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Ruta chalepensis (L.): Phytochemical study and bioinsecticidal effect against *Tribolium castaneum* (Herbst.)

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Abstract
Under favorable conditions, food stocks suffer attacks from insects, including *Tribolium castaneum* (Herbst.). The methods used to limit losses generally are based on chemical pesticides; however, the inconsiderate use of these insecticides causes various problems. Thus, the use of plants with insecticidal properties represents an alternative solution of chemical control. In this context, we evaluated the insecticidal potential of the powder of the aerial part of *Ruta chalepensis* L. on adult stage of *Tribolium castaneum* (Herbst.), without separation of sex. The result showed a total mortality obtained after 22 days with a dose of 2g. Indeed, the values of lethal Time TL50 and TL99 are very significantly different from one dose to another. However, aqueous extracts have no effect. The phytochemical study of *Ruta chalepensis* L. shows its richness in polyphenolic substances, triterpenes and alkaloids. The polyphenol content is related to the extraction method. The decoction shows more efficiency.

Keywords: *Ruta chalepensis* L., *Tribolium castaneum* (Herbst.), insecticidal activity, powder, aqueous extracts

1. Introduction
The food and nutritional security of populations is a major issue in some developing countries, in fact, in addition to food price spikes, losses due to attacks from pest populations: insects, fungi, bacteria and rodents are dramatic [1]. The damage caused by insects including *Tribolium castaneum* (Herbst.) represent a very important part, greatly reduce the quality and quantity of stock products [2]. These losses are estimated by 35% of world agricultural production according to the United Nations Food and Agriculture Organization (FAO) [3]. In other words, a significant part of what is produced does not reach consumers.
Faced with this scourge, the use of pesticides has been encouraged since the Second World War following the rise of the chemical industry and the development of new synthetic techniques [4]. However, these pesticides have caused long-term adverse side effects. In particular, the pollution of the environment, the development of resistant strains [5]. Also, health effects including central nervous system disorders, cancers and reproductive disorders [4].
Thus, the use of other biological alternatives is a necessity. Indeed, the development of new plant-based molecules as biopesticides is a strategy that has demonstrated its effectiveness in protecting seeds stored against insect pests [6-7].
In this context, the objective of this work is the phytochemical study of *Ruta chalepensis* L., thus, the evaluation of the insecticidal activity of its powder and its raw extracts against adults of *Tribolium castaneum* (Herbst.) to reduce losses of stored food.

2. Materials and methods
2.1 Biological material
The samples of the aerial part (stems, leaves and flowers) of the *Ruta chalepensis* L. are collected in May 2018, around Oulmes region (Central plateau of Morocco). Afterwards a botanical identification was carried out based on several morphological criteria and using various books of botany and medicinal plants.

2.2 The insect used
This study focused on the red flour Tribolium (*Tribolium castaneum* (Herbst.)) which is a small granivorous beetle belonging to the family of Tenebrionidae. The adult color is uniformly reddish brown and a length of 3 to 4 mm long. With a longevity of 2 to 8 months

Following the Abiotic conditions, the red Tribolium can invade the stocks of different foodstuffs (chickpea, wheat, rice and maize...).

2.3 Phytochemical screening
2.3.1 Qualitative analysis
The phytochemical tests to detect the presence of tannins, flavonoids, saponins, reducteurs compounds (mucilage), sterols and triterpenes and alkaloids were performed according to the method described by [8-9-10]. The tests were based on the visual observation of color change or formation of a precipitate after the addition of specific reagents.

2.3.2 Quantitative analysis
The amount of total polyphenols was determined using the Folin–Ciocalteu's method [11], Briefly, 0,1ml of extract was mixed with 2ml of aqueous Na_2CO_3 (2%, w/v), After 5 min, 0,1ml of 1/10 the Folin–Ciocalteu reagent were added. The mixture was incubated at ambient temperature, protected from light for 30 minutes. The absorbance was measured at 700 nm and the results were expressed in mg equivalent of gallic acid per g of extract (mg GAE/g of extract).

2.3.3 Moisture content
The water content of *Ruta chalepensis* L. was determined by the oven drying method at 105 ° C [12]. Indeed, the moisture content of the samples was determined by passing 100 g of the fresh material in the oven at a temperature of 105 °C for one hour. Three repetitions were performed for each sample to obtain an average representing the moisture content. The moisture content (H %) is calculated by the following formula:

$$\boxed{H (\%) = ((M_f – M_d) / M_f) * 100}$$

Where:
H: Moisture content expressed as a percentage; M_f: Masses of the fresh plant in g; M_d: Mass of the dry plant in g.

