STUDIES ON FIELD BIOLOGY OF THE BLISTER BEETLE, *MELOE PROSCARABAEUS* L. (COLEOPTERA: MELOEIDAE) IN FABA BEAN FIELDS AT EL-FARFRA OASIS, EGYPT.

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ABSTRACT

The blister beetle, *Meloe proscarabaeus* L., recorded a pest attacking faba bean was detected for the first time in El-Farafra oasis, New-Valley governerate (Egypt) in 2002. The life cycle and phenology of *M. proscarabaeus* were studied during two successive seasons (2004 & 2005) in faba bean fields. The pest develops one generation per year. Beetle emergence lasted from mid-November to the last week of March with adult peak on the end of January. Oviposition was detected from early January to the last week of the same month. Female laid 1998 – 2291 eggs (av. 2098 eggs) in the soil. Larval development is hypermetamorphic, with three distinct phases and seven larval instars. First and second instar larvae (triungulins) were recorded attached on faba bean leaves from February to March. Fully fed 5th instar larvae were showed excavated chambers in the soil. Seventh instar larvae go into aestivation in the soil from May to October (137 – 146 days). Pupal stage lasted 63.9 days and beetles emergence was recorded on mid-November. Developmental time from egg to adult emergence averaged 309.6 days. Location, mating and oviposition habits were illustrated and discussed.

INTRODUCTION

Blister beetles (Coleoptera : Meloeidae) are generally considered to be the most damaging field pests of leguminous field crops, especially alfalfa in Europe (Pinto, 1999; Kaszab, 1981; Bologna & Pinto, 1992), Asia (Saha, 1979; Prasad *et al.*, 1999; Ozbek & Szaloki, 1998 ; Balikai, 2000), Africa (Moor, 1978, Mathews *et al.*, 2001; Gahukar *et al.*, 1989) and USA (Kineney et al., 1998 ; Nead *et al.*, 1996 ; Ward, 1985). In Egypt, Alfieri (1976) recorded eleven species of *Meloe* as desert

insect fauna collected from different regions, and since that time no further information about blister beetles are explored. The blister beetle, *Meloe proscarabaeus* L. was first recorded as pest of agricultural crops in El-Farfra oasis by Ali *et al.* (2005 a & b) reported field observations on crop damage and food plants of adults.

Life cycle and behaviour of blister beetles species are not unique, but vary with the different species since the development of the most species is hypermetamorphic (Pinto *et al.*, 1996; Bologna & Marangoni, 1996, Ward, 1985). Therefore the present study was undertaken to clarify the biological aspects and behavior of *Meloe proscarabaeus* L. different stages under the normal circumstance of faba bean field.

MATERIAL AND METHODS

Studies on the field biology and sexual behavior of blister beetle, *Meloe proscarabaeus* L. were conducted in faba bean field (*Vicia faba* L.) at El-Farfra oasis, New valley governorate. In El-Farfra oasis as a whole adult has been recognized in the months November, December, January, February and March, freshly emerged beetles (5♀♀, 5♂♂) were kept inside wire-wooden cage (35 × 50×50 cm) fixed on faba bean plants and replicated 8 times throughout emergence time of beetle (mid- November – late February). Regular observation on adult behaviour, mating, feeding, egg-laying behaviour, number of deposited eggs, egg hatchability and life time of both sexes were recorded under field conditions.

To follow up the development of immature stages, oviposition sites of the newly emerged female beetles were recognized in the field and labeled ; these were separated into eight groups, three sites each, according to date of egg laying. Each site contained one egg mass (2000-2150 eggs). Glass cylinder cages, each were fixed on each site at the following dates: 3rd, 9th, 12th, 19th, 22nd, 26th January, 3rd and 6th of February. Daily observations of cages were continued until beetle emergence (mid- November). Observations involved the number of hatched 1st instar larvae, behaviour and developmental time of different larval instars, pupation and pupal period, mortality ratio of different immature stages and number of newly emerged beetles in each field site. The life span, sex ratio, fecundity and adult longevity of newly emerged beetles were calculated and determined.

