RESULTS AND DISCUSSION

Bacillus cereus is a Gram-positive endospore forming opportunistic human pathogen of the *B. cereus* species group.. This group comprises the six species *B. cereus, Bacillus mycoides, Bacillus pseudomycoides, Bacillus thuringiensis, Bacillus weihenstephanesis* and *Bacillus anthracis* **(Gordon *et al*., 1973; Lechner *et al*., 1998, Priest *et al*., 1988, Turnbull and Kramer, 1991)**. While a high degree of diversity concerning the virulence factors is found a close genetic relationship has been observed between all species of the *B. cereus* group **(Helgason *et al*., 2000)**. It was therefore suggested that the entire group represents a single species. *Bacillus thuringiensis*, is facultatively anaerobic, endospore forming bacterium. It is characterized by its ability to form parasporal crystalline inclusions toxic to larvae of different insect orders. These proteinaceous are the basis for the commercial use of *B. thuringiensis* as a bioinsecticide, and since the beginning of the 1950s, this bacterium has been used increasingly against various insect pests. **(Gert B. Jensen, *et al*., 2002)**.

1. Cytological studies:

1.1. Effect of Agerin on plant system:

Three doses of Agerin were tested on two plant species (*Vicia faba* & *Allium sepa*), as well as a control group.

1.1.1. Effect of Agerin on *Vicia faba*:

Agerin induced a chromosomal aberration and abnormal cell division of the three treatments of faba bean root cell as compared with control.

1.1.1.1. Effect of Agerin on the miotic behavior of *vicia afaba*

The aberrations caused by treating the root cells with Agerin are shown in Figure (1) and Table (1).

Treating the *Vicia faba* seeds with Agerin induced a wide range of mitotic abnormalities in almost all mitotic stages. As shown in Table (1), the frequency of mitotic abnormalities and mitotic index increased by increasing the concentrations of the insecticide. The maximum value of mitotic absnormalities was 52 % after treating with the highest concentration (C_3)

Table (1) also shows the mean and the standard error of the studied aberrations caused by treating the root cells with gerin. They are significantly different by Duncan's new multiple range test.

These results are in partial agreement with these obtained by **El-Ashry, (2003)**,who reported genotoxic effect of cadmium chloride ($CdCl_2$) in *Vicia faba* plant by studing its effect on root growth and mitotic division and abnormal mitosis.

Also, **Zhang and Yang (1994)** concluded that cadmium chloride concentrations reduced the miotic index and it had genotoxic effect at the chromosomal level in *Hordeum vulgare*.

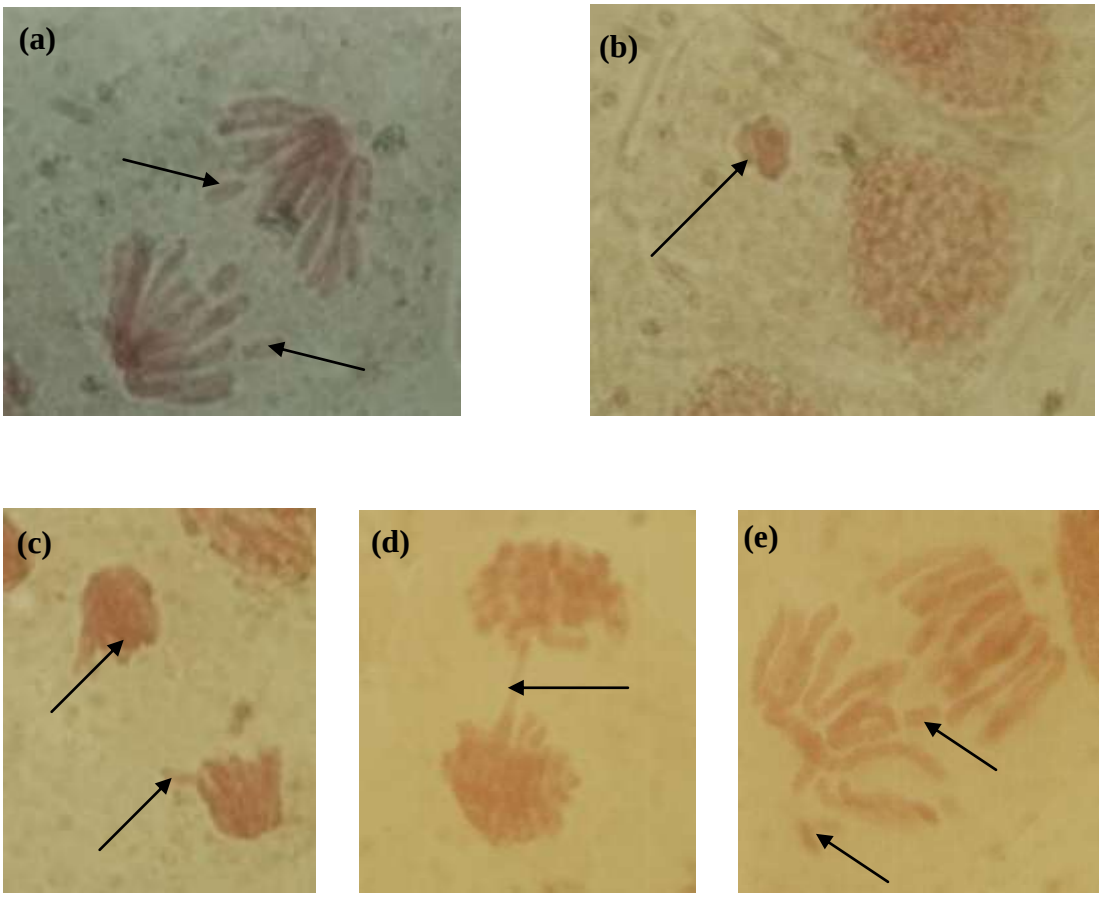

Fig. (1): Some types of abnormalities in mitotic division in the root-tips of *Vicia faba* treated with different concentrations of Agerin.

(a) Anaphase break.

(b) Interphase with micronuclei.

(c) Sticky telophase and fragmentation.

(d) Anaphase with bridge.

(e) Anaphase with break and fragmentation.

Table (1): Different types of abnormalities in root-tip cells after seed soaking for 24h of *Vicia faba* with different doses of agerin.

Doses gm/l	Total No. of counted cells	Chromosomal Aberration								Total No. of aberration cells	% of abnorm-alities	Mean ± Std. Error
		Break	Sticky	Bridge	Lagger chromosome	Micro nuclei	Ring	Disturbed chromosome	Fragmentation			
C_o	50	2	-	-	-	-	-	-	-	2	4	-1.1250 ± 1.12748
C_1 1.5 gm/l	50	3	2	3	1	1		1	-	11	22	1.1250 ± 1.12748(*)
C_2 2.5 gm/l	50	7		2	-	1		5	3	18	36	2.0000 ± 1.1274.8(*)
C_3 3.5 gm/l	50	10	3	4	-	-	1	6	2	26	52	3.0000 ± 1.12748

(*) Significant by Duncan test.

However**, Frantisek *et al.*, (1989)** disagreed with our result as they reported that using the *Drosophila* wing spot test. They have not found any genotoxic activity of *B. thuringiensis* β-exotoxin. Both the pure β-exotoxin and commercial microbial insecticide Biotoxibacillin containing β-exotoxin were negative in the induction of somatic mutations as well as miotic recombination.

