Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

E-ISSN: 2320-7078
P-ISSN: 2349-6800
JEZS 2019; 7(3): 915-919

Received: 04-03-2019
Accepted: 09-04-2019

S Abirami
Department of Nematology Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

K Poornima
Professor and Head, Department of Nematology Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

N Swarnakumari
Assistant Professor, Department of Nematology, Tamil Nadu Agricultural University, Tamil Nadu Agricultural University

Correspondence
S Abirami
Department of Nematology Tamil Nadu Agricultural University, Coimbatore, India

Effect of temperature and sonication on endospore attachment on *Meloidogyne incognita* (Kofoid and White) and compatibility of endospores of *Pasteuria penetrans* (Thorne) Sayer & Starr with bioagents

S Abirami, K Poornima and N Swarnakumari

Abstract
Pasteuria penetrans (Thorne) Sayer &Starr is a gram positive and endospore forming bacterial parasite of root knot nematode, *Meloidogyne* spp. Spore attachment on cuticle is the initial process of infection by *P. penetrans*. The parasitization of *P. penetrans* is influenced by external factors such as soil temperature and other microbes. To understand these factors, *invitro* experiments were conducted to assess the role of temperature and sonication on spore attachment. The compatibility of *P. penetrans* endospore with other bioagents such as *Purpureocillium lillacinum, Pochonia chlamydosporia* and *Bacillus thuringiensis* was also studied. The results showed that endospores of *P. penetrans* were compatible with other bioagents. Highest endospore attachment on juvenile was recorded at 25°C (85 per cent) and 30 min sonication (20khz) increased the spore attachment (89.76 per cent) on juveniles of *Meloidogyne incognita*.

Keywords: *Pasteuria penetrans*, endospores, temperature, *Purpureocillium lillacinum, Pochonia chlamydosporia, Bacillus thuringiensis*, Sonication

1. Introduction
Application of nematicides play a major role to control plant parasitic nematodes, worldwide. Indiscriminate application of chemicals leads to resurgence of sendentory endoparasite resulting in development of resistance against nematicides (Degenkolb and Vilcinskas, 2016) [4]. Hence there is an urgent need to look for an alternate strategy, biological control being one of them which involves controlling parasitic nematodes nematode antagonistic organisms. Among them, fungi and bacteria have large number of nematode antagonistic organisms such as egg parasitic fungi, *Pochonia chlamydosporia, Purpureocillum lilacinum*, bacterial biocontrol agents like *Bacillus* spp. and *Pasteuria penetrans* (Stirling *et al*., 1986) [13].
Pasteuria penetrans is an obligate and endospore forming parasite of root-knot nematode, *Meloidogyne* spp. The multiplication of this bacterium starts from attachment of endospores on juvenile cuticle and subsequently it moves inside root along with juveniles. After attachment endospores germinate and produce thalli and spread throughout the nematode body, forms sporangia that endogenously form single spores (Mankau, 1975) [8].
P. penetrans life cycle synchronizes with nematode life cycle Metchnikoff (1888) [9]. In the present study, a laboratory experiment was undertaken to assess the influence of temperature, sonication and other biotic factors on endospore attachment. The effects of these external factors on endospore attachment are discussed in this paper. This experiment was conducted with a view that the results will be useful to optimize the temperature and sonication period for culturing of *P. penetrans* and induce successful attachment on cuticle of *Meloidogyne* spp. juveniles.

2. Materials and Methods
Culturing of *P. penetrans* was obtained by crushing endospore infected females from roots. The identity of the spore was confirmed by morphological characters under compound microscope (Leica - 020-518500) (Fig 2). Approximately 50 egg masses of root-knot nematode, *M. incognita* was allowed to hatch in 100 ml of water and freshly hatched juveniles inoculated with endospores.

It was incubated at room temperature (28±1°C) for 24 h. Spore attachment was confirmed under compound microscope and spore encumbered juvenile were inoculated to tomato plant (PKM 1 variety) (Fig 1). After one month, roots were dissected and *P. penetrans* infected females were examined and used for further experiments.

