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Effect of Altering Parent Sex Ratio on Egg Laying and Subsequent Development in *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

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Abstract.- Different male and female sex ratios were compared in the parent population of *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) to standardize its laboratory rearing and to use it as fictitious host of beneficial insects. Egg laying and percent egg hatching was decreased significantly with the increase in number of males to one female as compared to pairing of one male and one female (control). The highest decrease in fecundity was 20% and in hatching 7% when five males were paired with one female. On the other side, egg laying and hatching was significantly decreased when female number was increased in pairing to one male. In 1:5 (male: female) egg laying per female and hatching percentage was decreased to 61% and 9.8% respectively, over control. There was a significant increase in egg laying and percent egg hatching when males and females were increased in equal ratio in pairing as compared to control. At 150: 150 ratio (male: female) egg laying was 2.5 fold over control, whereas percent hatching was 90% and at par to control. Changes in developmental parameters as adult's longevity, larval and pupal periods and F_1 male to female ratio were non-significant in all treated sex ratios.

Key words: *Plodia interpunctella*, pairing ratio, development egg laying, Indian meal moth.

INTRODUCTION

Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) has cosmopolitan habitat and consider as a common household pest of stored grains and value-added food products (Cox and Bell, 1991; Na and Ryoo, 2000; Sauer and Shelton, 2002). Many research scientists have studied different strains of *P. interpunctella* on variable diameters such as reproduction, development, biology and effect of different ratios of male and female adults on maintaining the colony of different household insect pests at different localities in the world (Savov, 1973; Brower, 1975; Hoppe, 1981; Proshold *et al*., 1982; Ellis and Steele, 1982; Henneberry and Clayton, 1984, 1985; Proshold, 1996; Fadamiro and Baker, 1999; Huang and Subramanyam, 2003).

Healthy female of *P. interpunctella* lays up to 350 oval shaped whitish eggs on surface of grains singly or in clusters with incubation period ranges is 2-4 days. Newly hatched larvae feed on fine material of the grain and their colour vary from off-white to pink, yellowish green or brownish, depending upon the nature of diet (Johnson and Wofford, 1991; Johnson *et al*., 1992; Rees, 2004). Larval stages are pest or feeding stages, appear in grains a wide variety of food such as grain products, beans, nuts, cereals, dried fruits, vegetables, dog food, candy, dried milk and many other food stuffs. Larvae quite often migrate a considerable distance from their food source while searching for a pupation site and are found on counter tops, walls and ceilings (Hamed *et al*., 2010). Having five larval instars, from 4^{th}-instar larvae of Indian meal moth starts spin a web and leave behind silken threads wherever they crawl (Allotey and Goswami, 1990). The webbing was visible and is often sufficiently abundant to attract attention (Nansen and Philips, 2003). Loosely clinging webbing on the grain is the characteristic of this pest (Arbogast, 2007). Last instar larvae spin a silken web within 3-5 days and pupate in that cocoon, as they mature it changes colour from light-brown to dark-brown pupae. After 6-10 days adult moths emerge out from cocoon and within an hour these fresh newly emerged adults are ready to search their mate. Adult female can lay several dozen to several hundred eggs and again life cycle will start. Throughout the

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world different products have been reported to be infested by *P. interpunctella*, damage directly effect on quality of stored food products (Johnson *et al*., 1992, 1995; Nansen *et al*., 2004). Loss by infestation not only affect on product it also decrease the indirect economic cost. When infestations are heavy there can be three to seven generations per year or more, depending on availability of food and favourable temperature.

A harmful and lethal effect of pesticides on natural enemies has decreased their populations in the fields (Trdan *et al*., 2012; Laznik and Trdan, 2014). To maintain the population of beneficial insects/ predators in the field, augmentative and inundative releases from mass reared laboratory population is imperative (Sattar and Abro, 2011). In controlled conditions mass rearing of beneficial insects needs an artificial diet because it is difficult to provide natural host throughout the year (Sattar *et al*., 2007). So for the survival, growth, development and reproduction of beneficial insects, diet requirement can be meet from the eggs of different insects such as *P. interounctella*. Present work is the contribution in biology study of *P. interounctella* potential of oviposition, developments with the objectives to study rate of multiplication in different and equal ratios of adults on fecundity and adults' emergence to get maximum population.

MATERIALS AND METHODS

Culture of *P. interpunctella*, was maintained on a standard artificial diet in biological control laboratories of NIAB under controlled temperature (25±2˚C), humidity (65±5% RH) and 14 h light: 10 h dark conditions. Experiment was conducted to study the effect of different and equal ratios of *P. interpunctella* moth on its egg laying and other developmental parameters. Newly emerged adults of age 1-2 hrs were used in the experiment. One to five freshly emerged males were allowed to mate with same age virgin female and vice versa. Similarly, equal number of males and females in ratios moths 1 to 5, 50, 100, 150 and in multiple pairs were allowed to mate oviposition and further development. Each treatment was replicated 10 times.

