Enhance the infectivity of Entomopathogenic nematode for the desiccation by novel methods.

Saheir F. El-Lakwah

Department of Pest Physiology, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.
sosoellakwah@yahoo.com

Abstract: New progenies of the three entomopathogenic nematodes species (*Steinernema riobrave*, *Steinernema rarum*, *Heterorhabditidsindica*) and the isolate *Heterorhabditids* sp. (Hp2) obtained from the continuous culturing of nematode density inside the host (full grown larvae of the greater wax moth, *Galleria mellonella*). The newprogenies have the ability to penetrate and caused high mortality to the host more than the original species and its efficacy were not affected by the desiccation. The 4, 5, 6th progenies of the four tested species recorded penetration rate range (78.33-96.33%) and 100% mortality to *Galleria mellonella* in the soil moisture 10, 5, 3%. That mean these new juveniles are an excellent biocontrol agent.
[Saheir F. El-Lakwah. **Enhance the infectivity of Entomopathogenic nematode for the desiccation by novel methods.** *J Am Sci* 2013;9(6):639-645]. (ISSN: 1545-1003). http://www.jofamericanscience.org. 81

Keywords: Entomopathogenic Nematode, efficacy, mortality, penetration rate, desiccation, *Galleria mellonella*.

1.Introduction

The success of entomopathogenic nematodes applications for insect control in soil and the survival of naturally occurring nematode populations depends on the Infective Juvenile's ability to disperse and persist until it can locate a host. Migration and persistence of IJ have been shown to be affected by numerous intrinsic factors (e.g., behavioral, physiological and genetic characteristics) and extrinsic factors (e.g., temperatures, soil moisture, soil texture, RH, UV radiation) (Kaya, 1990; Smits, 1996) and biotic nature [antibiosis, competition, and natural enemies (Kaya and Koppenhöfer, 1996). The most important soil factor is moisture (Grant and Villani, 2003a, b).

Several studies have indicated that the soil moisture range in which IJs can be active may differ among entomopathogenic nematode species (Koppenhöfer*et al*., 1995; Grant and Villani, 2003a). IJs cannot survive rapid desiccation in laboratory experiments under low RH regimes but they can persist considerable lengths of time in dry soil (Kung *et al*., 1991).

Some crops need to be a period of drought and the vast desert areas has a few water. Anbesse *et al*. (2013) provided applicable methods to improve and stabilize beneficial traits of heterorhabditid EPNs through selective breeding for desiccation tolerance in liquid culture. Therefore, it is important to enhance the infectivity of nematode species or strains in lower soil moisture conditions.

Wijbenga and Rodgers (1994) reported that the shelf life of an entomopathogenic nematode product depends on many factors such as initial condition of the nematodes, formulation, storage temperature, package size, the density of nematodes and nematode species. Effects of density should be taken into account in the laboratory culture and field release of entomopathogenic nematodes. Infectivity is commonly used to evaluate the efficacy of entomopathogenic nematodes as biocontrol agents.

El-Lakwah *et al*., (2008) established novel methods to enhance entomopathogenic nematodes efficacy and were obtained new progenies of *S. riobrave* and *H. bacteriophora*, with higher infectivity than in the original nematodes.

In this investigation we tested the effect of ranges of desiccation on the infectivity of several new progenies of *S. riobrave*, *S. rarum*, *Heterorhabditids* sp. (Hp2) and *H. indica* which were received by continuous culturing of nematode juveniles under optimum condition of temperature and nematode density.

2. Material and Methods

Three entomopathogenic nematode species and local isolate were used in the present search the four nematodes were obtained from the laboratory of Insect Parasitic Nematodes, Plant protection Research Institute, Agriculture Research Centre, Egypt. *S. riobrave*, *S. rarum* and *H. indica* imported from USA, Florida, *Heterorhabditids* sp. (Hp2) originally isolated from a soil sample El-kasasin-Ismailia-Egypt.

The samples of sandy loam soil were dried in oven for (3 hrs at 72 ºC), and aerated for at least 1 week before use. Three soil moistures adjusted by (W/V) 3, 5 and 10%.

Continuous culturing of nematode juveniles for several cycles under optimum conditions of temperature and nematode density inside the full grown larvae of the greater wax moth, *Galleria*

mellonella (El-lakwah, *et al*., 2008). Full grown larvae of *G. mellonella* kept in 1.5 ml Eppendorf tubes, lined with double layer filter paper (Whatman No. 1), were subjected to nematode infection, at a dose level of 20 IJs/larva in 300 µl of distilled water and kept at 25°C, in the dark. All dead larvae were placed in extraction dishes after 48 hours (White, 1927*)*.

