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Biochemical differences between the virgin queens and workers of the Ant, *Camponotus maculatus* (Fabricius)

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ABSTRACT

The present data showed that the activities of all the tested enzymes except acetylcholinesterase (α,β-esterases, acid phosphatase and glutathione S-transeferase) were significantly higher in the whole body homogenates of the virgin queens ant, *Camponotus* maculatus (Fabricius) than that recorded for the workers. Also, the concentration of total soluble proteins of the virgin queens was higher than of workers. These proteins were electrophoretically separated into 22 bands (258.6 to 35.4KDa) in the virgin queen samples while they were separated into 19 bands (216.7 to 35.4KDa) in the worker samples. Ten protein bands were common between the two castes (108.1, 103.9, 99.8, 94.6, 63.7, 61.3, 55.9, 48.1, 40.6 and 35.4KDa) and the remaining bands were characteristic for each caste .Finally, there was difference in the genomic DNA of the two studied castes.

Key words: Differentiation – Queen – Worker – Enzymes – SDS Electrophoresis –DNA– Ants

INTRODUCTION

Ants are social insects of the family Formicidae and, along with the related families of wasps and bees, belong to the order Hymenoptera. The highly organized colonies and nests of ants consist of millions of individuals. They are mostly sterile females (workers, soldiers, and other castes) with some fertile males (drones) and one or more fertile females (queens).Ants dominate most ecosystems, forming 15-20% of the terrestrial animal biomass .Their success has been attributed to their social structure, ability to modify their habitats, tap resources and defend themselves (Ant-Wikipedia ,the free encyclopedia).

Acetylcholinesterase is an enzyme that catalyzes the hydrolysis of neurotransmitter acetylcholine into choline and acetic acid .It has the potential for serving both as biochemical indicator of toxic stress and is also used a biomarker for heavy metal pollution (Sharaawi *et al.*,2002).

Esterases are enzymes involved in several important physiological processes in organisms, including reproduction, digestion, metabolism of juvenile hormone and detoxification of xenobiotics (Perez-Mendoza *et al*., 2002 and Shanmugavelu *et al.,* 2000).

Acid phosphatase is important in biological processes that need high level of energy, such as development, growth, gamete's maturation and histolysis(Ray *et al*., 1984). The selection of this enzyme as an indicator of sterility is based on it's importance in the reproductive system in insects (Moore and Frazier, 1976).

The primary function of glutathione S-transferase is generally considered to be the detoxification of both endogenous and xenobiotic compounds (Wilce and Parker, 1994).

Polyacrylamide gel electrophoresis has been used for separation of protein in different castes of honey bee (Chan *et al*., 2006).

A PCR based technique known as random amplified polymorphic DNA (RAPD) does not require knowledge of specific sequences, but rather uses random 10 base-pair primers. This method has become very useful for identification and differentiation of closely related insect species and populations (Williams *et al*., 1990).

The present work aimed to determine the biochemical differences between the virgin queens and workers of *Camponotus maculatus* (Fabricius) from Egypt through studying the activities of some of their vital enzymes (acetylcholinesterase, α, β-esterases, acid phosphatase and glutathione S- transferase). The concentration of their total soluble proteins was also measured. Moreover, their electrophoretic protein patterns and genomic DNA were studied.

MATERIALS AND METHODS

The virgin queens and workers of *Camponotus maculatus* were supplied by Prof. Dr. Reda Fadeel who collected them from 6th October governorate. These samples were homogenized in distilled water using a Teflon homogenizer surrounded with a jacket of crushed ice for 3 minutes. Homogenates were centrifuged at 6000 r.p.m. for 10 minutes, and the supernatants were used directly for determination of total soluble proteins and enzyme assays.

