

Biotic potential of *Tribolium castaneum* (Coleoptera: Tenebrionidae) on four types of wheat substrates

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Abstract:

This study investigates the influence of wheat substrates on the biotic potential of the insect *Tribolium castaneum*. Various substrates, including grains of durum wheat (*Triticum durum* Var. Irden) and their respective flours, as well as grains of soft wheat and their flours (*Triticum aestivum* Var. Merchouch), were utilized in the experiment. Results demonstrated that the fecundity of *T. castaneum* was similar across all four substrates, but soft wheat flour proved to be the most suitable oviposition medium. Egg fertility remained consistent across all substrates. The durations of pre-imaginal developmental stages varied according to the substrates, with shorter durations observed on wheat flours compared to whole grains. Mortality rates also fluctuated depending on the substrates, with wheat flours exhibiting lower mortality rates. Survival curves displayed similar patterns across all substrates. Therefore, soft wheat and durum wheat flours appeared to promote the development of *T. castaneum* in comparison to wheat grains.

Key Words:

Tribolium castaneum, Wheat substrates, Biotic potential, Fecundity, Fertility, Developmental durations

Introduction:

The red flour beetle, *Tribolium castaneum*, is a cosmopolitan pest of stored products [1, 2, 3]. It belongs to the family Tenebrionidae. Tenebrionidae is a vast and diverse group containing over 10,000 species, of which nearly 100 are pests of various stored products [4, 5]. The term

"Tenebrio" means seekers of dark places [6]. Damage caused by this pest typically results in both quantitative and qualitative losses [7, 8]. The product attacked by *T. castaneum* usually contains feces, carcasses, and exuviae. It also turns grey with an unacceptable pungent smell due to the production of benzoquinones, a defensive chemical material from their prothoracic and abdominal glands, rendering the product unfit for human consumption [9, 5, 10]. These are formidable pests, as they can thrive on dry grains [11]. Among others, cereals provide a favorable environment for their population. However, the source of infestation in stocks can vary. Infestation may begin at the field level for some insects, while it can also occur along the post-harvest chain followed by the commodity, and finally, it can take place in warehouses. Nevertheless, some substrates are more resistant than others to this insect. It is in this context that our study is conducted, which aims to evaluate the biotic potential of *Tribolium castaneum* on four types of wheat substrates: grains of durum wheat (*Triticum durum* Var. Irden) and their respective flours, as well as grains of soft wheat and their flours *Triticum aestivum* (Merchouch variety).

1 Materials and Methods:

1.1 *T. castaneum* Strain:

1.1.1 Adults:

The adult *T. castaneum* used in these tests were obtained from a laboratory colony reared on hard wheat flour *Triticum Durum* (Irden variety) under the following conditions: a temperature of $28 \pm 2^\circ\text{C}$, a relative humidity of $70 \pm 5\%$, and a photoperiod of 14 hours light: 10 hours dark (photophase: scotophase).

1.1.2 Sexing Technique:

To obtain virgin adults, sexing of the insect was performed at the nymphal stage. Based on the primary criteria of sexual dimorphism in nymphs, it was possible to separate male nymphs from female nymphs. Female nymphs can be distinguished from male nymphs by the genital papillae, located just in front of the urogomphi, which are more developed in females than in males [12]. Sexing *T. castaneum* as nymphs is necessary to ensure that no prior mating has occurred.

1.2 Substrate Preparation:

Four types of substrates were prepared: a substrate based on hard wheat grains (*Triticum Durum*, Irden variety), a second one based on soft wheat grains (*Triticum aestivum*, Marchouch variety), the third with hard wheat flour, and the fourth with soft wheat flour.

1.3 Biological Tests:

1.3.1 Influence of the Four Types of Substrates on the Fecundity, Fertility, and Development of *T. castaneum*:

To assess the influence of the four types of substrates on the development of *T. castaneum*, 5g of each substrate were placed in separate 9 cm diameter Petri dishes. Five newly emerged virgin males and five newly emerged virgin females (aged 3 days) were introduced into each of these Petri dishes. The number of eggs laid on these different types of substrates over 12 days was recorded and observed daily under a binocular microscope to determine the percentage of hatched eggs with their incubation period and the duration of development of the various larval stages. The nymphs were immediately collected and separated by sex. These observations continued until the emergence of adults.

