Al-Qadisiyah Journal For Agriculture Sciences (QJAS)
ISSN : 2618-1479 Vol.13, Issue. 1 ,(2023), pp. 113-122
https://jouagr.qu.edu.iq/

Ovicidal Activity and Phytochemical Analysis of some Spontaneous Plants Extracts Against *Ectomyelois ceratoniae* (Zeller) Lepidoptera: Pyralidae

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Abstract. The use of aqueous extracts as alternative solutions that significantly reduce to the damage caused by pests like *Ectomyelois ceratoniae*. This work presents in-vitro tests that are successfully applied to evaluate the ovicidal activity of four spontaneous plants aqueous extracts, as, *Rosmarinus officinalis*, *Peganum harmala*, *Thymus vulgaris* and *Artemisia herba-alba* againt the moth eggs. The results showed a highly significant effect for *T. vulgaris* and *A. herba-alba*, sometimes reaching 100%, followed by *R. officinalis*, then *P. harmala* which was not present any effect on this pest eggs. *T. vulgaris* and *A. herba-alba* showed a strong inhibition of egg hatching. It exceeds 93 % mortality for a concentration of 0.12 mL/mL. For *R. officinalis* at 0.18 mL/mL concentration present a 72% mortality. While at 0.12 mL/mL, the mortality was 40%. The lethal doses to inhibit 50 and 90 % of the eggs were estimated to confirm the efficiency of the aqueous extracts , stronger results shows, respectively (0.014 mL and 0.062 mL) from *T. vulgaris* and (0.019 mL and 0.069 mL) from *A. herba-alba*. This work originally explores the benefits of some kinds of plants that are largely available and inexpensive for the biotreatment of carob moth eggs.

Keywords. Aqueous extracts, Spontaneous plants, Ovicidal, *Ectomyelois ceratoniae*, Lethal dose.

1. Introduction

The date palm (*Phoenix dactylifera*) is the providential tree of hot Saharan regions where it is grown. It provides a wide range of products; most importantly the date fruit, as a food of high-energy value. For millions of people living in drylands, dates are anessential resource for their food security [1,2].

The cultivation of the date palm like all plants is confronted with several phytosanitary problems, such as *Fusarium oxysporumf.sp albedinis* diseases and pests such as the yellow mite *Oligonychus afrasiaticus*, the white scale *Parlatoria blanchardi* and the carob moth *Ectomyelois ceratoniae*. This last is currently considered as the most formidable predator of dates and as the main constraint to exploitation [3], it causes considerable damage to the crop both which decrease the yield product and the quality of the fruit, and also affects the fruits storage [4]. To overcome this issue, various control methods intensely developed to reduce the rate of infestations of this pest such as chemical treatment [5]. Unfortunately, the anarchic and abusive use of pesticides by farmers causes harmful effects on the environment and human health. In addition to these disadvantages, the chemical treatment is not sufficiently efficient with a time-consuming process [6,7,8].

There fore, developing an alternative method to treat the insect pest is of crucial importance because these methods are natural and meeting the anti-toxicological requirements, and falling within the context of protecting the environment. Among these methods, the use of natural pesticides that can treat and protect palm fruit without harmful effect and can adequately control the pest [9,10,11].

Herein, a special attention was paid to the secondary metabolites of plants due to their large disponibility and their effectiveness to control insects [12]. Accordingly, several plant species have been successfully used because of their excellent insecticidal properties. These natural products are increasingly sought after for sustainable agriculture [13].

For instance, the use of vegetal extracts of *Acorus gramineus rhizomes* can efficiently protect lepidopteran larvae [14]. On the hand, the treatment of insect's pest eggs by bioinhibitors has been intensely applied due to their high ovicidal activity [15,16,17,18].

The research team of [19] have applied another technique efficiently applied to control the ovicidal activity of the essential oil from *Cheilocostus speciosus* rhizome against *Helicover paarmigera* eggs.

While, [20] have prepared an ethanol extracts from 17 plants collected from the Pantanal wetland in the state of Mato Grosso do Sul, Brazil, to control the ovicidal activity of *Haemonchus placei*. Their method reaches to the yield of hatchability inhibition rates of 19.85%, 10.35% and 15.9% for the highest concentration tested.

Similarly, [21], have evaluated the efficacy of using foliage extracts of goat weed, *Ageratum conyzoides* on egg hatchability of diamond back moth, *Plutellaxy lostella*. Recently, the use of *Catharanthus roseus* extracts against *Earias vittella* is recently rested with a good ovicidal activity (70.47%) [22].

