

Virulence of entomopathogenic fungi (spore suspension) against 2nd Instar larvae of *Spodoptera littoralis*:-

For studying the toxicity of entomopathogenic fungi on the cotton leaf worm, leaf dipping technique was used. Aqueous suspension of entomopathogenic fungi were tested.

Data in table (1, 2, 3, 4, 5) show that the mortality percentage in the 2nd instar larvae of *S. littoralis* increased with increasing the time elapsed after treatment.

For *Metarhizium anisopliae*, data in table(1) and fig(1) show that the mortality percentage after two days of treatment was 2.60, 1.05, 4.16, 12.64, 19.38 % at the concentrations 2.8×10^5 , 2.8×10^6 , 2.8×10^7 , 2.8×10^8 , 2.8×10^9 spore / ml respectively. After four days post treatment it was 7.46, 6.60, 12.83, 26.35, 33.43 %, as well as after six days it was 12.52, 14.98, 21.64, 36.92, 43.14 % for the same concentrations, respectively. After eight days mortality percentages were 17.32, 24.06, 29.59, 45.15, 50.32 %, while after ten days it was 21.76, 32.77, 36.56, 51.70, 55.90 % at concentrations 2.8×10^5 , 2.8×10^6 , 2.8×10^7 , 2.8×10^8 , 2.8×10^9 spore / ml respectively. After twelve days mortality percentage increased to be 25.83, 40.70, 42.61, 57.02, 60.38 % for the same concentrations mentioned above respectively.

Table (1): Cumulative corrected mortality percentage of *S. littoralis* 2nd Instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *M. anisopliae* at different periods.

Conc (Spore/ml)	Cumulative mortality % indicated days after treatment					
	2	4	6	8	10	12
2.8×10^9	19.38	33.43	43.14	50.32	55.90	60.38
2.8×10^8	12.64	26.35	36.92	45.15	51.70	57.02
2.8×10^7	4.16	12.83	21.64	29.59	36.56	42.61
2.8×10^6	1.05	6.60	14.98	24.06	32.77	40.70
2.8×10^5	2.60	7.46	12.52	17.32	21.76	25.83

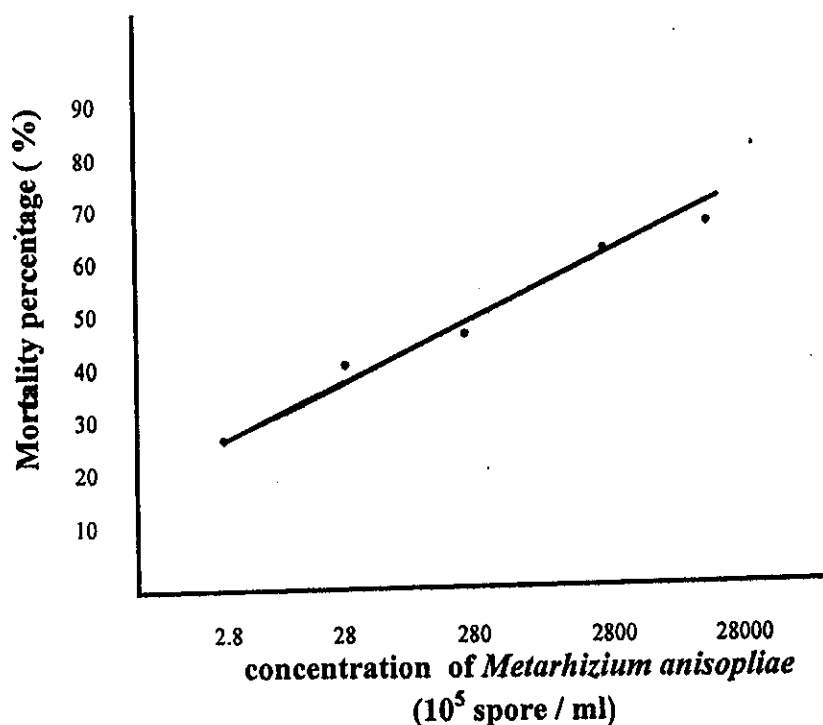


Fig. (1) : Toxicity regression line of *M. anisopliae* against 2nd instar larvae of *S. Littoralis* after 12 day of inoculation

Regarding *Metarhizium flavovrdiae*, data in table (2) and fig (2) show that the mortality percentage after two days of treatment was 10.46, 9.91, 4.31, 15.73, 12.58 % for the concentrations 2.7×10^5 , 2.7×10^6 , 2.7×10^7 , 2.7×10^8 , 2.7×10^9 spore / ml respectively. Through the period from the 4th to the 12th day, the mortality percentage increased with increasing the time elapsed post treatment. For example, mortality percentage was ranged between 20.20 - 26.10 % after 4 days post treatment, while it ranged between 27.83 - 36.54 %, 33.99 - 44.67 %, 39.09 - 51.16 % and 43.40-56.44 % after 6, 8, 10 and 12 days post treatment, respectively.

Table (2): Cumulative corrected mortality percentage of *S. littoralis* 2nd instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *M. flavovrdiae* at different periods.

Conc (Spore/ml)	Cumulative mortality % indicated days after treatment					
	2	4	6	8	10	12
2.7×10^9	12.58	26.10	36.54	44.67	51.16	56.44
2.7×10^8	15.73	26.58	34.33	40.29	45.08	49.06
2.7×10^7	4.31	14.22	24.42	33.54	41.41	48.11
2.7×10^6	9.91	20.63	29.26	36.26	42.03	46.89
2.7×10^5	10.46	20.20	27.83	33.99	39.99	43.40

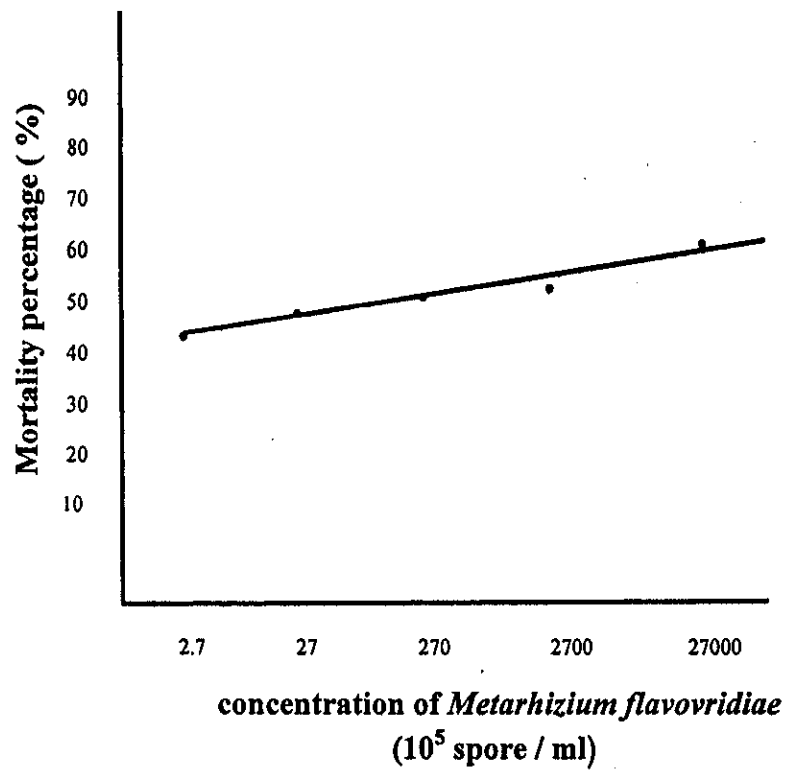


Fig. (2) : Toxicity regression line of *M. flavoviridae* against 2nd instar larvae of *S. Littoralis* after 12 day of inoculation

Taking into account the mortality percentage of *Pacilomyces farinosus* (Table: 3 and Fig: 3) after two days of treatment was 14.09, 13.60, 15.21, 5.75, 3.07 % for the concentrations 0.3×10^5 , 0.3×10^6 , 0.3×10^7 , 0.3×10^8 , 0.3×10^9 spore / ml respectively.

After four days post treatment it was 21.46, 21.31, 23.85, 15.76, 13.12 %, while it was 26.66, 26.81, 29.94, 25.16, 24.75 % after six days post treatment at the same concentrations respectively.

There were slight difference in mortality percentage was pronounced between the three highest tested concentrations after 8, 10 and 12 days of treatment. Also, at two lower concentrations, mortality percentage was almost the same after 8, 10 and 12 days.

Table (3): Cummulative corrected mortality percentage of *S. littoralis* 2nd instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *P. farinosus* at different periods.

Conc (Spore/ml)	Cummulative mortality % indicated days after treatment					
	2	4	6	8	10	12
0.3×10^9	3.07	13.12	24.75	35.53	44.84	52.69
0.3×10^8	5.75	15.76	25.16	33.29	40.21	46.13
0.3×10^7	15.21	23.85	29.94	34.65	38.47	41.69
0.3×10^6	13.60	21.31	26.81	31.10	34.62	37.60
0.3×10^5	14.09	21.46	26.66	30.70	34.00	36.80

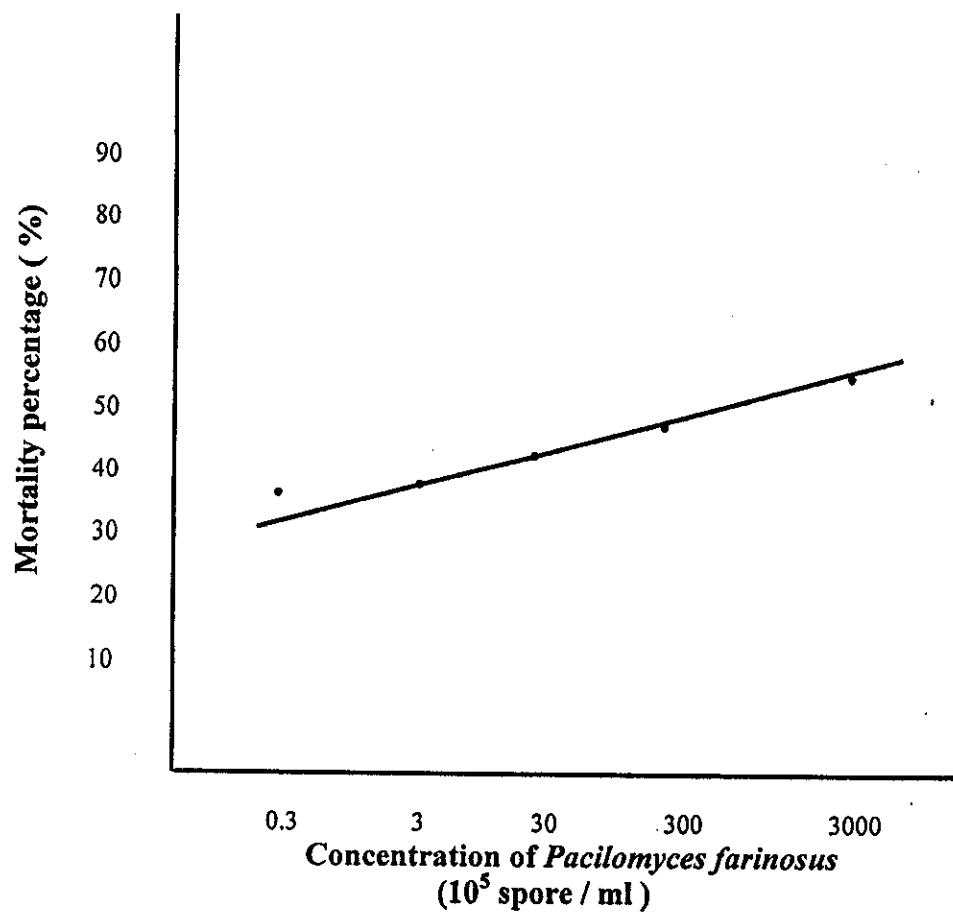


Fig. (3): Toxicity regression line of *P. farinosus* against 2nd instar larvae of *S. littoralis* after 12 day of inoculation

As for *Beauveria bassiana* (Table: 4 and Fig: 4) the mortality percentage increased with increasing the time elapsed after treatment at different concentrations. After two days, mortality percentages were almost lower than 10 %, then gradual increase in mortality percentage appeared at different time intervals. Mortality percentage ranged between 23.57-51.37 % after 12 days post treatment.

Generally, positive correlation was obtained between mortality percentage and tested concentrations.

Table(4): Cumulative corrected mortality percentage of *S. littoralis* 2nd instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *B. Bassiana* at different periods.

Conc (Spore/ml)	Cumulative mortality % indicated days after treatment					
	2	4	6	8	10	12
1.6×10^9	10.54	22.54	32.17	39.88	46.16	51.37
1.6×10^8	8.15	18.70	27.65	35.07	41.27	46.51
1.6×10^7	5.75	13.22	19.85	25.59	30.57	34.94
1.6×10^6	6.23	12.01	16.78	20.81	24.29	27.36
1.6×10^5	3.51	8.25	12.68	16.68	20.30	23.57

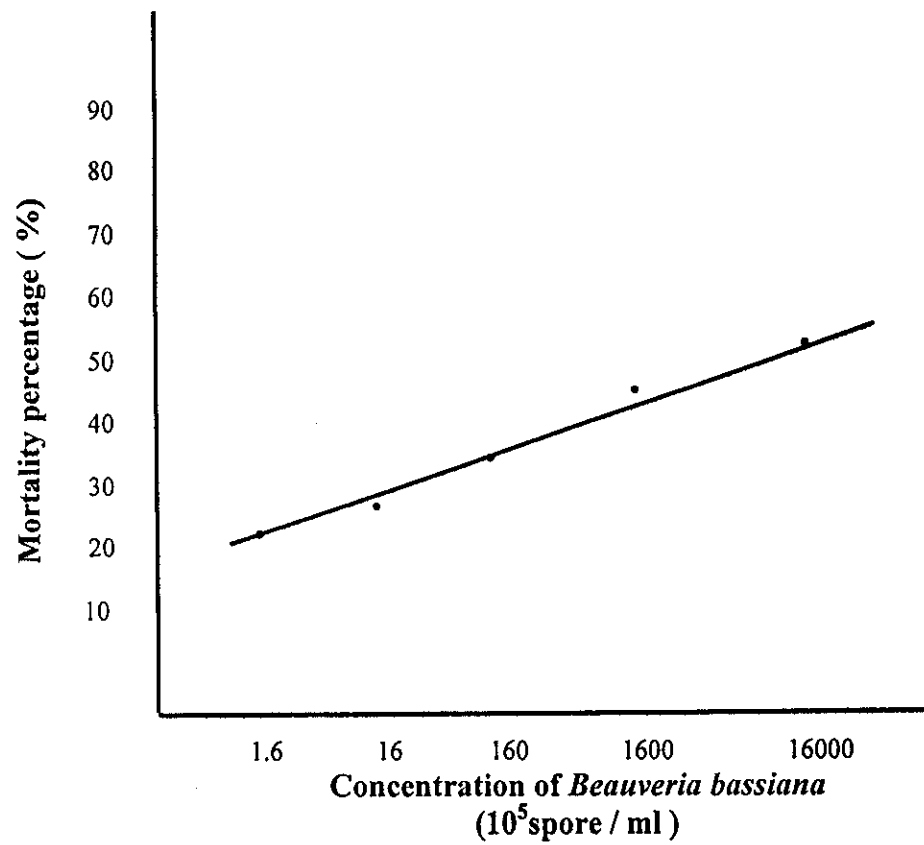


Fig. (4): Toxicity regression line of *B.bassiana* against 2nd instar larvae of *S. littoralis* after 12 day of inoculation

In addition to the mortality percentage of *Beauveria brongniartti* (Table: 5 and Fig: 5) after tow days post treatment was 5.98, 10.70, 9.36, 9.35, 11.69 % for the concentrations 0.9×10^5 , 0.9×10^6 , 0.9×10^7 , 0.9×10^8 , 0.9×10^9 spore / ml respectively.