After 10 days, the first generation of emerged juveniles was received in distilled water. Continuous culturing of nematode juveniles, for several cycles under the same conditions, was carried out to collect the new generations. Each generation was washed three times and concentrated to approximately 5000 IJs/ml. Penetration rate, Virulence (one -on -one) and mortalityof the four species were determined According to (Koppenhöfer and kaya, 1999).

1. Penetration Rate and mortality:

In each of the investigated nematode progenies, *G. mellonella* larvae were exposed to nematode infection, using 24-well tissue culture plates each 20 IJs/larva in 300 µl of distilled water were placed on the bottom of each well which was then filled with pre wetted soil. The soil water content (%w/v soil moisture) tested were 10%, 5% and 3%. A wax moth larva was placed in each well and the plate was covered with its lid and kept in the darkness incubator at 25°C. Mortality were taken after 48 hours and corrected according to Abbott's formula (Abbott, 1925). For 4-5days, according to the species of nematode, all dead larvae were washed twice with distilled water to remove any nematode juveniles that attached to them, dried and dissected under a stereomicroscope. The number of nematodes inside each larva was counted in at least 10 larvae for each generation for each soil moisture10%, 5% and3% in addition to a control (20IJ/larva in 300 µl of distilled water in 1.5 ml Eppendorf tubes, lined with double layer filter paper)and the penetration rate was calculated as percentage.

2. Virulence (one: one):

G. mellonella larvae were subjected to nematode infection as previously described in penetration rate determination, but at a dose level of 1 IJ/larva in 300 µl of distilled water and kept at 25°C, in the dark. For each of the tested nematode generations, 3-5 replicates, each of 6 larvae, for each soil moisture10%, 5% and3% in addition to a control (1IJ/larva in 300 µl of distilled water in 1.5 ml Eppendorf tubes, lined with double layer filter paper). Mortality records were taken after 48 hours and the Virulencewas calculated as percentage.

Statistical Analysis

The Percentage values in the present study were normalized using arcsine transformation. The significance of the main effects was determined by analysis of variance (ANOVA) using SAS program (SAS Institute,2002) The significance of various treatments was evaluated by Duncan's multiple range test (P<0.05) .

3. Results and Discussion

The mortality percentage of the *S. riobrave*, *S. rarum*, *Heterorhabditids* sp. (Hp2) and *H. indica* to *G. mellonella* in the control condition were presented in Fig.1A, and the original juveniles of these tested nematode recorded mortality (96.66, 80, 100 and 93.33%,respectively).The mortality increase through the progenies where the 1st progenies of the *S. riobrave*, *Heterorhabditids* sp. (Hp2) and *H. indica* recorded (100%) mortality to *G. mellonella* except species *S. rarum* recorded (100%) mortality at the 3rd progenies. The decreasing in the mortality percentage to *G. mellonella* by decreasing the soil moisture (10, 5, and 3%) showed in Fig.1B, C and D. But at the 3rd, 4th, 5th and 6th progenies of the *S. riobrave*, *S. rarum*, *Heterorhabditids* sp. (Hp2) and *H. indica*, it try to keep its efficacy where it recorded at the3rd progeny (100, 93.33,100 and 93.33% , respectively). And at the 6th progeny the tested nematode were not affected by desiccation where it recorded 100% mortality to *G. mellonella*

In Fig 3A,the penetration rate of the original progenies of the *S. riobrave*, *S. rarum*, *Heterorhabditids* sp. (Hp2) and *H. indica* to *G. mellonella* in the control condition were (39, 37.33, 58 and 31.66%,respectively), afterwards, it increased gradually through the 1st, 2nd, 3rd,4th, 5th and 6th progenies of the four tested nematode, Where it recorded the highest penetration rate at the 6th progenies of the four nematode in the control condition (100, 98, 99and 98.66%, respectively). Fig.3B, C and D presented the penetration rate of the origin and the 6 progeniesfor each tested nematodein the soil moisture (10, 5and 3%).The effect of desiccationappear on the decreased the penetration rate for all the progenies, the penetration rate of the6th progenies of the *S. riobrave*, *S. rarum*, *Heterorhabditids* sp. (Hp2) and *H. indica*in the 3% soil moisture were (91.66, 90, 87and 85%, respectively).Despite this it were more than the penetration rate of the original nematode in the control condition.

