



## Using statistical methods to determine spatio-temporal distribution of aphids and aphidophagous ladybirds in alfalfa fields

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**Abstract.** This study was conducted to determine distribution pattern and related parameters of spotted alfalfa aphid, *Therioaphis maculata* (Buckten), pea aphid, *Acyrtosiphon pisum* (Harris) and two coccinellid species (*Coccinella septempunctata* L. and *Hippodamia variegata* Goeze) in six alfalfa fields during two growing seasons, 2016 and 2017. Weekly sampling was started when alfalfa plants reached 10 cm in height and continued until harvest. Each field was divided into 20m×20m plots. Two samples per plot per sampling time were randomly taken using a 1 × 1 m quadrat, to count adult coccinellids. Twenty alfalfa stems (longer than 10cm) per plot were randomly cut and shaken eight times on a white pan to record the aphids' density. The aphids that fell into the pan were counted and recorded. The ratio of variance to mean, as well as Taylor's power law (TPL) and Iwao's patchiness index (IPI), were used to determine the spatial distribution patterns of the insects. The variance exceeded the mean on all sampling dates, which may imply an aggregative distribution. Aggregation was also confirmed by using regression coefficient of both TPL (parameter b) and coefficient of IPI ( $\beta$ ), which both were significantly above unity. Correlation between aphid counts and those of the coccinellids indicated that there was a positive but weak relationship between predator and prey densities in the fields, although a weak correlation may imply that factors other than prey accessibility contribute to the spatial distribution of the predators. Using different statistical methods provides useful information about the spatial distribution and dispersal pattern of aphids and natural enemies in alfalfa fields, and this information can be used in the biological control of pests.

**Keywords:** distribution patterns, Taylor's power law, Iwao's regression, aggregation

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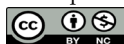
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## Introduction

Spotted alfalfa aphid *Therioaphis maculata* (Buckten) and pea aphid *Acyrtosiphon pisum* (Haris) are important aphid species that mainly attack alfalfa fields and cause injury to plants by feeding, injecting toxins, and transmitting plant pathogens (Khanjani, 2005; Hodgson, 2007; Canevari *et al.*, 2015). Beneficial arthropods associated with pest control in alfalfa fields can be categorized as generalists and specialists. *Coccinella septempunctata* L. and *Hippodamia variegata* Goeze (Coleoptera: Coccinellidae) are two generalist predator species that simultaneously occur in the fields. They are polyphagous and important biological control agents of aphids on miscellaneous crops (Caballero-Lopez *et al.*, 2012; Aleosfoor *et al.*, 2014; Shayesteh *et al.*, 2015).

Studying the spatio-temporal distribution of insects is attractive for entomologists for many reasons. The spatial distribution of an insect pest determines which sampling design (*i. e.* completely randomized, stratified, clumped, or systematic design) may be adopted to provide minimal sampling costs and maximum precision and accuracy

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(Buntin, 1994). The variance to mean ratio also determines the sample size in sampling programs. Furthermore, in pest management programs, knowing the spatial pattern of a pest directly affects decision making patterns such as sequential sampling (Bins, 1994; Southwood & Henderson, 2000) or monitoring efforts (Iranipour *et al.*, 2020). Spatial distribution patterns of insects have been studied using different methods. The traditional statistical tools classify insect distribution patterns as aggregated, uniform, or random categories, depending on mean to variance ratio and frequency distribution (Ferguson *et al.*, 1999; Duarte *et al.*, 2015). Spatio-temporal correlation between pest population densities and number of foraging natural enemies may indicate that if a natural enemy is able to regulate the pest population and to what extent. Such studies are beneficial in determining the role of a natural enemy as a regulatory factor in decreasing the pest population and predicting the result of releasing a natural enemy in augmentation programs (van Driesche & Bellows, 1996). This is often studied under a common topic of density dependence (Price *et al.*, 2011). In many studies, the correlation between natural enemy counts and pest densities are studied (e. g. Sunderland & Vickerman, 1980; Iranipour *et al.*, 2018).

