

RESEARCH ARTICLE

The Isolation of Some Types of Fungi from Hard Ticks and the Study of the Effect of Fungi and the Extract Ethanol of the Plant Nerium Olender in the Control it

Habeeb Waseel Kadhum Shubber, Esraa Fadhil Wathah, Walaa Yas Lahmood, Ali Bustan Mohsen Alwaaly

Biology Dept. College of Science, Al-Qadisiyah University, Iraq.

Abstract

The Fungi Isolated *Metathizium anisopliae*, *Pencillium* sp, *Beaveriabassina* and *Trichotheciumrosum* the last one was isolated for the first time from Hard Ticks. The two types of germinal suspension fungal '*Trichotheciumvosum* and ' exceed in different life stages of *Hyalommasppin* which the highest proportion of the eggmortalityis (92.15)% and (89.88) % successively, While in Un fed larvae is (100)% and engorged larvae is (92.4)% and it reached in un fed larvae (100)% and (80.4)% for the engorged larvae successively and it reached in un fed nymphs (86.2)% and in engorged nymphsit was (54.4)% and un fed nymphs (66)% while in engorged nymphsIt reached (20)% successively while the highest level is 10^8 while the lowest activity is the fungus *Pencillium* sp. While in the advanced stages was noticed as a perfect in planet extracts it reached the highest proportion of mortalityis (81.3)% in related to un fed adult and (72.4)% in related to engorged adult and the fungal filtrate exceeded the fungal extracts in causing mortality proportion.

Keywords: *Hard Ticks, Fungi, Plant extracts.*

Introduction

The *Hyalomma* considered as a member of the family of the hard ticks which is important transcriber for many of diseases for both Humans and other animals [1] like the Thyroid disease and Eastern fever [2] and it also causes many disease for the cattle and causes economic losses[3] and this is why it had to be resisted, but the use of chemical pesticides results for a lot of environmental problems one of them is the appearance of new pests because of losing the activity of Predators and Parasites. The use of the Chemical pesticides results the killing of other types which are unlikely to be killed.

Another problem is that the Chemical pesticides produce the chemical garbage in the agricultural product [4,5]. For these reasons it had to search another means and one of them is the biological resistance by using biological factors. Fungi and plants are considered one of these factors, in which it recorded a lot of fungal infections for the types of ticks such as *Beaveriaspp*, *Fusarium* sp, *Aspergillum*s and many other types [6]. For the importance of *Hyalommawe* in this

paper isolated many types of fungi and the effect of them as a biological factors in controlling the ticks which are *Trichotheciumrosum*, *Beaveriabassina*, *Metarhiziumanisopliae*, *Pencillium*sppand the extract ethanol of the plant *NeriumOlenderin* considering the plant extracts one of the means of eco friend[7].

Materials and Method

Collect samples of ticks: samples of ticks had been collected from many districts of Al-dewaniyah city and they had been classified according to taxonomic key [8, 9]. The way of [10, 11] has been followed in preparing of ticks farm and feeding the stages (larvae, Nymphs, adults) by using experimental rabbits.

Diagnose and Isolate the Fungi

Fungi had been isolated from the eggs, larvae and the adults of ticks according to the way [12] while the diagnose is according to the way [13] depending on the outside

appearance and the classify characterizations.

Preparing the Fungal Suspension

The fungal suspension had been prepared by using devises in which each capacity of these devises is (250)ML for each one of the fungus.(150)ML of Emersonypps broth had been put inside the devises and heated in(25)C° for a week with putting in mind shaking it daily to spread the fungal growth. After that they had been filtered by using a piece of gauze and put on slice counting spores in which it results the highest

Level (10⁸x 3 spore/ML) [14] and for the sake of getting lower level we followed the equation (Lacey, 1997) by which we get (10⁵,10⁶, 107,108) for each fungus.

The Effect of the Raw Suspension for the Fungi in Different Life Stages of Hyalomma spp

The Effect on the Eggs

The eggs that are put by filled female had been taken and adult by the above levels of extract fungus separately for each fungus by dipping it in the spore extract for a minute through using soft brush then it had been raised to filtering paper and put in other dishes and then in brooder in (28) Co and in humidity (85) % and counted the mortality after 5 days of the treatment.

The Effect on Unfed Larvae and Engorged Larvae

(100 – 150) sample had been taken, Un fed larvae had been dealt with by the above levels for each fungus separately through dipping it according to the way[15] ,while engorged larvae had been dealt with according to the way [16] and put in brooder in (28)C° and humidity 85% .