And the same for *Tuta absoluta* treated by using methanolic extracts of liana of *Caesalpinia welwitschiana* and leaves of *Caesalpinia.bonduc* with an efficieny of the most active (49.9% and 53% at 100 ng/ml, respectively). [23].

More recently, the ovicidal effect of extracts of *Calotropis procera* to the Pest *Cadra cautella.* The extracts displayed a good activity with 50% of *C. cautella* eggs not hatching at 10.000 ppm (1%) [24].

Recently, [25] have estimated the biological activity of plant extracts on the small tomato borer *Neoleucinodes elegantalis*. The rope tobacco extract was then selected to assess its effectiveness not only on eggs and the prepupal stage.

However, the use of natural biopesticides for the control of pest is limited and the inhibition eggs of carob moth have been not sufficiently studied even the considerable damage caused by this type of insect. This study aims to propose natural extract by proving the efficiency of using natural products extracted from four spontaneous plants *Rosmarinus officinalis*, *Peganum harmala*, *Thymus vulgaris* and *Artemisia herba-alba* with the analysis and evaluation of the ovicidal effect of their extracts.

In addition, the evaluation of the inhibition eggs of the carob moth under natural conditions is also proposed. The study shows that the method applied here is simple, fast-prepared aqueous extracts and natural product using disponible spontaneous plants, which achieve a good efficiency for the inhibition of *Ectomyelois ceratoniae* eggs.

2. Materials and Methods

The insect *(Ectomyelois ceratoniae)* used in this work was obtained from date palm fruit. The plants of *Rosmarinus officinalis, Peganum harmala, Thymus vulgaris* and *Artemisia herba-alba* were collected from different regions of southern Algeria. Before extraction, the aerial part of different species has been collected and washed with distilled water, then dried at room temperature in the shade and in the open air. Then, the plants were grinded using a Restsch-SM100 electric grinder still obtaining a powder. In order to avoid the effect of storage conditions on the activity of *Ectomyelois ceratoniae*, the infested date was directly obtained from the date palm.

2.1. Rearing of Carob Moth

The rearing of the moth was carried out under controlled conditions, a temperature of (27 ° C ± 2) and a humidity of (65% ± 10) and photoperiod of 16 light hours and 8 hours of darkness [26]. Also, in order to promote a favorable environment for the emergence of adults. Next, dates infested with the carob moth are placed in a 3D emergence cage (70cm× 60cm× 70cm).

After emergence of adults, was captured with a test tube and then placed in jars of (30cm H × 18cm W × 12cm W) to ensure the reconciliation of the mating process. After 24 hours, the eggs are obtained which are collected and then placed in petri dishes. Finally, only the fertile eggs (20 eggs each time) were used for experimental tests.

2.2. Preparation of Aqueous Extracts

The aqueous extract was prepared according to the method proposed by [27]. A 100 g of powder from each plant was mixed in a 1L of distilled water at boil temperature, then left to cool. After stirring for 30 minutes, the resulting mixture is filtered using whatman paper (3 mm).

2.3. The Treatment of Eggs and Mortality Rate Calculation

The eggs were treated by spraying the extracts with a small fine drop sprayer. Each petri dishes were daily observed with a binocular magnifying glass and cold light to determine the hatching rate and to assess the effect of treatment. To confirm the reliability of test, five similar samples were simultaneously tested under closed system. Finally, all data were recorded periodically after 7 days. The percentage of mortality was calculated using the following formula:

$$\text{Mortality \%} = (\text{Number of unhatched eggs} / \text{Total number of eggs}) \times 100$$

2.4. Statistical Analysis of Data

The statistical analysis was carried out with the software package by ANOVA variance analysis for the comparison between the extracts and their concentrations.

2.5. The Lethal Dose (LD)

The LD50 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 (mg). The formula of Schneider and the table of probits are used. Schneider formula:

$$MC = [(M2-M1)/(100-M1)] \times 100$$

MC: rate of corrected mortality; M2: rate of mortality in the treated population; M1: rate of mortality in the control population [28].

2.6. Phytochemical Test

The qualitative phytochemical analyses of aqueous extracts was based on the colorations and/or precipitation method by [29,30,31].

The experiments aim to determine and to specify only the bioactive molecules for inhibiting eggs with high yield. Therefore, a comparative study was done between the extracted components with high effect on carob moth eggs and the extracted components that show no significant effect on *Ectomyelois ceratonia* eggs.

