Original Research Article

Variation in calcium content of ginger germplasm inoculated with bio-agent, *Trichoderma viride* against root-knot nematode, *Meloidogyne incognita*

Abstract

The study was aimed to control nematodes in ginger crop in a sustainable way through inoculation of bio-agent, *Trichoderma viride*. The outcome of our research revealed that *Trichoderma viride* alone (T_2) was the most effective treatment among all. This bio-agent was lowered the root-knot nematode populations as suppressing the disease intensity and responsible for enhancement of plant growth, increase the availability of nutrients to host plant. *Meloidogyne incognita* alone (T_1) infected rhizomes resulted in highest reduction of magnesium content amounting 2.26 and 2.43 mg/100g dry weight whereas maximum content was recorded in *Trichoderma viride* alone (T_2) inoculated rhizomes as 2.84 and 3.08 mg/100g dry weight in suravi and suprabha over control (T_7) respectively. *Trichoderma viride* inoculated 15 days prior to *Meloidogyne incognita* (TV→MI) was found to be most efficient one among all combined treatments. Susceptibility towards nematode induced more nutrient deposition in rhizomes of ginger crop due to poor translocation process through xylem.

Key words: *Trichoderma viride,* bio-control, *Meloidogyne incognita*, Magnesium content, Ginger crop

1. Introduction

Ginger (*Zingiber officinalis* Rosc) is an herbaceous perennial plant cultivated as an annual cash crop. It is mostly used as a spice and also a major ingredient for production of various medicines. India is the leading country in ginger production, accounting for 30% of global production each year [14]. The extent of ginger production in India is predicted to be round 1.89 million metric tons in financial year 2021. This become a boom from the previous fiscal year's total of 760 thousand metric tons [11]. In the year 2017, as per the report of department of Agriculture, Govt of Odisha, a total of 1,33,680 tons of ginger was produced from 16,965 ha land [3]. The majority of production is concentrated to southern districts of Odisha like Koraput, Kandhamal, Keonjhar, Gajapati, Bargarh, Jharsuguda, and Mayurbhanj. The root knot nematode *Meloidogyne incognita* is reported as the most important and common nematode affecting ginger production in Odisha [7]. The utilization of the chemical nematicides is restricted now-a-days because of their impermanent impact, less accessibility, significant expense, less resistivity against nematode improvement, wellbeing and ecological dangers, residual toxicity, and unfavorable consequences for the useful microflora and fauna living in soil [1, 12]. Apart from chemical management of root-knot nematode, the use of biopesticides / bioagents proved as an effective alternative or supplemental management tool to replace chemical methods in recent years, as more emphasis has

been on eco-friendly pest management methods [10]. Different instruments like antibiosis, mycoparasitism, and enzymatic hydrolysis have been recommended for the bio-control action of *Trichoderma* spp. against nematode [9]. In mycoparasitism method, enzymes such as chitinases, glucanases, and proteases appear to play a significant role [4]. Along with direct antagonism, *Trichoderma* spp. used several other mechanisms to control *Meloidogyne*, such as fungal metabolite production and induced resistance [2, 8].

2. Materials and Methods

The designing and experimentation of the research work was performed in the net house of the Department of Nematology, OUAT, to investigate the variation of secondary nutrients such as calcium content in ginger rhizome when inoculated with bio-agent, *Trichoderma viride*, against root-knot nematode, *Meloidogyne incognita* in all feasible combinations of bio agent and test nematode. The soil was prepared by mixing soil, sand, and FYM (farm yield manure) in 2:1:1 ratio. The soil was packed in a gunny bag, autoclaved at 1.1 kg cm-2 pressure and subjected for cooling at room temperature. Clay pots with a diameter of 8 inches were thoroughly washed, sterilized with a 1 percent formalin solution, and air-dried. After air drying, the clay pots were filled with 2 kg of sterilized soil. In due time, the ginger rhizomes were surface sterilized with streptocycline and sixer solution for 10 minutes followed by half an hour in quinalphos at a rate of 0.2% each. For the experimentation, two ginger germplasms, Suravi (resistant) and Suprabha (highly susceptible) were planted in the clay pots filled with the soil. About 15 days after planting of the rhizomes, small openings of 2 cm profundity have been made in the soil near the base of the plant, and 2000 J_2 (second stage juveniles of root-knot nematode (*Meloidogyne incognita*)) inoculated in to the small holes of relevant treatments (T_1, T_3, T_5 and T_6) @ 2000 J_2 pot^{-1} (2 kg soil). In T_4, the test nematode *M. incognita* was inoculated one week after the inoculation of bio agent *Trichoderma viride*. 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. After, 60 days of planting, the ginger plants were harvested, washed thoroughly, and placed in labeled envelopes and dried in a hot air oven at 80˚C for 48-72 hours. After drying, the dry weight of each samples was measured. In ordered to determine the calcium content, powdered rhizome samples weighing 0.5 g were placed in a 100 ml conical flask and 10 ml of pure HNO_3 was poured to each and left overnight to assess the calcium content. The flasks were then heated until brown vapours began to emerge. Each flask was filled with 5 ml of di-acid mixture [HNO3: HClO4 (70 per cent) = 3:2 by volume] and heated until white vapours emerged, reducing the volume to 2 ml. One ml 6N HCL was added again, and the mixture was gently heated for one minute. After heating, 15 ml of warm distilled water was added to each flask, and the contents were transferred to a 50 ml volumetric flask, which was then rinsed twice with distilled water and the capacity was increased to 50 ml. Following Jackson's technique, the aliquot was filtered out using Whatman No.42 filter paper, and the filtrate was maintained for collecting digested plant tissues [5]. The digested materials were then analysed using the Versenate technique. Five milliliters of the Di-acid solution were pipetted into a

