The Pharma Innovation Journal 2022; 11(4): 1795-1798

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(4): 1795-1798

www.thepharmajournal.com
Received: 18-01-2022
Accepted: 28-02-2022

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Evaluation of different metabolites and native isolate of *Trichoderma* on *Meloidogyne graminicola* causing root knot disease in basmati rice

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Abstract
In the present investigation, an experiment was conducted to test the efficacy of metabolites (culture filtrate) of *Trichoderma* isolate S_{32} at different concentrations *viz*, 100%, 75%, 25%, 10% and 5% on larval mortality and egg hatching of *M. graminicola*. Among different concentrations, maximum (80%) larval mortality and minimum (18.33%) egg hatching was recorded at 100% concentration after 72 hours of inoculation. Minimum (15.00%) larval mortality and maximum (30%) egg hatching was recorded at 5% concentration whereas, in case of untreated control (5%) larval mortality and (70%) egg hatching was recorded after 72 hours of inoculation.

Keywords: *Trichoderma* isolate, culture filtrate, larval mortality, egg hatching etc.

Introduction
Rice (*Oryza sativa* L.) belongs to family Poaceae, is the most important cereal crop of kharif season. Basmati rice is a unique product of Indo-Gangetic planes of Himalaya, having unmatched quality characters and aroma which makes it an important export commodity among food grains exported from India. India is one of the top exporters of rice, both basmati and non-basmati rice, and the annual Indian basmati rice exports around 4.63 lac MT. India exports basmati rice to almost 132 countries across the world every year and the major export destinations for Indian basmati rice are Iran, Saudi Arabia, UAE and Iraq. (APEDA 2020-21) [2]. Basmati rice production is affected by biotic and abiotic stresses. In biotic stresses various pathogens i.e., nematodes, fungi, bacteria and viruses etc. affect basmati rice crop adversely. Among the diseases, root knot nematode is an important problem of rice. Nematode problem is increasing very fast in areas where farmers are continuously following rice-wheat cropping system. Basmati variety PB-1121, which is most popular among farmers is most susceptible for this disease (Khilari *et al.*, 2011) [8]. Rice root-knot nematode (*M. graminicola*) having established endoparasitic pest of nurseries and main crop. The main characteristic symptoms produced by *M. graminicola* are terminal hook shaped or spiral galls on the roots and other symptoms of damage include patches of stunted and yellowish plants. Presence of root galls and reduced root system ultimately causes significant decline in plant growth and grain yield (Khan *et al.*, 2012) [7]. The high population density of *M. graminicola* caused wilting of seedlings along with severe reduction in growth parameters while low population caused only reduction in growth parameters. Root-knot nematode alone is capable of causing up to 50% yield losses in rice in many production regions (Lorenzana *et al.*, 1998) [9].
It is therefore vital to control the nematode population of *Meloidogyne* spp. in farms to prevent the occurrence of a global food crisis. Due to the high economic impact of root-knot nematodes, companies, researchers, and farmers have developed several strategies for controlling them (Stirling, *et al.*, 1999) [16].
One strategy involves the use of chemical nematicides. However, these chemicals are detrimental to human health and pollute the environment, authorities and other concerned organizations increasingly discourage their application (Poveda, *et al.*, 2020) [13]. Biocontrol provides a long-term solution to crop pests, reducing the costs required for pest control on the farm. Additionally, the biocontrol agents do not cause environmental pollution; therefore, their application does not harm other organisms and humans in the environment (Admin, 2020) [1].

Further, unlike chemicals, pests do not develop resistance to biological control agents. Moreover, bioagents are specific to the target organism; therefore, they do not destroy other beneficial organisms in the process (Admin, 2020) [1].

It is possible to control root-knot nematodes at different stages of their lifecycle; therefore, the farmer can apply the biocontrol agent depending on the nematode stage and the agent's effectiveness at that stage (Bernard, *et al.,* 2017) [4]. *Meloidogyne* species have been successfully suppressed by using several biological control agents (Murslain *et al.,* 2014; Muhaeud-ud- Din *et al.,* 2018; Haque *et al.,* 2018) [12,11,6]. The fungal biocontrol agents, *Trichoderma* spp. promotes plant growth and has the ability to colonize root surfaces and cortex. Various mechanisms suggested for the biocontrol activity of *Trichoderma* spp. against phyto nematodes are antibiosis and enzymatic hydrolysis. All mechanisms, except competition, might potentially are involved in the nematode biocontrol process.

