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Manaswini Mahapatra
Department of Nematology, College of Agriculture, University Agriculture & Technology, Bhubaneswar, Odisha, India

Dhirendra Kumar Nayak
Department of Nematology, College of Agriculture, University Agriculture & Technology, Bhubaneswar, Odisha, India

Antaryami Mishra
Department of Soil Science and Agricultural Chemistry, College of Agriculture, Odisha University Agriculture & Technology, Bhubaneswar, Odisha, India

Corresponding Author
Manaswini Mahapatra
Department of Nematology, College of Agriculture, University Agriculture & Technology, Bhubaneswar, Odisha, India

Interaction effects of biological control agent, *Trichoderma viride* against root-knot nematode, *Meloidogyne incognita* on iron content of ginger rhizome

Manaswini Mahapatra, Dhirendra Kumar Nayak and Antaryami Mishra

Abstract
Research was conducted at the departmental net house to study the micronutrient, iron content variation by interaction effects of antagonistic fungi, *Trichoderma viride* and root-knot nematode, *Meloidogyne incognita* in all possible combinations for bio-control of the test nematode infecting ginger germplasm *viz.* suravi and suprabha. Antagonistic fungi, *Trichoderma viride* @ 6g pot^{-1} alone proved to be more effective among all treatments resulted in higher plant vigour, chlorophyll, and nutrient status (N, P, K, Fe, and Zn). Among all combined treatments, TV→MI (*Trichoderma viride* inoculated 15 days prior to *Meloidogyne incognita*) was found to be most efficient, acting as a plant growth stimulator in close association with plant rhizomes and suppressing the root-knot development of *M. incognita* in ginger plants.

Keywords: *Trichoderma viride, Meloidogyne incognita,* interaction, biocontrol, iron content, ginger crop

1. Introduction
Ginger (*Zingiber officinalis* Rosc) is a vital spice cash crop, and India is the leading producer, ranking first in the world. Plant-parasitic nematodes are the major limiting factor which causes a yield loss of 11-80 per cent (Mohapatra *et al.*, 1986) [9]. It was also observed that the nematodes form disease complexes in association with secondary pathogens, *viz.* bacteria, fungus, and viruses. The crop is susceptible to *Meloidogyne incognita* (Stirling and Nikulin, 1998) and root knot nematode is solely responsible for crop loss and damage. In ginger's case of root rot disease, root knot nematode enhances the disease severity by promoting the entry of pathogens followed by their multiplications *viz. Fusarium oxysporum*, *Phythium* spp. (Kaur and Sharma, 1988). Because of the global consideration of environmental degradation due to indiscriminate use of nematicides, researchers have been forced to discover non-chemical and eco-friendly approaches like the application of bio-control agents (Lamovsek *et al.*, 2013) [7] to suppress nematode population and sustainable use of nematicides to reduce crop loss. Antagonistic fungi, *Trichoderma* spp. are efficient bio-control agents against many fungal phytopathogens (soil-borne, foliar and postharvest etc.) and root-knot nematodes (Bandyopadhyay and Cardwell, 2003; Vinale *et al.*, 2008) [2, 17]. They are also responsible for increasing nutrient availability, promoting plant growth, enhancing disease resistance and improving crop production (Vinale *et al.*, 2004; Harman *et al.*, 2004) [16, 4]

2. Materials and Methods
2.1 Preparation and sterilization of soil mixture
The sandy loamy soil from the central university farm was collected and processed further to remove the rocks, pebbles and garbage. Two part of soil, one part of sand, and one part of FYM (farm yield manure) were mixed thoroughly, packed in a gunny bag and autoclaved at a pressure of 1.1 kgcm^{-2}. After sterilization, the soil mixture was cooled at room temperature for 24 hours. Earthen pots (8 inched) were washed thoroughly, sterilized with 1% formalin solution, and air-dried before filling with 2 kg of sterilized soil.

2.2 Growth and maintenance of test plant
Ginger rhizomes were surface sterilized with streptocycline, sixer solution for 10 minutes followed by half an hour in quinalphos at the rate of 0.2 per cent each and air-dried under

shade conditions. About two to three rhizomes were sown in each pot containing 2 kg sterilized soil and later thinning was done, keeping one healthy plant in each pot. To understand the variation of iron content in the nematode (*Meloidogyne incognita*) inoculated with *Trichoderma viride*; two varieties Suravi (Resistant) and Suprabha (Highly susceptible) were grown in pots.

