Original Research Article

Evaluation of total phenol content of some of the common paddy cultivars of odisha with the application of biofertilizer in assessing resistance to *Meloidogyne graminicola*.

Abstract

The quatiative 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*. The total phenol content of four paddy cultivars were studied under pot culture condition in the net house following CRD design under different treatments using biofertilizer *A. brasilense*. The objective of the work was to study the quantitative changes in phenolic compounds content and the effect of bioferilizer 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 were seen higher than the susceptible variety Bas-12 and highly susceptible variety Lalat under five different treatments. There were increase in percentage of change ($p \leq 0.05$) in T_3 in all the four varieties followed by T_2, and T_1 with respect to control T_5. The T_4 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 in India.

Key Words – 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 provides instant energy with a high carbohydrate content & is a staple food that is consumed by majority of Indian population. Paddy cultivation is an important part of Indian economy. India is the second largest producer of rice & largest

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Comment [Ap1]: Abstract has not described the contents of the research in detail so that readers do not get brief information about the results and benefits of the research

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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 (Plowright & Bridge, 1990; Soriano et. al. 2000). 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 (Bridge and Page, 1982; Yik and Birchfield, 1979). 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 of each four varieties were grown in earthen pots of 15 cm height x 15 cm dia, 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)

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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 ± 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 @ 1000J2 at 15DAT + *Azospirillum brasilense* @ 12kg/ha (after 7days of nematode inoculation) T_2 – *Azospirillum brasilense* @ 12kg/ha at 15DAT + Nematode @1000J2 (after 7days of *A. brasilense* application), T_3 – *Azospirillum brasilense* @ 12kg/ha only at 15DAT, T_4 – Inoculated (1000J2/plant) and 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.

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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. Result

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 varieties of paddy. The post-infection phenol content increase percentage were recorded highest in roots of MR varieties Manik and Abhisek 27.77% and 30.76% wrt control $T_{5.}$ In roots, the increased phenol content in T_3 were recorded 0.26, 0.19, 0.22, and 0.20mg/100g samples in Abhisek, Manik, Bas-12 and Lalat. In treatments T_1 (N→A) and T_2 (A→N) in all R, MR, S & HS varieties were observed increased resistance against the nematode as compared to the control. The phenolic compound content were recorded higher in resistance varieties than susceptible and highly susceptible varieties. Basically, shoots of control plants in all four cultivars were observed more content of phenolic compounds than their roots. But after *M. graminicola* invasion the amount increased in all the varieties in both the roots and shoots.

4. Discussion :

Patel et. al, 2018 studied that the resistant cultivars of tomato infected with *M. incognita* showed maximum increase in the amount of total phenol content than the susceptible cultivars. 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 (Giebel, 1974). Several studies have been found that resistant cultivars with nematode infection had higher amount of phenolic compounds (Ganguly and Dasgupta, 1982) and the infection with *Meloidogyne javanica* stunted the growth of *Carica papaya* and enhanced the level of O-dihydric phenols, total phenols and IAA in infected plants (Goel *et.al*, 1982). The resistance inducing plant defense mechanism were being activated due to the secretion of phenolic substances against nematode attack (Ohri and Pannu,2010).

Table 1- : Percentage of change in total starch content in four different paddy cultivars under different treatments against *Meloidogyne graminicola* :

Table-a : (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	0.60	27.65
T_5	0.18		0.49		0.13		0.47	
SE (±m)	0.0060		0.0276		0.0054		0.0259	
CD (0.05)	0.0219		0.0531		0.0148		0.0507	

Table-b :

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	39.28
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)	0.0057		0.0138		0.0055		0.0073	
CD (0.05)	0.0162		0.0398		0.0156		0.0193	