Material and Method

Preparation of culture filtrates of bio agent

The effect of culture filtrate of bio-agents on larval mortality and egg hatching were tested in-vitro. For obtaining the culture filtrates of bio-agent *Trichoderma* isolate S_{32} was cultured on Potato dextrose broth medium. Five mm disc of *Trichoderma* isolate S_{32} was used to inoculate the flasks containing 200 ml of Potato dextrose broth (PDB). The inoculated flasks were incubated at 26 ± 2 °C temperature in incubator shaker. After one week of incubation, cultures of *Trichoderma* isolates S_{32} filtered through Whatman No.1 filter paper. Filtrate was used in different concentration to see the inhibition effect on J_2 mortality as well as on egg hatching. Sterilized distilled water was used as a control.

Collection of juveniles (J_2)

The infected rice roots having galls developed by *Meloidogyne graminicola* were collected. The uprooted rice plant roots were washed under running tap water. Then galls were separated from the root, transferred to the watch glass and were teased with the help of needle. The crushed root galls with water suspension were observed in sterobinocular microscope for the confirmation that the galls contain eggs and juvenile stage of *M. graminicola.* The J_2 stage of *M. graminicola* were separated by spreading the suspension on to a double layer tissue paper placed over wire gauze and then submerged into water in petri plates.

Collection of eggs

Eggs were collected from the rice plants maintained as pure culture. The infected roots were dissected with a sterilized dissecting needle and egg were picked up from the galled root with help of dropper. The picked eggs were kept in sterilized cavity block containing sterilized water.

Mortality test

To study the effect of culture filtrates of *Trichoderma* on larval mortality, different concentrations (100, 75, 50, 25, 10 and 5%) of culture filtrate were prepared and separately poured into 5 cm petri dishes. Three replications was maintained for each treatment and control were maintained by adding 10 ml of sterilized distilled water only. All these petri plates were added by l ml suspension containing 20 freshly hatched J_2 of *M. graminicola* with the help of a flat tipped picking dropper. Petri plates were incubated at room temperature. Observations on larval mortality were recorded after 24, 48 and 72 hours of inoculation with the help of stereomicroscope. Per centage of larval mortality was calculated by following formula given by Ahmad *et al.* (2004) [3].

$$\text{Per cent larval mortalit} = \frac{\text{Total number of larvae killed}}{\text{Total number of larvae inoculated}} \text{X100}$$

Egg hatching test

To study the effect of *Trichoderma* culture filtrate on nematode egg hatching, different concentrations (100, 75, 50, 25, 10 and 5%) of culture filtrate were prepared and separately poured into 5 cm petri plates (3 replications). Control petri plates were maintained by adding 10 ml of sterilized distilled water only. All these petri plates were added by 1 ml suspension containing 20 fresh eggs of *M. graminicola* with the help of a flat tipped picking dropper. These petri plates were incubated at room temperature.

Observations on egg hatching were recorded at 24, 48 and 72 hours. Per cent egg hatching was calculated by using formula given by Ravichandra (2010) [14].

$$\text{Hatching percentage} = \frac{\text{No. of hatched juveniles}}{\text{No. of hatched} + \text{Unhatched egg}} \times 100$$

Results and Discussion

Effect of culture filtrate of *Trichoderma* on J_2 of *M. graminicola*

In this experiment maximum larval mortality 30% was recorded at 100% concentration at 24 hours of inoculation. Whereas at 100, 75, 50, 25, 10 and 5% concentration of culture filtrate to larval mortality was 80%, 61.65%, 40%, 25%, 18.35% and 15% respectively. In case of control 5% J_2 mortality was recorded at 72 hours of inoculation. Results clearly indicates that *Trichoderma* isolate S_{32} has inhibitory effect on nematode larvae. On the basis of results of several researchers it is already proved that *Trichoderma* spp. produced several metabolites which was nematicidal effect on the larvae of root knot nematode (*M. graminicola*). The experiment was conducted to observe the effect of secondary metabolites produced by the tested bioagent on rice root knot nematodes egg hatching and nematode larvae mortality. The tested bioagent is soil borne and pathogen rice root knot nematode also exclusively soil borne in nature. Antibiosis is one of the important mechanisms of biological control. Therefore, if a bioagent is good metabolite producer that have inhibitory effect on pathogen, then this bio agent may be certainly work as a effective bioagent. To test the antibiosis effect of selected native isolate of the *Trichoderma* S_{32} the present experiment was conducted.

