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Castor oil (*Ricinus communis*) insecticide activity and its effects on populations (*Brevicoryne brassicae and Coccinella algerica*)

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Dedication

Almighty God thank you for the power and courage that you gave me to complete this work.

To my mother:

Mother, I will never forget your wise counsel to my place. You're the one who said you don't thank your parents. Only, I can't find a way today to avoid you thank you for all you have done for us. Your concern has always been the success of your children. May your sacrifices, sorrows and your privations find their reward in the culmination of this modest work which is also the fruit of your perseverance, your courage and especially your patience. This work is also the fruit of your love, your blessings and especially your good education.

To my father:

I have always found with you understanding and support. Your prayers and your advice have never failed me throughout my studies. Find through this modest work, reward your affection.

To my brother and sisters:

Thank you for all your support in the hard time.

To my beautiful aunt:

Thank you for supporting me all of these years and taking my responsabilities off my chest.

To my friends:

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Summary

List of abbreviations

R. communis: *Ricinus communis.*

B. brassicae: *Brevicoryne brassicae.*

v/v: volume/volume.

LD: Lethal dose.

RNA: Ribonucleic acid.

FAO: Food and Agriculture Organization.

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Introduction

Introduction

The research of chemical agricultural products remains a significant scientific, technological, and environmental problem among the present important issues in phytopharmaceutical research.

Since the independence of European and African countries, agricultural production has been focused towards expanding due the population increase in order to attain sufficiency and supply food for the people. This encouraged the researchers to seek for ways to boost the crop's yields as synthetic pesticides. As a result, pesticide usage has increased in quantity, but quality has decreased over time, as seen by the appearance of various quality problems and health issues (diseases and epidemics).(Swagata and al,. 2021)

Pesticides are chemicals or mixes of chemicals that are mostly employed in agriculture or public health programs to protect plants from pests, weeds, and diseases, as well as humans from vector-borne diseases like malaria, dengue fever, and schistosomiasis. Typical examples include insecticides, fungicides, herbicides, rodenticides, and plant growth regulators. These products are also used for non-agricultural applications, such as improving and maintaining public urban green spaces and sports fields. (Polyxeni and al., 2016).

With the gradual increasing of regulations on the use of synthetic pesticides, natural regulators of pest organisms such as weeds and invasive plants will become more important. Weed biocontrol is growing in popularity as it becomes more resistant to chemical herbicides.

Pesticide residues can be discovered in a wide range of common foods and beverages. It's also worth noting that washing and peeling won't totally eliminate the residues. The majority of the times, the concentrations do not exceed the safe levels set by law. However, in the case of simultaneous exposure to two or more chemical substances have synergistic effects; these "safe limits" may understate the true health risk.(Usha Bajwa, 2011).

Pesticides can enter the body by skin contact, ingestion, or inhalation. The type of pesticide, the duration and method of exposure, as well as the individual's health status (e.g., nutritional inadequacies and healthy/damaged skin) all have a role in the potential health consequence. Pesticides can be digested, expelled, stored, or bioaccumulated in the body fat of humans and animals. Chemical pesticides have been linked to a variety of severe health

impacts and environmental problems, including dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine effects. High occupational, accidental, or intentional exposure is also a concern pesticide exposure can lead to hospitalization and death with the significant health consequences, highlight the urgent need for alternative solutions to be implemented health impacts, all biologists and farmers are seeking for alternate strategies to boost crop quantity and quality while reducing organic pesticide harm. The use of biological pesticides, such as animals, bacteria and medicinal plants, has contributed significantly to biological conflict, so that the desired goal is to eliminate all diseases, insects and harmful plants without damaging the crop.(Polyexeni and al,. 2016).

The term *biopesticides* defines compounds that are used to manage agricultural pests by means of specific biological effects rather than as broader chemical pesticides. It refers to products containing biocontrol agents – i.e., natural organisms or substances derived from natural materials (such as animals, plants, bacteria, or certain minerals), including their genes or metabolites, for controlling pests. According to the FAO definition, biopesticides include those biocontrol agents that are passive agents, in contrast to biocontrol agents that actively seek out the pest, such as parasitoids, predators, and many species of entomopathogenic nematodes. (M Sporleder and al, 2013).

Plants and its derivatives represent effective alternatives to synthetic chemicals and provide very promising results such as cumin, peppermint and castor.

Castor (*Ricinus communis L*.) is a species of annual or perennial flowering plant which belongs to the spurge family *Euphorbiaceae*, monotypic genus *Ricinus*, and Ricininae subtribe. It is a fast-growing and suckering shrubby tree which reaches the size of 5–12 m. It is commonly known as veranda (Bengali), arandi (Hindi), era-gach (Assamese), castor and castor oil plant (English), wonderboom (Dutch), ricin (French); Rizinus and Palma Christi (German), Fico d'inferno (Italy), and ricino (Portuguese). And also called "*Palma Christi*" because the red leaves resembled the palms of Christ and because of the plant's amazing healing powers.(Scarpa and Guerci, 1982)

In the tropics, the plant can reach the height of a small tree, being over 40 feet high with foliage 15 feet in diameter, and with a woody trunk often a foot in diameter. The plant grows rapidly, appearing to spring up spontaneously, growing along river banks and other areas that have suitable moisture. Because of its rapid growth and wide leaves that offer much needed

shade from the tropical sun sun, the plant is also known as the **"African Wonder Tree"**(Balint GA, 1974).

The long-stalked and simple glazing leaves are 15–45 cm long; alternate and palmate with 5–12 deep lobes with serrate leaf margins ; lobes acuminate, membranous, oblong to linear; 1–3-cm-long stipules united to a sheathing bud, deciduous; and petiole 3.5–50 cm long, round.(CABI, 2022).

Different leaf colors are observed in castor, which start off as dark reddish purple or bronze when young and turn into dark green, sometimes with a reddish tinge as they mature. In some varieties, the leaves are really green from the start, whereas in others, a pigment suppresses the green color of all chlorophyll-bearing parts, leaves, stems, and young fruits so that they remain a dramatic purple to reddish brown color throughout the whole life of the plant

The flowers are burgeoned in an erect terminal panicle-like inflorescence, which consists of cymes, usually glaucous, later-appearing lateral by overtopping, up to 40 cm long. The flowers are unisexual, regular, with short pedicel, 1–1.5 cm in diameter; the calyx with 3–5 lobes; corolla absent; male flowers toward the base of the inflorescence with many stamens in branched bundles; and female flowers relatively few in number and remain toward the apex of the inflorescence with early caducous sepals, three-celled superior ovary, usually soft spiny, style 3, red or green, 2-cleft. (CABI, 2022)

Fruits are ellipsoid to subglobose, usually three-lobed smooth or spiny capsule, 1.5–2.5 cm long, brown, dehiscing in three cocci each opening by a vulva and one-seeded .(Maroyi, 2016).

Seed is ellipsoid, 9–17 mm long, compressed with a brittle, mottled, glaring seed coat with distinct caruncle at the base, endosperm copious, white, and cotyledons thin. (Mayori, 2016).

Seedlings are grown by epigeal seed germination; cotyledons petioled, broadly oblong up to 7 cm long, flat with entire margins; and first leaves opposite. (Mayori. 2016).

The genetic diversity in castor is restricted due to its monotypic existence. Six subspecies viz. persicus, chinensis, zanzibarinus, sanguineus, Africans, Mexicans were identified based on eco-geographical grouping (Uguru and Abuka, 1998) (Bahia and al, 2008) (Bezerra and al, 2010). However, there is no difference in the chromosome number (2n=20) among the sub-species and they all can cross easily with each other (Uguru and Abuka, 1998; Nóbrega and al, 2010)

Introduction

Castor is distributed in tropical, subtropical, and warm-temperate climates across the world. It's prevalent on fellow land, roadside, and complexes in rural and urban settings, as well as near seasonally dry rivers at elevations of 400 to 2700 meters. Castor is thought to have originated in Northeastern Africa, namely Ethiopia and Somalia (Anjani, 2012), and it has four distinct centers of diversity: (a) Ethiopian-Eastern African, (b) Northwest and Southwest Asia, and Arabian Peninsula (c), Indian subcontinent, and (d) China (Severino and al, 2012)It has been naturalized all across Africa, from the Atlantic coast to the Red Sea, from Tunisia to South Africa, and on Indian Ocean islands. It's also frequently farmed and naturalized in America's and Asia's tropical and subtropical regions, as well as temperate Europe (Govaerts and al, 2014).

