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Influence of the Wheat and Corn Flour Types on the Biological Parameters on the Red Flour Beetle, *Tribolium castaneum* (Herbst)

Awadalla, S. S.[1]; Amira A. Abdel-Hady[1]; A. S. Hashem[2] and Eman S. Elsayed[1]

[1]Entomology Department, Faculty of Agriculture, Mansoura University, Mansoura, Egypt
[2]Stored Product Pests Research Department, Plant Protection Research Institute, Agricultural Research Center, Sakha, Kafr El-Sheikh

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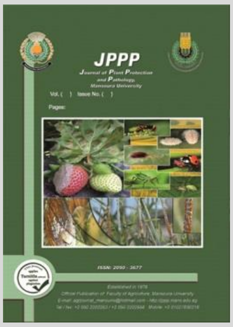

ABSTRACT

The red flour beetle, *Tribolium castaneum*, is an important pest in wheat and maize mills. The goal of this study to determine the level of risk via examining the survival rate, oviposition, and development phases of this pest. The shortest development durations were recorded when the larvae reared on the whole wheat flour followed by wheat flour without bran (22.8±0.31 and 24.7±0.32 days, respectively). The longest development durations were recorded when larvae reared on corn flour without bran followed by bran only (31.1±0.28 and 31.5±0.34 days, respectively). The highest larval survival rate was 95% when larvae reared on whole wheat flour, whereas the lowest one was 94% when larval reared on both of flour without bran and bran only. Meanwhile the lowest survival rates were 84% and 83% when larvae reared on corn flour without bran and bran only, respectively. The longest oviposition period was recorded on wheat flour without bran (280.5±5.70 days). The adult longevity was the longest on wheat flour without bran. The number of progeny produced by the first generation was higher on whole flour than flour without bran. The large differences between the various flour types suggest varying levels of risk by insect infestation. Understanding What insect diets successfully used is essential for creating management measures as customer interest in these alternative flours grows and they become more common in food facilities.

***Keywords*: red flour beetle; alternative flours; life history; egg development; stored products; insect pests**

INTRODUCTION

Numerous studies have been conducted on traditional cereal grains like wheat and rice to determine how insects can grow and develop on entire seeds, milled flours, milling fractions, and milling byproducts (Arthur *et al.*, 2019; and 2020). However, little is known about the capacity of stored product insects to thrive on these different flours and the potential problems these insects can cause to this quickly expanding industry as the variety of flour types utilised by the food industry continues to proliferate. The interactions between plants and phytophagous insects are initially mediated by chemical cues from plants, such as volatiles (Germinara *et al.*, 2008), in both natural and agronomic ecosystems. A lot of biological interactions take place in the silo, an airtight habitat, and these interactions result in significant economic losses (Cox and Collins, 2002). The red flour beetle, *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae), is observed to survive on a variety of commoditiessuch as wheat, maize and rice flour, as well as it considered a secondary pest feeder of stored products (Campbell *et al.*, 2010; McKay *et al.*, 2019). These insects thrive on a range of wheat strains and have high fecundity on wheat flour (Ali and Sarwar, 2011). These beetles were more prolific than those that fed on plain wheat flour, millet, barley, rice, sorghum, and soybean flour, which had the lowest fecundity (Naseri *et al.*, 2017).

The cuticle serves as the initial line of defence between organisms and their surroundings (Welti and Wang, 2004; Zunino and Zygadlo, 2005). According to Bargel *et al.* (2006), and Yeats and Rose (2013), this protects plants from biotic and abiotic variables and plays a role in plant-insect interactions that influence the behaviour of parasitoids and/or predators. In general, the cuticle is often made up of a complex mixture of long-chain non-polar substances like hydrocarbons, wax esters, aldehydes, ketones, long-chain alcohols, fatty acids, terpenoids, and sterols (Lemieux 1996). These substances have been shown to control developmental processes, alter relationships between species, and act as precursors of hormones and pheromones (Lucini *et al.*, 2006; Kosma *et al.*, 2010). Although many research (van Loon *et al.*, 1992; Li and Ishikawa, 2006; Braccini *et al.*, 2015) have reported on the association between plant foliar waxes and insects, little is known about the involvement of the kernel cuticle in grain-insect interactions. According to several authors (Niewiada *et al.*, 2005; Nawrot *et al.*, 2010), the cuticular waxes of the wheat grain are crucial for the oviposition and nutrition of *T.castaneum*.

