Insecticidal Mycotoxins against Cockroach *Periplanta americana* (Dictyoptera:Blattidae)

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Abstract

The study includes isolating and identifying the fungus that accompany *P. americana* It has been noticed that there are 12 types of fungi belonging to 8 genus of Deuteromycetes, Zygomycetes and Oomycetes through using the agriculture media P.D.A in laboratory .The results show the secondary metabolites of fungus *Aspergillus flavus* surpassed in a rate of 93.33 % at killing third instar nymphs of *P. americana* after mixing it with food the lowest mortality rate reached 26.66% under the influence of the fungus *Pethium sp.* at concentration 100% after 72 hours of treatment .The results show the secondary metabolites of the fungus *A. terreus* through spray over other treated fungi with a mortality rate of adults reached 86.66 % The lowest mortality rate was 20 % for the fungus *Pethium sp.* at concentration 100% after 72 hours of treatment. The results also show that the fungus *A.terreus* superseded the other treated ones through nurturing the adults of *P.americana* with mortality rate of 93.33%, whereas the fungus *A. plurisepta* has the lowest rate, reaching 73.33% at concentration 100% after 72 hours of treatment, while the concentration 25% of fungus *A. plurisepta* give the lowest rate. In addition , the activities of tow detoxifying enzymes GST and EST in the third instar nymphs and adults increased after infection with secondary metabolites of fungi , the highest activity appeared at 3 days and in the concentration 100% .

Keywords: *Fungus, Periplanta americana, adults*, nymph .

Introduction

P. americana is considered a major insect in Iraq, because it wide spreads in houses and accompanies human beings and their foods (Lee, 2000) . It is a dangerous insect that can carry a lot of serious diseases. It spreads in infected environments, so it carries many types of bacteria, such as Salmonella and Staphylococcus (Facozibe *et al*., 2010). Using chemical insecticides is considered the most important means to put an end to the spreading of this insect. However, overusing such insecticides led to disordering the environmental system besides the resistance these insects develop against such insecticides (Pesticide Resistance) (Khan,2011).Therefore, attention was shifted towards using alternatives that would achieve the same goal and have less impact on environment, one of these alternatives

is using Biological control (Lopes,2011). Entomopathogenic fungi have been recommended since they are effective low risk for human some of these fungi are *Fusarium solani* and *Trichoderma harizanium* using to control *P. Americana* (Abdul Wahid , 2012) . Therefore, the study aims at isolating some fungi that accompany *P. americana* and testing the impact the secondary metabolites of these fungi on third instar nymphs and the adults of cockroaches *P. americana* , and studying their impact in effectiveness of the enzymes , (GST) glutathione S-transferase and (EST) Esterase .

Materials and Methods

1-Insect culture

The adults of *P. Americana* were collected from the houses between July 2015 and September 2015.These cockroaches were raised in laboratory inside dimmed and up perforated plastic containers. The adult and non-adult insects were nurtured with (1:1) mixture of dried milk ,sugar and pieces of bread. Each culture was supplied with a 10 ml bottle filled with water and covered with a piece of cotton dangling towards the bottom of the bottle and serving as a source of water. Food and water were changed every three days. The containers were kept in laboratory conditions in terms of light and temperature (Ez Aldine, 2010) .

2- The Fungi

(12) types of fungi were embayed in the experiment .These fungi isolated from infected adults of *P.americana* derived from the houses . Before the experiment , the fungus were cultured and purificated on (P.D.A) Potato dextrose agar medium for 15 days at 25±2 C° the fungus identified in the microbiology laboratory of College Science, the isolated are kept in refrigerator for use in experiment .

3-Preparing the Results of secondary metabolites for Fungi

The media P.D.B was prepared and distributed in 250 ml flasks, and fertilized with 0.5 cm circles of 7-day age fungi colonies that grew in the media P.D.A. The flasks were incubated at 25±2 C° for two weeks. Then, filtration was conducted with a filter paper from What man Grade.1,and put onto Buchner funnel with air-evacuator assistance. The filtration was replicated using membrane filter (0.22 m), and the concentrations (25%, 50%, 75%, 100%) were attained (Singh and Prakash,2010) .

