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Evaluation of some agricultural waste extracts against mosquito larvae, and some types of microorganisms as insecticidal and antibiotic agents.

Jayda, G. Eldiasty[1,2]; Mosaad, M. Hassan[3] and Omnia, M.H.M. Kamel[1,4]

1- Biology Department, Faculty of Science, University of Tabuk, Saudi Arabia
2- Botany Department, Faculty of Science, University of Damietta, Damietta, Egypt
3 -Protective Medicine, Ministry of Health, Tabuk, Saudi Arabia & Department of Entomology, Faculty of Science, Ain Shams University, Cairo, Egypt
4- Department of Applied Chemistry, National Research Centre, Dokki, Cairo, Egypt
E-mail: gida_hamada@yahoo.com
galdiasti@ut.edu.sa,

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ABSTRACT

Extracts of pomegranate peel and apricot kernel were evaluated against bacteria, fungi and insect. Pomegranate extract appear potency when used against bacteria (*Escherichia coli, Pseudomonas aeruginosa, Morganella* sp, *Micrococcus* sp, *Staphylococcus aureus*), fungi (*Fusarium* sp., *Alternaria alternata*, *Penicillium* sp. And *Aspergillus niger*) and yeast (*Candida albicans*), but apricot was effective only on three types of fungi (*Fusarium* sp, *A. alternata* and *A. niger*) but not effective against any type of bacteria and yeast (*Candida albicans*) at concentrations which used in this study.

Evaluation of pomegranate and apricot extracts appear different degree of potency against *Culex pipiens* larvae, apricot extract more effective than pomegranate extract. The potency was increased when make binary mixture of both extracts. Apricot extract appear also effect on life cycle of mosquitoes by decrease pupation percent and adult emergence percent comparing with control.

INTRODUCTION

Mosquitoes (Diptera: Culicidae) are among the most serious insect pests of medical importance. They are vectors of various disease agents some of which cause millions of cases of illnesses and deaths in human and animal each year. Among these diseases, malaria, yellow fever, dengue and dengue hemorrhagic fever, filariasis and Rift Valley fever at endemic and epidemic areas in many countries (WHO, 1991, Lerdthusnee, *et al.,* 1995 and Madani, *et al.,* 2003). The use of chemical insecticides for controlling pests and vector of diseases is undesirable, save and yet effective methods of control are being sought. The use of natural products of plants origin is a new trend that preserves the environment and can be applied effectively by using techniques more sui Table for developing countries (Abbassy, 1998; Mohamed *et al.,* 2003 and Kamel *et al.,* 2005).

Infectious diseases are still one of the leading causes of death in the world. Although conventional drugs provide effective treatment for some infections, antibiotic resistance continues to grow among key microbial pathogens such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Streptococcus* spp and Enterobacteriaceae (Bax *et al*., 2000; Bhavnani and Ballow, 2000).

The plant fungal diseases have been controlled by chemical fungicides. The development of resistant strains of pathogens against various chemical fungicides (Lin, 1981 and Witte, 1998) and their harmful effects on soil biosphere and causing health hazards for humans and animals which found to pose carcinogenic risk due to their residual toxicity (Anonymous, 1998 and Sarmamy, 2001) make the use of these chemicals limited.

Plants are the gifts of nature used to cure number of human diseases (Deepa *et al*., 2012). Plant extracts show antibacterial effects (Sarmamy and Al-Juboory, 2005) and antifungal activity against wide range of fungi (Aba Alkhail, 2005; Basm and Khalil, 2007; Sarmamy and Saleem, 2009). Some authors using agricultural waste materials and plastic waste to decrease the cost of production of natural insecticides, antimicrobial agent and convert the waste material to benefit one (Bakr *et al*., 2008, El-Maghraby *et al.,* 2012 and Fahim, *et al.,* 2013).

The present study was undertaken to: a) test the potency of several waste plant extracts against the larval stage of mosquito (*Culex pipiens*) and some types of microorganisms (bacteria & fungus b) analyze the joint action toxicity resulting from mixing botanical extracts with conventional insecticides, c) evaluate the latent effect of waste extract on the developmental stages of *Culex pipiens*.

MATERIALS AND METHODS

Test microorganisms and culture preparation:

Gram negative bacteria *Escherichia coli*, *Pseudomonas aeruginosa*, *Morganella sp*. Gram positive bacteria *Staphylococcus aureus*, and *Micrococcus* sp. were obtained from "Culture Collection of Antibiotic Resistant Microbes (CCARM)" Military Hospital Tabuk. The yeast *Candida albicans* was obtained from (CCARM)" Military Hospital Tabuk, *Aspergillus niger* was isolated from infected onion bulbs, *Alternaria alternata* and *Fusarium sp*. Isolated from infected tomato fruits while *Penicillium sp*. isolated from infected citrus fruits and pure cultures of the isolated fungi were identified on the basis of morphological and microscopically characteristics according to the key of (Bessy, 1968 and Barnett and Hunter, 1972). The bacterial strains were cultured on nutrient agar (NA) medium at 37°C, and fungal strains on potato dextrose agar (PDA) medium at 28°C (Imtiaj and Lee. 2007). The yeast strain *Candida albicans* has been maintained at 4°C on Sabouraud Dextrose Agar (SDA) plates and subcultured at 25°C in Sabouraud Dextrose Broth (SDB) before each experiment to ensure viability and purity (Sha Fighi *et al*., 2012).

Antimicrobial assay

Determination of antibacterial and antifungal (yeast) *Candida albicans* activities:

Antimicrobial activity of the crude extracts was determined by Agar well assay methods as described by (Collins *et al*., 1995, NCCLS 1999, Moshi *et al*., 2006 and Rojas *et al*., 2006). The inoculum size of each group of bacteria and yeast were prepared by using nutrient broth to give a concentration of 1×10^8 bacteria and 1×10^6 yeast per milliter. The suspension (100µl) was spread onto the surface of Mueller Hinton Agar (MHA) medium. Wells (5 mm in diameter) were cut from the agar with a sterile borer and 50µl extract solutions were delivered into them. Negative controls were prepared using sterile distilled water, gentamycin, was used as positive reference standards to determine the sensitivity of each

microbial species tested. The treated plates were stored in a refrigerator at 4°C for at least six hours to allow diffusion of the extracts into the agar while arresting the growth of the test microbes (Ndyetabura *et al*., 2010). The plates were then incubated for 24-48 hours at 37°C. Antimicrobial activity was determined by measuring the diameters of inhibition zones (DIZ) in mm. All tests were performed in triplicates and the developing inhibition zones were compared with those of the reference discs. The means and standard deviations (±SE) of (DIZ) was done.

