Rhizospheric Bioweapons for Tuber Yield Enhancement in Chlorophytum Borivilianum against Meloidogyne Incognita Infestation

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Abstract
Chlorophytum borivilianum Santapau and Fernandez (Liliaceae) is a therapeutically important tuber crop, vulnerable to Meloidogyne incognita (Kofoid and White) Chitwood infestation. Therefore, the present study was designed to explore the efficacy of rhizospheric microbes viz. Bacillus megaterium, Pseudomonas fluorescens, Glomus intraradices, and Trichoderma harzianum CIMAP-RPN01, both singly and in combinations, for the successful management of M. incognita as well as to determine the comparative impact of different microbes on the growth/yield of C. borivilianum. Maximum reduction in M. incognita infestation was recorded in dual treatment viz. T. harzianum + P. fluorescens followed by that of T. harzianum + B. megaterium. This study proves the beneficial effects of rhizospheric microbes in galvanizing the quality and quantity yields of this medicinally important crop.

Keywords: Biocontrol; Chlorophytum borivilianum; Meloidogyne incognita; Rhizospheric microbes.

Introduction
The plant genera Chlorophytum Santapau and Fernandez (vern. Safed Musli) comprising approximately 256 species is a medicinally important crop from family Liliaceae with potent medicinal properties used as a revitalizer, remedy for diabetes, arthritis, as a curative for natal and post-natal problems, in the treatment of diarrhea, dysentery, gonorrhoea and leucorrhoea [1]. Because of its immense therapeutic properties, the plant is frequently symbolized as “divya aushadhi” in Ayurveda [2,3]. The genus is represented in India by 12 different species viz. C. heyneanum Wall, C. breviscapum Dalz., C. arundinaceum Baker, C. glaucum Dalz., C. tuberosum Baker, C. khasianum Hooker, C. attenuatum Baker, C. malabaricum Baker, C. undulatum Wall. syn. C. nepalense (Lindl) Baker, C. orchidarium Lindl., C. laxum Br. and C. borivilianum Santapau and Fernand [2], grown in the hilly areas of Uttar Pradesh, Gujarat, Rajasthan and Madhya Pradesh. It is a tuber crop with plant tubers containing 2-15% saponin accountable for enhancing vitality and immunity in human beings [4].

Despite of the vast diversity of plant species, C. borivilianum Santapau and Fernandez is the most important owing to its highest tuber yield along with the highest saponin/phytochemical constituents [5]. The dried tubers of C. borivilianum contain 42% carbohydrates, 8-9% proteins, 3-4% fibres and saponins (Yadav and Pandey, 2004) and therefore form an obvious choice for the commercial cultivation of the crop. Furthermore, plant tubers are also imperative as the plant primarily propagates through roots due to the poor seed setting and viability (<20%). Though the crop is affected by several diseases like leaf spot, anthracnose and wilt, the major threat to its commercial cultivation occurs due to the root-knot nematode, Meloidogyne incognita (Kofoid and White) Chitwood infestation [5]. M. incognita is a sedentary endoparasitic nematode, infesting the plant roots, thereby affecting growth and yield of the host plant. The nematode causes great loss to the agricultural economic sector as against the annual requirement of 3500 tonnes, India is barely able to contribute its share as 500-600 tonnes [6,7]. Furthermore, the over-exploitation of the plant due to its growing demands world over has placed it in the category of threatened species, on the verge of extinction [8-10]. The Medicinal Plant Board of India (MPBI) has also documented the plant as the sixth most important herb to be protected and promoted [11].

Therefore to meet the current industrial requirement for Chlorophytum roots, alternate strategies for the quality and quantity yield management are urgently required. Agricultural practices such as the use of insecticides or pesticides though have promising effects, yet the concentrated and continuous use of these chemicals disturbs soil health, leading to acidification, micro nutrient depletion, soil degradation, reduction in the activity of soil micro flora and fauna, poor crop health and lower yields [12,13]. Besides, the excessive use of these fertilizers may contribute to the environmental risks like increase in global temperature, ground and surface water pollution, etc. In this regard, it is desirable to have ecofriendly and cost-effective measures for agricultural sustainability [14,15]. Rhizospheric microbes serve as a better option for effective and less resource demanding measures having plant growth promoting and biocontrol potentials. Therefore, in the present investigation, attempts were made to determine the comparative impact of different microbes viz. Bacillus megaterium (NCBI-KC978881), Pseudomonas fluorescens (ATCC No. 13525), Glomus intraradices, and Trichoderma harzianum CIMAP-RPN01 (NCBI-KF157964), alone and in combination on the growth/yield of C. borivilianum along with their efficacy for protection against M. incognita infestation.

