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Biocidal Effect of Methanolic Extracts from the Fruits of *Capsicum frutescens* L. (Solanaceae) on the Larvae and Adults of *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae)

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Abstract

The efficacy was tested at concentrations 0, 0.025, 0.05, 0.075 and 0.1 g of powder per g of wheat grain and the methanolic extract at 0, 10, 20, 25 and 30 gl-1 of the fruits of *Capsicum frutescence* on the death of the first instar, the instar and adults of *Tribolium castaneum*. The results of the study indicated that there is a direct relationship between the percentages of larval and adult mortality and the concentrations of methanolic extract and powder used in the experiments.. The highest mortality rates were recorded in all stages of the insect during the first 24 hours after the treatment, and the mortality rate decreased significantly on the second and third days of the treatment compared to the mortality rates recorded on the first day, and the highest mortality rates were recorded in first stage larvae 70 % and fifth stage larvae 56.7 % during the first 24 hours of their treatment with a concentration of 30 $g.l^{-1}$ from the méthanolique extract of *Capsicum frutescence*, and at the same concentration the highest rates of cumulative mortality were recorded after 72 hours of treatment, were in the first stage larvae was 96.7% and in the fifth stage larvae 90%. At the same concentration, the highest rates of cumulative loss were recorded after 72 hours of treatment. They were 92% in first instar, 87% in fifth instar, and 67% in adults.

Key words: *Tribolium castaneum* larvae*; Capsicum frutescence*; methanolic extract; biopesticide.

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1. Introduction

The hot pepper plant *Capsicum frutescens* is one of the most widely distributed plants and is claimed in various regions of Morocco and its fruits are either red or green, with a very spicy taste and are used in many food industries and are used as popular prescriptions for treating many diseases [1]. Reference [2,3] used the extract of hot pepper against insects as it has a highly effective killer and repellent, especially against aphids, white fly and all insects with a soft body. He indicated that the addition of waxy substances to the pepper extract increases its effectiveness period to two weeks after treatment and increases its stability against rain and sunlight. The poor use of chemical pesticides in pest control has led to the emergence of many problems such as polluting the environment [4,5,6], the emergence of generations of pesticide-resistant insects [7,8], and their harmful and residual effect on treated plants, their high stability and slow decomposition in the environment [9]. For these reasons, the attention of researchers has recently turned to a return to the use of insecticides of plant origin [10]. The red rust beetle, *T. castaneum*, is considered one of the main storage insects and it is spread in all regions of the world, especially the warm regions of them live on all their phases on grains and their products, as well as dried fruits and vegetables, and they spread in mills, causing heavy economic losses. Several studies using plant extracts have been carried out to combat this pest [11,12,13,14,15]. Other studies of the fatal or repellant effect of chili fruit extracts on some insect pests [2,16]. Capsaicin is the material that is extracted from *Capsium annum* L., and used as a botanical insecticide [17]. Methanol extract of fruits and leaves of *C. frutescens* against second and third instar larvae of Aedes aegypti is observed by [18]. The study by [19] showed that that *Capsicum frutescens* have insecticidal effects on the maize weevil, S. zeamais. Reference [20] are found that the chili pepper fruit extract has any insecticidal effect on insects, specifically termites. The present study evaluated the efficacy of powder ant methanolic extract of *Capsicum frutescens* fruit against the adults and larvae of *T. castaneum.*

2. Materials and Methods

2.1. Tribulium castanium

2.1.1. Adults

The *Tribolium castaneum* species comes from a local market grain storage warehouse. It is maintained by mass rearing at a temperature of 28 ± 2 ° C and a relative humidity of 70 ± 5% and a photoperiod of 16: 8 hours (light: dark). To obtain a large population of *T. castaneum*, the adult individuals of the mixed sex were placed in 1 liter borosilicate glass jars containing a breeding medium composed of 95 g of hard wheat flour and 5 g of yeast. These jars are covered with mosquito net to allow insects to breathe. A week after laying the eggs, the adults are eliminated; the eggs laid evolved into first-generation adults 40-45 days after infection. In order to avoid the phenomenon of overpopulation, we proceed to a regular transfer of adults in new jars, thus making it possible to ensure new generations. We used adults of known age between 10 and 14 days in our trials.

