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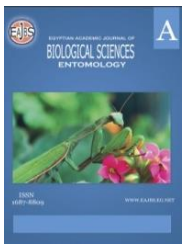

Influence of Temperatures on Storage of Formulated Entomopathogenic Nematodes

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ABSTRACT

Due to entomopathogenic nematodes EPNs represent excellent biological control agents for soil stages of several insect pests. This work aimed to study the influence of the different temperatures on the survival and vitality of EPN IJs after a long-term duration, and evaluate the suitability of EPNs, for formulation and storage on Hydrogel was used as a carrier medium. The new method was used for storage of IJs that was pieced in a square shape of Baby diapers which containing of Hydrogel (SAP), and fine cotton fibers to offer the moisture for IJs. Four EPNs were investigated, three of them were foreign, *Steinernema carpocapsae* (S.c.), *Steinernema glaseri* (S. g.), *Heterorhabditis bacteriophora* (H.b.), and one indigenous, *Heterorhabditis indica* (RM1). The tested EPNs divided into two main groups, the first group was stored at room temperature for 4 months, and the second was stored in the refrigerator for a long-term period extended up to 12 months. The survival and pathogenicity of the formulated EPNs were discussed. Results of the EPNs, which were stored at room temperature (group 1), showed that survival % of (RM1) and (S. g.) IJs were more than the others two (S. c.) and (H. b.). Also, (RM1) and (S. g.) achieved high pathogenicity % of the tested wax moth larvae, *Galleria mellonella,* more than (S. c.) and (H. b.). The data showed excellent success in case of storage of EPNs IJs at low temperature (group 2), where all EPNs remained survive with percentage 100% for 5 months, while the survival of (S. g.) was 100% after 10 months, followed by (RM1) with survival 100% after 8 months, and (S. c.) as a long-term for 7 months. All EPNs species showed high vitality and infected the *G. mellonella* larvae with pathogenicity % reached to 85 %, 78%, 76%, and 72% by (S. g.), (S. g.), (S. c.), and (H. b.), respectively as storage long-term period expanded to 12 months.

INTRODUCTION

Entomopathogenic nematodes (EPNs) (Steinernematidae and Heterorhabditidae) are very important biological agents against several species of soil pests (Georgia *et al*., 2006). Nematodes have a mutuality symbiosis with bacteria; *Xenorhabdus* for

steinernematids and *Photorhabdus* for heterorhabditids (Kaya and Gaugler, 1993). The infective juvenile (IJ) is the only free-living stage and has the ability to search and infect the insects. Entomopathogenic nematodes used as-an insecticides in pest management IPM programs because they are considered non-toxic to humans, relatively specific to their target pest(s), and they can- applied with standard pesticide equipment (Grewal *et al.*, 2005 and Shapiro-Ilan *et al*., 2006a). EPNs are used against numerous soil-inhabiting pests successfully (Shapero-Ilan *et al*., 2002) but their poor survivor's storage at room temperature is one of the main factors that prevent them from realizing their full potential as bioinsecticides (Grewal, 2002). non-feeding infective juveniles (IJs) of EPNs depend on stored reserves for their energy supply. Therefore, energy conservation is a vital factor to stay the life of IJs (Patel and Wright, 1997 a; and Qiu *et al*., 2000). High temperatures lead to an increase in the nematode's physiological activity, increase the consumption of stored energy, and result in limited shelf-life (Andalo *et al*., 2011). Two main important factors are affecting nematode's survival; (1): storage media and methods. Formulations that contain active mobile nematodes, based on sponge and vermiculite substrate, these methods require cooling during storage and transportation (Grewal, 2002 and Ramakuwela, *et al*., 2015). Formulations that reduce nematode mobility, such as alginate gels have been investigated (Georgis, 1990; Hussein and Abdel-Aty 2012). Formulations that reduce the IJ metabolism to partial dormancy have shown considerable promise for long-term storage such as anhydrous polyacrylamide gel (Bedding and Butler, 1994), powders (Bedding, 1988), granules (Connick *et al*., 1993). Exposure of EPNs to an osmotic solution could induce a dormant state similar to that induced by desiccation (Finnegan *et al.*, 1999). Chen and Glazer (2005) used gel granules in combination with the osmotic reduction of nematode metabolism. (Glazer and Salame 2002; Qiu *et al*., 2000). (2) Storage temperature: Low-temperature storage is the most common and important factor affecting nematode survival in formulations, but even at low temperatures, nematode species differ in storage long-term of optimum storage temperatures (Strauch *et al*., 2000; Andalo *et al*., 2011). Recently, research moved toward improving liquid formulations by using polymer gels (Andalo *et al*., 2010; Hussein and Abdel-Aty, 2012 and De Waal *et al.*, 2013).

