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Influence of Food Quality and Quantity on Cannibalistic Behavior in *Tribolium Castaneum* Adults Towards Larvae and Their Attraction to Preferred Food Sources

Hasnaa Talib Khudhier[1], Huda Noori Jawad[2], Hussain Ali Jabbar[3]
[1] Dept. of Biology, College of Science, University of Basrah, Iraq
[2] Dept. of Biology, College of Science, University of Basrah, Iraq
[3] Dept. of plant protection, Basrah Agriculture Directorate, Iraq

Email: huda.noori@uobasrah.edu.iq

Abstract. The current study aims to scrutinize the qualitative and quantitative impacts of three essential food types which are wheat flour, dry milk, and mays starch on the cannibalism process of the adult Tribolium castaneum and their larvae, and to identify the food type that is preferable for trapping and attracting adults during different periods of time. The results of the main effects showed that there are significant differences between the three food types in regard to their impact on the average survival rate. The average of surviving larvae is ranged from 9.3 larvae for wheat flour to 2.6 and 2.3 larvae for dry milk and mays starch treatments, respectively. The highest larval survival was at a concentration of 5 grams of wheat flour, while the lowest survival appeared to be at a concentration of 0.2 grams of dry milk and mays starch. During the first week, the highest number of surviving larvae was recorded, whereas the lowest one was observed in the fourth week; the survival rates were 7.0 and 2.0 respectively. In respect with the findings of the trapping and attraction of adult insects, wheat flour appeared to be the most attractive food type for adults, with an average of 50.2 insects if compared to 5.0 and 2.7 insects for dry milk and mays starch treatments, respectively. As for the average effect of time periods, the highest attraction took place on day five, with an average of 25.7 insects, while the lowest was recorded on day one, with an average of 10.6 insects. It was noticed that there was variation of the interaction effect; the highest average was observed on day five, while wheat flour treatment attracted 70.3 insects. On the contrary, the lowest effect for mays starch and dry milk treatments were recorded on the 4th and 5th days, with only an average of 2.5 insects attracted. Such findings highlight the significance of food type and food quantity in modifying predation behavior and adult attraction of Tribolium castaneum.

Highlights:

1. Survival: Wheat flour supports highest larval survival; dry milk and starch lowest.
2. Attraction: Wheat flour attracts most adults, peaking at 70.3 insects on day five.
3. Impact: Food type and quantity influence predation and adult attraction behavior.

Keywords: E Cannibalism, Tribolium castaneum, , Flour condition.

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Introduction

The red flour beetle Tribolium castaneum (Herbst) is considered as one of the most common pests infesting stored flour mills under adverse conditions. The insect belongs to the order Coleoptera and family is the Tenebrionidae [1]. The losses of stored food and their products can be attributed to variety reasons, the most significant of which being insect pests infestation [2]. Storage pests play a significant role in the deterioration and decay of wheat, which is avital worldwide, that is stored and consumed as flour [3]. Furthermore, dry milk and mays starch are considered basis nutritional staples for humans [4]. It is regarded as a secondary pest widely distributed in warm storage area, it is seldom found in intact grains. It predominantly colonizes flour dust derived from damaged grains, which have been compromised as a result of primary insect infestation [5]. In both its adults and larval stages, the insect consumes wheat flour, recognized for producing high – quality bread rich in protein, carbohydrates, essential vitamins and minerals [6]. In contrast, there are food substances that repel the insects, such as the spices, black peppercorn, laurel leaves and others [1,7].

The insect's larval stage is regarded the most harmful, the longest phase of its existence, and the most active and voracious feeder [8]. Older larvae can consume whole and intact grains, such as sesame seeds, resulting in both qualitative and quantitative losses [9]. The presence of the insect in all stages on infected grains and flour causes major damage since it gives the flour a harsh odor and a bitter flavor [10], It additionally secretes carcinogenic and toxic chemical compounds such as quinine, as well as corpses, feces, gaseous secretions, and shells from repeated molts [11], which promote the growth of many fungi, causing flour to spoil and turn gray, rendering it unfit for human and animal consumption [12]. [13] confirmed that severe infestations of the insect lead to the loss of nearly half a billion tons of globally stored grains. The numerical density and severity of the red flour beetle infestation are related to many physical factors that affect the growth and vitality of this insect, including temperature, relative humidity, light, competition, and type of food [14]. [15] indicated that the type of food has a significant impact on the insect's biology; as the protein content of the food increases, the numerical density of the insect also increases. The growth and reproduction of the insect are also affected by the duration and conditions of product storage [16]. Additionally, the insect is affected by competition for food as competition for food

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increases and the surrounding conditions become harsh, the insect's behavior towards self-cannibalism of its castes increases.