El-Sherbeny *et al.*, (2002) reported a gradual increase in the percentage of chromosomal abnormalities as the increasment of the dose and duration of treatment increased with cascade on faba bean plant.

Basyezynki *et al.*, (1980) showed that cycloheximie induced mitotic index.

Also **El-Ashry (2003)** showed a significant reduction of mitotic index with high concentrations of organophose phosphates (phosphamidon) (100 and 200 ppm) indicating mitotic inhibitions.

Sharma *et al.*, (1977) reported that the beta-exotoxin thuringiensin A and the protein subunit of the delta-endotoxin, both isolated from *Bacillus thuringiensis*, resulted in a depressive effect on mitosis in root-meristem cells of *Allium cepa*, possibly by prolonging the cell-generation time.

1.1.1.2. Effect of Agerin on the meiotic behavior of *Vicia faba*

The aberrations caused by spraying the flower buds with different doses of Agerin twice are shown in Figure(2) and Table (2) which demonstrate the cytological effect of Agerin insecticide after

spraying on the meiotic cell division of *Vicia faba* used in the present study. The frequency of abnormal cells generally increased as the concentration of the insecticide increased. The three applied concentrations of Agerin induced a considerable frequency of chromosomal aberrations in both the first and the second meiotic divisions. The maximum values of meiotic abnormalities was 23 which was observed with the highest concentration (C_3) as compared to the control value.

Table (2) represents the mean and the standard error of the studied aberrations which are significantly different by Duncan's New multiple range test ($p < 0.05$).

These results are in accordance with those of El **Ashry (2003)** who reported that phosphamidon produced several types of chromosomal abnormalities in either mitosis or meiosis. These results also agree with those of **Abd El-Salam *et al*., (2000)** who found that the different tested concentrations of the two insecticides (Pyrethroid and catabron) have mutagenic activity at the cytological level in six cotton varieties.

El-Sherbeny *et al*., (2002) also agreed with our results that Cascade induced a significant percentage of abnormalities on meiosis of pollen grains of variety of *Vicia faba* (G12a2) that Pollen mother cells (PMCs) have some types of chromosomal abnormalities such as stickiness, lagging, bridges and spindle disturbance.

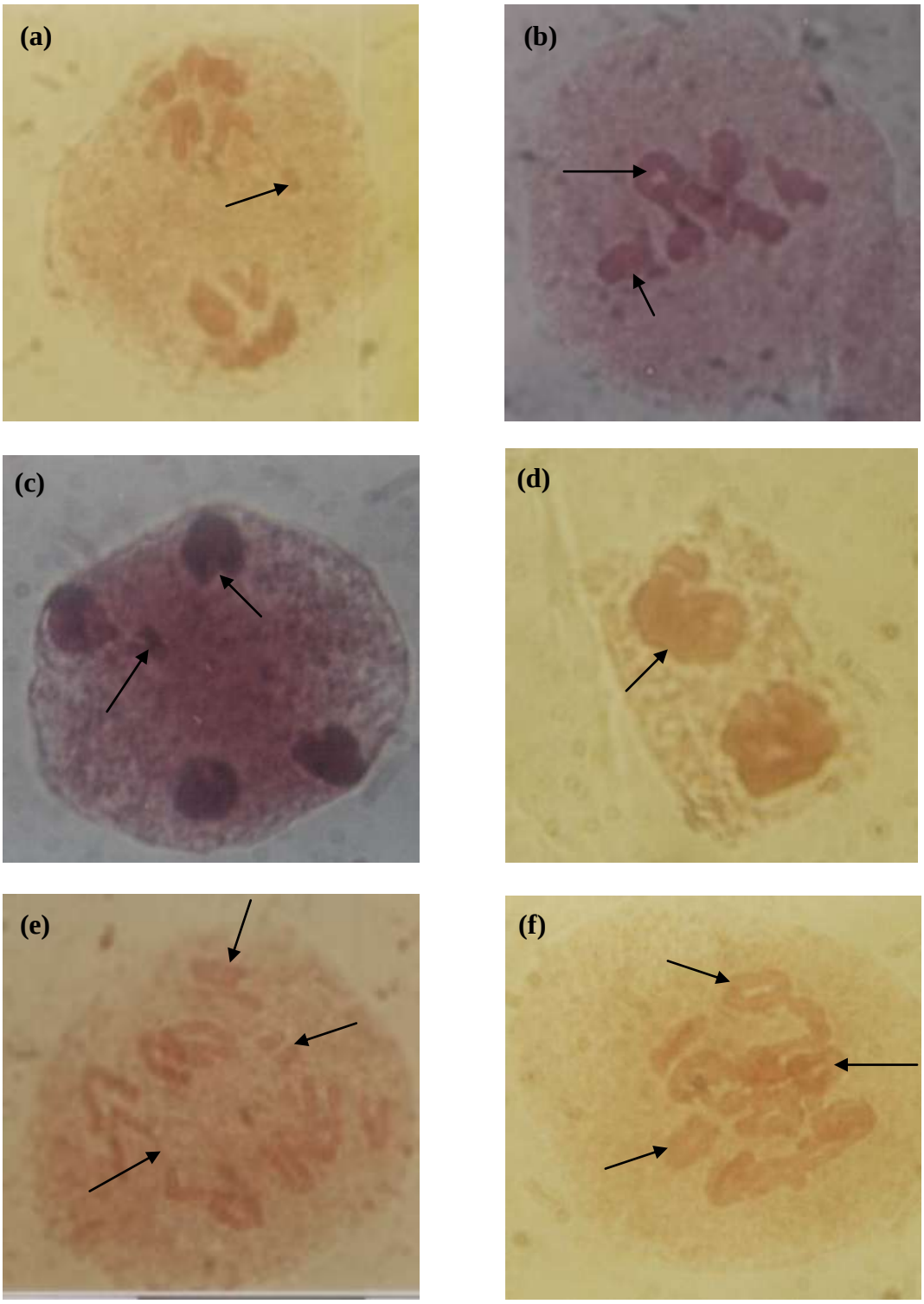

Fig. (2): Some types of abnormalities in mieosis division in the flower buds of *Vicia faba* treated with different concentrations of Agerin.

(a) Anaphase (I) with lagging chromosome.
(b) (Prophase (I) (Diakinesis) with sticky and ring chromosome.
(c) Telophase (II) with sticky and lagging chromosome.
(d) Telophase (I) with sticky.
(e) Anaphase (I) with bridge, break and laggard chromosome.
(f) Prophase (I) with ring chromosome.

Table (2): Different types of abnormalities in the meiosis of *Vicia faba* after spraying of the flower buds with different doses of agerin twice in the field.

Doses gm/l	Total No. of counted cells	Chromosomal Aberration								% of abnorm-alities	Mean ± Std. Error
		Break	Sticky	Bridge	Lagger chromosome	Micro nuclei	Ring	Disturbed chromosome	Fragmentation		
C_o	50	-	-	-	-	-	-	-	-	-	-1.3750 ± 0.68547
C_1 1.5 gm/l	50	2	3	2	2	-	1	-	1	22	1.3750 ± 0.68547(*)
C_2 2.5 gm/l	50	3	3	1	-	2	1	-	3	26	1.6250 ± 0.68547(*)
C_3 3.5 gm/l	50	6	3	2	-	1	2	3	6	46	2.8750 ± 0.68547(*)

(*) Significant by Duncan test.