Biochemical study

Enzyme assays:

- The activity of acetylcholinesterase (AChE) was assayed colorimetrically according to the method described by Simpson *et al***.** **(**1964**).** Acetylcholine bromide (AchBr) was used as a substrate.
- The activity of the enzyme was expressed as µg substrate hydrolyzed / min / g body weight.
- The activities of α and β-esterases were determined according to the method of Van Asperen (1962**)** using α-naphthyl acetate and β-naphthyl acetate as substrates, respectively. Naphthol produced as a result of substrate hydrolysis can be measured by the addition of diazoblue sodium lauryl sulphate solution which produces a strong blue colour in case of α-naphthol or strong red colour in the case of β-naphthol. The colour was measured spectrophotometrically (by Milton Roy Spectronic model 1201 spectrophotometer).
- The activity of the enzyme was expressed as µg α- or β -naphthol released /min. / g body weight.
- Acid phosphatase (AcP) activity was measured according to the method described by Powell and Smith (1954**).** In this method, the phenol released by enzymatic hydrolysis of disodium phenylphosphate, reacts with 4-aminoantipyrine, and by the addition of potassium ferricyanide, the characteristic brown colour is produced.

The produced color was measured immediately by spectrophotometer at 510nm. The enzymatic activity is expressed as µg phenol released/min/g body weight.

Glutatione S-transferase activity (GST) was assayed in the presence of 1,2-Dichloro-nitrobenzen as a substrate according to the method of Habig *et al*., (1974).

A unit of activity is defined as the amount of enzyme catalyzing the formation of 1 µmole of product per min.

The concentration of total soluble proteins was determined in whole body homogenates of virgin queens and workers according to the method of Bradford (1976).

Statistical analysis

The significance in variation of enzyme activities means of queens and workers was assayed according to Smith (2004).

Protein Electrophoresis

Sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) was used for separation of proteins of queens and workers according to Laemmli (1970).

Protein samples of whole body of virgin queens and workers (20 µg) were loaded together with Bio RAD molecular markers (208-37 Kda).The gel was stained by Coomasie blue followed by immersion in a destaining solution. The destained gel was photographed and quantitative analysis of protein patterns was done using a laser densitometer and a computerized Gel Document system.

Extraction and PCR amplification of genomic DNA

DNA was extracted from virgin queens and workers according to the manufacturer specifications of genomic DNA isolation kit of (Promega ,USA).The extracted DNA was then used as an amplification template for RAPD-PCR analysis, using 10 primers (OPH-03, OPH-02, OPH-09, OPG-17, OPB-10, OPB-03, OPF-09, OPG-06, OPG-04, and OPC-02).

The total reaction volume was 50 µl containing 100 ng DNA. 50 pmol primer, 200 µM $DNTP_4$ (dATP, dGTP, dCTA, & dTTP), 3 mM $MgCl_2$, and 0.5 Tag polymerase. Amplification was performed using DNA thermal cycler (Perkin Elmer Cetus, model 2400). The thermal conditions were as follows: 6 min. at 94°C (hot start), 40 cycles (1 min. at 94°C, 2 min. at 36°C, & 3 min. at 72°C), and a final extension (post extension) of 10 min. at 72°C. The PCR products were run out on 1.8% agarose gels and then stained with ethidium bromide. Separation was documented by photography, using Polaroid^R camera. The values of DNA fragment sizes (bps) as well as those of the DNA markers were automatically computerized.

Sequences of primers:

OPH-03 5'-AGACGTCCAC-3'
OPH-02 5'-TCGGACGTGA-3'
OPH-09 5'-TGTAGCTGGG-3'
OPG-17 5'-ACGACCGACA-3'
OPB-10 5' -CTGCTGGGAC-3 '
OPB-03 5'-CATCCCCCTG-3'
OPF-09 5'-CCAAGCTTCC-3'
OPG-06 5'- GTGCCTAACC-3'
OPG-04 5'- AGCGTGTCTG-3'
OPC-02 5'-GTGAGGCGTC-3'

RESULTS AND DISCUSSION

Enzyme assays:

The activities of acetylcholinesterase, α, β -esterases, acid phosphatase and glutathione S-transferase in the whole body homogenates of the virgin queens and workers ant, *Camponotus maculatus* have been studied.

Acetylcholinesterase:

It is well known that acetylcholinesterase (AChE) is the target site for organophosphorus and carbamate insecticides. Thus, the increase in its activity as shown in Table (1) might be required by the workers to maintain the colony and this may reflect the toxic stress that face the ants from application of insecticides used for control. This explanation was supported by Umit and Halil (2000) who stated that the activity of AChE in the resistant strain of *Anopheles sacharovi* collected from localities which receive intensive insecticides was higher than that collected from localities where insecticides are used in small quantity or not used at all.