Following these experiments, the following biodemographic parameters were determined:

- Fertility;
- Fecundity;
- Average duration of larval stage development;
- Average duration of nymph development;
- Number of survivors at each stage;
- Number of individuals lost at each stage;
- Sex ratio (R).

1.4 Data Analysis:

To investigate any significant effects of substrates on the biological and physiological parameters of *T. castaneum*, a statistical comparison using the student's t-test at a 5% significance level was conducted on continuous variables and using the χ^2 test on proportions. The existence of possible relationships between the studied parameters was detected using linear regression. The results are presented graphically illustrating the relevant biological parameters. Statistical analyses were performed using Excel software.

3 Results:

3.1 Effect of Substrates on *T. castaneum* Fecundity:

The fecundity of *T. castaneum* follows a similar pattern on all four types of substrates. Egg laying begins on the 4th day after females are introduced to soft wheat grains, hard wheat flour, and soft wheat flour. For hard wheat grains, egg laying begins on the 6th day. The maximum number of eggs recorded during the 12 days of testing varies by substrate. It is 11.79, 32.20, 33.53, and 42.79, respectively, on hard wheat grains, soft wheat grains, hard wheat flour, and

soft wheat flour. Considering the average fecundity during the experimental period, the substrate based on soft wheat flour is the most suitable egg-laying medium for *T. castaneum*. It is statistically higher in fecundity compared to the three other substrates. The number of eggs laid on soft wheat grains and hard wheat flour are statistically comparable and significantly different from the fecundity of the insect on hard wheat grains.

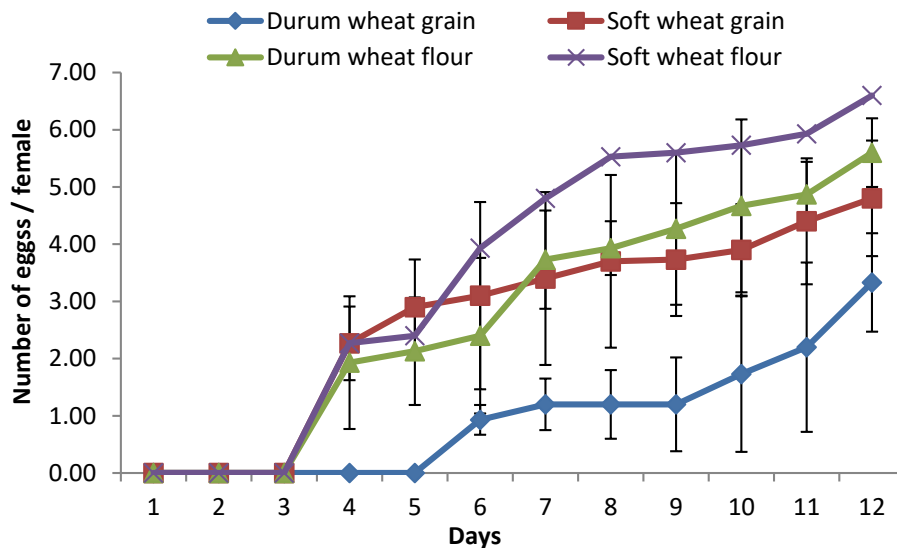


Fig 1: Chronological Evolution of the Fecundity of *Tribolium castaneum* Over 12 Days on Four Types of Wheat Substrates

Table 1: Fertility of *Tribolium castaneum* After 12 Days on Four Types of Wheat Substrates

| Types of Wheat Substrates | Numbers of female | Average ± Standard deviation |
|---------------------------|-------------------|------------------------------|
| Durum wheat grain | 5 | 11,79 ^{a*} ±3,34 |
| Soft wheat grain | 5 | 32,20 ^b ±2,81 |
| Durum wheat flour | 5 | 33,53 ^b ± 1,12 |
| Soft wheat flour | 5 | 42,79 ^c ±2,48 |

*: The average fertility rates assigned by the same letter do not differ statistically from each other (Student's t-test at 5%).

