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Do Egg Pods in the Desert Locust *Schistocerca gregaria* Display as Oogenesis Limiting Factor? 1-The Effect of Egg Pod Extracts on Reproductive Performance

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Abstract: The egg pod of *Schistocerca gregaria* as other *Acrididae* is consisted of two essential parts, the eggs and the froth plug above the buried egg mass. Froth and egg mass extracts were obtained by single solvent extractions. Solitary and gregarious ovipositing females were exposed to contaminated ovipositing sand cups or treated antennae with froth and egg extracts. The mean number of egg pods per female and the mean number of egg per pod were significantly reduced in all treatment by contaminated sand or antennae in both solitary and gregary females, by froth and egg extracts. The fecundity and fertility are remarkably reduced in all treated females compared with control. The ethanol solvent caused higher reduction in fertility percentages than hexane extracts in case of contaminated sand. The hatched nymphs per female were significantly diminished by treatments in all cases. Furthermore, the reproductive potential were lower than control in all treatments and the decrease ranged from 42.1 to 97.4%.

Key words: Egg pods, *Schistocerca gregaria*, aggregative pheromone, reproductive potential

INTRODUCTION

The conception of phase polymorphism in the migratory locust, *Locusta migratoria* and *Locusta pardulina* was put forward by Uvarov, in 1921, later it was extended to *S. gregaria*^[1,2]. It says that the morphological, physiological and behavioral differences between solitary and gregary locusts evolve by a gradual transition of a population from one phase to the other, elicited by external factors^[1-5]. In nature, some phase characteristic can change within hours whereas others need several generations^[6]. Several mediating factors have been implicated in the phase dynamic of *S. gregaria* including visual^[10], tactile^[7-9], chemical^[11,12], dietary^[13] and the previous phase history of the locust^[14].

Pheromone-mediated aggregation serves to bring together insects for protection, reproduction and feeding^[15]. Nolte and Ciworkers^[17] traced the source of pheromone in *S. gregaria* and *Locusta migratoria* to hopper faeces. Fuzeau-Braesch *et al.*^[16] analyzed samples of airborne volatiles collected from the cages of *S. gregaria* and *L. migratoria* in their gregarious phase and identified three compounds. Bioassays showed the mixture of three compounds elicited clumping behavior in both species but no attraction. They were thus referred to as cohesion pheromone. Obeng-ofori *et al.*^[18] showed

that there were two sets of releaser juvenile pheromone systems one specific to nymphs and other for adult stages.

MATERIALS AND METHODS

Stock colony and rearing conditions: The stock colony of *S. gregaria* was initiated using progenitors of wild strains indigenous to Aswan, Egypt. The insects have been reared and handled to satisfy the crowded breeding conditions described by Hunter-Jones^[19]. Newly-hatched hoppers were captured in wooden cages with wire-gauze sides (60x60x70 cm) at a rate of hundred hatching/cage. The bottom was furnished with sand layer of 20 cm depth and the leaves of the leguminous plant, *Sesbania aegyptiaca* were daily provided as feeding material in summer and *Alexandrianum trifolium* in winter. The cages are equipped inside with electric bulb in order to maintain an ambient temperature of 32±2°C and 30-50% RH.

Experimental insects

Gregarious-maintaining conditions: Experimental gregarious locusts were segregated from the general stock colony at the beginning of the first instar and held up in groups, each of 15 hoppers per cage. The cages are wooden-framed cube of 30 cm side, equipped with a zinc

bottom covered with thin layer of sand, glass-covered sides and a wire-gauze top provided with a little door. All cages were incubated at $32\pm 2^{\circ}\text{C}$ and $65\pm 5\%$ RH. Unconsummated food, dead locust and faeces were removed daily. The whole cage had to be thoroughly washed and effectively sterilized with an antiseptic agent every 4-6 weeks or whenever it became empty after terminating any experiment.

Solitarious-maintaining conditions: An egg pod from the gregarious stock colony was isolated and kept aside till hatching. Newly-hatched hoppers were distributed individually in cylindrical glasses and kept away from each other to prevent visual and tactile contact till emergence of the first generation of the transient adults. All glasses were incubated and maintained under the previous conditions of the gregarious colony in a separate adjusted temperature room. The isolation of the hatching of the second solitarious generation was repeated to obtain the second generation of adult. This operation was repeated again and the resulting nymphs and adults were kept under isolation condition for more than 3 years. Experimentation and all observations were only undertaken whenever the generally-known solitary morphogenetic characteristics.

