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Mass Production of *Steinernema* spp. on *In-vitro* Developed Solid Medium

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Abstract: The development of *in-vitro* solid Wouts agar medium for *Steinernema* spp. culture has been conducted. *Steinernema carpocapsae* DD136, *S. scapterisci*, *S. riobrave*, *S. carpocapsae* All strain, *Steinernema* sp. SII, *S. abbasi* and *S. glaseri* were propagated on modified Wouts solid medium for 6 generations. Quantitative evaluation has been carried out through nematode yielded number. They produced, 5, 8, 8.5, 5.5, 6, 4 and 3 million IJs per one Kg medium for the previously mentioned nematode spp., respectively The modified medium costs 15 LE/Kg (2.5$). Qualitative evaluation test for the produced nematodes has been carried out through the bioassay on some veterinary parasites and some agricultural pests.

Key words: Entomopathogenic nematodes · *In-vitro culture* · *Boophilus annulatus* · *Hyalomma dromedarii* · *Argas persicus* · *Musca domestica* · *Grellotalpa grellotalp*a · *Spodoptera littoralis*

INTRODUCTION

Entomopathogenic nematodes (EPNs) (genera *Steinernema* and Heterorhabditis) are biological control agents that are used to control a variety of economically important insect pests [1, 2]. These nematodes and their symbiotic bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp. for steinernematids and heterorhabditids, respictevely) form a remarkable complex capable of infecting a variety of insect pests [3].

Nematodes are motile, searching for their hosts using the sense organs. They are attracted to the host bio-secretions and have the ability to recycle and multiply in great numbers under suitable environmental conditions. However, under stressed environmental conditions, EPNs can look for and survive in sheltered microenvironments in which they usually encounter their hosts. They are virtually without competition from other biological agents for control of soil-inhabiting and plant-boring insects. Currently, more than forty countries are working to develop nematodes as biological insecticides. Nematodes are sold in the U.S., Europe, Japan and China for controlling of insect pests in high-value horticulture, agriculture, home and garden niche markets [4-6].

Entomopathogenic nematodes are cultured *in vivo* or *in vitro* for large-scale commercial production as well as for laboratory experimentation or field testing [7, 8]. The technology of *in vitro* mass rearing of entomopathogenic nematodes has developed long ago since the forties [9] At that time, entomonematodes have been produced by a variety of means; by insect infection or on artificial media (axenic or monoxenic culture) in solid and liquid phase fermentation. The means chosen have depended on the amounts of product required and the time, resources, as well as the knowledge available [10]. In addition, *In vitro* technology requires substantial capital investment in sterilization equipment, as well as considerable technical expertise. These disadvantages are offset by the ease of scale-up from laboratory bench to commercial scale that achieves substantial economies of scale [10]. The result is efficiency and therefore production costs of $12 *(Steinernema carpocapsae* Weiser) to $17 *(Heterorhabditis bacteriophora* Poinar) USD per billion infective juvenile nematodes [11]. Moreover, entomopathogenic nematodes are extraordinary lethal to many important soil insect pests, yet are safe for plants and animals. This high degree of safety mean that unlike chemicals or even *Bacillus thuringiensis*, nematode applications do not require masks or other safety equipment and re-enter time residues, groundwater contamination, chemical trespass and pollinators are not issues. Most biological agents required days or weeks to kill, yet nematodes, working with their symbiotic bacteria, kill insects in 24-48 hrs [12]. In addition, nematode production is easily accomplished for some species using standard fermentation in tanks up to 150000 liters.

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Nematodes do not require specialized application equipment, as they are compatible with standard agrochemical equipment including pressurized, mist, electrostatic fund and aerie sprayers. However, recent studies have shown that the pathogen nematodes can invade and kill some certain economic veterinary insects such as the sheep blowflies *lucillia cuprina*; *Chrysomia albecips* and flesh fly *Parasarcophaga* spp. [13-17]. Also, the engorged females of some tick species of both soft and hard ticks are susceptible to be infected with entomopathogenic nematodes [18-22]. They indicated that the full engorged female ticks of *Boophilus annulatus* (Say) and *Hyalomma dromedarii* (Koch) appeared to be the stage most susceptible to penetration and killing by the nematodes. Also, EPNs showed highly virulent towards mature and immature stages of soft ticks *Argas* (*Persicargas*) *persicus* (Oken) under laboratory condition [14, 23] Although, The association between entomopathogenic nematodes and their insect hosts under natural field conditions are poorly understood especially against veterinary and medical important parasites there was field application on *Boophilus annulatus* by El-Sadawy and Abdel-Shafy [21]. They revealed the possibility of using EPNs as spray on the infected caws. The present work aims to Mass production of different species/strains of entomopathogenic nematodes using Wouts modified *in vitro* solid culture. Quantity and quality evaluation of the produced species/strain of nematodes.

