ANTIMICROBIAL ACTIVITY OF THREE INSECT SPECIES, CRUDE EXTRACTS AGAINTS CERTAIN MICROBIAL AGENTS.

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Abstract

The aim of the present study was to investigate the antibacterial and antifungal activities of crude extracts of the desert locust, Schistocerca gregaria, the cotton leaf worm, Spodoptera littoralis and the oriental hornet, Vespa orientalis using different solvents; methanol, petroleum ether and chloroform against Gram-positive bacteria; Micrococcus luteus and Staphylococcus aureus, Gram-negative bacteria, Pseudomonas aeruginosa and Escherichia coli and fungi; Penicillium chrysogenum and Candida albicans.

Preparation of crude insect extracts from the whole body of the three species were firstly washed with 80 % ethanol as a disinfectant, then rinsed in distilled water, excess water was removed by filter paper, then homogenized in 10 ml of the first solvent, centrifuged at 13,000 r.p.m. for 30 min.at 4ºC, supernatant was removed and used as first solvent extract, residue was soaked in the second solvent then centrifuged, The previous step was carried out with the remain solvent as mentioned before. All solvent extracts were dried by evaporation of solvents for usage in the antimicrobial activity tests.

A strong effect against Gram-positive bacteria Micrococcus luteus and Staphylococcus aureus was observed by the extraction of desert locust by petroleum ether as an inhibition zone recorded 30±0.1mm and 32±0.4 mm, respectively. While the inhibition zone effect of the desert locust and the oriental hornet extracted by methanol was 23±0.2mm, 26±0.44mm and 24±0.26mm, 24±0.64mm, respectively. While the oriental hornet extracted by petroleum ether was 26±0.18 mm, 25±0.51mm. On the other hand, Escherichia coli inhibited by the oriental hornet extracts, while Pseudomonas aeruginosa inhibited by two oriental hornet extracted by methanol, petroleum ether and the desert locust extracted by chloroform, respectively. While all extracts except the oriental hornet extracted by chloroform have antifungal activity against Penicillium chrysogenum.

Keywords: *Antimicrobial activity, Crud extract, Insect, bacteria and fungi.*

Introduction

The growing problem of antibiotic resistance by antimicrobial, demands the research for novel compounds, especially from natural sources **(Pemberton, 1999; Costa-Neto, 2002, 2005a, b; Feng *et al*., 2009)** they evaluate the antimicrobial activities of insect body extracts by. Generally insects regarded as pests with little thought ever given to the benefits of insects in their lives not just as a source of honey as food, silk for clothing and pollination of plants they are especially important natural products being extracted from insects and the knowledge of their physiological processes is used to benefit people's lives **(Leather, 2009)**. In terms of huge numbers and diversity, it is not surprising that they should possess very effective immune systems producing powerful antimicrobial factors, for example insects feeding on decomposing corpses, as locust, cotton leaf worm and large numbers of social insects such as oriental hornets. Microorganisms such as bacteria and fungi are developing resistance to the current antibiotics. Available antibacterial and antifungal agents are very much costly and toxic too. So the current shift to the use of whole body insect extract as antibacterial and antifungal agents may be more effective, economic and safe.

More than 900 therapeutics products were isolated from insects. Many pharmacologically active components in insects include peptides, enzymes, and biogenic amines that cause toxic reactions. They are wide spectrum antimicrobial and anti-inflammatory **(Krell, 1996).** Some insects controlled microbial infections show a great interest in Public Health, for this reason the development of new and better antimicrobial compounds are in need.

The aim of this study was to monitor the antimicrobial potentiality of the desert locust, *Schistocerca gregaria,* the cotton leaf worm*, Spodoptera littoralis* and the oriental hornet*, Vespa orientalis* whole body extract dissolve in methanol, petroleum ether and chloroform against Gram-positive bacteria**,** *Micrococcus luteus, Staphylococcus aureus*, Gram-negative bacteria, *Escherichia coli*, *Pseudomonas. aeruginosa* and the fungi, *Candida albicans* , *Penicillium.chrysogen*.

