

STUDY OF SOME PHYSICO-CHEMICAL PROPERTIES AND INSECTICIDAL ACTIVITY OF SEED OIL OF *Citrullus colocynthis* Schrad. (Cucurbitaceae) ON *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae)

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Abstract. *Citrullus colocynthis* Schrad. (Cucurbitaceae) seed oil extract insecticidal activity was evaluated by direct spraying method on adults of *Tribolium castaneum* Herbst (Coleoptera: Tenebrionidae). *Citrullus colocynthis* was collected from *Oued Larneb* which is located in the Algerian Septentrional Sahara. The results showed notable mortality rates that varied from 28.89% to 100% and varied considerably depending on the applied dose and the duration of exposure. The lethal effect of this oil was comparable to that of the insecticide Decis® 50EC. The estimation of the lethal doses 50 and 90 allowed us to verify the toxicity of this oil which were of the order of 0.002 mL and 0.030 mL respectively after 18 hours of observation.

Keywords: Seed oil; *Citrullus colocynthis*; *Tribolium castaneum*; insecticide; lethal dose; Algerian Sahara

INTRODUCTION

In several African and Asian countries, food security is one of the issues and a challenge for any reliable and sustainable development policy [10]. The definition of a strategy that ensures food sovereignty is both an issue and a necessity. In these countries, particularly subject to climatic constraints including drought, biological disasters (invasion of insect pests) and political disturbances and conflicts, food security represents a major concern of populations and scientific communities [10, 11].

To cope with the demographic growth recorded in these countries, it is necessary to put in place a strategy that aims to increase agricultural production. The agricultural sector provides most of the human food is often subjected to different environmental constraints and also to pests which can have serious consequences on the quality and quantity of crops [10, 38].

Population growth and market economy are pushing the authorities to step up their efforts to increase the area cultivated and to diversify production systems to produce quality food [11, 12].

Both in fields and stores, agricultural products are under severe attack by pests and pathogenic agents. According to the Food and Agriculture Organization of the United Nations [8], losses due to insect pests correspond to 35% of world agricultural production. Facing this critical situation, the usage of chemical insecticides is the most used technique to control these pests [5].

The losses caused by these different pests are estimated at 300 million US dollars per year [32], which justifies the implementation of several means of control among which chemical control remains the oldest and the most effective. However, the problems of environmental pollution, the negative interference of pesticides on human health and the pressure exerted by the consumer for healthy organic farming practices have led to an increase in efforts to develop alternative

control methods with better harmony with the environment [37].

For some African localities, the high cost of synthetic chemical insecticides makes them inaccessible to farmers. Therefore, the enhancement of the insecticidal potential of many plants including Neem (*Azadirachta indica* Juss. (Meliaceae)), Castor (*Ricinus communis* L. (Euphorbiaceae)) and *Hyptis suaveolens* L. Poit. (Lamiaceae) are of capital interest [31].

Herbal preparations have been used successfully in the managing crop pests and are categorized as spectrum insect repellents and an obvious alternative to chemical pesticides [18, 22, 31].

In Algeria, few studies have been carried out on the insecticidal activities of plants even though several plants have been recognized to have insecticidal properties. Kemassi (2014) and Kemassi *et al.* (2015; 2019) [18, 22, 23], showed the insecticidal possibilities of some endogenous plants from the Algerian Sahara.

This study relates to evaluate of insecticidal effects of seed oil of Coloquint *Citrullus colocynthis* Schrad. (Cucurbitaceae) against the imagos of *Tribolium castaneum* L. (Coleoptera: Tenebrionidae).

MATERIALS AND METHODS

Insect test. *Tribolium castaneum* Herbst. (Coleoptera: Tenebrionidae), is a pest of stored foodstuff that is best known in tropical and subtropical regions. This insect is considered as a severe secondary pest which causes major damages to beaten stocked millet throughout the entire Sahel region [14, 33].