Arbab & Bakry (2016); Soemargono *et al.* (2011), and Touhidur *et al.* (2006) used statistical analysis to determine the spatial distribution patterns of *Parlatoria blanchardi* (Hemiptera: Diaspididae) on palm trees, *Bactrocera dorsalis* Complex (Diptera: Tephritidae) in mango orchards, and *Aphis gossypii* Glover and coccinellids on chilli, *Capsicum annuum* L. respectively. Population density and spatial distribution patterns of *Tuta absoluta* (Meyrick) and cabbage aphid *Brevicoryne brassicae* (L.) and its parasitoid *Diaeretiella rapae* (McIntosh) and *Hypera postica* (Gyllenhal) were studied by Ghaderi *et al.* (2018), Amini *et al.* (2014) and Moradi-vajargah *et al.* (2011), and their results indicated that these insects had aggregated distribution patterns on their hosts in the fields. The coefficients of the spatial pattern can be used for improving the sampling program to estimate the population density of insects. Spatial distribution patterns of insects are influenced by environmental factors such as habitat heterogeneity, environmental instability, competition, and predation and also by behavioral characteristics of the insects themselves. Determining constant or variable spatial parameters, and spatial similarities among habitats, provide information on insect behavior and relationships with their habitat (Vinatier *et al.*, 2011; Khaliq *et al.*, 2014). For instance, Iranipour *et al.* (2017) showed that all stages of *Calliptamus italicus* (Linnaeus) (Orthoptera: Acrididae) had an aggregation tendency, and suggested a combination of behavioral and environmental causes of aggregation on the basis of Blackith's  $\lambda$ . Moreover, Rakhshani *et al.* (2009) studied population dynamics of three main alfalfa aphids. Their results revealed that populations of aphids were mainly affected by alfalfa harvesting, ambient temperature, and coccinellid predators.

The aim of this study was to determine the spatial distribution and association of spotted alfalfa aphid *T. maculata* and pea aphid *A. pisum* and their coccinellid predators *C. septempunctata* and *H. variegata* in alfalfa fields by using indices of distribution and regression methods. Also, it was not known if distribution attributes are site-specific or a common model can well describe distribution pattern of alfalfa aphids and their predatory ladybirds.

## Materials and methods

### Study site

This study was conducted in six alfalfa fields, each with an area of 1.2 - 2 ha, located in the experimental farm of the Faculty of Agriculture, University of Tabriz (UTM Zone 38.020195 N, 46.425083 E), during 2016 and 2017 growing seasons. The fields were cultivated with Ghara Yonja c.v., the native cultivar of East Azarbaijan Province of Iran. Field borders and spatial locations of samples and traps were geo-referenced and saved in a hand-held GPS receiver (Model GPS-map 76CSx, Garmin, Olathe, Kansas, USA) in the UTM coordinate system.

### Sampling

All fields were divided into 20 × 20 m grids. Weekly sampling was started when plants' height reached approximately 10 cm and continued until harvest. The coccinellids were sampled using a 1 × 1 m quadrat. Two quadrats were thrown at each grid randomly, and the number of *C. septempunctata* and *H. variegata* adults were counted and recorded separately. To sample aphids, 20 stems per grid were cut randomly and shaken into a white pan and the number of spotted alfalfa aphid and pea aphid dropped in the pan were counted and recorded separately. The number of insects counted in the two quadrats were pooled and used in analysis.

## Statistical analysis

The spatial distribution of insects was determined using indices of distribution and two regression methods, Taylor's power law (Taylor, 1961) and Iwao's patchiness index (Iwao, 1968). The index of dispersion ( $I_D$ ), defined as variance to mean ratio ( $S^2/\bar{x}$ ), was used to decide if distribution is random ( $I_D = 1$ ), clumped ( $I_D > 1$ ), or regular ( $I_D < 1$ ). Departure from a random distribution was tested by:

$$I_D = (n-1) S^2/\bar{x} \quad (\text{Equation 1})$$

where  $S^2$  is the sample variance,  $\bar{x}$  is the sample mean, and  $n$  is number of samples taken per observation (number of stems in each grid). Whenever the  $I_D$  obtained from a sample data exceeded the critical value of a chi-square ( $\chi^2$ ) with  $n-1$  degrees of freedom and probability level of  $\alpha=0.05$ , a nonrandom distribution was concluded.

Mean crowding ( $x^*$ ) proposed by Lloyd was calculated as:

$$x^* = \bar{x} + (S^2/\bar{x}) - 1 \quad (\text{Equation 2})$$

As an index, mean crowding is highly dependent upon both the degree of clumping and population density. To remove the effect of density, Lloyd introduced the index of patchiness ( $I_P$ ). It was calculated as follows:

$$I_P = x^*/\bar{x} \quad (\text{Equation 3})$$

When  $I_P=1$ , population distribution will be random. However, it will turn to regular or aggregated if the index shifts to  $<1$  or  $>1$ , respectively (Lloyd, 1967).

## Regression methods

Taylor's power law (TPL) was used to model the relationship between variance and mean as follows:

$$S^2 = a \bar{x}^b \quad (\text{Equation 4})$$

where  $S^2$  is the variance,  $\bar{x}$  is the sample mean,  $a$  is a scaling factor related to sample size, and  $b$  is measure of the species aggregation. When  $b = 1$ ,  $< 1$  and  $> 1$ , the distribution is random, regular and aggregated, respectively. Through the use of a log transformation, we can estimate the coefficients with linear regression as:

$$\log(S^2) = \log(a) + b \cdot \log(\bar{x}) \quad (\text{Equation 5})$$

where  $a$  and  $b$  are the parameters of the model, estimated by linearization of the equation after a log-log transformation (Taylor, 1961).

