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Evaluation of Total Phenol Content of some Common Paddy Cultivars of Odisha with the Application of Biofertilizer in Assessing Resistance to *Meloidogyne graminicola*

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The total phenol content of four common paddy cultivars of Odisha namely Abhisek (R), Manik (MR), Bas-12 (S) and Lalat (HS) were studied under pot culture condition in the net house following CRD design under different treatments using biofertilizer A*zospirillum. brasilense* alone or in combinations against rice root-knot nematode *Meloidogyne graminicola*. The objective of the work was to study the quantitative changes in phenolic compounds content and the effect of *A.brasilense* in the induction of phenolic compounds on the test nematode. It was observed that there was a significant increase in total phenol content in both resistant and moderately resistant paddy cultivars Abhisek and Manik in treatment T_2 (*Azospirillum brasilense* @ 12kg/ha at 15DAT +

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M. graminicola @ 1000J2 after 7days of *Azospirillum* inoculation) were seen higher than the susceptible variety Bas-12 and highly susceptible variety Lalat as compared to control T_5 with increase percentage of 38.77%, 36.58%, 35.02% and 39.28% respectively in shoots. Similarly the trend was continued in roots containing total phenol content of 0.25mg/g in Abhisek, 0.18mg/g in Manik, 0.21mg/g in Bas-12 and 0.19mg/g in Lalat as compared to other treatments. There were increase in percentage of change ($p \leq 0.05$) in roots under treatment T_3 (*A* alone) in all the four varieties followed by T_2 (A→N), and T_1 (N→A) with respect to control T_5. Basically, shoots of control plants in all four cultivars were observed more content of phenolic compounds than their roots. But after *M. graminicola* invasion and the action of *A. brasilense* the amount increased in both the roots and shoots of all the cultivars. The T_4 (N alone) treatment in all the R, MR, S and HS varieties were recorded higher as compare to T_5 but less than the treatments T_3, T_2, and T_1 in both roots and shoots against the test nematode, which could exacerbating for eco-friendly RKN management approach.

Keywords: Biofertilizer (Azospirillum brasilense); Paddy (Oryza sativa); cultivars; Meloidogyne graminicola; phenolic substances.

1. INTRODUCTION

In Asia, paddy is also known as rice belongs to Poaceae family, one of the main food grains of India as well as south-east Asian countries. It is a staple food that consumed by majority of Indian population. Paddy cultivation is an important part of Indian economy. India is the second largest producer of rice & largest exporter in the world. Rice production increased from 53.6Mt to 120Mt (2020-21). Odisha is a leading producer of rice of India & has contribution of a sizable amount of rice grains to the central pool of food stocks.

Being an adaptable crop, it can be cultivated in a variety of climates, be it plains, the mountains & hence is a kharif and rabi crop. In odisha, there are three systems of paddy cultivation such as dry, semi-dry & wet. The dry system accounts for 18% of the rice area and rest is shared by semi-dry and wet systems (Pani & Patra, 2004)**.** Among ecto and endo plant parasitic nematodes, root-knot nematode *Meloidogyne spp.*,is an important poplyphagus group of plant parasitic nematode which attack paddy crop grown extensively under various agro-climatic zones of India as well as in most of the south and east Asian countries of the world. Paddy is adversely affected by the rice root-knot nematode *Meloidogyne graminicola* and major constraints in agricultural production that results yield loss ranges from 20-80% & 11-73% respectively under upland or intermittently flooded condition [1, 2]. Damage in paddy plant consists of various degree of stunting, lack of vigour, chlorosis of leaf, wilting under moisture stress, secondary infection by other pathogens produces disease complexes, malfunctioning of root system. The eggs are laid in the root cortex. The newly parasitic J2s migrate intercellularly in the rice root cortex towards the root tip, where nematode invade the vascular cylinder (De Waele and Elsen,2007) and a relatively fast life cycle on rice in red lateritic soil favouring soil temperature 22-29 degree celcius [3,4]. The sedentary nematode feeds from the giant cells, development of nematode especially pyriform shape of female nematode can result in tearing of the root and subsequent release of egg masses through this aperture into the soil and leads to a patchy appearences in paddy field. *M. graminicola* remain within their host for most of their life cycle and are thus protected from predators and potential pathogens by the immune system of the host.