2.3 Nematode inoculum

Second-stage juveniles (J_2) of *Meloidogyne incognita* were used as test nematode in the ginger crop. The egg masses of *Meloidogyne incognita* were collected from the heavily infected brinjal roots on which a pure culture of nematode was maintained by handpicking with the help of sterilized forceps. The collected egg masses washed thoroughly in distilled water and placed in 15 coarse mesh sieves with a double-layered tissue paper, placed in petri plates containing water in such a manner that they were touching the water. Then petri plates were incubated at 25 ˚C. Freshly hatched second-stage juveniles (J_2) were collected at 24 hours intervals and fresh water was added to the petri plates at each collection. The concentration of petri plate solution was adjusted to 200 ± 5 nematodes in 1ml of solution. About 10 ml of the liquid suspension containing 2000 freshly hatched juveniles was inoculated to specific earthen pots depending upon experimental treatments. After Fifteen days of sowing, two small holes of 2 cm depth were made in the soil close to the base of the plant, into which 2000 J_2/plant were inoculated in the relevant treatments (T_1, T_3, T_5 and T_6) @ 2000 J2 pot^{-1} (2 kg soil). For T_4, *M. incognita* was inoculated one week after the inoculation of *Trichoderma viride*.

2.4 *Trichoderma viride* inoculum

Twenty different rhizosphere soil samples from healthy pigeon pea, chickpea, black gram, and green gram plants grown on the university campus were collected, stored in sterile plastic bags, and airdried. Isolation was done by using serial dilution technique on Trichoderma Selective Medium (Elad and Chet, 1983; Morton and Stroube, 1955) [3, 10]. The nematode was cultured on potato dextrose agar (PDA, 20% potato, 2.0% dextrose, 2.0% agar, pH = 7.0). After that, cultures were purified using a single spore culture technique on PDA plates (point inoculation) and incubated for 24-48 hours at 27 °C. The identification was done based on their cultural and morphological characteristics like colony color, sporulation nature and growth, conidia and conidiophores characteristics through microscopic observation (Rifai, 1969) [12]. Isolates of *Trichoderma viride* were taken for this study. For soil application of *Trichoderma viride*, the bio-agent was added at the rate of 6 g pot^{-1}, maintaining the minimum CFU *i.e.* 2×10^6 per 1 g of formulation.

3. Experimental design

Trichoderma viride was applied around the culms of ginger in the presence or absence of nematode, *Meloidogyne incognita* in each possible combination. The whole experiment was finished in completely randomized design (CRD) with seven experimental treatments: (1) T_1- *Meloidogyne incognita* (MI) alone @ 2000 J2 pot^{-1} of soil, (2) T_2- *Trichoderma viride* (TV) alone @ 6 gm pot^{-1} (3) T_3- MI→TV (*Meloidogyne incognita* inoculated 15 days prior to *Trichoderma viride*), (4) T_4- TV→MI (*Trichoderma viride* inoculated 15 days prior to *Meloidogyne incognita*), (5) T_5- TV+MI (*Meloidogyne incognita* and *Trichoderma viride* inoculated simultaneously), (6) T_6- Chemical application with Carbofuran 3G @ 2.5 kgha^{-1} 15 days prior to *Meloidogyne incognita*, (7) T7- Untreated control. The pots were sprinkled with water passed through 500 mesh sieves up to the soil capacity. Each treatment was replicated 4 times.

4. Observations

The ginger plants were terminated 60 days after nematode inoculation to determine the iron content in the rhizome of the ginger crop. Each treatment plant was taken out from the pots, and soil particles adhering to rhizomes were removed by washing under tap water and adequately labeled. Dry weight of rhizomes was taken with the help of a physical balance. Rhizomes were kept in labeled envelopes for dry weight determination and dried in a hot air oven running at 80˚C for 48-72 hours before weighing. To determine iron content, powdered rhizome samples weighing 0.5 g were taken in a 100 ml conical flask and 10 ml of concentrated HNO_3 was added to each and kept overnight. The flasks then heated till brown fumes evolved. 5 ml of di-acid mixture [HNO_3: $HClO_4$ (70%): 3:2 by volume] was added to each flask and heated up again till white fumes evolved, reducing the volume to 2 ml. Again 1 ml of 6N HCL was added and heated gently for one minute. After heating, 15 ml of warm distilled water was added to each flask and the content was transferred to a 50 ml volumetric flask followed by twice rinsing with distilled water and volume was made up to 50 ml. The aliquot was then filtered out through Whatman No.42 filter paper and the filtrate were kept for the estimation of iron content (Fe) by adopting the procedure of Jackson (1973) [5]. The digested sample was introduced to AAS for iron content analysis after standardizing the AAS with respective standards.

