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# POTENTIAL OF RHIZOBACTERIA FOR THE BIOCONTROL OF *MELOIDOGYNE JAVANICA*

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## ABSTRACT

Root knot nematodes are production hazards to vegetable crops of the world. Present studies were conducted to search out antagonistic bacteria against these castrophic pathogens and to find out active metabolites secreted by the rhizobacterial strains. Fifteen Rhizobacterial strains were selected for antagonism against *Meloidogyne javanica.* Maximum mortality was observed by strain Rh37 which showed 53% juvenile mortality after 48 hr. as compared to control. Minimum mortality 3% was noted by Asr14 and R.E.4. Strains MR1, Mr53, AJ-3, Asr28 and Rh17 exhibited 43%, 28%, 25%, 24% and 23% mortality of *Meloidogyne javanica.* Mortality by remaining strains ranges from 9-23%. Active metabolites secreted by the rhizobacterial strains were found to be protease. The protease producing strains AJ-3 and Rh3 showed 25% and 53% juvenile mortality after 48 hrs. Hence, these studies will be helpful to manage tomato root knot.

**Keywords:** Rhizobacteria, *Meloidogyne javanica*, biocontrol, proteases

## INTRODUCTION

Root knot nematodes (*Meloidogyne* spp) are world wide in their distribution and attack a wide variety of crops (Goody *et al.,* 1965; Sasser and Freckman, 1987) and more than 3000 host species (Abad *et al*., 2003). Out of 81 *Meloidogyne* spp. identified so far (Karssen, 2002; Shahid *et al.,* 2009), *Meloidogyne javanica* is the most abundant and damaging nematode (Maqbool and Shahina, 2001). Various species of *Meloidogyne* induce major morphological and physiological changes within roots, attack nearly every crop sown, where not, only yield is greatly affected but quality is also reduced (Hondoo, 1996). In Pakistan, root knot nematodes, *Meloidogyne* spp. are recognized as important parasites of vegetable crops infecting more than 102 plant spp. (Zaki, 2000). Up to 81% frequency of infection has been recorded in tomatoes (Khan *et al*., 2005). Control of plant parasitic nematodes is difficult because of the enormous variety of suitable hosts. Chemical control of nematodes has been proved generally

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effective but highly expensive and often hazardous to use (Schneider *et al.,* 2003; Hasabo and Noweer, 2005) and highly toxic to mammals. (Rehman *et al*., 2009). For this reason, the researchers focused on using biocontrol agents in place of chemical agents during recent years. Biological control represents an effective alternative and practicable agricultural method for the control of plant parasitic nematodes (Hesch, 2004).

In general, the research on biological control of plant parasitic nematodes has concentrated on the use of microbial agents, in particular, the use of parasitic fungi and bacteria. (Jatala, 1986 and Gugino *et al.,* 2006). These agents have been used to control cyst and root knot nematodes, which include the most important nematode pests in the world agriculture. The objectives of present studies were to isolate rhizobacteria from infected plants of tomatoes and to evaluate them as potential biocontrol agents against *Meloidogyne javanica* and to understand the potential mechanism of biocontrol.

## MATERIALS AND METHODS

Twenty three samples of tomato plants infected with root knot nematodes (*Meloidogyne* spp.) were collected from Loralai (Bluchistan) on the basis of above ground symptoms. *Meloidogyne* spp. were isolated by Whitehead and Hemming tray method (Whitehead and Hemming, 1965). The isolated nematodes were inoculated onto brinjal plants for multiplication. When egg masses were formed, single egg masse was detached and inoculated in twenty three plants, each grown in earthen pots (15×14 cm). These pots were filled with 1.5 kg of sterilized soil (Loam and compost, 3.1. Ph 7.2). Females were taken from egg masses and were identified on the basis of perreneal pattern (Taylor and Sasser, 1978). Isolation of rhizobacteria was also done from the same soil according to (Kasimpur *et al.,* 2004). Soil from rhizosphere of tomato plants was taken and rhizobacteria were isolated. One gram of soil was mixed in the 20 ml test tube containing 9 ml autoclaved saline. The suspension was vortexed and dilutions were made up to $10^{-8}$. Each dilution measuring 0.1ml was spread on Lauria Bertani (L.B) plates and plates were incubated at 28 ± $3^0$C until colony development was observed.