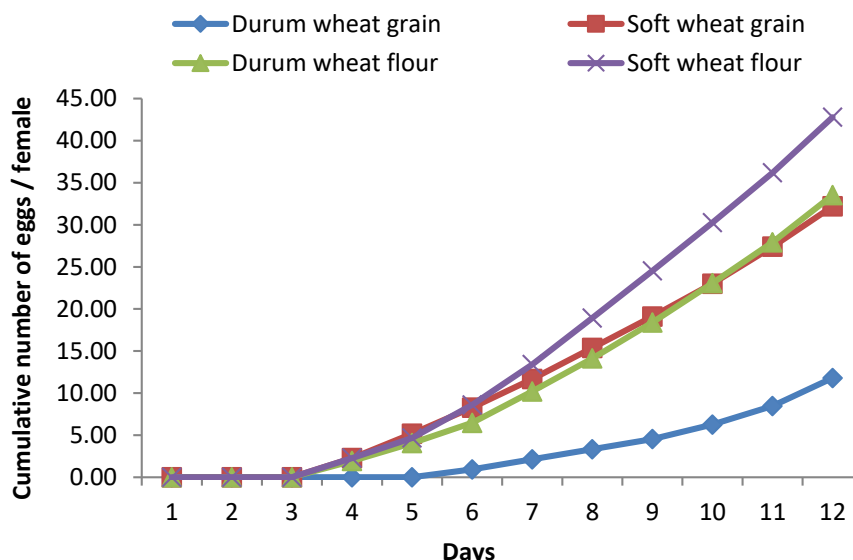


Fig 2: Chronological Evolution of Cumulative Oviposition of *Tribolium castaneum* Over 12 Days on Four Types of Wheat Substrates.

3.2 Effect of Substrates on *T. castaneum* Fertility

The fertility of eggs (= number of hatched eggs/number of eggs laid *100) is approximately 72.66%, 73.00%, 73.33%, and 75.33%, respectively, on hard wheat grains, soft wheat grains, hard wheat flour, and soft wheat flour (Table 2). These values do not differ statistically (χ^2 calculated = 2.79 < χ^2 (1; 0.05) = 3.84).

Table 2: Fertility of *Tribolium castaneum* over 12 days on the four types of wheat substrates

| Types of Wheat Substrates | Number of eggs | Number of eggs hatched | % hatching |
|---------------------------|----------------|------------------------|---------------------|
| Durum wheat grain | 150 | 109 | 72,66 ^{a*} |
| Soft wheat grain | 150 | 111 | 74,00 ^a |
| Durum wheat flour | 150 | 110 | 73,33 ^a |
| Soft wheat flour | 150 | 113 | 75,33 ^a |

*: Fertilities assigned by the same letter do not differ statistically from each other (χ^2 test at the 5% threshold).

3.3 Effect of Substrates on the Development of *Tribolium castaneum*

3.3.1 Effect on Embryonic Development

3.3.1.1 Effect on Egg Incubation Period

The average incubation periods for *Tribolium castaneum* eggs are 5.33 ± 0.96 , 5.77 ± 1.10 , 6.17 ± 1.42 , and 6.40 ± 1.85 days, respectively, on soft wheat flour, hard wheat flour, soft wheat grains, and hard wheat grains. They are statistically comparable across all four substrates tested (Table 3).

Table 3: Duration (days) of incubation of *Tribolium castaneum* eggs on the four wheat substrate types.

| Types of Wheat Substrates | N | Average \pm Standard deviation |
|---------------------------|-----|----------------------------------|
| Durum wheat grain | 150 | $6,40^a \pm 1,85$ |
| Soft wheat grain | 150 | $6,17^a \pm 1,42$ |
| Durum wheat flour | 150 | $5,77^a \pm 1,10$ |
| Soft wheat flour | 150 | $5,33^a \pm 0,96$ |

*: The means assigned by the same letter do not differ statistically from each other (Student test at the 5% threshold)

3.3.1.2 Effect on Egg Mortality

Not all eggs laid on each of the substrate's hatch; approximately 25 to 27% of the eggs die before hatching. The rates of embryonic mortality observed on hard wheat grains, soft wheat grains, and hard wheat flour are statistically comparable. When considered separately, all three of them are statistically higher than the mortality rate observed on soft wheat flour (Fig 3).