The mortality percentage ranged between 13.42-23.14 % after 4 days post treatment, while it ranged between 19.95-32.02 %, 25.56-39.06 %, 30.43-44.79 % and 34.69-49.57 % after 6, 8, 10 and 12 days, respectively.

Table (5): Cumulative corrected mortality percentage of *S. littoralis* 2nd instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *B. brongniartti* at different periods.

Conc (Spore/ml)	Cumulative mortality % indicated days after treatment					
	2	4	6	8	10	12
0.9×10^9	11.69	23.14	32.02	39.06	44.79	49.57
0.9×10^8	9.35	19.42	27.59	34.26	39.82	44.52
0.9×10^7	9.36	20.44	29.53	36.94	43.06	48.20
0.9×10^6	10.70	16.88	21.40	24.99	27.98	30.55
0.9×10^5	5.98	13.42	19.95	25.56	30.43	34.69

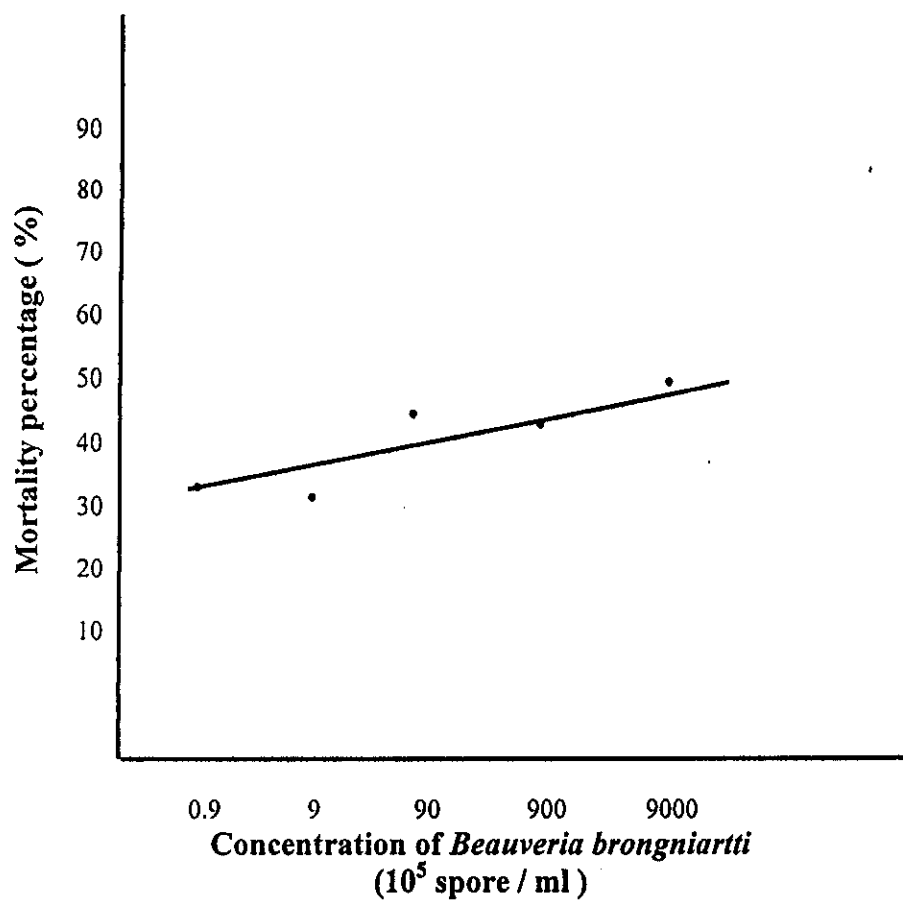


Fig. (5): Toxicity regression line of *B. brongniartii* against 2nd instar larvae of *S. littoralis* after 12 day of inoculation

Generally *M. anisopliae* was found to be the most effective isolate against 2nd instar larvae of *S. littoralis* followed by *M. flavoviridae*, *P. farinosus*, *B. bassiana* then *B. brongniartti* respectively.

Probit analysis show that LT₅₀ values for *M. anisopliae* (Table: 6 and Fig: 6) were 29.4, 14.7, 14.8, 9.4 and 7.8 days for the concentrations 2.8×10^5 , 2.8×10^6 , 2.8×10^7 , 2.8×10^8 , 2.8×10^9 spore / ml respectively, as for *M. flavoviridae* (Table:7 and Fig: 6) they were 15.7, 13.4, 12.6, 12.5, 9.4 days for the concentrations 2.7×10^5 , 2.7×10^6 , 2.7×10^7 , 2.7×10^8 , 2.7×10^9 spore / ml respectively.

LT₅₀ values for *P. farinosus* (Table:8 and Fig: 6) ranged between 11-27days for the concentrations 0.3×10^5 , 0.3×10^6 , 0.3×10^7 , 0.3×10^8 , 0.3×10^9 spore / ml respectively, Regarding *B. bassiana* (Table: 9 and Fig: 6) LT₅₀ values ranged between 11-39 days for the concentrations 1.6×10^5 , 1.6×10^6 , 1.6×10^7 , 1.6×10^8 , 1.6×10^9 spore / ml respectively, In addition to LT₅₀ values for *B. brongniartti* (Table:10 and Fig: 6) ranged between 12-22 days for the concentrations 0.9×10^5 , 0.9×10^6 , 0.9×10^7 , 0.9×10^8 , 0.9×10^9 spore / ml respectively.

Table (6): Virulence of *M. anisopliae* against the 2nd instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (Spore/ml)	LT ₅₀ (days)	95% (fiducial limit)		a	b
		Lower	Upper	Intercept	Slope
2.8 x 10 ⁹	7.8979	6.5692	10.0032	3.6998±0.1974	1.4487±0.2361
2.8 x 10 ⁸	9.4389	7.9628	11.9671	3.3457±0.2128	1.6969±0.2504
2.8 x 10 ⁷	14.8895	12.0226	21.2926	2.6691±0.2694	1.9873±0.3046
2.8 x 10 ⁶	14.7035	12.3502	19.5465	1.8889±0.3566	2.6649±0.3889
2.8 x 10 ⁵	29.4444	19.0676	79.2048	2.5560±0.3111	1.6637±0.3475

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (7): Virulence of *M. flavoviridae* against the 2nd instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (Spore/ml)	LT ₅₀ (days)	95% (fiducial limit)		a	B
		Lower	Upper	Intercept	Slope
2.7 x 10 ⁹	9.6105	8.0827	12.2788	3.3473±0.2130	1.6817±0.2506
2.7 x 10 ⁸	12.5292	9.6313	20.3381	3.6146±0.2052	1.2618±0.2428
2.7 x 10 ⁷	12.6250	10.6192	16.4557	2.6383±0.2651	2.1446±0.3004
2.7 x 10 ⁶	13.4714	10.6444	20.2115	3.2455±0.2243	1.5535±0.2611
2.7 x 10 ⁵	15.7700	11.8163	27.3581	3.3225±0.2227	1.4005±0.2597

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (8): Virulence of *P. farinosus* against the 2nd instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (Spore/ml)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
0.3 x 10 ⁹	11.2745	9.8198	13.7023	2.3796±0.2819	2.4906±0.3169
0.3 x 10 ⁸	13.5004	11.0328	18.7181	2.8512±0.2508	1.9010±0.2864
0.3 x 10 ⁷	19.0080	12.6708	50.6428	3.6562±0.2075	1.0507±0.2454
0.3 x 10 ⁶	24.7545	15.0539	94.6192	3.5991±0.2128	1.0052±0.2509
0.3 x 10 ⁵	27.1628	15.7402	132.6001	3.6375±0.2116	0.9501±0.2499

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (9): Virulence of *B. bassiana* against the 2nd instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (Spore/ml)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
1.6 x 10 ⁹	11.4376	9.3901	15.5778	3.2510±0.2208	1.6526±0.2578
1.6 x 10 ⁸	13.5283	10.8311	19.6460	3.0994±0.2333	1.6801±0.2697
1.6 x 10 ⁷	21.5037	15.1660	43.9351	2.9643±0.2550	1.5277±0.2916
1.6 x 10 ⁶	38.0946	21.0357	190.9748	3.1034±0.2547	1.1997±0.2931
1.6 x 10 ⁵	39.1842	22.1402	174.8575	2.7678±0.2931	1.4012±0.3310

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (10): Virulence of *B. brongniartii* against the 2nd instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (Spore/ml)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
0.9 x 10 ⁹	12.1999	9.7679	17.6746	3.3530±0.2168	1.5161±0.2539
0.9 x 10 ⁸	14.7900	11.4226	23.5566	3.2232±0.2274	1.5187±0.2642
0.9 x 10 ⁷	12.7867	10.2948	18.2983	3.1886±0.2266	1.6367±0.2632
0.9 x 10 ⁶	41.5471	20.6212	411.9550	3.4732±0.2255	0.9433±0.2643
0.9 x 10 ⁵	22.0220	15.3566	46.5762	2.9942±0.2530	1.4937±0.2897

LT₅₀ : Lethal time (time required to kill 50% of larvae).

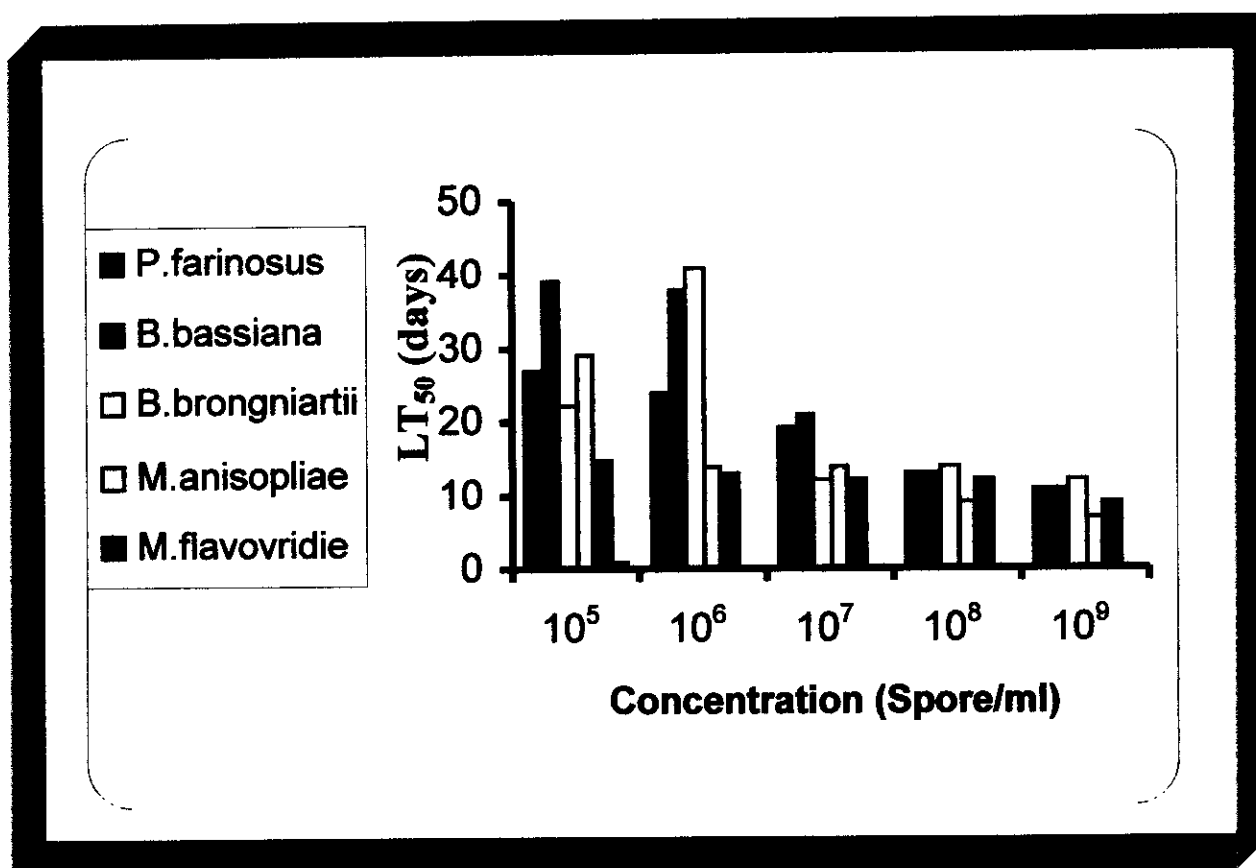


Fig (6): Relationship between different concentrations of the five tested fungi and LT₅₀ values (days)

Virulence of entomopathogenic fungi (spore suspension) against 4th instar larvae of *S. littoralis*:-

Data in table (11, 12, 13, 14, 15) show that the mortality percentage in the 4th instar larvae of *S. littoralis* increased with increasing the time elapsed after treatment.

For *M. anisopliae* data in table (11) and fig (7) show that the mortality percentage after four days of treatment was 4.25, 6.85, 7.33, 7.74, 7.13 % for the concentrations 2.8×10^5 , 2.8×10^6 , 2.8×10^7 , 2.8×10^8 , 2.8×10^9 spore / ml respectively. At the three highest tested concentrations, the mortality percentage was almost the same after 4 days post treatment but there were slight difference in mortality percentage between the two lower concentrations after 4 days. The mortality percentage ranged between 8.06 – 18.23 %, 12.04 – 30.52 %, 12.04 – 30.52 % and 19.71 – 55.00 % after 6, 8 and 10 days.

Table(11):Cummulative corrected mortality percentage of *S. littoralis* 4th instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *M. anisopliae* at different periods.