Edaphoclimatic conditions have been summarized according to (Rousset, 2008) as Follows:

• **Climate:** Tropical and subtropical, between 40ºN and 40ºS.

• **Soil:** The plant is demanding and fatigues the soil. It requires good topography; the maximum slope should not exceed 12%, and good sun exposure. It needs deep, fertile and well-drained siliceous or siliceous-clay soils. Alluvial soils are excellent for this plant. The ideal pH is between 6 and 7. Production is not good in wet and poor soils.

• **Temperature:** 20 to 30ºC.

• **Humidity:** Below 80%, ideal around 65%.

• **Precipitation:** 375 to 500 mm of rain during the vegetative period.

• **Altitude:** Between 300 and 1500 m.l (Mahdjouba, 2016)

Castor oil is listed as a remedy for numerous ailments and also became a valuable product for early civilizations in Northern Africa and the Middle East because mature castor seeds contain 40-60 % oil that is easy to remove from the bean mash.

Castor oil's medical usage declined after World War II. The usage of laxatives and antimicrobials has decreased dramatically with the introduction of gentler laxatives and antimicrobials. The oil is digested by lipases in the colon, which releases ricinoleic acid into the lumen. The laxative and labor-inducing actions of free ricinoleic acid are caused by alterations in intestinal villi (Cline and al, 1976;Goodman and al, 1975) .

Like all vegetable and animal oils, castor oil is a triglyceride, which is chemically a molecule of glycerol esterified with a fatty acid whose chains are composed of approximately 90% of ricinoleic acid [acid (9Z, 12R)-12 hydroxyoctadec-9-enoic].

Oleic and linoleic acids are the other two significant compounds, although they are present in much smaller quantities: they represent respectively about 2 and 6% of the chain of gras73 acids. The other compounds, very minority, are the palmitic acids, stearic and linolenic substances, each of which is less than 1% to 0.5%.

Seeds bark and leaves are all more or less toxic due to the presence of a glycoprotein lectin that inhibits intracellular protein synthesis by inactivation of ribosomes (Sijelmassi, 1991).

Castor has a molecular weight of approximately 64 kDa (approximately 570 amino acids) consisting of two subunits A and B that are connected by an intermolecular disulfide bridge (Olsnes and Kozlov, 2001). Subunit B (isoleucine) allows the fixation of ricin on the cell membrane, it has two fixation sites where certain glycan structures adhere to the surface of the cells (lectinic properties) and control the endocytosis in the cytosol of the target cells, whereas subunit A is an enzyme (RNA-N-glycosidase), which will inhibit protein synthesis by binding to endoplasmic reticulum ribosomes and adenine cleavage inactivation cell (Olsnes and Kozlov, 2001; Sijelmassi, 1991). This determines the toxic action of ricin at the cellular level. Because of the enzymatic properties of this toxin, it is thought that a single ricin molecule can, after translocation in the cytosol, kill the cell (Olsnes and Kozlov, 2001; Sijelmassi, 1991).

The concentration of ricin is highest in seeds, which also contain proteins, water and lipids. These seeds provide 60% of their weight in castor oil which is made up of 85% glycerides, ricinoleic acid and contains 1% vitamin E.

Ricinoleic acid damages the intestinal mucosa and produces considerable water and electrolyte losses, which is why it has such a strong and unpleasant purgative effect. At a given quantity and concentration, castor is one among the most poisonous natural poisons, (Sijelmassi, 1991). It inhibits the formation of more complex proteins in the intestinal wall, resulting in digestive system injury.

Ricinus communis appears different movements such as antimicrobial one against dermatophytic and pathogenic bacterial strains such as *Streptococcus progenies* and *Staphylococcus aureus*. The result uncovered that the petroleum ether and acetone extracts restrain microbial movement, while ethanolic extricats has antimicrobial action as it were on higher concentration (Islam and al, 2010).

The significant antioxidant capacity of *Ricinus communis* seeds at low concentration helps alleviate diseases induced by oxidative stress. The chemical compounds indicates that antioxidant actions are methyl ricinoleate, ricinoleic acid, 1, 2-octadecadienoic, and methyl

ester (Oloyede, 2012). Castor leaf and stem extracts include flavonoids that have antioxidant properties. (Singh and Gupta, 2010; Gupta and Sharma, 2006)

The oil derived from the seed of castor has the ability to prevent ulcers; however it is more effective against ulceration caused by pylorus ligation, aspirin, and ethanol in rats. The antiulcer effect of *Ricinus communis* is attributed to the drug's or corroborant's cytoprotective impact on the gastric mucosa, which improves mucosal protection. (Rakesh and Kabra, 2011)

The ethanolic extract of *Ricinus communis* root has a considerable effect in lowering fasting blood glucose levels. As a result, *Ricinus communis* is regarded as a potent diabetic phytomedicine. (Shokeen and Anand, 2008)

Castor root methanolic extract has anti-inflammatory and free radical scavenging properties, the extract enhances the free radical scavenging ability of the stable radical 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH), nitric oxide, and hydroxyl radical. (Ilavarasan and Mallika, 2006).

Our study focused on examining the castor(*Ricinus communis*) insecticidal activity and its effects on pests population's such as aphids of colza (*Brevicoryne brassicae*) and ladybugs (*Coccinella algerica*) which considered as the number one enemy of aphids, in purpose to see the effectiveness of the methanoic extract on targeted and non-targeted populations.

Materials and Methods

I. Objective

The objective of our work is to perform the insecticidal power of castor and their synergy on the aphids of cereals (wheat and colza). The extraction was carried out by a well-defined method: Soxhlet extraction to obtain the methanoic extract of castor.

II. Material et Methods

II.1. Material

II.1.1. Plant Material

The study focused on a species of aromatic medicinal plant; *Ricinus communis* (*Ricin commun*). We harvested the plants in March 2022 from three different locations of Guelma State with varying plant environments and pollution levels. (From ben djerrah (Bled Hamlaoui), belkhir and oued el maiz).

According (Anjani, 2011; Berman and al, 2010). The castor can be classified as:

- **Superdivision:** *Spermatophyta* - Seed plants
  - **Division:** *Magnoliophyta* - Flowering plants
    - **Class:** *Magnoliopsida* - Dicotyledons
      - **Subclass:** *Rosidae*
        - **Order:** *Euphorbiales*
          - **Family:** *Euphorbiaceae* - Spurge family
            - **Genus:** *Ricinus* L. - Ricinus
              - **Species:** *Ricinus communis* L. - Castor

Castor must be classified as Angiospermae, Eudicotyledone, Rosanae and Malpighiales. (Zimmerman and Smith, 1966).

Figure 01: *Geographical situations of the different RCM varieties (Google maps).*

II.1.2. Animal equipment

Aphids (*Brevicoryne brassicae*) and ladybugs (*Coccinella algerica*) in figure 02 were harvested in sampling manner from wheat on the experimental farm located at Belkheir locality (Road of Hadjar El Mengoub of hjer mangoub) (Boumaaza said). The insects in question were identified by Dr. Khaladi O.

Figure 02: Aphids (*Brevicoryne brassicae*) and ladybugs (*Coccinella algerica*) that were harvested.

II.1.3. Laboratory equipment

In Table 1, we have collected all the material needed for this study.

