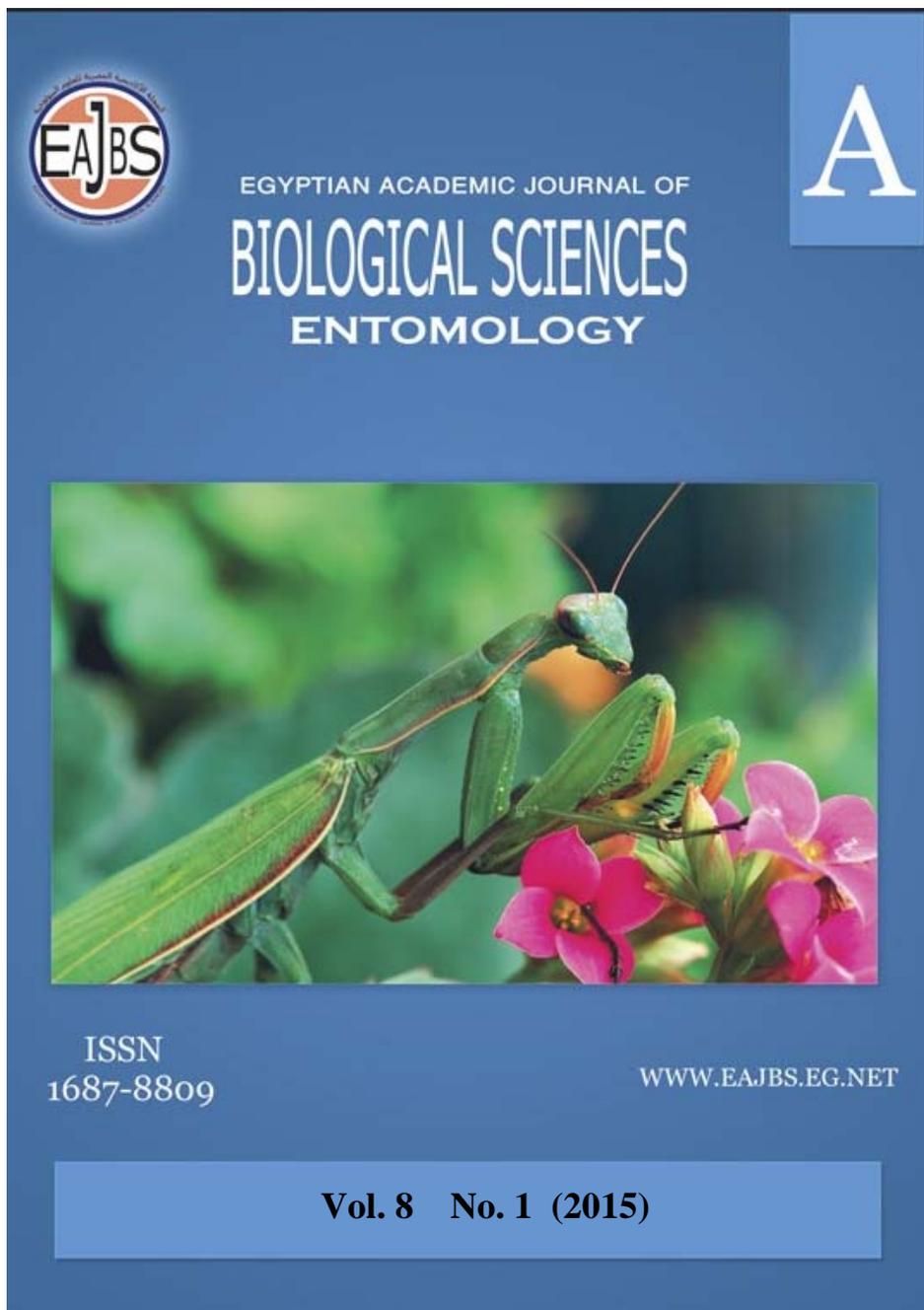


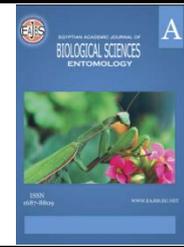
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## Effects of pyriproxyfen and chlorfluazuron on the external bacteria isolated from *Monomorium pharaonis* (L.) collected from different cities in Cairo governorate

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### ABSTRACT

The present study was examined the effect of two IGRs (pyriproxyfen & chlorfluazuron) on the external bacterial flora isolated from the Pharaoh ant workers *Monomorium pharaonis* (L.). The Pharaoh ant workers were collected from three different cities in Cairo governorate. Different concentrations of 0.1%, 0.5% & 1% pyriproxyfen and 0.001%, 0.01% & 0.1% chlorfluazuron were used.

Bacteria associated with Pharaoh ant workers (indoors & outdoors collected samples) were isolated before and after treatment with IGRs. It was found that, the external bacteria isolated from outdoors samples were less counted than those collected from indoors. The presence of some pathogenic bacteria was recorded. The used IGRs cause significant reduction in the count of bacteria in external isolates. Kinds of isolated bacteria were also affected by IGRs treatment and some kinds of them were disappeared in treated samples.

### INTRODUCTION

The Pharaoh ant; *Monomorium Pharaonis* (L.), is cosmopolitan in its distribution, having been carried by commerce to all regions of the world (Wheeler 1910). This ant was probably originated in the North Africa-Middle Eastern region and is a major indoor pest in most parts of the world (Edwards 1986).

Infestations usually occur in large office buildings and apartment complexes, factories, food establishment and hospitals (Edwards 1986). In hospitals, it causes problems by contaminating equipment, sterile packing penetrating intravenous solutions and feeding on dressed wounds.

When collecting food and water they keep to a definite track, rarely deviating from it. Nests are found in a variety of situations; behind tiles, in light fitting, in fuse boxes, indeed in any concealed part of buildings.