Iwao's patchiness regression method (IPI) was used to quantify the relationship between mean crowding index ( $x^*$ ) and mean density ( $\bar{x}$ ) using the following equation:

$$x^* = \alpha + \beta \bar{x} \quad (\text{Equation 6})$$

where  $\alpha$  indicates the tendency to crowding (when it is positive) or repulsion (when it is negative), and  $\beta$  reflects the distribution of population in space and is interpreted in the same manner as  $b$  of TPL (Iwao, 1968). Goodness of fit for each model was evaluated using coefficients of determination ( $R^2$ ).

Student's t-test was used to determine if line slope of regression exceeds one, in other words if variance exceeds mean:

$$t = \frac{\text{slope} - 1}{SE_{\text{slope}}} \quad (\text{Equation 7})$$

where  $SE_{\text{slope}}$  is the standard error of the slope for either TPL or IPI regression model. Calculated values are compared with critical t-values with  $n-2$  degrees of freedom. If  $t < t_{\alpha(2), n-2}$ , the null hypothesis (slope = 1) would be accepted and spatial distribution would be random. Otherwise, the null hypothesis would be rejected and if slope  $> 1$  or  $< 1$ , the spatial distribution would be aggregated or uniform, respectively.

### Test for coincidental regression

An analysis of covariance (ANCOVA) was adopted for testing hypothesis, if regression lines had the same parameters. It allowed the simultaneous testing of two hypotheses including  $H_0: \beta_1 = \beta_2 = \beta_3 \dots = \beta_k$  and  $H_1$ : elevations of all lines were the same as well as an alternative hypothesis. In simple terms, the null hypothesis meant that the lines had the same slopes and elevations and they were coincident. While the alternative hypothesis implies that the slope or elevation of at least one of the regression lines was different. The following F-statistic was used to test the null hypothesis of the population parameters of the six locations:

$$F = \frac{\frac{SS_t - SS_p}{2(K-1)}}{\frac{SS_p}{Df_p}} \quad (\text{Equation 8})$$

where  $SS_t$  and  $SS_p$  are total (data of the six fields as a single regression) and pooled (summed over the six lines) regressions with  $2(K-1)$  and  $Df_p$  degrees of freedom. As long as F-statistic is not significant, all sample regressions is assumed to estimate the same population and one can estimate general or common regression parameters (common slope as well as common height or y-intercept).

### Spatial correlation between predators and preys

The spatial correlation was evaluated between the aphids and their predators, *C. septempunctata* and *H. variegata*. A simple linear regression was adopted to investigate the spatial association between aphid counts as an independent variable and ladybird counts as a dependent variable (Rezaei, 1995). All analyses were done using SPSS ver. 26 (IBM Corp. released, 2019).

## Results

### Distribution indices

The results showed that the variance to mean ratios were greater than unity for all studied insects in all fields that may indicate aggregated distribution (Tables 1 and 2). Lloyd's mean crowding ( $x^*$ ) and index of patchiness ( $I_p$ ) supported this conclusion;  $I_p$  was  $> 1$  in all datasets of both aphids except one dataset belonging to *A. pisum* (Table 1). Both ladybirds *C. septempunctata* and *H. variegata* also showed aggregative spatial pattern with  $I_p > 1$  in 41 and 28 out of 42 datasets, respectively (Tables 2).

### Regression analyses

General pattern of both models of IPI and TPL for all the studied insects was tendency to crowding at higher densities in all fields (Table 3). Positive values for intercept and slopes higher than unity for both models (except in a few cases) may imply that variances exceed means even at low insect densities with an increasing trend of variance to mean ratio by increasing insect density.

### Common regression parameters

Differences in regression parameters estimated for one species in different fields led to testing the hypothesis of similarity of dispersal patterns among fields using covariance analysis. The results indicated that the distribution parameters of all insects in all six alfalfa fields were the same (both slopes and elevations) ( $F=0.290$ ,  $P=0.978$ ;  $F=1.945$ ,  $P=0.077$ ;  $F=0.130$ ,  $P=0.999$  and  $F=0.730$ ,  $P=0.691$ ; for *T. maculata*, *A. pisum*, *C. septempunctata* and *H. variegata*, respectively, by TPL;  $df=10,30$  in all analyses). Therefore, the data from all fields were pooled and analyzed together, and common parameters characteristic of each insect were estimated (Table 4). Once again, slopes higher than one (based on t-values) demonstrate a crowding tendency at higher densities in all insects. Also, a positive value for intercept in TPL may imply an aggregation even at lower densities. Small intercepts of TPL and negative values in IPI for *T. maculata* may show tendency to repulsion or random distribution at lower densities of this pest. High coefficients of determination of the pooled data may show a good fitness and suitable description of data by both models.