DOI: 10.33794/qjas.2022.135860.1083

3. Results and Discussion

3.1. Effect of Different Aqueous Extracts on the Carob Moth Eggs

The Table 1 showed the mortality rate of the eggs treated by different aqueous extracts. Exposure to this aqueous extracts in doses ranging from 0.024 to 0.18 mL/mL caused the inhibition of the treated eggs; the mortality rate increased by the increase of the applied dose. However, the extract of *P. harmala* in different concentrations did not have a significant ($p > 0.001$) effect on the eggs of the moth (table1). It is followed respectively by the *R. officinalis* plant with a significant effect ($p < 0.001$). On the other hand, the *T.vulgaris* and *A. herba-alba* plants had a very high and significant effect reaching 100%, was evident even at the lowest concentrations, with mortality rates above 80% and 66% respectively. The use of two different concentrations of the *T.vulgaris* mortality (0.06 mL/mL and 0.024 mL/mL) provides a good mortality rate than using *A. herba-alba*. Interestingly, the mortality rate obtained with using only a 0.024 mL/mL of *T. vulgaris* is similar to the use of 0.06 mL/mL of *A. herba-alba*.

The results show that, among the four plants tested: *T.vulgaris*, *A.herba-alba* and *R. officinalis* have a very effective effect on the mortality of carob moth eggs. These results show a difference in ovicidal efficacy

So, we can also say that the *T.vulgaris* and *A.herba-alba* have almost identical inhibition.

This mortality is probably due to the inhibitors (compounds) that contains these plants. Thus, we notice that the mortality rate of the C1 concentration of the *T. vulgaris* plant has a stronger effect than the C4 concentration of the *R. officinalis* species. Has the same mortality rate for the 0.06 mL/mL concentration of 77 % *A. herba-alba*.

Table 1. Mortality rate (%) of eggs treated with aqueous extracts.

Plants	Concentration	Mortality %	F-value	P-value
Peganum harmala	C_0	10±5	0, 319	0,862
	C_1	9±5,48		
	C2	9±4,18		
	C3	8±4,47		
	C4	8±2,74		
Rosmarinus officinalis	C0	10±5	7,256	0,001
	C1	42,33±6,81 55,0±8,66		
	C2	60,0±5,0		
	C3	95,0±5,0		
	C4			
Thymus vulgaris	C0	10±5	172,405	0,000
	C1	80±9,13		
	C2	86±2,50		
	C3	96,25±2,50		
	C4	100±0		
Artemisia herba-alba	C0	10±5	126,580	0,000
	C1	66,3±7,5		
	C2	77,0±5,7		
	C3	95,0±7,07		
	C4	100±0		

(C0=Control; C1= [0.024 mL/mL]; C2=[0.06 mL/mL]; C3=[0.12 mL/mL]; C4=[0.18 mL/mL]).

3.2. Estimation of the Lethal Doses of Aqueous Extracts

The Figure 1 represents the linear regression curves of the effects of the applied aqueous extracts doses on the mortality of the *Ectomyelois ceratoniae* eggs, allowed the estimation of the lethal doses

50 and 90 (LD50 and LD90) of this extracts. Showed low lethal dose values, which is explained by the high inhibition of this extracts againt *Ectomyelois ceratoniae* eggs. *T.vulgaris* and *A.herba-alba* showed very low lethal dose values, which is explained by the very high inhibition of this extracts with the carob moth eggs, followed by *R. officinalis* showed low lethal dose values, which is explained by the high inhibition of this extracts againt carob moth eggs. However, the *P. harmala* showed high lethal dose values, which is explained by the contact low toxicity of this extracts with the carob moth eggs. The lethal doses 50 (LD50) were reported for the different extracts of, *P. harmala, R. officinalis, T. vulgaris* and *A. herba-alba* were respectively estimated as (138.079, 0.119, 0.014, 0.019). Similarly, the estimated lethal doses 90 (LD90) were found (5932.646, 1.204, 0.062, 0.069, respectively. The values of lethal doses of the inhibition process are reported in (Table 2).

Table 2. Estimated lethal doses for different extract.