In Fig.2A, B, C and D, the virulence of the *S. rarum*, *Heterorhabditids* sp. (Hp2) and *H. indica* to *G. mellonella* where the original juveniles of the four nematode at the control condition recorded (37.5, 4.1, 45.83 and 75%, respectively) and these percentage decreased by the effect of desiccation on the four nematode where the original juveniles of the four nematode in the 3%soil moisture recorded (20.83, 4.16, 20.83 and 20.83%, respectively)

virulence to *G. mellonella*, while the enhancing progenies 4th, 5th and 6thfor all the tested nematode were not affected by the desiccation condition and recorded 100% virulence to *G. mellonella* in the soil moisture (10, 5and 3%).

Consumer acceptance of nematode products as biological control agents of insect is determined by their ease of use, efficacy and price. Efficacy is the most important factor determining the quality of nematode products. Temperature is the most important factor affecting nematode survival in formulations. Each species has an optimum storage temperature, which is well below the optimum temperature for activity and reproduction of the species and reflects the climatic conditions of its original locality. Dunphy and Webster (1986) found that the differences in virulence were influenced by temperature.

Density should be taken into account; Selvan*et al.* (1993) reported that the percentage penetration declined with increasing dose, so that, in the enhancing method continuous culturing of nematode juveniles (El-lakwah, *et al*., *2008*), was under optimum condition of temperature 25 °C and nematode density 20 IJs/larva. We established that, after several tests was carried out to choose the optimal temperature and nematode density.

We obtained new progenies. Its penetration rate and mortality to *G. mellonella* was higher than the penetration rate and mortality of the original species in the laboratory conditions of appropriate moisture (filter paper). The strong descendant came from the strong parent. Or, high penetration rate may be due to the low number of IJs which penetrated the *G .mellonella* larvae, thus having more amount of food, because of the suitable nutrient conditions. In that case, infective juveniles of nematode with high quality and efficacy are produced.

Water content of soil plays an important role, and even standardizing by water potential as done in the (Koppenhofer and Fuzy ,2006) study may not result in the exact same availability of water in the soil pores in different soils. So we used the same samples of sandy loam soil in this search. Nematode survival is affected by the interaction of soil type, moisture, and aeration (Wallace, 1971).

This research studied the effect of desiccation on the new progenies which already recorded high infectivity at the control condition. The observation in our result that these new progenies had resisted the desiccation.

Because nematode species may differ in their ability to adjust to low soil moisture, the effect of soil moisture on nematode may also differ among nematode specie(Kung *et al*., 1991; Grant and Villani, 2003b). On the other hand the effect of soil moisture may also differ among the activity of the nematode species as we observed in our result the % mortality and virulence were greater than the % penetration rate which were recorded by the same progenies of the species so we suggested that the penetration rate was affected by desiccation condition because penetration rate depended on the number of nematode enter the larva but the mortality of larva can occur by one IJ, so penetration rate was more effected by the soil texture and the low amount of water which prevent the IJ from moving. Nematode movement is optimal if the thickness of the water film is approximately half the thickness of the nematodes' body (Wallace, 1958). As the soil dries, the water film becomes thinner and larger pores drain of water which increasingly restricts nematode movements.

Grant and Villani, 2003a, who demonstrated that Insect mortality, was generally low in low-moisture, that agree with our result where thepenetration rate and %mortality to *G. mellonella* were recorded by the original juveniles of the most tested speciesdecreased withdecreased soil moisture.

The new progenies have an increase in the penetration rate and virulence than the original speciesand its infectivity was not affected by the soil moisture decreased. This was related to the efficiency of the enhancement which occurred to this progeny and to its parents, since each generation improvement of the individuals was preceded by the selection of the strongest and the fastest nematode which happened during the reproduction of individuals, thereby, producing generation after generation with highest mortality effect and penetration. Also at least in some situations, the nematodes become established recycle, and their offspring continue to control the target insect (Parkman *et al*., 1993), especially if this offspring descending from parents passing in the enhancing method that make them having high mortality to their host and high penetration rate.

As we see the desiccation affected on the infectivity of the four tested nematode but the new progenies obtained from the enhancing method can resisted the desiccationand recorded high infectivity so the information gained from these experiments – hopefully-may enable us to use the above strain and species more effectively in the field as a biological agent.

