Results

&

Discussion

RESULTS AND DISCUSSION

1. Survey of the two honeybee foulbrood diseases (American and European foulbrood) and accompanied *Varroa destructor* mites in certain apiaries at seven Egyptian different Governorates:

1.1. Survey of AFB and EFB in the considered apiaries:

1.1.1. Investigation of the brood combs morphology:

At the 34 apiaries which were visited and inspected, the following results were recorded:

al 665 honeybee colonies from 32 apiaries had appeared diseased symptoms distinguishable with the sealed broods, those symptoms (Figures No. 17-30) were:

- Odor of the colonies was strong when opening it and very bad liked of the rotten fish odor particularly in the late retrogressive cases.
- The adult bees in the diseased colonies were not abundant or presented with little amount.
- The sealed brood was discolored as very dark contrary to the normal color.
- The brood cells cappinges were sunken inner the cells.
- Most cappinges were punctured at more than one side.
- The broods were dead at older age or young pupae upright in the cells.
- Color of the dead brood was light brown or coffee brown to dark and sometimes black.
- The brood which recently dead was soft and make a sticky or a ropy threadlike material longer than 2cm. when inserting of a matchstick into the cell and then drawing out then the threadlike fall back.
- These dead broods exhibit pronounced glue to gluepot odor.

- Much of these dead broods were dried, make black scales lie flat on the lower side of cells and adhered tightly to the cells walls.
- In some cases, the fine threadlike tongues of dead pupae were observed.

All the symptoms which were observed and above-mentioned are acceptance with the descriptions of **AFB** disease by **Morse, 1980; Sanford, 1987; Shimanuki & Knox, 1991; Alippi, 1991; Ritter & Kiefer, 1993; Nordstrom & Fries, 1995; Goodwin *et al.*, 1996; Alippi, 1997; Hansen & Brodsgaard, 1999** and **Chantawannakul & Dancer, 2001.** So, it could be concluded that all the honeybee colonies which appeared those symptoms are infected with **AFB** disease.

12) 40 **honeybee colonies** from 6 **apiaries** had appeared diseased symptoms distinguishable with the sealed broods, those symptoms (Figures No. 31-32) were:

- Odor of the colonies was fairly bad when opening it and likes of the acidity or fermented odor.
- Abundance of the adult bees in the diseased colonies was a little decreasing.
- The unsealed and some sealed broods in advanced cases of the disease were discolored.
- The broods were dead at younger of unsealed larvae in coiled stage whereas appeared curled or twisted shape in different positions of the cells.
- Color of the dead larvae was yellowish white to orange or brown.
- The brood which recently dead was watery, slight sticky and sometimes made a short threadlike about as 0.5-1cm. by inserting of a matchstick into the cell and drawing out then the threadlike did not fall back again.
- The dead broods exhibited odor of slight sour to penetratingly sour.

- All those dead broods in the advanced cases were dried and made a brown scales appear in granular twisted shape not adhered tightly to cells walls whereas it could be moved easily.

All the symptoms which were observed and above-mentioned are acceptance with the descriptions of **EFB** disease by **Cantwell, 1974; Langdridge, 1979; Morse, 1980; Bailey, 1981; Farmnote, 1984; Sanford, 1987** and **Shimanuki & Knox, 1991.** So, it could be concluded that all the honeybee colonies which appeared those symptoms are infected with **EFB** disease.

1.1.2. Hoist Skim Milk test procedure:

All the scales or diseased larvae which were taken from the exhibited colonies to **AFB** symptoms gave a positive reaction with this test whereas the milk curdled in littler than 40sec. on the glass slide while those which were taken from the exhibited colonies to **EFB** symptoms did not curdle to the milk, and those reactions in the two cases agree with showing of **Chantawannakul & Dancer, 2001** to the test whereas the **AFB** bacterium has the ability on curdling the skim milk because it produces some proteolytic enzymes to milk proteins during its sporulation, but the **EFB** bacterium is non-spore bacterium due to that I confirm on sickness of the inspected colonies in the cases (a) and (b) with **AFB** and **EFB** diseases respectively.

- So that, numbers and percentages of the diseased honeybee colonies with both of **AFB** and **EFB** diseases were evaluated and registered in table No. (5) depending on results of the previous tests.
- Also, the inspection dates of all the apiaries were registered in table No. (5) and cleared on Diagram No. (1), whereas it is concluded from them that the **EFB** infections appear in the spring months only, while **AFB** symptoms can exhibit at any time over the year in the honeybee colonies.

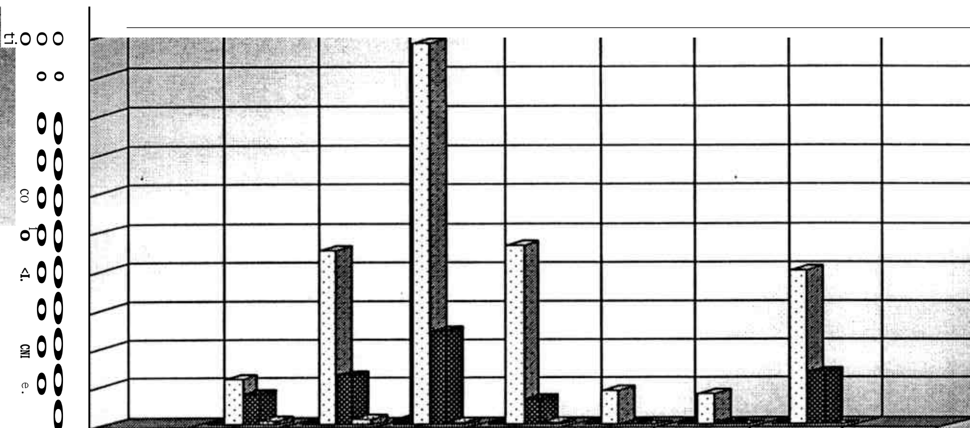
Table (5): Numbers and infection percentages of the honeybee colonies infected with the foulbrood diseases in certain Egyptian apiaries during 2004, 2005, 2006 and 2007 seasons.

	Governorate	Locality (apiary)	Inspection date month/year	Total No. of the inspected colonies	The infected colonies			
					AFB		EFB	
					No.	%	No.	%
	Cairo	Abbasia	2 / 2007	95	76	80	11	11.6
		Sixth Part (Nasr city)	2 / 2007	24	5	20.8	0	0
Total		**2 apiaries**		**119**	**81**	**68.1**	**11**	**9.2**
	Giza	Abu-Elnomros	9 / 2004	80	31	38.8	0	0
		Gesr-Elmanawat (Elhawamdia)	4 / 2005	11	3	27.3	0	0
		Elsahran (Elhawamdia)	4 / 2005	11	2	18.2	0	0
		Dokki	4 / 2006	32	2	6.3	7	21.9
		Elaresha	8 / 2006	25	16	64	0	0
		Kafr-Tohormos	10 / 2006	35	35	100	0	0
		Elgalatma (Elmanashi)	2 / 2007	112	17	15.2	9	8.04
		Geziret-Elbahr (Elmanashi)	3 / 2007	145	23	15.9	0	0
Total		**8 apiaries**		**451**	**129**	**28.6**	**16**	**3.6**

	Governorate	Locality (apiary)	Inspection date month/year	Total No. of the inspected colonies	The infected colonies			
					PO		PCI	
					NO.		NO.	%
	CY	Makint-Ezzibaq (Quha)	Z/ ZOOS	oo		S 1ℨ	o	o
		Tersa (Toukh)	3 / 2005	ZOO	SI	Z S•S I	o	o
		Kafr-Elwalaga	4 / 2005	ZO	177	17-7-1	o	o
		Kafr-Mansour	Z 00S	OV	FET	OC	o	o
		Kafr-Taha	9 / ZOOS	ZO	z	0I	o	CD
		Tannan	9 / ZOOS	E	cr	50 E	o	o
		Mazlaqan-Zeky (Toukh)	9 / ZOOS	SC	Z8	80	o	o
		East Agrarianism Association	I / Z00S	IZS	9L	60.8	o	o
		Kafr-Shokr	1 / 2006	OL I	ZO	II 8	o	o
		Mazlaqan-Peltan (Toukh)	/ 9002	SS	IO	Z 8I	o	o
		Saft-Elgezira	/ 900Z	ZZ	r-		o	o
		Halaba	OI /900Z	SI	Cr%	60	o	o
		Sendipis (Quha)	Z / LOOZ	ZO	cf)	7 1	o	OC
		Petmeda(Shebeen)	ℨ / LOOZ	ZO	.0	1:1ℨ	o	o
Total		14 apiaries		186	I SVZ	SZ	.o	19'0 I

	Governorate	Locality (apiary)		Total No. of the inspected colonies				
		Quisna	12 / 2004	30		I 36.7	0	0
		Ashmun	2 / 2005	37	27	73	0	0
		Elkawady	3 / 2005	280	[illegible]	0.71	0	0
	[illegible]	Elghannamia	3 / 2005	40	[illegible]	0	[illegible]	1 7.5 1
		Sarawa	10 / 2005	50	[illegible]		0	0
		Sheshae	10 / 2006	18	18	100	0	0
		Shatanof	3 / 2007	10	0	[illegible]	I	[illegible]
Totall		7 apiaries		465	66	14.2	[illegible]	[illegible]
1	Dacahlia	Met-Ghamr	4 / 2005	89	[illegible]	3.4	0	0
Totall		1 apiary		89	[illegible]	3.4	0	0
1	Shargia	ouIwuua	9 / 2005	80	[illegible]	6.1	0	0
Total I		1 apiary		80	[illegible]	6.1	0	0
	Faiume	Tamia	2 / 2007	400	136	34	0	0
		1 apiary		400	136	34	0	0

seasons.



	Cairo	Giza	Qualubia	Menoufia	Dacahlia	Sharqia	Faiume
Total No of inspected apiaries	[illegible]	[illegible]	14	7	1	1	1
Total No. of inspected colonies	11[illegible]	401	981	4[illegible]0	[illegible]	[illegible]	400
Total No. of AFB colonies	01	1[illegible]	245	66	3	5	1[illegible]
Total No. of EFB colonies	11	16	6	7	0	0	0

Governorates

2. Laboratory Experiments:

2.1. Diagnosis of the two foulbrood diseases:

2.1.1. Reinspection the morphology of the collected brood combs:

When the infected brood combs were subject to investigate for a second time in the laboratory, all the clinical symptoms of **AFB** or **EFB** disease which were observed in the field, were confirmed of as far as 32 samples with clinical symptoms of **AFB** disease (Figures No. 33-38) and 6 samples with **EFB** symptoms (Figure No. 39).

2.1.2. Reprocedure the Hoist Skim Milk test:

32 samples from the 38 diseased brood combs which were brought to the laboratory gave a positive reaction with **Hoist** test whereas color of the muddy mixtures in the tubes returned to clear after about 20min. incubation of them at 37°C., while 6 samples gave a negative reaction whereas persisted almost muddy (Figures No. 44-45), and according to **Holst, 1946** and **Shimanuki & Knox, 1991** that ***P. L larvae*** bacterium produces during the sporulation a high level of proteolytic enzymes for the skim milk so the milk proteins fall or precipitate in the tubes then the mixtures become clear in contrast of the non-spore bacterium of ***M. pluton,*** It could be concluded that the positive samples contain ***P. L larvae,*** the causative bacterium of **AFB** disease while the negative samples contain ***M. pluton,*** the causative bacterium of **EFB** disease.

2.1.3. Isolation and purification of the causative bacterium of both foulbrood diseases:

al From the bacterial suspensions which were prepared from both of broods and honeys of the 32 **AFB** samples, bacterial colonies appeared or grew on SBA medium after 96hrs. of incubation at 37±1°C. in some samples **(Anderson, 1990; Shimanuki &**

Knox, 1991; Hornitzky & Clark, 1991; Hornitzky & Nicholls, 1993; Alippi, 1997 and **Hornitzky,1998a and 1998b)** and after 7days of incubation in other cases **(Hoyo *et al.,* 2001).**

12) From the bacterial suspensions which were prepared from both of broods and honeys of the 6 **EFB** samples, bacterial colonies appeared or grew on **Bailey's** medium after 9 days of incubation at 34±1°C. **(Anderson, 1990** and **Djordjevic *et al.,* 1998).**

2.1.4. Identification tests of the isolated bacteria:

2.1.4.1. Morphology describe of the bacterial colonies:

al The bacterial colonies which appeared slowly on SBA medium (Figures No. 46-47) were single colonies measure about 4mm. in diameter, had irregular edges, flat, light grey, non-pigmented (this characteristic according to **Chantawannakul & Dancer, 2001;** is first difference between most non-pigmented subsp. ***Larvae*** and pigmented subsp. ***pulvifaciens*** which belong to ***Paenibacillus larvae*** bacterium), so it could be concluded that those colonies are belong to ***P. I. larvae*** bacterium according to **Drobnikova *et al.,* 1994; Nordstrom & Fries, 1995; Hornitzky, 1998b** and **Hoyo *et al.,* 2001.**

j21 The bacterial colonies which appeared very slowly on **Bailey's** medium (Figures No.48-49) were single colonies, very small measure 1-2mm. in diameter, biconvex, shape circular, have regular edges, white of color and sometimes grow deeply inner the medium, so it could be concluded that those colonies are belong to ***M. pluton*** bacterium according to **Anderson, 1990** and **Djordjevic *et al.,* 1998.**

2.1.4.2. Catalase production test:

The bacterial colonies which isolated on **SBA** medium gave a negative reaction with catalase test, so it could be though that the

reason is non-ability of the isolated bacterium on producing to catalase enzyme which analyses H202, however according to **Shimanuki & Knox, 1991** it could be concluded that the isolated bacterium is not ***Bacillus alvei*** or ***Bacillus laterosporus*** bacteria which are catalase +ve, but it is one of the two subspecies which belong to ***Paenibacillus larvae*** bacterium which all are catalase-ve.

2.1.4.3. Gram stain test:

By the microscopic examination to the stained films which were prepared from cultures of **SBA** medium, obtained data were:

- Flooded rod forms with the blue or violet color, measured as 1.5-6.0µm. long and as 0.5-0.6pm. wide (Figures No. 50-52).
- Blue rods during transformation to endo-spores (Figures No. 53-54).
- Very small and ellipsoidal bright spores measured as 1.2pm. length and 0.611m. diameter (Figures No.55-56).

However, pursuant to descriptions of all the researchers up to **Piccini & Zunino, 2001,** it could be concluded that those bacterial forms are the different stages of Gram +ve ***Paenibacillus larvae,*** and I confirm that it is subsp. ***larvae*** whereas Gram +ve rods of subsp. ***pulvifaciens,*** the causative bacterium of powdery scale disease according to **Shimanuki & Knox, 1991;** measure as 1.5-3.0µm. long and as 0.3-0.6 m. wide, but measures as 1.0 by 1.3-1.5µm. respect for the spores, and this is second difference between two subsp. ***larvae*** and ***pulvifaciens.***

2.1.4.4. Staining of the spores and vegetative cells with some other stains:

al Schaeffer & Fulton method (Zaki, 1988);

By the microscopic examination to the flooded films which prepared from cultures of **SBA** medium, I found all the endospores had only green color without any combined red crystal bodies

(Figure No. 57), so it could be confirmed that spores not belong to ***Bacillus thuringiensis*** bacterium which appears the red crystal bodies in this test according to staining method of Schaeffer & Fulton.

121 The negative staining;

With respect to the prepared films from cultures of **SBA medium, the rods were found** flooded with light violet color while the spores . remain bright on dark blue background (Figures No. 58-61). On the other hand, respect to the prepared films from cultures of Bailey's medium, cocci forms or sphericals appeared with white color or bright on black ground in pairs or chains (Figures No. 62-63) as well as descriptions of **Alippi, 1991** and **Djordjevic *et al.*, 1998.**

2.1.4.5. Nitrate reduction test:

After **72hrs. incubation at 37°C. to the tubes of nitrate** medium which were inoculated by cultures 24hrs. of **SBA medium,** light grey growths appeared in tubules as a sub-culture (Figure No. 64); then after addition of the reagent to media, a stable deep red dye formed immediately (Figure No. 65) as well as description of **Shimanuki & Knox, 1991; so it could be concluded that** cultures not belong to ***Bacillus alvei*** which is negative to this test but belong to ***Paenibacillus larvae*** bacterium.

2.1.4.6. Voges-Proskauer test:

The cultures of **SBA** medium gave a negative result with this test whereas color of the media did not turn to reddish, and according to **Alippi & Aguilar, 1998a,** those cultures are ***Paenibacillus larvae*** subsp. ***larvae*** bacterium.

