Journal of Plant Biochemistry & Physiology
ISSN: 2329-9029

Pandey and Saikia, J Plant Biochem Physiol 2014, 2:1
DOI: 10.4172/2329-9029.1000123

Research Article Open Access

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. fluoresces* 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, arthiritis, as a curative for natal and post-natal problems, in the treatment of diarrhea, dysentery, gonorrhea and leucorrhea [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. orchidastrum* 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. borvillianum* 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

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Received January 20, 2014; **Accepted** February 26, 2014; **Published** March 02, 2014

Citation: Pandey R, Saikia SK (2014) Rhizospheric Bioweapons for Tuber Yield Enhancement in *Chlorophytum Borivilianum* against *Meloidogyne Incognita* Infestation. J Plant Biochem Physiol 2: 123. doi:10.4172/2329-9029.1000123

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*

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

Figure 1: (A-E): Effect of M. incognita infestation on *C. borivilianum* tubers. shows the healthy plant tubers without any tissue loss whereas Figure 1(B-E) depict the effect *M. incognita* populations, with increasing period of infestation on the plant tubers. The extent of *M. incognita infestation* is shown by the complete loss of the plant tubers after four weeks of infestation (Figure 1E).

water and were transplanted singly into clay pots (7.0 kg soil capacity), containing a mixture of autoclaved soil (76% 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 @ 2kg 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.

Nematode isolation

The population of *M. incognita* was maintained on brinjal (*Solanum melongena L.*) grown in sterilized loamy-sand soil in earthen pots under glasshouse conditions. Mature egg masses were hand-picked from the roots, allowed to hatch and then juveniles (J2) were separated into a tray [19]. Populations of *M. incognita* were obtained by isolating from soil using Cobb's decanting and sieving technique followed by Baermann funnel method [20].

Culture and maintenance of bio control agents

Microbial cultures viz. *T. harzianum*, *B. megaterium, P. fluorescens* and *G. intraradices* are regularly maintained in the Microbial Technology and Nematology Department, CSIR-CIMAP, Lucknow. The fungal biocontrol agent was cultured using sand maize media while the bacterial isolates were cultured in Luria broth. For multiplication, the fungal cultures were kept in a BOD incubator for 96 h at 30 ± 1˚C and the bacterial cultures were placed on a rotary shaker at 28 ± 1˚C for 48 h at 200 rpm orbital shaking conditions. The fungal mycelial mat, after incubation and mass multiplication in the previously mentioned media was homogenized and suspended in 500 mL of 0.1 M phosphate buffer (K_2HPO_4; KH_2PO_4) at 1.2 x 10^6 colony forming units (CFU) mL^{-1}. The bacterial cultures, *B. megaterium* and *P. fluorescens*, as luria broth suspensions were centrifuged at 6000g for 10 min. The supernatant was discarded and the pellet containing bacterial cells was suspended in 500 mL of 0.9% saline to a final density of 2.3 x 10^8 (CFU) mL^{-1} for *B. megaterium* and 2.9 x10^8 (CFU) mL^{-1} for *P. fluorescens*. *G. intraradices* inoculum was propagated on maize roots (*Zea mays*) for 10 weeks in a 1:1 (v/v) mixture of sterilized sand and soil (5 kg) of low phosphorus content (7.5 kg ha^{-1}) and subsequently left to shade-dry for 2 weeks. The inoculum was based on root fragments colonized (70%) with *G. intraradices* and the sand–soil fraction with AM fungus propagules (spores and mycelium) from dry maize pot culture [21]. The roots in the pot culture were extracted from the soil, cut into 1 cm segments and thoroughly mixed with the sand–soil mix from the pot culture and stored at 5˚C until use. The inoculums potential (potential of a specific amount of inoculum to cause root infection under a standard set of conditions) of *G. intraradices* used in this study was 8.9 ± 1.3 infecting propagules g^{-1} sand-soil mixture. Bacterial agents were inoculated as 10 mL of 10^8 CFU/pot and the fungal isolate as 10 mL of 10^6 CFU/pot. Inoculums of arbuscular *mycorrhizal* fungi, G. *intraradices* used in the present experiment consisted of soil containing spores (8-10 spores g^{-1}).