Determination of antifungal activity:

Concentrations of 0, 2, 5, 10, 15 and 20% of the raw extracts were prepared and added to the sterilized (PDA) medium, mixed well then 20 ml of the mixture (PDA medium + plant extract) were poured in each 9 cm sterilized Petri dishes. The medium without extract was served as control 0%. Mycelial discs of fungi were prepared using a cork borer (5mm in diameter) from the margin of 5 days old culture of the tested fungi and placed at the center of Petri dishes after solidification of PDA medium. Each treatment was replicated three times. Plates were incubated in an incubator at 28 °C. Fungal growth after 48, 96 and 168 hrs was measured by taking the mean of the two diameters taken at right angles for each colony (Sarmamy *et al*., 2011).

Tested compounds:

The tested plant wastes were washed to avoid dusts and dirt then lift to dry under shade in the laboratory. Dried part of plants (Pomegranate peel and Apricot kernel) were cut into small pieces and ground in an electric grinder. Hundred grams of the resulting powdered materials of each plant were exhaustively extracted with ethanol absolute, following the method described by Kamel *et al.*, (2005b).

Tested mosquitoes:

***Culex pipiens* (Culicidae: Diptera).**

Provided by collecting from Tabuk area and transferred to the research laboratory of Biology Department-Science Collage-Tabuk University where self-perpetuating colonies were established and maintained during the present study, according to the method described by Kamel *et al.*, (2005a). Late third larval instars were used for toxicological studies.

Toxicological studies:

Efficiency of plant extracts:

Preliminary, toxicological bioassay tests were carried out to the selected plant extracts on tested insects as a modification for the method described by (Wright, 1971 and Kamel *et al*., 2005b) their LC_{50} and LC_{95} values were determined as well as their slope function, according to Finney, 1971 and WHO, 1981). Bioassay tests were carried out to Deltamethrin and Altosid on the tested insect.

Joint action of selected waste extracts:

The selected waste plant extracts were mixed with each other, and with either IGR or Pyrethroid at a level of their corresponding LC_{25} values. The tests were carried out as mentioned before. The combined action of the different mixtures was expressed as the co-toxicity factor which was estimated according to the equation given by (Kamel *et al.,* 2005a).

Effect of sublethal treatments of selected waste plant extract and Deltamethrin on some biological activities of *Culex pipiens*:

The 3rd instar larvae of *Culex pipiens* (500 larvae) were exposed to sub lethal dose of the most potent plant extract and Deltamethrin. Survived larvae after twenty-four hours post treatment were gently washed and transferred to labeled pans.

Treated larvae and controls were kept under laboratory conditions were maintained under laboratory conditions till adult emergence. The percentage of pupation and adult emergence were calculated.

RESULTS AND DISCUSSION

Antimicrobial studies:

The antimicrobial activity of the ethanolic extract of Pomegranate (*Punica granatum*) and Apricot (*Prunus armeniaca*) were determined against the linear growth of four fungi *Fusarium* spp, *Alternaria alternata*, *Aspergillus niger* and *Penicillium* spp. (Plate 1) and determination of (DIZ values) for five bacteria used *Escherichia*

coli, *Pseudomonas aeruginosa*, *Morganella sp*, *Micrococcus sp*, *Staphylococcus aureus* and yeast (*Candida albicans*) at different concentrations, (Plate 2).

Effect of Pomegranate (*Punica granatum*) extract on studied fungi

The different Pomegranate extract concentrations affected significantly the mycelial growth of the studied fungi. In the case of *Fusarium sp* and *Alternaria alternata* (Table 1) all pomegranate extract concentrations (0.5-5%) had antifungal activity against two fungi and reduced their mycelia growth (6.33-19 mm) and (7.33-13 mm) compared with control 34.66 mm and 30.66 mm after 3 days of inoculation. The highest antifungal activity was recorded at concentration of 2 % and no growth occurred at concentration 5% at 9 day for the two fungi respectively. Results presented in (Table 2) show that *Penicillium sp* was more sensitive more than *Aspergillus niger* to pomegranate extract. The mycelial growth was 22.33 mm and 59 mm, respectively in comparison with 83 mm and 85.66 mm for the control at 9 day of the two fungi, respectively.

Table 1: Effect of Pomegranate extract on the growth of *Fusarium sp* and *Alternaria alternata*. Each value is the mean of 3 replicates ± S.E.

Treatments		Pomegranate Extract (%)	
Time (day)	Conc. (%)	*Fusarium* sp Mycelial growth (mm)	*Alternaria alternate* Mycelial growth (mm)
		Mean ± S.E	Mean ± S.E
3	0	34.66 ± 0.88	30.66 ± 0.33
	0.5	19 ± 0.57	13 ± 0.57
	1	17.33 ± 0.33	9 ± 0.00
	1.5	8.66 ± 0.33	7.33 ± 0.33
	2	6.33 ± 0.66	0 ± 0.00
	5	0 ± 0.00	0 ± 0.00
6	0	76 ± 0.57	54.66 ± 0.33
	0.5	39.66 ± 0.33	33.33 ± 0.88
	1	24.66 ± 0.88	23 ± 1.52
	1.5	14 ± 0.57	17 ± 1.15
	2	8.5 ± 0.28	12.33 ± 0.33
	5	0 ± 0.00	0 ± 0.00
9	0	84.66 ± 0.33	85 ± 0.00
	0.5	55 ± 0.00	50.33 ± 0.33
	1	33.33 ± 0.88	28.33 ± 0.88
	1.5	22.33 ± 0.33	25.66 ± 0.33
	2	14 ± 0.57	19.33 ± 0.33
	5	0 ± 0.00	0 ± 0.00

Table2: Effect of Pomegranate extract on the growth of *Penicillium sp* and *Aspergillus niger*. Each value is the mean of 3 replicates ± S.E.

