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BIOACTIVITY OF SOME BIOCIDES ON THE COTTON LEAF WORM, SPODOPTERA LITTORALIS (BOISD.), (NOCTUDIAE: LEPIDOPTERA)

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ABSTRACT: ABSTRACT: The biocides, Protecto *(Bacillus thuringiensis kurstaki)*, Verosit (SLNPV) and Perovect (SLNPV+ B.T. *var. kurstaki*) were tested against the 2nd and 4th instar larvae of *Spodoptera littoralis* using semi-field trails in a cotton field. Cotton plants were sprayed with different rates from the tested biocides. Their efficacy and persistence were determined under field conditions. The mortality was higher on 2nd instar than 4th instar larvae as it is generally the most susceptible stage to bacterial endotoxins. Higher mortality rates were obtained at zero time against the 2nd and 4th instars than at fifth and seventh day after treatment of the three biocides. A significant difference appeared in mortality between 2nd and 4th instar larvae at zero time and seventh day after application in all cases, as well as in reduction among *S. littoralis* larval population in the cotton field.

Key Words: *Spodoptera littoralis, Bacillus thuringiensis,* NPV, Protecto, Verosit, Perovect.

INTRODUCTION

Cotton is one of the world's leading crops, and represents almost half the world's fiber market with cotton fiber production in 1997-1998 estimated by 18,888,000 tonns worldwide. Most losses are caused by six species of bollworms; cotton is attacked by close to 50 different insect pests. The conventional cotton growing is heavily dependent on chemical input; nearly a quarter of insecticides used around the world each year is applied on cotton (Pertak *et al.* 2001). The Egyptian cotton leaf worm, *S. littoralis,* is one of the most important insect cotton pests in Egypt. It causes considerable damage to cotton plants.

Bacillus thuringiensis has become the leading biopesticide science of the beginning of the 1970s, due to the lethality of the toxin to insects. At present, variety of microbial insecticides are commercially available for pest control and used extensively in forest, vegetables, and row crops. Even so, the microbial products in use represent only 1% of the worldwide insecticide sales (Rowe and Margarities 1987), Roberts et al. (1991), and Lambert and Perferone (1992). Two problems with the use of the biological agents are that they can be deactivated by UV light following application to the target zone (Prozsgay et al. 1987) or removed from the target site by rain.

(Behle et al., 1997) Formulations that protect the active agent from adverse environmental conditions should increase the amount of active agent that remains in the target zone and extend its residual activity. Resistance to conventional insecticides and concern about their environmental effects has led to increase in use of *Bacillus thuringiensis* for insect control. Also, in the last decade, there have been great steps forward in the development of B.t. as a biocide. These include the isolation and selection of new strains that are effective to a wider range of insect pests.

MATERIAL AND METHODS

Insects:

The second and fourth instar larvae of *S. littoralis* were collected from cotton field in Fayoum governorate and reared in the laboratory on cotton leaves. Larvae from the second generation were selected to be used. For the check, larvae were obtained from colony maintained in the Plant Protection Institute, Dokki, Giza. This colony was established from 5th instar larvae collected from cotton field in 2000. This research was carried out at plant Protection Department, Faculty of Agriculture, Al-Azhar University, Nasr City Cairo, Egypt.

Bacterial insecticides:

1- Protecto: *Bacillus thringiensis variety kurstaki* 32,000 IU/mg. (9.4% Active ingredient) .

2- Verosit: *S. littoralis virus* (SLNPV) 5x109 pibs/ml (4 % Active ingredient).

3- Perovect: SLNPV+ B.T. *var. kurstaki* 2+5 Active ingredient (w:w) produced by Plant Protection Research Institute, Ministry of agriculture, Doki, Giza.

Experimental design

A cotton field of 2 Faddens at Fayoum governorate was selected for the test. The whole field was divided into 36 plots and the different treatments were arranged according to the randomized complete block design. Each treatment had four replicates, and plots sprayed with water served as check. The Egyptian cotton cultivar Giza 75 was the planted variety in the present study.

