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EFFECT OF STARVATION ON MORTALITY AND TOTAL BODY WEIGHT OF LARVAE AND ADULTS OF *TRIBOLIUM CASTANEUM**

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Abstract: The sixth instar larvae of *Tribolium castaneum* showed 100% mortality after 16 days and adult beetles after 18 days of total starvation under laboratory conditions at 35 °C and 75% relative humidity. Rate of wet weight loss was greater in sixth instar larvae than adult beetles. Larvae reduced their wet weight sharply during initial 48 hours of starvation and thereafter maintained steady reduction. In contrast adults reduced their wet weight gradually throughout the experimetation. However, both stages of *T. castaneum* decreased their dry wieght abruptly during the initial 48 hours followed by steady reduction.

Key words: Starvation, *Tribolium castaneum*, red flour beetle.

INTRODUCTION

The ability to withstand starvation could have an important evolutionary cosequrnces, if stored grain insects are forced to undergo some periods of starvation (Sverdlov and Wool, 1975). Although the response of higher animals to such stress is fairly understood (Scharrer and Scharrer, 1963), very little informatioin is available on the effect of total starvation on physiological and bio-chemical systems of insects (Dahlman, 1973). In the absence of sufficient bio-chemical data on starved *Tribolium castaneum* (Sverdlov and Wool, 1975), and since insecticides were planned to be administered to these insects in this laboratory under starved conditions, it was felt necessary to generate basic data for *T. castaneum* under total starvation conditions.

Some of the previous studies in other laboratories describe the effects of starvation on the length of the interlarval period in the tsetse fly, *Glossina morsitans orientalis* (Saunders, 1972); on survivorship curves, weight loss and percent dry weight of larvae of tobacco hornworm, *Manduca sexta* (Dahlman, 1973); on growth development and survival in the red trunip beetle, *Entomoscelis americana* (Gerber, 1984) and on survival and maintenance of soldier proportion in laboratory groups of the *Formosan subterranean* termite, *Coptotermes formosanus* (Su and La-Fage, 1986).

Review of literature revealed that very little published information was available on these aspects of stored grain insects particularly *T. castaeum*. Sverdlov and Wool (1975) reported some aspects of survival of starved adult *T. castaneum*, whereas Malik and Galley (1976) studied combined effects of starvation and radiation on feeding activity of this beetle. Likewise Rosinski *et al.* (1979) studied the effect of starvation on trehalase activity of closely related stored grain insect, *Tenebrio molitor* and

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Buscarlet *et al.* (1986) demonstrated the effect of fasting and irradiation on free amino acids of *T. castaneum*. From this laboratory we have already reported effect of total starvation on mortality, total body weight, some enzyme activities and some biochemical components in 6th instar larvae of *T. castaneum* (Saleem and Shakoori, 1993) at 30±1 °C and 75% relative humidity.

MATERIALS AND METHODS

Rearing of beetles

The methods have already been described elsewhere (Saleem and Shakoori, 1986, 1993). The master culture of the red flour beetle, *T. castaneum* was obtained from the Food Storage Division of the Pakistan Agricultural Research Council (PARC), Karachi and was maintained in a temperature controlled laboratory at 30±1 °C with 60% relative humidity (p.h). The insects were reared in empty jam jars covered with muslin cloth and whole meal wheat four was used as the culture medium. Sixth instar larvae collected 28±1 days after egg laying and adult beetles collected after 10±1 days after emergence from pupae were used in the present study.

Determination of mortality and loss of body weight

Seventeen glass vials (diameter 2cm., height 4cm.) each containing 10 final instar larvae and 10 glass vials (diameter 2cm., height 4cm) each containing 10 adult beetles were weighed with and without insects and then placed in desiccators maintained at 75% r.h. with the help of saturated sodium chloride solution according to the procedure described by O'Brien (1948), Ernst (1957) and Minnick *et al.* (1973). The desiccators were in turn kept in a temperature controlled lab. maintained at 35±1 °C.

