

BIO-INSECTICIDAL EFFECT OF POLYPHENOL EXTRACTS OF ANVILLEA RADIATA AGAINST CEREAL APHID RHOPALOSIPHUM PADI

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Abstract: To reduce the abusive apply of artificial organic pesticides with discernment, biodegradable bio-pesticides origin from plants were used as an alternative phytosanitary method against crop pests. This experimentation allowed testing in vitro the bio-insecticidal activity of *Anvillea radiata* Coss and Dur (Asteraceae), endemic plant from the southeastern Algeria, against the cereal aphid *Rhopalosiphum padi* (Linnaeus, 1758). Three concentrations were tested (D1= 5mg/ml, D2= 2.5mg/ml and D3= 1.25mg/ml) with the butanolic and chloroformic extracts. The results showed a mortality rate of *R. padi* varying significantly by treatment and concentration according to the tested substances. The quantitative analysis of the two extracts shows that the crude butanolic extract is rich in polyphenols (348.935±7.456 µg EAG/mg MS) compared to the crude chloroformic extract (56.754±0.982 µg EAG/mg MS). Samely to the insecticidal effect tested, the concentration of 5 mg/ml presented an important insecticidal activity with the butanolic extract (62%) higher than the chloroformic extract (26%). Also, the lethal doses (LC50) of the crude butanolic extract were estimated by 23.07 mg/ml, 6.71 mg/ml and 5.70 mg/ml respectively after 24, 48 and 72hours of the treatment.

Keyword: Bio-insecticide, Cereal Aphid, *Anvillea radiata*, chemical compound, lethal dose.

I. INTRODUCTION

In Algeria, the harvested area of cereals was estimated by 3 421 833 ha in 2017 [1]. This crop remains insufficient and does not cover constantly increasing needs [2]. The cultivation of cereals in Algeria is most often faced with various difficulties which limit production. However, several pests confront this vegetable such as diseases and insect pests [3]. In the latter group, cereal aphids are in the first place. These Homopterans cause direct and indirect significant damage. They hurt the plant and take the sap to feed themselves. At the same time, they inoculate the plant with phytopathogenic agents such as barley dwarf yellowing virus. This disease can cause significant damage because the symptoms are not noticeable until very late time when no cure is possible. Despite the dangers it presents to humans and their environment, chemical intervention is the only way to control these arthropods. Nevertheless, in recent decades, other methods of control have emerged including natural products that are increasingly required as alternatives for various insecticides, fungicides, herbicides or even medical applications [4].

The possibility of using secondary metabolites of plants against harmful insects was the subject of many works. Noting the most recent studies in Algeria such as [5-14].

In this context the objective of our study is to evaluate the bio-insecticidal activity of the crude butanolic and chloroformic extracts of *Anvillea radiata*, a spontaneous plant from the south-eastern Algeria, against the larvae of the cereal aphid *Rhopalosiphum padi*. As well as to determine the lethal dose (LC50) of the crud extract tested in a specific time against this aphid.

II. MATERIAL AND METHODS

A. Description and origin of *Anvillea radiata*

Anvillea radiata Coss and Dur, vernacular name "Nogde", is particularly growing in North Africa (Algeria and Morocco) that is belonging to the family of Asteraceae [15]. This plant is a very branching shrub 20-50 cm, woody base

stem and branches, elongated triangle leaves, attenuated at the base in to a petiole, and lamina strongly dentate surrendered large , 4 to 5 cm in diameter including long ligules, surrounded by radiating upper leaves gradually passing the bracts, these tough, yellow-orange flowers all; glitter truncated at the top of the receptacle and a long silka chenes prismatic those from the periphery to three angle from the center of those surrendered four corner devices flowers ligules long, up to 25 mm [16].

Samples were collected in April 2016 from the region of Masaad in the southeastern Algeria. This plant was identified by Mr. EDDOUD Amar, Professor at the faculty of natural and life sciences in the University of Ouargla (Algeria). It was deposited in the herbarium of the Laboratory of Biogeochemistry and Desert environments (BMD), at the University of Ouargla with a reference number (0613/2016).

B. Screening of secondary metabolites

A quantity of three grams of *A. radiata* was placed separately in conical vials with 100 ml of the following solvents: methanol, water, chloroform, petroleum ether then stayed for 2 hours and filtered using filter paper. The filtrates were used for screening the secondary metabolites according to standard procedures followed by [17-20].

