RESULTS

IV. RESULTS

1. Susceptibility of *S. gregaria* to entomopathogenic nematodes:

The purpose of the following tests was to investigate the effect of nematodes on locust mortality and to determine the efficiency of the different species and isolates according to their LC_{50} values .

Concentrations of five indigenous heterorhabditid isolates (EKB20, EASD98, EBR5, EAM6 and EAS59) and one foreign steinernematid (*S. glaseri*) entomopathogenic nematodes were tested against all nymphal instars (from 1st to 5th) as well as the adults of *S. gregaria* . Mortality percentages of insects within 5 days of infection and medium lethal concentrations are given in the tables (1 to 7) and were graphically illustrated in figures (3-9) .

It was found that, infected nymphs or/and adults show no signs of infection until shortly before death when they became sluggish, fall on their side and make slow movements of the appendages until they die. The maximum mortality was induced after 48 hr of infection, then it began to decrease gradually until the end of the experiment in all nematode species.

Data presented in the tables (1-7) indicate that *S. gregaria* were susceptible to all the tested nematode species, however, the degree of susceptibility differed according to the nematode species and isolates used. A positive correlation was evident between percent mortality and the nematode concentration in the suspension (higher levels, however, caused higher mortality percentages than lower infection levels) in heterorhabditid infected insects. It was also evident that, the two isolates of *H. bacteriophora* (EKB20 & EASD98) were the most virulent nematodes on *S. gregaria* , all infection levels caused mortality of infected insects as 100% mortality was recorded at

Table (1): Percent mortality of 1st instar nymphs of *S. gregaria* infected with different concentrations of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EAS59, EBR5 and EAM6.

IJs/nym.	% mortality at the idicated days after treatment																			
	1 day		2 days						3 days						Total					
	KB20	AS98	S.g	KB20	AS98	AS59	BR5	AM6	S.g	KB20	AS98	AS59	BR5	AM6	S.g	KB20	AS98	AS59	BR5	AM6
50	0.00	0.00	0.00	20.0	10.0	0.00	0.00	0.00	0.00	3.30	0.00	0.00	0.00	0.00	0.00	23.3	20.0	0.00	0.00	0.00
100	0.00	0.00	0.00	40.0	40.0	0.00	0.00	0.00	0.00	10.0	6.70	0.00	0.00	10.0	0.00	50.0	46.7	0.00	0.00	10.0
200	0.00	0.00	0.00	66.7	60.0	10.0	10.0	20.0	3.30	20.0	20.0	10.0	3.30	13.3	3.30	86.7	80.0	20.0	13.3	33.3
300	60.0	50.0	10.0	40.0	50.0	23.3	10.0	33.3	10.0	0.00	0.00	10.0	10.0	10.0	20.0	100	100	33.3	20.0	43.3
600	80.0	50.0	20.0	20.0	50.0	33.3	30.0	30.0	10.0	0.00	0.00	10.0	13.3	16.7	30.0	100	100	43.3	43.3	46.7
900	---	---	20.0	---	---	50.0	40.0	40.0	23.3	---	---	20.0	26.7	13.3	43.3	---	---	70.0	66.7	53.3
1200	---	---	33.3	---	---	66.7	40.0	40.0	26.7	---	---	20.0	30.0	30.0	60.0	---	---	86.7	70.0	70.0
1500	---	---	40.0	---	---	70.0	50.0	66.7	40.0	---	---	30.0	50.0	20.0	80.0	---	---	100	100	86.7

* Doted lines not tested.

** Control mortality was zero % throughout the time of experiment.

Figure (3): Percent mortality of 1st instar nymphs of *S. gregaria* infected with different concentrations of *S. glaseri* and *Heterorhabditis* sp.

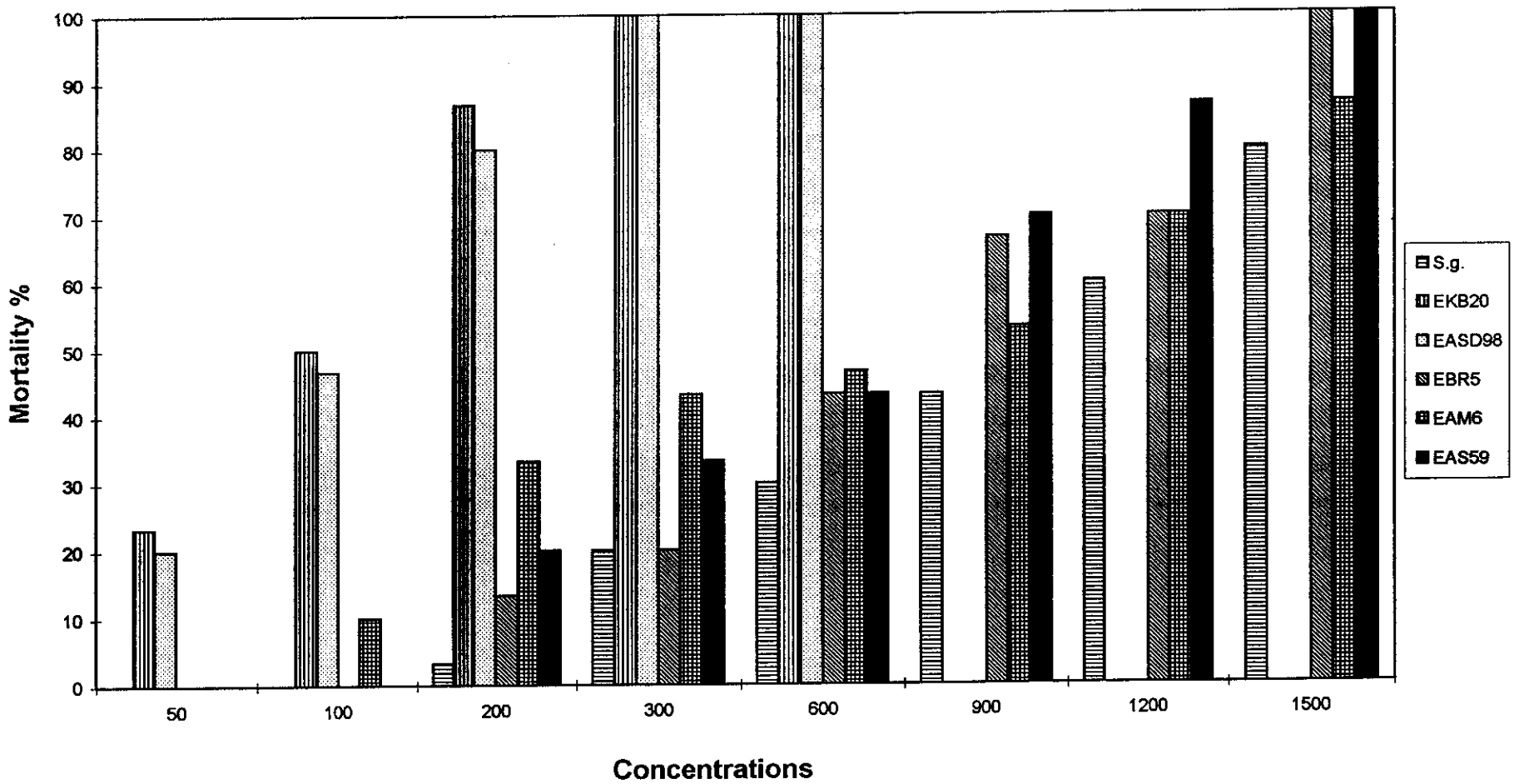


Table (2): Percent mortality of 2nd instar nymphs of *S. gregaria* infected with different concentrations of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EAS59, EBR5 and EAM6.

IJs/nym.	% Mortality at the indicated days after treatment																					
	1 day		2 days						3 days						4 days				Total					
	KB20	AS98	S.g	KB20	AS98	AS59	BR5	AM6	S.g	KB20	AS98	AS59	BR5	AM6	S.g	AS59	BR5	AM6	S.g	KB20	AS98	AS59	BR5	AM6
100	0.00	0.00	0.00	23.3	20.0	0.00	0.00	0.00	0.00	6.70	6.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	30.0	26.7	0.00	0.00	0.00
200	0.00	0.00	0.00	33.3	30.0	0.00	0.00	0.00	0.00	10.0	10.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	43.3	40.0	0.00	0.00	0.00
300	0.00	0.00	0.00	40.0	40.0	20.0	6.70	0.00	0.00	26.7	20.0	3.30	0.00	10.0	6.70	3.30	3.3	10.0	6.70	66.7	60.0	26.7	10.0	20.0
400	0.00	0.00	3..30	40.0	46.7	—	—	—	16.7	30.0	20.0	—	—	—	10.0	—	—	—	30.0	70.0	66.7	—	—	—
500	53.3	43.3	10.0	40.0	36.7	—	—	—	10.0	0.00	0.00	—	—	—	0.00	—	—	—	20.0	93.3	80.0	—	—	—
600	70.0	50.0	16.7	30.0	50.0	20.0	20.0	23.3	0.00	0.00	0.00	20.0	10.0	10.0	10.0	0.00	3.33	0.00	26.7	100	100	40.0	33.3	33.3
900	—	—	30.0	—	—	40.0	40.0	30.0	10.0	—	—	26.7	13.3	20.0	0.00	0.00	0.00	0.00	40.0	—	—	66.7	53.3	50.0
1200	—	—	30.0	—	—	40.0	40.0	30.0	20.0	—	—	30.0	20.0	26.7	6.70	0.00	0.00	0.00	56.7	—	—	70.0	60.0	56.7
1500	—	—	30.0	—	—	700.0	60.0	46.7	30.0	—	—	10.0	20.0	30.0	10.0	0.00	0.00	0.00	70.0	—	—	80.0	80.0	76.7

* Doted lines not tested.

** Control mortality was zero % throughout the time of experiment.

Figure (4): Percent mortality of 2nd instar nymphs of *S. gregaria* infected with different concentrations of *S. glaseri* and *Heterorhabditis* sp.

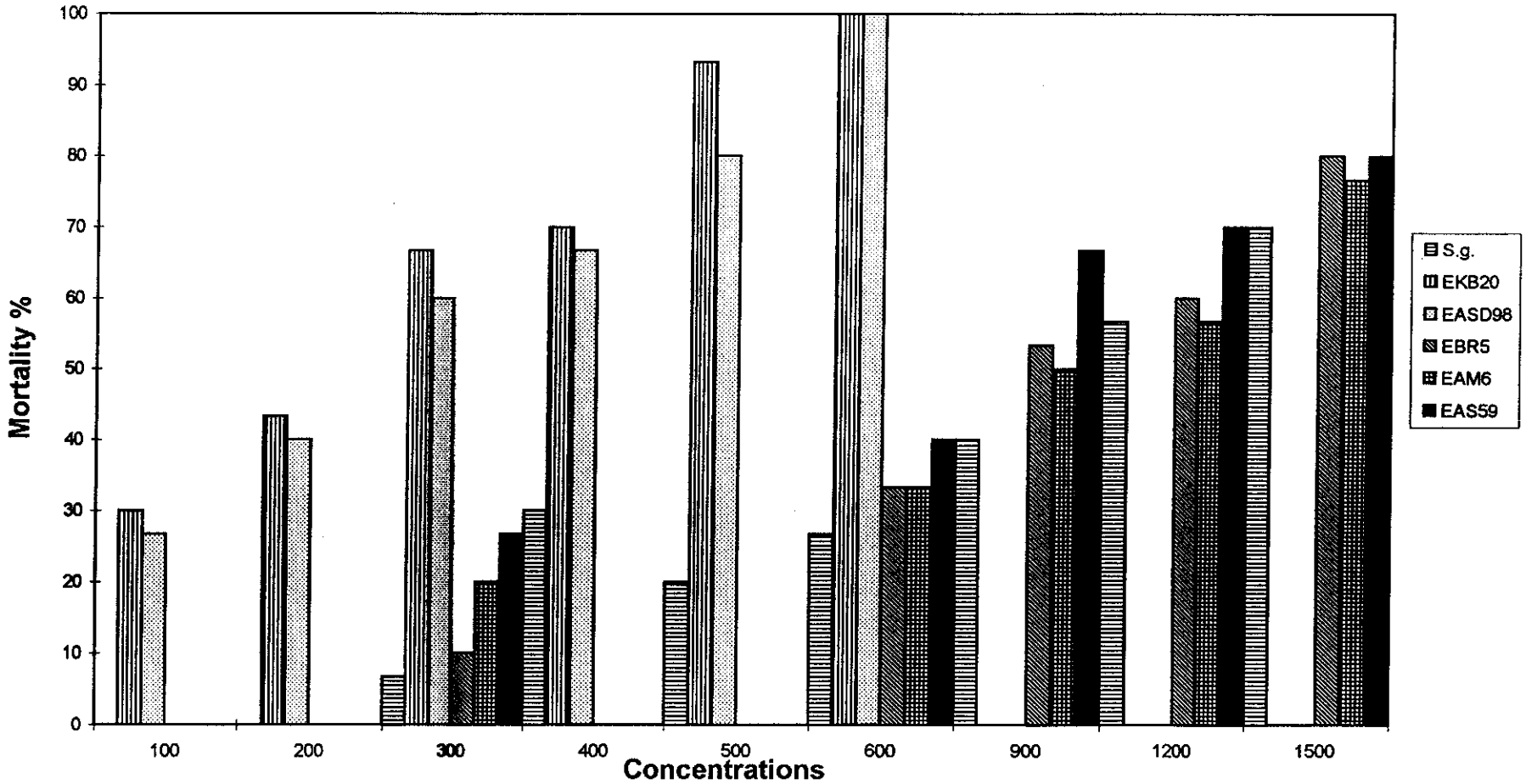


Table (3): Percent mortality of 3rd instar nymphs of *S. gregaria* infected with different concentrations of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EAS59, EBR5 and EAM6.

IJs/nym.	% mortality at the indicated days after treatment																					
	1 day		2 days						3 days						4 days			5days	Total					
	KB20	AS98	S.g.	KB20	AS98	AS59	BR5	AM6	S.g.	KB20	AS98	AS59	BR5	AM6	S.g.	BR5	AM6	S.g.	S.g.	KB20	AS98	AS59	ER5	AM6
100	0.00	0.00	—	20.0	13.3	—	—	—	—	6.70	10.0	—	—	—	—	—	—	—	—	26.7	23.3	—	—	—
200	0.00	0.00	—	20.0	20.0	—	—	—	—	13.3	3.30	—	—	—	—	—	—	—	—	33.3	23.3	—	—	—
300	0.00	0.00	10.0	30.0	30.0	10.0	0.00	0.00	00.0	20.0	16.7	6.70	0.00	10.0	0.00	10.0	0.00	3.30	13.3	50.0	46.7	16.7	10.0	10.0
600	10.0	0.00	0.00	50.0	46.7	16.7	20.0	0.00	10.0	20.0	30.0	10.0	0.00	0.00	0.00	6.70	20.0	0.00	10.0	80.0	76.7	26.6	26.7	20.0
1000	70.0	40.0	20.0	30.0	43.3	43.3	30.0	20.0	0.00	0.00	0.00	3.30	13.3	16.7	30.0	0.00	10.0	0.00	50.0	100	83.3	46.7	43.3	46.7
1500	90.0	90.0	30.0	10.0	10.0	40.0	40.0	36.7	10.0	0.00	0.00	26.7	30.0	16.7	26.7	0.00	10.0	0.00	66.7	100	100	66.7	70.0	63.4
2000	—	—	40.0	—	—	43.3	50.0	50.0	23.3	—	—	36.7	30.0	16.7	0.00	0.00	0.00	6.70	70.0	—	—	80.0	80.0	76.7
3000	100	90.0	40.0	0.00	10.0	60.0	70.0	53.3	40.0	0.00	0.00	26.7	30.0	30.0	6.70	0.00	0.00	0.00	86.7	100	100	86.7	100	83.3
4000	—	—	50.0	—	—	80.0	90.0	70.0	10.3	—	—	20.0	0.00	0.00	10.0	10.0	0.00	0.00	70.3	—	—	100	100	90.0

* Doted lines not tested.

** Control mortality was zero % throughout the time of experiment.

Figure (5): Percent mortality of 3rd instar nymphs of *S. gregaria* infected with different concentrations of *S. glaseri* and *Heterorhabditis* sp.

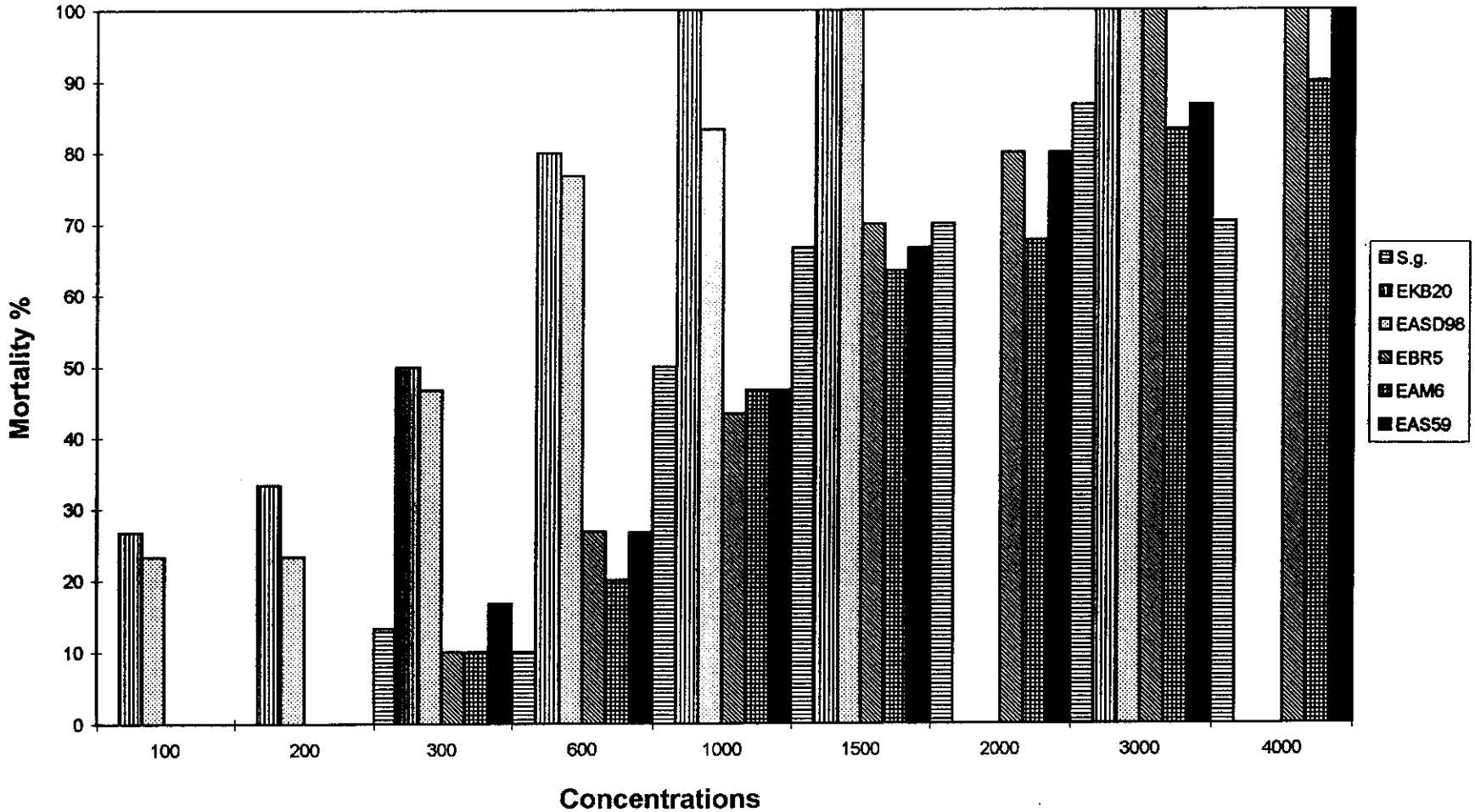


Table (4): Percent mortality of 4th instar nymphs of *S. gregaria* infected with different concentrations of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EAS59, EBR5 and EAM6.

IJs/ny.	% mortality at the indicated days after treatment																							
	1 day		2 days						3 days						4 days					5days	Total					
	KB20	AS98	S.g	KB20	AS98	AS59	BR5	AM6	S.g	KB20	AS98	AS59	BR5	AM6	S.g	KB20	AS.59	BR5	AM6	S.g.	S.g	KB20	AS98	AS59	BR5	AM6
300	0.00	0.00	0.00	10.0	0.00	0.00	0.00	0.00	0.00	3.30	10.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	13.3	10.0	0.00	0.00	0.00
600	0.00	0.00	0.00	30.0	30.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	10.0	0.00	0.00	0.00	0.00	0.00	40.0	30.0	0.00	0.00	0.00
1000	0.00	0.00	0.00	40.0	40.0	20.0	0.00	0.00	0.00	10.0	10.0	20.0	10.0	10.0	6.70	3.30	0.00	10.0	10.0	3.30	10.0	53.3	50.0	40.0	20.0	20.0
1500	0.00	0.00	0.00	53.3	40.0	40.0	20.0	16.7	10.0	20.0	36.7	0.00	10.0	10.0	10.0	6.70	3.30	10.0	10.0	10.0	30.0	80.0	76.7	43.3	40.0	46.7
2000	0.00	10.0	13.3	60.0	50.0	40.0	40.0	30.0	0.00	20.0	26.7	3.30	13.3	16.7	10.0	0.00	3.30	0.00	0.00	10.0	33.3	80.0	86.7	46.7	53.3	46.7
3000	60.0	10.0	36.7	40.0	90.0	56.7	40.0	36.7	10.0	0.00	0.00	3.30	20.0	20.0	10.0	0.00	0.00	0.00	0.00	0.00	56.7	100	100	60.0	60.0	56.7
4000	—	—	0.00	—	—	50.0	43.3	40.0	20.0	—	—	20.0	30.0	23.3	10.0	—	0.00	0.00	0.00	10.0	40.0	—	—	70.0	73.3	63.3

* Doted lines not tested.

** Control mortality was zero % throughout the time of experiment.

Figure (6): Percent mortality of 4th instar nymphs of *S. gregaria* infected with different concentrations of *S. glaseri* and *Heterorhabditis* sp.