Preparation of froth and egg extracts

Collection of egg pods: The ovipositing 8 days females were segregated from solitarious and gregarious colonies before the appearance of yellowing of their hind wings. Each female was confined with one male in small cage (20x20x20 cm) equipped with ovipositor cup. The sand for oviposition was sieved using wire mesh (2 mm²) and washed successively with hexane, ethyl acetate, methanol and finally with distilled water. It was then dried and heat sterilized by backing in an oven at 150°C for 24 h. Sterilized sand was moistened by adding 15 ml water 100 g⁻¹ of sand^[20]. The ovipositing cups were filled with the moist sand and offered to the ovipositing females. The fresh egg pods (one day after oviposition) were collected for froth and eggs extraction processes.

Extraction of froth and eggs: Froth and egg extracts were obtained by single solvent extractions^[20]. For single solvent extraction, eggs and froth derived from egg pods were allowed to dry at ambient temperature ($25-27^{\circ}\text{C}$) for 6 h. Each part was then placed in a dropping funnel (150 ml) and 6 ml of each solvent of hexane and ethanol were added. The solvents were evaporated and the sediments were dissolved in 6 ml of saline solution and used directly to contaminate the sterilized sand in the ovipositing cups. Amounts of extracts, corresponding to 2 pod equivalents were tested individually.

Ovipositing bioassays: The present study was carried out to follow up the effect of hexane and ethanol extracts of the two parts of egg pods (froth and eggs) on the reproductive potential and other related parameters of the ovipositing females.

The experimental work was designed out as follows:

- Exposing of solitarious ovipositing females to contaminated ovipositing sand cups with froth and egg extracts of the solitarious egg pods, respectively.
- Exposing of gregarious ovipositing females to contaminated ovipositing sand cups with froth and egg extracts of the solitarious egg pods, respectively.
- Exposing of gregarious ovipositing females to contaminated ovipositing sand cups with froth and egg extracts of their own egg pods, respectively.
- Exposing of solitarious ovipositing females to contaminated ovipositing sand cups with froth and egg extracts of gregarious egg pods, respectively.

The experiments were repeated as follows:

- 1st During the preoviposition and oviposition period (after first egg pod).
- 2nd Dipping the antennae of the ovipositing females in the extracts during the preoviposit on period.

Criteria used for evaluation

Reproductive performance of the ovipositing adults: Eight days old ovipositing gregary or solitary females were paired with 8 days old males from the same phase from the experimental gregarious or solitarious original culture and confined on contaminated ovipositing sand cups according to the previous design.

The number of egg pods per female, eggs per pod, hatched nymphs per pod, the period between two layings, the incubation period, mean number of ovarioles per ovary, fecundity and fertility percentages were calculated and recorded for each extract phase. The following parameters were determined according to Elsayed^[21]

Fecundity = Total number of eggs per females

Ovariole yield % = $\frac{\text{No. of eggs per pod}}{\text{No. of ovarioles per females}} \times 100$

Fertility % = $\frac{\text{No. of hatched eggs per pod}}{\text{No. of deposited eggs per pod}} \times 100$

Decrease in R.P.% = $\frac{100-(a \times b \times 100)}{A \times B}$, Where

A = mean egg hatch/control B = mean egg laid/control
a = mean egg hatch/treated b = mean egg laid/treated

$$\text{Phase dependent \%} = \frac{a}{(a+b)} \times 100 \quad \text{or} \quad \frac{b}{(a+b)} \times 100$$

a = main total treatment of solitary b = main total treatment of gregary

$$\text{Percentage of reduction from control} = \frac{(a-b)}{b} \times 100$$

a = treatment b = control

Statistical analysis: All data were subjected to analysis of variance. Means were compared using least significant difference (L.S.D.) at P=0.05 by using Microsoft excel software program.

RESULTS AND DISCUSSION

Effects on females treated during the pre-oviposition period

Effects on egg pods: The number of egg pods per female was significantly reduced in all treatments by contaminating sand or antennae, in both solitary and gregary females, by both hexane or ethanol and by froth and egg extracts. The reduction was pronounced by contaminating sand than antennae. The reduction in laid egg pods was higher in solitary treated females compared with treated gregary females. The percentage of reduction reached more than 40% in 11 treatments out of 16 in gregary females (Table 1). Significant differences were not always found among means of laid egg pods by using extracts of froth or eggs from solitary or gregary, except in few cases. The extracts of froth were mostly effective than those of eggs. Similarly, the contaminated sand than contaminated antennae and by using hexane (non-polar) as solvent compared with ethanol (polar).