The pest is maintained under semi-controlled conditions at the Pedagogical Laboratory/ University of Ghardaia (Algeria). The adults of *Tribolium castaneum* maintained in mass breeding came from a stock of contaminated semolina. The breeding was kept in plastic boxes (L=50cm, W=35cm and H=20cm) under

semi-controlled conditions (temperature $32 \pm 2^\circ\text{C}$, humidity $60 \pm 4\%$ and illumination 8h/16h).

Plant material. The plant specimen was *Citrullus colocynthis* seeds which is a perennial plant of the Cucurbitaceae family. It is a native plant of tropical Asia and Africa and is now widely distributed in the Saharo-Arab regions of Africa and in the Mediterranean region [13]. It is thus cultivated in tropical countries as a medicinal plant for the pulp of its fruits [35]. The Coloquint grows in sandy-clay soils of the Saharan regions [6, 7]. The fruits of the Coloquint are often used as deadly poison [39].

In high doses, this plant is highly toxic to animals and humans. In case of intoxication, syndromes of metabolic disorders are observed; gastrointestinal pain followed by diarrhea, vomiting, urinary retention, fatigue, hypothermia, disorder of cardiac functions and cerebral congestion producing a fatal collapse [8].

The seeds used for the experiments were collected in the region of *Oum Larneb* ($32^\circ14'39.1''\text{N}$ and $3^\circ47'50.3''\text{E}$) located in the municipality of Metlili in the district of Ghardaïa (Algerian northern Sahara).

***Citrullus colocynthis* seed oil preparation.** The seeds of *Citrullus colocynthis* were collected from ripe fruits which rinsed well and then dried in the oven that was set at 50°C for 24 h. Once dried, the seeds were crushed using a laboratory mixer with double tank to heat and cool this type of Mill. This mill has a capacity of 50 mL and an inlet of up to 7 mm.

For the extraction of oil from the seeds powder of the Coliquant, a 2.5 L capacity soxhlet was used. A quantity of 250 mL of hexane was used in the flask. In the cellulose cartridge, 70 g of seeds powder was deposited and the heating was ensured by a balloon heater set at a temperature of 50°C .

The extraction procedure took around 6 h. The oil separated from the solvent was carried out in a Hei-VAP Heidolph type rotary evaporator fitted with a water bath overheating protection system that was set at a temperature of 50°C and a rotation speed of 100 rotations/min.

Physico-chemical characterization of *Citrullus colocynthis* seed oil. Various indexes characterizing the obtained vegetable oil such as density, refractive index, hydrogen potential (pH), acidity index, saponification index, peroxide index and the esterification index were studied for *Citrullus colocynthis* seed oil.

Biological tests. The mode of application adopted for this study is the application by direct contact. It consisted of direct spraying of seed oil on 15 adults of *Tribolium castaneum* placed in a Petri dish lined with a filter paper. Each Petri dish receives 0.93 mL of the tested product at different doses. For each chosen dose, three repetitions were performed. To find the lethal doses 50 and 90, six concentrations of *Citrullus colocynthis* seed oil were chosen, designated 50%, 40%, 30%, 20%, 10% and 5%. The applied doses were prepared by mixing the seed oil of *C. colocynthis* with a mixture of DMSO and distilled water (v/v). After 4 h

of the treatment application, 5 g of semolina per Petrie dish was deposited. For this study, an insecticide of the synthetic Pyritrinoides group (Decis[®]EC50) was used as a positive control.

The Kinetic monitoring of the mortality was carried out in regular periods of time for 18 consecutive hours (2, 4, 6, 8, 12, 18 h). The insects of the different treated and control groups were maintained under the same conditions as those described for the breeding of insects.

Mortality rate. Mortality is the first criterion for judging the effectiveness of chemical or biological treatment. The percentage of mortality observed in control and treated adults is estimated by applying the following formula:

Observed mortality = [Number of deaths/Total number of individuals] \times 100 [30].

