Original Research Article

In vitro screening of different rhizobacterial strains against egg hatching of rice root-knot nematode, *Meloidogyne graminicola*

Abstract:

Meloidogyne graminicola is an economic yield loss causing major pest of rice globally. For managing this pest, biological control practices act as an alternate strategy, as it is the most effective and economic. Plant growth promoting rhizospheric bacteria (PGPR) are most significant because they benefit plants both directly and indirectly by enhancing plant growth and provides long lasting antagonistic effects against plant parasitic nematodes. Considering this an *in vitro* efficacy research experiment of nematoxicity of native rhizobacterial strains was conducted, against egg hatching of *M. graminicola* at S/2 and S/4 concentration levels of intact bacterial culture and cell free culture filtrates (CFCs). The hatching behavior of rice root-knot nematode eggs was observed on alternate days for ten days which resulted in inhibition of egg hatching by all bacterial cultures at both concentration levels. When compared to the untreated control, *Bacillus* spp. showed the most egg inhibition in the S/2 concentration of both culture filtrates i.e,86.8% and 84.8%. Similarly, in the S/4 level of concentration, most egg hating such as 83.7% and 79.8% was recorded in *Bacillus* spp. culture filtrates. The rate of hatching was inversely proportional to the concentration of strains at exposure time, decreasing as the concentration increased. The results of the experiment revealed the potential of rhizospheric bacteria which makes it more feasible and environmentally safe approach for the management of *Meloidogyne graminicola*

Keywords: *Bacillus* spp. ,*Meloidogyne graminicola*, *Pseudomonas putida ,* Rice root-knot nematode, rhizobacterial strains

Introduction:

Rice is one of the most essential food crops in the world and forms the staple food for more than half of the global population (McCouch *et al.*, 2016)**.** Asia provides 90% of the global rice production. In India, rice is grown on an area of 43.78 mha and is produced annually at a rate of about 127.9 million tons (Anoymous, 2022). Plant parasitic nematodes (PPNs) are widespread in nature and they can cause severe damages and yield losses to numerous agricultural, forestry, ornamental and officinalis plants (Mitiku, 2018; Sasanelli *et al*., 2018). *M.graminicola*, commonly called the rice root-knot nematode, is a prevalent pest at global level causing severe damages to cereals (Dutta, 2012) and infecting more than 100 plant species. *M. graminicola* is different from other species on the basis of the length of eggs, J2 length, a-value, hyaline tail portion; male length, distance up to excretory pore, spicules and gubernaculum lengths; female length and width, stylet length, , EPST (distance of excretory pore from anterior end / stylet length [females]) ratio, and vulval length (Salalia *et al.,*2017). Rice root-knot nematode produced characteristic symptoms, in the form of terminal hook shaped or spiral galls (Khan *et al.,* 2012). In general, plant-parasitic nematodes cause 21.3% yield losses adding up to Rs. 102,039.79 million (1.58 billion USD) every year. While, Rice root-knot nematode, *M. graminicola*, was economically most important causing yield losses of Rs. 23,272.32 million in rice (Kumar *et al*., 2020.). Because of the significance of their monetary effect, different management strategies have been emerging to control these plant-parasitic nematodes, such as application of live microbes (e.g., bacteria, fungi) and/or their secondary metabolites, etc. Bacteria eliciting the stimulation of plant defense mechanisms act as the most favorable implementation due to its higher efficiency than chemical pesticides or at least close to it. Moreover, bacterial application produces surplus positive effects on growth stimulation, increases yields and suppresses other pathogenic microorganisms. (Migunova and Sasanelli, 2021).

The soil bacteria that antagonistically colonize the rhizosphere directly or indirectly play an important role in promoting plant growth and development through the production and secretion of various chemicals called Rhizobacteria, plant growth promoters or Plant Growth Promotion Rhizobacteria (PGPR) (Ahemad and Kibret,2014; Raza *et a*l.,2016). Direct antagonistic effect can be achieved by parasitism, antibiosis, or competition for nutrients or infection sites. While indirectly , bacteria can enhance host defense mechanisms aggravating induced systemic resistance (Raymaekers *et al*., 2020). Number of bacterial species belonging to *Agrobacterium sp., Arthrobacter sp., Azotobacter sp., Clostridium sp., Desulfovibrio sp., Serratia sp., Burkholderia sp., Azospirillum sp., Bacillus sp.,* and *pseudomonas sp.* were described for management of nematodes (Khabbaz et al.,2019) through different mechanisms based on the

capability of microbes to compete effectively for ecological niche, colonize plant surface and produce nematicidal and antimicrobial compounds (antibiotics, toxins, siderophores, hydrolytic enzymes, etc.). Several experimentations have specified positive control of phytoparasitic nematode by bacteria, showing potential prospects for their application. *In vitro,* greenhouse and field experiments have revealed that bacteria can control the growth of ecto and endoparasitic nematodes by different mode of actions.