Field tests

The field tests were carried out in summer season during June 2001, the spray was done at June 17. In the same day, the sprayed leaves were selected randomly just after treatment (zero time) and brought to the laboratory. The larvae were counted in the field after 24 hrs, 3^{rd} , $5t^{h}$, 7^{th} days after treatment by selecting 4 meter randomly per plot (one meter in length) and counting all larvae found. Then larvae were compared in both treated and untreated check plants 3, 5 and 7 days after treatment. The reduction percent was calculated using Henderson's formula (Henderson and Tilton 1955).

Laboratory tests

Leaves from the outer, middle and lower parts of cotton plants were collected at different times (zero time, 3^{rd}, $5t^{h}$ and 7^{th} days) and put in paper bag and brought from the field after being sprayed by various aqueous concentration of Protecto, Perovect and Verosit.

The treated leaves were offered for second and fourth instar larvae in jars placed in a rearing room at 28°C . Food was renewed by untreated cotton leaves after 24hrs and the larvae were observed daily for toxicity symptoms injury and mortality. Larval mortality was assessed after 2, 4, 6 and 8 days until adult emergence. Larvae were considered dead if they did not move when prodded.

In 2001, application of the biocides occur at the rates of (200 liters of water per feddan) 150, 300, 600, 1200 and 2400 gm/ feddan for Protecto , Verosit and Perovect. Experiments with each concentration and the untreated check were replicated four times. As mortality percentages in check larvae in the laboratory ranged from 5.-16%, the obtained data were corrected according to the formula given by Abbott (1925).

Statistical analysis:

The collected data were statistically analysed according to Steel and Torrie (1980). The mean values were compared at the 5% level of significance by using the least significance difference (L.S.D.) test.

RESULTS AND DISCUSSION

The objective of experiment was to determine the efficacy and persistence of Protecto, Verosit and Perovect versus 2^{nd} and 4^{th} instar larvae of the cotton leaf worm, S. littoralis in the laboratory and under field conditions.

Table (1) represented the mortality percent of S. littoralis as 2^{nd} and 4^{th} instar larvae. Protecto acted more than Verosit and Perovect at zero time against 2^{nd} instar larvae where there were significant differences between the mean mortality percent (51.12, 46.0 and 42.5% respectively). The main effects of Protecto, Verosit and Perovect on larval mortality were as follows: 86.6, 92.5 and 80.0% respectively at higher rate (2400g/F). There were significant differences ($p > 0.05$) between the different rates. For the 4^{th} instar larvae, the data show that the biocide Verosit was more effective than Protecto and Perovect and there was significant difference between the Verosit and Protecto and between Verosit and Perovect but not between Protecto and Perovect. The 2^{nd} instar larvae of cotton leaf worm were more sensitive than 4th instar larvae. The accumulative mortality for 2^{nd} instar was higher than for 4th instar larvae, as they are generally the most susceptible stage to bacterial endotoxins (Homby and Gardner 1987).

Table (2) shows the bioactivity of the three tested biocides (Protecto, Versit and Protecto) assessed against 2^{nd} and 4th instar larvae of *S. littoralis* at third day after application in the field. The 2^{nd} and 4th instar larvae were affected by Perovect more than other biocides. The larval mortality percents were 65.0, 47.0 and 70.0 % as affected by Protecto, Verosit and perovect, respectively. No significant difference between the two instars for Perovect;. but there was significant difference between Protecto and Verosit against 2^{nd} and 4^{th} instars. The mean percent mortality rates against 2^{nd} instar larvae were 36.5, 35.5 and 31.0% for Perovect, Protecto ınd Verosit, respectively at third day after spraying. The mean values for mortality gainst 4th instar larvae were 34.0, 26.9 and 24.60% for Perovect, Verosit and 'rotecto, respectively at the third day after application.

After five days, significant differences ($P > 0.05$) in mortality levels were recorded between the various biocides. The results obtained in this assay indicate that, the Perovect is the most virulent against 2nd instar larvae of *S. littoralis* (Table 3) while Verosit is the least pathogenic virus against this instar. The data obtained in Table (3) showed that, B.T. (Protecto) caused highest mortality percent against 4th instar larvae. At the 7th day post application, the activity of biocides against 2nd and 4th instar larvae decreased gradually (Table 4). The mortality rates were 15.0, 12.5 and 17.5% (2nd instar) and 10.0, 8.0 and 11.5 % (4th instar) for Perovect, Protecto and Verosit, respectively.