2.1.4.7. Indole production test:

Also, the result in this test get out negative whereas no fushia

ring forms in test tubes, and exactly those cultures are ***Paenibacillus larvae*** sulbsp. ***larvae*** bacterium unanimously with **Alippi & Aguilar, 1998a** and **Piccini & Zunino, 2001.**

2.1.4.8. Growth on nutrient agar test:

The cultures which isolated on **SBA** medium did not success in appearance when re-growing or sub-culturing of them on nutrient agar and this result acceptances with reports of all researchers who tried useless for culturing of this bacterium on nutrient agar medium, while ***Paenibacillus larvae*** subsp. ***pulvifaciens*** can able to growth easily on this medium, and this is third difference between the two subspecies ***larvae*** and ***pulvifaciens.***

2.1.4.9. Microscopy examination by using the modified hanging drop test:

On contrast the negative result of the previous test, exactly same of the used bacterial suspensions gave a positive result in this test whereas appeared to a **Brownian movement** of the spores which means movement of spores around its axis or pivot in the local position due to its collision by molecules of the milieu. So, it could be confirmed that isolated bacterium on **SBA** medium is ***Paenibacillus larvae*** subsp. ***larvae*** in contrast ***Paenibacillus larvae*** subsp. ***pulvifaciens*** which is negative for **Brownian movement** test as fourth difference between them according to **Shimanuki & Knox, 1991.**

2.1.4.10. Artificial infection procedure ***(in Vitro):***

Both of the two larval groups which fed with the bacterial inoculum of ***P. L*** subsp. ***larvae*** or ***M. pluton*** in separately, exhibited most of clinical symptoms of **AFB** or **EFB** disease respectively comparative with those the accompanies which did not fed as control whereas became prepupae (Figure No. 67).

2.2. Confirmation of identify of the bacterial isolates at some specific microbial laboratories:

According to method of biochemical **API** tests which were performed in the **VACSERA** laboratories on pure culture isolated from exemplary **AFB** sample on slant of **SBA** medium at 12/1/2006 and submitted to them at 1/10/2007, the outcome or result was ***Paenibacillus larvae*** as it was cleared in the attached report.

R oldingeomputty for Biological products & Vaccines
Environmental Monitoring Iwo

Report form

1. Requester: *Moluzmecl El-Stied Mohamed Hassan*
2. Date of request: *1(10/2007*
3. Type of sample: *Pure Culture on slant* (s 6 e (4,_,L.,,)
4. Origin of sample: *Honey bee brood with clinical symptoms of APB disease*
5. Date of sampling: *12/1/2006* C,.4.
6. Media used: *MYPGP medium*

Profile:

Teat *It*	0	1	2	3	4	5	6	7	8	9
Test Name	0	GYL	ERY I	DARA	1 LARA	RIB	DXYL i	LKYL	ADO	MDX
Result	-	-	-	-	-	+				-

Test #	10	11	12	13 .	14	15	16	L7	18	19
Test Name	GAL	GM!	FRU	MNU	SHE	RUA	DUI.	1NO	MAN	SOR
Result	-		-			+	-			

Test*	20	21	22	23	24	25	26	27	28	29
Test Name	ADM	MDG	NAG '	AMY	ARB i	ESC	SAL	CEL :	MAL	LAC
Result	-				-			+		j

Tot *it*	30	31	32	33	34	35	36	37	38	39
i Test Name	610.1.	SAC	THE	INU	ML'/,	RAF	AMD	GLYG	XLT .	GEN
Result	-		+		+			-	-	-

Test *it*	40	41	42	43	44	45	46	f 47	48	49
Teat Name	rot	LYX.	TAG	DPW	LAX'	DARL	LARL I	GNT :	2KG	5KG
Result			-				-	-	-	-

UNPG I AD11	LUC	ODC	C1T	1125	URE^{-1} TDA	1ND	VP = GEL	OW

*identification of sample:

Paenibacillus larvae

Title	Name	Signature	Date
Environmental Monitoring Analyst (s):	***Micro. Mohamed Rabat***		2.7 *halv-7*
	Ph. Naha Ismail	01/1401,A T_{Sm}4	,2s-ri=tal
Technical Manager of Etnironmental i Monitoring unit:	***Dr. Momen Formal***	C-:- 2-4/-	***, a 1 Il.k.e-t ° 4***
1 QC General 3.1anager	*1144.47Vita/*	r•c^{i} a.'''	,:*iyi. t,* 7

Pane No Ill

2.3. Study of the relationship between *Varroa destructor* mites and infection with the foulbrood diseases:

2.3.1. Count of *Varroa destructor* mites numbers from the collected debrises:

In table No. (6) it is shown of higher and lower numbers of Varroa mites which were estimated from the collected debrises of the honeybee colonies with **AFB** clinical symptoms, while those numbers which belong to the colonies have EFB symptoms are shown in table No. (7), in addition to registering of Varroa numbers which were estimated from 5 **healthy honeybee** colonies/apiary in the two cases, whereas the following appears:

- In **AFB** case, the maximum number of Varroa was 65 **individuals** at one of the apiaries **(Gesr-Elmanawat** in **Giza).**
- In **EFB** case, the maximum number of Varroa was 57 **individuals** at one of the apiaries **(Elgalatma** in **Giza).**
- Generally, the minimum number of Varroa from the healthy colonies restricted • between 16-34 **individuals,** while the maximum numbers were 850 and 752 **individuals** at **Tersa** and **Elghannamia** apiaries respectively.
- Generally, the low numbers of Varroa from the healthy colonies approach or near to the high numbers of Varroa from whether **AFB or EFB** colonies.
- But the high numbers of Varroa from the healthy colonies exceed with very far o ff all the numerical levels of Varroa from whether **AFB or EFB** colonies.

Governorate	Locality (apiary)	No. of colonies for collecting debrises	Colonies with symptoms	
			Higher number	Lower number
	Makint-Ezzibaq			
	Kafr-Elwalaga			

Governorate	Locality (apiary)		No. of Varroa in collect debrises						
				with toms	Colonies without symptoms				
			Higher number	Lower number	Replicates				
Cairo	Abbasia	11+5	43	10	208	164	97	53	45
Giza	Elgalatma	9 + 5	57	18	127	80	64	53	34
Qualubia	Sendipis	6 + 5	14		92	85	59	40	36
Menoufia	Elghannamia	3 + 5				618		11	
	Shatanof	4 + 5	18		411	143	114	69	
Total	5 apiaries	58 colonies							

2.3.2. Isolation of the foulbrood bacteria from *Varroa destructor* mites:

In table No. (8), the results were as follows:

- All the inoculums which extracted from non sterilized Varroa of the colonies with **AFB** or **EFB** symptoms gave respectively, ***P. l. larvae*** or ***M. pluton*** growths on the suitable medium, and generally, mean of the bacterial colonies numbers was very low whereas varied between 3-5 **colonies** in **AFB** case and between 1-2 **colonies** in **EFB** case.
- All the inoculums which extracted from exo-sterilized Varroa of **the** colonies with **AFB** or **EFB** symptoms did not give any growth on the suitable medium.
- All the inoculums which extracted from whether non-sterilized or exo-sterilized Varroa of the healthy colonies did not give any growth on the suitable medium.

And dependency on previous results of the counting and bacterial isolation from Varroa, it could be concluded the following points:

- No relation presences between strength of AFB or **EFB** disease and Varroa numbers which existence in the honeybee colonies.
- Varroa mites are considered mere of feeble carrier to the pathogens on their bodies only if it found in the diseased colonies (Figure No. 69).
- Therefore, according to **Panizzi & Pinzauti, 1988; Sammataro, 1997 and Hansen & Brodsgaard, 1999,** no definite relation between **AFB** or **EFB** disease and ***Varroa destructor*** mite.

Symptoms of disease	Apiary	Extract inoculums from Varroa of the colonies with clinical symptoms of the disease								Extract inoculums from Varroa of the colonies without clinicalsymptoms of the disease							
		Varroa non sterilized				Varroa exo sterilized				Varroa non sterilized				Varroa exo sterilized			
		Replicate			Mean	Replicate			Mean	Replicate			Mean	Replicate			Mean
AFB - [nom,toms heated -SBA medium used	Gesr-Elmanawat (Elhawamdia)																
	Makint-Ezzibaq (Quha)																
	Tersa (Toukh)																
	Kafr-Elwalaga																
	Met-Ghamr																
EFB - Inoculums non heat - Ba ley's medium use	Abbasia																
	Elgalatma (Elmanashi)																
	Sendipis (Quha)																
	Elghannamia																
	Shatanof																

2.4. The inhibitory effect of some therapeutic materials on *Paenibacillus larvae larvae* bacterium growth under laboratory conditions *(in Vitro):*

As shown in table No. (9) that the untreated plates appeared high numbers of ***P. 1. larvae*** colonies after 96hrs. incubation at 37±1°C. then the growth spread and full over surfaces of the plates.

By opposite, all the therapeutics gave just disparate inhibition levels at the different concentrations that appear from the different numbers of ***P. 1. larvae*** colonies which were lower than those in the untreated plates.

Except **Peppermint powder** (Tables No. 12, 12') all therapeutic materials resulted a complete inhibition to ***P. L larvae*** growth.

Whereas the best inhibited concentrations for **Tylosin, Neomycin, Erythrocin** and **Primomycin** were 1.25, 1.5, 1.5, and 0.75m1 /100m1. respectively after both of 96hrs. and 7days incubation (Tables No.10,10').

And the best inhibited concentrations for **Peppermint, Clove** and **Cinnamon oils** were 0.25, 0.1 and 0.05m1 /100m1. respectively after both of 96hrs. and 7days incubation (Tables No.11,11'), concentrations of completely inhibition by using of **Clove** and **Cinnamon oils** were 1000 and as low as 10p.p.m. respectively, recommended by **Calderon *et al.*, 1994.**

But the best -inhibited concentrations for **Clove** and **Cinnamon powders** were 0.2 and 0.15gm /100m1. respectively after both of 96hrs. and 7days incubation (Tables No.12,12').

Finally, the best inhibited concentrations for **Zanzan fruits extract** and **Farozal Liquid compound** were 0.5 and 1.25m1 /100m1. respectively after 96hrs. incubation (Table No. 13), while

after 7days incubation the complete inhibition continued at 0.5 and 1.5m1 /100m1 for **Zanzan fruits extract** and **Farozal Liquid compound** respectively (Table No. 13'), concentration of completely inhibition by using both of the formulated or technical grade Azadirachtin «Neern plant» is 5ug / ml., recommended by **Williams** *et al.,* **1998.**

Table (9): Untreated Petri dishes (Control)

Replicate					
After 96hrs. of incubation at 37±1°c. «appearance time of the colonies»	**The bacterial growth is evaluated by numbers of the colonies**				
After 7days of incubation at 37±1°c.	**The bacterial growth is evaluated by area of the growth which covers the plate**				

Keys of tables :

< The bacterial growth is very too light as nebula or mist.

Table (10): Antibiotics group « after 96 hrs. of incubation at 37 ± 1° c. »

Therapeutic substance		Tylosin					Neomycin					Erythrocin					Primomycin				
	licate		)	w	.	vi	*..ı	t...)	(...4	A	(t	lA	l'4		A	Cil			(.0.1	.A	A
Cone by m11100m1. medium	0.05	x	...	x	x.p	x	27	25	30	I31	28	18	12	VD	FT				FIT	o	UT]
	0.1	x	xi	x	x	x	18	19	24	121	1 26	VD	110	114	17,7	FT,	177		01	LA	oo
	0.15	x	x	x	x	x	1 16	Ili	20	18	22	Z)	oo		cp,	Cr	LA	1	4	C:1	CT.
	0.2	x	x	x	x	x	113 1	14	.o		12	o\	L.>		3	.4..	Cs..)	Lo	--	ı-ı	ts...)
	0.25	x	x	x	x	x	..4	12	oo	1	.0	Is.)	4		1.0	..-.	Z'''	.	--	O	C...>
	0.5	x	)x	)	-x	-x	vo	c.,	--1	c%	oo		IV		.4	(...>	CD	O''	C>	Z''	C>
	0.75	4'	...	..	a	-	4=..	ts..)	—	Iv	Lo	—,	(..,>		h./	D''	O	O	CD	C>	CD
	r'	=	ot	ot	=	a	-	[.'	N.)	=	—,	o	.--		CD	CZ>	CD	CD	C>	O	O
	1.25	o	o	o	o	o	a-	o	c>	—	c)	=	.,		D'''	CD	C>	O	O	C>	CD
	1.5	x	x	x	x	x	co	o	O	O	O	O	CD		C>	CD	O	CD	CD	O	O

Table (10'): Antibiotics group o after 7days of incubation at 37 ± 1° c. >>

Therapeutic substance		Tylosin					Neomycin					Erythrocin					Primomycin				
	plicate	—	N	m		wa	,-(	N	M	et	kll	---i	N	M	•Zr	If)			M	Cr	in
tutimam •Ituo mpu Sq au03	Li) c;	x	x	x	x	x	r--	ILS'TI	8'L		7'£	L'17	1'£	6'17	17 t	9'£	Z	9'£	9'Z	Z	7
	,_ o	x	x	x	x	x		617	Z*9	S'	8'9	E7	9'Z	9'£	971		6'Z	9'Z		E'I	Z
	14 c;	x	x	x	x	x			Z'S	L'17	C'S	£7		.	9'I	£'I	£ 1	8'I	;	9 1	9'1
	el O	x	x	x	x	x	t'£	9'£	67	6'Z	1'£	9 1	8.0	£'1	8'I		S'0	8 0	£ 0	£'0	£'0
	n c)	x	x	x	x	x	8'1	E	1'Z	97	£ Z	S'0		£'0	8 0	E'0	c::	£'0	£'0	1'0	5'0
	if) c;	x	x	x	x	x		77	8'1		I 7	E'0	cò	O £	,_,	I 8 O	CD	..O	O	..0	CI
	1 2 c=	--.	177	177		170	-,		4	cò	8'0	O	8'O	O	5'0	O	O	CZ	O	O	O
	'-'	_c	O	O	_c	_c	£'0	£'0	cò	1'0	£'0	1	£'0	S'0	1'0	O	O	O	C>	C>	O
	.-'	c)	O	C)	O	O		I'0	1'O	£'0	O	1'0	£'0	£'0	1'0	..O	CD	C)	O	O	CD
	tn ee	x	x	x	x	x	c)	c)	c)	o	c)	c)	c)	C>	o	C>	O	O	O	C>	C>

ble (11): Volatile essential oils « after 96 hrs. of incubation at 37 ± 1° c. »

Conc. by m11100m1. medium	Ilicate														
		_–-	N	44	.f.,	Ut	I-+	t.,)	to.)	.A.	CJi		ICJ	C.44	41	VI
0.0 (It	a,	NJ t..)	26	20	28	h.)	O	C..)	--	O	O	O	O	O	O
0.[-I	O	-.	12	14	10	O	O	O	O	O	O	O	O	O	O
0.1i (11	00	—	VI	!O	10	O	O	O	O	O	O	O	O	O	O
0 ks j	c>	L.)	.--.	O	—	c>	O	O	O	O	O	O	O	O	O
0 t4 CA	O	O	O	c:	c:,	O	O	O	O	O	O	O	O	O	O
0 U1	O	C>	O	O	O	O	C>	O	O	O	O	O	O	O	O
0 .-.1 (.1k	c>	O	O	C>	O	O	=	O	(D	O	O	O	O	O	O
I•-'	O	O	O	O	C>	(c	O	O	O	O	O	O	O	O	O
tL i(A	O	O	O	O	O	c>	O	O	O	O	O	O	O	O	O
?- (A	O	C>	O	C>	C>	O	O	O	O	O	O	O	O	O	O

Table (11'): Volatile essential oils « after 7days of incubation at 37 ± 1° c. »																
Therapeutic substance		Peppermint					Clove					Cinnamon				
Replicate		1(	("4		'	V')	,-1	N	M	7t.	tf)	,--	<-4	M	-i"	1f)
utwatu •inooiltu fig •auoD	0 0 S		[illegible]	6 8			0 C	©	0.S	0 ꟻ	O.I	0	0	0	0	0
	O*I	Z•6	E.9	M	ꟻ 6	Z.6	0	0	0	0	0	0	0	0	0	0
	S	[illegible]	Z•9	1		Z.6	0	0	0	0	0	0	c>	0	0	0
	O	0.1	0.8			0 ꟻ	0	0	0	0	0	0	0	0	0	c=
	0 Z	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	O.S	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	SL'	0	0	0	0	0	0	0	0	0	0	0	c>	0	0	0
		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	SZ'	0	0	0	0	©	0	c	0	0	0	0	0	0	0	0
	S*1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	(12): Essential plants powders « after 96 hrs. of incubation at 37 ± 1° c. »															
	plicate	1-,	Z	W	4:.	c.n	0—	ts.)	co.)	4=.	u	)--i	IN)–	(.•,)	•P	VI
					185	87	VI	co		12		N.)	t...n	C...)	.-•	ts.)
					81	76	-1=.	-...)	h.)	(j	1...)	N.)	.—	O	N)	O
					78	ISL	O	O	O	.--	O	O	O	O	O	O
					65	59	O	O	O	O	O	O	O	O	O	O
					62	50	c)	O	O	O	O	O	O	O	O	O
		45			47	38	O	O	O	O	O	O	O	O	O	O
					32	35	c)	O	O	O	O	O	O	O	O	O
					30	26	c)	O	O	O	O	O	O	O	O	O
					16	12	©	O	O	O	O	O	O	O	O	O
					---1	ul	O	O	O	O	O	O	O	O	O	O