For isolation of bacteria from endosphere, roots of tomato plant were cut into pieces and were crushed in pestle and mortar. Further procedure was same as described above. Rhizobacterial strains were identified on the basis of cell morphology, colony morphology and gram staining, using Bergeys Manual for Determinative Bacteriology (Holt *et al.,* 1994). Fifteen strains were tested for their antagonistic activity against *Meloidogyne javanica* (Table 1). The inoculum was produced by transferring two loopful of the bacterial strain from five days old culture to 100 ml Kings-B liquid medium and incubated at room temperature on shaker for 48 hr. The bacterial cells were centrifuged at 8000 rpm for 15 min. The pellet was discarded and supernatant was collected in sterile beaker prior to use. Antagonistic were conducted by method as described by Hamid *et al.*, (2003). One ml of each bacterial supernatant and nematode suspension containing 25-40 juveniles were taken in glass cavities at 25-30$^o$C. Juveniles kept in sterile

distilled water served as control. Data regarding juvenile mortality were recorded after 24-48 hr. The juveniles were considered dead when they do not move on probing with a fine needle (Cayrol *et al*., 1989)**.** Data were analyzed by two way analysis of variance. Means were compared with Duncans Multiple range test (Duncans, 1951). Production of hydrogen cyanide (HCN) was observed according to the method of Lork (1948). Presence of chitinase was checked on chitin agar medium as described by Brien and Colwald, (1987) and detection for protease production was done as described by Denisci *et al* (2003) on Skim milk agar medium.

## RESULTS AND DISCUSSION

Twenty-three single egg masses were taken from individual brinjal plant and mature female from each single egg mass was extracted and identified on the basis of perennial pattern. Two types of *Meloidogyne spp*. were found i.e. *Meloidogyne javanica* and *M. arenaria.* Ninety eight 98% were found to be *Meloidogyne javanica* and 2% *M. arenaria.* Therefore, *Meloidogyne javanica* was selected for further studies**.**

Table 1. Nematicidal activity of rhizobacterial strains against *Meliodogyne javanica* juveniles (*in- vitro* assay).

| Strain | * Mortalities of nematodes (%) (mean ±SD ) | |
|---|---|---|
| | % Mortality after 24 hrs | % Mortality after 48 hrs |
| A.Sr14 | 1± 0.00i | 3±0.82h |
| R.E4 | 3±0.81h | 3±0.81h |
| A.J-3 | 16±1.82b | 25±1.61d |
| A.Sr13 | 6 ±1.63fg | 12±1.41f |
| Rh37 | 14±0.00cd | 53±0.00a |
| Rh33 | 3±0.79h | 3±0.79h |
| Mr1 | 24±1.83a | 43±1.41b |
| Rh46 | 7±1.64f | 9±0.57g |
| A.Sr28 | 15±0.00bc | 24±1.85d |
| Rh17 | 14±1.70cd | 23±0.76d |
| Mr53 | 13±0.75d | 28± 1.58c |
| Rh27 | 9±0.84dc | 11±0.00fg |
| A.Sr24 | 7±0.00ef | 9±0.90g |
| R-6 | 10±1.63d | 15±1.63e |
| R-7 | 5±0.75fg | 9±0.57g |
| Control | 6±0.70f | 10±0.78g |
| LSD (0.05%) | 1.636 | 2.62 |

Values are an average of three readings. ± Standard Deviation
* Mortality refers to the number of dead nematodes/ number of ones tested.

A total of 52 rhizobacteria were isolated from rhizosphere and endosphere of tomato plants. Among them 49 were from rhizosphere and remaining three were from endosphere. Among 52 isolates, fifteen representative strains for *Pseudomonas* and *Bacillus* were selected for antagonistic tests against *Meloidogyne javanica*. Rhizobacteria belonging to *Pseudomonas* and *Bacillus*

spp. have also been used in bioantagonism (Ismail *et al*., 1997, Aksoy and Mennan, 2004).