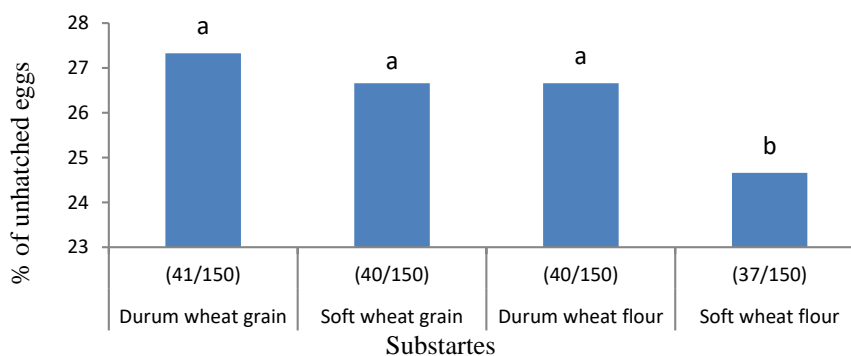


Fig 3: Mortality of *Tribolium castaneum* eggs on the four types of wheat substrates (Number of unhatched eggs/Total number of eggs laid).

The histograms labelled with the same letter are not statistically different from each other (χ^2 test at a 5% significance level).

3.3.2 Effect on Larval Development

3.3.2.1 Effect on Development Duration

The duration of development for the larvae of *Tribolium castaneum* varies among the considered substrates. It ranges from 26.01 ± 0.6 to 27.40 ± 1.0 days for soft wheat flour and

hard wheat flour and from 29.10 ± 1.4 to 29.9 ± 1.7 days for soft wheat grains and hard wheat grains (Table 4). Additionally, the development duration is significantly shorter on both types of flour compared to both types of grains.

Table 4: Duration (days) of larval development of *Tribolium castaneum* on the four types of wheat substrates.

| Types of Wheat Substrates | N | Average \pm Standard deviation |
|---------------------------|-----|----------------------------------|
| Durum wheat grain | 109 | $29,9^a \pm 1,7$ |
| Soft wheat grain | 111 | $29,1^a \pm 1,4$ |
| Durum wheat flour | 110 | $27,4^b \pm 1,0$ |
| Soft wheat flour | 113 | $26,1^b \pm 0,6$ |

*: Within the same column, the means assigned by the same letter do not differ from each other (Student’s test at 0.05)

3.3.2.2 Effect on Larval Stage

During their development, larvae of *T. castaneum* experience mortality rates that vary depending on the substrate on which they develop. On the substrates of hard wheat grains and soft wheat grains, the mortality percentages are approximately 80% of the recorded larval populations (Fig 4). On the other hand, on the substrates of hard wheat flour and soft wheat flour, the mortality rate fluctuates around 71% to 74%. It is statistically lower than that observed on the other two substrates (Figure 4). The substrates of hard wheat flour and soft wheat flour appear to be more suitable for the larval development of *T. castaneum*, while the other two substrates (Fig 4) seem less suitable for larval development.

