



The effect of different flours on *Tribolium confusum* and the effect of infestation on various food parameters

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Abstract

Tribolium confusum is a common associated pest that can be infested in a wide range of preserved products. The infestations causes to a significant loss of the food product. When infested six different food products (wheat 82%, El-Fayrooz white maize, crushed white maize, crushed barley, crushed oats, and crushed sorghum) by the confused flour beetle *T. confusum*. Crushed barley had the largest significant means of total *T. confusum* populations (larvae, pupa, and adult) with (200 individuals) followed by El-fayraoos white maize and wheat flour 82%. Conversely, Crushed oat and crushed sorghum had the lowest significant means of total populations (67 individuals/40 gm flour). Crushed barley flour had the highest percentage of weight loss (30.83%), while crushed sorghum and oat flours had the least weight loss. The findings reveal that insect populations and weight loss have a significant positive correlation. On other hand, the infestation effects on nutritional parameters of products, shown the moisture and ash of all infected samples were higher than the control. Crushed barley in control and infested samples had the highest percentage of ash, while wheat flour had the lowest. The infested samples, on the other hand, had a lower lipid content than the control samples; In comparison to the control, the protein level of all infested samples. This rise could be attributed to the nitrogenous waste left behind by insects. The highest percentage of carbohydrates loss in the infested samples was in crushed barley (85.43%); this could be due to that it was the most infested sample.

Keywords: *Tribolium confusum*, population's infestation, weight loss, chemical composition

Introduction

Tribolium confusum (Herbst) is the confused flour beetle an important secondary pest on stored grain products. It infests a wide range of crushed grains, including flours, nuts, biscuits, chocolate, peas, spices, and dried fruits. Because of its great reproduction capacity, it caused significant losses of stored products [1]. It is common in many places: homes, factories, mills, storage containers, warehouses, etc [2].

Infested flour has a greyish color and is moldy [3]. *Tribolium confusum*, a Tenebrionid flour beetle, has odor glands known as "melanotic stink glands," responsible for Quinoid secretions in the stored food they infect [3, 4]. These secretions have been shown to cause cancer in both people and animals. Hepatocellular carcinomas were caused by eating cookies manufactured with *Tribolium castaneum* infested flour [5]. Many fungi are related to whole flour that has been infested by the flour beetle [6].

The quality of food in terms of its content from carbohydrates, proteins, and other nutrients elements affects the development of beetles [7-11]. The products (wheat, white, maize, barley, oats, and sorghum) used in the research are of great economic importance to humans and animals. The use of wheat is directly related to endeavors by humans to avoid starvation, keep control of their food supplies and provide humans with nutrition and critical elements. Wheat is a primary ingredient of several processed foods [12]. It contains 75%–80% carbohydrates, 9%–18% protein, fiber, many vitamins (particularly B vitamins), calcium, iron, and many macro- and micro-nutrients. With a protein percentage of approximately 13%, it is the most important source of vegetable protein in human meals.

Barley is the 4th most essential cereal crop after wheat, rice and maize. It has a worldwide distribution and is extremely important in the production of animal feed and alcohol. Compared to other grains, it provides many health benefits since they contain a lot of antioxidants polyphenols. This is attributable in part to the dense high content of fiber-glucan. In addition to being used for brewing, barley has recently been used in human meals such as breakfast cereals, pasta, noodles, soups ect [13-15]. Oats are a source of high-value compounds such as beta-D-glucan for animal feed and human consumption.

Oats' popularity as a nutritious meal has improved. The traditional use of human diets in many nations has changed, which led to an increase in oat use and the goods made from them [16, 17].

The rate of loss from poor storage is more than 50 percent of the crop, especially in developing countries as a result of the less or absent of safe storage procedures in regions with hot climates and among retailers. Furthermore, the necessity of these crops as a major daily component of nutrition, whether for humans or animals, it represents the food security of any country and with the absence of food culture, the seriousness of this pest and its harmful health effects, this study was conducted.

This study aims to shed light on the harmful economic and nutritional effects resulting from infestation with this serious pest.