Conc (Spore/ml)	Cummulative mortality % indicated days after treatment			
	4	6	8	10
2.8×10^9	7.13	18.23	30.52	55.00
2.8×10^8	7.74	13.89	19.88	30.57
2.8×10^7	7.33	11.97	16.33	24.04
2.8×10^6	6.85	11.43	15.79	23.58
2.8×10^5	4.25	8.06	12.04	19.71

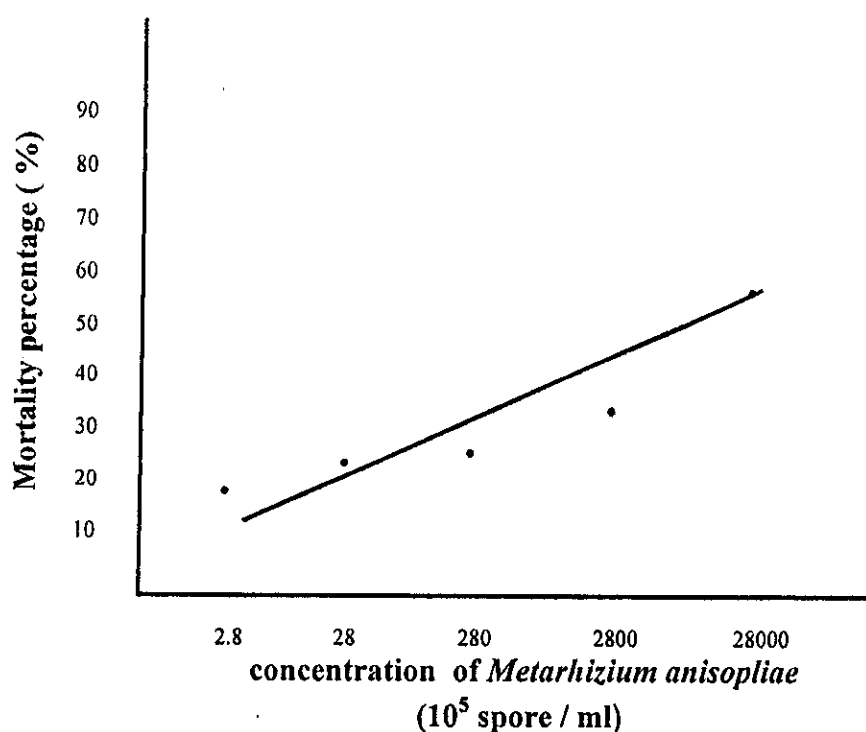


Fig. (7) : Toxicity regression line of *M. anisopliae* against 4th instar larvae of *S. Littoralis* after 10 days of inoculation

As for *M. flavovrdiae* (Table: 12 and Fig: 8) there were positive correlation between mortality percentage and time elapsed post treatment was pronounced through the period from 4th to 10th days. The mortality percentage ranged between 4.53 - 10.71 %, 10.91 - 23.04 %, 18.30 – 35.22 % and 32.89 – 54.98 % after 4, 6, 8 and 10 days at different concentrations.

Table(12):Cummulative corrected mortality percentage of *S. littoralis* 4th instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *M. Flavovrdiae* at different periods.

Conc (Spore/ml)	Cummulative mortality % indicated days after treatment			
	4	6	8	10
2.7×10^9	10.71	23.04	35.22	54.98
2.7×10^8	4.27	13.79	26.04	49.54
2.7×10^7	6.31	14.48	23.17	39.45
2.7×10^6	1.87	7.12	15.12	33.82
2.7×10^5	4.53	10.91	18.30	32.89

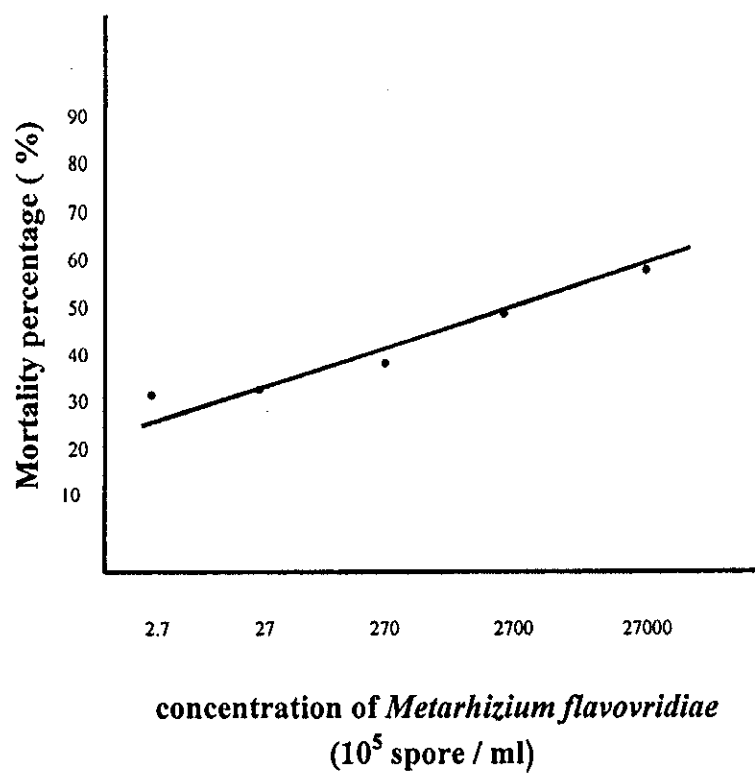


Fig. (8) : Toxicity regression line of *M. flavoviridae* against 4th instar larvae of *S. Littoralis* after 10 days of inoculation

Taking into consideration, the mortality percentage of *P. farinosus* as shown in table (13) and fig (9) after four days post treatment was 20.45, 24.70, 25.96 22.54, 27.36 % for the concentrations 0.3×10^5 , 0.3×10^6 , 0.3×10^7 , 0.3×10^8 , 0.3×10^9 spore / ml respectively.

The mortality percentage was almost the same at the four lower tested concentrations to be ranged between 25.97 - 28.95 %, 30.31 - 33.96 % and 36.91 - 41.50 % after 6, 8 and 10 days but it was more than 50 % at the higher concentration after 10 days.

Table(13):Cummulative corrected mortality percentage of *S. littoralis* 4th instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *P. farinosus* at different periods.

Conc (Spore/ml)	Cummulative mortality % indicated days after treatment			
	4	6	8	10
0.3×10^9	27.36	36.77	44.01	54.51
0.3×10^8	22.54	28.95	33.96	41.50
0.3×10^7	25.96	30.98	34.79	40.41
0.3×10^6	24.70	29.86	33.80	39.65
0.3×10^5	20.45	25.97	30.31	36.91

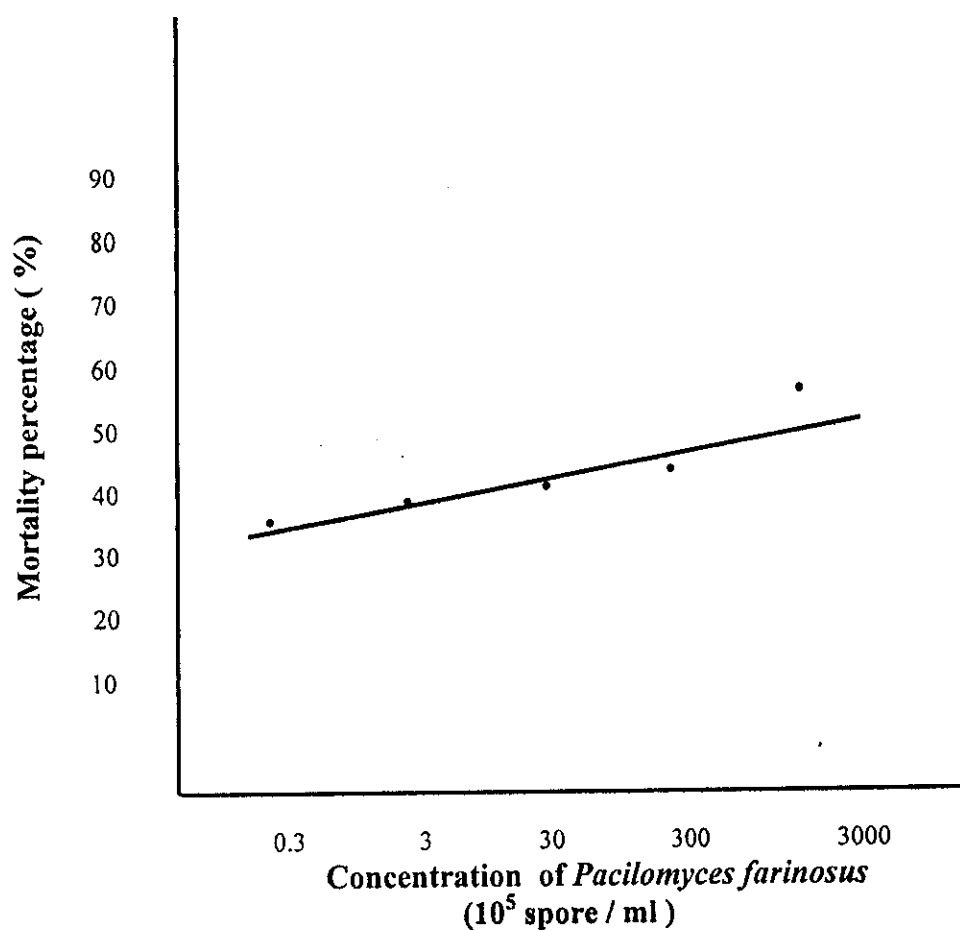


Fig. (9): Toxicity regression line of *P. farinosus* against 4th instar larvae of *S. littoralis* after 10 days of inoculation

Regarding *B. bassiana*, (Table: 14 and Fig: 10) the mortality percentage was lower than 10 % after 4 days then increased with increasing the time elapsed post treatment at different concentrations to be ranged between 5.05 - 18.95 %, 8.66 - 29.67 % and 16.59 - 48.15 % after 6, 8 and 10 days.

Table(14):Cummulative corrected mortality percentage of *S. littoralis* 4th instar larvae after feeding on treated castor bean leaves immersed in different concentration of *B. bassiana* at different periods.

Conc (Spore/ml)	Cummulative mortality % indicated days after treatment			
	4	6	8	10
1.6×10^9	8.58	18.95	29.67	48.15
1.6×10^8	5.17	13.35	22.90	41.15
1.6×10^7	3.58	9.04	15.62	29.22
1.6×10^6	4.35	9.36	14.91	25.85
1.6×10^5	2.11	5.05	8.66	16.59

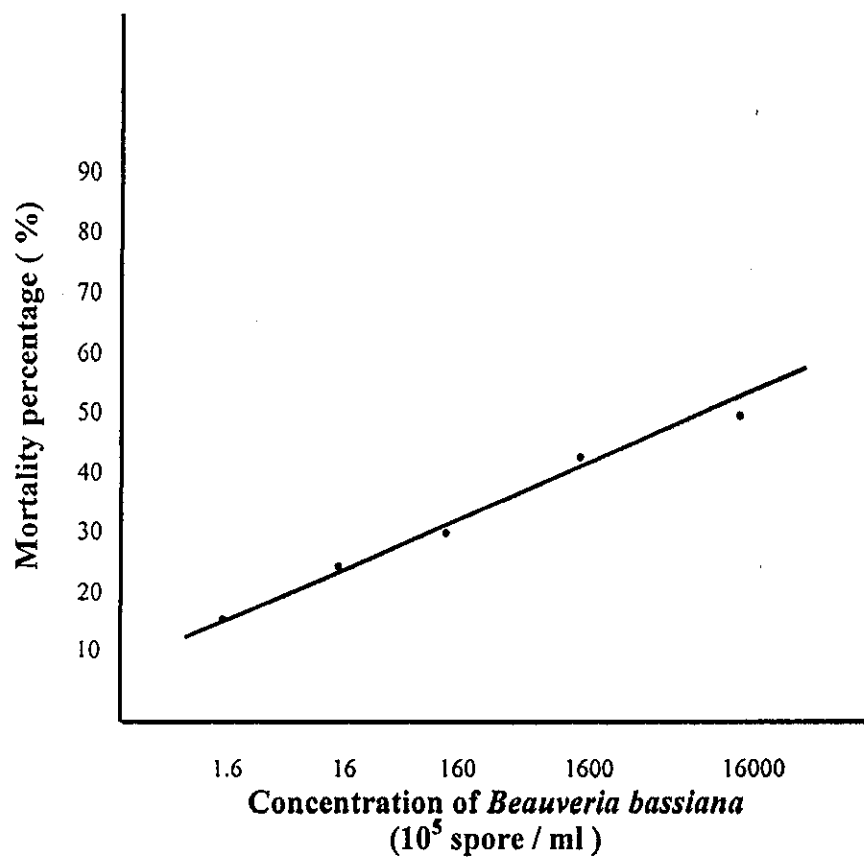


Fig. (10): Toxicity regression line of *B. bassiana* against 4th instar larvae of *S. littoralis* after 10 days of inoculation

In addition to the mortality percentage of *B. brongniartii* (Table: 15 and Fig: 11) ranged between 7.40 - 9.17 %, 12.95 - 20.14 %, 18.33 - 31.33 % and 27.93 - 50.29 % after 4, 6, 8 and 10 days.

There were similarity in the mortality percentage between the two highest tested concentrations was noticed after 4, 6, 8 and 10 days, but there were difference in mortality percentage at the three lower tested concentrations after the same days.

Table(15):Cummulative corrected mortality percentage of *S. littoralis* 4th instar larvae after feeding on treated castor bean leaves immersed in different concentration of *B. brongniartii* at different periods.

Conc (Spore/ml)	Cummulative mortality % indicated days after treatment			
	4	6	8	10
0.9×10^9	9.17	20.14	31.33	50.29
0.9×10^8	10.14	20.82	31.35	48.99
0.9×10^7	18.29	25.26	30.94	39.76
0.9×10^6	7.48	14.16	20.84	32.84
0.9×10^5	7.40	12.95	18.33	27.93

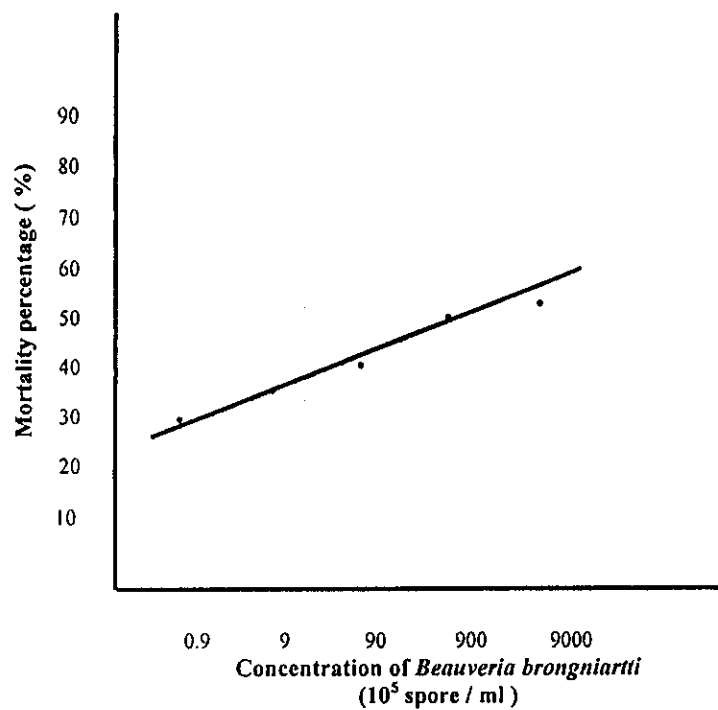


Fig. (11): Toxicity regression line of *B. brongniartii* against 4th instar larvae of *S. littoralis* after 10 days of inoculation

Generally *M. anisopliae* was found to be the most effective isolate against 4th instar larvae of *S. littoralis* followed by *M. flavovridae*, *P. farinosus*, *B. brongniartti* then *B. bassiana* respectively.

Data in table 16,17,18,19&20 and fig 12 show that LT₅₀ values for *M. anisopliae* (table: 16 and Fig: 12) were 35.1, 33.8, 33.6, 22.0, 10.5 days for the concentrations 2.8×10^5 , 2.8×10^6 , 2.8×10^7 , 2.8×10^8 , 2.8×10^9 spore / ml respectively, while LT₅₀ values for *M. flavovridae* (Table: 17 and Fig: 12) were 17.7, 15.8, 15.1, 12.0, 10.8 days for the concentrations 2.7×10^5 , 2.7×10^6 , 2.7×10^7 , 2.7×10^8 , 2.7×10^9 spore / ml respectively.