Effect on eggs per pod: This parameter may be considered the outward expression of the oogenesis function (Table 2). The number of eggs was significantly reduced in all cases. Here again, the effect of extracts in reducing the number of eggs per pod showed similar trends like number of pods per female. The reduction was higher in contaminated sand, eggs laid by solitary females, extract of froth and hexane solvent, as compared with contaminated antennae, eggs of gregary, extract of eggs and ethanol solvent. It may be of concern to add that the production of egg per pod like pods per female was higher in solitary females than gregary ones.

Effect on fecundity: The total eggs laid per female may be considered a concept of quantitative value expressing the limiting effect of treatments by the factors extracted from froth and eggs. Using these extracts in contaminating antennae and sand receiving the laying females showed

as previously mentioned in egg pods and eggs per pod (Table 1 and 2), the main following trends:

The percentage of reduction were pronounced and exceeded 50% in all cases and reached 86.92% in some cases (Table 3). The same trends concerning: hexane or ethanol, contaminating sand or antennae, solitary or gregary laying females, froth or egg extracts were stereotyped here again. It could be concluded that the factors extracted and used in treatment displayed a limiting action on the quantitative performance of egg production in locust ovipositing females.

Effect on egg yield per ovariole: To evaluate the response of the ovipositing females of the desert locust (Table 4). They were exposed together with males to contaminated sand and contaminated antennae with froth and egg extracts of both phases. The analyses of these results give the following trends:

The percentage of egg yield per ovariole in all treatment was remarkably lowered by the treatment of froth or egg extracts.

It may be of concern to note that the percentage egg yield per ovariole was remarkably higher in gregary phase than solitary phase in all treatments (Table 4).

It may be concluded that solitary ovipositing females were more sensitive to the treatments compared with their gregary counterparts in all the experimental cases. The percentages in reduction in egg yield per ovariole in solitary females were much higher than their counters. This may point to that solitary females have different biological characters in the physiology of egg production compared with gregary ones. The variation in reduction percentage for each treatment in solitary laying females was much more intensified compared with gregarious. For example the percentage reduction in solitary laying females ranges from 42.15 to 71.18 in hexane extract, compared with 38.65 to 55.16 in gregary (Table 4).

It is the first time in this experimental work that eggs of gregary phase surpassed solitary phase. The ovariole yield of solitary control was 88.90 and 93.90 for gregary control (Table 4).

Effect on the fertility: The treatment with foam and egg extracts lowered the fertility remarkably in all treatment compared with control. The fertility percentages ranged from 32.5 to 84.7.

The reduction percentages from control were higher in solitary ovipositing females than gregary ones. This may be explained, as previously mentioned, by the high sensitivity of solitarious than gregarious. The reduction percentages from control ranged from 12.7 to 66.74 (Table 5).

Table1: Effect on egg pods per female treated during the pre-ovipositing period

| Solitary phase | | | | | | | | |
|-------------------|--------------------------|--------------------------------------|--------------------------|--------------------------------------|--------------------------|--------------------------------------|--------------------------|--------------------------------------|
| Contaminated sand | | | | | Contaminated antennae | | | |
| Hexane | | Ethanol | | | Hexane | | Ethanol | |
| Treated phase | No. of egg pods ±S.D. | % of reduction from contamination | No. of egg pods ±S.D. | % of reduction from contamination | No. of egg pods ±S.D. | % of reduction from contamination | No. of egg pods ±S.D. | % of reduction from contamination |
| Extract of | | | | | | | | |
| Solitary froth | 3.70±0.6c | 38.33 | 4.0±0.00b | 33.33 | 3.7±1.00c | 50.00 | 3.80±0.36b | 36.66 |
| Solitary eggs | 4.25±0.6b | 29.00 | 4.0±0.00b | 33.33 | 3.0±1.00b | 50.00 | 3.33±0.6b | 44.50 |
| Gregary froth | 3.00±0.0d | 50.00 | 2.7±0.70c | 55.00 | 3.5±0.57b | 41.66 | 2.70±0.5c | 55.00 |
| Gregary eggs | 2.30±0.5e | 61.66 | 2.3±0.67c | 61.66 | 3.2±0.80b | 41.66 | 3.53±0.26b | 41.16 |
| Solitary control | 6.00±0.0a | 00.00 | 6.0±0.00a | 00.00 | 6.0±00.0a | 00.00 | 6.00±0.0a | 00.00 |
| LSD. 0.05 | 0.42 | | 0.6 | | 0.6 | | 0.63 | |
| Gregary phase | | | | | | | | |
| Solitary froth | 2.3±0.5c | 42.50 | 2.7±0.57b | 32.50 | 2.7±0.50b | 32.50 | 2.0±0.00c | 50.00 |
| Solitary eggs | 2.0±0.7c | 50.00 | 2.7±0.57b | 32.50 | 2.7±0.70b | 32.50 | 2.0±0.00c | 50.00 |
| Gregary froth | 3.0±0.0b | 25.00 | 3.5±0.60ab | 12.50 | 2.3±0.60b | 42.50 | 2.7±0.77c | 42.00 |
| Gregary eggs | 3.00±0.0b | 25.00 | 3.5±0.60ab | 12.50 | 2.3±0.40b | 42.50 | 3.3±0.57b | 42.00 |
| Gregary control | 4.0±0.0a | 00.00 | 4.0±0.0a | 00.00 | 4.0±0.00a | 00.00 | 4.0±0.00a | 00.00 |
| LSD. 0.05 | 0.6 | | 0.82 | | 0.8 | | 0.5 | |