Material and Methods

Insect Source

The *Tribolium confusum* adults were collected from a stock culture maintained at the Department of Plant Protection, Fayoum University

Source of Flour and Grains

For the experiment, six different flours were tested, including whole wheat flour (*Triticum aestivum* L.) with an extraction rate of 82%. It is produced at the Arab Republic of Egypt's mills and delivered to bakers. It is the Egyptian people's main source of daily bread. Commercial available white maize flour (El-Fayrooz) packaged and produced by El-Fayrooz Company for Food Industries, Arab Republic of Egypt, is widely used by housewives to manufacture bread and cakes and the work of various seasonings to cover meat and many kitchen uses. The grass family Poaceae includes white maize (*Zea mays* L.), barley (*Hordeum vulgare* L.), oat (*Avena sativa* L.), and sorghum (*Sorghum bicolor*). Grains were purchased from a feed store (Local Market) in Egypt's Fayoum governorate. They were crushed by an electric grinder.

Experiment Technique

In little glass jars (5 cm diameter and 12 cm length), 40 gm per each flour were packed. After that, ten pairs of adult mixed-sex beetles were put into each jar. The muslin cloth and rubber band have been used to cover the jars; three replicates of each flour, as well as a control jars without an insects, were created. The jars were incubated at $(28 \pm 2^{\circ}\text{C})$ in the laboratory incubator for three months of storage.

The jars containing each of the flours were sieved thoroughly at the finale of the storage time to separate the several stages of *T. confusum*, and they were counted.

Weight Loss of all Type Flours by Populations of *T. confusum*

The weight loss was checked after three months. The electric scale was used to weigh all types of flour at the start of experiment and again at the end of the storage period. To calculate the amount of weight consumed by the wider populations *T. confusum* ^[18] formula was used:

Weight loss = (Initial weight – Final weight) / Initial weight \times 100.

Otherwise; the different flours were processed so that the chemical composition could be determined.

Rate of Emergence Adults of *T. confusum*

Percentages of adults' emergence of *T. confusum* were calculated using the formula:

% Adults Emergence = (Sum of Adults / Sum Populations) \times 100

Chemical Composition

Following the method described by ^[19], were measured all of moisture, protein, fat, ash, and fiber content. The difference was used to compute carbohydrate content using the equation following.

$$\text{NFE (\%)} = 100 - (\% \text{ Moisture} + \% \text{ Protein} + \% \text{ Fat} + \% \text{ Ash} + \% \text{ fiber}).$$

Total Starch by Phenol-Sulfuric Acid Method, According to ^[20], starch reacts with a strong acid and heat to produce furan derivatives, which condense with phenol to form constant yellow-gold molecules that can be detected spectrophotometric ally at 490 nm.

Titrateable acidity: To assess total titrateable acidity, 10 g of powdered grains were added into the flask containing 90 mL of distilled water and quickly agitated for 30 minutes. After that, 0.5 mL of 1.0 % phenolphthalein indicators reagent was added, followed by titration with 0.1 M NaOH. The time it took for a pink tint to persevere for 30 seconds was used to assess the acidity.

Data Analysis

To find the least significant differences, the number of larvae, pupae, and adults that emerged from each of the six flours was analysed using the Duncan test at the 0.05 probability level. The mean and standard errors (SE) are shown for each value.

Chemical composition data: moisture, protein, fat, ash content, carbohydrate, and titrateable acidity obtained were statistically analyzed using one-way analysis of variance with Tukey's post hoc test was used to compare treatment means at 0.05 significant levels.

Furthermore, the data mentioned above were also subjected to correlation to identify any relationship between the chemical composition of the cereal flours and the biological activity of *T. confusum*. All analyses were conduct by using Statistical Software (spss) Version 21.0

Results and Discussion

Effect of different types of flours on *T. confusum*

T. confusum population growth in different flours after three months of storage

The illustrated and tabulated results (Table 1 & Fig., 1) showed high differences in the mean numbers of the beetle *T. confusum* stages for the six treatments (six different flour types), an average of 10 pairs of the beetle/40gm flour after storage for three months of infection.

The highest significant mean numbers of confused beetle larvae were recorded on El-fayraoz white maize and crushed barley flour 79.33 & 75.67 followed by wheat flour 82% and lowest significant mean numbers of larvae were recorded on crushed white maize, crushed oat, and crushed sorghum crushed flours, range of 25.33 to 22.33 larvae/ 40gm flour. The larvae mortality was little in all treatments.

The growth of the pupal stage of *T. confusum* in the different flour types shows that there was no significant difference between crushed barley, crushed white maize, and El-fayraoz white maize, but differ significantly higher from the sorghum, wheat, and oats flours.