Materials and Methods

Juveniles of *M. graminicola* were raised on rice plants (var. Pusa 1121) in earthen pots filled with steam-sterilized sandy loam soil under screen house conditions. The brown-colored galls of the infected rice roots were taken and washed under running tap water. Galls were teased in water under stereoscopic microscope for the collection of eggs and freshly hatched larvae for testing of the nematicidal activity of cultures and cell free culture filtrates (CFCs) of rhizospheric bacteria against egg hatching of *M. graminicola* at two different dilutions i.e., S/2 and S/4.

The rhizobacterial strains were isolated from field soil naturally infested with *M. graminicola*. Soil samples were collected from the screen house, infested fields of rice growing villages of Fatehabad and Sirsa districts. Isolation of rhizobacterial strains were done through serial dilution method, ten gram of soil from the infested sample was mixed in 90 ml sterile water (10^{-1} dilution). One ml of this dilution was added to 9 ml water to make 10^{-2} dilution and similarly dilutions up to 10^{-6} was prepared. A 100 µl was poured on nutrient agar (NA) plate and incubated at 27 ^{o}C for 48-72 hrs. The individual colonies were remarked for isolation. Colonies were picked by sterile inoculation needle in laminar flow and was multiplied in more Petri-plates of nutrient agar. Around 16 rhizobacterial isolates were isolated and purified. The isolates were maintained on nutrient agar slants and stored in refrigerator at 4 ^{o}C. Then 100 ml nutrient broth were prepared in Conical flasks of 250 ml capacity. Spores from formed colonies of each isolate of rhizobacteria was inoculated in nutrient broth and incubated at 28 ^{o}C for 48-72 hrs. The growth of rhizobacterial culture was measured by taking optical density (OD) at 600 nm with ultraviolet (UV) spectrophotometer. Total 50 ml of culture broth was centrifuged at 10000 rpm for 10 minutes, the supernatant was collected in laminar flow under sterile condition in sterile glass vials by filter sterilization using bacteriological filters of 0.2 µm size. The CFCs were diluted to S/2 and S/4 concentrations by adding sterile water/nematode suspension and pH of culture filtrate was measured by using pH meter. two different dilutions S/2 (S/2 = 5ml Culture Filtrate + 5ml nematode suspension) and S/4(S/4= 2.5ml Culture Filtrate +7. 5ml nematode suspension). Ten ml of suspension containing approximately 100 freshly hatched juveniles (J2)

of rice root-knot nematode, *M. graminicola* was taken in tissue culture plates. Measured quantity of isolates CFCs was added to each tissue culture plate to make the resultant dilutions S/2 and S/4. Water and broth were also taken as check. Each dilution was replicated thrice. These tissue culture plates were kept at room temperature. Larval mortality after 48hrs exposure of the larvae was recorded by calculating immobilized second stage juveniles under stereoscopic binocular microscope. Per cent larval mortality was calculated and data obtained was subjected to angular transformation and evaluated by applying CRD factorial design. Similarly, one hundred eggs were placed along with respective bacterial isolate/dilution in tissue culture plates; the plates were kept in BOD at 25□C ±1□C. Each dilution is replicated three times. The number of juveniles hatched after 2, 4, 6, 8 and 10 days were calculated under stereoscopic binocular microscope. Water and Nutrient broth were taken as check. Mean larvae hatched was calculated.

Results and Discussion

The rhizobacterial strains were tested for inhibition of hatching of eggs of *M. graminicola*. The eggs were exposed to intact bacterial and culture filtrates of bacterial strains at two different concentrations i.e., S/2 and S/4. The data on egg hatching was recorded on alternate days up to tenth day. The results on hatching inhibition by different intact bacterial culture at concentration level S/2 are presented in Table 1. All the bacterial cultures in general reduced the hatching of *M. graminicola* as compared to the check. Maximum hatching inhibition was observed where nematode eggs were exposed to the intact bacterial culture of *Bacillus* sp followed by *Pseudomonas putida* and *P. fluorescens*. Irrespective of bacterial isolates maximum inhibition in hatching was observed after 48hrs of exposure. The rate of hatching was inversely proportional to the concentration of bacterial culturate. The hatching increased with the increase in exposure period and continued till tenth day. The maximum hatching was observed in sterile water. The nutrient broth which was kept as check for testing nematicidal properties showed that nutrient broth alone did not affect much on hatching inhibition. The interaction between exposure period and isolates was also found significant. Among the different exposure period, maximum egg hatching inhibition was observed after 4th day of incubation which was statistically at par with that of the 2nd day. Similarly, the effect on different CFCs on hatching behaviour of *M. graminicola* at S/2 concentration level was also tested and results are presented in Table.2. The rate of hatching was inversely proportional to concentration of the strains with all exposure period, hatching decreased with the increase in the concentration. Maximum and significant inhibition was observed in *Bacillus* sp. followed by to *P. putida* at S/2 concentration. After 48 hrs exposure

