Evaluation of the Efficiency of some Methods in Biological Control of *Phlebotomus Papatasi* (Diptera:Psychoididae).

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Abstract

The research studies the biological efficiency of the fungus *Verticillium lacanii* and the alcoholic extract leaves *Nerium sp Capparis sp. and Lawsonia sp.*against fourth larval instar and adults of male and female *Phlebotomus papatasi*. The study was conducted at the laboratory condition at a temperature of 25±2℃, relative humidity. 65 ± 5% and a period of illumination of 12 hours/day. The suspension of *Verticillium lacanii* at a concentration of 1×10^8 spores/ml gave the highest mortality 86.74% for the fourth larval instar after 24 hours of treatment, while the suspension of *Verticillium lacanii* at concentration of 1×10^8 spores/ml gave the highest mortality of 78.16% for the male *Phlebotomus papatasi* after after 24 hours of treatment, the suspension of *Verticillium lacanii* at a concentration of1×10^8 spores/ml gave the highest mortality rate of 46.75% for female after 24 treatment. Alcoholic extract of *Capparis* gave highest mortality 89.57% for the fourth larval instar, 76.18% for males, and 87.71% for the female Phlebotomus papatasi after 24 hours at concentration 0.07 mg/l. The alcoholic extract of *Lawsonia sp.* showed the lowest mortality was 43.01% for the fourth larval instar, 29.21% for males, 31.86% for female sand fly *Phlebotomus papatasi* after 24 hours of treatment at concentration 0.01 mg/l respectively.

1. Introduction:

Among the living organisms that live parasitic as well as absorbing human and animal blood is a sand fly *Phlebotomus papatasi* [1]. The sand fly *Phlebotomus papatasi* is characterized by its small size (1.5- 5)mm in length, its general color is yellow or brown, and its body is covered with dense bristles [2]. A vector of the parasite that causes the Baghdad grain, Leishmania tropica. It is worth noting that this type is one of the most important species spread in Iraq [3].

The *Phlebotomus papatasi* is one of the medically important insects for humans, based on the type of the final host for feeding this insect and the specificity of the vector type [4]. As it feeds on blood and thus attacks humans and sucks their blood, causing allergic reactions, the sand fly *Phlebotomus papatasi* transmits a number of diseases, including cutaneous and visceral leishmaniasis, belonging to the order Diptera of the Psychodidae family [5]. The *Phlebotomus papatasi* was recorded in Iraq by [6]. An animal must be available for the purpose of infection, leishmaniasis in Iraq is transmitted by the type *Phlebotomus papatasi* [7]. It is considered of cutaneous leishmaniasis, and the optimal period for transferring the parasite in Iraq is between mid-August to mid-October 1972 [8].

The *Phlebotomus papatasi* is characterized by its small size (1.5-5)mm in length and its general color is yellow or brown, which is a fully metamorphic or holometabola insect, meaning that its life cycle passes through four stages, starting with the egg, the larva, the pupa, and finally the adult[9]. The larvae can be identified by the presence of thick bristles all rings of the body up to the head [10]. Fungicides and pesticides of plant origin are important as they are natural materials that decompose quickly in nature and do not pollute the environment [11]. The fungu Verticillium s lacanii is a fungi accompanying insects and helps to mortality insects through its spores that grow on the insect's wall, causing the decomposition of fatty, protein and chitin in the insect's body

wall through the secretion of special enzymes [12].

The study demonstrated the possibility of using plant extracts against living organisms [13]. The aim of the research is to study the effect of fungus Vertcillium lacanii and and Alcoholic extract of the leaves of N *Nerium sp Capparis sp. and Lawsonia sp* on larvae instar and adult male and female of the *Phlebotomus papatasi*.

2. Materials and Methods:

2.1 Insect Collection and Breeding:

Using the Light trap, *Phlebotomus papatasi* were collected from different areas in Saladdein Governorate and transferred to the laboratory after placing them in a cooling box containing a cotton layer saturated with water to supply the necessary moisture.Transparent plastic cans used for domestic use with screw-down lid sizes of 500 ml were used to raise immature stages, in addition to the males and females intended for laying eggs. Several holes with a diameter of 5 ml were made in the base of the box to supply moisture from the bottom.

