Biological Forum – An International Journal **14(2a): 533-538(2022)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Water Soluble Formulation of Nematode Antagonistic Bacterium, *Pasteuria penetrans* for the Management of Root-knot Nematode, *Meloidogyne incognita* in Tuberose

***M. Janani*[1*], *N. Swarnakumari*[1], *A. Shanthi*[1] *and M. Gnanachitra*[2]**
[1]*Department of Nematology, AC & RI, TNAU, Coimbatore (Tamil Nadu), India.*
[2]*Department of Agricultural Microbiology, AC& RI, TNAU, Coimbatore (Tamil Nadu), India.*

(Corresponding author: M. Janani)*
(Received 30 April 2022, Accepted 22 June, 2022)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: Root-knot nematode, *Meloidogyne incognita* is a serious damage causing nematode in both sub-tropical and tropical regions. It causes global crop losses in agricultural and horticultural crops. Recent reports indicate that this nematode become an issue to farmers due to its parasitism. *Pasteuria penetrans* is a potential hyperparasitic bacterium against *M. incognita*. Though it is a successful biocontrol agent against nematodes, mass production and commercial formulation of *P. penetrans* is still unavailable. Based on these information, a water soluble formulation of *P. penetrans* was prepared using emulsifiers and surfactants. This formulation was tested against *M. incognita* in tuberose under pot culture condition. Different concentrations of the formulation *viz*., 0.5ml, 1ml, 2ml and 2.5ml were prepared and tested its efficacy against *M. incognita*. Results showed that the formulation 2ml per plant recorded with highest reduction in number of gall (5.1/plant) as well as egg masses production (3.1/plant; 82% reduction over control). The same treatment showed highest plant growth parameters in terms of shoot weight as well as number of tubers. The outcome of this study showed that the formulation 2ml/plant possesses highest potential in suppressing nematode infestation in tuberose. Hence, this treatment was further were forwarded for field study.

Keywords: *Meloidogyne incognita, Pasteuria penetrans,* water soluble formulation; Shelf-life, Parasitization, Tuberose, Pot culture study.

INTRODUCTION

Root-knot nematode, *Meloidogyne* sp. is one of the most devastating pests of many field and horticultural crops (Khan, 2015). They exist in soil in areas with hot climates or short winters (Khan *et al*. 2021). About 2000 plants worldwide are susceptible to infection caused by root-knot nematodes and they contribute approximately 5% of global crop loss (Saha *et al.*, 2016). If root-knot nematodes become established in deep-rooted, perennial crops, control is difficult and options will be limited. Bio-control potential of *P. penetrans* against *Meloidogyne incognita* was documented (Mukhtar *et al.*, 2013). Tuberose (*Polianthes tuberose* L) is one of the most important tropical ornamental bulbous flowering plants cultivated for production of long lasting flower spikes. It is popularly known as Rajanigandha or Nishigandha. It belongs to the family Amaryllidaceae and it is native of Mexico. Tuberose can successfully be grown in pots, borders, beds and commercially cultivated for various uses (Johnson 1970). It's spikes produce about 20 fragrant white florets. Nowadays, root-knot nematode infestation that is particularly *M. incognita* in tuberose is a challenging one for farmers (Gowda and Chawla 2013). It is the most economically damaging genera of plant parasitic nematodes (Grace *et al.,* 2019). Root-knot nematode infection has been reported from all tuberose growing areas in India (Sellaperumal *et al.*, 2015). Most of the commercially grown varieties of tuberose have been reported to be susceptible to *M. incognita* (Chawla and Singh, 2006). Understanding the interaction between an obligate hyperparasitic bacterium, *P. penetrans* and its obligate plant-parasitic nematode host, *Meloidogyne* spp (Davies, 2009). So in such way, a biocontrol agent can be used to control its infestation and the disease caused by them (Abdullah, 2012; Grace *et al*., 2019).