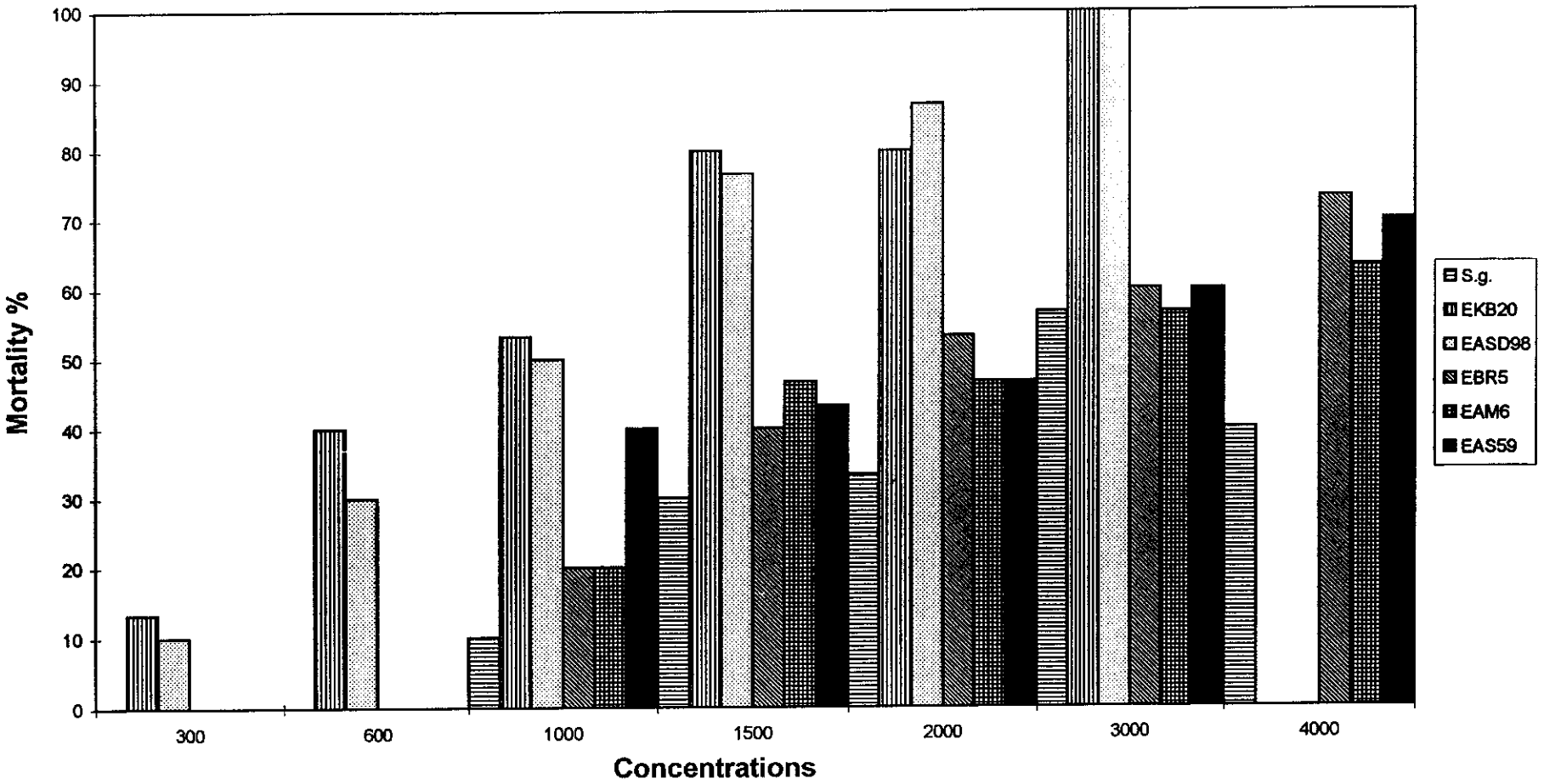


Table (5): Percent mortality of 5th instar nymphs of *S. gregaria* infected with different concentrations of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EAS59, EBR5 and EAM6.

IJs/ny.	% mortality at the indicated days after treatment																							
	1 day		2 days						3 days						4 days				5days	Total					
	KB20	AS98	S.g	KB20	AS98	AS59	BR5	AM6	S.g	KB20	AS98	AS59	BR5	AM6	S.g	AS59	BR5	AM6	S.g.	S.g	KB20	AS98	AS.59	BR5	AM6
300	0.00	0.00	0.00	30.0	30.0	0.00	0.00	0.00	0.00	6.70	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	36.7	30.0	0.00	0.00	0.00
600	0.00	0.00	0.00	40.0	40.0	10.0	20.0	0.00	0.00	16.7	13.3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	56.7	53.3	10.0	20.0	0.00
1000	0.00	0.00	0.00	70.0	60.0	20.0	20.0	0.00	0.00	13.3	20.0	10.0	10.0	16.7	10.0	0.00	3.30	0.00	6.70	16.7	83.3	80.0	30.0	33.3	16.7
1500	40.0	20.0	13.3	60.0	70.0	30.0	26.7	20.0	0.00	0.00	0.00	10.0	10.0	0.00	10.0	3.30	10.0	6.70	10.0	33.3	100	90.0	43.3	46.7	26.7
2000	—	—	10.0	—	—	40.0	40.0	30.0	0.00	—	—	16.7	20.0	10.0	16.7	0.00	0.00	0.00	10.0	36.7	—	—	56.7	60.0	40.0
3000	80.0	70.0	30.0	20.0	30.0	43.3	40.0	40.0	30.0	0.00	0.00	16.7	30.0	20.0	0.00	0.00	0.00	0.00	0.00	60.0	100	100	60.0	70.0	60.0
4000	—	—	30.0	—	—	56.7	63.3	40.0	0.00	—	—	20.0	20.0	33.3	20.3	—	0.00	0.00	0.00	50.3	—	—	76.7	83.3	73.3

* Doted lines not tested.

** Control mortality was zero % throughout the time of experiment.

Figure (7): Percent mortality of 5th instar nymphs of *S. gregaria* infected with different concentrations of *S. glaseri* and *Heterorhabditis* sp.

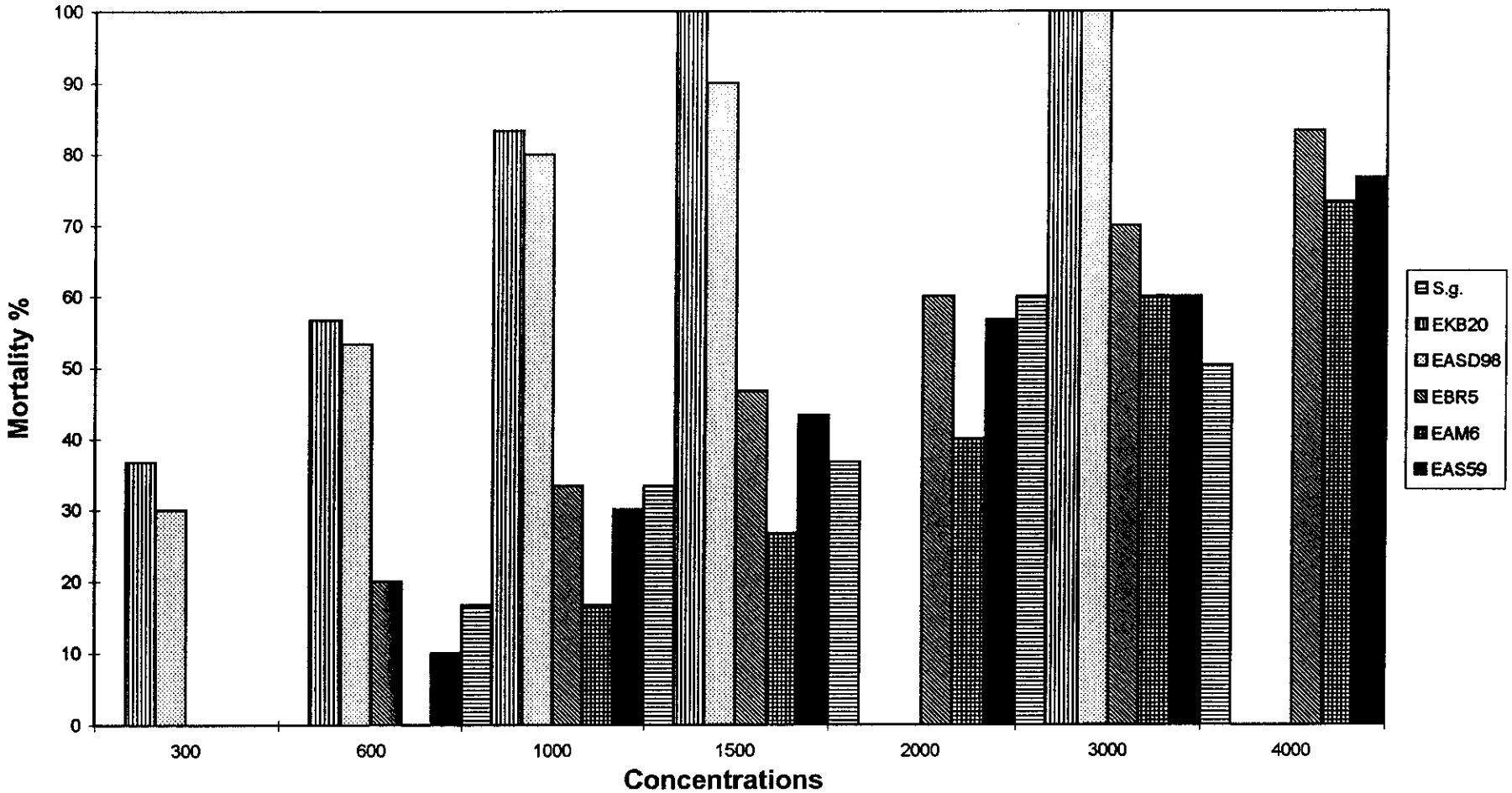


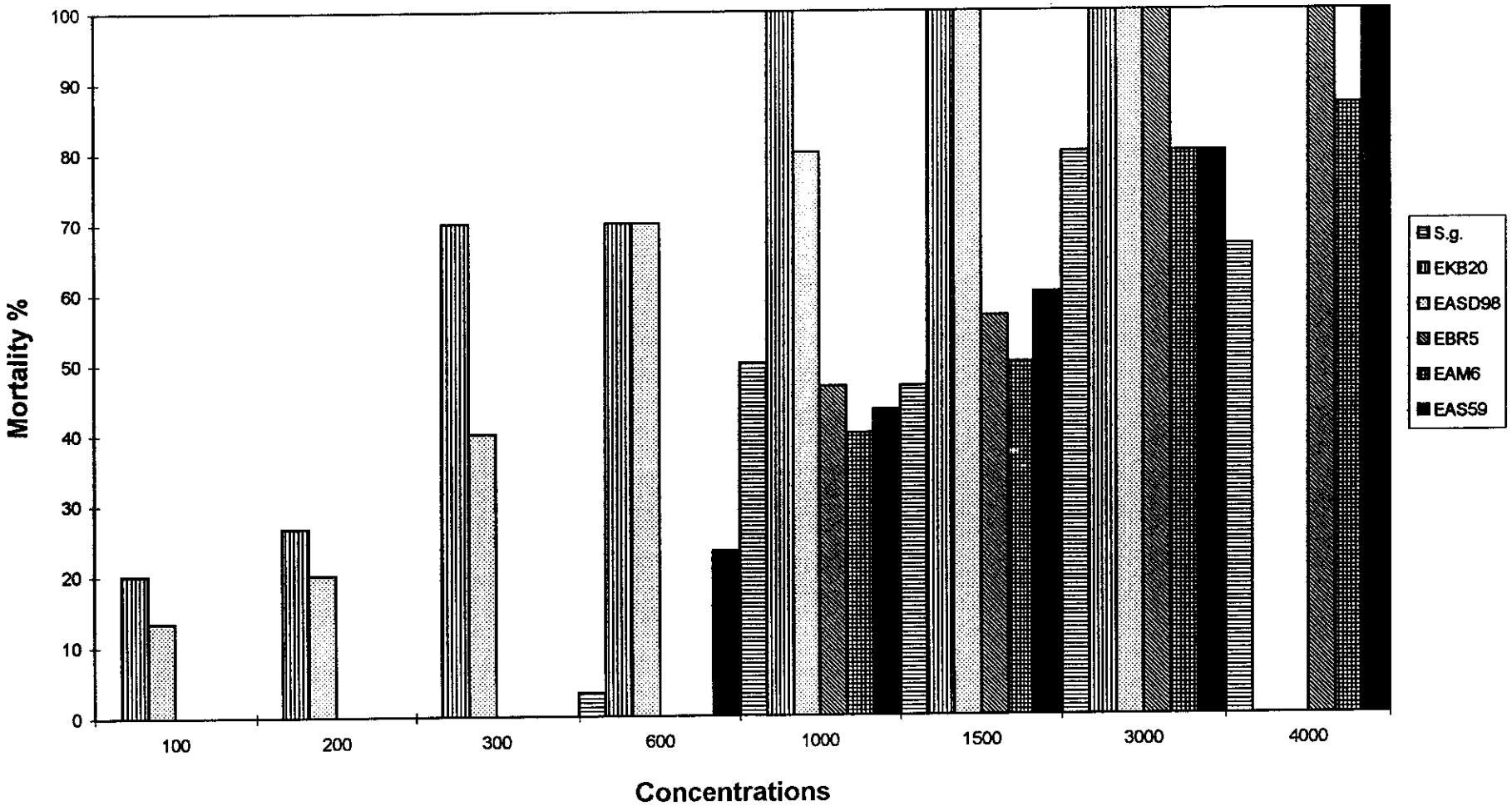
Table (6): Percent mortality of *S. gregaria* adults infected with different concentrations of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EAS59, EBR5 and EAM6.

IJs/nym.	% mortality at the indicated days after treatment																					
	1 day		2 days						3 days						4 days			5days	Total					
	KB20	AS98	S.g.	KB20	AS98	AS59	BR5	AM6	S.g.	KB20	AS98	AS59	BR5	AM6	S.g.	BR5	AM6	S.g.	S.g.	KB20	AS98	AS59	BR5	AM6
100	0.00	0.00	—	20.0	10.0	—	—	—	—	0.00	3.30	—	—	—	—	—	—	—	—	20.0	13.3	—	—	—
200	0.00	0.00	—	20.0	20.0	—	—	—	—	6.70	0.00	—	—	—	—	—	—	—	—	26.7	20.0	—	—	—
300	0.00	0.00	0.00	40.0	30.0	0.00	0.00	0.00	00.0	30.0	10.0	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	70.0	40.0	0.00	0.00	0.00
600	0.00	0.00	0.00	60.0	50.0	13.3	0.00	0.00	3.30	10.0	20.0	10.0	0.00	0.00	0.00	0.00	0.00	0.00	3.30	70.0	70.0	23.3	0.00	0.00
1000	20.0	0.00	30.0	80.0	70.0	40.0	30.0	20.0	0.00	0.00	10.0	3.30	10.0	10.0	10.0	6.70	10.0	10.0	50.0	100	80.0	43.3	46.7	40.0
1500	80.0	90.0	26.7	20.0	10.0	40.0	40.0	30.0	0.00	0.00	0.00	10.0	16.7	13.3	10.0	0.00	6.70	10.0	46.7	100	100	60.0	56.7	50.0
3000	90.0	100	60.0	10.0	0.00	50.0	70.0	60.0	10.0	0.00	0.00	30.0	20.0	20.0	0.00	10.0	0.00	10.0	80.0	100	100	80.0	100	80.0
4000	—	—	36.7	—	—	70.0	80.0	70.0	10.0	—	—	30.0	20.0	16.7	10.0	0.00	0.00	10.0	66.7	—	—	100	100	86.7

* Doted lines not tested.

** Control mortality was zero % throughout the time of experiment.

Figure (8): Percent mortality of *S. gregaria* adults infected with different concentrations of *S. glaseri* and *Heterorhabditis* sp.



infection levels of 300 infective juveniles/insect in the 1st nymphal instar and 600 IJs/insect in the 2nd instar for the two isolates, 1000 & 1500 IJs/insect in the 3rd instar & adult for EKB20 and EASD98 isolates respectively, 3000 IJs/insect in the 4th instar for the two isolates and 1500 &3000 IJs/insect in the 5th instar for EKB20 & EASD98 isolates respectively. The corresponding LC_{50} values were, 94 & 105 for 1st, 220 & 240 for 2nd, 270 & 280 for 3rd 740 & 860 for 4th and 430 &480 IJs/nymph for 5th instar and 280 & 380 IJs/adult for EKB20 & EASD98 respectively (table, 7). In contrast, the mortality percentages induced by the foreign nematode species *S. glaseri*, were irregular and range from, 6.7-70% in the 2nd instar, 10-86.7% in the 3rd instar, 10-56.7% in the 4th instar, 16.7-60% in the 5th instar and 3.3-80% in the adult stage . The highest concentration (4000 IJs/insect) of nematodes used against, 3rd, 4th, 5th nymphal instars as well as the adults induced low mortality. Mortality percentages of the 1st instar nymphs infected with *S. glaseri* were directly proportional to concentrations, as 80 % mortality was achieved at the highest concentration of 1500 IJs/ nymph. *H. indicus* exhibited moderate virulence against the pest whereas *S. glaseri* was, to some extent, the least virulent species among the tested nematodes. In general significant differences ($F= 5.78$, $P< 0.01$) were observed in pathogenicity among the tested nematodes and *Heterorhabditis* species were superior to *S. glaseri* for controlling the insect pest as indicated from LC_{50} values (table, 7).

It was also noticed that, the higher concentrations of nematodes killed the insects much more rapidly than the lower concentrations. Effect of *H. bacteriophora* (EKB20 & EASD98 isolates) was shown up within 24 hr at the higher levels of infection, while the lower levels caused mortality after 48 hr of infection. On the other hand the effect of *S. glaseri* started lately after 48 hr and stopped after 5 days at LC_{50}s of 860, 960, 1400, 2900, 2700, and 1700 IJs/insect for 1st-5th instar nymphs and adults respectively. *H. indicus* was moderate, started its effect after 48 hr of infection and stopped after 4 days.

Table (7): Relative efficiency of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EBR5, EAM6 and EAS59 against *S. gregaria*.

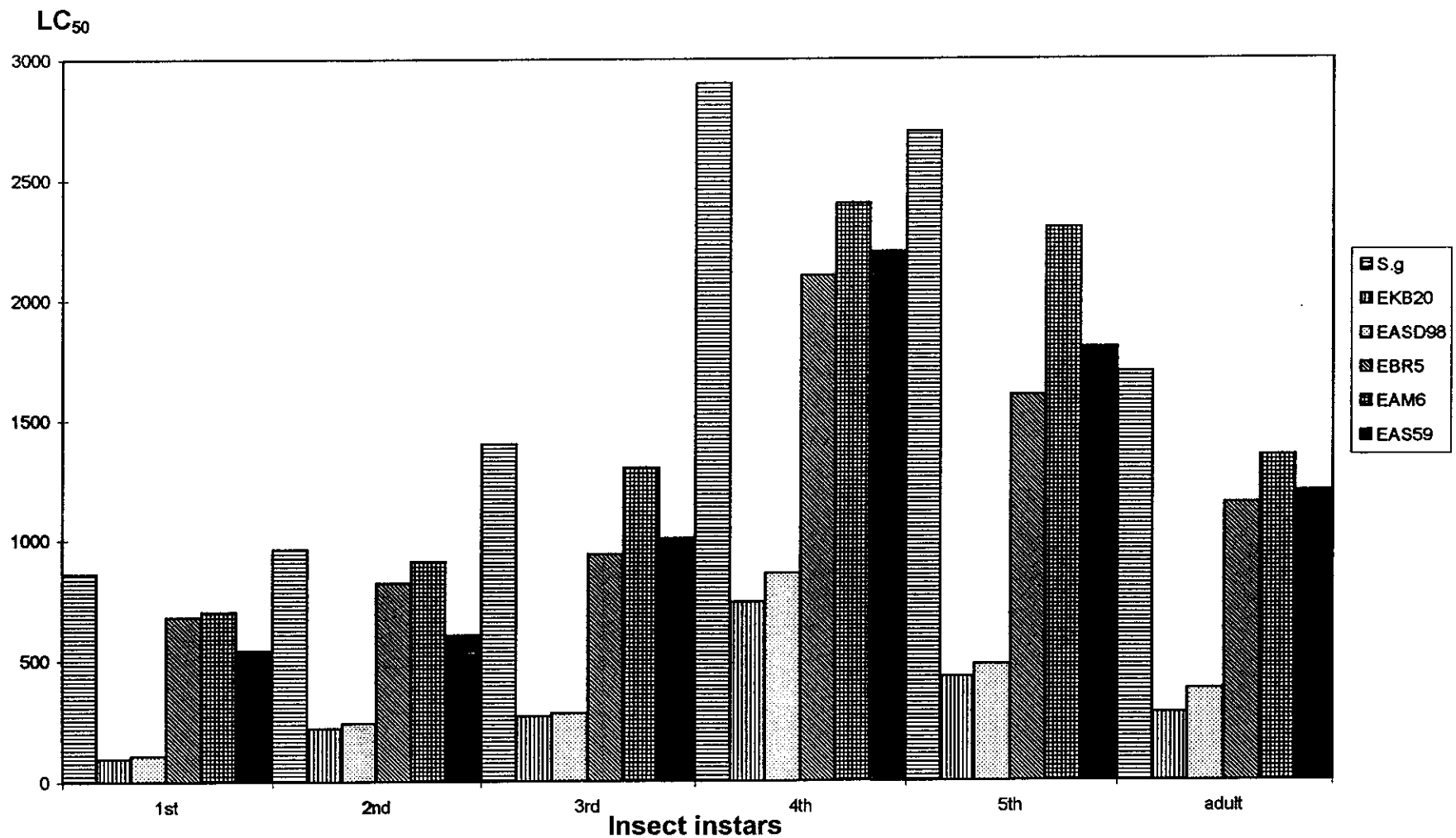
Host instar	S.g.			EKB20			EASD98			EBR5			EAM6			EAS59		
	LC_{50}	(b)*	(r)**	LC_{50}	(b)	(r)	LC_{50}	(b)	(r)	LC_{50}	(b)	(r)	LC_{50}	(b)	(r)	LC_{50}	(b)	(r)
1st	860	3.84	0.981	94.0	1.61	0.914	105	2.00	0.941	680	3.45	0.955	700	3.81	0.960	540	3.24	0.952
2nd	960	3.33	0.998	220	2.07	0.865	240	2.87	0.903	820	2.89	0.967	910	3.12	0.991	605	2.06	0.970
3rd	1400	2.90	0.894	270	2.48	0.937	280	2.51	0.944	940	2.88	0.948	1300	3.33	0.994	1005	3.21	0.956
4th	2900	0.26	0.188	740	2.04	0.91	860	2.26	0.971	2100	2.64	0.976	2400	3.41	0.999	2200	3.11	0.981
5th	2700	1.40	0.748	430	2.13	0.945	480	2.24	0.982	1600	2.51	0.987	2300	3.68	0.995	1800	2.87	0.995
adult	1700	3.27	0.854	280	2.32	0.919	380	2.63	0.939	1150	2.73	0.956	1350	3.12	0.993	1200	2.95	0.959
Total mean	1753 ± 146.8 a			339 ± 39.1 b			390.8 ± 31.0 b			1215 ± 89 c			1493 ± 96.5 a			1225 ± 92.3 c		

(b)* = Slope

(r)** = Correlation Coefficient

*** Means followed by the same letter within the row are not significantly different ($P > 0.05$) according to L.S.D.

Figure (9): Relative efficiency of *S. glaseri* and *Heterorhabditis* sp. against *S. gregaria*.