Understanding how *T. castaneum* lays eggs on various types of flours and how offspring evolve over time may help researchers determine the appropriateness of various substitute flours. The bran of wheat and corn, whole grain flour or white flour were all considered in the current study as potential diets for this insect. To assess how the source affected appropriateness, we procured these flours from the same two commercial sources whenever available. Here, this study focused on how many eggs produce by *T. castaneum* females on various types of flour and how

*** Corresponding author.**
E-mail address: amiraali@mans.edu.eg
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successfully their offspring mature. This study also examined the capacity to develop to the adult stage by putting single eggs on each flour and observing development under a more controlled set of circumstances.

MATERIALS AND METHODS

The experiments were carried out at the laboratory on the Economic Entomology Department, Faculty of Agriculture, Mansoura University, at 30±1°C, 65 ± 5 % R.H and continuous darkness. This beetle was reared for three generations on the wheat flour and the corn flour before starting the present experiment. Enough quantity on wheat and corn kernels were firstly sieved to remove stones, dust and different stages of the insects. The grains were sterilizer by freezing on (-20°C) for three days to killed any hidden insect stages (EL –sabaay 1998). Then the wheat and corn grains were milled and were sieved to divide into whole grain flour, white flour (flour without bran) and wheat bran or corn bran. The wheat flour types or the corn flour types were transferred to the laboratory in plastic buckets until using in the following experiments. Enough individuals of the unsexed emerged adults aged between 5 and 7 days old were maintained in ten glass jars (500 ml) and allow to mating and egg laying. The incubation periods were calculated and recorded for all treatments. The newly hatched insect larvae were placed in petri- dishes (9cm diameter) containing 5 grams on wheat flour types (whole grain flour or white flour or bran) as well as with corn flour types. Each petri - dish contained only two newly hatched larvae and each treatment was replicating fifty times. The immature stages duration, the larval and pupal stages survival and the adult emergence were calculated and recorded, for each wheat flour types and corn flour types. After the adult emergence, thirty unsexed pairs at the insect pest from each treatment was divided to three replicates (ten pairs for each replicate) and transferred to plastic jars containing the wheat flour types or corn flour types. The ovipositional periods, the adult longevity as well as number of F1 progeny were daily recorded for each flour type.

Statistical analysis:

Data of developmental times, survival, pre-oviposition, post-oviposition, and oviposition periods, female longevity of *T. castaneum*, were analyzed by one-way ANOVA, and the means were separated using Student-Newman-Keuls Test (Costat Software, 2004).

RESULT AND DISCUSSION

Non – choice test was carried out to determine the influence of wheat flour types on the developmental stages on *T. castaneum* under laboratory conditions (30±1°C and 65±5% R.H)

The present results illustrated in the Table (1) showed the influence of the wheat flour types on the developmental stages on the red flour beetles *T.castaneum* under laboratory conditions (30±1 °C and 65±5% R.H) , In respect to the incubation period for the insect pest on the wheat flour types it was 5.6±0.19 day on all types of flour with no with no significant difference.

Regarding to the larval stage on whole flour was the shortest duration followed by flour without barn and bran .and presented by 11.5±0.17 ,13.7±0.26 and 14.2±0.2, respectively. Statistical analysis revealed that, a highly significant differences were obtained between the larval stage duration according to different flour types Table (1).

On the other hand, the pupal stages duration was the shortest (5.4±0.33 days) on flour without bran followed by bran (5.5±0.32 days) and the longest pupal stage duration was recorded on whole flour (5.7 ±0.15 days) with no significant difference. (Table 1).

The obtained result arranged in Table (1) showed the total developmental stages duration according to the influence on the wheat flour types, the shortest developmental stages were recorded when the larvae reared on whole flour followed by flour without bran represented by 22.8±0.31 days, 24.7±0.32 days, respectively. Meanwhile, the longest developmental stages were recorded when the larvae reared on barn presented by 25.2±0.29 days. Statistical analysis revealed that a highly significant differences were obtained between the developmental stage durations according to wheat flour types.

Table 1. Influence of wheat flour types on development of the red flour beetle *T. castaneum* under laboratory conditions (30 ±1°c and 65 ±5 %).