LT₅₀ values for *P. farinosus* (Table: 18 and Fig: 12) ranged between 10 – 25 days for the concentrations 0.3×10^5 , 0.3×10^6 , 0.3×10^7 , 0.3×10^8 , 0.3×10^9 spore / ml respectively, while they ranged between 12 – 32 days for *B. bassiana* (Table: 19 and Fig: 12) at the concentrations 1.6×10^5 , 1.6×10^6 , 1.6×10^7 , 1.6×10^8 , 1.6×10^9 spore / ml respectively, in addition to *B. brongniartti* (Table: 20 and Fig: 12) they ranged between 11 - 25 days for the concentrations 0.9×10^5 , 0.9×10^6 , 0.9×10^7 , 0.9×10^8 , 0.9×10^9 spore / ml respectively .

Table (16): Virulence of *M. anisopliae* against the 4th instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (Spore/ml)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
2.8 x 10 ⁹	10.5713	9.8782	15.5046	1.6204±0.6086	3.1782±0.6567
2.8 x 10 ⁸	22.0846	14.0949	198.5495	2.4223±0.6164	1.9178±0.6680
2.8 x 10 ⁷	33.6309	16.8230	1.13197E+05	2.5443±0.6434	1.6085±0.6978
2.8 x 10 ⁶	33.8808	16.7658	2.80580E+05	2.6060±0.6337	1.5648±0.6884
2.8 x 10 ⁵	35.1764	17.6372	46649.8689	2.1794±0.7220	1.8242±0.7745

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (17): Virulence of *M. flavoviridae* against the 4th instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (Spore/ml)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
2.7 x 10 ⁹	10.8517	9.2310	14.5449	2.0327±0.5643	2.8556±0.6156
2.7 x 10 ⁸	12.0883	10.3918	15.9992	1.1227±0.6727	3.5822±0.7165
2.7 x 10 ⁷	15.1504	11.8061	29.2944	1.8791±0.6314	2.6439±0.6788
2.7 x 10 ⁶	15.8098	12.6345	27.9566	0.8198±0.8171	3.4866±0.8537
2.7 x 10 ⁵	17.7112	13.0834	45.0068	1.7303±0.6834	2.6195±0.7292

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (18): Virulence *P. farinosus* of against the 4th instar larvae of *S. littoralis* expressed as LT₅₀ values(days).

Conc (Spore/ml)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
0.3 x 10 ⁹	10.9828	7.6834	23.8274	3.4952±0.4884	1.4995±0.5454
0.3 x 10 ⁸	18.5796	11.0913	1.26083E+14	3.5655±0.5033	1.1304±0.5599
0.3 x 10 ⁷	23.2870	N.D*	N.D*	3.8479±0.4942	0.8427±0.5517
0.3 x 10 ⁶	23.7761	N.D*	N.D*	3.7843±0.4978	0.8834±
0.3 x 10 ⁵	25.3296	N.D*	N.D*	3.5542±0.5129	1.0300±0.5698

* Not detected

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (19): Virulence *B. bassiana* of against the 4th instar larvae of *S. littoralis* expressed as LT₅₀ values(days).

Conc (Spore/ml)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
1.6 x 10 ⁹	12.4734	10.2962	18.8368	1.9668±0.5895	2.7676±0.6388
1.6 x 10 ⁸	14.2940	11.5154	23.8658	1.5985±0.6551	2.9446±0.7006
1.6 x 10 ⁷	19.3750	13.8363	60.5636	1.6154±0.7243	2.6294±0.7689
1.6 x 10 ⁶	23.4380	15.0097	178.6775	1.9466±0.7023	2.2289±0.7505
1.6 x 10 ⁵	32.7771	17.5840	6240.8546	1.6300±0.8566	2.2236±0.9037

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (20): Virulence *B. brongniartii* of against the 4th instar larvae of *S. littoralis* expressed as LT₅₀ values(days).

Conc (Spore/ml)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
0.9×10^9	11.9296	9.9565	17.2461	1.9816 ± 0.5815	2.8036 ± 0.6314
0.9×10^8	12.2710	10.0789	18.8841	2.1517 ± 0.5713	2.6158 ± 0.6219
0.9×10^7	18.6705	11.7030	1716.1181	3.2816 ± 0.5203	1.3518 ± 0.5758
0.9×10^6	19.5847	13.3698	89.2160	2.3017 ± 0.6176	2.0886 ± 0.6681
0.9×10^5	25.2992	14.9925	627.0007	2.4662 ± 0.6256	1.8058 ± 0.6781

LT₅₀ : Lethal time (time required to kill 50% of larvae).

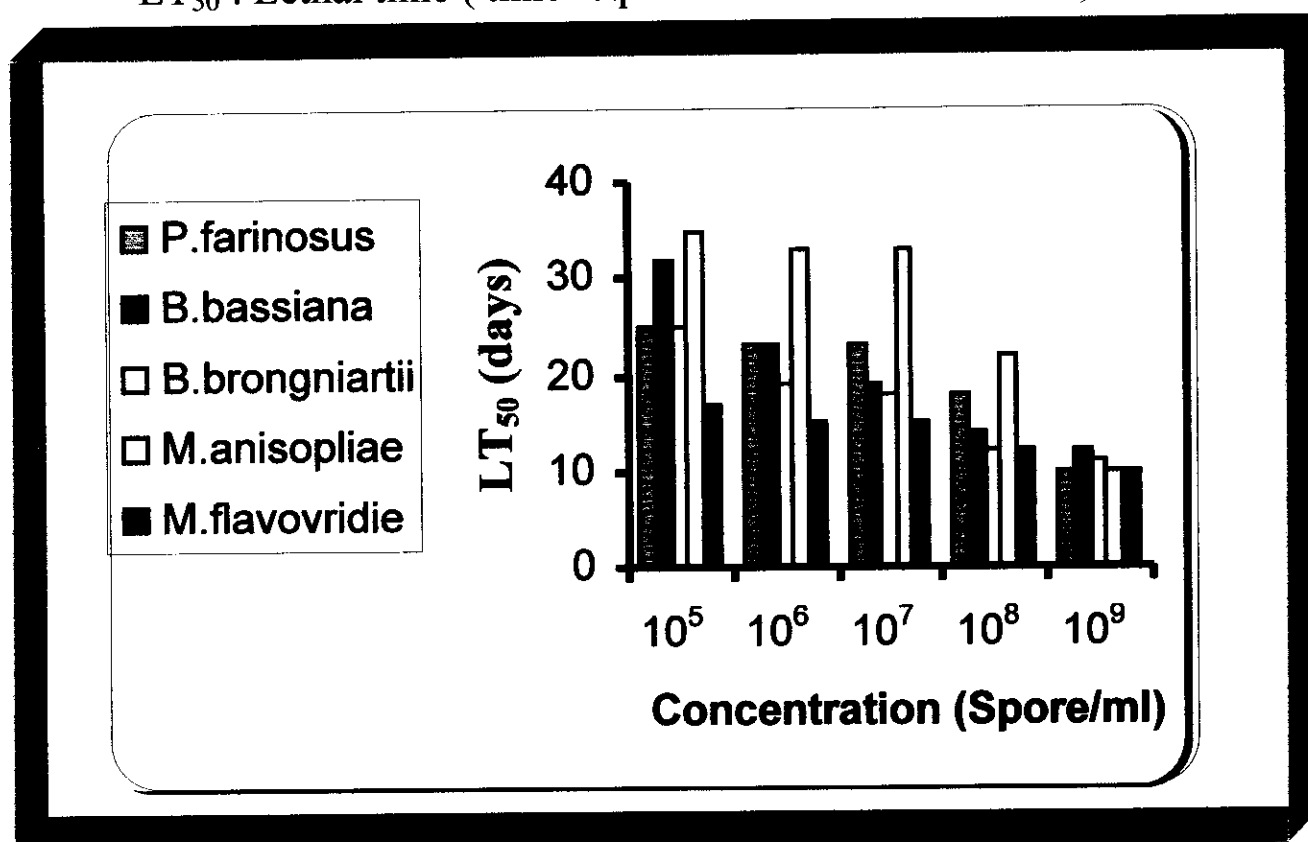


Fig (12): Relationship between different concentrations of the five tested fungi and Lt₅₀ values (days)

Virulence of entomopathogenic fungi (Metabolites) against 2nd instar larvae of *S. littoralis*:-

For *M. anisopliae* (Table: 21 and Fig: 13) the mortality percentage after two days of treatment was 8.81, 8.07, 7.87, 6.81, 6.28 % for the concentrations 100, 75, 50, 25, 10 % respectively.

After four days post treatment it was 18.47, 16.07, 14.49, 13.09, 12.23 %, then ranged between 17.16 – 26.38 %, 21.33 – 32.88 %, 24.94 – 45.33 % and 28.12 – 58.97 % after 6, 8, 10 and 12 days for the same concentration mentioned above respectively. There were similarity in the mortality percentage between the two lower tested concentrations after 2, 4, 6, 8, 10 and 12 days.

Table(21):Cummulative corrected mortality percentage of *S. littoralis* 2nd instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *M. anisopliae* at different periods.

Conc (%)	Cummulative mortality % indicated days after treatment					
	2	4	6	8	10	12
100	8.81	18.47	26.38	32.88	45.33	58.97
75	8.07	16.07	22.60	28.02	32.61	36.58
50	7.87	14.49	19.76	24.11	27.82	31.04
25	6.81	13.09	18.21	22.52	26.23	29.47
10	6.28	12.23	17.16	21.33	24.94	28.12

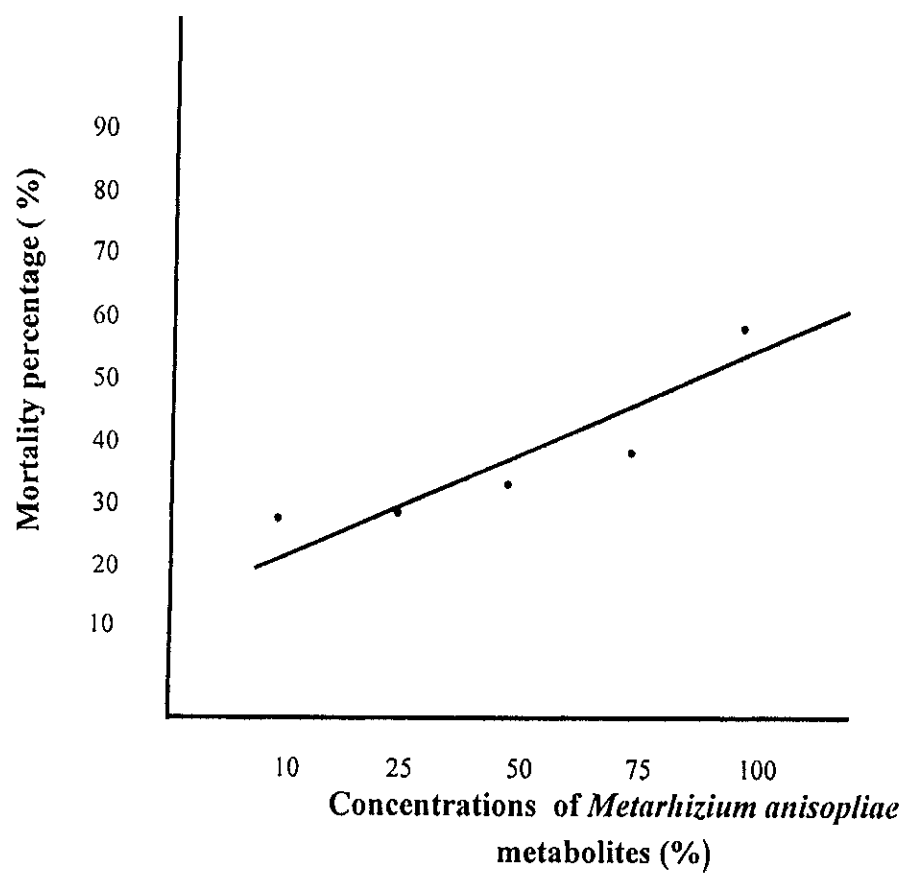


Fig. (13): Toxicity regression line of *M. anisopliae* against 2nd instar larvae of *S. littoralis* after 12 day of inoculation

Regarding *M. flavovrdiae* (Table: 22 and Fig: 14) the mortality percentage after two days of treatment was 23.18, 19.06, 16.73, 15.29, 14.84 % for the concentrations 100, 75, 50, 25, 10 % respectively, then gradual increase in mortality percentage appeared with increasing time elapsed post treatment. The mortality percentage ranged between 39.83 – 60.21 % after 12 days.

Table(22):Cummulative corrected mortality percentage of *S. littoralis* 2nd instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *M. flavovrdiae* at different periods.

Conc (%)	Cummulative mortality % indicated days after treatment					
	2	4	6	8	10	12
100	23.18	34.86	42.55	48.21	52.62	60.21
75	19.06	31.10	39.38	45.59	50.50	54.50
50	16.73	26.13	32.68	37.69	41.73	45.10
25	15.29	24.39	30.74	35.65	39.63	42.97
10	14.84	22.98	28.72	33.16	36.78	39.83

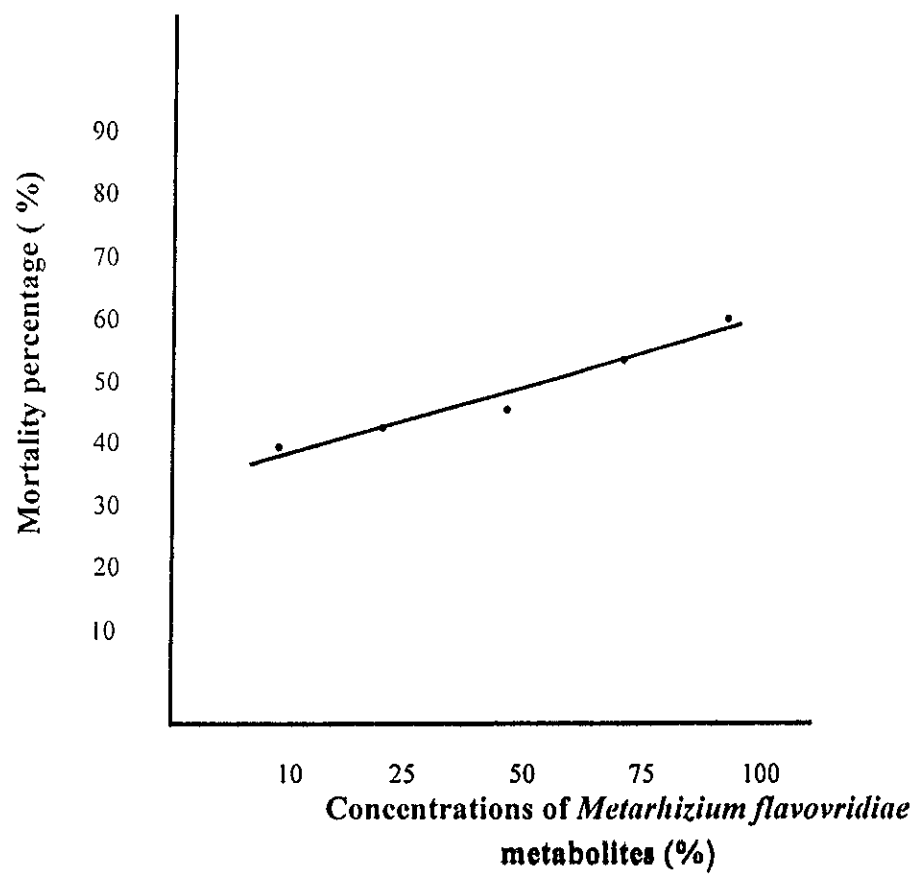


Fig. (14): Toxicity regression line of *M. flavoviridae* against 2nd instar larvae of *S. littoralis* after 12 day of inoculation

As for *P. farinosus* (Table: 23 and Fig: 15) the mortality percentage after two days post treatment was 22.70, 16.45, 12.23, 9.73, 6.73 % for the concentrations 100, 75, 50, 25, 10 % respectively. There were increasing in mortality percentage from 4th to 12th days ranged between 14.39 – 36.88 %, 20.91 – 46.30 %, 26.44 – 53.14 %, 31.20 – 58.39 and 35.33 – 59.58 % for the same concentration mentioned above respectively.