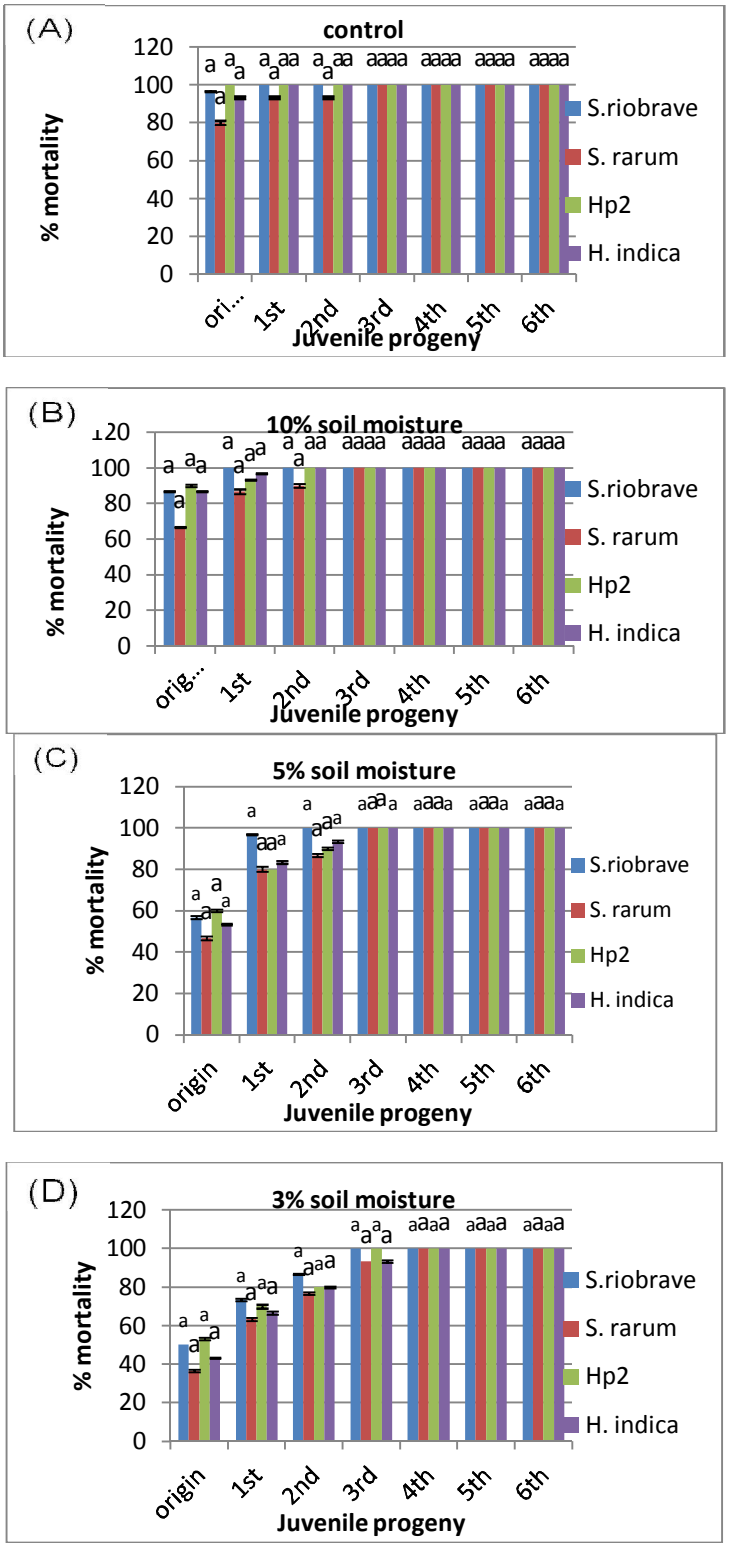

Fig (1): The mortality% of *G. mellonella* larvae subjected to Juvenile progeny of *Steinernema riobrave*, *Steinernema rarum*, *Heterorhabditids indica* and *Heterorhabditids sp.* (Hp2) at (A) control, (B)10% soil moisture, (C)5% soil moisture and (D)3% soil moisture. The statistics analysis for each species is made separately. Bars indicate Standard error of mean. Columns within each species annotated with the same letter are not significantly different (Duncan's multiple range; $P<0.05$).