Under in-vitro conditions, strains tested against *Meloidogyne javanica* showed 3-53 juvenile motility after 48 hrs. as shown in the Table 1. The mortality of juveniles may be due to controlled temperature, relative humidity, soil type and type of metabolites secreted by rhizobacterial strains as these proper conditions are impossible in-vivo. Our results are in accordance with Khurrum *et al.* (1997) and Nasima *et al.* (2002). Results for metabolite secretion showed that among four rhizobacterial strains tested for protease production, two rhizobacterial strains AJ3 and RH37 were found to produce proteases as shown in Fig-1. None of tested strains showed HCN and chitinase production. Protease producing strain were confirmed as *Bacillus subtillus* and *Pseudomonas fluorescence.*

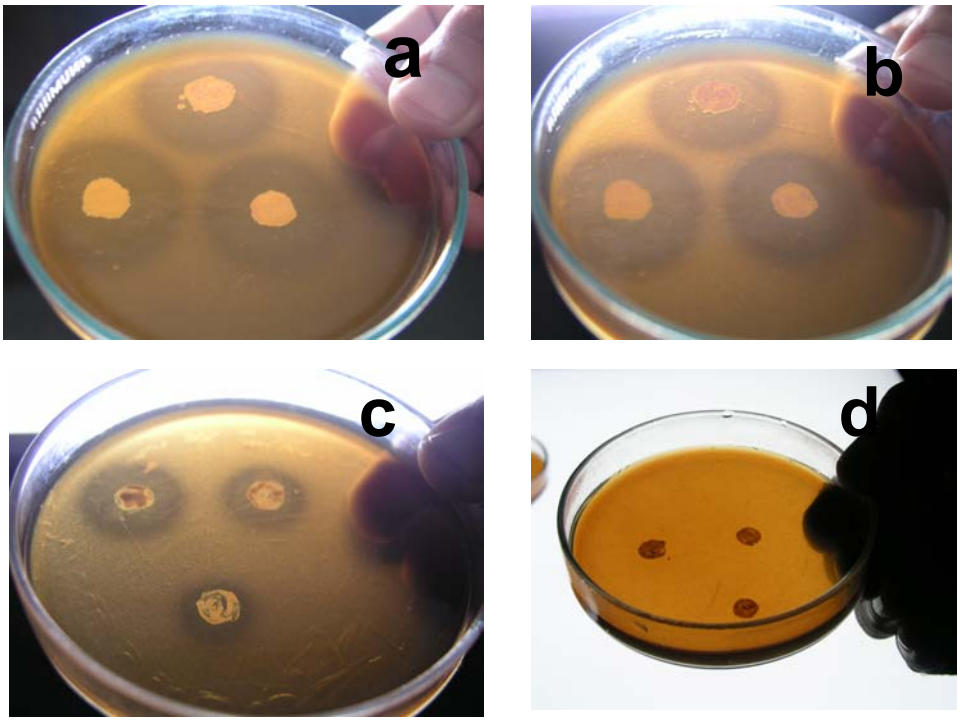


Figure 1. (a-d) (a), Protease production by reference strain *Pseudomonas fluorescence* CHA0 (b), Protease production by *Pseudomonas fluorescence* Rh37 (c), Protease production by *Bacillus subtillus* A-J3 (d), Negative strain showing no protease production.