RESULTS AND DISCUSSION

A. Adult Stage

1-Adult emergence

In 2002/2003, emergence of *M. proscarabaeus* beetles lasted from mid November to approximately end of March. Number of emerged adults increased gradually reaching maximum level in the last week of January (150 adults / 100 plants) after which it decreased reaching the minimum level (22 beetles / 100 faba bean plants) in the third week of March. Sex ratio was close to 1:1 with a slight predominance of females. Swarming of beetles was air and soil temperature depended on. Under the conditions of 19-21 ^{0}C, 18-22 ^{0}C air, and soil temperatures, about 50.3 % of adult population was emerged from the soil.

In the second season, 2003/ 2004, beetles emergence followed the same aforementioned trend with slight low number of emerged beetles. Whitehead (1991) reported that adult of *Meloe rugosus* have been recognized in the months of September, October, November, December, January, February and April and emergence took place in synchronous waves during September and October. However, the periodicity of *M. rugosus* adult emergence occurs in the same period of *M. proscarabaeus* three one-emergence waves were observed for the present species.

2-Mating

Pairs of beetles were several times observed in copulation during our periodic inspection of infested faba bean fields. Copulation occurred during day-light. Beetles that reached its sexual maturity (50 days post emergence) showed evidence of mating. It is true that males are attracted to females by sex pheromone emitted by females. Male was observed searching for the female at early morning and when they meet each other, courtship may start.

Primarily, male touched the antenna of the female partner faced her side and when female exhibits response, male touches her abdomen by its antenna. Repeated touches might take place until the female stopped calmly; this acts for about 90 minutes.

The male jumped on the female dorsal side rapidly and held female by thorax fore legs while female abdomen was held by male mid and hind legs (Fig.1). Because female abdomen is longer than that of male, female withdraws its abdominal segments (telescope movement) to cope male abdominal end. Male abdominal tip flexed below

the abdominal tip of the female, the highly chitinzed male genitalia protruding and a great part of it was inside the female body, widely opening the female genitalia aperture for the entrance of the apical fleshy part of aedeagus. After this, the male rolled up to the opposite direction (tail to tail position) and mating is carried out. The maximum period of the act of copulation lasted for about one hour. When the male and female are disturbed during copulation, they soon separate from one another. Selander (1964) and Bologna & Marangoni (1986) reached to similar sexual behavior on *M. proscarabeus* and *M. violaceus* when courtship was always short, but the subsequent dorsal phase was protracted; during this phase, males touched the antenna of their partners, and areas of cuticular pores on the antenna of these males appear to be connected with emission of contact pheromone.

3- Pre-oviposition period

Results in Table (1) reveal that duration of the preoviposition may vary according to emergence time of aestivated beetles and prevailing environmental conditions. Beetles emerged in mid-November started egg laying after 49 days post emergence, while about 48 – 57 (av. 51.3 days) days was acquired to beetles emerged from late November to nearly mid-December for oviposite. Weather conditions prevailed in November averaged 28.5 °C, 17.3 °C, 24.8 °C, 48.8 % while in December these values lowered to 24.0 °C, 10.5 °C, 20.3 °C and 57.5 % for maximum, minimum, soil temperatures and relative humidity, respectively.

So it could be stated that prevailing temperatures particularly soil temperature pronounsly affected the emergence time of beetles and consequently the duration of pre-oviposition. Inspection of eight groups of beetles differing in emergence dates and confined in field cage (one pair / cage) showed that duration of preoviposition period ranged between 48 and 57 days with an average of 50.4 ± 0.6 days. (Table 2).

4- Oviposition habits

Egg-laying by *M. proscarabaeus* has been observed once in January. Females spent three hours excavating an egg chamber of 5 x 5 cm diameter and 4 x 6 cm deep in the closed boundary strips of 10 cm height near to irrigation canals and this time seems to be acceptable since the sediments and soil texture at El-Farafra oasis are sandy requiring minimal excavation labor. A female of *M. rugosus* spent 30 hours excavating an egg chamber and that long time may be regarded to the soil texture of oviposition sites at Wiltshire (Whitehead, 1991). Female of *M. proscarabaeus* spent two hours searching for the appropriate ovipositing site and

used mandibles, fore and hind legs in excavating the oviposition chamber. Oviposition lasted four hours. Eggs are laid in a longitudinal mass.