Biological Parameters	Wheat flour types		
	Whole flour	Flour without bran	bran only
Incubation Period	5.6 ±0.16 a	5.6 ±0.16 a	5.6 ±0.16 a
Larval stage	11.5 ±0.17 b	13.7 ±026 a	14.2 ±0.2 a
Pupal stage	5.7 ±0.15 a	5.4 ±0.16 a	5.5 ±0.17 a
Total developmental stages	22.8 ±0.32 b	24.7 ±0.31 a	25.3 ±0.29 a

Means followed by different letter in a rows are significantly different at 5% probability level (ANOVA, Student- Newan-Keuls Test).

The present result illustrated in Table (2) showed the influence of the corn flour types on the developmental stages on the red flour beetles *T. castaneum* under the laboratory condition (30 ±1°c and 65±5 % R.H).

In respect to the incubation period for the insect pest on the corn flour types it was 5.5±0.17 days on all corn types with no significant difference. Regarding to the larval stage on whole corn flour was the shortest duration followed by flour without bran and bran, and presented by 17.1 ±0.23 .19.3 ±0.21 and 19.9 ±0.28 days, respectively. Statistical analysis revealed, that a highly significant differences were obtained between the larval stage duration according to different corn types Table (2).

Table 2. Influence of corn flour types different on development stages of the red flour beetle *T. castaneum* under laboratory conditions (30 ±1°c and 65 ±5%).

Biological Parameters	Corn flour types		
	Whole flour	Flour without bran	bran only
Incubation Period	5.5 ±0.17 a	5.5 ±0.17 a	5.5 ±0.17 a
Larval stage	17.1 ±0.23 b	19.3 ±0.21 a	19.9 ±0.28 a
Pupal stage	6.1 ±0.1 a	6.3 ±0.15 a	6.1 ±0.18 a
Total developmental stages	28.8 ±0.25 b	31.1 ±0.28 a	31.5 ±0.34 a

Means followed by different letter in a rows are significantly different at 5% probability level (ANOVA, Student- Newan-Keuls Test).

On the other hand, the pupal stage duration was the shortest (6.1±0.1) on the whole corn flour followed by (6.1±0.15) on the bran and (6.3±0.18) on the flour without bran with no significant difference Table (2). The obtained result arranged in Table (2) showed the total developmental stages duration according to the influence on the corn flour types of the developmental stages on the red flour beetles *T. castaneum* under laboratory condition (30 ±1°c and 65±5 % R.H).

The longest developmental stages were recorded when larvae reared on corn flour without bran followed by bran only represented by 31.1±0.28 and 31.5±0.34 days, respectively. Meanwhile the shortest developmental stages were recorded when the larvae reared on the whole corn flour and presented by 28.7±0.25 days. Statistical analysis revealed that, a highly significant differences were obtained between the developmental stages duration according to different types of corn flour.

The obtained data presented in Table (3) showed the survival rate of the red flour beetles when reared on the types of flour, it can be noticed that, the larval survival rate ranged between 95% and 94%, the highest larval survival rate was 95% when larvae reared on whole grain flour, while the lowest larval survival rate was 94% when larval reared on flour without bran and bran only. Moreover, the pupal survival rates were 100% on all types of flour. In addition, Table (3) the survival rates during the developmental stages for the larvae until emergence of the adult ranged between 95% to 94%. It can be noticed that, the highest survival rates when reared the insect pest on whole grain flour, respectively. Meanwhile the shortest survival rates were 94% when reared the insect pest on flour without bran and bran only.

The obtained data presented in Table (3) shown the survival rate of the red flour beetle *T. castaneum* when reared on the types of corn. It can be observed that, the larval survival rate ranged between 86% and 83% the highest larval survival rates were 86% when larvae reared on whole corn flour, while the shortest larval survival rates were 83% when larvae reared on bran only.

Moreover, the pupal survival rates were 100% on all types of corn flour. As shown in Table (3) the survival rates during the developmental stages for the larvae until emergence of the adults ranged between 86% and 83%. It can be observed that the highest survival rates when reared the insect pest on whole corn flour, respectively. Meanwhile the shortest survival rates when reared the insect pest on bran.

Table 3. Larval survival percentages of the red flour beetle *T. castaneum* at different flour types under laboratory conditions (30 ±1°c and 65 ±5 %).