**Table 1.** Estimated spatial distribution parameters (variance to mean ratios and Lloyd's mean crowding and patchiness indices) of *Therioaphis maculata* and *Acyrtosiphon pisum* in alfalfa fields.

Date	Field	<i>T. maculata</i>				<i>A. pisum</i>			
		$S^2/\bar{x}$	$I_D\#$	$x^*$	$I_P$	$S^2/\bar{x}$	$I_D$	$x^*$	$I_P$
04-Jul-2016	A	91.927	1746.605	133.01	3.161	5.671	107.748	0.621	0.43
10-Jul-2016	A	3.938	74.822	12.549	1.306	6.452	122.594	7.313	3.93
17-Jul-2016	A	8.671	164.744	16.06	1.914	2.908	55.252	4.936	1.63
08-Aug-2016	A	6.373	121.082	13.016	1.703	2.498	47.456	5.402	1.384
13-Aug-2016	A	5.683	107.968	17.659	1.361	4.683	88.979	9.207	1.667
20-Aug-2016	A	8.139	154.633	26.4	1.371	4.19	79.601	10.428	1.441
27-Aug-2016	A	20.058	381.093	41.843	1.836	5.748	109.219	12.272	1.631
05-Jul-2016	B	31.928	606.628	53.561	2.366	3.867	73.467	4.2	3.15
12-Jul-2016	B	16.089	305.697	27.589	2.207	8.467	160.867	8.467	8.467
18-Jul-2016	B	6.38	121.212	12.08	1.803	1.91	36.281	1.143	4.898
10-Aug-2016	B	1.1	20.9	1.6	1.067	3.246	61.675	6.779	1.495
14-Aug-2016	B	3.859	73.318	7.826	1.576	2.943	55.917	7.11	1.376
21-Aug-2016	B	8.827	167.72	17.894	1.778	12.977	246.565	31.177	1.624
28-Aug-2016	B	32.569	618.818	58.869	2.156	6.028	114.533	13.895	1.567
07-Jul-2016	C	11.413	216.844	20.968	1.986	3.207	60.934	2.818	4.612
14-Jul-2016	C	6.774	128.705	11.624	1.987	2.983	56.683	2.583	4.306
19-Jul-2016	C	7.123	135.328	10.178	2.51	1.596	30.323	1.818	1.488
09-Aug-2016	C	7.441	141.376	13.292	1.94	2.782	52.849	4.803	1.59
15-Aug-2016	C	11.739	223.036	34.973	1.443	5.692	108.157	12.182	1.627
22-Aug-2016	C	6.228	118.334	27.356	1.236	3.596	68.32	12.66	1.258
29-Aug-2016	C	8.916	169.41	25.576	1.448	9.868	187.483	24.336	1.573
01-Jul-2017	D	55.075	1046.416	84.205	2.795	32.065	609.242	76.863	1.678
30-Jul-2017	D	14.223	270.237	24.33	2.19	12	228.009	25.572	1.755
06-Aug-2017	D	20.309	385.877	34.381	2.281	11.835	224.863	43.478	1.332
12-Aug-2017	D	50.082	951.56	120.523	1.687	32.127	610.421	88.556	1.542
10-Sep-2017	D	8.341	158.484	18.329	1.668	7.366	139.961	21.866	1.411
16-Sep-2017	D	60.425	1148.071	126.056	1.892	17.556	333.562	76.461	1.276
23-Sep-2017	D	19.374	368.105	55.279	1.498	22.752	432.29	42.335	2.057
2017-Jul-02	E	19.856	377.27	46.985	1.67	24.133	458.535	75.3	1.443
2017-Jul-31	E	61.082	1160.561	70.018	7.047	31.731	602.896	46.052	3.006
2017-Aug-07	E	39.588	752.163	80.318	1.925	20.318	386.039	59.395	1.482
2017-Aug-13	E	96.039	1824.746	175.86	2.176	27.168	516.186	74.937	1.537
11-Sep-2017	E	20.594	391.281	53.978	1.57	37.424	711.048	76.218	1.915
17-Sep-2017	E	27.666	525.646	49.611	2.162	38.884	738.8	71.839	2.116
24-Sep-2017	E	10.257	194.876	18.718	1.978	33.159	630.015	61.223	2.106
03-Jul-2017	F	19.089	362.691	39.535	1.843	29.582	562.056	56.997	2.006
01-Aug-2017	F	33.248	631.717	76.111	1.735	30.654	582.434	59.092	2.007
08-Aug-2017	F	15.021	285.408	45.868	1.44	18.406	349.709	53.067	1.488
14-Aug-2017	F	25.573	485.896	49.82	1.973	23.436	445.285	51.18	1.781
12-Sep-2017	F	15.826	300.69	25.118	2.44	8.997	170.949	16.336	1.959
18-Sep-2017	F	8.501	161.512	35.131	1.271	8.701	165.319	33.039	1.304
25-Sep-2017	F	24.929	473.651	62.114	1.627	22.98	436.616	63.657	1.527

