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PERFORMANCE OF PESTICIDE AND BIOPESTICIDE ON GROWTH, YIELD AND FORSKOLIN CONTENT IN *COLEUS FORSKOHLII* INFECTED WITH *MELOIDOGYNE INCOGNITA*

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Abstract

A microplot experiment was carried out for assessing the performance of fungal biological control agents and chemical pesticides/nematicides on growth, yield and forskolin content in root-knot nematode infected coleus plants. Among the tested treatments, *Trichoderma viride, Paecilomyces lilacinus, Glomus fasciculatum* and neem oil seed cake treated plants exhibited significantly outstanding performance on growth and reduced disease incidence as compared to the ones treated with chemical pesticides and other treatments including control. Forskohlii content of biopesticides, *T. viride, P. lilacinus* and biofertilizers, *G. fasciculatum* and neem oil seed cake was estimated much higher against each of the chemical pesticide treated plants. However, both the biopesticides (*T. viride* and *P. lilacinus*) and biofertilizers (*G. fasciculatum* and neem oil seed cake) were statistically at par, particularly, in respect to forskolin content.

Forskolin (7 beta-acetoxy-8, 13-epoxy-1 alpha, 6 beta, 9 alpha-trihydroxy-labd-14-ene-11one) is the main active ingredient in the Ayurvedic herb *C. forskohlii* belonging to mint family that mainly grows in subtropical regions of India. This compound has been extensively investigated and widely used as medicine for use in the treatment of allergics, respiratory problems, cardiovascular diseases, glaucoma, psoriasis, hypothyroidism and weight loss. Forskolin increase Cyclic AMP and appears to have additional actions that are due to its ability to alter a number of membrane transport proteins (www.advance.health.com/coleus.html).

Among the commercially valuable medicinal plants, *C. forskohlii* (Briq.) has an important place in pharmaceutical industries due to its medicinal value. However, the roots of coleus, the main sources of forskolin, are often very badly affected by several soil inhabitants causing severe yield loss (Rajendran & Vadivelu, 1990). Among the soil inhabitants, plant parasitic root-knot nematode is a major constraint along with other soil borne pathogenic microorganisms like fungi and bacteria. Root injury caused by root-knot nematode larval penetration as primary invader is known to enhance easy entry for fungal and bacterial, the secondary pathogens (Pitcher, 1965). Due to the adverse effect of chemical

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pesticides on plant, soil and human health, an alternative eco-friendly biological integrated disease management package is tested in which fungal bio-control agent based biopesticides are used as core component. The observations were recorded in respect to yield and also quality of forskolin.

Materials and Method

Processing plant material and chemicals: Healthy cuttings of coleus plants were procured from the Amity Herbal Garden, Manesar, Haryana. For preparation of root powder of coleus plants were uprooted, shade dried and powdered for further analysis. All the other chemicals used were of analytical grade and purchased from Qualigens Fine Chemicals Mumbai and SRL chemicals, India.

Experiment: A microplot experiment was designed to compare the different pesticides against root-knot nematode, *M. incognita* affected coleus plants. The experiments were carried out on soil infested with initial population of about 5 larvae (J_2) per gram soil in 3m x 2.5 m microplot using randomized block design (RBD) with three replications for each treatment. Seedlings were transplanted singly on ridges with 60 cm spacing between row to row and 30 cm spacing between plant to plant.

Prior to transplantation of seedlings raised on sterilized soil, bare root dip treatment was done of about 10-12 cm long cuttings of healthy coleus plants comprising 3-4 pairs of leaves, with fungal bioagents and chemical pesticides separately in accordance with the treatments for 30 minutes. For the bare root dip treatments, fungal bioagents and chemical pesticides were dissolved as per their doses in sterile water by thorough mixing. Two weeks prior to transplantation *G. fasciculatum* was mixed in the soil along with neem oil seed cake in their respective treatments as follows: 1) *T. viride* alone @10g w/l + N; 2) *P. lilacinus* @ 10g w/w + N; 3) *G. fasciculatum* + N; 4) Neem oil seed cake alone (NOSC) @ 2% w/w + N; 5) Furadan + N; 6) Phorate + N; 7) Trizophos + N; 8) Control -1 infested with *M. incognita*; 9) Control-2 without infestation of *M. incognita*.