maximum inhibition in egg hatching was recorded irrespective of bacterial strains. Maximum hatching was recorded in sterile water (check) and nutrient broth alone irrespective of exposure period. The interaction between exposure period and bacterial isolates was also found significantly. Among the different exposure period, maximum egg hatching inhibition was detected after 2^{nd} day of incubation followed by 4^{th} and 10^{th} day. The effect of the different intact bacterial culture on rice root-knot nematode hatching behaviour at S/4 concentration level is presented in Table 3. The perusal of data indicated that the maximum hatching inhibition was observed in *Bacillus* sp. which was statistically at par with that the *P. rettgeri, vibrio* sp. and *P. putida (*81.5%) at S/4 concentration. Irrespective of bacterial isolates maximum hatching inhibition was recorded after 48 hrs exposure. The rate of hatching was inversely proportional to the concentration of bacterial isolates as it decreased with the increase in the concentration. Irrespective of exposure period maximum egg hatching was observed in sterile water and nutrient broth. The interaction between exposure period and isolates was also found significant. Among different exposure period, maximum egg inhibition was observed after 4^{th} day of incubation. After 4^{th} day of incubation egg hatching gradually decreased but continued till 10^{th} day of exposure. Perusal of data in Table 4 indicated that effects of the different CFCs on hatching behaviour of *M. graminicola* at S/4 concentration. The rate of egg hatching was inversely proportional to concentration of the strains at all exposure period as hatching decreased with the increase in the concentration. The results showed that in general all bacterial isolates were observed to cause significant inhibition in the egg hatching as compared to the untreated check. Maximum hatching inhibition was recorded in *P. rettigeri* followed by *vibrio* sp., *Bacillus sp*. and *P. putida* at S/4 after 48hrs of exposure period. Irrespective of exposure period maximum egg hatching was recorded in untreated check i.e., sterile water. The interaction between exposure period and isolates was found significant. Among the different exposure period, maximum egg inhibition was observed after 4^{th} day of incubation but continued till tenth day. Data revealed that in intact bacterial culture, maximum and significantly highest larval mortality i.e. 90.3% was observed, where *M.graminicola* larvae was exposed to *P. putida* followed by *P.rettgeri, Vibrio* sp.*,P. fluorescens* and *Bacillus* sp.in S/2 and S/4 concentration. All other intact bacterial culture significantly increased the juvenile mortality at both the concentrations as compared to untreated check where only mortality was observed. Among all the bacterial cultures, *P. putida* showed highest mortality effect at both concentrations and more mortality rate was found in S/2 concentration. All the other CFCs significantly increased the larval mortality at both the concentrations as compared to untreated check.

The results of the present studies also agreed with the findings of Kumar *et al.,* (2018) who found that the rhizobacterial cultures caused larval mortality at S/2 and S/4 concentrations in cultures and CFCs. The mortality of *M. graminicola* observed in the present studies might be due to production of antibiotics. The non-cellular extract exhibited high larvicidal properties. Nakayama *et al*. (1999) noted that PGPR antibiotics from culture filtrate or pure antibiotics had similar results. . The percentage mortality of juveniles increased along with increase in concentration of CFCs and exposure time. The results are in conformity with the results of Pankaj *et al*. (2011) who reported the effectiveness of *Bacillus* and *Pseudomonas* strains. The present study also agreed with the findings of Siddiqui and Mahmood (1999) that *M. incognita* egg hatching, juvenile mortality, and nematode reproduction were affected by the toxins produced in CFCs.

Table 1. Effect of native rhizobacterial strains (Intact bacteria) on egg hatching inhibition of *Meloidogyne graminicola* under *in vitro* conditions at S/2 concentration

