intercept. An equation for estimating pest sample size was developed by Karandinos (1976). Ruesink (1980), Wilson and Room (1982), and Wilson (1985) incorporated Taylor's power law into Karandinos' equation to form the sample size model used in this study:

$$N = a\left(\frac{t_{\alpha/2}}{d}\right)^2 \left(m^{b-2}\right)$$

where *N*= sample size, $t_{\alpha/2}$= *t*-student of table, *m*= Mean density, *d*= The range of accuracy, *a* and *b*= Taylor's coefficients.

Coefficient of Aggregation

Many indices used for describing species aggregation are based on the mean and variance of samples. These include the negative binomial common *k*, Lloyd's index of mean crowding and Iwao's patchiness regression. A simple method for calculating *k* is based upon the proportion of zeros obtained in samples. If *N* is the total number of samples and N_0 is the number of samples with zero individuals, *k* would be estimated by using the following interactive equation (Pedigo and Buntin, 1994):

$$\log(N/N_0) = \hat{k}\log(1+m/\hat{k})$$

Density Dependence in Prey-predator Interaction

To determine the type of interaction between prey and predator, analysis of simple linear regression was carried out between prey and predator population densities. If *P*-value> 0.05 (b= 0) predator would be density independent, but if *P*-value≤ 0.05 and *b*> 0 or *b*< 0, predator would act as density dependent and inverse density dependent in its predation activity, respectively.

RESULTS

Sampling Program

Data set from primary sampling were employed to calculate *RV*. Calculated *RV* and sample size for *A. gossypii* were 6.73% and 56 leaves, respectively.

Population Fluctuation

Population fluctuation of *A. gossypii, C. septempunctata* and *H. variegata* are shown in Figures 1-3. The population of *A. gossypii* and *H. variegata* was observed from the beginning of the sampling period (11th May), but no *C. septempunctata* population was recorded until 18th May when the eggplant started its vegetative stage. The results indicated that the highest population density of *A. gossypii, C. septempunctata* and *H. variegata* occurred in 8th June (11.62 per leaf), 17th August (0.36 per leaf) and 3rd August (2.11 per leaf), respectively. During the sampling season, populations of *C. septempunctata* and *H. variegata* had greater and more irregular fluctuations as compared to the aphid population (Figures 2 and 3).

Table 1. Estimated values of intercept and slope of *Aphis gossypii, Coccinella septempunctata* and *Hippodamia variegata* by regression analysis of Taylor's power law and Iwao's patchiness regression on eggplant in Varamin (Tehran Province) during 2006.

Species	Taylor's power law				Iwao's patchiness regression			
	a	*b*	r^2	P_{value}	*α*	*β*	r^2	P_{value}
Aphis gossypii	-0.194	2.020	0.797	0.000	-1.570	2.000	0.979	0.000
Hippodamia variegata	0.168	1.030	0.899	0.000	0.258	1.260	0.606	0.001
Coccinella septempunctata	0.106	1.060	0.993	0.000	0.045	1.430	0.529	0.101

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Table 2. Estimated parameters by Lloyd mean crowding, index of dispersion, Lloyd mean crowding to mean and common *k* for *Aphis gossypii, Coccinella septempunctata* and *Hippodamia variegata* on eggplant in Varamin (Tehran Province) during 2006.

Species	m	S^2	m^*	I_D	Z	m^*/m	k
Aphis gossypii	5.406	43.20	12.39	20944.2	18324.2	2.293	0.787
Hippodamia variegata	0.388	1.290	2.713	1299.82	909.823	6.988	1.665
Coccinella septempunctata	0.55	0.076	0.381	75.4221	19.4221	6.628	0.031

Spatial Distribution

Iwao's α and β and Taylor's *a* and *b* coefficients for each species on eggplant in 2006 are shown in Table 1. The results of Taylor and Iwao regression methods indicated that the spatial distribution patterns of the cotton aphid and its predators were aggregated.

The index of dispersion (I_D) on eggplant in 2006 showed that the spatial distribution of populations on eggplant was also aggregated. The I_D values for each population in all sampling dates were significantly greater than 1 (Table 2), which means this species exhibited aggregated behavior within the habitat.