These larvae and adult beetles were forced to undergo total starvation till 100% mortality was achieved. Mortality, dry and wet weight data was recorded after 24 and 48 hours for larvae and beetles, respectively.

First of all insects were weighed to obtain total body wet weight. Thereafter, one vial from each category was kept in an oven at 110 °C for 24 hours for estimation of thier total dry weight as described by Dahlman (1973). Hence total body water content was the difference of live wet weight and dry weight obtained by keeping the insects overnight at 110 °C (Woodring, 1984). The experiments lasted 16 and 18 days for larvae and beetles, respectively. That way 68 glass vials each containing 10 final instar larvae and 40 vials each containing 10 adult beetles were used in the present study. Each experiment had 4 replicates.

RESULTS

Mortality

All sixth instar larvae died after 16 days of total starvation. About 23% mortality occured within 24 hours, while 50% insects died between 3rd and 4th days of starvation. The daily mortality recorded is shown in Table I. Hence total starvation

caused the highest mortality during the early period of 48 hours of forced starvation, and then it showed gradual increase in mortality with the lapse of time period (Table I).

Table I. Effects of starvation on total body wet weight, total body dry weight, total body water content and mortality of 6th instar larvae of *Tribolium castaneum* at 35 °C and 75% r.h. Each value is a mean of 10 beetles

Days of starvation	Wet weight in mg (n=4)	% loss of wet weight	dry weight in mg (n=4)	% loss of dry weight	Total body water content in mg	% loss of total body water content	Dead larvae (n=4)	Mortality (%)
0	24.10±0.42	0	11.25±0.16	0	12.85	53.38	0	0
1	17.33±1.15	28.09	9.8±0.64	12.89	7.53	43.45	9	22.5
2	12.78±0.50	46.97	6.90±0.88	38.67	5.88	46.01	18	15.0
3	12.70±0.60	47.30	6.63±0.41	41.07	6.07	47.80	19	47.5
4	12.18±1.6	49.46	6.53±0.19	41.95	5.65	46.39	23	57.5
5	10.83±1.29	55.06	5.73±0.48	49.07	5.10	47.09	21	52.5
6	10.43±0.73	56.72	6.37±0.84	43.38	4.06	38.93	26	65.0
7	10.38±1.51	56.93	6.20±0.93	44.89	4.18	40.27	29	72.5
8	10.43±2.23	56.72	5.73±1.14	49.07	4.70	45.06	28	70.0
9	8.63±0.73	64.19	5.23±0.57	53.51	3.40	39.40	29	72.5
10	8.23±0.46	65.64	5.60±0.67	50.22	2.68	32.37	32	80.0
11	8.03±0.39	66.68	5.40±0.19	52.00	2.63	32.75	33	82.5
12	7.68±0.39	68.13	5.00±0.64	55.55	2.68	34.89	34	85.0
13	6.80±0.51	71.78	4.90±0.54	56.44	1.90	27.94	33	82.5
14	6.23±0.68	74.15	4.40±0.80	60.88	1.83	29.37	35	87.5
15	6.25±0.59	74.07	4.38±0.16	61.07	1.87	29.92	36	90.0
16	6.00±0.93	75.10	4.35±0.93	61.33	1.65	27.50	40	100.0

Table II shows the average number of beetles which died due to the forced starvation and thir corresponding percent mortality. No mortality was recorded during the first 144 hours of starvation. The 18% mortality occured first after 8 days of starvation, while 100% mortality was recorded after 18 days of deprivation of food. The percent mortality on day 10, 12, 14 and 16 was 42.5, 75.0, 95.0%, respectively.

Total body weight

Table I shows the effect of total starvation on total body wet weight of 6th instar larvae of *T. castaneum* kept at 35±1 °C and 75 r.h. The total body wet weight of 10 larvae recorded on day 0 was 24.10±0.42mg (n=4) which reduced 47% during the first 48 hours of total starvation. Thereafter, the reduction in body wight was gradual

till it reached its maximum on day 16. The 10 larvae weighed after 16 days of starvation as 6.00±0.93mg (n=4), showing thereby a 75% decrease in the total body wet weight.