4-The Impact of Fungal secondary metabolites on third instar nymphs through Nurturing

A mixture of 5 ml of fungal secondary metabolites: 5 gm of dried milk had been prepared and distributed in 5 cm aseptic plastic trays. The trays were put raising containers that have 10 nymphs in three replicates at 100 % concentration. On the other hand, the control was nurtured on 5 ml of liquid media: 5 gm dried milk. The mortality percentage was calculated after 72 hours of treatment (Lopes , 2011).Mortality rate was calculated with (Abbot, 1925) formula and was

corrected in accordance with the Orell and Schneider formula

$$\%\text{corrected mortality rate} = \frac{M.R.T - M.R.C}{100 - M.R.C} \times ١٠٠$$

M.R.T= Mortality Rate in Treatment

M.R.C = Mortality Rate in Control

5-The Impact of Spraying Fungal secondary metabolites on adults

Ten adults had been put in plastic containers (10cm× ١٠cm)whose tops were covered with tulle fabric, and sprayed with 100 ml of filtration in a concentration of 100% with a small aseptic sprinkler. control was sprayed only with distilled water. Then, the treated insects were transferred to other containers where this experiment were replicated three times. The treated containers were kept in incubations at 27±0.5 C° and in shades (Baggio *et al*., 2016) . Mortality rate was calculated .

6-The Impact of Fungal secondary metabolites on adults through Nurturing

Ten adults had been put for all replicate and for each concentration of secondary metabolites, and the applied test method mentioned in paragraph (4) (Lopes,2011). and calculated the mortality rate daily and for 3 days.

7-The Impact of Fungal secondary metabolites on Enzymes

Insect extract was prepared as 1/10 weight (gm)/volume (ml) phosphate buffer (PH = 7.5). followed Oppenorth (1985) method for measuring the enzyme's activity of (GST) and Han *et al*. (1998) for measuring the activity of (EST). Results were corrected and the experiment was replicated three times.

8-Statistical Analysis:

The Results were analyzed in accordance with treatment experiments system, and according to Completely Randomized Design (C.R.D.). Protected Least Significant Difference (L.S.D) was tested under probability level (0.05) to test the significance of differences within treatments.

Results and Argument

1. Fungi Separation and Identification

(12) types of fungi belonging to (8) species have been separated and identified in laboratory using the agriculture media P.D.A table (1) , The majority of these types belong to Deuteromyces compared to Zygomycetes and Oomycetes, because of their ability to produce numerous proliferation units, the small size of these units, the melanin some of them have that protects them in critical situations, and its ability to spread in large areas (Domsch *et al*., 1980). In addition, some of them secrete enzymes that help them survive most environments (Samson *et al*.,1988).The study conforms with Abdul Wahid (2012) who isolated the fungi *F. solani* and *T. harzanium* from *P. americana* adults, and isolated the fungus *Metahrizium anisoplia* of the same insect adults (Shahid *et al.*,2012).

Table (1) The fungi that isolated from adults of *P. americana*

The scientific name	Class
Aspergillus flavus	Deuteromycetes
A. terreus	Deuteromycetes
A. niger	Deuteromycetes
A. plurisepta	Deuteromycetes
A.parasitica	Deuteromycetes
Penicilium coryliphilum	Deuteromycetes
Fusarium moniliform	Deuteromycetes
Rizopus sp.	Zygomycetes
Trichoderma harizanium	Deuteromycetes
Mucor firiformis	Zygomycetes
Alternaria alternata	Deuteromycetes
Pethium sp.	Oomycetes

2.The Impact of secondary metabolites on the third instar nymph stage through Nurturing

Table (2) showed that the impact of Fungal secondary metabolites on the third nymph stage for *P. americana*, where the two fungi *A.flavus and A.terreus* showed the highest killing rate reaching to (93.33%) and (90%) respectively. Whereas the lowest killing rate was 26.66% for the fungus *Pethium sp.*at the concentration 100%, which emphasized the meaningful differences at a meaningful level of 0.05 as well as the positive correlation between duration and mortality rate. For instance, the mortality rate was 43.33% when nymphs exposed to the fungus *A.terreus* after 24 hours of treatment, and increased to 93.33% after 72 hours of treatment. This study conforms with what Kaken (1996) argues that the exposition of the third nymph stage of *P. americana* to secondary metabolism of the fungus *M. anisoplia*e at the concentration of 5.51 mg/l leads to its mortality rate of 45% over two days. The exposition of the fourth nymph stage for *P. americana* to secondary metabolism of the fungus *T. harizanium* at the concentration of 2 mg/l leads to its mortality rate of 86% after seven days (Lopes,2011) .