Among the number of management strategies of rice root-knot nematodes, though the easy, traditional and quick knock down effects of nematode control is mainly based on use of chemical nematicides*,* yet the harmful impacts on environment due to residual effects of toxic compounds, creates health hazard in human, increases risk of pollutions, imbalance between various ecosystems and pest resurgence after prolonged use .So a classical biological approach, which may emphasize on the non-conventional method of nematode management by using cost effective sources like Biofertilizer such as *Azospirillum brasilense* is an eco-friendly alternatives to stabilize production for low cost production. The notable biofertilizers of different strains are known to have a significant role in suppression of rice root-knot nematode disease and their effectiveness was reported in paddy by inducing the increasing in total phenol content of all types of paddy cultivars under different treatments conditions. Therefore the present experiment work was concentrated to exploit effectiveness of biomanagement in suppression of *Meloidogyne graminicola* infecting different paddy varieties by using biofertilizer.

2. MATERIALS AND METHODS

The experiment was conducted in the net house of the Department of Nematology, College of Agriculture, Orissa University of Agriculture and Technology (OUAT), Bhubaneswar during 2020 and 2021 in kharif seasons. The entire experiment was done under natural environmental condition and was designed in complete randomized design(CRD) comprising of five treatments with three replications. After screening and evaluation of different growth parameters of one hundred ten paddy cultivars by following RKI index (0-5scale), which were collected from different sources of odisha, four varieties were selected for biochemical analysis of total phenol content against the pathogen. In order to understand the basis of nematode resistance four varieties of paddy cultivars namely Abhisek (Resistant), Manik (Moderately resistant), Bas-12 (Susceptible) & Lalat (Highly susceptible).

2.1 Preparation of Soil and Sowing of Seeds

Five to six healthy seeds from each four varieties were grown in earthen pots of 15 cm height x 15 cm diamension sterilized with formaldehyde solution (1.0 %) and filled with autoclaved soil (15 lbs/20min). Before sowing the seeds were surface sterilized by treating them with 0.1 % $HgCl_2$ for 5 minutes, washed thoroughly with sterile water and air dried.

The soil to be filled in the pots were pulverised, mixed with N, P, K fertilizers @ 150:50:60 per hectare on soil weight basis and filled into the pots @ 1 kg/pot. The surface sterilized seeds were sown @ 4 to 5 seeds per pot. Each variety was replicated 3 times. Watering was done regularly after the emergence of seedlings. At 15 days after sowing the plants were thinned keeping one seedling per pot. Biofertilizer *A. brasilense* and nematodes were applied as per treatment details mentioned below. A small glass tube (2 cm long, 0.5 cm bore) was inserted into the soil near the base of each of the surviving seedlings. Two weeks after seedling emergence nematodes were counted under a stereoscopic microscope and released into the holes after removal of the glass tube @ 1000 $J_2 \pm 20$ per seedling in 10 ml. Sterile water in treatments T_1, T_2 at 7days prior or after the application of biofertilizer and at 15DAT in T_4 . For analysis of total phenol content the following treatments were followed. These pots were arranged on green house benches according to the treatments and replications. During the period of investigation, following Complete Randomised Design (CRD) with five treaments, each replicated thrice.The treatments are as follows:

T1 - Nematode *Meloidogyne graminicola* @ 1000J2 at 15DAT + *Azospirillum brasilense* @ 12kg/ha (after 7days of nematode inoculation)

T_2 – *Azospirillum brasilense* @ 12kg/ha at 15DAT + Nematode *Meloidogyne graminicola* @1000J2 (after 7days of *A. brasilense* application),

T_3 – *Azospirillum brasilense* @ 12kg/ha alone at 15DAT,

T_4 – Inoculated with *Meloidogyne graminicola* (1000J2/plant) alone at 15DAT

T_5 – Control.

Recording of observation after 45 days of transplanting and fresh root and shoot samples were collected for estimation of total phenol content.

2.2 Estimation of Total Phenolic Substances (Bray and Thrope, 1954)

Exactly 0.5 g plant samples were ground with a pestle and mortar in 10 ml of 80 per cent ethanol until it became a pulp. The homogenate was centrifuged at 5000 rpm for 20 minutes. The process was repeated with another 5 ml of 80 per cent ethanol. Both the supernatants were pooled and evaporated to dryness. The residue was dissolved in 10 ml distilled water. The aliquot was pipetted into test tubes with 0.5 ml each. The volume was made up to 3 ml with distilled water. Exactly 0.5 ml of folin-ciocalteu reagent was added into it. After 3 minutes 2ml of 20 per cent $Na_2 CO_3$ solution was added into each tube. The contents were mixed thoroughly, placed in boiling water for 1 minute and then cooled. Absorbance was measured at 650 nm in a colorimeter and compared with a blank. The above procedure was followed for extraction of phenol from both shoot and root samples of paddy varieties. A standard curve was prepared using different concentrations of catechol. Then the concentrations of the phenol in test samples was calculated by comparing with the standard curve and expressed as mg/g material (catechol) on fresh weight basis.