Hughes *et al.* (1989) decided to investigate whether particular ants carried any bacterial pathogens or opportunist. The role of insects as vector for several pathogenic and food spoilage microorganisms was covered by many authors (Alcamo & Frishman, 1980, McCoy *et al.*, 1988 and Ahmad *et al.*, 1995).

Other investigators discussed the essential symbiosis between microorganisms and several insects. For example the normal gut flora of termites and other xylophagous insects are important for survival of their host, since bacteria are involved in cellulose hydrolysis, maintaining the redox potential of the gut and preventing entry of the viable foreign bacteria (Veivers *et al.*, 1982).

Pharaoh ant control currently emphasizes the use of toxic bait (Edwards 1986). Insecticidal sprays and dusts are often ineffective because they only affect a small percentage of the workers that forage (Williams 1990) and may also cause budding, which amplifies the problem. Multiple nests within a relative small area also reduce the effectiveness of sprays. Unlike sprays which usually do not contact a nest toxic bait can be transported to the nest by foragers which feed on the bait and then distribute the toxin throughout the colony by trophallaxis (Vail and Williams 1994).

IGRs are used by many authors to control Pharaoh ants (Wilson and Booth 1981, Ryba *et al.* 1988, Vail and Williams 1995, Vail *et al.* 1996 and Oi *et al.* 2000). The advantages of using IGRs are that they are more likely to be distributed throughout the entire colony because they do not adversely affect workers (Williams, 1990). Also IGRs are more acceptable to the consumer since they are considered safe compound.

Shoemaker *et al.* (2000) reported the presence of *Wolbachia* in native South American population of the fire ant *Solenopsis invicta*, *Wolbachia* are cytoplasmically inherited bacteria that induce a variety of effects with fitness consequences on host arthropods, including cytoplasmic incompatibility, parthenogenesis, male-killing and feminization. They concluded that, *Wolbachia* may have significant fitness effects on these hosts (either directly or by cytoplasmic incompatibility) and therefore suggested that, these microbes potentially could be used in biological control programs to suppress introduced fire ant populations.

This study was designed to evaluate the toxicological activities of the two IGRs against the associated bacterial flora with Pharaoh ant workers.

## MATERIALS AND METHODS

### **Samples of Pharaoh ant workers collection and associated external bacterial isolation:**

Samples of Pharaoh ant workers were collected from indoors and outdoors houses of three districts, Abbassia, Heliopolis and Nasr-city, which represented three different levels of population at Cairo governorate, Egypt. The collected workers were transferred to the laboratory in sterile screw-capped glass bottle.

The external bacterial flora were isolated according the method described by Ahmad *et al.* (1995). Briefly, about 50% of the collected workers from each area were shacked well separately in sterile screw-capped glass tube containing 10 ml sterile saline solution (0.85 g/L NaCl) using agitator for 3 min., in order to suspend the external bacteria in the saline solution. The dilutions of each sample were streaked on different selective and non-selective microbiological media (Nutrient, Blood and McConkey agar) in order to isolate the out surface bacteria.

### **Bacteria associated with Pharaoh ant treated by IGRs:**

The other 50% of the collected workers were treated by different concentrations

of pyriproxyfen (0.1, 0.5, and 1.0%) and chlorfluazuron (0.001, 0.01, and 0.1%) for four days. The external bacteria were isolated as mentioned above.

#### Identification of the isolated Bacteria:

Gram stain and different selective media with different biochemical reactions were used for bacterial identification (Roche, 1974).

## RESULTS

### The viable count of the external bacteria associated with untreated Pharaoh ant workers

The viable count of bacterial isolates associated with ants collected from three different localities, Abbassia, Heliopolis and Nasr-city was examined both indoors and outdoors.

The data presented in Table 1 and Fig. 1 indicated that in case of indoor Pharaoh ant isolates, the count number of localities decreased significantly ( $p < 0.05$ ) from a mean of  $18.3 \pm 0.8$  to a mean  $14.1 \pm 1.04$  and  $6.7 \pm 1.03$  for Abbassia, Heliopolis and Nasr-city respectively.

In case of external bacteria isolates of outdoor Pharaoh ants, the data presented in Table 1 and Fig. 1 showed that no significant difference in bacterial counts from ants collected from Heliopolis and Abbassia, the mean bacterial counts were  $9.7 \pm 0.7$  and  $11.8 \pm 0.6$  respectively.

Table 1: Viable count of external bacteria both indoor and outdoor from Pharaoh ant collected from three different areas.

Area	Mean external bacterial counts/mg in thousands $\pm$ SE	
	Indoor	Outdoor
Abbassia	$18.3 \times 10^6 \pm 0.866$ aA	$9.7 \times 10^6 \pm 0.75$ aB
Heliopolis	$14.1 \times 10^6 \pm 1.0408$ bA	$11.8 \times 10^6 \pm 0.629$ aA
Nasr-city	$6.7 \times 10^6 \pm 1.0308$ cA	$5.3 \times 10^6 \pm 1.041$ bA

Mean values in vertical columns having different small letters are statistically significant ( $p < 0.05$ )

Mean values in horizontal rows followed by the same capital letters indicate no significant difference ( $p > 0.05$ ).