Significant differences were found in the mean numbers of adult *T. confusum* observed on crushed barley flour compared to other types of flour tested that recorded 200 insect / 40gm flour followed by wheat flour with 139 insects, showing the lowest significance of adults on crushed oat flour. Highly significant means of adult mortality of confused beetle on crushed barley flour with 15.33 insect / 40gm flour after three months infestations were observed. However, types of flours studied did not have a significant difference in adult mortality. The total population of *T. confusum* among different types of flours, the highest significant numbers of populations (200 individuals) was observed on barley flour compared to other types of flour tested, followed by El-Fayrooz white maize flour and wheat flour 82% where there were no statistical differences between both, but the oat flour recorded the lowest population with a mean 67 individuals /40 gm flour. The highest mean numbers of the total mortality of the confused beetle was shown in barley at 16.33 individuals; other flours studied had the lowest mortality rates, with no significant differences among them.

Table 1: Mean \pm (SE) at $P < 0.05$ of population of *T. confusum* reared on different products after three months of infestation.

Treatments	Mean of larvae		Mean of pupae	Mean of adult		Mean of total population	
	survive	Mortality		survive	Mortality	survive	Mortality
Control	ND	ND	ND	ND	ND	ND	ND
Wheat flour 82%	55.33 ^b \pm 4.33	0.33 ^a \pm 4.33	3.67 ^b \pm 1.03	139 ^b \pm 5.06	2.67 ^b \pm 5.06	198 ^b \pm 6.51	3 ^b \pm 6.51
El-Fayrooz white maize flour	79.33 ^a \pm 4.33	1.33 ^a \pm 4.33	11.66 ^a \pm 1.03	116 ^c \pm 5.06	3.33 ^b \pm 5.06	207 ^b \pm 6.51	4.67 ^b \pm 6.51
Crushed white maize	25.33 ^c \pm 4.33	1.66 ^a \pm 4.33	12 ^a \pm 1.03	92.67 ^d \pm 5.06	3.33 ^b \pm 5.06	130 ^c \pm 6.51	5 ^b \pm 6.51
Crushed barley	75.67 ^a \pm 4.33	1 ^a \pm 4.33	13.33 ^a \pm 1.03	200 ^a \pm 5.06	15.33 ^a \pm 5.06	289 ^a \pm 6.51	16.33 ^a \pm 6.51
Crushed oats	22.67 ^c \pm 4.33	2 ^a \pm 4.33	2.67 ^b \pm 1.03	42.33 ^f \pm 5.06	1.67 ^b \pm 5.06	67 ^e \pm 6.51	3.67 ^b \pm 6.51
Crushed sorghum	22.33 ^c \pm 4.33	0	4 ^b \pm 1.03	71.67 ^e \pm 5.06	2 ^b \pm 5.06	98 ^d \pm 6.51	2 ^b \pm 6.51
Duncan "F"	24.04**		13.77**	73.36**		102.32**	

Each mean (mean \pm SE) is based on three replications. (a,b,c,d) letters refer the means significant with the same column. ** refer to significant of "F-Test" with the same column

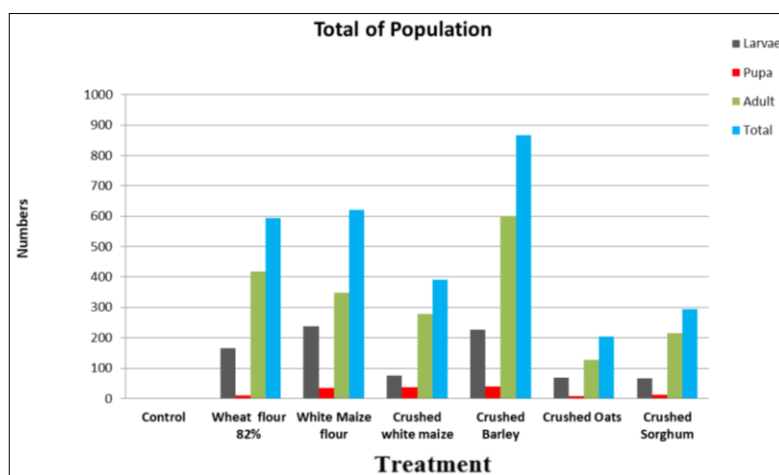


Fig 1: Total individual numbers of populations of *T. confusum* reared on different products after three months of infestation.

Rate of emergence adult of *T. confusum* at the different diets

Table 2 shows that adult emergence is high 73.13%, 71.28%, & 70.20% in crushed sorghum, white maize, and wheat, respectively, followed by crushed barley and oats flour, while El-Fayrooz white maize recorded the lowest percentage of adult emergence with 56.04%. It is clear from the above that although crushed barley and El-Fayrooz white maize flours recorded the highest means numbers of confused beetle, but crushed sorghum flour had the highest adult emergence, because it gives faster growth rates in the emergence of adults. In contrast, El-Fayrooz white maize flour had the lowest emergence of adults.