In contrast to the 6th instar larvae, ten adult beetles manifested gradual decrease in their total body wet weight from day 0 through day 18 from 20.25±0.42mg to 6.15±0.25mg showing about 70% loss in weight (Table II).

Table II. Effect of total starvation on total body wet weight, total body dry weight, total body water content and mortality of adult beetles of *Tribolium castaneum* at 35 °C and 75% r.h. Each value is a mean of 10 beetles

Days of Starvation	Wet weight in mg (n=4)	% loss of wet weight	dry weight in mg (n=4)	% loss of dry weight	Total body water content in mg	% loss of total body water content	Dead beetles (n=4)	% Mortality
0	20.25±0.42	0	9.73±0.27	0	10.52	51.95	0	0
2	18.63±0.50	8.00	3.70±0.40	10.58	9.63	51.69	0	0
4	16.20±0.19	20.00	7.70±0.19	20.86	8.50	54.47	0	0
6	16.28±0.54	19.60	8.15±0.39	16.24	8.13	49.94	0	0
8	14.20±0.65	29.88	7.33±0.30	24.66	6.87	48.38	7	17.5
10	11.45±0.78	43.46	6.43±0.16	33.91	5.02	43.84	17	42.5
12	8.93±0.28	55.90	5.73±0.43	41.11	3.20	35.83	30	75.0
14	6.88±0.18	66.02	5.23±0.26	46.25	1.65	23.98	38	95.0
16	6.30±0.15	68.89	5.25±0.35	46.04	1.05	16.67	38	95.0
18	6.15±0.25	69.63	5.20±0.44	46.56	0.95	15.45	40	100.0