1.1.2. Effect of Agerin on *Allium cepa*

Treatment with Agerin resulted in some abnormalities in the mitotic division of root tip cells of *Allium cepa*, these abnormalities are shown in Figure (3) and Table (3).

Agerin induced a wide range of mitotic abnormalities as the results showed a positive association between the doses and the percentage of abnormalities. The maximum percentage of mitotic abnormalities was observed after treatment with the highest concentration (C_3) (Table 3). However, no significant difference were observed between the effects of the different concentrations.

Linnainmaa *et al.* (1977) subjected β-exotoxin to four different nonmammalian tests. They did not observe any clastogenic effect in root tips of *Allium cepa*.

Negative results for mutagenicity of β-exatoxin were also obtained in the Ames test **(Cantwell *et al*., 1982, 1983).**

Lui *et al*. (1992) found that cadmium chloride (0.5-20 ppm) reduced the MI and induced various chromosomal aberrations in *Allium cepa* roots.

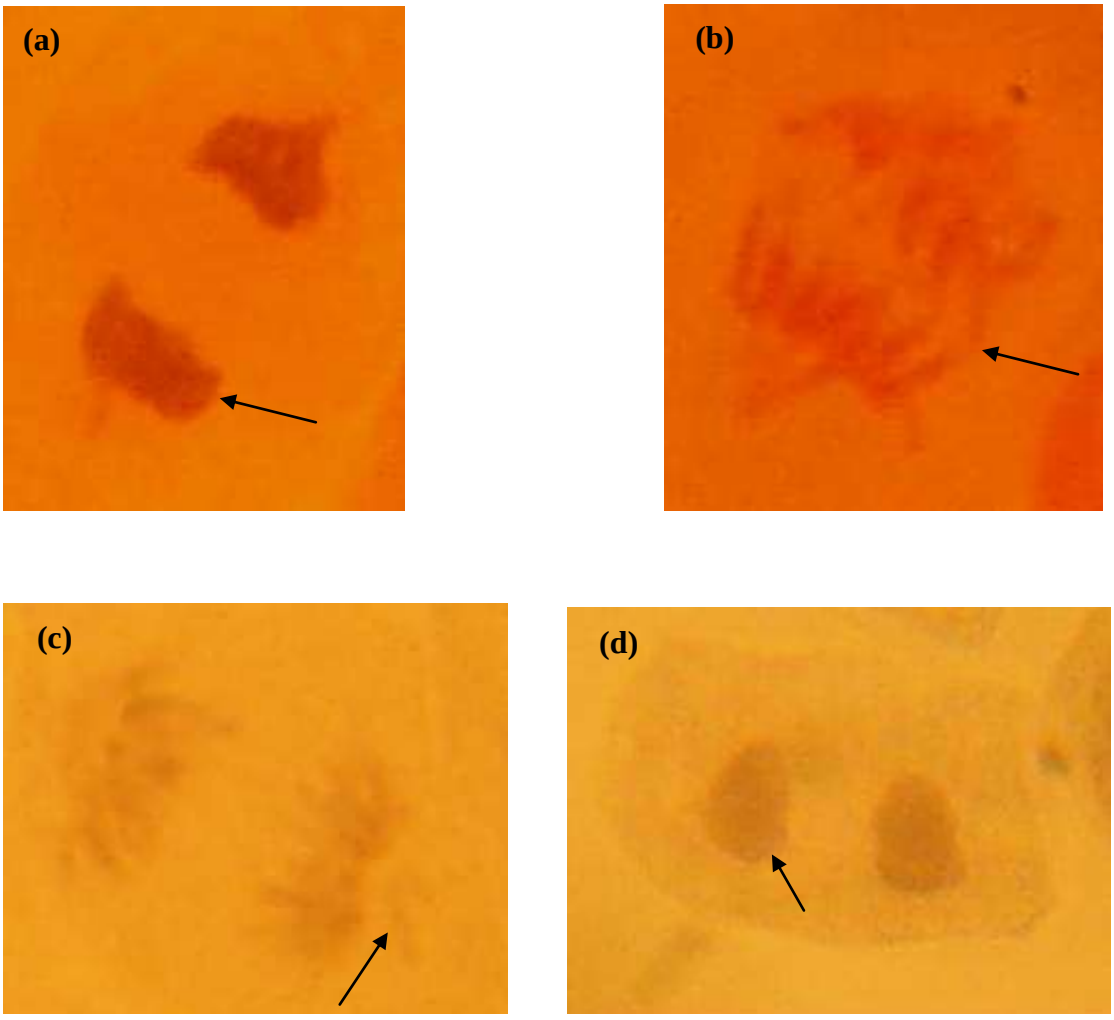

Fig. (3): Some types of abnormalities in mitosis division in the root-tips of *Allium cepa* treated with different concentrations of Agerin.

(a) Sticky anaphase.

(b) Anaphase with bridge.

(c) Late anaphase with lagging chromosome.

(d) Sticky telophase.

Table (3): Different types of abnormalities in root-tip cells of *Allium cepa* after treated for 24 h with different doses of the Agerin.

Doses gm/l	Total No. of counted cells	Chromosomal Aberration								Total No. of aberration cells	% of abnorm-alities	Mean ± Std. Error
		Break	Sticky	Bridge	Lagger chromosome	Micro nuclei	Ring	Disturbed chromosome	Fragmentation			
C_o	50	-	2	-	-	-	-	-	-	2	4	-1.0000 ± 1.96708
C_1 1.5 gm/l	50	2	5	-	-	-	-	2	1	10	20	1.0000 ± 1.96708
C_2 2.5 gm/l	50	3	14	1	-	-	-	-	-	18	36	2.0000 ± 1.96708
C_3 3.5 gm/l	50	4	17	3	-	-	-	7	-	31	62	3.6250 ± 1.96708

2. Biochemical genetic studies:

2.1. With *Vicia faba*:

2.1.1 Electrophoretic analysis:

The capability of Agerin to induce cytological aberrations, denoting its mutagenic effect, the Agerin also induced obvious alterations in the electrophoretic profiles of the seed proteins of *Vicia faba* (Fig. 4 and Table 4). The maximum number of bands was 17. Comparison between the treated samples and the control revealed the existence of some changes in the protein banding pattern among the treated samples. Band No. (4) was distinguished by its complete absence in C, C_2 and C_3 treatments, whereas it occurred in C1.

The figure also showed that band no. (3) was present in treatment C and C_1 and absent in C_2 and C_3.

However, band No (5) is distinguished by its appearance in C_2 and complete absence in C, $C_1$and C_3. But band no. (12) was present in C_1 and C_3 and disappeared in C and C_2.

It was also noted that band No. (8) is considered to be prominent band. The figure also showed that band no. (15) was absent in C and C_1 but appeared in $C_2$and C_3.

These results are in partial agreement with those of **Hassan (1996), Abdel-Salam *et al*. (1993), Muller and Gottschalk (1973) and Auerbach (1962)** in studies on some other pesticides and their effects on M_2 seed storage proteins of *Vicia faba* varieties.