MATERIALS AND METHODS

The greater wax, moth *Galleria mellonella* L. (Lepidoptera: Pyralidae), is a serious pest of beehives and stored bee wax. However, it is widely used in the mass production of biological control agents including the entomopathogenic nematodes (EPNs) (Metwally *et al.*, 2012)

Rearing of *Galleria mellonella*:

Galleria mellonella was reared by using an artificial medium (Han and Ehlers, 2000) in a plastic jar (22 cm l.* 9 cm d.) at room temperature (20- 25 °C) in the laboratory.

Rearing and Production of the Tested EPN Species:

A large scale of EPN IJs was produced in vivo, three foreign species Steinernema *carpcapsae* (S. c.); *Stienernema glasri* (S. g.); *Heterorhabditis bacteriophora* (H. b.), and native strain *Heterorhabditis indica* (RM1) isolated from Egypt and identified by (El-Assal, *et al.*, 2002) were cultured on *G. mellonella* larvae. All EPN species produced by inoculated with 200 IJs per last larval instars of greater wax moth and placed on filter paper (Whatman No.1) known white trap described by (Shapiro–Ilan *et al.*, 2003). Harvesting of IJs that were emerged and migrated from the cadavers larvae and moved to the water inside the white trap and collected them daily for one week (Woodring and Kaya, 1988). The collected IJs were rinsed with tap water and allowed to pass through a

sieve into tap water and left at room temperature for an hour. EPN IJs were stored in 0.1 % formalin with 25 ml of non-sterile water at a concentration 10000IJs/ml. Suspension of IJs has been stored in 500ml flat-angled tissue culture flasks at a concentration of 250000 IJs/flask. Three flasks were prepared for each tested EPN species and temperature.

Tested Storage Formula:

A carrier medium for EPNs storage formulation used by a new technique (Baby diapers) which are characterized by ease of use, handling, and facility of obtaining them from the market and pharmacy, in addition, they are sterilized so, they are ready to use. Baby diapers were used as a carrier medium for storage entomopathogenic nematodes. Baby diapers containing hidrogel which is a superabsorbent polymer (SAP) or anhydrous polyacrylamide gel that can absorb and retain extremely large amounts of liquids. Also, baby diapers contain cotton fibers which may reduce the adhesion between the particles of the hydrogel and offer moisture to nematode. Components of baby diapers were described by (Counts *et al*., 2017). Baby diapers prepared before used by removing the lateral elastic tapes and dividing them into squares (7 cm x 7 cm) and remove the thin waterproof layer and then each piece was placed in a plastic pot (11cm in diameter x 5cm in high). Nematodes suspension in 25 ml with 250000IJ/ flask added to the surface of the hidrogel by using a pipette and covers the plastic pot tightly. Three pots replicate for each species of tested nematode in addition to one-pot for control with the same concentration of IJs in the sterile water only.

Optimum Storage Temperature:

To determine the optimal storage temperature, tested EPN pots divided into two main groups, the first group was stored on the shelves, and left at room temperature for approximately 4 months. Tested optimum temperatures were recorded as shown in (Table 1). The second group was stored at (5-7°C) in the refrigerator for a long-term period extended up to 12 months. The Survivors were determined by counting every month, five replicates for each nematode species. The survival percentage of IJs was calculated by using the dilution method described by (Kaya and Stock, 1997), live and dead (motile juveniles only) were examined with a stereoscopic microscope.

Table 1: Temperatures were recorded during procedure of EPNs IJs storage duration.

No. Months	Minimum C° temp.	Maximum C° temp.	Average C° temp.
1th	9	18.9	13.6
2nd	9.7	20.4	14.9
3rd	11.6	23.5	16.9
4th	14.6	28.3	21.2

Infectivity of Formulated Nematodes Test:

The harvested nematode IJs from the carrier formulation (both of two groups that were stored at room temperature and in the refrigerator) were examined for estimating their vitality and ability to infect the last larval instar of *G. mellonella*. Infectivity of IJs was evaluated monthly by assaying formulated nematodes against 10 last instar larvae of the greater wax moth *G. mellonella* with concentration 500IJs/ml with 3 replicates for each treatment.

The Control experiments were inoculated with sterile water only. All experiments were placed inside (33.5 h.*20 w. cm) plastic jars and closed tightly and left at room temperature. Mortality was observed and recorded daily for three days.

Statistical Analysis:

Obtained data were analyzed as two-way ANOVA, using Proc ANOVA in SAS (Anonymous. 2003), and means were compared by LSD (P= 0.05 level) in the same program.