2.1.2. Larvae

Adult individuals of the same sex and of known age, between 10 and 14 days old were placed in 1 liter

borosilicate glass jars containing a rearing medium composed of 95 g of hard wheat flour and 5 g of yeast. These jars are covered with mosquito net to allow insects to breathe. A week after laying the eggs, the adults are eliminated; the eggs laid were placed in petri dishes (Ø 90 mm) containing no food. These petri dishes are kept under the same *T. castaneum* rearing conditions and inspected every 24 hours. We used in our tests fist instar and fifth instar of *T. castaenum* obtained respectively 2 days and 21 days after hatching of the eggs.

2.2. Preparation of the powder

The dried fruits of Capsicum frutescence were bought at the local market of Meknes (Morocco) and dried in an oven set at 40 ° C to constant weight; they were then crushed. The powder is passed through a 0.5 mm mesh sieve and then stored in cool glass vials, protected from light and air.

2.3. Extraction of the compounds from the fruits of Capsicum frutescens

The fruits used come from the cultivation of hot peppers, a local variety. 50g of *C. frutescens* fruit, dried to constant weight and crushed, is extracted with a soxhlet using 250 ml of absolute methanol for analysis (LABOSI) for 8 hours. The extract was then recovered after evaporation at 40 ° C under vacuum using a Bruchi-type rotary evaporator at speed 4. The dry extract was taken up in 1% methanol. The crude extract in solution was diluted according to the desired concentration for biological tests. For each concentration 3 repetitions were carried out.

2.4. Biological tests

2.4.1. Evaluation of the ingestion effect of Capsicum frutescence fruits

2.4.1.1. Effect of the methanolic extract of Capsicum frutescence fruits on T. castaneum adults

Twenty (20) individuals of *T. castaneum* aged 10 to 14 days are introduced into petri dishes (Ø 90 mm) each containing 20 g of cracked wheat kernels dipped beforehand in the methanolic extract at concentrations of 10 g, 20 g, 25 g, and 30 g of extract per liter of 1% methanol for 20 seconds and then dried in the open air for two hours to evaporate the methanol. The tests are repeated 3 times for each dose and for the control ($0g.l^{-1}$). The number of dead insects is calculated every 24 hours for 6 days after treatment. The petri dishes are kept under the same conditions for *T. castaneum* rearing.

2.4.1.2. Effect of the methanolic extract of Capsicum frutescence fruits on T. castaneum larvae

Twenty (20) larvae of *T. castaneum*, whether first or fifth instar, were added to each Petri dish (Ø 90 mm), each containing 2 grams of flour hard wheat mixed with yeast, in a ratio of (1: 20) previously sprayed with methanolic extract at concentrations of 10 g, 20 g, 25 g, and 30 g of extract per liter of 1% methanol and left to air dry for two hours to evaporate the methanol. The tests are repeated 3 times for each dose and for the control (0g/l). Percent larval mortality was calculated 24, 48 and 72 hours after treatment. The Petri dishes are stored under the same conditions for growing *T. castaneum*.

2.4.1.3. Effect of Capsicum frutescence fruit powder on the viability of T. castaneum larvae

Twenty (20) larvae of *T. castaneum*, whether of the first or fifth instar, are introduced into petri dishes (Ø 90 mm), each containing 2 grams of hard wheat flour mixed with yeast, in a ratio of (1: 20) previously mixed with different doses of powders of the fruits of *C. frutescence*. The doses used are 2.5%, 5%, 7.5% and 10% of the weight of the powder per weight of seeds, i.e. a powder weight of 0.05 g, 0.1 g, 0.15 g, and 0.2 g. respectively. Control batches (0%) are produced in parallel with the untreated hard wheat flour. The tests are repeated 3 times for each dose and for the control. The number of dead larvae is calculated 24, 48 and 72 hours after treatment. The petri dishes are kept under the same conditions for *T. castaneum* rearing.

2.5. Data analysis

The results of the various tests were subjected to statistical analysis with the Fisher test. This method makes it possible to compare the means two by two, for the different doses of powder and methanolic extracts, with the Statview V5.0 software. If the adjusted probability is less than 0.05, the difference is significant. If the adjusted probability is greater than 0.05, the difference is considered not significant. Mortalities have been corrected according to the method of [21].