The lethal dose (LD). The letters LD denote the "Lethal Dose", the LD_{50} is the quantity of a material administered in a single dose, which causes the death of 50% (half) of a treated group. The LD_{50} is a way of measuring the short-term toxic potential (acute toxicity) of a material. It is calculated from the regression line of the probits corresponding to the percentage of mortality corrected as a function of the logarithms of the applied concentrations. The formula of Schneider and the table of probits are used.

Schneider formula:

$$\text{MC} = \frac{(\text{M}_2 - \text{M}_1)}{(100 - \text{M}_1)} \times 100$$

where:

- MC: rate of corrected mortality;
- M_2 : rate of mortality in the treated population;
- M_1 : rate of mortality in the control population [18, 19, 25, 27].

Statistical analysis. The obtained results from the various experimental tests were interpreted statistically using the software "XLSTAT Version 2012". A multiple comparison analysis of variance of means which involved comparing the average of one group with the average of another. Fisher's Least Significant Difference (LSD) test was also performed. The ANOVA analysis of variance aims to compare the means of the results after the normality test. This statistical test monitors the level of significance and determine the influence of the studied factors or interferences between factors.

RESULTS

Extraction yield of *Citrullus colocynthis* seed oil. Despite its toxicity, in Saharan medicine, different parts of Coloquinte are used by local population. The extraction yield corresponds to the percentage of the weight of the extracted pure oil relative to the weight of the raw plant material used for the extraction. The mature seeds of *Citrullus colocynthis* contained about 19.93 % of oil. This yield value shows that the seeds of this Saharan plant have relatively medium oil content.

Physico-chemical analysis of *Citrullus colocynthis* seed oil. Coloquint seed oil are pale yellow

in color and viscous in appearance. The physico-chemical properties of Coloquint seed oil are listed in table 1.

Table 1. Physico-chemical properties of Coloquint seed oil

Coloquint seed oil properties	
Color	Pale yellow
pH	5.0
Density at 20 °C	0.386
Refractive index (I.R)	1.474
Saponification index (mg KOH / g)	171.105
Acidity index	8.415
Peroxyde index (meqO ₂ /kg)	0.4
Ester index (mg KOH/g)	162.69

Given the results of Table 1, the characteristics of edible oils are similar to those reported for coloquint seed oil, but it is rather essential to complete the study by their toxicity test. Coloquint seed oil had a density of 0.39 at 20 °C. For the refractive index measured, it was 1.474 at 20 °C. This oil had an acid pH with a pH value of 5.0. The saponification index was of 171.105 which shows that this oil is of good industrial quality. The value of the peroxide index of this oil was 0.4 meq O₂ / kg. The ester index is the difference between the saponification index and that of acid, we conclude that as much the saponification index is high and less the acid index is thus, that of ester is important. According to the obtained results, it was noted that the oil of the seeds of *Citrullus colocynthis* had a low value of the esterification index compared to other oils. This is logical because the obtained acid and saponification indexes from our oil were lower, which confirms the good industrial quality of the studied oil. The ester index of coloquint seed oil was 162.69 mg KOH/g.

Effect of *Citrullus colocynthis* oil on the mortality of *Tribolium castaneum*. The direct spraying of Coloquint seed oil on the imagos of *Tribolium castaneum*, caused different signs of intoxication. Exposure to this oil in doses ranging from 0.05 to 1 mL/mL caused the death of the treated individuals; the mortality rate increased by the increase of the applied dose. Analysis of the obtained results showed a non-significant difference between the values of the observed mortality rates in the group of treated insects with Coloquint seed oil and the individuals in the positive control group and a significant difference between the results was reported for individuals in the negative control group and individuals in the treated group with the oil of this Saharan plant at different concentrations (Fig. 1).

