

# Effect the Extracts of *Agaricus Bioporus* on Some Biological Aspects of *Musca Domestica* (Linnaeus1857) (Diptera: Muscidae)

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## ABSTRACT

The efficacy of *Agaricus bioporus* extract was studied using concentrations (1, 3, 5 and 7) mg / ml for the aqueous extract and concentrations (0.25, 0.5, 1 and 2) mg / ml for chloroform extract when treating the third larval stage and pupae, and noting its effect on some aspects of life *Musca domestica* in Karbala governorate for the month of September of 2018 to control domestic flies, and the results showed a decimation in the number of third-stage larvae and the role of the pupa treated with *A. bioporus* extracts and the emergence of an increase in the percentage of inhibiting the emergence of adults resulting from the treatment of the third larval stage and the role of the pupa. These extracts reduce the average lifespan of the insect under study.

**Keywords:** *Musca domestica*, Fungal Extracts, *Agaricus bioporus*

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## INTRODUCTION

The house fly insect *M. domestica* belongs to the order Diptera, Muscidae family. This family includes 170 genera belonging to 4200 species, many of which, such as house flies, are medically important for being a pathogen and a vector of pathogens. It represents a mechanical vector for many diseases to humans and animals [1, 2, 3, 4]. Because of the danger of continuing to use one type of toxins or insecticides, excessively or not properly studied, it will lead to environmental pollution and poisoning humans and domestic animals and enables insects to form strains and generations resistant to the action of chemical pesticides, which prompts those in charge of pest control to change and switch control methods to reduce the economic damage caused by its increase [5,6,7]. The use of natural plant extracts for some plants, including some fungal species, in controlling insects has proven effective as a pesticide for different insect roles [8,9]. *A. bioporus* is a large fungal species, Macrofungus, that is characterized by seeing its fruiting bodies with the naked eye, and includes edible species such as *A. bioporus*, which have a high nutritional value because they contain a higher protein content than most vegetables [10,11,12]. This fungus belongs to the subfamily Homobasidiomycetidae, under the section of the Basidiomycotina fungi, which contains more than 14,000 species [13,10]. Insects, repellent and attracting to laying eggs, or with disruptive or deterrent activities [14], and a study by [15] confirmed that this fungus contains the phenolic functional group upon its aqueous extraction. These compounds are important in controlling insects, so this study aimed to control the third-instar larval and the pupae stage of house flies by studying the effect of the aqueous extract and chloroform of mushrooms on some aspects of the life performance of the insect by treating the third-instar larvae and the pupal stage and calculating the percentage of larvae and pupae mortality Treatment with it, and the calculation of the rate of age of the immature roles and the adult role of the insect as a result of treatment with these extracts.

## MATERIALS AND METHODS

### 1- Collecting and diagnosing plant samples:

Samples of mushrooms were collected from the markets of Karbala governorate in March of 2018, dried and milled, put in a glass bottle and kept in the refrigerator until use.

### 2. Preparation of plant extracts:

Using the method [16], modified from [17] was adopted in the preparation of aqueous extract, but in the preparation of extracts of organic solvents, the organic solvent, Chloroform, was used in the preparation of Organic plant extracts where the [18] and modified from [17] was adopted in the extraction process for mushrooms.

When preparing the necessary concentrations for the study experiments, 10 grams of the dry matter of mushrooms were dissolved in 100 ml of distilled water, so the concentration became 10 g / ml, from which the concentrations (1, 3, 5 and 7) mg / ml were prepared, and the control treatment contained only distilled water.

To prepare chloroform mushroom extract by taking 2 grams of mushroom powder and dissolving in 2 ml of chloroform and 2 ml of ethyl alcohol and complete the volume to 100 ml of distilled water, from which the following concentrations were prepared (0.25, 0.5, 1 and 2) mg / ml, and the control treatment was used. 2 ml of solvent and 2 ml of ethyl alcohol to which 96 ml of distilled water are added.