2.1 Influence of biocontrol agents on *P. penetrans* spore attachment

Effect of biocontrol agents such as egg parasitic fungi, *Pochonia chlamydosporia*, *Purpureocillium lilacinum* and bacterial bioagent, *Bacillus thuringiensis* on endospores morphological characters was studied *in vitro*. Endospores of *P. penetrans* ($0.25x10^6$ endospores/ml) were mixed with conidial spores of *P. lillacinum*, *P. chlamydosporia* and *B. thuringiensis* at the concentration of $0.75x10^6$ separately in microfuge tube. Spores of *P. lillacinum* and *P. chlamydosporia* were collected in fungal culture (Talavera *et al*., 2002) [15]. Then the spore powder was incubated in microfuge containing *P. penetrans* endospores. Whole set of experiment was incubated at 25°C. The condition of endospores was observed at every 24h interval. After 72 h of incubation, 300 J2 of *M. incognita* was inoculated for each treatment and each treatment was replicated four times.

2.2 Influence of temperature on *P. penetrans* endospore attachment

A laboratory study to assess the effect of temperature on *P. penetrans* endospores and attachment on juvenile of *M. incognita* was conducted at different temperatures *viz*., 20°, 25°, 30°, 35° and 40°C.

P. penetrans infected gravid females were transferred to microfuge tube containing sterile distilled water and crushed to release endospores in 1 ml sterile distilled water. The spore load in microfuge tube was assessed and adjusted to have $1x10^6$ endospores/ml.

These microfuge tubes were incubated at 20°, 25°, 30°, 35° and 40°C. Each treatment was replicated five times. All treatments were incubated for 15 days in BOD. After that endospores were transferred to 15 ml glass vial containing 4 ml of water with 150 J2 of *M. incognita*. The microfuge tubes taken from each temperature were transferred to separate vials replication wise. Observation on spore attachment was recorded at every 24 h interval up to 72 h.

2.3 Influence of sonication on *P. penetrans* endospores attachment

One million endospores were collected in microfuge tube (1.5 ml capacity) containing one ml distilled water. The spore suspension was sonicated at 20kHz at different time intervals *viz.*, 5,10,15, 20, 25 and 30 min with a probe sonicator (Model No: FS-120) with 300V power supply. The effect of sonication on endospores was studied by incubating sonicated endospores with J2 of *M. incognita*. Spore attachment and number of spores per juvenile were observed after 72h.

2.4 Effect of different stresses on endospore viability

Effect of high temperature, sonication, bead beater (Qiagen: Tissuelyser II) and sterile river sand (0.1mm) on viability of endospore was studied. Endospores of *P. penetrans* ($2x10^6$/ml) were incubated at temperatures as high as 105° to 120°C for 20 min. The endospore of *P. penetrans* viability was also tested at different sonication time intervals. Two numbers of iron beads (2mm size) were utilized for endospore disruption at 10 to 20 min time interval using bead beater (Qiagen: Tissuelyser II)). River sand was sterilized and sand particles of 0.5mm size were separated using 20µ sieve. Endospore of *P. penetrans* was crushed mechanically using river sand. Observations were taken on structural changes in endospore surface and total endospore disruption.

3. Results and Discussion

3.1 Influence of biocontrol agents on *Pasteuria* spore attachment

The result an experiment was to study the compatibility of *P. penetrans* with other biocontrol agents showed that the *Pasteuria penetrans* endospores are more compatible with other bioagents such as *P. chlamydosporia*, *P. lilacinum* and *B. thuringiensis*. Per cent of spore attached juveniles was recorded at *P. penetrans* combine with other bioagents *viz.*, *P. chlamydosporia*, *P. lilacinum* and *B. thuringiensis* 82.00, 80.00 and 84.00 per cent respectively. Similarly, number of spores per juvenile recorded as 10.60, 11.20 and 9.60 endospores per juvenile. There was no significant difference between the treatments which indicate that other bioagents, metabolite and chemicals which exudates from spores of other organism did not influence endospores of *P. penetrans* (Table 1). Observation also showed that the conidial spores of *P. lilacinum* and *P. chlamydosporia* germinated but the hyphal structures did not affect the endospore structure.

The present study revealed that *P. penetrans* endospores were more compatible with other biocontrol agents used against *M. incognia.* Similar finding was observed in field experiment, when *P. penetrans* combined with *P. lillacinum* and *P. chlamydosporia* increased the growth parameters and reduces the incidence of *M. incognita* on chilli crop found by Chaudhary and Kaul, 2011 [1]. Application of mycorrhizal fungus *Glomus* sp. along with *P. penetrans* endospores reduced *M. incognita* population by 57% on tomato and there was no effect of the mycorrhizal treatment on *P. penetrans* attachment to *M. incognita* as observed by Talvera *et al.* (2002) [15]. Koshy (2003) [7] and (Davies, 2009) [2] reported that combination of bioagents with endospores of *Pasteuria* increased the vine length, number of leaves, shoot and root weight and yield of black pepper.