A mixture of borax gypsum mixed with water was poured into the base of the perforated canister is -23cm thick and left in the laboratory without a cover to dry, after that the inner part of the can cover was removed and kept in the form of a ring only closed after covering the mouth of the can with a tulle cloth with precise holes for ventilation in addition to making two holes, one of them on the wall of the package to enter the female feeding by the aspirator and closed with a cotton swab and the other in the nozzle covered with tulle, a cotton saturated with 30% sucrose solution was placed in it to feed the adults. The larval food was prepared by mixing equal weights of animal ration consisting of a mixture (wheat, yellow corn, soybeans and edible oil) and dry rabbit droppings.

It was ground by an Electrical Grinder, and then 5% of the beef liver powder was added to it and left for two weeks, as the beef liver powder helped accelerate the growth of the larvae. The final product was divided equally into small sterile tubes that were kept in the refrigerator to prevent the growth of fungi until use. The adult cage was prepared in the form of a square wooden frame with dimensions of 30×30×50cm, its floor is of hard wood, the wooden frame was completely covered with soft tulle fabric and one of its sides was covered With a white cloth for inserting and withdrawing specimens and the laboratory animal that represents the source of the blood meal through it, it was also re-wrapped with a layer of soft wire clip to ensure that the wholes would not escape if the tulle was damaged [13].

2.2 Preperaing Commenter Fugi:

The *Verticillium lacanii* were obtained from the Faculty of Agriculture, Tikrit University. *Verticillium lacanii* was grown on Potato Daxtrose Agar medium in pantry dishes until we prepared the suspension of *Verticillium lacanii* [14]. We used 50ml glass containers, and an area of 0.6cm was taken from growing on the Potat dextrose agar culture medium. Sterilized one week old, the dishes was in 9.4ml of sterile water and shaken for ten minutes to remove spores from their sporozoites. It incubated at 28°C for 7 days, and for distributing the fungal growth, it was stired continuously, papers No.1. Spores of *Verticillium lacanii* diameter was calculated using an erythrocyte counting slide[15]. Then concentrates were prepared,1×10^4, 1×10^6, 1×10^8spore/ml.

2.3 Biological Testing in the Fourth Larval Instar:

Five plastic boxes, each containing 10 fourth instar larvae, and for each concentration of the tested fungus *Verticillium lacanii* concentrations, four containers containing 50ml for each concentration of the fungus suspension, while the fourth contained sterile distilled water only and called the control factor, larvae were then transfered to containers of 100ml. A container of distilled water and larval food of 10mg/ml were placed in the incubator at a of 2± 0°C and a photoperiod of 12 h after which the mortality was calculated after 6 h, 12 h, 18 h, 24 h.

2.4 Biological Testing in Adults:

10 Adult replicate were then individually in a wide-container with a capacity of 1 liter and four replicates for each concentration in addition to the control factor. 30°C and a light period of 12 hours, after that the percentage of loss was after 6 h, 12 h, 18 h and 24 h.

2.5 Preparation of the Alcoholic Extract:

The leaves of Nerium sp were obtained from public gardens, while the leaves of *Capparis sp.* and Lawsonia sp. were obtained from the local markets of Samarra. The plant extracts were prepared based on the extracts of the leaves of the plan N.olender, C.spinosa and L. inermis converting it into a fine powder using an electric grinder and weighing 100g of dry powder for each plant and placing it in the Extraction thamble container, which was placed in the Soxhlet Extrador extraction device until the plants' colors are gone and for a different period of time from 18-26 hours, according to what each plant requires [16]. Then the water was evaporated from the extract under low pressure at a temperature of 40 C by a rotary evaporator device until the plant extract powder was obtained, which was kept in tight glass bottles and placed in the refrigerator until it was used in the study.