These protease producer strains show 25% and 53% juvenile mortality after 48 hrs. Previous studies revealed that microbial proteases may play important role in infection of hosts (nematodes) by degrading the host's protective barriers (Huang *et al*, 2004). It has also been observed that bacterial proteases can

dissolve and digest nematode cuticle or even kill hosts (Clarkson and Charnley, 1996; Meyer, 2003). Because cuticle of nematodes is composed of proteins and chitin, especially the outer part that is covered by a layer of proteinaceous membrane, which is an effective barrier to protect nematodes from damage (Tunlid *et al*. 1994). Therefore, rhizobacterial enzymes like collagenases, proteases, and chitinases have also been emphasized in biological control of nematodes. In present studies, protease producing strains, which lead to a reduction of nematode numbers in- vitro, were not found to produce chitinases and HCN. It can be assumed that other metabolites i.e. collaginases, elastases etc. (Cox *et al.,* 1981) which were not investigated here, may also affect nematode infectivity. However these preliminary results constitute a strong incentive for further experiment on the use of strains of rhizosphere in biocontrol of plant parasitic nematodes.

## CONCLUSION

It is concluded from present studies that amongst the fifteen rhizobacterial strains, selected for antagonism against *Meloidogyne javanica,* the maximum mortality was observed by strain Rh37 which showed 53% juvenile mortality after 48 hr. as compared to control. Minimum mortality 3% was noted by Asr14 and R.E.4. Strains MR1, Mr53, AJ-3, Asr28 and Rh17 exhibited 43%, 28%, 25%, 24% and 23% mortality of *Meloidogyne javanica.* Mortality by remaining strains ranges from 9-23%. Active metabolites secreted by the rhizobacterial strains were found to be protease. The protease producing strains AJ-3 and Rh3 showed 25% and 53% juvenile mortality after 48 hrs. Hence, these studies will be helpful to manage tomato root knot.

## REFERENCES

Aksoy, H. M. and S. Mennan. 2004. Biological control of *Heterodera cruciferae* (*Tylenchida:Heteroderidae*) Franklin 194 with *fluorescent pseudomonas* spp. Phytopathol., 152: 514-518.

Brien, M. O. and R. R. Colwal. 1987. A rapid test for chitinase activity that uses 4-methyl umbelliferyl N-acetyl-beta D-Glucosamine. Applied Environmental Microbiol., 53 (7): 1718-1720.

Cayrol D. J., C. Dijjian and L. Pijaroski. 1989. Study of nematocidal properties of the culture filtrate of nematophagous fungus *Pacilomyces lilacinus*. Review de Nematologie, 1331-336.

Cox, G. N., M. Kusch and R. S. Edgar. 1981. Cuticle of *Caenorhabditis elegans*: its isolation and partial characterization. J. Cell Biol., 90: 7-17.

Clarkson J. M and A. K. Charnley. 1996. New insights into the mechanisms of fungal pathogenesis in insects. Trends Microbial., 4: 197-203.

Denisci, D., A. Kazan, E. C. A. Abein and A. Erarsian. 2003. Newly isolated *Bacillus clausii* GMBAE42: an alkaline protease producer capable to grow under highly - alkaline conditions. Journal of Applied Microbiology, 96: 320-327.

Duncan, D. B. 1951. A significant test for differences between ranked treatment in analysis of variance. Verginia J. Sci., 2: 171-189.

Goodey J. B., M. T. Franklin and D. J. Hooper. 1965. Goodey's the Nematode Parasites of Plants Catalogued Under their Hosts. Common Wealth Agriculture Bureau Farnham. 3rd edition. p. 2146.

Gugino, B. K., G. S. Abawi and J. W. Ludwig. 2006. Damage and management of *Meloidogyne hapla* using oxamyle on carrot in New York. J. Nemotol., 3: 483-490.

Hasabo, S. A and E. M. A. Noweer. 2005. Management of root knot nematodes on egg plant and some plant extracts. Egyp. J. Phytopathol., 33: 65-72.

Hamid, M., I. A. Siddiqui and S. S. Shaukat. 2003. Improvement of *Pseudomonas fluorescens* CH0 biocontrol activity against root-knot nematode by addition of ammonium molybdate. Letters in applied Microbiol., 36: 239-244.