Table(23):Cummulative corrected mortality percentage of *S. littoralis* 2nd instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *P. farinosus* at different periods.

Conc (%)	Cummulative mortality % indicated days after treatment					
	2	4	6	8	10	12
100	22.70	36.88	46.30	53.14	58.39	59.58
75	16.45	26.21	33.05	38.29	42.51	46.03
50	12.23	22.20	29.72	35.67	40.55	44.66
25	9.73	18.51	25.41	31.01	35.70	39.70
10	6.73	14.39	20.91	26.44	31.20	35.33

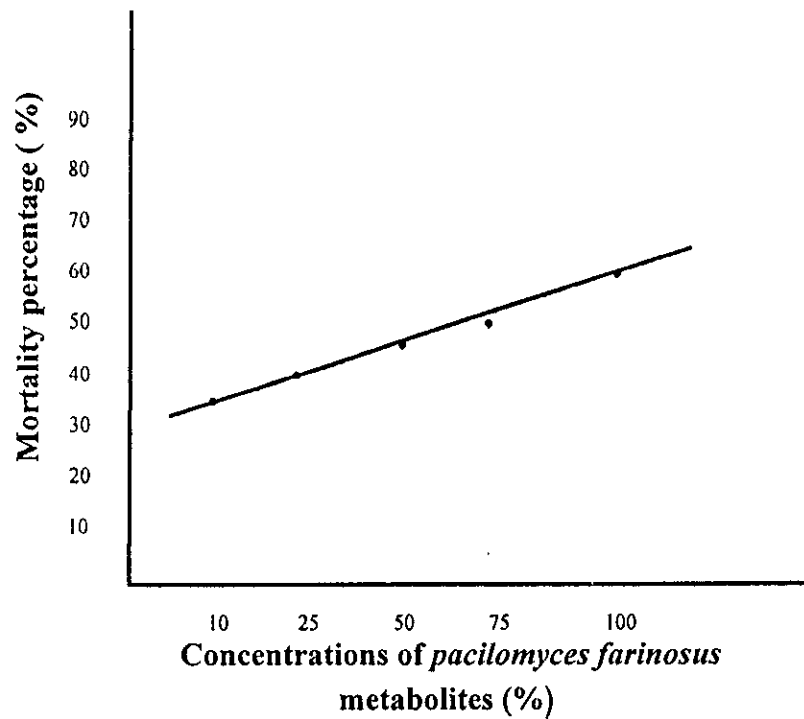


Fig. (15): Toxicity regression line of *P. farinosus* against 2nd instar larvae of *S. littoralis* after 12 day of inoculation

Taking into account the mortality percentage of *B. bassiana* as shown in table (24) and fig (16) after two days of treatment was 12.48, 9.64, 10.01, 7.53, 5.48 % for the concentrations 100, 75, 50, 25, 10 % respectively. The mortality percentage reached 50 % after 12 days at the four highest tested concentrations, generally it ranged between 15.05 – 26.73 %, 24.09 – 37.79 %, 31.97 – 46.38 %, 38.73 – 53.18 % and 44.53 – 55.68 % after 4, 6, 8, 10 and 12 days.

Table(24):Cummulative corrected mortality percentage of *S. littoralis* 2nd instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *B. bassiana* at different periods.

Conc(%)	Cummulative mortality % indicated days after treatment					
	2	4	6	8	10	12
100	12.48	26.73	37.79	46.38	53.18	55.68
75	9.64	22.07	32.33	40.62	47.37	52.95
50	10.01	21.73	31.23	38.90	45.18	50.41
25	7.53	19.15	29.40	37.93	45.01	50.93
10	5.48	15.05	24.09	31.97	38.73	44.53

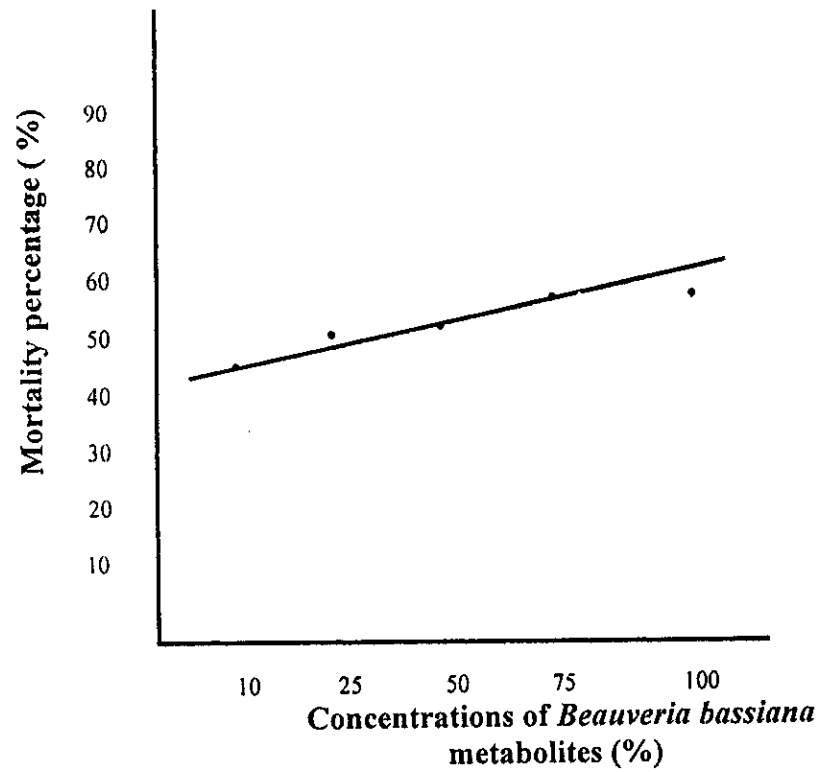


Fig. (16): Toxicity regression line of *B. bassiana* against 2nd instar larvae of *S littoralis* after 12 day of inoculation.

In addition to the mortality percentage of *B. brongniartti* (Table: 25 and Fig: 17) was lower than 10 % at the three lower tested concentrations after 2 days, also it was almost the same at the two highest tested concentrations after 12 days. The mortality percentage ranged between 36.22 – 57.77 % after 12 days.

Table(25):Cummulative corrected mortality percentage of *S. littoralis* 2nd instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *B. brongniartti* at different periods.

Conc (%)	Cummulative mortality % indicated days after treatment					
	2	4	6	8	10	12
100	17.10	30.96	40.84	48.26	54.08	57.77
75	10.06	23.66	34.85	43.78	50.96	56.81
50	7.59	17.86	26.73	34.15	40.38	45.68
25	5.54	14.42	22.66	29.83	36.02	41.38
10	3.70	10.90	18.21	24.92	30.91	36.22

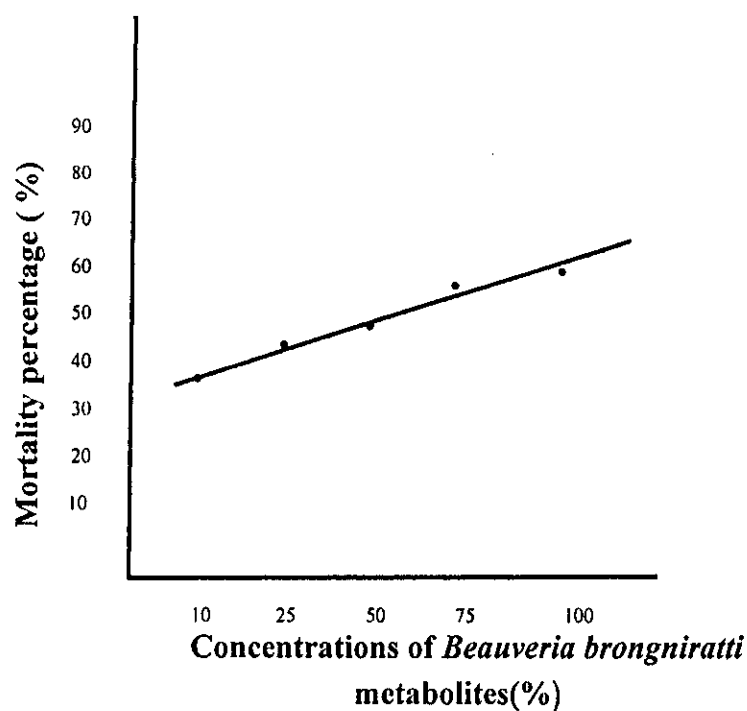


Fig. (17): Toxicity regression line of *B. brongniartii* against 2nd instar larvae of *S. littoralis* after 12 day of inoculation

Generally *M. flavoviridae* was found to be the most effective isolate against 2nd instar larvae of *S. littoralis* followed by *P. farinosus*, *M. anisopliae*, *B. brongniartii* then *B. bassiana* respectively .

Data in table 26,27,28,29&30 and fig 18 show that LT_{50} values for *M. anisopliae* (Table: 26 and Fig: 18) were 8.55, 21.45, 31.47, 33.20, 35.69 days for the concentrations 100, 75, 50, 25, 10 % respectively, regarding *M. flavoviridae* (Table: 27 and Fig: 18) LT_{50} values were 7.75, 9.77, 15.59, 17.48, 21.59 days for the same concentrations respectively.

As for *P. farinosus* (Table: 28 and Fig: 18) LT_{50} values ranged between 8 - 21 days for the concentrations 100, 75, 50, 25, 10 % respectively, in case of *B. bassiana* (Table: 29 and Fig: 18) they ranged between 9 - 14 days, while *B. brongniartti* (Table: 30 and Fig: 18) they ranged between 9 - 18 days for the same concentrations mentioned above respectively.

Table (26): Virulence of *M. anisopliae* against the 2nd Instar larvae of *S. littoralis* expressed as LT_{50} values (days).

Conc (%)	LT_{50} (days)	95% (fiducial limit)		a	b
		Lower	Upper	Intercept	Slope
100	8.5507	7.9704	26.0047	3.1931±0.2305	1.5102±0.2672
75	21.4539	14.7664	47.6672	3.1902±0.2368	1.3592±0.2738
50	31.4725	18.5420	122.8217	3.2304±0.2403	1.1814±0.2783
25	33.2024	19.3671	132.9200	3.1426±0.2486	1.2211±0.2866
10	35.6917	20.2955	157.7143	3.0997±0.2539	1.2240±0.2920

LT_{50} : Lethal time (time required to kill 50% of larvae).

Table (27): Virulence of *M. flavoviridae* against the 2nd instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (%)	LT ₅₀ (days)	95% (fiducial limit)		a	b
		Lower	Upper	Intercept	Slope
100	7.7531	6.9250	12.5802	3.9229±0.1920	1.1429±0.2306
75	9.7775	7.8178	13.9799	3.7420±0.1981	1.2704±0.2362
50	15.5994	11.0347	33.6236	3.7095±0.2035	1.0817±0.2413
25	17.4876	12.0216	41.4331	3.6541±0.2069	1.0830±0.2447
10	21.5993	13.7183	69.9488	3.6525±0.2089	1.0098±0.2469

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (28): Virulence of *P. farinosus* against the 2nd instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (%)	LT ₅₀ (days)	95% (fiducial limit)		a	b
		Lower	Upper	Intercept	Slope
100	8.0113	5.7761	8.7763	3.8374±0.1926	1.3745±0.2319
75	14.7096	10.6575	29.0324	3.6850±0.2039	1.1262±0.2417
50	15.1604	11.3217	26.6799	3.4379±0.2157	1.3230±0.2530
25	18.8480	13.3725	38.3966	3.3024±0.2268	1.3312±0.2639
10	21.9149	15.1839	47.3900	3.0708±0.2465	1.4389±0.2833

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (29): Virulence of *B. bassiana* against the 2nd instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (%)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
100	9.5097	7.6721	11.1732	3.3186±0.2132	1.7611±0.2510
75	10.8973	9.1046	14.2567	3.1654±0.2244	1.7686±0.2613
50	11.8317	9.6705	16.3043	3.2199±0.2232	1.6596±0.2600
25	11.6621	9.7412	15.3312	2.9972±0.2360	1.8774±0.2724
10	14.2017	11.4767	20.2205	2.8342±0.2536	1.8795±0.2893

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (30): Virulence of *B. brongniartii* against the 2nd instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (%)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
100	9.1516	7.1336	10.9303	3.5965±0.2016	1.5058±0.2399
75	9.7077	8.2804	12.0920	3.1610±0.2221	1.8630±0.2594
50	13.8989	11.0965	20.3319	3.0543±0.2369	1.7023±0.2732
25	15.9318	12.4496	24.6064	2.8732±0.2542	1.7690±0.2900
10	18.6407	14.1271	31.2047	2.6585±0.2796	1.8430±0.3150

LT₅₀ : Lethal time (time required to kill 50% of larvae).

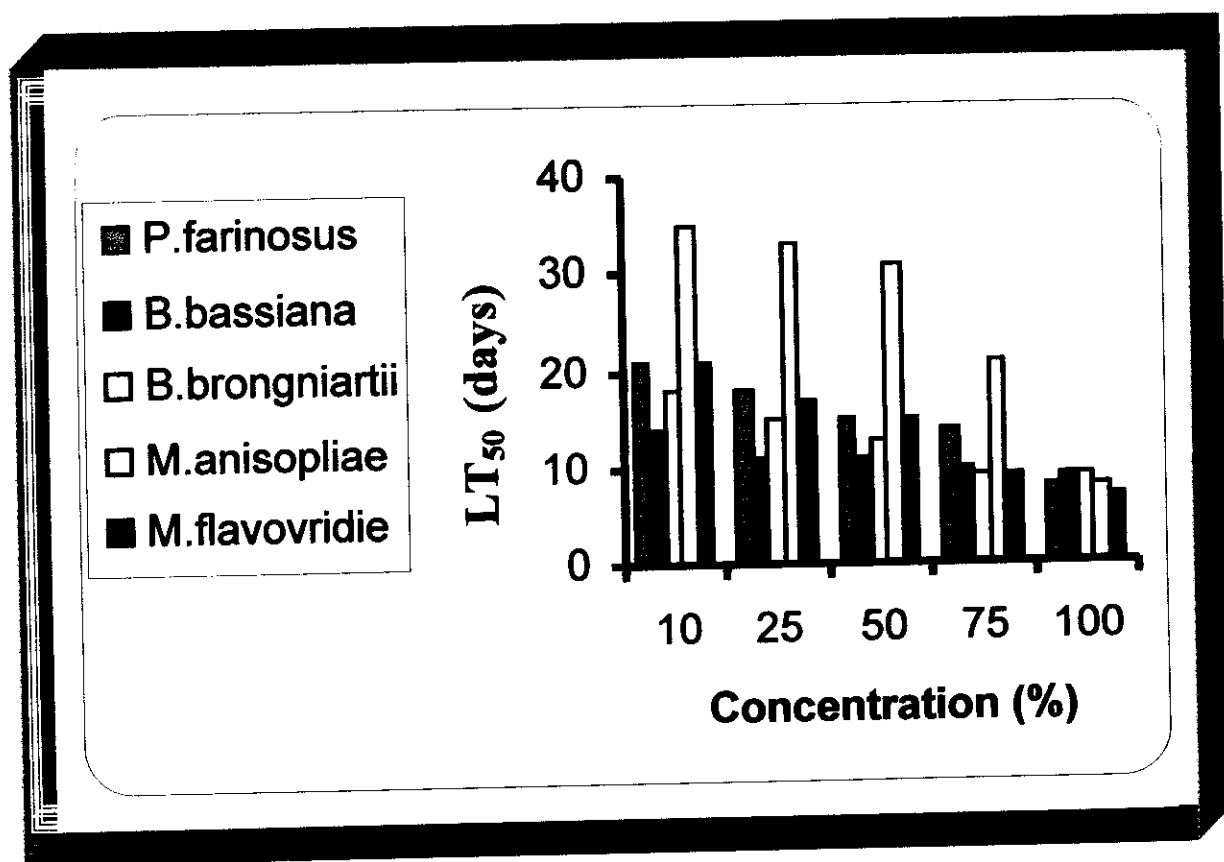


Fig (18) Relationship between different concentrations of the five tested fungi and LT_{50} values (days).

