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Effect of Temperature, Soil pH, Agitation Intervals and Soil Types on the Spore Attachment of *Pasteuria penetrans* to Root Knot Nematodes, *Meloidogyne javanica*

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Abstract: Studies were conducted to see the effect of various factors viz; temperature, soil pH, agitation intervals and soil types on the spore attachment of *Pasteuria penetrans* to *Meloidogyne javanica*. All the experiments were conducted in growth room where temperature was maintained at 28°C. Results revealed that the spore attachment increased with the increase in temperature, soil pH (from neutral to alkaline) and agitation intervals. It was observed that there was no effect of soil types on the spore attachment.

Key words: Temperature, agitation intervals, *Meloidogyne*, soil pH, soil types, spores of *Pasteuria penetrans*, attachment

Introduction

There are many kinds of micro organisms e.g. fungi, bacteria, viruses and nematodes which cause different diseases to many vegetable, field crops, fruits and ornamental plants. Nematodes in this regard are very important and play a key role.

Amongst the nematodes root-knot nematode *Meloidogyne javanica* (Treub, 1885; Chitwood, 1949) is the most destructive that tremendously reduces the quantity as well as the quality of the product. In agriculture soils the approximate distribution of *M. javanica* is 31% (Maqbool, 1986).

The worldwide distribution of root knot nematode and their involvement with other pathogens make them the most dominant disease producing agent. Most of the plant parasitic nematodes including *Meloidonyne* spp., live in the soil and attack the plants. There are various control measures of root-knot nematodes i.e. chemical, cultural and biological but the biological control proved to be much effective, cheap and safer because chemicals control are expensive, laborious to use and health hazardous as well as some of them are phytotoxic.

Bio-control of root-knot nematode is done by various means but with the help of a bacterium *Pasteuria penetrans* (Thorne, 1940; Mankau, 1975; Sayre and Starr, 1985) may be much helpful and cheaper. *Pasteuria penetrans* is a candidate bio-control agent of nematode, *Meloidogyne* spp. The potential of the bacterium, *P. penetrans* for the control of root-knot nematode has been reported by Mankau and Prasad (1977), Eddaoudi and Bourijate, (1998); Gowen *et al.* (1998).

Pasteuria penetrans is the most specific obligate parasite of nematodes but one of the major problem in using this bacterium as a biocontrol agent is the inability to culture this bacterium *in vitro* on any of the standard bacteriological media. So this study aims at to see the effect of various factors like, temperature, pH, agitation intervals and soil types on the attachment of spores to the cuticle of *Pasteuria penetrans*.

Materials and Methods

Effect of temperature on spore attachment: The studies were conducted in the Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan during 1992.

The *Pasteuria penetrans* culture was prepared by grinding dried root powder in water with a pestle and mortar and removing the root debris by pouring the slurry through a 25 μm sieve as described by Stirling and Watchel (1980).

Approximately 4500 larvae of *Meloidogyne jananica* held in 100 ml were added into a 250 ml conical flask containing 10 ml of *Pasteuria penetrans* suspension. The inoculated material was incubated at 20, 25 and 30°C for 24 h and the experiment was conducted in triplicate.

After the incubation period, attachment of *P. penetrans* to larvae of *M. javanica* was recorded. A small quantity of suspension was poured into the counting dish and was moved under the stereo microscope for the search of nematode. From each replication 6-7 nematodes were observed for spore attachment.

Effect of soil pH on spore attachment: Three soil samples were adjusted at three different pH levels i.e. pH, 5, 7 and 9 with the help of pH meter by adding HCl for acidity or NaOH for alkalinity. The soil pasts, which were prepared for pH adjustment, were placed on polythene sheets for the evaporation of moisture. After complete dryness the soil was grinded. The culture of *P. penetrans* was prepared by grinding air dried roots in the pestle and mortar and added to the soils adjusted at different pH levels @ 1 g kg^{-1}. The experiment was conducted in triplicates for each pH level. Dry soil weighing 500 gm mixed with *P. penetrans* culture was taken in plastic trays (12x20 cm^2) (depth 2 cm) lined with tissue paper. This plastic tray was then placed in a metaled tray. Adding tap water in the lower tray upto the bottom of upper plastic tray moistened soil samples.

Nematode culture containing 3000 *Meloidogyne juveniles* in 100 ml suspension was pipetted on the soil surface uniformly. Plastic trays were incubated at room temperature, 28°C, for 48 h. After the incubation period the spore attachment of *P. penetrans* to the larvae of *M. javanica* was recorded.

Water from lower trays was poured in beakers separately for each replication. This water was allowed to settle down for 2-3 h, then upper water was siphoned out gently leaving 100 ml water in each beaker. A small quantity of this water was poured into the counting dish and was moved under the stereo microscope for the search of nematodes.

From each replication of pH levels 6-7 nematodes were observed after making temporary slides in a drop of water and thus encumbered spores/J-2 were counted by using high power inverted microscope.

Effect of agitation intervals on spore attachment: The culture of *P. penetrans* was prepared by grinding dried roots in water with the help of a pestle and mortar and then removing the slurry through a 25 μm sieve as described by Stirling and Wachtel (1980).