MATERIALS AND METHODS

Isolation of Symbiotic Bacteria: About 20 sterilized infective juveniles (Ijs) of seven species of Steinernema (*Steinernema carpocapsae* DD136; *S.scapterisci*; *S.riobrave*; *S.carpocapsae* all strain; *Steinernema* sp S2; *S.abbasi* and *S.glaseri*) were infected of a greater wax moth larvae (*Galleria mellonella*). After 24h, a leg of surface sterilized *G.mellonella* larvae was removed and the released haemolymph was streaked on Nutrient-Agar (NA) plates containing 8% nutrient broth (LAB) and 1,2% agar. Plates were incubated at 25°C for 3 days. Then, the characteristic of bacterial colonies developed [24]; these were repeatedly subculture until a pure culture was obtained. Pure cultures were transferred to nutrient broth slants. Incubated at 25°C for 3days. Stored at 6°C.and subculture at least once a month to maintain viability. Bromothymol Blue (BTB) 0.025g and 0.04g Triphenyltetrazolium Chloride (TTC) were added to NA plates as an indicator.

Mass Culture Method: The medium depended Wouts solid medium with some modifications. It consisted of 16g Difco Bacto nutrient broth, 12g Difco Bacto agar, 6g Difco Bacto yeast extract, 7g soy flour, 5ml sun oil, 5ml glycerol, 0.5g Na Cl, 0.219g Ca Cl_2, 2g K_2HPo_4, 1.89g, KH_2HPo_4. These components are completed to 1 liter of distilled water. The previously mentioned substances were placed in Erlenmeyer flask 2 liters and cocked together on Bunsen flame to make it homogenous component. It was autoclaved for 20 min at 100 KP [25].

Culture in Petri Dish: To prepare mass production, modified Wouts medium was pored in 9-10cm sterilized Petri dishes. Inoculatation took place with pure colony of *Xenorhabdus* spp. which were prepared previously and incubated at 25°C. After 3days a layer of bacterial growth covered the plate. A small piece of agar was then removed from the center of the plate and an aqueous suspension (200IJs/g media) of surface sterilized IJs was placed in the resulting cavity. These juveniles moved into the bacterial growth (leaving dead specimens and contaminants behind), fed on the bacteria and developed into adult females within 3-5 days [25]. Every plate contains 20gm solid media. Nematodes were recovered after 3 weeks except *S.glaseri* which recovered after 15 days. Recovered nematodes had been counted and calculated the mean numbers. 5 Petri dishes were prepared for every nematode species.

Harvest Nematodes from Petri Dishes: A modified version of the White trap [26] was used. Petri dish cover was removed and the bottom of the Petri dish was placed into a larger dish, then water was added to the larger dish (20 Cm). Four pieces of filter paper were placed on the medium and allow a part of each filter paper to touch the water. The IJs will migrate into the water using the filter paper as a bridge. IJs were collected from the water as previously mentioned and counted. Calculation was carried out by mean of IJs/1ml suspension. Nematodes were stored in distilled water in tissue culture bottles on 4°C excepted *S.abbasi* on 25°C.

Bioassay Tests: The collected nematodes were tested against some veterinary important external parasites such as the engorged female of hard ticks (*Boophilus annulatus* and *Hyalomma dromedarii*); different stages of soft tick (*Argas persicus*) and 3rd instar larvae of

Musca domestica. Also, they were tested against some agricultural pests such as the adult of mole cricket (*Grellotalpa grellotalp*a) and 6th instar larvae of cotton leaf worm (*Spodoptera littoralis*). 2000, 1000, 500, 250, 125 IJs/pot were applied on the previously mentioned parasites and insects except the concentrations of *Spodoptera littoralis* which were 40, 20, 10, 5IJs/pot. Nematodes were suspended in 1ml tap water and weitted by water to make the humidity 15% (w/w). *B. annulatus, H.dromedarii, A. persicus* and *G. gryllotalpa* replicated 5 times and every pot contains 5 individuals. *M. domestica* and *S. littoralis* replicated 10 times with 10 larvae in every pot. All the experiments were applied in plastic pots 50C[3] full of 2g clean sand except *G.*in Petri dish 10Cm fu. They were incubated at 25°C for 3-4 days except for cotton leaf worm (48h). All cadavers of veterinary parasites and agricultural pests were placed on white trap [26]. The immigration of infected juveniles (IJs) had been observed.