TABLE (I)

Emergence and egg laying date of *M. proscarabaeus* adult during faba bean season 2002 - 2003, EL-Farafra Oasis.

Group	Date of beetles Emergence	Date of egg laying	Weather factors			
			Max temp. °C	Min temp. °C	Soil temp. °C	R.H %
I.	November,15,2003	January, 3 ,3004	29	16	26	47
II	November, 20	January, 9	28	15	25	49
III	November, 24	January, 12	29	15	26	49
IV	November, 30	January, 19	27	11	22	50
V	December, 4	January, 22	28	12	23	49
VI	December, 9	January, 26	25	11	24	60
VII	December, 13	January, 31	22	10	18	60
VIII	December, 24	February, 3	21	9	16	61

Female moves after egg lying and continues feeding until death. More than one egg chamber and one egg mass per female have never been witnesses in *M. proscarabaeus*. Similar oviposition habits are characteristic of *Meloe* spp. Selander and Fasula (2000) found eggs of Meloinae are deposited in masses in the ground or under stones, while beetles belonging to *Nemognathina* lay eggs on the food plants of adults; however females of our species never laid eggs on their food plants. Although females of *M. proscarabaeus* lay eggs once during January, females of *M. rugosus* deposit their eggs once in November and twice in December (Whitehead, 1991). The female of *Meloe variegates* digs holes between 2 and 3 cm deep in the ground and lays several batches of yellow eggs, (Bohac and Winkler, 1964).

5- Fecundity

Females of the blister beetles showed conspicuous variation in the number of eggs laid by the female according to insect spices, locality and environmental factors effects. Females of *M. proscarabaeus* deposited number of eggs varying according to season and thermal conditions. Females of 2003 population deposited number of eggs ranged between 1997.6 and 2290 eggs per female with an average of

2098.7 eggs/ female (Table, 2). The minimum and maximum numbers of eggs laid by 2004 females were 1975 and 2160 eggs/ female (av. 2044.4 eggs/ female). Generally the maximum number of deposited eggs (2290 eggs/ female) was shown by female beetles emerged at the end of November and the lowest (1997.6 eggs/ female) was achieved by females emerged at the end of December. Fecundity of *M. Proscarabeus* females seems to be very low if compared with number of eggs laid by *M. variegates* females (10000 eggs/ female) and that could be attributed to insect spices locality and environmental conditions. The vast numbers of eggs laid by spices of necessary to ensure the survival of the species for very few larvae survive to develop into adult beetles (Bohac and Winkler, 1964).

6- Oviposition period

Emergence of *M. proscarabaeus* female beetles took place from mid-November to nearly late December and they commenced egg deposition during early January to approximately the beginning of February. Observations of field cages containing one pair of newly emerged beetles (♀, ♂) revealed no distinct difference in oviposition period irrespective of difference in female emergence time. Generally, females spent 3.1-3.6 hours with an average of 3.3 h to lay its whole eggs (Tables 2 & 3). Prevailing environmental temperatures for egg depositing seem to have no effect on the duration of oviposition period and did not relate to this process.

7- Adult longevity

Duration of adult survival of the blister beetle *M. proscarabaeus* varied according to sex and emergence time of beetles. Generally, females survived longer thanmales. Female survival 100-128 days with an average of 118.5 ± 1.9 days, while this period lasted 87-116 days with an average of 103.5 ± 2.0 days in 2002/203 season. Results obtained in 2003/2004 did not significantly differ than the preceding season, female survival averaged 102.75 days while this period shortened to 94.13 days in case of male beetles. Beetles emerged in 30th November survived the longest period (128 and 116 days for female and male adults, respectively). Adult longevity showed gradual decrement in the successive dates of beetles. Selander and Fasula (2000) reported that adults of *Nemegnatha plazata* F. (Meloidae) commonly live three months or more which is nearly the same for *M. proscarabaeus* adults that deter mined in the present study. (Tables 2 & 3).