Per cent egg inhibition at S/2 concentration (C)						
Isolates	**Exposure periods (T)**					
	2nd Day	**4th Day**	**6th Day**	**8th Day**	**10th Day**	**Mean**
Enterobacter cloacae	88.6(70.3)*	79.0(71.2)	73.3(69.7)	61.6(80.6)	48.3(75.5)	70.2(73.4)
Heterorhabiditis bacteriophora	89.6(68.5)	80.6(68.8)	75.3(74.2)	64.0(78.9)	51.6(77.6)	72.2(73.6)
Heterorhabiditis indica	88.0(69.7)	77.3(70.3)	71.3(69.4)	58.3(71.8)	44.0(70.3)	67.8(70.3)
Bacillus sp.	97.0(69.1)	92.0(68.3)	87.6(70.3)	80.0(72.2)	77.3(69.1)	86.8(69.8)
Pseudomonas flourscence	93.6(70.9)	90.0(71.2)	83.6(70.0)	78.0(72.2)	75.3(71.5)	84.1(71.2)
Gluconacetobacter diazotrophicus	86.6(70.0)	75.3(64.6)	68.3(69.1)	54.0(62.7)	38.3(63.9)	64.5(66.0)
Azotobacter chroococcum	87.0(61.5)	76.0(73.5)	69.6(71.5)	56.0(60.2)	40.6(60.6)	65.8(65.5)
Pseudomonas putida	92.6(68.8)	86.6(68.5)	85.3(65.1)	83.0(62.0)	77.6(62.7)	85.1(65.4)
Providencia rettigeri	96.3(61.1)	86.3(65.1)	80.6(62.7)	75.0(60.6)	72.6(59.7)	82.2(61.8)
Vibrio sp.	95.3(63.1)	82.3(65.6)	74.3(61.3)	74.6(63.9)	68.3(64.3)	79.0(63.6)
RbMg-101	88.0(62.4)	78.0(66.1)	72.0(64.6)	59.6(62.7)	45.6(54.3)	68.6(62.0)
RbMg-102	88.6(61.5)	79.0(58.9)	73.0(60.2)	61.0(57.6)	47.6(69.5)	69.8(61.5)
RbMg -103	87.6(66.2)	76.6(55.7)	70.6(56.5)	57.6(67.5)	49.3(63.9)	68.4(62.0)
RbMg -104	90.3(59.7)	82.3(58.0)	77.0(58.6)	67.3(57.1)	56.0(61.3)	74.6(59.0)
RbMg -105	88.6(58.9)	79.0(57.1)	73.3(55.5)	61.3(59.1)	48.0(62.2)	70.0(58.6)
RbMg -106	87.3(57.2)	76.0(60.2)	70.6(60.4)	56.3(58.4)	41.0(62.7)	66.2(59.8)
RbMg -107	86.3(61.1)	74.6(58.6)	68.0(48.8)	53.3(57.1)	37.0(51.7)	63.2(55.5)
RbMg -108	88.6(53.1)	79.6(49.7)	73.6(63.4)	62.0(62.0)	49.0(47.2)	70.6(55.1)
RbMg -109	90.6(48.4)	83.0(65.6)	78.3(60.0)	69.0(59.7)	58.3(50.5)	75.8(56.9)
RbMg -110	87.3(51.3)	77.0(49.3)	70.6(55.1)	57.3(51.5)	42.6(48.6)	67.0(51.2)
RbMg -111	89.3(46.9)	80.6(51.9)	75.3(56.1)	64.0(49.2)	51.6(53.1)	72.2(51.4)
RbMg -112	89.6(53.7)	81.3(51.1)	75.6(56.6)	65.0(54.3)	53.0(51.3)	72.9(53.4)
RbMg -113	88.3(37.4)	78.6(49.4)	72.6(44.0)	60.6(45.9)	47.0(41.5)	69.4(43.6)
RbMg -114	90.6(61.5)	83.6(60.2)	79.0(38.2)	69.6(39.6)	59.3(61.7)	76.4(52.2)
RbMg -115	90.0(58.5)	81.6(55.7)	76.6(42.4)	66.0(43.6)	54.3(44.6)	73.7(49.0)
RbMg -116	88.3(48.4)	79.0(43.8)	73.0(39.8)	61.0(37.4)	47.3(44.4)	69.7(42.7)
Sterile Water	81.6(49.8)	66.0(40.7)	56.6(45.9)	37.0(46.7)	15.6(43.2)	51.4(45.2)
NB	87.3(50.3)	77.3(47.4)	70.6(43.4)	57.6(22.5)	42.6(40.7)	67.7(40.9)
Mean	(59.3)	(59.5)	(58.3)	(57.7)	(58.1)	
CD at 5%						
Isolates			(1.7)			
Exposure periods			(0.7)			
Isolates×Exposure periods			(3.8)			

* Figures in parenthesis are angular transformed values

Table 2. Effect of native rhizobacterial strains (culture filtrate) on egg hatching inhibition of *Meloidogyne graminicola* under *in vitro* conditions at S/2 concentration