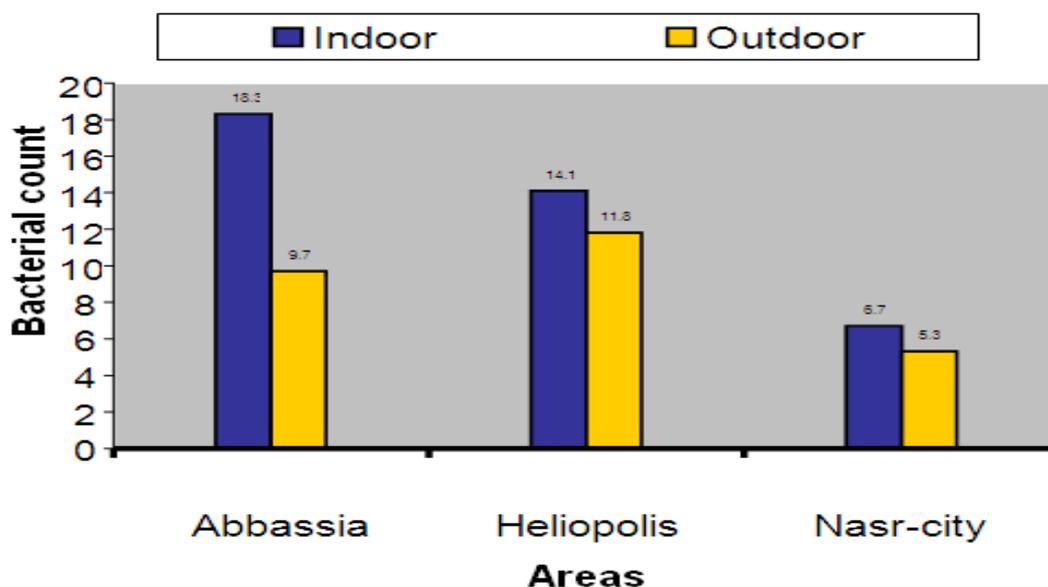


Fig. 1: Viable count of external bacteria both indoor and outdoor from Pharaoh ant collected from three different areas.

In Nasr-city it was significant decrease in bacterial counts to  $5.3 \pm 1.04$  than the other two localities.

Comparing bacterial counts between both indoor and outdoor Pharaoh ants isolates from the three areas, the data showed a significant decrease ( $p < 0.05$ ) in bacterial counts in Abbassia area from indoor and outdoor, it was  $18.3 \pm 0.8$  and  $9.7 \pm 0.7$  respectively while in both Heliopolis and Nasr-city, there were no significant differences between both indoor and outdoor bacterial count.

#### The effect of pyriproxyfen on the counts of bacteria associated with examined Pharaoh ant.

The external inhibitory action of pyriproxyfen on the bacteria associated with both indoor and outdoor Pharaoh ant collected from three different localities; Abbassia, Heliopolis and Nasr-city was studied. Different concentrations were used according to the mortality tests.

The data in Table 2 and Fig. 2 shows that at the different concentrations of pyriproxyfen no significant differences appear in bacterial count. This was clear in Abbassia, Heliopolis and Nasr-city.

But there was a significant difference between different conc's in comparison to its control in all the areas under investigation.

When we compare different areas with different conc's of pyriproxyfen, we notice that there is a significant difference between Nasr-City and the other two areas, which have no significant differences between them.

At conc. 1% of pyriproxyfen, it was  $13.1 \pm 0.913$ ,  $10.1 \pm 0.479$  and  $4.7 \pm 1.291$ , at conc. 0.5% of pyriproxyfen it was  $14.4 \pm 1.031$ ,  $10.9 \pm 0.854$  and  $5.1 \pm 1.472$  and at 0.1% of pyriproxyfen it was  $15.1 \pm 0.646$ ,  $11.5 \pm 1.108$  and  $5.4 \pm 1.472$ .

Table 2: Viable count of external bacteria associated with the treated indoor Pharaoh ant with pyriproxyfen collected from the three different areas.

% conc. of Pyriproxyfen	Mean bacterial counts/mg in thousands $\pm$ SE		
	Abbassia	Heliopolis	Nasr-city
<b>0.0</b>	$18.3 \times 10^6 \pm 0.866bA$	$14.1 \times 10^6 \pm 1.041bB$	$6.7 \times 10^6 \pm 1.031bC$
<b>0.1</b>	$15.1 \times 10^6 \pm 0.646aA$	$11.5 \times 10^6 \pm 1.108aA$	$5.4 \times 10^6 \pm 1.472aB$
<b>0.5</b>	$14.4 \times 10^6 \pm 1.031aA$	$10.9 \times 10^6 \pm 0.854aA$	$5.1 \times 10^6 \pm 1.291aB$
<b>1</b>	$13.1 \times 10^6 \pm 0.913aA$	$10.1 \times 10^6 \pm 0.479aA$	$4.7 \times 10^6 \pm 1.291aB$

Mean values in vertical columns having the same small letters indicate no significant difference ( $p > 0.05$ )

Mean values in horizontal rows having different capital letters are statistically significant ( $p < 0.05$ )

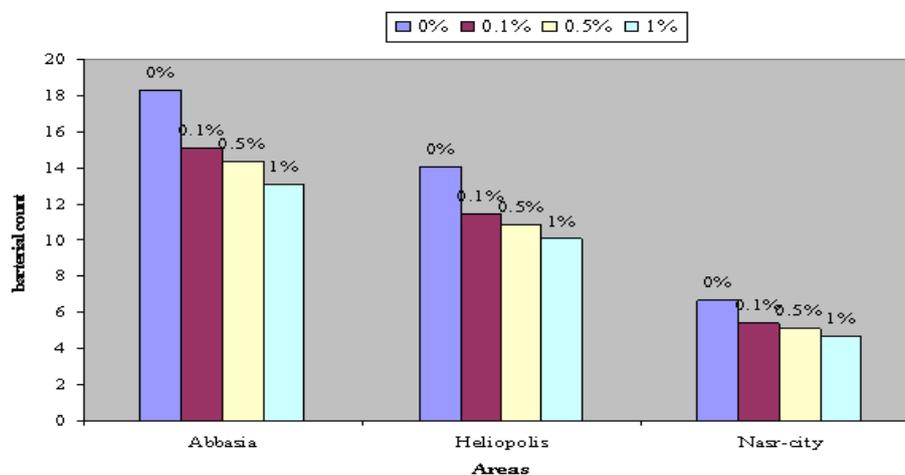


Fig. 2: Viable count of external bacteria associated with the treated indoor Pharaoh ant with pyriproxyfen collected from the three different areas.

In Table 3 and Fig. 3 the data shows no significant difference in external bacteria associated with treated Pharaoh ants with different concentrations collected from different areas.