Grains flour status		Larval stage	Pupal stage	Adult larval
Wheat	Whole flour	95.0	100.0	95.0
	Flour without bran	94.0	100.0	94.0
	bran only	94.0	100.0	94.0
Corn	Whole flour	86.0	100.0	86.0
	Flour without bran	84.0	100.0	84.0
	bran only	83.0	100.0	83.0

Data illustrated in Table (4) showed the influence at the wheat flour types on the ovipositional period's female longevity and the number of F1 progeny at the red flour beetle *T. castaneum* under laboratory condition at 30±1°c and 65±5 R.H.

Regarding to the pre-ovipositional period was ranged between 7.3 ± 0.15 days on flour without bran and 7.6±0.14 days on bran with no significant difference. In respect to the ovi-position period were the shortest on whole flour followed by bran presented by 245.3±6.69 and 274.5±5.78 days, respectively. Meanwhile, the longest ovipositional periods were recorded on flour without bran presented by 280.5±5.70 days.

Statistical analysis revealed that a highly significant differences were obtained between the ovipositional periods under the effect of different flour types. Regarding to post ovipositional periods were the shortest on bran followed by flour without bran and presented by (5.6±0.16 days) and (7.4±0.16 days), respectively. While the longest post-ovipositional periods were (12.4±0.16 days) on the whole corn flour with a significant difference.

The adult longevity was the longest on flour without bran presented by 295.2±5.79 days, respectively. Moreover, the shortest adult longevity was recorded on whole flour presented by 263.2±1.41 days, respectively. Statistical analysis revealed that a highly significant differences between the adult longevity under the effect of different flour types.

The number of progeny for the first generation were the highest on whole flour followed by flour without bran and presented by 652.9±1.05 individuals / females and 598.8±2.39 individuals / females, respectively. On the other hand, the lowest average number of progeny were recorded on bran only presented by 583.7±1.99 individuals / females. Statistical analysis showed that a highly significant differences between the numbers of progeny for the first generation under the effect of different flour types.

Table 4. Influence of wheat flour types on female longevity and number of F1 progeny of the red flour beetle *T. castaneum* under laboratory conditions (30 ±1°c and 65 ±5 %).

Biological Parameters	Wheat flour type		
	Whole flour	Flour without bran	bran only
Pre – ovipostion period	7.5 ± 0.17 a	7.3 ± 0.15 a	7.6 ± 0.22 a
ovipostion period	245.4 ± 1.42 b	280.5 ± 5.69 a	274.5 ± 5.79 a
Post –ovipostion period	12.4 ±0.16 a	7.4 ± 0.16 b	5.6 ± 0.16 c
–Female longevity	265.3± 1.39 b	295.2± 5.79 a	287.7± 5.80 a
No of F1 Progeny / Female	652.9±1.05 a	598.8±2.39 b	583.1±1.99 c

Means followed by different letter in a rows are significantly different at 5% probability level (ANOVA, Student- Newan-Keuls Test).

Data illustrated in Table (5) showed the influence at the corn flour types on the ovipositional periods female longevity and the number of F1 progeny at the red flour beetle *T. castaneum* under laboratory condition at 30±1°c and 65±5 R.H

Regarding to the pre-ovipositional period was ranged between 8.4 ± 0.16 days on flour without bran and 8.8±0.13 days on bran only with no significant differences. In respect to the ovi-position period were the shortest on flour without bran followed by whole corn flour presented by 280.5±5.93 and 281.6±7.71 days, respectively. Meanwhile, the longest ovipositional periods were recorded on bran presented by 282.1±6.40 days. Statistical analysis revealed that, there were no significant differences between the ovipositional periods under the effect of different corn flour types.

Regarding to the post ovipositional periods were the shortest on bran followed by flour without bran and presented by 5.6±0.16 days and 7.8±0.13 days. While, the longest post ovipositional periods were 10.7±0.22 days on whole corn flour, with a significant difference.

The adult longevity was the longest on whole corn flour presented by 301.1±5.85 days. Moreover, the shortest adult longevity was recorded on bran presented by 296.6 ± 0.76 days. Statistical analysis revealed that a no significant differences between the adult longevity under the effect of different flour types. The number of progeny for the first generation were the highest on whole corn flour followed by bran .and presented by 319.8±1.42 individuals/females and 274.3±1.76individuals/females, respectively.