$$\text{(Fe) mg/100 g dry weight} = \frac{AASR \times 50}{Sample\ \text{wt(g)} \times 10}$$

5. Statistical analysis and interpretation of data

Fisher's methods of analysis of variance at 5% level of significance were followed by ANOVA model. Further, the comparison of the treatment means was done by calculating standard error of mean (S.E.M) and Critical difference (C.D) in the following manner.

S.E (m)± for treatment = $\sqrt{EMS/R}$

CD at 0.05= $\sqrt{2}\times$ S.E (m) × t (0.05) at error d.f.

where, d.f.= degree of freedom

r= replication

EMS= Error means sum of square

S.E(m)± = Standard error mean

CD (0.05) = Critical difference at 5% level.

6. Results

In the present research, iron content in rhizome of treated ginger samples were increased in all treatments over control. Maximum increase was seen susceptible variety than resistant one. Maximum reduction of iron content was found in T_1 (MI) of both varieties having 13.15 % and 20.49 % in suravi and suprabha respectively over untreated control on dry weight basis. Highest increased iron content in inoculated ginger rhizomes was seen in T_2 (TV) amounting 1.91 and 2.08 mg/100g dry weight in suravi and suprabha respectively over check. Highest per cent of decrease was noticed in susceptible variety, suprabha and least in resistant variety, suravi compared to control.

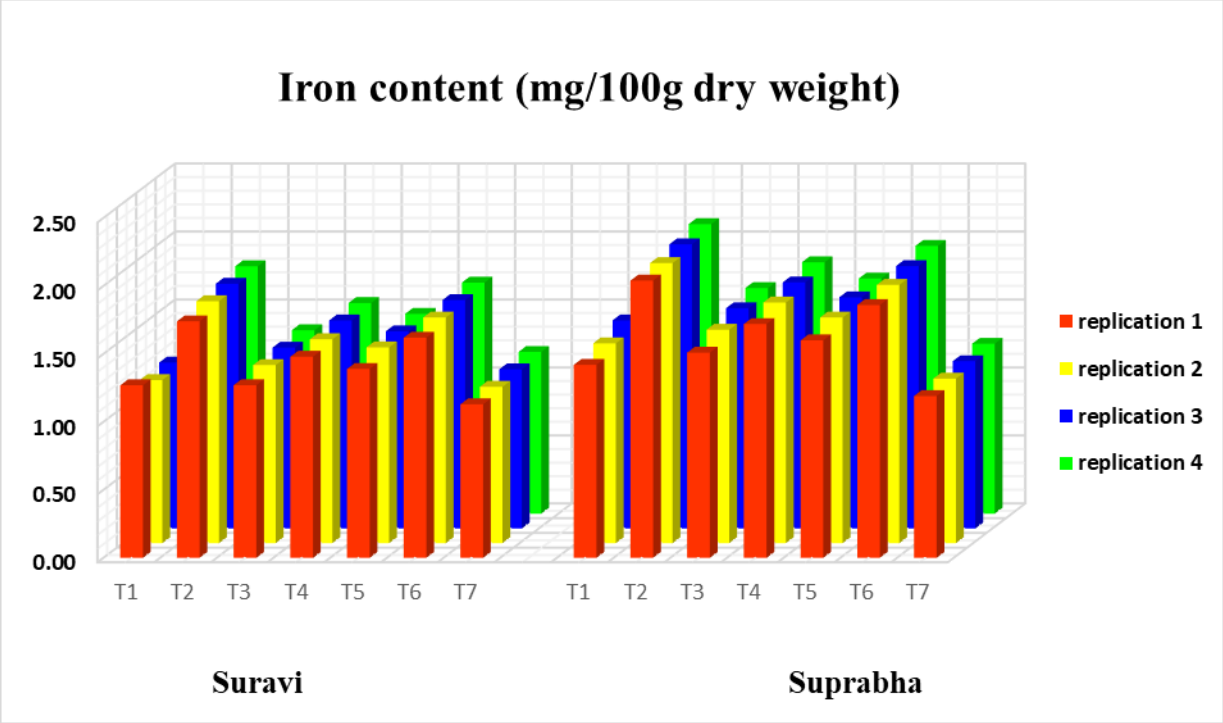

Fig 1: Iron content (mg/100g dry weight) in both Suravi and Suprabha germplasm

Table 1: Influence of *M. incognita* and *Trichoderma viride* either alone or in combination on Iron (Fe) content (mg/100g dry weight) of ginger rhizome