Coloquint seed oil applied at high concentrations cause appreciable mortality rates, close to those noted in the groups treated with the insecticide Decis® 50 EC. Their effectiveness against the imagos of *Tribolium castaneum* was particularly noted after 12 hours of exposure showing a comparable effect to that noted for the insecticide Decis® 50 EC, even at the low concentration of 0.25 mL/mL, where the oil seems effective. The exposure of the *Tribolium castaneum* for 18 hours to Coloquint seed oil at the low concentrations of 0.05 mL/mL and 0.11 mL/mL,

generated 80% and 100% of mortality, respectively. For the negative control, no mortality was recorded for the duration of the biological tests. As it is useful to point out that the used insecticide (Decis® 50 EC) as positive control is one of the most toxic formulations that is characterized by its shocking effects and the rapidity of its action with mortality occurs after only 2 hours of exposure. The comparison of the insecticidal power of Coloquint seed oil with an approved and effective insecticide against insects of different groups allowed us to judge the insecticidal potential of this oil against the imagos of *Tribolium castaneum*. After 2 hours of exposure to *Citrullus colocynthis* seed oil, mortality percentages of 82.22%, 80% and 77.78% were noted for those treated with concentrations of 1 mL/mL; 0.67 mL/mL and 0.43 mL/mL respectively. Although at low concentrations of 0.25 mL/mL, 0.11 mL/mL and 0.05 mL/mL, the reported mortality rates are around 68.89%, 77.78% and 28.89% respectively. These values of mortality rates increased according to the duration of exposure (4 h, 6 h and 12 h), and they reached 100% mortality even for low concentrations after 18 hours of exposure, except for the concentration of 0.05 mL/mL where an 80% mortality rate was observed.

Biocidal efficacy of *Citrullus colocynthis* seed oil on *Tribolium castaneum*. The efficiency concentration 50% (EC₅₀) is estimated by drawing the probit regression line corresponding to the percentages of the corrected mortalities as a function of the logarithms of the concentrations (mg) in the applied extracts. The toxicological parameter calculations are reported in (table 2).

The graphical representations of the linear regression curves of the effects of the applied Coloquint seed oil doses on the mortality of the *Tribolium castaneum* imagos (Fig. 2 A-E), allowed the estimation of the lethal doses 50 and 90 (LD₅₀ and DL₉₀) of this oil extract. The results grouped in Table 2 showed low lethal dose values, which is explained by the high contact toxicity of this oil extract with the imagos of *Tribolium castaneum*. The lethal doses 50 reported for the different exposure durations of 2 h, 4 h, 6 h, 12 h and 18 hours were of the order of 0.10 mL/mL, 0.048 mL/mL, 0.010 mL/mL, 0.009 mL/mL and 0.002 mL/mL, respectively and for the estimated lethal doses 90 (LD₉₀) were 1.64 mL/mL, 1.28 mL/mL, 1.02 mL/mL, 0.20 mL/mL and 0.030 mL/mL, respectively.

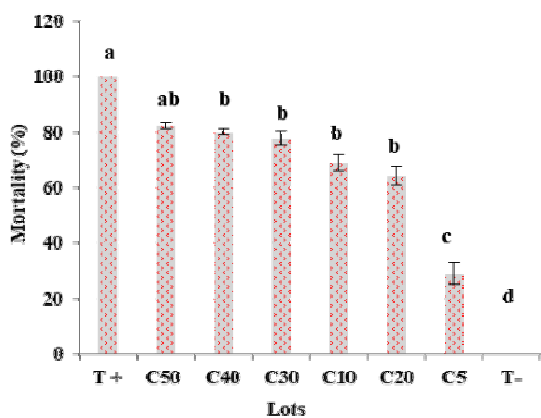
DISCUSSION

Extraction yield of *Citrullus colocynthis* seed oil.