The Impact of fungal secondary metabolites on Table (2) the third instar nymph

Fungi	Mortality rate %			Average of metabolites
	24 hour	48 hour	72 hour	
A.flavus	٤٣,٣٣	80.00	٩٣,٣٣	72.22
A.terreus	٤٠,٠٠	٧٣,٣٣	٩٠,٠٠	67.77
A.niger	٣٦,٦٦	٧٠,٠٠	٨٠,٠٠	62.22
A.plurisepta	٣٣,٣٣	63.33	٧٦,٦٦	57.77
P.coryliphilum	٢٦,٦٦	56.66	٧٣,٣٣	52.21
F. moniliform	٢٣,٣٣	٥٠,٠٠	٧٠,٠٠	47.77
A.parasitica	٢٣,٣٣	٣٦,٦٦	٦٣,٣٣	41.10
Rizopus sp..	٢٠,٠٠	٣٠,٣٣	٥٣,٣٣	34.55
T. harizanium	١٦,٦٦	26.66	٤٦,٦٦	29.99
M.firiformis	١٣,٣٣	٢٠,٠٠	٤٠,٠٠	24.44
A. alternata	١٠,٠٠	20	٣٣,٣٣	21.11
Pethium sp.	٦,٦٦	16.66	٢٦,٦٦	16.66
Control	0.00	0.00	0.00	٠,٠٠
Average of time	٢٤,٤٤	٤٥,٣٠	٦٢,٢١	43.98

L.S.D (0.05) = ٢,١١

3.The impact of spraying the secondary metabolites on adults

Table (3) showed the secondary metabolism of the fungus *A.terreus* over the other treated in meaningful differences at a killing rate of 30%, whereas the metabolism of *Pethium sp.* had the lowest mortality rate reaching 3.33% during 24 hours. In terms of duration, the metabolism of *A.terreus* had the highest killing rate of 86.66%, and the lowest killing rate was achieved by the metabolism of *Pethium sp.* which reached 20% after 72 hours of treatment. This shows that the longer the period of exposition of the insect to the fungal metabolism, the higher the mortality rate. These results are close to what Inglis (2011) concludes after treating *P. americana* adults with secondary metabolism of the fungus *T.harizanium* at the concentration of 1 ml, where the killing rate reached 70% during fife days. Pete and Gregory (2011) added that using the metabolites of *M.anisoplia* against *Blatella germinica* adults led to its mortality rate of 93.3% after seven days of treatment. Narean *et al.*(2014) achieved the highest mortality rate ranged between 54.98-63.45 after one day of treatment after using the fungus *Hirstella thonopsoni* at the concentration of 3 mg/ml against *P. americana* adults. Most metabolites of the fungi had apparent impact on the rates of immortality, but in different ways. The result of that would be the ability of certain fungi to secrete analyzing enzymes and mycotoxin that affect the activities of insects. They may draw back the fabrics or kill them, or they may affect of evolution and development of the insect (Charnley,2003). This study showed that the secondary metabolites of the fungal had toxic effects on *P. americana* its may be due to surface structure and the chemical composition of insect cuticle (Golebiowski *et al.*, 2011) .

Table(3) The impact of spraying the secondary metabolites on adults

Fungi	Mortality rate %			Average of metabolites
	24 hours	٤٨ hours	72 hours	
A.terreus	30.00	٧٠,٠٠	٨٦,٦٦	62.22
A.flavus	٢٦,٦٦	٦٠,٠٠	٧٦,٦٦	54.44
A.niger	٢٣,٣٣	٥٦,٦٦	٧٠,٠٠	49.99
A.plurisepta	٢٠,٠٠	٤٣,٣٣	٦٦,٦٦	43.33
P.coryliphilum	٢٠,٠٠	٤٠,٠٠	٦٠,٠٠	40.00
F. moniliform	١٣,٣٣	٣٣,٣٣	٥٣,٣٣	33.33
A.parasitica	١٦,٦٦	٢٣,٣٣	٤٦,٦٦	28.88
Rizopus sp..	١٣,٣٣	٢٠,٠٠	٤٠,٠٠	24.44
T. harizanium	١٠,٠٠	٢٠,٠٠	٣٦,٦٦	22.22
M.firiformis	٦,٦٦	١٦,٦٦	٣٠,٠٠	17.77
A. alternata	٣,٣٣	١٦,٦٦	٢٦,٦٦	15.55
Pethium sp.	٣,٣٣	١٠,٠٠	٢٠,٠٠	11.11
Control	0.00	0.00	0.00	0.00
Average of time	14.35	31.53	47.17	33.60