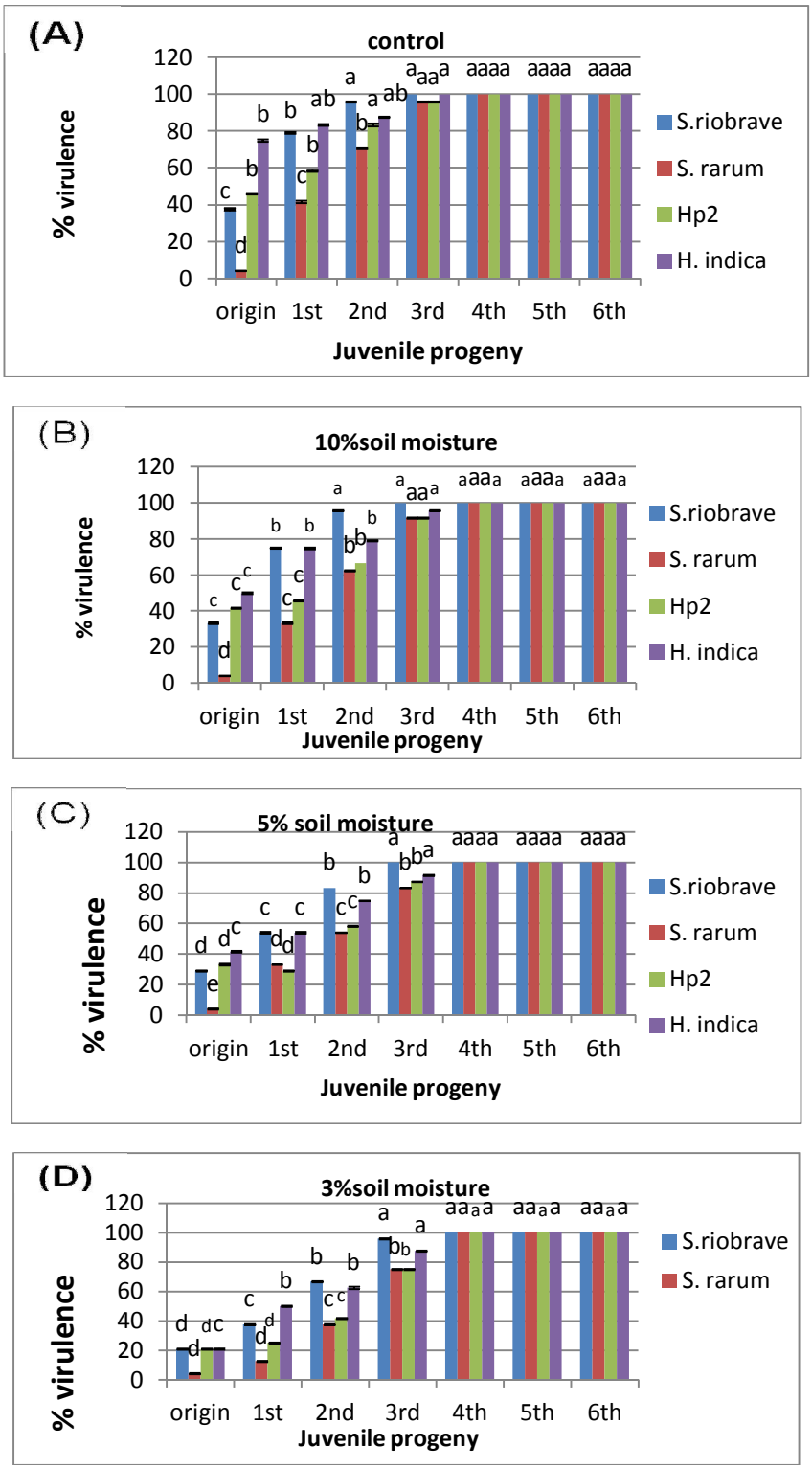

Fig (2): the virulence% of Juvenile progeny of *Steinernema riobrave*, *Steinernema rarum*, *Heterorhabditids indica* and *Heterorhabditids sp*. (Hp2) to *G. mellonella* larvae at (A) control, (B)10% soil moisture, (C)5% soil moisture and (D)3% soil moisture. The statistics analysis for each species is made separately. Bars indicate Standard error of mean. Columns within each species annotated with the same letter are not significantly different (Duncan's multiple range; $P<0.05$).