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

S.No.	Treatments	Fleshy roots (No.)	Root Length (cm)	Root diameter (mm)	Fresh tuber weight (g)
1	T1	6.33^{e}	13.00^{c}	8.77^{f}	72.60^{g}
2	T2	3.67^{f} (-42.02)**	7.33 (-43.61)**	6.57^{g} (-25.08)**	38.87^{h} (-46.55)**
3	T3	6.67^{de} (+5.37)	11.00^{f} (-15.38)	9.00^{f} (+2.62)	79.33^{e} (+9.26)
4	T4	7.67^{bc} (+21.16)	13.67^{c} (+5.15)	10.70^{d} (+22.0)	91.50^{c} (+26.03)
5	T5	6.67^{de} (+5.37)	11.67^{f} (-10.23)	9.87^{e} (+12.54)	74.93^{f} (+3.20)
6	T6	7.33^{cd} (+15.79)	14.33^{c} (+10.23)	11.40^{c} (+29.98)	88.00^{d} (+21.21)
7	T7	6.67^{de} (+5.37)	13.00^{c}	10.20^{de} (+16.3)	80.93^{e} (+11.47)
8	T8	8.33^{ab} (+31.59)	14.33^{c}(+10.23)	13.13^{a} (+49.71)	99.17^{b} (+36.59)
9	T9	9.33^{a} (+47.39)	18.67^{a} (+43.61)	12.50^{b} (+42.53)	118.30^{a} (+62.94)
10	T10	8.67^{ab} (+36.96)	17.00^{b}(+30.76)	11.83^{c} (+34.89)	100.33^{b} (+38.19)
11	CV%	8.09	5.78	3.44	1.25
12	LSD	0.98	1.32	0.61	1.80
13	MSS (P < 0.05)	66.80	265.20	104.02	12073.01

*The Duncan Test at a level of 1% of probability was applied. The averages followed by the same letter do not differ statistically between themselves.
** Values in parentheses indicate percentage reduction over Untreated-Uninoculated control. Each value is an average of three replicates.

Table 2: Effect of different treatments on the plant growth parameters.

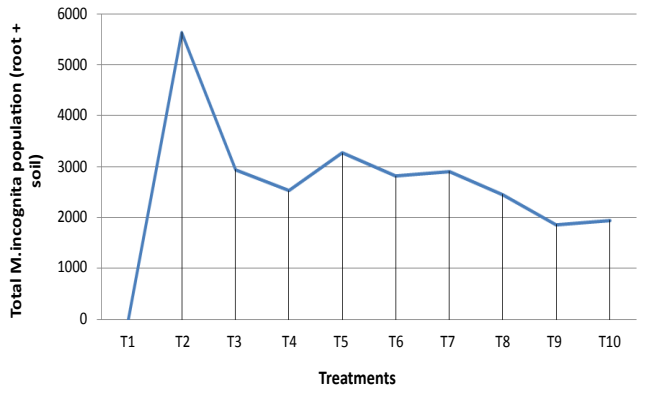

Figure 2a: Influence of different treatments on total population of M. incognita in *C. borivilianum.*

combinations were able to manage the *M. incognita* populations in an effective manner and thereby increasing the plant growth. Among the single treatments, *T. harzianum* (T4) showed better results for the parameters like number of tubers and tuber weight, depicting a marked enhancement of 21.16 and 26.03% respectively over the untreated- *M. incognita* inoculated control. The treatment (T6) having *P. fluorescens* inoculations showed better response for enhancing the tuber length (10.23%) and tuber diameter (29.98%) against the inoculated control.

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 potent 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 statement 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 affectivity 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.

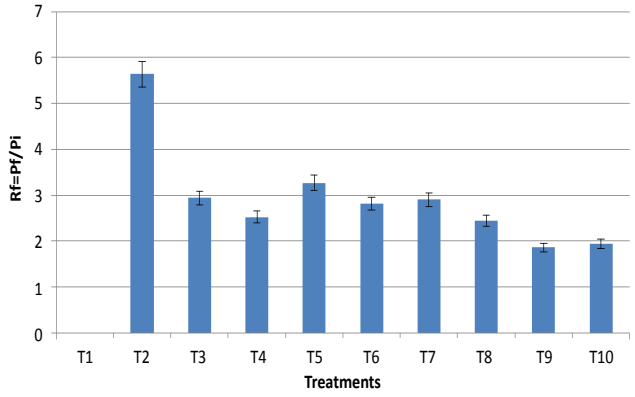

Figure 2b: Effect of different treatments on reproduction factor of *M. incognit.*

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 nematode parasites limiting production, with infestations reported on *Mentha arvensis*, *Ocimum sanctum*, *Papaver somniferum*, *Bacopa monnieri*, *Withania somnifera* and *Artemesia 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, Zn, 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 management of nematodes, the use of chemical nematicides is prevalent, and carbofuran is the only effective nematicide available in market till date. These synthetic nematicides besides rising the costs of production also presents risks to the environment and non-target organisms [29]. For this reasons, alternative methods of control are the need of the hour [30]. In this sense, the application of microbial inoculants as biocontrol agents can represent the substitution of conventional pesticides and become an alternative method to the nematodes control [14].