Statistic Analysis: All data were subjected to statistical analysis including the calculation of the mean and standard error (mean ±SE). Differences between treated groups were tested for significance using a one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Differences were considered significant at $P<0.05$ level [27] using SPSS version 10 computer program.

RESULTS

In vitro solid culture of seven species or strains of *Steinernema* were reared for 6 generations on modified Wouts medium. They were recovered after 21 days except *S. glaseri* which recovered after 15 days. Infected juveniles (IJs) yields were fluctuated according to the nematode species. The IJs means numbers were 5000, 8000, 8500, 5500, 6000, 4000 and 3000 IJs per one gram medium for *S. carpocapsae* DD136, *S. scapterisci*, *S. riobrave*, *S. carpocapsae* All strain, *Steinernema* sp. SII, *S. abbasi* and *S. glaseri* (Table 1). This medium costs 15 LE/Kg (2.5$) without package costs. The seven *Steinernema* species/strains were applied against the cattle tick, *B. annulatus*, camel tick *H. dromedarii*, fowl tick *A. persicus*, house fly *M. domestica*, mole cricket *G. gryllotalpa* and cotton leaf worm *S. littoralis*. These nematodes exhibited vital effects on both tested veterinary parasites and agricultural pests. Mortality percentages elevated with increasing the concentration of nematode species/ strains in all treated parasites and pests. The two species of hard tick *B. annulatus* and *H. dromedarii* showed high susceptibility to the three nematode species *S. carpocapsae* DD136, *S. abbasi* and *Steinernema* sp.SII. Statistic analysis revealed that no significant variance among them in high concentrations 2000 and 1000 IJs/ml and significant differences in concentrations 500, 250 and 125IJs/ml (Tables 2 and 3).

Table 1: Mass production of *Steinernema* species

	DD136	Ss	Sr	Sc All	SII	Sa	Sg
No of IJs/g	200	200	200	200	200	200	200
Wt. of M6/g	20-25	20-25	20-25	20-25	20-25	20-25	20-25
Duration/day	21	21	21	21	21	21	15
Temperature °C	25	25	25	25	25	25	25
Population/1gm media	5000	8000	8500	5500	6000	4000	3000

DD136 = *Steinernema carpocapsae* DD136; Ss = *S.scapterisci*; Sr = *S.riobrave*; Sc All = *S.carpocapsae* all strain; SII = *Steinernema sp* SII; Sa = *S.abbasi*; Sg = *S.glaseri*

Table 2: The mortality percentages, LC_{50} and LC_{90} of the cattle tick *Boophilus annulatus* females infected with different *in-vitro* culture entomopathogenic nematodes

	Concentrations (Ijs/ml) Mortality percentage (Mean +SE)						
Nematode strain	2000	1000	500	250	125	LC_{50}	LC_{90}
S. carpocapsae DD 136	84.0±5.0a	80.0±4.2a	68.0±3.3b	56.0±2.7b	40.0±4.2b	194	3032
S. abbasi	96.0±2.7a	80.0±4.2a	72.0±3.3ab	56.0±2.7b	40.0±7.3b	197	1505
S. scapterisci	56.0±2.7b	48.0±3.3b	48.0±3.3c	36.0±2.7c	28.0±3.3c	1002	>10000
Steinernema sp. SII	92.0±3.3a	84.0±2.7a	80.0±4.2a	72.0±3.3a	56.0±2.7a	76	1581
S. carpocapsae All	60.0±4.2b	52.0±3.3b	32.0±3.3d	20.0±4.2d	16.0±2.7d	1124	>10000
S. riobrave	40.0±4.2c	36.0±5.0c	16.0±5.0e	16.0±2.7d	00.0±0.0e	3344	>10000
S. glaseri	36.0±6.5c	24.0±2.7d	20.0±0.0e	16.0±2.7d	00.0±0.0e	7875	>10000
F value	32.917	41,556	55,563	56,417	33,389		
P value	0,000	0,000	0,000	0,000	0,000		

R= 5 ΣR = 25 No mortality was recorded in the control group.