α and β-esterases:

The activities of α- and β-esterases in the virgin queens were significantly higher than that detected for the workers. The activities were 797±58.1 µg α-naphthol/g/min. and 862.2±102.6 µg β- naphthol/g/min. for the virgin queens compared with 32.1±4. 7µg α -naphthol/g/min and 25.7±3.5 µg β -naphthol/g/min. for the workers, respectively (Table 1) These enzymes are involved in insecticide degradation, their increased activity being the main mechanism in organophosphorus resistance, in several organisms (Vaughan and Hemingway, 1995; Vaughan *et al*., 1997). Thus, it could be suggested that queens produced high levels of esterases to minimize the effect of toxic materials (insecticides).i.e to become more resistant to organophosphorus insectsides as reported by Lima Catelani, (1996) and Sousa-Polezzi (2002) who found an increase in the synthesis of some esterases in *A. aegypti* resistant to organophosphorus insectisides.

Acid phosphatase:

Results in Table (1) revealed a significant higher activity of acid phosphatase (AcP) in the virgin queen samples than that detected for the workers. The activity was 9.9±1.6 µg phenol/g/min. for the virgin queen samples compared with 1.8±0.3 µg phenol/g/min. for the worker samples.

Table 1: The activities of acetylcholinesterase (AChE), α, β - esterases acid phosphatase (AcP) glutathaione S- transferase and the concentration of total proteins of virgin queens and workers ant, *Camponotus maculatus*.

Parameter	Mean ± SD		$F_{1,4}$	Significance
	Worker	Virgin Queen		
Acetylcholinesterase	1376.6±215.1	1128.5±126.4	2.97	Not Sign.
α - Esterase	32.1±4.7	797 ±58.1	517.45	1%
β - Esterase	25.7±3.5	862.2±102.6	199.25	1%
Acid phosphatase	1.8±0.3	9.9±1.6	76.20	1%
Glutathione S- transferase	1.64±0.3	58.9±7.2	187.43	1%
Total soluble Proteins	13.1±2.1	43.7 ±14.1	13.92	5%

Enzymes activities were expressed as follows:
The activity of AChE was expressed as µg ACh Br/gm/min.
The activity of α and β-esterases were expressed as µg α or β naphthol /min./gm body weight .
The activity of AcP was expressed as µg phenol /min. /gm body weight.
The activity of glutathaione S -transferase was expressed as M of product/min.
The concentration of total soluble proteins was expressed as mg/g body weight.

This result agrees with that reported by El Sherif *et al.,* (1990) who found a highest level of AcP. in the reproductive caste of termites while the soldiers and workers exhibited lower levels of activity. These results may be attributed to the importance of AcP in reproducing individuals (Moore and Frazier, 1976) .Moreover,the enzyme enhances the addition of phosphate ions to the phosphate pool for synthesis of high energy needed to gamete's maturation (Bogitsh ,1974) and DNA

synthesis (Moore & Fraizer, 1976). On the other hand, the lowest level of AcP activity observed in workers and soldiers of termites are explained by being sterile castes.

Glutathione S-transferase:

The activity of glutathione S-transferase (GST) 0f the virgin queens was significantly higher than that detected for workers (Table 1). The activity of the GST enzyme was 58.9±7.2 M product/min for virgin queens while it was 1.64±0.3 M product/min for the workers.

GST could be considered an important component of the defense of herbivorous insects against both exogenous and endogenous oxidative radicals as reported by (Krishnan and Nodrik, 2006).Therefore, lack of glutathione has been shown to leave the body more vulnerable to damage. Thus, virgin queens produced high amount of this enzyme to protect their bodies from wearing by these free radicals..Moreover,GST enzyme has a significant role in the detoxification of organophosphorus and pyrethroid insecticides (Hooper-Bùi *et al., 2005)* .Thus, the higher level of the activity of this enzyme recorded in the virgin queens in the present study might be a prerequisite for the queens to defend themselves against the above mentioned insecticides. This explanation may be supported by Umit and Halil (2000) who found that the activity of GST enzyme was higher in *Anopheles sacharovi* collected from localities receiving intensive insecticide application than in localities where insecticides are used in smaller quantities or not at all.