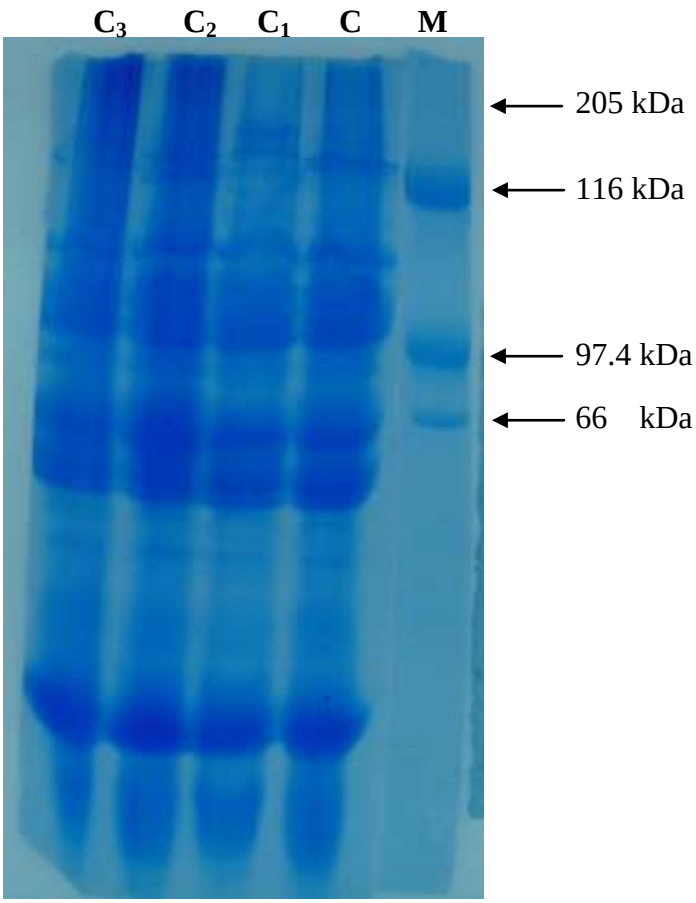

Fig. (4): SDS-PAGE bands of total protein for the control and the three treatments with Agerin in *Vicia faba* (from right to left).

Table (4): SDS-PAGE bands of total protein for the control and the three treatments of *Vicia faba* treated with different concentrations of Agerin.

Band No.	M.wt.	C	C_1	C_2	C_3
1	205	-	-	-	-
2		+	-	+	+
3		+	+	-	-
4	116	-	+	-	-
5		-	-	+	-
6		+	+	+	+
7		+	+	+	+
8	97.4	+	+	+	+
9		+	+	+	+
10		+	+	+	+
11		+	+	+	+
12		-	+	-	+
13		+	+	+	+
14		+	+	+	+
15	45	-	-	+	+
16		+	+	+	+
17		+	+	+	+

Abd El-Salam *et al*. (1993) suggested that the genes encoding protein bands in the high molecular weight region might be more sensitive to the mutagenicity than the genes encoding protein bands in the other regions.

However, disappearance of bands could be also explained on the basis of mutational events at the regulatory genes that suppress transcription while the appearance of new bands could be explained on the basis of mutational events at the regulatory system of an unexpressed gene (5) that activate it **(Abdel Salam *et al*., 1993).**

El-Ashry, (2003) mentioned that the induction of bridges, laggards and micronuclei would lead to a loss of some genetic material. This conclusion was in agreement with the results obtained by Gottschalk and Wolf (1993). These results are in agreements with those obtained by **El-Sherbeny *et al*. (2002).**

Attalla, *et al*. (2002) conducted an electrophoresis analysis of endotoxin protein of this *B. thuringiensis*. The results indicated the occurrence of 21 different proteins. Among them, one toxic protein of 71 kDa was specific against *lepidopterous* larvae and another protein of 27 kDa was specific against *dipterous* larvae. No specific toxic protein against *coleopterous* larvae was found.

2.1.2. Isozyme analysis:

Three isozyme systems, i.e., esterase, glutamate oxaloacetate transaminase (GOT) and peroxidase, were investigated in the present study to detect the possible effect of Agerin on *Vicia faba* and mice.

2.1.2.1. Esterase isozyme:

Development of esterase patterns were done for the three treatment groups used in *Vicia faba*, Fig(4) and Table(5).

Banding patterns of esterase isozyme was as follow:

- Band No. (1) occurred in the 3rd concentration and was completely absent in the other groups.
- Band No. (2) was found in C and C_3 and disappeared in C_1 and C_2.
- Band No. (3) occurred in the treated groups and was absent in the control group.
- Band No.(4) was found in the control group and absent in all treated groups.
- Band No. (5) was found in C, C_1 and C_2 and disappeared in C_3.

Tan-WeiJia *et al.*, (1997) reported that the toxicity of fenvalerate to susceptible and field-resistant strains of 2nd instar larvae of *Helicoverpa armigera* was increased by 12.9 and 34.4 fold when the pest was exposed to *Bacillus thringiensis* at the same time, respectively. Although the difference of alpha-naphthylacetate esterase and acetylcholinesterase activities was not significant, the values of Km and V_{max} of alpha-naphylacetate esterase in the field strain after *Bacillus thringiensis* treatment declined and affinity to special inhibitors of acetylcholinesterase in this strain increased, compared with those without *Bacillus thringiensis* treatment. However, there was significant difference between activities of glutathione-S-transferase in the larvae with or without *Bacillus thringiensis* treatment. PAGE analysis showed that all the activities

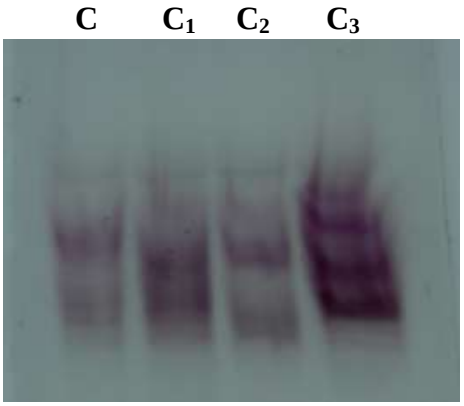

Fig. (5): Electrophoretic patterns of esterase isozyme for the control and the three treatments with Agerin in *Vicia faba* (from left to right).

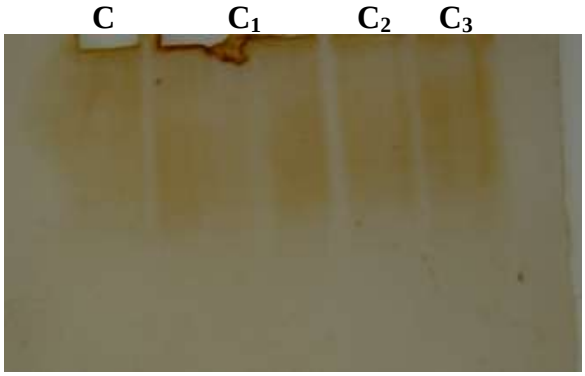

Fig. (6): Electrophoretic patterns of peroxidase isozyme for the control and the three treatments with Agerin in *Vicia faba* (from left to right).