The data in Table (4) showed that, there was significant difference between the tested biocides against 2nd or 4th larvae of *S. littoralis* at 7th day after treatment. The results obtained in previous assays indicated that, the virus-bacteria complex (Perovect) caused the highest mortality percent against 2nd and 4th instar larvae of *S. littoralis* at the higher rate (2400g/F). after different periods. Efficacy of the biocides in insect-kill could be arranged in the order: Perovect > Protecto > Verosit. There was significant difference in larval mortality values between zero time and 7th day; and this agree with Jacqueline *et al.* (1996). Ridway *et al.* (1996) detected significant effects of Dipel 2X (B.T. *Kurstaki*)(10G contains 1600 IU/mg) on percentage of mortality 5 days after treatment but not after 10 days against the European corn borer, *Ostrinia nubilalis* (Hubner). After 7 days of exposure under field conditions *B. thuringiensis* treated cabbage plants provided 30 ±28.0 % mortality of cabbage looper larvae *Trichoplusia ni* (Hubner) (Behle *et al.* 1997).

The data in Table (5) clear that the presence of *S. littoralis* larvae before and after spry by the biocide compounds in the cotton field. The reduction rates in *S. littoralis* larval population after spray by Protecto, Verosit and Perovect at higher rate (2400 g/F) were 660.0 ± 36.05, 540.33±85.48 and 540.0±26.45 %, respectively after seven days compared with respective values of 1031.66±59.22, 880.0±26.45 and 1050.0±50.0% before spray. There were significance differences between different rates in different periods for biocides ($p > 0.05$). Salama *et al.* (1990) found that the effective threshold rate of application of Dipel 2X to control A. ipsilon on some vegetable crops was 250 g/F. Gadallah *et al.* (1990) reported that, the Dipel was the most toxic compound followed by SAN 415, Thuricide, and Florbac XLC as B.T.-biocides against *S. littoralis*.

Data in table (6) represents the reduction percent among *S. littoralis* larvae in cotton field treated by biocides. Means of residual activity of Protecto, Perovect and Verosit at the higher rate (2400 g/F) were 33.0, 30.5 and 28.0 %, respectively This results agree with pervious data recorded by (Barker 1998). He also reported that, B.T. toxin-caused delays in larval development could further increase mortality because of increased larval exposure to predators, parasites, disease, and adverse weather conditions.

The efficacy of *B. thuringiensis* was decreased when compared for other formulations of *B. thuringiensis* like Dipel 2X and MVP II, (Mohamed 1997). This may be because the *S. littoralis* larvae tolerate the toxicity of *B. thuringiensis.* Lower mortalities of *S. littoralis* by low concentration of *B. thuringiensis* can be explained by physiological defense mechanisms. For example, damaged midgut epithelial cells of the rice moth *Corcyra cephalonica* (Stainton) (Lep., Pyralidae)

larvae are replaced, and the newly made cells are protected from toxicity of B. *thuringiensis* subsp. *Kurstaki* by a quickly produced mucous layer covering their surface (Chiang *et al.*, 1986). Larvae of S. littoralis ingested high dose of ?-endotoxin, exhibited reduced toxicities in the presence of particles (Ben-Dov *et al.*, 2003), this may explain the lower mortality of higher rates (1200, 2400 g/F) after 3rd day for biocides.

Environmental conditions in the field adversely affect the insecticidal activity of *B. thuringiensis* (McGuire *et al.* 1994 and McGuire and Shasha,1995). This could be the explanation for the decreasing of the reduction percent of *S. littoralis* treated by biopesticide in the field.

Table (1). Bioactivity of some biocides against 2nd and 4th instars larvae of *S. littoralis* at zero time after spraying in cotton field.