The observations made on the control experiments in the present study revealed no mortality of the pest used after 5 days from setting up the experiment.

2. Rate of *S. gregaria* mortality by entomopathogenic nematodes (LT_{50}):

Results obtained in table (8) indicated that, significant differences were observed in the rate of insect mortality among the tested nematode species ($F= 60.5$, $P < 0.001$). *H. bacteriophora* (EKB20 isolate) was the quickest to cause insect mortality followed by EASD98 isolate, LT_{50} values were, 15, 16, 16.5, 23, 19.5 & 19 hr for EKB20 isolate and 17, 19, 18.5, 32, 21 & 19 hr respectively for EASD98 isolate. While *S. glaseri* was the slowest, LT_{50}s were, 43, 61, 47, 68, 54 & 49 hr for 1st instar to adults respectively. *H. indicus* exhibited moderate effect. Rate of insect mortality also differed among nematode isolates of the same species, for example, the LT_{50} values of *H. bateriophora* (EBR5 & EAM6 isolates) were higher than that of the other two isolates.

3. Establishment:

From table (9) it can be stated that, the number of infective juveniles that successfully established in *S. gregaria* depended on host instar, and differed significantly ($F= 5.12$, $P < 0.01$) among nematode species and isolates. *S. gregaria* nymphs and adults proved to be more susceptible to invasion by *H. bacteriophora* than the other species tested; a mean of 13.7/3rd, 27.6/4th & 36/5th instar and 41.7 nematodes/adult were found in cadavers treated with EKB20 isolate. Followed by EASD98 isolate, where the number of IJs established per cadaver was 11.1, 24.6, 28.2 and 40.5 nematodes for 3rd, 4th, 5th instar and adult respectively.

Table (8): Estimated hours to kill 50 % (LT_{50}) of *S. gregaria* infected with *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EBR5, EAM6 and EAS59.

Host instar	LT_{50s} in hours					
	S.g.	**EKB20**	**EASD98**	**EBR5**	**EAM6**	**EAS59**
1st	43.0	15.0	17.0	39.0	40.0	37.0
2nd	61.0	16.0	19.0	52.0	55.0	44.0
3rd	47.0	16.5	18.5	41.0	45.0	40.0
4th	68.0	23.0	32.0	58.0	61.0	55.0
5th	54.0	19.5	21.0	52.0	53.0	49.0
adult	49	19.0	19.0	46.0	47.0	43.0
Total mean	53.7 ± 3.8 a	18.2 ± 1.18 b	21.1 ± 2.24 b	48.0 ± 2.98 a	50.2 ± 3.1 a	44.7 ± 2.6 c

* Means followed by the same letter within the row are not significantly different ($P > 0.05$) according to L.S.D.

Figure (10): Estimated hours to kill 50% (LT_{50}) of *S. gregaria* infected with *S. glaseri* and *Heterorhabditis* sp.

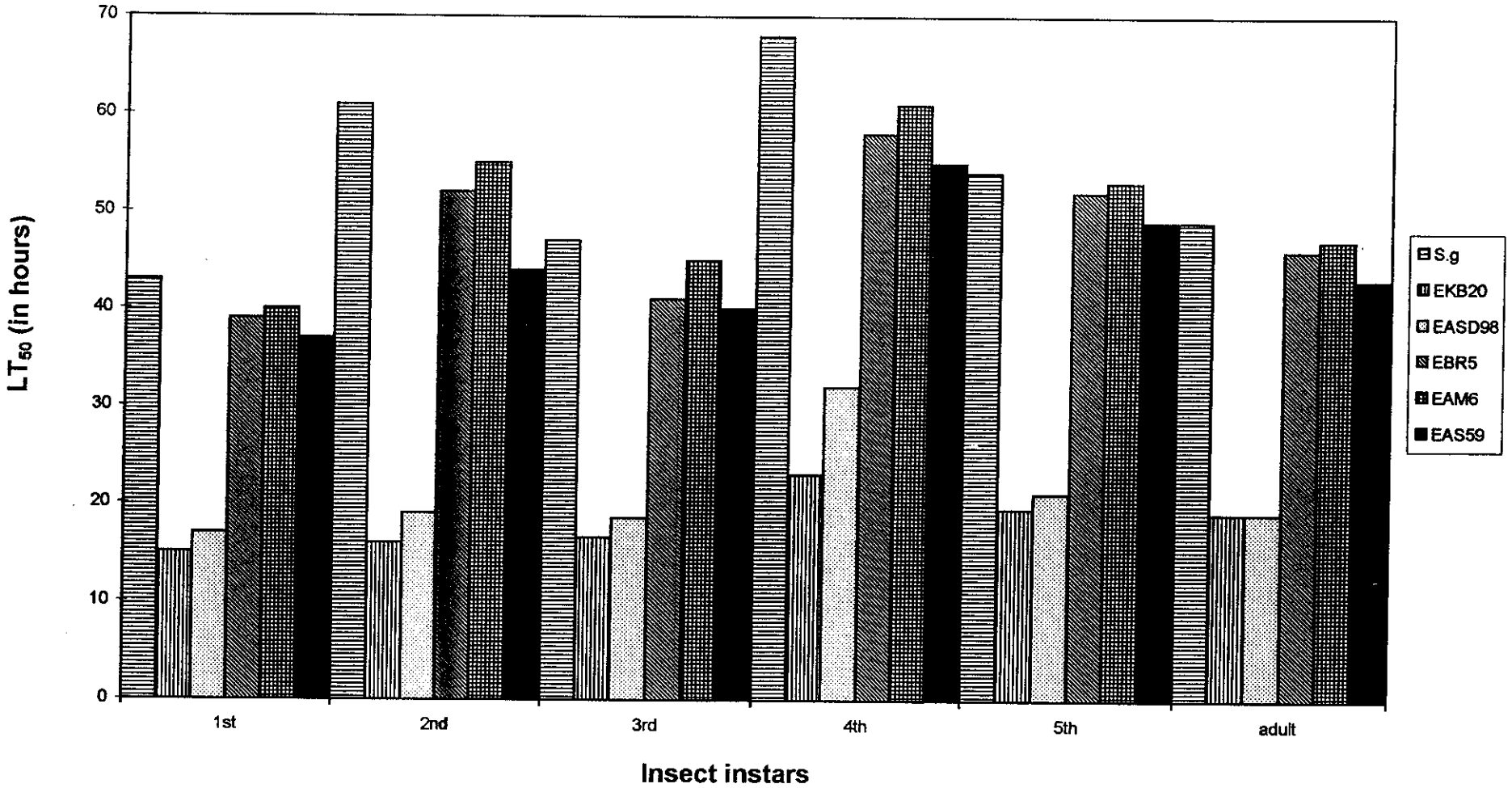


Table (9): Establishment of *S. glaseri* and *Heterorhabditis* sp. in *S. gregaria* nymphs and adults at an inoculum level of 3000 IJs/insect.

Host instar	Mean number of nematodes established /cadaver ± S.E.					
	S.g.	EKB20	EASD98	EBR5	EAM6	EAS59
3rd	1.60 ± 0.10	13.7 ± 2.01	11.1 ± 1.10	7.60 ± 1.80	7.40 ± 0.90	4.40 ± 0.50
4th	5.90 ± 0.30	27.6 ± 1.90	24.6 ± 1.60	9.20 ± 1.30	7.70 ± 0.40	6.20 ± 0.70
5th	8.00 ± 1.01	36.0 ± 3.01	28.2 ± 2.02	19.80 ± 0.90	9.10 ± 1.03	8.80 ± 1.10
adult	12.6 ± 2.10	41.7 ± 2.90	40.5 ± 3.20	23.1 ± 1.70	18.8 ± 1.05	15.3 ± 2.20
Total mean	7.0 ± 2.3 a	29.8 ± 4.1 b	26.1 ± 3.05 b	14.9 ± 1.3 c	10.8 ± 2.1 a	8.7 ± 2.4 a

* Means followed by the same letter within the row are not significantly different ($P > 0.05$) according to L.S.D.

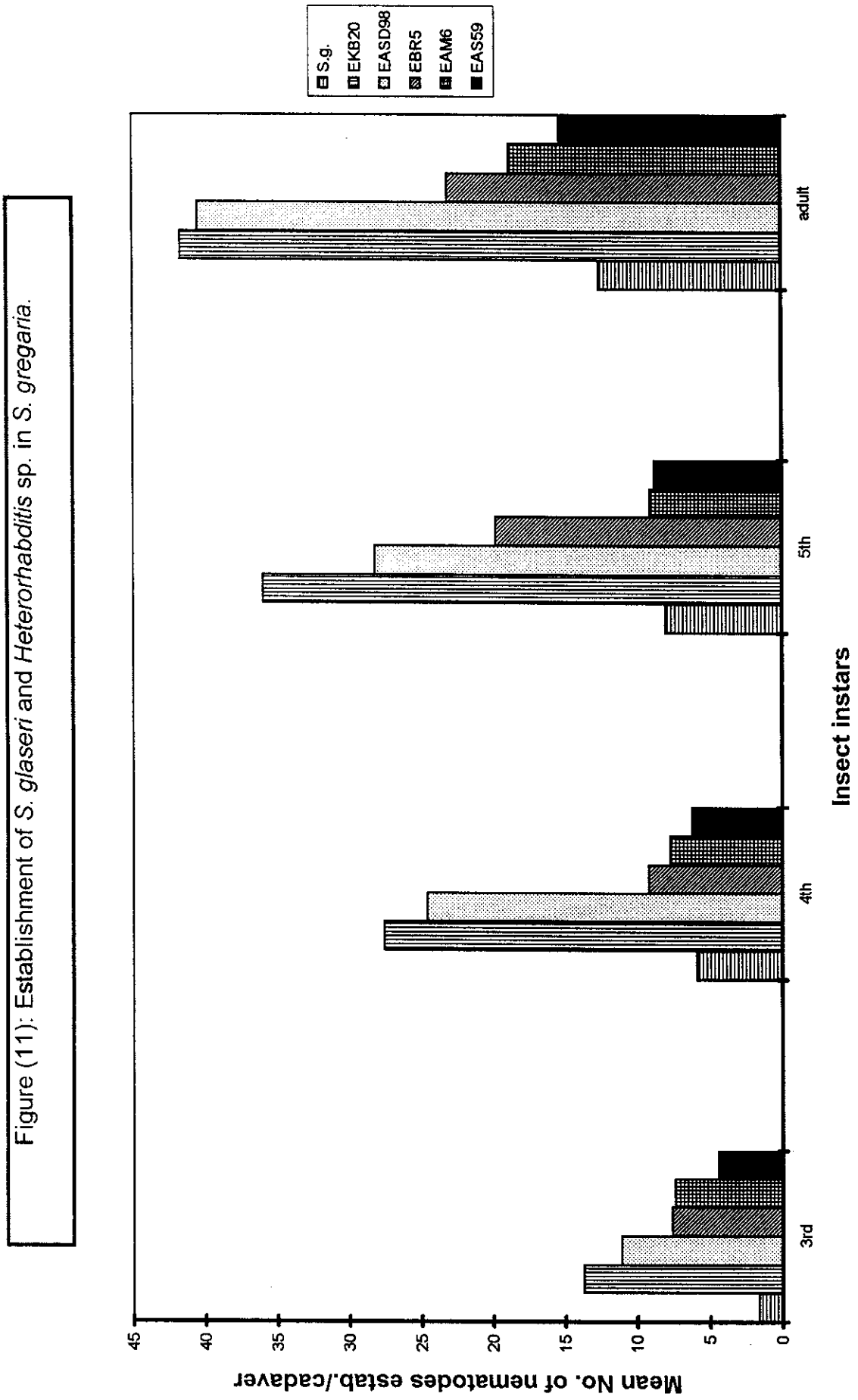


Figure (11): Establishment of *S. glaseri* and *Heterorhabditis* sp. in *S. gregaria*.

Establishment was excellent in adult, poorer in 4th and 5th instar nymphs and much reduced in the 3rd instar especially in nymphs treated with *S. glaseri*, the number of nematodes established was 1.6/nymph.

4. Nematode propagation:

Data in tables (10-13) show the number of infective juveniles (IJs) per cadaver of *S. glaseri* and *Heterohrabditis* sp. that reproduced and migrated after 20 days of treatment by different concentrations.

The number of IJs emerging from *S. gregaria* nymphs and adults varied significantly among the tested nematode species. *H. bacteriophora* reproduced better than *H. indicus* and *S. glaseri*. Results show also that, the yield of nematodes per cadaver at different inoculum levels was irregular, and the highest inoculum levels produced small yield of nematodes in some cases.

Superior merit was manifested by the nematode isolate EKB20 for the highest numbers of its emerging IJs , one adult cadaver could produce from about 668880 to > 1,500,000 infective nematode juveniles. From 382800 to 688000, 288000 to 551300 & from 31900 to 91500 IJs per 5th, 4th and 3rd instar cadaver respectively, such attribute of the nematode isolate should be considered in making final determination for the isolate might be selected for further improvement. **The isolate EASD98 produced the second highest** numbers of IJs : more than 850000 infectives per adult cadaver were produced at an infection level of 300 IJs /insect. 613180 IJs/5th, 423760 IJs /4th and 52640 IJs /3rd instar nymph cadaver at an inoculum level of 1000 IJs /nymph.

In contrast, *S. glaseri* showed the least reproductive potential, the maximum number of IJs produced was 71500/adult cadaver and 56980/5th instar cadaver at infection levels of 1000 & 1500 IJs /insect respectively, and 38300 & 4100 per 4th and 3rd instar cadavers resp. at an infection level of 3000 IJs /nymph.

Table (10): Rate of migration of infective juveniles (IJs) of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EBR5, EAM6 and EAS59 emerging from the 3rd nymphal instar of *S. gregaria* at different inoculum levels.

IJs/nymph	Mean number of IJs emerging from one cadaver ± S.E. X 10^3					
	S.g.	EKB20	EASD98	EBR5	EAM6	EAS59
300	2.00 ± 0.21	59.00 ± 3.40	24.36 ± 1.56	21.00 ± 3.1	6.500 ± 0.08	9.00 ± 1.04
600	3.50 ± 0.17	70.00 ± 2.76	20.25 ± 2.10	22.35 ± 2.7	25.67 ± 3.2	14.55 ± 0.99
1000	3.10 ± 0.45	91.50 ± 1.99	52.64 ± 3.02	30.17 ± 1.87	20.80 ± 2.78	19.50 ± 2.1
1500	3.23 ± 0.33	84.00 ± 3.11	41.50 ± 1.87	29.31 ± 1.49	27.88 ± 1.85	17.80 ± 1.75
3000	4.10 ± 0.89	31.90 ± 4.04	25.95 ± 2.40	50.93 ± 2.2	36.12 ± 3.01	24.20 ± 2.68
Total mean	3.186 ± 0.51 a	67.20 ± 9.4 b	32.94 ± 2.4 c	30.8 ± 4.7 cd	23.4 ± 3.3 de	17.0 ± 2.2 e

* F= 35.11, P < 0.001

** Means followed by the same letter within the row are not significantly different (P > 0.05) according to L.S.D.

Figure (12): Rate of migration of IJs of *S. glaseri* and *Heterorhabditis* sp. emerging f rom 3rd instar nymphs of *S. gregaria*.

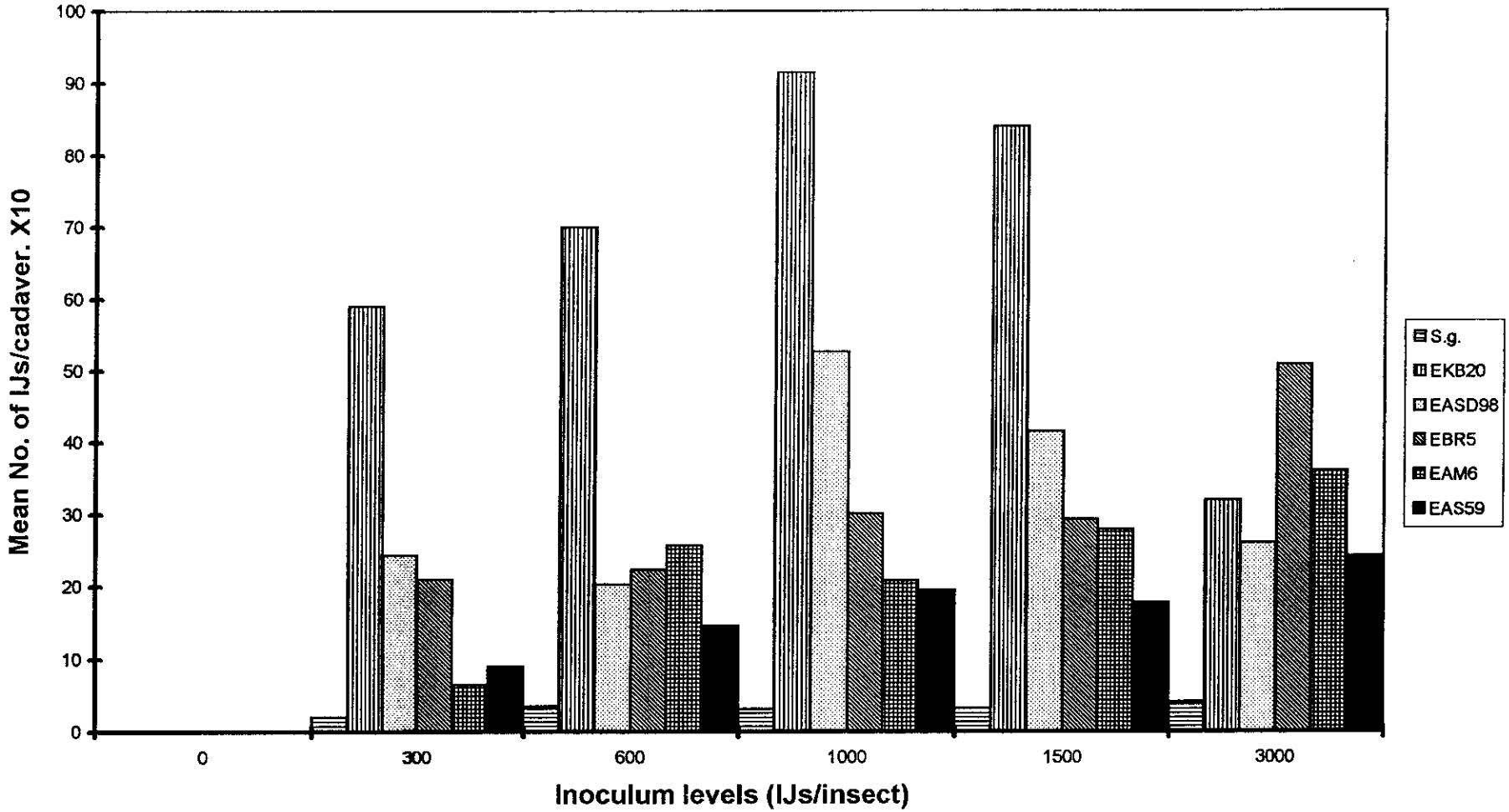


Table (11): Rate of migration of infective juveniles (IJs) of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EBR5, EAM6 and EAS59 emerging from the 4th nymphal instar of *S. gregaria* at different inoculum level.

IJs/nymph	Mean number of IJs emerging from one insect cadaver ± S.E. X 10^4					
	S.g.	EKB20	EASD98	EBR5	EAM6	EAS59
300	0.00	28.800 ± 2.3	25.600 ± 1.9	0.00	0.00	0.00
600	0.00	32.315 ± 2.1	31.478 ± 3.7	0.00	0.00	0.00
1000	2.10 ± 0.80	54.000 ± 3.4	42.376 ± 2.5	11.277 ± 1.30	11.700 ± 0.67	3.045 ± 0.05
1500	3.56 ± 0.40	55.130 ± 1.9	38.557 ± 1.3	12.158 ± 2.40	11.845 ± 1.8	4.252 ± 0.11
2000	3.70 ± 0.88	44.198 ± 2.5	39.440 ± 5.6	12.242 ± 0.99	12.331 ± 2.2	4.510 ± 1.02
3000	3.83 ± 0.20	39.838 ± 3.7	30.910 ± 2.4	17.302 ± 3.10	13.512 ± 3.4	4.936 ± 0.80
Total mean	2.19 ± 0.7 a	42.38 ± 4.35 b	34.73 ± 2.55 b	8.83 ± 1.86 c	8.23 ± 2.56 ac	2.790 ± 0.9 a

* F= 40.86, P < 0.001

** Means followed by the same letter within the row are not significantly different (P > 0.05) according to L.S.D.

Figure (13): Rate of migration of IJs of *S. glaseri* and *Heterorhabditis* sp. emerging from 4th instar nymphs of *S. gregaria*.