porcelain basin. To neutralize the acidity, 10 percent sodium hydroxide was added drop by drop, with an additional 5 ml added as an excess to keep the pH at 12. In 0.02 N EDTA, a pinch of murexide indicator was applied until the colour changed from pinkish red to blue. The percentage of calcium content was calculated by the following equation.

$$\text{Percentage of calcium in the given sample on moisture free} = 0.0004 \times \mathrm{B} \times \frac{\mathrm{V}}{5} \times \frac{100}{\mathrm{W}} \times \frac{100}{100 - \mathrm{M}}$$

Where,

Weight of Plant sample taken = W g

Volume of Di-acid extract prepared = V ml

Volume of Di-acid extract pipette out for titration = 5 ml

Volume of 0.02 N EDTA used for Ca alone = B ml

1 ml of 0.02 N EDTA =0.0004 g of Ca

Moisture content of the sample = M

3. Experimental design

In each conceivable combination, *Trichoderma viride* was treated around ginger culms in the presence or absence of the test nematode *Meloidogyne incognita*. The experiment was set up using seven experimental treatments in a completely randomized design (CRD): (1) T_1- *Meloidogyne incognita* (MI) alone @ 2000 J_2 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- Carbofuran 3G @ 2.5 kg / ha 15 days prior to *Meloidogyne incognita*, (7) T_7- Untreated control. During the experimentation, watering was performed water passed through 500 mesh sieves up to the soil capacity. Each treatment was replicated 4 times.

4. Statistical analysis

The ANOVA model was used to follow Fisher's methods of analysis of variance at a 5% level of significance. Furthermore, the treatment means were compared using the following formulas: standard error of mean (S.E.M) and critical difference (C.D).

S.E (m) ± for treatment = √EMS/R

CD at 0.05= √2× S.E (m) × t (0.05) at error df

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.

5. Results and Discussion

The calcium (Ca) content of the treated ginger rhizomes was higher than the uninoculated control. Treatment with *Trichoderma viride* alone resulted in an increase in secondary plant nutrients such as calcium, followed by carbofuran 3G induced plants, and then *Trichoderma viride* inoculated 15 days before *Meloidogyne incognita*. Only *Meloidogyne incognita* (T_1) infected rhizomes showed the greatest reduction, with 2.84 and 3.07 mg/100g dry weight in suravi and suprabha, respectively, over control (T_7). The susceptible variety (suprabha) had the highest rise in calcium content in the rhizome compared to the resistant variety (suravi). The calcium content in T_2 (TV) increased by 37.27 percent and 26.56 percent over control in suprabha and suravi, respectively. The same result was also reported by Waghmare [13] who found that, plant nutrients like, N, P, K, Ca, and Mg were dramatically reduced in shoots and increased in roots with increase of inoculum levels. The root-knot nematode was controlled with carbofuran at 1 and 2 kg a.i. /ha, *Trichoderma harzianum* at 1 and 2 per cent w/w, and neem seed oil as a seed treatment for mungbean against *M. incognita*. The plant nutrition status demonstrated that the shoot has more content nutrition such as N, P, K, Ca, and Mg than the infected control plant, however the root has lower levels of these elements. Mohanty *et al.* [6] investigated the effects of *R. reniformis* inoculation on cowpea root samples and discovered that the four macronutrients, namely N, K, Ca, and Mg, increased in infected root samples compared to control samples.