It is a process that occurs when the insect is exposed to harsh conditions represented by a lack of food [17] or being in a stressful food environment, which drives the insect to this behavior to withstand those conditions and survive until a suitable food environment becomes available [8,18].

As declared by the study of [19] that the condition of Flour has a nutritional property that are distinguished by elements which are rich in carbohydrates, proteins, and essential enzymes that form a balanced mixture for the beetle's growth and reproduction, such as gluten and starch, which support the stages of development of the beetle larvae and its life cycle better than other food types. Studies have also revealed that the beetle's life cycle is shorter in the flour environment, which adds to higher reproduction rates and population density. The physical properties of wheat flour, such as moisture and pH, where wheat flour retains high moisture and appropriate acidity, enhance enzymatic activity and metabolic processes, thereby encouraging the beetle's survival and continuing reproduction, as opposed to its survival in a dry environment like dry milk or mays starch.

The Aims of the Study

This study attempts to arrive at the following objectives:

1. The study aimed to determine the preferred type of food for the insect reared on three types of food materials (wheat flour, dry milk, and mays starch) using the cannibalism process, as well as to understand how the insect behaves when it is starving by consuming its larvae, which is considered the most dangerous stage.
2. The study also sought to clarify the mechanism of attraction by employing sticky traps to assess insect food preferences by counting the number of attracted beetles to three different food samples and determining the type of food that repels the insect, highlighting the possibility of the insect infesting two important types of food, dry milk and mays starch

Methods

The process of sampling collection involves the following procedures:

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1. Identifying and breeding insects: flour beetles of contaminated flour from shop, bakeries and houses were collected. Prof. Dr. Kazem Saleh Al-Hadlak of the Department of biology, College of Science, University of Basra, identified the chosen study species, the red flour beetle Tribolium castaneum. The insects were grown in the insect research laboratory, department of biology, in one-liter plastic bottles containing 500 grams of white flour each. The bottles were covered with light fabric with fine openings to allow air in and allow the insects to breathe, and then secured with rubber bands to prevent them from escaping. The upbringing was carried out under standard laboratory circumstances so that they might be used in the study and research experiments.
2. Sampling collection was done by using the sieving method by means of which the holes of the sieve was tiny the adults and larvae of the Tribolium castaneum insect to pass through it.
3. Preparation of food samples: -

   In line with the method of [20], the three types of food samples necessary for the study (wheat flour, dry milk, mays starch) were prepared and placed in the freezer for 24 hours at a temperature of (-20) to ensure their sterilization and safety from all potential insect stages.
4. Distribution of adults and larvae on the three types of food:

   The 81 plastic containers were used with a capacity of (100) gram and dimension (5x5) cm and put them into three groups, each holding a different sort of food. Each type of food was divided into 27 plastic containers with three concentrations (5, 2.5, and 0.5 grams). Each concentration consisted of three treatments: the first (5 larvae + 5 adults), the second (10 larvae + 10 adults), and the third (15 larvae +1 5 adults). The adults had previously been starved, and all containers were held for one month at laboratory temperatures (26 ±2 – 30 ±2) C◦ and humidity (60 ±5 – 65 ±4 %). Weekly monitoring was carried out to count and report the number of larvae devoured in each treatment.
5. Sticky traps were distributed in three groups and locations around the department of biology laboratories, under controlled temperature and

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humidity conditions. Each group had three traps, each carrying a different type of food (wheat flour, dry milk and mays starch), for a total of nine traps [21] The insect's appealing and repelling meals were determined by releasing huge numbers of adult red flour beetles from a laboratory-reared farm into the sticky traps, with an identical spacing of 50 cm between each trap. The quantity of insects in each type of food was counted and recorded on a daily basis for five days. Each of the three groups was deemed a replicate for the experiment, with fresh.

Result and Discussion

Statistical analysis results of Table No. (1) indicates that there are significant differences ($p<0.05$, $df=107$, $F=128.1$) in the effect of food quality on Cannibalism of the larvae by adults of Tribolium castaneum, as the highest survival mean reached 9.3 larvae when feeding on wheat flour, with a significant difference from the dry milk and mays starch treatments, which reached 2.6 and 2.3 larvae, respectively. As for the effect of the time period ($p<0.05$, $df=3$, $F=1131.1$), the highest survival mean reached 7.0 larvae in the first week and the lowest rate was 2.0 larvae in the fourth week.