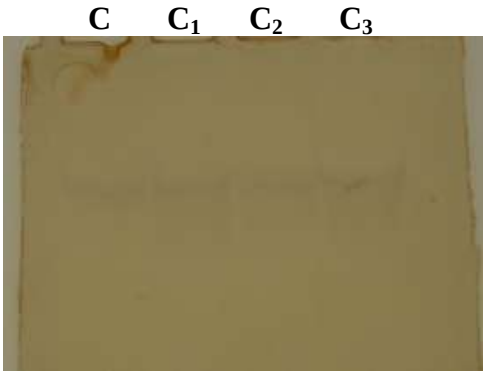

Fig. (7): Electrophoretic patterns of GOT isozyme for the control and the three treatments with Agerin in *Vicia faba* (from left to right).

Table (5): The presence of three isozyme (esterase, peroxidase and GOT) bands for the three treatments of *Vicia faba* and the control.

Band No.	C	C_1	C_2	C_3
	Esterase			
1	-	-	-	+
2	+	-	-	+
3	-	+	+	+
4	-	-	-	+
5	+	+	+	-
	peroxidase			
1	smear	smear	smear	smear
	GOT			
1	+	+	+	+

+; Presence of band

-; Absence of band

of esterase isoenzymes in the larvae treated with *Bacillus thringiensis* were lower than those without *Bacillus thringiensis* treatment, and the band (E6) of esterase isoenzymes, one of the acetylcholinesterase isoenzymes, in the field strain with *Bacillus thringiensis* treatment disappeared on electrophoresis spectrum by special inhibitors.

Yoo-ChongMyung *et al.* (1995) studied the changes in haemolymph esterase which was induced by forced feeding into the oral cavity and direct injection into the haemocoel with *Bacillus thuringiensis* (subsp.) *kurstaki* delta-endotoxin of last-instar larvae of Helicoverpa assulta. The enzyme activity was increased to some extent by toxin treatment, and the effect was greater in the group injected directly. A carboxylesterase (E6) and a cholinesterase (E10) were induced characleristicaly in the 2 treated groups, which seemed to be related to protection from the toxin.

Zhang-ShaoYan *et al*.,(2004) reported that to determine the effects of Bt toxic protein on the development and activities of some enzymes in *H. armigera*, the insect was reared on diets containing Bt protoxin with increasing Bt concentrations (0, 0.5, 2.0, 4.0, 6.0, 8.0, 10.0 and 12.0 micro g/g). The pupal weights and increments of larval weights in H. armigera reared on diets with Bt protoxin with incrasing Bt concentrations decreased, but the larval mortality enhanced significantly. Activities of alpha-naphthylacetate esterase and acetylcholinesterase also increased with the increase of Bt protoxin concentration.

2.1.2.2. Glutamate oxaloacetate transaminase (GOT) isozyme:

Transaminase are involved in the cell in the formation of amino acids for which the cell can synthesize the 2-keto acid. The existence of GOT isozyme forms has to do with the cellular function include gluconeogensis, i.e., synthesis of sugar (glucose) to replenish and keep blood sugar at a normal level when there has been no recent intake or carbohydrates in food (mainly in liver cells), (Champe and Harvey, 1987).

Electrophoretic patterns of GOT isozyme in *Vicia faba* are shown in Figure (7) and Table (5) found to be exhibit only one band. This band was presented in all treatments. No differences were observed between treated concentrations and their control.

2.1.2.3. Peroxidase:

Figure (6) and Table (5) represent banding patterns of peroxidase enzyme in *Vicia faba* of the three treatments as well as the control group. As shown in the figure there is no clear bands but only shading in all group.

2.2. With Mice:

2.2.1.Electrophoretic analysis

Figure (8) and Table (6) represent liver protein banding patterns for the treated male with the three doses (C_1, C_2, C_3 and Control. From the figure the maximum number of bands was 25. Bands No. (4,8, 15, 16and 22) were absent in the control and present in each of three concentrations used. Bands No (11, 23and 24) were found in the control and disappeared from the three treatments. It

also showed that band No. (4, 8) were prominent bands while bands No. (13, 14, 15, 19, 21, 2, 3, 6, 7, 10, 11 and 22) are found in all doses as well as the control.

Also, band No. (17) was present in C, C_2 and C_3 but absent in C_1 and vice versa in band (19).

Johari and Sadagapau (1989) reported that a marked decrease was found in total proteins when they studied the effect of diets of aflatoxin on the performance of different categories of pourtry and the perimissible dietary level of aflatoxin on hybrids.

On the other hand, these results was contradicting with those of **Ankrah *et al*. (1993)** who reported that serum total protein and albumin levels were not affected by the exposure to aflatoxin B_1 and G_1 in mice *via* their feed.

Joanne *et al.*, (2005) identified current control of the sheep blowfly (*Lucilia cuprina*) relies on chemical insecticides, however, with the development of resistance and increasing concerns about human health and environmental residues, alternative strategies of control this economically important pest are required. In this study, they have identified several isolates of *Bacillus thuringiensis* (Bt) collected from various Australian soil samples, that produce crystals containing 130 and 28 kDa proteins. These isolates were highly toxic to feeding larvae in both *in vitro* bioassays and *in vivo* on sheep. By N-terninal amino acid sequencing, we identified the smaller crystal band (28 kDa) as a cytological (Cyt) protein. Upon solubilization and proteolytic processing by trypsin, the 130 kDa crystal protein

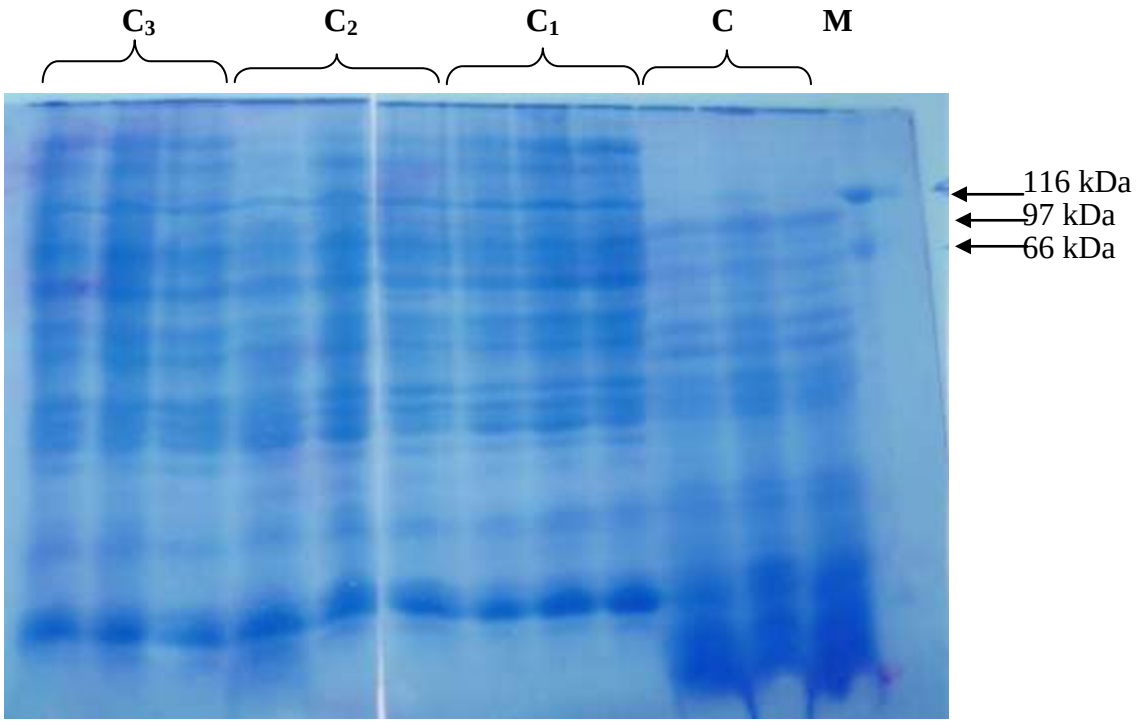

Fig. (8): SDS-PAGE bands of total protein for the control and the three treatments with Agerin in mice (from right to left).

