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Bio-Efficacy Of Flavonoid Extracts And Plant Powders Of Two Artemisia Against Tribolium Castaneum (Coleoptera: Tenebrionidae)

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Abstract
This study aims to estimate, in laboratory conditions, the insecticidal effect (leaf powders and flavonoid extracts) of two medicinal plants: Artemisia herba-alba and Artemisia campestris against the pest of stored cereals Tribolium castaneum. The treatments were administered by direct contact to adults of T.castaneum. The vegetable powder was tested with five doses 1, 2, 3, 4, 5% and four doses of the flavonoids are tested 10, 30, 60, 80µl. The toxicity of the crude phytopreparations was estimated by evaluation of mortality, LD50, LD95, TL50. All tested products cause total mortality in adults of T. castaneum. A.herb- alba vegetable powder is more active than A. campestris powder. For flavonoid extracts, it is the butaolic fraction which is the most effective.
Keywords: *Artemisia herba alba, Artemisia campestris, vegetable powder, Flavonoid extract, Tribolium castaneum, Insecticidal activity.*

INTRODUCTION

Stored food constitutes the group of agricultural products most traded on international markets. As a result, we are obliged to fight against parasitic species which compete for food with humans [1]. Phytophagous insects are considered a plague that threatens human food resources. In developing countries, particularly in Asia and Africa, the damage caused by insects is important because of the climatic conditions suitable for their development.

Stored food insects including Tribolium castaneum represent a very important part of stored food pests. They can cause significant losses by reducing the quality and quantity of stored products [2]. According to the Food and Agriculture Organization of the United Nations (FAO), losses due to pests correspond to 35% of world agricultural production [3]. Due to the high outbreaks of these insects, the control using synthetic chemical pesticides is the most widely used method. Although pesticides are effective, their collateral effects on the environment are undoubtedly invaluable and the fate of these chemicals in ecosystems remains poorly understood [4]. To-date, more than 5000 flavonoids are documented and their antimicrobial/insecticidal activities demonstrated. Flavonoids from the roots of Pueraria lobata have exhibited antityrosinase activity, and isoflavonoids isolated from Gliricidia sepium do act as anti-termite compounds against Glyptotermes dilatatus . Flavonoids of Thea sinensis and Sophora flavescens have shown promising antimicrobial activity against gram-positive bacteria, Staphylococcus aureus and Bacillus subtilis [5].Recent research has shown that plant extracts have several properties that allow them to be part of alternative strategies aimed at limiting the use of synthetic organic pesticides in agriculture. In this context, we propose to study, during this research work, the bioinsecticidal effect of plant powders and flavonoids of two species of Artemisia growing spontaneously in the region of Djelfa on adults of Tribolium castaneum. Artemisia campestris L. is an important crop widely used in the south Mediterranean basin as a food spice, and also in folk medicine [boukhql]. The benefcial effects on the health of this plant have prompted us to explore its secondary metabolites to provide important information on its chemical content. Among The objective of this study was to extract phenolic compounds from the aerial part from A. campestris and to determine the chemical composition of methanol fraction.

MATERIALS AND METHODS

1. Plant material

The aerial parts of Artemisia herba alba and Artemisia campestris were collected in the region of Moudjebara and Amra wilaya of Djelfa in February 2017. Table 1 records the data on the selected stations.

The criteria for choosing plant material are based on the availability of the plant in Algeria and on its use in traditional local pharmacopoeia. In order to preserve as much as possible the integrity of its chemical composition, the aerial part of each plant was dried in the shade and protected from heat and light for fifteen days before use. All parts of each plant species was grounded to fine powder and preserved in jars.