P. penetrans is a potential biocontrol agent and studied by several authors (Chen and Dickson 1998; Swarnakumari and Sivakumar 2005). It is a good bacterial parasite of nematode. It was first described as

P. romosa a parasite of water fleas, *Daphina magna* (Skerman *et al.*, 1980) followed by many scientists renamed differently, finally it was reported as *Pasteuria penetrans* (Sayre and Starr 1985). Juvenile of *Meloidogyne* nematode hatch from eggs and have a short-free living second stage (J_2 stage) in soil after which they invade the rhizosphere and attach to host-plants. The form galls around developing juveniles where they complete their life cycle (Danlei *et al.* 2004). A study by Chen and Dickson (1997) developed binomial sampling plans to estimate *P. penetrans* endospore attachment to J_2 *M. arenaria*. Ayanaba (1993) invented the slow-release bio-degradable granules containing endospores of *P. penetrans* in order to suppress nematode reproduction. Swarnakumari (2021) have entrapped *P. penetrans* endospores in alginate beads and tested its efficacy in cucumber. EC formulation of this bacterium was developed and tested by Srishalini *et al.* (2021). In the current study a water soluble formulation was developed and tested in tuberose. The methodology adopted and results obtained are described in this paper.

MATERIALS AND METHODS

Pure culture maintenance of *P. penetrans* on root-knot nematode *Meloidogyne incognita.* Pure culture of *P. penetrans* was maintained in bhendi (variety Co 4) plants. Egg masses of *M. incognita* were collected from the infected field (Fig. 1) and incubated at room temperature (27±2 ℃) in water for hatching. After egg hatching endospores of *P. penetrans* were added to the suspension containing J_2 of *M. incognita* and incubated for 3 days for spore attachment. Then the endospore attached J_2 were inoculated into the bhendi plants and maintained in the sterile pot mixture (sand 1: redsoil 2: FYM 2). These plants were pulled out 30 days after inoculation to confirm the multiplication of *P. penetrans* (Fig. 2). These cultures were used for experimental purpose. After that bhendi plants were uprooted and infested gravid female of *M. incognita* were dissected to record the presence of endospores.

Preparation of water soluble formulation of *P. penetrans.* Endospores of *P. penetrans* were collected and stored in sterile microfuge tubes containing sterile water (2ml) by crushing infected female nematodes manually. Water soluble formulation was prepared by using lecithin (Van Nieuwenhuyzen, 1976), Triton –X, tween 80, glycerol, tween 20. Lecithin (1ml) was added to the hot water and mixed thoroughly. Tween 80 (3ml), glycerol (1ml) Triton- X (3ml) and tween 20 (3ml) were added to this prepared mixture (Fig.3). Finally 1 ml of endospore suspension (1×10^6/ml) was added to this mixture and stored in a glass vial at room temperature (27±2 ℃).

Assessment of shelf-life of the formulation. The formulation was transferred into sterilized microfuge tubes and stored in room temperature (27±2 ℃). Observations on viability of endospores and condition of the formulation were recorded on 30th and 50th day after inoculation (DAI).

Testing the efficacy of water soluble formulation of *P. penetrans* against *M. incognita* in tuberose. Seed tubers of tuberose plant were planted in pots filled with sterile pot mixture (sand : vermicompost: red soil - 2:1:1). Various concentrations of each formulation *viz.*, 0.5ml, 1ml, 2ml and 2.5ml were inoculated separately to pots. A chemical check was maintained with Nimitz (1.5g/plant) and the fungal bioagent, *Pochonia chlamydosporia* (1ml/plant) as positive check. One set of pots were maintained without any treatment as untreated (Control) for comparison. Three replications were maintained for each treatment. Healthy J_2 of *M. incognita* were collected from freshly hatched eggs and were inoculated into the pots (100 J_2/pot). Observations were taken on nematode infestation in soil and root and plant biometric characters were also recorded.

Statistical analysis. The data obtained from various experiments described above were analyzed using ANOVA DMRT (Panse and Sukhatme 1954) and analyzed using AGRES statistical software.

RESULTS AND DISCUSSION

Properties of the formulation. The physical characters of the formulation showed that the formulation was dull white in colour and was stable upto 2 months. The formulation was also water soluble. It contained an average of 1.5×10^6 spores/1.5ml (Table 1). Endospores retained their shape and were intact in the formulation. Findings of Raut *et al.* (2012) also revealed that the formulation was more effective and consistent. Zhou *et al*. (2010) confirmed that the role of lecithin in formulation preparation and they have a synthesized a lecithin-based nanoemulsion and measured particle size, viscosity, stability and skin hydration. Swarnakumari (2021) have entrapped *P. penetrans* endospores in alginate beads and tested its efficacy in cucumber. EC formulation of this bacterium was developed and tested by Srishalini *et al.* (2021). These findings were in agreement with the current observations. Results of these experiments confirmed that the water soluble formulation was effective against *M. incognita* in tuberose.