Per cent egg inhibition at S/2 concentration (C)						
Isolates	**Exposure periods (T)**					
	2nd Day	**4th Day**	**6th Day**	**8th Day**	**10th Day**	**Mean**
Enterobacter cloacae	89.0(70.6)*	79.0(71.2)	73.3(69.7)	61.6 (80.2)	41.6(73.7)	68.9(73.1)
Heterorhabiditis bacteriophora	89.6(68.5)	80.6(69.1)	75.3(78.7)	64.0 (77.5)	45.6(78.6)	71.0(74.5)
Heterorhabiditis indica	88.0(69.7)	77.3(70.6)	71.3(36.4)	58.6 (71.8)	36.3(67.5)	66.3(63.2)
Bacillus sp.	96.6(69.1)	92.6(68.3)	85.3(70.6)	76.6 (72.2)	73.0(69.1)	84.8(69.8)
Pseudomonas flourscence	92.0(70.9)	88.0(71.5)	80.0(70.0)	73.3(72.8)	70.0(71.5)	80.6(71.4)
Gluconacetobacter diazotrophicus	86.6(70.0)	75.3(64.8)	68.3(69.4)	54.3(62.7)	30.0(63.9)	62.9(66.2)
Azotobacter chroococcum	87.3(61.5)	76.0(74.9)	69.6(69.7)	56.0(60.2)	32.6(60.6)	64.3(65.4)
Pseudomonas putida	95.6(66.6)	84.0(66.1)	82.6 (61.7)	79.3 (62.0)	73.3(62.7)	83.0(63.8)
Providencia rettigeri	95.3(28.8)	83.3(65.1)	76.3 (62.7)	69.3 (60.6)	66.3(59.7)	78.1(55.4)
Vibrio sp.	96.0(63.1)	77.6(65.6)	67.3 (61.3)	68.0 (63.9)	59.6(64.3)	73.7(63.6)
RbMg-101	88.0(62.4)	78.0(66.1)	72.0 (64.6)	59.6 (62.7)	38.6(54.3)	67.2(62.0)
RbMg-102	89.0(61.5)	79.0(58.9)	73.0(60.2)	61.0(57.6)	40.3(67.5)	68.4(61.1)
RbMg -103	37.0(63.4)	23.3(55.7)	29.3(56.5)	42.3(65.4)	65.0(60.9)	39.4(60.4)
RbMg -104	90.3(55.3)	82.3(58.0)	77.0(58.6)	67.3(32.7)	49.6(61.3)	73.3(53.2)
RbMg -105	85.3(58.9)	79.0(56.7)	73.3(55.5)	61.3 (59.1)	42.0(62.2)	68.2(58.5)
RbMg -106	87.3(57.2)	76.0(60.2)	70.0(60.4)	56.3(58.4)	33.0(62.7)	64.5(59.8)
RbMg -107	86.3(61.1)	74.6(58.6)	68.0(49.0)	53.3(57.1)	28.3(51.7)	62.1(55.5)
RbMg -108	89.0(53.1)	79.6(49.9)	73.6(61.1)	62.0(58.9)	41.6(47.4)	69.2(54.1)
RbMg -109	90.6(48.4)	83.0(62.9)	78.3(56.4)	69.0(55.5)	52.0(50.5)	74.6(54.7)
RbMg -110	87.3(51.3)	77.0(40.5)	70.6(55.1)	57.3(51.5)	35.6(48.6)	65.6(49.4)
RbMg -111	89.3(46.9)	80.6(51.9)	75.3(56.1)	64.3(49.2)	45.3(53.3)	71.0(51.5)
RbMg -112	90.0(53.7)	81.3(51.1)	75.6(56.8)	65.0(54.3)	46.0(51.3)	71.6(53.4)
RbMg -113	88.3(37.5)	78.6(49.4)	72.6(40.1)	60.6(42.4)	40.3(36.6)	68.1(41.2)
RbMg -114	91.3(58.7)	83.6(56.7)	79.0(32.6)	70.0(34.4)	54.0(58.9)	75.6(48.3)
RbMg -115	90.0(54.5)	81.6(50.5)	76.6(38.3)	66.0(39.1)	49.3(53.9)	72.7(47.3)
RbMg -116	88.3(44.7)	79.0(40.3)	73.0(34.6)	61.0(31.1)	40.3(39.9)	68.3(38.1)
Sterile Water	82.0(46.1)	66.0(36.5)	57.0(42.2)	37.3(42.5)	8.6(39.3)	50.2(41.3)
NB	87.6(47.3)	77.3(44.6)	70.6(39.2)	57.6(15.5)	34.3(35.0)	65.5(36.3)
Mean (C×T)	(57.2)	(58.4)	(56.0)	(55.4)	(57.4)	
CD at 5%						
Isolates			(3.2)			
Exposure periods			(1.3)			
Isolates×Exposure periods			(7.3)			

* Figures in parenthesis are angular transformed values

Table 3. Effect of native rhizobacterial strains (Intact bacteria) on egg hatching inhibition of *Meloidogyne graminicola* under *in vitro* conditions at S/4 concentration