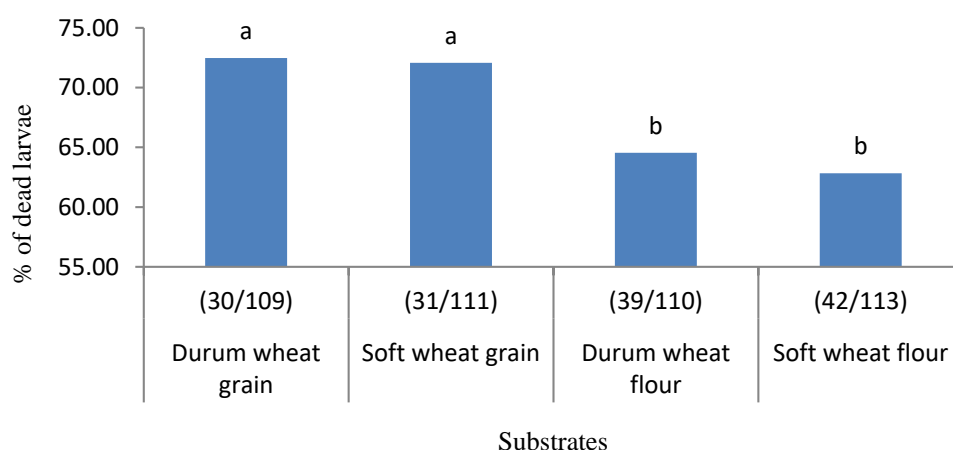


Fig 4: Larval mortality observed on the four types of wheat substrates. The histograms marked with the same letter are not statistically different from each other (χ^2 test at the 5% significance level).

3.3.3 Effect on Nymphal Development

3.3.3.1 Effect on Development

Table 5 shows the average development durations of nymphs of *Tribolium castaneum* on hard wheat grains, soft wheat grains, hard wheat flour, and soft wheat flour. The results indicate that the nymph development periods are 6.13 ± 0.85 , 6.45 ± 0.96 , 6.39 ± 0.84 , and 6.51 ± 0.85 days, respectively, on soft wheat flour, hard wheat flour, soft wheat grains, and hard wheat grains. However, the nymph development durations do not differ significantly among them (Table 5).

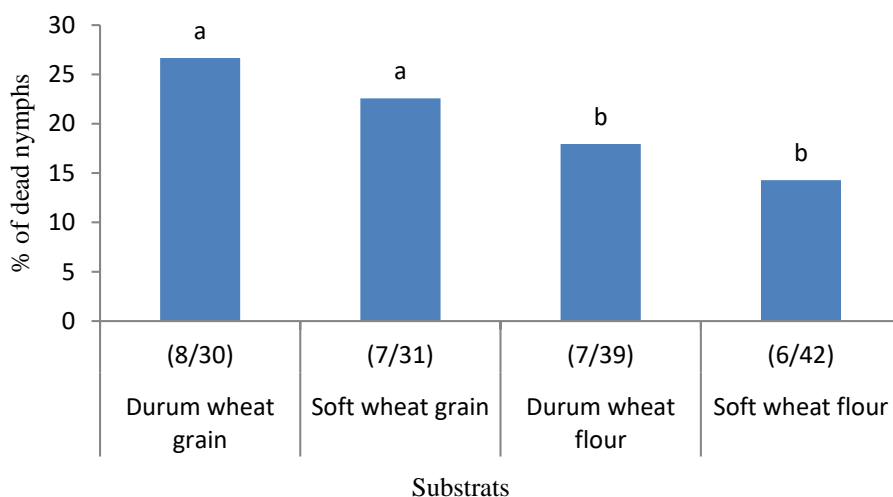


Fig 5: Nymphal mortality observed on the four types of wheat substrates.

The histograms labelled with the same letter do not differ statistically among themselves (χ^2 test at the 5% significance level).

3.3.4 Effect on adult emergence

The percentages of *T. castaneum* adults emerged after pupation are 77.33% and 77.42%, respectively, on hard wheat grains and soft wheat grains, and these rates do not differ statistically between them. As for the rates of adult emergence on hard wheat flour (82.05%) and soft wheat flour (85.71%), they are statistically higher than those on the two types of wheat grains (Fig 6).

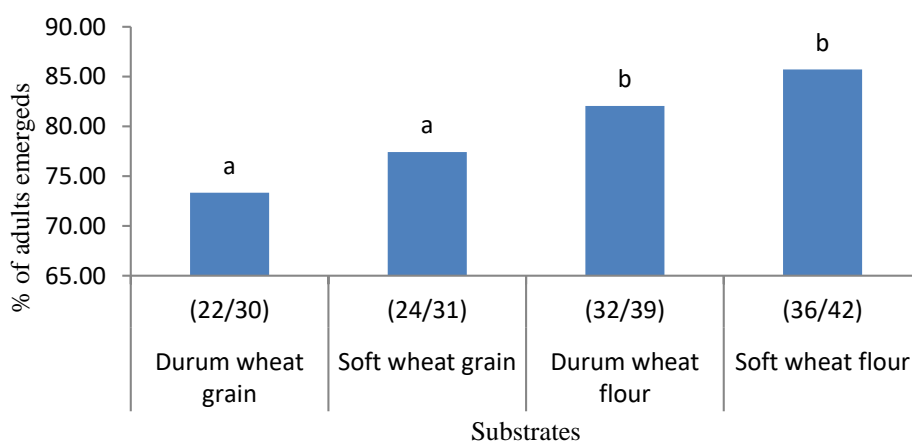


Fig 6: Adult offspring obtained on the four types of wheat substrates.