On the other hand, the lowest average number of progeny were recorded on flour without bran presented by 268.9±1.12 individuals/females. Statistical analysis showed that a highly significant differences between the number of progeny for the first generation under the effect of different corn flour types.

Table 5. Influence of corn flour types on female longevity and number of F1 progeny of the red flour beetle *T. castaneum* under laboratory conditions (30 ±1°c and 65 ±5 %).

Biological Parameters	Corn flour type		
	Whole flour	Flour without bran	bran only
Pre – ovipostion period	8.5 ± 0.17 a	8.4 ± 0.16 a	8.8 ± 0.13 a
ovipostion period	281.9 ±5.81 a	280.7 ± 7.71 a	282.2 ± 0.71 a
Post –ovipostion period	10.7 ± 0.22 a	7.8 ± 0.13 b	5.6 ± 0.16 c
Female longevity	301.1 ± 5.85 a	296.9 ± 6.74 a	296.6 ± 0.76 a
No of F1 Progeny / Female	319.8 ± 1.42 a	274.3 ± 1.76 b	268.9 ± 1.12 c

Means followed by different letter in a rows are significantly different at 5% probability level (ANOVA, Student- Newan-Keuls Test).

DISCUSSION

The quantity of eggs deposited and offspring born varied significantly depending on the type of flour used. It is likely that volatile olfactory signals, and/or textural and chemical gustatory cues are what cause the variations in oviposition. The texture of low-risk flours was noticeably different from other evaluated flours, which had a more powdered, finer texture or were greasy with a semi-flaky feel. Additionally, nutritional factors like sodium, fibre, or protein can influence the probability of infestation. According to several reports (Chapman, 2003; Ukeh *et al*., 2010; Kostromytska *et al*., 2018), the search for food in phytophagous insects, such as *Sitophilus* spp. or *Tribolium* sp., involves two complementary stages: the insect response to the volatile organic compounds (VOCs) released by the food source and the surface-testing behaviour of the insects using their gustatory and tactile receptors. This would enable the argument to be made that the epicuticle might take part in these complementing search phases. The findings of the current investigation support this theory because *T. castaneum* favoured maize kernels without the epicuticle and hence caused less harm to them. The development of insects has been demonstrated to be significantly impacted by the chemical and physical characteristics of these flours. For instance, brewer's yeast added to maize and wheat flour dramatically increased output compared to corn and wheat flour alone (Sokoloff *et al*., 1966). Astuti *et al*. (2020) demonstrated that in the flours they tested, flours with low protein content (5%) are unsuitable for *T. castaneum* development but also that high protein content in flours like soybean flour (>25%) is unsuitable for development, which is similar to our results showing a positive association with egg count and protein. The *trade-off* between proteins and carbs in flours for growth and fecundity was also described by Wong and Lee (2001).

According to our research, kernels lacking the epicuticle suffered less damage than those with it. Tipping *et al.* (1988) founded that few egg plugs were seen in kernels with smooth surfaces, indicating that the resistance of kernels is related to the surface relief. This is consistent with the epicuticle's thickness decreasing following the extractive procedure we performed in our study, which we linked to the potential disappearance of wax clusters and, consequently, the grain surface's roughness. The findings here so imply that cleaning the epicuticle with an organic solvent reduced the kernel's roughness, making it less alluring to insects. However, the presence of phagostimulatin compounds, such as sitosterol, stigmasterol, octadecanoic acid, and heptacosane, which were found in the maize kernel cuticle, may be a complement to testing the surface using the gustatory and tactile receptors (Doss and Shanks, 1984; Harada, 1985; Lawrance, 2016). This shows that the cuticular relief and chemical makeup may work together to contribute to the maize cuticle's attractiveness. The microclimate of *T. castaneum* has been demonstrated to be significantly impacted by particle size, which we hypothesised to play a role in our series of tested flours but which was not specifically evaluated here. However, traditional grains of all kinds with comparable nutritive qualities and textures are found in flours that are highly susceptible to infection. The highest offspring emergence rates were observed in the flours made from rye, wheat and millet, whereas buckwheat had fairly high progeny emerging rates. Sorghum, maize and rice all had relatively few offspring emerge, which shows that the flour may include nutrients or chemicals that alter growth to the adult stage after egg laying (Arthur *et al*., 2019).