In comparing the different concentrations of pyriproxyfen in each area, it was noticed that, there was no significant difference between them, however in comparing areas with each other, it was clear that, there is a significant difference between Nasr-City and the other two areas at all different concentrations of pyriproxyfen.

It was  $6.9 \pm 0.85$ ,  $8.9 \pm 1.49$  and  $3.9 \pm 0.853$  at conc. 1% of pyriproxyfen in Abbassia, Heliopolis and Nasr-city respectively, it was  $7.5 \pm 0.407$ ,  $9.3 \pm 1.04$  and  $4.2 \pm 0.479$  at conc. 0.5% of pyriproxyfen and it was  $8.0 \pm 0.408$ ,  $9.7 \pm 1.04$  and  $4.4 \pm 5.369$  at 0.1% of pyriproxyfen.

The 1% conc. of pyriproxyfen showed the highest effect on the bacterial count associated with the tested Pharaoh ant in all examined areas.

Table 3: Viable count of external bacteria associated with the treated outdoor Pharaoh ant with Pyriproxyfen collected from three different areas.

% conc. of Pyriproxyfen	Mean bacterial counts/mg in thousands $\pm$ SE		
	Abbassia	Heliopolis	Nasr-city
<b>0.0</b>	$9.7 \times 10^6 \pm 0.75$ aA	$11.8 \times 10^6 \pm 0.62$ aB	$5.3 \times 10^6 \pm 1.04$ 1aC
<b>0.1</b>	$8.0 \times 10^6 \pm 0.408$ aA	$9.7 \times 10^6 \pm 1.04$ aA	$4.4 \times 10^6 \pm 5.369$ aB
<b>0.5</b>	$7.5 \times 10^6 \pm 0.407$ aA	$9.3 \times 10^6 \pm 1.04$ aA	$4.2 \times 10^6 \pm 0.479$ aB
<b>1</b>	$6.9 \times 10^6 \pm 0.85$ aA	$8.9 \times 10^6 \pm 1.49$ aA	$3.9 \times 10^6 \pm 0.853$ aB

Mean values in vertical columns having the same small letters indicate no significant difference ( $p > 0.05$ ). Mean values in horizontal rows having different capital letters are statistically significant ( $p < 0.05$ ).

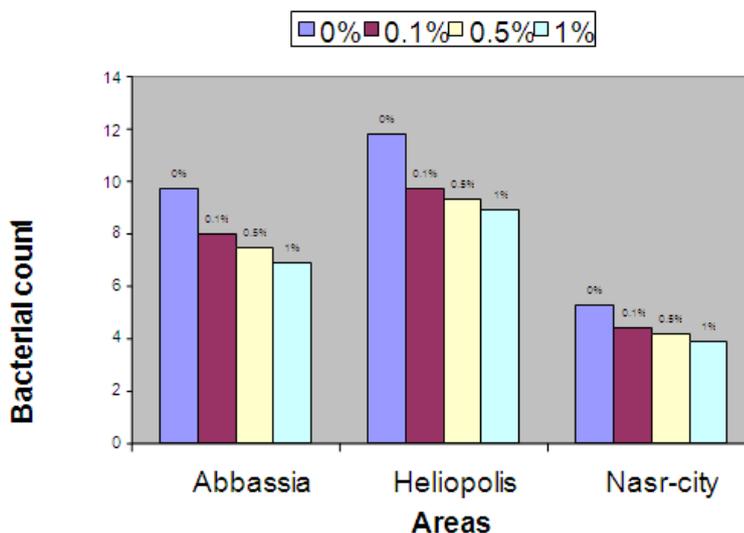


Fig. 3: viable count of external bacteria associated with the treated outdoor Pharaoh ant with pyriproxyfen collected from three different areas.

The data in Table 4 shows no significant differences are observed between indoor and outdoor bacterial count in both Heliopolis and Nasr-city at different conc. of pyriproxyfen.

In Abbassia bacterial count in outdoor was significantly lower than that in the indoor, it was  $13.1 \pm 0.913$  to  $6.9 \pm 0.85$  at conc. 1%,  $14.4 \pm 1.031$  to  $7.5 \pm 0.407$  at conc. 0.5% and  $15.1 \pm 0.646$  to  $8.0 \pm 0.408$  at conc. 0.1%.

Table 4: Comparison between both indoor and outdoor external bacterial count associated with Pharaoh ant treated by pyriproxyfen and collected from three different areas.

Area	% Conc. of pyriproxyfen	Mean bacterial counts/mg in thousands $\pm$ SE	
		Indoor	Outdoor
Abbassia	0	18.3 x 10 <sup>6</sup> $\pm$ 0.866 A	9.7 x 10 <sup>6</sup> $\pm$ 0.75 B
	0.1	15.1 x 10 <sup>6</sup> $\pm$ 0.646 A	8.0 x 10 <sup>6</sup> $\pm$ 0.408 B
	0.5	14.4 x 10 <sup>6</sup> $\pm$ 1.031 A	7.5 x 10 <sup>6</sup> $\pm$ 0.407 B
	1	13.1 x 10 <sup>6</sup> $\pm$ 0.913 A	6.9 x 10 <sup>6</sup> $\pm$ 0.85 B
Heliopolis	0	14.1 x 10 <sup>6</sup> $\pm$ 1.041 A	11.8 x 10 <sup>6</sup> $\pm$ 0.629A
	0.1	11.5 x 10 <sup>6</sup> $\pm$ 1.108 A	9.7 x 10 <sup>6</sup> $\pm$ 1.04 A
	0.5	10.9 x 10 <sup>6</sup> $\pm$ 0.854 A	9.3 x 10 <sup>6</sup> $\pm$ 1.04 A
	1	10.1 x 10 <sup>6</sup> $\pm$ 0.479 A	8.9 x 10 <sup>6</sup> $\pm$ 1.49 A
Nasr-city	0	6.7 x 10 <sup>6</sup> $\pm$ 1.031 A	5.3 x 10 <sup>6</sup> $\pm$ 1.041 A
	0.1	5.4 x 10 <sup>6</sup> $\pm$ 1.472 A	4.4 x 10 <sup>6</sup> $\pm$ 5.369 A
	0.5	5.1 x 10 <sup>6</sup> $\pm$ 0.913 A	4.2 x 10 <sup>6</sup> $\pm$ 0.479 A
	1	4.7 x 10 <sup>6</sup> $\pm$ 1.291 A	3.9 x 10 <sup>6</sup> $\pm$ 0.853 A

Mean values in horizontal rows followed by the same capital letters indicate no significant difference ( $p > 0.05$ ).