# significant in all cases ( $P < 0.0001$ ). df is 20 for all insects in each field.

**Table 2.** Estimated spatial distribution parameters (variance to mean ratios and Lloyd's mean crowding and patchiness indices) of *Coccinella septempunctata* and *Hippodamia variegata* in alfalfa fields

Date	Field	<i>C. septempunctata</i>				<i>H. variegata</i>			
		$S^2/\bar{x}$	$I_D\#$	$x^*$	$I_P$	$S^2/\bar{x}$	$I_D$	$x^*$	$I_P$
04-Jul-2016	A	5.769	110.123	17.018	1.392	5.304	100.775	7.165	1.351
10-Jul-2016	A	2.619	49.768	4.703	1.525	2.355	44.739	2.077	0.882
17-Jul-2016	A	1.583	30.083	0.667	8	0.917	17.431	1.945	2.12
08-Aug-2016	A	1.119	21.262	1.833	1.069	1.921	36.504	1.231	0.641
13-Aug-2016	A	1.776	33.741	2.038	1.615	2.341	44.484	1.556	0.665
20-Aug-2016	A	1.051	19.961	0.813	1.066	1.976	37.548	1.143	0.578
27-Aug-2016	A	1.568	29.797	2.711	1.265	1.536	29.192	0.941	0.612
05-Jul-2016	B	2.97	56.429	11.437	1.208	3.762	71.471	4.528	1.204
12-Jul-2016	B	15.701	298.321	22.568	2.869	9.937	188.811	12.204	1.228
18-Jul-2016	B	4.689	89.096	7.823	1.893	3.047	57.887	2.88	0.945
10-Aug-2016	B	2.256	42.856	1.556	5.185	5.915	112.393	10.682	1.806
14-Aug-2016	B	1.252	23.795	0.952	1.361	1.367	25.967	0.5	0.366
21-Aug-2016	B	1.733	32.933	1.333	2.222	2.058	39.108	2.125	1.032
28-Aug-2016	B	4.55	86.452	9.183	1.63	1.633	31.02	3.766	2.307
07-Jul-2016	C	2.555	48.536	8.249	1.232	2.389	45.389	2	0.837
14-Jul-2016	C	2.101	39.925	2.526	1.773	1.658	31.496	1.308	0.789
19-Jul-2016	C	0.854	16.227	1.382	0.904	2.028	38.528	1.25	0.616
09-Aug-2016	C	1.747	33.191	1.964	1.614	1.415	26.883	0.5	0.353
15-Aug-2016	C	2.02	38.38	2.424	1.726	1.387	26.357	0.6	0.433
22-Aug-2016	C	1.496	28.427	1.56	1.466	1.619	30.752	1.214	0.75
29-Aug-2016	C	5.719	108.667	8.038	2.422	2.865	54.44	3.333	1.163
01-Jul-2017	D	7.928	150.638	10.845	2.769	5.056	96.068	6.878	1.36
30-Jul-2017	D	1.988	37.774	2.417	1.692	4.853	92.208	7.062	1.455
06-Aug-2017	D	1.46	27.741	1.805	1.342	5.731	108.886	9.421	1.644
12-Aug-2017	D	5.773	109.691	10.571	1.823	7.039	133.735	13.217	1.878
10-Sep-2017	D	2.253	42.799	2.348	2.144	3.071	58.343	3.416	1.112
16-Sep-2017	D	5.774	109.702	10	1.913	7.368	139.992	13.678	1.856
23-Sep-2017	D	6.443	122.411	7.764	3.345	5.726	108.79	7.523	1.314
2017-Jul-02	E	3.952	75.094	6.657	1.797	3.127	59.414	4.242	1.357
2017-Jul-31	E	9.359	177.821	10.359	5.179	3.62	68.779	4.21	1.163
2017-Aug-07	E	3.932	74.707	6.419	1.841	14.15	268.857	18.227	1.288
2017-Aug-13	E	2.184	41.501	3.761	1.46	2.856	54.272	4.536	1.588
11-Sep-2017	E	2.585	49.109	2.264	3.332	3.256	61.872	3.333	1.024
17-Sep-2017	E	5.327	101.219	7.609	2.318	3.545	67.353	6.75	1.904
24-Sep-2017	E	2.132	40.517	2.056	2.227	6.277	119.262	7.2	1.147
03-Jul-2017	F	3.923	74.529	5.907	1.979	7.328	139.232	10.636	1.451
01-Aug-2017	F	4.195	79.703	6.795	1.887	4.663	88.596	8.063	1.729
08-Aug-2017	F	3.818	72.542	6.464	1.773	14.218	270.141	18.608	1.309
14-Aug-2017	F	3.293	62.565	5.662	1.681	5.638	107.118	9.007	1.598
12-Sep-2017	F	3.234	61.449	3.219	3.269	4.541	86.285	4.711	1.037
18-Sep-2017	F	4.56	86.64	8.498	1.721	5.763	109.493	7.932	1.376
25-Sep-2017	F	1.959	37.223	2.082	1.854	1.313	24.955	1.098	0.836

