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Studies on Biology and Morphometrics of *Etiella zinckenella* (Lepidoptera) on Lentil under Laboratory Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. Author GK designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SSY and GK managed the analyses of the study. Authors Manisha and Sindhu managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: *Etiella zinckenella* (Treitschke), an important and destructive pest of lentils and peas owing to its feeding habit.
Aim: The present study aims to investigate the biology and morphometrics of E*tiella zinckenella* (lepidoptera) on lentil under laboratory conditions.
Study Design: Laboratory experiment carried out on biological parameters of *Etiella zinckenella* in the department of Entomology, CCSHAU and Hisar.
Results: Study revealed that a single female could lay 46-65eggs (average 56.3 eggs) preferably singly on pods, flowers, calyx and leaves of lentil. Incubation period of the eggs varied from 5.18 to 5.38 days (average 5.24 days). After egg hatching, larvae passed through 5 larval instars. Total larval and pupal period was observed on an average 16.9 and 13.38 days, respectively. Adult longevity of male and female was found on an average 4.1 and 5.6 days respectively. *Etiella zinckenella* completed its total life cycle in 37-45 days.

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Conclusion: Biology of an insect pest is a condition precedent to find out its management strategies. Hence, observation on nature and behaviour of each developmental stage of this pest can be further utilised in the effective management at its damaging stage under field conditions.

Keywords: Etiella zinckenella; adult longevity; incubation; larval period; oviposition; pupal period.

1. INTRODUCTION

Lentil is a rich source of proteins (25 per cent) and is considered as an important source to overcome the problem of malnutrition due to protein gap. In India, it is grown on an area of 1.60 million hectares with an average production of 0.94 million tonnes, and productivity level of 591 kg /ha [1]. In Haryana, it is cultivated on an area of 4.1 thousand ha with production and productivity of 3.2 thousand tonnes and 780 kg/ha, respectively [2]. Poor crop management along with abiotic and biotic stresses are the major constraints in its production. Among the biotic constraints, insect pests play a major role in its yield reduction. However *Aphis craccivora* Koch [3] and *Etiella zinckenella* Treit. Singh and Dhooria [4] have been reported as major insect pests of lentil in India. *Etiella zinckenella* infests lentil at flowering and pod formation stages and is considered as main reason of low productivity, besides reduction in yield and quality of the grains is also affected. In India, it has been reported to infest 11.4 and 50.9 per cent of lentil and pea pods, respectively, resulting in yield losses of 10.6 and 23.9 per cent [4]. Sandhu and Verma [5] observed that 12 to 15 per cent pods of lentil were 2 infested by *E. zinckenella*. Infestation of this pest has been reported up to17.5 per cent in Haryana [6]. On the basis of biological parameters viz. number of eggs, higher hatching, shorter larval period with lower larval and pupal mortality and higher per cent adult emergence, we can use the most effective management practices at the most damaging stage of insects.

2. MATERIALS AND METHODS

2.1 Laboratory Culture of *E. Zinckenella*

Investigations on various biological aspects of *E. zinckenella* were studied in the laboratory, Department of Entomology at 28±10C and 60-70% humidity on lentil (cv. Garima). Initially a good number of full grown larvae of *E. zinckenella* were collected during March, 2016 from lentil and field pea plots. These were further reared in battery jars (20×15 cm), under the normal laboratory conditions to obtain the adults by supplying fresh pods of lentil to developing larvae till pupation. The freshly formed pupae were transferred into Petri dishes for the emergence of adults. One pupa was kept in each Petri dish which was provided for easy emergence of the moth. The newly emerged moths were released in pairs of opposite sex in another specimen jar covered with muslin cloth and fastened with rubber bands. These jars were provided with cotton soaked in 5 per cent honey solution for adults. A tender twig of Lentil in a small 50 ml beaker having water was placed inside the specimen jar to receive the eggs from these adults. The eggs thus laid on the leaves, walls of jar and the muslin cloth were removed gently with the help of a moist camel hair brush and kept in the Petri dishes for the study of incubation period and viability of the eggs.