Table 1: Geographic and bioclimatic parameters of the harvesting stations

Plants	Stations	Longitude (North)	Latitude (East)	Altitude (m)	Bioclimatic stage
A. herba-alba	Moudjebara	34° 30' 15.45"	3° 28' 18.74"	1040	Semi-arid to cool winter
A. campestris	Amra	34° 22' 24.67"	3° 8' 44.55"	1142	Semi-arid to cool winter

2. Insecte test

Tribolium castaneum is a pest of stored food, it is found in all parts of the world (cosmopolitan). It exists where the stored grain exists in the form of grains or flour. It is very abundant in the tropics. In cold climates, it is present only in high temperature storage [6]. From the age of three days, the female lays about 500 to 800 eggs daily which hatch after five days at 30 ° C. The larvae circulate freely in the infested foodstuffs and pupate there without cocoons. At 30 ° C, larval life lasts about three weeks and the adult emerges from the nymph six days after formation. The longevity of the insect varies from 2 to 8 months depending on the abiotic conditions [7]. Tribolium castaneum is able to infest wheat, corn, barley, sorghum, millet, cassava, peanuts, cotton, castor, cocoa [8].

3. Rearing the insect

The mass rearing of insects is carried out in the natural and life sciences laboratory of the biology department of Ziane Achour University of Djelfa, in a glass jar on durum wheat semolina, the jar is placed in a controlled oven. At a temperature of 25C ° and a relative humidity of 65 to 70% and a photoperiod 12h / 12h, which constitutes the optimal conditions for the development of this insect [9].

4. Preparation of plant extracts

Following the protocol described by Merghem [10], the crushed plant material (50 g) is subjected to extraction by maceration in an ethanol / water mixture (48/12: v / v) for 72 hours with renewal of the solvent. Every 24 hours. The maceras are combined, then they are filtered and then evaporated using a rotary evaporator. The dry residue is taken up in boiling distilled water to undergo decantation. After decanting overnight, the clear phase is recovered. After the recovery of the limpid phase will undergo clashes by solvents of increasing polarity. This step makes it possible to separate the flavonoids according to their structure and their degree of polymerization by confronting them with several solvents ranging from less polar to more polar. The aqueous phase is successively confronted with the following solvents: Petroleum ether, Chloroform, Ethyl acetate, 1-butanol.

5. Biological tests

5.1. Evaluation of the effect of leaf powder on adults of Tribolium castaneum

Five (5) pairs of Tribolium castaneum are introduced into Petri dishes containing 25g of wheat semolina mixed with the powder of the leaves of each aromatic plant in five chosen doses (1%, 2%, 3%, 4%, and 5%) the weight of the powder per weight of seeds [11].

5.2. Evaluation of the effect of flavonoids by contact on adults of Tribolium castaneum

Weights of 25g of wheat semolina introduced into Petri dishes are treated with the flavonoids of Artimisia herba-alba and Artimisia compestris at doses of 10 µl; 30µl; 60µl; 80µl using micropipettes [12].

The experimental follow-up is carried out for 20 days by noting daily the number of dead individuals and any behavioral abnormalities observed.

6. Exploitation of results

6.1. Mortality rate

Mortality is the primary endpoint of the effectiveness of a chemical or biological treatment. The percentage of mortality observed in control adults treated with plant extracts is estimated by applying the following formula [13]:

Observed mortality = [Number of deaths / Total number of individuals] × 100

6.2. EC50 efficiency concentration

The EC50 is one way of measuring the short-term toxic potential (acute toxicity) of a material. For the present study, the Probit method is followed [14].

6.3. Mortality time

Lethal times 50; correspond to the time required for 50% of individuals in a population to die following treatment with a given substance. It is calculated from the regression line of the probits corresponding to the percentage of mortality corrected for the logarithms of treatment time [8].

7. Statistical analyzes

In order to evaluate the results obtained for each parameter studied, we used a statistical test using SPSS © software version 22.0.0 for Windows ™.

Significant differences between groups were statistically analyzed by analysis of variance (ANOVA) using the Global Linear Model (GLM). P values less than 0.05 are considered significant. The Duncan test was used to compare the mean values of mortality under the effect of doses and fraction [16].