3.2 Influence of temperature on spore attachment

Incubation of endospores at 25°C showed more number of spore encumbrance and which was 10.5 spores/juvenile followed by 20° and 30° C. At 72 h, the number of encumbered juveniles and number of spores per juvenile were 8.5 and 7.75 respectively. The number of endospores encumbered and average number of spores attached per juveniles were lower at higher temperature of 35° and 40°C. After 28 days of inoculation, encumbered juveniles penetrated root system and produced galls successfully and *P. penetrans* developed inside the nematode body and mature endospores were produced. More number of infected females (30.5) was observed at 25°C followed by 30° and 20°C which showed 18.75 and 13 females/root respectively (Fig 3). Number of endospore was significantly more at 20° followed by 25°and 30°C *viz.*, $1.8x10^6$, $1.3x10^6$ and $0.8x10^6$ (Table 2).

In the present study, when the temperature increase the spore attachment on juvenile of *M. incognita* also increased and temperature had influence on attachment of endospores to nematodes. The result was similar to that of Javed *et al.* (2002) [6] and Davies *et al.* (1988) [3] who found that spore

attachment was more at 30°C. Feritas *et al.* (1997) [5] and Swarnakumari *et al.* (2016) [14] reported that incubating endospores at 30 ° to 70 °C for 5 h per day over 10 days resulted in reduction of endospore attachment on juveniles of *M. arenaria* race 1. In contrast, Williams *et al.* (1989) [16] reported that endospores of *P. penetrans* attachment on juveniles were stable when the endospore exposed at 60°C for 15 min.

3.3 Influence of sonication on *P. penetrans* spore attachment on juveniles

When the endospores were exposed to ultra sound with a wave length of 20Khz for at 30 min, 89.76 per cent spore attachment was recorded and number of spores per juvenile was also more (46.50 spores / juvenile) followed by 25 and 20 min which recorded 82.67 and 81.61 per cent spore encumbrance respectively and the number of spores per juvenile were 40.50 and 32.75 endospores per juvenile (Table 3). The endospores when incubated without sonication, attachment were lower compared to other treatments. Among the sonication periods, 5 and 10 minutes recorded lowest endospore attachment. Endospore sonication for 5 and 10 min showed lower spore attachment of 31.34 and 56.00 per cent and the number of endospores attached also were optimum of 9.00 and 12.25 spores per juvenile (Fig 4).

Influence of sonication on spore attachment was recorded at 25 and 30 min sonication and none of the juvenile penetration was observed and more numbers of infected females were observed at 5 min and 10 min sonication. Similarly, spore load per female was also same ($1.8X10^6$) with 5 and 10 min of sonication followed by 15 min sonication recorded 4.50 infected females per root system and spore load of $1.0X10^6$. Sporangial cell wall plays a significant role in spore attachment on juveniles. When the endospores are exposed to ultra sound wave length, sporangial cell wall gets disrupted. Similar finding was reported by Stirling *et al.* (1984) [12] who observed that 30 min sonication increased spore attachment on juveniles of *M. javanica*. Orui (1997) [10] reported that spore attachment increased at 30 min of sonication. Even though, number of spore attachment on juveniles increased by sonication for 30 min, it may reduce the penetration into the root system and interfere with the sinusoidal movement of J2 of *M. incognita*. Same finding was reported by Persidis *et al*., 1991 [11].

3.4 Effect of different stresses on endospore morphology

Endospore of *P. penetrans* structural condition was not influenced by higher temperature. Likewise there was no impact of sonication in endospore cell wall. Even though endospore shrinkage was observed from 50min sonication upto 60 min, cell wall was intact and no disruption was observed. Bacteria endospore with iron beads in bead beater machine and manual crushing with river sand did not affect the endospore at different time interval (Table 4).

The results of current study showed that high temperature (autoclaving temperature) 121°C for 20 min did not affect *P. penetrans* endospore viability. Heat exposed endospores were morphologically similar to normal endospore which indicates that high temperature does not effect endospores viability as reported by Williams *et al.*, 1989 [16]. Sonicated endospores from the current research work was viable as that of normal endospores upto 45min, but sporangial cell wall disruption was observed at 50 min. This observation was similar to Orui (1997) [10] who reported that endospores of *P. penetrans* morphologically varied with normal endospore when they were exposed to 50 min sonication.