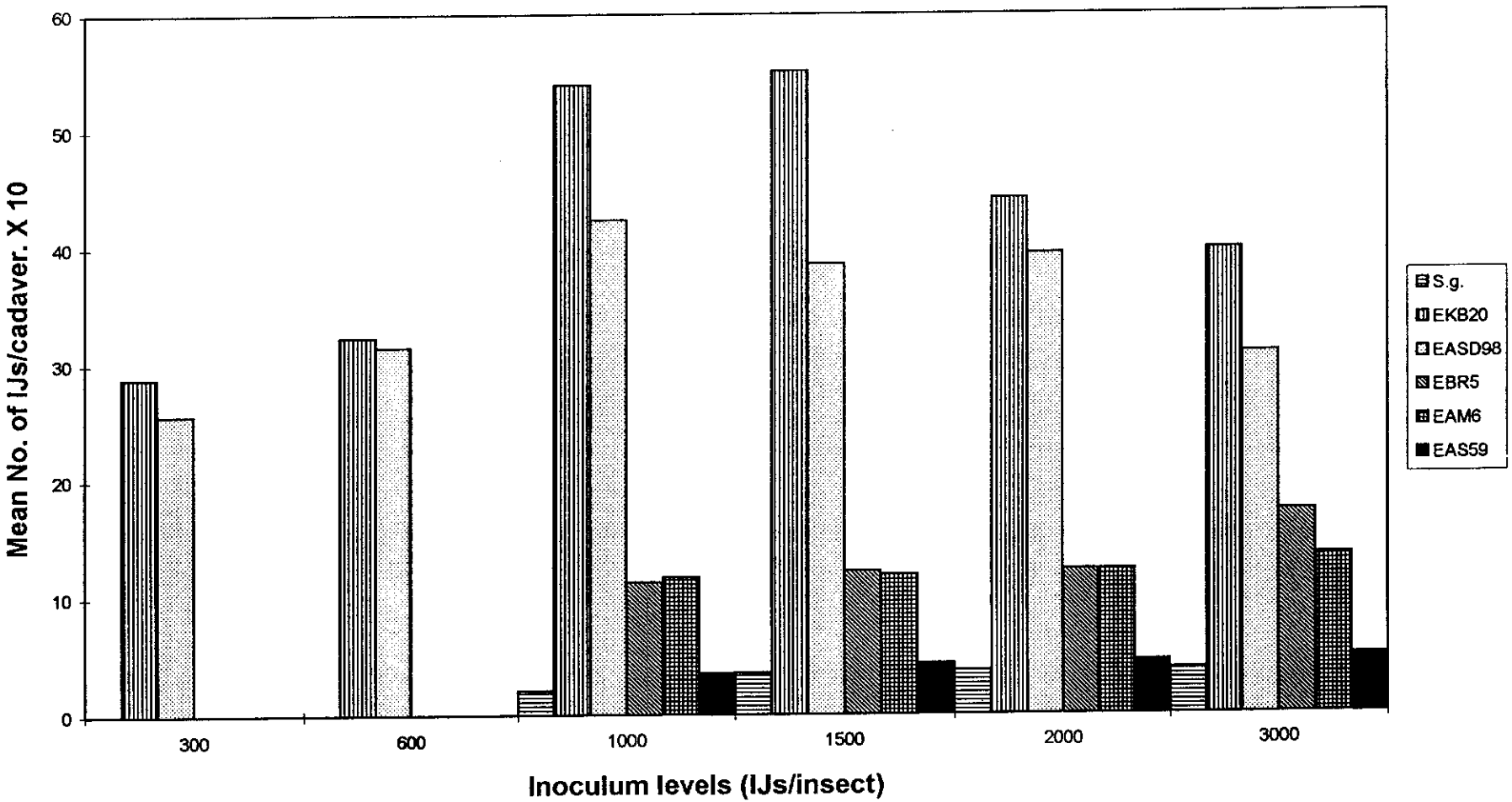


Table (12): Rate of migration of infective juveniles (IJs) of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EBR5, EAM6 and EAS59 emerging from the 5th nymphal instar of *S. gregaria* at different inoculum levels.

IJs/nymph	Mean number of IJs emerging from one insect cadaver ± S.E. X 10^4					
	S.g.	**EKB20**	**EASD98**	**EBR5**	**EAM6**	**EAS59**
300	0.00	38.28 ± 3.1	34.667 ± 1.9	0.00	0.00	0.00
600	0.00	54.52 ± 2.65	38.920 ± 2.43	14.618 ± 1.5	0.00	5.80 ± 1.01
1000	4.210 ± 0.33	66.70 ± 4.6	61.318 ± 2.13	17.970 ± 3.12	12.403 ± 1.43	7.32 ± 0.45
1500	5.698 ± 1.2	68.80 ± 3.21	47.407 ± 1.89	18.141 ± 2.4	12.685 ± 0.77	6.875 ± 0.29
3000	5.130 ± 0.7	57.72 ± 1.78	45.264 ± 4.31	19.235 ± 0.88	15. 01 ± 2.30	7.688 ± 0.99
Total mean	3.00 ± 1.3 a	57.2 ± 5.5 b	45.5 ± 4.6 c	13.99 ± 3.64 d	8.00 ± 3.3 ad	5.54 + 1.4 a

* F= 41.42, P < 0.001

** Means followed by the same letter within the row are not significantly different (P > 0.05) according to L.S.D.

Figure (14): Rate of migration of IJs of *S. glaseri* and *Heterorhabditis* sp. emerging from 5th instar nymphs of *S. gregaria*.

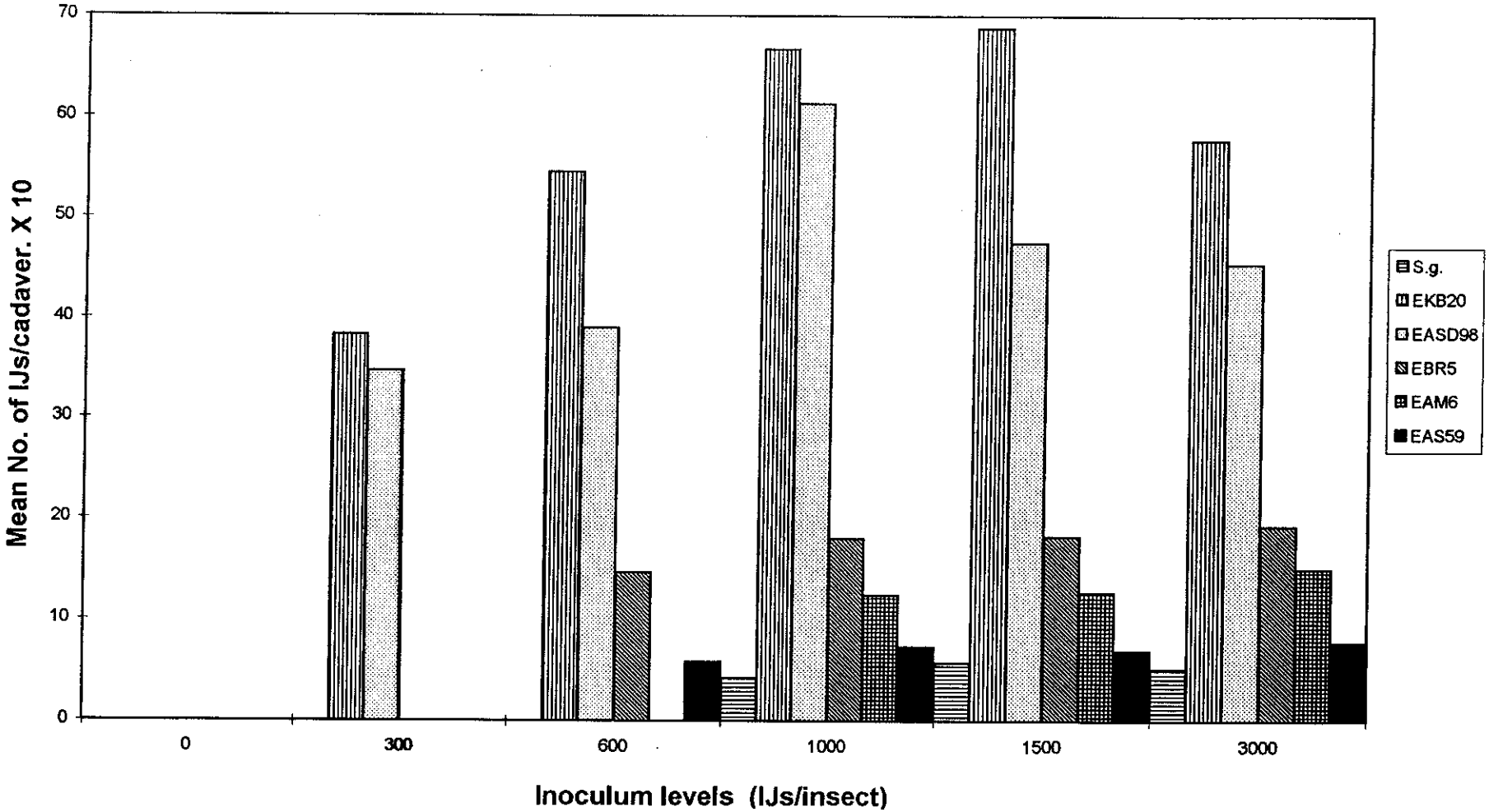


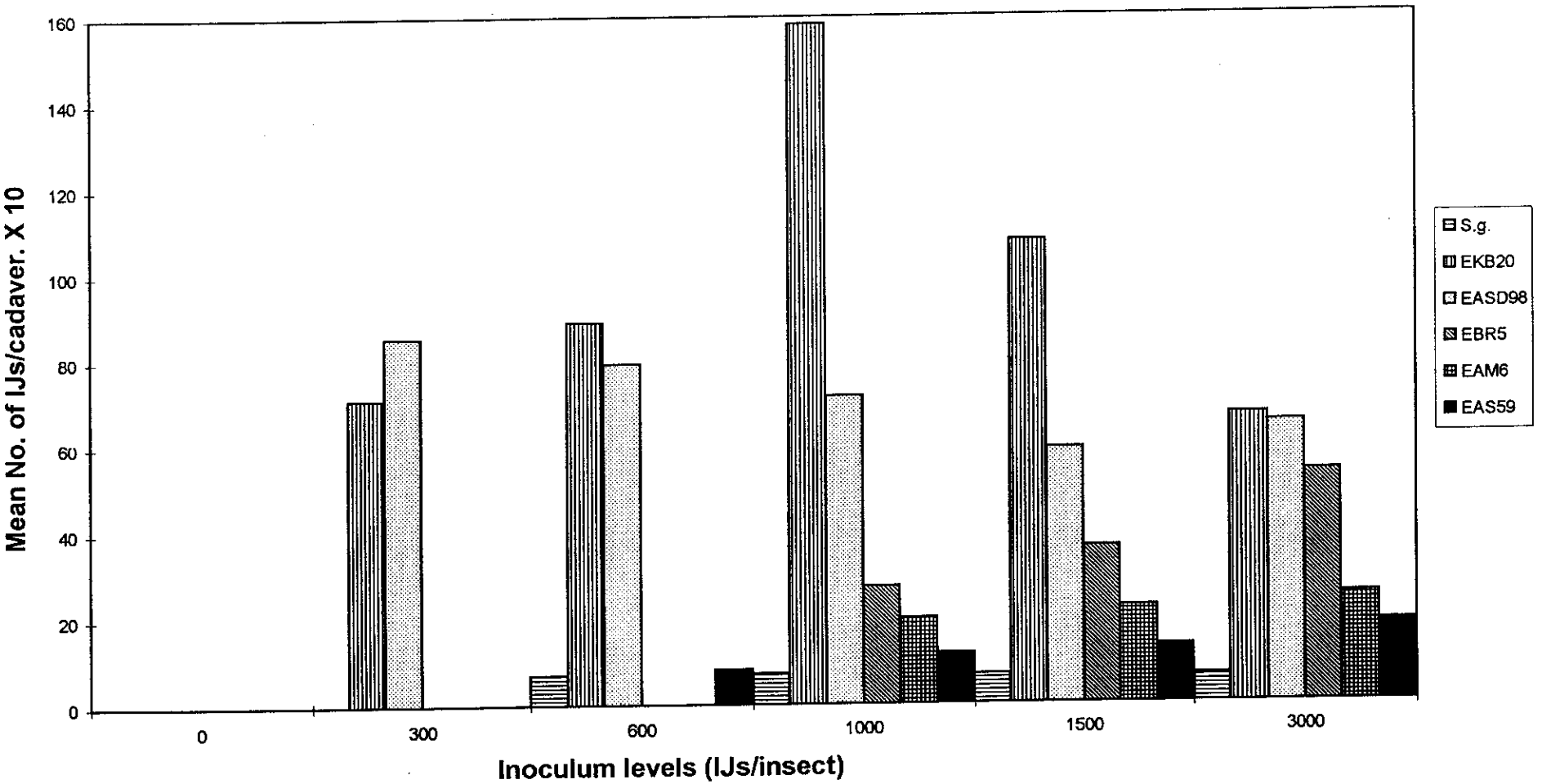
Table (13): Rate of migration of infective juveniles (IJs) of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EBR5, EAM6 and EAS59 emerging from *S. gregaria* adults at different inoculum levels.

IJs/adult	Mean number of IJs emerging from one insect cadaver ± S.E. X 10^4					
	S.g.	EKB20	EASD98	EBR5	EAM6	EAS59
300	0.00	71.022 ± 4.5	85.372 ± 3.45	0.00	0.00	0.00
600	6.98 ± 0.57	88.902 ± 2.89	79.137 ± 2.99	0.00	0.00	8.135 ± 1.02
1000	7.15 ± 1.3	158.25 ± 3.12	71.47 ± 4.01	27.075 ± 2.3	19.794 ± 2.1	11.552 ± 0.96
1500	6.73 ± 0.89	107.66 ± 4.21	59.079 ± 2.46	36.131 ± 1.1	22.226 ± 1.3	13.324 ± 0.44
3000	6.30 ± 0.45	66.888 ± 1.99	64.93 ± 1.87	53.345 ± 3.1	24.954 ± 4.3	18.514 ± 2.14
Total mean	5.43 ± 1.38 **a**	98.54 ± 6.8 b	71.99 ± 4.8 c	23.3 ± 3.8 d	13.4 ± 2.5 e	10.30 ± 3.1 a

* F= 20.01, P < 0.001

** Means followed by the same letter within the row are not significantly different (P > 0.05) according to L.S.D.

Figure (15): Rate of migration of IJs of *S. glaseri* and *Heterorhabditis* sp. emerging from *S. gregaria* adults.



The reproductive potential of *H. indicus* (EAS59 isolate) was mediate between *H. bacteriophora* and *S. glaseri.*

It was also noticed that, the number of emerging IJs varied greatly among the different nematode isolates of the same species.

5. Effects of temperature:

5.1. Effect of temperature on survival:

Analysis of variance showed significant differences ($F= 9.72$, $P < 0.01$) in survival among the tested nematodes and the isolate EBR5 was the most affected followed by EAM6 isolate table (14). The rate of nematode survival was related to temperature used as well as to the exposure time except for *S. glaseri* which was not affected by temperature throughout the experiment (it recorded 100% survival). 100% of the infective juveniles (IJs) of all nematode species were survived after exposure to -5 ^{0}C for 30 minutes except those of EBR5 isolate where only 70% remain alive. All IJs of EASD98 and EAS59 survived after increasing the exposure time to 60 min. while the EKB20, EBR5 and EAM6 isolates were recorded 75, 36 and 46% survival respectively. By increasing the exposure time to 150 min., only EASD98 isolate was recorded 99% survival, while the other nematode species showed a considerable reduction in their survival rates.

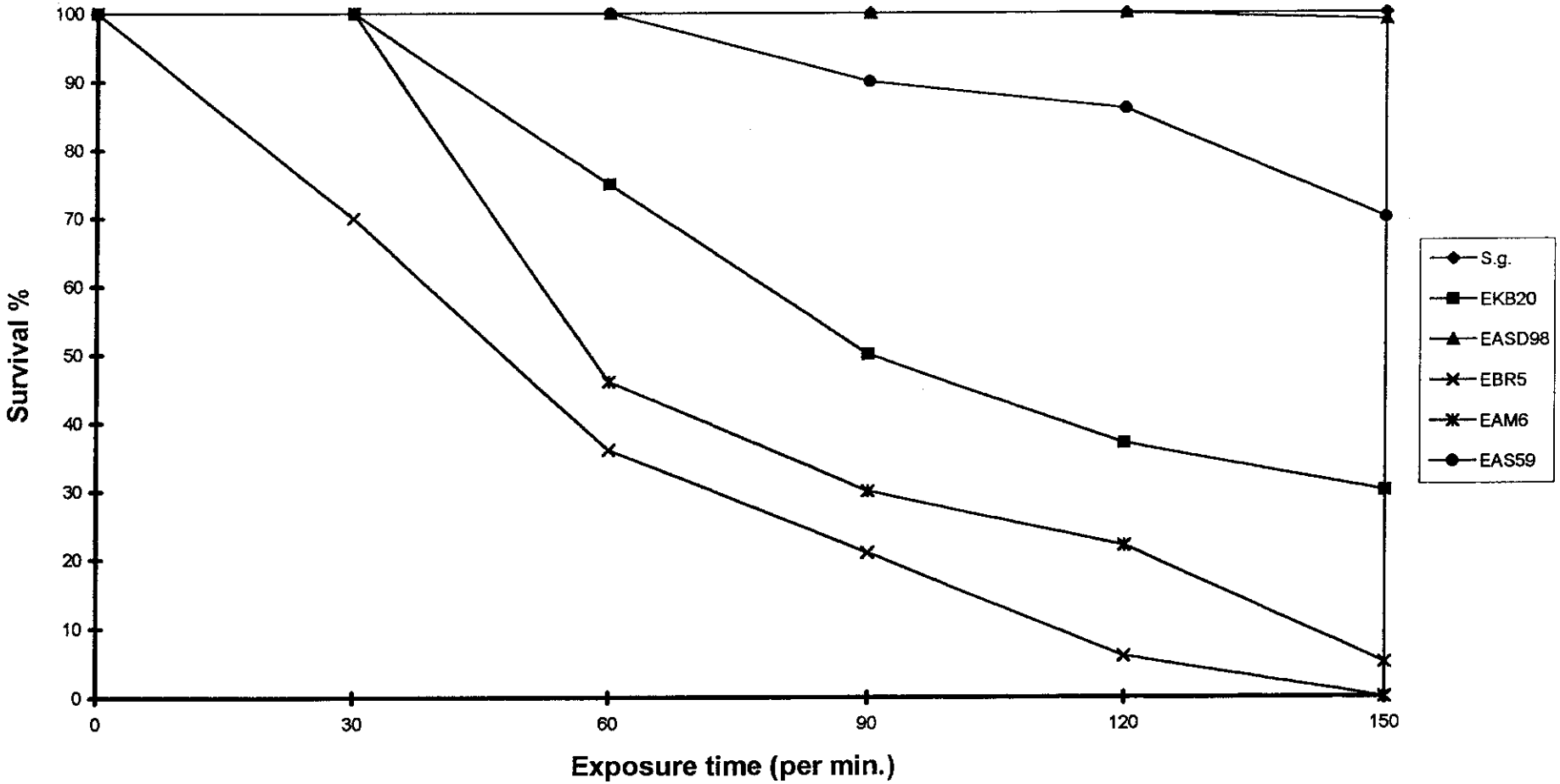
Survival of IJs of *S. glaseri* was not affected by temperature when exposed to temperatures ranging from 0-40 ^{0}C for 5 days (table, 15) except at a temperature of 40 ^{0}C, 71% of IJs were survived after 5 days. By increasing the exposure time, a reduction in the rate of survival was obtained, and was much evident at 40 ^{0}C after 15 days of exposure, it was recorded 3% survival.

Table (14): Effect of -5 ^{0}C on the survival of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EAS98, EBR5 EAM6 and EAS59 at different exposure times per minutes

exposure time/min	Survival % at - 5 ^{0}C					
	S.g	**EKB20**	**EASD98**	**EBR5**	**EAM6**	**EAS59**
30	100	100	100	70	100	100
60	100	75	100	36	46	100
90	100	50	100	21	30	90
120	100	37	100	6	22	86
150	100	30	99	0	5	70

* Significant differences in survival were observed among the tested nematodes at the tested temperature ($F = 9.72$, $P < 0.01$).

Figure (16): Effect of -5 ^{0}C on the survival of *S. glaseri* and *Heterorhabditis* sp. at different exposure times.



Table(15): Effect of different temperatures on the survival of *S. glaseri* at different time intervals (per day).

temp.(°C)	Survival %					
	1 day	2 days	3 days	5 days	10 days	15 days
0	100	100	100	100	100	98
10	100	100	100	100	98	91
20	100	100	100	100	95	75
25	100	100	100	100	90	77
30	100	100	100	100	88	74
35	100	100	100	100	76	45
40	100	100	100	71	18	3

Table (16) illustrated that, EASD98 and EAS59 isolates were not affected by temperatures ranging from 0-30 ^{0}C for 48 hr , while the nematode survival was decreased to 93 & 49% for EASD98 and to 80 & 20% for EAS59 isolate as the temperature increased to 35 & 40 ^{0}C respectively. A full mortality percentages were recorded for the 3 nematode isolates, EKB20, EASD98 & EAS59 at the highest tested temperature (40 ^{0}C) after 5 days, while the two isolates EBR5 & EAM6 were recorded full mortality percentages at the highest two temperatures (35 & 40 ^{0}C) after 72 & 48 hr respectively.

From tables (15 & 16) it was clear that, significant differences ($F= 6.51$, $P < 0.01$) were observed in survival among the tested nematodes at the tested temperatures and the most affected isolates were EBR5 and EAM6. Survival of heterorhabditid nematode isolates was greater at the higher temperatures from 20-30 ^{0}C than at 0 and 10 ^{0}C throughout the experiment. Optimal survival was achieved at 25 ^{0}C followed by 30, 20, 10 &0 ^{0}C. In contrast, *S. glaseri* survival was greater at the lower temperatures, 0 and 10 ^{0}C than at the higher ones throughout the 15 days of experiment.

5.2. Effect of temperature on pathogenicity:

As shown from table (17) significant differences ($F= 3.41$, $P < 0.05$) in pathogenicity of different nematode species and isolates at the tested temperatures were observed. Pathogenicity was increased as the incubation temperature increased from 15-30 ^{0}C but declined at the higher temperatures. These tests showed a trend similar to survival, *S. glaseri* infected hosts at the widest temperature range (10-37 ^{0}C), pathogenicity was greater at higher temperature ranges (20-37 ^{0}C) than at the lowest tested temperature of 10 ^{0}C, while *H. indicus* (EAS59 isolate) infected hosts at the narrowest range (15-30 ^{0}C). *H. bacteriophora* (EKB20 & EASD98 isolates) killed insects between 15 and 35 ^{0}C. In general, heterorhabditid nematode pathogenicities were greater

Table (16): Effect of different temperatures on survival of the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EBR5, EAM6 and EAS59 at different time intervals (per days).

Temp. (°C)	Survival % after the indicated days of exposure																											
	EKB20						EASD98						EBR5						EAM6						EAS59			
	1d	2d	3d	5d	10d	15d	1d	2d	3d	5d	10d	15d	1d	2d	3d	5d	10d	15d	1d	2d	3d	5d	10d	15d	1d	2d	3d	5d	10d	15d
0	80	50	30	12	0	0	100	100	90	77	26	0	76	49	15	0	0	0	78	54	41	11	0	0	100	100	82	40	6	0
10	100	95	86	75	59	10	100	100	95	80	70	21	100	93	61	60	50	0	100	96	85	77	65	20	100	100	90	60	54	0
20	100	100	95	85	80	78	100	100	100	98	96	80	100	100	100	88	75	68	100	100	89	75	68	61	100	100	96	90	86	76
25	100	100	100	100	96	93	100	100	100	100	96	91	100	100	100	96	89	88	100	100	100	93	91	89	100	100	100	100	92	87
30	100	100	100	98	90	81	100	100	100	100	95	89	100	100	90	86	77	69	100	100	86	79	70	68	100	100	100	95	91	85
35	70	34	15	5	0	0	98	93	87	48	9	0	46	31	0	0	0	0	23	6	0	0	0	0	95	80	45	13	1	0
40	50	13	1	0	0	0	80	49	6	0	0	0	30	0	0	0	0	0	21	0	0	0	0	0	61	20	4	0	0	0

* Significant differences in survival were observed among the tested nematodes at the tested temperatures ($F = 6.51$, $P < 0.01$)

Figure (17): Effect of temperature on survival of *S. glaseri* and *Heterorhabditis* sp. after 15 days of exposure.