Table (1) Effect of food quality and quantity on Cannibalism of the larvae by adults of T. castaneum

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food quality	Food quantity/g	Added larvae	Remaining larvae Time/week				Mean food quality
			1St	2Sd	3Th	4Th	9.3
Wheat flour	0.2	15	11.3	8.3	6.3	3.6	
		10	8.6	7.3	5	2.3	
		5	5.0	4.6	3.6	3.0	
	Mean		8.3	6.7	5.0	3.0	
	2	15	14.3	12.6	8.6	8.0	
		10	10.0	9.3	7.0	6.6	
		5	5.0	5.0	5.0	4.6	
	Mean		9.7	9.0	6.8	6.4	
	5	15	15	14.6	14.3	13.6	
		10	10.0	9.6	9.3	9.0	
		5	5.0	5.0	5.0	5.0	
	Mean		10.0	9.7	9.5	9.2	
Dry milk	0.2	15	7.6	4.0	9.7	0	2.6
		10	6.0	3.3	7.1	0	
		5	3.0	1.6	4.5	0	
	Mean		5.5	3	7.1	0	
	2	15	8.6	3.6	0	0	
		10	5.6	3	0	0	
		5	2.6	1.6	0.3	0	
	Mean		5.6	2.7	0.1	0	
	5	15	8.6	5.0	0.3	0	
		10	7.3	3.3	0.6	0	
		5	3.3	2.6	0.6	0	
	Mean		6.4	3.6	0.5	0	
mays starch	0.2	15	7.3	3.0	0.3	0	
		10	5.3	3.3	0.0	0	2.3
		5	3.3	1.6	0.6	0	
	Mean		5.3	2.6	0.3	0	
	2	15	8.0	3.6	0.2	0	
		10	6.0	2.6	0.2	0	
		5	3.7	2.7	0.5	0	
	Mean		5.7	3.0	0.3	0	
	5	15	8.0	3.6	0	0	
		10	7.0	3.0	0	0	

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5	3.5	2.5	0.3	0
	6.5	3.4	0.1	0
Mean				
Mean /Week	7.0	4.8	2.5	2.0

Figure (1) shows significant differences ($p<0.05$, $df=4$, $F=98.7$) in the effect of food quality and quantity on Cannibalism of the larvae by adults of Tribolium castaneum, with the highest survival mean reaching 9.6 larvae when fed 5 g. of wheat flour and the lowest survival mean reaching 2.0 larvae when fed 0. 2 g. of mays starch.

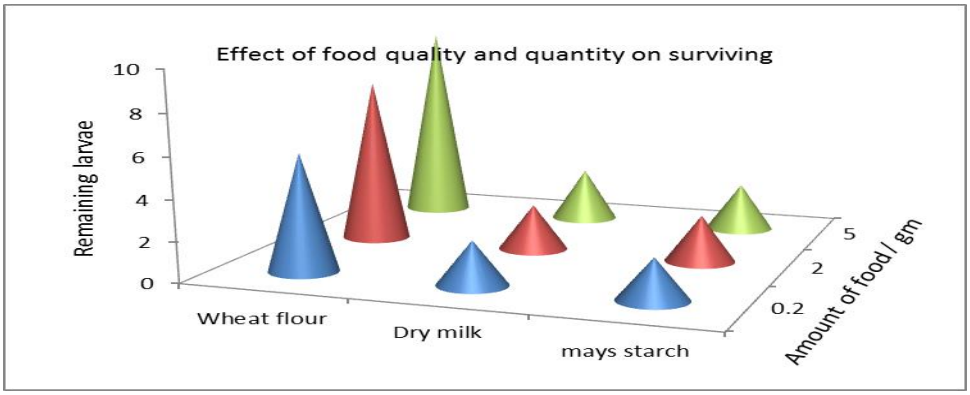

Figure (1) The effect of food quantity on Cannibalism of the larvae by adults of Tribolium castaneum

Figure (2) shows the effect of food quality and insect density on Cannibalism of the larvae by adults of Tribolium castaneum, ($p<0.05$, $df=4$, $F=182.7$) with the highest survival mean of 10.9 larvae when there were 15 insects in the wheat flour treatment and the lowest survival mean of 1.3 larvae when there were 5 larvae in the dry milk treatment.