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

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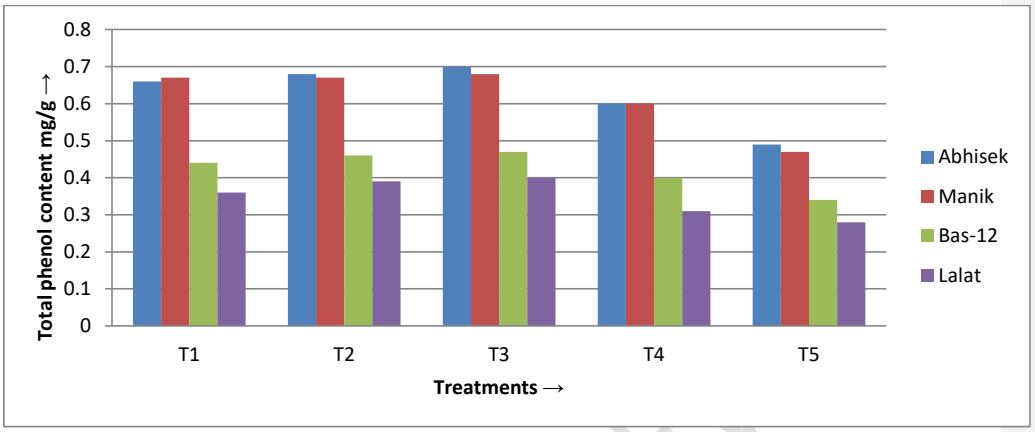
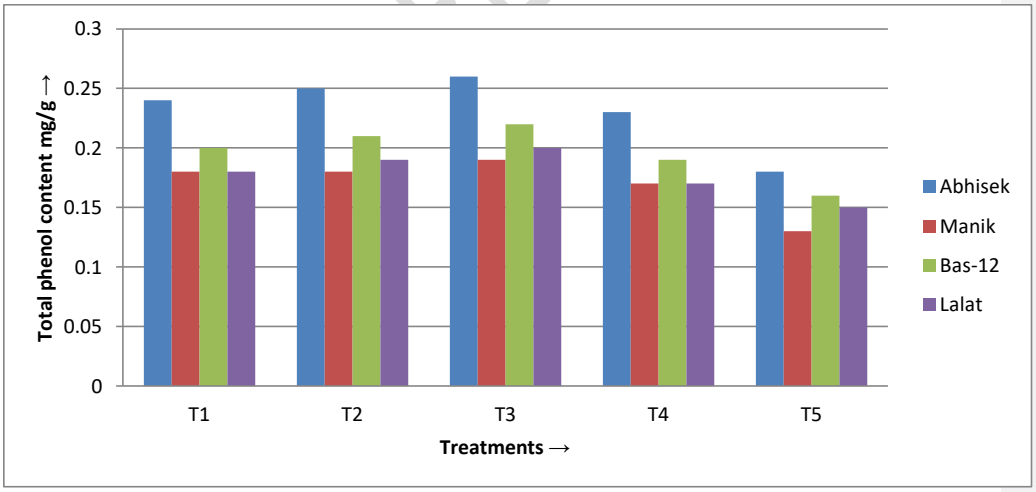


Fig.2 - Total phenol content in roots (mg/g) of different paddy cultivars under five different treatments on fresh weight basis



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. (Williamson and Gleason 2003, 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 (McClure and Mende, 1998; Jaubert *et.al*. 2002; Bellafiore *et.al*.2008). Pathogen invasion enhances the transcription of m-RNA resulting increase amounts of PAL enzymes that helps in synthesis of more number of phenolic compounds (Taiz and Zeiger, 2002). Barkovskii A., 1995 noticed that *Azospirillum* strains were able o sustain some of its activities using phenolics as an alternative electrons acceptor under low oxygen content caused by the pathogen. Inoculation of *Azospirillum brasilense* showed positive outcomes on the growth of tomato plant against pathogen (Licea and Quiroz, 2020).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 (Robson & Robson, 2015) 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 heir 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 HS variety Lalat 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.

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