Table (11'): Volatile essential oils « after 7days of incubation at 37 ± 1° c. »

Therapeutic substance	Peppermint					Clove					Control				
Replicate															
tumpauututooNtu Aq •auoD															
SWO															
O,T															
SVO															
V0															
S*O															
O*S															
SL*0															
SZT															

(12): Essential plants powders « after 96 hrs. of incubation at 37 ± 1° c. »

Therapeutic substance		Peppermint					Clove					Cinnamon				
	plicate	I--L	N	teJ	416	U1	|--I	t..)	to4	.16	(A	o--	t•-)		46	
Conc. by gm1100m1. medium	o c.	90	91	83	85	87	li1	co	11	12	16	IV	01	La (k.)	7	NU)
	o.	79	86	80	81	76	4=	..l	2	5	3	t's.)	7	0	t•J	0
	o |1.' U1	67	70	78	178	83	0	CD	0	7.	0	0	0	0	0	0
	o t,,	62	61	67	65	59	0	0	0	0	0	0	0	0	0	0
	© U1	53	57	58	62	50	0	CD	0	0	0	0	0	0	0	0
	o. U.1	45	50	44	47	38	0	0	0	0	0	0	0	0	0	0
	c' U1	40	38	37	32	35	0	0	0	0	0	0	0	0	0	0
	0-	36	32	28	30	26	CD	0	0	0	0	0	0	0	0	0
	is.) U1	21	20	17	16	12	0	0	0	0	0	0	0	0	0	0
	ch		12	Ch	--I	In	0	0	0	0	0	0	0	0	0	0

Table (12'): Essential plants powders « after 7days of incubation at 37 ± 1° c. »

Therapeutic substance	Peppermint					Clove					Cinnamon				
I Replicate															
tumpatu luipoi\tu2 Sci •auop															

.3): Extracts & Compounds « after 96 hrs. of incubation at 37 ± 1° c. »

Conc. by rn11100m1. medium		h-+	kJ	CA)	A	til	I.+	N.)	C/4	A	;;;;
	C) u)	cō	La	29	25	20	56	61	54	7T	C1 00
	„	LA	La	20		21	38	42		41	45
	(A	I :.	oo	ı,c)	13		36	31	33	40	w
	i,„	CO	LA	...I	NI	-4,	27	34	29	26	::
	is.) vi	O	=	(...)	?	.-'	14	16	20	17	N.)
	.1,	O	O	O	O	O	12	15	10		13
	S.. v	O	O	O	O	O	01	4.)	00	--.1	--I
	-,	O	O	O	O	O	ts.)	vi	,--•	-4.	=
	is. cil	O	O	O	O	O	O	O	O	O	O
	,-•	O	O	O	O	O	O	O	O	O	O

Table (13'): Extracts & Compounds « after 7days of incubation at 37 ± 1° c. »

Therapeutic substance		Zanzan extract					Farozal Liquid compound				
	icate	1.1	N	M	·.1.	kAn		N	M	·v	I.O
Lung:law •itupoiltu Sq -auoD		9Z 2.	8S'8	VS' L	S'9	Z'S	9171.	6'g I	t7	8'171	L LI
	I'O	6'E	8£'£	Z'S	9117	917'S	88'6	6'0 I	1'01	Z7 I	L'
	SVO	98'Z	80'Z	17E7	8E'E	t7E'Z	9£ 6	90'8	8S'8	17'0 I	ZE'S
	Z*O	80'Z	£'I	Z8' I	ZS'0	170' I	ZO' L	t78'8	175'L.	9L'9	917'S
	SZ'O	o	E 1'0	6E'0	...c.	£I'0	179'E	91'17	Z'S	Z1717	L'
	S*O	o	o	O	c,	C	Z I E	6'E	9'Z	17E7	8£ £
	SL'O	o	o	c)	c)	o	9S' I	8L'0	807	Z8' I	Z8'1
	v-4	o	o	c)	c,	o	ZS'0	E1	9Z*0	170 I	9Z O
	SZ' I	c)	o	c)	c)	c>		6E*0	9Z 0	.o	E I '0
		c>	c>	o	c>	o		o	o	o	o

Therefore, it could be concluded from the previous results that the therapeutics already arranged with respect to their effect fitness on ***P. L larvae*** bacterium growth in the laboratory by minimum inhibition concentration (MIC) as follows:

Cinnamon oil by MIC as 0.05m1 /100m1. medium.

Clove oil by MIC as 0.1m1 /100m1. medium.

Cinnamon powder by 'WIC as 0.15gm /100m1. medium.

Clove powder by MIC as 0.2gm /100m1. medium.

Peppermint oil by MIC as 0.25m1 /100m1. medium.

Zanzan fruits extract by MIC as 0.5m1 /100m1. medium.

Primomycin by MIC as 0.75m1 /100m1. medium.

Tylosin by MIC as 1.25m1 /100m1. medium.

Each of **Neomycin, Erythrocin** and **Farozal liquid compound by MIC** as 1.5m1 / 100m1. medium.

On the other hand, it was noticed that MIC levels for complete inhibition of ***P. L larvae*** bacterium growth to the applied therapeutics were progressive and converged.

3. Field Experiments:

3.1. Control of the two foulbrood diseases at honeybee colonies *(in Vivo):*

3.1.1. Effect of the tested therapeutic materials on disappearance or recurrence of AFB and EFB diseases:

Table No. (14) shows this effect to ten therapeutic materials which are named in same the table, whereas all the honeybee colonies were exhibitors to symptoms of **AFB** disease before the treatment except sure 3 colonies which belong to the shaking experiment, while after last therapeutic addition the following was:

- The treated colonies with the third, fifth, sixth and seventh therapeutics **[Clove oil, Clove «Shaking experiment», Cinnamon oil,** and **Cinnamon]** did not exhibit any foulbrood symptoms until 117days.
- The treated colonies with the second, fourth and eigth therapeutics **[Primomycin, Clove** and **Zanzan extract]** did not exhibit any foulbrood symptoms until 104 days, whereas at 117days in the treatments of **Primomycin** and **Zanzan extract,** two replicates exhibited **AFB** symptoms, while one replicate of **Clove** treatment exhibited **EFB** symptoms at 117days.
- The treated colonies with the first and ninth therapeutics **[Tylosin** and **Farozal liquid compound]** did not exhibit any foulbrood symptoms until 78 days, whereas in both of the two treatments, **AFB** and **EFB** symptoms began successively appear in most of the treated replicates (colonies) from 91 days to last checkup day.
- In the **untreated** colonies **(control** replicates), **AFB** symptoms remained existence between the increase and decrease in strength of the disease dependency on adult bees verve and queens activity.

Inspection period				After last treatment by											
	Odays											39day			
Replicate / Treatment						3	1	2	3	1	2	3	1		
ylosin (pollen substitute)	^	^													
rrnnomyein (sugar syrup)	^	^													
Cloveoil (pollen substitute)	^	^													
Clovesugar syrup)	^	^													
Clove (sugar syrup) (Shaking)															
Cinnamon oil (pollen substitute)	^	^													
Cinnamon(sugar syrup)	^	^													
z.,anzan extract (sugar syrup)	^	^													
	^	^													
un-treated(Control)															

A ; American foulbrood disease (AFB).
E ; European foulbrood disease (EFB).
_ ; Nothing or No diseased symptoms or Healthy.

Table (14): Continued

Inspection period															
Treatment / Replicate															
Tylosin (pollen substitute)	–	–		–	–	–	–	–			–				
Primomycin (sugar syrup)	–	–		–	–	–	–	–			–				
Clove oil (pollen substitute)	–	–		–	–	–	–	–			–				
Clove (sugar syrup)	–			–	–	–	–	–			–				
Clove (sugar syrup) (Shaking)	–	–		–	–	–	–	–			–				
Cinnamon oil (pollen substitute)	–			–	–	–	–	–			–				
Cinnamon (sugar syrup)	–	–		–	–	–	–	–			–				
Zanzan extract (sugar syrup)	–	–		–	–	–	–	–			–				
Liquid Farozal (sugar syrup)	–	–		–	–	–	A	–			–				
un-treated (Control)	A			–	A		A	A			A				

Keys of Table :
A ; American foulbrood disease (AFB).
E ; European foulbrood disease (EFB).
_ ; Nothing or No diseased symptoms or Healthy.

- From the previous results it became a clear that 3rd, 6th and 7th treatments are superior on the remainder treatments in lengthening to absence or disappearing period of the disease, and those results cleared that natural materials especially essential oils have a high inhibition qualification to the foulbrood symptoms.
- participation of the 5 6 treatment with the 3 previous treatments in the qualification on hiding the foulbrood symptoms for along 117days, rather its superiority on other antibiotics and natural treatments, is somewhat accordant with **Hansen & Brodsgaard, 1999,** whereas reported that the shaking method to the diseased colonies accompanied with the other control manners and beekeeping management procedures succeed in treating the diseased colonies and hiding of foulbrood symptoms by efficacy reaches to 99.99% or in the lower cases to 80% .
- On the other hand, the results of **Cinnamon** treatment approach from results of **Al Hojaymi, 2005,** who examined the **Cinnamon hexanean extract** and found that it is superior on **OTC** because it led to disappearance of **AFB** disease through 6 days after the first sprinkling and all during of the experiment period which was 33 days.

3.1.2. Effect of the tested therapeutic materials on brood rearing activity:

First: Table No. (15) shows the means and increase percentages of sealed brood area which estimated for evaluating efficiency of each treatment, and by used **Co-statistics program** for get the low significant difference **(L.S.D. at 5%)** to each measurement, the following was found:

1) The **untreated** replicates were low significance for along the experiment periods until 78 days, whereas became non-significant at day 91 by brood mean 36.5inch^2 at **L.S.D.** equal 56.179 .

2) All the treatment gave good significances with varying degrees until the last measurement time which 91days in comparison with each calculated **L.S.D.** value at significance level 5% .

3) The brood rearing activity reached to its higher means at the day 91, whereas get in the lesser treatment **(Clove — Shaked colonies)** to triple of **untreated** replicates, and in the greater treatment **(Cinnamon oil)** to about 8.5 times of **untreated** replicates.

4) Overall, the averages of sealed brood area for all treatments were a good with existence of an intra-closed ranges or non-significant ranges between replicates of some treatments as well as showed in table No. (15) by the linked marks which placed on the average values whereas;

   - **Clove oil, Cinnamon oil** and **Cinnamon** treatments get closed significant and all had a high significance, so they took mark **a .**

   - **Primomycin, Clove** and **Zanzan extract** treatments get closed significant but all were far than the previous 3 **treatments** in the significance, so they took mark **b .**

   - **Tylosin, Clove «Shaked colonies»** and **Farozal** treatments get closed-significant but all are very far than the first 3 treatments and in the same time they somewhat approximate

from the second 3 treatments in the significance, so they took mark be .

- Finally, the **untreated** replicates get out non-significant, so this treatment took mark c .

Second: Diagram No. (2) shows the different variations or the fluctuations in means of the sealed brood area to each treatment for along experiment period, and the distinctions between efficacies of all treatment during that period from December 2006 to March 2007, whereas:

1) From 13 to 26 days, the **untreated** colonies dropped by a slight amount and continued in decreasing by the same rate for along experiment period, while all the different treatments **1-9** began suddenly in rising from different low levels by a large amount with the following arrangement; **Cinnamon oil, Cinnamon, Clove oil, Zanzan extract, Clove, Primomycin, Farozal liquid compound, T:ylosin,** and **Clove «Shaked colonies»** to date 26 days and when this date **Tylosin** took the seventh rank and **Farozal** became in the eigth position by exchange of the places.

2) From 26 to 39 days, the treatments continued in the rising but by amount are less than the previous time except the **Clove «Shaked colonies»** treatment which raised by same the previous rate, and all **9 treatments** remained to keep with the same previous arrangement.

3) From 39 to 52 days, the **9 treatments** decreased by a slight amount with keeping on the same previous arrangement.

4) From 52 to 65 days, the treatments much dropped by the same previous rate and **Farozal** exchanged again the places with

Tylosin, then the new arrangement of the treatments became as follows; **Cinnamon oil, Cinnamon, Clove oil, Zanzan extract, Clove, Primomycin, Farozal liquid compound, Tylosin,** and **Clove «Shaked colonies»** in the last.

5) From 65 to 78 days, all the treatments returned to increase by a simple degree except **Clove oil** treatment which remained in decrease but by a very simple amount, with keeping of all **9 treatments** on the previous new arrangement.

6) Finally, from 78 to 91 days, all **9 treatments** more increased and by faster rate with keeping on the previous arrangement.

Third: From the average values (7times) which registered in table No. (15) and cleared on Diagram No. (3) it is showed that the **10 treatments** already occupied different ranks, whereas the **L.S.D.** at 5% = 52.240 as follows:

- Rank 1; **Cinnamon oil** treatment (in pollen substitute) had a high significance with average of 271.9 inch^2 as sealed brood area / colony and by increase percentage equal to 538.6% from average of the **untreated** colonies which equal to 42.6 inch^2.

- Rank 2; **Cinnamon** treatment (in sugar syrup) had a high significance with average of 255.5 inch^2 as sealed brood area / colony and by increase percentage equal to 500.1% from average of the **untreated** colonies which equal to 42.6 inch^2.

- Rank 3; Clove **oil** treatment (in pollen substitute) had a high significance with average of 213.0 inch^2 as sealed brood area / colony and by increase percentage equal to

400.3% from average of the **untreated** colonies which equal to 42.6 inch^2.

- Rank 4; **Zanzan fruits extract** treatment (in sugar syrup) was significant wiith average of 146.5 inch^2 as sealed brood area / colony and by increase percentage equal to 244.2% from average of the **untreated** colonies which equal to 42.6 inch^2.

- Rank 5; **Clove** treatment (in sugar syrup) was significant with average of 126.5 inch^2 as sealed brood area / colony and by increase percentage equal to 197.2% from average of the **untreated** colonies which equal to 42.6 inch^2.

- Rank 6; **Primomycin** treatment (in sugar syrup) was significant with average of 116.0 inch^2 as sealed brood area / colony and by increase percentage equal to 172.5% from average of the **untreated** colonies which equal to 42.6 inch^2.

- Rank 7; **Farozal liquid compound** treatment (in sugar syrup) was significant with average of 110.7 inch^2 as sealed brood area / colony and by increase percentage equal to 160.0% from average of the **untreated** colonies which equal to 42.6 inch^2.

- Rank 8; **Tylosin** treatment (in pollen substitute) was significant with average of 108.5 inch^2 as sealed brood area / colony and by increase percentage equal to 154.9% from average of the **untreated** colonies which equal to 42.6 inch^2.

- Rank 9; **Clove «Shalced colonies»** treatment (in sugar syrup) was significant with average of 73.4 inch^2 as sealed

brood area / colony and by increase percentage equal to 72.5% from average of the untreated colonies which equal to 42.6 inch^2.

- Rank 10; Untreated colonies were non significant with average of 42.6 inch^2 as sealed brood area / colony.