#significant in all cases ( $P < 0.0001$ ). df is 20 for all insects in each field.



**Table 3.** Regression analyses of both TPL and IPI regression models for *Therioaphis maculata*, *Acyrtosiphon pisum*, *Coccinella septempunctata* and *Hippodamia variegata* in alfalfa fields.

Field		<i>T. maculata</i>					<i>A. pisum</i>				
		$a \pm SE$	$b \pm SE$	$R^2$	$t$	$Pvalue$	$a \pm SE$	$b \pm SE$	$R^2$	$t\#$	$Pvalue$
A	TPL	-0.690±0.461	2.482±0.386	0.891	3.83	0.012	0.700±0.152	0.893±0.244	0.727	-0.434	0.681
	IPI	-22.805±8.88	3.423±0.425	0.928	5.690	0.002	3.831±1.327	0.945±0.269	0.710	-0.202	0.847
B	TPL	0.207±0.060	2.222±0.059	0.996	20.64	0.00	0.550±0.118	1.264±0.159	0.926	1.66	0.157
	IPI	-3.247±1.531	2.359±0.101	0.990	13.36	0.000	2.037±1.432	1.450±0.169	0.935	2.651	0.045
C	TPL	0.769±0.158	1.444±0.148	0.922	0.97	0.37	0.424±0.096	1.301±0.136	0.948	2.21	0.077
	IPI	6.181±1.710	1.102±0.113	0.945	0.905	0.406	0.947±0.829	1.418±0.108	0.971	3.85	0.01
D	TPL	0.254±0.344	1.811±0.228	0.926	3.54	0.016	0.305±0.452	1.625±0.301	0.853	2.07	0.092
	IPI	8.034±9.664	1.679±0.230	0.913	2.944	0.032	6.941±7.026	1.325±0.177	0.917	1.82	0.127
E	TPL	0.806±0.546	1.486±0.389	0.754	1.28	0.256	1.800±0.370	0.782±0.238	0.684	-0.90	0.413
	IPI	10.274±15.499	1.862±0.391	0.818	2.20	0.078	38.220±8.62	0.761±0.222	0.701	-1.071	0.290
F	TPL	0.794±0.562	1.327±0.393	0.698	0.85	0.430	0.350±0.490	1.647±0.343	0.821	1.88	0.118
	IPI	7.358±8.257	1.421±0.273	0.843	1.53	0.184	6.367±9.696	1.461±0.325	0.801	1.418	0.215
Field		<i>C. septempunctata</i>					<i>H. variegata</i>				
		$a \pm SE$	$b \pm SE$	$R^2$	$t$	$Pvalue$	$a \pm SE$	$b \pm SE$	$R^2$	$t$	$Pvalue$
A	TPL	0.234±0.083	1.242±0.133	0.945	1.81	0.128	0.334±0.107	1.083±0.212	0.839	0.40	0.711
	IPI	0.040±0.213	1.3869±0.04	0.995	8.88	0.00	0.607±0.662	1.760±0.483	0.726	1.573	0.176
B	TPL	0.385±0.121	1.421±0.183	0.922	2.30	0.70	0.440±0.102	1.373±0.194	0.908	1.92	0.112
	IPI	0.630±2.658	1.757±0.495	0.715	1.52	0.187	0.910±1.659	1.898±0.585	0.677	1.534	0.185
C	TPL	0.170±0.320	1.467±0.123	0.811	1.51	0.189	0.350±0.050	1.206±0.090	0.972	2.28	0.071
	IPI	0.627±0.960	1.306±0.315	0.774	0.97	0.37	0.384±0.220	1.953±0.315	0.884	3.022	0.030
D	TPL	0.252±0.118	1.824±0.244	0.917	3.36	0.199	0.472±0.043	1.459±0.070	0.988	6.48	0.00
	IPI	0.687±1.442	1.937±0.409	0.817	2.88	0.570	2.124±0.505	1.578±0.107	0.977	5.38	0.00
E	TPL	0.463±0.130	1.338±0.317	0.780	1.06	0.995	0.436±0.172	1.563±0.399	0.753	1.40	0.217
	IPI	2.206±2.40	1.421±0.911	0.327	0.462	0.662	-0.796±2.76	2.896±0.923	0.662	2.053	0.095
F	TPL	0.397±0.059	1.347±0.124	0.958	2.77	0.038	0.362±0.128	1.783±0.238	0.917	3.28	0.021
	IPI	1.111±0.480	1.494±0.148	0.952	3.32	0.020	-0.498±2.50	2.693±0.670	0.763	2.57	0.05