2.2 Egg

Ten pairs of newly emerged males and females were released individually into a separate specimen jars containing fresh twig of lentil for egg laying. For recording, the pre-oviposition, oviposition and post-oviposition periods. The twigs were replaced daily. The cotton swab was dipped in 5 per cent honey solution, squeezed and kept in hanging position inside each specimen jar which serve as food for the moths. The swabs were replaced daily. The pre-oviposition period was recorded by counting the number of days between the emergence of an adult female and the start of egg laying. Oviposition period was recorded by observing the duration between the laying of first to the last egg. Post-oviposition period was recorded by counting number of days between last egg laying till the death of female. Further, the females, which were kept to record the oviposition period were further observed for finding out total fecundity. Fecundity means the egg laying capacity of female. Ten females were taken for recording the total number of eggs laid after mating. From the twigs used for fecundity study, the leaves having eggs were plucked and the number of eggs on these leaves were counted. The lentil twig with counted number of eggs (10) was placed on a blotting paper in a Petri dish. To the petiole of this leaf, a moist cotton swab was

wrapped which maintained the required humidity for hatching and also prevented the drying up of the eggs and the leaf. Hatching of the eggs were recorded on these eggs kept in five such replicates after every 24 hrs. The number of eggs hatched per day were counted, converted into per cent hatchability of eggs and continued till the last hatching. Average incubation period was calculated from these eggs batches.

2.3 Larva

To study the duration and number of larval instars, 15 newly hatched larvae were taken and were placed in a separate specimen tube individually. The lentil twig with pods was supplied as food for developing larvae. To find out duration of different larval instars, the left over exuviae were recorded daily using stereoscopic binocular microscope and for rest of the instars, the exuviae were observed either with hand lens or naked eyes.

2.4 Pupa

For the determination of pupal period, 5 groups (replications) of pupae (each group containing 10 pupae) were kept in separate Petri dishes and observed daily for adult emergence. Average pupal period was worked out on the basis of days taken by the adults to emerge out of pupae and converted into percentage

2.5 Adult

Twenty newly emerged moths were paired (male and female) and each pair was kept in a specimen jar where cotton swab soaked in 5% honey solution was placed. The lentl twig with pods were also supplied as food for adults. The adults were observed and their mortality was recorded daily. Data thus obtained was converted into adult longevity. For determining the sex ratio, 5 batches of pupae were observed, each batch containing 15 pupae. These batches were observed for adult emergence. Adult emerged were identified by killing them in killing bottle and differentiated into males and females on the basis of morphological features. Mating time of adults was also observed for 10 pairs of the insects. Each pair was kept in a specimen jar where cotton swab soaked in 5% honey solution and plant twig with pods were supplied as food for adults. Mating period was recorded by observing the duration of mating. Total life cycle of *Etiella zinckenella* was recorded by observing time duration between egg deposition to adult emergence. These observations were taken in 10 replications.

2.6 Morphometrics

Length and width of different stages were measured in the first generation, with the help of ocular and stage micrometer by observing 10 individuals of each stage viz egg, first to fifth instar, pupa and adult (male & female) under stereoscopic zoom binocular microscope.

2.7 Statistical Analysis

The data collected during present studies were statistically analyzed. Data on various biological parameters of *Etiella zinckenella* were subjected to statistical analysis by calculating mean value and Standard deviation.

3. RESULTS AND DISCUSSION

On perusal of data presented in Table 1, biological parameters were studied which are given as follows:

3.1 Pre-oviposition, Oviposition and Post-oviposition Period

Results presented in Table 1 indicate that the pre-oviposition, oviposition and post-oviposition period ranged from 1 to 2 days (average 1.3 days), 2 to 3 days (average 2.3 days) and 1 to 2 days (average 1.4 days), respectively. Several authors several authors [7,8] reported that pre-oviposition period ranged from 2 to 5 and 1 to 9 days, respectively hence support the present investigations. Jaglan and Sucheta [9] reported that the oviposition and post oviposition periods varied from 2-3 days and 1-2 days, respectively on lentil which are in conformity with the present investigation.