Observations recorded in terms of plant growth, yield, forskohlii content and disease incidence including number of galls, egg-masses / plant, eggs / egg-mass and soil population after 160 days at termination of experiment. Cobb's Decanting and sieving method followed by a modified Baermann's funnel technique was used for estimation of soil population (Southey, 1970).

Biochemical analysis: Thin layer chromatography was performed with aluminum backed precoated plates of silica gel 60F254 (E. Merck, Darmstadt, Germany) in toluene: ethyl acetate (80:20, v/v) as mobile phase.

Anisaldehydesulphuric acid was used as the spray reagent. Melting point was checked with Toshniwal melting point apparatus (Mumbai, India) with 2 mg of sample in a melting point capillary tube. UV spectrum was recorded on Hitachi U-2001 Spectrometer (Tokyo, Japan). 0.8 mg of sample was dissolved in few ml of methanol by gentle shaking, the final volume was made up to 10 ml with methanol in a volumetric flask.

Extraction, isolation and estimation of forskohlii: 500 g of powdered stem material (particle size 100-1000Zm obtained by sieving) was extracted with chloroform (3 x 500 ml) under continuous stirring at room temperature (30° C ± 5°C) for 2 h for each extraction. The chloroform extract was filtered and concentrated to dryness under reduced pressure (474mbar) at 40° C to obtain 10.3 g of semisolid residue. A portion of this residue (2g) was chromatographed on 60 g of silica gel (230-400 mesh) packed as a slurry in toluene in a column (100 cm X 2.5 cm i.d.). It was eluted with toluene (100%), toluene: EtOAc (80:20). Fractions of toluene: EtOAc (80:20) were concentrated under reduced pressure (240-77 mbar) at 40° C to obtain a solid residue (500 mg). This solid residue on repeated crystallization with EtOAc: nhexane (1:15, v/v) yielded forskohlii (100 mg) (Dubey *et al.*, 1981; Schaneberg *et al.*, 2003).

Estimation of pesticide residues: The plant samples (5 g) were taken at harvest of the crops separately and extracted thrice with 150, 100 and 75 ml portion of acetone in a Waring blender, partitioned with 100, 75 and 50 ml portions of chloroform and cleaned up using charcoal florisil (1:3) column (32x2 cm), the chloroform extract was vacuum-evaporated to dryness at 40°C using a Buchi type rotary evaporator and dissolved in acetone for analysis.

Samples were analyzed on Hewlett Packard 6890 gas chromatograph equipped with flame photometric detector and Hp-5 (5% phenyl dimethylpoysiloxane) megabore column (15x0.53 mm id and 5µg film thickness). Temperature © of injection port was 260, detector 270 and column 180. The flow rates of nitrogen hydrogen and air were 60, 150 and 110 ml / min, respectively. The standard solution was prepared from technical grade furadan, phorate and trizophos (93.1, 93.4 to 93.1% purity, respectively) obtained from FMC Corporation, USA.

Results and Discussion

Effect on plant growth and forskolin content: The plants inoculated with 4 different biopesticides separately showed more vigorous growth than the 3 chemical pesticides (Table 1 and 2). Among different treatments, the highest plant growth was observed in *Trichoderma viride* treated plants followed by neem oil seed cake, *Paecilomyces lilacinus, Glomus fasciculatum,* furadan,