The Effect on Unfed Nymphs and Engorged Nymphs

(50-100)samples of un fed nymphs and engorged nymphs had been taken and dealt with fungal extract through dipping them according the way [15] and put in brooder in (28) C°and humidity 85% .

The Effect on Unfed and Engorged Adults

(10-15) samples had been taken and dealt with as the same way of nymphs except replacing nymphs by adults and counted the mortality proportion after 5 days from the treatment.

Preparing the Raw Filtrate for Fungi

Potato dextrose broth has been put in to glass flask. Three flasks used for each treatment and put in steamy device in (121) C° and in pressure [15] pound/inch² for half an hour. The flasks had been left to be cold and inoculate them by culture media on which fungus had grown. A drop of (0.5) was added for each of fungus at the age of seven days, and then three drops of lactic acid had been put for each flask. These flasks had been put in a brooder in (25) Co with shaking it for (3-4) days to spread the fungal growth. After 28 days the flasks had been leached by leaching paper (NO.1) and again leached by leaching paper (0.45) Micro Milli by using air vacuum device [17]. The concentration (100, 75, 50, 25) had been used for each fungus separately by using distilled water for the sake of using the filtrate in the experiments.

Preparing the Extract Ethanol for Nerium Olender

The way [18] had been followed in preparing the extract ethanol of the plant and the concentration (60, 40, 30, 15) Mg/ML had been prepared from it.

The Effect of Filtered Fungal and the Extract Ethanol of Nerium Olenderon the Adult Stages of HyalommaSpp

(10-15) sample of unfed and engorged adult had been dipped in the concentration that are already prepared from filtered fungal and extract plant separately and according to(15). The mortality proportion had been accounted after 5 days from the treatment.

Statistical Analysis

The experiments had been designed according to the pattern factorial experiments with completely randomized design (CRD) and the mortality proportion had been corrected according to the equation Abbott Formula [19].

$$\text{The corrected mortilaty} = \frac{\text{mortilaty proportion in treatment} - \text{mortilaty proportion in control}}{100 - \text{mortality proprtion in control}} \times 100$$

It had been chosen the lowest significant difference (L.S.D.) under prospect level (0.05). To identify significant differences, the corrected mortality proportion had been turned to angle value to get it in to statistical analysis [20]

Results and Discussion

The Isolated Fungi from the Adult Stages of the Hard Ticks Hyalomma Spp

The following Fungi: *Beaveriabassina*, *Metarhiziumanisopliae*, *Pencillium spp* and *Trichotheciumrosom* had been isolated (the last one had been isolated for the first time from *Hyalommasp* from hard tick's adults

(male and female). It had been test their diseases in uninfected members of ticks and these ticks proved their sufficient, then they had been isolated from dead infected to insure the fungi according to the way [21]. There are a lot of studies that prove the existence of fungi in different types of hard ticks, like [22, 23] that proved the isolation of different types of fungi from hard ticks like *Rhizopus*, *aspergillums*, *Pencillium* from *Hyalommaanatolicum* gender, and *Amblyomalepidium*.

The Isolation of Fungal Suspensions in the Different Life Stages of Hyalomma spp

The Effect on Eggs

Table1: the effect of the fungal suspension on eggs mortality of *Hyalomma spp*

concentrations	Rate of Motility %			
	<i>Trichotheciumrosom</i>	<i>Metarhiziumanisopliae</i>	<i>Beaveriabassiana</i>	<i>PencilliumSp</i>
10 ⁵	32	36	29	20
10 ⁶	40	35	27	25
10 ⁷	85.32	66.39	51.33	49.23
10 ⁸	92.15	83.88	72.47	63.21

L.S.D=5.3

The above results showed the excellence of fungal suspension of both *Trichotheciumrosom* and *Metarhiziumanisopliae* above other understudy fungi as their mortality proportion in the concentration 10⁸ reached 92.15% and 89.88% successively while the lowest mortality proportion is of *Pencilliumspin* which it reached 20% in 10⁵ concentration. The fungi's ability to get in and break through the chorion layer is the cause of hatching eggs which results the

highest mortality proportion [24, 25] refer to the activity of the fungal suspension *scopulariopsisbrevicaulis* for hatching the eggs of *Hyalommaanatolicum* in 44.13 % proportion. [26] Clarify that the two fungi *Beaveriabassina* and *Metarhiziumanisopliae* caused the lack of effectiveness for the eggs of *Rhipicephalusappendiculatus*.