Morisita's index of all spices was significantly greater than 1.96 in all sampling dates (Table 3). It indicated that spatial distribution of all species was aggregated.

The m^*/m value for each population in all sampling dates was significantly greater than 1 (Table 2), which was indicated of aggregated pattern in all the examined species.

The sample size was re-calculated using *k* and Taylor's coefficient (*a* and *b*) (Table 5). These values of sample size can help improve sampling program of *A. gossypii* and of its two predators.

Coefficient of aggregation was calculated for all species, as shown in Table 2.

Density Dependence in Prey-predator Interaction

As *P*-value of the linear regressions between population density of aphid-coccinellid was greater than 0.05, then *b*= 0, which showed the predation of both coccinellids on *A. gossypii* as density independent (Table 4).

DISCUSSION

When the weather was warmer in July, the population density of cotton aphid decreased and reached zero in late-July and in early-August. Furthermore, the severe decline in

Table 3. Morisita's index and *Z* values for *Aphis gossypii, Coccinella septempunctata* and *Hippodamia variegata* on eggplant in Varamin (Tehran Province) during different sampling dates of 2006.

Date	I_δ (*A. gossypii*)	Z (*A. gossypii*)	I_δ (*C. septempunctata*)	Z (*C. septempunctata*)	I_δ (*H. variegata*)	Z (*H. variegat*)
11-May	950.00	74140.60	0.00	-1250.00	0.00	0.00
15-June	362104.00	89540.50	718.22	10758.33	0.00	-3375.00
13-Jul	4080.00	4076.94	49080.00	49069.90	120.00	105.00
17-Aug	0.00	-17.50	0.00	0.00	1075.20	147380.00
14-Sep	0.00	-17.50	8824.24	173690.00	0.00	0.00
11-Oct	0.00	-17.50	0.00	0.00	1.87	0.00

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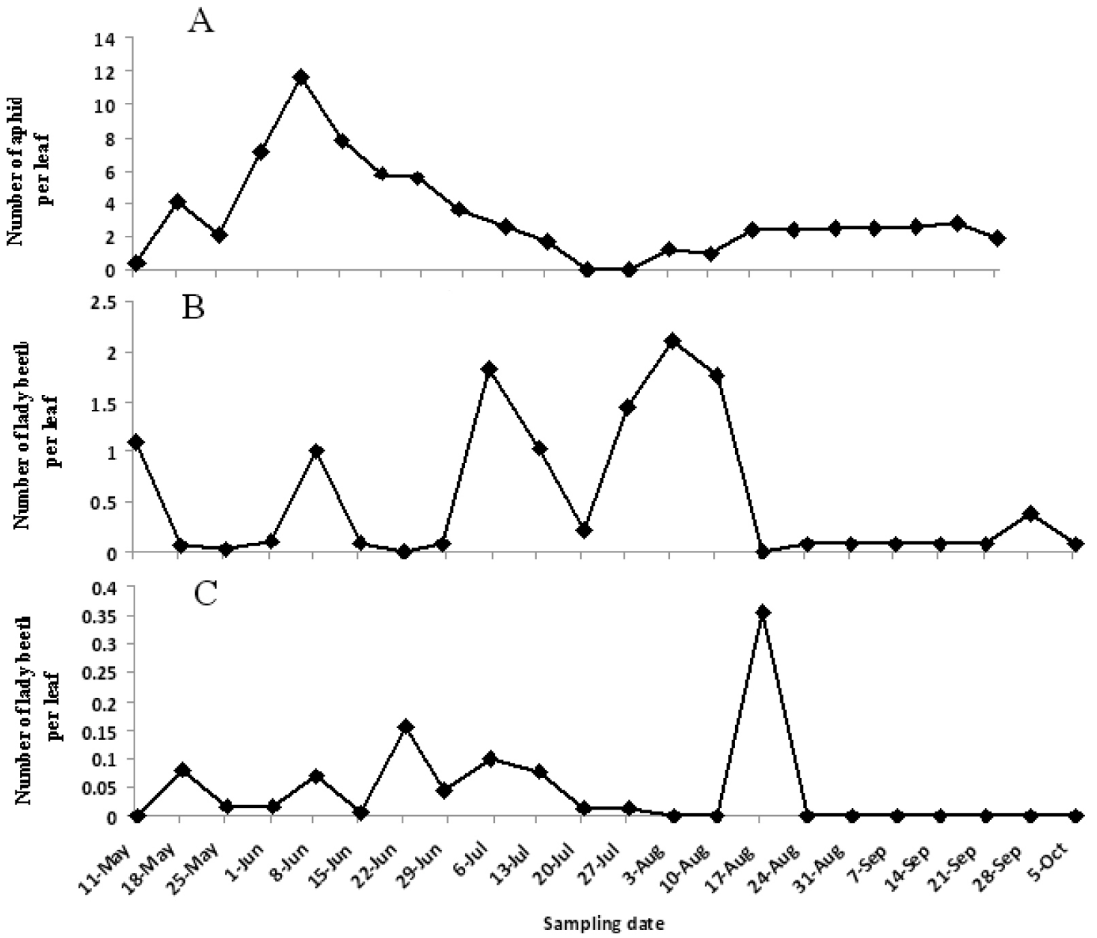