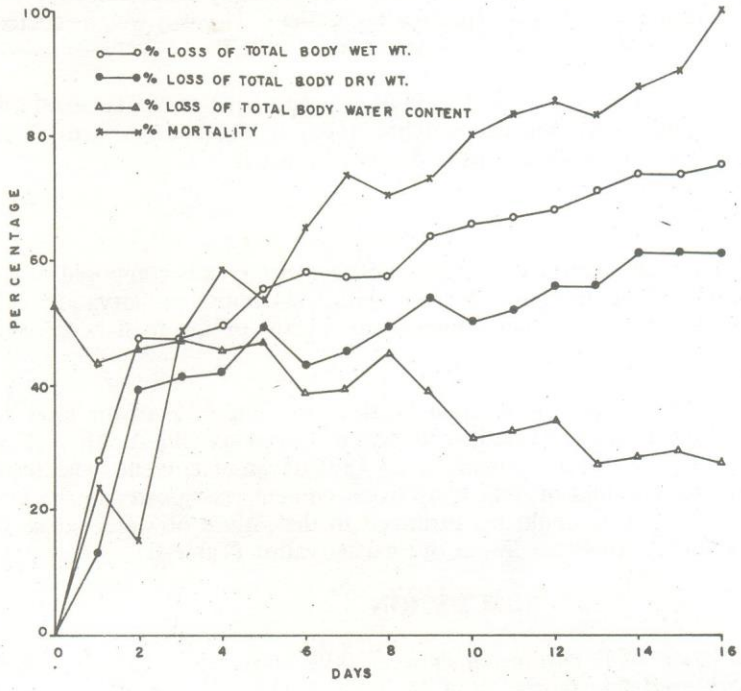
Total body dry weight

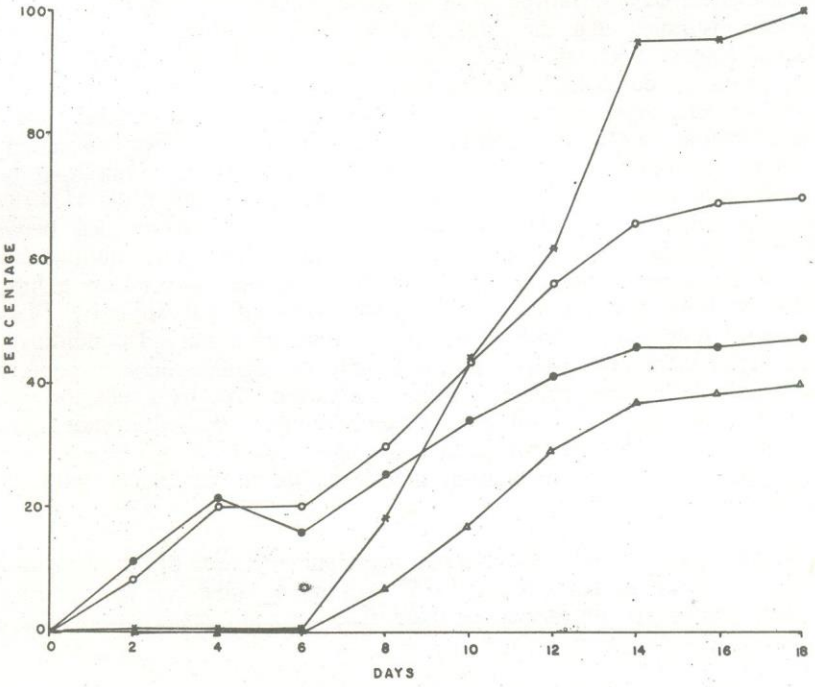
Table I shows the effect of starvation on the total body dry weight of sixth instar larvae of *T. Castaneum*. Fifty three percent of the body weight was lost after drying of day 0 larvae at 110 °C (from 24.10±0.42 to 11.25±0.16 mg for 10 larvae). The loss of dry body weight is reduced after starvation. The wet weight is lost by 75% and dry weight by 61% after 16 days of starvation. Under total starvation conditions the maximum reduction of 38.67% was noted during the first 48 hours of starvation (*i.e.* from 11.25±0.16 to 6.90±0.88mg per 10 larvae at day 0 and day 2, respectively). On day 16 the dry weight was 4.35±0.93mg for 10 beetles, which was 61.33% less than that of day 0 larvae.

Fig. 1. Effects of total starvation on mortality and body weight loss at 75% relative humidity and 35 °C of 6th instar larvae of *Tribolium castaneum*

Fig. 2. Effects of total starvation on mortality and body weight loss at 75% relative humidity and 35 °C of adult beetles of *Tribolium castaneum*

EFFECT OF STARVATION ON *TRIBOLIUM CASTANEUM*





The total body wet weight decreased 52% after drying of day 0 adult beetles at 110 ^{o}C (from 20.25±0.42 mg to 9.73±0.27mg for 10 beetles). The wet weight decreased 70% and dry weight by 47% after 18 days of starvation (Table II).

The reduction in dry weight was gradual until it reached the maximum of 42.45% on day 16 when compared with their dry weights on day 0. Percent loss in dry weight of adult beetles from day 2 through day 18 is shown in Table II.

Total body water content

Fifty three percent of the total body weight of 6th instar larvae is composed of water at day 0. The water content decreased 43.45% within 24 hours of starvation, while after 16 days of starvation, the water content was 27.5% of the total body weight (Table I).

The total body water content of the adult beetles constitute 52% of the total body weight. The water content decreased during 28 days of forced starvation. After 18 days of total starvation, the adult beetle contained 15.45% of water content. The results, therefore, indicated that the loss of total body water content was greater in the larvae than in the adult beetles. This could be attributed to the failure of water conserving mechanism in larvae under stress conditions of total starvation (Table II).