Multi-purpose equipment	**single-use equipment**	**Solvents**
Refrigerator Oven (27C°37C°) – incubator Professional balance Micropipettes Spatula Chronometer (T, H%) Soxhlet Rota-vapor Flask 200 ml Flask 250 ml Becher	Distilled water Pasteur pipettes Sterilized kneading boxes Test tube Syringe	Ether petrol Methanol Acetone

Table 01: *Sampling and laboratory equipment.*

II.2. Methods

II.2.1. Drying:

In order to gets the best drying of castor leaves there are three ways either natural drying between sunlight and dark room for 5 to 7 days. Drying at 60°C for 48 hours or 72 hours at 105°C in the oven. (Figure 03 shows dried castor leaves).

Figure 03: *Dried castor leaves (personal, 2022)*

II.2.2. Crushing:

The plant material consists of the leaves of *Ricinus communis (L.).* The dried leaves are then ground with an electric crusher until a powder is obtained. (Figure 4 shows the crushing process).

Figure 04: *crushing the castor dried leaves.(personal, 2022).*

II.2.2.1. Humidity test:

The moisture content of the plants is determined by the drying process in the oven; we introduced 30gr of plant material (castor) into an oven that was raised to 60°C for 48h (Stran and al., 2007). At the exit of the oven, the plant material is left in a desiccator for 15 min ambient before being weighed; this allows expressing the water content.

The moisture content (H %) is given according to the following formula:

$$H\% = (M_0 - M_1) / M_0 \times 100$$

H%: moisture content expressed as a percentage.

M_0: mass of the sample (fresh leaves) before the drying (in gr)

M_1: mass of the sample (dry sheet) after boiling (in gr).

III. Soxhlet extraction:

The principle is the same as for any extraction, but here there is the problem of the diffusion of the solvent in the solid phase, which can be very slow. A very large number of successive extractions must be carried out to obtain a satisfactory separation.

The Soxhlet extractor is a device specially designed for continuous solid-liquid extraction (Penchev, 2010). This device bears the name of its inventor: Franz von Soxhlet.

The solvent (5 to 10 times the amount of the solid sample to be extracted) is boiled and then condensed with the ball condenser in the siphon tank, containing the solid to be extracted in a thick paper cartridge. The contact between the solvent and the product to be extracted is hard during the accumulation of the solvent in the tank, and then when the solvent reaches a certain level; it initiates the siphon and returns to the flask by dragging the dissolved substance. This cycle may be repeated several times, depending on the ease with which the product diffuses into the solvent (Penchev, 2010).

This extraction method requires a pro-treatment for the mixture obtained by Soxhlet; in practice, a rotary evaporator is used to separate the extract and the extraction solvent (Penchev, 2010).

III.1. Operating mode:

To perform the extraction, we first dry the leaves (the Castor 4 days) and grind before use.We treated 10gr of the sample to be extracted (castor) powder by a solvent (ether petrol) that allows the leaves to be loosened, and then let dry for 10 min at room temperature (the treatment is done under the hood). (Figures 05 reveal the treatment of RCM with ether petrol).

Figure 05: *The treatment of castor with ether petrol. (personal, 2022).*

After delipidation, the sample is inserted into a cartridge placed in the Soxhlet topped with a refrigerant carried by a flask containing 250 ml of extraction solvent (We have a hydroalcoholic methanol mixture – water (v/v): 60%). (Figures 5 characterize the insertion and treatment of soxhlet sample with methanol solvent).

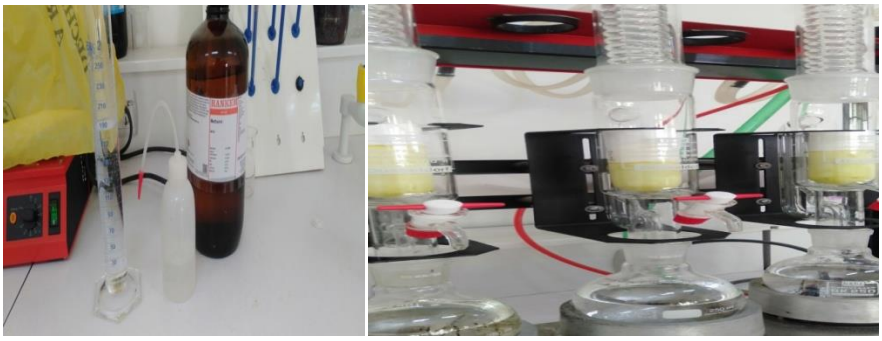

Figure 06: *The insertion of soxhlet sample with methanol solvent and treated castor. (personal, 2022).*

We started the system, heating the flask to the boiling temperature of the solvent 64.7°C, causing it to evaporate; the distillation column generates solvent vapors that are condensed in the refrigerant. When the cartridge is full, the solution obtained (solvent and solute) automatically empties by siphoning (leaching) and then returns to the boiler where the solvent is again boiled. This pure and hot solvent feeds the cartridge containing the inert solid and solute. The operation is repeated several times until the total exhaustion of the plant (exhaustion for 6 cycles). (Figure 7 indicates the castor extraction by soxhlet).

Figure 07: *Soxhlet hydro-alcoholic extraction (personal, 2022).*

The treatment time is different depending on the solvent; the contents of the flask (solvent plus solubilized matter) are concentrated using Rotavapor to remove the solvent.

The methanoic extract was prepared at the rate of 10g of vegetable powder per 250ml of the solvent, following the protocol summarized in Figure 8.

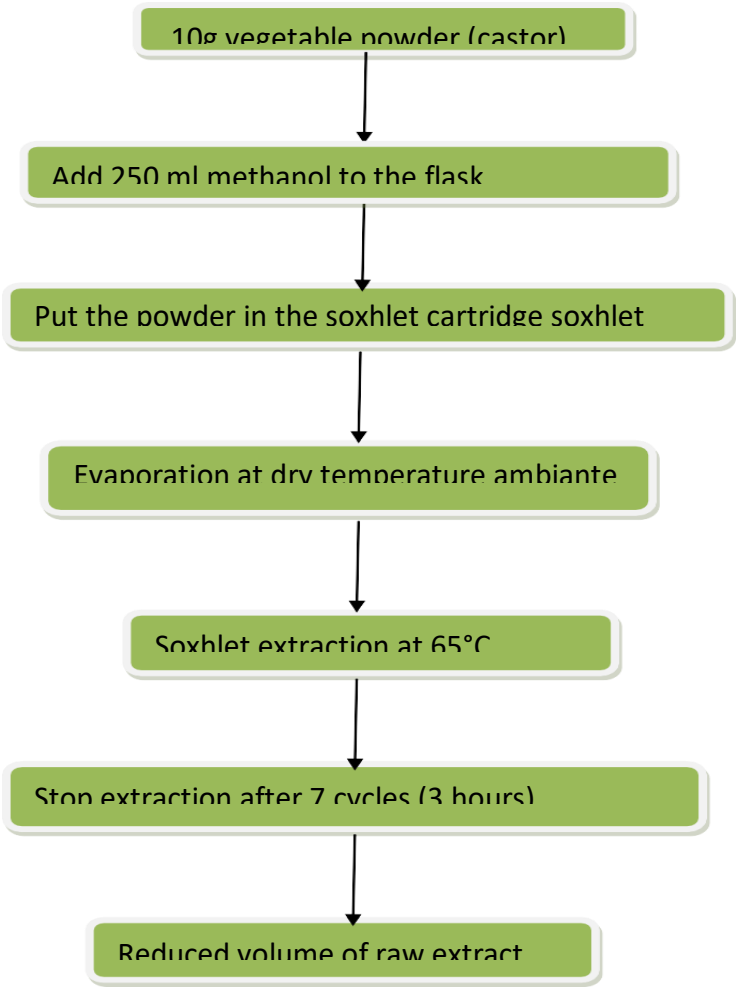

Figure 08: *Soxhlet extraction protocol.*

IV.1. The rotary evaporator

The rotary evaporator uses a fast and efficient separation technique: it allows the extraction of a solvent whose boiling temperature is lowered by working under reduced pressure (Hireche, 2013) , The rotary evaporator used in the experiment is of the Buchi R-210 type, its characteristics are shown in the table (Annex 3).