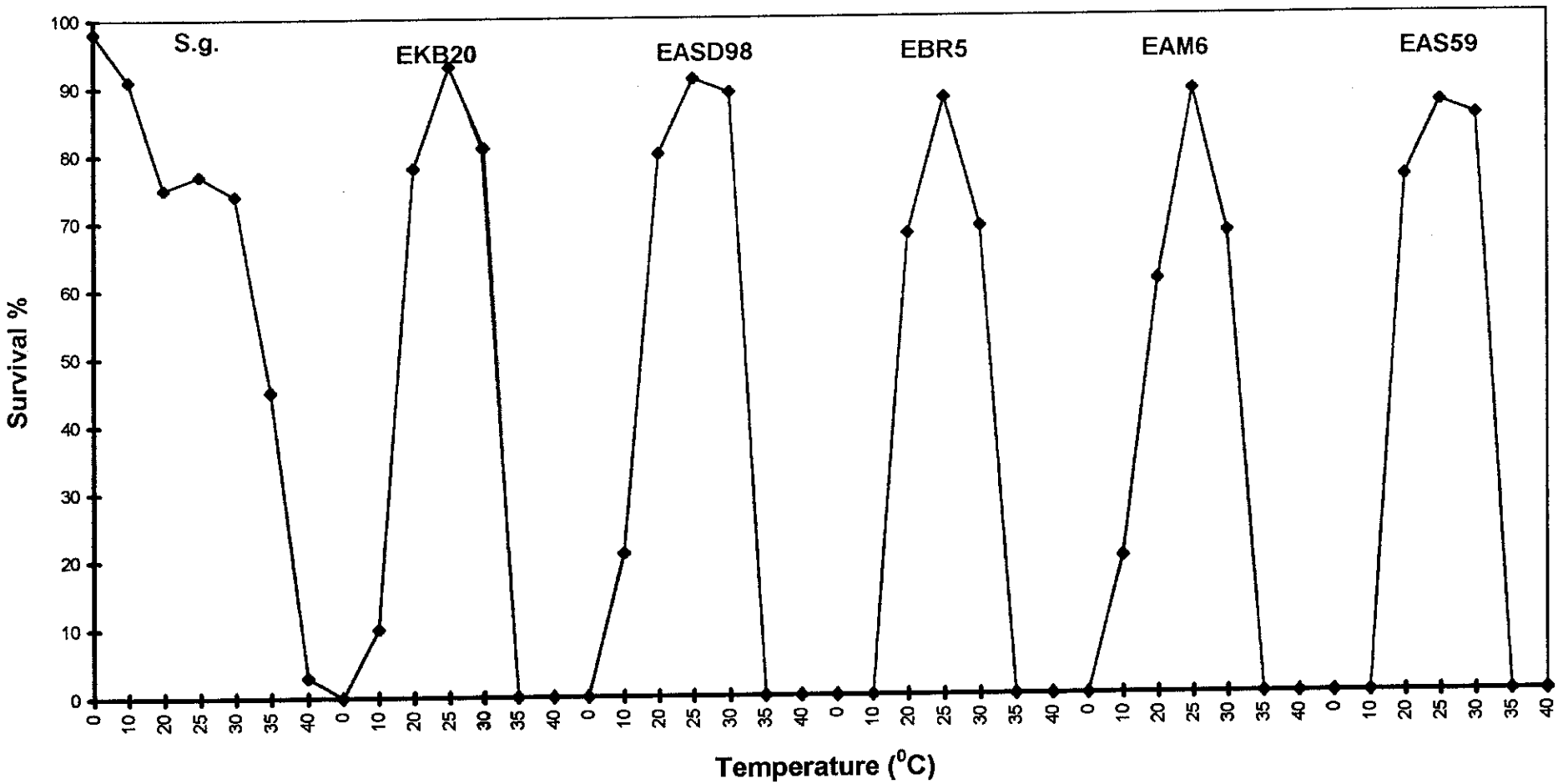
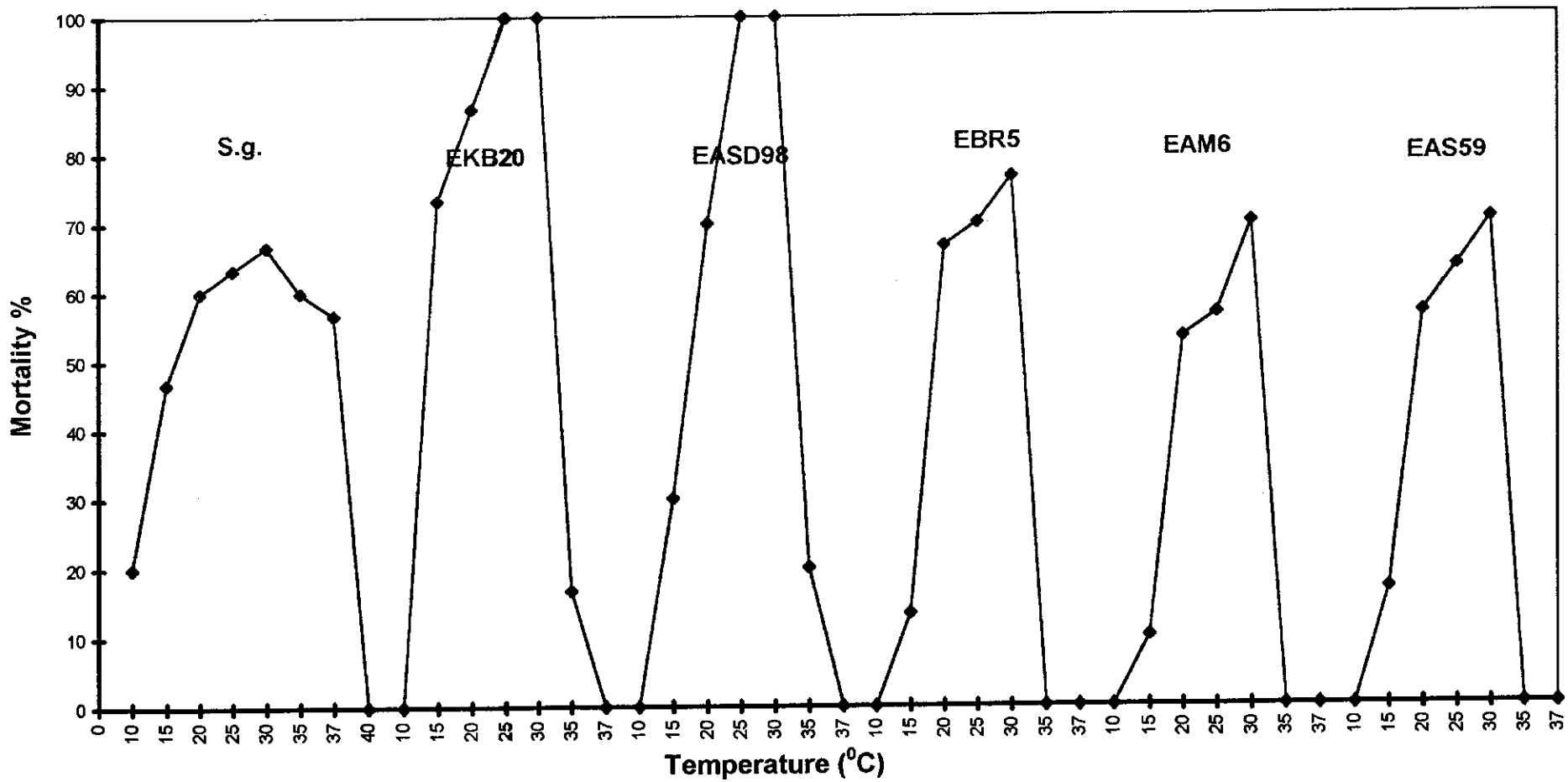


Table (17): Infectivity of *S. glaseri* and the Egyptian heterorhabditid nematode isolates, EKB20, EASD98, EBR5, EAM6 and EAS59 to 5th instar nymphs of *S. gregaria* at different temperatures.

Temp (^{0}C)	Mortality %					
	S.g.	EKB20	EASD98	EBR5	EAM6	EAS59
10	20.0	0.00	0.00	0.00	0.00	0.00
15	46.7	73.3	30.0	13.3	10.0	16.7
20	60.0	86.7	70.0	66.7	53.3	56.7
25	63.3	100	100	70.0	56.7	63.3
30	66.7	100	100	76.7	70.0	70.3
35	60.0	16.7	20.0	0.00	0.00	0.00
37	56.7	0.00	0.00	0.00	0.00	0.00

* Significant differences in pathogenicity were observed among the tested nematodes at the tested temperatures ($F = 3.41$, $P < 0.05$)

Figure (18): Pathogenicity of *S. glaseri* and *Heterorhabditis* sp. to 5th instar nymphs of *S. gregaria* at different temperatures.



at temperatures from 15-30 ^{0}C than at a highest temperature of 35 ^{0}C. Optimal pathogenicities were obtained at 30 ^{0}C followed by 25 ^{0}C.

It was also indicated from table (17) that, heterorhabditid nematodes tested could not induce nymphal mortality at a temperature of 10 ^{0}C.

5.3. Effect of temperature on time required to kill 50% of insects (LT_{50}):

Data presented in table (18) indicated that, the rate of *S. gregaria* mortality increased significantly as the incubation temperature increased from 15-30 ^{0}C. Effects of temperature on the time required for dauer juveniles to kill 50% of the nymphs were different between steinernematid and heterorhabditid nematodes tested, for instance, at 25 & 30 ^{0}C *H. bacteriophora* (EKB20 isolate) killed the hosts most rapidly, the LT_{50} values were 40 & 19.5 hr compared with 56 & 50 hr for *S. glaseri* respectively. In contrast, *S. glaseri* was quicker to cause insect mortality at 15 ^{0}C, the LT_{50} value was lower than that of EKB20 isolate, it was 86 hr compared with 102 hr for EKB20 isolate.

5.4. Effect of temperature on establishment:

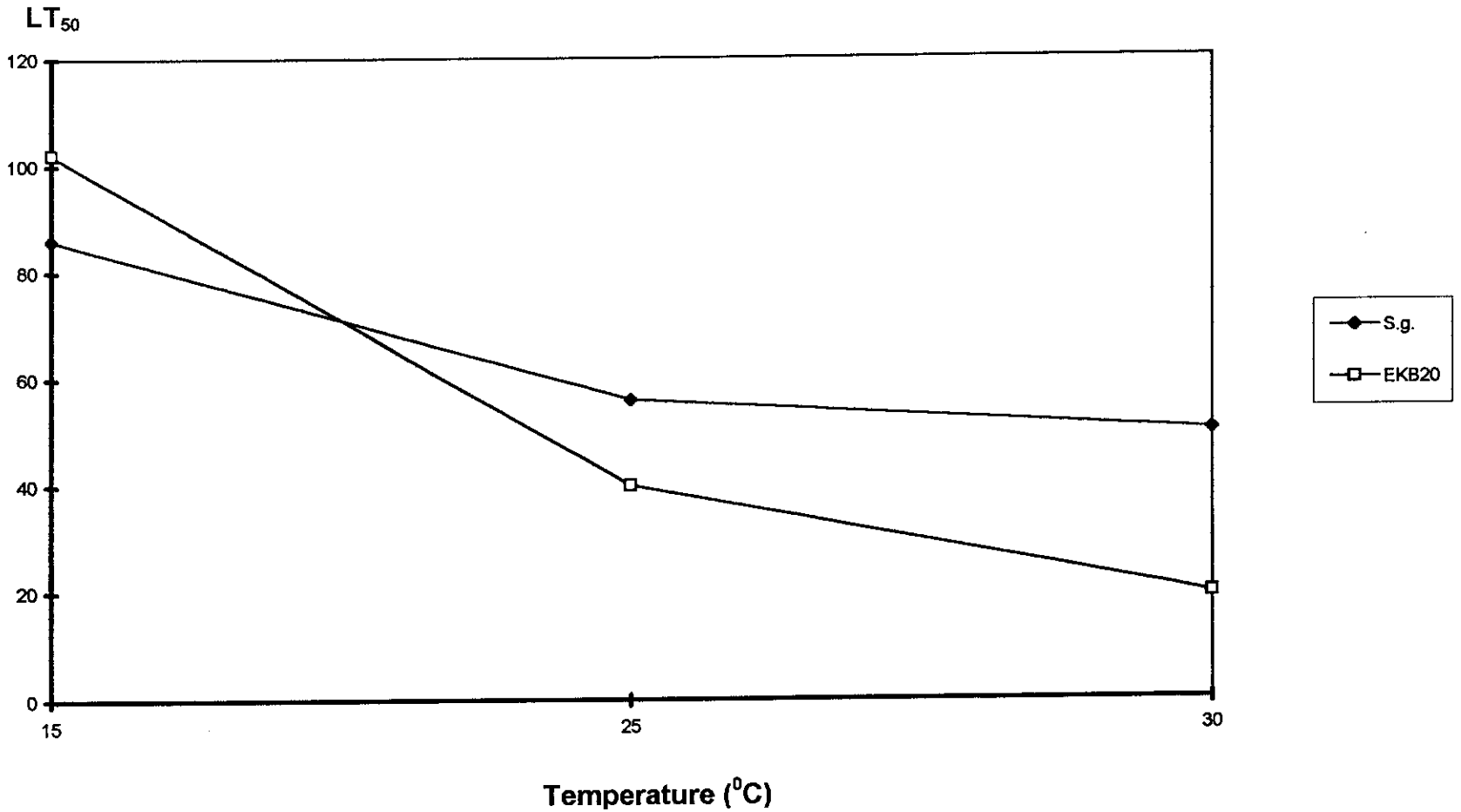
Results in table (19) indicated that, temperature ranges for establishment of *S. glaseri* (NC strain) in *S. gregaria* nymphs were broader (15-37 ^{0}C) than that of *H. bacteriophora* (EKB20 isolate) which was established in hosts between 15-32 ^{0}C. The number of infective juveniles (IJs) that successfully established in nymphs depended on temperature and differed significantly in both nematode species. IJs of *H. bacteriophora* were the most effective at establishing in *S. gregaria* at 20, 25 & 30 ^{0}C; a mean of 30, 36 & 31 nematodes were found per nymph respectively compared with 8, 9 & 12 nematodes for *S. glaseri*. In contrast, *S. glaseri* infective juveniles that established within hosts were higher than that of *H. bacteriophora* at 15 & 32 ^{0}C. IJs of *S. glaseri* failed to develop within dead nymphs at 10 ^{0}C while

Table (18): Effect of temperature on the rate of *S. gregaria* mortality by the entomopathogenic nematodes, *S. glaseri* and *H. bacteriophora* (EKB20 isolate): estimated hours to kill 50% (LT_{50}) of the nymphs.

Nematode strain	Temp. (°C)	Mortality % after the indicated hours of treatment										LT_{50} (hr)
		12hr	18hr	24hr	36hr	48hr	60hr	72hr	84hr	96hr	108hr	120hr	
S.g.	15	0.0	0.0	0.0	0.0	0.0	0.0	15	46.7	76.7	0.0	0.0	86.0 ± 2.12 a
	25	0.0	0.0	0.0	0.0	20	70	83.3	0.0	0.0	0.0	0.0	56.0 ± 1.70 b
	30	0.0	0.0	0.0	0.0	40	80	86.7	0.0	0.0	0.0	0.0	50.0 ± 2.10 b
EKB20	15	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	40	56.7	73.3	102.0 ± 2.69 a
	25	0.0	0.0	0.0	30	80	80	100	0.0	0.0	0.0	0.0	40.0 ± 1.56 b
	30	0.0	40	80	90	100	0.0	0.0	0.0	0.0	0.0	0.0	19.5 ± 0.78 c

* LT_{50} values followed by the same letter within a column at the same nematode species are not significantly different ($P > 0.05$) according to L.S.D.

Figure (19): Effect of temperature on estimated hours to kill 50% (LT_{50}) of *S. gregaria* nymphs infected with *S. glaseri* and *H. bacteriophora* (EKB20 isolate)



those of *H. bacteriophora* could not established at 35 ^{0}C, nevertheless dead IJs were located in the haemocoel of the nymphs after death, which confirms the ability of the IJs to penetrate the host.

5. Effect of temperature on span of nematode life cycle:

From table (19) it was shown that, the span of life cycle of the nematodes tested declined significantly as the incubation temperature increased from 15-25 ^{0}C. *H. bacteriophora* was quicker to complete reproduction in the hosts, at 20 & 25 ^{0}C, infective juveniles started to emerge from infected cadavers after 16 & 12 days from exposure to nematodes respectively. Whereas *S. glaseri* was slow to complete reproduction in its hosts, time taken to 1st emergence was 18 & 14 days respectively. In contrast, at 15 ^{0}C *S. glaseri* was quicker to complete their life cycle in the host cadavers. Time taken to 1st emergence was 35 days compared with 37 days for *H. bacteriophora* (EKB20 isolate).

5.6. Effect of temperature on nematode reproduction:

S. glaseri possessed a broader thermal niche breadth for reproduction (15-32 ^{0}C) than *H. bacteriophora* (15-30 ^{0}C). Significant differences were found in reproductive potential of the two different nematode species (table, 19), *H. bacteriophora* showed a higher reproductive potential than *S. glaseri*. At 20-30 ^{0}C reproduction was excellent, more than 570000 infective juveniles of *H. bacteriophora* were produced per nymph at 25 ^{0}C, but it was poorer at 15 ^{0}C. *H. bacteriophora* failed to emerge from cadavers at 32 ^{0}C although three nematodes were established at this temperature. Reproduction of IJs of *S. glaseri* was better at 25 & 30 ^{0}C, the maximum number was 60000 IJs/ cadaver at 30 ^{0}C, but at 15 ^{0}C reproduction was negligible.

Table (19): Effect of temperature on the establishment, span of life cycle and reproductive potential of *S. glaseri* and *H. bacteriophora* (EKB20 isolate) in 5th-instar nymphs of *S. gregaria*.

Temp.(°C)	Mean No. of nem. estab./cadaver ± S.E.		Mean No. of days taken to 1st emerg. ± S.E.		Mean No. of IJs emer. /cad. ± S.E. X 10^4	
	S. g.	EKB20	S.g.	EKB20	S.g.	EKB20
10	0.00 ± 0.00	0.00	0.00	0.00	0.00	0.00
15	6.00 ± 1.01 a	5.00 ± 0.58 a	35.0 ± 2.35 a	37.0 ± 1.56 a	0.60 ± 0.14 a	1.20 ± 0.11 a
20	8.00 ± 0.59 ab	30.0 ± 0.60 b	18.0 ± 1.47 b	16.0 ± 1.12 b	3.20 ± 0.29 b	48.95 ± 1.79 b
25	9.00 ± 2.03 b	36.0 ± 1.50 c	14.0 ± 1.01 c	12.0 ± 1.00 c	5.13 ± 0.37 c	57.72 ± 1.6 c
30	12.0 ± 0.10 c	31.0 ± 2.10 bc	---	---	6.00 ± 0.88 c	40.00 ± 2.05 d
32	7.00 ± 0.58 a	3.00 ± 0.50 a	---	---	4.00 ± 1.00 bc	0.00
35	5.00 ± 1.17 a	0.00	---	---	0.00	0.00
37	2.00 ± 0.60 d	0.00	---	---	0.00	0.00

* Doted lines not tested

** Means with the same letter within a column are not significantly different (P > 0.05) according to L.S.D.

Figure (20): Effect of temperature on the establishment of *S. glaseri* and *H. bacteriophora* (EKB20 isolate) in 5th instar nymphs of *S. gregaria*.