### The effect of chlorfluazuron on the counts of associated bacteria of examined Pharaoh ant

The external inhibitory action of pyriproxyfen on the bacteria associated with both indoor and outdoor Pharaoh ant collected from three different localities; Abbassia, Heliopolis and Nasr-city was studied. Different concentrations were used according to the mortality tests.

It was noticed that, there was no significant difference in bacterial count at all different concentrations except for 0.1% conc. This was clear in Abbassia, Heliopolis and Nasr-city. It was obvious that the bacterial count was increase with decrease the the conc. of chlorfluazuron in all the three areas (Table 5 & Fig. 4).

Table 5: Viable count of external bacteria associated with the treated indoor Pharaoh ant by chlorfluazuron collected from three different areas.

% conc. of chlorfluazuron	Mean bacterial counts/mg in thousands $\pm$ SE		
	Abbassia	Heliopolis	Nasr-city
0.0	18.3x10 <sup>6</sup> $\pm$ 0.866bA	14.1x10 <sup>6</sup> $\pm$ 1.041bB	6.7x10 <sup>6</sup> $\pm$ 1.031bC
0.001	17.1x10 <sup>6</sup> $\pm$ 1.601bA	13.6x10 <sup>6</sup> $\pm$ 1.25bB	6.4x10 <sup>6</sup> $\pm$ 1.24bC
0.01	15.2x10 <sup>6</sup> $\pm$ 1.25bA	11.7x10 <sup>6</sup> $\pm$ 1.547bB	5.6x10 <sup>6</sup> $\pm$ 1.03bC
0.1	12.5x10 <sup>6</sup> $\pm$ 0.629aA	9.7x10 <sup>6</sup> $\pm$ 1.108aB	4.6x10 <sup>6</sup> $\pm$ 1.031aC

Mean values in vertical columns having the same small letters indicate no significant difference ( $p > 0.05$ )

Mean values in horizontal rows having different capital letters are statistically significant ( $p < 0.05$ )

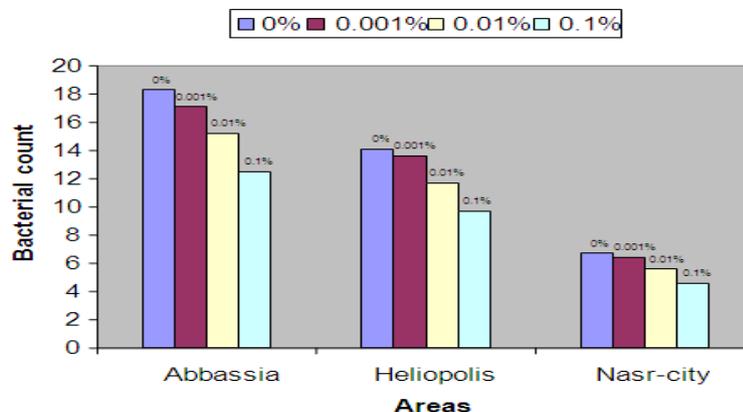


Fig. 4: Viable count of external bacteria associated with the treated indoor Pharaoh ant by chlorfluazuron collected from three different areas.

In Abbassia it was  $12.5 \pm 0.629$ ,  $15.2 \pm 1.25$ ,  $17.1 \pm 1.601$  and  $18.3 \pm 0.866$  at 0.1%, 0.01%, 0.001% and 0.0% respectively.

In Heliopolis it was  $9.7 \pm 1.108$ ,  $11.7 \pm 1.547$ ,  $13.6 \pm 1.25$  and  $14.1 \pm 1.041$  at 0.1%, 0.01%, 0.001% and 0.0% respectively.

And in Nasr-city it was  $4.6 \pm 1.031$ ,  $5.2 \pm 1.03$ ,  $6.4 \pm 1.24$  and  $6.7 \pm 1.031$  at 0.1%, 0.01%, 0.001% and 0.0% respectively.

In comparing the mean bacterial count of the same conc. in the three different areas, the data in Table 5 shows significant differences. It was noticed that, the bacterial count decrease significantly from Abbassia to Heliopolis and Nasr-city.

At 0.1% the bacterial count was  $12.5 \pm 0.629$ ,  $9.7 \pm 1.108$  and  $4.6 \pm 1.031$  in Abbassia, Heliopolis and Nasr-city respectively.

At 0.01% the bacterial count was  $15.2 \pm 1.25$ ,  $11.7 \pm 1.547$  and  $5.2 \pm 1.03$  in Abbassia, Heliopolis and Nasr-city respectively.

At 0.001% the bacterial count was  $17.1 \pm 1.601$ ,  $13.6 \pm 1.25$  and  $6.4 \pm 1.24$  in Abbassia, Heliopolis and Nasr-city respectively.

The outdoor treated Pharaoh ants show no significant difference in external bacterial count at all different concentrations this was clear in Abbassia, Heliopolis and Nasr-city (Table 6 & Fig. 5).

Table 6: Viable count of external bacteria associated with the treated outdoor Pharaoh ant treated by chlorfluazuron collected from three different areas.

