



Assessment of *Earias vittella* (Fabricius) Egg Distribution in Cotton Crop at Multan, Pakistan: A Comprehensive Guideline for Developing a Pest Scouting Method

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ABSTRACT

The knowledge of oviposition preference by female insects is a basic requirement for developing insect pest scouting methods to determine the potential damage and to apply control measures for key pest like *Earias vittella* in cotton. Field experiments were conducted to assess the distribution of *E. vittella* eggs within the cotton plant. Out of 240 plants inspected, *E. vittella* females laid 204 and 194 eggs on 40.9% and 36.9% of the plants in 2011 and 2012, respectively. Spearman Rank Correlation showed that a significant and positive relationship (association) existed between the number of infested plants or plant parts and number of eggs laid. In every week, an average of 6.1 ± 1.2 plants were infested with 13.6 ± 2.7 eggs in 2011 and 5.5 ± 1.1 plants with 12.9 ± 2.2 eggs in 2012. Four and three peak egg-laying periods were observed in 2011 and 2012, respectively. Taylor's power law of regression showed an aggregate spatial pattern distribution of eggs for *E. vittella* in plants in both years. Variance-to-mean ratio, the chi-square test index, and Lloyd index of patchiness indicated uniform eggs distribution for both years. Within the plants, the most preferred oviposition sites were just below the terminal portion on such structures as the leaves, terminal buds and NE leaves. The current study regarding the site selection for oviposition provides information that can be used to develop guidelines for *E. vittella* management in cotton production.

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MTJ, SAS, MAS and MB planned the study. MTJ conducted the field experiments. MTJ, SAS and MB did the statistical analysis. MTJ wrote the article with input from all co-authors.

Key words

Egg-laying, Main stem leaves, Oviposition, Site preference, Sympodial branches.

INTRODUCTION

The spotted bollworm, *Earias vittella* Fab (Lepidoptera: Noctuidae), is an important pest of cotton worldwide. It is widely distributed in Pakistan and India where it causes huge damage to cotton (Aziz *et al.*, 2012). *E. vittella* infests different parts of cotton plants leading to shedding of squares, flowers and bolls (Dhawan *et al.*, 1990), resulting in about 50% losses in cotton seed yield (Sidhu and Sandhu, 1977; Hasan and Ansari, 2010). The pest remains active throughout the year on different alternate hosts and produces 6-8 generations (Vennila *et al.*, 2005). In Pakistan, the initial attack by *E. vittella* has been observed on cotton during June and July, with maximum infestation occurring during August and September (Syed *et al.*, 2011).

Various attempts have been made in the past to control *E. vittella* under integrated pest management (IPM) strategies such as by cultivation of resistant cultivars (Sharma and Agarwal, 1983; Drake, 1991; Dhillon and Sharma, 2004), application of non-host plant extracts, use of bio-control agents and applications of bio-rational and conventional insecticides (Aziz, 2010; Badiyala, 2013; Rahman *et al.*, 2015). An important reason for the failure of *E. vittella* control strategies is poor understanding of the ecological linkage between the pest and its host. Site preference for egg laying/oviposition is an important ecological interaction between phytophagous insects and their host plants and therefore depends on host quality (Batáry *et al.*, 2008). Such factors can lead to variation in performance and survival of insect progeny for eggs deposited in different locations within a plant (Holland *et al.*, 2004). A study of the vertical distribution of eggs within plant aimed at the determining their location in the plant is crucial to minimize the time spent during the sampling process and augments the reliability of the

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IPM (Renwick and Chew, 1994). Despite the economic importance of *E. vittella* moth, its host selection and within-plant distribution are poorly understood.

The knowledge of oviposition site selection is a basic requirement in developing a pest monitoring strategy (Ross and Ostlie, 1990) and the application of control measures. Several reports on within-plant distribution of different lepidopteran insects like *Heliothis* spp. (Wilson *et al.*, 1980; Duffield and Chapple, 2001), *Alabama argillacea* (Hübner) (Fernandes *et al.*, 2007) in cotton, *Grapholitha packardii* (Zeller) in blue barriers (Mallampalli and Isaacs, 2002) and leaf miner, *Tuta absoluta* (Meyrick) in processing tomatoes (Torres *et al.*, 2001) are available.