2.3.4 Preparation of the water extracts
Different types of extracts have been prepared from powder of the *Ruta chalepensis* L.:

Decoction
We performed a 10% aqueous decoction.10g of powder were put in 100 ml distilled water and boiled for 1 hour. The decocted cooled was filtered.

2.3.4.1 Maceration
An aqueous maceration was also performed on 10 g of powder with 100 ml of water during 24 h, the extract was filtered.

2.3.4.2 Infusion
The *Ruta* powder (10 g) is infused in boiling water (100ml) for 15 minutes, and then filtered.
The final extracts were concentrated on rotary evaporator 55 °C, and then they are stored at 4 °C in glass vials, sterile and hermetically sealed. The yield of different extracts is calculated by the following formula:

$$\boxed{\text{Yield } \%= (M_{ext}/ M_{sam})*100}$$

With: M_{ext}: Mass of extract after evaporation of the solvent in g and; M_{sam}: Dry mass of the plant sample in gram [13].

2.4 Insecticidal activity
2.4.1 Toxicity by contact of the powder on filter paper
The method of contact toxicity is based on the use of four doses 0.5, 1, 1.5 and 2 g to evaluate the insecticidal potential of the powder tested. Each quantity of this powder was spilled evenly over the paper filter, which have 9 cm of diameter (63.58 cm² surface area) and placed in a plastic petri dish of the same diameter. If we want to express the different quantity of powder (0.5, 1, 1.5 and 2 g) per unit area of filter paper (63.58 cm²) this corresponds to doses 0.009, 0.015, 0.023 and 0.31 g/cm² respectively. Subsequently, 20 non-sexed adult insects (without male and female sex separation) were introduced into each petri dish containing a treated washer. The dishes are immediately closed by parafilm and are then placed at a temperature between 28 and 30 °C [14]. Furthermore, three repetitions were performed with an untreated control box.

2.4.2 Toxicity by contact of the water extracts on filter paper
The method is the same as that described previously for the powder; however, the treatment of *Tribolium castaneum* (Herbst.) was carried out by the raw aqueous extracts (decocted, infused, macerated) with the following volumes: 100 µl, 200 µl and 300 µl [15], which corresponds respectively to the following doses 1.72, 3.15, 4.72 µl/cm².

2.4.3 Corrected morality calculation
The percentage of corrected mortality of control adult insects and treated with the powder is calculated using Abbott's formula [16]

$$\boxed{Pc = Po- Pt /100-Pt \times 100}$$

With Pc: corrected mortality in percentage; Pt: mortality observed in the control and Po: mortality observed in the test.

2.4.4 Calculation of TL 50 and TL90
The importance of the toxicity of the powder tested against the adults of *Tribolium castaneum* treated is estimated by the determination of the LT50 and LT99 (Lethal Time of 50 % and 99 % of the individuals), being based on the right regression of mortality rates over time [17].

2.4.5 Statistical Analysis
The evaluation of the lethal effect of powder on adults of *Tribolium castaneum* (Herbst.) was performed using analysis of variance (Anova). The means and standard deviations were calculated using Graph Pad software and the Tukey test at the 5 % probability level. Thus, the values of $p \leq 0.05$ are considered statistically significant.

3. Results & Discussion
3.1 The water content of *Ruta chalepensis* L.
Determining the humidity of the aerial part of *Ruta chalepensis* L. revealed a rate of 60 % so more than half of the fresh weight of the plant is water (Figure1). Indeed, several factors could influence the water content as the age of the plant, the soil conditions and plant shelf life after harvest.

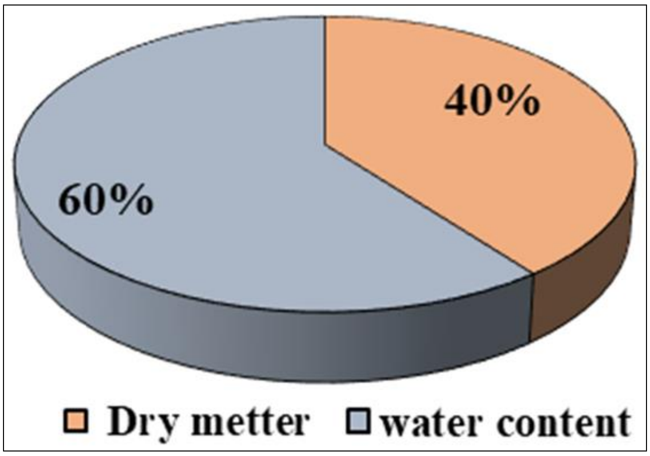

Fig 1: The water content of *Ruta chalepensis* L.