8- Food and feeding habits

Adult of *M. proscarabaeus* were observed feeding on leaves and flowers of faba bean (*Vicia faba*), peas, alfalfa, Egyptian clover, onion and the wild weed

Meliolotus indica, (Ali *et al.* 2005b). Ozbek and Szaloki (1998) identified *Micromerus erivanicus* (Meloidae: Coloptera) as a pest of flowers of *Vicia* spp. and *Onobrychis sativa* and *Mylabris quadripunctata* (Meloidae) as a pest of soft wheat grains in Turkey. Adults of *Meloe varigatus* was recorded as insect pest of sugar beet, cabbage and winter rye (Stebnicka, 1987). Immediately after the emergence of *M. proscarabaeus* adults, beetles were seen moving in swarms from range- land to faba bean fields where they disperse and starting feeding for a period of up to 50 days. Faba bean was seeded in early November and beetles emergence time convened with arising of faba bean seedling of about 10-12 days with 2-3 leaflets. These young plants are the most preferable food.

TABLE (II)

Average no. of eggs/female and sexual reproduction periods of M. proscarabaeus recorded under field conditions (Average ± s.e.Temp. 20 0C, relative humidity 38 %).

Group	No -eggs per female	Hatchability %	Pre-oviposition (days)	Oviposition (hours)	Post Oviposition Period. (days)	Adult longevity	
						Female	Male
1	2026.6	90.7	49	3.1	86	123	102
2	2051.6	95.5	50	3.6	90	125	104
3	2173.3	92.0	49	3.00	80	119	110
4	2290.6	93.0	50	3.4	93	128	116
5	2227.0	95.7	48	3.2	92	122	111
6	2000.0	99.5	57	3.1	90	119	103
7	2022.6	98.6	49	3.3	87	112	95
8	1997.6	91.0	51	3.5	77	100	87
Average ±s.e.	2098.7±25.6	94.5 ±0.7	50.4 ±0.6	3.3 ±0.04	86.8 ±1.2	118.5 ±1.9	103.5 ±2.0

a: Average of 24 observations.

Newly emerged beetles have strong mandibles and long fore legs were seen masticate and attack faba bean seedling feeding on leaves and plant stems and finally completely destroy the whole plants. Through feeding, beetles secrete a yellow fluid from coaxial and antennal joints; this fluid blistering noninjured leaves which bear brownish and finally die. Plants severely attacked may fail to produce flowers and beans, so the crop yield is completely lost. Feeding of beetles occurs

during the whole day-light and never seen feeding during day-night. Coleman (1983) recorded a female *M. proscarabaeus* grazing on *Ranuchus* sp. in Cornwall. Ward (1985) reported that blister beetles feed on plant materials, particularly flowers of such plants as alfalfa, careless weed (Pigweed), punchrevine (goathead), peanuts, soybeans and many other species. It seems that faba bean is not recorded in the diet of *Meloe* as indicated by Pinto (1980), therefore our record of this food plant as a diet of *M. proscarabaeus* becomes first record. Beetles were generally more numerous on the field side nearest to range-land and irrigation canals.

TABLE (III)

Percentage of immature mortality of *M. proscarabaeus* developed under field conditions (Average ± s.e. Temp. 20 C & R.H 38 %).

Group	No. of 1st instars hatched larvae	No. of emerged beetles	Immature mortality (larva+ pupa)
1	1837.6	107.3	94.1
2	1960.0	117.6	94.0
3	2000.0	127.0	93.6
4	2132.3	129.6	93.9
5	2133.3	136.6	93.5
6	1990.0	95.0	95.2
7	1995.0	40.0	97.9
8	1820.0	10.0	99.4
Average ± s.e.	1982.5±25.8	95.4±10.3	95.2±0.4

9-Significance of cantharidin

Field and laboratory examinations proved that adults of *M. proscarabaeus* exuded copiously a yellow fluid from the joints of their legs (Fig.1 d) this fluid is often secreted by reflexive bleeding when an adult beetle is pressed or rubbed. This phenomenon was commonly noticed in most spices of meloid beetles (Whitehead, 1991; Ward, 1985; Edwards *et al.* 1989). Like other blister beetle species, *M. proscarabaeus* contains a large quantity of oily, yellowish hemolymph which they exude when disturbed. This fluid was identified as cantharidin (a bicyclic terpenoid $C_{10}H_{12}O_4$). It is found in hemolymph and gonads of beetles, *Lytta vesicatoria* (L.) (Meloidae) contains more cantharidin than any other member of the family; cantharidin is found mainly in the elytra but it has also been shown to exist in the genetalia and the hemolymph (Bohac and Winkler, 1964).