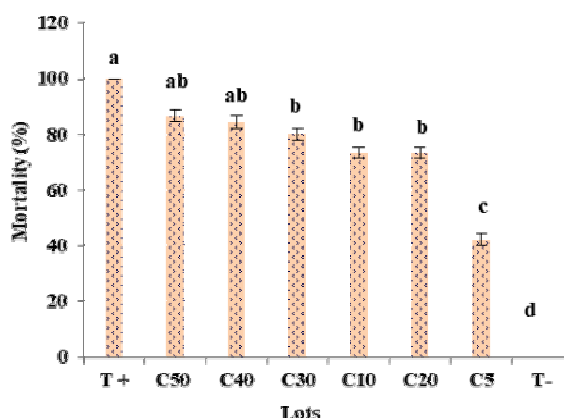
The values of the extraction yield of *Citrullus colocynthis* seed oils are generally average. This is close to that noted for *Pergularia tomentosa* L. (Asclepiadaceae) harvested in the northern Algerian Sahara which content in seed oil of 22.37% [2]. Yanif *et al.* (1999) noted that the Coloquint oil yield of the seeds collected in the Negev desert and the Arava

valley (border of Jordan and in the Sinai desert (Egypt), were between 17% and 19% [39]. These variations are maybe due to the changes in the environmental conditions which influence the degree of ripeness of the fruits. Also, Salvador *et al.* (2001),

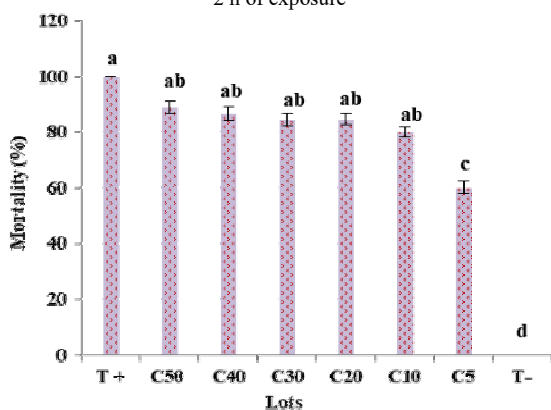
reported that the content of a dense oil in fruit varies considerably depending on the period of maturity; it is high at the beginning of the ripening stage and it drops slightly when the fruit exceeds maturity [34].



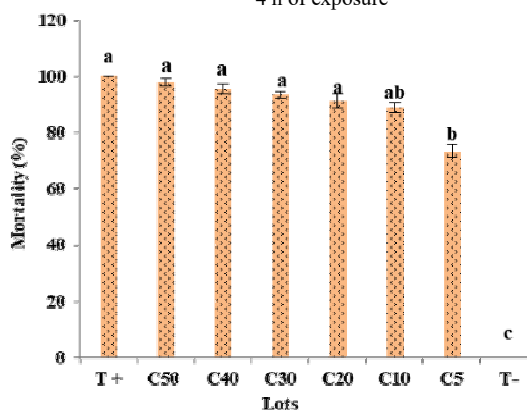
A: Mortality rates observed in different control and treated groups after 2 h of exposure



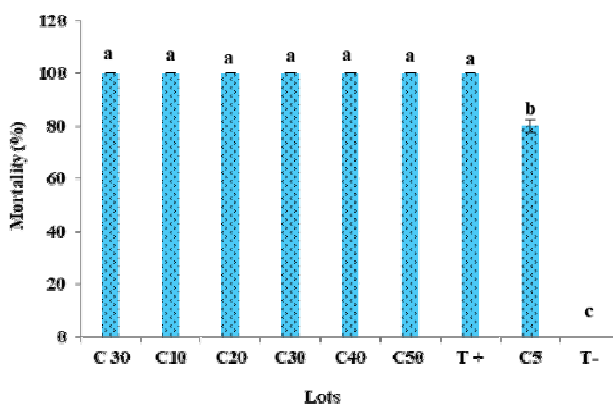
B: Mortality rates observed in different control and treated groups after 4 h of exposure



C: Mortality rates observed in different control and treated groups after 6 h of exposure



