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Bacterial symbionts of entomopathogenic nematodes against *meloidogyne incognita* (kofoid & white, 1919) (tylenchida: Heteroderidae)

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Abstract
The root-knot nematode (RKN), *Meloidogyne incognita* (Kofoid & White, 1919) (Tylenchida: Heteroderidae), is a significant pest that is widely distributed around the world and reduces crop yields. Unfortunately, the majority of the powerful chemicals used to control RKN are extremely poisonous, very expensive, and have harmful effects on the environment. Utilising biocontrol agents is one of the potential viable alternatives. Entomopathogenic bacteria are one of the most effective and eco-friendly biocontrol agents for treating the root-knot nematode. This study's goal was to assess the efficiency of culture broth, cell pellet suspensions and cell-free Xenorhabdus and Photorhabdus filtrates bacteria against root-knot nematodes. Under laboratory conditions, culture broth, cell pellet suspensions, and cell-free filtrates of *Photorhabdus and Xenorhabdus* bacteria strains obtained from various Egyptian nematodes were tested for their ability to repress the activity of the root-knot nematode, *M. incognita*. Increasing the density of all seven strains of bacteria increased the mortality rate in juvenile Meloidogyne incognita (P<0.05) for all strains and exposure durations. *P. luminescens* (EGAP2) had the highest density (4 x 10^7 cells/mL) of the strains tested, and it killed more than 97% of the root-knot nematode J2s after 48 hours of exposure. There was also a significant difference in after 48 hours of exposure, the efficacy of cell-free filtrates was determined (P 0.05). Secondary metabolite concentrations at 100% were more effective than other concentrations, with significant differences (P <0.05). The data clarified that all symbiotic bacterial cell pellet Cell-free filtrates and suspensions exhibited potent activities to reduce the root-knot nematode. Their nematicidal activity was concentration-dependent. In conclusion, bacterial symbionts of entomopathogenic nematodes can be an optimal option for biocontrol of the root-knot nematode.

Keywords: *Meloidogyne incognita*, *Photorhabdus*, *Xenorhabdus*, biological control

Introduction
The Tylenchida: Heteroderidae root-knot nematode (RKN), *Meloidogyne incognita* (Kofoid & White, 1919), is a significant pest that is widely dispersed around the world and causes yield losses. Such a nematode is common in tropical areas and has been linked to a variety of plant types in numerous nations. Unfortunately, the majority of powerful chemicals used to control RKN are extremely toxic, expensive, and have adverse effects on the environment. The use of biocontrol agents is one of the potential effective alternative methods. bacteria that coexist in close proximity to entomopathogenic nematodes create substances having antibacterial and antifungal properties. *Xenorhabdus* spp (Thomas & Poinar, 1979) [13] (Enterobacterales: Morganellaceae) and *Photorhabdus* spp (Boemare, 1993) [3] (Enterobacterales: Morganellaceae) were cultivated *in vitro* and It was examined *in vivo* how their filtrates affected the mortality of *M. incognita* second stage juveniles (J2s). After 24 hours, the mortality of the juveniles was assessed. The mortality rates of the filtrates from the *in vivo* and *in vitro* cultures were 80% and 20%, respectively. As a result, the method employed to cultivate the bacteria had an impact on the quality of compounds with nematicidal activity, illustrating the differences between *in vivo* and *in vitro* cultivation and the significance of the insect host. (Andaló *et al.*, 2012) [2]. M. incognita was nematotoxic to the cells and toxins of Xenorhabdus and Photorhabdus species. Toxins and bacterial cell suspensions with higher concentrations worked better. in suppressing juveniles are more active than the lower concentrations (Aatif *et al.*, 2012) [1]. Authors also documented that percentage juvenile's Root-knot nematode (RKN) immobilisation increased with concentration at concentrate 4 x 10^7 cell.ml^{-1} *Xenorhabdus* spp. and *Photorhabdus* spp. cell suspensions completely *M. incognita* juveniles become immobile after 48 hours of exposure. Both bacterial toxins and bacterial cell suspensions rendered young people immobile and caused = 90% immobilization of juveniles. The free living juveniles of *M. incognita* (J2) were highly susceptible to the secondary metabolites crude extracts. A 75% mortality of J2 was observed at the highest concentration (4mg/ml) for all tested bacterial crude extracts (Orozco, 2012) [9]. The nematotoxic properties of Photorhabdus luminescens' cell-free (Thomas & Poinar, 1979) [13] (Enterobacterales: Morganellaceae) (strain: TT01), *Xenorhabdus budapestensis* (Lengyel, 2005) [7] (Enterobacterales: Morganellaceae) (strain: AF 2013 or EMA) and *X. szentirmaii* (Lengyel, 2005) [7] (Enterobacterales: Morganellaceae) (strain: EMC), isolated from entomopathogenic nematodes *Heterorhabditis bacteriophora* (Poinar, 1976) (Rhabditida: Heterorhabditidae) and *Steinernema bicornutum* and *S. rarum*, respectively examined on *M. incognita* at 6, 12, 24, and 48 hours after exposure to incognita (J2s), as well as the mortality rate. The rate of larval death appeared to be dose/dependent, according to the data. After 48 hours of exposure, the highest dose of TT01 CFCM had a 100% fatality rate. Nematode management employing antagonistic bacteria has been accomplished without endangering the environment. (Nour El-Deen *et al.*, 2014) [8].