Shelf- life assessment. Viability of endospores in the formulation was observed on 30 and 60 days after storage. There was no change in the physical condition of the formulation for 2 months (60 days). The parasitization potential of *P. penetrans* on J_2 of *M. incognita* was normal on 30 and 60 days after storage at room temperature.

Pot culture. Water soluble formulation of *P. penetrans* with different concentrations was applied to tuberose plants. A check of an untreated control was also maintained for comparison. The whole plants were uprooted 45 days after planting (Fig. 6). Growth

parameters with each treatment were observed and recorded (Table 3, Fig. 5) This was in accordance with Weibelzahl-Fulton *et al.* (1996) who tested the efficacy of *P. penetrans* against *M. incognita* and *M. javanica* in tobacco plant. Application of water soluble formulation with concentration of 2ml /plant recorded the lowest gall formation that was 66% reduction over control. The same treatment was on par with *P. chlamydosporia* (49.3% reduction over control) followed by Nimitz (48.3%). Whereas, formulation at 1ml and 2.5ml / plant was on par with each other and reduced the gall formation by 46% when compared to control. Highest reduction of egg masses was recorded in 2.5ml (3 egg masses /plant that is 82.3% reduction over control). Application of *P. chlamydosporia* recorded 4 egg masses / plant that was 76.4% reduction over control followed by Nimitz (Table 2, Fig. 4). Highest reduction of J_2 population in soil was observed in pots received 2ml of the formulation followed by *P. chlamydosporia.* Number of infested females was higher in plants applied with the formulation 2ml followed by 2.5ml. Tzortzakakis *et al.* (1997) conducted the interaction study between *P. penetrans* and *M. incognita* under pot culture condition. The spore load was sufficient in females and successfully controlled the *M. incognita.*

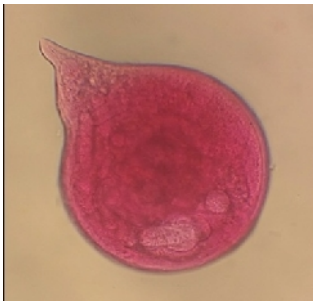

Fig. 1(a) Adult Female of *M. incognita*

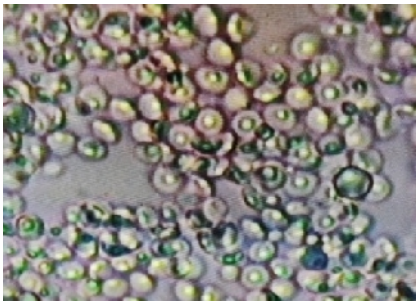

Fig. 1(b) *P. penetrans* endospore

Fig. 2 (a) Preparation of base material

Fig. 2(b) Addition of endospores

Fig. 2. Preparation of water soluble formulation.

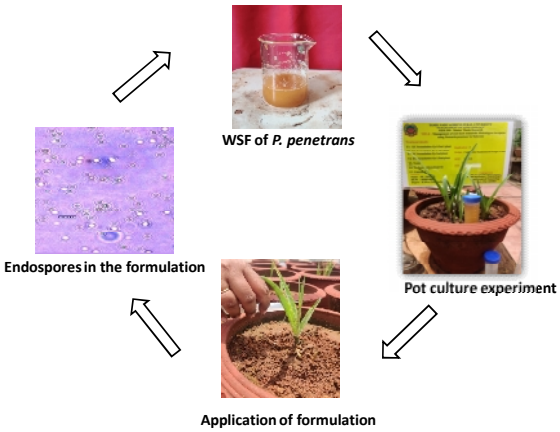

Fig. 3. Flow chart of activities of pot culture experiment.