In conclusion, the present study revealed that endospores of *P. penetrans* attachment on juvenile and development of *P. penetrans* was more in temperature 20°-30°C. When endospores sonicated at 30 min will increase spore attachment. But number of spores increase per juveniles will reduce nematode movement and penetration into root system. Endospores did not influenced by other bioagents producing metabolites and chemical compounds which means *P. penetrans* endospores more compatible with other biocontrol agents.

Table 1: Influence of biocontrol agents on *Pasteuria penetrans* spore attachment on *M. incognita* juvenile

Treatment	Per cent spore attached juveniles (%)*	No. of spore attached / juvenile**	Avg. no. of infected females / 5g root**	No. of endospores / female
P. chlamydosporia + *P. penetrans*	82.00 (70.08)	10.60 (3.15)	9.00 (3.02)	$1.2 x10^6$
P. lilacinum + *P. penetrans*	80.00 (65.91)	11.20 (3.31)	9.40 (3.06)	$1.8 x10^6$
B. thuringiensis + *P. penetrans*	84.00 (68.98)	9.60 (3.05)	9.40 (3.06)	$2.1 x10^6$
T4-control (*P. penetrans*)	86.00 (68.30)	10.20 (3.16)	9.80 (3.12)	$1.8 x10^6$
SEd	8.91 (NS)	0.27 (NS)	0.07 (NS)	-
CV	20.89 (NS)	13.47 (NS)	3.81 (NS)	-
CD (p=0.01%)	26.17 (NS)	0.79 (NS)	0.21 (NS)	-

Values in parentheses are arc sine (*) and square root (**) transformed values.
Values with same alphabets are not significantly different by DMRT

Table 2: Influence of temperature on *Pasteuria penetrans* spore attachment on *M. incognita* juvenile

Soil temperature / time in hour	Per cent encumbered juveniles (%)*	Avg. no. of spores attached / J2**	Avg. no. of infected females / 5g root**	No. of endospores / female
20°C	80.00^{ab} (63.80)	8.5^{a} (3.40)	13.00^{c} (3.59)	$0.8x10^6$
25°C	92.50^{a} (78.60)	10.5^{a} (3.28)	30.50^{a} (5.50)	$1.8x10^6$
30°C	85.00^{a} (70.37)	7.75^{a} (3.34)	18.75^{b} (4.35)	$1.3x10^6$
35°C	42.50^{c} (44.99)	6.5^{a} (3.30)	9.25^{d} (3.02)	$0.6x10^6$
40°C	12.50^{c} (20.46)	0.75^{b} (0.80)	8.75^{d} (2.94)	0.00
SEd	6.64	0.33	0.22	-
CV	19.01	16.59	0.75	-
CD (0.01%)	19.58	0.98	9.28	-

Values in parentheses are arc sine (*) and square root (**) transformed values.
Values with same alphabets are not significantly different by DMRT

Table 3: Influence of sonication on *P. penetrans* spore attachment on *M. incognita* juveniles

Sonication/min	Per cent encumbered juveniles (%)*	Avg. No. of spores attached / J2*	Avg. no. of infected females / 5g root*	No. of endospores / female
T1- 5 min	31.34^{c} (39.83)	9.75^{d} (3.11)	9.00^{a} (3.07)	1.8 x10^{6}
T2- 10 min	56.00^{b} (48.45)	12.25^{d} (3.13)	8.75^{a} (2.78)	1.8 x10^{6}
T3- 15 min	58.67^{b} (60.44)	26.00^{c} (4.88)	4.50^{b} (2.21)	1.0 x10^{6}
T4- 20 min	81.61^{b} (64.70)	32.75^{c} (5.63)	1.50^{c} (1.40)	0.5 x10^{6}
T5- 25 min	82.67^{a} (65.51)	40.50^{b} (6.11)	0.00^{d} (0.70)	0.00
T6- 30 min	89.76^{ab} (71.41)	46.50^{a} (6.95)	0.00^{d} (0.70)	0.00
Control (not sonicated)	19.37^{d} (26.49)	9.00^{d} (2.97)	9.75^{a} (3.11)	1.2 x10^{6}
SEd	2.08	0.27	0.15	-
CV	8.03	8.30	10.54	-
CD (p=0.01%)	6.19	0.77	0.43	-

Values in parentheses are arc sine (*) and square root (**) transformed values.
Values with same alphabets are not significantly different by DMRT

Table 4: Effect of different stresses on *P. penetrans* endospore morphology

Factors		Factors	
Temperature	**Reaction**	**Sonication**	**Reaction**
105°C	+	35 min	+
110°C	+	40 min	+
115°C	+	45 min	+
120°C	+	50 min	-
		55 min	-
		60 min	-
Factors		**Factors**	
Beat beater	**Reaction**	**Sterile river sand**	**Reaction**
5 min	+		+
10 min	+		
15 min	+		
20 min	+		