RESULTS

A. Storage at Room Temperature:

Survival Percentage of Formulated Nematodes:

The survival percentage recorded 98.92 and 98.70 (at 9°C- 18.9°C with average 13.6°C) after the first month of storage period with *S. glaseri* and *H. indica* (RM1), respectively. No significant difference between *S. glaseri* and *H. indica* (Fig. 1). Also, the IJs survival % of *S. carpocapsae* and *H. bacteriophora* was 92.98 and 91.42, respectively, at the same previous temperature. There was a significant difference between the *S. glaseri* and *S. carpocapsae* and there was also a significant difference between *H. indica* (RM1), *H. bacteriophora*, and *C.carpocapsae*. While, there was no significant difference between *S. carpocapsae* and *H. bacteriophora*, at all room temperatures. Also, the survival % of IJs recorded 94.89 and 92.5 of IJs (at 9.7°C- 20.4°C with average 14.9°C), after two months of storage period with *H. indica* (RM1) and *S. glaseri*, respectively, while they were 86.66% and 82.75% with *S. carpocapsae* and *H. bacteriophora*, respectively. After the 3rd month, the survival% of IJs was 84.52 and 82.08 (at 11.6°C- 23.5°C with average 16.9°C) with *S. glaseri* and *H. indica*, respectively and it was 77.5% and 74.25% with *S. carpocapsae* and *H. bacteriophora*, respectively. With the gradually increasing temperature up to 20°C at the end of the fourth month, the survival rate declined to 57.65% and to 68.53% with *S. carpocapsae* and *H. bacteriophora*, while the two species *H. indica* (RM1) and *S. glaseri* were 73.46% and 70.58%, respectively. In general, there was no significant difference between two EPN species *S. glaseri* and *H. indica*, and also between *S. carpocapsae* and *H. bacteriophora*, while there was a significant difference between *H. indicia* (RM1) and both species *S. carpocapsae* and *H. bacteriophora* at all 4 months along the storage period (F= 24.87 of TRT F=172.47 of the month, df=3,3; p<.0001). After a few days of storage IJs in distilled water (control) at 13.6°C, all of the nematode juveniles were stopped moving and died.

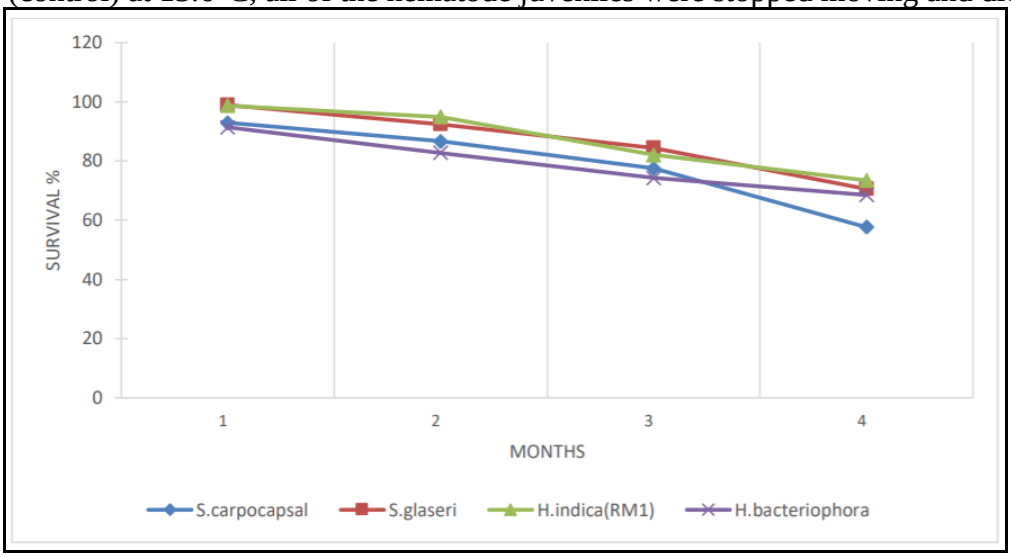

Fig. 1. Survival % of EPNs after storage period (Months).