Materials and Methods

Collection of insects:

Collection of locust: The desert locust, *Schistocerca gregaria* was reared according to **(Hunter-Jones, 1961)** in wood formed cages (60 x 60 x 70 cm) with10-15% humidity, photoperiod (12 L: 12 D) and an ambient temperature (32±2oC) and feeding on fresh clean leaves of clover *Trifolium alexandrinum* . The desert locust reared at laboratory of Entomology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt.

Collection of cotton leaf worm: The cotton leaf worm, *Spodoptera littoralis* was reared according to **(Bergomaz and Boppre´, 1986)**. Egg patches were kept in the laboratory under constant laboratory conditions of 27 + 2oC and 70 + 5% R.H. The hatched larvae provided daily with fresh castor bean leaves *Ricinus communis*.The cotton leaf worm was reared at the laboratory of Entomology Department, Plant Protection Research Institute, Ministry of Agriculture, Giza, Egypt.

Collecting of oriental hornet: The oriental hornets *Vespa orientalis* were collected by sweep net from White Canyon, Taba, South of Sinai. The collected specimens where identified according the key of Goulet and Huber (1993).

Extraction:

Preparation of crude insect whole body were extracted by three different solvents according to the methods of **Miyanoshita *et al*., (1996) and Meylaers *et al*, (2002**).

Microbe's culture:

Bacterial culture: Nutrient agar medium, gm/L**,** consisted of: peptone, 5.0; beef extract, 3.0; and agar-agar, 15.0 the pH was adjusted to 7.0 before sterilization. **(Tadashi, 1975)**

Fungal culture: Sucrose-Nitrate agar medium, gm/L consisted of: sucrose, 30; NaNO3, 2.0; K2HPO4, 1.0; mgSO4. 7H2O, O.5; agar, 15.0 and distilled water, 1000 ml. The pH value was adjusted at 7-7.3 before sterilization **(Tadashi, 1975).**

Determination of the antibacterial inhibition zone:

The synthesized compounds were evaluated for their antimicrobial activity using the agar diffusion technique **(Lu *et al*, 2007).**

Results

Antimicrobial activity:

Data recorded that, the desert locust, *Schistocerca gregaria,* the cotton leaf worm*, Spodoptera littoralis* and the oriental hornet*; Vespa orientalis* extracts showed variable antimicrobial potentiality against Gram-positive; bacteria, *Micrococcus luteus* and *Staphylococcus aureus*, Gram-negative bacteria; *Escherichia coli* and *Pseudomonas Aeruginosa*. Fungus; *Candida albicans* and *Penicillium chrysogen* strains. The control was DMF (Dimethylformamide solvent) showed no antimicrobial activity against the tested microorganisms.

Antibacterial activity on Gram- positive:

Data given in table (1) showed that the antibacterial activity of the desert locust, the cotton leaf worm and the oriental hornet extracted by three different solvents against *Micrococcus luteus*. Data showed that the highest effect attained by the cotton leaf worm extracted by petroleum ether recording 31±0.18mm and the desert locust extracted by petroleum ether recording 30±0.1mm.While the rest of extracts showed a variable efficacy rate. The oriental hornet extracted by methanol was 26±0.44mm> the oriental hornet extracted by petroleum ether was 26±0.18mm>the cotton leaf worm extracted by methanol was 24±0.1mm> the locust extracted by methanol and chloroform were 23±0.2mm> 21±0.6mm > the cotton leaf worm extracted by chloroform was 20±0.5mm > the oriental hornet extracted by chloroform was 18±0.34mm. On the other hand the antibacterial activity of attained by the desert locust, the cotton leaf worm and the oriental hornet extracted by three different solvents against *Staphylococcus aureus* showed that the highest effect was the locust extracted by petroleum ether (32±0.1mm), followed by the cotton leaf worm extracted by petroleum ether (31±0.18mm).While the rest of extracts showed a variable efficacy rate results as the oriental hornet extracted by petroleum ether was 25±0.51mm> the oriental hornet extracted by methanol was 24±0.64mm> the locust extracted by methanol was 24±0.26mm> the cotton leaf worm extracted by methanol was 22±0.18mm> and chloroform was20±0.26mm> the locust

extracted by chloroform 20±0.21mm > the oriental hornet extracted by chloroform was 17±0.3mm.