D: Mortality rates observed in different control and treated groups after 12 h of exposure

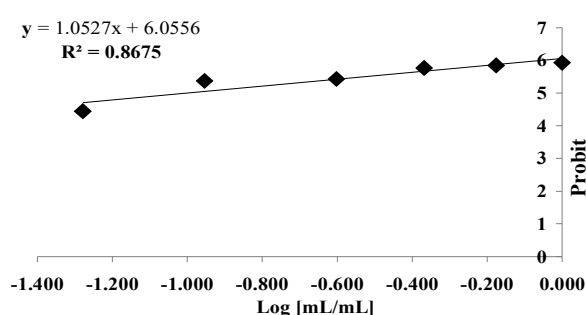


E: Mortality rates observed in different control and treated groups after 18 h of exposure

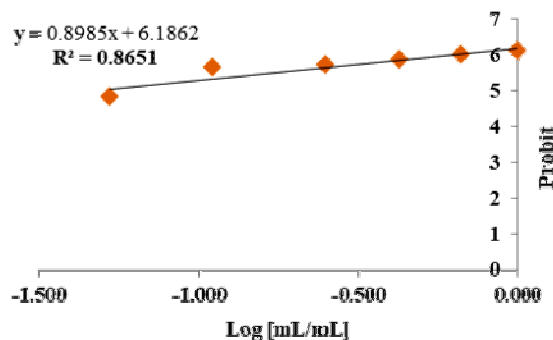
Figure 1. Variation in mortality rates of *Tribolium castaneum* in control and treated groups with *Citrullus colocynthis* seed oil. (C50=[1 mL/mL]; C40=[0.67mL/mL]; C30=[0.43 mL/mL]; C20=[0.25mL/mL]; C10=[0.11 mL/mL]; C5=[0.05 mL/mL]; T+= Decis®EC50; T-= Water +DMSO (v/v).

Table 2. Estimated lethal doses for *Citrullus colocynthis* seed oils based on exposure time (h: time hour)

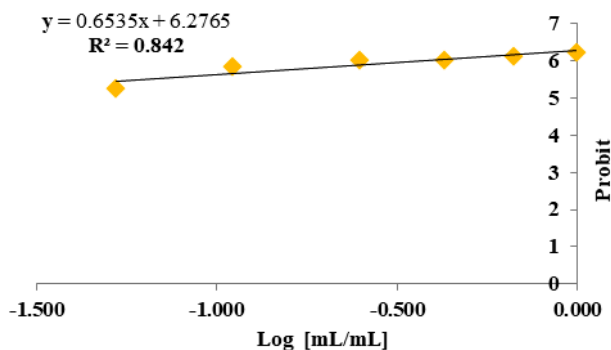
Exposure time (hours)	Regression equation	Regression coefficients	Lethal doses (LD) [mL/mL]	
			LD50	LD90
2 h	$y = 1.0527x + 6.0556$	$R^2 = 0.8675$	0.100	1.64
4 h	$y = 0.8985x + 6.1862$	$R^2 = 0.8651$	0.048	1.28
6 h	$y = 0.6535x + 6.2765$	$R^2 = 0.8420$	0.010	1.02
12 h	$y = 0.9478x + 6.9351$	$R^2 = 0.9412$	0.009	0.20
18 h	$y = 1.0823x + 7.9282$	$R^2 = 0.5244$	0.002	0.030



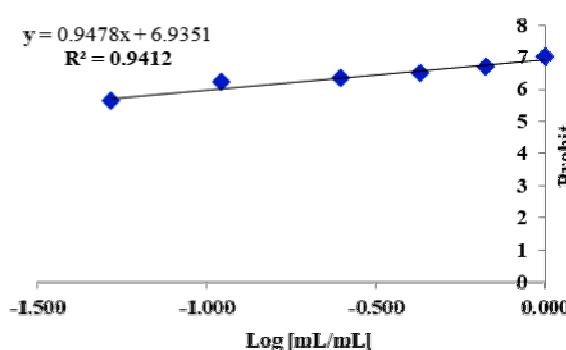
A. Effect of the concentration of *Citrullus colocynthis* seed oil on the mortality of *T. castaneum* imagos after 2 h of exposure