Table 2: Total individual numbers of populations of *T. confusum* reared on different products after three months of infestation.

Treatments	No. of larvae		No. of pupae	No. of adult		Total population		% Emergence adult
	survive	Mortality		survive	Mortality	survive	Mortality	
Control	ND	ND	ND	ND	ND	ND	ND	ND
Wheat flour 82%	166	1	11	417	8	594	9	70.20
El-Fayrooz white maize flour	238	4	35	348	10	621	14	56.04
Crushed white maize	76	5	36	278	10	390	15	71.28
Crushed barley	227	3	40	600	46	867	49	69.2
Crushed oats	68	6	8	127	5	203	11	62.56
Crushed sorghum	67	0	12	215	6	294	6	73.13

Weight loss (%) in the different products by confused beetle

Results shown in (Tables 3 & 5) indicate that after a storage period of 3 months, the weight loss by *T. confusum* on different types of products occurred more in crushed barley flour with a mean 12.33 gm percentage 30.83% while El-Fayrooz white maize, wheat 82%, and crushed maize were recorded moderate percentages of weight loss of 19.17%, 11.67%, & 11.67% respectively. On the other hand, the minimum weight loss was noted in crushed sorghum and oats flours with 3.33 & 2.67 gm/40gm flour.

The results in Table 5 shown insect populations and weight loss have significant positive correlations of 0.829, 0.897, and 0.926 for larval, adult, and total populations of *T. confusum*, respectively.

It is worth noting that there is no weight loss in the control because no insect infestations were recorded in the control at the end of the storage period.

Table 3: Weight loss in different products by confused beetle infestation (a,b,c,d) letters refer the means significant with the same column.

Product	Standard weight	weight loss (gm)	Mean \pm SE	% weight loss
Control	120	0	0	0
Wheat flour 82%	120	14	4.67 ^{bc} \pm 0.1	11.67 %
El-Fayrooz white maize flour	120	23	7.67 ^b \pm 0.1	19.17 %
Crushed white maize	120	14	4.67 ^{bc} \pm 0.1	11.67 %
Crushed barley	120	37	12.33 ^a \pm 0.1	30.83 %
Crushed oats	120	8	2.67 ^c \pm 0.1	6.67 %
Crushed sorghum	120	10	3.33 ^c \pm 0.1	8.33 %

Table 4: Mean \pm (SE) at P<0.05 of chemical composition & acidity of different products after three months infestation by *T. confusum*.

Type	Status	Ash	Lipid	Protein	Fiber	Carbohydrate	Starch	Starch loss ratio %	Moisture	Titrateable acidity
Wheat flour 82%	Control	0.780 \pm 0.09	1.79 \pm 0.14	7.62 \pm 0.32	11.63 \pm 0.415	66.33	42.13	61.24	11.85 \pm 0.28	15.94 \pm 1.02
	Infested	0.683 \pm 0.02	1.36 \pm 0.36	9.68 \pm 0.43	6.46 \pm 1.066	74.14	25.80		7.68 \pm 0.22	87.32 \pm 0.78
El-Fayrooz white Maize flour	Control	1.50 \pm 0.09	1.68 \pm 0.05	12.43 \pm 0.73	4.01 \pm 0.415	71.41	42.70	55.17	8.96 \pm 0.43	42.21 \pm 0.83
	Infested	1.22 \pm 0.03	1.40 \pm 0.03	15.19 \pm 0.52	7.68 \pm 1.066	66.87	23.56		7.63 \pm 0.56	133.58 \pm 1.69
Crushed white maize	Control	1.22 \pm 0.05	2.21 \pm 0.19	13.48 \pm 0.63	10.82 \pm 0.415	63.53	60.75	47.45	8.74 \pm 0.32	44.61 \pm 0.71
	Infested	1.07 \pm 0.15	1.65 \pm 0.08	15.86 \pm 0.41	16.31 \pm 1.066	57.63	32.72		7.48 \pm 0.24	84.48 \pm 0.82
Crushed barley	Control	2.37 \pm 0.09	2.23 \pm 0.14	10.46 \pm 0.42	4.45 \pm 0.415	72.7	50.79	85.43	7.79 \pm 0.29	29.11 \pm 0.91
	Infested	2.02 \pm	1.88 \pm	15.63 \pm	22.18 \pm	51.21	43.39		7.08 \pm	137.12 \pm