3.2 Fecundity and Adult Longevity of *Etiella zinckenella*

Observation taken on eggs showed that, eggs were laid on muslin cloth, paper strips as well as on the plant parts supplied for food in the battery jars. Mostly eggs were laid singly on pods flowers, calyx and leaves of lentil. The freshly laid eggs of lentil pod borer were oval in shape and whitish in colour which changed to orange before hatching. A single female laid 46 to 65 eggs (average 56.3eggs) during her total life time. None of the females deposited any egg on the first day of emergence, i.e all females started

Table 1. Developmental period of different life stages of *Etiella zinckenella*

Sl. no.	Parameters observed		Range	Mean±S.D
1.	Pre-oviposition period (days)[1]		1-2	1.3 ± 0.5
2.	Oviposition period (days)[1]		2-3	2.3 ± 0.5
3.	Post oviposition period (days)[1]		1-2	1.4 ± 0.5
4.	Fecundity[2]		46-65	56.3 ± 6.2
5.	Incubation Period (days)[3]		5.18-5.38	5.24 ± 0.09
6.	Percent hatchability (days)[3]		74-92	81.6±6.8
7.	Larval instar period (days)[4]			
	1st instar		1-2	1.53 ± 0.5
	2nd instar		2-3	2.6 ± 0.5
	3rd instar		3-4	3.5 ± 0.4
	4th instar		3-4	3.5 ± 0.4
	5th instar		5-6	5.5 ± 0.5
7.	Total larval period (days)[4]		15-19	16.9 ± 1
8.	Total pupal period (days)[5]		12.7-13.9	13.38 ± 0.7
9	Adult emergence		80-100	88±8.4
9.	Adult longevity (days)[5]			
	Under Laboratory conditions	Female	5-6	5.6 ± 0.5
		Male	3-5	4.1 ± 0.7
10.	Sex- ratio (Male : Female)[5]		1.0:(1.0-1.7)	1.0 : 1.4
11.	Mating period (mins)[2]		43-63	51.5 ± 9.9
14.	Total life cycle (days)[2]		37-45	40.9 ± 2.2

Table 2. Sex ratio of *Etiella zinckenella*

Pupae observed*	Total no. of adults emerged	Total no. of males	Total no. of females	Per cent males	Per cent females	Sex Ratio
Mean ± S.D	44	25	19	56.7 ± 4.3	43.3 ± 4.3	1.0: 1.4

Table 3. Morphometrics of different developmental stages of *Etiella zinckenella* fed on lentil

Sr. no.	Number observed	Developmental stage	Average length (mm)	Range	Average width (mm)	Range
1.	10	Egg	0.51±0.04	0.47-0.57	0.35±0.03	0.30-0.40
Larva						
2.	10	1st instar	0.89±0.03	0.85-0.95	0.15±0.02	0.12-0.17
	10	2nd instar	2.83±0.12	2.67-2.98	0.78±0.05	0.70-0.87
	10	3rd instar	6.22±0.09	6.14-6.33	1.49±0.05	1.43-1.56
	10	4th instar	11.29±0.03	11.24-11.34	2.21±0.04	2.14-2.25
	10	5th instar	15.33±0.02	15.28-15.38	2.89±0.03	2.84-2.92
Pupa						
3.	10	Female pupa	8.74±0.11	8.61-8.97	2.61±0.10	2.51-2.84
	10	Male pupa	8.80±0.23	8.72-9.14	2.67±0.13	2.48-2.77
Adult						
4.	10	Female	11.34± 0.18	10.78-11.48	21.13±0.26	20.79-21.23
	10	Male	12.20± 0.16	11.56-12.52	22.09± 0.29	21.92-22.29