Thus, knowledge about *E. vittella* site selection with in the cotton plant is needed in order to develop an acceptable sampling programme and control plan for this pest. Chakravarthy (1985) and Sharma and Agarwal (1983) recommended the whole plant as a sampling unit for eggs assessment. Though theoretically feasible, one would spend several hours in sampling for eggs on whole plants in the field. Therefore, within-plant eggs distribution is an important characteristic of ecological communities that provide farmers with guidelines to control *E. vittella* (Debouzie and Thioulouse, 1986; Arbab and McNeill, 2014). The egg distribution for other cotton bollworms was adequately described by the spatial distribution (Nachapong, 1980). Analysis of the spatial distribution of insects allows for an estimation of pest densities and identifies key timing for pest management decisions that allow growers to minimize costs of control (Khaing *et al.*, 2002).

Therefore, current information for the assessment of eggs within the plant as well as within the field is of utmost importance to monitor *E. vittella* population in the crop. The present study described how the eggs were distributed within cotton plants (intra plant distribution), a critical aspect in devising effective control measures against the pest.

MATERIALS AND METHODS

Field experiments were conducted at the Central Cotton Research Institute [30°12'N latitudes and 71°26'E longitudes and 123 m altitude/elevation], Multan, Pakistan, with plot position as North-South over two seasons (2011 and 2012) to collect data about the preferred oviposition sites of *E. vittella* in unsprayed cotton crops. The commercial cotton variety, CIM-496, was sown during the 2nd week of May with row to row and plant to plant distances of 76 cm and 22.9 cm, respectively. The cotton was planted to three plots (representing three replications) each measuring 30 m × 10 m. Experimental plots were separated by 2 m wide fallows. Standard agronomic

practices were performed in growing the cotton crop.

Data on egg-laying (No. of eggs), was recorded on a weekly basis starting from the 1st week of July (about 45 days after sowing) till the last week of October during both years on: (i) main stem leaves (MSL) (at base of sympodial branch), sympodial branches (S. Br) and (ii) Terminal bud (Ter. bud), newly emerged leaves (NE), leaves (all types), squares, flowers and bolls.

During every sampling, 15 plants (N=15) were randomly selected and examined individually for eggs. Egg-counting was done with the aid of a 10 X magnifying glass.

Percent increase in S. Br over MSL was calculated as follows:

$$\% \text{ increase} = \frac{\text{No. of eggs on S.Br} - \text{No. of eggs of MSL}}{\text{No. of eggs on S.Br} + \text{No. of eggs of MSL}} \times 100$$

Number of eggs on each leaf node of MSL and on each S. Br was total egg found on respective part during whole season.

Statistical analysis

Data on egg counts was analyzed by General linear Model by using Statistix 8.1 (Statistix, 2008). The Least significant difference (LSD) was used separate the means of various parameters used for eggs and larval population on the cotton plant (Gomez and Gomez, 1984).

One sample T-test with Spearman Rank Correlation for number of eggs and infested plants within the plant, Taylor's power law based on regression techniques (Taylor, 1984), index of dispersion (ID) (Elliott, 2003) and index of patchiness (IP) (Lloyd, 1967) were used to test the dispersion of the pest because the use of a single index can lead to incorrect conclusions (Myers, 1978).

Spearman rank correlation

The Spearman's rank-order correlation is the nonparametric version of the Pearson product-moment correlation. Spearman's correlation coefficient, (ρ , also signified by r_s) measures the strength and direction of association between two ranked variables likewise total number of eggs and the infested cotton plants within field (Fauvergue *et al.*, 1994).