Virulence of entomopathogenic fungi (Metabolites) against 4th instar larvae of *S. littoralis*:-

For *M. anisopliae* (Table: 31 and Fig: 19) the mortality percentage after four days of treatment was 11.19, 9.54, 7.93, 5.71, 5.04 % for the concentrations 100, 75, 50, 25, 10 % respectively. There were difference in mortality percentage at the three lower tested concentrations after 4, 6, 8 and 10 days. Although the mortality percentage was different at the two highest tested concentrations after 4, 6 and 8 days but it was almost the same after 10 days.

Table(31):Cummulative corrected mortality percentage of *S. littoralis* 4th instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *M. anisopliae* at different periods.

Conc (%)	Cummulative mortality % indicated days after treatment			
	4	6	8	10
100	11.19	22.09	32.57	42.81
75	9.54	18.45	27.19	42.19
50	7.93	15.41	22.91	36.29
25	5.71	12.58	20.06	34.22
10	5.04	10.97	17.48	30.06

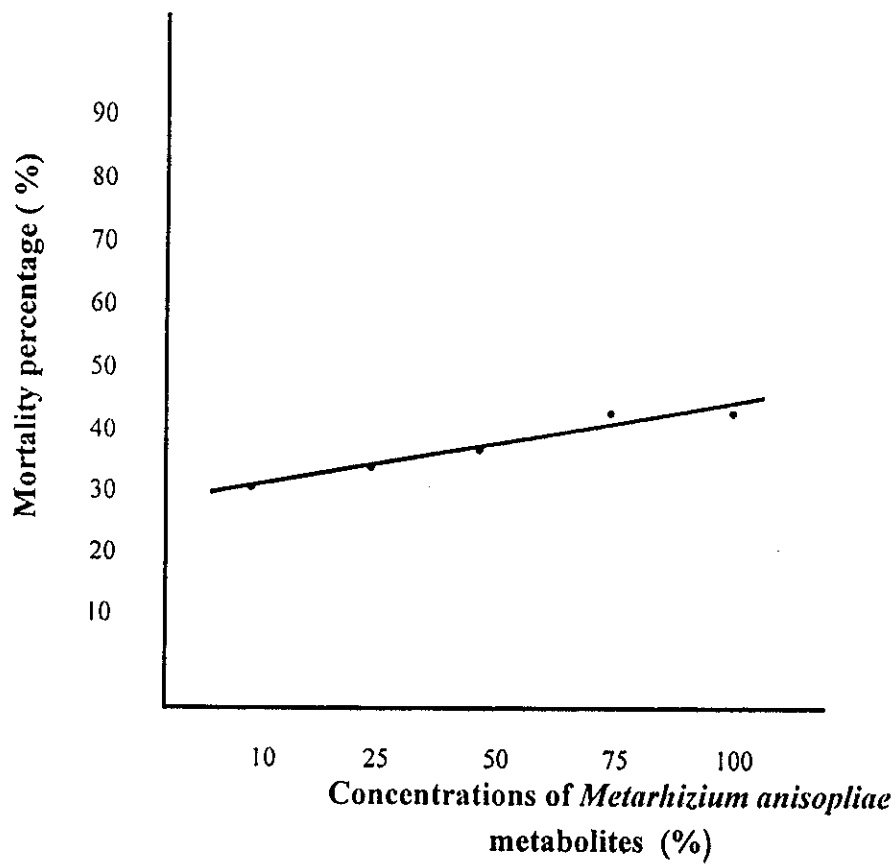


Fig. (19): Toxicity regression line of *M. anisopliae* against 4th instar larvae of *S. littoralis* after 10 days of inoculation.

Regarding *M. flavovrdiae* data in Table (32) and Fig (20) show that the mortality percentage after four days of treatment was 21.59, 17.63, 12.67, 10.15, 4.26 % for the concentrations 100, 75, 50, 25, 10 % respectively, then increased to ranged between 10.86 – 32.37 %, 18.72 – 41.14 % and 34.42 – 54.17 % after 6, 8 and 10 days.

Table(32):Cummulative corrected mortality percentage of *S. littoralis* 4th instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *M. flavovrdiae* at different periods.

Conc (%)	Cummulative mortality % indicated days after treatment			
	4	6	8	10
100	21.59	32.37	41.14	54.17
75	17.63	28.07	36.94	50.60
50	12.67	20.95	28.38	40.60
25	10.15	17.39	24.13	35.64
10	4.26	10.86	18.72	34.42

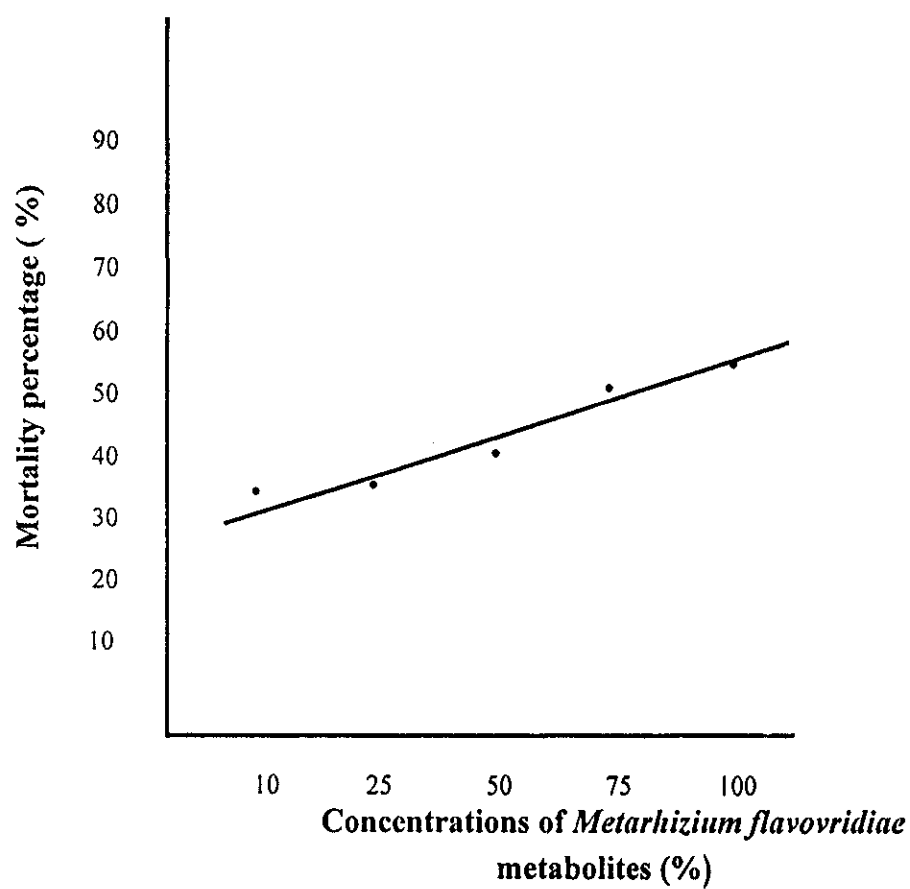


Fig. (20): Toxicity regression line of *M. flavoviridae* against 4th instar larvae of *S. littoralis* after 10 days of inoculation

As well as *P. farinosus* (Table: 33 and Fig: 21) the mortality percentage increased from the 4th to the 10th day to be ranged between 2.64 – 14.68 %, 5.92 – 22.34 %, 9.76 – 35.92 % and 17.86 – 45.00 % after 4, 6, 8 and 10 days.

Table(33):Cummulative corrected mortality percentage of *S. littoralis* 4th instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *P. farinosus* at different periods.

Conc (%)	Cummulative mortality % indicated days after treatment			
	4	6	8	10
100	14.68	22.34	35.92	45.00
75	7.29	12.65	17.83	27.09
50	4.73	9.48	14.52	24.27
25	5.02	9.27	13.61	21.79
10	2.64	5.92	9.76	17.86

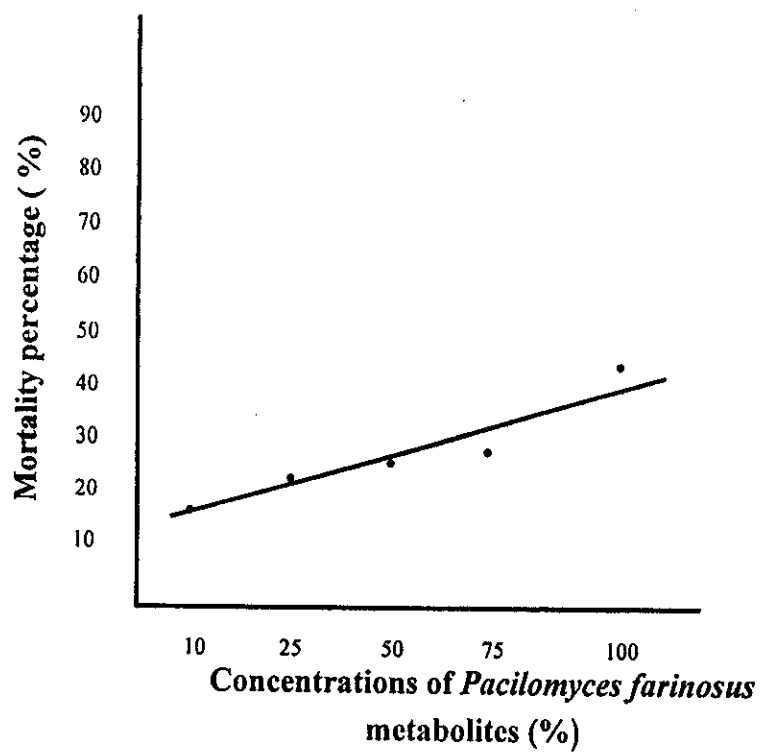


Fig. (21): Toxicity regression line of *P. farinosus* against 4th instar larvae of *S. littoralis* after 10 days of inoculation

Taking into account *B. bassiana* as shown in table: 34 and fig: 22 the mortality percentage was lower than 10 % at the two lower tested concentrations after 4 and 6 days but it ranged between 10.40 – 12.46 % and 17.96 – 21.12 % after 8 and 10 days for the same concentrations. The mortality percentage ranged between 26.45 – 38.78 % after 10 days at the three highest tested concentrations.

Table(34):Cumulative corrected mortality percentage of *S. littoralis* 4th instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *B. bassiana* at different periods.

Conc (%)	Cumulative mortality % indicated days after treatment			
	4	6	8	10
100	10.83	18.81	26.24	38.78
75	8.81	14.59	19.96	29.29
50	6.99	12.22	17.30	26.45
25	4	8.07	12.46	21.12
10	3.26	6.66	10.40	17.96

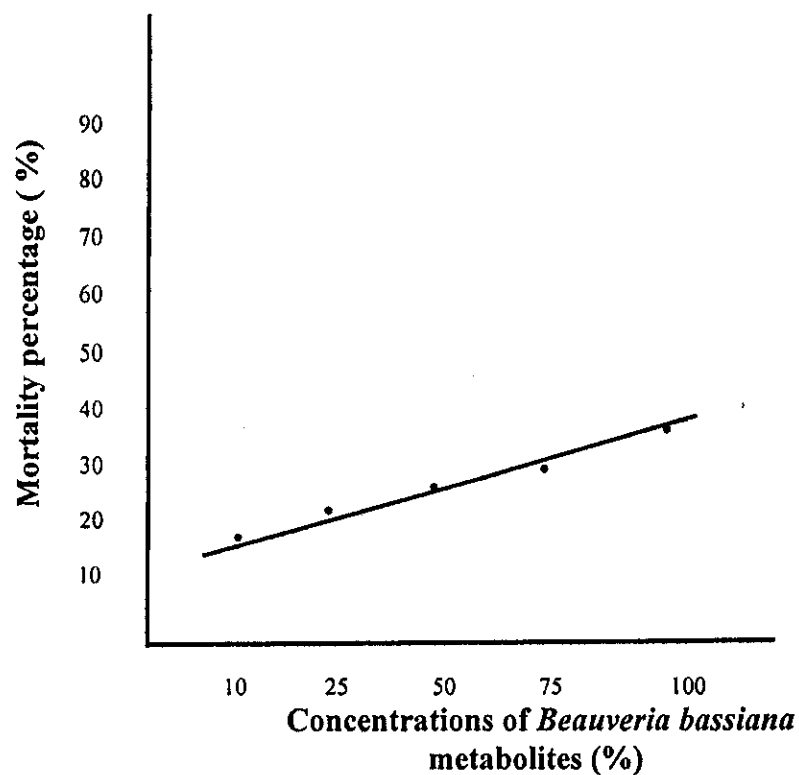


Fig. (22): Toxicity regression line of *B. bassiana* against 4th instar larvae of *S. littoralis* after 10 days of inoculation

In addition to the mortality percentage of *B. brongniartti* (Table: 35 and Fig: 23) after four days post treatment was 15.82, 12.56, 7.85, 5.58, 4.57 % for the concentrations 100, 75, 50, 25, 10 % respectively. Through the period between the 6th and the 10th day post treatment there were gradual increase in mortality percentage ranged between 8.30 – 22.37 %, 12.09 – 27.83 % and 19.28 – 36.48 % after 6, 8 and 10 days.

Table(35):Cummulative corrected mortality percentage of *S. littoralis* 4th instar larvae after feeding on treated castor bean leaves immersed in different concentrations of *B. brongniartti* at different periods.