Table (15): E

Measurements	13 days		26 days		inch) after last treatment 39 days		by days	
Treatments (with their ranks)	Brood area	% Increase	Brood area	% Increase	Brood area	% Increase	Brood area	% Increase
	53.0	8.9	113.7	146.2	115.0	161.4	102.0	
	55.3	13.7	117.0	153.4	117.3	166.7	104.8	
	103.2	112.0	235.3	409.7	237.3	439.4	221.0	
	60.3	24.0	121.0	162.1	123.0	179.6	117.0	
	16.3	-66.5	47.5	2.9	78.7	78.8	71.0	
	134.7	176.7	283.3	513.7	288.7	5561		
Cinnamon 21	121.7	150.0	270.3	485.5	271.3	516.7	265.8	
	65.3	34.2	147.3	219.1	149.0	238.6	137.2	
	53.5	9.9	108.0	133.9	110.0	150.0		
Un-treated 10	48.7		46.2		44.0			
L.S.D at %5			22.122		31.916		27.668	

Table (15): Continued

	• • • •							Total	
		.5 t.					*uaiu* pooig	a2giany	3suanui %
							V6SL.	9 S 801	617S1
Primomycin 6 I	Z*L6	Z'Ogl	0711	ELL'	g'80Z	ZIL+7	ZZI8	W9I I	S'LL/
	COIZ	5*ZI717	0'80Z	8'51V	£'5L,Z	£1759	8'061'I	nit	£'001
. Clove 5	Z1701	£'891	O'LZI	61712	0'£Z	17'8E5	S'S88	S'9ZI	VL6I
6 OuPluilS) a& ID	C£9	0179	0 68	L'OZI	81171	0 50£	0 1715	9 ⁊ EL	S'ZL
Cinnamon oil 1	5 1IZ	Z665	£176Z	8•6z9	5'1717£	8 £178	1903.0	WILZ	913£S
Cinnamon 2	L'EgZ	£'£55	EV8Z	0'509	O'IZE	5'6LL	1788.2	S SSZ	1'005
Zanzan extract 4	(ra ⊥	8 ZSZ	0'9171	0 Z6Z	8*E17Z	0'895	L'SZOI	146.5 b	ZI7IZ
	CZ6	C8£1	£'801	9'891	g'1 OZ	17517	9'17LL	110.7 be	0'091
Un-treated 10	£8'8		£'017		5'9£		Z98'0	a 9711	
	28.514		33.326						

Diagram (2): Means of brood area by inch ^{2} after last treatment every 13 days show the efficacy differences among the different used therapeutics.

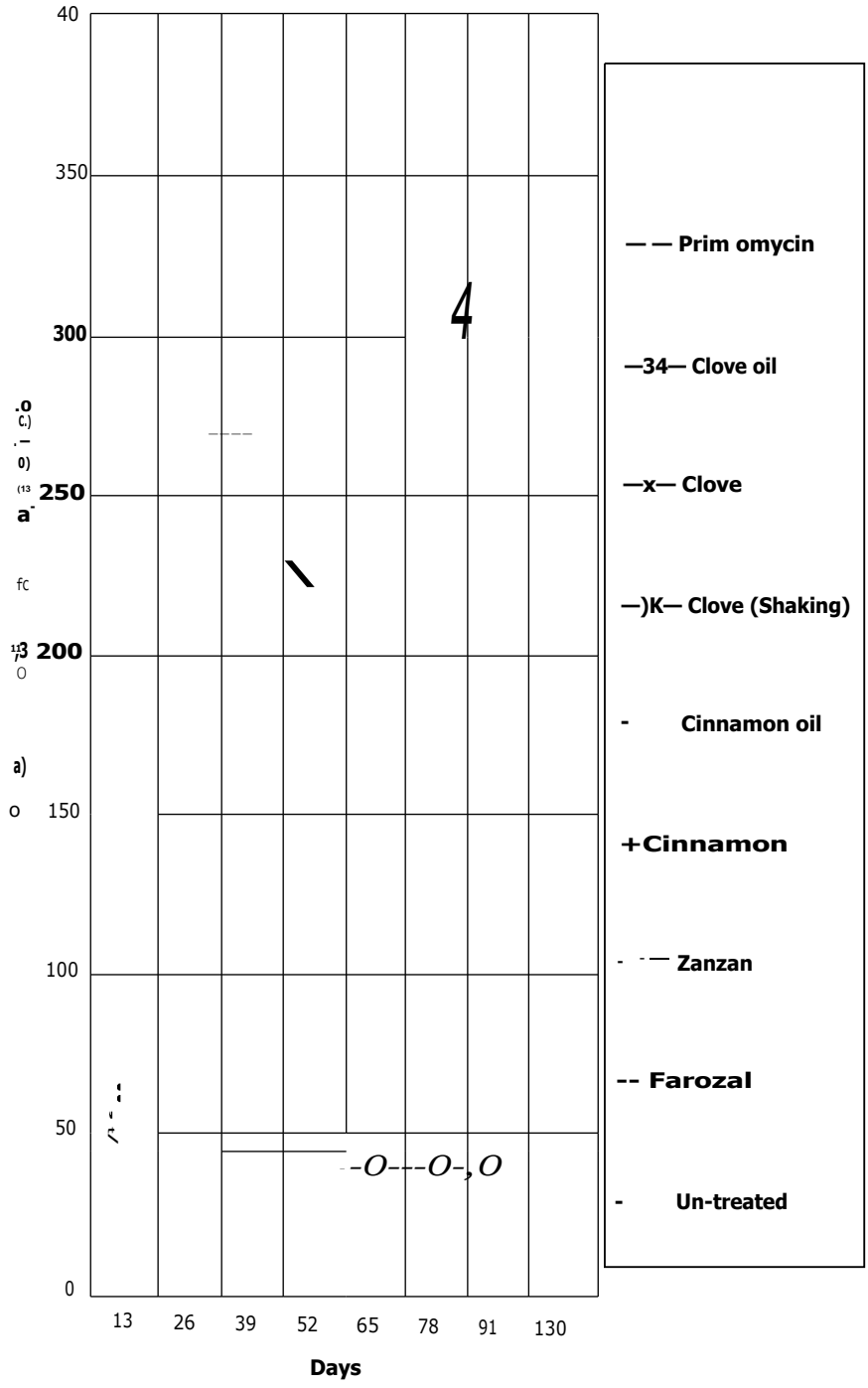
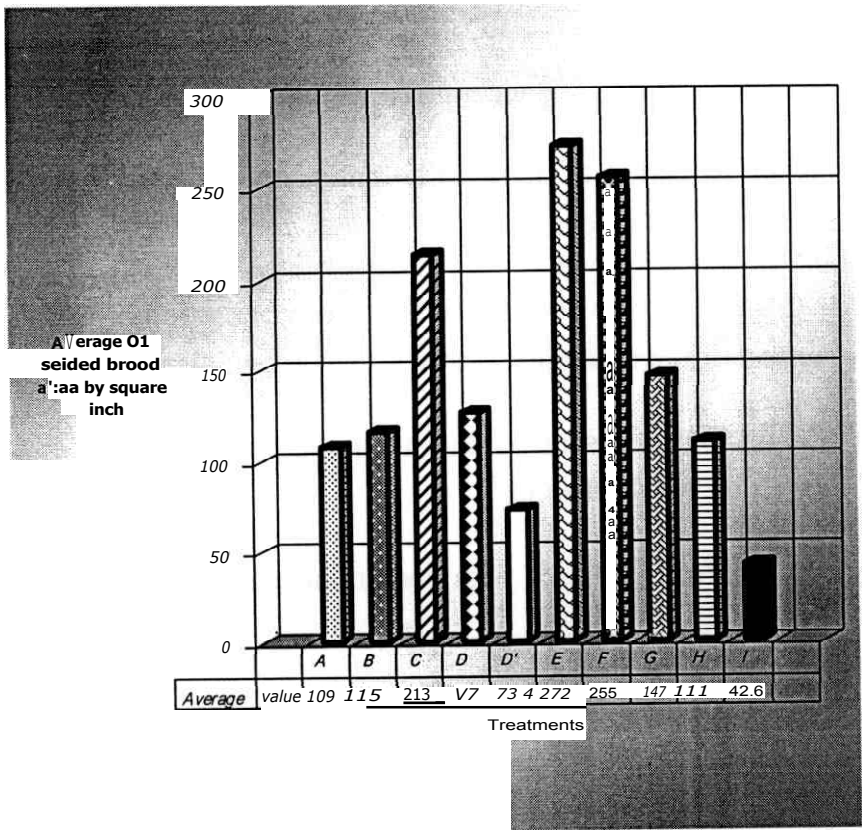


Diagram (3): Effect of certain control agents of AFB disease on brood rearing activity (inch^2) in the tested honeybee colonies during winter and spring 2006/2007 season.



A - - - Tylosin
B Primomycin
C - - - Clove oil
D Clove
- - - Clove (Shaked colonies)
E Cinnamon oil
F - - - Cinnamon
G Zanzan fruits extract
H Farozal Liquid compound
I - - - un-treated (Control)

4. Recommendations:

First: - for beekeepers:;

1) The colonies must be opened during spring and autumn, and examine of all the brood combs for any abnormalities whereas the prevent or control **AFB and EFB** based on the early detection and on the accurate identification of the disease.

2) When detection of diseased colonies, do not mix contaminated materials with clean others.

3) Do not move to the infected apiaries with foulbrood for not spread of the contagion between the different apiaries.

4) Do not buy hives or second-hand equipment without checking carefully for foulbrood.

5) Do not feed the bees with contaminated honey or pollen with foulbrood pathogens.

6) Do not exchange of the brood combs among the different colonies without checking for foulbrood symptoms.

7) Do not leave honey combs exposed to robbing whereas they help in spreading of the infection if they are contaminated.

8) Do not incorporte swarms from unknown origin into the apiary.

9) It must be disinfect of the contaminated hives by washing in water bath containing on **formic acid** or **sodium hypochlorite** or any suitable chemical disinfectant with using of hard emery to scrape the inner wood.

10) Must complete to disinfect of the hives by scorching carefully.

11) Must treat all colonies of the diseased apiary by the therapeutic.

12) It could be recommended that using of any available natural therapeutic from the therapeutics which used in this field study for control AFB and EFB diseases especially **Cinnamon,** whereas they can be used by maximum 1.5m1. or **gm.** (oil or powder therapeutic) / 100m1. or **gm.** nutrient material as far as **6 times** by break period **one week.**

13) While for prevent appearance of the foulbrood symptoms, I recommend with use of the therapeutic as **twice** by break period **4 days** especially between the flowering seasons and after extraction of the honey.

14) I recommend with wholly stop on using of any **veterinary** antibiotics whether for control or prevent the foulbrood diseases because they lead to bad changes in characteristics and quality of the honeybee products especially the honey, on the other hand, they lead to induce of immunity at the bacterial pathogens opposite any treatment.

15) In case of extreme necessity, it can be use of the specific **human** antibiotics but with exchange between them.

16) I recommend with the **shaking method** to the bees as a control manner to the foulbrood diseases especially **AFB,** whereas this manner prevent the foulbrood appearance for along about one year, but it requires too much of the labor and the patience.

Second: - for researchers;

1) When appearance or presence clinical symptoms of the foulbrood diseases especially **AFB** in a what honeybee colony, it is un- necessary to isolation of the causative bacterium for recognition with existence of the foulbrood disease in the colony because the known clinical symptoms especially those belong to **AFB** disease are not induced excepting by only one bacterium which is *Paenibacillus larvae* by confession of all researchers in beekeeping and microbiology fields.

2) But in case of the colonies without foulbrood symptoms, it is necessary to examine of some samples from the broods and honey if the purpose was determination to presence or absence of the spores in the healthy colony.

3) In case of the package bees swarms, it must non satisfy with the simple checks for *Nosema apis* spores rather it is very necessary to examine of the adult bees for presence of the foulbrood spores and try isolation from them but that needs to saving of the suitable abilities and subsidization.

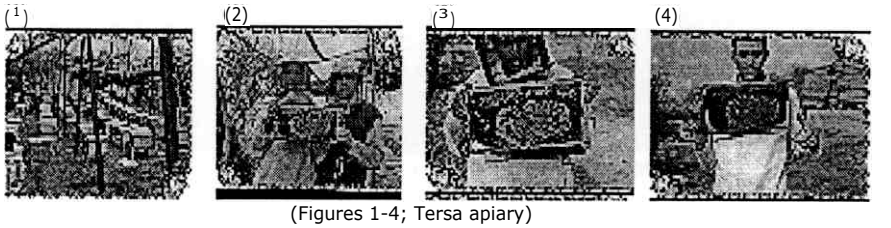
4) Also, it is must that made of the previous examine on samples directly taken from the swarms in the field, not which are received to the diagnosis laboratories whereas the workers in those packages often lose the spores so the test result get out contrary to the actual.

5) Also, it is really necessary isolation of the causative bacteria for the foulbrood diseases if the purpose is procedure to biochemical or microbial tests on those bacteria in the laboratory.

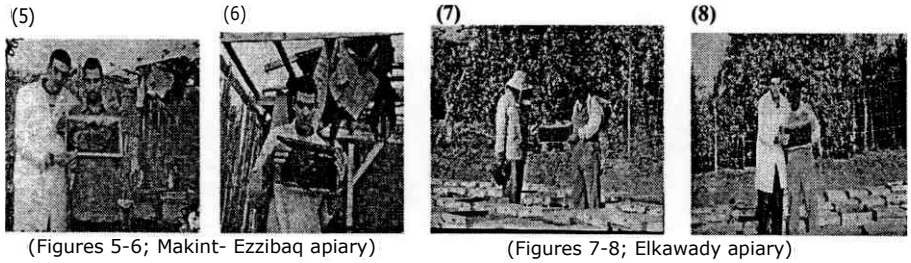
5. Photographs:

5.1. « Survey Part »

(Figures 1 — 16 ; Some of the field visitations performed for survey of the infected honeybee colonies with the foulbrood diseases).



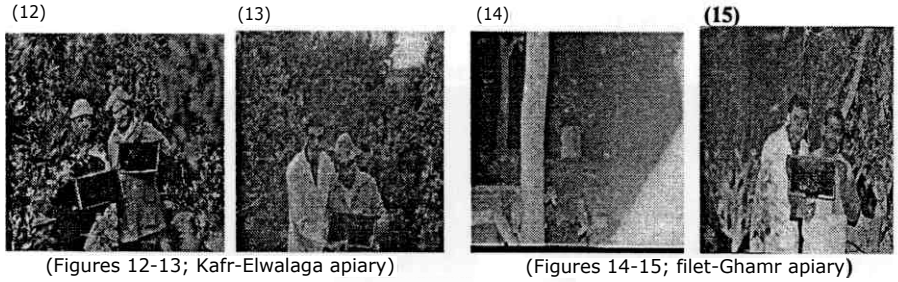
(Figures 1-4; Tersa apiary)



(Figures 5-6; Makint- Ezzibaq apiary)

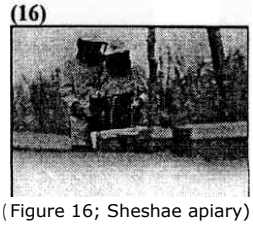
(Figures 7-8; Elkawady apiary)

(Figures 9-11; Gesr-Elmanawat apiary)



(Figures 12-13; Kafr-Elwalaga apiary)

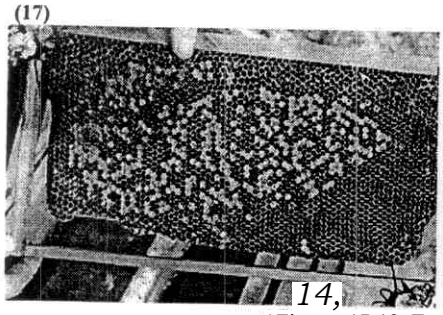
(Figures 14-15; filet-Ghamr apiary)



(Figure 16; Sheshae apiary)

(Figures 17 —32; The clinical symptoms of AFB and EFB diseases in the infected honeybee colonies at some visited apiaries, whereas each group show the infected comb shape then the diseased larvae characters).