Detection of **Alkaloids** was made respectively by the reagent tests of Mayer (HgCl_2 , KI and H_2O), Dragendorff [$\text{Bi}(\text{NO}_3)_2$, KI and H_2O] and Wagner (KI, I₂ and H_2O) on the extracts. The presence of alkaloids was notified by the appearance of a red-orange precipitate, for Mayer and Dragendorff reagents, and brown, red or black for Wagner's reagent. Concerning the phenols, they were detected by the FeCl_3 test carried out on the extracts and confirmed their presence by the formation of intense color.

The detection of **tannins** was made by Alkaline Reagent test; 2ml of extracts that were treated with a solution of sodium hydroxide. The appearance of yellow to red color indicates the presence of tannins.

Flavonoids were characterized from the reagents Zinc-HCl reduction test. It consists to add a pinch of Zinc dust and a few drops of HCl to all the extracts. Formation of deep red color indicates the presence of Flavonoids. In lead-acetate test, 2ml of all the extracts were added to few drops of basic lead acetate solution. The formation of reddish-brown precipitate indicates the presence of flavonoids. Also in Shinoda's test, 1-2ml of all the extracts was added to a small piece of magnesium paper with few drops of HCl. The appearance of red color indicates the presence of flavonoids.

Identification of **Coumarins** was made by adding 2ml of all the extracts, in separate tubes, and covered with a piece of paper soaked in NaOH and heated. The production of a yellow fluorescence under UV light indicates the presence of coumarins.

Concerning **sterols** test performed by Salkowski's, 2ml of all the extracts were added to 5ml of chloroform. A volume of 1ml of H_2SO_4 was added carefully along the walls of the tube containing the mixture (chloroform + H_2SO_4). The formation of reddish color in the lower layer indicates the presence of steroids.

About **terpenoids** Screening Test, 2ml of all extracts were added to 1% of HCl and stayed for 5 to 6 hours. Then, these extracts were treated with 1ml of Trim-Hill reagent, containing 10ml of acetic acid, 1ml of 0.2% copper sulfate in water and 0.5ml of concentrated hydrochloric acid, and heated in a boiling water bath for 5 to 10 minutes. Formation of a bluish green color indicates the presence of terpenoids.

C. Extracts preparation

The aerial part of *A. radiata* was dried in shade, in a dry and ventilated place at room temperature in the laboratory. Then they were grounded to a fine powder of homogeneous particle size of 0.5mm diameter. The obtained powder was stored in glass vials, hermetically preserved, protected from light and moisture.

A quantity of 1900 g of *A. radiata* powder was macerated in petroleum ether for 24 hours, then the extract was recovered by filtration and the residue was macerated 3 times (48 h x 3) in a hydro-ethanolic mixture. After concentrating the combined extracts, consecutive liquid-liquid extractions were performed on the aqueous phase with chloroform (CHCl_3) and then with ethyl acetate (AcOEt) and finally with butanol (BuOH) [21]. Obtained extracts were tested on the *R. padi* aphid with three doses D1= 5mg/ml, D2= 2.5mg/ml and D3= 1.25mg/ml.

D. Determination of total phenolic compounds

Total phenolic content was determined by using Folin-ciocalteu reagent with a spectrometric method [22]. A volume of 100 μl of prepared extracts with concentrations of 0.1mg/ml for each and same for standard gallic acid with concentrations of (100-50-12-5-8-6) $\mu\text{g/ml}$, were transferred into a test tube and mixed with 1ml of Folin-ciocalteu reagent (previously diluted 10-fold with deionized water). Mixture could stay at room temperature for 5min. A quantity of 0.8ml of 7.5% (w/v) Sodium Carbonate solution was added to mixture that was gently stirred. After staying at room

temperature for 30min, the absorbance was measured at 765nm using Unicam helios Gamma spectrophotometric UV-Visible. Gallic acid was used as a standard for the calibration curve. Total phenolic content was expressed as gallic acid equivalents dry matter (μg per mg).

E. Breeding of *Rhopalosiphum padi*

The biological material was chosen by the team of plant protection laboratory from the National Institute of Agronomic Research of Algeria (INRAA) of Touggourt. The cereal aphid *R. padi* Linnaeus, 1758 (Homoptera: Aphididae) was selected between two other aphid species, *Sitobion avenae* and *Schizaphis graminum*, because of the considerable damage caused by this aphid to cereal growing in the southeastern regions of Algeria.