Total soluble proteins

Table (1) shows that the concentration of total soluble proteins detected from the whole body homogenates of virgin queens was higher than that detected from the workers. It was 43.7±14.1mg/gbody weight compared to 13.1±2.1mg/g body weight.

Wilkinson (1976) stated that proteins help insects to synthesis microsomal detoxifying enzymes. Therefore, the decrease in the concentration of total proteins detected from the whole body homogenates of worker samples in the present study may reflect the decrease in activity of these enzymes that may lead to their death from application of insecticides. Proteins also involved in the defense against reactive oxygen substance (ROS) have major effects on longevity in many if not all organisms (Nystrom, 2005, Finkel and Holbrook, 2000). Therefore, the presence of high concentration of proteins in the virgin queens protect them from these substances to maintain their life.

It has been reported that lack of sufficient ROS defense mechanisms in foraging bees reflects reduced somatic maintenance. This effect was directly and positively linked to the circulating vitellogenin protein levels in the bees. Vitellogenin was shown to exhibit characteristic antioxidant function being preferentially carbonylated by ROS (Wolschin and Amdam, 2007)

Finally, the detected high concentration of total soluble proteins in the virgin queen samples in the present study may be explained as mentioned by Hahn *et al.,* (2004) who stated that the storage protein content has evolved by the queens of harvester ant of genus *Pogonomyrmex* in concert with colony –founding strategies

Protein Electrophoresis:

Electrophoretic fractionation of the whole body homogenates of the virgin queen samples revealed the presence of 22 protein bands with molecular weights ranged from 258.6 to 35.4 KDa .While there were 19 protein bands ranged from 216.7 to 35.4 KDa in the worker samples.Ten of these bands (108.1, 103.9, 99.8, 94.6, 63.7, 61.3, 55.9, 48.1, 40.6 and 35.4 KDa) were common between the aforementioned two castes of *C. maculatus*.The remaining bands were characteristics for each caste. They were twelve in the virgin queens samples (258.6, 228.8, 210.9, 189.1,154.1,

140.1,101.4 ,82.4,66.3,53.4 ,42.3 and 38.7 KDa).While they were nine for the worker samples (216.7 ,208 ,191.7 ,162.8, 138.3, 89.1, 86.2, 68.1 and 57.8 KDa) (Table 2 and Fig. 1 (A) & (B)).

Chan *et al*., (2006) also found differences in electrophoretic separation of haemolymph proteins between workers and queens of *Apis mellifera.*

Myles and Change (1984) studied the differences in electrophoretic protein profiles in all castes of the termite, *Neotermes connexus* . They attributed their results to cuticular pigmentation and sclerotization which in nymphs markedly differ from reproduction castes.

Table (2): Relative molecular weights of different protein bands detected in the whole body homogenates of the virgin queens (q) and workers (w) of *Camponotus maculatus*.

Band no.	Molecular Weights (KDa)	*Virgin queens*	Workers
1	258.6	1	0
2	228.8	1	0
3	216.7	0	1
4	210.9	1	0
5	208	0	1
6	191.7	0	1
7	189.1	1	0
8	162.8	0	1
9	154.1	1	0
10	140.1	1	0
11	138.3	0	1
12	108.1	1	1*
13	103.9	1	1*
14	101.4	1	0
15	99.8	1	1*
16	94.6	1	1*
17	89.1	0	1
18	86.2	0	1
19	82.4	1	0
20	68.1	0	1
21	66.3	1	0
22	63.7	1	1*
23	61.3	1	1*
24	57.8	0	1
25	55.9	1	1*
26	53.4	1	0
27	48.1	1	1*
28	42.3	1	0
29	40.6	1	1*
30	38.7	1	0
31	35.4	1	1*
Total brand	31	22	19

(*) means common protein bands between the virgin queens and workers of ant, *Camponotus maculatus*.