(17)



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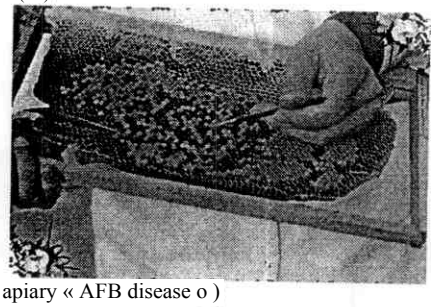
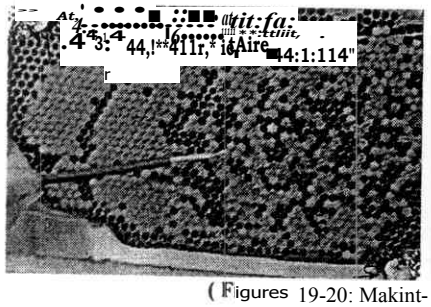
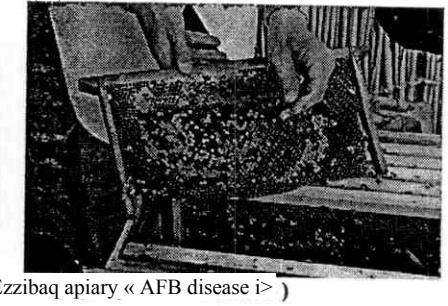


(Figures 17-18; Tersa apiary « AFB disease o)

(19)

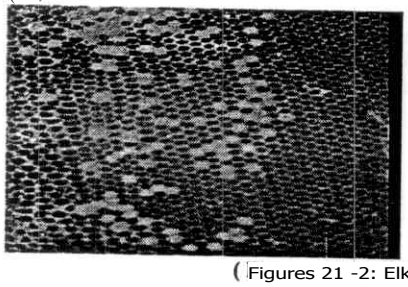


(20)



(Figures 19-20: Makint- Ezzibaq apiary « AFB disease i>)

(21)



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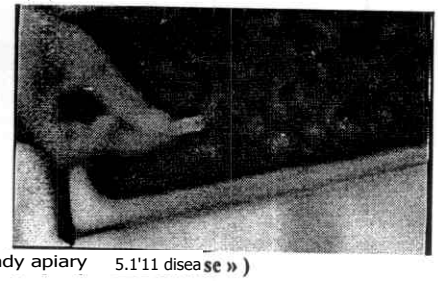
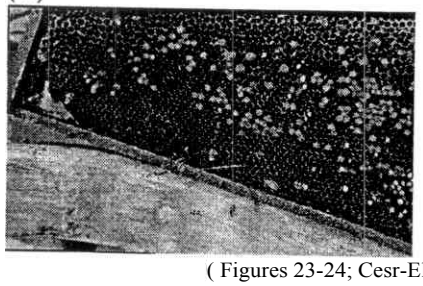
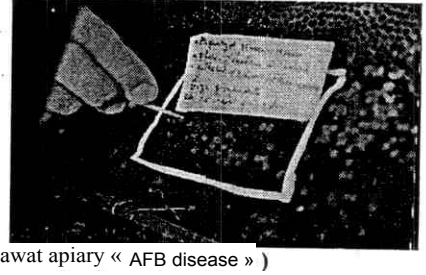


(Figures 21 -2: Elkawady apiary 5.1'11 disease »)

(23)

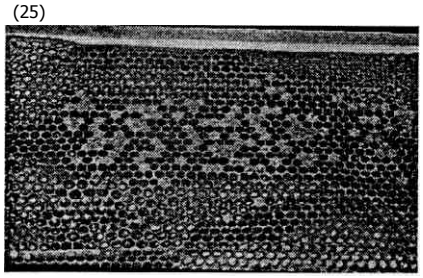


(24)

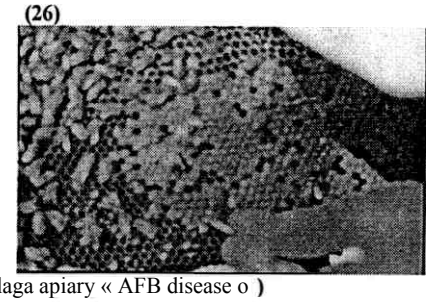


(Figures 23-24; Cesr-Elmanawat apiary « AFB disease »)

(25)

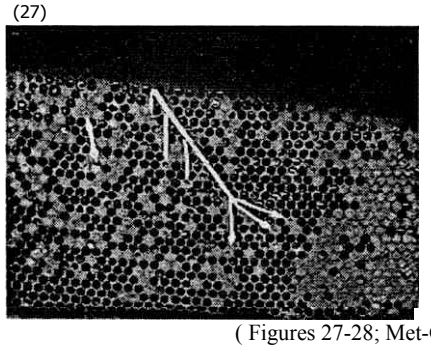


(26)



(Figures 25-26; Kafr-Elwalaga apiary « AFB disease o)

(27)



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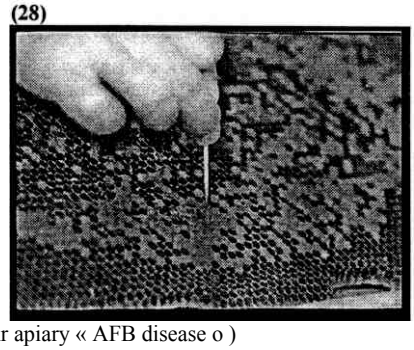
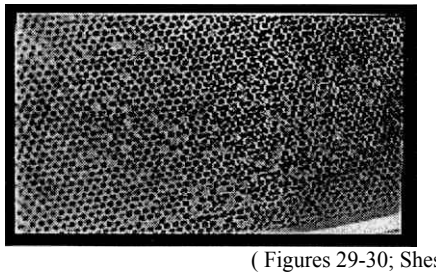


(Figures 27-28; Met-Ghamr apiary « AFB disease o)

(29)



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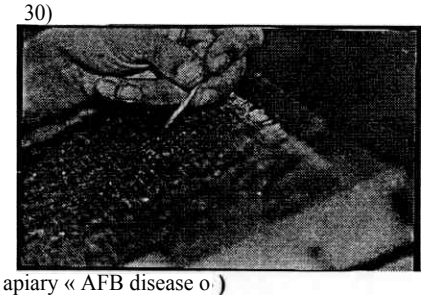
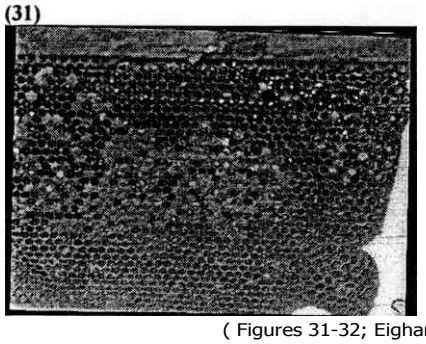
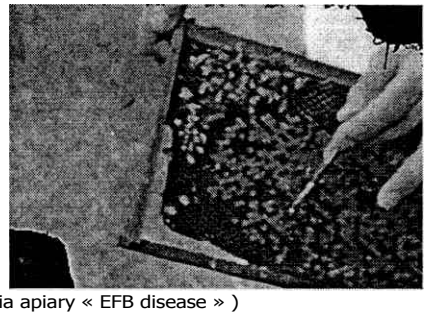


(Figures 29-30; Sheshae apiary « AFB disease o)

(31)



(32)

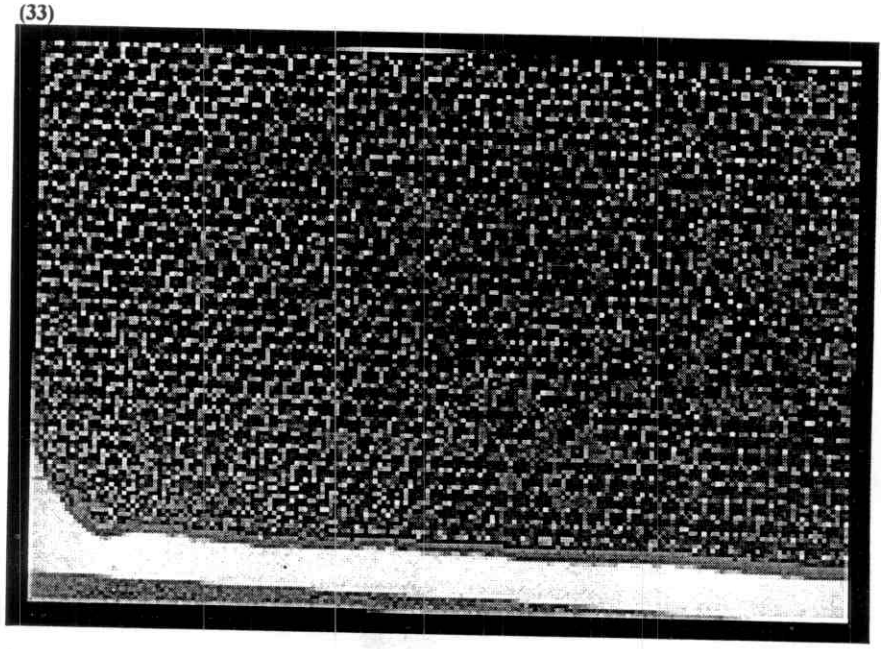


(Figures 31-32; Eighannamia apiary « EFB disease »)

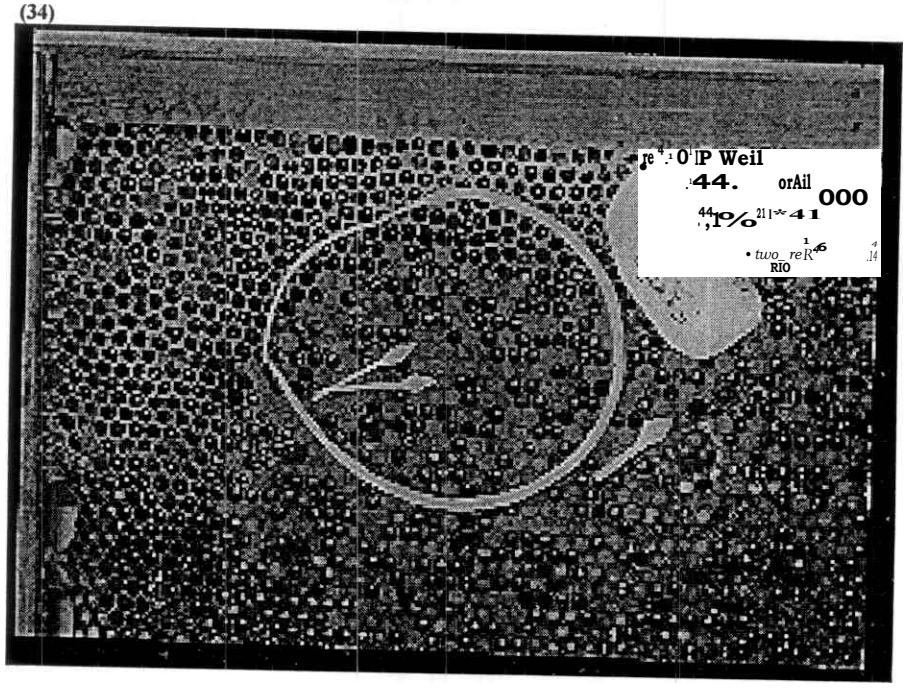
5.2. « Laboratory Experiments Part »

(Figures 33 — 38; Some of the collected brood combs as diseased samples, which really showed AFB.symptoms when *were* reinspected in the laboratory).

(33)

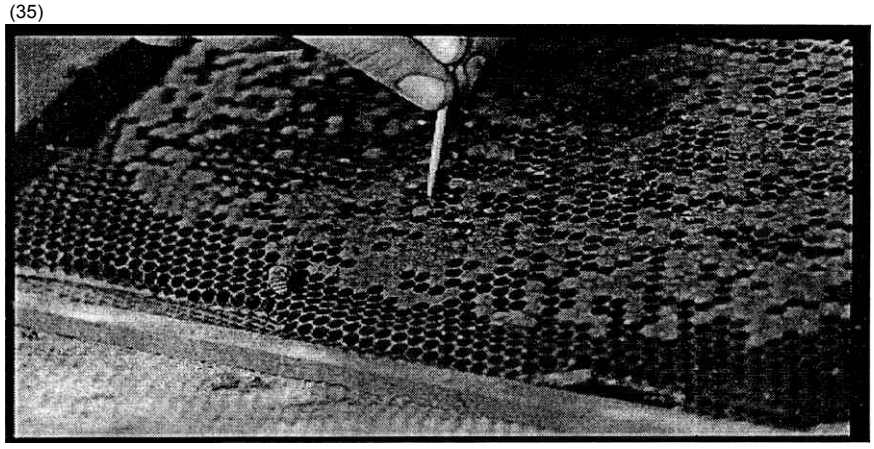


(34)

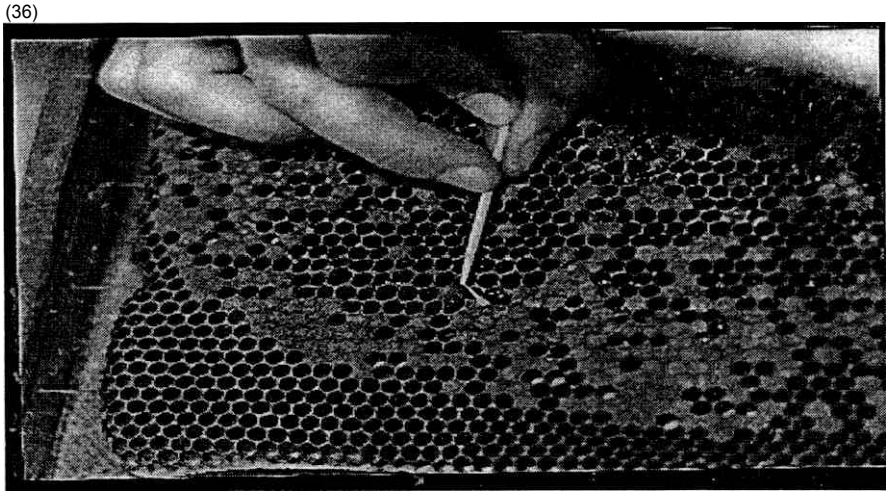


(Figures 33-34; AFB Symptoms are discolored cells, sunken , punctured cappings, and the gluepot odor needs to smell) .

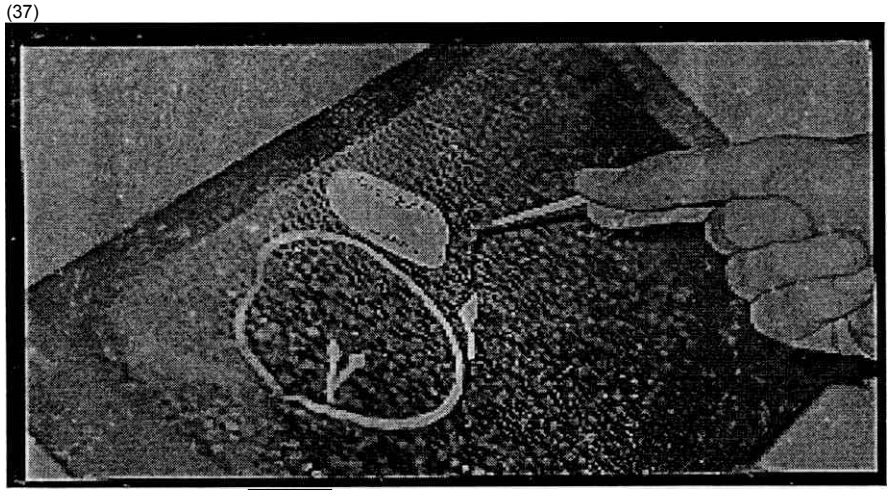
(35)



(36)

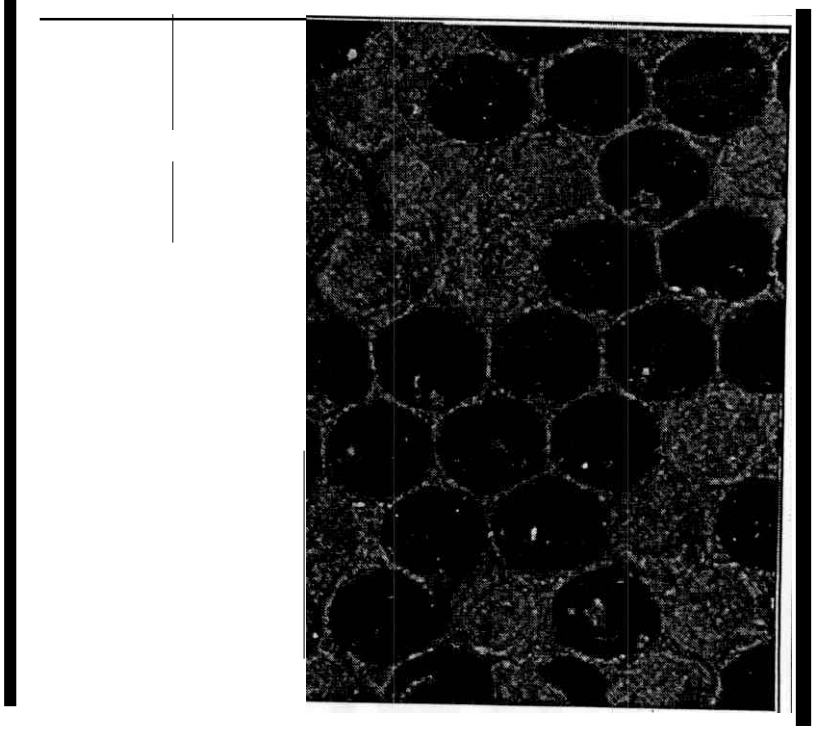


(37)



(Figures 35-37; Show an one important character of AFB Symptoms, that is the recently dead larva is soft and make a sticky or a ropy threadlike material longer than 2cm. and falls back when inserting of a matchstick into the cell and then drawing out) .