Treatments		Pomegranate Extract (%)	
Time (day)	Conc. (%)	*Penicillium sp* Mycelial growth (mm)	*Aspergillus niger* Mycelial growth (mm)
		Mean ± S.E	Mean ± S.E
3	0	52.66 ± 1.45	65 ± 2.88
	5	47 ± 1.52	61.33 ± 0.88
	10	5.5 ± 0.28	39 ± 0.57
	20	0 ± 0.00	8 ± 1.15
	30	0 ± 0.00	0 ± 0.00
6	0	71.33 ± 0.88	77.66 ± 0.33
	5	65 ± 1.73	71.33 ± 0.88
	10	18.33 ± 0.33	65 ± 2.51
	20	0 ± 0.00	24 ± 1.73
	30	0 ± 0.00	0 ± 0.00
9	0	83 ± 0.00	85.66 ± 0.33
	5	78.33 ± 0.88	80 ± 0.00
	10	22.33 ± 0.66	73.33 ± 0.33
	20	0 ± 0.00	59 ± 1.52
	30	0 ± 0.00	0 ± 0.00

Time of incubation affected the growth of mycelia significantly. (Figs. 1 and 2) show that all concentrations of pomegranate extract at the three times affected mycelia growth of *Fusarium sp* and *Alternaria alternata* and the inhibitory effect significantly increased with the increase in the concentration. It seems that the interactions between time of incubation and extract concentrations (Figs. 3 and 4). At day 3, it was observed that pomegranate extract significantly reduced the liner growth of *Penicillium sp* and *Aspergillus niger* with the increase in the concentrations. The linear growth continued to increase after 6 days of inoculation with different concentrations and at day 9, the highest antifungal activity was recorded at concentration of 10 % and 20 % for the two fungi, respectively. It seems that *Penicillium sp* and *Aspergillus niger* are more sensitive to high concentrations of pomegranate extract while no growth occurred at 20% and 30% of the two fungi, respectively.

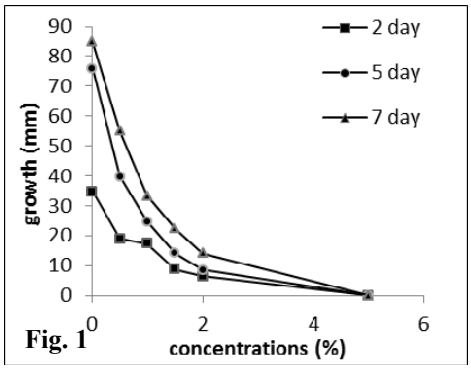


Fig. 1

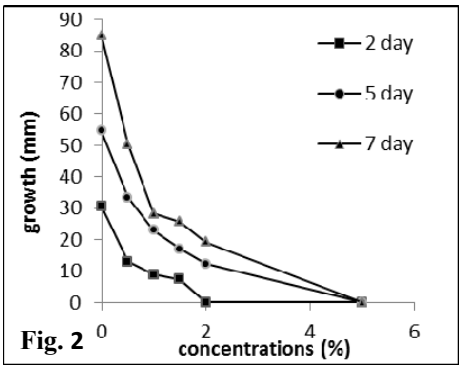
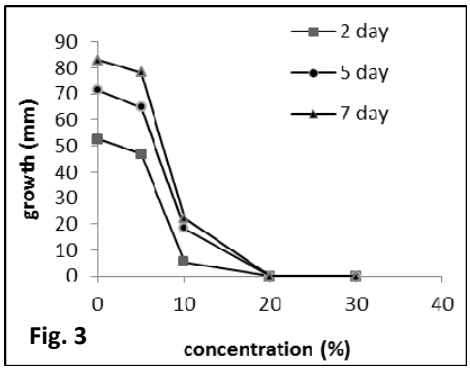


Fig. 2



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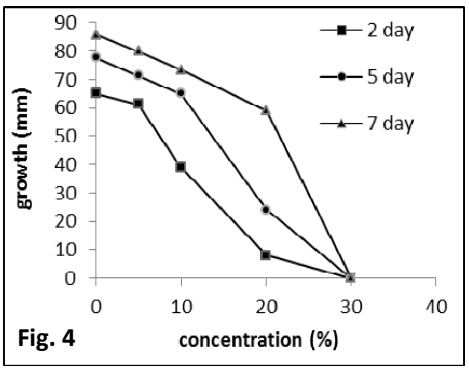


Fig. 4

Figs. 1-4: Effect of interactions between conc. of pomegranate extract and time of incubation on mycelial growth of *Fusarium sp* (Fig. 1), *Alternaria alternata* (Fig. 2), *Penicillium sp* (Fig. 3) and *Aspergillus niger* (Fig. 4).

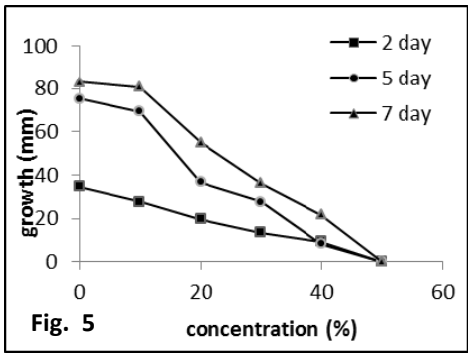
Effects of Apricot (*Prunus armeniaca*) extract on studied fungi

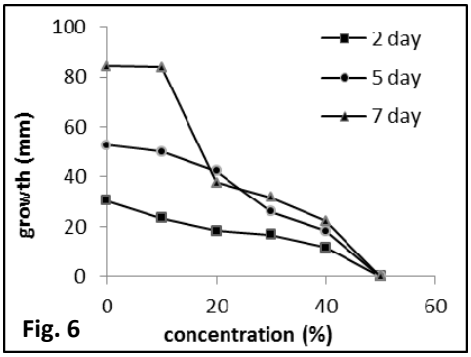
Results presented in (Table 3) show that the extract of apricot at all concentrations affected significantly the growth of mycelia of *Fusarium sp*, *Alternaria alternata* and *Aspergillus niger* except *Penicillium sp* was resistant to apricot extract at all tested concentrations. The mycelial growth of previous sensitive fungi reduced to (8.33-69.33 mm), (18.33-50 mm) and (47.66-74.33 mm) at 6 day in comparison with 75.33 mm, 52.66 mm and 75 mm for control of three fungi, respectively.