3.2 Phytochemical study of *Ruta chalpensis* L.

In order to screen the different classes of secondary metabolites present in the aerial parts of *Ruta chalpensis* L., a phytochemical screening was carried out. The results of the chemical characterization have demonstrated the presence of several chemical compounds known to have interesting biological activities (antibacterial activity, antifungal activity, antioxidant activity...). These include polyphenolic substances, including catechin tannins, flavonoids (flavones), sterols, triterpenes and alkaloids. These detected chemical families are in agreement with other works [18-19-20], on the same specie (*Ruta chalepensis* L.), in Egypt, Turkey and Saudi Arabia.

In addition, previous research has allowed the isolation of alkaloids, coumarins and flavonoids present in *Ruta chalepensis* L. [21-22]. These phenolic or terpene compounds would be in favor of antioxidant, anti-inflammatory, and antibacterial properties and therefore largely responsible for the use of this plant in the plant protection products industries.

Again, the yield of the crude aqueous extracts of the aerial part of *Ruta chalpensis* L. varies depending on the method of Preparation. Thus, the decoction represents the most profitable mode with a percentage equal to 2.18 %, which exceeds those obtained, by the other modes, infusion (1.85 % and maceration (1.54 %). For its part, the quantitative study of these extracts by means of the colorimetric and spectrophotometric assay revealed that the polyphenol content is also related to the extraction method (Figure 2). The decoction (63.21 mg / g of extract) shows more efficiency, in comparison with the infusion (61.33 mg / g of extract) and the maceration for the extraction of these active principles.

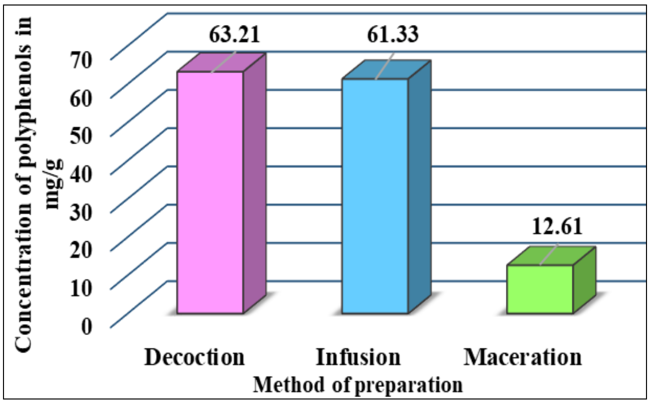

Fig 2: Concentration of polyphenols according to the method of preparation

3.3 Insecticidal activity

The results of the evolution of adult mortality rates of *Triboluim castaneum* (Herbst.), depending on the time and dose of the powder used (Figure 3), reveal an important insecticidal power of *Ruta chalepensis* L, even at low doses 0.5 g.

Moreover, the percentage of mortality increases as the dose used increases, and reaches 100 % after 22 days for the dose 2 g. This leads us to infer that the insecticidal effect of *Ruta chalepensis* L. powder on adults of *Triboluim castaneum* (Herbst.) Is closely related to the dose used and is therefore dose / dependent or dose / effect.