IV.1.A. The principle of rotary evaporator

The mixture of solvent and solute is placed in the right balloon. The balloon is immersed in a water bath. It is tilted and rotated to create a film of liquid and thus increase the evaporation surface of the solvent. The pressure inside the assembly is lowered by means of a water tube which increases the evaporation rate. After condensation in the refrigerant, the solvent is recovered in the left balloon (Ould Amar, 2013).

In our extraction, we used methanol as a solvent that evaporates through the steam rota at 65°C; the raw extract obtained is kept in a dark bottle "under the cover of light" well closed at a temperature less than 6°C until it is used. (Figure 9 reviews the separation of the solvent and pure oil by rota-evaporator).

Figure 09: *The separation of the solvent and pure oil by rota-evaporator (personal.2022).*

Evaporation of the solvent was carried out according to the following protocol (Figure 10 illustrate the Rota-evaporator protocol):

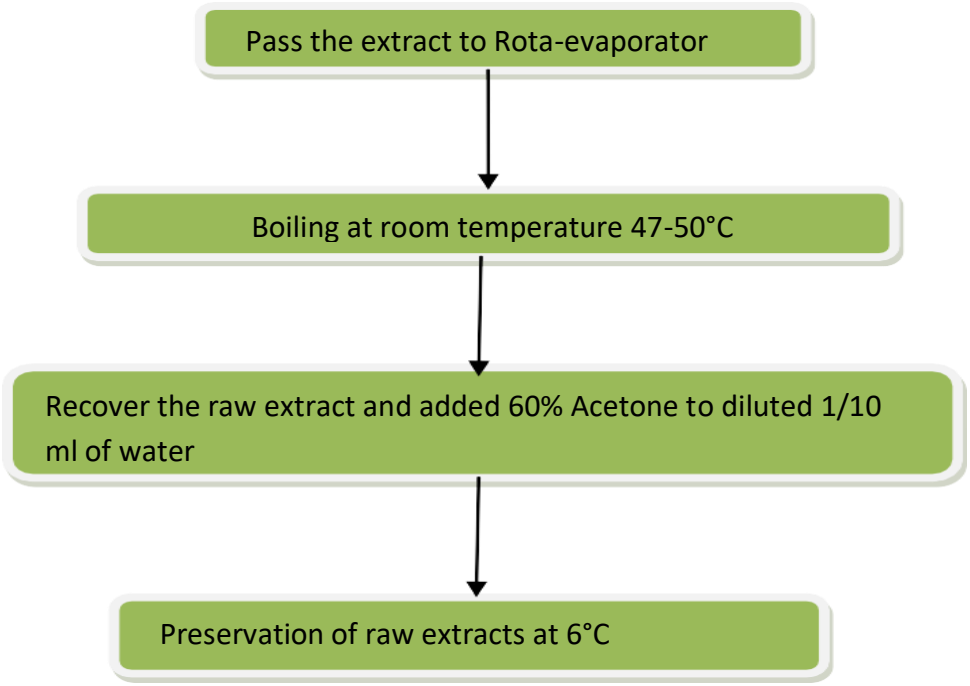

Figure 10: *Rota-evaporator Extraction Protocol.*

IV.II. The extraction yield

After each extraction step, the extraction yield is calculated; the yield expressed as a percentage of the weight of the starting material is determined by the following relationship:

Where: $$R = M_{ext} \times 100 / M_{éch}$$

R: yield in %.

M_{ext}: is the mass of the extract after evaporation of the solvent in gr.

M_{ech}: is the mass of the plant sample in gr (clemence and dongmo, 2009).

V. Insecticide activity

The *Ricinus communis* (hydro-alcoholic extract) insecticide activity test on the Aphids was inspired by the technique of the World Health Organization (WHO, 1963). The tests were carried out at the plant protection laboratory.

VI. Preparation of dilutions

The dilution preparations were obtained according to the following protocol: very low to very concentrated concentrations were chosen in order to test the insecticide potency of these substances.

- For each of the common castor hydro alcoholic extracts and the synergic extract (50% common castor), solutions of a volume of 20 ml of increasing concentrations are prepared by dilution in distilled water of 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40% and a positive control with 60% acetone.

- And negative control with distilled water. (Figure 11 shows different diluted product).

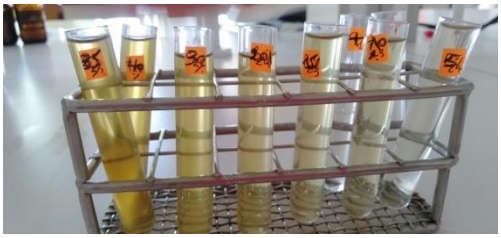

Figure 11: *Different prepared diluted castor extracts (personal, 2022).*

VII. Contact test

A volume of each prepared dilution is evaluated by spraying on the healthy leaves of bigaradier (which will serve as a food carrier for aphids) containing 5 aphids, which have been collected with a brush and placed in 9 cm diameter and 1.8 cm height aerated petri dish boxes containing soaked filter paper of the same diameter as the petri dish, The latter keeps the moisture and freshness of the leaf as long as possible. (Figure 12 display prepared petri dished for the test).

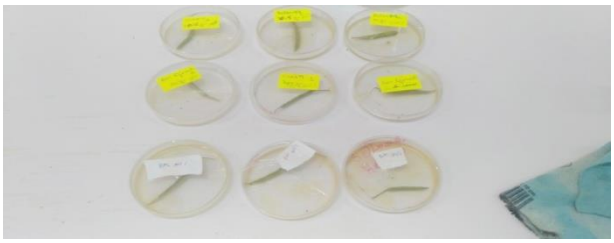

Figure 12: *Petri Dishes prepared for the test (personal, 2022)*

The same number of aphids was placed in the control boxes (positive and negative); the negative control is sprayed just with distilled water, and the positive control was sprayed with diluted acetone.

For each treatment, tests were repeated 5 times and controls were used as references.

After treatment, the boxes are well closed by the para-film

Each treatment of the test was performed on aphids also by modifying the food carrier.

The test was carried out under temperature conditions of 22± 3°C and a relative humidity of 48±9°C, natural photoperiod of 8/16.

The test was performed also on ladybugs following the same steps (step by step).

VIII. Parameters studied

We chose essentially only one parameter: the effect of the different concentrations of the extracts at the chronological scale (after 24h, 48h, 72h and 168h) for the follow-up

VIII.1. The mortality rate

The mortality rate (%) is determined for each treatment after 24h, 48h, 72h and 7d after spraying.

A product is said to be effective by the assessment of mortality; the counting of the dead individuals in a population treated by a toxicant is not the actual number of individuals killed by that toxicant, taking into account the natural mortality observed in the negative control. Post-exposure mortality should be corrected using the Abott formula (Bouras and Benhamza 2013):

$$\mathbf{Mc\% = [(M_0\% - M_T\%) / (100\text{-}M_T\%)] \times 100}$$

Mc%: corrected mortality expressed in %.

M_0%: mortality observed after spraying.

M_T%: mortality observed in the control.

VIII.2. DL50, DL90 and DL100 Determination

The effectiveness of a toxicant is measured by its DL50, DL90 and DL100, which represent the amounts of toxic substance causing death of 50%, 90% and 100% of individuals in the same batch respectively. They are derived from the equation of the regression line (y=ax+b) corresponding to the mortality rates corrected for treatment concentrations (wabo – sanded, 2005).