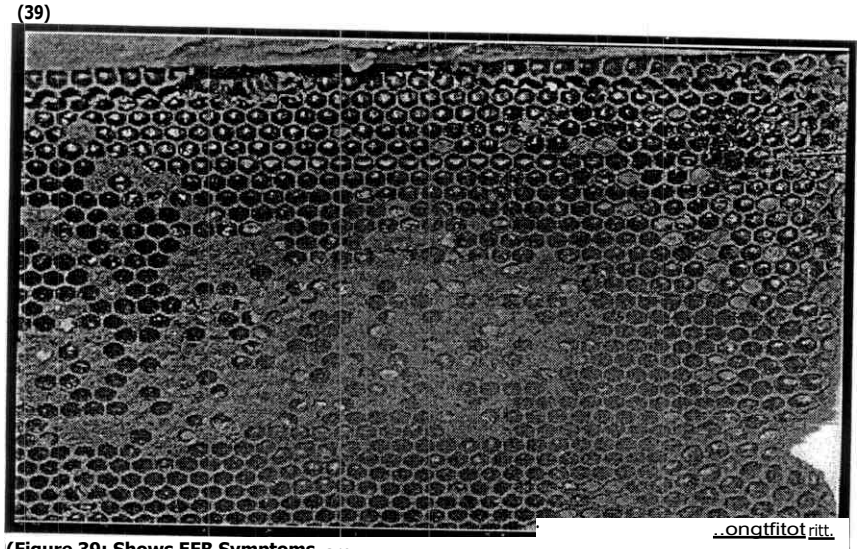
38



(Figure 38; Shows an another one important character of AFB Symptoms, that is the black scales which lie flat on the lower side of cells and adhere tightly to the cells walls) .

(Figures39; One of the collected brood combs as diseased samples, which really showed to EFB symptoms when was reinspected in the laboratory).

(39)



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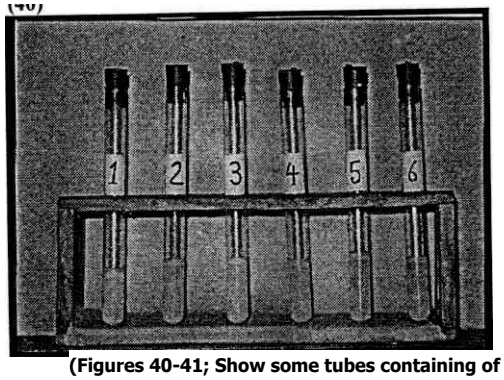
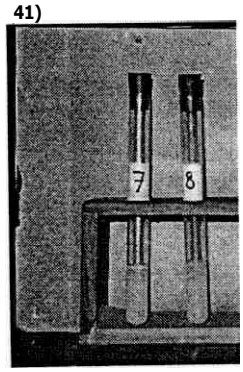
(Figure 39; Shows EFB Symptoms are **the dead larvae is yellowish white to orange or brown,** uncapped, **twisted shape not adhere tightly to cells walls and the acidity odor needs to smell).**

(Figures 40-45 ; Steps of the Hoist Skim Milk test in the laboratory).

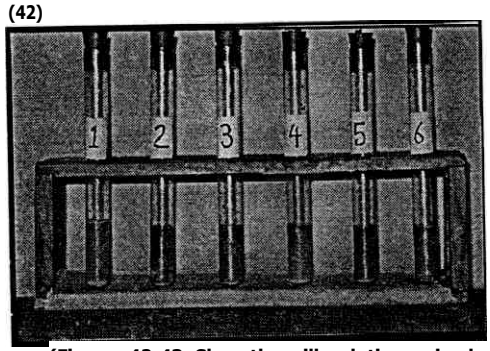


41)

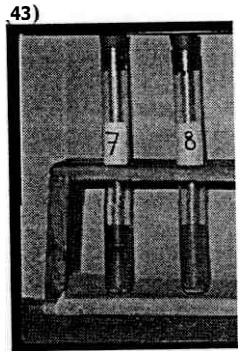


(Figures 40-41; Show some tubes containing of 3-4 ml. skim milk solution only).

(42)

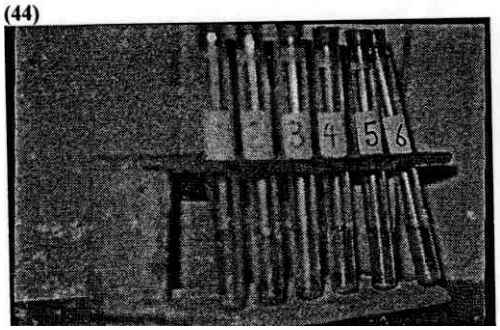


43)

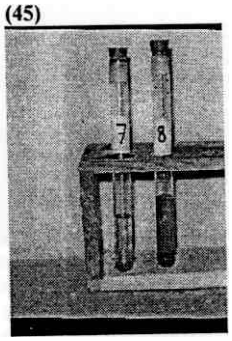


(Figures 42-43; Show the milk solutions mixed with scales or ropy material from some diseased samples before the incubation , whereas tubes belong to the following apiaries: 1- Abu-Onnomros , 2- Makint-Ezzibaq , 3- Tersa , 4- Elkawady , 5- Gesr-Elmanawat, 6- Kafr-Elwalaga , 7- Met-Ghamr and 8- Elghannamia) .

(44)



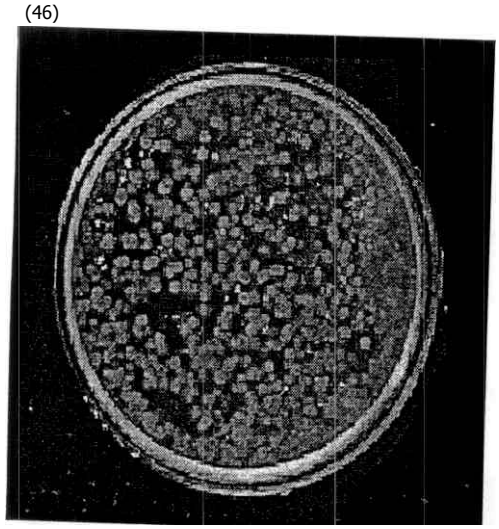
(45)



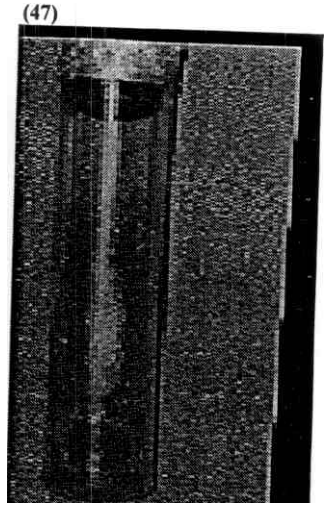
(Figures 44-45; show the different reactions to Hoist test after the incubation at 37°C. for 20 min., whereas the tubes 1-7 gave positive so those are diseased by AFB , while the tube 8 gave negative so that is diseased by EFB) .

(Figures 46-49 ; Shape of 1he bacterial colonies isolated from some diseased brood samples) .

(46)

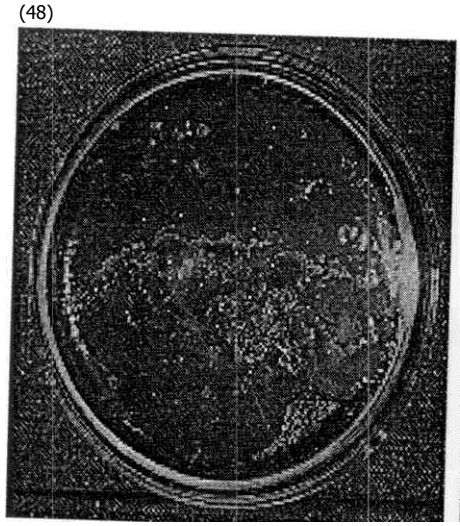


(47)



(Figures 46-47; Show the colonies shape of *Paenibacillus larvae* subsp. *larvae,* the causative bacterium of AFB disease, which isolated from one diseased brood sample and grew on SBA medium).

(48)

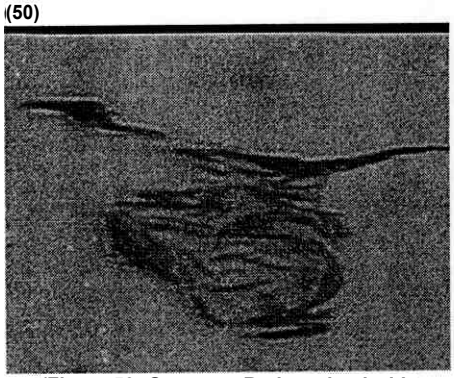


(49)

(Figures 48-49; Show the colonies shape of *Melissococcus pluton,* the causative bacterium of EFB disease, which isolated from one diseased brood sample and grew on Bailey's medium « in first plate bacterium is mixed with spreader growth of *Bacillus alvei* , but in second plate bacterium *M. pluton* is pure) .

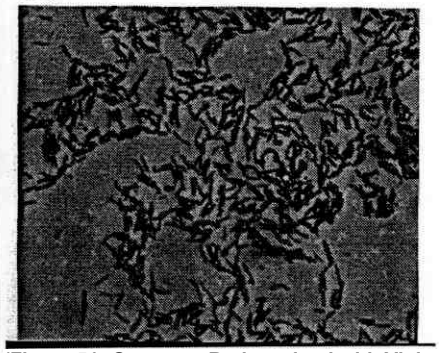
(Figures 50-56; Vegetative rods, transformation and spores stages of *Paenibacillus larvae* subsp. *larvae* **from colonies of the isolated bacterium tested by Gram stain) .**

(50)



(Figure 50; Gram +ve Rods stained with Gentian dye , Magnification 1000 once) .

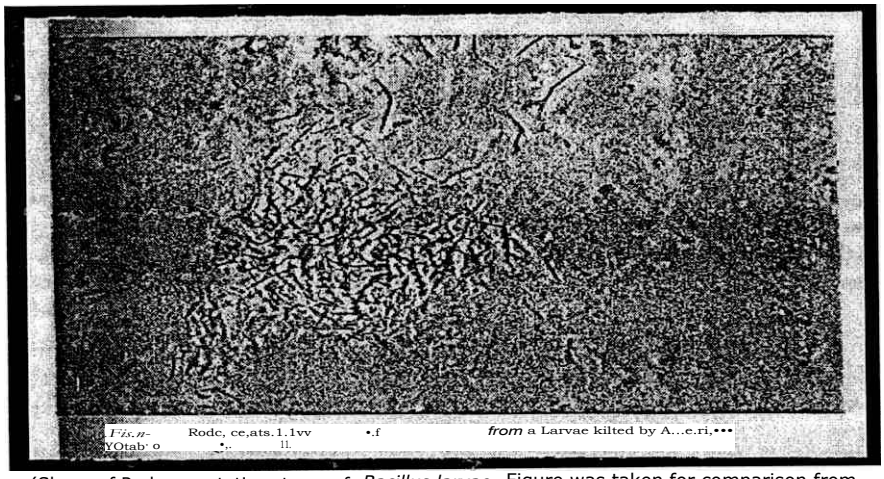
(51)



(Figure 51; Gram +ve Rods stained with Violet Crystal dye , Magnification 1000 once) .

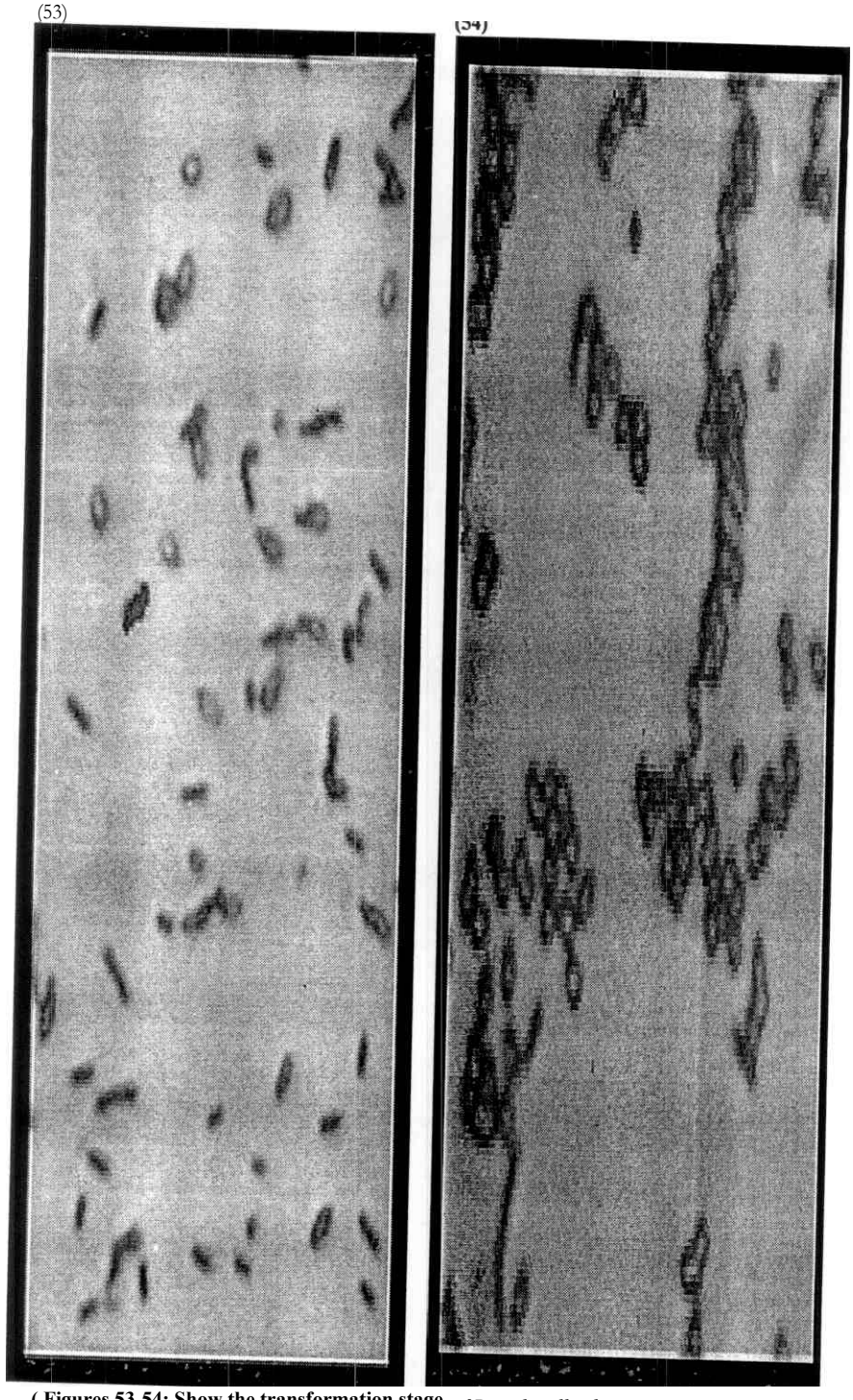
52)

(Figure 52; Gram +ve Rods stained with Violet Crystal dye , Magnification 1600 once) .



(Shape of Rods, vegetative stage, of *Bacillus larvae,* Figure was taken for comparison from book « Honey Bee Pests, Predators **And Diseases »** Edited by Roger A. Morse, 1980) .

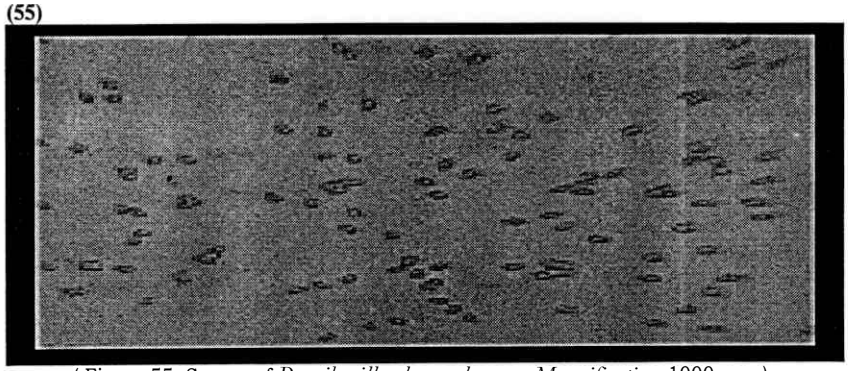
(53)



(Figures 53-54; Show the transformation stage *of Paenibacillus larvae* subsp. *larvae* **bacterium from the rods or vegetative cells to endo-spores, Magnification 1600 once) .**

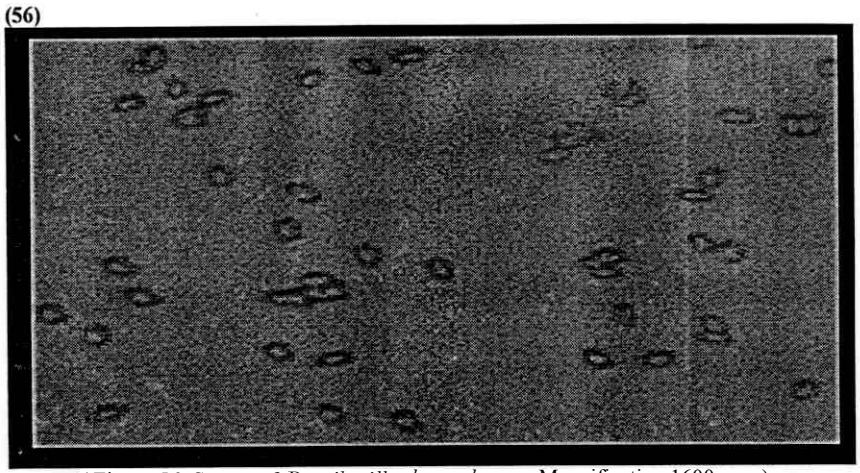
(Figures 55-56; Show the spore stage of *Paenibacillus larvae* subsp. *larvae* bacterium).