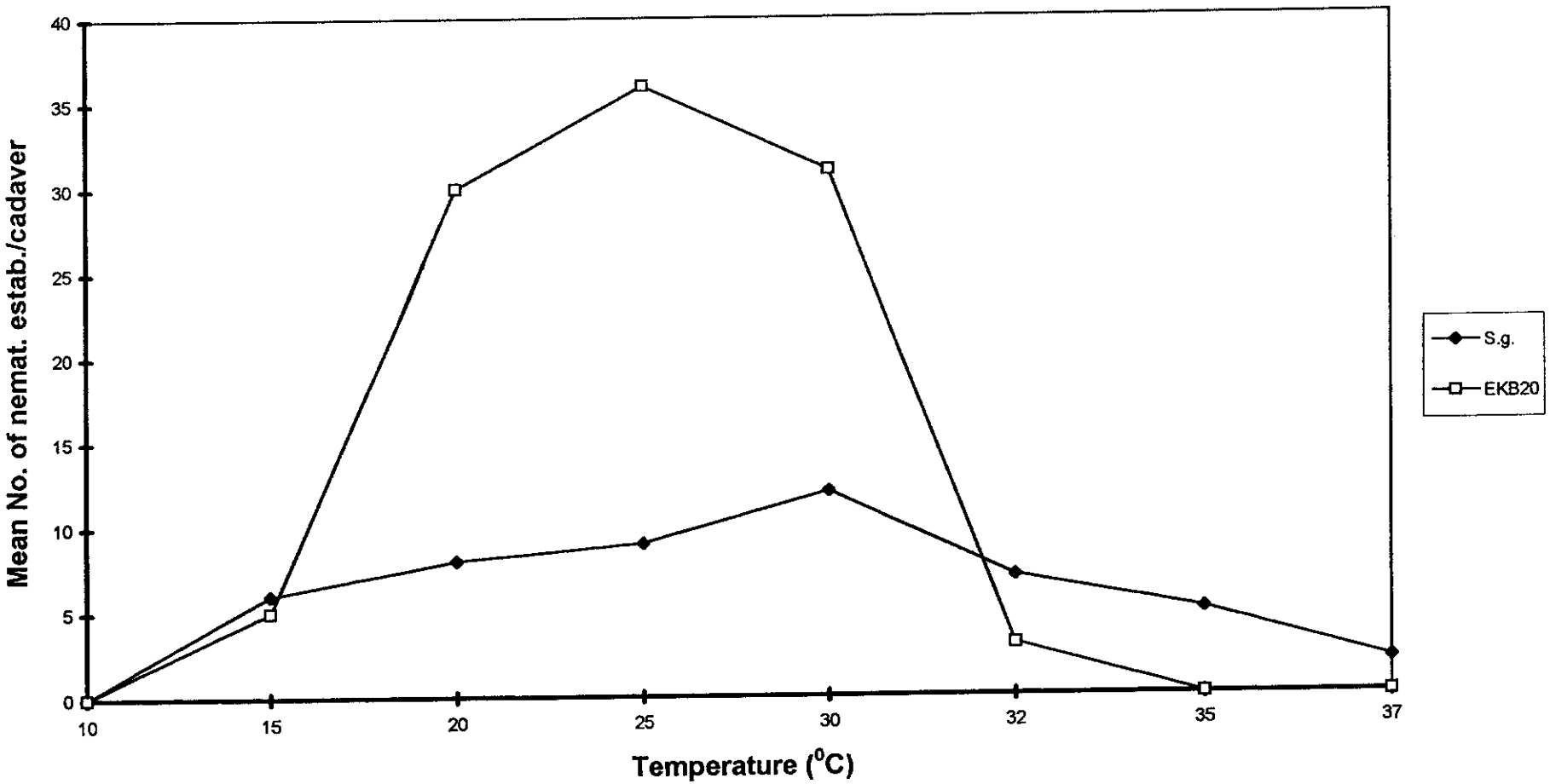
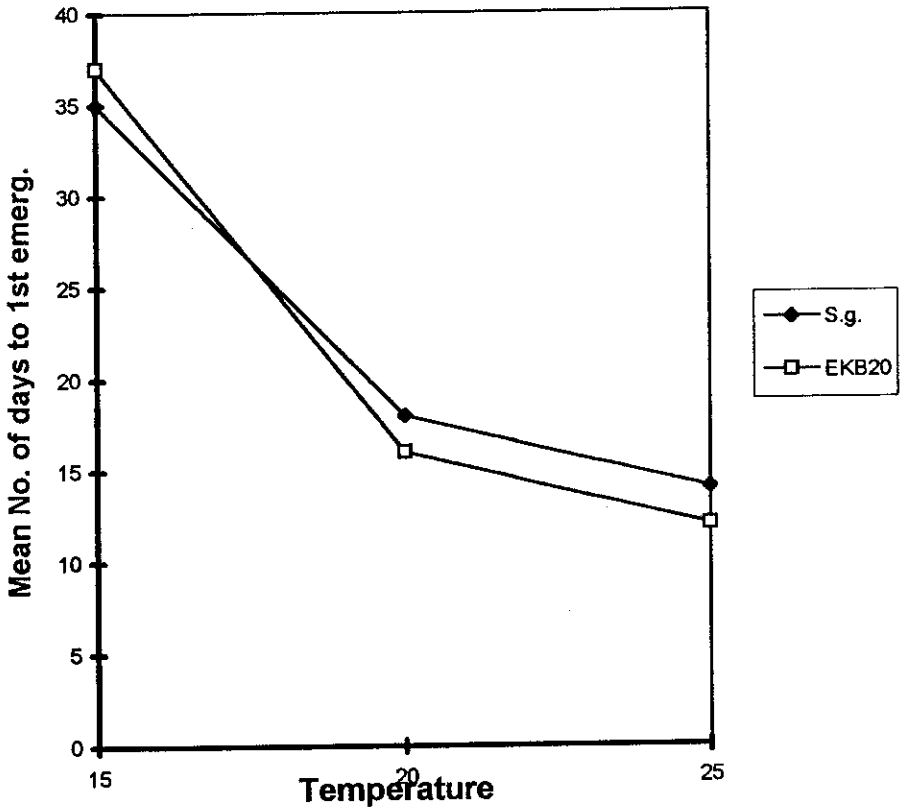
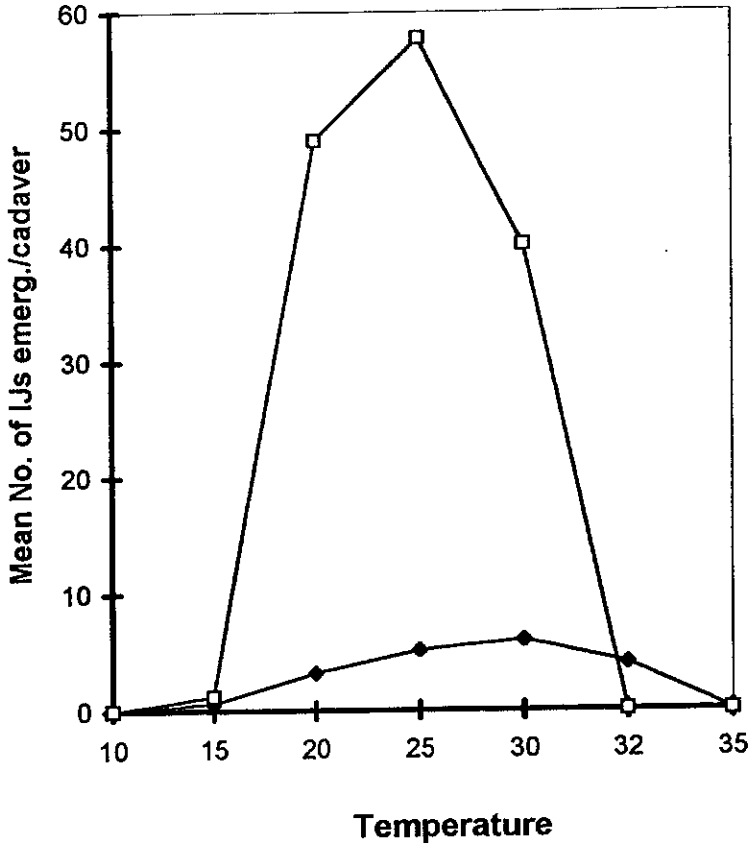


Figure (21): Effect of temperature on span of life cycle (A) and reproductive potential (B) of *S. glaseri* and *H. bacteriophora* (EKB20 isolate).



(A)



(B)

From the above mentioned results, (table, 19), it was noticed that, *H. bacteriophora* had a lower optimum temperature (i.e. temp. at which maximum number of IJs were produced per host), it was 25 ^{0}C, while *S. glaseri* had a higher one (30 ^{0}C).

6. Effects of relative humidity:

6.1. Nematode survival at different relative humidities:

The effect of different relative humidity (RH) levels on the viability of *S. glaseri* and *H. bacteriophora* (EKB20 isolate) infective juveniles is presented in table (20). Significant differences were observed in survival of *S. glaseri* and *H. bacteriophora* ($F= 8.96$, $P< 0.05$) at the five ranges of RH ($F= 6.85$, $P< 0.05$) over the 12 hr test period. The rate of nematode mortality was related to the RH levels. A gradual reduction in nematode survival was recorded at 80% and 60% RH, whereas at 40% and 20% RH a drastic mortality was observed within 2 hr of exposure.

S. glaseri survival decreased significantly as RH decreased from 100 to 20% (Figure, 22A). Survival remained at 100, 68 and 30% after 12 hr at 100%, 80% and 60% RH respectively. Nematodes survived for 6 hr at 40% RH and 4 hr at 20% RH.

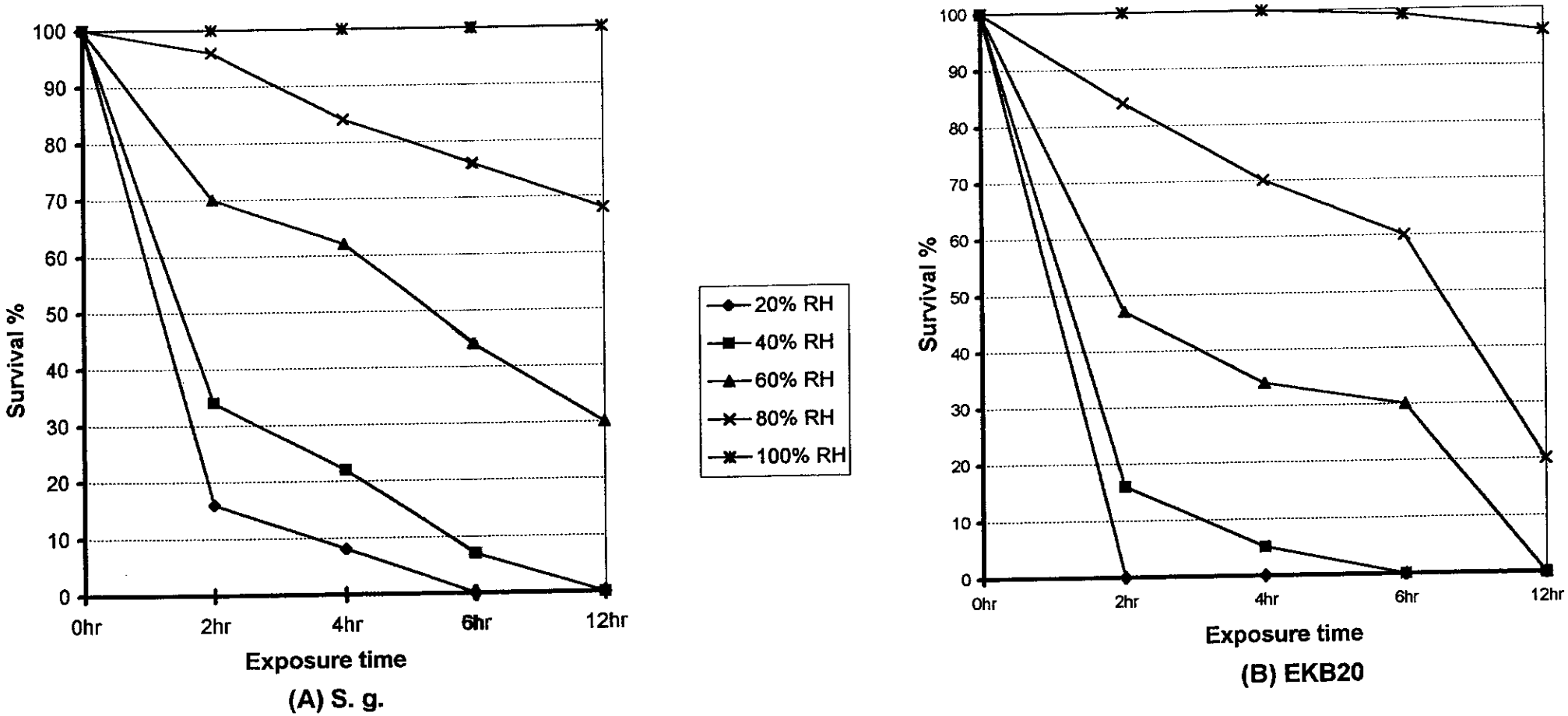
H. bacteriophora survival decreased significantly as RH decreased from 100 to 80% during the test period. Nematodes survived 12 hr at 100% and 80% RH, 6 hr at 60% RH and 4 hr at 40% RH (Fig., 22B). Following a 12 hr exposure period 96% of the infective juveniles survived at 100% RH. Complete mortality (zero % survival) was recorded within 2 hr of exposure at 20% RH.

Table (20): Effect of different relative humidities on the survival of *S. glaseri* and *H. bacteriophora* (EKB20 isolate) incubated at 25 ^{0}C.

Nematode strain	Exposure time	Survival % at the indicated RHs				
		20	**40**	**60**	**80**	**100**
S.g.	**2hr**	**16.0**	34.0	70.0	96.0	100
	4hr	**8.00**	22.0	62.0	84.0	100
	6hr	**0.00**	7.00	44.0	76.0	100
	12hr	**0.00**	0.00	30.0	68.0	100
	Total mean	6.0 ± 0.8 a	15.8 ± 3.6 b	33.6 ± 5.2 c	81.0 ± 5.9 d	100 ± 0.00 e
EKB20	**2hr**	**0.00**	16.0	47.0	84.0	100
	4hr	**0.00**	5.00	34.0	70.0	100
	6hr	**0.00**	0.00	30.0	60.0	99.0
	12hr	**0.00**	0.00	0.00	20.0	96.0
	Total mean	**0.00**	5.25 ± 1.7 a	27.8 ± 6.1 b	58.5 ± 7.3 c	98.8 ± 0.90 d

* Means with the same letter within a row are not significantly different (P > 0.05) according to L.S.D.

Figure (22): Effect of different RHs on the survival of *S. glaseri* and *H. bacteriophora* (EKB20 isolate) incubated at 25 C^0.



6.2. Nematode pathogenicity at different relative humidities:

Significant differences in pathogenicity of these two nematode species ($F= 5.99$, $P< 0.05$) at the tested RH ($F= 5.36$, $P< 0.05$) were also observed (table 21). Pathogenicity results were again closely correlated to nematode survival. *S. glaseri* caused 56.7% nymphal mortality of *S. gregaria* at 100% RH after the 1st two hours of exposure. Nematodes retained pathogenicity of 50, 16.7 and 3.3 *S. gregaria* mortality after 12 hr at 100%, 80% and 60% RH respectively. Nematodes did not cause lethal host infection after, 4 hr at 40% RH and 2 hr at 20% RH (Fig., 23A).

H. bacteriophora retained full pathogenicity of *S. gregaria* nymphs at 100% RH during the test period. Nematodes caused 6.7% lethal host infection after 12 hr at 80% RH but did not infect hosts after 6 hr at 60% RH and 2 hr at 40% RH (Fig., 23B).

Pathogenicity of these two nematode species decreased significantly as RH decreased from 100 to 20% throughout the test period. It was also observed that, pathogenicity decreased as exposure time increased from 2 to 12 hr except at 100% RH, *S. glaseri* pathogenicity was irregular whereas *H. bacteriophora* pathogenicity was unaffected at all tested exposure times.

Table (21): Effect of different relative humidities on the pathogenicity of *S. glaseri* and *H. bacteriophora* (EKB20 isolate) to 5th instar nymphs of *S. gregaria*.

Nematode strain	Exposure time	Mortality % at the indicated RHs				
		20	40	60	80	100
S.g.	2hr	3.30	6.70	20.0	46.7	56.7
	4hr	0.00	3.30	16.7	40.3	53.3
	6hr	0.00	0.00	13.3	40.0	56.7
	12hr	0.00	0.00	3.30	16.7	50.0
	Total mean	0.83 ± 0.25 a	2.5 ± 0.6 a	13.3 ± 3.6 b	35.9 ± 6.9 c	54.2 ± 1.6 d
EKB20	2hr	0.00	3.30	16.7	70.0	100
	4hr	0.00	0.00	10.0	46.7	100
	6hr	0.00	0.00	3.30	43.3	100
	12hr	0.00	0.00	0.00	6.70	100
	Total mean	0.00	0.83 ± 0.25 a	7.5 ± 2.1 b	41.7 ± 4.5 c	100 ± 0.0 d

* Means with the same letter within a row are not significantly different (P > 0.05) according to L.S.D.

4. Oenocytoids (OEs):

The OEs are large cells, round or oval, showing characteristic spindle-shaped profiles, with darkly staining nucleus and clear uniform, weakly acidophil cytoplasm (figure, 31A).

The same categories of haemocytes were found by **Hoffmann *et al.* (1969)**.

Counts of the different types of haemocytes are shown in tables (22 and 23) and graphically illustrated in figures (24 and 25). Data in these tables showed clear changes in the relative numbers of circulating haemocytes following *S. glaseri* and *H. bacteriophora* infection.

At the beginning of the experiment, the prohaemocytes comprised 23.4 % of the total cell population in the control nymphs, this ratio was gradually decreased to 20.1, 19, 18.7, 17.3, 16 and 15.3 % with the progress of time. In the treated nymphs, PRs were sharply decreased as a result of the nematode infection. This reduction reached its maximum at the end of the experiment (after that time all nymphs infected with *H. bacteriophora* were dead). It reached 1.5 % and 1.3 % in nymphs infected with *S. glaseri* and *H. bacteriophora*, respectively, at 30 hr post-infection .

The dominant cell type in the normal nymphs was the plasmatocytes, it comprised 51.2 % of the total cell population. PLs (phagocytic cells) which may play a role in the defensive reactions in the haemolymph of the insects were found to increase post infection. This increase reach a maximum just before the death of the nymphs . Plasmatocyte counts were very close to the normal counts from 2 to 18 hr post-infection with *S. glaseri*, but increased significantly ($P< 0.001$) at 24 and 30 hr post-infection, it was 63.9 and 74% of the total cell population compared with 55.5 and 56 % of the untreated control nymphs respectively. EKB20 caused a significant ($P< 0.05$) increase in PLs number at 6 hr post-infection and a very highly significant ($P < 0.001$) increase

Table (22): Differential haemocyte counts at different times post-infection of 4th nymphal instar of *S. gregaria* with 3000 dauer stage juveniles of *S. glaseri* / nymph.

Time post infection	Percentages of haemocytes mean ± S.E.							
	Prohaemocytes		Plasmatocytes		Granulocytes		Oenocytoids	
	control	test	control	test	control	test	control	test
0hr	23.4 ± 0.68		51.2 ± 0.81		18.1 ± 1.01		7.3 ± 0.46	
2hr	20.1 ± 1.03	16.5 ± 0.07*	51.4 ± 0.07	52.50 ± 2.01	20.0 ± 0.48	19.0 ± 0.03	8.5 ± 0.61	12.00 ± 0.92*
6hr	19.0 ± 0.13	14.15 ± 0.1***	51.9 ± 0.26	53.25 ± 0.77	20.4 ± 0.31	20.1 ± 0.01	8.7 ± 0.11	12.5 ± 0.76*
12hr	18.7 ± 0.02	3.00 ± 0.03***	52.0 ± 0.27	52.30 ± 1.04	20.5 ± 0.16	31.7 ± 0.07***	8.8 ± 0.76	13.0 ± 0.01**
18hr	17.3 ± 0.05	2.70 ± 0.15***	53.1 ± 0.32	53.50 ± 2.11	21.0 ± 0.29	29.5 ± 0.15***	8.6 ± 0.12	14.3 ± 0.03***
24hr	16.0 ± 0.61	1.71 ± 0.03***	55.5 ± 0.01	63.9 ± 0.04***	20.5 ± 1.1	26.43 ± 0.1***	8.0 ± 0.22	8.00 ± 2.10
30hr	15.3 ± 1.01	1.5 ± 0.01***	56.0 ± 0.02	74.0 ± 0.03***	20.0 ± 2.18	21.00± 0.39	8.7 ± 0.09	3.50 ± 0.15***

* Significant = P < 0.05
** Highly significant = P < 0.01
*** Very highly significant = P < 0.001

Figure (24): Differential haemocyte counts at different times post-infection of *S. gregaria* 4th instar nymphs with *S. glaseri*.

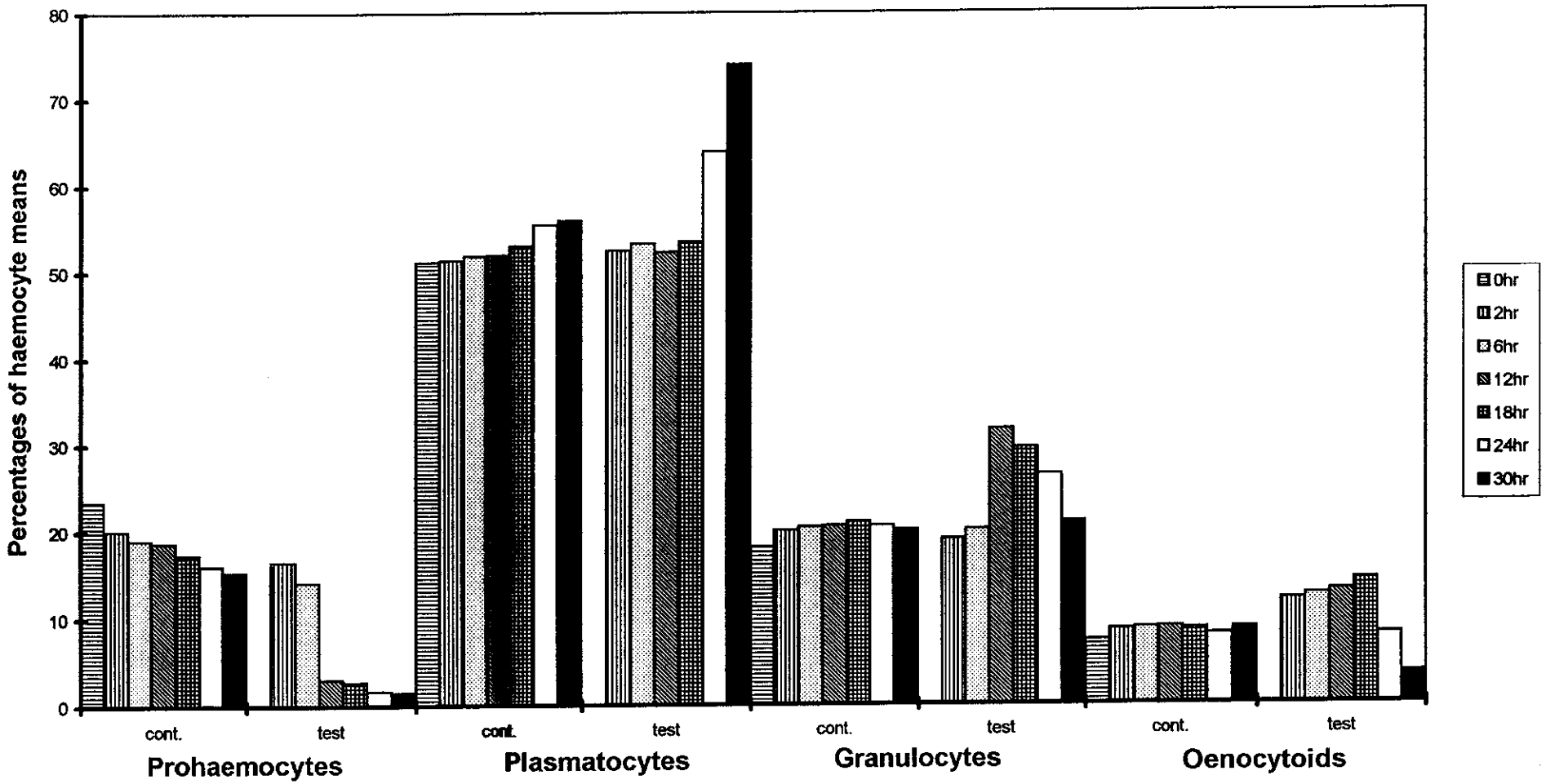
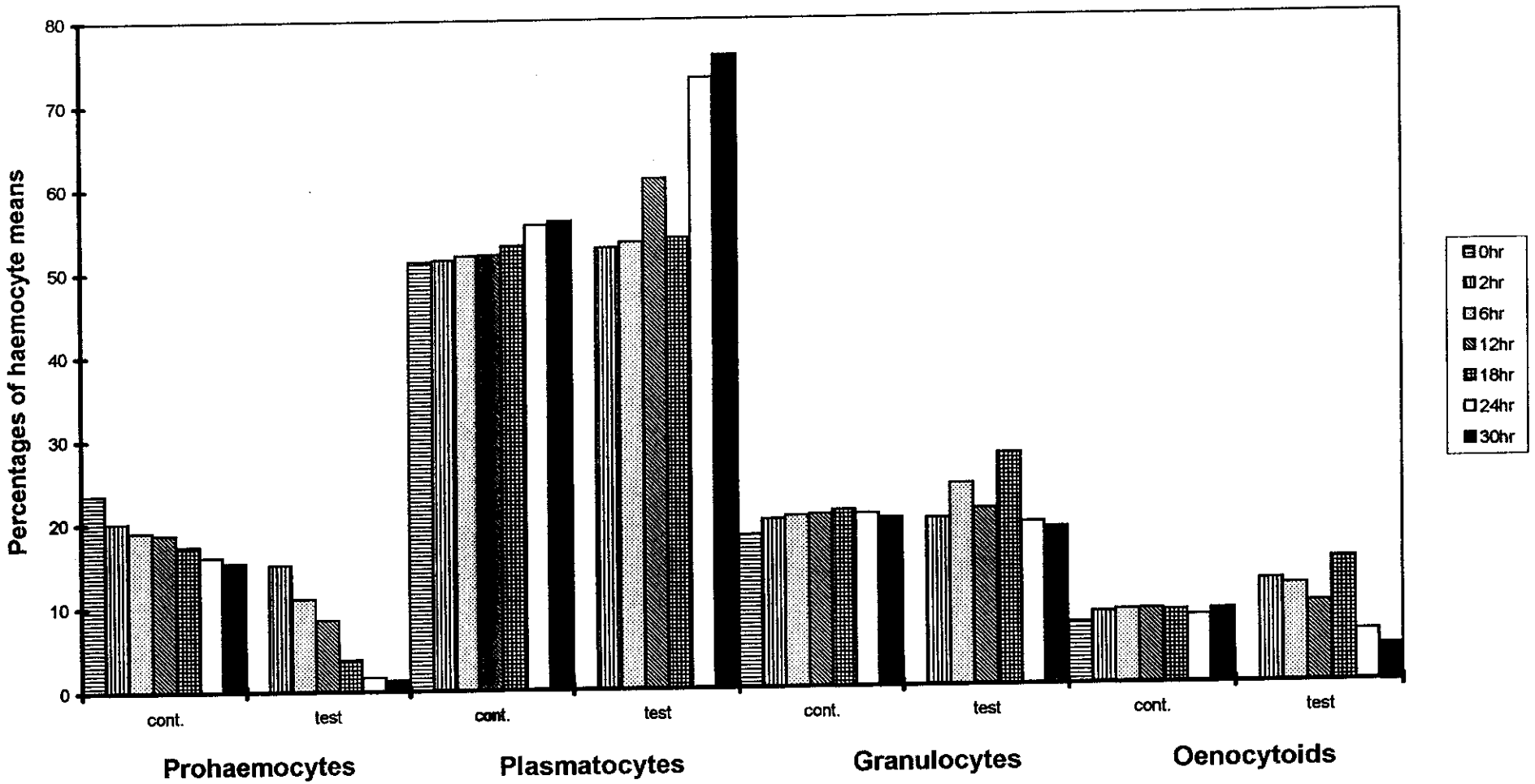