Taylor's power law based on regression techniques

The Taylor's power law based on variance-mean relationships, ($s^2 = ax^b$) has been used widely (Arbab and McNeill, 2014) to infer the type of spatial distribution (uniform, random or aggregated) of the counts of *E. vittella* eggs and larval population in the cotton field. The equation is solved with linear regression as:

$$\text{Log}(s^2) = \text{log}(a) - b \text{log}(x)$$

Where, s^2 and x are the sample variance and sample mean,

respectively; the slope (b) is a measure of aggregation (dependent upon species behavior or the environment); and the intercept (a) is a scaling factor. When $a=b=1$, $a>1$, $b<1$, and $a<1$, $b>1$, the distribution is random, uniform, and aggregated, respectively (Ifoulis and Savopoulou-Soultani 2006).

Index of dispersion, $ID = S^2 / \bar{x}$; (where S^2 is sample variance and \bar{x} is sample mean) is known as the variance to-mean ratio is commonly considered to be a useful index to describe the spatial distribution of a given insect within the field. Where $ID=1$ population is random, <1 regular, and >1 aggregated (Kuno, 1991).

Modified the equation performing chi-square test for the variance-to-mean ratio to determine its significant departure from 1.0 as:

$$d(\text{Chi square test index}) = \sqrt{(2 \times \chi^2)} - \sqrt{2(N-1)} - 1$$

Whereas, Chi-square, $\chi^2 = (S^2/\bar{x}) \times (N-1)$.

If $d<1.96$, agreement with a random distribution and <-1.96 , a uniform distribution, and if $d>1.96$, an aggregated distribution is suspected.

The index of patchiness:

$$IP = \frac{\bar{X} + \left(\frac{S^2}{\bar{X}}\right) - 1}{\bar{X}}$$

This index was proposed by Lloyd (1967) to measure the pattern intensity of the pest that is unaffected by thinning (the random removal of points) or plants density variation. If $IP=1$, random, <1 uniform, and >1 aggregated.

RESULTS

Oviposition site preference for sympodial branches and main stem leaves

A total of 240 plants were observed during 2011 and 2012. *E. vittella* females oviposited 204 eggs on ninety-two plants (40.9%) in 2011 and 194 eggs on eighty-three plants (36.9%) in 2012. Test for association between the

number of infested plants and eggs laid as determined with help of SRC (Spearman rank correlation) showed significant relationship 2011: $\rho=0.876$; $P\text{-value}=0.00$ and 2012: $\rho=0.969$; $P\text{-value}=0.00$ for the females to select the plants for egg laying. The female moth selected 6.1 ± 1.2 plants for 13.6 ± 2.7 eggs per week in 2011 and 5.5 ± 1.1 plants for 12.9 ± 2.2 eggs in 2012 (Table I). During the crop growing season, four peak egg laying periods (two each in August and September in 2011 and three, one in August and two in September in 2012) were observed (Fig. 1).

Table I reveals that the number of eggs laid on MSL (main stem leaves) and S. Br (Sympodial branches) varied significantly ($F_{(1, 27)} = 10.3$ and $P\text{-value}= 0.0034$, respectively in 2011 and $F_{(1, 27)} = 20.8$; $P\text{-value} = 0.0001$ in 2012). The females laid 3.6 ± 0.72 (26.47%) and 2.9 ± 0.52 (22.16%) eggs on MSL of 2.3 ± 0.49 (15.6%) and 2.3 ± 0.42 (15.1%) plants in 2011 and 2012 per week, respectively. Association between MSL and number of plants $\rho = 0.65$; $P\text{-value}= 0.01$ (2011) and 0.86 ; $P\text{-value}= 0.00$ (2012) was significantly positive. The results further revealed that an average of 10.0 ± 2.49 eggs per 6.1 ± 1.20 plants in 2011 and 10.1 ± 1.84 eggs per 5.5 ± 1.08 plants, were distributed among S. Br of infested plants. Positively high SRC, (2011: $\rho = 0.88$; $P\text{-value}=0.00$ and 2012: 0.95 ; $P\text{-value}=0.00$) for sympodial branches also reveal female moth selection of fruiting branches for oviposition in the field (Table I).