### 3- Insect collection and diagnosis

Larvae were collected from poultry fields in Karbala governorate, where the excrement containing the roles of the immature insect of domestic flies was taken, and the pupae resulting from the third larval stage were isolated and were diagnosed according [19].

### 4- Breeding of the insect

The domestic fly insect was raised in the laboratory in laboratory conditions, where the pupae were placed in a wooden cage with dimensions of (40X40X40 cm), closed with metal wire on four sides and with tulle on the fifth side and a wooden base, and inside Petri dishes were placed containing milk with sugar in proportion 5% to feed adults, and a layer of cotton cloth was placed over the milk so that insects could stand on it and did not stick to the milk when laying eggs or when feeding,

After mating the insects and laying eggs in clumps containing approximately 100 eggs in each block, these eggs were separated by a brush, and according to the [20] method, the eggs were transferred to a special medium prepared for the growth of larvae consisting of 600 grams of dung horse manure as it was dried And grind it and sterilize it with Autoclave and add to it 200 g of malt wort (which is in the final stage of beer preparation which is ground, sterile and ready for use) and 22 g of yeast, and 20 ml of NaOH were added, then 1200 ml of distilled water were added. To the previous components.

**5-The effect of mushroom aqueous extract and chloroform on the third-instar larval of the insect: -**

Put 12 gm of industrial food when raising larvae in plastic petri dishes with a capacity of 100 ml and add 5 ml of each concentration of mushroom extracts at a rate of three replicates for each concentration. As for the control treatment, only distilled water was used, or the same volume of solvent was used with distilled water. Petri

dishes were covered with a piece of gauze during the experiment, and the results were recorded every 24 hours until the emergence of adults. And her life was followed up. As for the effect of the extracts on the pupae, 10 pupae, aged 24 hours, were used for each concentration.

And the pupae were placed in a piece of gauze and immersed for 10 seconds in each concentration of aqueous extract and chloroform of the mushroom in three replications and left to air dry and then placed in test tubes and covered with a piece of gauze [21] . As for the control treatment, the solvent was used only for the purpose of comparison and it was kept under breeding conditions. The mortality rates for the third larval stage and the pupae and until they reach the adult stage, and the calculation of the growth period for the third-instarlarval and adult stage , and the rates representing the percentage of mortality were corrected according to [22].

$$\text{The Percentage of Corrected mortality} = \frac{\%mortality\ at\ treatment - \%mortality\ at\ control}{100 - mortality\ at\ control} * 100\%$$

The percentage of inhibition of emergence of adults was calculated through the percentage of inhibition of emergence (IE%). According to the [23].

$$(IE\%) = 100 - (T \times 100 / C)$$

Where T = the percentage emergence in the treatment.

C = the percentage of emergence in the control treatment.

The results of the study experiments were analyzed according to the global experiments model and with a complete randomization design. The mortality ratios were adjusted according to [22,24], and the corrected values were converted to angle values for inclusion in the statistical analysis.

**RESULTS**

**1- The effect of cold water and chloroform extracts of *A. bioporus* in mortality percentage of third stage larvae and pupae of *M. domestica***

The results in Table (1) indicated that there was a direct relationship between the concentrations used and the percentage rate of mortality. This relationship continued

with the difference in the type of solvent used in the extraction. 1mg/ml and it increased to reach 56.67% at a concentration of 3 mg / ml, and this increase continued to reach 73.37 and 100% at concentrations 5 and 7 mg / ml, but when using the same extract with the treatment of virgins, there was the same relationship, that is, the higher the concentration, the greater the percentage of pupae death, as the percentage of mortality reached In it, it reached ( 13.34, 23.34, 33.34 and 40) %, respectively, in the aforementioned concentrations, compared to the control treatment, which amounted to 0% for both treatments. As for when using chloroform extract for *A. bioporus*, when treating third stage larvae, the percentage of mortality was (23.34, 43.34, 50.00 and 73.34)%, respectively, in concentrations (0.25, 0.5, 1 and 2) mg / ml, but when pupae were treated with the same extract it was The percentage of depreciation (11.00, 17.67, 26.34 and 31.00)% at the same aforementioned concentrations compared to the control treatment, which amounted to 0% for both treatments.