To estimate the lethal concentration 50 (EC50), it was carried out to transform the percentages of corrected mortalities into probits, and to transform the doses applied into a decimal logarithm: These transformations allow us to establish equations of the regression lines of log dose as a function of probits [15]. To estimate the TL50 in the use of plant extracts, regression lines were constructed by drawing up the mortality rate (given in Probits) as a function of the treatment time (taken as a logarithm) [16].

RESULTS

1. Insecticidal effect of Artemisia herba alba powder and Artemisia campestris powders on adults of Tribolium castaneum (by contact)

From the results presented in Figure 2, we observe that adult mortality of T. castaneum is proportional to exposure time. A.herba alba powder induces adult mortality from 2nd day for all doses tested. Total adult mortality of T. castaneum is recorded on 20th day except for the 5% dose where 100% mortality was observed on 15th day. The vegetable powder of A. campestris at a dose of 3%, 4% and 5% causes the mortality of adults of T. castaneum since the 4th day (Figure 3). However, mortality did not begin until 8th day for the 1% and 2% dose. The mortality of 100% of adults of T. castaneum is recorded from the 17th day for the 4% and 5% doses and on the 18th day for the 3% dose.

Analysis of variance for the dose of A. herba alba powder and A. campestris powder did not reveal a significant difference between the doses (p = 0.997, p = 0.215). The percentage of insect mortality does not vary considerably when the dose is changed.

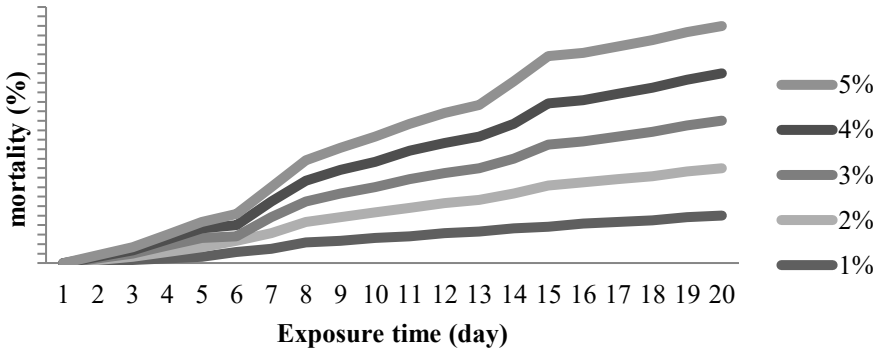

Figure 2: Evolution of adult mortality of Tribolium castaneum as a function of time and powdered doses of A. herba alba.

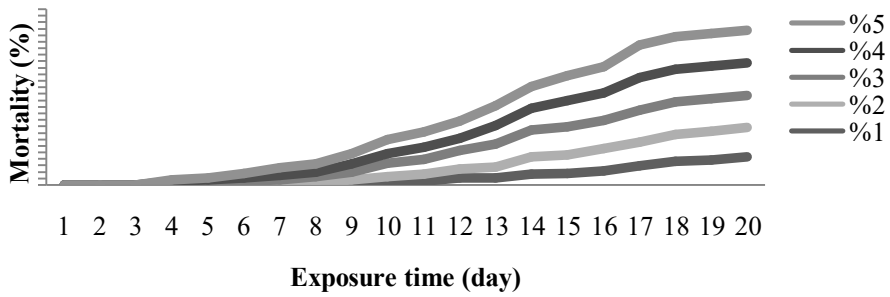

Figure 3: Evolution of adult mortality of Tribolium castaneum as a function of time and doses of powder A. campestris.