The harmful effects of RKNs on tomatoes were reduced by IJ aqueous suspensions, EPN applications made on infected cadavers, and particularly X. bovienii supernatant treatments. In comparison to the control, which used solely RKNs, the treatment resulted in decreased RKN egg masses, increased plant height, and higher fresh and dry weights. M. incognita J2 was suppressed by crude extracts of P. l. sonorensis (CH35 strain), and nematode mortality was concentration-dependent. (Orozco *et al*., 2016) [10].

Materials and methods

The experiments were carried out in the laboratory; EPNs bacterial cell suspension and cell-free filtrate of *Photorhabdus* and *Xenorhabdus* bacteria strains obtained from various Egyptian nematodes were evaluated to test their antagonistic effects on the root-knot nematode (RKN), *M. incognita.*

Microorganisms

Photorhabdus luminescens subsp. laumondii HP88, MH368153, MH368154, MH368155, MH368156, and MH368157 of the species Photorhabdus luminescens (EGAP1), Photorhabdus luminescens (EGAP2), and Photorhabdus luminescens (EGAP5) MH3681and *X. nematophilus* strains obtained from Pest Physiology Dept., Plant Protection Research Institute, Agricultural Research Centre, Egypt.

Root-knot nematodes

Meloidogyne incognita used in the present study was kindly supplied by the Plant Pathology Research Institute, ARC, Egypt.

Effect of bacterial cell suspension on root-knot nematode

There was only one colony of each bacterium. Transferred to 3-ml of LB medium as a vaccination for 50 culture in ml. The inoculum was shaken for the most aeration at 28°C for 24 h, Afterward, the mixture was transferred to 250-ml Erlenmeyer flasks with 50 ml of LB media, shaking at 150 rpm for 2 days. sterilised distilled water was used to dilute the bacterial suspension to provide densities (4 x 10^4, 4 x 10^5, 4 x 10^6 and 4 x 10^7 cell.ml^{-1}) of *X. nematophilus*, *P. luminescens*, *P. luminescens subsp. laumondii* HP88, *P. luminescens* (EGAP1*), P. luminescens* sGAP2), *P. luminescens* (EGAP3*), P. luminescens* (EGAP4), *P. luminescens* (EGAP5), *and P. luminescens* (EGAP2).Five ml of root-knot nematode suspension, carrying approximately 100 (J2s) of RKN, poured *M. incognita*, on different density of bacteria (on Petri plates with a 9 cm diameter) to increase the volume to 10 ml. The experiment had three replicates. Plates were covered with top and kept at 25o C in an incubator. After counting the number of dead nematodes at 24 and 48 h. specifically; J2s were touched with a small needle while motionless to confirm mortality. The Duncan's multiple range test (P0.05) was used to assess the relevance of different treatments. Costat, a programme created by Berkeley, California-based Cohort Software Inc., was used for all analyses. The percentage of Root-Knot nematodes *M. incognita* was calculated by the following formula:

$$\% \text{ Mortality} = \frac{\% \text{ Mortality in treatment} - \% \text{ Mortality in control}}{100 - \text{Mortality in control}} \times 100$$

Effect of bacterial cell free-filtrate on Root-knot nematode

On the other hand different dilutions (100%, 50%, 25% and 12.5%) of EPNsB cell-free filtrates created by incorporating the required water quantity. Afterward, 5 ml of root-knot suspension and transporting of nematodes approximately 100(J2s) of RKN, *M. incognita*, was poured on different dilutions of each cell-free filtrate On Petri dishes with a 9 cm diameter, to increase the volume to 10 ml. The experiment had three replicates. Plates were then covered with top and kept at 25º C in an incubator. The numbers of following the recording of dead nematodes 24 and 48 h. specifically; J2s. without any move were touched with a tiny needle to confirm mortality. The The significance of different it was treatments assessed by Duncan's (P 0.05) different range test. The software programme "Costat," produced by Cohort Software Inc. in Berkeley, California, was used for all analyses.