Table (1): Antibacterial activity indicated by growth-inhibition zone, mm (Mean± SD) with different crude of the desert locust, the cotton leaf worm and oriental hornets extracted by methanol, petroleum ether and chloroform against (Gram-positive) bacteria.

Samples	Gram-positive bacteria (Mean± SD)	
	Micrococcus luteus	***Staphylococcus aureus***
Lm	23±0.2	24±0.26
Lp	30±0.1	32±0.4
Lc	21±0.6	20±0.21
Cm	24±0.1	22±0.18
Cp	31±0.24	31±0.18
Cc	20±0.5	20±0.26
Om	26±0.44	24±0.64
Op	26±0.18	25±0.51
Oc	18±0.34	17±0.3
DMF	NA	NA

Lm: locust methanol extracts.
Lc: locust chloroform extract.
Lp: locust petroleum ether extract.
Cm: cotton leaf worm methanol extract.
Cp: cotton leaf worm petroleum ether extract.
Cc: cotton leaf worm chloroform extract.
Om: oriental hornet methanol extract.
Oc: oriental hornet chloroform extract.
Op: oriental hornet petroleum ether extract.
DMF: Dimethylformamide control.
SD: standard deviation.
NA: negative activity.

Antibacterial activity on Gram-negative:

Data given in table (2) showed the antibacterial activity of of the desert locust the cotton leaf worm and the oriental hornet extracted by three different solvents against *Escherichia coli*. Data showed a strong effect of the oriental hornet extracts which recorded that the oriental hornet extracted by methanol was 37±0.1mm > petroleum ether was 29±0.6mm> and chloroform was19±0.45mm.While the rest of extracts showed no action. On the other hand the antibacterial activity against *Pseudomonas aeruginosa* showed a promising effect on the oriental hornet extracted by methanol was 18±0.17mm> the locust extracted by chloroform was 14±0.5mm> the oriental hornet extracted by petroleum was 13±0.35mm.while the rest of extracts showed no action.

Table(2): Antibacterial activity indicated by growth-inhibition zone , mm (Mean± SD) with different crude extracts of the desert locust, the cotton leaf worm and oriental hornets extracted by methanol, petroleum ether and chloroform against (Gram-positive) bacteria.

Samples	Gram-negative bacteria (Mean± SD)	
	Escherichia coli	***Pseudomonas. aeruginosa***
Lm	NA	NA
Lp	NA	NA
Lc	NA	14±0.5
Cm	NA	NA
Cp	NA	NA
Cc	NA	NA
Om	37±0.1	18±0.18
Op	29±0.6	13±0.35
Oc	19±0.45	NA
DMF	NA	NA

Lm: locust methanol extracts.
Lc: locust chloroform extract.
Lp: locust petroleum ether extract.
Cm: cotton leaf worm methanol extract.
Cp: cotton leaf worm petroleum ether extract.
Cc: cotton leaf worm chloroform extract.
Om: oriental hornet methanol extract.
Oc: oriental hornet chloroform extract.
Op: oriental hornet petroleum ether extract.
DMF: Dimethylformamide control.

SD: standard deviation.
NA: negative activity.

Antifungal activity on yeast:

Data given in table (3) showed the antifungal activity of the desert locust the cotton leaf worm and the oriental hornet extracted by three different solvents against *Candida albicans* showed no action. On the other hand *Penicillium chrysogenum* fungal strain showed excellent effect of two oriental hornet extracts recorded that the oriental hornet extracted by methanol was 27±0.4mm and petroleum ether was 25±0.54mm. While the locust extracted by chloroform recorded was 25±0.11mm. And the rest of extracts showed a variable efficacy rate results as the locust extracted by methanol was19±0.15mm > and petroleum ether was 18±0.21mm> the cotton leaf worm extracted by petroleum ether was18±0.13mm> the cotton leaf worm extracted by chloroform was18±0.6mm> methanol was16±0.32. And Oriental hornet chloroform extract showed no action.