RAPD-PCR analysis:

The results recorded in Table 3 and illustrated in Figs. (2 , 3 & 4) reveal distinguishable differences in Genomic DNA between the virgin queens and workers of *Camponotus maculatus*.

Distinct DNA fragments that amplified by the tested primers are referred to as RAPD markers. They are considered to be queen specific if they were found only in the virgin queen samples as shown in Table (3) under the column with symbol (1). Likewise, markers are considered to be worker specific if found only in worker samples as shown in Table (3) under the column with symbol (2).

Table 3:DNA profile of virgin queen (1) and worker (2) of *Camponotus maculatus* using RAPD-PCR technique and OPH-03, OPH-02, OPH-09 and OPG-17 primers

	Primers								
Band	**Approx. band**	**OPH-03**		**OPH-02**		**OPH-09**		**OPG-17**	
No.	**size in bp**	**(1)**	**(2)**	**(1)**	**(2)**	**(1)**	**(2)**	**(1)**	**(2)**
1	1839					1	0		
2	1491			1	0	0	1	1	0
3	1415					1	0		
4	1148					1	0		
5	981	1	0					0	1
6	931			1	0			1	0
7	893					1	0		
8	756	0	1			0	1		
9	717			0	1			0	1
10	681			1	0			0	1
11	613	1	0					1	1
12	524							0	1
13	497	1	0						
14	472							1	0
15	448	0	1						
16	425	1	0						
17	363					1	0		
18	327					0	1		
19	295							0	1
20	280	1	0			1	0		
21	252							0	1
22	227	1	0			1	0		

Table 3 Cont.: DNA profile of virgin queen (1) and worker (2) of *Camponotus maculatus* using RAPD- PCR technique and OPB-10, OPB-03, OPF-09 and OPG-06 primers

	Primers								
Band No.	Approx. band size in bp	OPB-10		OPB-03		OPF-09		OPG-06	
		(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
1	1936					1	0		
2	1838			1	0				
3	1744					0	1		
4	1491	0	1						
5	1343			1	0				
6	1274	0	1						
7	1210							0	1
8	1148					0	1		
9	1034			1	1	1	0		
10	931	0	1					0	1
11	884	0	1	1	0				
12	839							1	1
13	796			0	1				
14	756	1	0						
15	717					1	0		
16	681	0	1					1	0
17	613			1	0				
18	497					1	1		
19	383							0	1

Table 3 Cont.: DNA profile of virgin queen (1) and worker (2) of *Camponotus maculatus* using RAPD- PCR technique and OPG-04, and OPC-02primers

	Primers				
Band	**Approx. band size in**	**OPG-04**		**OPC-02**	
No.	**bp**	**(1)**	**(2)**	**(1)**	**(2)**
1	756			1	0
2	719			0	1
3	704			1	0
4	635	1	0		
5	609			1	0
6	561	1	1		
7	527	0	1		
8	506	0	1	1	1
9	497	1	0		
10	475			0	1
11	465	0	1		
12	437			1	1
13	411	1	1		
14	378			0	1
15	341	0	1		
16	334			0	1
17	314			0	1
18	295	0	1	0	1
19	280			1	0
20	266			0	1
21	245	1	1		

Out of 10 primers screened, 6 were found to reveal bands which were observed in the two studied castes. primer OPG-17 generated one band with 613 bp, primer OPB-03 generated one band with 1034 bp, primer OPF-09 generated one band with 497 bp, primer OPG-06 generated one band with 839bp, Primer OPG-04

generated 3 bands (561, 411 and 245 bps), primer OPC-02 generated 2 bands (506 and 437 bps), (Table,3 and Figs. 2, 3 and 4).

The number of fragments (or loci) amplified with RAPD primers depends on factors such as the size, composition of the genome and the reaction conditions (Williams *et al.*, 1990).

Polymorphisms generated with RAPD primers result from success or failure of the primer to bind, creating the presence or absence of a particular amplified band, and from insertions or deletions that change the length of the amplified region. In general most RAPD markers are of the first type and are dominant in expression, whereby a diploid with two copies of a RAPD marker cannot be distinguished from those containing one copy (Tingey *et al.*, 1992 and Williams *et al.*, 1990).