Conc (%)	Cummulative mortality % indicated days after treatment			
	4	6	8	10
100	15.82	22.37	27.83	36.48
75	12.56	19.05	24.71	34.00
50	7.85	13.72	19.38	29.41
25	5.58	10.33	15.15	24.14
10	4.57	8.30	12.09	19.28

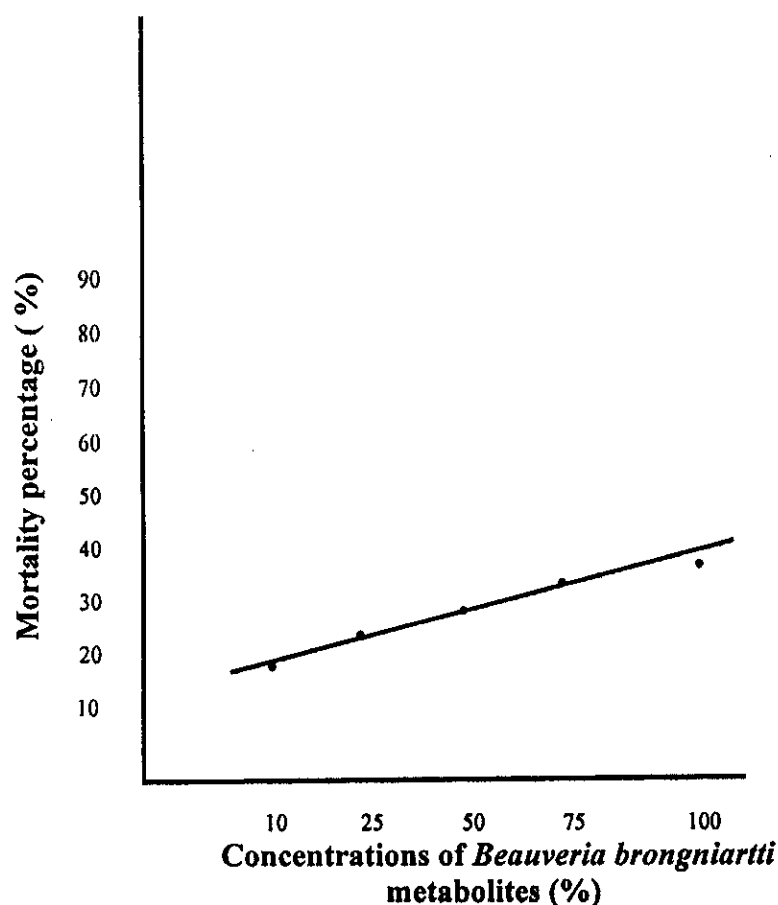


Fig. (23) Toxicity regression line of *B. brongniartii* against 4th instar larvae of *S. littoralis* after 10 days of inoculation

Generally *M. flavoviridae* was found to be the most effective isolate against 4th instar larvae of *S. littoralis* followed by *p. farinosus*, *M. anisopliae*, *B. bassiana* then *B. brongniartii* respectively .

Data in table 36,37,38,39&40 and fig 24 show that LT_{50} values for *M. anisopliae* (Table: 36 and Fig: 24) were 14.02, 14.57, 17.26, 17.56, 20.04 days for the concentrations 100, 75, 50, 25, 10 % respectively, while LT_{50} values for *M. flavoviridae* (Table: 37 and Fig: 24) were 10.54, 11.79, 16.02, 18.75, 16.75 days for the same concentrations respectively. Regarding *P. farinosus* (Table: 38 and Fig: 24) LT_{50} values ranged between 14 - 32 days for the concentrations 100, 75, 50, 25, 10 % respectively, as for *B. bassiana* (Table: 39 and Fig: 24) they ranged between 16 – 35 days, in addition *B. brongniartti* (Table: 40 and Fig: 24) they ranged between 21 - 38 days for the same concentrations mentioned above respectively.

Table (36): Virulence of *M. anisopliae* against the 4th instar larvae of *S. littoralis* expressed as LT_{50} values (days).

Conc (%)	LT_{50} (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
100	14.0217	9.8903	18.5930	2.2547±0.5611	2.5395±0.6124
75	14.5785	11.2469	30.1046	2.2890±0.5813	2.3296±0.6317
50	17.2684	12.5032	51.1030	2.2539±0.6068	2.2195±0.6568
25	17.5623	12.8766	46.8345	1.9406±0.6500	2.4582±0.6976
10	20.0477	13.8588	78.7476	1.9473±0.6731	2.3445±0.7208

LT_{50} : Lethal time (time required to kill 50% of larvae).

Table (37): Virulence of *M. flavoviridae* against the 4th instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (%)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
100	10.5456	8.4026	18.4858	3.0899±0.5038	1.8670±0.5596
75	11.7909	9.3193	22.1959	2.8786±0.5196	1.9797±0.5740
50	16.0211	11.5293	55.3513	2.7171±0.5529	1.8950±0.6058
25	18.7566	12.7585	97.5002	2.5848±0.5795	1.8970±0.6317
10	16.7536	12.7300	36.5896	1.6133±0.6895	2.7667±0.7343

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (38): Virulence of *P. farinosus* against the 4th instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (%)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
100	14.4374	11.7939	129.2273	2.9612±0.5396	1.6423±0.5937
75	26.5380	15.3064	1059.8214	2.4794±0.6289	1.7702±0.6817
50	26.3749	15.7186	488.9356	2.1005±0.6934	2.0402±0.7435
25	32.3587	16.9621	9533.1600	2.2682±0.6907	1.8092±0.7433
10	32.4982	17.4438	5057.3194	1.7831±0.8087	2.1278±0.8573

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (39): Virulence of *B. bassiana* against the 4th instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (%)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
100	16.6792	11.9646	56.0766	2.5646±0.5701	1.9927±0.6221
75	25.1902	14.7280	973.9752	2.6291±0.6024	1.6920±0.6558
50	27.1486	15.4995	1273.3758	2.4542±0.6354	1.7757±0.6881
25	30.3917	16.7661	2188.2806	2.0527±0.7272	1.9877±0.7778
10	35.5735	17.9018	41565.9137	1.9869±0.7714	1.9425±0.8226

LT₅₀ : Lethal time (time required to kill 50% of larvae).

Table (40): Virulence of *B. brongniartii* against the 4th instar larvae of *S. littoralis* expressed as LT₅₀ values (days).

Conc (%)	LT ₅₀ (days)	95% (fiducial limit)		a Intercept	b Slope
		Lower	Upper		
100	21.3991	12.7297	4704.9391	3.1698±0.5347	1.3757±0.5901
75	22.2276	13.4151	922.2418	2.9253±0.5586	1.5403±0.6130
50	23.7037	14.5174	367.2847	2.4818±0.6162	1.8317±0.6685
25	28.5660	16.0741	1507.9118	2.2871±0.6700	1.8634±0.7220
10	38.3324	18.0985	1.47888E+06	2.2759±0.7121	1.7202±0.7660

LT₅₀ : Lethal time (time required to kill 50% of larvae).

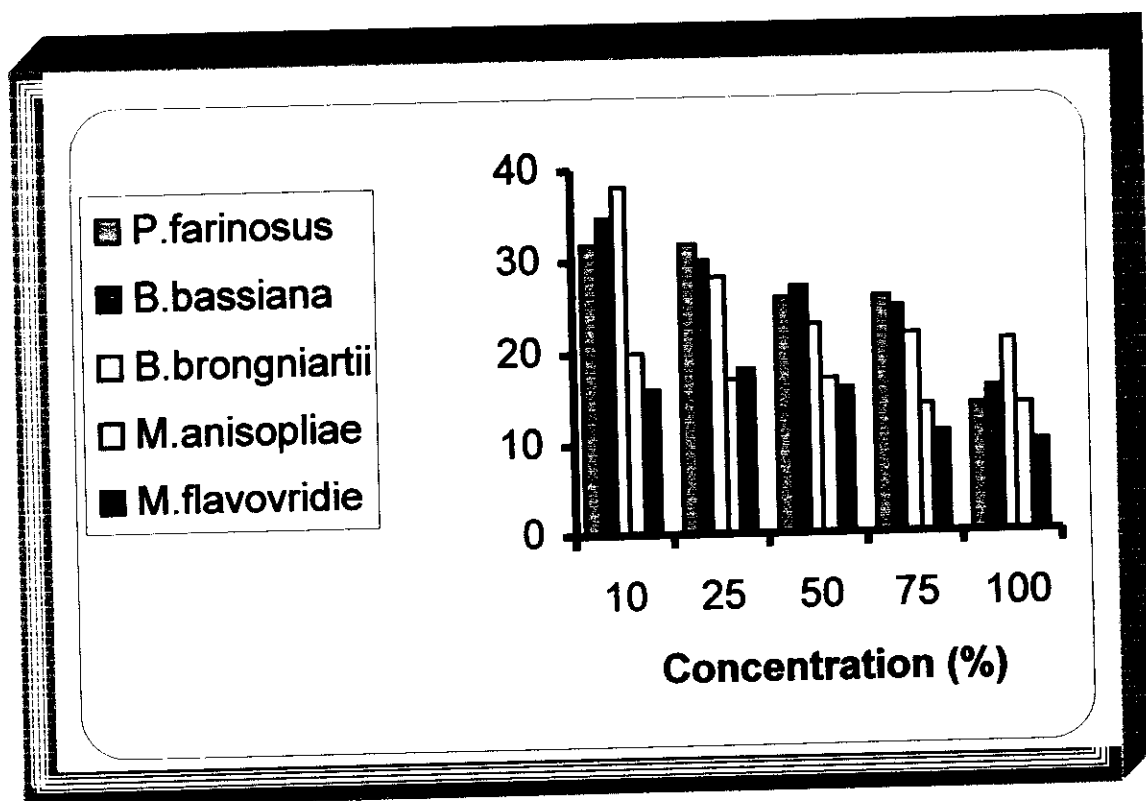


Fig (24) Relationship between different concentrations of the five tested fungi and LT_{50} values (days).

Fig (25): Malformations induced by *M. anisopliae* in several stages of cotton leaf worm *S. littoralis*.



Normal larvae



Malformation showing intermediate stage between 5th and 6th larval instars showing old and dark exuvium



Normal pupae



Pupal-moth intermediate stage (pupae failed to be moth), malformed pupae with small fragment of moth



Normal adult



Malformation of moth included crumpled wings and frizzled abdomen



Fig (26): Malformations induced by *M. flavoviridae* in several stages of cotton leaf worm *S. littoralis*.



Normal larvae



Malformation showing small crumpled and frizzled larvae



Normal pupae



Malformation showing abnormal pupae attached with moth



Malformed pupae showed body shrinkage



Normal adult



Malformation showing small size moth without wings

Fig (27): Malformations induced by *B. brongniartii* in several stages of cotton leaf worm *S. littoralis*.



Normal larvae



Malformation showing small and frizzled larvae



Normal pupae



Malformation showing moth failed in emerging from pupal case



Normal adult



Malformation showing small moth with twisted wings

Fig (28): Malformations induced by *B.bassiana* in several stages of cotton leaf worm *S. littoralis*.



Normal larvae



Malformation showing frizzled and dark larvae



Normal pupae



Malformed pupae showed the growth of the fungus on the surface of pupae



Normal adult



Malformation show frizzled moth

Fig (29): Malformations induced by *P. farinosus* in several stages of cotton leaf worm *S. littoralis*.



Normal larvae



Malformation showing dwarfism larvae



Normal pupae



Malformation showing intermediate stage between pupae and moth (Pupal-moth)



Molecular characterization

a- Random Amplified Polymorphic DNA- Polymerase Chain Reaction (RAPD – PCR) profiles

The five primers amplified 227 DNA fragments including 45 non polymorphic fragments, thus 182 fragments (80%) were polymorphic in one or the other of the 5 genotypes (table: 41).

The different primers revealed different levels of polymorphism among the 5 entomopathogenic fungi genotypes (Fig 30, 31, 32, 33 and 34).

The highest number of amplified fragments was 59 with the primer **OPE 20**, while the lowest was 25 with the primer **OPA 20** (Table 41).

The number of polymorphic amplicons per primer ranged from 30 (Primer **OPA 13**) to 49 (Primer **OPE 20**).

The average number of amplified fragment per primer across the 5 genotypes was 45 and the average of polymorphic amplified fragment was 36. If the primer producing the highest number of reproducible polymorphic amplicons (high level of polymorphism) were considered the best, then this will apply to **OPE 20**, **OPD 08**, **OPD 07**, **OPA 13** and **OPA 20** since they produced 49, 44, 44, 30 and 15 polymorphic amplicons from total of 59, 54, 44, 45 and 25 fragments respectively (table : 41).

1- OPA 13 primer

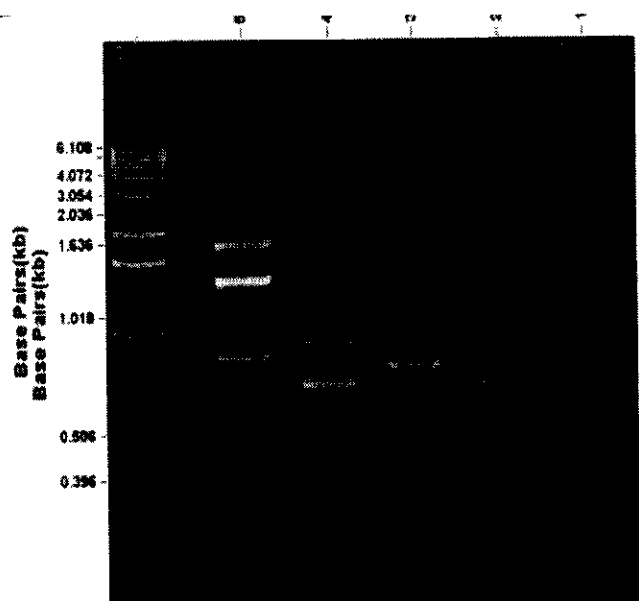


Fig (30)

2-OPA 20 primer

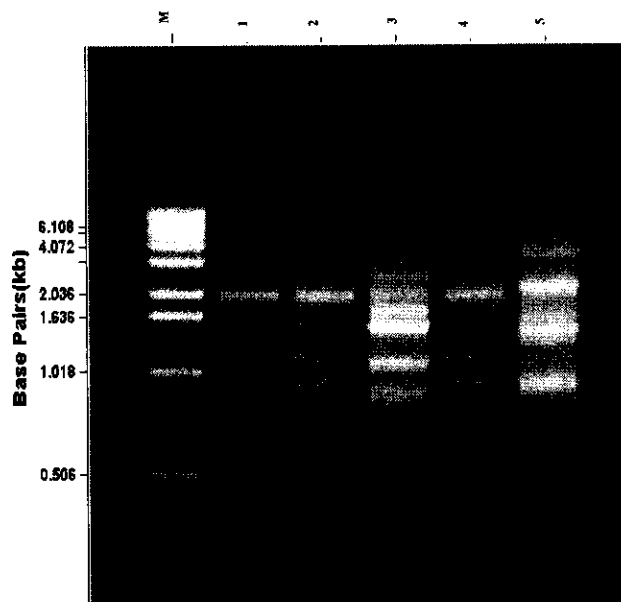


Fig (31)

3-OPD 07 Primer

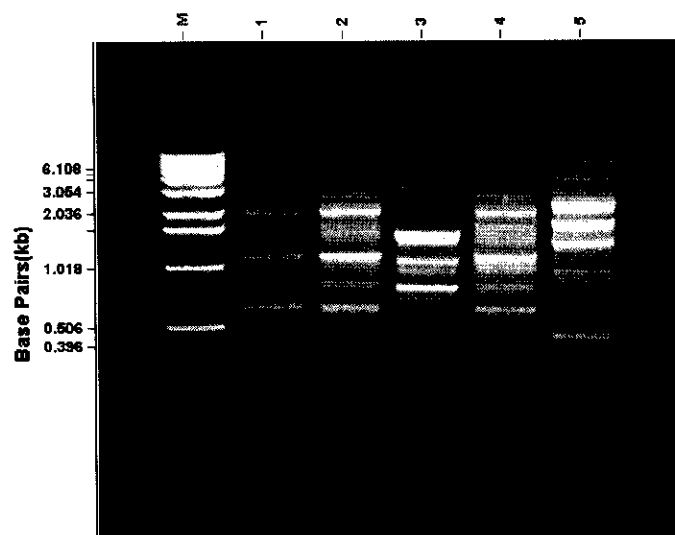


Fig (32)

RAPD profiles of different isolates of entomopathogenic fungi using the primer OPA 13, OPA 20 and OPD 07. Lane 1, *Beauveria bassiana*; Lane 2, *Beauveria brongniartii*; Lane 3, *Pacilomyces farinosus*; Lane 4, *Metarhizium flavovirdiae*; Lane 5, *Metarhizium anisopliae*; Lane M, DNA marker.