Figure 1. Population fluctuation of *Aphis gossypii* (A), *Hippodamia variegata* (B) and *Coccinella septempunctata* (C) on eggplant in Varamin (Tehran Province) during 2006.

A. gossypii population from mid-June could have been mainly due to the predators' action or plant-aphid interaction. The population density of the pest increased again from mid-August to late-September (Figure 1). Because of the weather conditions or plant-predator interactions on the population of *C. septempunctata* and *H. variegata*, these predators had greater and more irregular population fluctuations as compared to the pest during the sampling season. Population density of *H. variegata* per sample unit was higher than that of *C. septempunctata* and was also established earlier on eggplant [Figures1(B and C)], suggesting that *H. variegata* may be a more effective agent than *C. septempunctata*.

Celini and Vaillant (2004) used different statistical methods including Taylor's power law, Iwao's patchiness regression and Nachman's model to estimate the spatial distribution pattern of *A. gossypii* on cotton in Bangui, Central African Republic. In our study, the spatial distribution pattern of the cotton aphid and of its predators, using regression methods (Taylor and Iwao), was aggregated. The results from index of dispersion, Morisita's coefficient and Lloyd mean crowding revealed that the spatial distribution of all examined species was aggregated, suggesting that the presence of an individual insect at one point led to an increase in the probability of anotherindividual being nearby, the habitat

Table 4. Statistics of the linear regression between the mean population density of *Aphis gossypii, Coccinella septempunctata* and *Hippodamia variegata* on eggplant in Varamin (Tehran Province) during 2006.

Species	*a*	*b*	r^2	P_{value}
A. gossypii- H. variegata	-0.366	0.325	0.225	0.141
A. gossypii-C. septempunctata	-0.133	0.082	0.214	0.296

occupation probabilities not being the same by individuals. This result is similar to that obtained by Celini and Vaillant (2004). The value of α was significantly greater than 0 for the predators, indicating that colonies or clumps constituted the basic components of these populations with the patch size decreasing throughout the development. The data proved to be well fitting Iwao's model (r^2= 0.979) for *A. gossypii*, but they better fitted Taylor's model as compared to Iwao's for *C. septempunctata* (r^2= 0.993) and *H. variegata* (r^2= 0.899).

Since Morisita's coefficient estimates the spatial distribution of each date, using the mean and variance of each sampling date separately, therefore it seems to be more accurate than the index of dispersion. Despite many entomologists utilizing Taylor's indices in their works; Morisita's index gave a higher coefficient of determination and a better interpretation of the dispersion of the species. Spatial distribution of the studied insects (using different analytical methods) was obtained as either in an aggregated or regular pattern, suggesting that the different statistical methods lead to varying results and accuracy in calculating spatial distribution of an organism.

For an applied population biologist, knowledge of a population aggregation is necessary to develop sequential, binomial or any other sampling plan. A change in the aggregation trend of a species often requires an alternation in the sampling plan for an accurate population count (Slone and Croft, 2001). Comparison of aggregation among populations is only appropriate when data are collected under similar circumstances. Our study will allow population biologists to quantify the effect of such natural processes as predation on population distribution.