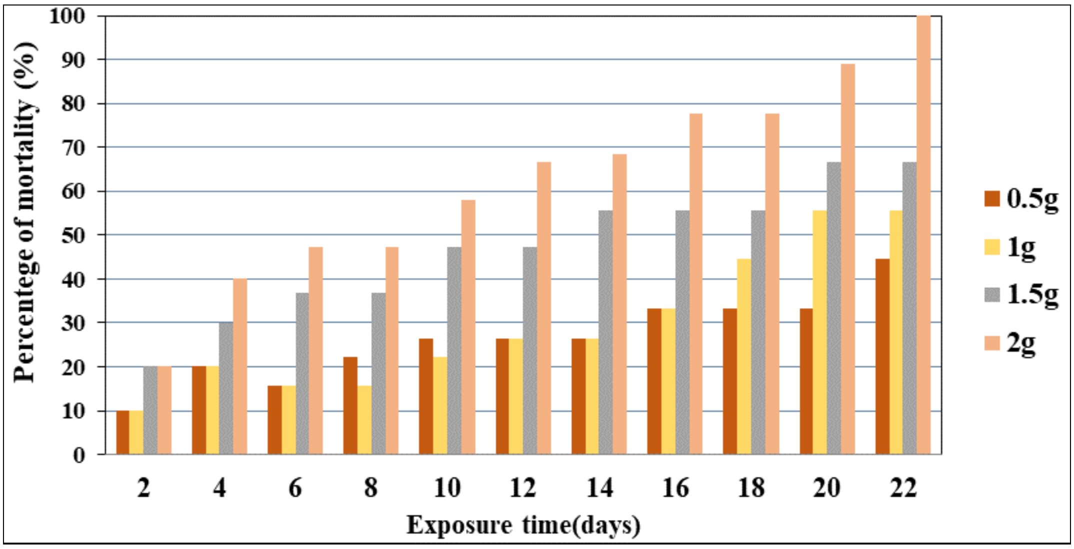

Fig 3: Evolution of the adult mortality rate of *Tribolium castaneum* (Herbst.) in the presence of powder of *Ruta chalepensis* L. depending on the exposure

Additionally, the dose 1.5 g of the powder causes the death of 55.56 % strength after 14 days. Thus, to achieve the same result, adults *Tribolium castaneum* (Herbst.) should be exposed for 20 days at a dose of 1g. So, the more the dose decreases, the more the exposure time needed to achieve the same results increases. The same remark was raised

concerning the whole range of doses used. Thus, the exposure time is dose dependent.
To better confirm this, we calculated the TL50 and TL90 lethal times after which 50% and 99% of the individuals of *Tribolium castaneum* (Herbst.) die. In the light of the results recorded in Table 1, it appears that the values TL50 and TL99 are very significantly different from one dose to another (*p*<0, 05). Indeed, the shortest TL50 TL99s are recorded by 2 g of the powder of *Ruta chalepensis* L. On the other hand, the lowest dose of 0.5 g corresponds to the highest lethal times. We can conclude that the lethal times vary according to the dose, since the smallest lethal time corresponds to the highest dose.

Table 1: LT50 and LT99 (in days) of adults of *Tribolium castaneum* (Herbst.) exposed to powder of *Ruta chalepensis* L.

Doses	0, 5g		1g		1,5g		2g	
Lethal time	LT50	LT99	LT50	LT99	LT50	LT99	LT50	LT99
	24,75± 0,01^{a}	49,00± 0,15^{b}	19,79± 0,02^{c}	39,20± 0,17^{d}	12,66± 0,81^{e}	32,66± 0,02^{f}	8,63± 0,02^{g}	21,78± 0,01^{h}

Values are means ± standard deviation of three separate experiments. Means followed by different letters are significantly different at *P*<0.05 (Tukey test).

Indeed, the evaluation of the insecticidal activity of the powder of *Ruta chalepensis* L. against *Tribolium castaneum* (Herbst.) shows a strong correlation between the mortality rate, the dose used and the exposure time. Therefore, there is an insecticidal effect dose-time dependent. In contrast, tests conducted with crude aqueous extracts that are not endowed with an effect with the doses used. In contrast, tests conducted with crude aqueous don't have any effect on the doses used.
The toxic potency of *Ruta chalepensis* L. against *Tribolium castaneum* Herbst. It will be due to its richness in various secondary metabolites, like other species of the Rutaceae family. Indeed, triterpenes are known by their insecticidal and insect repellent properties [6-7- 9-23]. Alkaloids have repellent or anti-palatability properties for a large number of insect pests [24]. Also, the growth, development and fecundity of several insect pests are influenced by tannins [25], that affect the number and size of eggs, which would weaken the survival and health of the next generation [26].
These compounds, and in addition to being endowed with an activity against certain target insects, they are biodegradable in non-toxic products favoring their application in biological control, thus the creation of new classes of less dangerous insecticides [27- 28].

4. Conclusions
Plant allele chemical molecules could be a healthy alternative to synthetic products to fight against pests of foodstuffs. *Ruta chalepensis* L. is a species rich in secondary metabolites that attribute insecticidal properties. The contact toxicity tests carried out with *Ruta chalepensis* L. powder against adults of *Tribolium castaneum* Herbst, showed their effectiveness contrary to the tests carried out by the raw aqueous extracts. It would therefore be interesting to deepen the studies, in order to characterize the molecules susceptible to the observed insecticidal activity, by evaluating their effects on the different stages of development, thus, the physiological activity of the target insect.

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