Rate g/f	2nd instar								
	Protecto			Verosit			Perovect		
	LM%	PM%	AM%	LM%	PM%	AM%	LM%	PM%	AM%
150	13.3	0.0	0.0	10.0	0.0	0.0	7.5	5.0	0.0
300	32.5	0.0	0.0	30.0	0.0	0.0	25.0	0.0	5.0
600	53.3	0.0	0.0	40.0	0.0	5.0	45.0	0.0	0.0
1200	69.9	0.0	0.0	57.5	5.0	0.0	55.0	5.0	2.5
2400	86.6	0.0	0.0	92.5	0.0	0.0	80.0	5.0	0.0
Mean	51.12			46.0			42.5		
Rate g/f	4th instar								
	Protecto			Verosit			Perovect		
	LM%	PM%	AM%	LM%	PM%	AM%	LM%	PM%	AM%
150	12.5	0.0	2.5	12.5	0.0	0.0	10.0	5.0	0.0
300	30.0	0.0	00	27.5	0.0	0.0	25.0	0.0	0.0
600	35.0	0.0	0.0	35.0	2.5	0.0	35.0	0.0	2.5
1200	42.5	2.5	0.0	55.0	0.0	0.0	37.5	5.0	0.0
2400	62.5	0.0	0.0	70.0	0.0	2.5	65.0	5.0	0.0
Mean	36.5			40.0			34.5		

LM%= larval morality percent, PM%= pupal mortality percent,
AM%= adult mortality percent
L.S.D. at 0.05
For compounds = 3.21
For rates = 4.1
For interaction between instars X rates = 5.87

Table (2). Bioactivity of some biocides against 2nd and 4th instars larvae of *S. littoralis* at 3rd day after spraying in cotton field.

Rate g/f	2nd instar								
	Protecto			Verosit			Perovect		
	LM%	PM%	AM%	LM%	PM%	AM%	LM%	PM%	AM%
150	7.5	2.5	0.0	15.0	0.0	0.0	5.0	5.0	0.0
300	12.5	2.5	2.5	25.0	2.5	0.0	20.0	2.5	5.0
600	42.5	5.0	0.0	25.0	5.0	2.5	37.5	5.0	0.0
1200	50.0	5.0	2.50	42.5	10.5	0.0	50.0	5.0	5.0
2400	65.0	2.50	5.0	47.5	10.0	2.5	70.0	2.5	0.0
Mean	35.5			31.0			36.5		
Rate g/f	4th instar								
	Protecto			Verosit			Perovect		
	LM%	PM%	AM%	LM%	PM%	AM%	LM%	PM%	AM%
150	5.0	5.0	0.0	5.0	0.0	0.0	10.0	2.5	0.0
300	15.0	0.0	00	17.5	5.0	0.0	22.5	0.0	0.0
600	35.5	0.0	0.0	27.0	2.5	2.5	30.0	7.5	2.5
1200	30.0	7.50	2.5	40.0	7.5	0.0	50.0	0.0	0.0
2400	37.5	2.5	2.5	45.0	10.0	2.5	57.5	10.0	0.0
Mean	24.6			26.9			34.0		

L.S.D. at 0.05
For instars = 4.72
For compounds = 3.34
For rates = 4.31

Table (3). Bioactivity of some biocides against 2nd and 4th instars larvae of *S. littoralis* at 5th day after spraying in cotton field.

Rate g/f	2nd instar								
	Protecto			Verosit			Perovect		
	LM%	PM%	AM%	LM%	PM%	AM%	LM%	PM%	AM%
75	5.0	0.0	0.0	2.5	0.0	0.0	7.5	0.0	0.0
150	10.0	2.5	00	15.0	2.5	0.0	15.0	0.0	0.0
300	32.5	2.5	0.0	32.5	2.5	2.5	42.5	2.5	0.0
600	42.0	5.0	2.5	32.5	7.5	0.0	50.0	2.5	0.0
1200	52.5	2.5	5.0	42.5	2.5	2.5	57.5	5.0	0.0
Mean	28.4			25.0			34.5		
Rate g/f	4th instar								
	Protecto			Verosit			Perovect		
	LM%	PM%	AM%	LM%	PM%	AM%	LM%	PM%	AM%
75	2.5	0.0	2.5	2.5	0.0	2.5	5.0	0.0	0.0
150	10.0	0.0	0.0	10.0	2.5	0.0	7.5	0.0	2.5
300	32.5	2.5	0.0	17.5	5.0	2.5	25.0	0.0	2.5
600	45.0	2.5	0.0	20.0	7.5	0.0	27.5	5.0	0.0
1200	52.5	2.5	0.0	30.0	5.0	5.0	37.5	5.0	0.0
Mean	28.5			16.0			20.5		

L.S.D. at 0.05
For instars = 2.72
For compounds = 3.93
For interaction between instars and compounds = 3.58

Table (4). Bioactivity of some biocides against 2nd and 4th instars larvae of *S. littoralis* at 7th day after spraying in cotton field.