Table 4. Head capsule width and pupal weight of *Etiella zinckenella* fed on lentil

Sr. no.	Insect stage	Number observed	Mean±SD	Range
Larvae*				
1	1st instar	10	0.10±0.02	0.08-0.12
2	2nd instar	10	0.50±0.04	0.45-0.53
3	3rd instar	10	0.82±0.06	0.77-0.89
4	4th instar	10	0.94±0.03	0.89-0.98
5	5th instar	10	1.20±0.04	1.17-1.23
Pupae**				
6	Male pupae	10	0.042±0.01	0.036-0.051
7	Female pupae	10	0.040±0.01	0.037-0.044

**Width of Head capsule in mm. **Weight of Pupae in grams*

ovipositing one day after emergence. Abul-Nasr and Awadalla [7] reported 47 to 178 eggs per female which are in conformity with this investigation. The data pertaining to adult longevity of *Etiella zinckenella* revealed that longevity of male moths varied from 3 to 5 days (average 4.1 days) and longevity of female moths varied from 5 to 6 days (average 5.6 days). Hence males are short lived than females. Naito et al. [10] reported the adult longevity at 30^0C temperature as 5.7 days which supports the present investigation. Singh and Dhooria [4] also reported the longevity of adults 5.2 days on lentil and 7.42 days on pea, respectively which is in close agreement with present investigation.

3.3 Incubation Period and Hatchability of Eggs

The incubation period was counted as the duration in days after egg laying till their hatching. For finding out the incubation period five batches, having 50 eggs were observed. Average incubation period was calculated and per cent hatchability was calculated on the basis of the number of eggs hatched successfully into first instar larvae. It is evident from table that incubation period ranged from 5.18 to 5.38 days (average 5.24 days) These studies are in agreement with the findings of several authors [11,4,12] also observed incubation period of 4 to 6 days, 5.3 days and 3 to 16 days, respectively. Data pertaining to per cent hatchability varied from 74 to 92 days (average 81.6 days) and these studies are in agreement to the findings of Singh and Dhooria [4] who reported that egg viability on an average 92.8 per cent. Several authors [13,14] who observed egg hatchability of 69 and 70 per cent, respectively also give partial support to the present investigation.

3.4 Larval Period

Observations on duration, number of instars and total larval period were recorded on 15 freshly hatched larvae. The data revealed that the larvae passed through five instars before entering pupal stage. Newly hatched larva was tiny, yellowish in colour with with black shinning head. First instar larvae lasted for 1 to 2 days (average 1.53 days) and second instar larvae were creamish to brownish in colour and blackish head, which remained for 2 to 3 days (average 2.6 days) to become third instar. The third instar larvae were much longer than preceding instars and took 3 to 4days (average3.5 days). Fourth instar was having yellowish head with black spots on vertex and lasted for 3 to 4 days (average 3.7 days). Fifth instar was pink in colour with yellowish head. Each thoracic leg of larva was five segmented and the abdominal prolegs were present on third to sixth and tenth abdominal segments and it remained for 5 to 6 days (average 5.5 days). The total larval period ranged from 15 to 19 days (average 16.9 days). The first instar completed its stage comparatively in shorter period than rest of the instars. Morphological features investigated in the present studies are in corborration with the findings of several authors [11,7,15] who reported larval period of *E. zinckenella* on different host plants as 11, 10 to 17, and 15 to 23 days, respectively.

3.5 Pupal Period

Pupation took place on glass jars, soil, muslin cloth and tissue paper in each of five observations, ten individuals were studied and pupal periods were calculated. Based on morphological characters, it was found that pupa were dark brown in colour with six hook shaped fine spines at the posterior end. Sexes were differentiated at pupal stage on the basis of differences in slit distance .Observations revealed that average pre-pupal period ranged from 1.9 to 2.3 days (average 2.04 days) and data in table revealed that average pupal period varied from 12.7 days to 13.9 days (average 13.38 days). Observations on pre-pupal period coincides with several authors [7,4] who observed that pre-pupal period was 2 days and 2 to 4 days, respectively and data in table revealed that average pupal period varied from 12.7 days to 13.9 days (average 13.38 days) which was in confirmation with [15] who reported that pupal period ranged from 10 to 13 days in March under laboratory conditions.