		0.13	0.13	0.67	1.066				0.28	0.68
Crushed oats	Control	1.90 ± 0.02	1.29 ± 0.06	12.46 ± 0.42	16.14 ± 0.415	58.53	50.74	53.37	9.68 ± 0.46	21.88 ± 1.00
	Infested	1.52 ± 0.06	1.096 ± 0.10	18.81 ± 0.68	38.77 ± 1.066	32.31	27.079		7.49 ± 0.27	91.31 ± 1.3
Crushed sorghum	Control	1.55 ± 0.03	2.76 ± 0.12	10.90 ± 0.65	3.63 ± 0.415	72.43	68.97	64.87	8.73 ± 0.25	42.84 ± 0.68
	Infested	1.26 ± 0.12	2.49 ± 0.09	15.13 ± 0.39	19.94 ± 1.066	53.88	39.41		7.30 ± 0.27	82.17 ± 0.85

Table 5: Simple correlation (r) between means populations of confused beetle *T. confusum* and chemical composition & weight loss of after three months of infestation

Treatments	Ash	Fat	Protein	fiber	Moisture	Carbohydrate	Starch	Acidity	weight loss
Larvae (r)=	0.194	- 0.167	- 0.300	- 0.506	- 0.086	0.583	- 0.707	- 0.110	0.829*
Adult (r) =	0.221	0.196	- 0.479	- 0.469	- 0.151	0.595	- 0.403	- 0.182	0.897*
Total insect (r) =	0.231	0.092	- 0.400	- 0.515	- 0.170	0.621	- 0.509	- 0.127	0.926**

** refer to significant of (r) test with the same column

Effect of flour beetle, *T. confusum* on different products (diets)

Chemical composition and titratable acidity before and after infestation by flour beetle, *T. confusum*

Proximate analysis showed that the contents of moisture, ash, lipid, and protein determined in control and infested samples are shown in Table 4. All infested samples had lower moisture levels than the control.

As was the ash %, the highest ash content was in the control and infested crushed barley samples, and it was 2.37% and 2.02%, respectively, and the lowest ash content was in wheat flour 82%, and it was 0.787 and 0.683, respectively. Whereas the lipid content of the infested samples was reduce than control, which were 1.79%, 1.68%, 2.21%, 2.23%, 1.29, and 2.76% in wheat flour 82%, commercial white maize flour, crushed white maize, crushed barley, crushed oats, and crushed sorghum respectively; it was 1.36%, 1.40%, 1.65%, 1.88%, 1.09%, and 2.49% in those infested samples. Crushed sorghum had the highest lipid percentage (2.76%) and the highest lipid percentage loss (90%), while crushed white maize had the lowest lipid percentage loss (74%). This could be attributed to *T. confusum* consuming a large bit of the lipid in infested samples.

In contrast to the lipid content, all infested samples had higher protein content than the control. This increase may be due to the nitrogenous waste left by the flour beetle, *T. confusum*, and the insect body parts in the samples where the control samples had the lowest percentage of protein, which is 7.62%, 12.43%, 13.48%, 10.46%, 12.46%, and 10.90% in wheat flour 72%, commercial white maize flour, crushed white maize, crushed barley, crushed oats, and crushed sorghum, respectively, while the same infested samples rose to 9.68%, 15.19%, 15.86%, 15%, 63%, 18.81%, and 15.13% respectively.

The percentage of fiber in the control samples, as shown in Table 1, varied, so the samples with the highest percentage of fiber were crushed Oats (16.14%), followed by wheat flour 82% (11.63%) and then Crushed white maize (10.82%), while the lowest samples with a decrease in the percentage of fiber were crushed Sorghum (3.63%). While the fiber % increased in all samples treated with insect compared to the control samples, except for the wheat flour 82% sample, in which the ratio of fiber reduced to 6.46%, and it was the lowest percentage of fiber in the samples treated with the insect, while the highest percentage of fiber was 38.77% in crushed Oats, followed by 22.18% in crushed Barley, then 19.94% in crushed Sorghum. This could be due to insects consuming a lot of carbs.

As it is clear from Table (4) that the percentage of carbohydrates decreased in all samples treated with the insect, except for wheat flour 82%, in which the percentage of carbohydrates increased from 66.33 to 74.13 in the control sample. As for the rest of the samples treated with the insect, Crushed Oats had the lowest decrease in carbohydrate percentage, dropping from 58.53% in the control sample to 32.32 %, followed by crushed Barley, and the value decreased from 72.43% in control sample to 51.21%, then crushed Sorghum, where the percentage decreased from 72.43% in control sample to 53.88%.