IV. Statistical analysis

The results obtained from the insecticide activity test are analyzed statistically with the ANOVA software for Microsoft Excel 2007 by the analysis of the variance with two classification criteria with a threshold of significance (P=5%), in order to determine the links between the effect of extract concentrations and aphid mortality. The meaning of the Bouras and Benhamza (2013) codes is as follows;

0***: highly significant.

0.001**: very significant.

0.01*: significant.

0.05: moderately significant.

0.1: not significant.

Resultats and Discussion

I. Resultats

I.1. Moisture content of plant material

Plants are essentially made up of water. The water content of a plant can vary depending on the organ, depending on the type of plant considered. Water and mineral nutrients are what we call sap. But, unlike animals, plants do not have a pump to circulate this sap: it is the foliar perspiration that causes it to rise along the stems, from the roots to the leaves. Because under the action of the heat provided by the solar radiation, the leaves of the plants sweat. This very important phenomenon is called evapotranspiration.

Plants lose a lot of water by evapotranspiration. The remaining water contributes to the photosynthesis of the organic substances that plants need to develop.

The results of our analyses for the various samples tested revealed a high humidity level for *Ricinus communis* with a rate of 65-70% (Fig. 13), which means that for castor, more than half of the weight of the fresh plant consists of water.

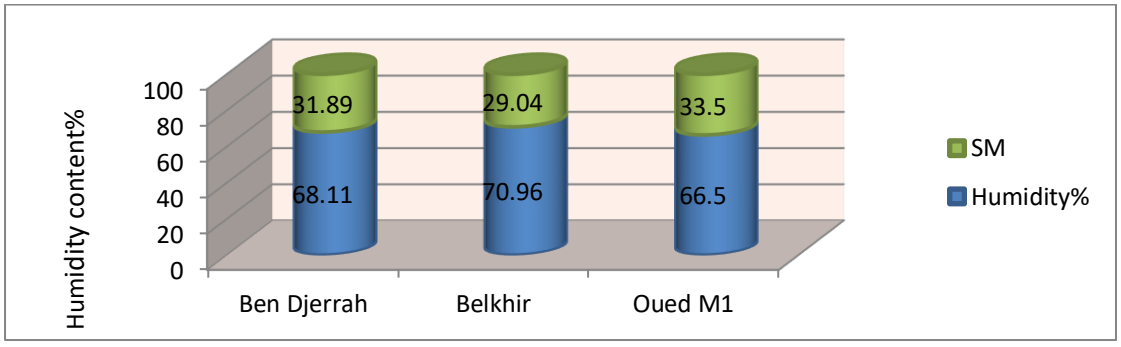

Figure 13: *Humidity of R. communis.*

While (Sadok and Bentounes, 2016) recorded a lower humidity 33% , and (Mahdjouba, 2016) found a result close to the previous 38%.

The high humidity gives us an idea of the degradation of the active ingredients of the plant (Bourkhis 2009).

II. Studies of extracts

II.1.1. Yield

The yields of the different extracts are defined as the ratio of the quantity of the extracted plant substances to the quantity of the plant material used.

II.1.2. Hydro-alcoholic extract

The extraction by the device of Soxhlet allows the total exhaustion of the active principles in the vegetable matter; this is represented by the variation of the color in *Ricinus communis* from dark brown to light (figure 14).

Figure 14: *The extraction cycles by the Soxhlet extractor of the RCM oil (personal, 2022)*

The yields obtained from the hydro-alcoholic extracts of the dry leaves of *R. communis* are 22.5% and 46.96% respectively. These values are much closer to those obtained by Neggaz (2015) following the extraction of the fresh leaves which allowed to have respective yields of 61.29 and 39.27%, while a yield of 35% was noted by Osman (2014) during the extraction of fresh R. communis leaves(**figure 15**). **Table 02** presents the yield for every variety of *Ricinus communis*.

Space	**The Yield %**
Ben Jarrah	46,96**%**
Belkhir	15,55**%**
Oued el maiz	31,07**%**

Table 02 *presents the yield for every variety of Ricinus communis.*

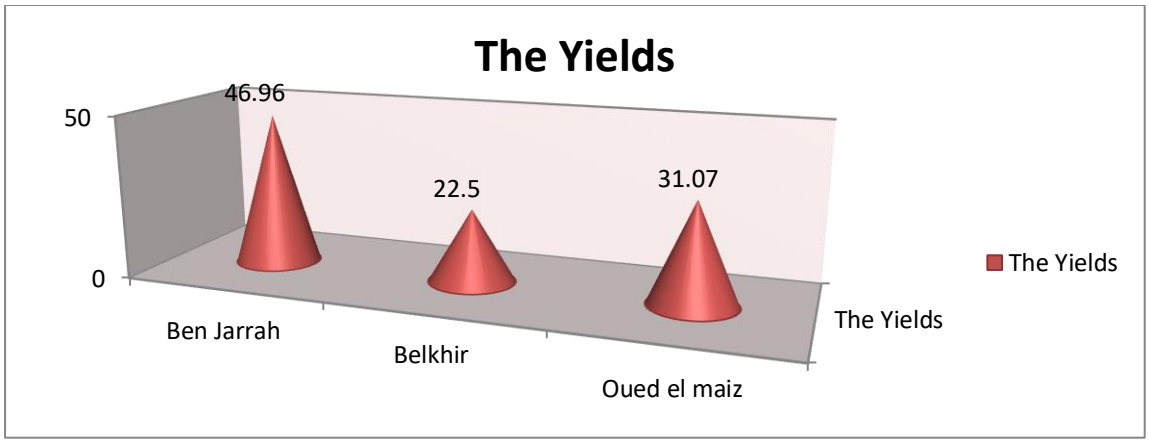

Figure 15: *The yields for every variety of Ricinus communis.*

According to the results shown in the table 02 and the figure 15, there is a difference in the yield between the three categories where we find the category of Ben djarrah the most recovered, noting that in the region the least contaminated among them. So we confirm that the pollution with all its sorts affect on the yield

These results are consistent with those obtained by Ait Taadaouit and al. (2011), which explain the selection of methanol as an extraction solvent. According to the literature, methanol has been recommended and frequently used for the extraction of active ingredients, especially phenolic compounds (Falleh et al., 2008).

Indeed, methanol is used for extraction; it can prevent certain active principles of the plant such as phenolic compounds from being oxidized by enzymes.

III. Insecticide activity

According to Regnault-Roger (2002), the toxicity of hydro-alcoholic extracts from toxic plants such as *Ricinus communis* has a confirmed insecticide effect on *Brevicoryne brassicae* with toxicity variations that are related to chemical composition.

According to Noudjou (2007), the combined effect of several terpenic compounds (synergy effect of compounds) is reported in insecticide activity.

Aphids are exposed to various treatments based on hydro-alcoholic extracts (polyphenolic) (the common castor and synergic extract). The follow-up was done in 24 hours, 48 hours, 72 hours, and 7 days.

Aphid mortality was recorded 24 hours after exposure for all extracts, while mortality in the positive and negative controls was only recorded after 48 hours of exposure.

According to Hullé and al. (2010), conditions conducive to aphid development show that the rate of development and fecundity depends on temperature. The minimum degree of their development is 4°C on average. Below this threshold, they no longer multiply. Between 4°C and 22°C, they multiply faster as the temperature rises. Beyond 22°C, their development slows down again. To become an adult, a female aphid needs an average of 120°C. Indeed, in the world of insects, the generation time is very short.

III.1. Treatment with polyphenolic extract of *Ricinus communis* on *Brevicoryne brassicae*:

The histogram representation in Figure 16 shows the evolution of the cumulative mortality rates of Aphids relative to the control as a function of the dose of the polyphenolic extract of the *R. communis* leaves used and the time. There was a significant insecticide effect after 72 hours for the eight doses selected compared to controls. In fact, the negative and positive controls did not record any mortality until after 48 hours of treatment, where rates of 20% and 40% were recorded after 72 hours and the 7th day a mortality of 40% and 60% respectively.