Aphid of cereals was collected during March to May 2017 and breeding in the plant protection laboratory at the experimental station of INRAA. The obtained samples were maintained under natural laboratory conditions, at an average temperature of approximately 27° C in petri dishes. A strainer of 0.5mm of diameter was used to separate aphids (larvae and adults) from plants.

F. In vitro larvicidal bio-assay

Ten (10) individuals of second stage (L2) of *R. padi* were recovered with a brush and placed separately in Petri dishes (10 repetitions) that their covers were perforated to allow insects breathing and prevent moisture condensation. Fresh cereal leaves were considered as nutrient medium. The extract of *A. radiata*, previously prepared, was applied by spraying individuals of *R. padi* with a concentration of 5mg/ml.

Mortality rates of *R. padi* populations were calculated after 24h, 48h and 72h of treatment application. The lethal concentration ($LC_{50\%}$) was obtained by transforming doses into decimal logarithms. Moreover, percentages of corrected mortality were transformed into probits by $y = a + bx$ by determine the dose corresponding a probit of 50% of the mortality. Control and treated observed mortality were calculated by using the following formula of [23]:

$$\text{Mortality rate (\%)} = \frac{\text{Number of dead individuals}}{\text{total number of individuals}} \times 100$$

The observed mortalities values were corrected by using the formula of [24] considering natural mortality observed in the control groups.

$$M_c = \frac{(M_2 - M_1)}{(1 - M_1)}$$

Where:

M1: percentage of mortality in the control group;

M2: percentage of mortality in the treated group;

Mc: corrected mortality percentage.

I. Statistical analyses

Results are evaluated statistically using R software (v.3.5.2). Analysis is started with normality test (Schapiro's test) then completed with ANOVA at level of $p = 0.05$. Furthermore, a Post-hoc test (Turkey's test) is conducted with an Agricola 1.3.1 package.

II. RESULTS AND DISCUSSION

Preliminary phytochemical screenings of methanol, chloroform, petroleum ether and aqueous extracts from the aerial parts of *A. radiata* were carried out to study main bioactive components that could constitute the main factor behind the plant bio-insecticidal activity. These results were announced in the table below.

The obtained results showed the presence of flavonoids, tannins, coumarins, phenols and terpenoids in the methanolic and aqueous extracts of *A. radiata* (Table 1). It is also noted that the petroleum ether extract does not contain any of these families of compounds. These results were similar to those obtained by [25], [15] who noted that *A. radiata*, collected from the region of Bechar in the southeastern Algeria, has an important presence of alkaloids, saponins, terpenes, tannins, steroids and cardenolids. Based on results of total phenol assays, we note that butanolic extract contains a significant amount of total phenols in the order of $348,935 \pm 7.456 \mu\text{g EAG/mg MS}$, while chloroformic extract presents a lower value of $56,754 \pm 0.982 \mu\text{g EAG/mg MS}$.

TABLE (1): QUALITATIVE PHYTOCHEMICAL SCREENING RESULTS OF *ANVILLEA RADIATA* EXTRACTS

	Methanol	Water	Chloroform	Petroleum ether
Alkaloids	-	-	-	-
Flavonoids	+	+	+	-
Tannins	+	+	-	-
Coumarins	+	+	-	-
Sterols	-	-	+	-
Phenols	+	+	-	-
Terpenoids	+	+	-	-

+: Presence; -: Absence.

Several studies confirmed the biological activity of plant's compounds. For this, [26] noted that polyphenols cause disturbance of insect's natural motor skills and their toxicity is positively correlated with the attractiveness of these compounds. Not only insects, but also phytopathogenic fungi, the activity of polyphenols against these micro-organisms allow plants creating barriers and do better resistance against them. Concerning coumarins, [27] as well [28] announced that these compounds have many biochemical and pharmacological activities that depend on structure and nature of the substituent. Also, it should be noted that terpenoids have an anti-inflammatory, antimalarial, antiviral and antibacterial properties [29]. Flavonoids are biosynthesized by many plants to control bacterial infections through interaction with the cell wall of bacteria and proteins [30]. The results of the bio-insecticidal activity of the crude extracts of *A. radiata* against the 2nd larval stage of the cereal aphid *R. padi* are presented in Fig. 1.