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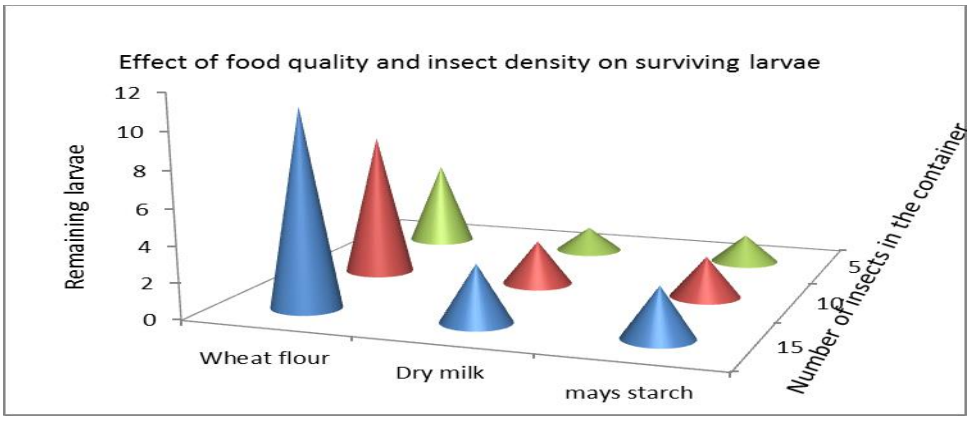

Figure (2) The effect of food quality and insect density on cannibalism of the larvae by adults of Tribolium castaneum

Table (2) Effect of food quality on insects caught through a period of time

Food Days	Wheat flour	Dry milk	Mays starch	Mean
1	23.6	5.0	3.3	10.6
2	40.6	5.0	3.3	16.3
3	53.6	5.3	3.0	20.6
4	63.0	5.0	2.0	23.3
5	70.3	5.0	2.0	25.7
Mean	50.2	5.0	2.7	19.3

Lsd.(0.05)to food=2.9 , time=5.0 , interaction =7.1

Effect of food quality on insects catch through a period of time

The results of the statistical analysis in Table (2) indicate that there are significant differences in the effect of food quality and time period on insect hunting, where the highest mean was 50.2 insects for the wheat flour treatment and the lowest mean was 2.7 insects for the mays starch treatment. As for the effect of time period, the highest mean was 25.7 insects on the fifth day and the lowest mean was 10.6 insects on the first day, while the effect of interaction reached the highest mean of 70.3 insects on the

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fifth day of the wheat flour treatment and the lowest mean of 2.0 insects per day on the fourth and fifth days of the corn treatment.

Discussion

According to the results provided in Table (1), wheat flour provides the most favorable nutritional environment for the growth, reproduction, and rearing of adult red flour beetles, T. castaneum, as well as their larval stage [22, 15]. This habitat resulted in the larvae remaining to uneaten by the adult beetles, with a survival rate of 9.3 larvae, as opposed to growing them in two alternative nutritional settings, one containing dry milk and the other having mays starch, with survival rates of 2.6 and 2.3, respectively. The statistical evidence revealed no significant difference between the two substances, dry milk and corn starch, in terms of their effects on the phenomena of adult insects cannibalism larvae. The results also revealed that raising the insect in its adult and larval stages on these two substances (dry milk and mays starch) caused the adult insects to devour their larvae in a significant and noticeable manner, indicating that these two substances are not preferred by the insect, so the insect resorts to devouring its stages in order to survive and continue life. This goes in line with the findings of [23,8], who reported that the process of devouring the red flour beetle T. castanum increases in flour that is conditioned with repellents as compared to fresh flour.

[24] confirmed that the red flour beetle's survival time and the increase of population density are based on its presence in a favorable environment having a desirable nutritional content for the insect, as well as the temperature and humidity of the environment. [25,11] noticed that most beetles favored second-grade flour for feeding, then first-grade flour, Madia, and bran. [26] talked about the effect of the molecular size of corn flour types on the population density of similar flour beetles; they also confirmed that the effect was high in coarse corn flour with a high rate of loss of the material's molecular size, while the density and loss rate decreased in soft and white corn flours.