After 72 hours of treatment exposure, the doses studied showed an important insecticide activity for doses of 10%, 15%, 20% and 30% since respective mortality rates of 60%, 80% and 100% were noted (Fig. 16). While, Neggaz (2015) recorded only 20%, 35% and 40% mortality for the respective doses of 10%, 20% and 30%.

The 35% dose gave the best result after a 24-hour exposure time with a mortality rate of 80%, while the 30% and 40% doses caused a total mortality of 100% after 72 hours of exposure (Fig. 16).

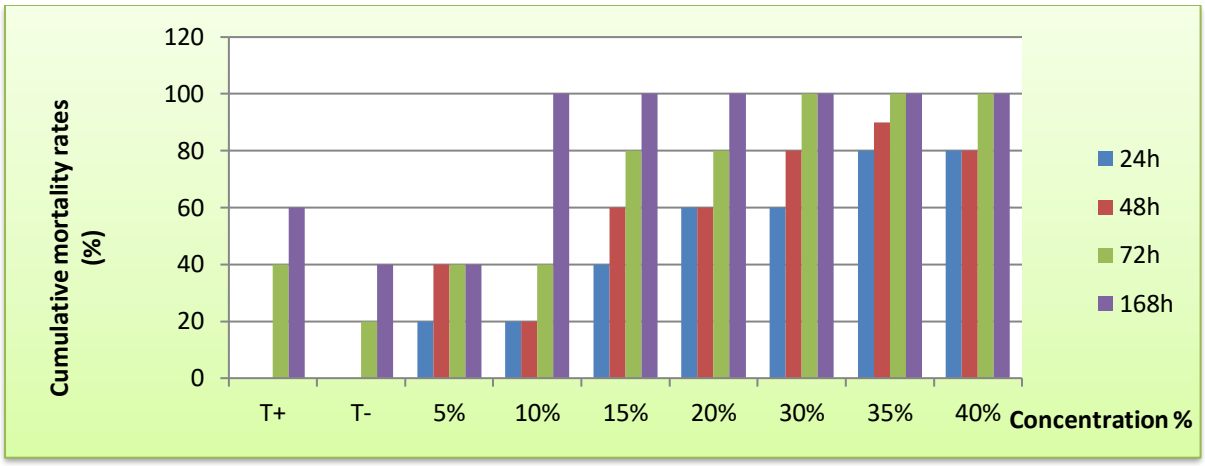

Figure 16: *The evolution of the cumulative mortality rate of R. communis on B. brassicae.*

For the cumulative corrected mortality of *R. communis* on *B. brassicae* shown in Figure 17, the high doses are 30% and 40% leading to total mortality of individuals on the third day of treatment.

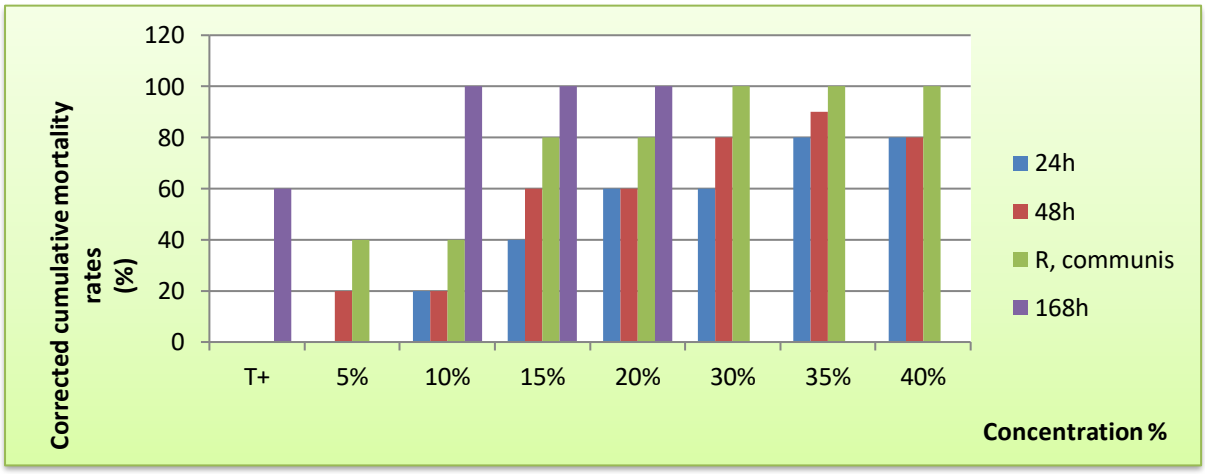

Figure 17: *The evolution of the the corrected cumulative mortality rate of R. communis on Brevicoryne brassicae*

- **Lethal doses 50, 90 and 100**

The lethal doses of the insecticide activity of *R. communis* on Aphids (Brevicoryne brassicae) were determined from the equation of the regression line shown in Figure 18, which corresponds to the concentration-corrected mortality; these doses are shown in Table 03.

Table 03: Lethal dose values of R. communis on B. brassicae.

LD50	6.90%
LD90	33.22%
LD100	100.00%

The lethal doses of the dry leaf extract of *R. communis* studied yielded results approximately close to that of *R. communis* for the mortality of 50% of the individuals tested where a dose of 6.90% was recorded. In addition, for the LD90 a low dose of 33.22% is required while for 100% mortality, a dose of 35% and 40% is required in contrast to the lethal doses obtained by Neggaz (2015) of the extract of fresh leaves which presents a low toxicity by the LD50 (13.32%) and the LD90 (82.72%). Also the results noted by (Sadok and bentounes, 2016) marked(LD_{50}: 9,90%, DL_{90} : 29,22%, DL_{100}: 34,43%) .

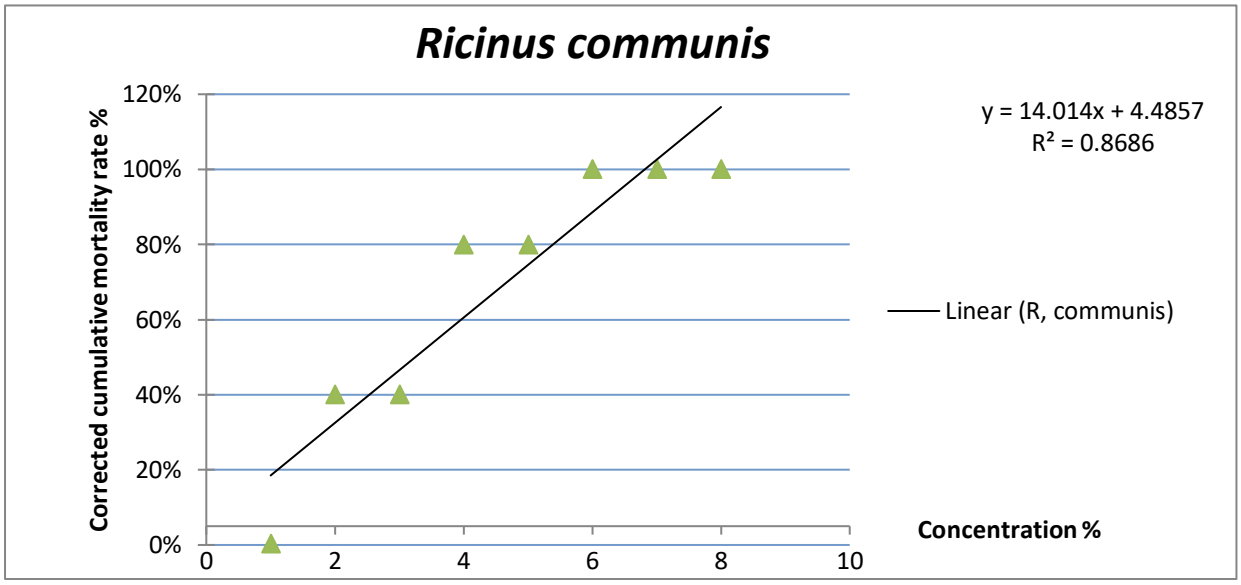

Figure 18: *The evolution of the corrected mortality of the polyphenolic extract of R. communis on B. brassicae.*

All of these results in figure 18 reveal that spraying aphids with castor polyphenolic extract is a good natural control method that should be suggested in the management of aphid populations.

