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Selection of the Most Suitable Host for the Mass Production of *Pasteuria penetrans* an Obligate Parasite of Root-knot Nematode *Meloidogyne javanica*

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Abstract: Selection of the most suitable host for the mass production of *P. penetrans* revealed that brinjal was the most suitable host followed by mungbean, mash and cowpea. It was due to more invasions sites present in the brinjal roots, which provided more penetration of nematodes in the roots. The relationship of quantity of roots and amount of spores revealed that within the host more amount of roots produced more endospores but amongst the hosts it did not happen.

Key words: Mass production, *Pasteuria penetrans*, *Meloidogyne javanica*, host, root-knot nematodes

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 nematodes 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 soil and attack the plants.

Very little work has been done on the biological control of root-knot nematode *Meloidogyne javanica* with the bacterium *Pasteuria penetrans* in Pakistan because *P. penetrans* is an obligate parasite of root-knot nematode and can not be grown on artificial bacteriological media.

No work has been done in Pakistan on the mass production of *P. penetrans* that can successively be used for biological control of this disease. However foreign literature provided little information about the mass production of *P. penetrans* and this is clear from the previous studies that mass production can only be done on those plants which are the susceptible hosts of root knot nematodes, *Meloidogyne jananica*.

Our studies aimed to select the most suitable host plants having more invasion sites in their roots and offering more penetration to root-knot nematodes, *M. javanica* parasitized by *P. penetrans* and ultimately helping in the mass production of *P. penetrans* spores.

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 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 *et al.*, 1988) 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).

Pasteuria penetrans is the most specific obligate parasite of nematodes but one of the major problem in using this bacterium as a bio-control agent is the inability to culture this bacterium *in vitro* on any of the standard bacteriological media. So this study aims at the mass culture of this bacterium on different host plants and selecting the most suitable host which can harbor more number of *Meloidogyne* females parasitized by *P. penetrans* and thus be able to produce more number of endospores.

Materials and Methods

These studies were conducted in Department of Plant Pathology, University of Agriculture, Faisalabad, Pakistan during 1992.

Four different susceptible hosts i.e. brinjal, cowpea, mungbean and mash were used for the mass production of *P. penetrans*. Nursery of brinjal plants was raised in microplots, whereas 3-4 seeds of remaining three hosts were sown directly in pots, which were thinned to one plant per pot after germination.

For brinjal three weeks old seedlings were transplanted in earthen pots (10x15 cm^2) containing formalin sterilized sandy loam (1:2) soil. One plant per pot was planted. One week after transplanting these plants was inoculated with freshly hatched 2^{nd} stage juveniles of *Meloidogyne javanica* after having the attachment with *P. penetrans*.

The inoculating material was pipetted around each plant through holes. Pots were completely randomized in the glasshouse and for each host four replications were maintained. Tap water was added to irrigate young seedlings throughout the period of experiment. After 4 weeks, plants alongwith soil were removed from the pots and their roots were carefully washed in running water. Data was recorded as the quantity of roots and number of endospores per mg of the root system.

These roots were chopped, sundried weighed and finally grinded in the pestle and mortar.

Pasteuria penetrans suspension was made by grinding the roots in 100 ml water and then removing the root debris by pouring the slurry through a 25 μm sieve as described by Stirling and Wachtel (1980).

Suspension was taken in the haemocytometer with the help of a micropipette, covered with the cover glass and was moved under the microscope for the search of *P. penetrans* spores, which were then counted and spore load was determined as per mg of the root powder.

Relationship between the quantity of roots and amount of *Pasteuria penetrans* spores produced on the roots of different host plants was determined.

Fresh and dry weight of roots was done. Then amount of *P. penetrans* spores produced was estimated as per mg of the roots and relationship between quantity of roots and the spores produced on them was determined.

Data was analyzed by using SAS as statistical package and means were compared by using DMR test (Gomez and Gomez, 1984).

Results and Discussion

From the comparison of treatment means it was clear that brinjal was the best host which has produced 106250 spores mg^{-1} followed by mungbean, mash and cowpea which have produced

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Table 1: Selection of most suitable host for the mass production of *Pasteuria penetrans*

Name of hosts	Mean spore produced per mg of roots
Brinjal	106250.00a
Mungbean	61875.00b
Cowpea	48125.00c
Mash	58750.00b
S.E.	1957.89

Figures sharing similar letters do not differ significantly at 5% level of significance.

61875, 58750 and 48125 spores mg^{-1} of the roots respectively (Table 1). These results indicated that brinjal was the most susceptible followed by mung and mash, which were almost equally susceptible and cowpea was least susceptible to *M. javanica*.

Relationship between the quantity of roots and amount of *Pasteuria penetrans* spores produced on them indicated that within a host more roots have produced more number of *P. penetrans* spores. Whereas amongst different hosts it did not happen, which showed that spore production was not related to the amount of roots but it was related to the invasion of nematodes in the roots. From the results it was also clear that brinjal has more invasion sites in the roots which provided more opportunity to the nematodes to invade the roots and has produced more number of spores. Whereas mung and mash have produced almost equal number of spores showing that they have almost same invasion sites but cowpea instead of having more root weights has produced less amount of spores as compared to other hosts which indicated that cowpea has less invasion sites in the roots and mung bean and mash instead of having less root weights as compared to cowpea has produced more amount of spores.

Host plant and temperature affect the development of *Pasteuria penetrans*. Parasitized females in okra roots contained twice as many endospores as those in tomato and eggplants (Giannakou *et al.*, 1999).

Crop rotation systems seem to have an effect on the build up of *P. penetrans* populations, since continuous cultivation with susceptible crops increases the number of spores in soil (Madula *et al.*, 1994). Most reports on the suppression of plant parasitic nematodes by the use of *P. penetrans* were from monoculture cropping systems where the host was consistently available to the parasite (Sikora, 1992).

From the results of the most suitable host for the mass production of *Pasteuria penetrans* it has been concluded that the mass production was more in case of brinjal and less in case of mung, mash and cowpea respectively. It was due to more penetration of female nematodes carrying *P. penetrans* in case of brinjal which provided more invasion sites in the roots while other hosts provided less invasion in their roots.

According to David and Triantaphyllou (1967) many factors may also influence spore production e.g. poor host nutrition or increased competition will influence nematode reproduction and cause more males to develop and if few females develop there will be less production of spores.

According to Sayre *et al.* (1988) mature bacterial stages occur more frequently in adult female nematodes and not to the larvae. It has also been concluded that within a host more number of roots has produced more spores per mg but among different hosts the amount of the spores produced is not affected by the amount of roots.

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