% conc. of chlorfluazuron	Mean bacterial counts/mg in thousands $\pm$ SE		
	Abbassia	Heliopolis	Nasr-city
0.0	$9.7 \times 10^6 \pm 0.75aA$	$11.8 \times 10^6 \pm 0.629aB$	$5.3 \times 10^6 \pm 1.04aC$
0.001	$9.3 \times 10^6 \pm 0.645aA$	$9.8 \times 10^6 \pm 0.358aA$	$5.1 \times 10^6 \pm 1.25aB$
0.01	$8.0 \times 10^6 \pm 0.817aA$	$9.2 \times 10^6 \pm 1.25aA$	$4.6 \times 10^6 \pm 0.346aB$
0.1	$6.7 \times 10^6 \pm 1.04aA$	$8.2 \times 10^6 \pm 0.853aA$	$3.8 \times 10^6 \pm 1.44aB$

Mean values in vertical columns having the same small letters indicate no significant difference ( $p > 0.05$ )  
 Mean values in horizontal rows having different capital letters are statistically significant ( $p < 0.05$ )

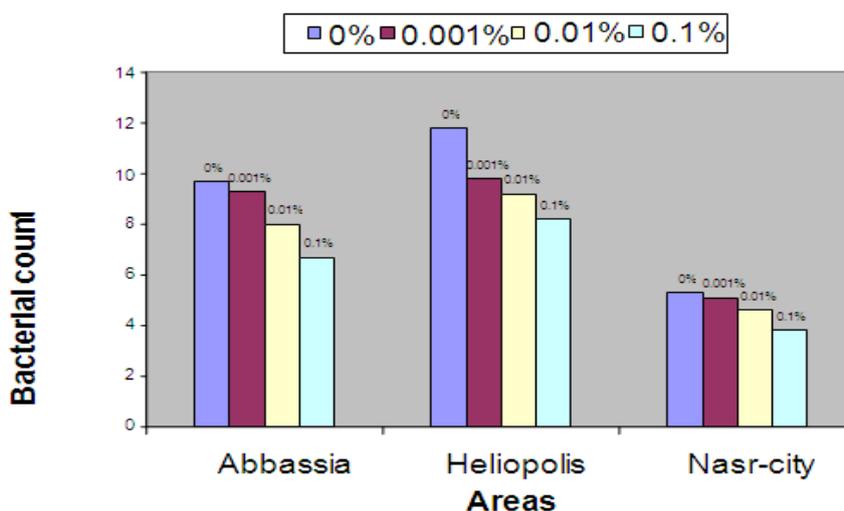


Fig 5: viable count of external bacteria associated with the Treated outdoor Pharaoh ant treated by chlorfluazuron collected from three different areas.

It was obvious that, no significant difference in bacterial count between the three concentrations and also between the different concentrations and the control. That was clear in Abbassia, Heliopolis and Nasr-city.

In comparing the three different areas with different concentrations of chlorfluazuron it was noticed that, there was no significant difference between Abbassia and Heliopolis at the different concentrations, but in comparing Abbassia and Heliopolis areas with Nasr-city area, there are significant difference between them at the different concentrations of chlorfluazuron.

The mean bacterial count of Nasr-city decreases significantly compared to the two other areas at different concentrations of chlorfluazuron.

At conc. 0.1% of chlorfluazuron the count was  $6.7 \pm 1.04$ ,  $8.2 \pm 0.853$  and  $3.8 \pm 1.44$  in Abbassia, Heliopolis and Nasr-city respectively.

At conc. 0.01% of chlorfluazuron the count was  $8.0 \pm 0.817$ ,  $9.2 \pm 1.25$  and  $4.6 \pm 0.346$  and at 0.001% of chlorfluazuron it was  $9.3 \pm 0.645$ ,  $9.8 \pm 0.358$  and  $5.1 \pm 1.25$ .

The data in Table 7 shows that there was no significant difference between indoor and outdoor mean bacterial count in both Heliopois and Nasr-city at different conc. of chlorfluazuron.

Table 7: Comparison between both indoor and outdoor external bacterial count associated with treated Pharaoh ant by chlorfluazuron collected from three different areas

Area	% Conc. of chlorfluazuron	Mean bacterial counts/mg in thousands $\pm$ SE	
		Indoor	Outdoor
Abbassia	0.0	$18.3 \times 10^6 \pm 0.866$ A	$9.7 \times 10^6 \pm 0.75$ B
	0.001	$17.6 \times 10^6 \pm 1.601$ A	$9.3 \times 10^6 \pm 0.645$ B
	0.01	$15.2 \times 10^6 \pm 1.25$ A	$8.0 \times 10^6 \pm 0.817$ B
	0.1	$12.5 \times 10^6 \pm 0.629$ A	$6.7 \times 10^6 \pm 1.04$ B
Heliopolis	0.0	$14.1 \times 10^6 \pm 1.041$ A	$11.8 \times 10^6 \pm 0.629$ A
	0.001	$13.6 \times 10^6 \pm 1.25$ A	$9.8 \times 10^6 \pm 3.58$ A
	0.01	$11.7 \times 10^6 \pm 1.547$ A	$9.2 \times 10^6 \pm 1.25$ A
	0.1	$9.7 \times 10^6 \pm 1.108$ A	$8.2 \times 10^6 \pm 0.853$ A
Nasr-city	0.0	$6.7 \times 10^6 \pm 1.031$ A	$5.3 \times 10^6 \pm 1.041$ A
	0.001	$6.4 \times 10^6 \pm 1.24$ A	$5.1 \times 10^6 \pm 1.25$ A
	0.01	$5.6 \times 10^6 \pm 1.03$ A	$4.6 \times 10^6 \pm 3.46$ A
	0.1	$4.6 \times 10^6 \pm 1.031$ A	$3.8 \times 10^6 \pm 1.44$ A

Mean values in horizontal rows followed by the same capital letters indicate no significant difference ( $p > 0.05$ )

But in comparing indoor and outdoor mean bacterial count in Abbassia, significant differences are found.