phorate and trizophos. The *Trichoderma* inoculated plants showed better performance as reported by Sukhada (1999). Maximum number of tubers, highest tuber length and highest weight of fresh and dry tubers was recorded in *T. viride* inoculated coleus plants as compared to other treatments. It may be attributed to the secretion of enzymes and plant growth hormones by *T. viride* in and around the rhizosphere of plants (Chet, 1987). Senthamarai *et al.*, (2006 a) also reported that fungal biocontrol agents show better performance over chemical pesticides on plant growth of coleus. The increased plant vigour with healthy root system in *T. viride* treatment was also observed in respect of forskohlii content in comparison to all others treatments which may be attributed to the hormonal effect on plants (Table 1). The application of *T. viride and G. fasciculatum* was, however, recorded almost at par in an increase of forskohlii over control (Earanna *et al.*, 2001). The present investigation thus confirmed the role of fungal biocontrol agents and *G. fasciculatum* in the improvement of both tuber yield and forskohlii content in *C. forskohlii* (Saleem *et al.*, 2005). Similar results were reported by Dharana *et al.*, (2006) for estimation of forskohlii in coleus plants treated with different biological control agents and found that all the biocontrol agents when used alone or in combination help in enhancing the growth and vigour of coleus plants to a great extent.

Effect on development of eggs, eggs / egg-masses and soil nematode population: Considering the effect of root-knot infected plants for each of the above treatments in coleus minimum no. of galls, no. of egg-masses/plants and no. of egg/egg-mass were recorded in *T. viride* treated root. Significant reduction was found in adult female and final nematode population in soil treated with *T. viride* (Table 2). Among all the treatments, both biopesticides and biofertilizers like plant vigour and forskohlii content exhibited better performance to manage the soil nematode population over the chemical pesticides.

The present investigation shows significant performance of the fungal bioagents, VA-Mycorrhiza and oil seed cake possessing antagonistic properties in reducing the nematode population while improving plant health. This experiment promises ideal management components against root-knot nematode infecting the coleus, would help in promoting organic farming of medicinal plants at growers' field. In support of present findings Boby & Bagyaraj (2003) reported several biocontrol agents including both fungal and bacterial to be most effective used alone and also in integration with *Glomus mosseae* or oil seed cake against the rot wilt complex infecting coleus plants while fungicide Emisan (0.2 %) was not as effective as the biocontrol agents in controlling the pathogen. Senthamarai *et al.*, (2006 b) also evaluated the effect of carbofuran on plant growth and yield of tubers of coleus. It is evident that the nematode acts as a plant growth inhibitor by forming galls near the vascular bundles in roots which is also supported by several histopathological and biochemical changes in plant

Table 1. Effect of bioinoculants and chemical pesticides on growth and yield of tubers and forskolin contents in *Coleus forskohlii*.

Treatments	Shoot		Root		Tuber length (cm)	Tubers / plant	Tuber yield (q/ha)		Forskolin yield (mg/plant)
	Length (cm)	Weight (g)	Length (cm)	Weight (g)			Fresh	Dry	
T-1	64 (8.1)	223 (14.9)	26 (5.1)	84 (9.2)	12 (3.5)	4.0 (2.11)	127 (11.2)	16.3 (4.1)	29.4 (5.2)
T-2	58 (7.6)	211 (14.5)	23(4.8)	73 (8.5)	8.0 (2.9)	3.0 (1.87)	124 (11.1)	14.6 (3.9)	22.3 (4.7)
T-3	62 (7.9)	216 (14.7)	25 (5.0)	80 (8.9)	10 10.3)	3.6 (2.04)	130 (11.4)	17.3 (4.2)	27.4 (5.2)
T-4	56 (7.5)	211 (14.5)	22 (4.7)	71 (8.4)	9.0 (3.0)	3.3 (1.95)	124 (11.1)	14.0 (3.8)	27.7 (5.3)
T-5	61 (7.8)	217 (14.7)	23 (4.8)	73 (8.6)	8.3 (3.0)	2.6 (1.77)	112 (10.6)	12.6 (3.6)	20.5 (4.5)
T-6	62 (7.8)	213 (14.6)	21(4.7)	71 (8.4)	7.6 (2.8)	2.3 (1.68)	111 (10.5)	12.3 (3.5)	19.3 (4.4)
T-7	63 (7.9)	215 (14.6)	23 (4.9)	73 (8.6)	8.0 (2.9)	2.3 (1.68)	111 (10.5)	12.6 (3.6)	19.9 (4.4)
T-8	24 (4.9)	181 (13.4)	14 (3.8)	97 (9.6)	6.0 (2.5)	1.0 (1.22)	116 (10.8)	12.6 (3.6)	12.5 (3.6)
T-9	65 (8.0)	218 (14.7)	21(4.6)	77 (8.8)	3.0 (3.0)	2.6 (1.78)	108 (10.4)	11 (10.8)	6.4 (4.1)
S.E	-	0.00	-	0.03	-	-	0.03	0.03	0.02
LSD (p 0.05)	0.15	0.10	0.20	0.29	0.28	0.28	0.08	0.15	0.19