Materials and Methods
Plant materials and growth conditions
Healthy plant tubers of C. borivilianum were obtained from the National Germplasm Repository for Medicinal and Aromatic Plants at the Central Institute of Medicinal and Aromatic Plants (CSIR-CIMAP), Lucknow, India. The tubers were surface sterilized by soaking in 1% (v/v) sodium hypochlorite solution for 5 min, washed with distilled water and then planted in plastic pots filled with farm soil. The pots were kept in a growth chamber at 25 ± 2°C temperature and 80 ± 10% relative humidity. The plant were subjected to different treatments viz. control, T. harzianum, P. fluorescens, T. harzianum + P. fluorescens, T. harzianum + B. megaterium, P. fluorescens + B. megaterium.
water and were transplanted singly into clay pots (7.0 kg soil capacity), containing a mixture of autoclaved soil (70% sand, 8% silt, 16% clay, pH 7.7) and composted farm manure in 5:1 ratio. Tubers after six days were inoculated with 1000 J2-juveniles (Pi) of *M. incognita*. Plants were maintained in a glasshouse at 30±2°C and 13 h day length until the experiment was terminated six months after inoculation. Table 1 depicts the various treatments of biocontrol agents used in the experiment. These microbes had earlier shown better potentials for plant growth promotion through the substantial management of phytonematodes in various pot and field trials and were therefore chosen for the present study. Three replicates were established per treatment, and arranged in a completely randomized block design. The following three treatments serving as controls were also maintained: untreated - uninoculated, untreated - *M. incognita* inoculated and chemical treated (carbofuran @ 2 kg a.i. ha⁻¹) - inoculated. The microbial agents were applied four days before *M. incognita* inoculation. The treated pots were irrigated as needed to maintain soil moisture. There were three replicates per treatment, and the pots were arranged in a completely randomized block design. After six months tubers were harvested and their length, diameter, fresh weight and tuber number were determined. The soil and root samples from each treatment were processed by sieving and decanting method [16,17] to access the populations of *M. incognita* in soil and tuber samples. Roots were rated for galling severity according to Krusberg and Nielson [18] on a scale of 0-4.

### Table 1: Experimental lay-out for the glasshouse experiment.

| S.No. | Treatments | Experimental details |
|-------|------------|----------------------|
| 1     | T1         | Untreated- uninoculated control |
| 2     | T2         | Untreated- inoculated control |
| 3     | T3         | Chemical control (carbofuran) + *M. incognita* |
| 4     | T4         | *T. harzianum* + *M. incognita* |
| 5     | T5         | *G. intraradices* + *M. incognita* |
| 6     | T6         | *P. fluorescens* + *M. incognita* |
| 7     | T7         | *B. megaterium* + *M. incognita* |
| 8     | T8         | *T. harzianum* + *G. intraradices* + *M. incognita* |
| 9     | T9         | *T. harzianum* + *P. fluorescens* + *M. incognita* |
| 10    | T10        | *T. harzianum* + *B. megaterium* + *M. incognita* |

### Reference

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### Statistical analysis

Analysis of variance (ANOVA) techniques were used for the statistical analysis of data. Duncan multiple tests applicable to RCBD were performed by ASSISTAT software version 7.6 beta. Duncan’s test was performed to test the significant differences among treatments. Least Significant difference (LSD) was calculated at 5% probability level (P=0.05) for comparing the significance of difference between any two treatment mean (15).

### Results

The incidence of root knot nematode disease caused by *M. incognita* has earlier been reported [6]. The infested fleshy roots were generally deformed and large numbers of eggs and larvae were detected inside the fleshy roots. Figure 1A-1E depicts the devastating effect of *M. incognita* populations on *C. borivilianum* crop. Since tubers are the plant parts of economic interest, a definite awareness and serious efforts are required to manage *M. incognita* problem for economic stability of this crop.