3. RESULTS

It was observed that there were increase in total phenol content of both the roots and shoots due to infestation caused by *M. graminicola* in all the

four cultivars of paddy under five different treatments. The phenolic compound content were recorded higher in resistance varieties than susceptible and highly susceptible varieties in both roots and shoots in post-infections.

3.1 Total Phenol Content in Shoot

There were maximum accumulation of total phenol in shoots of plants under T_3 (A only) treatments recorded as 0.70, 0.68, 0.4and 0.40 mg/g in Abhisek, Manik, Bas-12 and Lalat respectively over control. All inoculated Plant shoots were observed higher phenol content as compared to control. The treated plant shoots in T_2 (A→N) were showed higher phenolic substances such as 38.77, 36.58, 35.02 and 33.28 percent as compared to T_5 followed by treatment T_1 (N→A).

Table 1. Percentage of change in total phenol content in four different paddy cultivars under five different treatments against *Meloidogyne graminicola*:

Table 1. (Average of three replications)

Varieties	Total phenol content in mg/g on fresh weight basis							
	Abhisek (R)				Manik (MR)			
Treatments	Root	% of change	Shoot	% of change	Root	% of change	Shoot	% of change
T_1	0.24	33.33	0.66	34.69	0.18	38.46	0.67	36.73
T_2	0.25	38.88	0.68	38.77	0.18	38.35	0.67	36.58
T_3	0.26	44.44	0.70	42.85	0.19	46.15	0.68	44.68
T_4	0.23	27.77	0.60	22.44	0.17	27.65	0.61	29.77
T_5	0.18		0.49		0.13		0.47	
SE (±m)	0.06		0.02		0.05		0.02	
CD (0.05)	0.20		0.05		0.14		0.15	

Table 1 (Continued)

Varieties	Total phenol content in mg/g on fresh weight basis							
	Bas-12 (S)				Lalat (HS)			
Treatments	Root	% of change	Shoot	% of change	Root	% of change	Shoot	% of change
T_1	0.20	25.00	0.44	29.41	0.18	20.00	0.36	28.57
T_2	0.21	31.25	0.46	35.02	0.19	26.66	0.39	33.28
T_3	0.22	37.50	0.47	38.23	0.20	33.33	0.40	42.85
T_4	0.19	18.75	0.40	17.64	0.17	13.33	0.31	10.71
T_5	0.16		0.34		0.15		0.28	
SE (±m)	0.05		0.13		0.05		0.07	
CD (0.05)	0.16		0.39		0.15		0.19	

***T_1** –N @1000J2→Azsp @12kg/ha, **T_2** –Azsp @ 12kg/ha→ N @1000J2, **T_3** - Azsp @ 12kg/ha, **T_4** –Inoculated (N @1000J2), **T_5** – Control*

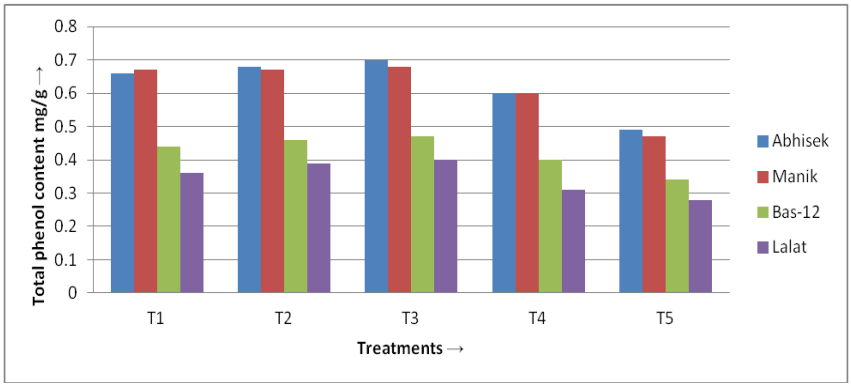

Fig. 1. Total phenol content in shoots (mg/g) of different paddy cultivars under five different treatments on fresh weight basis