Table 3: The mortality percentages, LC_{50} and LC_{90} of the camel tick *Hyalomma dromedarii* females infected with different *in-vitro* culture of entomopathogenic nematodes

	Concentrations (Ijs/ml) Mortality percentage (Mean +SE)						
Nematode strain	2000	1000	500	250	125	LC_{50}	LC_{90}
S. carpocapsae DD 136	92.0±3.3a	80.0±0.0a	76.0±5.0a	56.0±2.7a	40.0±0.0a	187	1723
S. abbasi	88.0±3.3a	52.0±3.3c	40.0±4.2c	36.0±6.5b	24.0±2.7b	547	5207
S. scapterisci	76.0±5.0b	56.0±2.3c	24.0±5.0d	20.0±6.0c	16.0±2.3bc	854	6096
Steinernema sp. SII	92.0±3.3a	80.0±0.0a	56.0±2.7b	12.0±3.3c	12.0±3.3c	491	1644
S. carpocapsae All	76.0±5.0b	68.0±3.3b	52.0±3.3b	40.0±0.0b	16.0±5.0bc	493	4400
S. riobrave	44.0±2.3c	24.0±5.0d	12.0±3.3e	8.0±3.3c	8.0±3.3c	3481	>10000
S. glaseri	24.0±5.0d	20.0±6.0d	12.0±3.3e	12.0±3.3c	12.0±3.3c	>10000	>10000
F value	42,609	47,400	38,400	19,364	11,475		
P value	0,000	0,000	0,000	0,000	0,000		

R= 5 ΣR = 25 No mortality was recorded in the control group.

Table 4: The mortality percentages, LC_{50} and LC_{90} of the fowl tick *Argas persicus* stages infected with different *in-vitro* culture of entomopathogenic nematodes.

Nematode strain	Concentrations (Ijs/ml) Mortality percentage (Mean +SE)						
per tick stage	2000	1000	500	250	125	LC_{50}	LC_{90}
Female							
S. carpocapsae DD 136	96.0±1.6ab	92.0±1.3a	90.0±3.0ab	84.0±1.6a	70.0±2.1b	31	613
S. abbasi	98.0±1.3a	96.0±1.6a	94.0±1.6a	90.0±3.0a	78.0±1.3a	21	320
Male							
S. carpocapsae DD 136	86.0±2.7c	66.0±1.6c	60.0±2.1d	40.0±2.1c	16.0±2.7e	427	2803
S. abbasi	90.0±3.0bc	80.0±3.0b	56.0±1.6d	42.0±2.5c	30.0±2.1c	319	2201
Nymph							
S. carpocapsae DD 136	90.0±3.0bc	82.0±2.5b	76.0±1.6c	70.0±2.1b	34.0±1.6c	169	1628
S. abbasi	96.0±1.6ab	90.0±3.0a	86.0±2.7b	90.0±2.1a	24.0±1.6d	163	745
F value	4,100	22,654	53,578	103,269	170,723		
P value	0,003	0,000	0,000	0,000	0,000		

R= 5 ΣR = 50 No mortality was recorded in the control group.

Table 5: The mortality percentages, LC_{50} and LC_{90} of the house fly *Musca domestica* larvae infected with different *in-vitro* culture of entomopathogenic nematodes

	Concentrations (Ijs/ml) Mortality percentage (Mean +SE)						
Nematode strain	2000	1000	500	250	125	LC_{50}	LC_{90}
S. carpocapsae DD 136	81.0±3.1ab	58.0±2.5b	40.0±3.0c	32.0±2.9b	16.0±1.6b	620	4733
S. abbasi	55.0±2.7c	40.0±3.0c	26.0±3.1d	10.0±2.1c	4.0±1.6d	1524	>10000
S. scapterisci	89.0±3.1a	84.0±4.0a	74.0±3.4a	62.0±2.5a	40.0±2.6a	168	1807
Steinernema sp. SII	78.0±3.9b	55.0±2.7b	31.0±2.8d	16.0±3.4c	12.0±2.5bc	823	4636
S. carpocapsae All	81.0±3.1ab	60.0±2.6b	45.0±3.1bc	25.0±2.7b	10.0±2.1bcd	642	3561
S. riobrave	75.0±3.1b	60.0±2.6b	49.0±2.3b	26.0±3.1b	8.0±2.5cd	670	4141
F value	12,958	23,307	32,901	41,968	34,477		
P value	0,000	0,000	0,000	0,000	0,000		

R= 10 ΣR = 100 No mortality was recorded in the control group.

