



## STUDYING IMPACT OF THE VARIOUS DILUTIONS OF THE FAGOUS INFILTRATION FOR *ASPERGILLUS FLAVUS* IN KILLING THE FIRST AGE ADULTS AND LARVAE OF *PLODIA INTERPUNCTELLA* (HUBNER).

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### Abstract

The recent study included the impact of various concentrations from the fagous infiltration of *Aspergillus flavus* in killing the first age larvae and adults of *Plodia interpunctella*. After 24, 48 hours of treatment, the results showed the activity of all concentrations in giving a ratio of killing the first age larvae and adults of *Plodia interpunctella*. These results showed that highest significant difference among the treatments was at (100%) concentration in which the ratio of killing the first age of the larvae (96%, 100%) after 24,48 hours of treatment respectively. The lowest ratio of death of the first age larvae at the 25% concentration. Their killing ratio reached at (8%, 48%) after 24, 48 hours of treatment respectively. Concerning the death of adults, the higher significant difference in killing the adults was at 100% concentration in which the ratio amounted to (92%, 100%) after 24, 48 hours of treatment respectively. The lowest significant difference to death of adults was at the 25% concentration after 24,48 hours of treatment that reached at (12%, 24%), respectively.

**Keywords :** *Plodia interpunctella*, *Aspergillus flavus*, fagous.

### Introduction

*P. interpunctella* also named Indian meal moth is one of the store house dangerous pests. Indian-meal moths feed on large of plants, grains and other human food products and dates, dried fruits, nuts and maize meal well-known Indian meal after which it is taken its name. It can also infest a wide range of food stuffs like flour, vegetable, grapes, dried raisins in branches of some trees and left over of fruit like strawberry, peach and apricot pulps and dates on tree-palm (Ismail, 2016). The young larvae in the first and second instars inserted themselves in to very narrow clefts where they climbed or crept and released then the silks. The economical damage of *P. interpunctella* resulted in feeding directly the stored grains, fruits and dates as well as the seed embryo. The first and second instars of larva is the most dangerous phase of this insect because it fed on all stored stuffs and it discharged its moulting shells and nitrogenous residues on the infested stuffs it is also increased the moisture content in the stored materials and then they decayed. So the stored food became unacceptable taste and invalid for human consumption. The human and animal that feed these stuffs infested by this insect hurt his healthy condition and had a dangerous infection like the tissue fibrosis (Charles *et al.*, 2016). In view of the risk of *P. interpunctella* on the public health, the economical losses due to destroy the stored stuffs and the modern attitudes to stop using the chemocides that polluted the environment when they used to kill the store house insects because their heavy damages in future, the modern studies aimed to use the very biological techniques to kill this pest, and other many pests. In these technique they used the plant extracts and fagous infiltration to limit the proliferating and spreading this insect. In our current study, The different concentration of the *A. flavus* infiltration used for its high toxic effect in killing the first age of adults and larvae of this insect. The *A. flavus* fungus contains aflatoxins, the most dangerous toxicity that decayed the insect bodies and the various living organism. B1G1, B2G2 are the most important kinds of toxins produced by this fungus. They are cancerogenic materials damaged all tissues of living organism and the body wall (Alkaylani, 2011; WHO, 2018).

### The aim of the study

Studying the effect of the various dilutions of the *A. flavus* on the first age larvae and adults insect *P. interpunctella* (Hunber).

### Materials and Methods

#### Method of gathering the insect samples

The samples of the adults and first age larva period for *P. interpunctella* have gathered from date stores by hand method form in Samarra city in 31/7/2018 (Abukhashim, 1992).

#### The culture Media used for growing fungi

Prepare the growth by melting (39)gram of PDA mixture in a distill water bath in a conical flask of (2) litres and put it in water bath and add to it (250)mg of chloramphenicol put the medium in an autoclave in 121C and pressure of 15 pound /inch for 20 minutes. This is used for isolating and purifying the fungi to use in the next experiments (Abdulhamid, 1988).