Seasonal variation in *E. vittella* eggs laying

Significant seasonal variation in egg-laying on MSL (2011; ($F_{(3, 12)} = 8.02$; $P\text{-value} = 0.0034$ and 2012; $F_{(3, 12)} = 4.39$; $P\text{-value}=0.027$) and S. Br (2011; ($F_{(3, 12)} = 13.26$; $P\text{-value}=0.0004$ and 2012; $F_{(3, 12)} = 7.4$; $P\text{-value} = 0.0046$) was observed. The most eggs laid on S. Br (20.8 and 13.5 in 2011 and in 13.5&15.5 in 2012 per week) and MSL (3.5 and 7.0 in 2011 and 4.0 and 4.3 in 2012) were recorded in August and September (Table II).

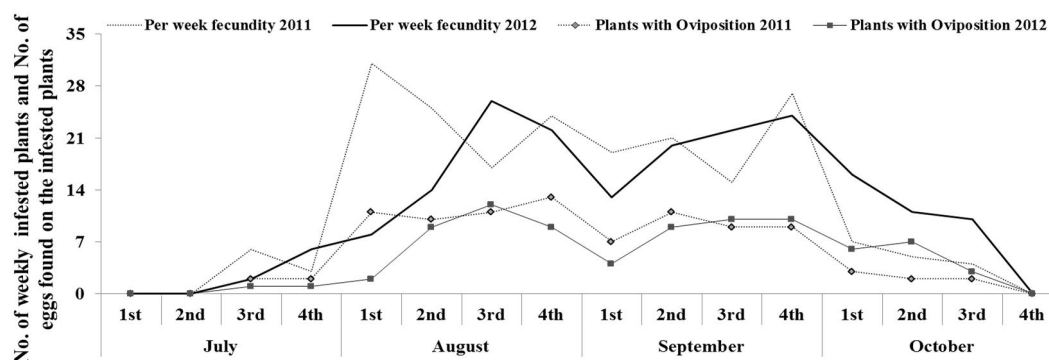


Fig. 1. Number of cotton plants (out of 15 plants) infested with oviposition and number of eggs of *E. vittella* on the infested plants recorded on weekly basis from July-Oct during 2011 and 2012.

Table I.- Number of infested plants out of 240 plants inspected, number of eggs of *E. vittella* during each season and Spearman Rank Correlation of infested plants with eggs.

Year		No. of infested plant		No. of eggs		*SRC (ρ)
		Total (%)	Mean No. of infested plants/week	Total (%)	Mean No. of eggs/week	
2011	Total	92 (40.9)	6.1 ± 1.20	204	13.6 ± 2.70	0.88; <i>P</i> -value = 0.00
	MSL	(15.6)	2.3 ± 0.49	(26.47)	03.6 ± 0.72	0.65; <i>P</i> -value = 0.01
	S. Br	(40.9)	6.1 ± 1.20	(73.53)	10.0 ± 2.49	0.88; <i>P</i> -value = 0.00
2012	Total	83 (36.9)	5.5 ± 1.10	194	12.9 ± 2.20	0.97; <i>P</i> -value = 0.00
	MSL	(15.1)	2.3 ± 0.42	(22.16)	02.9 ± 0.52	0.86; <i>P</i> -value = 0.00
	S. Br	(36.9)	5.5 ± 1.08	(77.84)	10.1 ± 1.84	0.95; <i>P</i> -value = 0.00

*SRC, Spearman rank correlation.

Table II.- Mean number of eggs/week laid by *E. vittella* females on MSL and S. Br of 15 plants during different months in 2011 and 2012.

Year	2011		2012	
	MSL	S. Br	MSL	S. Br
July	1.3	01.0	1.3	00.8
August	3.5	20.8	4.0	13.5
September	7.0	13.5	4.3	15.5
October	1.8	02.3	1.3	08.0

Distribution of eggs on plant structures

Preferred nodes on the main stem for oviposition

The female moth preferred the upper ten S. Br for egg-laying. The number of eggs laid were varied significantly from Ter. Bud to the S₁₀ (in 2011: $F_{(10, 151)} = 4.03$; *P*-value=0.0001 and in 2012: $F_{(10, 151)} = 4.03$; *P*-value=0.0001). Amongst these branches, the most preferred was S₁ (just below the top) where most (30-40 eggs) eggs were laid. On the rest of the branches, *i.e.* Ter. Bud and S₁ to the S₈, oviposition ranged from 25 to 10 eggs (Fig. 2).