2.6 Toxicity Test of Alcoholic Extracts:

The fourth larval instar of the insect in the laboratory was treated with concentrations 0.01, 0.03, 0.04, 0.07 mg/ml, fourth larval stage was controlled by soaking the previously prepared larval food with the concentration under study of alcoholic extract and left in the laboratory for a whole night to dry, then transferred 5 Larvae of the fourth larval age +and

in three replications using a paintbrush moistened with water to avoid damage to the larvae and then transferred to the previously prepared control pots, then add 1-2 g of larval food to each container containing larvae. The larvae were treated with different concentrations of extract by spraying 5 ml of extract by hand sprayer. As for the control treatment, it was treated with *Nerium sp Capparis sp. and Lawsonia sp* distilled water and placed in the incubator at a temperature of 27±1°C and a relative humidity of 75-80%. The results were recorded after 24 hours of treatment by examining the larvae using a dissecting microscope and a flashlight light source to stimulate the larvae to move in the case of being alive. mortality percentage rates for all treatments compared to the control treatment after 6, 12, 18 and 24 hours of treatment.

3. Results and Discussion:

3.1 Fourth Larvae Instar of *Phlebotomus Papatasi* Effected BY Various Concentrations:

Table 1 shows the fourth larval instar of the sand fly *Phlebotomus papatasi* affected by different concentration of the Verticillium lacanii. *Verticillium lacanii* at 1×10^4 spores/ml gave the highest percentage 76.46% for the fourth larval instar after 24 h, while *Verticillium lacanii* gave the lowest death rate of 46.73% after 6 h of treatment, lacanii at 1×10^6 spores/ml had the highest mortality 81.83% for the fourth larval instar after 24 h, while the Verticillium lacanii gave the lowest of 53.52% after 6 h, and the *Verticillium lacanii* at 1×10^8 spores/ml gave the highest percentage The mortality of 86. 74% for the fourth larval after 24 h, while the s fungus *Verticillium lacanii* gave the lowest 59.73% after 6 of treatment.

Table 1. Fourth Stage Instar of *Phlebotomus Papatasi* Effected by Various Concentrations.

Fungi	Phase	Concentration Spores/ml	%				Average mortality
			6 h	12 h	18 h	24 h	
V. lacanii	Fourth	1×10^4	46.73	55.93	64.23	76.64	60.96 C
		1×10^6	53.52	58.37	69.12	81.83	65.71 B
		1×10^8	59.73	64.36	73.14	86.74	70.99 A
		Average mortality of concentration	53.32 c	59.55 c	68.83 b	81.73 a	

*Similar capital letters in one line horizontally mean no significant differences.

3.2 8.2 Male of *Phlebotomus papatasi* effected by various concentrations:

Table 2 shows male *Phlebotomus papatasi* affected by different concentration of the Verticillium lacanii. *Verticillium lacanii* at the 1×10^4 s pores/ml gave 66.17% for the male after 24 h, while V. lacanii gave the lowest percentage of 39.63% after 6 h of treatment, and the suspension gave the *Verticillium lacanii* at of1×10^6 spores/ml had the highest mortality of 71.56% for the male after 24 h of tretment, while *Verticillium lacanii* gave the 43.27% after 6 and *Verticillium lacanii* at1×10^8 spores/ml the highest mortality 78.16% for the male after 24 h while the *Verticillium lacanii* gave the lowest percentage of 54.38% after 6 h of treatment.

Table 2. Male of *Phlebotomus Papatasi* Effected by Various Concentration.

Fungi	Phase	Concentration Spores/ml	%				Average mort1×ality
			6 h	12 h	18 h	24 h	
V. lacanii	Male	1×10^4	39.63	45.32	54.46	66.17	51.44 C
		1×10^6	43.27	52.48	61.25	71.56	57.14 B
		1×10^8	54.38	61.35	71.39	78.16	66.32 A
		Average mortality of concentration	54.76 c	53.05 c	62.36 b	71.9 a	

*Similar capital letters in one line horizontally mean no significant differences.