#### Potato dextrose broth (PDB)

Prepare the medium from cooking (200 gram potato) of potato cutting into small piece with (500ml) of distil water for (2) minutes in a beaker. The cooked potato filtered with a piece of steril gauze and add a 20 grams of dextrose and complete the volume to a litre with distilled water and implant the supernatant in glass flasks of (250) ml and a rate of (150)ml per flask. The media were sterilized by an autoclave at 121C, and pressure of 15 pound /inch for 20 minutes. Use the medium to prepare the fungal supernatant of the fungus *A. flavus* (Abdulhamid, 1988).

#### Potato dextrose broth (PDB)

Prepare the PDB and put it in a conical flask of 250 ml with amount of 15 ml /flask and then add chloramphenicol of 25 mg /ml and incubate each flask three tablets each diameter of 5mm with a cork borer from the edge of the fungal colonies purified on the middle of PDA and extract after 7 days for *A. flavus*. The flasks are incubated at  $\pm 25^{\circ}\text{C}$

considering shaking the flasks each (3-4) days to distribute the fungal growth and after (28) days the vaccine was filtered by using filtration papers and by the air discharge. The re-filtration was done by the accurate filterer. The concentrations (25%, 50%, 75%, 100%) are Prepared from the fungal supernatant *A. flavus* the concentrations of the supernatant are used in the following experiments (Amin, 2007).

Evaluation of the toxin efficacy of different dilutions taken from the fungal supernatant for *A. flavus* isolated in laboratory on growing and developing the adults and first age larva for an insect *P. interpunctella*.

The effect of the fungal supernatant for *A. flavus* on the adults and first age larva for an insect *P. interpunctella* under ( $\pm 27$ ) C and relative humidity 5%. The 25, insects are taken for each concentration of the fungal supernatant for two fungi (25%, 50%, 75%, 100%) at a rate 5ml by using a half liter plastic sprayer. The insects that were put in sterile plastic cans were sprayed at a distance (5cm). The number of dead adults is calculated in each concentration and for each fungal supernatant respectively.

### The statistical Analysis

The data analyzed using (ANOVA) Analysis one way variation by Minitab and the mathematic means of the treatment were Compared by using Duncan's Multiple Range test at level of probability  $P < 0.05$  (Al-Rawi&Abdul-Aziz,1980).

## Results and Discussion

**Table 1 :** Impact of different concentrations taken from *A.flavus* on killing the first age of larvae of the *P. interpunctella* (lepidoptera) after 24, 48 hours of treatment.

% concentration <i>Aspergillus</i> filtration	Percentage of the first age larva		Average
	After 24 hours of treatment	After 48 hours of treatment	
25%	8Aa	48Ba	28aC
50%	40Ab	68Cb	54bB
75%	72Ac	88cD	80cB
100%	96Ad	100Bd	98Cd
Average	54 Aa	76 Bb	0.0

\* The Similar Small Letters In On Column Mean That No Significant Differences Between Them.

\* The Similar Large In On Line Mean That No Significant Differences Between Them.

The results in the table no. (1) Showed that the ratio of killing for the *A. flavus* infiltrated concentrations at (25%) concentration reached at (8%, 48%) (ratio of death) after 24, 48 hours of treatment in comparison with the control sample in which there is no ratio of death in the first age larvae. At the (50%) concentration, ratio of killing is (40%, 68%) after 24, 48 hours of treatment. At (75%) concentration, ratio of killing is (72%, 88%) after 24, 48 hours of treatment. At (100%) concentration, the death ratio amounted to (96%, 100%) after 24, 48 hours of treatment, respectively. We can conclude that the more increasing in the concentration the more killing ratio is the accumulated impact of the toxic materials for the *A. flavus* infiltration in the insect digestive canal led to break down the enzymes responsible for toxicity and its metabolism, well known (MFO) mixed function oxidase. It is also noticed that the duration of exposing to the fungus infiltration has an effective impact in the insect