Pathogenicity of formulated nematodes:

Pathogenicity of IJs, *S. glaseri*, and *H. indica*, recorded 100% mortality of *G. mellonella* larvae which were stored at room temperature (9°C-18.9°C with average

13.6°C) for 30 days, followed by 98% with *S. carpocapsae* and 96% with *H. bacteriophora* (Fig. 2). Moreover, *H. indica* (RM1) recorded 100% mortality after 2 months of storage temperature (9.7°C-18.9°C with average 13.6°C), *S. glaseri* 98%, *S. carpocapsae* 96% and *H. bacteriophora* 92% after the same long-term of storage and temperature. Also, after 3 months at (11.6°C- 23.5°C with an average of 16.9°C) the mortalities were 76, 74, 68, and 66% occurred by *H. indica* (RM1), *S. glaseri, S. carpocapsae* and *H. bacteriophora*, respectively. At the end of the experiment, *H. indica* (RM1) and *S. glaseri* recorded 68% and 66% mortality. Meanwhile, the lowest mortality% recorded by the EPN species *H. bacteriophora* and *S. carpocapsae* was (40%) and (46%) against *G. mellonella* larvae after 4 months. While *H. indica* (RM1) and *S. glaseri* recorded 68% and 66% mortality at the same temperature (14.6-28.3°C with average 21.2°C) (Fig. 2). There is no significant difference between *S. glaseri* and *H. indica* (RM1) and also between *S. carpocapsae* and *H. bacteriophora*. There were significant differences between both *H. indica* (RM1) and *S. glaseri* and also between *S. carpocapsae* and *H. bacteriophora.* There was a significant difference among the constantly changing throughout the four months temperatures (F=12.72 of TRT, F= 156.61 of month df= 3,3; P<.0001).

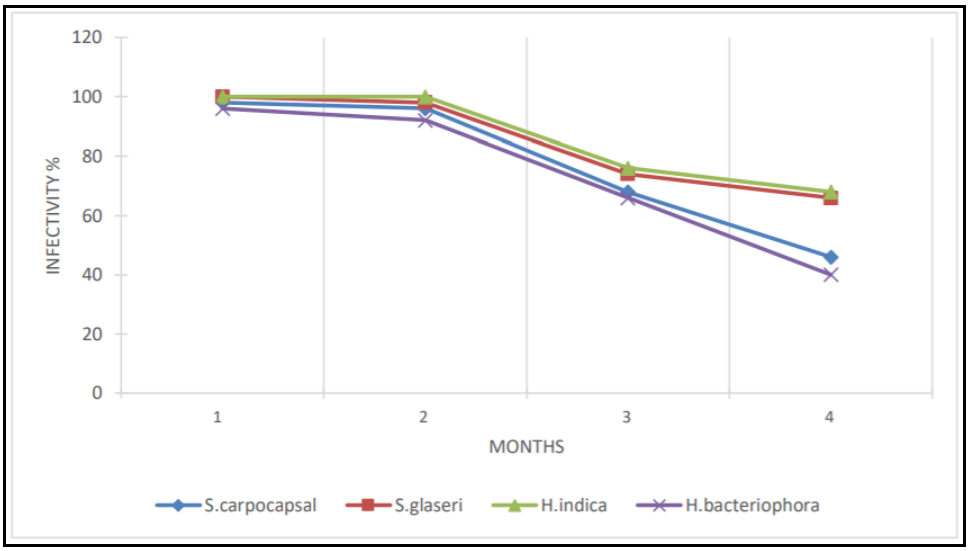

Fig.2. Infectivity % of EPNs after storage period (Months) at room temperature.

B.Storage at Refrigerator (5-7°C):

Survival Percent of Formulated Nematodes:

The survival % recorded (100) at (5-7°C) after 6 months (Fig. 3). No change recorded as the storage duration after 6 months of storage duration for all nematode species except *H. bacteriophora* recorded 98%. No significant difference between all EPNs. *S. glaseri* recorded the highest percentage survival reached 100% which expanded over 10 months and decreased to 98.8% and 98.5% after 11 and 12 months at the end of long-term duration. The lowest survival % was (90%) recorded by *H. bacteriophora*, while *H. indica* (RM1) and *S. carpocapsae* recorded 94% and 92.6%, respectively. (F=25.31 of TRT, F= 20.09 of month df= 3,11; P<.0001). There was a significant difference between *S. glaseri* and the three other EPN species. No significant difference between *H. indica* (RM1) and *H. carpocapsae*. No significant difference between EPN species survival after the first 5 months of the storage period. There was no significant difference among the three species *H. indica* (RM1), *S. glaseri*, and *S. carpocapsae* after

6, 7, and 8 months. There was a significant difference among all species of EPN species in the last four months. The survival % was significantly different between *H. bacteriophora* and the other three species continued into the last month of the long-term storage period. Control recorded survival % at temperature (5-7°C) was 100% of *H. indica* (RM1) after one month, 82% after 2 months, all species died after 4 months except *S. glaseri.* Only *S. glaseri* survived with 100% after one month decreased to 90% after 2 months, 76% after 3 months, and dropped to 35 after 5 months.