The histograms labeled with the same letter do not differ statistically among themselves (χ^2 test at the 5% significance level).

In terms of the developmental success from egg to adult in *T. castaneum*, the substrates, hard wheat flour, and soft wheat flour, provide a suitable egg-laying medium and a more favorable trophic source. The percentages of *T. castaneum* adults emerged after pupation are 14.66% and 16%, respectively, on hard wheat grains and soft wheat grains. These rates do not differ statistically between them and are significantly lower than the percentages of adult emergence on hard wheat flour (21.33%) and soft wheat flour (24%) (Fig 7).

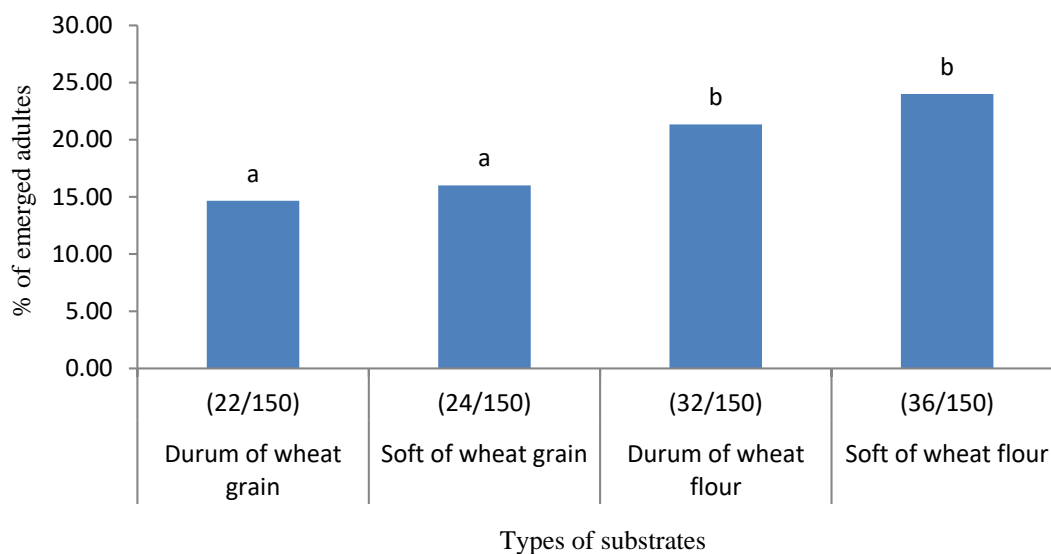


Fig 7: Adult offspring obtained after oviposition on the four types of wheat substrates.

The histograms labelled with the same letter do not differ statistically among themselves (χ^2 test at the 5% significance level).

3.3.5 Effect on Sex Ratio

At the end of emergence, the sex ratio observed per substrate is 0.57, 0.60, 0.60, and 0.63, respectively, for hard wheat grains, soft wheat grains, hard wheat flour, and soft wheat flour. Across these four types of substrates, the sex ratio is statistically similar.

3.3.6 Effect on the Biedemographic Parameters of *T. castaneum* Developed on the Four Wheat Substrates

Under laboratory conditions (RH = 70 ± 5% and T°C = 28 ± 2°C), *T. castaneum* completes its developmental cycle, from egg laying to the emergence of the first-generation adults, in approximately 37 to 42 days across the four tested substrates. The pre-imaginal development occurs in 37.56 days on soft wheat flour, 39.62 days on hard wheat flour, 41.66 days on soft wheat grains, and 42.81 days on hard wheat grains (Table 6). Similar to other biological

parameters, soft wheat flour provides the most suitable developmental substrate for *T. castaneum*. The duration of the consuming stages, corresponding to the damage periods, represents about 70% of the total duration of the development cycle across the four types of substrates; this is mainly due to the larval stages.