generation in case. Thus, exposing the first age larvae of the Indian-meal moth to the various fungus infiltration concentration led to malfunction in the larva moulting, therefore the first age larvae couldn't transform to the second age larva, because of the mechanical damage due to exposing the larva to wetting in fungus infiltration. This results in decaying its tissues in the digestive canal breaking the windpipe. Thus, the results of our study are consistent with the finding of (Hamoudi & Al-rahmanny, 2013), who confirm that the higher the concentration of infiltration and the duration of exposure, the killing rate for the first age larvae of the *Culex pipiens* mosquito has increased the fungal filtrate generates very high rates of killing and deformation as a result of the interaction of toxic compounds of the filtration of fungal with the vital system of the cells of the digestive canal with some compounds within the digestive canal, such as fat to metabolize the gut tissue and break the trachea. The results of (Al-Zubaydy *et al.*, 2010) study affirmed the activity of the *A. niger* infiltration in the bio-performance for the *Bemisia tabaci* adults and nymphs. The nymph phase is more sensitive than in the adults due to incomplete its defense devices. The increasing in death ratio, because of spraying by *A. niger* infiltration may return to the fungus toxic kind secreted by these fungi that affected in the bioactivity of its bowels. This is complied with our study results that assured the fungus toxin activity for the fungus infiltration in the larva instar of the Indian-meal moth. Our results in this study agreed with that of (Al-Jubouri, 2007) that proved that the fungus infiltration affected on the insect immature phases more than the adults. The immature instars are more sensitive and influenced by the fungus infiltration than the insect's adults phases because of in completing in its defense devices. The fungus infiltration; therefore influenced in the bio-activities for living organisms. They may stop mechanism of some tissues or killed them or affect in growing and developing the insect. This confirms in our study that showed that the fungus infiltration had an important role in inhibiting the process of growing and developing the first age of larva of the Indian-meal moth to the second age of larva. So its living cycle did not complete.

**Table 2 :** Impact of various concentrations taken from the fungus infiltration *A.flavus* on killing *P. interpunctella* adults (lepidoptera) after 24,48 hours of treatment.

% concentration <i>Aspergillus</i> filtration	Percentage of adults mortality		Average
	After 24 hours of treatment	After 48 hours of treatment	
25%	12Aa	24Ba	18aC
50%	48Ab	76Cb	62bB
75%	68Ac	88cD	78cB
100%	92Ad	100Bd	96Cd
Average	55Aa	72Bb	0.0

\* The Similar Small Letters In On Column Mean That No Significant Differences Between Them.

\* The Similar Large In On Line Mean That No Significant Differences Between Them.

The results in the table 2 showed that the ratio of killing for the concentrations of the fungus infiltration reached 12% at (25%) concentration after 24 hours of treatment, whereas the death ratio arrived at (24%) after 48 hours of treatment compared with the control sample in which there is no death ratio in the adults. At 50% concentration, the death ratio is (48%) after 24 hours of treatment, whereas the killing ratio amounted to (76%) after 48 hours of treatment at the same

concentration. At the 75% concentration, the death ratio showed (68%) after 24 hours of treatment, whereas the ratio of death is (88%) after 48 hours of treatment. But at (100%) concentration the death ratio reached at (92%) after 24 hours of treatment whereas the death ratio is up to (100%) after 48 hours of treatment, respectively. We therefore can concluded that the more increasing in the concentration the more killing ratio is. The infiltration in the digestive canal of this insect led to break down the enzymes responsible for removing the toxicity, well know by (MFO). It is also noticed the continuous exposing to the fungus infiltration has an effective impact in the insect generation in case. Thus, exposing *P. interpunctella* to the different concentrations taken from the fungus infiltration led to malfunction in flying process of this insect, because of the mechanical damage due to exposing the wing veins to wetting .This stops the flying process. Thus, our study results are consistent with (Alwan, 2017) study in which he mentroned to the activity of the *Aloe vera* gel and extract in stopping the flying process of *P. interpunctella* when the insect scales wet by *Aloe vera* extract .Study results also showed that the exposing factor for the fungus infiltration had more effective than the used dose. Thus our study results are agreeable with (Alshukry, 2000) results .He concluded that the continuous exposing *Culex pipiens* to the plant extract led to accumulate the toxic materials for these extracts in the digestive canal of this insect. It is noticed, through our study, breaking down the insect's body wall and breaking its windpipe and blocking its aspiration holes and decaying its neck section and then it died. This returns to the effect of the fungus infiltration that led to weaken in the in specification of the enzymes responsible for removing the toxicity. This results in making the nervous shocks in the insect and then its died. The fungus toxins in this infiltration combined with the insect body's wall cell cytoplasm and consequently the insect is poisoned and died. In addition, this *A. flavus* fungus has capacity of producing the protease, lipase, chitinase that played an important role in breaking down the insect's body wall so our study results complied with the study results of (Hamoudy & Al-rahmanny, 2015) that the different weight activity of *Alternaria alternata* proved killing *Musca domestica* adults .The fatal influence of this fungus returned to its fungus toxin that combined with the insect body wall cytoplasm and consequently led to poison it and then died.