Table (23): Differential haemocyte counts at different times post-infection of 4th nymphal instar of *S. gregaria* with 3000 dauer stage juveniles of *H. bacteriophora* (EKB20 isolate)/ nymph.

Time post infection	Percentages of haemocytes mean ± S.E.							
	Prohaemocytes		Plasmatocytes		Granulocytes		Oenocytoids	
	control	test	control	test	control	test	control	test
0hr	23.4 ± 0.68		51.2 ± 0.81		18.1 ± 1.01		7.3 ± 0.46	
2hr	20.1 ± 1.03	15.1 ± 0.55**	51.4 ± 0.07	52.8 ± 1.15	20.0 ± 0.48	19.9 ± 0.02	8.5 ± 0.61	12.2 ± 0.26**
6hr	19.0 ± 0.13	11.0 ± 0.78***	51.9 ± 0.26	53.4 ± 0.41*	20.4 ± 0.31	24 ± 0.02***	8.7 ± 0.11	11.6 ± 0.39**
12hr	18.7 ± 0.02	8.50 ± 0.07***	52.0 ± 0.27	61.0 ± 0.03***	20.5 ± 0.16	21.0 ± 1.21	8.8 ± 0.76	9.50 ± 0.21
18hr	17.3 ± 0.05	3.80 ± 1.01***	53.1 ± 0.32	53.9 ± 1.040	21.0 ± 0.29	27.6 ± 0.01***	8.6 ± 0.12	14.7 ± 0.05***
24hr	16.0 ± 0.61	1.70 ± 0.04***	55.5 ± 0.01	73.0 ± 0.01***	20.5 ± 1.1	19.3 ± 2.01	8.0 ± 0.22	6.00 ± 0.09*
30hr	15.3 ± 1.01	1.3 ± 0.02***	56.0 ± 0.02	75.7 ± 0.02***	20.0 ± 2.18	18.7 ± 1.09	8.7 ± 0.09	4.3 ± 0.01***

* Significant = $P < 0.05$
** Highly significant = $P < 0.01$
*** Very highly significant = $P < 0.001$

Figure (25): Differential haemocyte counts at different times post-infection of *S.gregaria* 4th instar nymphs with *H. bacteriophora* (EKB20 isolate).



at 12 hr, then it sharply dropped at 18 hr (53.9 %) but their level was still higher than that of the controls which was, 53.1 %, and returned to increase again to reach a maximum (75.7 %) at 30 hr post-infection compared with 56 % of the control.

At 2 and 6 hr post *S. glaseri* infection, the numbers of granulocytes were nonsignificantly decreased but sharply increased over the level of the normal insects at 12 hr post-infection, then it began to decrease again at 18 hr till the end of the experiment but their level was still higher than that of the controls, while it was fluctuated in nymphs treated with *H. bacteriophora* along the time of the test, it showed a very highly significant increase ($P< 0.001$) at 6 and 18 hr post-infection, but nonsignificantly decreased ($P> 0.05$) at 2, 24 and 30 hr post-infection.

Control nymphs did not show any significant change in the percentage of the oenocytoids along the time of the experiment. Oenocytoid numbers increased from 2 to 18 hr post-infection with the two nematodes, *S. glaseri* and *H. bacteriophora*, and reached the maximum at 18 hr post-infection, it was 14.3 and 14.7 % respectively compared with 8.6 % of the control, then it dropped at 24 and 30 hr post-infection.

7.2. Phagocytosis:-

The nematode *H. bacteriophora* released its associated bacteria, *Photorhabdus luminescens* into the haemolymph of *S. gregaria* nymphs at 6 hr post-infection, at this time the phagocytic response started weakly, while *S. glaseri* released its associated bacteria *X. poinarii* after that time. At 12 hr post infection the phagocytic response increased significantly and reached its maximum at 30 hr post-infection, at this time 28 and 35% of the total cell population of *S. glaseri* and *H. bacteriophora* infected nymphs respectively were phagocytosed bacterial cells (table, 24)

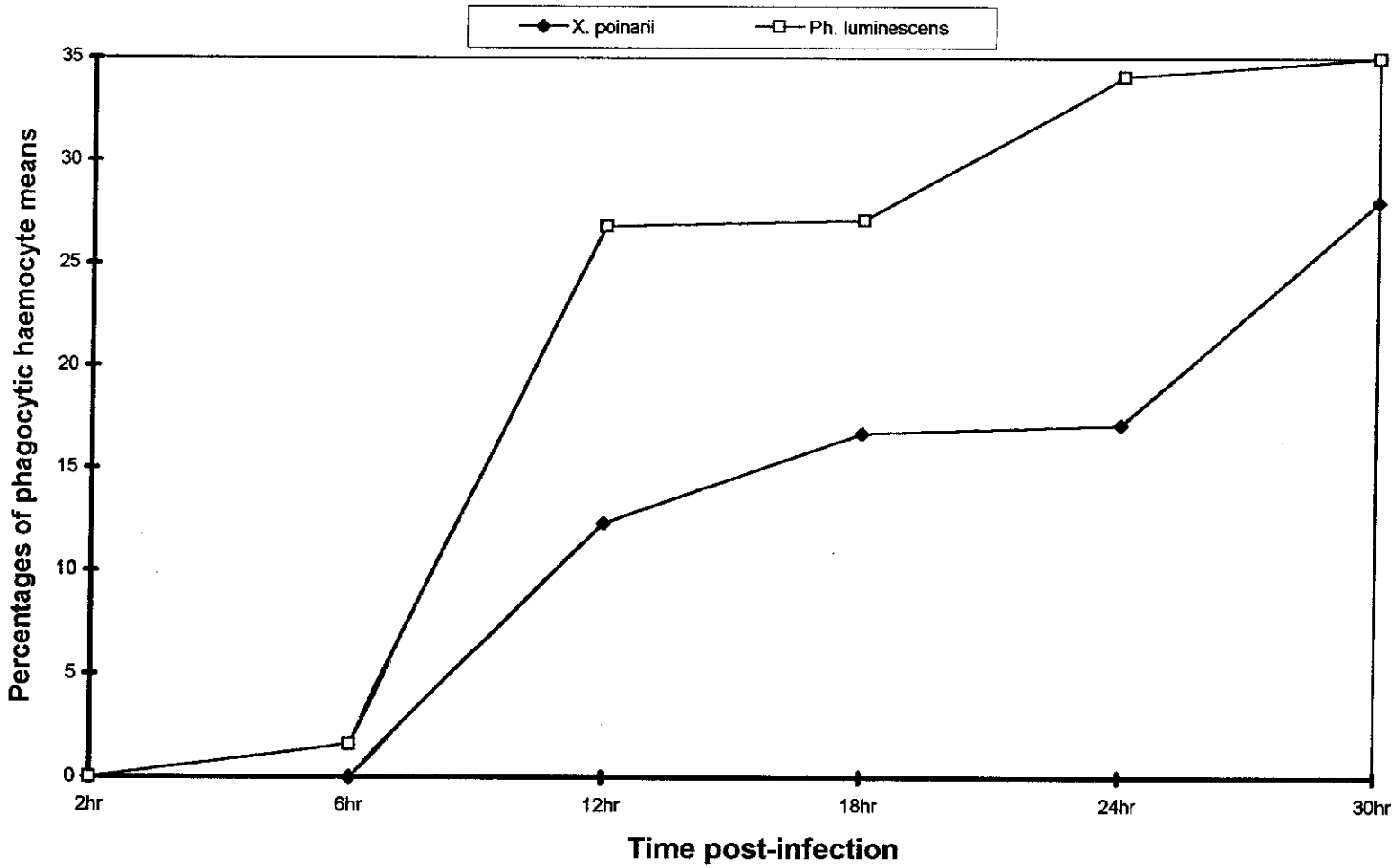
Table (24): Phagocytosis of *X. poinarii* and *P. luminescens* by the haemocytes of *S. gregaria* 4th-instar nymphs at different times post-infection with *S. glaseri* and *H. bacteriophora* (EKB20 isolate) respectively.

Bacteria species	Percentages of phagocytic haemocytes mean ± S.E. at the indicated hours					
	2hr	6hr	12hr	18hr	24hr	30hr
X. poinarii	0.00	0.00	12.3 ± 0.7	16.7 ± 0.74*	17.1 ± 1.05*	28.0 ± 2.04**
P. luminescens	0.00	1.6 ± 0.4	26.8 ± 1.2***	27.1 ± 0.9***	34.1 ± 0.38***	35.0 ± 3.1***

* Significant = $P < 0.05$ ** Highly significant = $P < 0.01$ *** Very highly significant = $P < 0.001$

\# Significant differences in phagocytosis were observed between the two nematode species ($P < 0.001$).

Figure (26): Phagocytosis of *X. poinarii* and *P. luminescens* by haemocytes of *S. gregaria* infected with *S. glaseri* and *H. bacteriophora* resp.



7.3. Total haemocyte counts (THCs):

The effects of infection with *S. glaseri* and *H. bacteriophora* on the total haemocyte counts are summarized in table (25). As shown from the table, THCs of *S. gregaria* infected with *S. glaseri* were decreased from 2-18 hr post-infection, and suddenly increased at 24 hr post-infection, to reach 14.6x1000 haemocyte/mm^3 compared with 2.483x1000 haemocyte/mm^3, and decreased again at 30 hr post-infection but not returned to the level present in control insects. THCs began to increase approximately 6 hr post *H. bacteriophora* infection, reaching a peak at 18 hr after infection, it was 12.225x1000 haemocyte/mm^3 compared with 2.371x1000 haemocyte/mm^3 of the untreated nymphs, and decreased again till the nymphal death.

Haemocyte deformations :

The damage of haemocytes as a result of nematode infection became pronounced after twelve hours from infection of the nymphs. At this time pathological vacuoles were formed especially in the prohaemocytes. All types of haemocytes were severely affected after thirty hours from infection and shortly before death of the nymphs. The damage at this time was more pronounced in the plasmatocytes.

On the basis of light microscopy observations, the haemocytes in the haemolymph of infected nymphs were morphologically described as follows:

Figure (28): Prohamocytes:

Fig. 28B: Deformed prohaemocyte, with large pathological vacuoles and fine cytoplasmic projections .

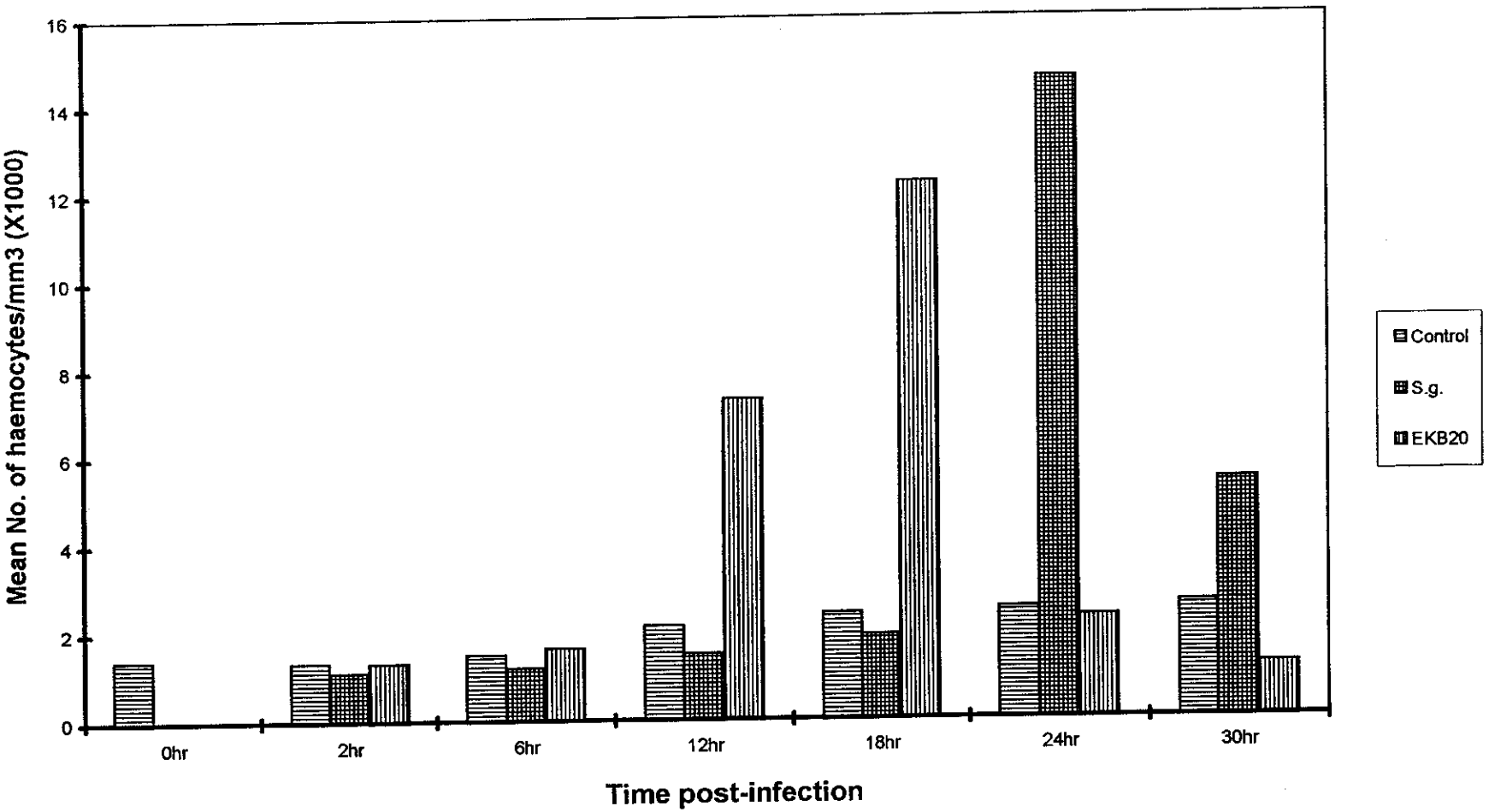
Fig. 28C: Infected prohaemocyte, with cutting cytoplasm and extruding outside the cell from one side.

Table (25): Total haemocyte counts at different times post-infection of 4th nymphal instar of *S. gregaria* with 3000 dauer stage juveniles of *S. glaseri* and *H. bacteriophora* (EKB20 isolate)/ nymph.

Time post-infection	Mean number of haemocytes/mm^3 ± S.E. (x 1000)		
	Control	S.g.	EKB20
0hr	1.337 ± 0.209		
2hr	1.341 ± 0.101	1.1120 ± 0.260	1.3150 ± 0.18
6hr	1.534 ± 0.230	1.2120 ± 0.109*	1.6250 ± 0.33
12hr	2.125 ± 0.330	1.538 ± 0.101**	7.30 ± 0.28 ***
18hr	2.371 ± 0.109	1.865 ± 0.240**	12.225 ± 0.275***
24hr	2.483 ± 0.250	14.60 ± 0.304***	2.300 ± 0.06*
30hr	2.562 ± 0.320	5.35 0± 0.207***	1.15 ± 0.108***

* Significant = $P < 0.05$
** Highly significant = $P < 0.01$
*** Very highly significant = $P < 0.001$

Figure (27): Total haemocyte counts at different times post-infection of *S gregaria* nymphs with *S. glaseri* and *H. bacteriophora (* EKB20 isolate).



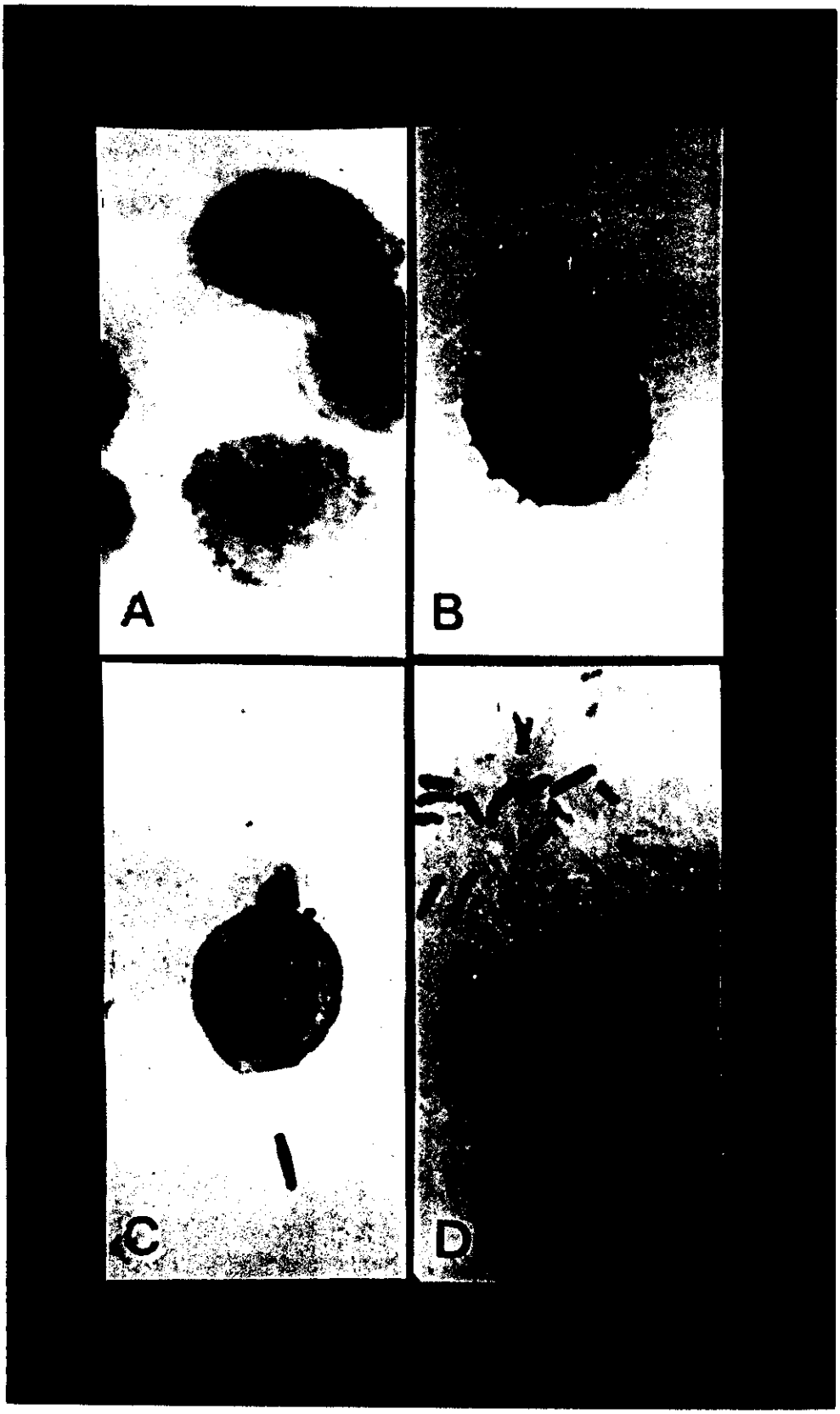


Figure (28)

Fig. 28D: Infected prohaemocyte surrounded by bacteria, with small pathological vacuoles in the cytoplasm and fine ruptures in the cell wall.

Figure (29): Plasmatocytes

Fig. 29B: A plasmatocyte developed cytoplasmic processes which are so fine that, they are visible only by phase contrast. These delicate filaments are in movement so that the bacteria in the haemolymph adhere to them.