Rate G/f	2nd instar								
	Protecto			Verosit			Perovect		
	LM%	PM%	AM%	LM%	PM%	AM%	LM%	PM%	AM%
150	2.5	2.5	0.0	5.0	0.0	0.0	5.0	0.0	0.0
300	12.5	0.0	0.0	7.5	0.0	0.0	7.5	0.0	0.0
600	10.0	0.0	2.5	7.5	0.0	2.5	12.5	0.0	2.5
1200	15.0	0.0	2.5	7.5	0.0	0.0	15.0	2.5	0.0
2400	15.0	2.5	2.5	12.5	2.5	0.0	17.5	2.5	2.5
Mean	11.0			8.0			11.5		
Rate G/f	4th instar								
	Protecto			Verosit			Perovect		
	LM%	PM%	AM%	LM%	PM%	AM%	LM%	PM%	AM%
150	5.0	2.5	0.0	5.0	0.0	2.5	2.5	0.0	2.5
300	5.0	2.5	0.0	7.5	0.0	0.0	7.5	0.0	0.0
600	10.0	0.0	0.0	10.0	0.0	0.0	17.5	2.5	0.0
1200	12.5	0.0	0.0	10.0	2.5	2.5	15.0	0.0	2.5
2400	17.5	0.0	0.0	7.5	5.0	2.5	15.0	7.5	0.0
Mean	10.0			8.0			11.5		

L.S.D. at 0.05
For rates = 4.25

Table (5). The reduction of *S. littorals* larvae before and after spray by some Biocide compounds in cotton field.

Comp-ounds	Rate G/f	Before spray	After spray				Mean
			24h.	3d.	5d.	7d.	
Protecto	0.0	1083.3 ±76.37	1116.66 ±76.37	1158.31± 52.04	1416.66 ±76.37	1416.66± 104.08	**1277.07**
	150	883.33 ±28.86	850.0 ±10.0	843.33 ±5.77	841.0 ±3.6	723.3 ±64.29	**814.40**
	300	960 ±52.91	926.66 ±25.16	890.0 ±26.45	814.66 ±69.17	806.66 ±70.23	**859.49**
	600	913.33 ±15.27	870.0 ±20.0	846.66 ±40.41	823.33 ±25.16	746.66 ±37.85	**821.66**
	1200	1026.66 ±55.07	943.33 ±40.41	909.66 ±17.61	853.33 ±50.33	720.0 ±26.45	**856.58**
	2400	1031.66 ±59.23	947.66 ±35.16	898.33 ±10.40	736.66 ±55.07	660.0 ±36.05	**810.66**
	Mean	**962.99**	**904.53**	**877.59**	**813.79**	**731.33**	**832.52**
Verosit	150	853.33 ±55.07	1023.33 ±60.06	990.0 ±21.79	946.66 ±25.16	921.66 ±29.29	**970.41**
	300	853.33 ±55.07	853.33 ±55.07	850.0 ±50.0	816.0 ±15.27	796.66 ±15.27	**828.99**
	600	960.0 ±36.05	956.66 ±37.85	910.0 ±26.45	880.0 ±20.0	836.66 ±32.14	**895.83**
	1200	750.0 ±50.0	716.66 ±37.85	665.0 ±39.68	653.33 ±55.07	590.0 ±34.64	**656.24**
	2400	880.0 ±26.45	838.33 ±37.52	811.66 ±62.51	763.33 ±60.27	540.33 ±85.48	**738.41**
	Mean	**859.33**	**877.66**	**845.33**	**811.86**	**737.06**	**817.97**
Pervect	150	880.0 ±26.45	866.66 ±30.55	806.66 ±15.27	776.66 ±25.16	761.66 ±16.07	**807.91**
	300	900.0 ±100	910.0 ±36.05	893.33 ±40.41	853.33 ±40.41	793.33 ±11.54	**862.49**
	600	886.66 ±32.14	823.33 ±32.14	760.0 ±30.0	741.66 ±43.68	703.33 ±15.27	**757.08**
	1200	950.0 ±50.0	915.0 ±30.41	876.66 ±15.27	800.0 ±30.0	696.66 ±35.11	**822.08**
	2400	1050.0 ±50.0	960.0 ±52.91	813.33 ±11.54	763.33 ±15.27	540.0 ±26.45	**769.16**
	Mean	**933.33**	**894.99**	**830.59**	**786.99**	**698.99**	**803.74**