#.  $t$  is 2.57 for all insects,  $df = 6$ .

Comparison between population parameters of insects suggests that *T. maculata* tend to crowd more intensively at higher densities (steeper slope) than *A. pisum*, although it is more sporadic at lower densities (smaller intercept). Based on TPL parameter estimates, the two ladybirds are more similarly distributed in habitat.

### Spatial correlation between predator and prey

Pairwise (one aphid- one predator) correlation analyses showed a positive, even though weak, association between *T. maculata* and coccinellids, ( $F = 14.295$ ,  $P = 0.0001$ ,  $df = 1,40$ ,  $r = 0.5131$  for *T. maculata* - *C. septempunctata*;  $F = 9.274$ ,  $P = 0.004$ ,  $df = 1,40$ ,  $r = 0.433$  for *T. maculata* - *H. variegata*), whereas *A. pisum* showed no correlation with *C. septempunctata* ( $F = 0.027$ ,  $P = 0.868$ ,  $df = 1,40$ ,  $r = 0.026$ ) and a significant negative correlation with *H. variegata* ( $F = 12.005$ ,  $P = 0.001$ ,  $df = 1:40$ ,  $r = -0.480$ ).

**Table 4.** Common parameter estimates of both TPL and IPI regression models for *Therioaphis maculata*, *Acyrtosiphon pisum*, *Coccinella septempunctata* and *Hippodamia variegata* in alfalfa fields.

insect	model	a ±SE	b±SE	R2	F#	Pvalue	t*	Pvalue
<i>T. maculata</i>	TPL	0.076±0.135	1.895±0.105	0.890	323.984	0.0001	8.503	0.0001
	IPI	-13.35±5.680	1.920±0.194	0.709	97.60	0.0001	4.735	0.0001
<i>A. pisum</i>	TPL	0.430±0.067	1.551±0.057	0.947	717.053	0.0001	9.518	0.0001
	IPI	2.792±1.685	1.526±0.064	0.933	561.389	0.0001	8.17	0.0001
<i>C. septempunctata</i>	TPL	0.335±0.042	1.414±0.081	0.882	300.976	0.0001	5.087	0.0001
	IPI	0.909±0.556	1.568±0.142	0.752	121.509	0.0001	3.993	0.0002
<i>H. variegata</i>	TPL	0.465±0.031	1.422±0.058	0.936	588.95	0.0001	7.202	0.0001
	IPI	0.575±0.513	2.124±0.168	0.799	159.043	0.0001	6.676	0.0001

#. df is 40 for all insects in each field. \*: For all insects in each field ( $\alpha$ :0.021, df:40).

## Discussion

According to the results, all studied aphids and coccinellids tend to have aggregative spatial distribution patterns. The variance-to-mean ratio as well as regression methods (IPI and TPL) confirmed this conclusion. Aggregation is a common phenomenon in the majority of insect species. Different causes of aggregation in insect communities have been presented by many researchers. For example, heterogeneity in the environmental factors such as temperature, humidity, water accessibility, daylight duration, soil fertilization, plant quality, plant growth rate; and behavioral causes such as attraction to common stimuli, reproduction of sedentary insects around a limited area, and interaction between these factors can influence the spatial distribution pattern of insects (Depickère *et al.*, 2004; Mahdavi *et al.*, 2015). Soleimani *et al.* (2015), Rakhshani *et al.* (2009), and Shayestehmehr *et al.* (2017, 2019) also reported aggregative distribution in different insect populations).

Another objective of this study was to test if common regression parameters (both slopes and elevations) can be defined to describe the spatial distribution of insects, independent of location. This hypothesis was confirmed by analysis of covariance of the data and led to the consideration of spatial distribution as a behavioral characteristic of the insects themselves rather than a site-specific characteristic of the locations. Also, it leads to the use of common parameters to predict population occurrence in a unit of habitat independent of its location (Zhao *et al.*, 2015; Malaquias *et al.*, 2017; Pezzini, *et al.*, 2019). In similar studies, researchers found common parameters describing population distribution (Mahdavi *et al.*, 2015; Miri *et al.*, 2017; Zarei Sarchogha *et al.*, 2018; Weber *et al.*, 2018; Tavakolizadeh *et al.*, 2019).