Treatments	Suravi (R)		Suprabha (HS)	
	Iron content (mg/100g dry weight)	Change over control (%)	Iron content (mg/100g dry weight)	Change over control (%)
T_1 (MI)	1.31	13.15	1.47	20.49
T_2 (TV)	1.91	64.87	2.08	70.70
T_3 (MI→TV)	1.40	20.91	1.59	30.33
T_4 (TV→MI)	1.63	40.52	1.79	46.52
T_5 (TV+MI)	1.51	30.17	1.67	37.09
T_6 (carbofuran 3G)	1.72	48.06	1.92	57.17
T_7 (Control)	1.16		1.22	
SE(m) ±	0.012		0.013	
CD (0.05)	0.036		0.040	

(+) Increase

T_1- *Meloidogyne incognita* (MI) alone @ 1000 J_2kg^{-1} of soil, T_2- *Trichoderma viride* (TV) alone @ 3gkg $^{-1}$ of soil, T_3- MI→TV (*Meloidogyne incognita* inoculated 15 days prior to *Trichoderma viride*), T_4- TV→MI (*Trichoderma viride* inoculated 15 days prior to *Meloidogyne incognita*), T_5- TV+MI (*Meloidogyne incognita* and *Trichoderma viride* inoculated simultaneously), T_6-Carbofuran 3G, T_7- Untreated Check.

7. Discussion

An increase in iron (Fe) concentrations was seen in nematode inoculated and both nematode & *Trichoderma viride* inoculated ginger rhizomes over control. Minimum increase was observed in only nematode inoculated plants followed by MI→TV, TV+MI, TV→MI and carbofuran 3G applied. Compared with resistant and susceptible varieties, maximum reduction was seen in resistant variety than susceptible variety. The highest accumulation of iron content was noticed in treatment inoculated with only *Trichoderma viride* (T_2). General reduction of plant growth and chlorotic effects on foliage, particularly in infected plants, can be correlated with a decrease in the concentration of iron. Change in concentration of iron in the synthesis of chlorophyll could influence the photosynthetic-related physiological process of the host. Our results showed that *Meloidogyne incognita* infestation restricted the availability of iron content in ginger rhizomes and could not translocate the deposited iron content in rhizomes through the xylem. Also, in the treatments of bio-agent (alone and combinations) and induced nematicides, *Meloidogyne incognita* infestation was suppressed, leading to improved growth of the ginger crop. This research supported the similar previous findings of Patil (2014) [11] who reported that the Fe and Zn contents in the rice leaves were significantly reduced in a non-basmati Pusa-44 and a basmati Pusa Sugandh-5 cultivar of rice infected with *M. graminicola* under both pot and field conditions. Mohanty *et al*., (1999) [8] reported that the inoculation of *R. reniformis* in cowpea roots reduce micro nutrients viz., Zn, Cu, Fe, Mn in inoculated plants over the control. Sreekanth *et al.* (2006) [14] observed that several micronutrients like copper, iron, manganese and zinc contents were decreased in infected plants of African marigold than healthy plants due to *Tylenchulus semipenetrans* infection. Several studies have demonstrated that *Trichoderma spp*. frequently enhances root colonization and development, crop productivity, resistance to abiotic stresses and uptake and use of nutrients (Siddiqui *et al.*, 2001; Harman *et al*., 2004; Affokpon *et al*., 2011) [13, 4, 1].

8. Conclusion

The biological control method for plant parasitic nematodes is a potential method for controlling the nematode population in a comparison with the toxic chemical nematicides. Therefore, various effective strategies had been formulated by the researchers so far. The Antagonistic fungi, *Trichodrma viride* has been proposed to be the best eco-friendly alternative to protect plants against biotic stress. The integrated treatments of bio control agent *Trichodrma viride* and nematode *Meloidogyne incognita* resulted in better plant growth and reduced disease intensity. The increase in plant growth due to nutrient supplementation by inoculated organisms, which enhance their efficiencies like phosphorous availability to the host plant and effective pathogen suppression by *Trichoderma viride.*

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