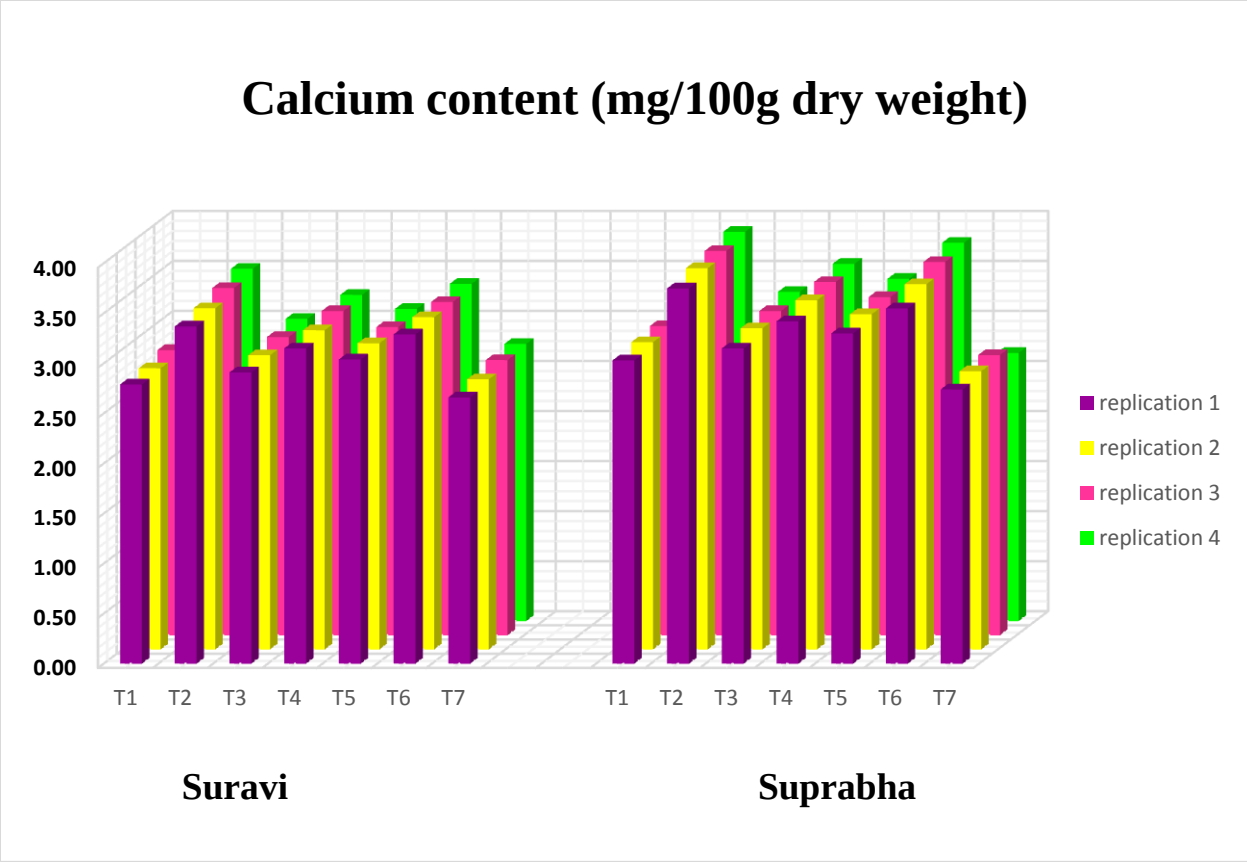

Fig. 1. Calcium content (mg/100g dry weight) of ginger germplasm in each treatment and replication

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

Treatments	SURAVI (R)		SUPRABHA (HS)	
	Calcium content (mg/100g dry weight)	Change over control (%)	Calcium content (mg/100g dry weight)	Change over control (%)
T_1 (MI)	2.84	4.23	3.07	11.73
T_2 (TV)	3.44	26.56	3.78	37.27
T_3 (MI→TV)	2.96	8.92	3.17	15.18
T_4 (TV→MI)	3.21	18.01	3.50	27.36

T_5 (TV+MI)	3.08	13.05	3.36	22.27
T_6 (carbofuran 3G)	3.33	22.33	3.62	31.55
T_7 (Control)	2.72		2.75	
SE (m)±	**0.007**		**0.028**	
CD (0.05)	**0.019**		**0.082**	

(+) Increase

T_1- *Meloidogyne incognita* (MI) alone @ 1000 J_2 kg^{-1} of soil**,** **T_2-** *Trichoderma viride* (TV) alone @ 3g kg^{-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.

Conclusion

Bio-agent, *Trichodrma viride* has been proposed to be the best eco-friendly alternative to protect plants against plant parasitic nematode attack. All the combinations of bio-agent resulted in better plant growth and reduced disease intensity. Among nematode alone & both nematode and *T. viride* inoculated treatments, maximum reduction in rhizomes were recorded in *M. incognita* alone (MI) treated plants whereas *Trichoderma viride* alone (TV) treated ginger crop detected having maximum rhizome calcium content over control. The increase in plant growth due to nutrient supplementation by inoculated organisms, which may enhance their disease resistant capacity to the host plants. Thus effective pathogen suppression occurred.

Data Availability Statement

The data presented in this study are available on request from the corresponding author.

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