A comparison of *k* values among the three species indicated that the aggregation of *C. septempunctata* was more pronounced than those of the other species because of low value of *k* index in *C. septempunctata*. This is due to the higher differences between the variance and the mean of the ladybird sampling data. Since the sample size that was estimated through common *k* was based on just the mean, this value was not influenced by variance, and therefore it did not present a reliable sample size. When sample size was estimated through Taylor's power law coefficients, the number of samples benefitted from a higher precision.

The regression between predators and prey densities was not significant, suggesting

Table 5. Calculated sample size of *Aphis gossypii, Coccinella septempunctata* and *Hippodamia variegata* populations on eggplant based on, common *k* and Taylor's power law coefficients in Varamin (Tehran Province) during 2006.

Species	Sample size (N)	Common *k*	Taylor's power law coefficients
Aphis gossypii	55.71	93.15	12.84
Hippodamia variegata	55.71	125.9	23.72
Coccinella septempunctata	55.71	933.8	99.53

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density independent predation due to predators' polyphagous behavior and as well, due to the existence of alternative preys among other phytophagous insects and mites on eggplant. This result revealed that the population distribution of the predators is not tightly linked to the prey distribution, a characteristic which would create refuges for the prey and consequently increase the persistence of the system. Bianchi and Warf (2003) showed C. *septempunctata* to be an important predator of aphids in arable crops depending on non crop landscape elements such as hedgerows resorted to hibernation.

A knowledge of spatial distribution parameters of the cotton aphid and its natural enemies can be employed to outline a sampling program and to estimate the population density of these insects for use in integrated pest management programs (through implementation of conservation and/or augmentation techniques).

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تغییرات جمعیت و اثرات متقابل شته *Aphis gossypii* و کفشدوزک های شکارگر *Coccinella septempunctata* و *Hippodamia variegate* در شرایط طبیعی

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چکیده

تغییرات جمعیت و الگوی توزیع فضایی شته سبز پنبه *Aphis gossypii* Glover و دو کفشدوزک شکارگر آن .*Coccinella septempunctata* L و *Hippodamia variegata* Goeze از ۲۱ اردیبهشت تا ۱۳ مهر ماه ۱۳۸۵ در شهرستان ورامین استان تهران بر روی بادمجان مطالعه شد. در این تحقیق همچنین نوع وابستگی تغذیه (شکار) کفشدوزک ها به تراکم جمعیت شته به عنوان یکی از ملاک های ارزیابی کارایی شکارگرها در شرایط طبیعی مورد کنکاش قرار گرفت. تعداد ۶۳ واحد نمونه برداری (برگ) با حداکثر خطای ۶/۷۳٪ به عنوان اندازه مناسب نمونه در نظر گرفته شد. برای تعیین الگوی توزیع فضایی حشرات مورد مطالعه از شاخص پراکندگی، روش رگرسیونی (تیلور و آیوائو)، شاخص موریسیتا و شاخص للوید استفاده شد. نتایج نشان داد که اوج جمعیت *A. gossypii*، *C. septempunctata* و *H. variegata* به ترتیب در ۱۵ خرداد ماه (۱۱/۶۲ عدد در هر برگ)، ۲۶ مرداد ماه (۰/۳ عدد در هر برگ) و ۱۲ مرداد ماه (۲/۱۱ عدد در هر برگ) ظاهر می شود. تمام روش های مورد استفاده موید تجمعی بودن الگوی توزیع فضایی شته و هر دو کفشدوزک شکارگر بود. رگرسیون خطی بین تراکم جمعیت شته و شکا ر گر نشان داد که هر دو شکارگر در تغذیه از طعمه خود بصورت مستقل از تراکم طعمه عمل می کنند. تعیین پارامتر های الگوی توزیع فضایی آفت و شکارگرهای آن می تواند علاوه بر طراحی برنامه های مناسب نمونه برداری، در ارزیابی میزان کارایی دشمنان طبیعی نیز مورد استفاده قرار گیرد.

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