2. Insecticidal effect of Artemisia herba-alba extracts on adults of Tribolium castaneum

From the results obtained we observed that the mortality varies according to the fraction and the dose (Figure 4). Analysis of variance revealed a significant difference between doses ($p = 0.000 < 0.05$). Duncan's test classifies doses into two groups A, B. Dose 10, 30 µl in group A and group B contains dose 3 and 4 (60, 80 µl). It is the chloroform fraction that causes mortality that exceeds 90% for all doses of 10, 30, 60 and 80µl. Analysis of variance for the fraction factor also revealed a very highly significant difference between the fractions ($p = 0.000 < 0.05$). according to Duncan's test at the 5% threshold, insects treated with the ethyl acetate fraction and the petroleum ether fraction are classified as homogeneous group (group A) while the chloroform fraction is classified in the second group (group B) and the n-butanol fraction consists of the third group (group C). The mortality of the insects was total and that for the treatment by the dose of 60 and 80µl. It can be noted that for all fractions, the mortality rate is proportional to the dose. As it can be observed that the percentage of mortality is 100% for the insects treated with the 80 µl dose for all the fractions.

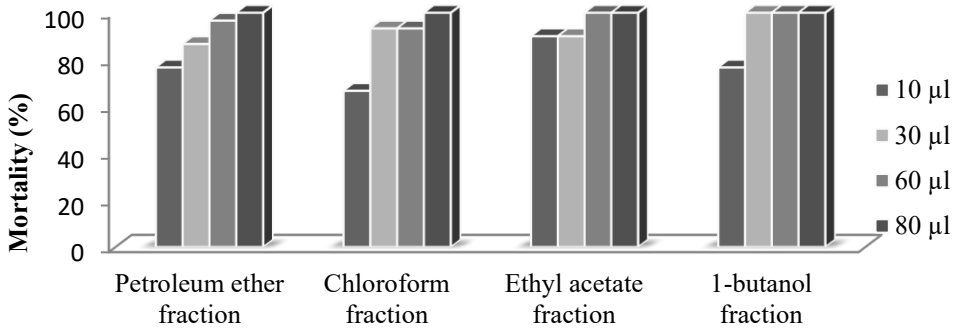

Figure 4: Percentage of mortality (%) of adults of Tribolium castaneum after treatment with alcoholic fractions of A. herba-alba

3. Percentage of mortality (%) of adults of Tribolium castaneum after treatment with the alcoholic fractions of A. campestris

All the fractions induce insect mortality exceeding 80% with all the doses. It is the n-butanol fraction that causes mortality in excess of 90% for all doses.

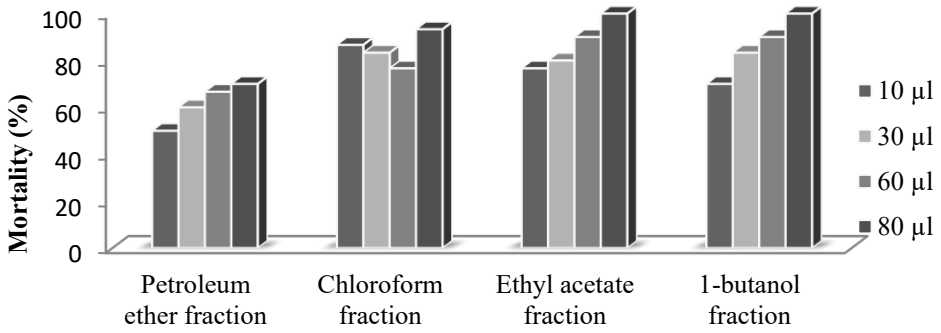

Figure 5: Percentage of mortality (%) of adults of Tribolium castaneum after treatment with the alcoholic fractions of A. campestris

Duncan's test at the 5% cut-off grouped the fractions into two homogeneous groups. Insects treated with the petroleum ether fraction, the n-butanol fraction and the ethyl acetate fraction were classified in a single group (group A), the second group (group B) is the insects treated with the chloroform fraction. Regarding the dose factor, ANOVA showed the existence of a very highly significant difference between the doses ($p = 0.000$). According to the Duncan test, insects treated with the 10, 30, 60 µl dose are classified as a homogeneous group (group A) and the insects treated with the 80 µl dose is the second (group B).