In Abbassia mean bacterial count decreases significantly from indoor to outdoor, it decreases from  $12.5 \pm 0.629$  to  $6.7 \pm 1.04$ , and from  $15.2 \pm 1.25$  to  $8.0 \pm 0.817$  and from  $17.6 \pm 1.601$  to  $9.3 \pm 0.645$  at conc of 0.1%, 0.01% and 0.001% of chlorfluazuron respectively.

#### **Kinds of bacteria associated with Pharaoh ants**

The external bacteria associated with Pharaoh ants were isolated from untreated and treated workers with different concentrations of pyriproxyfen and chlorfluazuron (IGRs) both indoors and outdoors from three different areas representing three different levels of populations; Abbassia, Heliopolis and Nasr-city.

#### **Kinds of external bacteria associated with untreated Pharaoh ants**

In our presented studies some light was thrown on scanning the microorganisms on the outer surface of collected samples which may be transmitted by them from one place to another carried by our tested ant and also on gut flora which may help in some biological aspect for tested ant in this study.

The kinds of external bacteria isolated from untreated Pharaoh ant workers collected from both indoors and outdoors of Abbassia, Heliopolis and Nasr-city were classified according to their gram stain into gram-positive and gram-negative bacterial

groups, the types were recorded in Table 8.

It was noticed from this table that, no differences in external bacterial kinds isolated from both indoors and outdoors samples which collected from the three different areas. Some of these bacteria were pathogenic including *Klebsiella sp.* and *Pseudomonas sp.* while others were commensals including *Anthracooid sp.*, *Diphtheroid sp.*, *Micrococci sp.*, *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*.

Table 8: External bacterial isolates from normal Pharaoh ant workers collected from three different areas indoors and outdoors.

Area	External bacterial isolets					
	Indoor			Outdoor		
	Name	Characters	Type	Name	Characters	Type
Abbassia	<i>Anthracooid sp</i>	Gram+ve bacilli	Comm.	<i>Anthracooid sp</i>	Gram+ve bacilli	Comm.
	<i>Klebsiella sp</i>	Gram-ve bacilli	Path.	<i>Klebsiella sp</i>	Gram-ve bacilli	Path.
	<i>Micrococci sp</i>	Gram+vecocci	Comm.	<i>Micrococci sp</i>	Gram+vecocci	Comm.
	<i>Pseudomonas sp</i>	Gram-ve bacilli	Path.	<i>Pseudomonas sp</i>	Gram-ve bacilli	Path.
	<i>Diphtheroidsp</i>	Gram +ve bacilli	Comm.	<i>Diphtheroidsp</i>	Gram +ve bacilli	Comm.
	<i>Staph. epidermidis</i>	Gram-vecocci	Comm.	<i>Staph. epidermidis</i>	Gram-vecocci	Comm.
	<i>Staph. saprophyticus</i>	Gram-vecocci	Comm.	<i>Staph. saprophyticus</i>	Gram-vecocci	Comm.
	Heltopolis	<i>Anthracooid sp</i>	Gram +ve bacilli	Comm.	<i>Anthracooid sp</i>	Gram +ve bacilli
<i>Klebsiella sp</i>		Gram-ve bacilli	Path.	<i>Klebsiella sp</i>	Gram-ve bacilli	Path.
<i>Micrococci sp</i>		Gram+vecocci	Comm.	<i>Micrococci sp</i>	Gram+vecocci	Comm.
<i>Pseudomonas sp</i>		Gram-ve bacilli	Path.	<i>Staph. saprophyticus</i>	Gram-vecocci	Comm.
<i>Staph. epidermidis</i>		Gram-vecocci	Comm.			
<i>Staph. saprophyticus</i>		Gram-vecocci	Comm.			
Nasr-city	<i>Anthracooid sp</i>	Gram +ve bacilli	Comm.	<i>Anthracooidsp</i>	Gram +ve bacilli	Comm.
	<i>Klebsiella sp</i>	Gram-ve bacilli	Path.	<i>Klebsiellasp</i>	Gram-ve bacilli	Path.
	<i>Pseudomonas sp</i>	Gram-ve bacilli	Path.	<i>Pseudomonas sp</i>	Gram-ve bacilli	Path.
	<i>Diphtheroid sp</i>	Gram +ve bacilli	Comm.	<i>Diphtheroidsp</i>	Gram +ve bacilli	Comm.
	<i>Micrococci sp</i>	Gram+vecocci	Comm.	<i>Staph. saprophyticus</i>	Gram-vecocci	Comm.
	<i>Staph. saprophyticus</i>	Gram-vecocci	Comm.			

Comm.: Commensals Path. : Pathogenic

### Bacteria associated with treated Pharaoh ants

In this study the viable effects of two insect growth regulators (pyriproxyfen and chlorfluazuron) on the bacterial persistence on or in workers body were studied.

#### Kinds of bacterial isolates after treatment by pyriproxyfen

The kinds of the external bacterial isolates from treated Pharaoh ant workers by different conc. of pyriproxyfen (IGRs) and collected from the same three areas under investigation both indoors and outdoors were represented as follows.

#### Kinds of external bacteria isolated from treated Pharaoh ant by pyriproxyfen.

Kinds of external bacterial isolates from both indoors and outdoors treated workers represented in Table 9, revealing that, there was no differences in bacterial kinds between both indoor and outdoor isolates at nearly all different concentrations, and also there was no difference occur in comparing to the untreated samples.

Table 9: External bacterial isolates from Pharaoh ant workers collected from three different areas indoors and outdoors and treated by pyriproxyfen.

Area	External bacterial isolates					
	Indoor			Outdoors		
	0.1%	0.5%	1.0%	0.1%	0.5%	1.0%
Abbassia	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>
Heliopolis	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. saprophyticus</i>
Nasr-city	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Micrococci sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Micrococci sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Micrococci sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. saprophyticus</i>

### Kinds of bacterial isolates after treatment by chlorfluazuron

The kinds of the external bacterial isolates from treated Pharaoh ant workers by different concentrations of chlorfluazuron (IGRs) and collected from the same three areas under investigation both indoors and outdoors were represented as follows.