Table (6): SDS-PAGE bands of total protein for control and three treatments of Mice treated with different doses of Agerin.

Band No.	M.wt.	C			C_1			C_2			C_3		
1		+	+	+	+	+	+	+	+	+	+	+	+
2		+	+	+	+	+	+	+	+	+	+	+	+
3		+	+	+	+	+	+	+	+	+	+	+	+
4		-	-	-	+	+	+	+	+	+	+	+	+
5		+	+	+	+	+	+	+	+	+	+	+	+
6		+	+	+	+	+	+	+	+	+	+	+	+
7		+	+	+	+	+	+	+	+	+	+	+	+
8		-	-	-	+	+	+	+	+	+	+	+	+
9		+	+	+	+	+	+	+	+	+	+	+	+
10		+	+	+	+	+	+	+	+	+	+	+	+
11		+	+	+	-	-	-	-	-	-	-	-	-
12		+	+	+	+	+	+	+	+	+	+	+	+
13		+	+	+	+	+	+	+	+	+	+	+	+
14		+	+	+	+	+	+	+	+	+	+	+	+
15		-	-	-	+	+	+	+	+	+	+	+	+
16		-	-	-	+	+	+	+	+	+	+	+	+
17		+	+	+	-	-	-	-	+	+	+	+	+
18		+	+	+	+	+	+	+	+	+	+	+	+
19		-	-	-	+	+	+	-	-	-	-	-	-
20		+	+	+	+	+	+	+	+	+	+	+	+
21		+	+	+	+	+	+	+	+	+	+	+	+
22		-	-	-	+	+	+	+	+	+	+	+	+
23		+	+	+	-	-	-	-	-	-	-	-	-
24		+	+	+	-	-	-	-	-	-	-	-	-

yielded among others, a truncated 55-60 kDa toxin moiety which exhibited larvicidal activity against sheep blowfly. The amino-terminal sequence of the trypsin-resistant protein band revealed that this *Bt* endotoxin was encoded by a new cry gene. The novel cry protein was present in all the strains that were highly toxic in the larval assay. They also identified from one of the isolates, a novel secretory toxin with larvicidal activity.

(Peter *et al*., (1985) discussed that alkaline-dissolved crystal δ-endotoxin from *"Bacillus thruingiensis*" var. *israelensis* (serovar H14) was injected into "mice" and seven species of insects representing the orders *Lepidoptera, Orthoptera, Coleoptera, Hemiptera,* and *Diptera*. High *in vivo* "toxicity" at 1 to 5 ppm (μg toxin/g body wet wt), was observed with mice and some insects, including some that are not sensitive to the toxin when administered orally. Neuromuscular effects were observed when the toxin was injected directly into the body cavity of the test animals. Biochemical studies suggested that different protein fragments within the crystal δ-endotoxin may be responsible for the majority of the mosquito larvacidal activity and the neurotoxic symptoms observed in larvae of *Trichoplusia ni.*

Mayes *et al.* (1989) studied that solubilized crystal polypeptide preparations of *Bacillus thringiensis* subsp, *israelensis* (BTI) were fractionated by immunoaffinity chromatography using a bound monoclonal antibody formed against the 28 K crystal polypeptide. The 28 K polypeptide was confirmed to be hemolytic

and to possess low mosquitocidal activity against *Aedes aegypti* larvae. By comparison, the 28 K polypeptide was more potent than the solubilized BTI crystals in male Swiss Webster "mice" as the LD 50 values were ($P < 0.05$) 0.77 and 2.33 mg prote4in/kg body wt, respectively. Acute administration of the 28 k polypeptide (mg/kg ip) produced severe hypothermia and bradycardia in the "mouse". No evidence for cooperativity between the 28 K and other crystal polypeptides was observed. Preliminary histological examination of the "mouse" heart exposed to the 28 K polypeptide did not reveal any specific lesion, suggesting that the deficient cardiac performance might be a secondary physiological response. Gross pathological examination of "mice" as well as Sprague-Dawley rats acutely treated with equivalent doses of solubilized BTI crystal preparations revealed focal to segmental reddened and edematous areas within the small intestine. Histopathology indicated that the major lesion was in the jejunum. Contrary to expectations from in vitro hemolysis assays, cytolysis of "mouse" red and white blood cells was not detectable after in vitro exposure to the BTI solubilized proteins. The present results indicate that the 28 K polypeptide is the mammalian toxic component of BTI crystals.

2.2.2. Isozyme analysis

2.2.2.1. Esterase isozyme:

The electrophoretic patterns of liver esterase for male mice using the three treatments (C_1, C_2, C_3) and the control are presented in Figure (9) and Table (7).

Electrophoretic patterns of liver esterase isozyme exhibited a maximum number of four bands as shown in Figure (9). However, not all bands necessarily appeared in all individuals. Band No.(4) appeared in the individuals of the control group and completely disappeared in the individuals of the treated groups.

Kutynyuk *et al.*,(1991) found that birds homozygous for ES-1 had 5-10 % higher resistance to the mycotoxin (T-2) than heterozygote comparable results were also reported by **Ghohsh *et al.*, (1990)** who found that the percentage of acid alpha-naphthyl acetate esterase reacting lymphocytes was significantly decreased in chicks fed with two levels of AFB_1.

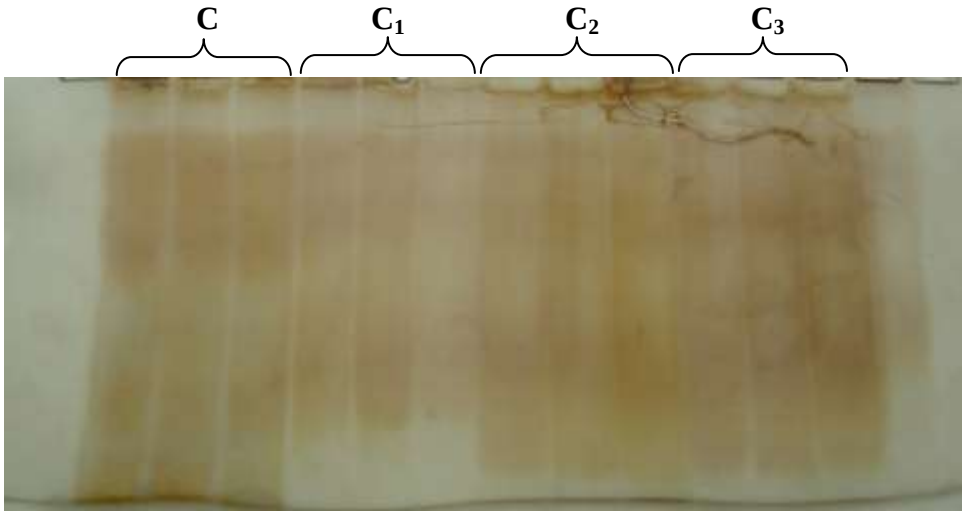

Fig. (9): Electrophoretic patterns of esterase isozyme for the control and the three treatments with Agerin in mice (from left to right).