3.6 Percent Adult Emergence

The per cent adult emergence ranged from 80 to 100 per cent (average 88 per cent). The present studies are in agreement with the findings of Abul-Nasr and Awadalla [7] who reported that per cent adult emergence was 50 to 100 per cent. Singh and Dhooria [4] also recorded 79.17 per cent adult emergence on lentil and 100 per cent on pea also support the present investigation.

3.7 Sex Ratio and Mating Period

The male and female moths emerged from the pupae were segregated on the basis of structure of antennae and shape of abdomen. It is evident from the table that males outnumbered the females. The average percentage of males and females was 56.7and 43.3, respectively. Average female to male ratio was 1.0 to 1.4. The present investigation derive support from Abul-Nasr and Awadalla [7] who also observed that the males outnumbered the females as they constituted 53 per cent of population. Singh and Dhooria [4] also observed 54.4 per cent of emerged moths as males and support the present investigation. The mating occurred only at night or in dark places or early hours in the morning. These studies were carried with 10 pairs of adults. The data presented in table indicate that mating period of *Etiella zinckenella* varied from 43 to 63 minutes (average 51.5 minutes). It was observed that that most of the adults paired on first night after emergence and remaining did on second night. The unmated females did not lay any egg. The insects were found copulating in captivity. The present studies are in agreement with the studies of Abul-Nasr and Awadalla [7].

3.8 Total Life Cycle

Results given in table showed that the life cycle of this insect ranged from 37 to 45 days (average 40.9 days). Singh and Dhooria [4] observed that the *E. zinckenella* completes its life cycle in average (45.84 days), which is in close agreement with the present investigation.

3.9 Morphometrics of Various Developmental Stages

3.9.1 Egg

The freshly laid eggs of *E. zinckenella* were whitish in colour which changed to orange colour before hatching. They were oval in shape. Length of egg ranged from 0.47 to 0.57 mm (average 0.51 mm) and width of egg ranged from 0.30 to 0.40 mm (average 0.35 mm). Abul-Nasr

and Awadalla [7] observed the size of egg as 0.6 to 0.7 mm in length and 0.30 to 0.45 mm in width while Singh and Dhooria [4] reported average length and width as 0.51 mm and 0.34 mm, respectively. So, the present findings are in agreement with those of above authors.

3.9.2 Larva

First instar larva was yellowish in colour with black shining head. First instar larvae when reared on lentil pods ranged from 0.85 to 0.95 mm in length (average 0.89 mm) and 0.12 to 0.17 mm in width (average 0.15 mm).