(+) no effect on spore morphology
(-) Shrinkage of exsporium of endospores

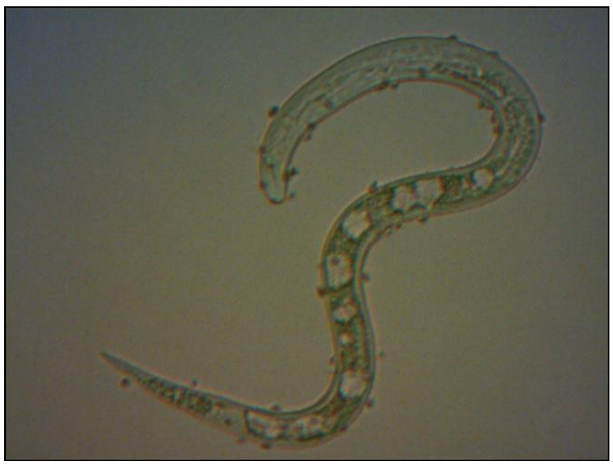

Fig 1: *Pasteuria penetrans* encumbered J2 of *Meloidogyne incognita*

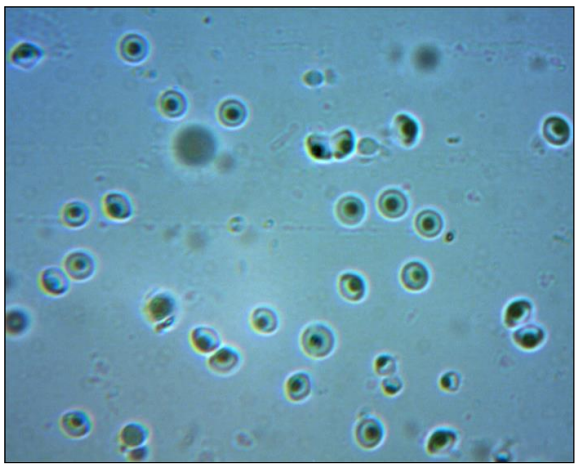

Fig 2: Fully matured endospores of *Pasteuria pene*

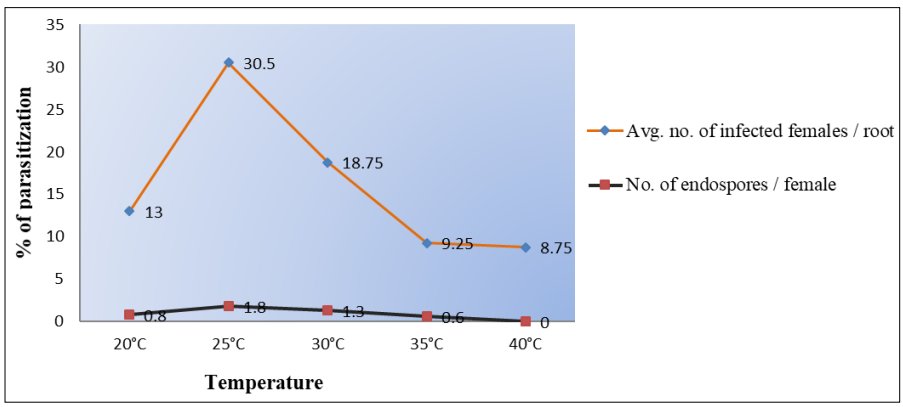

Fig 3: Influence of temperature on endospore encumberance in *M. incognita*

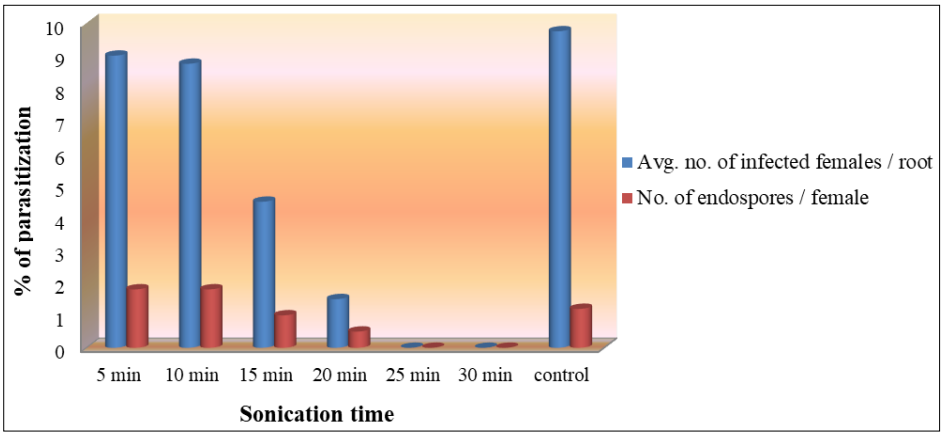

Fig 4: Influence of sonication on endospore encumberance in *M. incognita*

References

1. Chaudhary KK, Kaul RK. Compatibility of *Pasteuria penetrans* with fungal parasite *Paecilomyces* against root knot nematode on Chilli (*Capsicum annuum* L.). Journal of Experimental Biology and Agricultural Sciences. 2011; 1(1):36-42.
2. Davies KG. Understanding the interaction between and obligate hyperparasitic bacterium, *Pasteuria penetrans* and its obligate plant-parasitic nematode host. Advances in Parasitology. 2009; 68:211-245.
3. Davies KG, Kerry BR, Flynn CA, *et al*. Observations on the pathogenicity of *Pasteuria penetrans*, a parasite of root-knot nematodes. Annals of Applied Biology. 1988; 112(34):491-501.
4. Degenkolb, Thomas, Andreas Vilcinskas. Metabolites from nematophagous fungi and nematicidal natural products from fungi as an alternative for biological control. Part I: metabolites from nematophagous ascomycetes. Applied microbiology and biotechnology. 2016; 100(9):3799-3812.
5. Freitas LG, Mitchell DJ, Dickson DW, *et al*. Temperature effects on the attachment of *Pasteuria penetrans* endospores to *Meloidogyne arenaria* Race 1. Journal of Nematology. 1997; 29(4):547-555.
6. Javed N, Khan HU, Hussain Z, Ashfaq M, *et al*. Effect of Temperature, Soil pH, Agitation Intervals and Soil Types on the Spore Attachment of *Pasteuria penetrans* to Root Knot Nematode, *Meloidogyne javanica.* Pakistan Journal of Plant Pathology. 2002; 1(2):66-67.