3.3 8.3 Female of *Phlebotomus papatasi* affected by various concentration:

Table 3 shows the female *Phlebotomus papatasi* affected by different concentration of Verticillium lacanii. *Verticillium lacanii* at the 1×4^4 spores/ml gave the percentage 59.48% for the female after 24 h, while the s *Verticillium lacanii* gave the lowest percentage of 42.13% after 6 hours of treatment, and the s *Verticillium lacanii* at 1×6^{10} spores/ml had the percentage 69.41% for the female after 24 hours, while *Verticillium lacanii* gave the lowest percentage 49.62% after 6 h and *Verticillium lacanii* at 1×8^8Spores/ml the 75.46% for the female after 24 hours, while *Verticillium lacanii* gave the lowest percentag 51.83% after 6 hours of treatment .Mortality percentag increased with the increase in concentration. This study agree with [12]. which showed that the use of different concentrations of fungi Vertecillium lacannii leads to a clear mortality percentage in female and male insects, the results of the tests are shown Vertecillium lecanii fungi. at concentration 2×6^7 spore/ml highest mortality ratio 89.63% for the second larval age. similar study [17] with the current study, in which it was proven that the use of the fungal suspension

Metarhizium anisoplia led to mortality percentage 93% of males and mortality percentage 96% of females, meaning that the effect was more on females than males. *Similar capital

Table 3. Female of *Phlebotomus Papatasi* Effected by Various Concentration.

Fungi	Phase	Concentration Spores/ml	%				Average mortality
			6 h	12 h	18 h	24 h	
V. lacanii	female	1×10^4	42.13	45.72	54.96	59.48	52.8 C
		1×10^6	49.62	52.48	61.25	69. 41	58.19 B
		1×10^8	51.38	61.35	71.89	75.46	65.02 A
		Average mortality of concentration	47.71 c	53.18 c	62.71 b	68.11 a	

letters in one line horizontally mean no significant differences.

3.4 The Plant Extracts Efficacy of the Four Larval Instar after 24 Hours of Treatment:

The results show the effect of alcoholic extracts in mortality percentage fourth larvae instar as shown in Table 4. The ethyl alcoholic of the extracts of *Capparis sp.* caused the highest mortality percentage 89.57% at the higher user concentration of 0.07 mg/ml while the lowest mortality percentage was 62.32% at user concentration 0.01 mg/ml also the results o show that plant leaves extracts of Nerium sp it caused mortality percentage 84.21% at the concentration of 0.07mg/ml and caused mortality percentage 54.83% at 0.01, also the results show that the plant leaves extracts of *Lawsonia sp.* it caused mortality percentage 74.19% at the concentration of 0.07 mg/ml and it caused mortality percentage 43.01% at 0.01 mg/ml. All alcoholic extracts of the plants used showed significant differences after treatment.

Table 4. The plant Alcoholic Extracts Efficacy of the Fourth Larval Instar.

Plant Extracts	Concentration mg/ml				Average Mortality of Each Plant
	0.01	0.03	0.05	0.07	
Capparis sp.	62.32	67.83	71.15	89.57	71.73 A
Nerium sp.	54.83	60.71	69.96	84.21	67.42 B
Lawsonia sp.	43.01	59.35	65.29	74.19	60.46 C
Average mortality of each concentration	53.38 D	62.63 C	68.81 B	73.65 A	

*Similar capital letters in one line horizontally mean no significant differences.

3.5 8.5 The Plant Alcoholic Extracts Efficacy of the Male *Phlebotomus Papatasi*:

The results show the effect of alcoholic extracts in mortality percentage of male as shown in Table 5. The ethyl alcoholic of the extracts of *Capparis sp.* caused the highest mortality percentage 76.18% at the higher user concentration of 0.07mg/ml while the lowest mortality percentage was 39.43% at user concentration 0.01 mg/ml also the results o show that plant leaves extracts of Nerium sp. it caused mortality percentage 67.21% at the concentration of 0.07mg/ml and caused mortality percentage 33.42% at 0.01, also the results o show that the plant leaves extracts of Lawsonia sp. it caused mortality percentage 61.64% at the concentration of 0.07 mg/ml and it caused mortality percentage 29.21% at 0.01 mg/ml. All alcoholic extracts of the plants used showed significant differences after.

Table 5. The plant Alcoholic Extracts Efficacy of the Fourth Larval Instar.

Plant Extracts	Concentration mg/ml				Average Mortality of Each Plant
	0.01	0.03	0.05	0.07	
Capparis sp.	39.43	55.23	69.12	76.18	60.99 A
Nerium sp.	33.24	51.92	60.52	67.21	53.22 B
Lawsonia sp.	29.21	44.35	55.16	61.64	47.59 C
Average mortality of each concentration	33.69 D	50.51 C	61.62 B	65.43 A	

*Similar capital letters in one line horizontally mean no significant differences.