(55)

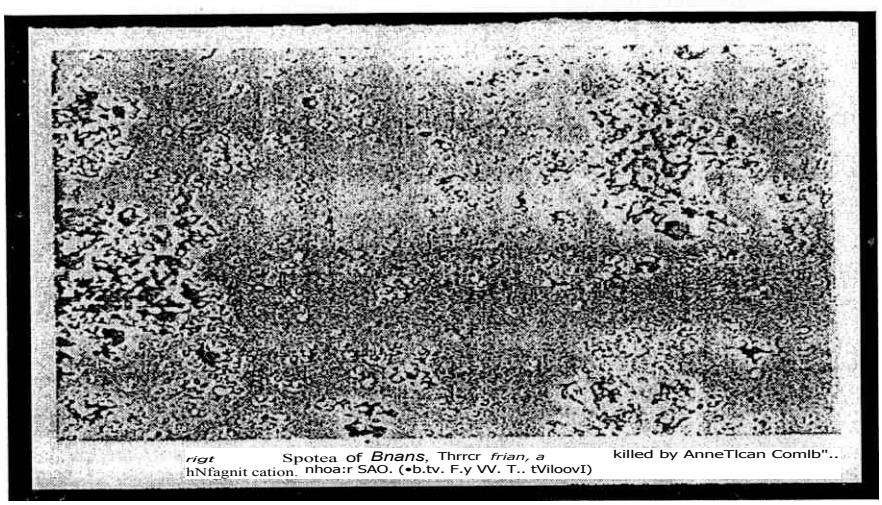


(Figure 55; Spores of *Paenibacillus larvae larvae , Magnification* 1000 once).

(56)



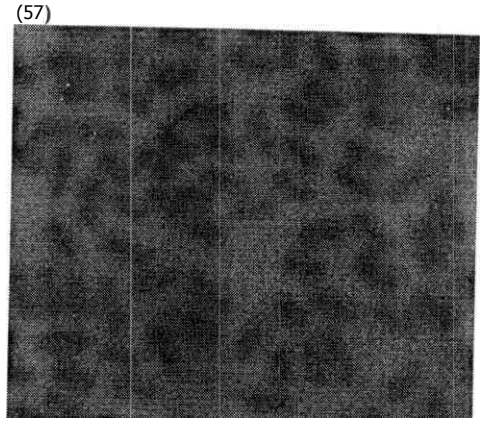
(Figure 56; Spores of *Paenibacillus larvae larvae ,* Magnification 1600 once) .



(Shape of *Bacillus larva* Spores , Figure was taken for comparison from book « Honey Bee Pests, Predators And Diseases o Edited by Roger A. Morse, 1980) .

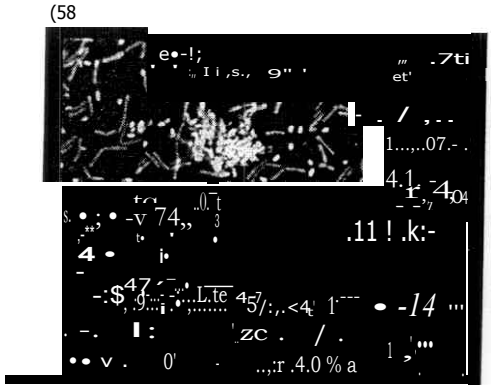
(Figures 57-63; Pigmentation of the bacterial organisms with some other dyes).

(57)

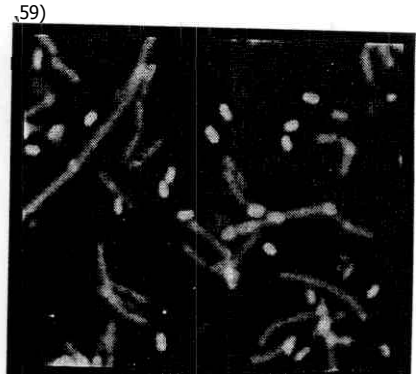


(Figure 57; The ellipsoidal spores of *Paenibacillus larvae larvae* are stained only with Malachite green dye « Schaeffer & Fulton method o , Magnification 1800 once) .

(58

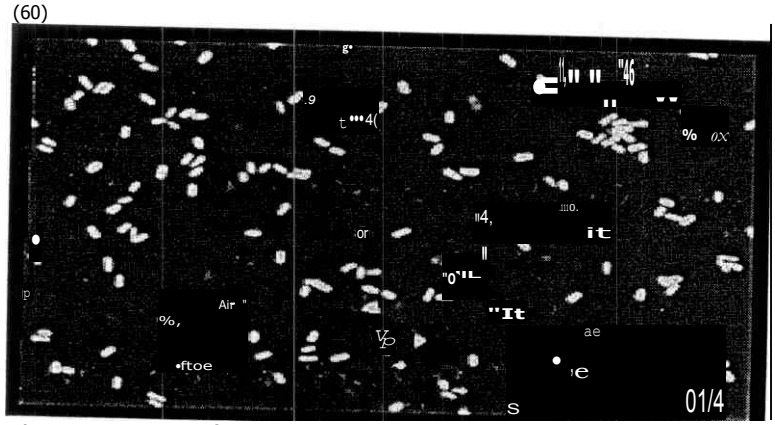


,59)



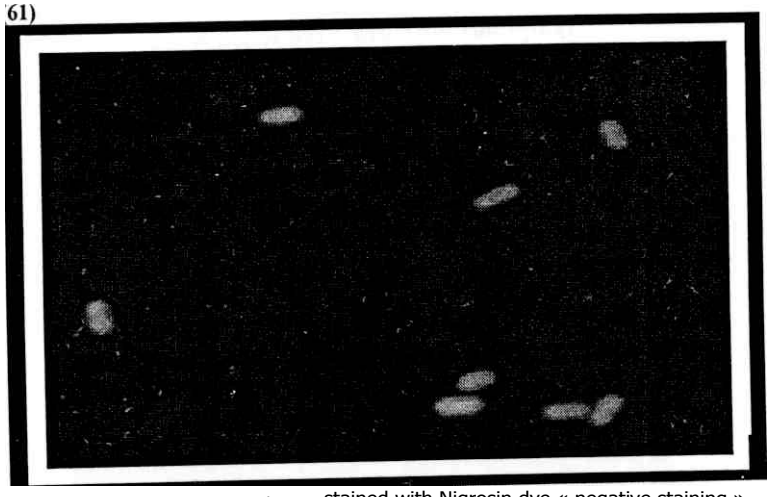
(Figures 58-59; Rods and Spores of *P. L larvae* stained with Nigrosin dye « negative staining » Magnification 1000 once. Magnification 1600 once.

(60)



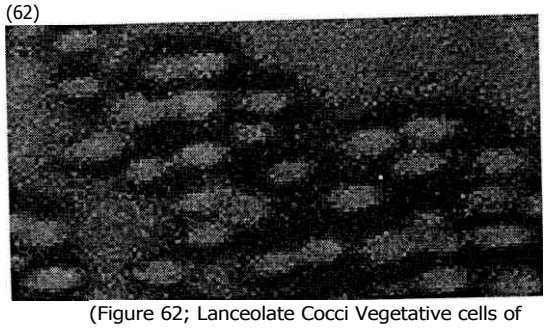
(Figure 60; Spores of *P. I. larvae* stained with Nigrosin dye « negative staining o , Magnification 1600 once) .

(61)

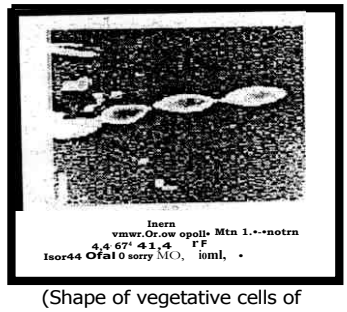


(Figure 61; Spores of *P. l. larvae* stained with Nigrosin dye « negative staining » , Magnification 1800 once).

(62)

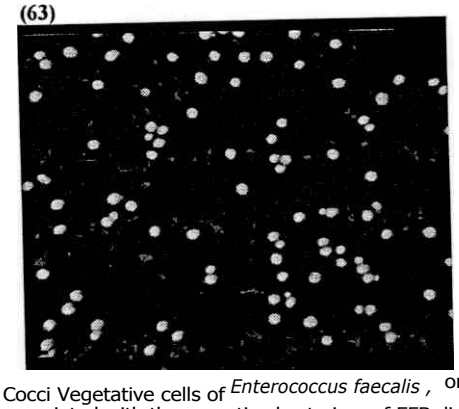


(Figure 62; Lanceolate Cocci Vegetative cells of *Melissococcus pluton* , the causative bacterium of EFB disease, are stained with Nigrosin dye, Magnification 1000 once).



(Shape of vegetative cells of *Melissococcus pluton* , Figure was taken for comparison from paper of Alippi, 1991 .

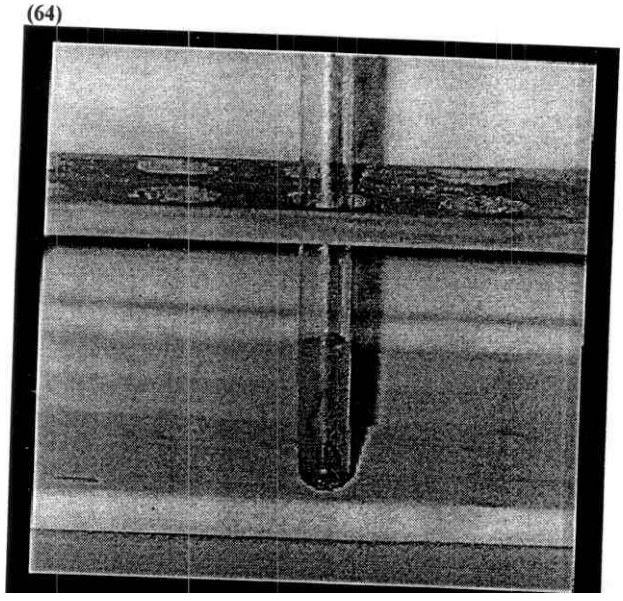
(63)



(Figure 63; Spherical or Cocci Vegetative cells of *Enterococcus faecalis* , one from the bacteria which are associated with the causative bacterium of EFB disease, are stained with Nigrosin dye, Magnification 1000 once).

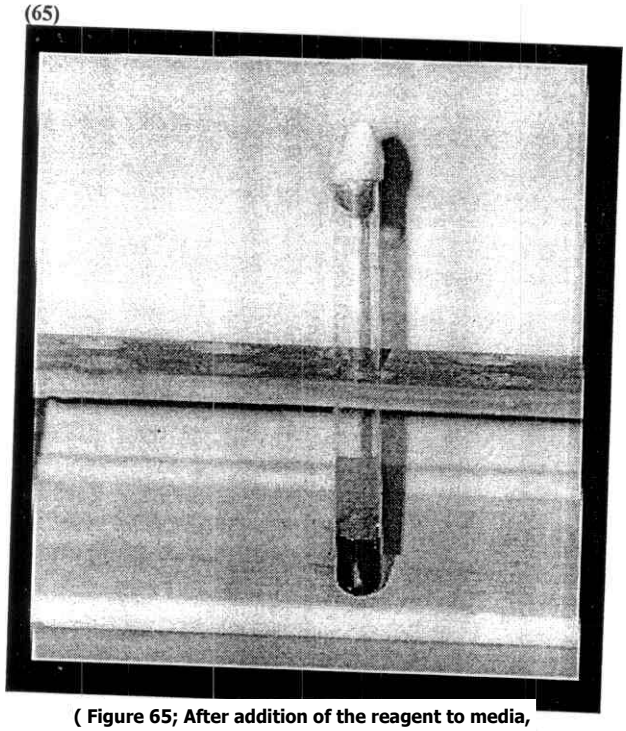
(Figures 64-65; Result of bacterial isolation from brood sample with AFB symptoms for Nitrate reduction test).

(64)



(Figures 64; The bacterium is growing in Nitrate broth medium after 72hrs. incubation at 37°C.).

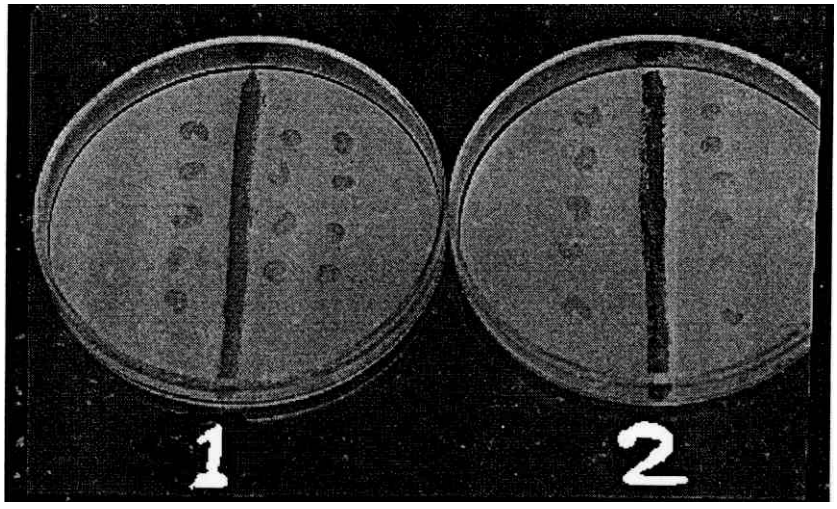
(65)



(Figure 65; After addition of the reagent to media, a stable deep red dye formed immediately as indicator on reduction of Nitrate to Nitrite).

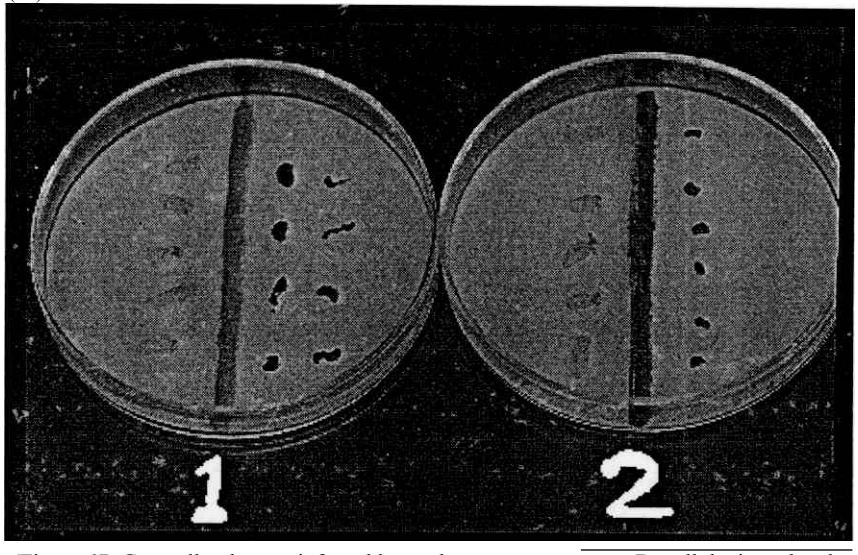
(Figures 66-67; Some steps of the artificial infection procedure *(in Vitro))* .

(66)



(Figure 66; In the right half of whether plates 1 and 2, some larvae did not inoculated for control; But in the left half of plate 1, some inoculated larvae by prepared inoculum of isolated bacterium from one brood sample with AFB symptoms ; While in the left half of plate 2, some inoculated larvae by prepared inoculum of isolated bacterium from one brood sample with EFB symptoms ; « After infection immediately) .