Lethal Exposure time doses (LD) [mL/mL]				
Extracts	**Regression equation**	**Regression coefficients**	**LD50**	**LD90**
P. harmala	y = 0,233x + 3,320	R^2 = 0,785	138,079	5932,646
R. officinalis	y = 1,275x + 6,179	R^2 = 0,730	0,119	1,204
T. vulgaris	y = 1,998x + 8,701	R^2 = 0,805	0,014	0,062
A. herba-alba	y = 2,316x + 8,972	R^2 = 0,840	0,019	0,069

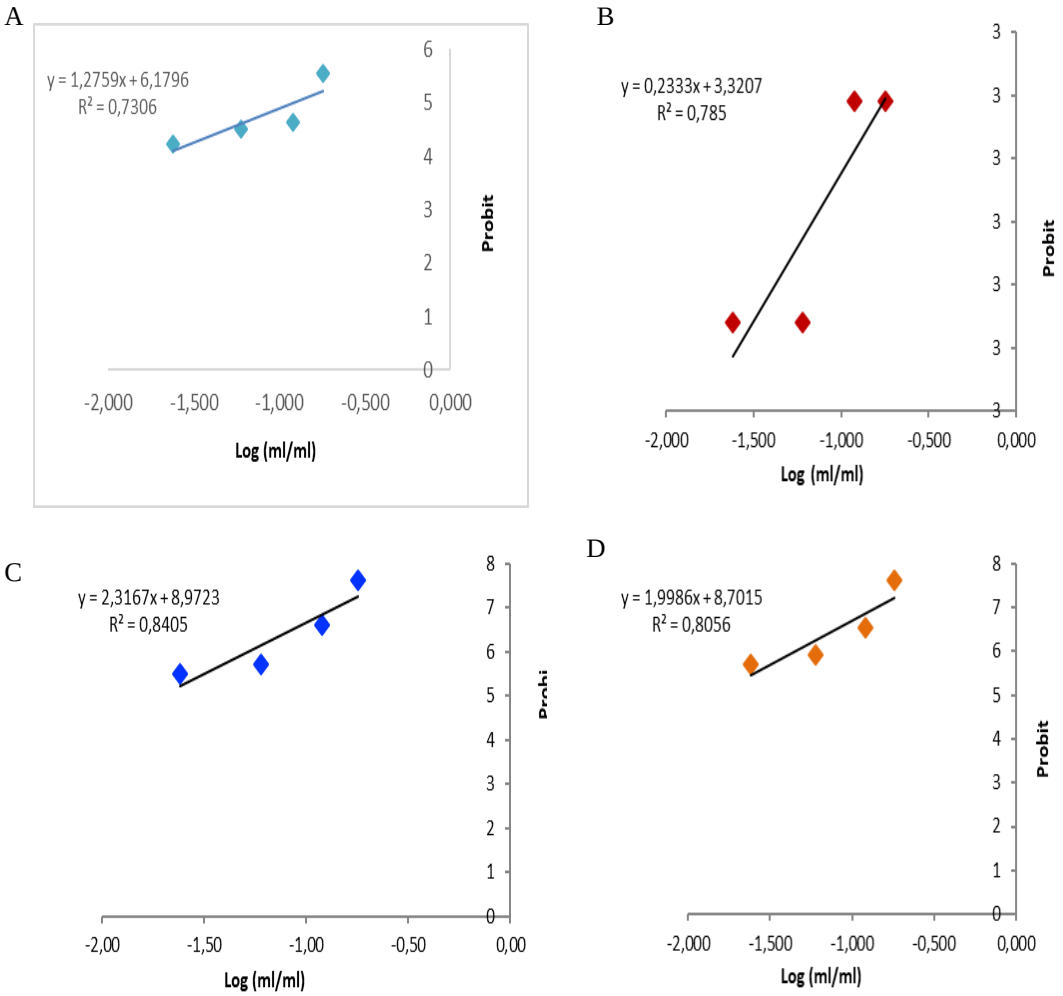

Figure 1. Effect of the extract's concentration on the mortality of *ectomyelois ceratoniae* eggs. A) *R.officinalis* aqueous, B) *P.harmala, C) A.herba-alba, D) T.vulgaris.*

3.3. Phytochemical Screening of the Aqueous Extracts

As shown in the table 3, the results of the phytochemical screening revealed that the extracts studied contain several chemical components including, alkaloids, flavonoids, tannins, quinones, terpenoids, steroids, phenols, coumarin and saponins. Therefore, the, except *P. harmala R. officinalis*, the extract of theses specie, exhibits an ovicidal effect. While the aqueous extracts of *R. officinalis, T. vulgaris* and *A. herba-alba* contain some tannins, phenols and coumarin which were not found in the *P. harmala* extract.

It was also demonstrated that the *T.vulgaris* and *A. herba-alba* contain high level of tannins (that reaches 100 % of mortality), and a low presence in *R. officinalis* displaying strong inhibition effect. Hence, the extracted compounds can be used as promising bioproducts for inhibiting eggs.

Table 3. Phytochemical screening of the aqueous extracts studied.