L.S.D. at 5% for: time investigation = 19.44, rates = 21.29,
Compounds = 15.05
Interactions between time investigation and rates = 47.62
Interactions between time investigation and compounds = 33.67
Interactions between rates and compounds = 36.88
Interactions between time investigation and rates and compounds = 82.48

Table (6) reduction percent of *S. littorals* larvae treated by some Biocide compounds in cotton field.

Compounds	Rate g/f	24h	3d	5d	7d	Means of residual activity
Protecto	150	7.0	14.0	28.0	39.0	22.0
	300	7.0	15.0	37.0	37.0	24.0
	600	8.0	15.0	32.0	39.0	23.5
	1200	12.0	19.0	37.0	47.0	28.75
	2400	12.0	20.0	47.0	53.0	33.0
Verosit	150	-15.0	-7.0	17.0	18.0	17.5
	300	3.0	8.0	28.0	30.0	17.25
	600	4.0	13.0	31.0	34.0	20.5
	1200	8.0	19.0	34.0	41.0	25.5
	2400	8.0	15.0	35.0	54	28.0
Perovect	150	5.0	16.0	34.0	35	22.5
	300	3.0	8.0	29.0	34.0	18.5
	600	11.0	21.0	37.0	40.0	27.25
	1200	7.0	15.0	37.0	45.0	26.0
	2400	12.0	29.0	43.0	38.0	30.5

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النشاط الحيوى لبعض المبيدات الحيوية علي دودة ورق القطن اسبودبترا ليتورالس الليليات : حرشفيات الأجنحة

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استخدمت فى هذه الدراسة ثلاث مركبات حيوية وهى البروتكتو (بسيلس ثيرونجنسس كورستاكى) ومركب فيروست (بوليهيدروس نووى) ومركب بيروفكت (فيرس + بكتريا) ضد يرقات العمر الثانى والعمر الرابع لدورة ورق القطن المصرية باستخدام الاختبارات الحقلية المعملية . تم تجريب خمسة معدلات من المركبات السابقة الذكر وتم تقدير كفاءة النشاط الابادى لهذه المركبات وكذلك الأثر المتبقي تحت الظروف الحقلية والظروف المعملية .

أثبتت النتائج أن نسب الموت كانت عالية في يرقات العمر الثانى مقارنة بنسب الموت في يرقات العمر اليرقى الرابع وذلك بعد الرش مباشرة ، وكانت نسب الموت فى العمر الثاني كالتالى ٨٦,٦ ، ٩٢,٥ ، ٨٠,٠ ٪ لكل من البروتكتو والفيروست والبيروفيكت على التوالى أم نسب الموت ليرقات العمر اليرقى الرابع كانت كالتالي : ٦٢,٥ ، ٧٠، ٠ ,٦٥ علي التوالي وذلك فى المعدل الأعلي (١٢٠٠ جم / فدان) . بعد اليوم الثالث من الرش سجلت نسب الموت في العمر اليرقي الثانى لكلا من المركبات السابقة كالتالي : ٧٠,٠، ٤٧,٥ ٠ ,٦٥٪ والعمر اليرقي الرابع كما يلى :٥٧,٥ ، ٤٥,٠ ، ٣٧,٥ ٪ على التوالي . سجلت أقل معدلات للموت للمركبات المختبرة بعد سبعة أيام من الرش ضد كلا من يرقات العمر الثانى والرابع .

أوضحت النتائج أن هناك اختلافات معنوية بين المعدلات المختلفة المستخدمة والأعمار اليرقية وكذلك بين أوقات أخذ العينات المختلفة لكل المركبات المستخدمة . وأوضحت النتائج الحقلية ان اعلى معدل للموت بالنسبة لليرقات كانت بعد سبعة أيام من الرش فى المعدل الأعلى (٢٤٠٠ جم / الفدان) وكانت كما يلي ٥٤,٠ ، ٥٣,٠ ، ٣٤,٠ ٪ لكل من البروتكتو والفيروست والبيروفكت .