**Table 1:** Effect *A. bioporus* extracts in mortality percentage of third stage larvae and pupae of *M. domestica*

chloroform extract			water extract		
Concentration mg/ml	% mortality of larvae	% mortality of pupae	Concentration mg/ml	% mortality of larvae	% mortality of pupae
control	0	0	control	0	0
0.25	23.34	11.00	1	40.00	13.34
0.5	43.34	17.67	3	56.67	23.34
1	50.00	26.34	5	73.34	33.34
2	73.34	31.00	7	100	40.00
L.S.D. the interaction between the extracts and the concentrations was 15.5 in the percent of mortality in three-instar larval death.					
L.S.D. the interaction between the extracts and the concentrations was 4.8 in the percent of mortality in pupae.					

When the mortality percentages were observed, the effect of cold-water extracts and chloroform was observed for the treatment of the third larval instar, and they were

more effective when the pupae were treated with the same extracts. The results of the statistical analysis indicated the significant differences in the results

obtained. These results are in agreement with [25] the increased concentration of the extract led to an increase in the mortality rates of the immature stages of the treated insect. A positive relationship was observed between the concentration of the extract and the percentage of destruction, and Confirmed [26] the effect of *Azadirachta excelsa* Jack leaf extract on the percentage of three-instar larval mortality of *M. domestica* by (20, 50, 60 and 94)% at concentrations (50, 100, 200 and 400) ppm, respectively, and it was the most effective. Of the remaining three plant extracts, *Pimpinella anisum* L. had a lethal effect on the phases of the house fly, but by 50% at a concentration of 400ppm in seven days of treatment, while *Cinnamomum zeylanicum* L. had a weak effect in the phases as the death rate did not exceed 40% while it was not *Solanum nigrum* L. leaf extract has no effect.

A study showed [21] that when ethanol extracts of *Calotropis procera* leaves were used on pupae of *M. domestica*, the percentage of pupae perishing was (3.33, 13.33, 30, and 96.66%), while in the extract of petroleum ether, the percentage of pupae perished was ( 0, 0, 10, and 33.33) % at concentrations of 0.25, 0.5, 1 and 2%,

respectively, compared to the control treatment, which represented 0% for pupae' mortality.

**2- The effect of cold water and chloroform extracts of *A. bioporus* in percentage of inhibition of adult emergence when treating third stage larvae and pupae of domestic flies *M. domestica***

The effect of water *A. bioporus* extracts and chloroform was observed in Table (2) significantly on the percentage of inhibiting adult emergence of *M. domestica* using different concentrations. The percentage of adult emergence was when treating larvae of the third-instar larval with aqueous extract (46.67, 70.00 and 80.00) As for when pupae were treated, the percentage inhibition of eruption was (13.34, 26.67, 36.67 and 43.34%) in concentrations (1, 3, 5 and 7) mg / ml, but when the larvae were treated with chloroform extract, the percentage inhibition of emergence of adults was (23.34, 43.34, 50.00, and 73.34%), but when the pupae were treated, the percentage of inhibition of eruption was (11.00, 17.67, 26.34 and 31.00)% in concentrations (0.25, 0.5, 1 and 2) mg / ml compared to the control treatment, which amounted to 0% for all Transactions.

**Table 2.** Effect of cold water and chloroform extracts of *A. bioporus* on the percentage of inhibition of adult emergence when treating third-instar larvae and pupae of *M. domestica*

chloroform extract			water extract		
Concentration mg/ml	%IE of adults when treating third-instar larvae	%IE of adults when treating pupae	Concentration mg/ml	%IE of adults when treating third-instar larvae	%IE of adults when treating pupae
control	0	0	Control	0	0
0.25	23.34	11.00	1	40.00	13.34
0.5	43.34	17.67	3	56.67	23.34
1	50.00	26.34	5	73.34	33.34
2	73.34	31.00	7	100	40.00
L.S.D. value of interaction between the extracts and the concentrations and the percent inhibition of emergence of adults when treating third-instar larvae 9.8					
L.S.D. value of interaction between the extracts and the concentrations and the percent inhibition of emergence of adults when treating pupae 4.2					