Caging of adults

Newly emerged moths of *P. interpunctella* were kept in glass jars (10x2.5 cm) and offered with droplets of 10% honey solution (Honey: Islamic Shahad Distributors®, Pakistan. Glass jars were covered with white nylon net of 30 meshes and tighten with rubber bands. Large size glass jars (12x5 mm) were used to pair equal ratios in treatments 50, 100, 150 and multiple numbers.

Collection of eggs

Glass jars were checked daily to count the eggs laid by females. For this eggs were shake slightly by top to downward on coloured paper to pass through the mesh and were collected with the help of fine camel hair brush on coloured paper and were counted under the stereo microscope (AmScope). Counted eggs were placed daily in covered glass Petri dishes (2.5cm x 4.5cm) for hatching and record of incubation period.

Larval feeding

Newly hatched small tiny larvae visible to the naked eye were shifted to glass jars (10×5 mm) having 30g standardized larval rearing diet: Wheat brain 100g, honey 25ml, L-Ascorbic acid [AnalaR 500g BDH laboratory supplies] 0.75g, methylparaben (Methyl 4-hydroxybenzoate, Nipagin) 0.75g and yeast (Saf-instant® S.I. Lesaffre) 20g. Corrugated paper spools were placed above the diet in each jar for pupation sites of wandering larvae.

Pupal collection

The pupae inside the cocoons were collected with the help of fine tip forceps and separated into males and females by observing characters described by Butt and Cantu (1962). Male and female pupae were placed in separate glass Petri dishes and were checked twice daily for record of percent pupal survival.

Adults emergence

Newly emerged virgin moths were recorded daily sex wise to observe male to female ratio. Virgin moths (males and females) were paired to determine their percent longevity; number of eggs laid and eggs viability.

Data record and statistical analysis

Data were recorded every 24 h in each replicate of all treatments by carefully examining the jars and Petri dishes. The data on the number of eggs laid, percent eggs hatching, percent adults' emergence and sex ratio were subjected to one-way analysis of variance using DMR test (Steel and Torrie, 1980) to determine the treatments differences.

RESULTS AND DISCUSSION

Results showed that eggs laid by *P. interpunctella* in all treatments of (Table I) were significantly different (*F*= 118.214, df= 8, *P*= 0.00). The highest number of eggs was recorded in 1: 3 male to female ratio (411.3 eggs) and 1: 2 (400.5 eggs). Whereas, in equal ratio ***i.e.***, 1:1 (male: female) the maximum number of eggs were 198.80 per female. Allotey and Goswami (1990) and Arbogast (2007) studied the effect the biology of this pest and recorded the percent fecundity and survival of different life stages at similar temperature and diet conditions. Significant difference was observed in hatching percentage of eggs (*F*= 10.35, df= 8, *P*= 0.00). The highest hatching (88.90%) was achieved in 1:1 ratio followed by 88.7 in 1:2 and 87.5 in 1:3 ratio and the lowest (82.26%) in 4:1 (male: females), respectively. There is no significant difference in percent adults emergence (*F*= 0.8910, df=8, *P*= 0.5280). However, the maximum and minimum percent emergence was recorded in treatments 1:1 (80.80) and 1:5 (76.70) male to female ratio.

Results on the basis of equal sex ratio and by increasing the adults from minimum to maximum, showed that the fecundity, hatching, survival and all developmental parameters are extremely dependent on their number and pairing time Table II. Fecundity (*F*= 3538.41, df=8, *P*= 0.00), hatching percent (*F*= 11.18, df= 8, *P*= 0.00) and percent adults emergence (*F*= 9.22, df= 8, *P*= 0.00), as number of adults increased from 2 to 5 fold by increasing the numbers of pairs. Consistent results were reported by Johnson *et al*. (1992, 1995). By increasing number of adults the fecundity increased and in biology study number of adults increase it is also possible to conclude that the results achieved with increasing number of adults where food play an important role Nansen *et al*. (2004). A more promising approach is to classify the food types based on different criteria however, biological parameters do not extensively vary when reared (Nansen and Phillips, 2003).

Data on developmental time and mortality of *P. interpunctella* is lacking in literature, estimation of development time, survival, temperatures and mortality could help in modelling of lifecycle. Fadamiro and Baker (1999) and Ellis and Steele (1982) evaluated that fecundity was highest when adult moths were in same number and age whereas, Henneberry and Clayton (1984, 1985) studied that the fecundity and egg viability were significantly and positively correlated with the number of females.