4. Biocidal efficacy of the vegetable powder on adults of Tribolium castaneum

To estimate the lethal concentration 50 (EC50) from which 50% of the mortality is obtained, the percentages of mortalities in probits were transformed, and the doses applied into decimal logarithm: These transformations we allow the establishment of straight line regression equations of the dose as a function of the probits.

Table 5. Regression equation, regression coefficient and values of LD50 and LD95 for vegetable powders.

	Regression equation	Regression coefficient	DL 50 mg/g	DL95 mg/g
A.herba-alba powder	y=1,36+0,74x	R^2 = 0,77	2,24	44,96
A.campestris powder	y=0,86+1,78x	R^2 = 0,54	13,64	34,44

In view of the results of (Table 5), it is noted that the concentrations which cause the mortality of 50% and 95% of the insects treated with the vegetable powder of A.herba-alba are of the order of: LD50 = 0.24 µg / g and LD90 = 44.96 µg / g. For the vegetable powder of A. campestris, it appears that the lethal doses which cause the mortality of 50% and 90% of the images are of the order of 13.64 µg / g and 34.44 µg / g respectively. We can say that the powder of A.herba-alba was more toxic than that of A. campestris.

5. Biocidal efficacy of A.herba-alba extracts on adults of Tribolium castaneum

From the table 3 we note that LD50, LD95, TL50 vary from one fraction to another. For A.herba-alba the petroleum ether fraction is the most toxic LD50 = 0.03 µg / g. but for the A. campestris is the fraction of Chloroform that appears to be the most toxic with a value of LD50 = 0.01 µl / g.

From the TL50 values of each fraction, it appears that the fraction of 1-butanol from A. herba alba appear to be more toxic, and it shows a particular rapidity of action against adults of Tribolium castaneum. According to all the results obtained, we were able to classify the fractions of A.herba alba of decreasing order according to their effectiveness against this pest as follows: The petroleum ether fraction> chloroform fraction > fraction of 1-butanol> fraction of ethyl acetate. Whereas for A. campestris: Chloroform fraction> ethyl acetate fraction petroleum ether fraction> 1-butanol fraction.

Table 6. Regression equation, regression coefficient, and LD50 and LD95 values for flavonoid extracts.

	Regression equation	regression coefficient	LD50 µl/g	LD95 µl/g	LT 50 (day)
petroleum ether fraction of A. herba alba	y=0,35+0,49x	R^2=0,99	0,03	1,91	11,46
Chloroform fraction of A. herba alba	y=0,92+1,45x	R^2=0,85	0,23	1,80	11,14
ethyl acetate fraction of A. herba alba	y=2,77+1,41x	R^2=0,96	3,54	46,89	14,21
fraction of 1-butanol from A. herba alba	y=3,47+3,22x	R^2= 0.98	0,48	1,40	5,98
petroleum ether fraction of A. campestris	y=0,73+0,47x	R^2= 0,84	1,39	4274,00	12,68
Chloroform fraction of A. campestris	y=0,02+0,43x	R^2=0,156	0,01	3787,77	8,96
ethyl acetate fraction of A. campestris	y=1,19+1,05x	R^2=0,156	0,55	22,77	11,34
fraction of 1-butanol from A. campestris	y=2,37+1,4x	R^2=0,87	1,98	28,05	12,43

6. Comparative analysis of treatments

The analysis of variance model G.L.M. (Table 7) showed that the dose and the fraction factor which have a very significant effect in percent mortality (p = 0.001, p = 0.000). Too the interaction (species * fraction) has a very highly significant effect in mortality (p = 0.000). The factor species did not has a significant effect in mortality (p = 0,249).

Table 7: Model G.L.M. applied to the insecticidal power of the treatments.