Table 6: Duration (days) of development of different stages of *Tribolium castaneum* on the four types of substrates.

| Types of substrats | Eggs | | Larvae | | Nymphs | | Eggs – Adults | |
|--------------------------|------|--------------------------|--------|--------------------------|--------|---------------------------|---------------|---------------------------|
| | N | Avg. ± S.d.* | N | Avg. ± S.d | N | Avg. ± S.d | N | Avg. ± S.d |
| Durum wheat grain | 150 | 6,40 ^a ± 1,85 | 109 | 29,9 ^{a*} ± 1,7 | 30 | 6,51 ^{a*} ± 0,85 | 22 | 42,81 ^a ± 1,55 |
| Soft wheat grain | 150 | 6,17 ^a ± 1,42 | 111 | 29,1 ^a ± 1,4 | 31 | 6,39 ^a ± 0,84 | 24 | 41,66 ^a ± 1,15 |
| Durum wheat flour | 150 | 5,77 ^a ± 1,10 | 110 | 27,4 ^b ± 1,0 | 39 | 6,45 ^a ± 0,96 | 32 | 39,62 ^a ± 0,96 |
| Soft wheat flour | 150 | 5,33 ^a ± 0,96 | 113 | 26,1 ^b ± 0,6 | 42 | 6,13 ^a ± 0,85 | 36 | 37,56 ^a ± 1,90 |

*: Avg = Average; And = standard deviation; *: Within the same column, the means assigned by the same letter do not differ from each other (Student’s test at 0.05).

3.3.7 Effect on the Life Table

The survival curves of *T. castaneum* exhibit the same profile across the four types of wheat substrates (Figure 9). They belong to the Type III survival curve according to [13]. High mortality occurs during the juvenile life of the species. The substrates, hard wheat grains, and soft wheat grains appear to be less suitable for the development of *T. castaneum*. The other two substrates provide comparable survival rates (Figure 8).

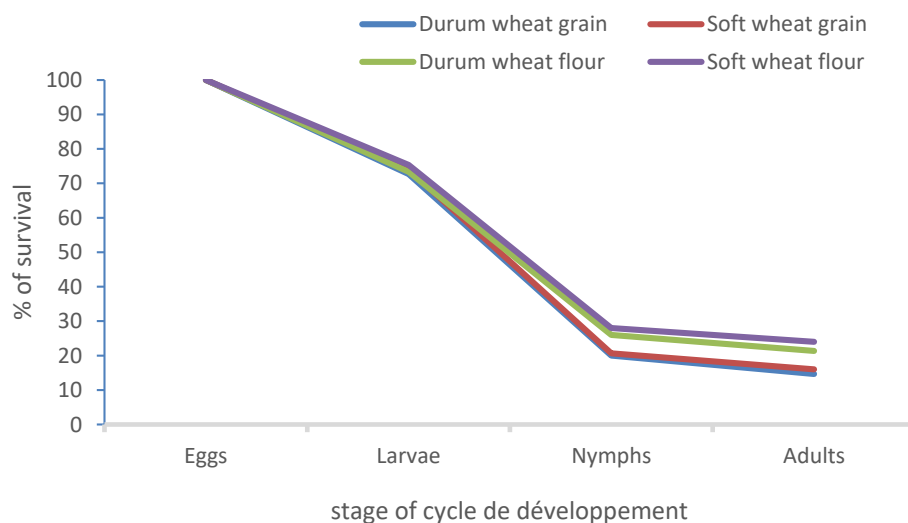


Fig 8: Survival curves of *T. castaneum* on the four types of wheat substrates.