The observed difference in genomic DNA in the present study between the virgin queens and workers may be explained as the two studies castes resulted from hybridization between two colonies as reported by Julian *et al*., (2002) in the two seed harvester ants, *Pogonomyrmex rugosus* and *Pogonomyrmex barfatus*. They said that within area of sympatry of these two ant species, workers displayed bands that were absent in their alate queen sisters .The most parsimonious explanation for these genotype differences is that the workers and queens come from different patrilines. Because Hymenoptera are haplo-diploid, workers from the same father share the markers specific to that patriline. Males are produced parthenogenetically, and their genotypes reveal the queen's genotype. Therefore, markers absent in male but present in workers are inherited patrilinially.Moreover, the difference in genomic DNA between the two studies castes may be explained as mentioned by Fournier *et al.*,(2005) who stated that, in the little fire ant , *Wasmannia auropunctata*, female sexuals are produced by a meiotic parthenogenesis from unfertilized eggs, workers from fertilized eggs, and males from fertilized eggs after the elimination of the maternal nucleus, resulting in a complete separation of male and female gene pools.

In conclusion, since the numbers of queens in the ant's colony is one or few, the present study showed that the queens produce high levels of insecticide detoxifying enzymes (α , β-esterases, acid phosphatase and glutathione S- transferase) as a mean of defensive mechanism against the insecticides that are used to control them .This study may also answer the question why insecticide treatment sometimes don't work .

Further molecular studies must be performed concerning expression of genes between the two castes to know the reliable cause of genetic difference between them.

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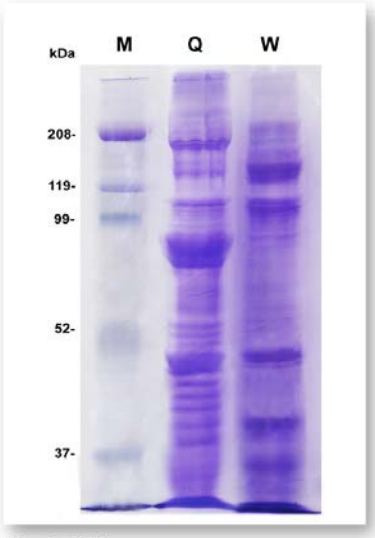

Fig. 1-(A)

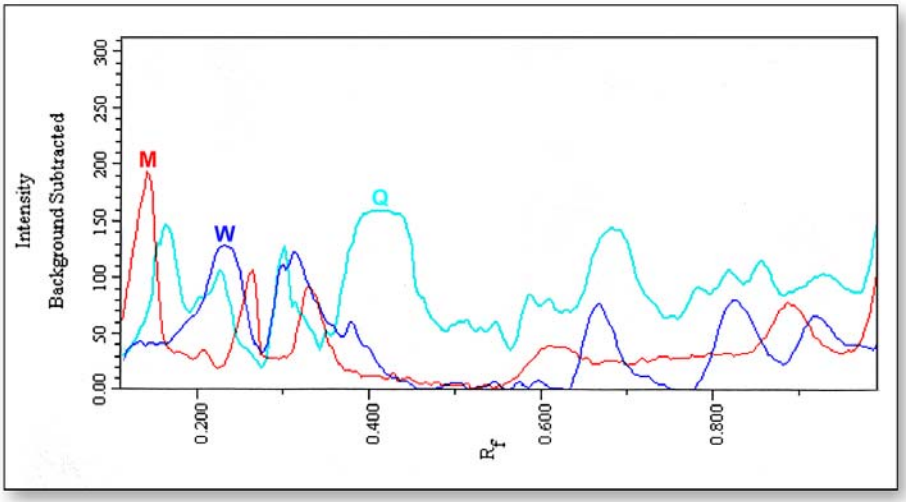

Fig. 1-(B)

Fig. 1: (A). The SDS PAGE gel showing the protein banding patterns of the virgin queens (q) and workers (w) of ***Camponotus maculatus.***
(B). Densitometer tracing of the protein profiles for the above mentioned two castes. The results are computerized using the gel Documentation system (GDS).