It was noted through the results in Table (2) that the cold water extract and chloroform of mushroom mushrooms in both treatments of the third stage larvae were more effective in the percentage of inhibiting the emergence of adults of the insect than the treatment of the pupae of the same extracts, and the results of the statistical analysis indicated that there were significant differences in the results that were made Obtained .

Confirmed [26] the effect of *Azadirachta excelsa* Jack leaf extract in the percentage of inhibiting the emergence of adult domestic flies, *M. domestica* by 94% at 400 ppm during seven days of treatment and it was more effective than the remaining three plant extracts. A study showed [21] that when ethanol extract of *Calotropis procera* leaves was used on pupae of *M. domestica*, the percentage of adult exit for *M. domestica* was (96.66, 86.66, 70 and 3.33%), while in the extract of petroleum ether the percentage was ( 100, 100, 90 and 66.66 ) % at concentrations (0.25, 0.5, 1 and 2%), respectively, compared to the control treatment, which represented 100%.

**3- The effect of water and chloroform extracts of *A. bioporus* on the longevity of immature and adult stages when treated third-instar larvae**

Table (3) shows the effect of an aqueous mushroom extract and chloroform on the growth period of third-

instar larvae, pupae and adults. In the chloroform extract, the mean ages were (1.2, 2.1, 1.5 and 3.9) days at a concentration of 2 mg / ml, respectively, compared to the control treatment, which was (2.8, 3.3, 4 and 13) days.

In the same table show an increase in the age of the larva in the concentration (1) mg / ml and the pupa in the concentration (5) mg / ml. It was noted that the ages of males and females decreased for all concentrations and the death of all larvae when treated with a concentration of 7 mg / ml when the larvae were treated with water extract. In the cold of mushroom mushrooms, as for the chloroform extract of mushroom mushrooms, a decrease in the life span of the roles resulting from both treatments was observed, and the results of statistical analysis indicated that there were significant differences in the results obtained.the results conform with[27] shows a decrease in the age of the fourth instar larvae and pupae and adult stage of insect in most of the extract concentrations. A studying showed[28] the effect of *Amanita muscaria* extract on the ages of *M. domestica*, confirmed the age of larvae to adults calculated in days (14.55, 14.85, 14.41, 14.38 and 14.63) days at concentrations (10, 20 and 30). And 40 and 50%, respectively, compared to the control treatment, which amounted to 14.42 days.

**Table 3.** The effect of cold-water extract and chloroform of *A. bioporus* on longevity of immature and adult stages that resulted from treatment of third stage larvae of *M. domestica*

	Concentration mg/ml	larva longevity (day)	pupa longevity (day)	male longevity (day)	Female longevity (day)
cold water extract	Control	2.8	3.3	12	13
	1	.18	2.2	4.2	8.6
	3	1.4	1.8	3.2	6.1
	5	3.1	1.4	2.1	5.3
	7	-	-	-	-
chloroform extract	Control	2.8	3.3	12	13
	0.25	2.8	2.6	3.9	8.1
	0.5	3.0	2.4	4.1	6.6
	1	2.4	2.4	2.7	5.8
	2	1.2	2.1	1.5	3.9
L.S.D. for interaction between the extracts and the concentration in age larvae 0.90 L.S.D. for interaction between the extracts and the concentration in age of pupae 0.785 L.S.D. for interaction between the extracts and the concentration in age of male 0.547 L.S.D. for interaction between the extracts and the concentration in age of female 0.81					

**4- Effect the water and chloroform extracts of *A. bioporus* on longevity of immature and adult stages when treating pupae of *M. domestica***

It is noted in Table (4) the effect of the aqueous mushroom extract and chloroform on the period of pupal growth and adults, as the average age of the pupal and

adult females when using the aqueous extract was (2.0, 1.5 and 5.9) days at a concentration of 7 mg / ml, either in the chloroform extract. The duration of ages was (2.1, 1.5 and 5.7) days at a concentration of 2 mg / ml, respectively, compared to the control treatment (3.0, 3.5 and 13) days.