The effect of food type and time period on the trapping and attraction of red flour beetles with sticky traps are shown in table 2. The study showed that there were significant differences in the average number of trapped insects for the three food types which are wheat flour, dry milk, and mays starch; a finding that indicates the beetle's preference and attraction to the wheat flour treatment, with an average of (50.2) insects

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compared to the dry milk and mays starch treatments which had the lowest average number of insects trapped, with an average of (2.7). This shows that wheat flour is the most desired and appealing material to the beetle, which is consistent with [27,]. This demonstrated that wheat flour had the largest numerical density of the red flour beetle, making it the optimum material for feeding and growing the insect's numbers, as opposed to other items such as bulgur, grains, cracked wheat, semolina, and bulgur, which had the lowest numerical density. The current study looked at dry milk and mays starch as repellents and less desirable materials for the insect. In study conducted by [26,27] to understand the development and survival of the confused flour beetle on 14 different types of food, including flour, spices, nuts, and various medicinal plants, it was discovered that mint, garlic, rue, thyme, rosemary, and eucalyptus are insect-repelling substances that caused the beetle to stop feeding and eventually die. As reflected in the statistics shown in table (2), the time period influences average insect capture and attraction rates, the highest average (25.7 insects) was recorded on the fifth day, while the lowest average (10.6 insects) occurred on the first day. The interaction effect produced the highest average (70.3 insects) on the fifth day for the wheat flour treatment, and the lowest average (2.0 and 5.0 insects) on the fourth and fifth days for the mays starch and dry milk treatments respectively. This indicates that the time period has its own effect on the average number of the insects which are trapped and attracted to the wheat flour treatment. As the time period increases, the number of insects that ate attracted to wheat flour increases too; they increase more than the dry milk and mays starch treatments over a longer length of time [15]. The results shown in figure (1) express how food quality and quantity having an effect on cannibalism of adult red flour beetle (Tribolium castaneum) larvae. The results indicated that the maximum larval survival rate was 9.6 larvae if treated with 5 grams of wheat flour. And the lowest was 2.0 larvae when treated with 0.5 grams of mays starch and dry milk. The statistical study revealed substantial variations in the quality and quantity of wheat flour on larval survival rate when compared to mays starch and dry milk which showed no significant changes between them. During the consumption period, the larvae consumed the most wheat flour, demonstrating a concentration effect the concentration began at 0.5 grams, then decreased to 2 grams, and finally to 5 grams of wheat flour [5]. [19] investigated the phenomenon of cannibalism in the larvae of the stray insect flour beetle, Tribolium

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confusum, when raised on a mixture comprising whole flour and yeast, as well as starving them. They confirmed that the occurrence of adult insects preying on their stages and larvae feeding on eggs, pupae, and each other was more noticeable when they were famished and food was scarce. The current results in Figure (2) showed that the storage period had an effect on the survival rate of larvae, as it reached its highest survival rate during the first week of storage and began to decrease during the second week and then the third week until it recorded the lowest survival rate in the fourth week of storage, where the effect was clear in the phenomenon of adults devouring larvae during a longer storage period, which is consistent with the study of [22]. The most recent results revealed no significant changes in the amount of mays starch and dry milk on the phenomena of larval cannibalism by adults, however storage time had a definite influence on cannibalism in these two materials.

In a study by [15], it was observed that intraspecific self-predation is a natural behavior that occurs in certain biological communities, but only under conditions of severe stress, such as extreme food scarcity, lack of an optimal food environment, intense competition, and harsh breeding conditions. Similarly, [25] noted that the self-predation behavior in the red flour beetle T.castaneum increased significantly when raised in an environment with oat flour, which was considered a stressful and inadequate medium for rearing the insect. In contrast, survival rates were higher and self-predation decreased when the beetles were reared in an environment containing wheat

Conclusion

The study carried out by defining the phenomena of cannibalism in the rusty grain beetle flour and comprehending its attraction to the type of food it favors the most, with substantial variations discovered between the three forms of food: wheat flour, mays starch, and dry milk. The biostatistical analysis showed that there were significant differences in regard to the effect of food quality on Cannibalism of the larvae by adults of Tribolium castaneum. The analysis also demonstrated that there are substantial variations in the quality and amount of wheat flour, as well as its influence on preventing cannibalization of larvae when compared to alternative food sources. Not only this but it also verified that cannibalism is a normal process observed in biological communities due to a severe nutritional deficit. Based on such statistical background, it could be

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concluded that food type and food quantity have significant effect on modifying predation behavior and adult attraction of Tribolium castaneum. The results also indicated that Tribolium castaneum had attraction towards its favorite food which was wheat flour. Meanwhile, it had less attraction towards other types of food which were dry milk and mays starch

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