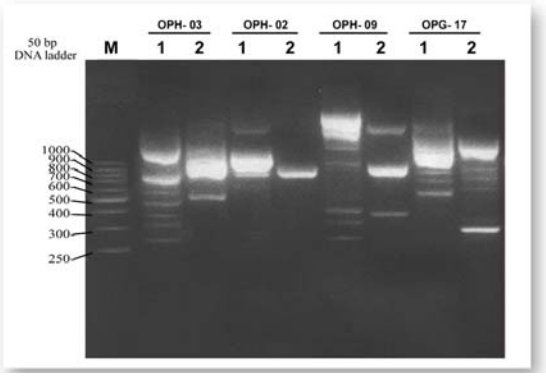

Fig. 2: Agrose – gel electrophoresis of genomic DNA of virgin queens (1) and workers (2) of *Camponotus maculatus* using RAPD-PCR technique and the primers OPH-03, OPH-02,OPH-9 and OPG-17.

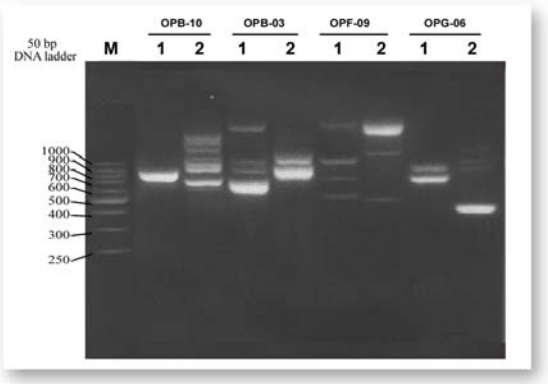

Fig. 3: Agrose – gel electrophoresis of genomic DNA of virgin queens (1) and workers (2) of *Camponotus maculatus* using RAPD-PCR technique and the primers OPB-10, OPG – 06.

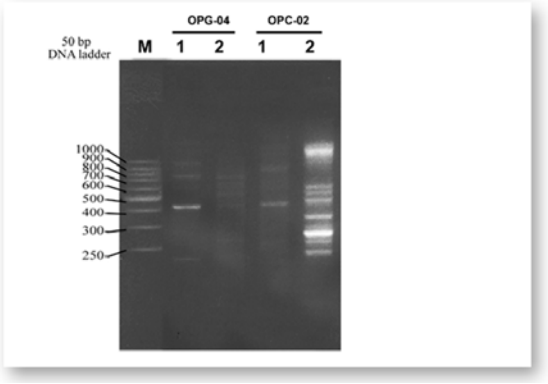

Fig. 4: Agrose – gel electrophoresis of genomic DNA of virgin queens (1) and workers (2) of *Camponotus maculatus* using RAPD-PCR technique and the primers OPG-04and OPC– 02.

ARABIC SUMMERY

الاختلافات البيوكيميائية بين الملكات البكر والشغالات للنمل (كمبونوتس ماكيولاتس)

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استهدف البحث دراسة الاختلافات البيوكيميائية بين مللكات النمل البكر(كمبونوتس ماكيولاتس) والشغالات . أوضحت الدراسة ارتفاع معنوى فى أنشطة كل الانزيمات المختبرة ما عدا استيل كولين استريز (خمائرβ و α - الفوسفات الحامضى وجلوتاثيون) فى المستخلص الكلى لجسم ملكات النمل البكر عن مثيلاتها فى الشغالات. كذلك ارتفاع فى تركيز البروتينات الكلية المذابة للملكات البكر عن مثيلاتها فى الشغالات وهذه البروتينات تم فصلها بالتفريد الكهربائى إلى 22 شريط بروتين (258.6 إلى 35.4 كيلو دالتون) فى عينات الملكات البكر بينما تم فصل 19 شريط بروتين (216.7 إلى 35.4) فى عينات الشغالات. وكان هناك عشر أشرطة بروتينية مشتركة فى كلا الطائفتين (108.1 ، 103.9 ، 99.8 ، 94.6 ، 63.7 ، 61.3 ، 55.9 ، 48.1، 40.6 ، 35.4 كيلو دالتون) وبقية الأشرطة كانت مميزة لكل طائفة وأخيراً كان هناك فرق فى الحامض النووى (DNA) للطائفتين المختبرتين.