Per cent egg inhibition at S/4 concentration (C)						
Isolates	**Exposure periods (T)**					
	2nd Day	**4th Day**	**6th Day**	**8th Day**	**10th Day**	**Mean**
Enterobacter cloacae	86.0(68.0)	74.3(68.5)	67.3(67.1)	52.6(80.2)	36.0(73.7)	63.2(71.5)
Heterorhabiditis bacteriophora	86.6(65.8)	76.3(66.4)	69.3(72.5)	55.3(77.5)	40.0(76.2)	65.5(71.7)
Heterorhabiditis indica	85.0(67.1)	72.3(68.0)	64.3(66.9)	48.6(69.7)	30.6(67.7)	60.2(67.9)
Bacillus sp.	96.6(66.6)	90.0(65.65)	84.6(68.2)	75.3(70.3)	72.0(67.4)	83.7(67.6)
Pseudomonas flourscence	92.0(68.8)	87.6(68.8)	80.0(67.4)	73.0(70.6)	69.3(69.4)	80.4(69.0)
Gluconacetobacter diazotrophicus	83.3(67.7)	69.3(61.5)	61.0(66.9)	43.6(59.5)	23.67(60.9)	56.2(63.3)
Azotobacter chroococcum	84.0(58.2)	70.6(71.5)	62.6(69.4)	45.6(56.3)	26.3(57.1)	57.8(62.5)
Pseudomonas putida	91.0(66.1)	83.3(68.3)	81.6(70.6)	79.3(58.6)	72.3(59.3)	81.5(64.6)
Providencia rettgeri	95.3(57.8)	86.3(62.0)	82.6(59.5)	74.6(48.7)	74.3(56.1)	82.6(56.8)
Vibrio sp.	94.3(59.7)	89.0(62.7)	80.6(57.8)	74.0(60.8)	71.3(61.1)	81.8(60.4)
RbMg-101	85.0(59.1)	73.0(63.2)	65.6(61.5)	50.0(59.3)	32.6(49.6)	61.2(58.5)
RbMg-102	86.0(32.1)	74.0(55.1)	66.67(56.3)	52.0(53.3)	34.6(67.0)	62.2(52.8)
RbMg -103	84.6(63.4)	71.6(51.3)	63.6(52.3)	47.3(64.6)	29.0(65.5)	59.2(59.4)
RbMg -104	88.0(63.9)	78.0(54.1)	72.0(54.7)	59.3(52.9)	45.3(58.0)	68.5(56.7)
RbMg -105	85.6(55.1)	74.3(52.3)	67.3(50.7)	52.0(55.1)	35.3(57.8)	62.9(54.2)
RbMg -106	84.3(52.9)	56.3(56.1)	62.6(56.7)	46.0(54.5)	27.0(59.3)	55.2(55.9)
RbMg -107	83.0(57.2)	69.0(54.5)	60.0(42.8)	42.3(37.0)	31.0(46.5)	57.0(47.6)
RbMg -108	86.3(48.0)	74.6(44.2)	67.3(60.2)	53.0(58.7)	36.3(41.3)	63.5(50.5)
RbMg -109	88.6(42.5)	79.0(62.9)	71.6(59.7)	61.6(59.3)	48.0(44.9)	69.8(53.9)
RbMg -110	85.3(46.1)	71.6(43.4)	63.6(50.3)	47.3(46.1)	29.0(42.6)	59.4(45.7)
RbMg -111	87.0(40.5)	76.3(46.7)	69.0(51.7)	55.6(43.4)	40.0(48.2)	65.6(46.1)
RbMg -112	87.0(48.8)	76.6(45.7)	70.0(52.3)	56.6(49.6)	41.3(45.5)	66.3(48.4)
RbMg -113	85.3(28.1)	73.6(46.5)	66.3(36.6)	51.3(39.1)	34.3(33.6)	62.2(36.8)
RbMg -114	89.0(58.0)	79.6(56.3)	74.0(29.0)	62.6(30.8)	49.3(58.2)	70.9(46.5)
RbMg -115	87.6(59.5)	77.3(57.6)	70.6(34.7)	58.0(36.0)	43.3(32.5)	67.4(44.1)
RbMg -116	85.6(42.2)	74.0(36.3)	66.3(31.2)	51.0(33.6)	34.67(37.0)	62.3(36.1)
Sterile Water	77.3(43.8)	58.0(32.4)	46.3(39.1)	22.6(39.9)	8.6(35.7)	42.6(38.2)
NB	84.6(44.5)	28.3(41.1)	36.3(36.0)	52.6(16.8)	70.6(57.2)	54.5(39.1)
Mean (C×T)	(54.7)	(55.8)	(54.4)	(52.9)	(54.6)	
CD at 5%						
Isolates			(2.0)			
Exposure periods			(0.8)			
Isolates×Exposure periods			(4.6)			