Preferred parts of the plants for oviposition

Similarly, the number of eggs on leaves at various nodes of the main stem starting from the top part downwards ($F_{(7, 112)} = 2.037$; *P*-value= 0.046 in 2011; $F_{(7, 112)} = 2.87$; *P*-value=0.0085 in 2012) also varied significantly and eggs were not found below the leaf of sixth node (Fig. 3). Eggs on plant structures likewise Ter. bud, NE leaves, leave, squares, flowers and bolls were varied significantly (2011: $F_{(5, 87)} = 8.84$; *P*-value= 0.00 and 2012: $F_{(5, 87)} = 3.33$, *P*-value = 0.0085). The most preferred part of the plant for egg laying were as in descending order; leaves, Ter. bud, NE leaves, squares, flowers and bolls (Fig. 4).

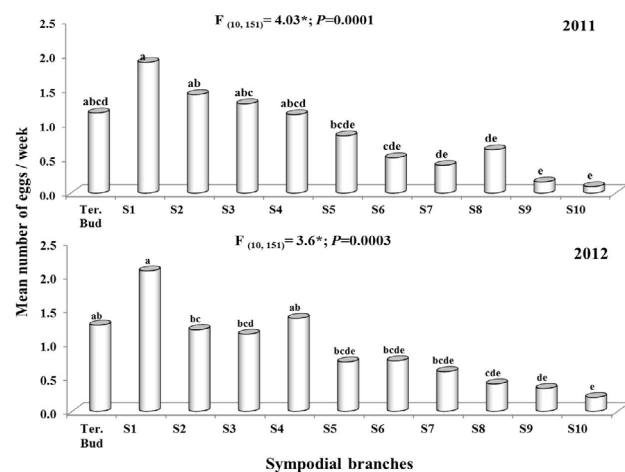


Fig. 2. Distribution of *E. vittella* eggs recorded weekly on the symphyllal branches (S. Br) starting from terminal bud (Ter. Bud) to vertically downward on the main stem during 2011 and 2012. No letters in common show significant difference in number of eggs found among S. Br in the whole season.

Dispersion pattern of *E. vittella* eggs in the field

Taylor's power law regression showed significantly positive relationships between the log (variance) and log (mean) of eggs of *E. vittella* per plant in both years. The intercept "a" of Taylor's power law was <1, and the distribution coefficient "b">1, indicating an aggregate spatial pattern distribution of eggs in both years (Table III). In contrast, the values obtained from the variance-to-mean ratio (ID) for testing distribution pattern of eggs in cotton field under unsprayed condition were <1 (ranging from 0.38 to 0.55) in each of the 2 years and were combined. Also, d, the chi-square test index, for all the data set was < -1.96 (-3.31, -2.94 and -3.42) and also Lloyd index of patchiness (IP) was < 1 (0.50, 0.29 and 0.30) suggesting that distribution was uniform (Table IV).

Table III.- Parameters for spatial distribution of *E. vittella* eggs derived from Taylor's power law with linear regression method.

Year	<i>a</i>	<i>b</i>	<i>R</i> ² Adj	Distribution pattern
2011	0.98 ± 0.12; <i>P</i> = 0.00	1.83± 0.39; <i>P</i> = 0.0003	0.68	Aggregate
2012	0.92 ± 0.12; <i>P</i> = 0.00	1.80± 0.44; <i>P</i> =0.0011	0.51	Aggregate
2011 and 2012	0.90 ± 0.12; <i>P</i> = 0.00	1.98± 0.5; <i>P</i> = 0.0014	0.50	Aggregate

a, the intercept is a scaling factor; *b*, the slope for measure of aggregation.

Table IV.- Mean, variance, Chi-square and indices for *E. vittella* eggs distribution in field conditions.