The ability of the insect to create offspring is an another biological aspect that has an impact on plants. According to the findings of our study, flies exposed to kernels without an epicuticle produced much fewer progeny than those exposed to kernels with one. This is in line with the findings of Nawrot *et al*. (2010), who found that the insect *S. granarius'* reproduction rate was considerably lower in washed wheat grains than in the corresponding controls. Additionally, according to Howard (2001), the parasitoid *Pteromalus cerealellae* (Boucek) may find the oviposition sites by sensing the cuticle hydrocarbons of the insect *Sitotroga cerealella* (Olivier). Therefore, a decrease in oviposition stimulating substances, such as hydrocarbons, during the cuticle washing may account for the lower number of offspring reported in insects exposed to kernels without an epicuticle (Niewiada *et al*., 2005).

Combining the aforementioned findings, it is possible to hypothesise that the insect's ability to reproduce is influenced by the relief and chemical makeup of the epicuticle of maize and/or wheat kernels. Furthermore, despite the two types' different chemical compositions, the insect reaction for all evaluated parameters did not show a difference between them. Understanding how insects select their nesting locations can also help managers manage mixed storage facilities, where products like wheat flour and corn flour are kept next to one another. In these situations, one may presume that if the wheat flour has insects, the corn flour must also have insects, necessitating insect treatment for all of these flours.

The maize flour probably won't be contaminated and won't require treatment, though. Additionally, it might be advantageous to store expensive flours close to flours with a greater risk of infestation so that pests will choose the cheaper flour as their "bait" rather than the more expensive flour. Future choice studies will reveal more details on the choices these insects make when choosing where to lay their eggs as well as more details on how to behaviorally control these pests. Additionally, a reduced risk strategy can be adopted by knowing which elements of these flours can operate to either hinder or encourage oviposition and the growth of insects in these flours.

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تأثير أنواع دقيق القمح ودقيق الذرة على المقاييس البيولوجية لخنفساء الدقيق الصدئية ***Tribolium Castaneum*** **(Herbst)**

سمير صالح عوض الله [1] ، أميرة على على عبدالهادى[1] ، أحمد سراج الدين هاشم [2] و إيمان سامى مصطفى السيد[1]

[1]كلية الزراعة –جامعه المنصورة – قسم لحشرات الأقتصادية
[2]قسم افات الحبوب والمواد المخزونة -معهد وقاية النبات -مركز البحوث الزراعية بسخا - كفر الشيخ

الملخص

تعتبر خنفساء الدقيق الصدئية من الأفات الهامة على دقيق القمح والذرة . الهدف من هذه الدراسة تحديد معدل البقاء ووضع البيض وأنماط النمو لهذه الافة لتحديد مستوى الضرر. سجلت أقصر فترة نمو للأطوار الغير كاملة عندما تم تربية اليرقات على دقيق الحبه الكاملة يليها على دقيق القمح بدون النخالة حيث سجلت 22.8 ± 0.31 و 24.7 ± 0.32 يوما على التوالى . و سجلت أطول فترة نمو للأطوار الغير كاملة عندما تم تربية اليرقات على دقيق الذرة بدون نخالة يليها نخالة الذرة فقط حيث سجلت 31.1 ± 0.28 و31.5 ± 0.34 يوما على التوالى . وكانت أعلى نسبة بقاء لليرقات 95% عندما تم تربيتها على دقيق الحبة الكاملة للقمح ، بينما كان أقل معدل بقاء 94 % عندما تم تربية اليرقات على دقيق القمح بدون نخالة أو النخالة . بينما كان أقل معدل للبقاء هو 84 ، 83% . عندما تم تربية الحشرة على دقيق الذرة بدون نخالة والنخالة فقط على التوالى . علاوة على ذلك سجلت أطول فتره لوضع البيض على دقيق القمح بدون نخالة حيث كانت 280.5 ±5.70 يوم وكانت أطول فترة لحياة الحشرة الكاملة المرباة دقيق القمح بدون النخالة وكان أعلى عدد لذرية الجيل الأول على الدقيق الحبة الكاملة يليها الدقيق بدون نخالة . الفروق الواضحة بين أنواع الدقيق تؤكد أن هناك مستويات مختلفة لخطورة الإصابة لهذه الحشرة . الوعى الجيد لأنواع الغذاء الذى تنجح الحشرة فى إصابتها ضرورى لتصميم برامج مكافحة وذلك بسبب إقبال المستهلك لأنواع الدقيق البديلة .