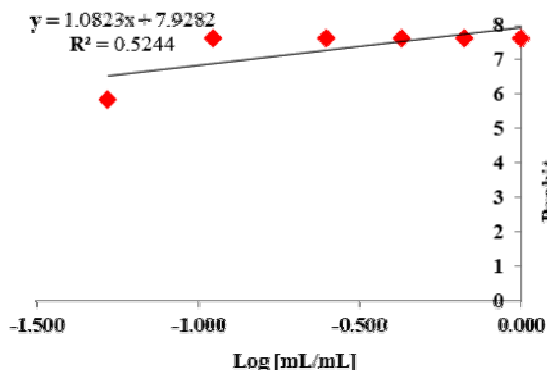
B. Effect of the concentration of *Citrullus colocynthis* seed oil on the mortality of *T. castaneum* imagos after 4 h of exposure



C. Effect of the concentration of *Citrullus colocynthis* seed oil on the mortality of *T. castaneum* imagos after 6 h of exposure



D. Effect of the concentration of *Citrullus colocynthis* seed oil on the mortality of *T. castaneum* imagos after 12 h of exposure



E. Effect of the concentration of *Citrullus colocynthis* seed oil on the mortality of *T. castaneum* imagos after 18 h of exposure

Figure 2. Effect of the variation in the concentration of *Citrullus colocynthis* seed oil and the duration of exposure on the mortality of *Tribolium castaneum* imagos

Physico-chemical analysis of *Citrullus colocynthis* seed oil. The values obtained in the study of the physico-chemical properties of Colocynth seed oils are comparable to those of oil seeds cited in other studies. The refractive index is considered as a criterion of the purity of oil; it varies depending on the wavelength of the incident light as well as the temperature at which the analysis is carried out. This index is proportional to the molecular weight of the fatty acids as well as to their degree of unsaturation. The refractive index of Colocynth seed oil is within the range established by *Codex Alimentarius* [9]. These values were similar to the results of vegetable oils that are rich in linolenic acids, where R = 1.468 to 1.490 [29]. The obtained acidity index values were similar to those cited in the literature; we recorded a value of 8.415 compared to 8.02 noted by Akpambang *et al.* (2008) for *Citrullus colocynthis* seed oil which were

purchased from the local market in Akure, Ondo State (Nigeria) [4]. Hassimi *et al.* (2007) [17] reported an acid index of around 4.06 for seed oil of *Citrullus colocynthis* obtained from the Sahara of Niger. The oil of Coloquint seeds harvested in Sudan had a saponification index of the order of 206 [28]. Giwa *et al.* (2010) [15] had declared a saponification index near the latter of the order of 204.44 mg KOH/g.

It has been shown that fresh oil has a peroxide index of fewer than 10 meq O₂/kg and they become rancid when the peroxide index is found in the range of 20 to 40 meq O₂/kg [29]. The peroxide index of this oil was approximately similar to the results obtained by Akpambang (2008) [4], with a peroxidation index of 1.72 meq O₂/kg for Coloquint oil from Ondo State (Nigeria). It has been shown that fresh oil has a peroxide index of fewer than 10 meq O₂/kg and they

become rancid when the peroxide index is found in the range of 20 to 40 meq O₂/kg [29].