Males have highest levels of cantharidin and males transfer it to females during copulation. Adult of blister beetles feed on leaves, flowers of alfalfa in the United States causing leaf loss which is not economic importance. The real problem with blister beetles lies in their toxicity to livestock, especially horses, when accidentally consumed in feed due to their containing cantharidin (Schoeb, 1978 & Schmitz, 1989).

Many of the common species of blister beetle contain cantharidin (Spanish-fly), a substance that will cause blisters when applied to the skin (Beasley *et al.*, 1983). Ward (1985) reported that cantharidin is a stable chemical and long-term health threat to nearly all livestock, particularly horses that are fed contaminated alfalfa hay. Research reports indicate cantharidin toxicosis can be induced in dairy and beef cattle, goats and sheep; other reports include rabbits, hedgehogs, mice and dogs. Cases of human death also have been reported. However, horses appear to be more susceptible to the toxic effects of this potent chemical than other livestock (Ray *et al.*, 1980) and (Graziano *et al.*, 1987). Although the topic effect of cantharidin to all livestock and human, blister beetles use this fluid and related analogs as defensive compounds against larger herbivores and predators.

According to the previously mentioned significance of blister beetles in different countries, the presence of *M. proscarabaeus* as a new pest in El-Farafra oasis may bring us to ring bell about the great damage and losses that threat our legume crops and livestock in such new reclaimed and cultivated areas. Today, the toxic properties of cantharidin are more widely recognized and its use is largely restricted to veterinarians, who employ it as counterirritant and blistering agent (Kineney *et al.* 1998).

B. Immature stages

1- Egg stage

Eggs are laid in masses in the ground or on the food plants of adults. Egg laying by *Nemognatha plazata* Fab. (Coleoptera: Meloidae) similarly deposited in the ground or under stones (Meloinae) or on the food plants (Selander, 1991). 2

2- Hatching

The results given in (Table 2) show that percentage of egg hatching ranged between 90.7% and 99.5% with an average of 94.5%. Variation observed in percentages of egg hatching could be ascribed to variation in environmental temperature. Eggs laid in the last week of December at air temperature average 21.2

^{0}C gave the highest value of egg hatchability (99.5%) while those deposited at the first week of January (17.5 ^{0}C) showed the lowest rate of egg hatchability (90.7%).

3- Incubation period

Incubation period slightly varied according to the time of egg laying. The shortest period (23 days) was recorded for beetle laid egg in January (mean temperature 17 ^{0}C and 30 % relative humidity). On the other hand, the longest incubation period (30 days) existed for eggs deposited in December (21 ^{0}C & 36 % R.H.). In general, incubation period averaged 27.7 ± 0.5 days.

4- Larval stage

As presented in Table (4) larval development is hypermetamorphic with three distinct phases and seven larval instars. The first phase or as called triungulin includes the 1st and 2nd larval instars. Larvae of this stage are active with long-legged (Fig.3a). They reach its feeding site, feeding on faba bean leaves and usually aggregated in clusters (Fig.3a). Duration of the 1st and 2nd triungulin larvae averaged 14.2 ± 0.2 days. After feeding to repletion, the larvae, with ecdysis, becomes scarabaeiform and enters a period of rapid growth that lasts until the end of the 5th instar. This phase includes 3rd, 4th and 5th instars. These instars lasted 13.2, 13.8 and 13.6 days, respectively. Fully fed 5th instars larvae were shown excavated chambers in the soil apart from the feeding site where they moult and develop into the 6th instar which lasts for 13.5 days. In instar seven, the larva typically becomes a heavy sclerotized and immobile and remained for 137 – 146 days (average 139.5 ± 0.6 days) in the soil at 10 – 15 cm. depth depending on the structure, temperature and moisture of the soil. It seems that this instar undergoes into aestival diapause during the months May, June, July, October, under the stress of environmental conditions (air temp. 40 ^{0}C, soil temperature 43.5 ^{0}C, soil moisture 50 %).