Table 1: Assessment of shelf-life of water soluble formulation entrapped with endospores of *P. penetrans.*

Treatments	% of parasitization		Average no. of spores / J_2	
	30th day	60th day	30th day	60th day
T1 - WSF *P. penetrans* 0.5 ml	80.00 (31.81)	82.00 (31.24)	8.1 (9.44)	8.5 (9.67)
T2 - WSF *P. penetrans*1 ml	85.00 (31.06)	81.00 (31.12)	11.6 (11.22)	11.1 (11.03)
T3 - WSF *P. penetrans* 2 ml	89.00 (32.1)	90.00 (33.1)	13.2 (12.04)	12.9 (11.93)
T4 - WSF *P. penetrans* 2.5 ml	82.00 (32.4)	84.00 (30.99)	11.3 (11.06)	11.2 (11.03)
SEd	7.91	8.48	0.68	0.65
CD (p = 0.05)	NS	NS	NS	NS

* Figures in parenthesis are Arc-sine transformed values WSF – Water Soluble Formulation

Table 2: Effect of water soluble formulation of *P. penetrans* on *M. incognita* infestation in soil and roots of tuberose.

Treatments	No. of galls/root system	No. of egg masses /root system	No. of infested females/root system	No. of endospores/ female	No. of J_2 in soil /100 cc
T1- WSF *P. penetrans* 0.5 ml / plant	10.00^b (1.81)	16.00^b (2.29)	7.10 (1.51)	1.2×10^6	82.00 (5.19)
T2- WSF *P. penetrans* 1 ml / plant	8.00^b (1.62)	8.00^a (1.62)	3.90 (1.13)	1.4×10^6	69.00 (4.77)
T3- WSF *P. penetrans* 2 ml / plant	5.10^a (1.29)	3.00^a (1.00)	3.60 (1.10)	2×10^6	60.00 (4.35)
T4- WSF *P. penetrans* 2.5 ml / plant	8.00^b (1.62)	3.00^a (1.00)	3.70 (1.10)	1.7×10^6	74.00 (4.84)
T5 - Nimitz 1.5g / plant	7.80^b (1.60)	7.00^a (1.80)	-	-	70.00 (4.79)
T6- *P. chlamydosporia* 1 ml / plant	7.70^b (1.59)	4.00^a (1.13)	-	-	61.00 (4.34)
T7 - Untreated (Control)	15.10^c (2.23)	17.00^b (2.35)	-	-	168.00 (7.45)
SEd	0.39	0.95	0.44	-	7.99
CD (p=0.05)	0.84	2.05	NS	-	17.55

Figures in parenthesis are square root transformed values. In a column, means followed by alphabet are significantly different from each other at 1% level by DMRT. WSF – Water Soluble Formulation

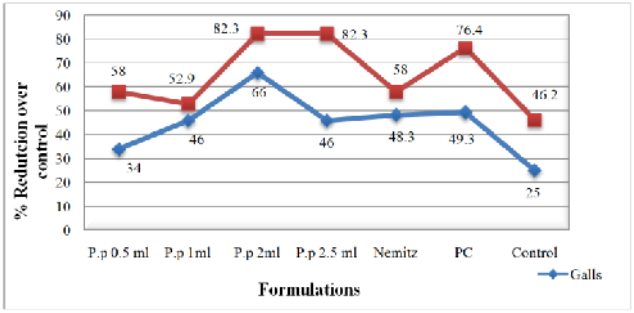

Fig. 4. Effect of water soluble formulation of *P. penetrans* on nematode infestation in roots of tuberose.

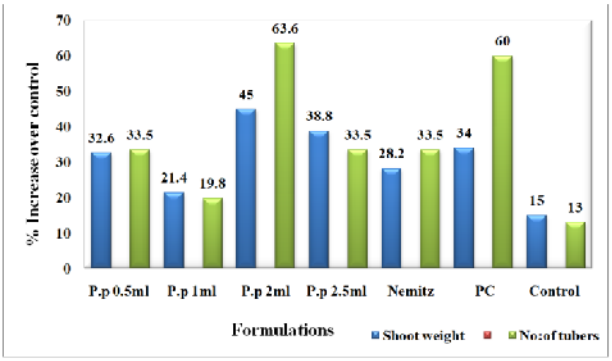

Fig. 5. Effect of water soluble formulation of *P. penetrans* on shoot weight and number of tubers.