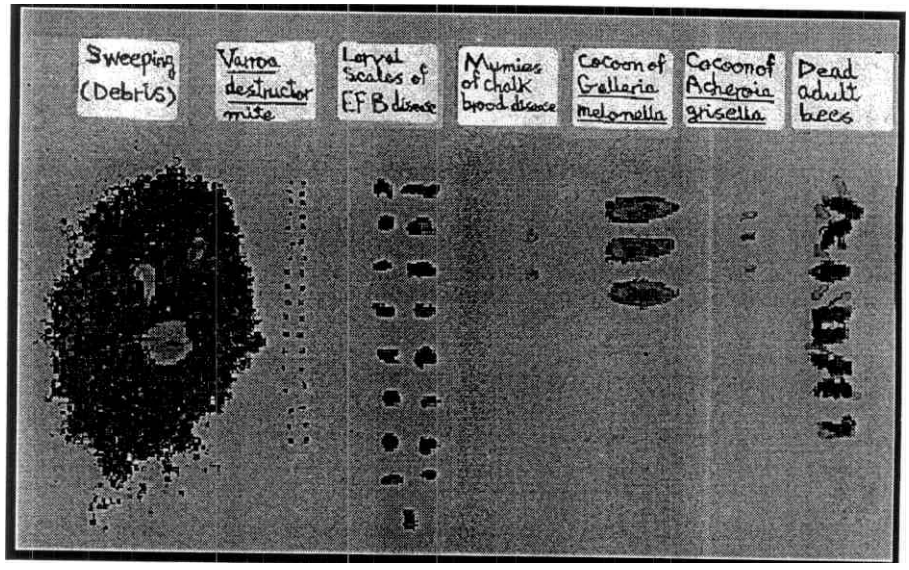
(67)



(Figure 67; Generally, the non infected larvae became to prepupae; But all the inoculated larvae gave sonic foulbrood•ymptoms, whereas in plate 1 the result symptoms near to the AFB symptoms, while near to EFB symptoms in plat 2 ; « After about 7days of incubation at 34°C inner the incubator)

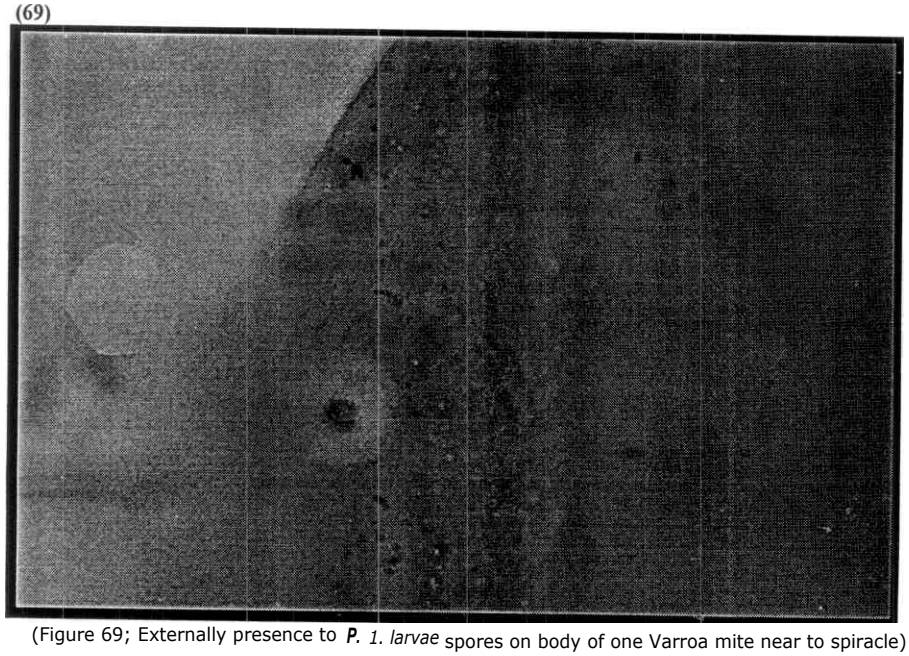
(Figures 68 — 69; Some of the procedures made for study of the relationship between Varroa mites and the foulbrood diseases) .

(68)



(Figure 68; Counting of Varroa mites in the collected debris from one honeybee colony, then classification of the reminders to groups before removed) .

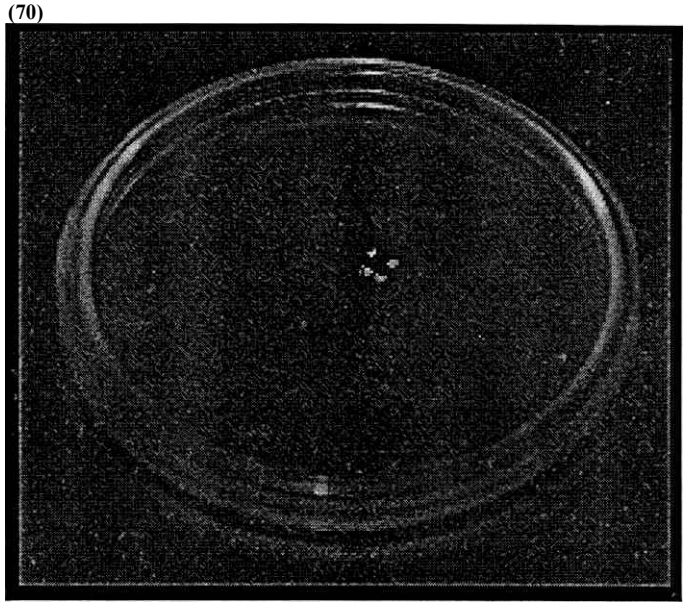
(69)



(Figure 69; Externally presence to ***P.*** *1. larvae* spores on body of one Varroa mite near to spiracle) .

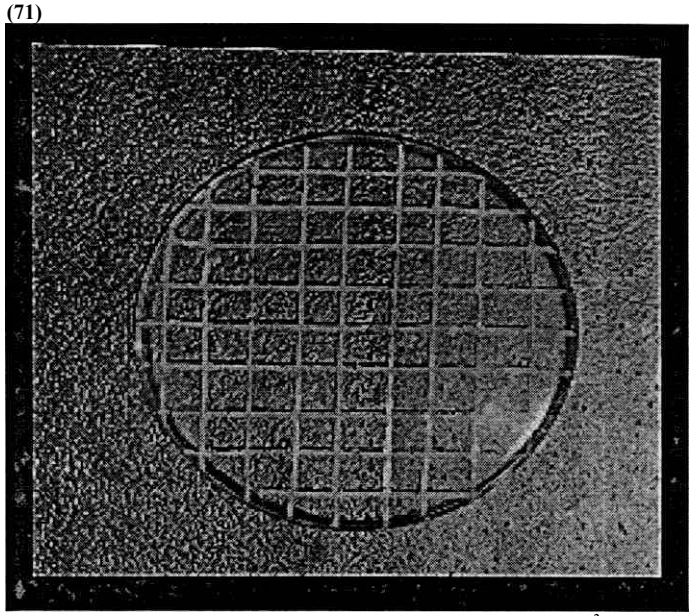
(Figures 70 — 71; Some works of the inhibited examinations to bacterium in the laboratory « *in Vitro »)* **.**

(70)



(Figure 70; Some bacterial colonies belong to *P. L larvae* bacterium are outgrowths on MYPGP medium in one of the laboratory treatments) .

(71)

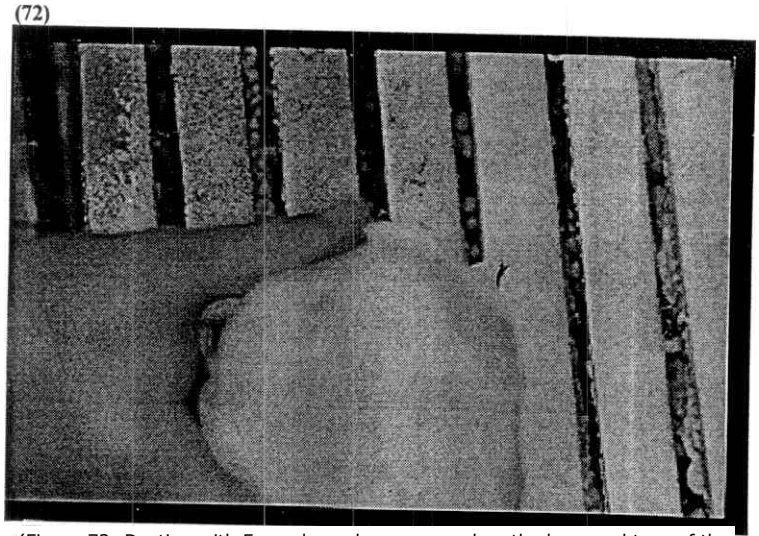


(Figure *71; The dish cover* 9cm. diameter which divided to 1cm^2 squares, and used in counting to the bacterial colonies numbers in the treated plates) .

5.3. « Field Experiments Part »

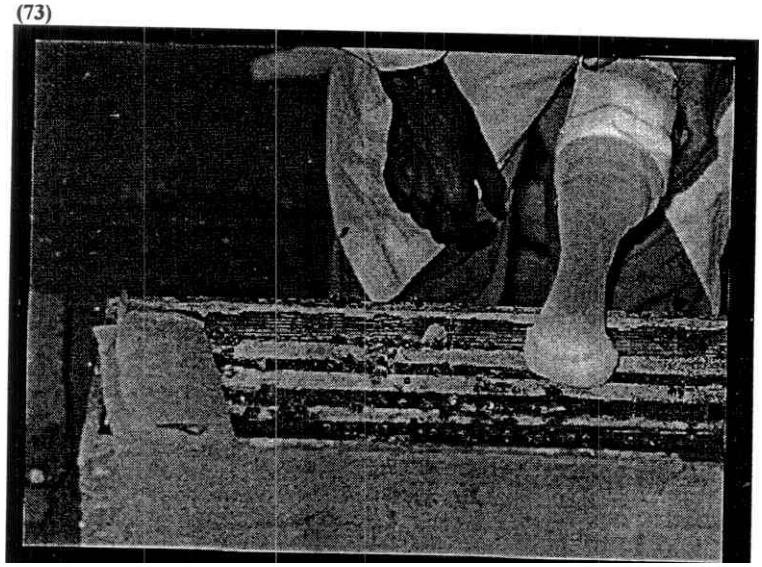
(Figures 72 — 80; Some of the beekeeping management procedures performed in the field for controlling of A FB disease and treatment of the infected honeybee colonies) .

(72)



(Figure 72; Dusting with Farozal powder compound on the bees and tops of the brood combs in the temporary hives before returning to the disinfected hives for decreasing the Varroa numbers which may be presence).

(73)

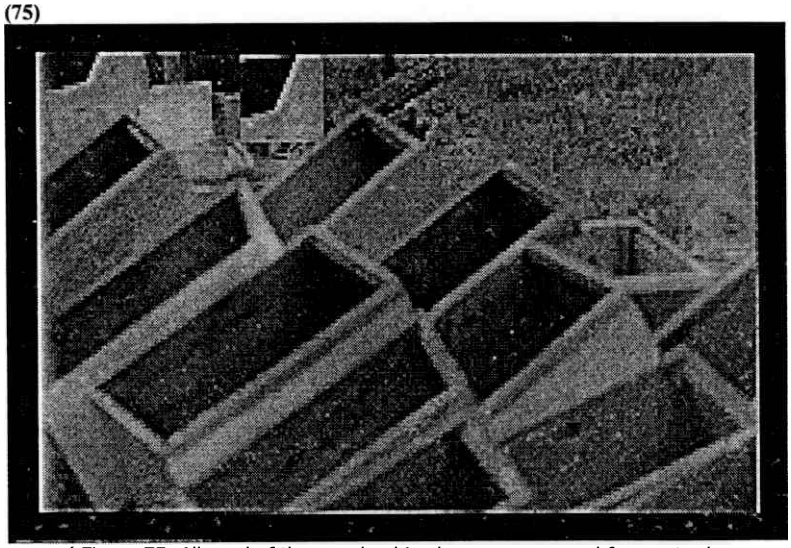


(Figure 73; Dusting with Farozal powder accompanied with putting of pierced sacks containing on saturated cardboard by Formic acid 65% in the temporary hives before returning to the disinfected hives for decreasing the Varroa numbers which may be presence) .

(74)

(Figure 74; Disinfecting of The removed wooden hive boxes, covers and frames by washing in a water bath containing of chemical disinfectants, and scrubbed by use of a metal emery or a hard brush) .

(75)

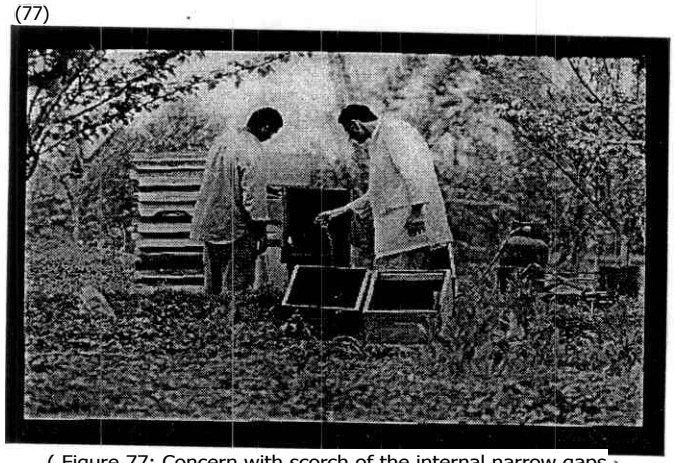


(Figure 75; Allowed of the wooden hive boxes, covers and frames to dry in the air and sun rays for along 2-3 days) .

(76)

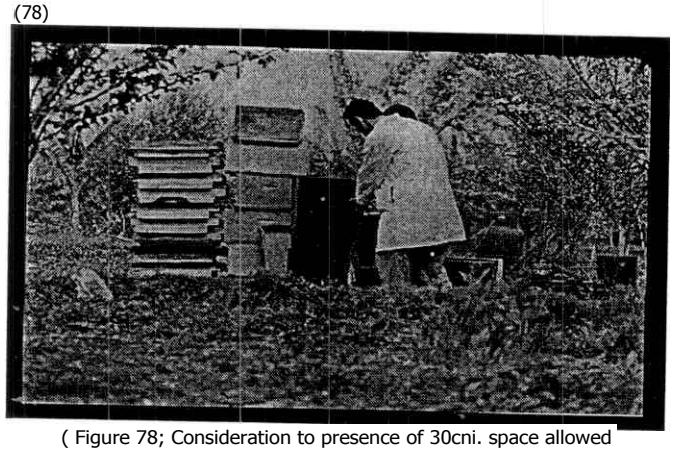
(Figure 76; The dry wooden boxes, covers and frames were whole Scorched with a blowtorch) .

(77)



(Figure 77; Concern with scorch of the internal narrow gaps) .

(78)



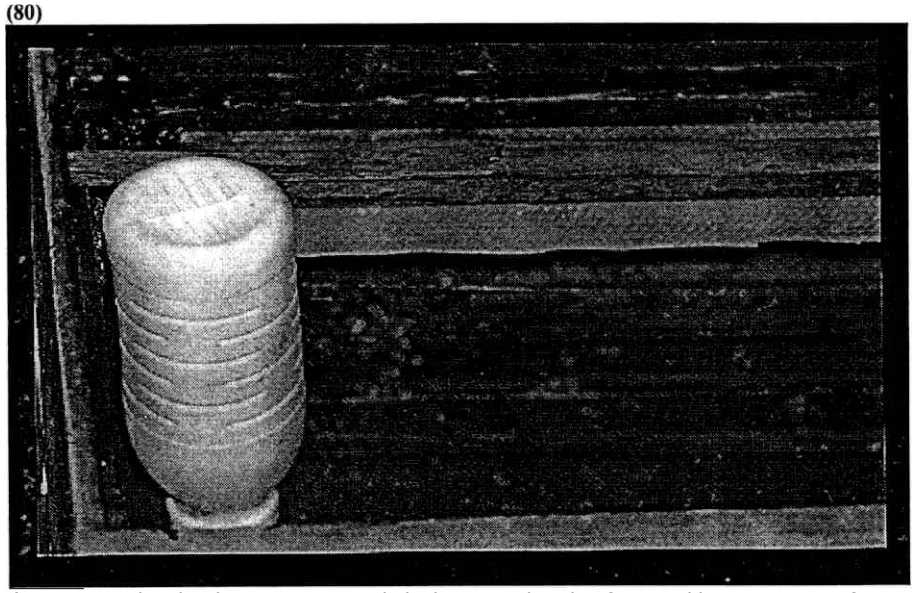
(Figure 78; Consideration to presence of 30cni. space allowed between surface of the wood and the blowtorch) .

(Figures 76-78; Show the scorch method and must that the beekeeper learns and practices of the control manners).

(79)

(Figure 79; Only the adult bees were shaked in a disinfectanted box over 2 wire frames without wax foundations after confinement of the queen inner introducing cage, then entrances of the hive were good closed for along 2 days without any feeding) .

(80)



(Figure 80; The alive bees were again shaked into another disinfectanted box over 2 wire frames with wax foundation stripes, and allowed the bees to feeding by a slowly feeder with sugar syrup containing on the selected therapeutic « Clove n , then the queens were liberated from the cage after 1day and the dismissal orifice was opened also).

(Figures 79 — 80; Show some steps of the shaked experiment which was performed on 3 infected honeybee colonies « replicates » with AFB disease at Sheshae apiary) .