Table 2: Effect on egg pods per female treated during the pre-ovipositing period

| Solitary phase | | | | | | | | |
|-------------------|--------------------------|--------------------------------------|--------------------------|--------------------------------------|--------------------------|--------------------------------------|--------------------------|--------------------------------------|
| Contaminated sand | | | | | Contaminated antennae | | | |
| Hexane | | Ethanol | | | Hexane | | Ethanol | |
| Treated phase | No. of egg pods ±S.D. | % of reduction from contamination | No. of egg pods ±S.D. | % of reduction from contamination | No. of egg pods ±S.D. | % of reduction from contamination | No. of egg pods ±S.D. | % of reduction from contamination |
| Extract of | | | | | | | | |
| Solitary froth | 45.7±3.6c | 51.79 | 55.4±9.7b | 41.43 | 55.70±2.3d | 41.36 | 51.13±4.7b | 36.66 |
| Solitary eggs | 43.4±3.1c | 54.21 | 43.8±1.9c | 53.69 | 60.32±7.7cd | 36.37 | 55.2±6.3b | 44.50 |
| Gregary froth | 61.7±6.3b | 34.91 | 36.4±3.4c | 61.52 | 69.15±0.5b | 27.08 | 57.6±3.6b | 55.00 |
| Gregary eggs | 30.8±2.2e | 67.51 | 43.6±1.0c | 53.91 | 63.50±1.8bc | 33.02 | 53.7±5.3b | 41.16 |
| Solitary control | 94.8±3.4a | 00.00 | 94.8±3.4a | 00.00 | 94.8±3.4a | 00.00 | 94.8±3.4a | 00.00 |
| LSD. 0.05 | 6.2 | | 7.6 | | 7.0 | | 7.5 | |
| Gregary phase | | | | | | | | |
| Solitary froth | 38.6±1.6c | 53.71 | 39.20±2.2cd | 52.99 | 61.26±8.6bc | 26.50 | 61.8±6.7c | 25.80 |
| Solitary eggs | 47.2±3.0b | 43.40 | 34.20±2.9d | 58.99 | 63.0±2.6b | 24.46 | 62.7±6.6b | 24.60 |
| Gregary froth | 50.7±1.8b | 39.20 | 52.40±8.9b | 37.17 | 54.2±3.2c | 35.09 | 58.2±3.2bc | 30.21 |
| Gregary eggs | 51.8±5.3b | 37.88 | 47.29±6.3bc | 43.29 | 55.0±4.5c | 34.05 | 53.4±7.6c | 35.97 |
| Gregary control | 83.4±2.8a | 00.00 | 83.40±2.8a | 00.00 | 00.0 | 00.00 | 83.4±2.8a | 00.00 |
| LSD. 0.05 | 8.2 | | 8.32 | | 7.6 | | 9.0 | |