5- Pupal stage

In September, the 7th aestivated larva resumes activity and, with ecdysis, the larva becomes again scrabaeiform and transforms to pupa free in the soil (Fig.3c). The duration of the pupal stage ranged between 60.3 and 69.3 days with an average 63.9 ± 0.7 days (Table 4).

6- Total developmental time

Adults of the blister beetle, *M. proscarabaeus* appeared at mid November after elapsding of 288.5 – 316.1 days with an average 309.6± 2.0 days.

TABLE (IV)

Mean durations (x ± s.e. in days) of immature stage of *M. proscarabaeus* (L.) developed under field conditions (Temp.20 °C & 55.4 % relative humidity).

Group	Mean duration in-days										
	Incubation period	1st	2nd	3rd	4th	5th	6th	7th	Total	pupa	Total duration
		Triungulin larvae		Active larvae			In-active larvae				
1	29.3	14.3	15.0	14.0	14.6	15.0	13.0	139	254.2	65.0	314.2
2	30.0	15.0	14.0	15.0	14.3	15.0	14.3	137	254.6	66.0	315.6
3	29.0	14.6	15.0	14.0	14.3	15.0	14.0	140	253.9	69.3	314.2
4	29.6	14.0	14.6	15.0	14.3	14.0	15.0	146	255.5	60.6	316.1
5	28.3	15.0	14.0	14.6	15.0	14.0	15.0	140	251.9	67.0	311.9
6	27.0	14.0	15.0	14.3	14.0	13.6	14.6	137	249.5	61.3	310.8
7	25.0	14.6	14.0	13.3	13.0	12.0	12.6	139	243.5	62.0	305.5
8	23.0	12.0	12.3	11.6	11.0	10.3	10.0	138	228.2	60.3	288.5
Average ± s.e.	27.7 ±0.5	14.2 ±0.2	14.2 ±0.2	13.2 ±0.2	13.8 ±0.2	13.6 ±0.3	13.5 ±0.3	139.5 ±0.6		63.9 ±0.7	309.6 ±2.0

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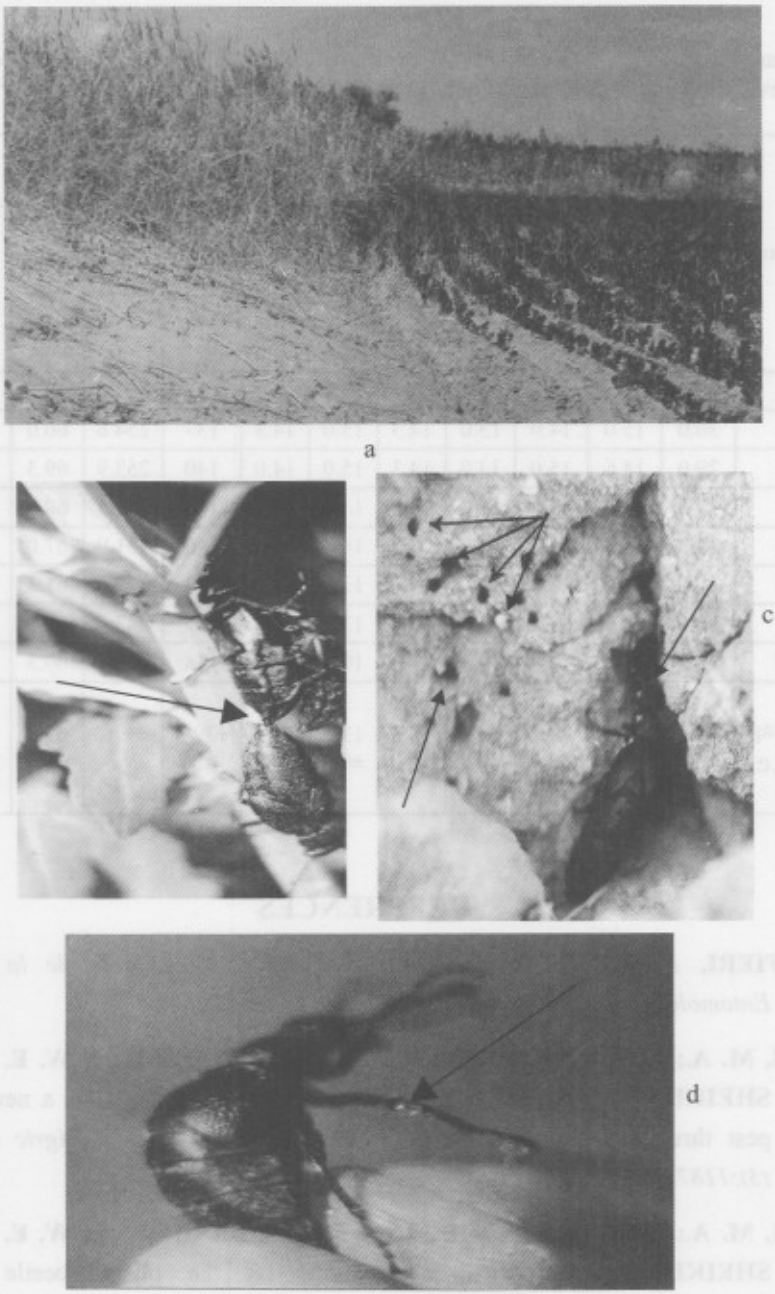