Statistical analysis

Every experiment was conducted in triplicate and set up using a totally random design. Arcsine transformation was used to normalise data that were given as percentage values. Analysis of variance (ANOVA) was used to establish the main effects' significance. The Duncan's multiple range test was used to determine the significance of various treatments (P 0.05). Costat, a programme from Berkeley, California's Cohort Software Inc., was used for all analyses. California. All data were recorded as means of three replications.

Results and discussion

Nematicidal activity of bacterial cell suspensions and cell- free filtrate on root-knot nematode (*M. incognita*)

a. Nematicidal effect of bacterial cell suspension

This study involved to examine impact of various cell suspensions with different densities *i.e*., 4 x 10^4, 4 x 10^5, 4 x 10^6 and 4 x 10^7 cell.ml^{-1} prepared from pure cultures *X. nematophilus*, *P. luminescens subsp. laumondii HP88*, *P. luminescens*, *P. luminescens* (EGAP1), *P. luminescens* (EGAP2), *P. luminescens* (EGAP3), *P. luminescens* (EGAP4), and *P. luminescens* (EGAP5) on (J2s) of RKN. Mortality rates (%) were calculated after 24h and 48h of exposure (Table 1). For all strains and exposure durations, increasing the density of all seven strains of bacteria increased mortality rate in juveniles *M. incognita* (P<0.05). Among the examined strains, higher density (4x10^7) only from *P. luminescens* (EGAP2), strain caused mortality in > 97% of the J2s of the root-knot nematode after 48 h of exposure, while the survival of the J2s root-knot nematode was increased to its maximum at the lower cell density inoculum (4x10^4) of *X. nematophilus* resulting in as low rate mortality as 4.7% after the same exposure period. Other cell densities of *P. luminescens* (EGAP2), (4 x 10^6, 4 x 10^5 and 4 x 10^4) caused 78%, 37.3% and 7% mortality in the juveniles.

Intensity of the population of the in comparison to the control, plant-parasitic nematodes dramatically decreased in all treatments. There were significant differences among the strains (Fig. 1). Also there were significant between the four densities (Fig. 2) and between the different days (Fig. 3) (df =6, F= 30.34,P=0.0000), (df= 3, F=2238.7,P=0.0000) and (df= 1, F=3260.95,P=0.0000).

Significant variations were seen in interactions between the

various strains and concentrations (df = 18, F = 3.151, P = 0.0001), also there was significant among the concentrations additionally, various days (df = 3, F = 564.75, P = 0.0000), However, there were no appreciable variations in the strains' interactions with one another and the various days. (df = 6, F = 1.15, P = 0.3357). The interactions between the strains, the varying densities, as well as varying days did not show any discernible changes. (df = 18, F = 1.54, P = 0.0872).

Table 1: Impact of various density of EPNs bacteria on % mortality of *M. incognita* at 24 and 48 h

Bacteria species	Density (cell.ml^{-1})	Mortality (%) after 24 h.	Mortality (%) after 48 h.
Photorhabdus luminescens (EGAP1)	4 x 10^4	3.3± 1.5	4.7± 1.2
	4 x 10^5	9± 2.6	19.3± 2.5
	4 x 10^6	19±2.1	60.7± 2
	4 x 10^7	24 ±2.6	86.3± 2.6
Photorhabdus luminescens (EGAP2)	4 x 10^4	5.7± 3.1	7± 3.6
	4 x 10^5	18.3± 4.2	37.3± 3
	4 x 10^6	32.7 ±2	78± 2
	4 x 10^7	41±3.6	97.7± 2.5
Photorhabdus luminescens (EGAP3)	4 x 10^4	3±1	5.3±2.5
	4 x 10^5	9.7±1.5	25± 3
	4 x 10^6	20.3± 2	69± 2.1
	4 x 10^7	29.3±2.1	91± 3.6
Photorhabdus luminescens(EGAP4)	4 x 10^4	6.7± 2.5	7.7± 2.1
	4 x 10^5	16± 3	34.3± 3
	4 x 10^6	28.7± 3	76.7± 4.2
	4 x 10^7	35.7± 2.1	93.7± 2.5
Photorhabdus luminescens(EGAP5)	4 x 10^4	5.3 ±3.1	6.3± 2.9
	4 x 10^5	14.3± 3	36 ± 3.5
	4 x 10^6	31 ± 4	75.3± 2.1
	4 x 10^7	38.7± 3.1	94.3± 4
Photorhabdus luminescens subsp. laumondii HP88	4 x 10^4	2.3± 2.1	5± 2
	4 x 10^5	11 ± 3.2	39.7±3.5
	4 x 10^6	23 ± 4	65.3± 2.1
	4 x 10^7	28±3	90 ± 4
Xenorhabdus nematophilus	4 x 10^4	2± 2	4.3± 1.5
	4 x 10^5	13± 3.6	30 ± 3.6
	4 x 10^6	29.3± 3.1	71 ± 2
	4 x 10^7	31.3± 2.6	92± 2.6