Mean of 3 replications; Figure in parenthesis are sin transformed value n+0.5; T-1= *T. viride*; T-2= *P. lilacinus*; T-3= Neem oil seed cake; T-4= *G. fasciculatum*; T-5= Furadan; T-6= Phorate; T-7= Trizophos; T-8= Control; T-9= Normal control.

Table 2. Effect of bioinoculants and chemical pesticides on galls, eggs/egg-masses, egg-masses/plant and soil nematode population infecting coleus plants.

Treatment	Galls/plant	Eggs/egg-mass	Egg-masses/plant	Females/g root	Soil nematode population
T-1 (*Trichoderma viride*)	441.67 (21.03)	134.67(11.63)	40.0(6.36)	89.0 (9.46)	791.67(28.14)
T-2 (*Paecilomyces lilacinus*)	418 (20.46)	158.33 (10.07)	50.0 (7.11)	101 (10.07)	1263.33 (35.55)
T-3 (Neem oil seed cake)	431.33 (20.78)	150.33 (121.60)	45.0 (6.75)	96 (9.82)	1087.67 (32.98)
T-4 (*Glomus fasciculatum*)	422 (20.55)	181.33 (12.28)	52.0 (7.25)	110.67 (10.54)	1336.67 (36.56)
T-5 (Furadan)	496.3 (22.29)	171.67 (13.21)	48.0 (6.96)	116.67 (10.83)	1738.33 (41.70)
T-6 (Phorate)	466.33 (21.61)	175.67 (13.27)	54.0 (7.38)	121.67 (11.05)	2043.33 (45.19)
T-7 (Trizophos)	488 (22.11)	163.33 (12.80)	57.0 (7.66)	126.33 (11.26)	2323.67 (48.21)
T-8 (Control)	1823.33 (42.71)	277.0 (16.45)	116.33 (10.81)	263.67 (16.19)	7081.0 (84.14)
T-9 (Normal control)	0.00 (0.71)	0.0 (0.71)	0.00 (0.71)	0.0 (0.71)	0.00 (0.71)
Mean ±S.E	0.01	0.5	0.00	0.10	0.11
LSD (P=0.05)	0.15	0.26	0.10	0.20	1.39

Mean of 3 replications; Figures in parenthesis are sin transformed value n+0.5

cells through developing the giant cells in vascular regions (Jones & Northcote, 1972).

Quantity of pesticide residues: Among all the treatments, residual particles were estimated more (0.499 µm) of Phorate than Furadan (0.421 µm) and Triazophos (0.449 µm) while in biological treated plants shown nil content of pesticides. Concentration of residual particle of Phorate was very high in plants tissues as normal recommended limit while concentration of residual particle of Furadan and Trizophos in treated plants was estimated also slightly more of normal recommended concentration (Anon., 1991). Zoi *et al.*, (2008) estimated the some toxic chemicals in groundwater. Meher *et al.*, (2008) also estimated some toxic nematicides from apple fruits and plants which were also found with more concentration as compared to recommended concentration limit.

Besides the better performance of sustainable eco-friendly management components in yield gain of the forskohlii as compared to the toxic chemical pesticides (phorate and trizophos), the quality and the medicinal value is reported to be superior and less costly. It is, therefore, advisable to encourage use of eco-friendly and a sustainable management method with safe components instead of using toxic chemicals to avoid bad quality.

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