Table 6: The mortality percentages, LC_{50} and LC_{90} of the mole cricket *Gryllotalpa gryllotalpa* adult infected with different *in-vitro* culture of entomopathogenic nematodes

	Concentrations (Ijs/ml) Mortality percentage (Mean +SE)						
Nematode strain	2000	1000	500	250	125	LC_{50}	LC_{90}
S. carpocapsae DD 136	88.0±3.3a	80.0±4.2b	52.0±3.3c	28.0±3.3bc	8.0±3.3	481	1852
S. abbasi	88.0±3.3a	76.0±5.0b	52.0±3.3c	20.0±0.0cd	4.0±2.7	544	1846
S. scapterisci	96.0±2.7a	92.0±3.3a	80.0±4.2a	52.0±3.3a	12.0±3.3	276	877
Steinernema sp. SII	92.0±3.3a	84.0±2.7ab	68.0±3.3b	16.0±2.7de	4.0±2.7	461	1315
S. carpocapsae All	88.0±3.3a	76.0±2.7b	48.0±3.3c	24.0±2.7bcd	8.0±3.3	525	2018
S. riobrave	76.0±5.0b	52.0±3.3c	28.0±3.3d	8.0±3.3e	0.0±0.0	956	3487
S. glaseri	96.0±2.7a	80.0±4.2b	72.0±3.3ab	32.0±3.3b	8.0±3.3	383	1236
F value	3,978	11,167	26,348	24,188	1,875		
P value	0,002	0,000	0,000	0,000	IS		

R= 5 ΣR = 25 No mortality was recorded in the control group. IS=Insignificant

Table 7: The mortality percentages, LC_{50} and LC_{90} of the cotton leaf worm *Spodoptera littoralis* larvae infected with different *in-vitro* culture of entomopathogenic nematodes

	Concentrations (Ijs/ml) Mortality percentage (Mean +SE)					
Nematode strain	40	20	10	5	LC_{50}	LC_{90}
S. carpocapsae DD 136	90.0±2.6ab	60.0±2.6b	26.0±3.1d	10.0±2.1abc	16	44
S. abbasi	89.0±3.1abc	78.0±3.9a	44.0±1.6b	8.0±2.5bc	12	34
S. scapterisci	80.0±2.1cd	20.0±2.1c	5.0±1.7e	5.0±1.7cd	27	69
Steinernema sp. SII	94.0±1.6a	86.0±3.4a	65.0±1.7a	16.0±3.4a	9	25
S. carpocapsae All	84.0±4.0bcd	55.0±2.7b	35.0±1.7c	13.0±2.6ab	16	58
S. riobrave	86.0±3.4abcd	60.0±3.6b	25.0±2.7d	6.0±1.6cd	17	46
S. glaseri	78.0±3.9d	53.0±2.6b	10.0±2.1e	0.0±0.0d	22	52
F value	3,392	53,196	91,583	5,739		
P value	0,006	0,000	0,000	0,000		

R= 10 ΣR = 100 No mortality was recorded in the control group.

Also, no significant variance between *S. scapterisci* and *S. carpocapsae* All strain or *S. riobrave* and *S. glaseri* in high concentrations 2000 and 1000 IJs/ml and great variance in other concentrations applied to *B. annulatus* (Table 2). These four nematodes showed great variances in all concentrations *versus H. dromedarii* (Table3). Table 4 indicates that, there were no significant differences between the two species *S. carpocapsae* DD136 and *S. abbasi* in all concentrations *versus A. persicus* females and slightly differences in males. But there were significant differences in nymphs. The five tested nematodes in table 5 did not act as the same for *M. domestica* as they showed great significant variances in all nematode concentrations. Also in the seven tested nematode species against *G.* and *S. littoralis*, they exhibited significant variances in all concentrations (Tables 6 and 7).