The Effect on Unfed Larvae and Engorged Larvae

Table 2: the effect of the fungal suspension on eggsmortality proportion of unfed larvae and engorged larvae

concentration	Rate of Mortality							
	<i>TrichotheciumRosum</i>		<i>MetarhiziumAnisopliae</i>		<i>BeaveriaBassiana</i>		<i>PencilliumSp</i>	
	unfed larvae	engorged larvae	unfed larvae	engorged larvae	unfed larvae	engorged larvae	unfed larvae	engorged larvae
10 ⁵	68.3	49.2	55.2	13	26.2	5.2	18.2	0.5
10 ⁶	74.2	61.2	88.1	53.6	82.1	44.2	54.1	39.4
10 ⁷	89.4	76.1	86.5	48.1	72.5	34.1	63.4	45.1
10 ⁸	100	92.4	100	80.4	98.2	66	96.3	60

L.S.D=9.02

The above table refers to the activity of fungal suspension in UN fed and Engorged larvae. in which it shows the excellence of the fungal suspension *Trichotheciumrosom* in which its highest mortality proportion is 100% for Un fed stage and 92.4% for Engorged stage in the concentration 10⁸ while the lowest mortality proportion is of *Pencillium* which reached 18.2% for Un fed stage and 0.5 for Engorged stage in

concentration 10⁵. Un fed larvae doomed proportion is higher than engorged larvae maybe because of the features that the cuticle layer has in the two types of larvae and not because the blood meal that they are feeding on it (27). This result was compatible with study of [15] when he studied the effect of *B. bassina* on the larvae of *R.appendiculatns*. In this part, [24] insure that Un fed larvae are more sensitive and

effective on *M.anisopliae* from other stages and themortality proportion for the larvae of *Boophilusannulatus* reached to 90% after incurred to *M. flavoridine*.

The Effect on Unfed Nymphs and Engorged Nymphs

Table 3: the effect of the fungal suspension on mortality eggs proportion of un fed nymphs and engorged nymphs

Concentration	Rate of Mortality							
	<i>TrichotheciumRosum</i>		<i>Metarhiziumanisopliae</i>		<i>BeaveriaBassiana</i>		<i>Pencilliumsp</i>	
	unfed nymphs	engorged nymphs	unfed nymphs	Engorged Nymphs	unfed nymphs	engorged nymphs	unfed nymphs	engorged nymphs
10 ⁵	34.4	10	20	6.5	12	5	8	1.1
10 ⁶	41.6	18	26.8	11	21	5	18.2	3.2
10 ⁷	70.1	47	54	19	35.7	12.5	25.8	3.1
10 ⁸	86.2	54.4	66	20	57.5	18.5	32	5.1

L.S.D.6.66

The above results show that the fungus *Trichotheciumrosum* is the most active from other fungi in which the highest effect in the concentration 10⁸ is 86.2% for un fed nymphs and 74.4% for engorged nymphs and that engorged stage has more resistance from un fed stage. The fungal ability to breakthrough cuticle layer, cause laziness, inactivity, slow down its mobility and death and this is why the fungi are considered efficient in their impact [28].

doomed than engorged nymphs and engorged larvae in which their highest doomed proportion reached 80% in treating it by the fungi *M. anisopliae*, *B. bassiana* and *Paeclomyces Fumosorsus*. This study was compatible with the study of (15) when he studied the nymphs of *R.appendiculatus*. When unfed nymphs of *R.sanguineus* were injected by the fungi *Metarhiziumsp*, black spots were shown on the cuticle after a week from injecting it by [24].

Both (Huschmidetal, 2013)and (Harteltetal, 2008) referred that Un fed nymphs and un fed larvae for *Ixodesricinus* were more

The effect of the fungal suspension on eggs the effect of the fungal suspension on eggs

Table 4: the effect of the fungal suspension on eggsmortality proportion of un fed adult and engorged adult

Concentration	Rate of mortality							
	<i>TrichotheciumRosum</i>		<i>Metarhiziumanisopliae</i>		<i>BeaveriaBassiana</i>		<i>PencilliumSp</i>	
	unfed adult	engorged adult	unfed adult	engorged adult	unfed adult	engorged adult	unfed adult	engorged adult
10 ⁵	0	0	0	0	0	0	0	0
10 ⁶	6	2	5	1	0	0	0	0
10 ⁷	10	4	5	1	2	0	0	0
10 ⁸	15	7	8	3	4	1	0	0

L.S.D =4.54

The above table shows the effect of fungal suspension in adult stages in which we noticed that the fungal suspension didn't affect the adult stages in the concentration 10⁵ for all fungal suspension.