Phytochemical Components	*Thymus vulgaris*	*Artemisia herba-alba*	*Rosmarinus officinalis*	*Peganum harmala*
Alkaloids	++	++	+++	+++
Flavonoids	+	++	++	++
Tannins	++	++	+	-
Quinones	++	++	-	+
Terpenoids	+++	+++	+++	+++
Steroids	+++	+++	+++	+++
Phenols	++	++	+++	-
Coumarin	+++	++	+	-
Volatile oils	++	++	+++	++
Saponins	-	-	-	-

Key: +++ = Strongly present; ++= Moderately present; += weakly present; - = Absent.

4. Discussion

Different extracts where successfully used for the treatment of carob moth eggs. As presented by [32], the *Zanthoxylum armatum* extract can inhibit the *Spodoptera litura* by soxhlet extraction method at room temperature with a good hatchability of 74 %. [33] have extracted an essential oil using clevenger-type apparatus under 29°C temperature and a 60±05 % of relative humidity. The resulted oil was considered as good solvent for the treatment of the ovicidal activity (100 %) against *Callosobruchus maculates*.

Another extraction method recently applied to extract five plant leaf (*Argemone mexicana* , *Clerodendrum viscosum, Clerodendrum inerme* , *Argemone mexicana* , *Vitex negundo*). The ethanol extracts were used to evaluate the ovicidal activity against *Spodoptera frugiperda and Spodoptera litura* using dipping method. The extract of *Argemone mexicana* showed the strongest ovicidal activity against *Spodoptera frugiperda* eggs with LC_{50} value of 11.72 mg/ml. In the case of *Spodoptera litura*, the plant extract of *Clerodendrum inerme* (LC_{50} =20.46 mg/ml) and *Argemone mexicana* (LC_{50} =21.82 mg/ml) revealed the highest ovicidal activity [34]. The bioactivity of *Melia azedarach* extracts was tested to treat the Tomato Leaf miner, *Tuta absoluta* eggs, which displays a medium yield mortality that vary between 39 to 41% [35]. Recently, an egg mortality rates of 71 to 100 % at 48th hour for 50 µl L^{-1} air doses of an essential oil of *Rosmarinus officinalis* L. in the Control of *Callosobruchus maculatus* [36].

One of the latest methods applied to evaluate the ovicidal activity against *Helicoverpa armigera* was based on the use of methanol extracts todifferent leaf L. And *Chromolaena odorata* extracts of (L) *Leonotis nepetifolia*, showing a(highest ovicidal activity (61.33±0.57% and 63.45±0.77%]37[. In ،the other workthe o vicidale ffects ofe ssentialo*Xanthogaleruca* ils Extracted from T. vulgaris on the *luteola* showed the highest ovicidal toxicity with the lowest LC50 of 122.8 ppm.]38[

The phytochemical screening showing thatof plant extracts (*Artemisia herba-alba*, *Rosmarinus officinalis*, *Thymus vulgaris*) containsalkaloids, coumarins, quinones and flavonoids : , were] confirmed by other studies39,40,41[.]42extracts contains: alkaloids, *Peganum harmala* showed that [coumarins, and flavonoids free.

In our present work, we have obtained the best mortality yield of 100% of inhibition against *Ectomyelois ceratoniae* eggs by using aqueous extract of two plants including, *Thymus vulgaris* and *Artemisia herba-alba.* Interestingly, the experimental method was simple fonctionnalisable at room temperature and uses only a concentration of 0.18 mL/mL of the extracts to reach the above-mentioned mortality yield. In addition, the operational relative humidity was set to equal 65 % and without any additional chemicals.

Conclusion

This paper demonstrates the high ovicidal properties of *Thymus vulgaris* and *Artemisia herba-alba* as plants easily harvested. The plants show very high rate of mortality and specific inhibition of hatching *Ectomyelois ceratoniae* eggs. In addition, the study also proves the efficiency of the aqueous extracts from *Rosmarinus officinalis* while the use of *Peganum harmala* has no significant effect on the eggs of dates moth. As results, the mortality rate of the obtained extracts is demonstrated to be a concentration-dependent parameter. Interestingly, the use of *Thymus vulgaris* and *Artemisia herba-alba* extracts reaches a 100 % of mortality at only a C4 of mass concentration. These extracts containing tannins, phenols and coumarin according to the phytochemical test, which explain the high efficiency of the plants in the protection of the date palm. The product obtained could be considered as good inhibitors of hatching eggs and, therefore, can be used for protecting date palm and other related vegetal plants.

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