Rhizospheric microorganisms prove to be a prospective source of new natural products for exploitation in agriculture and are recognized as a promising group in requisites of diversity and pharmaceutical potential [31]. In the present study, rhizospheric microorganisms with potent plant beneficial activities were used in single/ combined treatments for the crop boost-up in *C. borivilianum*. The microbes were also evaluated for their biocontrol potentials against *M. incognita* and *T. harzianum* was found to be the most efficient single treatment in controlling the *M. incognita* populations. The combination treatments for *T. harzianum with P. fluorescens* (T9) *and T. harzianum* with *B. megaterium* (T10) however, were having a better biocontrol potential than single treatment inoculations (Figure 2a and 2b). Biocontrol activity or the reduction of inoculum density of a pathogen by the microorganisms is achieved by mechanisms viz. competition, hyperparasitism, induced resistance etc. In this study, *Trichoderma* sp. chiefly operate by mechanisms such as mycoparasitism and production of volatile and non-volatile antibiotics. At CIMAP, earlier our group has identified a strain of *T. harzianum* that has been used to produce a disease-free, healthy nursery with a reduction in root-knot nematode infection and improving the growth and yields of *M. arvensis* [32].

Rhizospheric microbes destroy nematodes continuously in virtually all soils because of their constant association with nematodes in the rhizosphere. A large number of bacteria viz. *Rhizobium* sp., *Bradyrhizobium japonicum*, *Pseudomonas* (*P. fluorescens and P. aeruginosa*) and *Bacillus* (*B. subtilis*) have shown antagonistic effects against nematodes. Several microbial mechanisms such as phenolic compounds, organic acids and secondary metabolites [28,33] may be involved in the suppression of nematode soil populations and enhancement of plant growth and yield. This study revealed a drastic reduction in RKI and increase in total weight of the plant. Except for the treatments having *G. intraradices*, either in single or dual combinations, all the treatments were able to reduce the disease incidence in *C. borivilianum*. The combination treatment of *T. harzianum with P. fluorescens* (T9) showed the best biocontrol effect, suggesting for the use of microbial consortia for better management of *M. incognita* and plant growth promotion.

The present experimentation evaluates the effect of microbial bioinoculants on the growth and yield attributes of Safed Musli *(C. borivilianum)*. The study demonstrates that microorganisms greatly alter the growth/ yield parameters and also possess effective root-knot nematode suppression potentials. Results from the present experiment demonstrate the nematicidal potential of the biocontrol agents tested, especially *T. harzianum* and *P. fluorescens*, which resulted in increased tuber yield of *C. borivilianum*. Rhizospheric microbes, especially in combination treatments were found to be efficient in the management 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.

Acknowledgment

The authors are thankful to the Director CSIR-CIMAP, Lucknow, India for his constant encouragement and support during the course of the investigation.

References

1. Yadava, A, Pandey R (2004) *Chlorophytum borivillianum* Baker: A potent medicinal plant. Indian Forest 130:340–342.
2. Purohit SD, Dave A, Tiagi YD (1994) *Chlorophytum borivlianum* Sant. and Fern. An (Liliaceae) interesting species from the Aravallis in Rajasthan. Rheedea 4:113-115.
3. Oudhia P (2001) Problems perceived by safed moosli (*Chlorophytum borivillianum*) growers of Chhattisgarh (India) region: a study. J. Med. Aromatic Plant Sci. 22/23: 396-399.
4. Kaur R, Arora S, Thukral AK (2009) Enhancing seed germination of Chlorophytum borivilianum Sant. Et Fernand. With PGRs, steroidal hormones and Zinc. Res. J. Seed Sci. 2: 32-39.
5. Bordia PC, Joshi A, Simlot MM (1995) "Safed Musli" in Advances in Horticulture: Medicinal and Aromatic Plants. Malhotra Publishing House: New Delhi, pp. 440-449
6. Pandey R (2003) Root-Knot disease of Safed musli. Ind. J. Nematol. 33: 64.
7. Kothari SK, Singh K (2004) Safed musli (*Chlorophytum borivillianum*) revisited. J. Med. Aromatic Plant Sci 26:60-63.