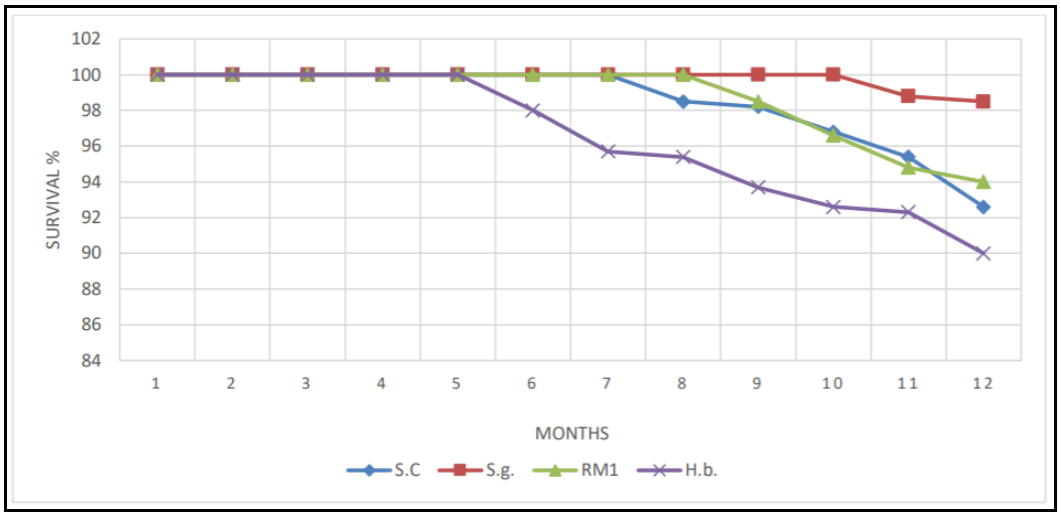

Fig. 3. Survival percentage % of EPNs after storage period /month

Infectivity of Formulated Nematodes:

The mortality % of the insect larvae reached (100) by infecting all EPNs species after 4 months of storage duration (Fig. 4). No significant difference among all EPNs species (F=10.98 of TRT, F=48.06 of month df=3,11; P<.0001). Also, *S. carpocapsae*, *S. glaseri*, and *H. indica* (RM1) recorded 100% mortality after 9 months and 96% by *H. bacteriophora*. Finally, the lowest mortality% was (72%) after 12 months caused by H. *bacteriophora* with significantly different from the other three species S. glaseri, *H. indica* (RM1), *and S. carpocapsae* which recorded 85%, 78%, and 76%, respectively.

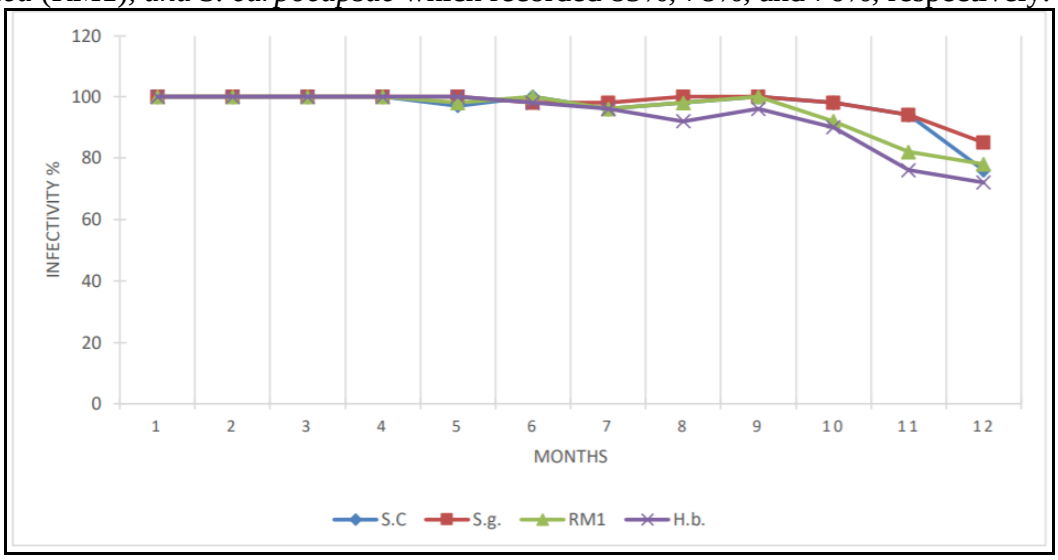

Fig. 4. Infectivity % of EPNs after storage period (months)