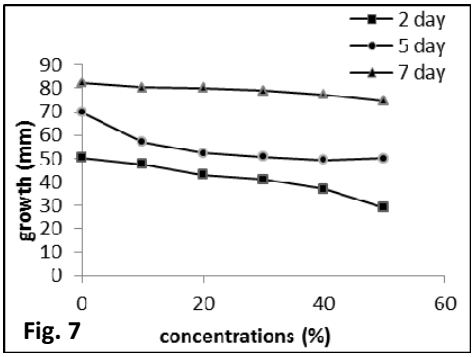
Figs. (5-8) show the interaction between time of incubations and apricot extract concentrations, at day 9, the highest effect was in concentration 40 % with 21.66 mm, 22.33 and 52.33 mm compared with 83 mm, 84.33 mm and 85 mm for control of three fungi, respectively. Apricot extract at 50 % concentration, continued to be most effective inhibitor of the growth of the studded fungi while no growth occurred.

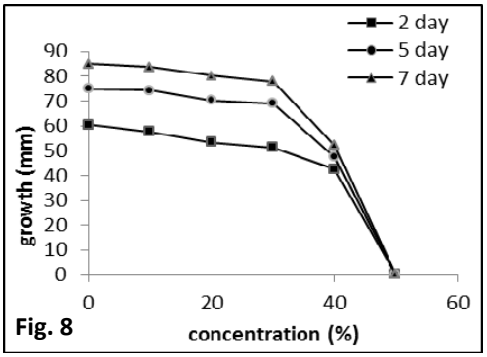
Table 3: Effect of Apricot extract on the growth of *Fusarium sp*, *A. alternata*, *A. niger* and *Penicillium sp*. Each value is the mean of 3 replicates ± S.E.

Treatments		Apricot Extract (%)			
Time (day)	Conc. (%)	*Fusarium sp* Mycelial growth (mm)	*A. alternata* Mycelial growth (mm)	*Aspergillus niger* Mycelial growth (mm)	*Penicillium sp* Mycelial growth (mm)
		Mean ± S.E	Mean ± S.E	Mean ± S.E	Mean ± S.E
3	0	34.66 ± 0.33	30.33 ± 0.33	60.33 ± 0.33	50.33 ± 0.33
	10	22.66 ± 0.66	23.33 ± 0.33	57.66 ± 0.33	47.66 ± 0.88
	20	19.66 ± 1.20	18.33 ± 0.88	53.33 ± 0.88	43 ± 1
	30	13.33 ± 0.88	16.66 ± 0.33	51.33 ± 0.88	41 ± 0.58
	40	9.33 ± 0.33	11.66 ± 0.33	42.66 ± 0.88	37 ± 0.00
	50	0 ± 0.00	0 ± 0.00	0 ± 0.00	29 ± 0.57
6	0	75.33 ± 0.88	52.66 ± 0.33	75 ± 0.00	69.66 ± 0.33
	10	69.33 ± 0.33	50 ± 0.00	74.33 ± 0.33	57.33 ± 0.00
	20	36.66 ± 0.33	42.33 ± 0.88	70.33 ± 0.33	52.33 ± 0.88
	30	27.66 ± 0.33	26 ± 0.57	69 ± 0.58	50.66± 0.33
	40	8.33 ± 6.33	18.33 ± 0.88	47.66 ± 0.33	49.33 ± 0.33
	50	0 ± 0.00	0 ± 0.00	0 ± 0.00	50 ± 0.00
9	0	83 ± 0.00	84.33 ± 0.66	85 ± 0.58	82.33 ± 0.33
	10	80.66 ± 0.88	84 ± 0.57	83.66 ± 0.33	80.33 ± 0.33
	20	55 ± 0.57	37.66 ± 0.33	80.33 ± 0.00	80 ± 0.00
	30	36.33 ± 0.33	31.66 ± 0.66	78 ± 0.57	79 ± 0.57
	40	21.66 ± 1.20	22.33 ± 0.67	52.33 ± 0.33	77.33 ± 0.88
	50	0 ± 0.00	0 ± 0.00	0 ± 0.00	74.66 ± 0.33









Figs. 5-8: Effect of interactions between conc. of Apricot extract and time of incubation on mycelia growth of *Fusarium sp* (Fig. 5), *Alternaria alternata* (Fig. 6), *Penicillium sp* (Fig. 7) and *Aspergillus niger* (Fig. 8).

Extract of Pomegranate were more effective on studied fungi than apricot extract and caused significant reduction in mycelial growth for the fungi. This may be due to the differences between the chemical components such as volatile oils, tannin and amygdalin (Mertens *et al*., 2006 and Paaverurve & Raal, 2010). The antimicrobial activities of phenolic compounds may involve multiple modes of action. For example, degrade the cell wall, interact with the composition and disrupt cytoplasmic

membrane, damage membrane protein, interfere with membrane integrated enzymes (Nychas, 1995; Sha Fighi *et al*., 2012 and Hassan *et al.,* 2013). The results are in agreement with the study of (Sarmamy *et al*., 2011) who concluded that ethanol extract of pomegranate was more effective on *A. niger* and *Penicillium sp*.

Effects of Apricot (*Prunus armeniaca*) and pomegranate (*Punica granatum*) extract on bacteria and *Candida albicans:*

The antibacterial activity of seven different concentrations of Apricot and pomegranate extract (1,2,5,10,20,30,40 and 50 %) and Gentamycin (10 µl), the later used as positive control against *Escherichia*. *coli*, *Pseudomonas aeruginosa*, *Morganella sp*, *Micrococcus sp*, *Staphylococcus aureus* and antifungal activity of *Candida albicans* were recorded after 24 hr. of incubation. In this study it is observed that apricot extract did not show any antibacterial effects against used bacteria or antifungal effect against *Candida albicans* however, pomegranate extract inhibited the growth of all used bacteria and *Candida albicans* and the inhibition zones ranged between 12-33 mm. Table (4) shows that high antibacterial effect was recorded on *Morganella sp* and *Micrococcus sp*, followed by *Staphylococcus aureus* and less effect was recorded for *Escherichia coli, Pseudomonas aeruginosa* and *Candida albicans*. As shown in (Fig. 9 and Plate 2). The inhibition zone increased proportionally with the increase in pomegranate concentration.

Table 4: Bacteria and *Candida albicans* sensitivity to different concentrations of Pomegranate extract. Each value is the mean of 3 replicates ± S.E.