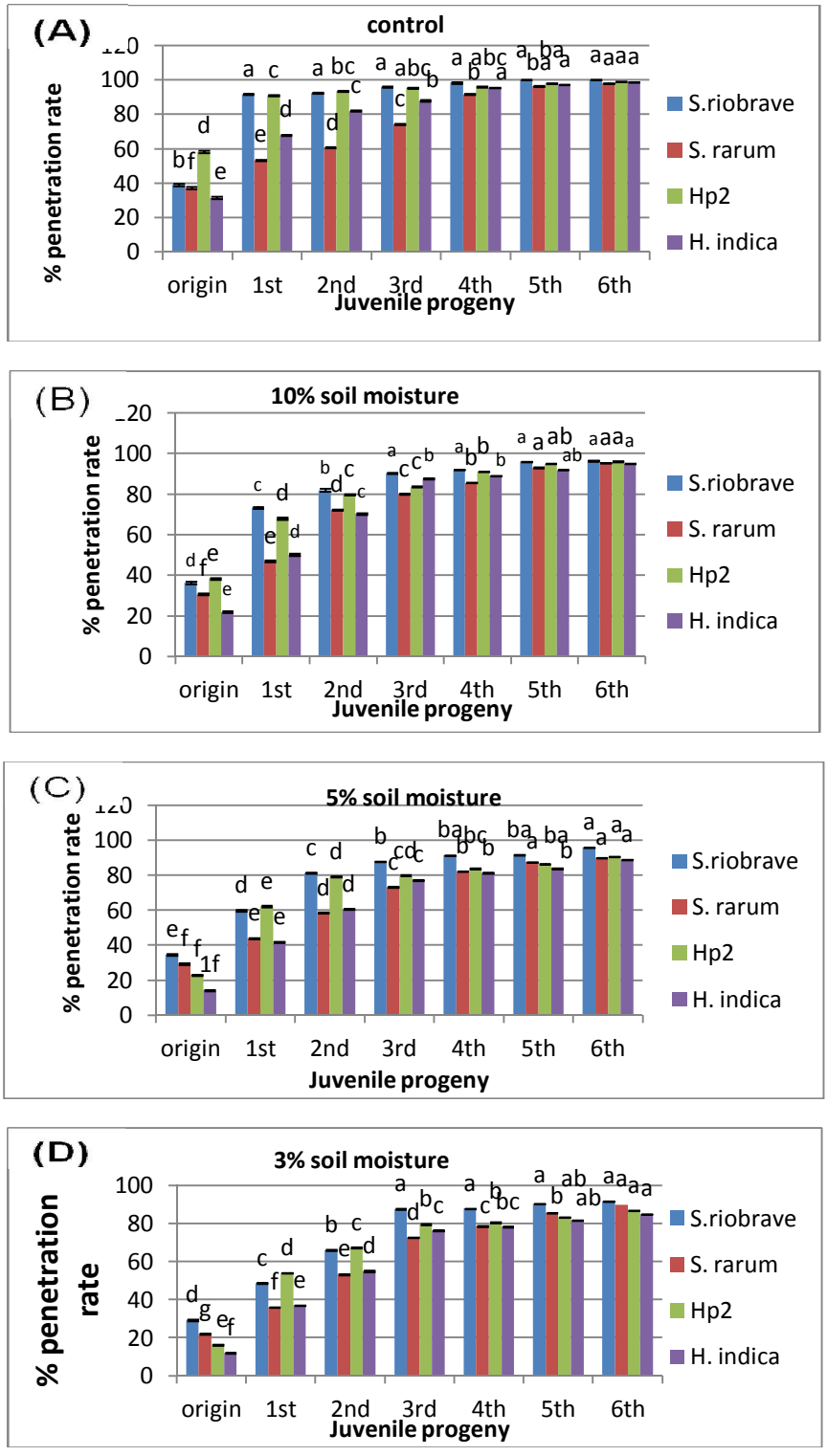

Fig (3): The penetration rate% of Juvenile progeny of *Steinernema riobrave*, *Steinernema rarum*, *Heterorhabditids indica* and *Heterorhabditids sp*. (Hp2) to *G. mellonella* larvae at (A) control, (B) 10% soil moisture, (C)5% soil moisture and (D)3% soil moisture. The statistics analysis for each species is made separately. Bars indicate Standard error of mean. Columns within each species annotated with the same letter are not significantly different (Duncan's multiple range; $P<0.05$).