### Kinds of external bacteria isolated from treated Pharaoh ant by chlorfluazuron

Kinds of external bacterial isolates from both indoors and outdoors treated workers are represented in Table 10. It was clear from this table that, there was no differences in bacterial kinds between both indoor and outdoor isolates at nearly all different concentrations when compared with the untreated one, also no difference noticed between them.

Table 10: External bacterial isolates from Pharaoh ant workers collected from three different areas indoors and outdoors and treated by chlorfluazuron.

Area	External bacterial isolates					
	Indoor			Outdoor		
	0.001%	0.01%	0.1%	0.001%	0.01%	0.1%
Abbassia	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>
Heliopolis	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. epidermidis</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Micrococci sp</i> <i>Pseudomonas sp</i> <i>Staph. saprophyticus</i>
Nasr-city	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Micrococci sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Micrococci sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Micrococci sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. saprophyticus</i>	<i>Anthracoïd sp</i> <i>Klebsiella sp</i> <i>Pseudomonas sp</i> <i>Diphtheroid sp</i> <i>Staph. saprophyticus</i>

Some of commensals bacteria are found in soil, water or in the air, and may cause diseases for immuno-compromised patients (pyogenic infection) such as meningitis, endocarditis, infection of prosthetic valves and artificial joints, on the other hand, the pathogenic species such as *Klebsiella* sp and *Pseudomonas* sp cause severe infection such as wound infection, burn infection, respiratory tract infection, eye infection, septicemia, meningitis and urinary tract infection (UTI), *Staph. aureus* cause eyelid infection, surgical wound infection, endocarditis on normal heart valve, metastatic abscesses, pneumonia and empyema in postoperative patients or following viral respiratory infection, osteomyelitis and arthritis especially in children. *Strept. faecalis* cause urinary, biliary and cardiovascular infection, Shanson (1999).

## DISCUSSION

### **The viable count of the external bacteria associated with untreated Pharaoh ant workers**

In our study Pharaoh ant workers were collected from three different areas representing different standard level of population (Abbassia, Heliopolis and Nasr-city) using artificial baits (peptone, glucose & agar) which was the most palatable bait used for workers ant.

The results obtained from this investigation have shown that, the ant surface microflora count is a reflection of the environment in which they survive. In general the results showed that the outdoor microflora counts of the ant workers are less in count than that of the indoor microflora count in all three areas. This may be due to the fact that, the surface of the outdoors ant exposed to various weather conditions such as sun's ultraviolet radiation which has a great effect on bacteria as suggested by Ahmad *et al.* (1995). The indoor conditions of relative humidity and temperature are suitable for microfloral growth. The ants' habit of regurgitating food in the form of pellets to be stored and later fed to pre-adult stages also provides fresh nutrients for microfloral growth (Beatson 1972).

### **The viable count of the external bacteria associated with Pharaoh ant workers both indoors & outdoors after treatments with IGRs (pyriproxyfen & chlorfluazuron)**

The present study was carried out to clarify the effect of different concentrations of IGRs (pyriproxyfen & chlorfluazuron) on the associated bacteria with Pharaoh ant workers both outdoors and indoors.

The results indicate that, there are significant decreases in microbial counts after treatment with IGRs in all cases studied with increasing the concentrations that was clear in Abbassia area than the other two areas. This may be due to that, Pharaoh ant are social insects so that bait with IGRs can cause local eradication, and the workers are able to bring the bait with IGRs back to the nests and distributing it among all the other members of the colonies. This may be affect not only on members of the colony but also on the microflora associated with it.

It is known that IGRs did not kill adult worker ants quickly, thus more surviving worker ants were available to distribute the bait to all colonies located at different nest sites. Thus, from a single bait source, the slow acting bait toxicant provided gradual, but long term control, (for ants worker & carried microflora) whereas the fast-acting bait toxicant provided rapid, localized control for a shorter duration (Oi *et al.* , 2000).

### **Kinds of microflora associated with Pharaoh ant workers**

The bacterial isolates were classified according to their gram stain into gram-positive & gram-negative bacterial groups. The very heavy & diverse bacterial floras which have been isolated from the surface of the tested Pharaoh ant workers revealed the high contamination of this insect which carries a considerable potential risk of pathogens.

The out-surface microbial flora associated with this insect under investigation is a reflection of the environment in which they survive whereas the internal flora show the difference in feeding sources of this insect (these results agree with previous findings reported by Beatson, (1973) who demonstrated that, Pharaoh ant workers have the ability to transmit a variety of bacterial pathogens. Hughes *et al.*, (1989) found also that Pharaoh ants have the capacity to carry and transmit a variety of pathogenic bacteria.

The results obtained from these investigations have shown that Pharaoh ants have the capacity to carry and transmit a variety of pathogenic bacteria. These results therefore confirm previous findings reported by other investigator (Alekseev *et al.* 1972, Beatson 1972 & 1973, Cartwright 1973, and Hughes *et al.*, 1989).

The bacterial isolates sub-cultured and identified, included, *Anthracoïd* sp, *Klebsiell* sp, *Micrococci* sp, *Pseudomonas* sp, *Diphtheroid* sp, *Staph. epidermidis*, *Staph. saprophyticus*, *Staph. aureus* and *Strept. faecalis*.

Some of these of bacterial species are pathogenic organisms such as *Klebsiell* sp and *Pseudomonas* sp, while others were commensals including, *Anthracoïd* sp and *Diphtheroid* sp. (Hughes *et al.*, 1989).

Many ants live in symbiosis with microorganisms present in their mid-gut (Boursau and Gross 2000). These authors found that the analysis of the parasitic and mutualistic interactions in these organisms will allow interesting insights into the evolution of symbiosis and possibly led to novel strategies of pest control.

Mutualism between insects and microorganisms is clearly one of the motor of evolution of insects and represent one of the keys to the enormous success of these huge groups of animals (Boursau and Gross 2000).

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