The association between coccinellid predators and aphid prey species was closely agreed upon by previous reports of Pons *et al.* (2009). In the current study, *T. maculata* showed a positive association with both predators, but *A. pisum* showed a negative correlation with *H. variegata*. These results indicate that more factors than the spatial occurrence of prey are needed to explain the occurrence of coccinellids. For example, in addition to feeding aphids, which are their main food source, ladybirds can also consume other food resources including pollen, nectar, and fungal spores, and the occurrence of these resources can influence coccinellids' presence (Al-Deghairi *et al.*, 2014; Kumar & Ahmad, 2016). Another important factor may be microclimate variations. Different responses of the aphids and ladybirds to environmental variables such as moisture and temperature may affect the distribution, abundance, and feeding behavior of the predator (Winder *et al.*, 2001; Nelson *et al.*, 2004; Elliot *et al.*, 2002). Differences in the motivation of predator and prey at different times of residence in a patch and depletion of the prey patches by feeding are other sources of change in predator communities that may alter abundances instantaneously and do not allow a parallel change of abundance of the two organisms (Bayoumy *et al.*, 2015; Samaranyake *et al.*, 2019).



Based on the mathematical indices of distribution and regression models, aphids and coccinellids exhibited generally an aggregated pattern in the alfalfa fields, and common parameters were estimated for predicting the distribution of the insects in a unit of habitat in the field condition. This information can be used in the pest management, conservation of natural enemies, and biological control of the pests. For example, based on the results of this study, one can choose an appropriate sample size to obtain a given level of precision or standard error acceptable in his/her study, referring to aphid density and estimating the mean/variance ratio using common parameters of TPL. Also, one can provide a sequential sampling program using parameters of Iwao's patchiness parameters.

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## استفاده از روش‌های آماری برای تعیین پراکنش زمانی-مکانی شته‌ها و کفشدوزک‌های شته‌خوار در مزارع یونجه

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### مکیده

این مطالعه به منظور تعیین الگوهای پراکنش و فراسنجه‌های مربوط به شته خالدار یونجه، *Therioaphis maculata* (Buckten) شته نخود، *Acyrtosiphon pisum* (Haris) و دو گونه کفشدوزک *Coccinella septempunctata* L. and *Hippodamia variegata* (Goeze) طی سال‌های زراعی ۹۵ و ۹۶ در شش مزرعه‌ی یونجه انجام شد. با رسیدن ارتفاع گیاهان یونجه به ۱۰ سانتی‌متر، نمونه‌برداری‌های هفتگی شروع و تا برداشت چین آخر ادامه یافتند. هر مزرعه به واحدهای ۲۰×۲۰ متر تقسیم و در هر واحد دو کادر ۱×۱ متری به صورت تصادفی انداخته شد و تعداد حشرات کامل کفشدوزک‌های داخل کادر شمارش شدند. برای شمارش شته‌ها، از بوته‌های داخل کادر، ۲۰ ساقه در هر شبکه انتخاب و از ته بریده شدند. سپس ساقه‌های بریده شده داخل یک تشت سفید تکان داده شدند و شته‌های افتاده در تشت شمارش و ثبت شدند. از نسبت واریانس به میانگین و همچنین قانون توان تیلور (TPL) و شاخص رگرسیون آیوانو (IPI) برای تعیین الگوهای پراکنش فضایی حشرات استفاده شد. در تمام تاریخ‌های نمونه برداری، مقدار واریانس از میانگین بیشتر بود، که می‌تواند بر پراکنش تجمعی دلالت داشته باشد. الگوی تجمعی پراکنش حشرات مورد مطالعه با نتایج به دست آمده از TPL (فراسنجه b) و ضریب رگرسیون IPI (B)، که مقدار هر دو به طور معنی‌داری بیش از یک بود، نیز تأیید شد. همبستگی بین تعداد شته‌ها و کفشدوزک‌ها نشان داد که بین شکار و شکارگر رابطه مثبت اما ضعیف وجود دارد، این همبستگی ضعیف ممکن است حاکی از آن باشد که عواملی غیر از دسترسی به شکار، در پراکنش مکانی شکارگرها نقش دارند. استفاده از روش‌های آماری مختلف، اطلاعات مفیدی در مورد پراکنش و الگوی مکانی حشرات و دشمنان طبیعی در مزارع یونجه فراهم می‌کند و از این اطلاعات می‌توان در کنترل بیولوژیکی آفات استفاده نمود.

**کلمات کلیدی:** پراکنش مکانی، قانون توان تیلور، رگرسیون آیوانو، تجمع

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