3. Results

3.1. Effects of methanolic extract of Capsicum frutescens fruits on the viability of T. castaneum adults

The results in table 1 indicate that adult mortality rates increase with increasing concentrations of methanolic extract. Adult mortality rates increased significantly after 24, 48, 72 hours of treatment with methanolic extract concentrations compared to control adult mortality rates. The highest percentage of adult mortality was 38.33% when treated with 30 $g.l^{-1}$ after 24 hours of treatment. The highest mortality rates in adults occurred after 24 hours of treatment with all concentrations of methanolic extract, and mortality rates were significantly reduced on the second, third and fourth days of treatment compared to the rates of mortality recorded on the first day. This one vary from about 0 to 1.6% with 0 $g.l^{-1}$, from 10 to 3.33% with 10 $g.l^{-1}$, from 13.33 to 10% with 20 $g.l^{-1}$, from 31.67 to 10% with 25 $g.l^{-1}$, and from 38.33 to 8.33% with 30 $g.l^{-1}$.

a, b, c Within the same concentration, the means affected by the same lowercase letter do not differ statistically between them according to the Fisher test at the probability threshold (5%); A, B, C Within the same period, the means affected by the same capital letter do not differ statistically between them according to the Fisher test at the probability threshold (5%).

Table 1: Mean mortality (± SD) of adults of *Tribolium castaenum* due to methanolic extract from *Capsicum frutescens* fruits.

Hours after Treatment	Concentrations (g. l^{-1})				
	0	**10**	**20**	**25**	**30**
24	$0,00^{aA}$ ± 0,00	$10,00^{aB}$ ± 0,00	$13,33^{aB}$ ± 2,89	$31,67^{aC}$ ± 6,24	$38,33^{aC}$ ± 2,50
48	$0,00^{aA}$ ± 0,00	$6,67^{bB}$ ± 2,89	$13,33^{aC}$ ± 2,89	$16,67^{bC}$ ± 5,77	$18,33^{bC}$ ± 2,36
72	$0,00^{aA}$ ± 0,00	$5,00^{bB}$ ± 0,00	$11,67^{aC}$ ± 2,36	$11,67^{bC}$ ± 2,36	$11,67^{cC}$ ± 2,36
96	$0,00^{aA}$ ± 0,00	$5,00^{bB}$ ± 0,00	$10,00^{aC}$ ± 0,00	$10,00^{bC}$ ± 0,00	$11,67^{cC}$ ± 2,36
120	$1,67^{bA}$ ± 0,00	$3,33^{cB}$ ± 0,00	$10,00^{aC}$ ± 0,00	$10,00^{bC}$ ± 0,00	$10,00^{cC}$ ± 0,00
144	$1,6^{bA}$ ± 0,00	$3,33^{cB}$ ± 0,00	$10,00^{aC}$ ± 0,00	$10,00^{bC}$ ± 0,00	$8,33^{dC}$ ± 2,36

3.2. Effect of the methanolic extract of Capsicum frutescens fruits on the viability of T. castaneum instar

The results of the second table indicate that the mortality rates of the first and fifth instar treated with methanolic extract of *C. frutescence* increased with increasing concentration. The proportion of deaths from the first instar larvae is statistically higher than that from the fifth instar taken alone. Also the mortality rate of the treated instar is significantly higher compared to the mortality rate of the instar of the control group (0 $g.l^{-1}$). The highest mortality percentages which were recorded after 24 hours of treatment with the concentration of 30 $g.l^{-1}$ are 70% and 56.7% of the first and fifth instar, respectively. The mortality rates were significantly reduced on the second and third days of treatment, compared to the mortality rates on the first day and this for all the concentrations used in the experiment.

Table 2: Mean mortality (± SD) of the first and fifth instars of *Tribolium castaenum* due to methanolic extract from *Capsicum frutescens* fruits