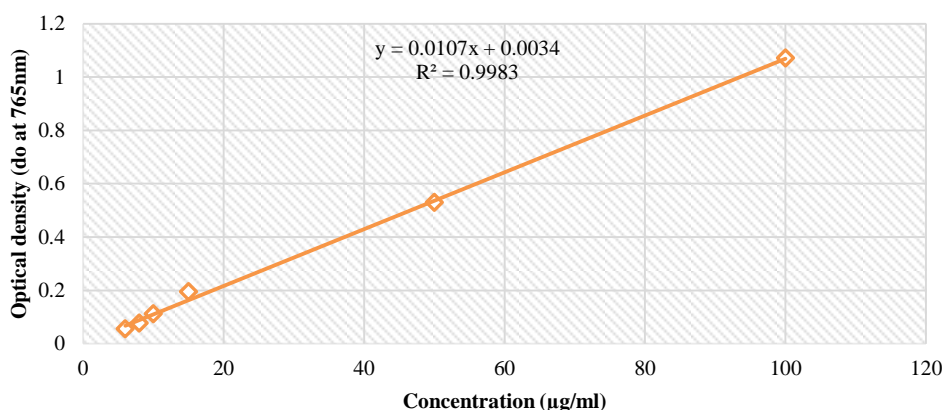


Fig. 1: CALIBRATION LINE FOR TOTAL PHENOLS PERFORMED BY GALLIC ACID

The bio-insecticidal activity of *A. radiata* crude extracts against *R. padi* larva showed that the highest mortality rates were obtained by the butanolic crud extract with $9.4 \pm 1.49\%$, $50.5 \pm 2.59\%$ and $62.7 \pm 3.09\%$ were respectively obtained after 24h, 48h and 72h of treatment application (Fig. 2). Unlike the chloroformic crud extract, lower mortality rates were recorded with values of $23.1 \pm 1.77\%$, $24 \pm 1.59\%$ and $26 \pm 2.26\%$ recorded respectively with the same periods. On the other hand, the mortality in control dishes was taken as a correction control. Statistical analyses confirmed the result of these compounds with a significant effect ($p=0.0107$) which proved that the mortalities caused by both of extracts have no relationship with time. This explains why these extracts have a striking effect on individuals. Also, it should be noted that at the end of the experimentation, the survived individuals showed different abnormalities and handicaps under the effect of *A. radiata* extracts. According to the results obtained in the present study, the significant effect of the butanicol extract of *A. radiata* was richer with chemical compounds comparing to the chloroformic one. Our results were confirmed by several studies on the compounds of this botanical species noting that its bio-insecticidal activity may be due to its high content of polyphenols, flavonoids, phenolic acids and germacranolides [31], [32], [4].

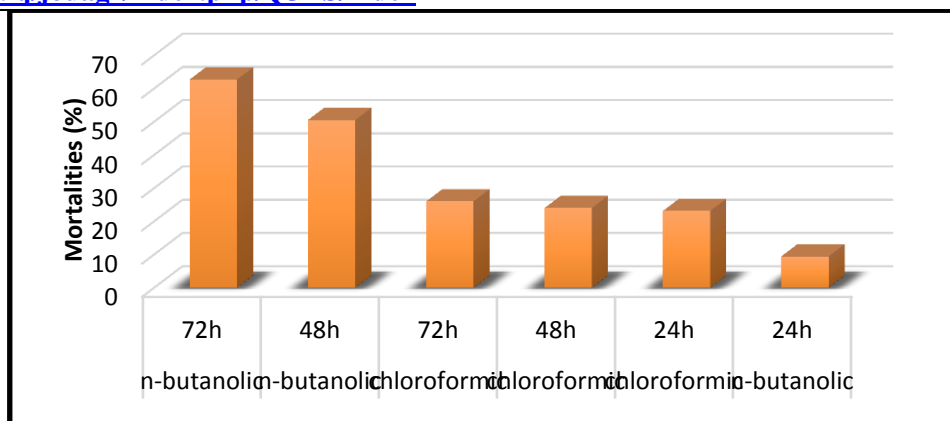


Fig. 2: EVOLUTION OF CORRECTED MORTALITY RATE OF *RHOPALOSIPHUM PADI* DEPENDING ON TIME VARIATIONS (24, 48 AND 72H) FOR THE CRUDE EXTRACTS OF BUTANOL AND CHLOROFORM

On the other hand, after 24h the chloroformic extract showed mortality rates of $15.5 \pm 2.14\%$, $5.5 \pm 1.14\%$ and $1.3 \pm 0.41\%$ with the three examined doses (D1= 5mg/ml, D2= 2.5mg/ml and D3= 1.25mg/ml). While after 48h, the mortality rates increased only in the first dose with $40 \pm 2.82\%$ until the end of the experimentation, after 72h, with $45.7 \pm 3.06\%$ (Fig. 3).