Toxicity lines cleared the virulence of tested entomopathogenic nematodes which are reared on modified Wouts medium. It was obviously that *S. carpocapsae* DD136 was the promising one against all tested veterinary parasites and agriculture pests. Whereas, their LC_{50} were 194, 187, (31, 427 and 169), 620, 481 and 16 IJs/ml for *B.annulatus*, *H. dromedarii*, *A. persicus* (female, male and nymph), *M. domestica*, *G. gryllotalpa* and *S. littoralis* respectively. Nematode species *S. abbasi* comes second in virulence arrangement. Their LC50 were 197, 547 (21, 319 and 163), 1524, 544 and 12 IJs/ml for the previously mentioned pests, respectively. The Egyptian native isolate *Steinernema* sp. SII could be considered the promising one against *B.annulatus* and *S.littoralis* if compared with the other nematode species. It recorded LC50 76 and 9 IJs/ml for previously mentioned pests respectively. Also, results of *S.scapterisci* were

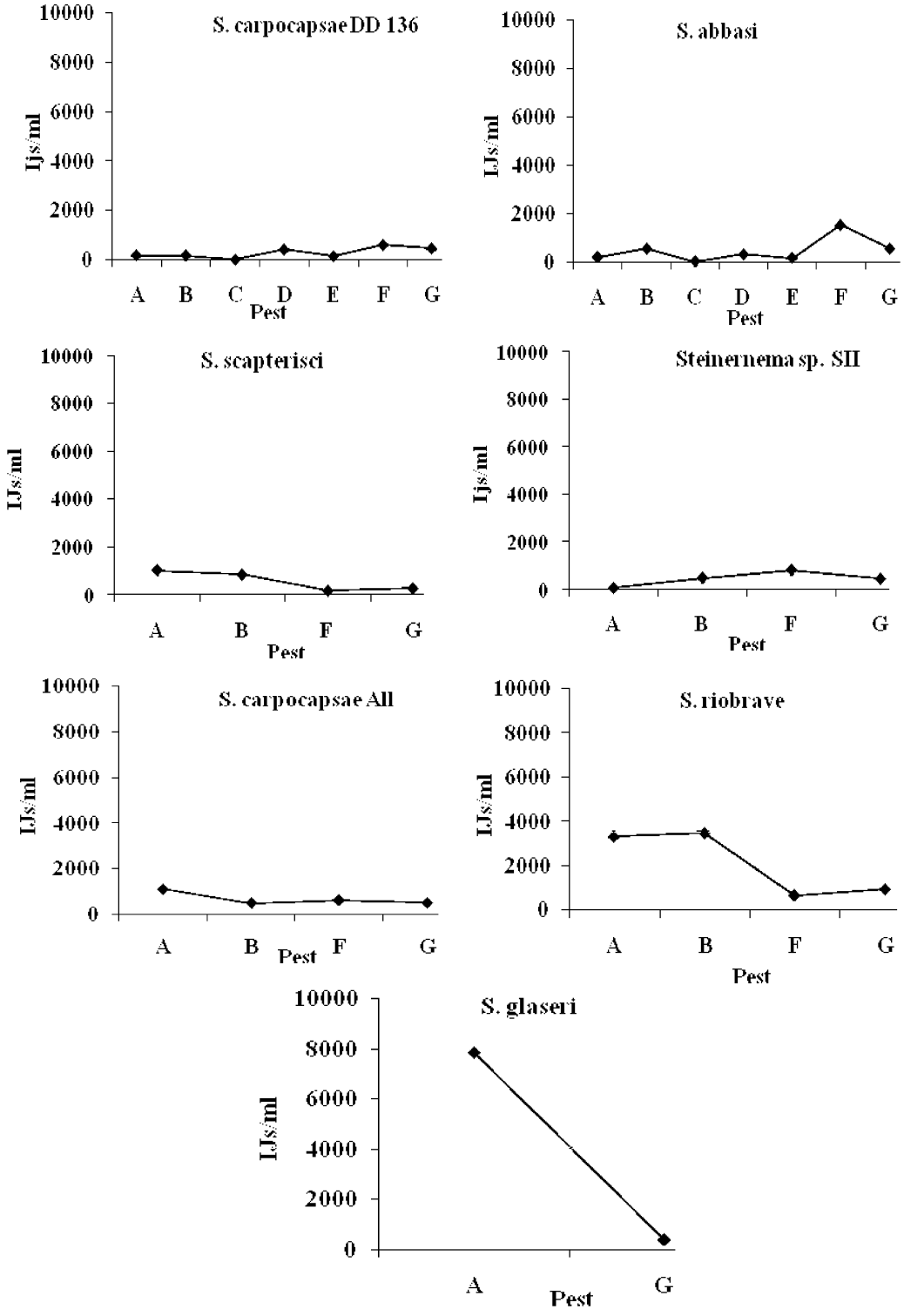

Fig. 1: The toxicity of entomopathogenic nematodes against veterinary, medical and agriculture pests; A) *B. annulatus*, B) *H. dromedarii*, C) *A. persicus* (female), D) *A. persicus* (male), E) *A. persicus* (nymph), F) *M. domestica*, G) *G. gryllotalpa*