Fig. 29C: Spindle-shaped plasmatocyte, its cytoplasm extended into two arms, engulfed a bacterial cell.

Fig. 29D: Round plasmatocyte, with cytoplasmic arms or pseudopodia (amoeboid-shape), its cytoplasm containing small inclusions.

Fig. 29E: Fusiform plasmatocyte, with a central nucleus and terminal filaments, try to phagocytoze large number of bacterial cells.

Fig. 29F: Phagocytic spindle-shaped plasmatocyte, with displaced nucleus and its cytoplasm engulfing large number of bacterial cells.

Fig. 29G: Phagocytic pear-shaped plasmatocyte, bigger in size with a multiplicated nucleus as a result of nematode infection.

Fig. 29H: Deformed plasmatocyte, with divided nucleus at the terminal of the cell. Large pathological vacuoles were formed in the cytoplasm.

Fig. 29I: Phagocytic spindle-shaped plasmatocyte, which was bigger in size, with multiplicated nucleus. Very large pathological vacuoles were formed in the nucleus.

Fig. 29J: Diseased plasmatocyte, invaded (attacked) by bacterial cells, with damaged cytoplasm, ruptures of the cell wall, containing small inclusions and pathological vacuoles.

Fig. 29K: Damaged plasmatocyte, with ruptured cell wall, destructed cytoplasm, and surrounded by bacteria.

Fig. 29L: Severely damaged plasmatocyte, containing only the nucleus and rudiments of the cytoplasm which was damaged by bacteria.

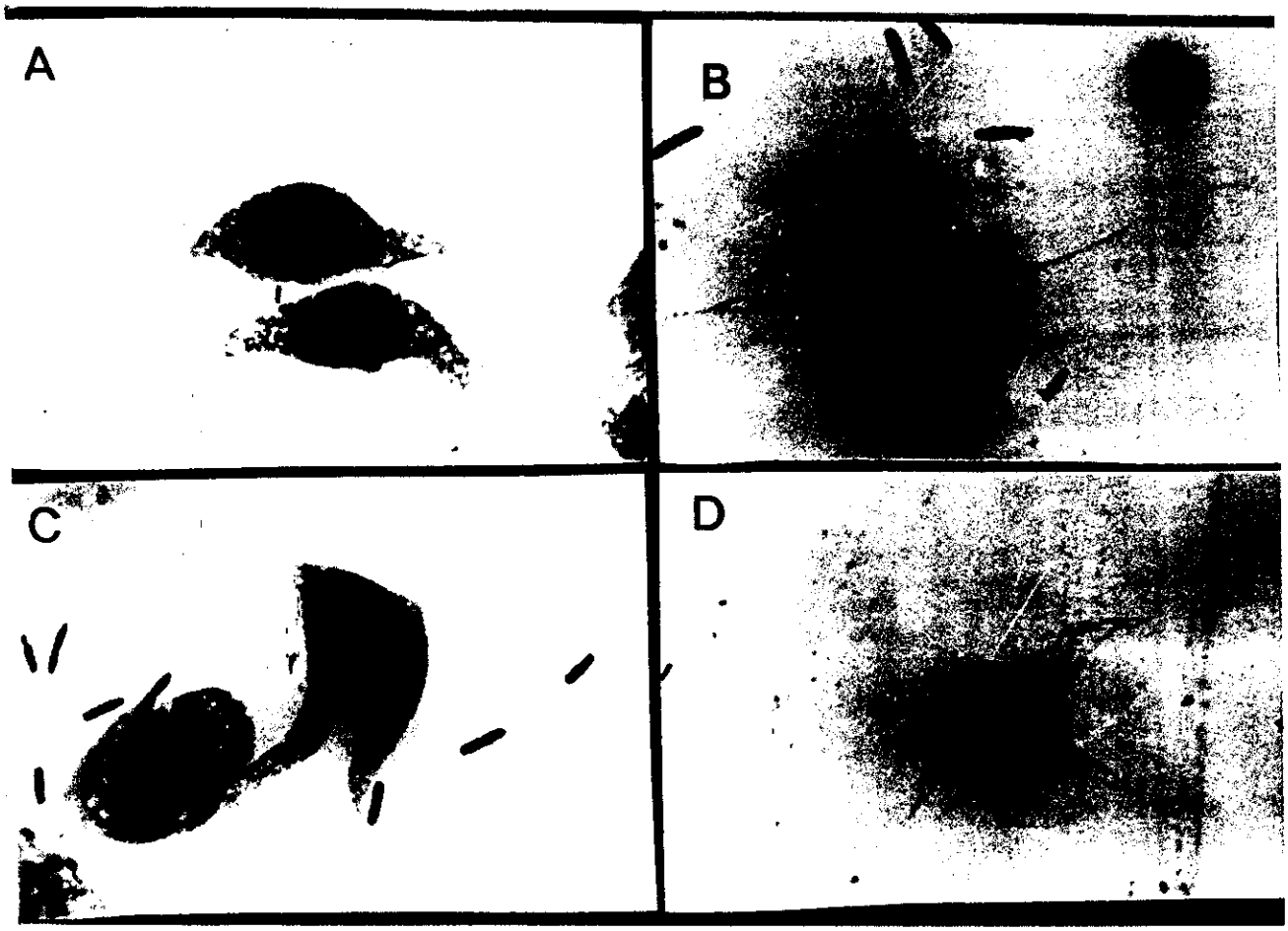


Figure 29(A, B, C & D)

Figure (30): Granulocytes

Fig. 30B: Treated granulocyte, elaborated fine cytoplasmic filaments (hyphae) by which the cell contact with other cells to form capsules around foreign bodies.

Fig. 30C: Treated granulocytes that aggregated to form a capsule as a response of infection.

Fig. 30D: Infected granulocyte, discharged its granules to form sticky substance entrapping large number of bacterial cells (nodule formation)..

Fig. 30E: degranulated granulocyte, the external and internal membranes were ruptured , with irregular vacuolated cytoplasm and damaged nucleus.

Figure (31) Oenocytoids

Fig. 31B: Infected oenocytoid contained two bacterial cells and surrounded by bacteria.

Fig. 31C: Deformed oenocytoid with extruding cytoplasm and divided nucleus.

Fig. 31D: Damaged oenocytoid, was not clearly distinguished, the external and internal membranes were ruptured, the cell cytoplasm was irregular around the destructed nucleus which divided into four parts by the action of bacteria.

Figure (30): Granulocytes (GRs). **A-** Normal GR. **B-** Treated GR elaborated fine cytoplasmic filaments. **C-** Infected GRs that aggregated to form a capsule around a foreign body. **D-** Damaged GR with discharged cytoplasm. **E-** Degranulated GR with irregular vacuolated cytoplasm and damaged nucleus. (X2000)

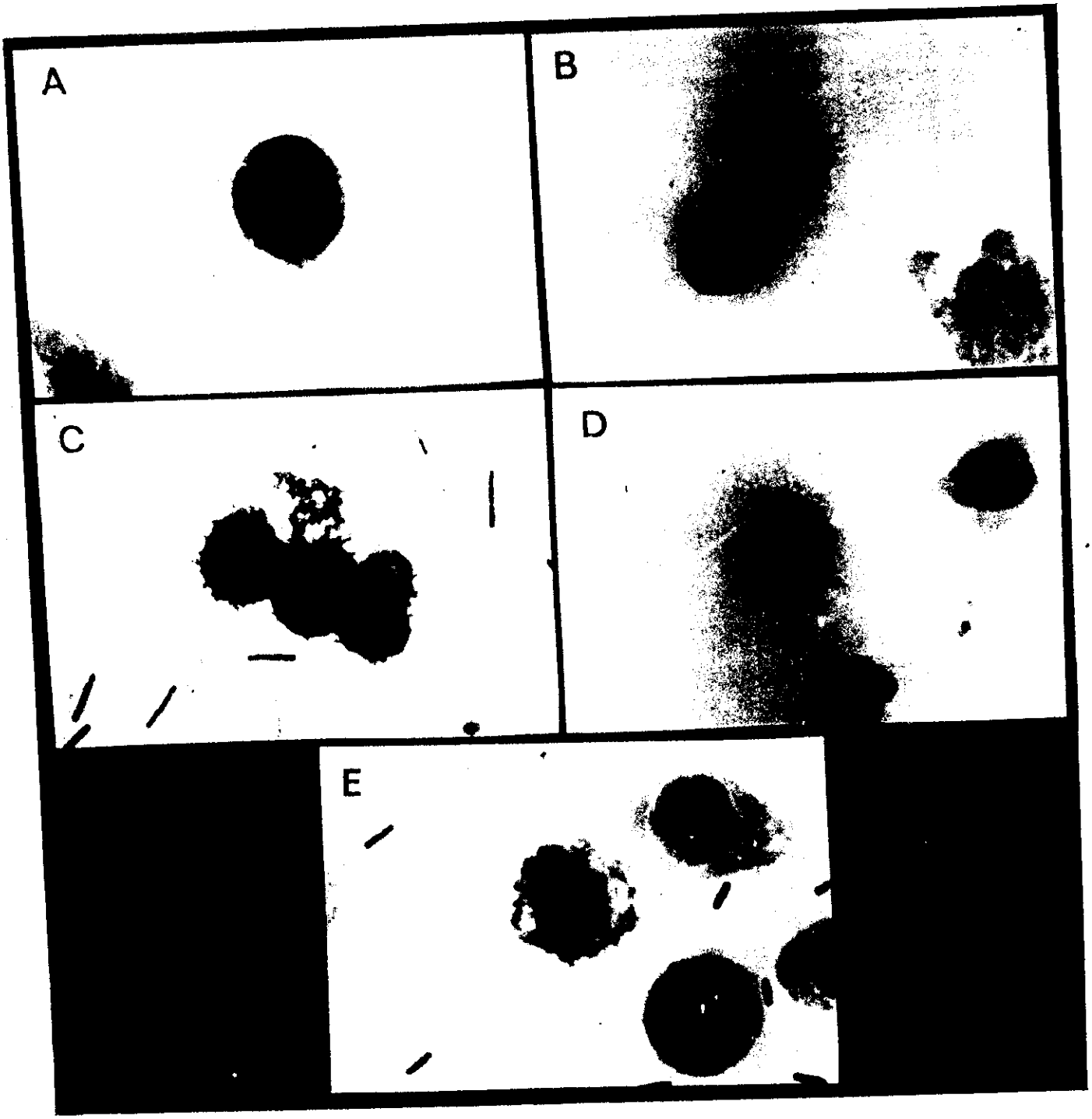


Figure (30)

Figure (31): Oenocytoids (OEs). **A-** Normal OE. **B-** Treated OE containing two bacterial cells. **C-** Deformed OE with extruding cytoplasm and divided nucleus. **D-** Damaged OE, its cytoplasm was irregulated around the destructed nucleus which divided into four parts. (X1000)

Figure (31)

8. Humoral immune response:

Table (26) depicts the antibacterial activity elicited in the haemolymph of *S. gregaria* nymphs at different time intervals after infection with the nematodes, *S. glaseri* and *H. bacteriophora* (EKB20 isolate). Significant differences ($P < 0.01$) were observed in antibacterial activities induced by the two nematode species.

The agar- well diffusion technique showed cleared zones surrounding the wells in case of bacterial lytic activity (figure, 32B). The bacterial lytic activities in *S. glaseri* infected nymphs were increased significantly from 2-12 hr post-infection and reached the maximum at 12 hr, at this time the zone width was 14.5 mm, but these activities declined again. *H. bacteriophora* induced weak lytic activities at 2 and 6 hr post-infection, the recorded zone widths were 5 and 7.5 respectively, these activities were arrested thereafter. The haemolymph was brownish in colour and full of bacteria.

9. Effect of nematodes on haemolymph protein content:

5 days old 4th-instar nymphs of *S. gregaria* were infected with about 3000 infective juveniles per nymph of *S. glaseri* and *H. bacteriophora* (EKB20 isolate). Samples of the haemolymph were collected and the mean values of haemolymph protein content were estimated every 6 hr for 24 hr post-infection.

The mean values of the haemolymph protein content are presented in table (27) and graphically illustrated in figure (33). The statistical analysis of the data indicate that, the infection with the nematodes caused a decrease in the haemolymph total proteins. Non significant reduction was observed at 6 hr post-infection, while the actual depletion of haemolymph total proteins began after twelve hours from the time of infection, where the total protein was decreased from 121.33 to 112 mg/ml. haemolymph in *S. glaseri* infected

Table (26): Antibacterial activity on agar-coated petri dishes of sera from *S. gregaria* nymphs infected with *S. glaseri* and *H. bacteriophora* (EKB20 isolate) at different time intervals.

Time post-infection (hr)	Dimention of zone of antibacterial activity (mm)	
	S. glaseri	*H. bacteriophora*
Control	0.0	0.0
2	7.50 ± 0.40	5.00 ± 0.21
6	12.1 ± 0.01*	7.50 ± 0.82
12	14.5 ± 0.90**	4.80 ± 0.05
18	10.2 ± 1.10	3.00 ± 0.11
24	7.00 ± 0.31	1.20 ± 0.01*
30	4.50 ± 0.50*	0.50 ± 0.01**
Total mean#	55.8 ± 3.22	22.0 ± 1.21

* Significant = $P < 0.05$

** Highly significant = $P < 0.01$

\# Significant diferences were observed between the two nematode species ($P<0.01$)

Figure (32): Agar-well diffusion test demonstrating antibacterial activity of *S. gregaria* serum applied to well 12 hr post-infection.

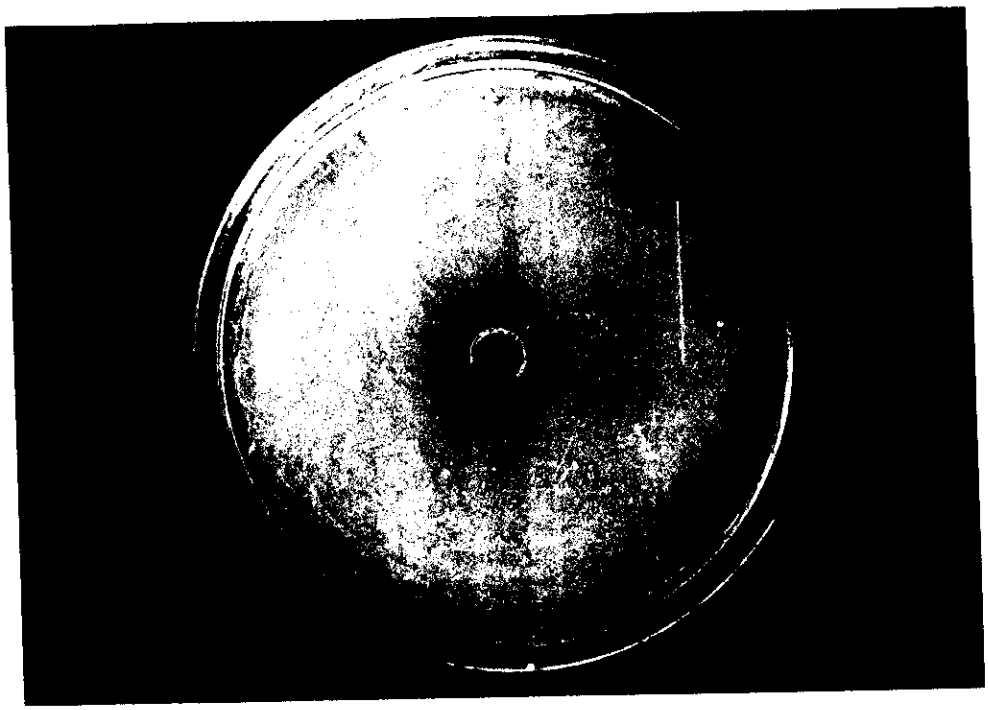


Fig. 32A: Control

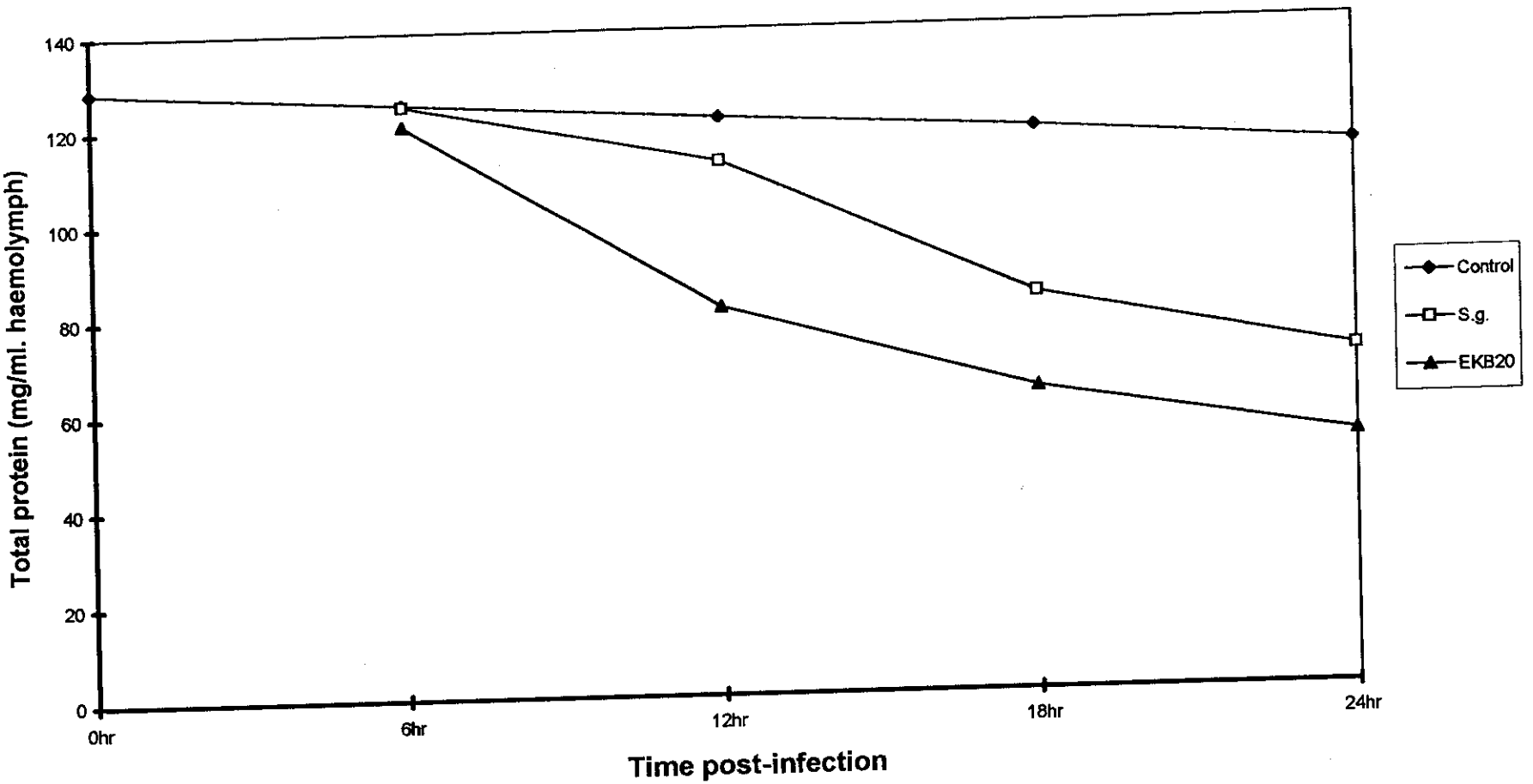
Fig. 32B: Treated

Table (27): Effect of *S. glaseri* and *H. bacterophora* (EKB20 isolate) on haemolymph total protein of 4th-instar nymphs of *S. gregaria*.

Time per hours	Total protein (mg/ml. haemolymph)				
	Control	*S. glaseri*	Reduction %	*H. bacteriophora*	Reduction %
0	128.50 ± 2.43	---		---	
6	125.03 ± 1.03	124.62 ± 2.300	0.33	120.5 ± 3.6400	3.60
12	121.33 ± 0.94	112.00 ± 1.2**	7.70	81.30 ± 3.4***	33.1
18	118.00 ± 1.37	83.000 ± 2.6***	29.66	63.14 ± 1.93***	46.5
24	113.81 ± 1.18	70.330 ± 0.83***	38.4	52.40 ± 2.04***	53.8

* Significant = $P < 0.05$
** Highly significant = $P < 0.01$
*** Very highly significant = $P < 0.001$

Figure (33): Effect of *S. glaseri* and *H. bacteriophora* (EKB20 isolate) on the haemolymph total protein of *S. gregaria* 4th instar nymphs.



nymphs and to 81.3 mg/ml. in *H. bacteriophora* infected nymphs. The highest decrease of total protein was detected after 24 hr from infection when the nymphs approached death, at this time the recorded reduction in protein content was 38.4 and 53.8% in *S. glaseri* and *H. bacteriophora* infected nymphs respectively. It is also clear from table (27) that, there was a gradual decrease of the total protein in control nymphs.

10. Semifield experiment:

5th-instar nymphs of *S. gregaria* were infected with a suspension of *H. bacteriophora* (EKB20 isolate) either by spraying or by contaminating the diet offered to the nymphs at a rate of 3000 IJs/nymph. Mortality percentages of nymphs within 7 days of infection and the number of cadavers emerging infective juveniles are given in table (28).

Data presented in the table indicate that *S. gregaria* nymphs were susceptible to infection with the nematode suspension under semifield conditions. Spraying trials gave better and faster results than contaminating the diet trials. While application of nematode suspension by spraying method gave 59.7% nymphal mortality after 3 days of application we gained 36.7% mortality by the other method after 7 days.

Results also show that, The number of cadavers emerging infective juveniles was higher (80%) in spraying method than in contaminating the diet method which recorded 55% positive cadavers.

Table (28): Mortality percent of 5th-instar nymphs of *S. gregaria* infected with *H. bacteriophora* (EKB20 isolate) at a rate of 3000 IJs/nymph under semifield conditions*.

days post - infection	Mortality %		Temperature (^{0}C)		Relative humidity %	
	Spraying	Feeding	at morning**	at night**	at morning	at night
1d	0.0	0.0	29	27	62	59
2d	34.0	0.0	30	29	60	58
3d	16.7	0.0	29	28	59	63
4d	0.0	10.0	31	29	74	72
5d	0.0	0.0	30	30	76	78
6d	0.0	16.7	31	30	73	57
7d	0.0	10.0	28	27	64	69
Total mortality %	59.7%	36.7%				
Positive cadavers***	80%	55%				

* It was observed that temperature has reached ca 40 ^{0}C or more during mid day time.

** Data were recorded at both 9 AM and PM.

*** Positive cadavers are those which produced infective juveniles.