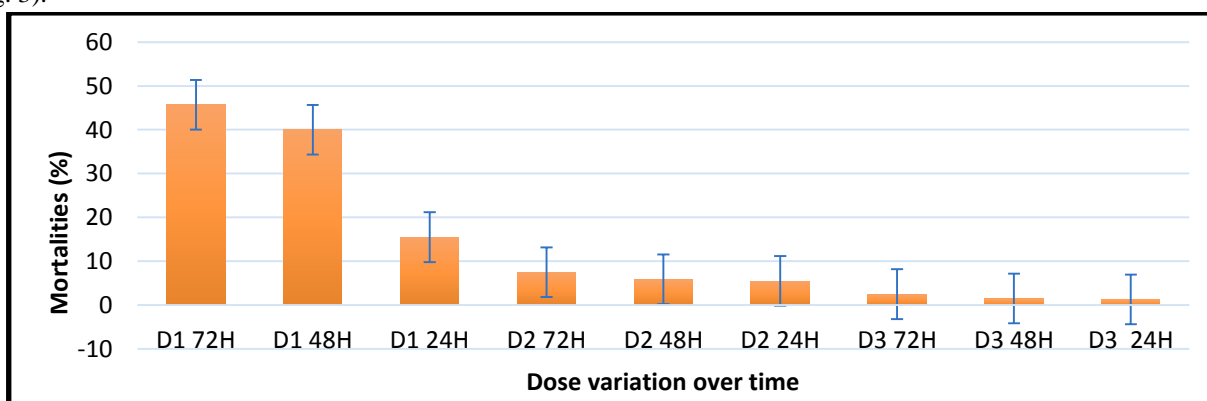


Fig. 3: EVOLUTION OF CORRECTED MORTALITY RATE OF *RHOPALOSIPHUM PADI* DEPENDING ON DOSES D1, D2 AND D3 FOR 24, 48 AND 72H

Concerning the lethal dose (LC_{50}) examined with butanolic extract of *A. radiata*, the regression equation was performed to calculate this dose for each time. This test showing the mortality of 50% of aphid's individuals recorded values of 23.07 mg/ml, 6.71 mg/ml and 5.07mg/ml after 24h, 48h and 72h, respectively (Table 2). Examination of these data revealed that the obtained values of LC_{50} decrease over time that explains the real and high efficiency of crude butanolic extract. This is certainly due to the chemical composition of the used botanical species as well as to the used solvent in the extraction which were able to extract various substances from different phytochemical classes.

TABLE (2) : VALUES OF DL_{50} APPLIED ON *R. PADI* ACCORDING TO THE TIME

Time (h)	LD_{50} values (mg/ml)
24	23.07
48	6.71
72	5.7

In fact, this *Asteracea* biocidal effect is presented by several authors around the world. Noting, [33] who argued the antibiotics and antioxidant effect of the aqueous extract of this plant by reducing about 15% of the spread of multi-resistant bacteria such as *Staphylococcus aureus* Gram (+) and *Escherichia coli*. In addition, [34] also demonstrated that *A. radiata* presents antioxidant and antibacterial activity due to same compounds recorded in our experiment. Same author reported that the highest quantities of those molecules do exist on flowers and vary according to the used solvent. According to the study conducted by [35] on the same species of aphid, they noted that the application of linalool and cinnamaldehyde on *R. padi* was very toxic. Not only aphids, but also, this plant is characterized by another compounds

against micro-organism where [36] demonstrated the antifungal activity of *A. radiata* against several fungi such as *Aspergillus* spp., *Penicillium expansum*, *Fusarium oxysporum* and *Alternaria* sp. Furthermore, [15] recorded a significant effect of the extract of this plant on the phytopathogenic agent of date palm *Fusarium oxysporum* f. sp. *albedinis*.

III. CONCLUSION

The bio-insecticidal effect of an endemic plant from the southeastern Algeria against the cereal aphid *R. padi* was conducted in the present study. The screening of this botanical species allowed identifying its chemical compounds that are flavonoids, tannins, coumarins, phenols and terpenoids. The toxicity of this plant extract was higher with the butanicol extract that was richer than the chloroformic one. It should be recommended more deep analysis and experimentations in the field to multiply this botanical species and synthesize identical active substances that are exist in it. The present study showed significant data on this kind of biological treatment against the cereal aphid. According to the obtained results, *A. radiata* revealed the presence of substances that could constitute a good method to control *R. padi* and might be introduced as an eco-friendly way to control this in sustainable organic agriculture.

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