8. Khanam Z1, Singh O, Singh R, Bhat IU (2013) Safed musli (*Chlorophytum borivilianum*): a review of its botany, ethnopharmacology and phytochemistry. J Ethnopharmacol 150: 421-441.
9. Thakur GS1, Bag M, Sanodiya BS, Debnath M, Zacharia A, et al. (2009) Chlorophytum borivilianum: a white gold for biopharmaceuticals and neutraceuticals. Curr Pharm Biotechnol 10: 650-666.
10. Nayar MP, Sastry AR (1988) Chlorophytum borivillianum, in M.P., Red Data Book of Indian Plants, Botanical Survey of India: Calcutta, India, 2, pp. 375-378.
11. Narasimham KRSL, Ravuru BK (2003) A note on some endangered medicinal plants as NTFPs of Eastern Ghats, Andhra Pradesh. Eptri Envis Newsletter 9:11-12.
12. Wahla V1, Maheshwari DK, Bajpai VK (2012) Nematicidal fluorescent pseudomonads for the in vitro and in vivo suppression of root knot (Meloidogyne incognita) of *Capsicum annuum L*. Pest Manag Sci 68: 1148-1155.
13. Dong LQ, Zhang KQ (2006) Microbial control of plant-parasitic nematodes: a five-party interaction. Plant Soil. 288: 31-45.
14. Saikia SK, Tiwari S, Pandey R (2013) Rhizospheric biological weapons for growth enhancement and Meloidogyne incognita management in Withania somnifera cv. Poshita. Biol. Cont. 65:225–234.
15. Almaghrabi OA1, Massoud SI, Abdelmoneim TS (2013) Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. Saudi J Biol Sci 20: 57-61.
16. Cobb NA (1918) Estimating the nema population of the soil; Agric. Tech. Bur. Pl. Ind. U.S. Dep. Agric, No. 1, pp.48.
17. Southey JF (1985) Laboratory methods for work with plant and soil nematodes. Mins. Agric. Fish. Food HMSO, London pp 202.
18. Krusberg LR, Nielsen LW (1958) Pathogenesis of root-knot nematodes to Porto Rico variety of sweet potato. Phytopathol 48:30-39.
19. Sasser JN, Freckman DW (1987) a world perspective on nematology: the role of the society. In: Veech J Dickson DW (ed.) Vistas on Nematol. 7-14.
20. Baermann G (1917) Eine einfache Methode zur affindung von Ankylostomum (Nematoden) Larven in Endproben. Geneesak. Tijdschr.Nederl. Indie 57:131-137.
21. Bagyaraj DJ (1991) Ecology of vesicular arbuscular mycorrhiza. In: Hand book of Applied Mycology-Soil and Plants, Vol. I Eds.
22. Chitwood DJ (2002) Phytochemical based strategies for nematode control. Annu Rev Phytopathol 40: 221-249.
23. Liu QL1, Thomas VP, Williamson VM (2007) Meiotic parthenogenesis in a root-knot nematode results in rapid genomic homozygosity. Genetics 176: 1483-1490.
24. Sharma P, Pandey R (2003) Biological control of root-knot nematode, Meloidogyne incognita in the medicinal plant Withania somnifera and the effect of biocontrol agents on plant growth. Afr. J. Agricul. Res. 4: 564-567.
25. Saikia SK, Tiwari S, Pandey R (2012) Rhizospheric innovations for growth enhancement and Meloidogyne incognita management in *Mentha arvensis cv*. Kosi. Int. J. Environ. Sci. Eng. Res. 3:26-34.
26. Pandey R, Gupta A, Singh HN, Kalra A (2009) Phytonematode management through Bacteria: An Underground Battle for existence In Recent Advances. Biopesticides Biotechnol Applications (Edited by J.K. Johri, NBRI, Lucknow), pp. 1-26.
27. Sikora RA, Niere B, Kimenju J (2003) Endophytic Microbial Biodiversity and Plant Nematode Management in African Agriculture. In: Neuenschwander P, Borgermeister C, Langewald J (Eds), Biological control in IPM systems in Africa. Cabi, U.K p-407.
28. Khan MR, Patra A, Panja BN, Chaudhuri S (2008) Identification of pathotype and pathogenicity of root knot nematode (Meloidogyne javanica) infecting safed musli (Chlorophytum borivillianum). Ann. Plant Protec. Sci. 16:542-544.
29. Campos VP, Souza JT, Souza RM (1998) Controle de fitonematoides por meio de bacterias. Revisao Anual de Patologia de Plantas - Passo Fundo pp-285-327.
30. Neves WS, Freitas LG, Dallemole-Giaretta R, Fabry CFS, Coutinho MM, et al. (2005) Atividade de extratos de alho (Allium sativum), mostarda (Brassica campestris) e pimenta malagueta (Capsicum frutescens) sobre eclosao de juvenis de Meloidogyne javanica. Nematologia Brasileira, 29: pp-273-278.
31. Masadeh B, Alten HV, Gisela GS, Sikora RA (2004) Biocontrol of root-knot nematodes using the arbuscular mycorrhizal fungus Glomus intraradices and the antagonist Trichoderma viride in two tomato cultivars differing in their suitability as hosts for the nematodes. J. Plant Dis. Prot. 111:322–333.
32. Kalra A, Singh HB, Pandey (1996) Strain of Trichoderma harzianum useful as nematode inhibitor, fungicide and plant growth promoter and a process for the isolation there of. United States Patent No. 6475772.
33. Spencer M, Ryu CM, Yang KY, Kim YC, Kloepper JW, et al. (2003) Induced defense in tobacco by Pseudomonas chlororaphis strain O6 involves at least the ethylene pathway. Physiol. Mol. Plant Pathol 63:27-34.