**Table 4.** Effect of cold-water extract of *A. bioporus* on the longevity of pupa and adult that resulted from treatment of pupae of house flies *M. domestica*

	Concentration mg/ml	pupa longevity (day)	male longevity (day)	female longevity (day)
cold water extract	Control	.03	12	13
	1	3.1	3.6	9.1
	3	2.2	3.5	8.1
	5	2.3	2.1	6.3
	7	2.0	1.5	5.9
chloroform extract	Control	3.0	12	13
	0.25	2.6	3.9	8.6
	0.5	2.4	3.2	8.1
	1	2.4	2.2	2.7
	2	2.1	1.5	5.7
L.S.D. for interaction between the extracts and the concentration in age of female 0.843 As for the age of the pupae and the male, there is no moral difference				

It was noted from Table No. (4) that the ages of pupae treated with cold water extract and chloroform of mushroom mushrooms decreased, and the ages of males and females resulting from the treatment of virgins for both treatments, and the results of statistical analysis indicated that there was a significant difference in the results obtained.

A studying showed[28] the effect of *Amanita muscaria* extract on the ages of *M. domestica*, confirmed the age of larvae to adults calculated in days (14.55, 14.85, 14.41, 14.38 and 14.63) days at concentrations (10, 20 and 30).

And 40 and 50%, respectively, compared to the control treatment, which amounted to 14.42 days.

**DISCUSSION**

It was observed when using aqueous extracts and chloroform for *Agaricus bioporus* that the percentage of mortality of the third-instar larval of house flies was higher than the percentage of mortality in cold water extract and chloroform when treating insect pupae, and the reason for the increase in the mortality rates of larvae treated with mushroom extracts may be due to the way the insect was exposed to the extract. As the feeding



factor is a direct factor that helps the insect to grow and increase the size of the body, and this increase appears with an increase in the cuticle area resulting from the absorption of food during the larval stage, as it is another factor that stimulates the continuation of development, growth and increase in size during the life cycle. The activity that the insect passes through from the egg to the adult, and in the case of normal feeding, the larvae devour large quantities of food in order to store a large part of it in their tissues for use in the pupal and adult stages to meet their necessary requirements, especially the basic units for building the body wall and the completion of the growth of the reproductive organs and in reproduction and before they stop. From feeding, to later turn into a pupal, its flexible structure increases as a result of the feeding process, so it grows in size and stops at a late third instar, so it wanders to search for a suitable place in preparation for entering the wandering stage or post feeding larva, and since the insect growth requirements used in the study work with ingestion, that is, it has an infectious effect in addition to the effect on skin contact, and thus an effect of the mushroom extracts occurred when treating the third stage larvae, which led to the larva being exposed to the largest amount of toxic substance by two paths, which are through feeding and contact with the skin, unlike the method of exposure of the pupae that the toxic substance reached the extract used by studying only by touching and not reaching the toxic substance through feeding, because the role of the pupa does not feed, which led to the arrival of a lesser amount of the toxic substance and thus a lower percentage of mortality than in the larvae [29]. The effect of these extracts on insect stages because they contain phenol compounds, toxic substances or other effective compounds that act as feeders, causing the insect to die [30]. The effect of the aqueous extract was higher than the effect of the chloroform extract, the reason may be due to the presence of water receptors in the wall of the insect, which causes easy penetration of water into the wall of the body and thus exposes the insect to the largest amount of toxic substance as a reaction takes place inside the insect's body, compared to the solvent of chloroform that is found. Difficulty penetration into the body of the insect through the body wall.[31]say there was relationship between the concentrations used and the percent of mortality, This relationship differed according to the type of solvent used in the extraction.

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