Bacteria	Pomegranate extract (%)								
	Inhibition Zone (mm)								
	+ve Control	**-ve control**	**1**	**2**	**5**	**10**	**20**	**30**	**40**
Escherichia. coli	38.16 ± 0.16	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	12.33 ± 0.33	20.66 ± 0.33	21.26 ± 0.14	23.33 ± 0.33
Pseudomonas aeruginosa	21.67 ± 0.33	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	5.5 ± 0.28	20.33 ± 0.33	22.66 ± 0.33	22.67 ± 0.33
Morganella sp	29 ± 0.0	0 ± 0.00	17.33 ± 0.33	20.5 ± 0.28	21.33 ± 0.33	25 ± 0.00	26.67 ± 0.33	30 ± 0.00	30.66 ± 0.33
Micrococcus sp.	26.66 ± 0.33	0 ± 0.00	17 ± 0.00	20.66 ± 0.33	22 ± 0.00	25.33 ± 0.33	31 ± 0.00	33 ± 0.57	33.67 ± 0.33
Staphylococcus aureus	23 ± 0.00	0 ± 0.00	0 ± 0.00	17.33 ± 0.33	23.33 ± 0.33	27 ± 0.00	30.33 ± 0.33	31.66 ± 0.33	32 ± 0.00
Candida albicans	28.83 ± 0.17	0 ± 0.00	0 ± 0.00	0 ± 0.00	0 ± 0.00	4.33 ± 0.33	12.33 ± 0.33	13 ± 0.00	17.33 ± 0.33

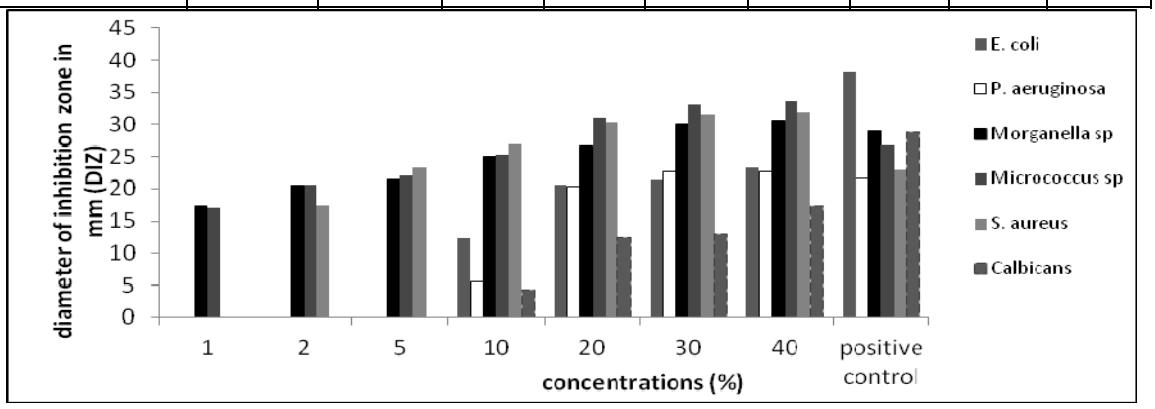


Fig. 9: Effect of different concentrations of Pomegranate extract on bacterial cultures and *C. albicans.*

The high inhibitory effect was observed with extract concentration between (5-40 %) of *Morganella sp*, *Micrococcus sp* and *Staphylococcus aureus* with DIZ values of (21.33-30.66 mm), (22-33.67 mm) and (23.33-32 mm), respectively. The DIZ values for *Escherichia coli* and *Pseudomonas aeruginosa* were between (12.33-23.33 mm) and (5.5-22.67 mm) at concentration of (10 - 40 %), respectively. For *Candida albicans* the DIZ values were between (4.33 – 17.33 mm) at concentration (10 – 40 %).

These findings are in accordance with the observations of (McCarrell *et al*., 2008), who found that aqueous extract of pomegranate rind inhibits growth of *S. aureus* and *P. aeruginosa*. Similarly, (Al-Zoreky, 2009) has reported that metanolic extract of pomegranate fruit peels is a potent inhibitor for *S. aureus*, *Listeria monocytogenes, E. coli* and *Yersinia enterocolitica*. Ahmet Duman *et al*., 2009 also reported the in vitro antibacterial activity of pomegranate extract against bacteria *P. aeruginosa, S. aureus, E. coli* showing inhibition zones ranging from 13-26 mm, however Similar results were recorded in our study. (Hassan *et al*., 2013) reported that *Punica granatum* significantly contributed to antibacterial activity, these included *E. coli, Staphylococcas aureus* and *Streptococcus faecalis*. (Nuamsetti *et al*., 2012) found that extract of Pomegranate fruit peels exhibited inhibitory activity against bacteria *Bacillus subtilis, Salmonella typhimurium* and *E. coli*. Moreover, Punicalagin showed strong activity against *Candida* spp. (Endo *et al*., 2010). In contrast, the study of (Abtahi *et al*., 2008) reported the antibacterial activity of bitter apricot extract against several bacterial strains and this might be brought by amygdalin, alkaloids, flavanoids, tannins and phenolic compounds.

In the present study, extract of pomegranate has been tested against bacteria (*S. aureus* and *P. aeruginosa*) as well as against pathogenic yeast, *C. albicans*. The *S. aureus* is responsible for a wide variety of diseases, including pneumonia, skin and soft tissue infections, and diabetic foot infections (Shorr, 2007). Similarly, *P. aeruginosa* is a common pathogen associated with burn wound infections, keratitis, and respiratory tract infections (Marquart *et al*., 2005). Under the conditions employed here, the bacteria were found to be sensitive to extract.

Insecticidal studies:

Evaluation of the larvicidal activity of waste plant extracts:

The waste plant extracts studied are available in large quantities to reduce the cost of production. The other principal criterion in the selection of plant species was their continuous usage due to their medicinal properties. Improvement the insecticidal properties was achieved on the following experiments.

Larvicidal activity of waste plant extracts against *Culex pipiens* larvae:

The results are represented in Table (5) and the regression lines are presented in (Fig. 10). The confidential limits of each of the tested plant extract were statistically calculated for LC_{50} and LC_{95} at P= 0.05. The LC_{50} values for ethanolic extract of pomegranate peel and apricot kernel are 1253.9 and 138.7ppm, respectively.