Source	Sum of squares type III	Df	Mean square	F	Signification
Day	662928,988	1	662928,988	4098,934	,000
Species	1666,713	1	1666,713	10,305	,249

Fraction	27482,667	3	9160,889	56,642	,000
Dose	25340,682	3	8446,894	52,228	,000
species * fraction	39471,027	3	13157,009	81,351	,000
species * dose	255,754	3	85,251	,527	,977
fraction * dose	8674,014	9	963,779	5,959	,644
species * fraction * dose	7388,886	9	820,987	5,076	,749

DISCUSSION

The use of plants or plant extracts (roots, leaves, bark and fruits) to protect crops against insect pests during storage is an ancient practice that is widespread in Africa and Asia [17]. According to the same authors, the branches and fresh leaves of aromatic plants are used to protect stored corn kernels and beans and cowpea kernels from attack by various insects stored in traditional storage containers. Even after a long period of storage, the dry leaves of these plants continue to emit strong persistent aromatic odors linked to their composition in essential oils which may be at the origin of their bio-activity.

Powders obtained by grinding the different organs (flowers, seeds, bark, roots and leaves) of dried plants have been tested against several pests of stored foodstuffs [18]. Work by Quarles [18] has shown that Chenopodium ambrosioides powders have a broad spectrum of action against insects but also nematodes, fungi and viruses. The toxic and repellent effect of C. ambrosioides and Eucalyptus saligna powders against cowpea weevil (C. maculatus) was noted by the work of Tapondjou [7].

The work of Ghandi [19], on the bio-efficacy of powders from the leaves of a Lythraceae (Punica granatum) and a Rutaceae (Murraya koenigii) against a pest of stored food stuffs, Tribolium castaneum, revealed a high mortality of adults and a delay in the development of the insect as well as a significant reduction in the population. These powders contain complex chemical compounds that may show higher bioactivity compared to plant constituents isolated by extraction methods.

The work of Kellouche [20] carried out on the cowpea weevil (C.maculatus) showed that the powders of aromatic plants belonging to the Myrtaceae, Moraceae, Oleacea and Rutaceae families significantly reduce the longevity of adults and / or the fertility of females. In addition, Righi [21], by testing the powders of the leaves and flowers of some spontaneous plants (Thyme, Santoline and Anagyrefétide) as well as those of the chickpea, observed a reduction in longevity, fecundity, fertility. , as well as the weight and the length of the adults of the chickpea weevil (Chinese weevil) C. chinensis in particular for the powders of the leaves which showed a bio-insecticidal effect even at very low doses.

Bouchikhi [22] studied and compared the biocidal effect of powders of ten aromatic plants including Lamiaceae, Rutaceae, Cistaceae and Asteraceae on adults of the bean weevil and moth (Tineola bisselliella). He has shown that they exhibit insecticidal properties on A.obtectus and insecticidal and larvicidal properties on T. bisselliella, as well as reducing the fertility of females of both species.

Delimi [1] studied the insecticidal effect of the essential oil extracted from Artemissia herba alba on the population of insect pests of stored food Ephestia kuehniella (Lepidoptera). They showed that the mortality rate of adults compared to controls was significantly affected during five days of exposure. In addition, the administration of the essential oil to pupae prolongs their pupal development and significantly disrupts the reproduction of exuviated adults.