Nematicidal effect of the EPNsB cell-free filtrate

The aforementioned experiment was repeated using cell free supernatant from pure broth cultures of the examined EPNsB strains. The juveniles were subjected to various dilutions of the cell free supernatents i.e., 100%, 50%, 25%, and 12.5 %. According to data *P. luminescens* (EGAP1), *P. luminescens* (EGAP2), *P. luminescens* (EGAP3), *P. luminescens* (EGAP4), and *P. luminescens* (EGAP5), *P. luminescens subsp. laumondii* HP88 and *X. nematophilus* can produce lethal secondary metabolites killing the nematode juveniles. The efficiency of the treatments varied significantly on secondary metabolites after 48h exposure at P<0.05. (100%) concentrations of secondary metabolites were more efficacious as compared to other concentrations and showed significant differences (P<0.05). Mortality of juveniles increased with increasing in concentrations of the secondary metabolites. 48 hours later. mortility of juveniles of RKN was influenced greatly by the rising concentration (P<0.05). Juvenile's mortality was less at the last concentrations as shown in Table (2).Higher concentration (100%) from *P. luminescens* (EGAP2), death rates were highest under strain. (96.3%) 48 hours following the exposure, while, the lower concentration (12.5%) of *X. nematophilus* and *P. luminescens* (EGAP1) gave the least effect (5%) subsequent to the same exposure period. Other concentrations of *P. luminescens* (EGAP2), (50%, 25% and 12.5%) caused 77%, 36% and 7.7% mortality of juveniles. In comparison to the control, the number of plant-parasitic nematodes dramatically decreased in all of the treatments.

There were high significant differences between the strains (Fig. 4), the four concentrations (Fig. 5) and between the different days (Fig. 6) (df =6, F= 24.97,P=0.0000), (df= 3, F=1784.6,P=0.0000) and (df= 1, F=2729.3,P=0.0000). On the other side, for interactions between the strains and the various concentrations, significant variations were observed. (df = 18, F = 2.44, P = 0.0023), also there was significant between concentrations and the different days (df = 3, F = 470.94, P = 0.0000), However, there were no appreciable variations in the interactions between the strains and the various days. (df = 6, F = 0.913, P = 0.4878), Although interactions between the strains did not show any discernible differences, the different concentrations and the different days (df = 18, F = 1.28, P = 0.2108). Our findings indicated that several treatments had a significant negative effect on *M. incognita* nematode. Evaluation of the treatments showed that *P. luminescens* (EGAP2) bacteria at 10^7 CFU and the 100% concentration of their secondary metabolites had the highest effect on percentage increase of mortality of *M. incognita*. The other strains and their secondary metabolites gave variable results.

Table 2: Effect of different concentrations of EPNsB cell-free filtrates on % mortality of *M. incognita* at 24 and 48 h.