Table 3: Effect of water soluble formulation of *P. penetrans* on plant biometric characters.

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)	No: of tubers	Tuber weight (g)
T1- WSF *P. penetrans* 0.5 ml / plant	18.2	49	8.9	1.93	2.00^{bc} (1.38)	38
T2- WSF *P. penetrans* 1 ml / plant	15.9	42	10.2	1.46	1.66^{bc} (1.27)	35
T3- WSF *P. penetrans* 2 ml / plant	20	60	13.5	4.03	3.66^{a} (1.91)	46
T4- WSF *P. penetrans* 2.5 ml / plant	16.2	54	12.4	2.09	2.00^{bc} (1.41)	30
T5 - Nimitz 1.5g / plant	16.3	46	13.2	1.81	2.00^{bc} (1.38)	28
T6- *P. chlamydosporia* 1 ml / plant	17.3	50	13.3	2.15	3.33^{a} (1.79)	42
T7 - Untreated	11.15	33	3.2	2.07	1.33^{c} (1.13)	27
SEd	-	-	-	-	0.7	-
CD (P=0.05)	-	-	-	-	1.52	-

*Figures in parenthesis are Square root transformed values. In a column, means followed by alphabet are significantly different from each other at 1% level by DMRT. WSF – Water Soluble Formulation

Fig. 6. Effect of water soluble formulation of *P. penetrans* on plant biometric characters.

CONCLUSION

P. penetrans is a successful and potential biocontrol agent which is very effective in controlling the most damage causing root-knot nematode *M. incognita* in tuberose plants. Water soluble formulation of *P. penetrans* was developed and tested against *M. incognita* in tuberose. Application of this formulation at the rate of 2ml / plant showed highest reduction in nematode infestation that was below economic thresh hold level. Hence this formulation may be further refined and tested in other crops in future.

Acknowledgement. The authors extent their gratitude to the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore for providing laboratory facilities and would like to thank DST-SERB, New Delhi for financial support to carry out this research work.
Conflict of interest. None.

REFERENCES

Abdullah, S. (2012). *Somatic embryogenesis and regeneration of Polianthes tuberosa L./Sakinah binti Abdullah* (Doctoral dissertation, University of Malaya).

Ayanaba, A. (1993). *U.S. Patent No. 5,248,500*. Washington, DC: U.S. Patent and Trademark Office.

Chawla, G., Singh, K. P., & Srivastava, A. N. (2006). Study on managing Root-knot Nematode (Meloidogyne Incognita (Kofoid & White) Chitwood) Infection on Tuberose *(Polianthes Tuberosa* Linn) Through Treatment of Mother Bulbs. *Journal of Ornamental Horticulture*, *9*(4), 290-292.

Chen, Z. X., & Dickson, D. (1998). Review of Pasteuria penetrans: biology, ecology, and biological control potential. *Journal of nematology*, *30*(3), 313.

Danlei, L., & Shengfu, Y. (2004). Preliminary study on tuberose root-knot disease. *Yunnan Nong ye da xue xue bao= Journal of Yunnan Agricultural University*, *19*(6), 670-672.

Davies, K. G. (2009). Understanding the Interaction Between an Obligate Hyperparasitic Bacterium, *Pasteuria penetrans* and its Obligate Plant-Parasitic Nematode Host, *Meloidogyne* spp. *Advances in Parasitology*, *68*, 211-245.

Gowda, M., & Chawla, G. (2013). Distribution of root-knot nematode (*Meloidogyne incognita)* in tuberose (*Polianthes tuberosa* L.) bulbs and comparison of extraction methods. *Indian Journal of Nematology*, *43*(2), 189-194.

Gowda, M., Gajbhiye, V. T., & Chawla, G. (2014). Carbosulfan absorption by *Polianthes tuberosa* L. bulbs and its effect on root-knot nematode, *Meloidogyne incognita*. *Indian Journal of Nematology*, *44*(2), 164-171.

Grace, G. N., Shivananda, T. N., Rao, M. S., & Umamaheswari, R. (2019). Management of nematodes using liquid formulations of *Purpureocillium lilacinum* in tuberose.

Johnson, A. W. (1970). Pathogenicity of four root-knot nematode species to *Polianthes tuberosa*. *Journal of nematology*, *2*(2), 191..