Fig. (1): Emergence and copulation behaviour of the blister beetle, *M. proscarabaeus* in a faba bean field. (a): site of beetle Emergence ; (b): mating; (c): emergence hides; (d): Cantharidin fluid excreted by adult beetles.

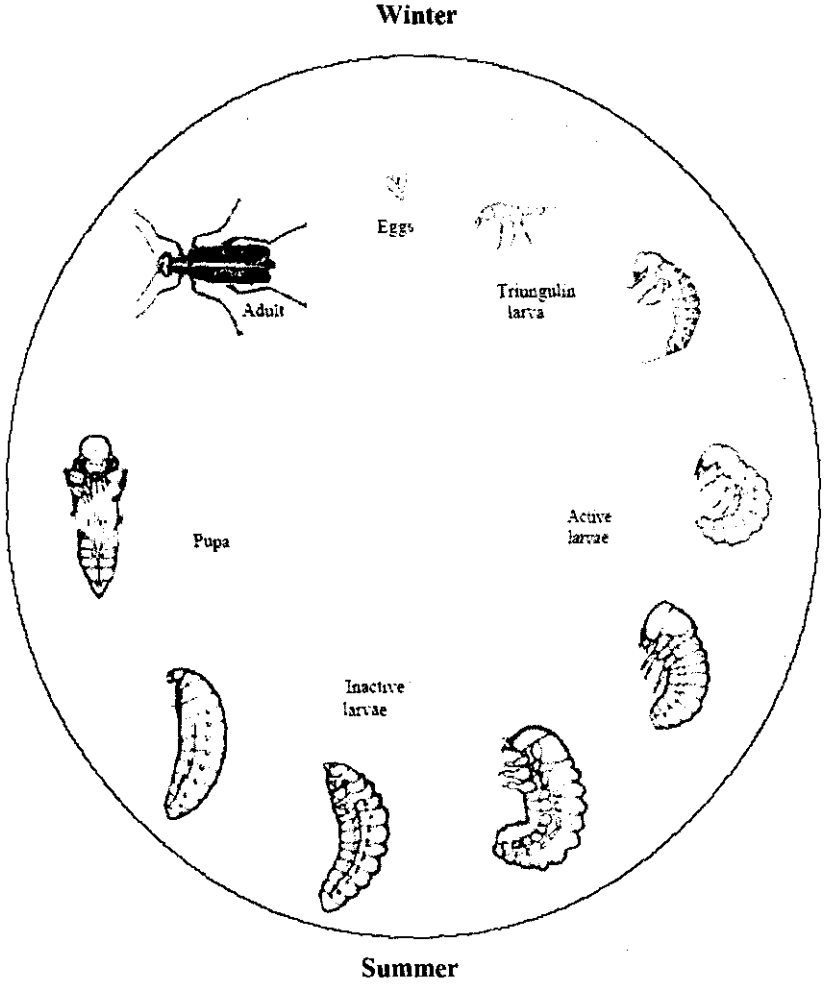

Fig. (2): Typical life cycle of on Meloidae. blister beetle. (After Sorensen and Baker1983).