Pairing time of adult moths play vital role in ovipositoion as shown in Table I. As number of males increases pairing time decreases which affect on fecundity per female. Huang and Subramanyam, 2003 reported that reproductive performance of *P. interpunctella*, fecundity and egg viability were significantly and positively correlated with the number of spermatophores/female and egg viability decreased by 22% per day only when females were delayed from mating. Hamed *et al*. (2010) investigated that males continued to produce progeny up to age of 3 days when they were mated with freshly emerged females. Almost the similar males to females ratio numbering 1.17:1.00 and 1.18:1.00 was achieved from treatments having 1and 2 days old males mated with newly emerged females.

When number of females increases with single male of the same age the pairing time recorded with two female adults in increasing numbers (Table I). The maximum time 18.10 minutes in two adult females, with one adult female whereas in same treatment maximum time 16.50 minutes with the second adult female. However, in other treatments with increasing number of females there is no pairing time was recorded with third fourth and fifth adult females. Our results are in the line to the results reported by Brower (1975) that the number of times a female mated was not related to the number of eggs laid. However, the treatment in which single male with increasing number of

Table I.- **Effect of adults pairing in different ratio and in equal ratio on fecundity and emerging sex-ratio of *Plodia interpunctella.***

Male: Female	Pairing time (minutes)	Total No. of eggs laid	Hatching (%)	Adult recovery (%)	Progeny Sex-ratio	
					Male	Female
Adult pairing in different ratio						
1: 1	16.5	198.80±0.25 b	88.90±0.22 a	80.80±0.20 a	1.11	1.00
2: 1	15.3	175.20±0.47 bc	86.00±0.23 b	78.20±0.11 ab	1.12	1.00
3: 1	15.5	177.80±0.38 bc	83.00±0.16 c	77.50±0.26 ab	1.17	1.00
4: 1	14.0	157.70±0.39 c	82.30±0.09 c	78.20±0.08 ab	1.21	1.00
5: 1	13.5	159.50±0.31 c	82.70±0.13c	78.00±0.16 ab	1.19	1.00
1: 2	18.1, 16.6	400.50±0.31 a	88.70±0.21 ab	78.80±0.17 ab	1.09	1.00
1: 3	16.2, 16.0, 0	411.30±0.03 a	87.50±0.17ab	78.00±0.14 ab	1.12	1.00
1: 4	15.7, 14.8, 0, 0	393.60±0.41 a	82.30±0.21c	77.70±0.12 ab	1.19	1.00
1: 5	15.8, 13.5, 0 ,0, 0	382.90±0.09 a	81.20±0.23c	76.70±0.18 b	1.26	1.00
Adult pairing in equal ratio						
1: 1		202.40±0.21 e	88.90±0.28 a	80.80±0.24 a	1.11	1.00
2: 2		388.50±0.22 e	90.00±0.22 a	75.50±0.16 bc	1.19	1.00
3: 3		962.00±0.22e	83.80±0.48 b	74.10±0.14 bc	1.21	1.00
4: 4		1281.40±0.21 e	80.30±0.30 b	67.30±0.14 d	1.22	1.00
5: 5		1666.80±0.41 e	82.50±0.24 b	72.20±0.13 c	1.16	1.00
50: 50		32050.00±0.14 d	89.60±0.18 a	78.90±0.27 ab	1.14	1.00
100: 100		50800.00±0.36 c	89.40±0.16 a	80.30±0.17 a	1.12	1.00
150: 150		76750.00±0.21 b	89.70±0.25 a	80.50±0.18 a	1.14	1.00
Multiple pairing		142580.00±0.00 a	92.50±0.36 a	81.90±0.15 a	1.24	1.00

Means (±SE, n=10) with similar alphabets in a column are statistically similar at P < 0.05.

females' direct effect the efficiency of ovipositor as the result more fecundity rate was recorded. The increased egg production in multiple pairing may be due to the transfer of nutrient secretions, in organic ions, pigments, proteins, juvenile hormones and ecdysteroids by males to females during copulation (Benz, 1969; Henneberry and Clayton, 1984; Park *et al*., 1998). Hamed *et al*. (2010) concluded that survival to adult decreased with the delay in age for mating of both sexes. Fecundity by *P. interpunctella* was highest when there was no mating delay for females and it was reduced where mating was delayed for females only. In first part, it is noted that increasing number of males with single female effect the pairing time and compete with other adult males, in result less number of eggs was recorded. Whereas, in the second part of study, where adults in bulk it is too difficult to note the actual pairing time of adults. Therefore, oviposition in *P. interpunctella* appears to be stimulated directly or indirectly by the transform of sperm by male to female (Norris, 1933). Hence, the results suggest that number of adults disrupt pairing may be effective behavioural strategies for managing this important pest.

These investigations will be useful to save commodities, stored goods and reduce consumer complaints as well as study is valuable for mass culturing of other beneficial insects in laboratories (Lum and Arbogast, 1980). Developing colony of *P. interpunctella*, as fictitious host, eggs are utilize as a source of food of other beneficial insects in different experiments. To minimize the negative and harmful effects of pesticides on natural enemies it is necessary to increase the population of biological control agents.

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