Approximately 4500 larvae of *M. javanica* held in 100 ml were added to a 250 ml conical flask containing 10 ml of *P. penetrans* suspension. The aliquots were arranged in triplicate for each agitation interval. The spore suspension with juveniles was agitated for three different time intervals i.e. 3, 6 and 12 h.

After stipulated agitation intervals a small quantity of suspension was poured into the counting dish or a watch glass and was moved under the high power inverted microscope for the search of nematodes. From each replication at least 6-7 nematodes were observed for the attachment of spores to the nematode body.

Effect of soil type on spore attachment: The soil samples of different soil types were prepared as under:

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75% loam soil + 25% sand
50% loam soil + 50% sand
25% loam soil + 75% sand

Each soil type weighing 500 g was mixed with *P. penetrans* roots @ 0.5 g in 500 g of soil and taken in the plastic tray of 12x20 cm^2 size (depth 2 cm) lined with tissue paper. This plastic tray was then placed in a metaled tray. Adding tap water in the lower tray moistened soil. Nematode culture containing about 3000 *Meloidogyne juveniles* held in 100 ml was pipetted on the soil surface uniformly. Three replications for each soil type were maintained and incubated at room temperature (28°C) for two days. After the incubation period, juveniles were collected from suspension and observed for attachment of spores as mentioned earlier.
Data was analyzed by using SAS as statistical package and means were compared by using DMR test (Gomez and Gomez, 1984).

Results and Discussion

Effect of temperature on spore attachment: Comparison of treatment means indicated that the attachment was increased with the increase in the temperature level. It was clear that maximum mean spore attachment (7.72 spores/J-2) was at 30°C, whereas less spore attachment (5.44 spores /J-2) was at 25°C, but minimum (3.89 spores/J-2) spore attachment was at 20°C. Thus it revealed that *P. penetrans* spores attached more frequently to *M. javanica* at 30°C than at 20°C (Table 1).

Table 1: Effect of various factors on spore attachment of *Pasteuria penetrans* to *Meloidogyne javanica*

Level of temp. °C	No. of spores attached	Level of pH	No. of spores attached
20	3.89c	5	1.00c
25	5.44b	7	5.44b
30	7.72a	9	6.28a
Agitation intervals (h)	**No. of spores attached**	**Soil types**	**No. of spores attached**
3	2.83c	75% loam + 25% sand	4.8a
6	5.44b	50% loam + 50% sand	5.0a
12	10.67	25% loam + 75% sand	4.9a

Data are means of three replications, Figures sharing similar letters do not differ significantly at 5% level of significance.

From the comparison of treatment means it revealed that the spore attachment was increased with an increase in soil pH. There was maximum (6.28 spores/J2) mean spore attachment at pH 9 and significantly less (5.44 spores/J-2) spores were attached at pH 7 but minimum spore attachment (1.00 spore/J-2) was at pH 5. It indicated that bacterial spores attached more frequently to *M. javanica* juveniles at alkaline pH 9 than at acidic pH (5).
From results it was indicated that the spore attachment has increased with the increase in the agitation intervals. It showed that maximum (10.67 spores/J-2) spore attachment was at 12 h agitation intervals but comparatively less and minimum spore attachment of 5.44 and 2.83 spores/J-2 has occurred at 6 and 3 hours agitation intervals respectively.
Spore attachment in respect of different soil types was non-significant which means that there was no effect of soil types on the spore attachment. It indicated that the attachment increased with the increase in temperature level. These results were similar to those obtained by Stirling (1984) and Davies *et al.* (1988), Giannakou *et al.* (1998) who also found greater attachment at 25 and 30°C. According to Striling (1981) *P. penetrans* spores attach more readily to the nematodes at 22.5-30°C than at 15°C. According to O' Brein (1980) the attachment probably involves chemical interaction between spores and the cuticle of nematodes and these interactions may involve factors like lectins.
It is clearly indicated that spore attachment increased when pH increased from neutral to alkaline but decreased with the decrease in pH from neutral to acidic which meant that acidic pH has a repellent effect on the bacterial spores and lessened the attachment.

Effect of agitation intervals on the spore attachment of *P. penetrans* to *M. javanica* revealed that there was maximum attachment in the spore suspension added with *Meloidogyne juveniles* agitated for 12 h and less attachment was recorded at 6 and 3 h agitation. It was due to the fact that bacterial spores are immobile (Wallace, 1966) and have an outer layer called exosporium which inhibit the attachment and during shaking the exosporium was broken down and helped in the attachment. Moreover during agitation there was more chance for the nematodes and the endospores to come closer to each other which also facilitated the attachment. According to Stirling *et al.* (1986) if the suspension of spores and nematodes are continuously bubbled the levels of spore attachment are even better because of the reason described earlier.
Studies made to observe the effect of soil types on the spore attachment of *P. penetrans* to *M. javanica* revealed that there was no effect of soil types on the spore attachment because spore attachment in all types of soils tested was almost the same. The results are conformity with Dutky and Sayre (1978) who found no relationship between the soil pore size and the nematode attachment to the bacterial spores. Spaull (1984) found more parasitized females (62.2%) in sandy soil and 53.3% from loam sand.

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