Nowadays, there is great interest in the research of botanical pesticides, due to their minor toxic effects on the environment and humans. In this regard, further research on the insecticidal activity of A. campestris L. have been undertaken. The methanolic extract of its stem was found to exhibit the highest larvicidal activity, with 100% mortality of Culex quinquefasciatus (mosquito larvae), and the estimated LD50 value was approximately 23 ppm [23]. However, the larval mortality induced by the ethanolic extract was quite low and only killed 33.6% of Culex pipiens L. mosquito larvae [24]. However, the lifespan of the insects Spodoptera littoralis and Bruchus obtectus was moderately reduced, in response to essential oil treatment, with an average inhibition of 50% [25]. Another research study indicated different larvicidal efficacy, mainly represented by the repellent effect on the larvae of Tribolium castaneum after 2 to 24 hours of exposure to hexane and acetone extracts from the aerial part of the plant. [26]. The essential oil of Artemisia herba-alba has been tested against three sucking insect pests under laboratory and greenhouse conditions. These pests included Bemisia tabaci, Aphis gossypii and Thrips tabaci. The results showed that the LC50 of A. herba-alba was 0.042% for eggs and 0.074% for the immature stages of B. tabaci. In addition, the oil showed high toxicity to A. gossypii with an LC50 of 0.023 and 0.085%. Artemisia herba-alba was more toxic to T. tabaci and A. gossypii than B. tabaci in the laboratory test while T. tabaci was sensitive (LC50 0.011 and 0.038%). The oil was effective in controlling the insects tested on cucumber plants in greenhouses. This treatment

resulted in a reduction of 85.41, 83.57% in the population of B. tabaci, 90.44, 88.00% for A. gossypii and 87.45, 84.45% for T. tabaci [27. 28]. A study was designed to evaluate the effect of essential oils extracted from Rosmarinus officinalis and Artemisia herba-alba on Acanthoscelides obtectus (Coleoptera: Bruchidae) under laboratory conditions. The doses used were 1 to 5 µL / 30 g of seeds for the essential oil of each plant. The results show that the two essential oils tested were very toxic to adults of A. obtectus, and they also cause a significant decrease in fertility by weevils. The LD50, calculated after 48 hours of exposure, showed that the essential oil extracted from Rosmarinus officinalis was the most toxic for adults with an LD50 = 0.59µL / 30g of seeds, while the LD50 = 1.69µL / 30g of seeds for Artemisia herba-alba [35]. According to our results we can note that the flavonoids of A. herba alba and A. campestris have proved to be very effective, whatever the concentration used, the mortality of the insects was total and that within 20 days following the treatment. The flavonoids and plant powders of A.herba alba and A. campestris were found to be toxic to the species of Tribolium castaneum, the absence of mortality at the control level shows that our test remains reliable for the study of the insecticidal effect of the flavonoids and the plant powders tested.

The insecticidal activity of the flavonoid and powders of these plants does not require a long time to manifest; it is almost identical for the two species of Artemisia. All of our tested products of two Artemisia (flavonoids, vegetable powders) cause a maximum mortality of 100%. The results show that the two aromatic plants tested are effective against Tribolium castaneum, these plants exhibit insecticidal properties on Tribolium castaneum. The effectiveness of these powders varies depending on the dose used and the length of exposure. All these tests carried out can confirm that the treatment of foodstuffs with flavonoid and vegetable powder from aromatic and medicinal plants can be very effective in controlling pests of stored foodstuffs. These flavonoids contain chemically very interesting products. The insecticidal activity of plants of P. harmalaa towards T. castaneum was studied. It was found that the powder of this plant gave a good result for its toxicity on individuals of Tribolium castaneum, this efficacy is confirmed by the death of larvae and adults of this pest. Death of all individuals (100%) is attributed to the 30% dose for both stages of development. Nowadays, the research of botanical pesticides is creating great interest, due to their minor toxic effects on the environment and humans.

CONCLUSION

Food pests, mainly Coleoptera, can cause the total loss of a stock. The search for alternative methods of protecting foodstuffs derived from the know-how of the elders, then the use of phytopesticides, products of local biodiversity, is now a promising alternative. Phytopesticides formulated from aromatic condiment plants are a serious avenue. The study of the toxicity of plant powders and flavonoid extracts on adults of Tribolium castaneum shows that these extracts have a particular toxicity against adults of Tribolium castaneum; a percentage of mortality of 100% is reported in the individuals treated with the pure extracts. In contact testing, we found that the flavonoid extracts tested revealed an insecticidal effect on the lifespan of adults of T. castaneum. However, the chloroform fraction of A. campestris was found to be the most active.

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