Table 3: Effect on fecundity of female treated during the pre-ovipositing period

| Solitary phase | | | | | | | | |
|-------------------|--------------------|--------------------------------------|--------------------|--------------------------------------|-----------------------|--------------------------------------|--------------------|--------------------------------------|
| Contaminated sand | | | | | Contaminated antennae | | | |
| Hexane | | Ethanol | | | Hexane | | Ethanol | |
| Treated phase | Fecundity ±S.D. | % of reduction from contamination | Fecundity ±S.D. | % of reduction from contamination | Fecundity ±S.D. | % of reduction from contamination | Fecundity ±S.D. | % of reduction from contamination |
| Extract of | | | | | | | | |
| Solitary froth | 168.00±32b | 70.43 | 222.00±39b | 68.27 | 204.30±34.5b | 64.04 | 185.0±16.6b | 67.44 |
| Solitary eggs | 187.00±18b | 67.10 | 98.30±29d | 86.70 | 182.30±47.0b | 67.92 | 183.3±31.0b | 67.74 |
| Gregary froth | 187.00±45b | 67.10 | 175.00±5.0c | 69.20 | 242.00±60.0b | 57.92 | 153.7±35.0b | 72.95 |
| Gregary eggs | 74.30±35c | 86.92 | 74.30±45d | 86.92 | 223.00±42.0b | 60.75 | 177.7±21.0b | 68.72 |
| Solitary control | 568.23±45a | 00.00 | 568.23±45a | 00.00 | 568.23±45.0a | 00.00 | 568.23±45.0a | 00.00 |
| LSD. 0.05 | 57.34 | | 46.84 | | 7.60 | | 49.36 | |
| Gregary phase | | | | | | | | |
| Solitary froth | 117.7±28c | 66.25 | 105.0±37.0c | 68.27 | 158.0±33.0b | 52.26 | 121.0±15.7b | 63.44 |
| Solitary eggs | 76.70±28c | 76.82 | 90.0±13.2e | 72.81 | 167.0±35.5b | 49.54 | 125.0±13.3b | 62.14 |
| Gregary froth | 152.0±1.5b | 54.07 | 179.0±4.6b | 45.92 | 125.0±23.6b | 62.23 | 137.0±40.0b | 58.61 |
| Gregary eggs | 155.0±10.7b | 53.17 | 163.0±11.1b | 50.75 | 128.0±31.8b | 61.32 | 122.3±17.0b | 63.05 |
| Gregary control | 331.0±37a | 00.00 | 331.0±37a | 00.00 | 331.0±37.0a | 00.00 | 331.0±37.0a | 00.00 |
| LSD. 0.05 | 38.9 | | 38.0 | | 51.0 | | 42.7 | |

Table 4: Effect on egg yield per ovariole of female treated during the pre-ovipositing period

| Solitary phase | | | | | | | | |
|-------------------|------------------|-----------------------------------|------------------|-----------------------------------|-----------------------|-----------------------------------|------------------|-----------------------------------|
| Contaminated sand | | | | | Contaminated antennae | | | |
| Hexane | | Ethanol | | | Hexane | | Ethanol | |
| Treated phase | % Ovariole yield | % of reduction from contamination | % Ovariole yield | % of reduction from contamination | % Ovariole yield | % of reduction from contamination | % Ovariole yield | % of reduction from contamination |
| Extract of | | | | | | | | |
| Solitary froth | 38.13 | 57.11 | 46.18 | 48.05 | 48.20 | 45.78 | 44.32 | 50.14 |
| Solitary eggs | 33.33 | 62.50 | 36.46 | 58.98 | 49.80 | 43.39 | 45.13 | 49.23 |
| Gregary froth | 51.42 | 42.15 | 30.33 | 65.88 | 57.60 | 35.20 | 79.80 | 10.23 |
| Gregary eggs | 25.70 | 71.10 | 25.70 | 71.10 | 53.00 | 40.38 | 44.75 | 49.66 |
| Solitary control | 88.90 | 00.00 | 88.90 | 00.00 | 88.90 | 00.00 | 88.90 | 00.00 |
| Gregary phase | | | | | | | | |
| Solitary froth | 42.10 | 55.16 | 43.32 | 53.86 | 66.00 | 29.71 | 69.00 | 26.51 |
| Solitary eggs | 52.54 | 44.04 | 38.00 | 59.53 | 70.00 | 25.54 | 70.00 | 25.45 |
| Gregary froth | 56.00 | 40.36 | 58.29 | 37.92 | 60.10 | 35.00 | 36.60 | 61.02 |
| Gregary eggs | 57.60 | 38.65 | 52.80 | 43.76 | 62.00 | 33.97 | 59.40 | 36.74 |
| Gregary control | 93.90 | 00.00 | 93.90 | 00.00 | 93.90 | 00.00 | 93.90 | 00.00 |