Concentrations (g. l^{-1})	**After 24 heures**		**After 48 heures**		**After 72 heures**	
	First instar	**Fifth instar**	**First instar**	**Fifth instar**	**First instar**	**Fifth instar**
0	$3,33^{aA}$ ± 2,55	$6,66^{aB}$ ± 2,55	$3,33^{aA}$ ± 2,55	$0,00^{aC}$ ± 0,00	$0,00^{aC}$ ± 0,00	$0,00^{aC}$ ± 0,00
10	$28,33^{bA}$ ± 2,55	$13,33^{bB}$ ± 0,96	$16,66^{bB}$ ± 2,55	$6,66^{bC}$ ± 2,55	$11,66^{bB}$ ± 2,55	$6,66^{bC}$ ± 2,55
20	$46,66^{cA}$ ± 2,55	$26,66^{cB}$ ± 0,96	$18,33^{bC}$ ± 2,55	10^{cD} ± 0,00	$16,66^{bC}$ ± 2,55	10^{bD} ± 5,00
25	$56,66^{dA}$ ± 2,55	$43,33^{dB}$ ± 2,55	$21,66^{bC}$ ± 2,55	$20,00^{dC}$ ± 0,00	$5,00^{cD}$ ± 0,00	$3,33^{cE}$ ± 0,96
30	$70,00^{eA}$ ± 2,89	$56,66^{eB}$ ± 2,55	$16,66^{bC}$ ± 2,55	$26,66^{eD}$ ± 0,96	$11,66^{bE}$ ± 2,55	$13,33^{bE}$ ± 0,96

a, b, c Within the same concentration, the means affected by the same lowercase letter do not differ statistically between them according to the Fisher test at the probability threshold (5%); A, B, C Within the same period, the means affected by the same capital letter do not differ statistically between them according to the Fisher test at the probability threshold (5%).

3.3. Effects of Capsicum frutescens powder fruits on the viability of T. castaneum larvae

The daily observations of the first of the fifth instar of *Tribolium castaenum* feeding on wheat flour mixed with the powder of the fruit of *Capsicum frutescens*, allowed us to record variable responses according to the concentration considered, the instar and the time of exposure. As shown in table 3, after 72 hours the powder causes low larval mortality. The highest mortality rates are around 10% for the first instar and 5% for the fifth instar after 72 hours of exposure. This rate remains statistically significant compared to the control batches (0 $g.g^{-1}$).

Table 3: Mean mortality (± SD) of instars and adults of *Tribolium castaenum* due to methanolic extract from *Capsicum frutescens* fruits.

Concentrations ($g.g^{-1}$)	**After 24 heures**		**After 48 heures**		**After 72 heures**	
	First instar	**Fifth instar**	**First instar**	**Fifth instar**	**First instar**	**Fifth instar**
0	$0,00^{aA}$ ± 0,00	$0,00^{aA}$ ± 0,00	$0,00^{aA}$ ± 0,00	$0,00^{aA}$ ± 0,00	$3,33^{bA}$ ± 2,36	$0,00^{aA}$ ± 0,00
0,025	$0,00^{aA}$ ± 0,00	$0,00^{aA}$ ± 0,00	$0,00^{aA}$ ± 0,00	$0,00^{aA}$ ± 0,00	$5,00^{bA}$ ± 0,00	$0,00^{aA}$ ± 0,00
0,050	$0,00^{aA}$ ± 0,00	$0,00^{aA}$ ± 0,00	$5,00^{bB}$ ± 0,00	$0,00^{aA}$ ± 0,00	$8,33^{cB}$ ± 0,00	$5,00^{bB}$ ± 0,00
0,075	$5,00^{aB}$ ± 0,00	$0,00^{bA}$ ± 0,00	$6,66^{aB}$ ± 0,00	$5,00^{aB}$ ± 0,00	$10,00^{cC}$ ± 2,36	$5,00^{aB}$ ± 0,00
0,100	± 0,00	± 0,00	± 2,36	± 0,00	± 0,00	± 0,00

a, b, c Within the same concentration, the means affected by the same lowercase letter do not differ statistically between them according to the Fisher test at the probability threshold (5%); A, B, C Within the same period, the means affected by the same capital letter do not differ statistically between them according to the Fisher test at the probability threshold (5%).

3.4. Cumulative mortality of Tribolium castaenum instar and adults treated with methanolic extracts of Capsicum frutescens fruits

The results of table 4 of the cumulative mortality in the first, fifth instar and adults of *Tribolium castaenum* after 72 hours of treatment with different concentrations of methanolic extract of hot pepper indicated that the cumulative mortality rates of the three stages of the insect increased significantly with the increase in the concentration of methanolic extract compared to the control group. Results of table 4 It is clear that the first

instar were the most sensitive and had the highest percentages of cumulative mortality compared to the fifth instar and adults and in all the extract substrates used in the experiment Followed by the fifth instar larvae and then the adults. Therefore, the adults are considered to be the most resistant to the methanolic extract compared to the larvae. The reason for the high resistance shown by the red rusty flour beetle adults to the methanolic extract of hot pepper compared to the larvae of the first and fifth instars may be due to the fact that the adults cover their body with a hard structure that protects them from the extract in their structure, which reduces its effect on them. This avoids the presence of plant pesticides on their food, unlike the larvae that are eager to feed them, especially the first-stage larvae, which need large amounts of food for the purpose of completing their growth.