Table 5: Larvicidal activity of tested plants against *Culex pipiens* larvae:

Plant	LC_{50} (Co. Limits)	LC_{95} (Co. Limits)	Slope Function
pomegranate peel	1253.9 (1177.7 – 1335.1)	2330.9 (2052.2 – 2647.8)	6.1
apricot kernel	138.7 (123.6 – 155.7)	429.2 (338.6 – 544.7)	3.4

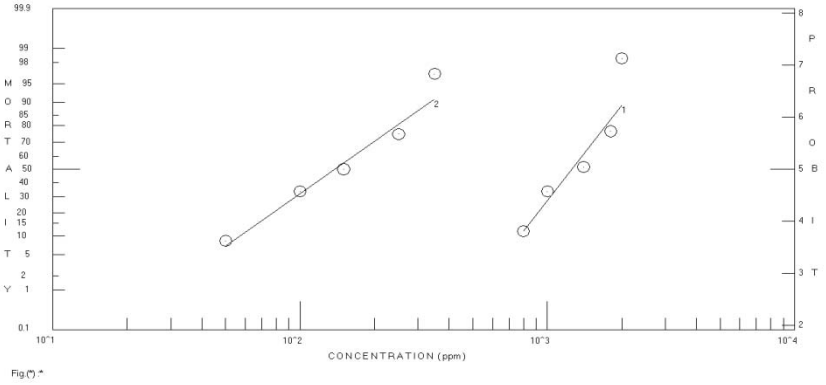


Fig. 10: Susceptibility of *Culex pipiens* larvae to waste extracts

The present data showed that despite the differences in potency of pomegranate peel and apricot kernel. They were found to possess parallel regression lines. This may suggest that these extracts have the same mode of action against the tested insect larvae (Busvine, 1971). The results showed in (Table 6) and (Fig. 11) represent the susceptibility of *Culex pipiens* to traditional insecticides (Deltamethrin & Altosid), although the potency of extracts are less than chemical insecticides but they more safe (Mann and Koufman, 2012) and conversion of waste material to natural beneficial insecticide (El-Maghraby, *et al.*, 2012).

Table 6: Larvicidal activity of Deltamethrin and Altosid against *Culex pipiens* larvae**:**

Insecticide	LC_{50} (Co. Limits)	LC_{95} (Co. Limits)	Slope Function
Deltamethrin	0.00031 (0.00027 – 0.00036)	0.0011 (0.00087 – 0.0015)	2.9
Altosid	0.0031 (0.0027 – 0.0036)	0.013 (0.0097 – 0.018)	2.6

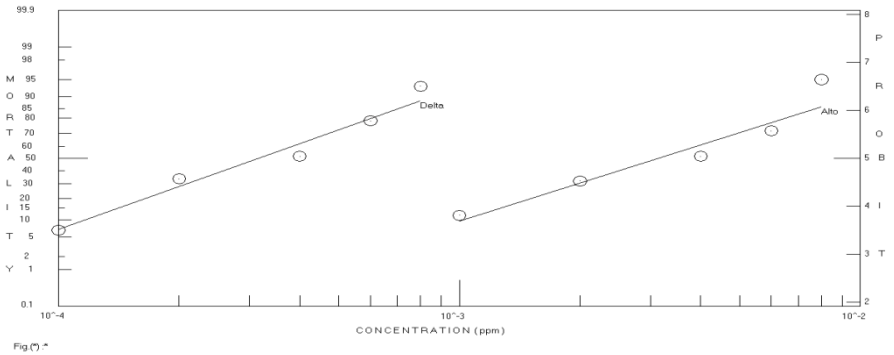


Fig. 11: Susceptibility of *Culex pipiens* larvae to Deltamethrin and Altosid insecticides

Joint action of waste plant extracts on *Culex pipiens* larvae:

The waste plant extracts were individually mixed with each other or with Altosid and Deltamethrin in a ratio of 1:1 at the level of LC_{25} of each. Thus, a total of 5 binary mixtures are prepared. The data in the (Table 7) and (Fig. 12) indicated that, potentiation effect was induced in all mixtures except Pomegranate extract with Altosid insecticide give additive action. Similarly variations on the joint action effect on the plant extracts and insecticides were demonstrated by (Barakat *et al.*, 1984 -85;

Mesbah *et al*., 1990 and El-Bokl and Moawad, 1997). Moreover, several authors have used the plant extracts to synergize the insecticidal activities of the traditional insecticides (Mohan *et al*., 2010 and Mansour *et al*., 2011).Variation in the joint action effect of the different insecticides binary mixtures could be attributed to the site and mode of action of the mixture components. Therefore the potentiation and additive effects may be attributed to that the mixture components which have the same site or/and mode of actions (El-Bokl and Moawad, 1997).

Table 7: Joint action analysis for the waste plant extracts with Deltamethrin or Altosid (IGR) or with each other mixed at LC_{25} levels.

Mixture components	Observed mortality%	Co-toxicity factor	Joint action
Pomegranate & Apricot	75	50	Potentiation
Pomegranate & Deltamethrin	90	80	Potentiation
Pomegranate & Altosid	60	20	Additive
Apricot & Deltamethrin	95	90	Potentiation
Apricot & Altosid	65	30	Potentiation

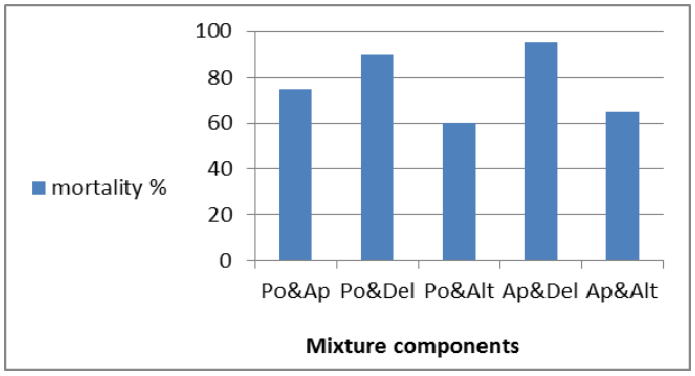


Fig. 12: Joint action analysis for the waste plant extracts together either with Deltamethrin or Altosid (IGR) at LC25 levels.