Khan, A., Aiman, S. I., Baber, Y., Hassan, F., Usman, H. M., Sohail, M. A., & Abbas, A. (2021). An overview of root-knot nematodes and their management.

Khan, M. R. (2015). Nematode diseases of crops in India. In *Recent advances in the diagnosis and management of plant diseases* (pp. 183-224). Springer, New Delhi.

Kumari, N. S., & Sivakumar, C. V. (2005). Biological control potential of the obligate parasite *Pasteuria penetrans* against the root-knot nematode, *Meloidogyne incognita* infestation in Brinjal. *Communications in Agricultural and Applied Biological Sciences*, *70*(4), 905-908.

Mukhtar, T., Arshad Hussain, M., & Zameer Kayani, M. (2013). Biocontrol potential of *Pasteuria* penetrans, *Pochonia chlamydosporia*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Meloidogyne incognita* in okra. *Phytopathologia Mediterranea*, 66-76.

Panse, V. G., & Sukhatme, P. V. (1954). Statistical methods for agricultural workers. *Statistical methods for agricultural workers.*

Raut, S., Bhadoriya, S. S., Uplanchiwar, V., Mishra, V., Gahane, A., & Jain, S. K. (2012). Lecithin organogel: A unique micellar system for the delivery of bioactive agents in the treatment of skin aging. *Acta Pharmaceutica Sinica B*, *2*(1), 8-15.

Saha, T., & Khan, M. R. (2016). Plant and soil nematodes associated with tuberose in West Bengal, India. *Bangladesh J. Bot*, *45*(5), 1015-1025.

Sellaperumal, C., Chawla, G., & Singh, K. P. (2015). Effect of Hot Water Treatment on Root-Knot Nematode, *Meloidogyne incognita* (Kofoid & White) Chitwood Infecting Tuberose (*Polianthes tuberose* Linn.) Bulbs. *Indian Journal of Nematology*, *45*(1), 113-118.

Skerman, V. B. D., McGowan, V., & Sneath, P. H. A. (1980). Approved lists of bacterial names. *International journal of systematic bacteriology*, *30*(1), 225-420.

Srishalini.S, Swarnakumari.N, Jothi.G, Thirubhuvanamala.G (2021). Efficacy of formulation of nematode antagonistic bacterium, *Pasteuria penetrans* (Thorne) Sayre and Starr, 1985 against root-knot nematode, *Meloidogyne incognita*. *Journal of biological control*, *35*(3), 146-152

Swarnakumari. N. (2021). Combined effect of *Pochonia chlamydosporia* formulation and *Pasteuria penetrans* in suppressing *Meloidogyne incognita* infestation in cucumber grown under protected condition. In: Proc. 5 th National symposium on plant protection in horticulture (NSPPH – 2021): challenges and roadmap ahead. P.50.

Tzortzakakis, E. A., DE. R. CHANNER, A. G., Gowen, S. R., & Ahmed, R. (1997). Studies on the potential use of *Pasteuria penetrans* as a biocontrol agent of root-knot nematodes (*Meloidogyne* spp.*). Plant Pathology*, *46*(1), 44-55.

Van Nieuwenhuyzen, W. (1976). Lecithin production and properties. *Journal of the American Oil Chemists' Society*, *53*(6Part2), 425-427.

Weibelzahl-Fulton, E., Dickson, D. W., & Whitty, E. (1996). Suppression of *Meloidogyne incognita* and *M. javanica* by *Pasteuria penetrans* in field soil. *Journal of Nematology*, *28*(1), 43.

Zhou, H., Yue, Y., Liu, G., Li, Y., Zhang, J., Gong, Q., & Duan, M. (2010). Preparation and characterization of a lecithin nanoemulsion as a topical delivery system. *Nanoscale research letters*, *5*(1), 224-230.

How to cite this article: M. Janani, N. Swarnakumari, A. Shanthi and M. Gnanachitra (2022). Water Soluble Formulation of Nematode Antagonistic Bacterium, *Pasteuria penetrans* for the Management of Root-knot Nematode, *Meloidogyne incognita* in Tuberose. *Biological Forum – An International Journal*, *14*(2a): 533-538.