L.S.D (0.05) = ٢,٧١

4. The Impact of concentrations of some fungal secondary metabolism on adults through Nurturing

Table (4) showed that the secondary metabolism of the fungus *A.terreus* showed the highest mortality rate reaching 93.33% at concentration of 100%, whereas the lowest mortality rate was achieved by the fungus *A.plurisepta* reaching 73.33%. Mortality was eliminated at control treatment. It points at the meaningful differences at meaningful level of 0.05 as well as at the positive correlation between concentration and mortality rate. Further, this type of correlations is also apparent between duration and mortality rate. For instance, the mortality rate was 83.33% at concentration of 100% and after 24 hours of treatment with the metabolite of *A.terreus*, but it increased to 93.33% after 72 hours. The current results conform with what Lopes (2011) concludes, after exposing *B.germinca* adults to metabolism of the fungus *Fusarium solani* at concentration of 1 ml when this led to its mortality rate of 86% after seven days. Golebiowsk (2011) argues that the exposition of *B.germinca* to metabolism of *B.bassiana* at concentration of 0.06 ppm led to its mortality rate of 80% in ten days . Inglis (2011) found that exposing P. americana to the leaky fungus *T. harzanium* at concentration of 1 ml led to its mortality rate of 100% after seven days. From this, we conclude that it is possible to use fungi as poisoning baits resisting *P. american.* In addition, these fungi, including *A.flavys* which is known for its production of aflatoxins, affect insects when they nurturing on them and cause them to die (Shahid *et a*l.,2012) . This results conforms to what Abdul Wahid (2012) concluded , that using poisoning baits having secretions of the fungi *pennicillium* , *Aspergillus* and *fusarium* led to the mortality of *P. americana* . However , in comparison with impact of spraying it was found that the fungal metabolites uses as nurturing were more efficient in control of *P. americana* , The result of that would be to amount of the spraying metabolites that reach to insect had lowest compared with the nurturing (Khalaf and Aylan ,2011)

. Table(4) The Impact of concentrations of some fungal secondary metabolism on adults through nurturing

Fungi	Mortality rate %								Average of metabolites
	٢٤ hours				48 hours				
	concentrations								
	٢٥	٥٠	٧٥	١٠٠	٢٥	٥٠	٧٥	١٠٠	
A.terreus	٢٣,٣٣	٣٠,٠٠	٥٠,٠٠	٨٣,٣٣	33.33	63.33	80.00	٩3.33	45.83
A. niger	١٦,٦٦	٢٦,٦٦	٤٠,٠٠	٨٠,٠٠	30	56.66	76.66	٨٠,٠٠	50.83
A.plurisepta	١٣,٣٣	٢٠,٠٠	٣٦,٦٦	٧٣,٧٧	23.33	46.66	66.66	٧٣,٣٣	44.21
Control	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Average of concentration	13.33	19.16	31.66	59.27	21.66	41.66	55.83	39.16	46.95

L.S.D (0.05) = 4.02

5.The impact of secondary metabolites on the Activity of EST Enzyme

The results of the study showed meaningful differences in activity of the EST compared to control group, table (5) .The highest activity was at 3 days ,This increase in the activity of the enzyme may relate to cells destructions resulted from being exposed to the effect of poison and the release of enzyme form these cells. This is what Fan *et al*.(2013) argued when exposing the larvae of *Dendrolimus tabulaeformis* to *P. americana*. The largest increase in the activity of this enzyme when the larvae are exposed to metabolism of *A. flavus* at concentration of 100% , whereas the activity of the enzyme was less when treating the adult insects table(6). The reason of this may lie in that poison permeability into the insect bodies the insect peeled and the establishing of body wall after peeling, because the insect will be in critical case between peeling the old skin and building the new one , EST has been to be most importan enzyme protect insect against attack from metabolites of entomopathogenic fungi (Ez Aldine, 2010 ; Gunase *et al*., 2011) .

6.The Impact of secondary metabolites on the Activity of GST Enzyme

The results in table (5) showed that the impact of secondary metabolism of the fungi on the activity of enzyme GST was meaningful, as the enzyme activity increased in all treatments, especially the metabolites of fungus *A. flavus* was used. The reason of this increase may relate to the increase of the needs of the insect for this enzyme to remove the poisonous of these combinations (Zibae *et al*., 2009). Whereas when treating the adults table(6) . the nymphs were more affected than the adults, which points at that the age of insect has a great role in determining the efficiency of fungi(Fan *et al* ., 2013) .

Table (5)The Impact of secondary metabolites on the on the Activity of EST and GST Enzymes of third instar nymp

Fungi	EST	GST
A.flavus	0.07±5.90	0.12±6.02
A.niger	0.22±5.85	0.14±5.90
A.plurisepta	0.20±5.70	3.23±5.80
P.coryliphilum	0.09±3.90	0.16±5.75
Control	0.22±4.20	0.24±4.20

L.S.D (0.05) = 1.62

Table (6)The Impact of secondary metabolites on the on the Activity of EST and GST Enzymes of adults

Fungi	EST	GST
A.flavus	0.50±3.45	0.22±4.20
A.niger	0.36±2.90	0.22±3.22
A.plurisepta	0.05±2.86	0.49±2.90
P.coryliphilum	0.22±2.27	0.25±2.74
Control	0.11±1.17	0.12±1.17

L.S..D (0.05) = ٠,٨٣

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