placed it in the promising group against *M.domestica* and *G. grellotalpa*, whereas their LC50 were 168 and 276 IJs/ml respectively. No virulence differences can be found in LC50 results of *S. carpocapsae* DD136 and *S. carpocapsae* All strain against *S. littoralis* (LC50,16 and 16 IJs/ml), respectively. Slightly differences in virulent were found between the previously mentioned nematode species and *S. riobrave* in LC50 results against *S. littoralis* and *M. domestica* (Fig. 1 and table 7). The agriculture pest *G. gryllotalpa* exhibited high susceptibility to both nematode species *S. scapterisci* and *S. glaseri*, which needed low number of IJs to kill 50 percent of its population (276 and 383 IJs/ml respectively). On the other hand, *S. littoralis* seemed more tolerance to these nematode species, whereas their LC50 were 27 and 22 IJs/ml respectively (Fig. 1 and table 7). However, mortality percentage and toxicity line of LC50 revealed poor virulent of the nematode species *S. glaseri* against the two species of veterinary parasites *B. annulatus* and *H. dromedarii*. They need large number of IJs (7875 and >10000 respectively) to kill 50 percent of their population (Fig. 1). No developmental stages were observed in hard and soft ticks. Negligible numbers of IJs immigrated from the house fly if compared to the great number of IJs immigrated from the mole cricket and cotton leaf worm.

DISCUSSION

Nematodes were first grown *in-vitro* on a solid medium axenically [28]. Thereafter it was realized that growth increased with the presence of bacteria [9, 29]. The importance of the natural symbiont was recognized [30] and monoxenic culture has been the basis for *in-vitro* culture since [25,31, 32]. To create monoxenic cultures surface-sterilized nematodes were added to a lawn of bacterial symbionts 25,31]. Solid culture was first accomplished in two-dimensional arenas, e.g., Petri dishes, containing various media such as those based on dog food, pork kidney, cattle blood and other animals products [32]. Wouts [25] developed an improved medium (less expensive and more consistent from batch to batch) [7].

In the present work, seven species/strains of nematode belong to *Steinernematidae* are successfully propagated on modified Wouts medium. Mass culture method of Wouts medium consisted of 0.44g Difico Bacto nutrient broth, 0.16g Difco Bacto yeast extract, 7.2g soy flour, 5.2g corn oil and 27g water. These components were cooked into smooth roux. To create a large surface area, the roux was squeezed thoroughly into 9g of damp polyurethane foam chips [25]. In this paper, the modification was don by adding nutrient broth, soy flour, sun oil, glycerol, agar, Nacl, $Cacl_2$, K_2HPo_4 and KH_2HPo_4 which mixed in one liter of distilled water. Nematode species are maintained for 6 generations on this medium. Because infected juveniles are nonfeeding [33], the presence of high level of stored energy compounds such as lipids is essential. Modified Wouts agar medium contains vegetable oil which provides nematodes with lipids. Also, glycerol is a simple poly component which provides nematode by energy. Addition of some minerals enhances suitable neutral condition with Ph 7.2. for nematode nutrition.

Quantitative and qualitative biochemical analyses of *in vivo* and *in vitro* produced nematodes could be an accurate method for establishing nematode quality [34]. There are some factors affecting yield such as nematode inoculum size in some strains but not others [35-37]. For example, *S. carpocapsae* (*Agriots* strain) produced optimum yields at an intermediate inoculum size (2,000 IJs/g medium) [36],Whereas *S. carpocapsae* (CB2B strain) and *H. bacteriophora* (H06) were not affected by inoculum size [35]. Bacteria inoculum size does not appear to be important in yield determination [35, 36]. Inoculum size was defined in the present study and fluctuated among yield of the seven nematode species. Whereas, 35g/litter of the modified Wouts solid medium produced 5, 8, 8.5, 5.5, 6, 4 and 3 million/Kg medium of *S. carpocapsae* DD136, *S. scapterisci*, *S. riobrave*, *S. carpocapsae* All, *Steinernema* sp. SII, *S. abbasi* and *S. glaseri*, respectively. Comparing the present results with those of Tabassum and Shahina, [38] whereas they followed successfully techniques described by Bedding [39]; they produced 5-7, 4-5 and 1-2 million infective juveniles of *S. pakistanense, S. asiaticum* and *S. feltae*, respectively in a single 500ml flask containing 80g of chicken offal medium. Longer culture times can provide higher yields but nematode mortality may also increase with time [35, 36] and culture time must be weighed against the cost of space. The present results confirmed that, because when the culture time was increased from three to four weeks, nematodes mortality were increased. Media composition can have a substantial effect on nematode yield. Other medium ingredients that may have a direct effect on nematode yield include protein source and salts [40]. In the present work, more than one species of nematodes was produced on dog food medium (PDF) but, they all failed to reproduce, so we try the Wouts agar medium was tried with some modifications.