4. Discussion

The four types of wheat substrates serve as suitable breeding and development media for *T. castaneum*. Considering the average fecundity of the insect, soft wheat flour and hard wheat flour appear to be more favorable substrates. The number of eggs laid on these flours may be linked to the physicochemical nature of the substrate. [14] reported that fecundity, development, and survival of *T. castaneum* were significantly higher in corn flour than in grains. Additionally, [15] and [16] found that texture played an important role and could impact the microclimate of *T. castaneum*. The differences in fecundity observed on the tested substrates in our conditions may also be explained by the presence of resistance factors in hard wheat grains (*Triticum durum*) compared to soft wheat grains (*Triticum aestivum*). This was evident in *T. castaneum* when it was made to lay eggs on other substrates such as corn, where the fecundity of *T. castaneum* was significantly higher in corn flour than on grains. It should also be noted that the finest flour in our case (soft wheat flour) had a higher risk of infestation.

It has been demonstrated that the chemical properties of flours have a significant impact on insect development. For example, corn and wheat flours supplemented with brewer's yeast are significantly better in terms of productivity than corn and wheat flour without yeast [17]. It has been shown that low-protein flours were not suitable for *T. castaneum* development, and high protein levels in flours such as soybean flour (>25%) were also unsuitable. This is consistent with [18], [19], and [20], who reported that *T. castaneum's* growth and development are more pronounced on high-protein foods. Low levels of riboflavin may be a factor in increasing development times or the absence of *T. castaneum* adult emergence [17, 21, 16]. Other studies have shown that high sodium levels result in decreased larval development [22]. [23, 4] found that higher levels of fiber and protein also tend to increase the number of eggs laid and the adult emergence rate. It is also not excluded that texture and chemical composition operate simultaneously to influence egg-laying behavior.

Regarding egg viability, there is no difference in the hatching percentages of eggs laid on each of the tested substrates, and the same applies to the egg incubation period. The duration and success of pre-imaginal stage development vary from one substrate to another. Similar observations were made by [24]. The extended duration of development and mortality of pre-imaginal stages on hard wheat grains and soft wheat grains may be due to the presence of physical barriers, such as the hardness of the seed coat, as observed in *T. castaneum* on corn grains. Studies by [25, 26, 27] have shown that larval survival was low and development was delayed in grains. [28] reported that seed hardness was negatively correlated with the number of eggs and F1 offspring emerged from *Rhizopertha dominica*. The development of *Tribolium* on wheat cultivar grains revealed that the pest's development was influenced by wheat variety

and grain size [29]. Other factors can also negatively affect pre-imaginal stage development, such as low carbohydrate content [30].

The type of flour had a significant effect on the number of eggs laid and adult emergence. The differences in egg-laying are likely due to volatile olfactory cues, texture cues, and/or gustatory cues. *Tribolium castaneum* prefers to feed and lay eggs on high-nutritional quality foods [31, 32, 33]. Low nutritional quality of foods can inhibit certain insect activities, particularly feeding [34, 35].

Overall, it is worth noting that the sources of these flours are a component to consider. This research suggests that there are several factors that can lead to a decrease in the risk of infestation by insects. A more detailed analysis of the components of these products will allow for a finer analysis of specific nutritional components crucial for the growth of *T. castaneum*. Further research needs to be completed to determine other nutritional factors and physical characteristics that can inhibit both egg-laying and the development of these pest insects.

In our study, we examined the relative risk of *T. castaneum* on four wheat substrates, but other species of stored product pests may have different patterns in terms of the risk they pose to these products. Substrate types and competition models between species may also differ [36, 37]. Other species may have very different responses to these tested substrates compared to *T. castaneum*. Strain-specific feeding preferences and toxicity effects for a single species could have a significant impact on infestation risks [38, 39]. Furthermore, understanding how insect communities interact with food sources is critical as the type of product and its abundance change. Changes in the composition of the insect community could lead to an increased risk of product infestation as other minor pests become more problematic.

5. Conclusion

Our study provides detailed insights into the behavior of *T. castaneum* on commercially available soft wheat and hard wheat grains and flour. We observe a significant risk of infestation by this pest in both flour and wheat grains. The results suggest that wheat grains inhibit the development of *T. castaneum*, making them more resistant to insect attacks. Therefore, it is advisable to store wheat in the form of grains for extended post-harvest storage, as this minimizes losses caused by this pest insect. On the other hand, wheat in the form of flour is susceptible to damage by *T. castaneum*, and long-term storage of flour is not recommended. Targeted treatments can now be implemented by managers to effectively control insect infestations in post-harvest grain storage.

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