4-OPD 08 Primer

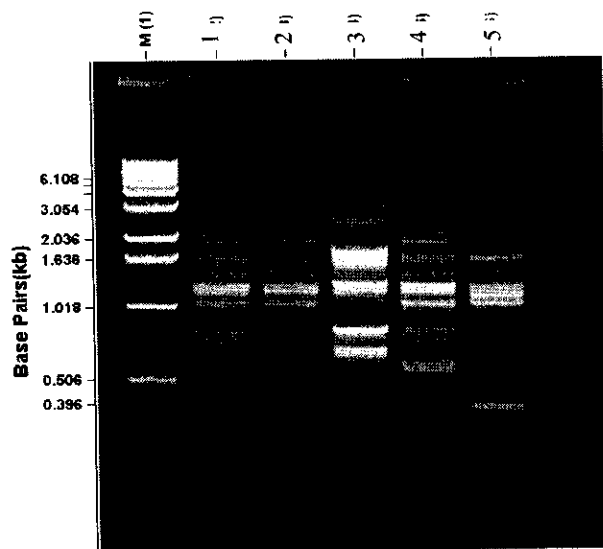


Fig (33)

5-OPE 20 Primer

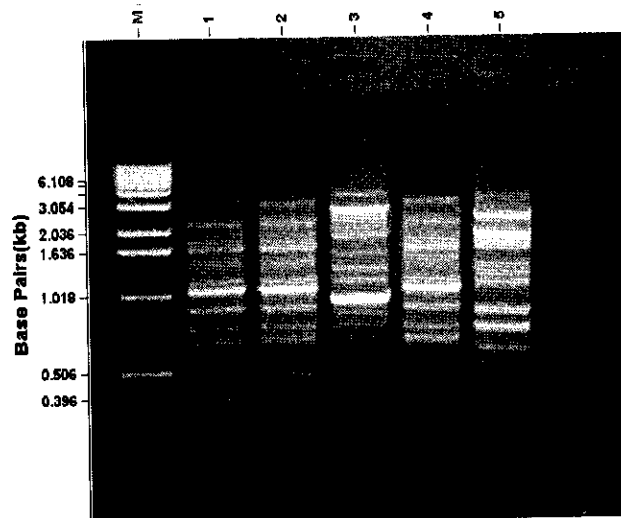


Fig (34)

RAPD profiles of different isolates of entomopathogenic fungi using the primer OPD08 and OPE 20. Lane 1, *Beauveria bassiana*; Lane 2, *Beauveria brogniartti*; Lane 3, *Pacilomyces farinosus*; Lane 4, *Metarhizium flavovrdiae*; Lane 5, *Metarhizium anisopliae*; Lane M, DNA marker.

Table (41) Number of amplicons and the number of polymorphic bands releaved by each RAPD – primer for the five tested fungi.

Primers	Number of amplicons	Polymorphic amplicons	Percentage of polymorphism %
OPA 13	45	30	66
OPA 20	25	15	60
OPD 07	44	44	1
OPD08	54	44	81
OPE 20	59	49	83

b- Sodium Dodecyle Sulfate Polyacrylamide Gel Electrophoresis (SDS – PAGE) analysis of total cellular proteins

Total cellular proteins of entomopathogenic fungi isolates *B. brongniartti*, *B. bassiana*, *M. anisopliae*, *M. flavovirdiae* and *P. farinosus* were fractionated on denaturing gels by electrophoresis (Sodium - Dodecyl Sulfate Polyacrylamide Gel Electrophoresis, SDS – PAGE (Fig 35)) (Laemmli, 1970). Comparison of protein patterns from *B. brongniartti* and *B. bassiana* revealed that 10 common bands { No. 1, 5, 6, 8, 9, 11, 12, 13, 15, 19 with Rf (0.037, 0.278, 0.324, 0.377, 0.412, 0.486, 0.53, 0.561, 0.652, 0.921), (0.036, 0.283, 0.327, 0.375, 0.408, 0.49, 0.535, 0.561, 0.648, 0.917) and MW (183.617, 102.972, 92.79, 78.009, 69.487, 56.425, 49.972, 45.819, 35.524, 17.44 KDa), (184.437, 101.773, 91.103, 77.837, 69.676, 57.15, 49.979, 46.332, 36.911, 17.3 KDa)} and band No 2 with M.W (136.21 KDa) and Rf (0.16) was characteristic band for *B. bassiana*, while bands No 3, 4 and 10 with M.W (123.908, 111.657, 62.98 KDa) and Rf (0.198, 0.238, 0.447) were characteristic bands for *B. brogniartti* (Table 42,43 and 44).

In case of *M. anisopliae* and *M. flavovrdiae* there were 3 common bands { No. 1, 15, 19 with Rf (0.039, 0.65, 0.915), (0.036, 0.652, 0.915) and MW (184.092, 36.808, 17.245 KDa), (182.15, 37.095, 17.085 KDa)} respectively, and bands No 5, 9, 10, 11, 12, 16, 17 and 18 with M.W (100.511, 72.096, 64.538, 58.109, 52.017, 32.065, 27.395, 24.667 KDa) and Rf (0.278, 0.406, 0.448, 0.488, 0.527, 0.702, 0.755, 0.791) were characteristic bands for *M. flavovrdiae*, while bands No 7 and 8 with M.W (87.261, 79.024 KDa) and Rf (0.336, 0.369) were characteristic bands for *M. anisopliae* (Table 42,43 and 44).

In addition to, bands No. 1, 15, 19 were detected in *B. brogniartti*, *B. bassiana*, *M.anisopliae* and *M. flavovrdiae* with Rf {(0.037, 0.652, 0.921), (0.036, 0.648, 0.917), (0.039, 0.65, 0.915), (0.036, 0.652, 0.915) and MW (183.617, 35.524, 17.44 KDa), (184.437, 36.911, 17.3 KDa), (184.092, 36.808, 17.245 KDa), (182.15, 37.095, 17.085 KDa)} respectively. Band No. 8 with Rf { (0.377), (0.375), (0.369), (0.381) and MW (78.009 KDa), (77.837 KDa), (79.024 KDa), (77.927 KDa) } was detected in *B. brongniartti*, *B. bassiana*, *M.anisopliae* and *P. farinosus* respectively, while band No. 4 with Rf { (0.238), (0.24) and MW (111.657 KDa), (110.088 KDa) } was only found in *B. brongniartti* and *P. farinosus*. Bands No. 5, 9, 11, 12 were found in *B. brogniartti*, *B. bassiana* and *M. flavovrdiae* with Rf { (0.278, 0.412, 0.486, 0.53), (0.283, 0.408,

0.49, 0.535), (0.278, 0.406, 0.488, 0.527) and MW (102.972, 69.487, 56.425, 49.972 KDa), (101.773, 69.676, 57.15, 49.979 KDa), (100.511, 72.096, 58.109, 52.017 KDa) } respectively. Band No. 6 was found in *B. brongniartti*, *B. bassiana* and *P. farinosus* with Rf { (0.324), (0.327), (0.329) and MW (92.79 KDa), (91.103 KDa), (88.124 KDa) } for the same isolates respectively, but band No. 10 with Rf {(0.447), (0.448) and MW (62.98 KDa), (64.538 KDa) } was detected in *B. brongniartti* and *M. flavovordiae* (Table 42,43 and 44) .

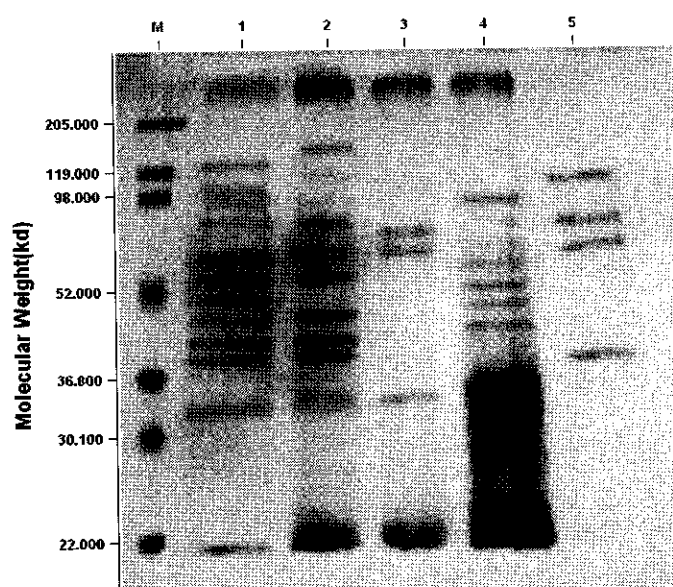


Fig (35) Electrophoretic patterns of total cellular protein of different isolates of entomopathogenic fungi. The number beside the gel indicate molecular masses of standard marker proteins. Lane 1, *Beauveria brongniartii*; Lane 2, *Beauveria bassiana*; Lane 3, *Metarhizium anisopliae*; Lane 4, *Metarhizium flavoviridae*; Lane 5, *Pacilomyces farinosus* ; Lane M, Standard molecular masses.

Table (42) Scored bands of the five tested entomopathogenic fungi *B .brongniartti* (1), *B. bassiana* (2), *M. anisopliae* (3), *M. flavovrdiae*(4) and *P. farinosus*(5)

	<i>Marker(KDa)</i>	<i>Lane 1</i>	<i>Lane 2</i>	<i>Lane 3</i>	<i>Lane 4</i>	<i>Lane 5</i>
1	183.574	1	1	1	1	0
2	136.21	0	1	0	0	0
3	123.908	1	0	0	0	0
4	110.872	1	0	0	0	1
5	101.752	1	1	0	1	0
6	90.673	1	1	0	0	1
7	87.261	0	0	1	0	0
8	78.199	1	1	1	0	1
9	70.42	1	1	0	1	0
10	63.759	1	0	0	1	0
11	57.228	1	1	0	1	0
12	50.656	1	1	0	1	0
13	46.075	1	1	0	0	0
14	43.642	0	0	0	0	1
15	36.584	1	1	1	1	0
16	32.065	0	0	0	1	0
17	27.395	0	0	0	1	0
18	24.667	0	0	0	1	0
19	17.268	1	1	1	1	0

Table (43) Molecular weight of SDS protein bands detected in the five tested entomopathogenic fungi *B. brongniartii* (1), *B. bassiana* (2), *M. anisopliae* (3), *M. flavovirdiae* (4) and *P. farinosus*(5)

<i>Marker</i>	<i>Lane 1</i>	<i>Lane 2</i>	<i>Lane 3</i>	<i>Lane 4</i>	<i>Lane 5</i>
<i>(M. W)</i>	<i>(M. W)</i>	<i>(M. W)</i>	<i>(M. W)</i>	<i>(M. W)</i>	<i>(M. W)</i>
205	183.617	184.437	184.092	182.15	110.088
119	123.908	136.21	87.261	100.511	88.124
98	111.657	101.773	79.024	72.096	77.927
52	102.972	91.103	36.808	64.538	43.642
36.8	92.79	77.837	17.245	58.109	
30.1	78.009	69.676		52.017	
22	69.487	57.15		37.095	
	62.98	49.979		32.065	
	56.425	46.332		27.395	
	49.972	36.911		24.667	
	45.819	17.3		17.085	
	35.524				
	17.44				

Table (44) Relative fragmentation (Rf) values of SDS protein bands detected in the five tested entomopathogenic fungi *B. brongniartti* (1), *B. bassiana* (2), *M. anisopliae* (3), *M. flavovirdiae*(4) and *P. farinosus*(5)

<i>Lane 1</i>	<i>Lane 2</i>	<i>Lane 3</i>	<i>Lane 4</i>	<i>Lane 5</i>
<i>Rf</i>	<i>Rf</i>	<i>Rf</i>	<i>Rf</i>	<i>Rf</i>
0.037	0.036	0.039	0.036	0.24
0.198	0.16	0.336	0.278	0.329
0.238	0.283	0.369	0.406	0.381
0.278	0.327	0.65	0.448	0.595
0.324	0.375	0.915	0.488	
0.377	0.408		0.527	
0.412	0.49		0.652	
0.447	0.535		0.702	
0.486	0.561		0.755	
0.53	0.648		0.791	
0.561	0.917		0.915	
0.652				
0.921				

Densitometric scanning of SDS PAGE protein (Table 45) revealed that:-

Band No. 9 (MW 69.487 KDa with Rf 0.412) for *B. brongniartti* had higher concentration which represented 158.95, while band No. 19 {MW (17.3 KDa), (17.245 KDa), (17.085 KDa) with Rf (0.917), (0.915), (0.915) }for *B. bassiana*, *M. anisopliae* and *M. flavoviridis* had higher concentration which represented 171.38, 145.94, 228.68 respectively. In addition to, band No. 4 (MW 110.088 KDa with Rf 0.24) for *P. farinosus* had higher concentration which represented 120.13.

Table (45) Amount of SDS PAGE protein bands (%) of the five tested entomopathogenic fungi *B. brongniartii*(1), *B. bassiana* (2), *M. anisopliae*(3), *M. flavovirdiae*(4) and *P. farinosus*(5)

<i>Marker</i>	<i>Lane 1</i>	<i>Lane 2</i>	<i>Lane 3</i>	<i>Lane 4</i>	<i>Lane 5</i>
<i>(amount%)</i>	<i>(amount%)</i>	<i>(amount%)</i>	<i>(amount%)</i>	<i>(amount%)</i>	<i>(amount%)</i>
166.75	128.24	150.95	133.59	132.12	120.13
149.45	119.03	104.42	103.15	107.76	116.94
151.93	110.36	97.05	102.94	100.41	112.02
130.22	118.02	126.47	87.12	112.23	113.3
145.7	123.15	140.27	145.94	109.35	
147.2	156.88	141.33		114.79	
140.38	158.95	151.77		180.39	
	157.76	150.83		171.94	
	149.59	142.53		157.36	
	148.92	119		152.35	
	147.7	171.38		228.68	
	130.08				
	117.59				

Table (46) Similarity matrix calculated by DICE Computer Package between the five tested entomopathogenic fungi *B. brongniartti* (1), *B. bassiana* (2), *M. anisopliae* (3), *M. flavovrdiae*(4) and *P. farinosus*(5)

Lane	1	2	3	4	5
1	1				
2	83	1			
3	44	5	1		
4	67	64	38	1	
5	35	27	22	0	1

Table (46) show that the mean values of the similarity index of the five different isolates ranged from 83% - 22%. Data in this table demonstrated that similarity value between *M. flavovrdiae* and *P. farinosus* was zero this means that there was no similarity between them but similarity value between *M. anisopliae* and *P. farinosus* was 22% this means that there was small similarity between them, on the other hand similarity value between *M. anisopliae* and *M. flavovrdiae* was 38%, while it was 83% between *B. brongniartti* and *B. bassiana* and this indicated that the higher similarity was found between *B. brongniartti* and *B. bassiana*.