DISCUSSION

The sixth instar larvae of *T. castaneum* showed 100% mortality in 16 days, whereas the adult beetles achieved this mortality in 18 days at 35±1 °C and 75% r.h. The results, therefore, revealed that the adult beetles survived longer than their fully developed larval stages. Such an ability is not surprising as the adult beetles are relatively less prone to desiccation by certain devices such as waxy epicuticle, the tracheal system and the impressive ability of the excretory system to produce vey dry products (Nicolson et al., 1974). The ability to restrict water loss varies. This statement could be further confirmed from the aforementioned results that percent total body water loss in the 6th instar larvae during starvation period ranged from 41.40% to 98.68% while its range in the adult beetles under similar conditions was between 3.61% to 90.97%. Further, loss of body water in the adult beetles was continuous and slow throughout the experimental period. In contrast the 6th istar larvae exhibited abrupt decrease in body water during the first 48 hours of starvation by 54.24% followed by steady reduction of body water in the remaining starved conditions till death occured. Presumably the beetles are well able to regulate their haemolymph composition during desiccation produced under starvation. We have not, however, followed changes in the organic constituents of haemolymph so that we cannot rule out the possibility that such changes contribute to death or that other events are responsible. The results of the present experimentation, therefore, are in accordance with those described by Nicolson *et al.* (1974).

The results of the present study revealed that mean survival time of 6th instar larvae was smaller than those of the adult beetles of *T. castaneum*. Likewise the rate of wet weight loss was greater in the immature stage than in adult stage throughout the

experimental period. Moreover the larvae reduced their wet weight during the experiment. The more rapid larval wet weight loss can be correlated with the higher initial larval mortality than.

The percent dry matter of larvae and adults, however, manifested almost similar pattern of reduction. Both stages decreased their dry weight abruptly during the initial 48 hours of starvation followed by steady reductions. This could be due to the utilization of energy reserves such as trehalose, glycogen, lipids and proteins for sustenance of life during starvation.

The findings of these experiments, therefore, revealed that the relative loss of solids and water (as indicated through the reduction of wet weight and total body water loss) occured at approximately equal rates in the 6th instar larvae and adult beetles during the first 48 hours of starvation, and thereafter all these parameters revealed gradual reduction till death. The increase with time of percent dry matter and percent weight loss prior to death presumably suggests the failure of water conserving mechanism in larvae than adults under stress conditions induced by total starvation. Dahlman (1973) also reported similar results in one of his studies in connection with the effects of starvation on survival, weight loss and percent dry weight loss of larvae of tobacco hornworm, *Manduca sexta*.

The results of similar experiment reported earlier from this lab (Saleem and Shakoori, 1993) revealed that 10 day old adult beetles, when kept under starved conditions at 30±1 °C and 60% r.h., survived longer *i.e.* up to 34 days. In contrast 30 day old beetles survived up to 18 days when kept at 35 ^{0}C and r.h. of 75%. This could be either attributed to the age of beetles (as younger beetles survived longer than elders) or to the temperature requirements (as the beetles sustained longer at the optimum temperature of 30 °C than 35 °C). Requirements of optimum temperature of 30 °C was confirmed from another experiment also reported from this laboratory. (Saleem and Shakoori, 1986), where the 6th instar larvae survived longer at this temperature than at 35 °C. Likewise age of the beetles may also play an important role in their survival as the 10 days old beetles may possess more energy reserves than those of elder ones for the sustenance of life. In a similar study Gray (1948) described that several factors affect the rapidity of growth of flour beetles, the more important of which are temperataure, relative humidity and the food medium. He further reported that temperature is probably the most important factor, while relative humidity has little effects than temperature (Gray, 1948). Holsapple and Florentine (1972) reported about the thermal perception of red flour beetle, *T. castaneum*. Likewise Edwards (1976) carried out experiments on age of *T. castaneum* and correlated it with its susceptibility against synthetic juvenile hormone and Heller-Haupt and Varma (1982) studied the effect of age on susceptibility of two species of African ticks (*Ixodidae*) to synthetic pyrithroid insecticides.

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