M. anisopliae and *M. flavoridive* prevent the females of *R. sanguines* from putting eggs, while(26) referred to the clear activity of *B.bassiana* in adult stages of *R. appendiculatus*.

The *PencilliumSp* didn't affect the adult stages in all tested concentrations. The fungus unable to effect the adult stages because ofits inability of germination and breakthrough the hard ticks, so the fungi are unable to injured and unable to excretion the dissection enzymes [29, 30]

The doom proportion of un fed adult of *Ixodesscapularis* was (96) %Laboratory and (53) % in the field by treating it with *Metarhiziumanisopliae* [31,24]

Studied the activity of *B. bassiana* and noticed there were no mortality in adult female but hatching eggs proportion that they put was zero.[24] clarified that the fungi

Insure that the two fungi *M.anisopliae* and *B. bassianacause* high mortality reached to (90-100) % after 10 days from the treatment. The effect of fungal filtration and extract ethanol of *Nerium Olender* against the adults of *Hyalommasp*

Table5: the effect of fungal filtration and extract ethanol of NeriumOlender against the adults of Hyalomma spp

Concentration	Rate of mortality%							
	<i>Trichotheciumrosum</i>		<i>Metarhiziumanisopliae</i>		<i>Beaveriabassiana</i>		<i>Pencilliumsp</i>	
	unfed adult	engorged adult	unfed adult	engorged adult	unfed adult	engorged adult	unfed adult	engorged adult
25	29.2	17.2	20	15.4	19.4	12.2	14.2	8
50	35.2	29	35	27.4	22.4	16.4	22.2	16.2
75	48.4	32.3	40	28	30.8	24.7	28.2	
100	61.4	52.4	56.1	47	50.1	42.2	48.2	25.2
L.S.D=2.75								
Extract ethanol of <i>Nerium Olender</i>	Concentration Mg/MI							
	15		30		40		60	
	unfed adult	engorged adult	unfed adult	engorged adult	unfed adult	engorged adult	unfed adult	engorged adult
Rate of mortality%	32.4	25.4	59.7	49.2	72.3	69.4	81.3	72.4
L.S.D= 2.34								

The above results clarify the efficiency of the extract ethanol of Nerium Olender in adult stages, the leach-ate also effect the adult stages in which the fungus *Trichotheciumrosum* recorded the higher doom pro-portion than other fungi, which reached (61.4)% in concentration (100) for un fed adult, while engorged adult reached 52.4% in the same concentration. The reason behind the efficiency of the extract plant is that the ability of the extract material to reduce the total sugar and protein level which cause the mortality for the insect [32].

The filtrate are metabolic products have the ability to interlink with immune-ne system, which reduce the activity, stop mobility and the quick death for the host and this is why this filtrate are efficient [33].

In this side [25] referred that the fungal leachate of *S. brevicornis* has microbial toxins which effect the adult stages of hard ticks *Hyalommaanatolicum* causing death for un fed adult in 77.5% proportion and it effects in engorged adults the females' fertility in putting eggs in 50.2% proportion and it also reduces hitching eggs proportion which are put by the females in 66.86%. [34].

Found that the hard ticks adults *Hyalommamarginatum* doom proportion reached 100% in treating it by ethyl acetate extract of *S. arachnoids* roots. [35] Showed the efficiency of plant extract of *Rhanteriumepapposum*,

Achilleafragrantissima and *Eragrostispoaeodes* cause the death of the adults after (30-60) minute from exposing them to the extracts.

While the following plant extract *Artemisiaherbaalba*, *Haloxylonsalicornicum*, plant *agocoronopus*, *Moltkiopsis ciliate* and *Lasiurushirutus* result stopping the process of putting eggs from filled female.

(Ilhametal, 2014) referred to the efficiency of the raw extract ethanol of the leaves of *Annon squamosal* against the adults of *Hyalommaanatolicum* in which it cause death for all of them in 100% proportion in (10,7.5)%.

Conclusion

- The plant extracts were somewhat similar to the secondary products of fungi in their effect on ticks
- The fungal filtration of fungi had an effect on the engorged and non-engorged dult stages.
- The superiority of *Thrichotheciumvosum* on the remaining fungus was under study in the effect on various stages of the tick.
- The non-engorged stages of the tick were more affected from engorged stages

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