Bacteria species	Dilution (%)	%Mortality after 24 h.	% Mortality after 48 h.
Photorhabdus luminescens (EGAP1)	12.5	3.7±2	5±1.7
	25	8±3.6	19.3±3.6
	50	18±2	59.7±3
	100	23.3±1.5	84±3
Photorhabdus luminescens (EGAP2)	12.5	6.3±2.3	7.7±2.9
	25	17.3±2.5	36±2.6
	50	31.3±4.2	77±2.5
	100	40±2	96.3±1.5
Photorhabdus luminescens (EGAP3)	12.5	3.7±2	5.7±2.5
	25	9±2.6	24±2.6
	50	19±2.6	68.7±4
	100	28.7±3.2	89.7±1.5
Photorhabdus luminescens (EGAP4)	12.5	7±3	8±2.6
	25	14±3	33.3±4
	50	27.7±0.6	75.7±2.1
	100	34±2	92.7±3.1
Photorhabdus luminescens (EGAP5)	12.5	6±2	7±1.7
	25	13.3±2.6	35.3±2.6
	50	29.7±2.1	74.3±2.6
	100	37.7±4.2	93±2
Photorhabdus luminescens subsp. laumondii HP88	12.5	3.3±0.5	5.7±1.2
	25	10±2.3	38.3±2.3
	50	22.3±3.1	64±3.1
	100	27±2.1	88.7±2.3
Xenorhabdus nematophilus	12.5	3±2.6	5±2.6
	25	12.3±2.9	29.3±2.3
	50	28.3±2.1l	69.3±2
	100	30.3±3	91.3±3.2

The destructive plant parasite nematodes known as root-knot nematodes (RKNs) seriously injure their plant hosts. Bacterial nematicides have gained popularity as a reliable biocontrol tool in the long-term management of RKNs. Here, we determined the activity of bacterial symbionts of entomopathogenic nematodes, *Photorhabdus* and *Xenorhabdus* bacteria against *M. incognita.*

Our results showed that the application of both filtrates with and without suspended bacterial cells of EPNsB reduced plant-parasitic nematode populations overall. Among the tested strains, the highest nematicidal activity of more than 96% mortality was obtained from *P. luminescens* (EGAP2) in $4x10^7$cell.ml^{-1} and its crude cell-free filtrate after 48 h of exposure time when compared to other treatments. In the same way, similar nematocidal action of bacterial culture against recently emerged J2s of *M. incognita* within a 24- to 48-hour exposure time was reported by Aatif *et al*. (2012) [1] [1]. The inhibition of M. incognita J2 by crude extracts of P*hotorhabdus l. sonorensis* (CH35 strain) was concentration-dependent. It was observed that different concentrations of the tested strains of EPNs affected the J2s of *M. incognita.* However, a similar pattern was seen in their effectiveness against RKN in their cell-free supernatant. It is most likely the harmful effects of EPN bacteria's toxins on nematodes (Orozco *et al*. 2016) [10]. At higher concentrations, an irreversible (nematocidal) effect was observed with time, whereas a nematostatic effect was noticed at lower concentrations. The crude filtrate obtained without dilution from *P. luminescens* (EGAP2) surpassed all previous records for *M. incognita* mortality (96.3%) after 48 h of exposure. These findings are consistent with those of Nour El-Deen *et al*. (2014), who investigated the nematicidal activity of *Xenorhabdus budapestensis* (EMA), *Photorhabdus luminescens* (TT01), and *Xenorhabdus szentirmaii* (EMC) filtrates, which were isolated from entomopathogenic nematodes Heterorhabditis Also, the cell suspension of $4x10^7$ cell.ml^{-1} or the crude cell-free filtrates without dilution from all tested EPNsB strains (EGAP1, EGAP2, EGAP3, EGAP4, EGAP5, HP88, and BA2) achieved more than 84% of 48 hours after exposure, *M. incognita* mortality. In the same way, Aatif *et al*. (2012) [1] elucidated that the concentration of 4 x 10^7 cell.ml^{-1}, *Xenorhabdus* spp., and *Photorhabdus* spp. The M. incognita juveniles were totally immobilised after 48 hours of exposure to cell suspensions. Juveniles were equally immobilised by bacterial toxins and bacterial cell suspensions., resulting in juveniles are immobile to a greater than 90% degree. In addition to Samaliev *et al*., (2000) [12], who reported showed M. javanica's hatching was totally prevented by X. nematophila.

The current study's findings are consistent with those obtained by Reghunath *et al*., (2015) [11], who achieved 100% mortality in *M. incognita* after a 24 h exposure time to bacterial cultures from entomopathogenic nematodes. However, the compared to other cultures, culture demonstrated low mortality bacteria in their purest form. Nematicidal action increased as bacterial cell concentration in a cultural broth increased. There was no recorded mortality was observed in the control group. These results correspond to those mentioned by Huangin *et al.,* (2010) [5], who revealed that when bacterial concentration increased, gall and egg mass development significantly decreased.