* Figures in parenthesis are angular transformed values

Table 4. Effect of native rhizobacterial strains (culture filtrate) on egg hatching inhibition of *Meloidogyne graminicola* under *in vitro* conditions at S/4 concentration

Per cent egg inhibition at S/4 concentration (C)						
Isolates	**Exposure periods (T)**					
	2nd Day	**4th Day**	**6th Day**	**8th Day**	**10th Day**	**Mean**
Enterobacter cloacae	85.6(67.7)	73.0(68.3)	65.6(66.4)	50.6(78.1)	24.3(72.0)	59.8(70.5)
Heterorhabiditis bacteriophora	86.3(65.1)	74.3(65.8)	67.6(70.3)	53.3(76.2)	29.6(74.0)	62.2(70.3)
Heterorhabiditis indica	84.0(66.9)	70.6(67.1)	62.6(66.4)	46.0(69.4)	22.0(67.5)	57.0(67.4)
Bacillus sp.	95.3(65.8)	87.6(65.1)	81.3(67.4)	69.3(70.0)	65.6(66.4)	79.8(66.9)
Pseudomonas flourscence	90.33(68.2)	84.3(68.5)	74.3(67.4)	65.3(70.0)	61.0(68.8)	75.0(68.6)
Gluconacetobacter diazotrophicus	82.3(67.1)	68.0(60.8)	59.0(66.4)	40.6(58.7)	16.0(59.5)	53.2(62.5)
Azotobacter chroococcum	83.3(57.1)	69.0(69.4)	60.6(66.6)	43.0(55.5)	18.6(56.1)	54.9(60.9)
Pseudomonas putida	88.6(65.1)	82.3(67.8)	77.3(70.6)	73.0(57.8)	70.6(58.4)	78.4(63.9)
Providencia rettgeri	94.3(56.7)	85.6(61.3)	79.6(58.5)	76.6(56.1)	72.6(54.9)	81.8(57.5)
Vibrio sp.	92.3(58.8)	89.0(62.2)	77.0(56.7)	76.3(59.5)	72.0(60.2)	81.3(59.5)
RbMg-101	84.6(58.2)	71.6(62.5)	64.0(60.7)	47.6(58.2)	20.6(48.4)	57.7(57.6)
RbMg-102	85.0(56.9)	72.6(54.1)	65.0(55.3)	49.6(52.3)	22.6(64.4)	59.0(56.6)
RbMg -103	84.0(59.6)	70.0(50.1)	61.6(51.1)	45.0(61.5)	21.0(63.1)	56.3(57.1)
3RbMg -104	87.6(61.6)	77.0(53.1)	71.0(53.7)	57.6(51.7)	34.6(57.4)	65.6(55.5)
RbMg -105	85.3(53.9)	72.6(51.1)	65.3(49.5)	49.6(54.3)	25.0(58.0)	59.6(53.4)
RbMg -106	83.3(51.7)	69.0(55.5)	60.6(55.9)	43.3(53.5)	23.3(58.7)	55.9(55.1)
RbMg -107	82.3(56.6)	67.0(53.7)	58.0(41.3)	39.6(51.9)	16.6(45.3)	52.7(49.7)
RbMg -108	85.3(46.9)	73.3(42.6)	66.0(56.4)	51.0(53.9)	27.6(39.6)	60.6(47.9)
RbMg -109	88.3(40.9)	78.3(58.6)	72.0(61.1)	60.0(60.9)	37.6(43.6)	67.2(53.0)
RbMg -110	84.0(44.7)	70.0(42.1)	61.6(49.4)	45.0(44.7)	20.0(41.1)	56.1(44.4)
RbMg -111	86.3(38.9)	74.3(45.5)	68.0(50.7)	53.6(42.1)	29.0(47.0)	62.2(44.9)
RbMg -112	86.6(47.6)	75.3(44.4)	68.6(51.3)	54.6(48.4)	33.3(44.6)	63.7(47.3)
RbMg -113	85.3(25.4)	72.3(42.1)	64.6(28.8)	49.0(32.7)	26.0(27.3)	59.4(31.2)
RbMg -114	88.3(54.1)	78.6(51.3)	73.0(23.1)	61.0(25.2)	40.3(57.1)	68.2(42.2)
RbMg -115	87.0(58.4)	76.0(58.0)	69.6(25.9)	56.0(26.0)	34.0(27.0)	64.5(39.1)
RbMg -116	85.0(35.5)	72.3(29.8)	65.0(28.7)	49.3(23.6)	22.6(30.9)	58.8(29.7)
Sterile Water	76.3(37.3)	56.0(26.3)	43.6(32.1)	19.0(34.9)	10.6(30.5)	41.1(32.2)
NB	84.0(39.2)	70.3(35.6)	62.0(27.2)	45.0(18.1)	28.6(32.3)	58.0(30.5)
Mean (C×T)	(53.8)	(54.07)	(52.1)	(51.6)	(51.9)	
CD at 5%						
Isolates			(2.8)			
Exposure periods			(1.2)			
Isolates×Exposure periods			(6.3)			