Table 5: Effect on fertility percentage of female treated during the pre-ovipositing period

| Solitary phase | | | | | | | | |
|-------------------|-------------|-----------------------------------|-------------|-----------------------------------|-----------------------|-----------------------------------|-------------|-----------------------------------|
| Contaminated sand | | | | | Contaminated antennae | | | |
| Hexane | | Ethanol | | | Hexane | | Ethanol | |
| Treated phase | % Fertility | % of reduction from contamination | % Fertility | % of reduction from contamination | % Fertility | % of reduction from contamination | % Fertility | % of reduction from contamination |
| Extract of | | | | | | | | |
| Solitary froth | 47.40 | 46.19 | 37.90 | 56.98 | 60.00 | 31.89 | 70.40 | 20.54 |
| Solitary eggs | 32.50 | 63.11 | 53.70 | 39.04 | 58.10 | 34.05 | 70.20 | 20.21 |
| Gregary froth | 57.80 | 34.39 | 29.30 | 66.74 | 84.70 | 0.39 | 69.40 | 21.22 |
| Gregary eggs | 35.70 | 52.40 | 35.70 | 59.47 | 81.50 | 07.49 | 48.00 | 45.22 |
| Solitary control | 88.10 | 00.00 | 88.10 | 00.00 | 88.10 | 00.00 | 88.10 | 00.00 |
| Gregary phase | | | | | | | | |
| Solitary froth | 68.10 | 20.10 | 49.32 | 42.13 | 67.30 | 21.03 | 75.00 | 12.00 |
| Solitary eggs | 52.72 | 38.14 | 46.00 | 46.02 | 75.00 | 12.00 | 80.30 | 05.78 |
| Gregary froth | 53.70 | 36.99 | 46.00 | 44.02 | 60.00 | 29.60 | 74.40 | 12.70 |
| Gregary eggs | 34.40 | 59.63 | 54.30 | 43.76 | 71.00 | 16.69 | 65.10 | 23.61 |
| Gregary control | 85.23 | 00.00 | 85.23 | 00.00 | 85.23 | 00.00 | 85.23 | 00.00 |

Table 6: Effect on hatchability of female treated during the pre-ovipositing period

| Solitary phase | | | | | | | | |
|-------------------|---------------------------|-----------------------------------|-------------------------------|-----------------------------------|-------------------------------|-----------------------------------|-------------------------------|-----------------------------------|
| Contaminated sand | | | | | Contaminated antennae | | | |
| Hexane | | Ethanol | | | Hexane | | Ethanol | |
| Treated phase | No. of hatched \pm S.D. | % of reduction from contamination | No. of egg hatched \pm S.D. | % of reduction from contamination | No. of egg hatched \pm S.D. | % of reduction from contamination | No. of egg hatched \pm S.D. | % of reduction from contamination |
| Extract of | | | | | | | | |
| Solitary froth | 47.4 \pm 1.5 | 45.70 | 37.9 \pm 4.3 | 56.80 | 71.8 \pm 2.0 | 18.20 | 80.6 \pm 7.1 | 8.20 |
| Solitary eggs | 33.6 \pm 1.0 | 61.50 | 53.6 \pm 2.5 | 38.90 | 56.2 \pm 0.9 | 36.00 | 68.6 \pm 8.2 | 21.80 |
| Gregary froth | 57.8 \pm 2.1 | 33.80 | 29.4 \pm 0.4 | 66.50 | 84.7 \pm 5.2 | 3.50 | 69.4 \pm 7.2 | 21.00 |
| Gregary eggs | 42.2 \pm 3.1 | 51.60 | 37.8 \pm 2.8 | 56.90 | 81.5 \pm 11.3 | 7.10 | 76.7 \pm 6.4 | 12.60 |
| Solitary control | 87.3 \pm 1.6 | | 87.3 \pm 1.6 | | 87.3 \pm 1.6 | | 57.8 \pm 1.6 | |
| LSD. 0.05 | 7.9 | | 5.2 | | 2.5 | | 9.3 | |
| Gregary phase | | | | | | | | |
| Solitary froth | 58.1 \pm 4.1 | 22.40 | 58.4 \pm 6.7 | 33.50 | 65.3 \pm 1.9 | 25.60 | 75.0 \pm 6.7 | 14.50 |
| Solitary eggs | 33.3 \pm 1.5 | 62.10 | 46.1 \pm 6.2 | 47.50 | 74.9 \pm 7.0 | 14.60 | 80.2 \pm 5.3 | 8.60 |
| Gregary froth | 53.6 \pm 5.2 | 38.90 | 63.9 \pm 7.2 | 27.20 | 62.7 \pm 1.6 | 28.50 | 70.8 \pm 4.1 | 19.60 |
| Gregary eggs | 34.4 \pm 2.7 | 60.80 | 54.5 \pm 5.6 | 37.90 | 71.6 \pm 4.5 | 18.40 | 61.4 \pm 3.4 | 30.10 |
| Gregary control | 87.8 \pm 3.5 | | 87.8 \pm 3.5 | | 87.8 \pm 3.5 | | 87.8 \pm 1.4 | |
| LSD. 0.05 | 4.3 | | 7.5 | | 12.8 | | 8.5 | |