3.6 The Plant Alcoholic Extracts Efficacy of the Female *Phlebotomus Papatasi*:

The results show the effect of alcoholic extracts in mortality percentage fourth stage larvae as shown in Table 6. The ethyl alcoholic of the extracts of *Capparis sp.* caused the highest mortality percentage 87.17% at the higher user concentration of 0.07mg/ml while the lowest mortality percentage was 43.32% at user concentration 0.01 mg/ml also the results o show that plant leaves extracts of Nerium sp. it caused mortality percentage 74.02% at the concentration of 0.07mg/ml and caused mortality percentage 39.22% at 0.01, also the results o show that the plant leaves extracts of Lawsonia sp. it caused mortality percentage 68.65% at the concentration of 0.07 mg/ml and it caused mortality percentage 31.86% at 0.01 mg/ml. All alcoholic extracts of the plants used showed significant differences after 24 treatment. The effective chemical compounds of plants used in affecting the fourth larval instar, male and female sandflies, greatly affect the process of digesting nutrients and their absorption in the stomach and intestines of sandflies, which leads to the death of larvae and adults.

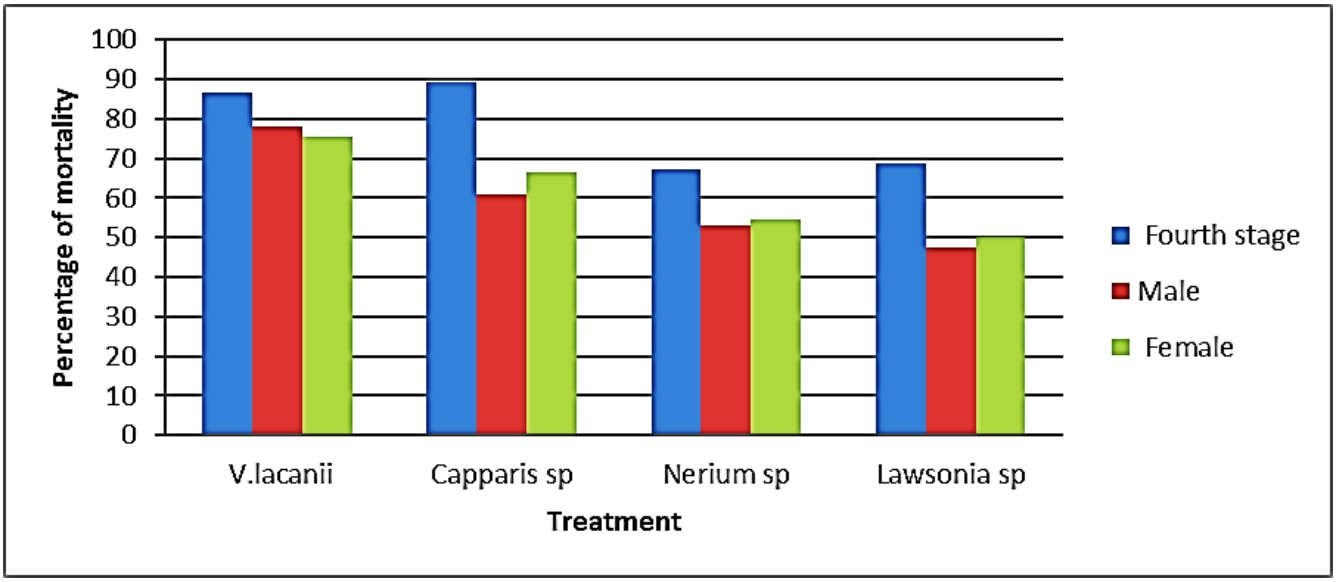

Figure 1. Comperating percentage mortality of *Phlebotomus papaptasi* treatment after 24h.

This study agrees with the study of [18] ethyl elcoholic extracts of the Atriplex helmius was 83.16% after three days at 20mg/ml while 78.15% after one days. Study of [19] proved that alcoholic extract of Azadiracta indicaled to a 100% killing rate on bugs male Culex lectularius 3 minutes, 5 minutes and 7 minutes.