Effect of larval treatment with Apricot kernel extract of the development of *Culex pipiens*:

The purpose of this experiment is evaluating the potency of extract by latent effect on developmental stages of *Culex pipiens*. The results represent in (Table 8) showed decrease in pupation and adult emergence in Apricot kernel extract and Deltamethrin treatments comparing with control. Both treatments showed highly significant reduction in the percentage of pupation.

Table 8: Efficiency of Apricot kernel extract and Deltamethrin on pupation and adult emergence of *Culex pipiens*:

Contribution	Control	Apricot extract	Deltamethrin
Pupation	476.6± 5.2* 95.32%	209.7 ± 2.6* 55.92% 0.05** $2.6X10^{-29}$***	191.7 ± 2.6* 51.12% 0.05** $8.2X10^{-30}$***
Adult emergence	470 ± 2.4* 98.61%	39.6 ± 3.5* 18.88% 0.3** $1.6X10^{-30}$ ***	152.3 ± 2.6* 79.45% 0.8** $1.7X10^{-34}$***

*= Mean & Standerd Erro **= F value ***= T value

Estimation of adult emergence percentage is based on the ratio of the total number of emerged adults to the total number of pupae. The results showed highly significant reduction in Apricot extract while showed significant reduction in Deltamethrin treatments comparing with control.

Effect of Apricot kernel ethanolic extract on developmental stages of *Culex pipiens* is resembled to Deltamethrin effect on pupation but the apricot extract more effective on adult emergence than Deltamethrin. These results may be due to the similarity between deltamethrin structure and some components of extract which phytochemical analyzed by (Talabani *et al*., 2012).

The authors concluded that the pomegranate extract is the promising agent against bacteria and fungi while the apricot extract is effective for mosquitoes and some pathogenic fungi. The authors recommended that the promising extracts need more investigations to know the mode of action and their physiological effect on target mosquito and microorganism. Also, semi field studies operate to know their effect on mosquitoes breading sites.

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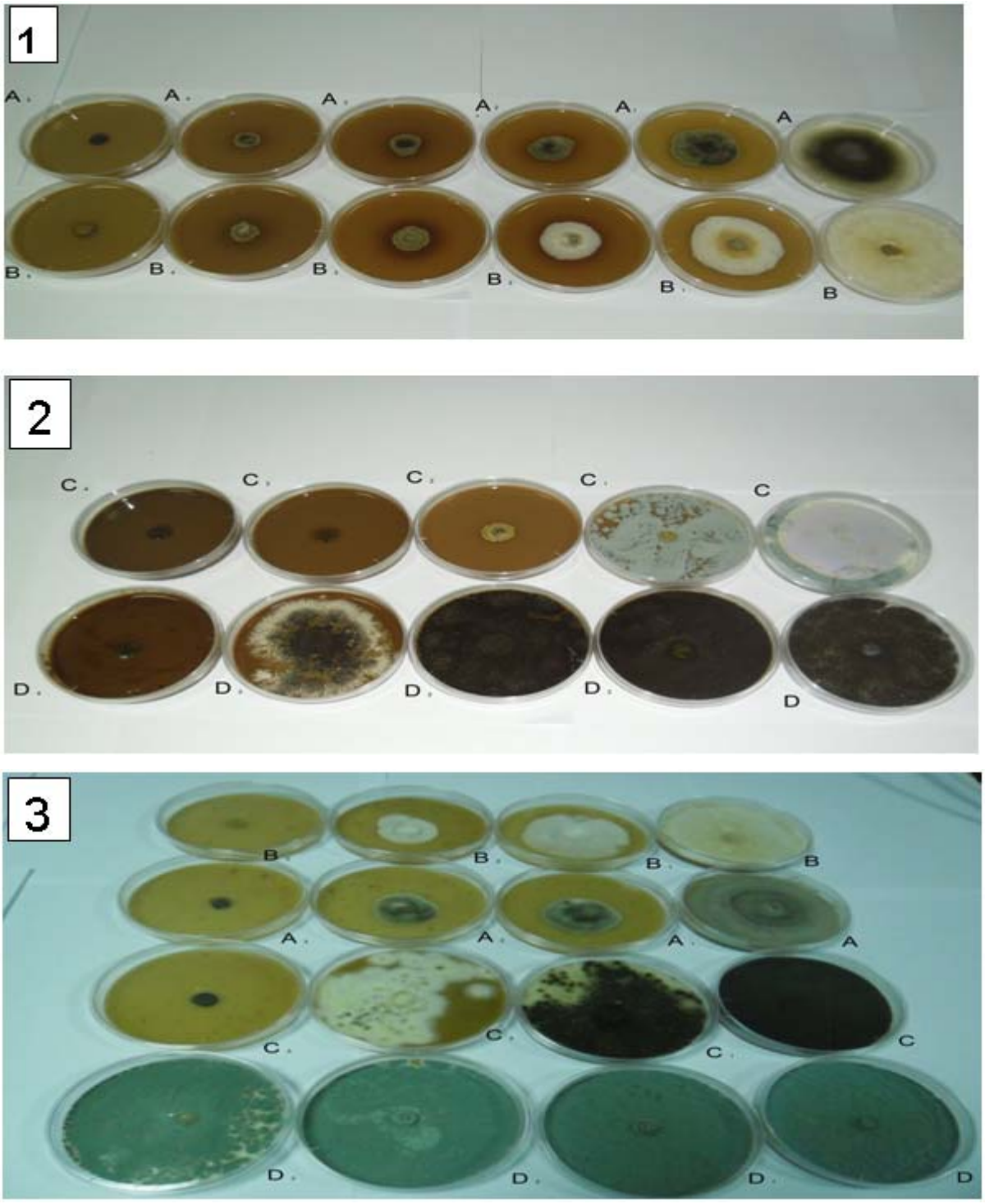

Plate 1: Inhibitory effect of pomegranate extract (1 and 2) and Apricot extract (3) on the mycelial growth of *Alternaria alternata* (A), *Fusarium sp* (B), *Penicillium sp* (C) and *Aspergillus niger* (D). A, B, C, D indicates control (fresh PDA) of respective fungi. A1-A5, B1–B5, C1-C5 and D1–D5 contain different concentrations of extracts as shown in Tables (1-3).