Siddiqui *et al.* (2001) [15] reported *Trichoderma* species caused significant effect on larval death of *M. javanica* and also found that culture filtrates of *T. harzianum* at 48 h caused 41% mortality of *M. javanica* compared to *T. viride* (31%). Similar results was also reporter by Hanwai (2016) [5] he reported that mortality of juveniles of *M. javanica* was affected significantly by the concentrations and exposure time in all treatments. They referred that Per cent juvenile mortality in the culture filtrates of the fungi and plant extract was directly proportional to concentration of the culture filtrates, plant extract and the duration of exposure. Maximum mortality (34.8%) was observed in 100% concentration of the

fungal filtrate of *P. lilacinus* after 48 hours of exposure whereas it was minimum (5.3%) in 25% concentration of the fungal filtrate of *P. lilacinus*, *T. hamatum* after 24 hours of inoculation.

Table 1: Effect of culture filtrate of *Trichoderma* isolate S_{32} on larval mortality of *M. graminicola*

Treatment Details (Filtrate concentrations)	at 24 Hours		at 48 hours		at 72 hours	
	Number of dead J_2	% Mortality	Number of dead J_2	% Mortality	Number of dead J_2	% Mortality
T1- 100%	6.00	30.00	9.00	45.00	16.00	80.00
T2- 75%	3.33	16.65	6.00	30.00	12.33	61.65
T3- 50%	2.67	13.33	4.33	21.65	8.00	40.00
T4- 25%	2.33	11.67	3.33	16.65	5.00	25.00
T5- 10%	2.00	10.00	2.67	13.35	3.67	18.35
T6- 5%	1.33	6.67	2.00	10.00	3.00	15.00
T7- Control	0.33	1.67	1.00	5.00	1.00	5.00
CD (5%)	1.280	-	0.611	-	1.158	-

Effect of *Trichoderma* on egg hatching of *M. graminicola*

The results obtained in this study clearly indicates that the metabolites of bioagents have inhibitory effect on the egg hatching of *M. graminicola*. After 24 hours of inoculation minimum egg hatching 8.33% was recorded at 100% and 75% concentration. In case of control maximum egg hatching 31.67% was recorded at 24 hours. After 48 hours of inoculation minimum egg hatching 11.67% was recorded at 100 and 75% concentration. After 72 hours of inoculation minimum egg hatching 18.33% was recorded at 100% concentration. Inhibitory effect of secondary metabolites of bioagents on nematode egg hatching may help in management of nematode population in soil.

Mahfouz *et al.* (2010) [10] evaluated efficacy of *Trichoderma* isolates against root knot nematode of rice in tomato cultivar and found that three fungal isolates (f_1, f_2, f_3) of *Trichoderma* inhibited egg hatching (20%, 27% and 31%) and J_2 mortility (59%, 62% and 61%) of *Meloidogyne incognita*. The f_1 isolate has the highest influence at 7 days of inoculation. The percentage of J_2 immobility increased with increase of the exposure period. Similar results were obtained by Hanawi (2016) [5] he found maximum per cent of unhatched eggs (70.3%) at 100% concentration of the filtrate of *P. lilacinus* while it was 67.9%, 54.4% in the treatment of *T. erecta* extract and *T. hamatum* filtrate respectively at the same concentration.

Table 2: Effect of culture filtrate of *Trichoderma* isolate S_{32} on egg hatching of *M. graminicola*.

Treatment Details (Filtrate concentrations)	at 24 Hours		at 48 hours		at 72 hours	
	Number of egg hatched	% egg hatching	Number of egg hatched	% egg hatching	Number of egg hatched	% egg hatching
T1- 100%	1.67	8.33	2.33	11.67	3.67	18.33
T2- 75%	1.67	8.33	2.33	11.67	4.00	20.00
T3- 50%	2.33	11.67	3.00	15.00	4.67	23.33
T4- 25%	3.00	15.00	3.33	16.67	5.00	25.00
T5- 10%	3.00	15.00	4.33	21.67	5.67	28.33
T6- 5%	3.33	16.67	5.00	25.00	6.00	30.00
T7- Control	6.33	31.67	9.00	45.00	14.00	70.00
CD (5%)	1.091	-	0.945	-	1.158	-

Conclusion

Based on the results of present investigation, it can be concluded that *Trichoderma* isolate S_{32} is effective in increasing larval morality and in reducing egg hatching of *M. graminicola.* It indicated that the presence of this isolate of *Trichoderma* in the soil may be helpful as a bio agent in the management of rice root knot nematode.

Acknowledgment

The author is grateful for the financial support granted by the DST/Inspire fellowship, Department of Science and Technology, Government of India, New Delhi for conduction of research on “Biological control of root knot nematode by using native isolates of *Trichoderma*” along with this the author also wishes to thank HOD, Department of Plant Pathology, S.V.P. University of Agriculture and Technology, Meerut-250110 (U. P.), for providing need-based facilities for conducting this research work.

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