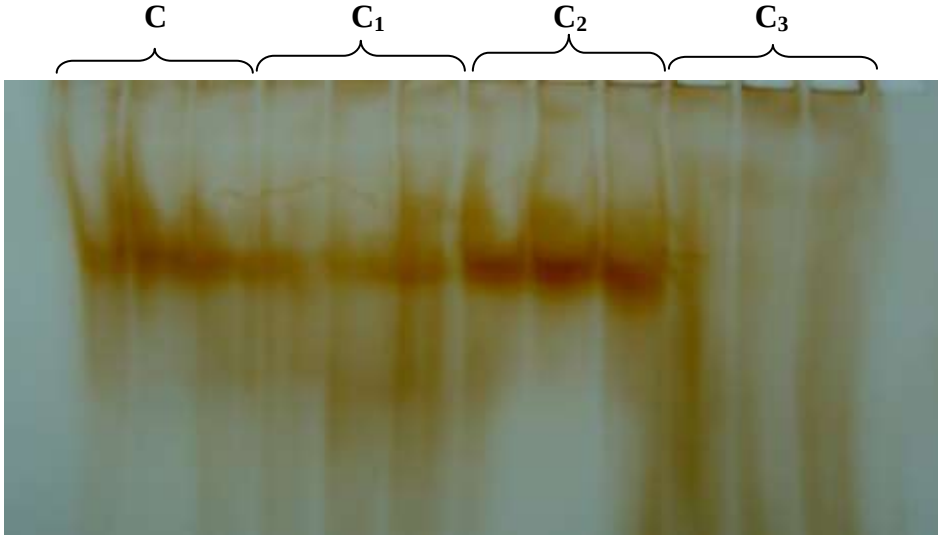

Fig. (10): Electrophoretic patterns of peroxidase isozyme for the control and the three treatments with Agerin in mice (from left to right).

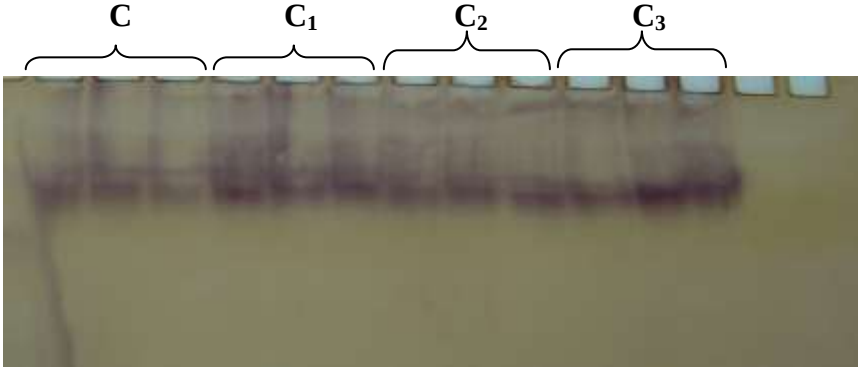

Fig. (11): Electrophoretic patterns of GOT isozyme for the control and the three treatments with Agerin in mice (from left to right).

Table (7): The presence of three isozymes (esterase, GOT and peroxidase) bands for the three treatments of Mice and the control.

Band No.	C			C_1			C_2			C_3		
	Esterase											
1	+	+	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	+
4	+	+	+	-	-	-	-	-	-	-	-	-
	GOT											
1	+	+	+	+	+	+	+	+	+	-	-	-
2	+	+	+	+	+	+	+	+	+	+	+	+
	Peroxidase											
1	+	+	+	+	+	+	+	+	+	+	+	+

+; Presence of band

-; Absence of band

2.2.2.2. Peroxidase isozyme:

The electrophoretic patterns of liver peroxidase for the four treatments (C_1, C_2, C_3 and Control) of the male mice used are presented in Figure (11) and Table (7).

From the Figure there is only one band in all treatments and the control group.

These results disagreed with those obtained by **Suneja *et al.*, (1989)** where they studied the effect of liver lipid peroxidation in rats at 8, 16 and 24 hours after feeding a single dose aflatoxin T-2 (2.0 mg/kg) and at 7, 14 and 21 days after daily feeding of toxin (0.75 mg/kg). Feeding a single dose caused a significant increase in liver lipid peroxidation in rats at 8, 16 an 24 hours post treatment. Also, significant increase was noticed at 14 and 21 days after dialy feeding aflatoxin.

2.2.2.3. Glutamate oxaloacetate transaminase (GOT) isozyme:

Figure (10) and table (7) represent actual banding patterns of liver glutamate oxalaoacetate transaminase (GOT) of the three treatments and the control for the male mice used with slight differences in their band intensity.

Band No. (1) is completely absent in the 3rd concentration (C_3) comparing with the other concentrations and the control group.

These results are in agreement with those of **Toskulkao *et al.*, (1991)** who indicated that pretreatment with ethanol at 24 and 48 hours prior to AFB, single interaperitoneal admnstration in male

Wister rats caused a significant increase in the activity of plasma glutamic oxaloacetic transaminase.

2.2.3. DNA analysis:

The general concession is that mutation in the P^{53} tumor suppressor gene in closely linked with the high incidence of several types of mammalian cancer including human and mice **(Hollstein *et al.*, 1991).** Cancer is now recognized as a genetic disorder at the cellular level that involves the mutation of a small numbers of genes. Many of these genes normally act to suppress or stimulate progression through the cell cycle, and loss or inactivation of these genes causes uncontrolled cell division and tumor formation Mutation in P^{53} is a G to T transversion in the 3^{rd} nucleotide of codon 249 **(Hsu *et al.*, 1991).** This specific G to T transversion is consistent with the occurrence of DNA damage induced by AFB_1 since the mutagenic metabolite induced this type of base change **(Foster *et al.*, 1983).** The same patients of AFB_1 adducts in liver DNA and serum albumin, and the polymorphism of glutathione-s-transferase μ, and enzyme was reported to be involved in the detoxification of AFB_1 **(Leu *et al.*, 1991).**

Fig. (12) shows that while the control treat exhibited the two characteristic fragments of the normal p53 at (~160 bp and 130 bp), each of the three concentrations of Agerin exhibited only one band which indicates the occurance of mutation due to the Agerin effect. Such potential mutation could reflect (a) serious hazards to those handling this bioinsecticide.