The percentage of starch in the samples in Table 4 was varied. The highest percentage of starch in the control samples was in crushed sorghum (68.96%), and the lowest percentage was 42.13% in wheat flour 82%. In comparison, the highest percentage of starch loss in the infested samples was in crushed barley (85.43%), this may be because it was the most infested samples, Whereas the lowest percentage (47.44%) was found in wheat flour 82%. The titratable acidity measures the extent of the spoilage of flour due to the growth of insects in it, and the increase in their number, and the higher the total acidity in flour. In comparison to infested samples, all control samples had the lowest titratable acidity values, as shown in Table 4, where crushed barley had the highest value of titratable acidity (137.12%) followed by El-fayrooze white maize flour, with a value of 133.58%. At the same time, the lowest value was 82.17% for crushed sorghum.

Simple correlation (r) between means populations of *T. confusum* and chemical composition of diets different tested

Table 5 shows a negative correlation between contents protein, moisture, starch, fiber and acidity in the various flours and mean numbers of larvae, adults, and total populations, with an increase in any of these resulting in a

decrease in beetle populations. In addition, the contents of the different diets of carbohydrates and fats had a positive correlation with insect counts, except for the effect of fat on larvae, which has a negative effect with -0.167 .

Discussions

Any nutrition product's moisture content indicates its susceptibility to infestation. In addition, the susceptibility and development of flour beetles are influenced by the quality of the diet. Among the two maize varieties, the maize yellow is more susceptible to infestation by *T. castaneum* larvae than the white maize [10, 9, 21]. millet flour, wheat flour, and sorghum had the highest counts of larvae, pupa, and adults, whereas maize had the lowest counts of larvae.

Our study illustrated that fine flour of white maize is more susceptible to *T. confusum* infestation and higher number populations than crushed white maize (grit flour). However, oats and sorghum had lower numbers of *T. confusum* than other products tested. According to [22], *T. castaneum* progeny were more abundant on fine flour than on grit flour. Furthermore, fine flour from millet or maize and grit maize flours were the most sensitive flours. Maize and millet flours are substantially more sensitive to *T. castaneum* and have a higher population count than sorghum and wheat flours. *Tribolium castaneum* larvae had a shorter developmental time, and females had lower fecundity on broken wheat and broken maize than on semolina. Only other commodities such as broken wheat and broken maize allow it to survive [23].

Tribolium confusum can grow completely on broken barley, and the larval development time is greatly reduced. In addition, the reproductive and the finite rate are significantly higher than cracked white rice [24]. It matches our results were crushed barley recorded highest numbers of population compare to other product tested.

There is a positive correlation between carbohydrates and protein in our study, which is consistent with [25]. who measured biological parameters (first emergence, body weight development rate, and number of progeny) of *T. castaneum*, after allowing unsexed adults to feed and oviposit for one week on the three food groups (high-carbohydrate, high in proteins and composite feeds), then removed them. It was observed that no progeny developed when the protein-rich meals were consumed. When fed a high-carbohydrate and composite diet, the control diet (wheat flour with 5% yeast) and wheat bran had the quickest emergence of first adults, the heaviest weight, and the most progeny. According to [8], the amount of protein in each diet was positively linked ($r=0.97$) with the number of adults and inversely correlated ($r=0.93$) with the carbohydrate content. After four months of storage, wheat lost the most weight (18.8%), followed by cashew nuts (15.6%), almonds (14%), walnuts (11.2%), and peanuts (8%), in that order [11].

The ecdysial of larvae caused by the infestation of this beetle pollutes the flour and leaves a foul odor on the flour and all of its products. In addition, it was noted that the infested flour has a higher gelatinization than the uninfested flour, demonstrating the effect of infestation on flour and makes it unsuitable for the bread-making process [26].

Conclusions

We concluded that nutrients contents of tested different flours had directly affects the development of the confused beetle, *T. confusum* founded negative correlation between contents protein, moisture, starch, fiber and acidity in the different diets and total populations, in contrast the contents of the different diets of carbohydrates and fats had a positive correlation with insect counts. In addition, the insect infestation has a considerable impact on the quality of the product, as it loses its nutritional value and becomes unsuitable for manufacture.

Acknowledgements

Not applicable

List of Abbreviation

Not applicable

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare no conflict of interest

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

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