Year	Mean ± SE	Variance	DF	P-value	Chi-Square (χ^2)	ID < 1 uniform	d < -1.96 uniform	IP < 1 uniform
2011	0.91 ± 0.18	0.50	11	0.9997	1.43	0.55	-3.31	0.50
2012	0.86 ± 0.15	0.34	11	0.9980	2.13	0.39	-2.94	0.29
2011 and 2012	0.88 ± 0.15	0.34	12	1.0000	0.56	0.38	-3.42	0.30

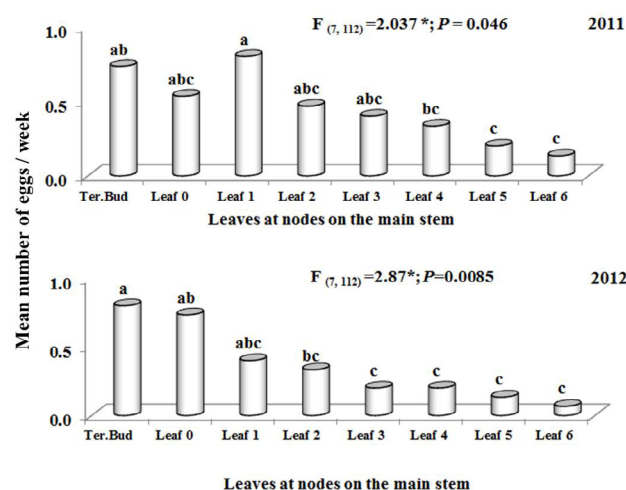


Fig. 3. Mean number of eggs on leaves of different nodes of the main stem during 2011 and 2012. No letters in common on the respective columns show significant difference among leaves on various nodes on main stem during the crop season 2011 and 2012.

DISCUSSION

Egg distribution of *E. vittella* in cotton field

E. vittella egg deposition began in the month of July about 45 days after cotton sowing. It was observed that this pest shifted to cotton from neighboring okra fields in July and remained active in cotton till the crop was harvested in October-November, with the peak pest populations during August-September. This observation was similar to the findings of Solangi *et al.* (2016) and Syed *et al.* (2011).

Mechanism of plant selection by *E. vittella* in the field

In the present study, *E. vittella* eggs distribution was

studied in relation to selection of suitable host cotton plants in the natural field conditions. Out of 225 plants observed, *E. vittella* eggs were found on only 36-41%, plants with infested MSL on 15-15.6% and S. Br on 36-41% plants. Most of the plants were without damage by *E. vittella*, except a few plants, which were previously damaged in old bolls (visual observation). More eggs were found on the plants sampled from the central part of a plot. This oviposition behavior indicates that *E. vittella* female moths has the ability to discriminate between a good and a bad or an unsuitable host plant as it directly concerns to the survival of the offspring (Renwick and Chew, 1994; Awmack and Leather, 2002; Binyameen *et al.*, 2013). Like other lepidopteran pests, *E. vittella* foraged to select suitable areas and hosts to avoid the risk of predation and/or parasitism (Ramaswamy *et al.*, 1987; Tamhankar *et al.*, 1993), and competition (Tingle and Mitchell, 1992; Hartlieb and Rembold, 1996; Suverkropp *et al.*, 2008; Zakir *et al.*, 2013).

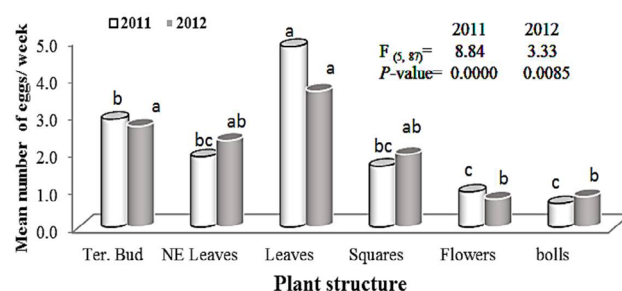


Fig. 4. Number of eggs on various structures of cotton plants during 2011 and 2012. Different letters on the respective blank columns or shaded column show significant difference among the plant structures during that respective year.