Qualitative evaluation had been carried out through virulence of produced nematodes against some veterinary and agricultural parasites and pests. The three nematode species *S. carpocapsae* DD136, *S. abbasi* and *Steinernema* sp. SII were the promising isolate against *H. dromedarii* and *B. annulatus* where, they could kill the engorged females but, they failed to replicate inside their haemolymph. This finding was confirmed by the results of El-Sadawy and Habeeb [20]; El-Sadawy and Abdel Shafy [21]; El-Sadawy *et al.* [22] and Glazer *et al.* [41]. The engorged females of soft tick *A. persicus* were the most susceptible stage to *S. carpocapsae* DD136 and *S. abbasi*. These results agree with those of Hassanain *et al.* [42]. It was observed that a period of two hours of *in vitro* exposure was sufficient for the engorged *R. (Boophilus) microplus* females to be infected by the entomopathogenic nematode *S. glaseri* CCA strain [43, 44]. In the present work, the results disagree with these observations. The tested *S. glaseri* did not show significant virulence against *H. dromedarii* and *B. annulatus*.

Taylor *et al.* [45] determined the potential for entomopathogenic nematodes to control flies in cattle feedlots by 40 strains for virulence toward 3rd. instar larvae of house flies (maggots) *Musca domestica*. Ten strains of *Steinernema* infected maggots, of which 7 strains (4 *S. carpocapsae* (Weiser), 2 *S. feltiae* (Filipjev) and 1 *S. scapterisci* Nguyen and Smart) caused significant mortality. *S. scapterisci*, *S. carpocapsae* DD136 and the native species *Steinernema* SII were the most virulence ones. *Steinernema scapterisci* is specific to mole crickets *G.gryllotalpa*. The nematode was successfully introduced by inoculative applications in golf courses and pastures [46, 47] in Florida Since 1993. It confirmed our results whereas *S. scapterisci* was the most virulence one against *G. gryllotalpa*. Moreover, studies with *S. scapterisci to* control mole crickets in pastures documented the efficacy of the nematode as a biological control agent [46, 48, 49]. It was determined that infection of mole cricket nymphs was substantially less than for adults [50-52]. Small nymphs were not infected at all by nematodes [48]. Our results agreed with those of Barbara [53]; Barbara and Buss [54] as they indicated that the adult stage of mole crickets should be targeted for nematode application. When the seven *Steinernema* spp. had been applied on the adult stage of mole cricket *G. gryllotalpa*. Bioassay test on the studied *Steinernema* spp. against *S. littoralis* revealed that the native isolates *steinernema* sp. SII was the most effective nematode followed by *S. abbasi* and *S. carpocapsae* DD136. This result partially agrees with those of Abdel-Razek [55] Abdel-Razek and Abd-Elgawad [56] whereas they studied the infectivity of *S. carpocapsae* All and *S. riobrave* on cotton leaf worm *Spodoptera littoralis*, they gave 100% mortality within 24 h post exposure. *S. riobrave* did not show much efficacy in our results, it gave 86% on 40 inoculums level 48 h post exposure. It may be du to the immune response because we collected our tested insects from the field.

In conclusion, the modified Wouts solid medium was successfully used to propagate 7 species/strains of *Steinernema* nematodes for 6 generations. This medium costs 15 LE. (2.5$) to produce one kg. Quantitative and qualitative tests were carried out through nematode yield number and bioassay tests. The bioassay tests were conducted against some veterinary parasites and agricultural pests. Most of *Steinernema* spp. showed significant virulence against the tested hosts.

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