* Figures in parenthesis are angular transformed values

Effects of the different intact bacterial culture and CFCs on hatching behavior of rice root-knot nematode at different concentration level i.e., S/2 and S/4 and showed that all bacterial culture significantly inhibited the egg hatching as compared to the untreated check. Maximum inhibition was observed in *Bacillus* sp. followed by *P. putida* and *P. fluorescens* at S/2 concentration, after 48hrs exposure. The rate of hatching was inversely proportional to concentration of the strains at all exposure periods. Reducing the concentration of the CFCs might have diluted the quantity of toxic metabolites present exhibiting positive influence on egg hatching of *M. graminicola*. Irrespective of exposure period maximum egg hatching was observed in sterile water. The interaction between exposure period and isolates significantly inhibit the egg hatching. Sharma *et al.* (1998) observed that delayed nematode egg hatch of *Meloidogyne* spp. due to culture supernatants of *Pseudomonas* spp. Chitinase is an essential element of nematode egg shells. CFCs of Pseudomonas bacterial strains show nematicidal activity which destroys the J2 of *M. javanica*. Unboiled culture filtrates showed nematicidal activity affecting juveniles 67 and 82% at 24 and 48 hrs of incubation, while boiled culture filtrates lost nematicidal activity (Nasima *et al*., 2002). Bansal and Bajaj (2003) also observed that, *G.diazotrophicus* produces the volatile fatty acids which are known to interrupt the normal nematodes developments likewise these organic acids also reduces egg hatching by damaging embryogenesis o*f M.incognita* Seenivasan *et al.* (2012) revealed that CFCs of PGPR strains PF1, TDK1, and PY15 of *P. fluorescens* showed the similar type of results against rice root-knot nematode under *in vitro* conditions. Present studies also agreed with findings of Kumar *et al.* (2018) that *Bacillus* strain inhibit the egg hatching rate of *M. graminicola* populations can be affected by different mode of actions of the rhizobacteria those are competition for essential nutrients, ISR, promoting plant growth, interfering with plant-nematode recognition, antagonist by producing enzymes, toxins and other metabolic by-products (Mendoza *et al.,* 2008; Tian *et al.,* 2007). *Pseudomonas* spp. were effective in bio-control of root-knot disease of pea by producing a wide-ranging antibiotic, growth promoting hormones, HCN, siderophores and phosphorous solubilized (Siddiqui *et al.,* 2009). *P. fluorescens* prompted the defense activity of correlated enzymes, like chitinase which is a hydrolytic enzyme that degrades chitin (a polymer of b-1, 4-linked N-acetylglucosa-mine) and peroxidase, in rice roots (Seenivasan *et al.,*2012). They observed that exposure to a 250-ppm aqueous solution of these volatile fatty acids completely suppressed egg hatching within 48hrs. Similar results have been found in our *in vitro* studies using CFCs obtained from bacterial strains*. Bacillus* spp. considerably produces volatile organic chemicals that are toxic to *M. graminicola* juveniles was observed by Aeron *et al.,* 2020). The 17 rhizobacteria isolates were evaluated by Sri Sudewi et al. in 2021, Three isolates were positively creating hydrogen

cyanide, and other isolates were capable of producing indole-3-acetic acid, which led to a considerable increase in germination compared to the control. Kumar *et al*. in 2020 showed that, the larval mortality of Meloidogyne graminicola, was significantly increased by all of the tested bioagents, including *Bacillus subtilis, Pseudomonas fluorescens, Paecilomyces lilacinus*, and two isolates of Trichoderma (Trichoderma isolates S13 and S7). A minimum (48.33%) percentage of eggs hatched when the investigated bioagents were diluted to 50% Under in vitro conditions.

Conclusion:

PPNs cause extensive damage to crop agroecosystems, resulting in severe yield losses; to control these pests, numerous synthetic pesticides are used for population reduction. Such pesticides have a significant impact on population reduction while also polluting the soil environment. To counteract the effects of pesticides, the use of biological control agents may be the best option a better alternative. Plant growth-promoting rhizoshperic bacteria (PGPR) are capable of acting as a biological control agent against plant parasitic nematodes as well as stimulating plant growth. At both dilutions, S/2 and S/4, all bacterial cultures (intact and culture filtrates) hindered egg hatching *in vitro* and throughout the entire exposure time When compared to the untreated control, *Bacillus sp*. caused the most egg hatching inhibition at S/2 and S/4 after 48 hours of treatment.

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