III.2. The effect of the treatment of *Ricinus communis* on ladybugs *Coccinella algerica*:

As it is indicated in the figure 19, there was an important mortality rate after 72 hours of treatment by the *R. communis* extract on *C. algerica*, we marked a 80% moratlity rate after 72

hours for 10% and 20% doses, while the 5% and 15% marked a weak mortality rate comparative to the positif and negative one, Although (Sadok and Bentounes, 2016) did not recorded any mortality for all used doses.

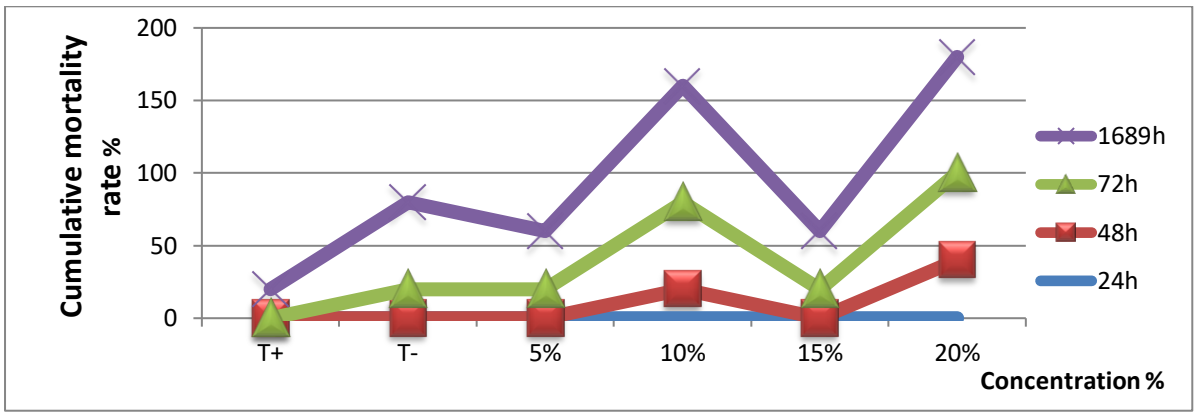

Figure 19: *The evolution of the cumulative mortality rate of R. communis on C. algerica.*

Comparing the cumulative mortality rate with the cumulative corrected mortality rate shown in Figure 20, we find that doses 10 and 20% are the most effective with a corrected mortality rate of 95.65% after 72 hours to 7 days exposure.

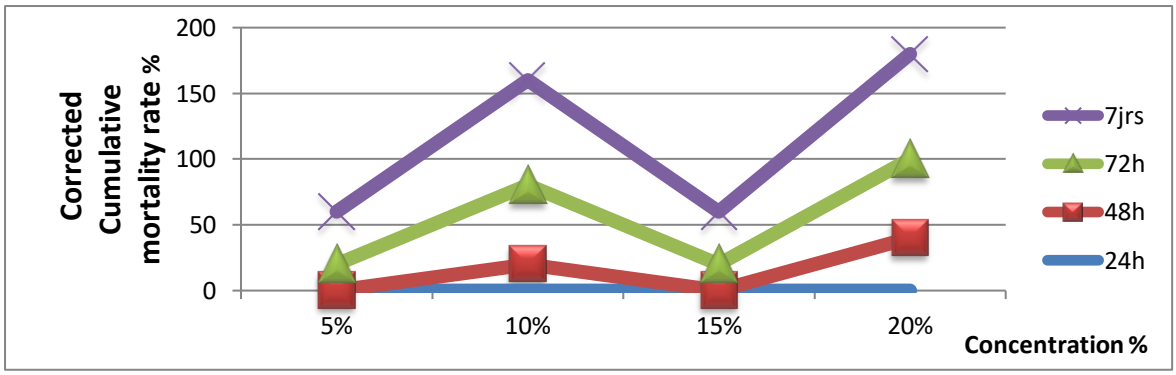

Figure 20: *The evolution of the corrected cumulative mortality rate of R. communis on C. algerica.*

- **Lethal doses 50, 90 and 100**

From the equation of the linear regression line represented in figure 21, corresponding to the mortality rate corrected for the concentrations of the polyphenolic extract of *R. communis* on *C. algerica*, the lethal doses referred to in Table 04 have been determined.

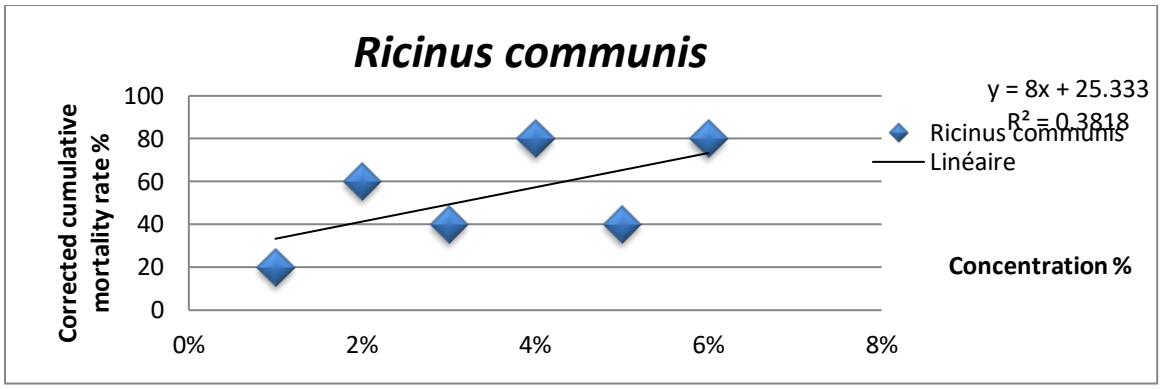

Figure 21: *The evolution of the corrected mortality of the polyphenolic extract of R. communis on C. algerica.*

Table 04: Lethal dose values of the polyphenolic extract on *C. algerica*.

DL_{50}	3, 08 %
DL_{90}	08, 08%
DL_{100}	09, 33%

According to the lethal doses obtained, polyphenolic extract of *R. communis* leaves requires a low dose to cause 50% , 90% and 100% mortality to cause total mortality of all individuals tested, with 03,08 % for LD50 , 08.08 % for LD90 and LD100 for 9,33 % highlighting the latter's toxicity.

For the development of castor-based insecticides, the findings acquired in the short or long term might be noteworthy and encouraging.

IV. Statistical analysis

The analysis of the variance with two classification criteria indicates a non-significant difference for the treatment factor (extract *R. communis*) (F=20.75 and P=5.17) and a highly significant difference for the concentration factor (Controls, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%) (F = 27.41 and P = 6.33).

The classification obtained by the Newman-Keuls test (Annex 8) groups the negative control and the positive control (Acetone 60%) in the same group, showing that acetone has a toxic effect of around 25%.

Group A corresponds to the most effective doses 40%, 35%; group B corresponds to the doses 05%, 10%, 15%, 20% and 30%.

The treatment factor by the plant extract is not significant, these results can be justified by the presence of molecules acting by biologically similar mechanisms in raw extracts for example the group of polyphenols which is present in almost all raw plant extracts.

Conclusion

Cereals are of critical importance to a wide number of countries due to their economic value, particularly the significant revenue they create on the one hand, and the jobs they provide on the other, as well as the operating and processing goods in many derivatives.

The major objective of this research was to identify an alternative to synthetic insecticides by examining polyphenolic extracts and essences of medicinal plants as insecticides made entirely of natural and biological ingredients.