Effect of *Citrullus colocynthis* oil on the mortality of *Tribolium castaneum*. The evaluation of the percentage of mortality in the *Tribolium castaneum* imagos treated with *Citrullus colocynthis* seed oils makes it possible to verify the insecticidal effects of this oil. The use of products of natural origins for the control of crop pests emanates from the sensitivity of these pests to extracts or derivatives of certain plants, where many well-known studies have shown the insecticidal possibilities of herbal preparations [16, 18-24]. According to Merabti et al. (2015), the aqueous extracts of *Citrullus colocynthis* fruits have larvicidal effects on L₄ larvae of *Culex pipiens* L. and *Culisetalon giareolata* L. (*Diptera: Culicidae*) [26]. Other studies on insecticidal activity against *Tribolium castaneum* like Ons Majdoub et al. (2014) showed that adults of *Tribolium castaneum* were more sensitive to the essential oil of *Ruta chalepensis* L. (Rutaceae) which 14% of treated individuals die after 24 hours of treatment [25]. The insecticidal activity of these oil was screened against the mentioned pests and have been proved to be more active on adults of this insect (LC₅₀ = 176.075 µL/L air and LC₉₀ = 291.9 µL/L air) than the larvae (LC₅₀ = 415.348 µL/L air and LC₉₀ = 685.907 µL/L air) [25]. Abdul Rahuman et al. (2008) found an insecticidal activity against larvae of mosquitoes (*Aedes aegypti*, *A. stephensi* and *Culex quinquefasciatus*) of oleic and linoleic acids extracted from *Citrullus colocynthis* [1].

Biocidal efficacy of *Citrullus colocynthis* seed oil on *Tribolium castaneum*. The lethal dose 50% (LD₅₀) and 90 (LD₉₀) are estimated to measure the degree of insecticidal efficacy of this seed oil. According to the literature, *Citrullus colocynthis* seed oil seems to be toxic to harmful insects. Soufi (2016) [36] reported the insecticidal effect of the seed oil of this plant on the white scale of the date palm plants where she noted a lethal dose 50 of 30.41 µL/cm² and 0.0643 mg/cm² for the seed oil and the aqueous pulp extract of the same plant after a 24 hours follow-up period. In Acheuk et al. (2018) [3] study on the insecticidal activity of the raw ethanolic extract of *Halocnemum strobilaceum* (Chenopodiaceae) against adults of the red flour beetle *Tribolium castaneum* they showed a calculated lethal dose 50 of around 225.4 µg/insect after 24 hour of exposure. Mostafa et al. (2012) [27] marked probits analysis for the estimation of LC₅₀ values, the 95% confidence limits and the regression equation at 24, 48 and 72 hour for the mortality of *Tribolium castaneum*. The LC₅₀ values of hexane extracts from *Cucumis sativus*, *Azadirachta indica*, *Tamarindus indica* and *Psidium guajava* after 24 hours of treatment were 20.64 µg/cm², 234.57 µg/cm², 732, 53 µg/cm² and 1944.40 µg/cm², after 48 hour of treatment were 24.43 µg/cm², 91.80 µg/cm², 178.74 µg/cm² and 1944.40 µg/cm² and after 78 hour of treatment were 10.74 µg/cm², 155.13 µg/cm², 58.36 µg/cm² and 774.22 µg/cm² respectively [27]. The values of the lethal doses

50 and 90 reported by Kemassi et al. (2019) [23] are in the order of 0.0158 mg/mL and 0.0322 mg/mL for aqueous leaf extract of *Euphorbia guyoniana* B. & R. (Euphorbiaceae) respectively, and 0.0186 mg/mL and 0.0394 mg/mL for aqueous root extract of *Euphorbia guyoniana* respectively in his study on the *Tribolium castaneum*.

The study of the toxicity of *Citrullus colocynthis* Schrad. seed oil, allowed us to highlight the insecticidal potential even at low concentrations against the imagos of *Tribolium castaneum*. Similarly, it should be noted that the observation of signs of poisoning in insects exposed to the oil of this plant after 2, 4 and 6 hours of exposure including movement disorders implies the neurotoxic effect of this seed oil this extract. Therefore, this vegetable oil can constitute a source for a new natural insecticidal product.

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