7. Koshy PK, Sosamma V, Samuel R. Bio- control of *Radopholus similis* on black pepper, *Piper nigrum* L. under field conditions. Indian Journal of Nematology. 2003; 33:43- 46.
8. Mankau R. *Bacillus penetrans* n. comb. Causing a virulent disease of plant-parasitic nematode. Journal of Invertebrate Pathology. 1975; 26:333-339.
9. Metchnikoff ME. *Pasteuria ramosa* un representant des bacteries a division longitudinale. Ann. Inst. Pasteur. 1888; 2:165-170.
10. Orui Y. Effect of spore sonication on attachment and host-attachment range of *Pasteuria penetrans* to the root-knot nematode. Applied Entomology and Zoology. 1997; 32(1):101-107.
11. Persidis A, Lay JG, Manousis T, Bishop AH, Ellar DJ. Characterization of potential adhesions of the bacterium *Pasteuria penetrans*, and of putative receptors on the cuticle of *Meloidogyne incognita*, a nematode host. Journal of Cell Science. 1991; 100:613-622.
12. Stirling GR. Biological control of *Meloidogyne javanica* with *Bacillus penetrans*. Phytopathology. 1984; 74(1):55-60.
13. Stirling GR, Bird AF, Cakurs AB, *et al*. Attachment of *Pasteuria penetrans* spores to the cuticles of root-knot nematodes. Review of Nematology. 1986; 9:251-260.
14. Swarnakumari N, Umamaheswari R, Sivakumar CV. An *in-vivo* method for mass multiplication of bacterial parasite, *Pasteuria penetrans* of root-knot nematode, *Meloidogyne* sp. Pest Management in Horticultural Ecosystems. 2016; 22(1):80-83.
15. Talavera M, Itou K, Mizukubo T. Combined application of *Glomus* sp. and *Pasteuria penetrans* for reducing *Meloidogyne incognita* (Tylenchida: Meloidogynidae) populations and improving tomato growth. Applied Entomology and Zoology. 2002; 37(1):61-67.
16. Williams AB, Stirling GR, Hayward AC, Perry J. Properties and attempted culture of *Pasteuria penetrans*, a bacterial parasite of root-knot nematode (*Meloidogyne javanica*). Journal of Applied Bacteriology. 1989; 67:145-156.