Table 7: Effect on decrease in reproductive potential of females treated during the pre-ovipositioning period

| Solitary phase | | | | |
|-------------------|--------------------------------------|--------------------------------------|--------------------------------------|--------------------------------------|
| Contaminated sand | | Contaminated antennae | | |
| Hexane | Ethanol | Hexane | Ethanol | |
| Treated phase | % Decrease in reproductive potential | % Decrease in reproductive potential | % Decrease in reproductive potential | % Decrease in reproductive potential |
| Extract of | | | | |
| Solitary froth | 85.86 | 85.57 | 71.10 | 74.00 |
| Solitary eggs | 91.95 | 86.67 | 72.10 | 72.50 |
| Gregary froth | 73.26 | 94.75 | 42.10 | 71.00 |
| Gregary eggs | 95.74 | 91.47 | 53.13 | 71.40 |
| Average | 86.70 | 89.62 | 59.61 | 72.20 |
| Gregary phase | | | | |
| Solitary froth | 80.12 | 58.11 | 51.6 | 51.30 |
| Solitary eggs | 97.40 | 91.63 | 50.0 | 46.40 |
| Gregary froth | 76.90 | 67.70 | 69.0 | 60.31 |
| Gregary eggs | 84.46 | 80.10 | 63.5 | 70.50 |
| Average | 84.72 | 74.39 | 58.5 | 57.13 |

Un-expectedly, the froth extract caused lower reduction than egg extract in case of hexane solvent in both solitary and gregary females and in contaminated sand. The ethanol solvent caused higher reduction in fertility percentages than hexane solvents in case of contaminated sand.

The reduction percentages from control were lower by contaminating antennae than by contaminating sand. This may confirm previous results about the two methods of contamination. Any how, the antennae proved to be a chemical receptor mediating the process.

Effect on the hatchability: The hatched eggs per female as an expression of the success of the embryonic development are presented in Table 6. The treatment of females during the pre-oviposition period caused failure of hatching giving striking effect of the extracted materials from both froth or eggs of solitary or gregary egg pod.

The hatched eggs per female were significantly diminished by treatments in all cases. The effect was more intensive and serious, as the reduction in hatchability reached 66.5% in solitary females treated with ethanol extract (gregary froth).

The reduction, of hatchability from control, however, was higher by contaminated sand than by contaminated antennae, by ethanol than by hexane and in solitary phase laying females than their gregary counterparts. This tendency confirm apparently the precedent trends as the reduction of hatchability is the other hand of success of hatchability, i.e., the occurring injury to the biological parameter. The hatched eggs per female in contaminated antennae are higher than those of contaminated sand. The hatched eggs were higher by using ethanol extract, froth and gregary laying females than using their opposite counterparts.

These results, however, point to that the factors extracted which played an effective role on reducing pods per female and number of eggs per pod which are quantitative expression of the treatments may be considered having dual action and affecting quality of eggs shown throughout diminishing of hatching and unsuccessful embryonic development.

Effects on the decrease in the reproductive potential: In the light of the objective of this work, it seems reasonable to consider that any factor resulting in decrease of the reproductive potential of the desert locust, is a promising control agent. This work is addressed to ask about the role of egg pods in limiting the production of oocytes. As shown by Tanaka *et al.*^[22] the oogenesis was inhibited by using trehalose inhibitor, validoxylamine A, against the migratory locust *L. migratoria*, it was accumulated in their bodies and suppressed oocytes development. This effect was through affecting JH biosynthesis by corpora allata, vitellogenin synthesis by the fat body and uptake of yolk material by the ovary.

The oocyte growth in locust is controlled by complex regulators. Cerstiaens *et al.*^[23] reported a clear effect of an insect neuropeptide from the *L. migratoria* was shown to be a potent gonadostimulie in *L. migratoria*. This gonadotropic action on oocyte growth implies complex regulation of oogenesis in *L. migratoria*. The experimental work was planed to use the extracts of the egg pods (froth and eggs) to contaminate sites of oviposition and the antennae of the depositing females. The pods were taken from solitary females and gregary ones.