Corresponding author
Saheir F. El-Lakwah
Department of Pest Physiology, Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza, Egypt.
sosoellakwah@yahoo.com

References:

1. Abbott, W.S. 1925: A method for computing the effectiveness of an insecticide. J. Econ. Entomol., 18: 265-267.
2. Anbesse S, Sumaya NH, Dörfler AV, Strauch O, Ehlers RU. 2013: Selective breeding for desiccation tolerance in liquid culture provides genetically stable inbred lines of the entomopathogenic nematode *Heterorhabditis bacteriophora*. Appl. Microbiol. Biotechnol. Jan; 97(2):731-739.
3. Dunphy, G.B. and Webster, J.M. 1986: Temperature effects on the growth and virulence of Steinernemafeltiae strains and *Heterorhabditis heliothidis*. J. Nematol, 18 (2): 270-272.
4. El-Lakwah S. F., El-Assal F. M., El-Mahdi M. M. 2008: Novel Methods for Enhancing Entomopathogenic Nematode Efficacy.Egypt. J.Agric.Res,86 (1)
5. Grant, J.A., Villani, M.G., 2003a: Soil moisture effects on entomopathogenic nematodes. Environ. Entomol. 32(1) 80–87.
6. Grant, J.A., Villani, M.G., 2003b: Effects of soil rehydration on the virulence of entomopathogenic nematodes. Environ. Entomol. 32, 983–991.
7. Kaya, H.K., 1990: Soil Ecology. In: Gaugler, R., Kaya, H.K. (Eds.), Entomopathogenic Nematodes in Biological Control. CRC Press, Boca Raton, FL, pp. 93–116.
8. Kaya, H.K. and Koppenhöfer, A.M., 1996: Effects of microbial and other antagonistic organism and competition on entomopathogenic nematodes. Biocontr. Sci. Technol. 6, 333–345.
9. Koppenhofer A.M. and Fuzy E.M. 2006: Effect of soil type on infectivity and persistence of the entomopathogenic nematodes *Steinernema scarabaei, Steinernema glaseri, Heterorhabditis zealandica*, and *Heterorhabditisbacteriophora*. Journal of Invertebrate Pathology 92 :11–22.
10. Koppenhöfer A M and Kaya H K 1999: Ecological characterization of *Steinernemararum*. Journal of Invertebrate Pathology 73: 120-128.
11. Koppenhöfer A M, Kaya H K, Taormino S P 1995: Infectivity of entomopathogenic nematodes (Rhabditida: Steinernematidae) at different soil depths and moistures. J Invertebr Pathol., 65: 193-199.
12. Kung SP., Gaugler R. and Kaya H. K. 1991: Effects of soil temperature, moisture, and relative humidity on entomopathogenic nematode persistence, Journal of Invertebrate Pathology, Vol, 57 Issue2, 242-249.
13. Parkman, J. P., J.H. Frank, K. B. Nguyen, and G. C. Smart, Jr. 1993: Dispersal of Steinernemascapterisci (Rhabditida: Steinernematidae) after inoculative applications for mole cricket (orthoptera: Gryllotalpidae) control in pastures. Biological Control. 3: 226-232.
14. SAS Institute 2002: SAS/stat user's guide version 9.1.3. SAS institute, Cary, NC.
15. Selvan, S., Gaugler, R., and Lewis, E.E. 1993: Biochemical energy reserves of entompathogenic nematodes. J. Parasitol.79: 167-172.
16. Smits, P., 1996: Post-application persistence of entomopathogenic nematodes. Biocontr. Sci. Technol. 6, 379–387.
17. White, G.F. 1927: A method for obtaining infective nematode larvae from culture. Science, 66: 302-303.
18. Wijbenga, J. and Rodgers, P.B. 1994: Storage of Heterorhabditismegidis UK211: Effect on performance and the physiological state of the nematode. Proc.Vith Colloq. Invertebe. Pathol. And Micorobial Control. Montpellier, France. 28 (2): 1-6.
19. Wallace, H.R., 1958: Movement of eelworms. Ann. Appl. Biol. 46, 74–85.
20. Wallace, H.R., 1971: Abiotic influences in the soil environment. In: Zuckerman, B.M., Mai, W.F., Rohde, R.A. (Eds.), Plant Parasitic Nematodes, vol. I. Academic Press, New York, pp. 257–280.

5/11/2013