### Conclusions

- Notice that the different concentration impact of the *A. flavus* infiltration was differented. The 100% concentration gave the highest ratio of killing the Indian meal-moth adults and larvae from the first age.
- There is a direct relationship between the exposing factor for the fungus infiltration and killing.
- Using *A. flavus* infiltration is an alternative and ideal technique in the biological control.

### Recommendations

- We commend making other detailed studies for other fungi and knowing their toxicity applied on the different insects and their various phases.

- Acomparative study should do between the toxic activity that produced by these fungi and the chemocides for insects to know which is more effective in the insect resistance.

### References

- Abdulhamid, M.A. (1988). Fungi and fungal toxins .publishing house of university. Cairo. Egypt. 2.
- Abukhashim, N.K. (1992). Practical insects science. second edition. Alfateh University. pp: 74.
- Al-Jubouri, A.N. (2007). Insulation and diagnosis of fungi associated with some insect species and assessment of their parasitic and parasitic ability against insect *Aphid nerii* (Homoptera: Aphididae). Master thesis. Technical College of Almsyb. Bord of Technical education.pp:1.
- Alkaylani, M.I. (2011). Foundations of modern fungi. Ibn alatheer house for printing and silk .University of Mosul. print: 1.
- Al-Rawi, Kashia, M. and Abdul-Aziz, K. (1980). Design and analysis of Statistical experiments. Dar Alkutub for printing & publishing. University of Mosul, 55.
- Al-shukri, B.M. (2000). Effect of horn leaves extracts *Fabricella lutea staph* in some aspects of mosquitos life *Culex pipiens* (Diptera: culicidae) Master thesis. Baghdad University, College of Science.
- Alwan, S.H. (2017). The effect of different concentrations of the peel and gel of *Aloe Vera* plant leaves extract in the decimation of India meal moth adults *Plodia interpunctella*. Dept of Biology, College of Education, University of Sammara. International Journal for Technology 12(1): 52-54.
- Alzubaidy, A.N.; Alsalami, W.M. and Naeas, H.A. (2010). Effect different dilutions from filtrate fungal *Aspergillus niger* in Nympha and adult insect *Bemicia tabaci* (Homoptera) .Al forat Journal of Agricultural sciences. 2(3): 167-183.
- Amin, M.K. (2007). Isolation and identification soil fungi in different regions from Basrah city and its morbidity test an insect whitfly *Bemicia tabaci* and *Aphis fabae* Ph.D. Thesis. Since college University of Basrah.pp2-3.
- Charles, W.; Rutsckhy E. and Calvin, D. (2016). Insect the Indian meal moth *Plodia interpunctella* .They pennsylvania. state University, 1-3.
- Hamoudi, A.M. and Al-Rahmanny, A.H. (2013). Study the effect of the different dilutions from the fungous filtrate of the fungus *Aspergillus flavus* on growth and development of the aqueous Phases in the matured insect of mosquito *Culex pipiens pipiens* L. Culicidae (Forsk.) Second international scientific conference. Agricultural of College. University of karkuk.
- Hamoudi, A.M. and Al-Rahmanny, A.H. (2015). Effect of contaminating the different weights of the fungus *Alternaria alternata* on Adults *Musca domestica* (Diptera: Muscidae). Second International conference. University of Kufa, College of Education, Department of Biology, 1-12.
- Ismaeil, A.Y. (2016). Canned palm pests, *Plodia interpunctella* (Indian moth) 148-147.
- WHO (2018). What is the aflatoxin, 1-2