Under the effect of 32 treatments plus 2 as control, a consistent action was met in the studied parameters. This action was limiting oogenesis and diminishing egg production in both phases. Such conclusion is valid through the precedent topics and a highly significant effect of the egg pod factors was dominating 32 treatments.

Now it may be useful to generalize the over all effect of these egg pod factors in order to approach other side of the problem. The effects on decrease of the reproductive potential of females treated during pre-oviposition period are presented. The reproductive potential of each treatment was related to its phase control. The evaluation of the data presented in Table 7 arise the following major trends:

The reproductive potential were lower than control in all treatments and the decrease ranged from 42.10 to 97.4%.

The percentages decrease was higher in solitarious than in gregarious in all treatment. The average decrease for solitarious were 81.71, 89.62, 54.61 and 72.23 in

treatments of hexane, ethanol in sand; hexane, ethanol in antennae, respectively. The counterpart averages for gregary females were, respectively 81.72, 74.39, 58.53 and 57.13. This trend confirms others for the same relation in the previous topics.

Contamination of sand with pod factors had more influence than contamination of antennae. The role of antennae as chemoreceptors mediating the effects of the various releasing and priming pheromones were studied by Njagi *et al.*^[24], Ingell *et al.*^[25], Oching *et al.*^[26] and Picimbon *et al.*^[27]. Its phase dependent variations were recorded by Eid *et al.*^[28]. This lesser effect of contaminating antennae used with virgin sand compared by contaminated sand may be explained by two suggestions, the first is that additional chemoreceptive sites on body of the females were deprived from the 'pod factors', the second is that contaminating antennae was practiced only, once, but contaminated sand provides a flow of "pod factors" for long time and refreshing the receive. Hexane solvent in sand, rather with antennae caused higher decrease in the percentage of the reproductive potential.

The extract of pods of a given phase was more effective when applied to the females of the other phase, showing thus a phase dependent effect.

This phase dependent effect motivated reconsidering the previous results in search for any role of phase. When the induction of a given item is related, to the sum of solitary and gregary induction's of the item the following relatives are reached.

| Phase | Phase dependant % (pre-ovipositing) | | | | | | | |
|---------------------|-------------------------------------|--------------|---------------|--------------|-----------------------|--------------|---------------|--------------|
| | Contaminated sand | | | | Contaminated antennae | | | |
| | hexane | | ethanol | | hexane | | ethanol | |
| | Solit -ary | greg -ary | Solit -ary | greg -ary | Solit -ary | greg -ary | Solit -ary | greg -ary |
| Egg pod | 56.3 | 43.7 | 51.1 | 48.8 | 56.5 | 43.5 | 57.2 | 42.8 |
| No. of eggs | 49.1 | 50.9 | 51.0 | 49.1 | 51.6 | 48.4 | 48.0 | 52.0 |
| Hatched eggs/pod | 49.5 | 50.5 | 42.3 | 57.7 | 53.4 | 46.5 | 48.5 | 25.1 |
| Fertility | 55.9 | 44.1 | 50.6 | 49.4 | 59.5 | 40.4 | 58.0 | 42.0 |
| Fecundity | 45.4 | 54.6 | 44.4 | 55.5 | 51.0 | 49.0 | 46.7 | 53.3 |
| Ovariole yield | 41.6 | 58.3 | 41.4 | 58.1 | 44.7 | 55.3 | 47.6 | 52.3 |

From these relatives it seems reasonable that the gregary females by both solvents in sand were higher than solitary females especially in hatched eggs per pod, fertility and ovariole yield. However, solitarious in the treatments were higher than gregarious in number of pods, number of eggs and fecundity.

In conclusion, the phase of locusts manifested its physiological potency in the quantitative characters in

gregary females. It could be concluded that the extracts emitted pheromonal factor; which affected the egg production. This assumption may be accepted in the light of the findings of many authors.

Behavioral experiments have shown that adult gregarious locust aggregate in response to pheromone blends emitted by sexually mature gregarious male locust. Female and male gregarious second to fifth instar nymphs have been shown to produce and respond to a nymphal pheromone^[16,18,29] faecal and nymphal volatiles^[30] egg laying attractions^[31] and putative sex-pheromone^[32] have also been shown to be behaviorally active for adult. These results and its biological significance should be discussed again in following paper.

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