Table 4: Cumulative mean (± SD) of instars and adults mortality of *Tribolium castaenum* due to methanolic extract from *Capsicum frutescens* fruits.

Concentrations (g. l^{-1})	Percentage of cumulative larval mortality after 72 hours		Percentage of cumulative adult mortality after 72 hours
	First instar	Fifth instar	Adults
0	$6,67^{aA}$ ± 2,36	$6,67^{aA}$ ± 2,36	$0,00^{bA}$ ± 0,00
10	$56,67^{aB}$ ± 4,08	$21,67^{bB}$ ± 2,89	$23,33^{bB}$ ± 4,71
20	$81,67^{aC}$ ± 2,36	$46,67^{bC}$ ± 4,71	$35,00^{cC}$ ± 0,00
25	$85,00^{aC}$ ± 4,08	$73,33^{bD}$ ± 4,71	$60,00^{bD}$ ± 8,16
30	$91,67^{aC}$ ± 4,71	$86,67^{aE}$ ± 2,36	$66,67^{bD}$ ± 2,36

a, b, c Within the same concentration, the means affected by the same lowercase letter do not differ statistically between them according to the Fisher test at the probability threshold (5%); A, B, C Within the same period, the means affected by the same capital letter do not differ statistically between them according to the Fisher test at the probability threshold (5%).

4. Discussion

A Significant insecticidal activity against larvae and adults of *Tribolium castaneum* was observed with crude methanol extract of *Capsicum frutescens* fruit at concentrations of 30g.l^{-1}. This result coincides with that reported by [22] using *Capsicum frutescens* giving a considerable reduction in *C. maculatus* infestations at all

stages of development, The cause of high mortality in adults of *T. castaenum* treated with concentrations of methanolic hot pepper extract may be due to the presence of compounds with insecticidal properties. Reference [2] reported that the chili fruit contains 0.93 to 1.34 mg of capsaicin and that its action may be repellent or toxic, and [23] reported a mean mortality of S. zeamais after 28 days of treatment with three doses (0.2, 0.4 and 0.6g of *Capsicum frutescens* / 20g corn seeds). [24] have also shown the antiparasitic activity of capsaicin in plant culture. Hot pepper extract has been effective in reducing the density of spider mites and aphids [25,26], and capsaicinoids extracted from capsicum have a biocidal effect on A. cytisorum, acting in a relatively short time. On the other hand, chili-based fumigants have also been reported to have insecticidal, repellant and anti-appetite effects on a wide range of insect pests [27,28]. The cause of death of the first and fifth instar larvae of *T. castaenum* after its release in durum wheat flour treated with the methanolic extract of *Capsicum frutescens* may be due to the anti-appetite action of the extract on larvae or to toxicity by ingestion of capsaicin and its derivatives if they have fed on treated durum wheat flour. Reference [29] pointed out that certain plant extracts, contain toxic substances witch lead to damage the epithelial layer that lines the middle gastrointestinal tracthus obstructing the secretion of digestive enzymes and therefore the death of the larvae. The present study showed that the highest mortality rates in first and fifth instar larvae occurred after 24 hours of treatment compared to the lowest rates recorded on the second and third days of treatment, Reference [30] attributed this to the loss of plant extracts of their effectiveness by their rapid degradation in the environment.

5. Conclusion

The results obtained in this study show that the powder and methanolic extracts of *C. frutescens* have a toxic power on adults and instar of *T. castaenum*, with a more pronounced activity of methanolic extracts. In the short and medium term, the powder with 0.1 $g.g^{-1}$ of sheaths and the methanolic extracts at 30 $g.l^{-1}$, can be integrated into a management program for *T. castaenum*, a pest of cereals stored especially in a situation at risk of resistance. to other products. However, it remains to assess their possible effects on natural auxiliaries associated with stored cereals. Indeed, the most interesting organic products, used to protect stored cereals, are those which have a minimal impact on all the components of the storage areas. Moreover, knowing that plant extracts lose their biological activity under radiation solar [31], modes of action, methods of application and the impact of factors physics on the degradation of botanical compounds tested should also be investigated.

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