### Growth promoting parameters

To estimate the beneficial effects of the symbiotic association (rhizospheric microbes and *C. borivilianum*), several plant growth promoting parameters viz. number of fleshy tubers, tuber length, tuber diameter and fresh tuber weight (g) were observed. Except for *G. intraradices* treatment, all the treatments, both singly and in
The combined treatment of *T. harzianum* with *P. fluorescens* (T9) was found to be effective for most of the observed parameters, significantly enhancing all the growth parameters. The treatment (T9) depicted best potentials for enhancing the number of tubers, tuber length and fresh tuber weight, recording an overall increase of 47.39, 43.61 and 62.94 percent respectively against the untreated- *M. incognita* inoculated control (Table 2). Furthermore, the same treatment was also found to be the most potential for the management of *M. incognita* populations. The combination treatment of *T. harzianum* and *P. fluorescens* showed maximum enhancement of growth parameters along with better management of *M. incognita* which suggests for the use of microbial consortia rather than single inoculation. When the main effects were significant, differences among factor levels were tested for significance using the mean sum of square at *P* = 0.05. All the treatments were statistically significant for the observed growth parameters as is evident by the MSS (mean sum of square) values (Table 2). The treatments exhibited a variable effect in single and combined treatments and the combination treatments were found to be more effective. A lower least significant difference (LSD) value at 5% probability (*P* >0.05) for all the observed growth parameters confirms the efficacy of the bio-inoculants for plant growth promotion as well as for *M. incognita* management (Figure 2a).

**Effect of bio-inoculants on the reproduction potential of *M. incognita***

Figure 2a represents the total *M. incognita* population (root + soil) in various treatments. All the treatments showed good reduction in *M. incognita* populations as compared to untreated- *M. incognita* inoculated control with the best reduction results observed in the combination treatment of *T. harzianum* with *P. fluorescens*. The single treatment of *G. intraradices*, although found to be effective for growth promotion effect, did not respond well for the control of *M. incognita* populations. Figure 2b shows the effect of microbial treatments on the reproduction factor (RF) of the root knot nematode. The results show that microbial agents completely counteract the negative impact of the nematode and reduce nematode population and root knot index, and were equally effective to carbofuran treatment.

**Discussion***

Medicinal and aromatic plants constitute a large segment of the flora, which provide raw materials for, cosmetic, fragrance and pharmaceutical industries. *C. borivilianum*, commonly christened as "Safed Musli" because of the white milky texture of its tubers after peeling, is a threatened/ endangered plant species in India. The fibrous roots of the plant are modified into fascicular roots (tubers) comprising the part of economic importance since its tubers are widely used as an important ingredient in the herbal Ayurvedic tonics. *C. borivilianum* is an important medicinal plant well known for its therapeutic potentials as aphrodisiac, immuno-modulator, adaptogen and antidiabetic.
The infestation of root knot nematode, *M. incognita* is a major yield constraint and visible threat to the effective commercialization of this valuable crop. Therefore, reliable and advanced information on the economic importance of this plant is needed to ensure that the developmental policies and investments take account on full costs and benefits of alternative land uses for economical purposes. The awareness for pests and pathogens, cultivation practices and proper orientation of this plant should be given major priority.

Plant parasitic nematodes cause great losses to the agricultural economy (estimated to be approximately 12% of the agricultural production), representing about 100 billion dollars in annual damage worldwide [22,23]. The intricate association of plant parasitic nematodes causes significant damage to almost all the medicinal plants studied till date [24,25]. The magnitude of crop damage due to nematode–plant interactions has established *M. incognita* as the most important plant parasitic nematodes limiting production, with infestations reported on *Mentha arvensis*, *Ocimum sanctum*, Papaver somniferum, *Bacopa monnieri*, Withania somnifera and *Artemisia annua* [26,27]. The present study provides an insight to the extent of damage caused due to *M. incognita* in *C. borivilianum*. The study also evaluates the rhizospheric microbes for the successful and efficient management of this phytopathogen. *M. incognita*, being a sedentary endoparasite of root, causes maximum damage to the plant roots, thereby having a significantly negative impact on the agricultural economy of the crop.

*C. borivilianum*, an important medicinal herb, is reported to be a susceptible host for *M. incognita* [28,6]. The root knot infested plants are stunted with smaller and chlorotic leaves, fewer rootlets and root-hairs and the roots bear small knots/galls. *M. incognita* infestation results in unthrifty growth and yellowing of leaves due to the reduced uptake of nutrients such as P, K, Mn and Cu etc. [26]. The complete eradication of *M. incognita* from agricultural soils is nearly impossible due to the polyphagus nature of this pest. In the approaches to the eradication of *M. incognita* populations and at the same time were also beneficial in enhancing the plant growth parameters in *C. borivilianum*. It was observed that all the microbial treatments, except for the *G. intraradices* treatment, were capable enough for the suppression of *M. incognita* populations. Our study emphasizes the use of rhizospheric microbes which apart from providing benefits of supporting better plant growth/ yield also reduces losses from root-knot nematode infestation in crop plants. Present results presume potential importance in developing new biocontrol based root-knot nematode management technology in the era of environment friendly organic farming. With this experiment as primary step, further study is needed to develop microbial consortium for commercially grown medicinal plants.
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