Distribution of E. vittella eggs on plant

Significantly more eggs on S. Br as compared to MSL shows the preference of female moth for outer border of the plants for oviposition. In the current study, the terminal buds and leaves on the main stem down to the sixth node, were preferred as oviposition sites by *E. vittella* females (Lundgren and Fergen, 2006). Further on the sympodial branches on the main stem starting from 1st node going downward, the first ten branches were likely selected by *E. vittella* females with the first eight branches more preferred for egg deposition. In addition, the branches were more desirable sites for oviposition than leaves on the main stem. These results revealed that like other lepidopteran pests (Zalom *et al.*, 1983), *E. vittella* prefer upper plant canopy up to the tenth node and also exterior margin (branches) of the cotton plants. It was also reported earlier by Chakravarthy (1985) that the *E. vittella* female moths most preferred the top 50 cm of the plant canopy for oviposition. This reflects the hypothesis “preference performance”, presented by Heisswolf *et al.* (2005) to enhance the performance of *E. vittella* offspring.

Distribution pattern of E. vittella eggs in the cotton fields

Association between host plants selection and eggs deposition in the cotton determined with SRC reveals significantly positive and random correlation. A similar study was conducted by Pai (2016) and determined various associations between honey bees and different flora. Taylor’s power law of dispersion determines weak correlation with the slope “b” > 1, reveals clumped or aggregated eggs on the plant in the cotton field. Sardana and Tewari (1990) also observed clumped distribution of *E. vittella* eggs in okra fields using the statistics of negative binomial distribution and the variance to mean ratio, k and chi-square values. Among the other indices, index of dispersion, chi-square test index and Lloyd index of patchiness ($ID < 1$, $d < -1.96$ and $IP < 1$, respectively) indicated that eggs were distributed uniformly in the cotton field. Chakravarthy (1985) noted that the eggs and larvae of *Earias* spp. followed a uniform distribution on cotton varieties. However, dispersion of *Earias* eggs on cotton varieties was due to environmental heterogeneity. Very few (5 to 15%) lepidopteran can lay eggs in large clusters; most Lepidoptera lay single eggs and develop as solitary larvae (Lacey and Kaya, 2007). Taylor (1984) and Southwood and Henderson (2009) stated that change in distribution from clumped/aggregated to random or regular resulted from the alteration of the size of the area occupied by the insects related to that of the sample or decreased and continuous population density, the dispersion was effectively random. Nachapong (1980) previously stated that the distribution of cotton bollworm was dependent on

both egg laying habits of the female moths and variation in growth among the cotton plants.

Present result further implies site preference for egg laying within plant based on fresh fruiting parts (Sharma and Agarwal, 1983; Dhillon and Sharma, 2004) depending upon female choice (Heisswolf *et al.*, 2005). It was observed that *E. vittella* female moths equally preferred terminal bud, unexpanded (tender terminal) leaves, fresh leaves, squares, flowers and bolls for sites of oviposition. There was slight variation in the number of eggs deposited on these structures as more eggs were laid on leaves (tender as well as fresh leaves) followed by squares, bolls and flowers. However, Sharma and Agarwal (1983) found *E. vittella* eggs deposited on the leaves, squares, and Dhillon and Sharma (2004) recorded ovipositions on buds and bolls.

CONCLUSIONS

The knowledge of distribution pattern and site selection for egg-laying are basic requirements to develop a management strategy, such as pest monitoring (Ross and Ostlie, 1990) and applications of control measures (Spangler and Calvin, 2001). The current study provides guidelines for both short and long term management of the seasonal outbreaks and detection of *E. vittella* initial infestation, respectively as an integral part in the IPM strategies. For instance, upper canopy of ten nodes is determined as the most important area of the plants for control measures of *E. vittella* at egg laying stages through augmentative releases of egg parasitoids such as *Trichogramma chilonis* or by ovicidal application. *E. vittella* females prefer the upper canopy of the cotton plants for oviposition as it contains all different types of fruiting bodies ranging from squares to mature green bolls as well as leaves. Moreover, the results from the current study indicate that regular pest scouting should be done after the plant starts its reproductive stage.

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Statement of conflict of interest

The authors have declared no conflict of interests.

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