Plants produce a variety of secondary metabolic chemicals. In insects, these molecules can have a variety of effects, including repellent, attractive, developmental disruptor, reproductive inhibitor, and so on. On the target organs, their toxicity might be direct or indirect (sensory organs, nervous systems, endocrine system, digestive system, reproductive system, etc.)

The results indicate that this methanoic extract has a significant insecticide effect on *Brevicoryne brassicae*, with an LD50 of 6.90 % extract controlling 50% of the population of this insect with a 100% mortality rate after 72 hours for the (10,15,20,30 %) doses.

These extracts were also tested on the ladybug *Coccinella algerica*, which is one of aphids' natural adversaries and must be protected. We find that doses 10 and 20% are the most effective with a corrected mortality rate of 95.65% after 72 hours to 7 days exposure and with an LD50 of 03.80%.

Based on the results of the tests, we assume that using plant extracts as an alternative to synthetic pesticides in the protection of the cerape is a potential possibility.

Résumé

Notre étude est fondée sur l'effet bio-insecticides des extraits polyphénolique de *Ricinus communis* sur les pucerons de colza *Brevicoryne brassicae*, les extraits ont également été expérimentés sur les coccinelles *Coccinella algerica* qui sont des ennemis naturels du puceron. Les résultats obtenus ont révélé des taux de mortalité de 96% ,100% respectivement pour *R. communis*. Les doses 30, 35 et 40% ont été les plus efficaces avec une mortalité de 100%. Les doses 10% et 20% a provoqué la mortalité des coccinelles à 96%. Selon les résultats obtenus, on remarque que l'extrait polyphénolique de *R. communis* (DL50= 6,90%) pour les pucerons et (DL50= 03,80%) pour controler 50% de population.

Mots clés : Bioinsecticide- Polyphénolique- *R. communis- B. brassicae- Coccinella algerica. -Mortalité.*

Abstract

Our study is based on the bio-insecticide effect of polyphenolic extracts of *Ricinus communis* on *Brevicoryne brassicae* rapeseed aphids, the extracts have also been tested on ladybugs *Coccinella algerica* which are natural enemies of the aphid. The results obtained showed mortality rates of 96% and 100% respectively for *R. communis*. Doses 30, 35 and 40% were most effective with 100% mortality. Doses of 10% and 20% resulted in 96% mortality of ladybugs. Based on the results obtained, we note that the polyphenolic extract of *R. communis* (DL50= 6.90%) for aphids and (DL50= 03.80%) to control 50% of population.

Key words: Bioinsecticidal- Polyphénolic- *R. communis- Brevicoryne brassicae- Coccinella algerica. -Mortality.*

الملخص

تستند دراستنا إلى تأثير المبيدات الحشرية الحيوية لمستخلصات البوليفينوليك من Ricinus communis على B. brassicae التي تعد أعداء طبيعيين للمن. Coccinella algerica ، وقد تم اختبار المستخلصات أيضًا على الخنافس R. communis. أظهرت النتائج التي تم الحصول عليها معدلات وفيات بنسبة 96٪ و 100٪ على التوالي في الجرعات 30 و 35 و 40٪ الأكثر فعالية مع معدل وفيات 100٪. أدت جرعات 10٪ و 20٪ إلى وفيات 96٪ من R. communis (DL50 = 6.90٪) للمن و (DL50 = 03.80٪) للسيطرة على 50٪ من السكان.

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Annex

Annex 01

A

B

C

D

E

F

A, B: *Leaves of Ricinus communis L.*
C: *Fruits of Ricinius communis L.*
D: Ricinus communis (castor bean) seeds.
E: *Flowers of Ricinus communis L.*
F: *Seedlings of Ricinus communis L.*

Annex 02

Figure 01: Examples of castor bean plants with different height. (Photos by : Máira Milani, Embrapa Cotton)

Figure 02: Examples of different colors in the fruits of castor bean. (Photos by: Máira Milani, Embrapa Cotton).

Annex 03

BUCHI R-210 Rotary Evaporator Data Sheet

Reference	BUC-23011A000
Display	Rotation Speed/ vapor temperature
Dimensions(W*H*D)	550*575*415 mm
Glass assemblies	A, V, C, S, E, CR, By
Weight	16-18 kg (depending on the glass assembly).
Connection voltage	100-240 V+- 10%
Frequency	50/ 60 Hz
Power consumption	Max. 60W
Degree of protection	IP21
Pollution degree	2
Rotation Speed range	20- 280 rpm
Flask size	50- 4000 ml
Max. Flask content	3 kg
Flask temperature	20-180°C

Annex 04

Table 01: Preparation of the raw extract of Ricinus communis:

EX / [c]	Castor extract	DW	Acetone 60%	final volume
10%	2ml	8ml	/	20ml
20%	4ml	6ml	/	20ml
30%	6ml	4ml	/	20ml
40%	8ml	2ml	/	20ml
T^-	00ml	20ml	/	20ml
T^+	00ml	8ml	12ml	20ml

Annex 05

Table 02: The evolution of the cumulative mortality rate of R. communis on B. Brassicae.

period / C	24h	48h	72h	168h
T+	00,00	00,00	40,00	60,00
T-	00,00	00,00	20,00	40,00
5%	20,00	40,00	40,00	100,00
10%	20,00	20,00	40,00	100,00
15%	40,00	60,00	80,00	100,00
20%	60,00	60,00	80,00	100,00
30%	60,00	80,00	100,00	
35%	80,00	90,00	100,00	
40%	80,00	80,00	100,00	

Table 03: The corrected cumulative mortality rate of R. communis on B. Brassicae.

period / C	24h	48h	72h	168h
T+	00,00	00,00	40,00	60,00
5%	20,00	40,00	40,00	100,00
10%	20,00	20,00	40,00	100,00
15%	40,00	60,00	80,00	100,00
20%	60,00	60,00	80,00	100,00
30%	60,00	80,00	100,00	
35%	80,00	90,00	100,00	
40%	80,00	80,00	100,00	

Annex 6

Table 04: The evolution of the cumulative mortality rate of R. communis on C. algerica.

time / C	24h	48h	72h	168h
T+	00,00	00,00	00,00	20,00
T-	00,00	00,00	20,00	60,00
5%	00,00	00,00	20,00	40,00
10%	00,00	20,00	60,00	80,00
15%	00,00	00,00	20,00	40,00
20%	00,00	40,00	60,00	80,00

Table 05: The evolution of the corrected cumulative mortality rate of R. communis on C. algerica**.**

time / C	24h	48h	72h	1689h
T+	00,00			20,00
5%	00,00		20,00	40,00
10%	00,00	20,00	60,00	80,00
15%	00,00		20,00	40,00
20%	00,00	40,00	60,00	80,00

Annex 7

Table 5: ANOVA variance of polyphenolic extracts (F1: Treatment; F2: Concentration)

	SCE	Ddl	MC	Test-F	Prob (5%)	E.T.	C.V
Total	42475	35					
F1	25900	08,00	3237,5	20,7507418	5,17		2,35508149
F2	12830,555	03,00	4276,85185	27,4124629	6,333		3,00878657
residual Var.	3744,444	24	156,018519				

Table 06 : F1 Mediums (Processing)

1(F1n1)	2	3	4
40	47,77	66,66	88.89

Tableau 07 : F2 Mediums (Concentration)

1(F2n1)	2	3	4	5	6	7	8	9
25	15	40	40	70.00	75.00	85.00	92.50	90

Table 08: Comparison of F2 averages by NEWMAN – KEULS test

F2	Libel	Medium	HOMOGENOUS GROUP		
1	**F2(n1)**	25			**C**
2		15			**C**
3		40.00		**B**	
4		40,00		**B**	
5		70,00		**B**	
6		75,00		**B**	
7		85,00		**B**	
8		92,50	**A**		
9		90,00	**A**		