DISCUSSION

The successful trials of EPNs production on large scale both in vivo or in vitro methods were encouraged plenty of researchers and scientists towered to find optimal storage methods. So, several attempts and procedures were used by different formulations and carriers media to keep IJs of EPNs alive, effective, and able to infect and control the target pest. Temperature is an important factor to keep IJs, storage stability, survival, and, more infectiveness under room temperature or in a refrigerator. The study aimed to upgrade the storage stability of the three foreign species; *S. carpocapsae, S. glaseri* , *H. bacteriophora,* and the 4th Egyptian strain *H. indica* (RM1). The survival of *H. indica* (RM1) extended for more than 4 months at room temperature followed by *S. glaseri, S. carpocapsae* and *H. bacteriophora*. In general, survival% and duration of storage decreased with increasing temperature up to 16.9°C. Similar results agreed with Ramakuwela *et al.* (2015), who used sponges as a carrier and they stated that the optimum temperature for *S. innovationi* was 15°C. Also, Hussein and Abdel-Aty (2012) investigated the influence of formulations and shelf- life at room temperature 25°C on two native strains of EPNs *H. bacteriophora* (BA1) and *S. carpocapsae* (BA2). By comparing the two formulated EPNs with Hydrogel, it was found that the storage potential of *S. carpocapsae* (BA2) juviniles was superior to that of *H. bacteriophora* (BA1) and *S. carpocapsae* (BA2) was more virulent than *H. bacteriophora* (BA1) to the larvae of the wax moth *G. mellonella*. The storage period of both two strains was extended to 50 days, the survival % of (BA1) decreased to 79.2 after 40 days, while, the survival % of (BA2) ranged from 93.4 to 37.9 for the storage periods 10 days and 50 days, respectively. Grewal (1998) mentioned that the flowable gel formulation was developed to improve the ease of use by the consumer, but nematode shelf-life at room temperature 25°C in the flowable gel was shorter than the alginate gel. The survival storage of *S. carpocapsae* recorded (1.0-1.5) months at room temperature and (3.0-5.0) months in the refrigerator at 5°C with flowable gel. While the survival was stored for (3.0-4.0) months at 25°C and for (6.0-9.0) months at 5°C with alginate gel. These results agreed with Goudet *et al*., (2010); Andalo *et al*., (2011). Gulcu and Hazer (2012) mentioned that EPN species differ distinctly in optimum storage temperature. On the other hand, the EPNs species that were stored at 5-7°C showed stability survival percent 100% along 10 months with *S. glaseri* and after 5 months with *H. bacteriophra.* Survival percent of all strains reached up to 90% extended to 12 months. Moreover, the infectivity percent was recorded 100% after 6 months of storage duration with *S. carpocapsae* and *H. indica* (RM1). At the end of the storage period, the highest mortality percent was 85% with *S. glaseri* and the lowest was 72% by *H. bacteriophora.* On the contrary, Ramakuwela *et al*., (2015) recorded that survival of *S. innovation* was better at 10°C and 15°C compared with all other temperatures which were >15°C and it was lowest at 5°C.

Low temperature reduces the metabolism of EPNs IJs, and the growth of contaminants include bacteria, yeast, and fungi that compete for available oxygen consumption in the storage medium, thereby causing harmful environmental conditions for the nematodes IJs (Georgis and Kaya, 1998; and Strauch *et al.,* 2000). The suggestion of application using the baby diapers pieces caring with nematode IJs which can reach to millions without losing a large number of IJs. Moreover, they can distribute the pieces of baby diapers on the surface of the soil on the farm to control the soil pests. The hydrogel which mixed with IJs also uses as a carrier formulation to spray the nematode on the foliage of plants. Future research will have to create methods and formulation materials that have more properties to provide the nematode IJs that exist in a large amount of oxygen, rich moisture, and packing technology to preserve optimum conditions as long as

storage and transportation.

REFERENCES

Andalo V.; Cavalcanti RS.; Molina JP. & Moino A. Jr. (2010). Substrates for storing entomopathogenic nematodes (Rhabditida:Steinernematidae,Heterorhabditidae). *Scientia Agricola,* 67:342–347.

Andalo V.; Moino A. Jr.; Maximiniamo C.; Campos VP. & Mendonca L. A. (2011). Influence of temperature and duration of storage on the lipid reserves of entomopathogenic nematodes. *Revista Colombiana de Entomologia*, 37 (2):203–209

Anonymous (2003). SAS Statistics and graphics guide, release 9.1. SAS Institute, Cary, North Carolina 27513, USA.