Hesch, 2004. The biocontrol fungus *Pochonia chlamydospora* shows nematodes host preference at the interspecific levels. Molecular Research, 108: 161-169

Holt, J. G., N. R. Kriig, P. H. A Sheath and Williams. 1994. Bergeys Manual for Determinative Bacteriology Baltimore: William and Wikins, U.S.A. pp.178-229

Hondoo, 1996. Role of nematodes, nematicides and crop rotation on productivity and quality of potatoes & sweet potatoes, peanut grain and sorghum. Journal of Nemotology., 28 (3): 389-399.

Huang, X. W., B. Y Tian, Q. H. Niu, J. K. Yang, L. M. Zhang and K. Q. Zhang. 2004. An extra cellular protease from *Brevibacillus laterosporus* G4 without parasporal crystal can serve as a pathogenic factor in infection of nematodes. Res. Microbiol., 156: 719-727.

Ismail, A. E. and M. Fadel. 1997. Suppressive effects of some native isolates of *Bacillus spp* on *Meloidogyne incognita* and *Tylenchulus semipenetrans*. Egyp. J. Biol. Pest Cont., 7 (2): 53-60.

Jatala, P. 1986. Biological control of plant parasitic nematodes. Ann. Rev. Phytopathol., 24: 453-489.

Karssen, G. 2002. The plant parasitic nematodes genus *Meloidogyne goldi,* 1892 (*Tylenchida*) in Europe. Leiden. Boston Koln., pp. 157.

Kasempur, M. N. 2004. Biological control *of Rhizoctonia solani*, the causal agent of rice sheath blight by antagonistic bacteria in greenhouse and field condition. Plant Path. J., 3 (2):88-96.

Khan, H. U., T.Mukhtiar and R. Ahmad. 2005. Geographical distribution of root knot nematodes (*Meloidogyne spp*) in the Punjab Province of Pakistan. Pak. J. Nematol., 23 (I): 133-140.

Khurrum S., S. Jehanara and Ihtisham-ul-Haq. 1997. Nematicidal activity of some medicinal plants. Pak. J. Nemotol., 15 (2): 101-105.

Lork, H. 1948. Production of hydrocyanic acid by bacteria. Physiol plant., 1: 142-146.

Maqbool, M. A. and F.Shahina. 2001. Biodiversity of Nematode Fauna in Pakistan. NNRC, University of Karachi, p. 1809.

Meyer, S. L. F. 2003. United States Department of Agriculture research service research Programs on microbes for management of plant-parasitic nematodes. Pest Manag. Sci., 59: 665-670.

Nasima, I., I. A. Siddiqui., S. S. Shaukat and M. J. Zaki. 2002. Nematicidal activity of some strains of *Pseudomonas spp*. Soil Biology and Biochemistry. 34: 1051-1058.

Rehman, A. U., N. Javed, R. Ahmed and M. Shahid. 2009. Integration of bio-products and *Pasturia penetrans* for the management of root knot nematodes over three crop cycles of tomatoes. Pak. J. Nemotol., 27 (2):325-336.

Schneider, S. M., E. N. Rosskopf and M. Mazzola. 2003. Research on alternatives to methyle bromide pre- plant and poat-harvest. Pest Manag. Sci. 59: 814-826.

Shahid, M., A. U. Rehman, S. H. Khan, K. Mehmood and A. U. Khan. 2009. Management of root knot nematodes infecting brinjalby biopesticides, chemicals, organic amendments and bio-control agents. Pak. J. Nemotol., 27 (2):159-166.

Taylor A. L., J. N Sasser. 1978. Biology, Identification of Root Knot Nematodes (*Meloidogyne species*). Raleigh, NC, USA, North Carolina State University, Graphics, pp. 111.

Tunlid, A. S., B. E. K. Rosen and L. Rask. 1994. Purification and characterization of an extracellular serine protease from the nematode trapping fungus *Arthrobotrys oligospora*. Microbiology.140: 1687-1695

Whitehead, A. G. and J. K. Hemming. 1965 Comparison of some quantitative methods of extracting small vermiform nematodes from soil. App. Biol., 55: 25-38.

Zaki, M. J. 2000. Bio-management of root-knot nematodes problem of vegetables. DFID, UK Research Project Report. Department of Botany. University of Karachi.

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