Second and third larval instars were creamish to brownish in colour with blackish head. Second and third instars ranged from 2.67 to 2.98 mm (average 2.83 mm) and 6.14 to 6.33 mm (average 6.22 mm) in length and 0.70 to 0.87 mm(average 0.78 mm) and 1.43 to 1.56 mm(average 1.49 mm) in width, respectively. Fourth larval instar was having yellowish head with black spots on vertex. Forth larval instar ranged from 11.24 to 11.34 mm (average 11.29 mm) in length and 2.14 to 2.25 mm (average 2.21 mm) in width. The fifth instar was dark pink in colour with yellowish head. Each thoracic leg of larva was five segmented and abdominal prolegs were present on third to sixth and tenth abdominal segments. One pair of thoracic spiracles were located on lateral sides from first to eighth segments. Fifth instar ranged from 15.28 to 15.38 mm (average 15.33 mm) in length and 2.84 to 2.92 mm (average 2.89 mm) in width. Head capsule width of 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} instar when reared on variety Garima ranged 0.08 to 0.12 mm(average 0.10 mm), 0.45 to 0.53mm (average 0.50 mm), 0.77to0.89 mm (average 0.82 mm), 0.89 to 0.98 mm (average 0.94 mm) and 1.17 to 1.23 mm (average 1.20 mm), respectively. The present findings on size of instars are in accordance with that of Singh and Dhooria [4] who reported first instar was on an average 0.87 mm in length and 0.13 mm in width whereas, full grown larva measured 15.25 mm in length and 2.93 mm in width. Head capsule width of 1^{st}, 2^{nd}, 3^{rd}, 4^{th} and 5^{th} instar when reared on lentil variety Garima ranged 0.08 to 0.12 mm (average 0.10 mm), 0.45 to 0.53 mm (average 0.50 mm), 0.77 to 0.89 mm (average 0.82 mm), 0.89 to 0.98 mm (average 0.94 mm) and 1.17 to 1.23 mm (average 1.20 mm), respectively.

3.9.3 Pupa

The pupa of *E. zinckenella* was of obtect type. The freshly formed pupa was greenish in colour which changed to brownish after 6-7 hours. Before one to two days of adult emergence, pupae became dark brown in colour. The pupa had well defined dark brown coloured spiracles situated dorsally on second to eighth abdominal segments. Six hook-shaped fine spines were present at the posterior end of the abdomen. The sexes at pupal stages were determined on the basis of distance between anal and genital opening. In male pupa, the genital opening was present on ninth abdominal segment while in female this opening was located on eighth abdominal segment. The anal opening was present on tenth abdominal segment in both the sexes. Therefore, more distance between the anal and genital opening was present in female pupae than male pupae. Length of male pupae ranged from 8.72 to 9.14 mm (average 8.80 mm) and in case of female pupae it ranged from 8.61 to 8.97 mm (average 8.74 mm). Width of male pupae ranged from 2.48 to 2.77 mm (average 2.67 mm) and in case of female pupae it ranged from 2.51 to 2.84 (average 2.61 mm). Weight of male ranged from 0.036 to 0.051 g (average 0.042 g) and weight of female pupae ranged from 0.037 to 0.044 g (average 0.040 g). The present investigations with respect to size and colour of pupa are in agreement with Singh and Dhooria [4] who reported that pupa was 8.64 mm in length and 2.79 mm in width.

3.9.4 Adult

The male and female moths were greyish in colour. The female moths were slightly smaller than male moths. The compound eyes were prominent and were bigger in males with more ocular distance than female moths. The forewings were longer and narrower than the hind wings which were hyaline and provided with long hairs at their inner margins. There was a white band along the costal margin of each forewing. The sexes in adult stages were distinguished on the basis of structure of antenna and sizes of abdomen In case of male moth, the pedicel of each antenna was broadened at the base having a projection at its inner margin and in case of female the antennal projection was absent. The abdomen of male moth was lesser in width and and provided with yellowish irregular tufts of hairs at anal end. In female moth, the abdomen was more wider than that of male and with tufts of yellowish hairs of regular length at the posterior end. The male moths ranged 11.56 to 12.52 mm (average 12.20 mm) in length and 21.92 to 22.29 mm (average 22.09 mm) in width. The female moths ranged from 10.78 to 11.48

mm (average 11.34 mm) in length and 20.79 to21.23 mm (average 21.13 mm) in width. The present findings are in agreement with that of Singh and Dhooria [4] who observed female moth measuring 20.08 mm in wing expanse and 11.4 mm in length and a white stripe was present on the forewing along the costal margin and adults were grayish in colour and sexes were distinguished on the basis of structure of antennae.

4. CONCLUSION

Hence, the observations obtained on the nature and behaviour of *E. zinckenella* from the present study can be further incorporated in the plan of its management, at the most susceptible stage of its life cycle, with the most effective conservation of biodiversity.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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