Bedding, R. A. (1988). Storage of entomopathogenic nematodes. Patent No.WO 88/0866

Bedding, R. A. & Butler, K. L. (1994). Method for storage of entomopathogenic nematodes. Patent No.WO 94/05150

Chen, S. & Glazer I. (2005). A novel method for long-term storage of the entomopathogenic nematode *Steinernema feltiae* at room temperature.Biological control 32:104-110.CONNICK, W. J., Jr., NICKLE, W. R. & VINYARD B. J. (1993). "Pesta": New granular formulations for *Steinernema carpocapsae*. *Journal of Nematol*ogy, 25, 198-203.

Counts,J.; Weisbrod A. & Yin S. (2017). Common diaper ingredient questions: Modern disposable diaper materials are safe and extensively tested. Vol: 56 issue: 5_suppl, : 23-27.

De Waal JY.; Malan AP. & Addison MF. (2013). Effect of humidity and a superabsorbent polymer formulation on the efficacy of *Heterorhabditis zealandica* (Rhabditida: Heterorhabditidae) to control codling moth, *Cydia pomonella* (L.). *Biocontrol Science and Technology*,23(1):37–41.

El-Assal, F. M.; M. H. El-Bishry & Abd El-Rahman, R. M. (2002). Four new isolates of the entomopathogenic nematodes Heterorhabditis spp. from Egypt: Identification, Virulence and Penetration Rate. *Egyptian Journal of Biological Pest Control*, 12 (1): 15-24.

Finnegan, M.; Downes M. J.; O'regan M. & Griffin C. T. (1999). Effect of salt and temperature stresses on survival and infectivity of *Heterorhabditis* spp. infective juveniles. *Nematology*, 1 (1): 69-78.

Georgis, R. (1990). Formulation and application technology. In: Entomopathogenic Nematodes in Biological Control. (GAUGLER, R. & KAYA, H. K., eds.) CRC Press, Boca Raton, Florida, 173-191.

Georgis, R. & Kaya, H.K. (1998). Formulation of entomopathogenic nematodes. H. D. Burges Formulation of microbial biopesticides beneficial microorganisms, nematodes and seed treatments. Kluwer Academic Publishers Drodrecht, The Netherlands. 289-308 PP

Georgis, R.; Koppenhöfer A. M.; Lacey L. A.; Belair G.; Duncan L. W.; Grewal P. S.; Samish M.;Tan L.; Torr P . & Van Tol, R.W. H. M. (2006). Successes and failures in the use of parasitic nematodes for pest control. *Biological Control*, 38: 103-123.

Glazer, I. & Salame L. (2000). Osmotic survival of the entomopathogenic nematode *Steinernema carpocapcae*. *Biological Control*, 18,251-257.

Goud, S.; Huger PS. & Prabhuraj A. (2010). Effect of temperature, population density

and shelf life of EPN *Heterorhabditis indica* (RCR) in sodium alginate gel formulation. *Journal of Biopesticides,* 3 (3):627–632

Grewal, PS. (1998). Formulations of entomopathogenic nematodes for storage and application. *Jap. Journal of Nematology,*28: 68– 74.

Grewal, PS. (2002). Formulation and application technology. pp. 265–287 *in* R. Gaugler, ed. Entomopathogenic Nematology. Wallingford: CABI Publishing.

Grewal, PS.; Ehlers R-U. & Shapiro-Ilan DI. (2005). Nematodes as Biocontrol Agents. CABI, New York, NY.

Han, RC. & Ehlers RU. (2000). Pathogenicity, development, and reproduction of *Heterorhabditis bacteriophora and Steinernema carpocapsae* under axenic in vivo conditions. *Journal of Invertebrate Pathology, 75:55-58.*

Hussein, M. A. & Abdel -Aty M. A. (2012). Formulation of two entomopathogenic nematodes at room temperature. Journal of Biopesticides. 5 (supplementary):23–27.

Kaya, H.K.& Gaugler R. (1993). Entomopathogenic nematodes. *Annals Revsiw of Entomol*ogy, 38:181–206.

Kaya, H.K. & Stock, P. (1997). Techniques in insect nematology. p. 281-324. In: Lacey, L., ed.

Metwally, H.M.S; Hafez G.A.; Hussein M.A.; Salem H.A. & Saleh M.M.E. (2012). Low Cost Artificial Diet for Rearing the Greater Wax Moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae) as a Host for Entomopathogenic Nematodes. *Egyptian Journal of Biological Pest Control,* 22(1), 15-17.

Patel, M. N. and Wright D. J. (1997) a. Glycogen: its importance in the infectivity of aged *Steinernema carpocapsae*. *Parasitology,* 114: 591 - 596.