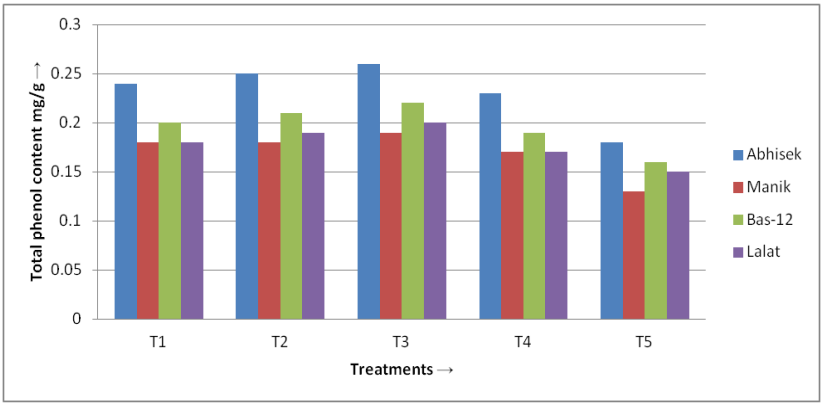

Fig. 2. Total phenol content in roots (mg/g) of different paddy cultivars under five different treatments on fresh weight basis

3.2 Total Phenol Content in Root

The post-infection phenol content increase percentage were recorded highest in roots of R and MR varieties Abhisek, Manik 27.77% and 27.65% wrt control T_5. In roots, the increased phenol content in T_3 were recorded 0.26, 0.19, 0.22, and 0.20mg/g samples in Abhisek, Manik, Bas-12 and Lalat. In treatments T_1 (N→A), T_2 (A→N) and T_3 (A only) in all R, MR, S & HS varieties were observed increased resistance against the nematode as compared to the control. The significant increase in phenolic compound amount were highest in Abhisek 0.25mg/g fresh weight followed by 0.19mg/g, 0.21mg/g and 0.19mg/g of sample in Mani, Bas-12 and Lalat respectively wrt control T_5.

4. DISCUSSION

The quantiative and qualitative changes in plant-derived phenolic substances plays a vital role in induction of plant defense mechanism against various pathogens including rice root-knot nematode *Meloidogyne graminicola* by using biofertilizer *Azospirillum brasilense*. It was studied that the resistant cultivars of paddy infected with *M.graminicola* showed maximum increase in the amount of total phenol content than the susceptible cultivars [5-7]. There is a distinct correlation between the degree of plant resistance to nematode pathogens and the amount of phenolic compounds present in plant tissues, which is the best known factors involved in the susceptible-resistance response [8]. Several studies have been found that resistant cultivars with nematode infection had higher amount of phenolic compounds [9] and the resistance inducing plant defense mechanism were being activated due to the secretion of phenolic substances against nematode attack [10], which was further enhanced by *Azospirillum spp.*

The mode of action of phenolic compounds may be related to the modification in the nematode physiology. RKNs secret a pool of substances into the plant cell membrane that to induce nematode feeding site formation [11]. (Cailaud *et.al*.2008). It has been studied that such secretion may be induced by some phenolic compounds such as resorcinol, catechol, hydroquinone, p-coumaric acid and caffeic acid [12]. Pathogen invasion enhances the transcription of m-RNA resulting increase amounts of PAL enzymes that helps in synthesis of more number of phenolic compounds [13]. It was noticed that *Azospirillum* strains were able to sustain some of its activities using phenolics as an alternative electrons acceptor under low oxygen content caused by the pathogen [14-16]. Inoculation of *Azospirillum brasilense* showed positive outcomes on the growth of plant against pathogen. It helps in reduction of nitrite in mineral decomposition processes and the plant growth promoting bacteria PGPB induces phenolic compounds such as IAA results plant growth. *A. brasilense* has been found in the intercellular spaces of vascular tissues of stems and roots [17] and also enhances plant hormone synthesis (Baars *et al.* 2018).

5. CONCLUSION

However, it is yet to study that those types of ISR in plant defense mechanism whether the phenolic compounds could lead the nematode to secrete their substances elsewhere rather than roots by preventing their establishment of feeding sites in such types of plant roots. Biofertilizer enhances the nematode control potential of the plant varieties by inducing the phenol content

that unlikely to increase the nematode mortaliy. The nematicidal effects of *Azospirillum brasilense* were seen significantly effective in R & MR varieties such as Abhisek and Manik as well as in another two varieties like Bas-12(S) and Lalat (HS) under different treatment conditions. Biological management approach is a promising and active area of nematological research at the present time. It is an essential step towards improving the level of reliability and biocontrol activity of bio-inoculants that enhances the natural regulations of plant parasitic nematodes below threshold damage level. In *M. graminicola* prone field it could be a cost effective eco-friendly nematode management approach in sustainable agriculture era.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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