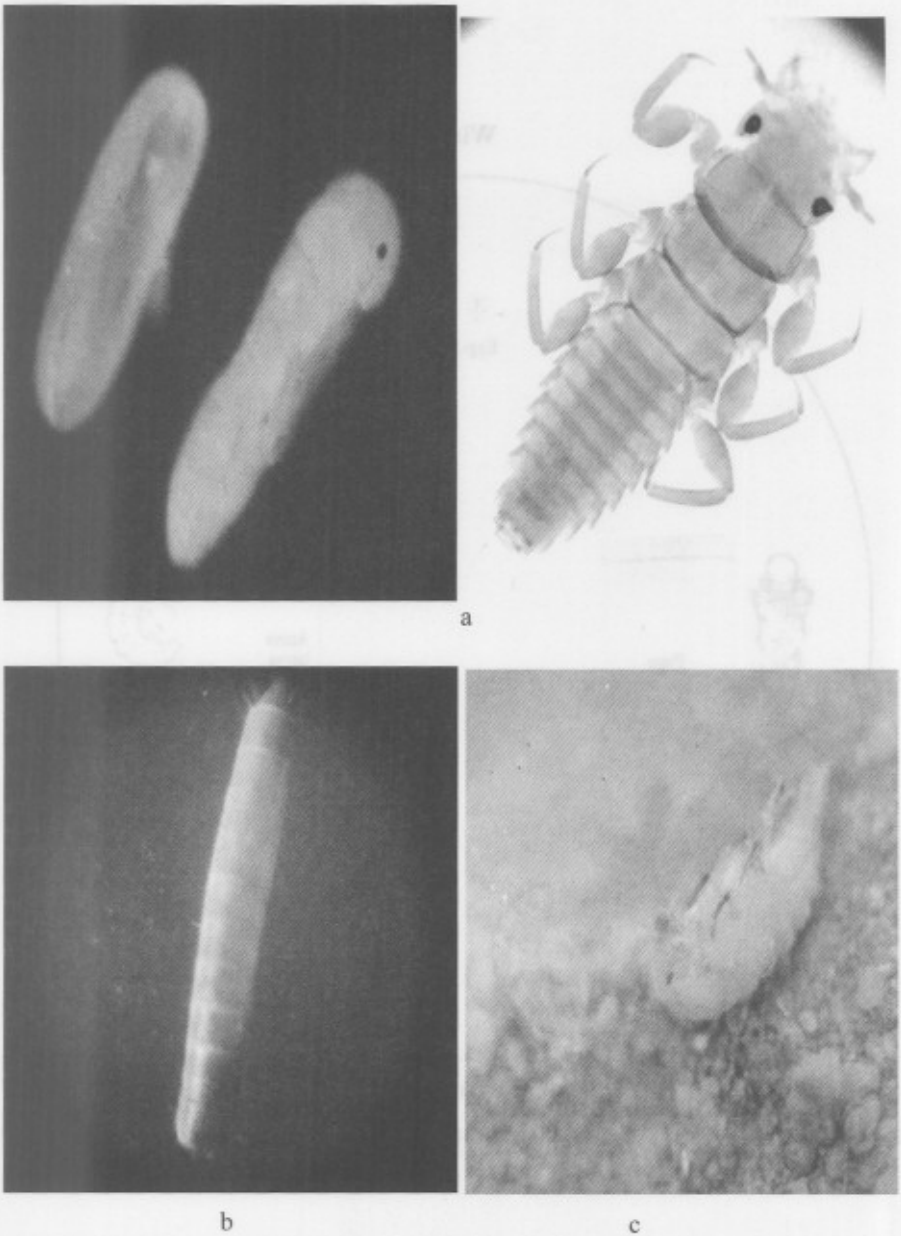

Fig. (3): Immature stages of the blister beetle *M. proscarabaeus*
(a):1st in star larva (Triangle).
(b):4th in insect larva (Inactive larva).
(c): pupa stage in the soil.

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