Table 6. The plant Alcoholic Extracts Efficacy of the Fourth Larval Instar.

Plant Extracts	Concentration mg/ml				Average Mortality of Each Plant
	0.01	0.03	0.05	0.07	
Capparis sp.	43.32	59.00	76.25	87.17	66.43 A
Nerium sp.	39.22	42.15	67.37	74.02	54.68 B
Lawsonia sp.	31.86	46.35	58.16	68.65	50.05 C
Average mortality of each concentration	38.13 D	49.16 C	54.34 B	76.61 A	

*Similar capital letters in one line horizontally mean no significant differences.

3.7 Comperating Mortality Percentage of Treatment after 24h:

Figure 1 showed a comparison of the highest mortality percentage of the fungi *Verticillium lacanii* against *Phlebotomus papaptasi* after 24 h of treatment, was 86.74% for the fourth larval stage, while was 78.16% for the male of as well as was 75.46% for for the female. The highest mortality was 89.27% of the plant extracts *Capparis sp.* for the fourth larval stage of *Phlebotomus papaptasi*, as while as was 67.21% for the plant extracts N. oleander for the male while was 68.65% for the plant extracts *Lawsonia sp.*for the female.

4. Conclusion:

The results of the current study showed that the fungus *Verticillium lacanii* caused a highest mortality percentage of the fourth larval instar compared to male and female, as well as the alcoholic extract of the leaves of the plant *Capparis sp.* caused highest mortality percentage of the fourth larval instar, and that could be used in the future in biological control of sand fly *Phlebotomus papaptasi*.

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تقييم كفاءة بعض الطرق في السيطرة البيولوجية على ذبابة الرمل *Phlebotomuspapatasi*(*Diptera* : *Psychoididae*)

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الخلاصة

تضمن البحث دراسة الكفاءة الحيوية للفطر *Verticilliumlacanii* والمستخلص الكحولي لأوراق نباتات *NeriumspCapparisspandLawsoniasp* في السيطرة البايولوجية على الطور اليرقي الرابع وبالغات ذكر وانثى ذبابة الرمل . *Phlebotomuspapatasi* أُجريت الدراسة تحت ظروف المختبر بدرجة حرارة 2±25 مْ، رطوبة نسبية % 5±65 وفترة إضاءة 12 ساعة أعطى عالق الفطر *Verticilliumlacanii* بتركيز 1×10^{8} بوغ أعلى نسبة هلاك % 86.74 للعمر اليرقي الرابع بعد 24 ساعة من المعاملة، أعطى عالق الفطر *Verticilliumlacanii* بتركيز 1×10^{8} بوغ أعلى نسبة هلاك %78.16 لذكور ذبابة الرمل *Phlebotomuspapatasi* بعد 24 ساعة من المعاملة و أعطى عالق الفطر *Verticilliumlacanii* بتركيز 1×10^{8} بوغ أعلى نسبة هلاك %75.46 . لإناث ذبابة الرمل بعد 24 من المعاملة. أما المستخلص الكحولي لنبات *Capparissp* بلغت اعلى نسبة هلاك %89.57 للطور اليرقي الرابع، %76.18 للذكور، %87.71 لإناث ذبابة الرمل *Phlebotomuspapatasi* بعد 24 ساعة عند التركيز 0.07 ملغم المستخلص الكحولي لنبات *Lawsoniasp* أظهر اقل نسبة هلاك %43.01 للطور اليرقي الرابع، %29.21 للذكور، %31.89 لإناث ذبابة الرمل *Phlebotomuspapatasi* بعد 24 ساعة من المعاملة عند التركيز 0.01 ملغم .

الكلمات الدالة : معلق، مستخلص كحولي، *Verticilliumlacanii*,*Phlebotomus papatasi*

التمويل: لا يوجد.

بيان توفر البيانات: جميع البيانات الداعمة لنتائج الدراسة المقدمة يمكن طلبها من المؤلف المسؤول.

اقرارات:

تضارب المصالح: يقر المؤلفون أنه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: لم يتم نشر المخطوطة أو تقديمها لمجلة أخرى، كما أنها ليست قيد المراجعة.