Table (3): Antifungal activity indicated by growth-inhibition zone, mm (Mean± SD) with different crude extracts of the desert locust, the cotton leaf worm and oriental hornets extracted by methanol, petroleum ether and chloroform against two fungus strains.

Samples	Fungus (Mean± SD)	
	Candida albicans	***Penicillium chrysogenum***
Lm	NA	19±0.15
Lp	NA	18±0.21
Lc	NA	25±0.11
Cm	NA	16±0.32
Cp	NA	18±0.13
Cc	NA	18±0.6
Om	NA	27±0.4
Op	NA	25±0.54
Oc	NA	NA
DMF	NA	NA

Lm: locust methanol extracts.
Lc: locust chloroform extract.
Lp: locust petroleum ether extract.
Cm: cotton leaf worm methanol extract.
Cp: cotton leaf worm petroleum ether extract.
Cc: cotton leaf worm chloroform extract.
Om: oriental hornet methanol extract.
Oc: oriental hornet chloroform extract.
Op: oriental hornet petroleum ether extract.
DMF: Dimethylformamide control.
SD: standard deviation.
NA: negative activity.

Discussion

In agreement with the present result **Mohtar *et al.* (2014),** showed that the insect crude extracted by methanol have antibacterial activity against Gram-positive bacteria namely; *Staphylococcus aureus*. The positive results of the desert locust, *Schistocerca gregaria* and the oriental hornet *Vespa orientalis* extracts in the present study against *Micrococcus luteus* were corresponded with that by **Valachova *et al.*, (2014)** where they observed a strong antibacterial activity of *Lucilia sericata* maggots crude extract against *Micrococcus luteus*.

In present study, the *Pseudomonas aeruginosa* and *Escherichia coli* as Gram- negative bacteria were inhibited by methanol oriental hornet extract; this result was hassling with the effect of whole body of the supermeal worm, *Zophobas morio* larvae extracted by methanol against *Pseudomonas aeruginosa* and *Escherichia coli* by **Mohtar *et al.* (2014).** In addition, the *Pseudomonas sp.* bacteria also inhibited by whole body of the black soldier fly larval, *Hermetia illucens* extract by **Park *et al*. (2015), w**hile **Meylaers *et al*., (2004)** disagreed with the present result. Where they showed that whole body housefly maggots extract didn't show any activities against *Escherichia coli.*

The present study has also shown that the *Penicillium chrysogenum* was sensitive strain to the different insect whole body extracts. In agreement with these results, **Meylaers *et al*., (2004)** observed the antifungal activity of housefly maggots whole body extracted by methanol against *Sachloroformharomyces cerevisiae*.

It was observed that the most effective extracts was the oriental hornet extracted by methanol then petroleum ether followed by a strong action

of desert locust extracted by chloroform while the methanol and petroleum ether extracts showed mild activity against fungi *Penicillium chrysogenum*.

Generally the present study showed that, the whole body of the desert locust, the cotton leaf worm and the oriental hornet extracted by methanol, petroleum ether and chloroform caused antibacterial and antifungal activities against the different microorganisms tested. While **Hou *et al*., (2007)** reported that the extract of the housefly larvae showed higher activity against Gram- positive bacteria than Gram-negative bacteria and did not show any antifungal activity.

Finally the activity of many insect body extracts against bacteria and fungi have also been recorded. For example: the flesh fly, *S. peregrina* **(Yamada and Natori, 1994),** the European bumble bee, *Bombus pascuorum* **(Rees *et al*., 1997),** the saw fly, *Acantholyda parki* **(Leem *et al*., 1999),** the mosquito vector, *Anopheles gambiae* **(Vizioli *et al*., 2001)** and the wax moth, *Galleria mellonella* **(Cytrynska *et al*., 2007).**

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