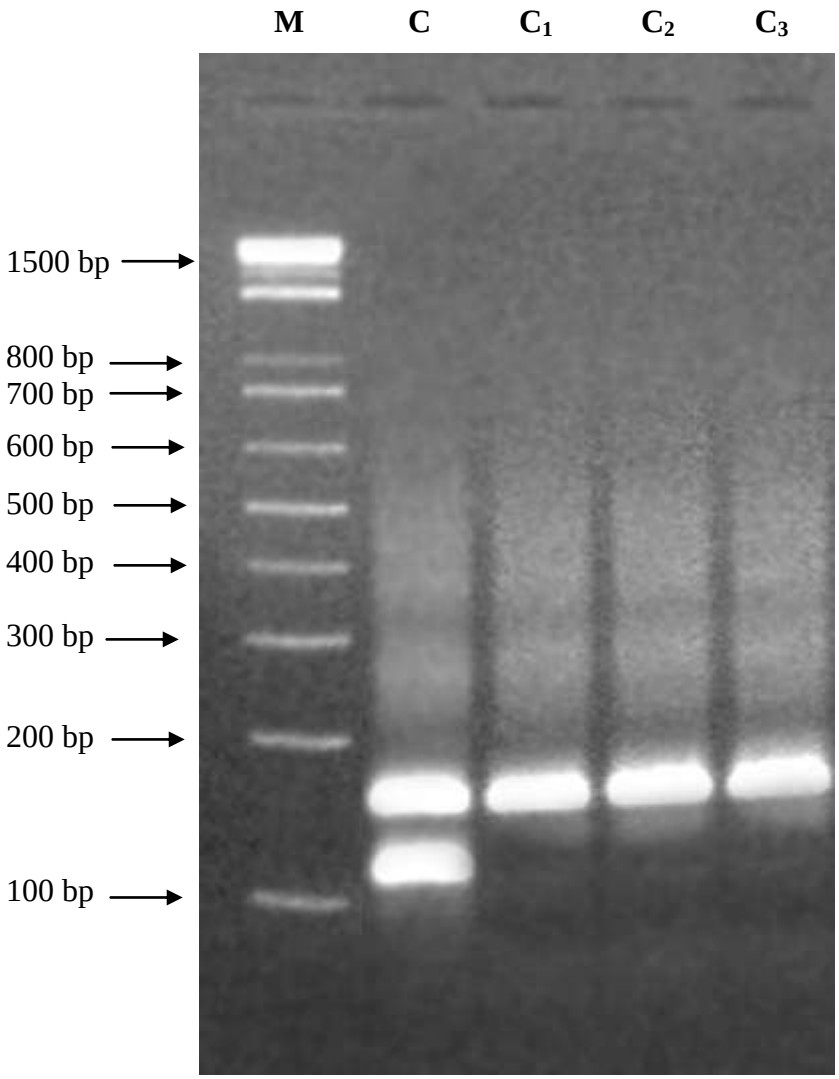

Fig. (12) PCR product of p53 gene amplified with OLF primers for the three groups treated with the three doses of Agerin and the control.

These results are in agreement with Hussein and **Cerutti (1993)** who investigated the mechanism of formation of mutation in codon 249 of the P53 tumor suppressor gene in hapatocarcinogenesis. They suggested that both mutability on the DNA level and altered function of the mutant serine 249 P^{53} protein are resbonsible for the observed mutational hot spot in p53 in hepatpocellular carcinoma (HCC) from AFB_1, contaminated areas.

Also **Bagchi *et al.*, (2000)** found that, p53-deficient mice are more susceptible to all four xenobiotics than C57BL/6NTac mice, with dose-dependent effects being observed. Specifically, at a 0.50 LD (50) dose, naphthalene and Cr (VI) induced the greatest toxicity in the liver tissue of mice, and naphthalene and endrin exhibited the greatest effect in the brain tissue. At this dose, TCDD, endrin, naphthalene, and Cr (VI) induced 2.3- to 3.7-fold higher increases in hepatic lipid peroxidation and 1.8- to 3.0-fold higher increases in brain lipid peroxidation in p53-deficient mice than in C57BL/6NTac mice. At a 0.10 LD(50) dose, TCDD, endrin, naphthalene, and Cr(VI) induced 1.3- to 1.8-fold higher increases in hepatic lipid peroxidation and 1.4- to 1.9-fold higher increases in brain lipid peroxidation in p53-deficient mice than in C57BL/6NTac mice. Similar results were observed with respect to DNA fragmentation and cytochrome c reduction (superoxide anion production). For example, at the 0.10 LD(50) dose, the four xenobiotics induced increases of 1.6- to 3. 0-fold and 1.5- to 2.1-fold in brain and liver DNA fragmentation, respectively, and increases of 1.5- to 2.3-fold and 1.4- to 2.5-fold in brain and liver cytochrome c reduction

(superoxide anion production), respectively, in p53-deficient mice compared with control C57BL/6NTac mice. These results suggest that the p53 tumor suppressor gene may play a role in the toxicity of structurally diverse xenobiotics.

Wu *et al.*, (2000) indicated that deltamethrin leads to the persistent increase of p53 and Bax expression and transient elevation of Bcl-2 expression, resulting in an increased ratio of Bax to Bcl-2, which may contribute to apoptotic cell death in rat brain following deltamethrin treatment.

Fu *et al*., (2005) findings were in accordance with this study where their results show that MC-LR can increase expression of p53 and Box significantly both *in vitro* and *in vivo*, however, MC-LR can only decrease the expression of Bel-2 significantly in vitro and there is no difference observed *in vivo*. It can be concluded that the expression of p53, Bel 2 and Bax are involved in the regulation of MC-LR induced apoptosis.

Wang *et al.*, (2005) mentioned that expression of p53 and Bax in each treatment group increased significantly compared with that in control group ($p<0.05$), with the exception of 0.1 microg/kg LR exposure group. Moreever, with exposurelevels increasing the expression of p53 and Bax increased gradually; while no changes of the expression of Bel-2 were observed. Conclusion p53 and Bax may play important roles in microcystin LR induced apoptosis, but Bel-2 seems not be involved in this process.

Also **Farazi *et al.*, (2006)** reported that, in the setting of intact telomeres, p53 mutation had no effect on hepatocarcinogenesis, whereas in the setting of telomere dysfunction, p53 mutation enabled advanced hepatocellular carcinoma of the wild-type p53 alleile in the late generation mTerrt(-/-)p53(+/-)mice, suggesting that reduced levels of p53 potently enable hepatocellular carcinoma progression in the setting of telomere dysfunction. Thus, this study supports a model that, in the face of chronic liver damage, attenuated p53 function and telomere-induced chromosomal instability play critical and cooperative roles in the progression of hepato cellular carcinoma.

Penttinen *et al.*, (2007) illustrated that, the spores of co-cultivated microbes evoked DNA damsge, p53 accumulation and cytotoxicity at a lower dose than the other exposures, and at the highest dose there was a 2.5-fold increase in DNA damage compared to control. In addition the spores of *Streptomyce californicus* alone induced a 1.5-fold increase in DNA damage compared to control, dose dependent p53 accumulation and also extensive cytotoxicity. In contrast the mixture of separately cultivated spores or spores of *Stachybotrys chartarum* alone did not induce DNA damage with any tested dose although they triggered significant cytotoxicity and a slightly increased p53 level. Their results suggest that te detected genotoxic responses are the result of DNA damage in RAW264.7 cells by some genotoxically active metabolite (s) and the production of this compound was stimulated in *Streptomyces calionicus* when it was co-cultivated with *Stachybotrys chartarum.*

Mobio *et al*., (2003) showed that, the results suggest a possible loss of protective mechanisms (such as p53-dependent apoptosis cycle arrest) in FB_1-damage MEF cells and confirm that cells lacking of mechanisms governed by p53 gene would susceptible to neoplastic cascade or mutation following DNA lesions induced by this mycotoxin.

Tong *et al*., (2006) suggested that the human DNA context of the p53 gene alone may not be the sole determinant of AFB1-induced mutagenesis. Furthermore, humanized p53 appears not to be as effective as murine.