Qiu, L., Lacey M. J. & Bedding R. (2000). Energy metabolism and its relation to survival and infectivity of infective juveniles of *Steinernema carpocapsae* under aerobic conditions. *Nematology,* 2: 551 - 559.

Ramakuwela, T.; Hatting J.; Laing MD.; Hazir S. & Thiebaut N. (2015). Effect of storage temperature and duration on survival and infectivity of *Steinernema innovationi* (Rhabditidae: Steinernematidae). *Journal of Nematology,* 47(4):332-336.

Shapiro-Ilan, D. I.; Gouge D. H. & Koppenho¨fer A. M. (2002 b). Factors affecting commercial success: Case studies in cotton, turf and citrus. pp. 333–356 in R. Gaugler, ed. Entomopathogenic Nematology. Wallingford, UK: CABI Publishing.

Shapiro-Ilan, D. I.; Lewis E. E.; Tedders W. L. & Son Y. (2003). Superior efficacy observed in entomopathogenic nematodes applied in infected-host cadavers compared with application in aqueous suspension. *Journal of Invertebrate Pathology,* 83:270–272.

Shapiro-Ilan, D. I.; Gouge D. H.; Piggott S. J. & Patterson Fife J. (2006 a). Application technology and environmental considerations for use of entomopathogenic nematodes in biological control. *Biological Control,* 38:124–133.

Strauch, O.; Niemann I.; Niemann A.; Schmidt A. J.; Peters A. & Ehlers R.-U. (2000). Storage and formulation of the entomopathogenic nematodes *Heterorhabditis indica* and *H. bacteriophora*. *Biocontrol,* 45:483–500

Woodring JL. & Kaya HK. (1988). Steinernematid and heterorhabditid nematodes: a handbook of techniques southern cooperative series bulletin 331. Fayatteville: Arkansas Agricultural Experiment Station.

ARABIC SUMMARY

تأثير درجات الحرارة على تخزين النيماتودا الممرضة للحشرات المُعدة على بيئة حاملة

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إستهدفت هذه الدراسة تأثير درجات الحرارة المختلفة على مدى بقاء الأطوارالمعدية للنيماتودا الممرضة للحشرات حيّة وفعالة وكذلك تقييم مدى ملاءمة تركيب المادة المستخدمة في التخزين كحامل للأطوار المعدية لإستمراريتها وفاعليتها. تمت الدراسة على أربعة أنواع من النيماتودا الممرضة للحشرات EPNs ثلاثة أنواع منها مستوردة تم توطينها في البيئة المصرية *Steinernema carpocapsae* (S.c.) و *Steinernema glaseri* (S. g.) و*Heterorhabditis bacteriophora*(H.b.) وسلالة واحدة مصرية *Heterorhabditis. indica* (RM1)وقد تم إستخدام طريقة جديدة كبيئة حاملة للأطوار المُعدية وهي عبارة عن قطع مربعة من حفاضات الأطفال Baby diapers و التي تحتوي على مادة الهيدروجل Hydrogel المُكونة من حبيبات البوليمرات تتميز هذه المادة بشدة إمتصاص السوائل (SAP) و بذلك توفر الرطوبة اللازمة لبقاء الأطوار المُعدية حيّة و فعّالة. كما تحتوي على الألياف القطنية الدقيقة والتي تقوم أيضا بتوفير قدرا من الرطوبة. أُجريت الدراسة بتقسيم النيماتودا المراد تخزينها إلى قسمين حيث تُركت المجموعة الأولى على أرفف المعمل تحت درجات حرارة الغرفة وحُفظت المجموعة الثانية على درجة حرارة منخفضة. و قد أظهرت نتائج المجموعة الأولى تفوق النوعين (RM1) و (S. g.) من حيث نسب البقاء أحياء وأيضا حققا نسب إصابة مرتفعة عند إجراء إختبارات العدوى ليرقات دودة الشمع الكبيرة Galleria *mellonella*مقارنة بنسب البقاء% ونسب الموت% للنوعين (S. c.)، (H. b.) بينما أظهرت النتائج نجاحاً في حالة التخزين على درجة الحرارة المنخفضة وظلت جميع الأنواع حيّةً بنسبة 100% لفترة تخزين خمسة أشهرو امتدت إلى عشرة أشهربالنسبة إلى النوع (S. g.) تبعها النوع (RM1) بنسبة بقاء 100% استمرت ثمانية أشهر، ثم النوع (S. c.) لمدة سبعة أشهر. كما أظهرت جميع الأنواع فاعلية و قدرة على الإصابة بلغت 85% ، 78%، 76% و 72% للأنواع (RM1) ، (S.g.) ، (S. c) و (H. b.) على التوالي، بعد فترة تخزين 12شهر.