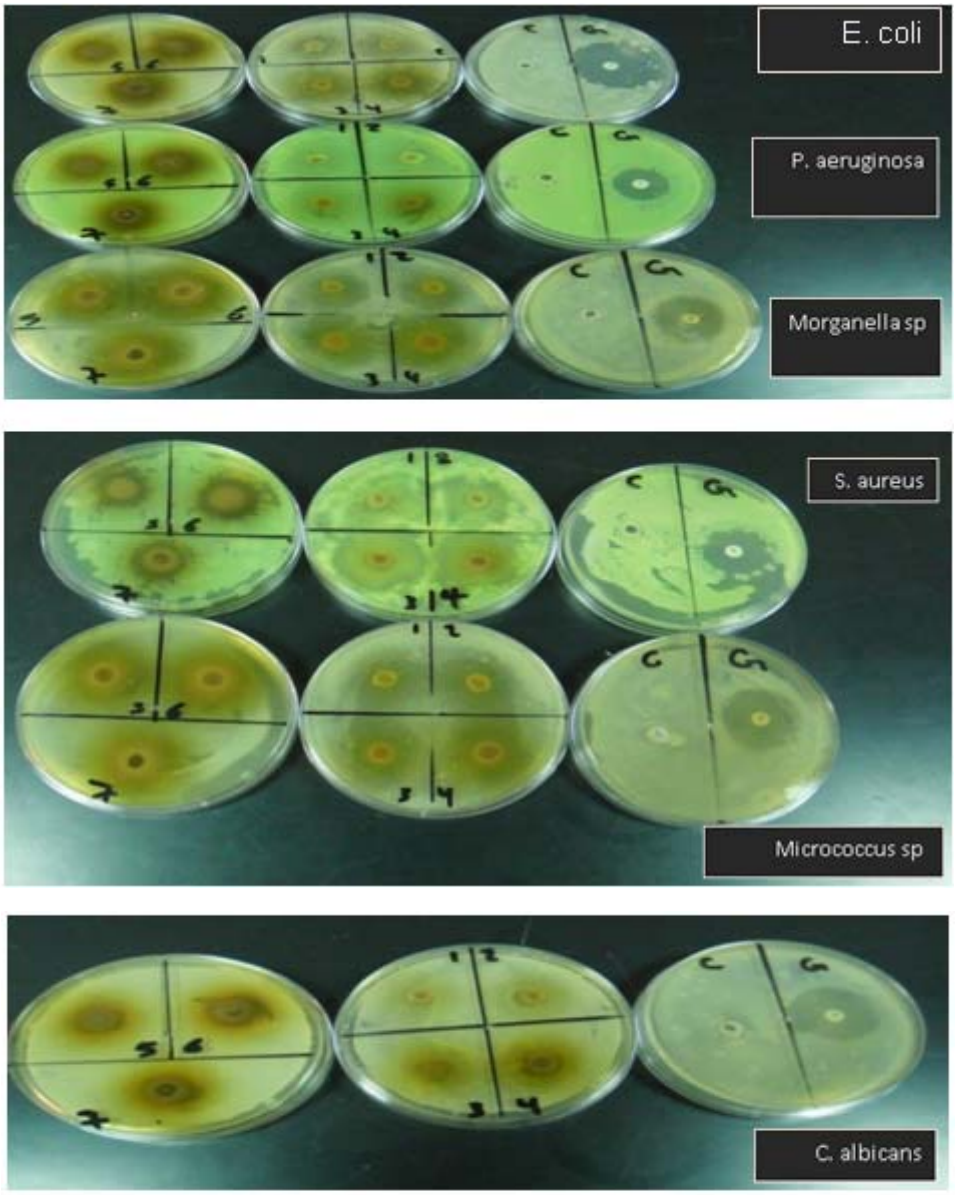

Plate 2: Inhibition zones of Pomegranate extract on bacteria and *C. albicans*. G and C indicate positive and negative control. (1-7) indicate different concentrations of Pomegranate extract on tested bacteria as shown in Table (4).

ARABIC SUMMARY

تقييم مستخلصات بعض المخلفات الزراعية ضد يرقات البعوض وبعض الكائنات الدقيقة الممرضة

جيدى الدياسطي[1,2]؛ مسعد حسن[3]؛ أمنية كامل[1,4]

1- قسم الاحياء، كلية العلوم، جامعة تبوك، المملكة العربية السعودية

2- قسم النبات – كلية العلوم – جامعة دمياط ، ج م ع

3- الطب الوقائي، وزارة الصحة، تبوك، المملكة العربية السعودية وقسم علم الحشرات، كلية العلوم، جامعة عين شمس، القاهرة، ج م ع

4- قسم الكيمياء التطبيقية، المركز القومي للبحوث، الدقي، القاهرة، ج م ع

تم تقييم مستخلصات كحولية من قشر الرمان ونواة المشمش كمضادات بكتيرية وفطرية وايضا كمبيدات حشرية ولقد اثبت مستخلص قشر الرمان كفاءة كمضاد بكتيري لكل انواع البكتريا المستخدمة في هذه الدراسة وهي (ايشريشيا كولاي – بسيدوموناس أرجينوسا – مورجانيلا – ميكروكوكس – ستافيلوكوكس) واثبت كفاءة كمضاد فطرى على (نوع فيوزاريوم- الترناريا الترناتا- اسبيرجيللوس نيجر – نوع البنسليوم) وعلى الخمائر مثل فطر كانديدا البيكانس في حين لم يظهر مستخلص نواة المشمش كفاءة كمضادات بكتيرية وفطر الخميرة كانديدا البيكانس للتركيزات المستخدمة في هذه الدراسة إلا كمضاد فطرى على (نوع فيوزاريوم- الترناريا الترناتا- اسبيرجيللوس نيجر) .

وعند تقييم مستخلصات قشر الرمان ونواة المشمش كمبيدات حشرية على يرقات البعوض كيوليكس بيبينز وجد ان المستخلصين لهما تأثير مميت على يرقات البعوض بدرجات متفاوتة. وجد ان مستخلص نواة المشمش اكثر كفاءة من مستخلص قشر الرمان وادى خلط المستخلصين معا إلى زيادة كفاءة المخلوط.

ووجد ايضا تأثير لمستخلص نواة المشمش على دورة حياة البعوض وذلك بانخفاض نسبة التعزر ونسبة خروج الحشرة الكاملة مقارنتا بالعينة القياسية.