Conclusion

Data from the present work point out the importance of the bacterial symbionts of the entomopathogenic nematodes as potential sources of organic bioactive substances that might be employed in biological control methods. Further studies are required to evaluate these biological control bacterial agents in large-scale production and field application.

List of abbreviations

RKN: The root-knot nematode
J2s: second stage juvenile
EPNs: Entomopathogenic nematodes
EPNsB: Entomopathogenic nematodes bacteria
ANOVA: Analysis of Variance
LB: Luria-Bertani

References

1. Aatif HM, Javedv N, Khan SA, Ahmed S. Virulence of *xenorhabdus* and *photorhabdus* bacteria and their toxins against juvenile's immobilization of *meloidogyne incognita*. Pak. J. Phytopathol,2012:24(2):170-174.
2. Andaló V, Rocha FS, Maximiniano C, Jr AM, Campos VP. *In vivo* and *in vitro* study of the effects of entomopathogenic bacteria and their filtrates on *Meloidogyne incognita*. Int Res J Mic,2012:3(1):005-009.
3. Boemare NE, Akhurst RJ, Mourant RG. DNA relatedness between *Xenorhabdus* spp. (Enterobacteriaceae), symbiotic bacteria of entomopathogenic nematodes, and a proposal to transfer *Xenorhabdus luminescens* to a new genus, *Photorhabdus* gen. nov. Int J Syst Bacteriol.,1993:43:249-255.
4. Bozhüyük KAJ, Zhou Q, Engel Y, Heinrich A, Pérez A, Bode HB. Natural Products from *Photorhabdus* and other Entomopathogenic Bacteria. In: "The Molecular Biology of *Photorhabdus* Bacteria" (Ed. ffrench-Constant R.), Spr Int Pub,2016:1:55-79.
5. Huangin H, Xu CK, Ma L, Zhang KQ, Duan CQ, Mo MH. Characterisation of volatiles produced from *Bacillus megaterium* YFM3.25 and their nematicidal activity against *Meloidogyne incognita*. Eur. J. Plant Pathol.,2010:(126):417-422.
6. Kepenekci I, Hazir S, Lewis E. Evaluation of entomopathogenic nematodes and the supernatants of the *in vitro* culture medium of their mutualistic bacteria for the control of the root-knot nematodes *Meloidogyne incognita* and *M. arenaria*. Pest Manag. Sci.,2015:(72):327-334.
7. Lengyel K, Lang E, Fodor A, Szállás E, Schumann P, Stackebrandt E. "Description of four novel species of Xenorhabdus, family Enterobacteriaceae: Xenorhabdus budapestensis sp. nov., Xenorhabdus ehlersii sp. nov., Xenorhabdus innexi sp. nov., and Xenorhabdus szentirmaii sp. nov". *Systematic and Applied Microbiology*,2005:28(2):115-22. doi:10.1016/j.syapm.2004.10.004.
8. Nour Eldeen A, Fodor AH, Amal A, El-Barty F. Nematicidal activity of entomopathogenic bacteria against root- knot nematodes, *Meloidogyne incognita* in-vitro. Int J Adv Res,2014:2(6):708-713.
9. Orozco R. Characterization of The Entomopathogenic Bacterium *Photorhadus luminescens* Sonorensis, and Bioactivity of its Secondary Metabolites. PhD Thesis, Fac. Science, Arizona Univ. USA, 2012, 90.
10. Orozco RA, Molnar I, Bode H, Stock SP. Bioprospecting for secondary metabolites in the entomopathogenic bacterium *Photorhabdus luminescens* subsp. *sonorensis*. J Invert Pathology,2016:(141):45-52.
11. Reghunath SR, Siji, JV, Mohandas C, Nambisan B. Evaluation of culture filtrate of an entomopathogenic bacterium for nematicidal properties against Root-Knot nematode, *Meloidogyne incognita*. J Crop,2015:41(1):32-36.
12. Samaliev HY, Andreoglou FI, Elawad SA, Hague NGM, Gowen SR. The nematicidal effects of the bacteria *Pseudomonas oryzihabitans* and *Xenorhabdus nematophilus* on the root-knot nematode *Meloidogyne javanica*. Nematol.,2000:(2):507-514.
13. Thomas GM, Poinar GO. *Xenorhabdus* gen. nov., a genus of entornopathogenic, nematophilic bacteria of the family Enterobacteriaceae. Int. J. Syst. Bacteriol,1979:(29):352-360.