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Research Article

Effect of *Trichoderma harzianum* isolated from different agroclimatic zones of Karnataka on growth and biochemical parameters of *Nigella sativa* L. a medicinal plant

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ABSTRACT: *Trichoderma* species are the fungi that are present in nearly all soils and other diverse habitats. Among *Trichoderma* genera *Trichoderma harzianum* is both plant growth promoter as well as antagonist against many pathogenic fungi. An effort was made to isolate ten isolates of *T. harzianum* from soil samples collected from different agroclimatic zones of Karnataka. A total of ten *Trichoderma* isolates representing each agroclimatic zone was isolated using potato dextrose agar medium and purified. The colony morphology and microscopic observation confirmed that all isolates belongs to the *T. harzianum*. A pot culture experiment was carried out to study the effect of the ten strains of *T. harzianum* on the medicinal plant *Nigella sativa* L. under glasshouse conditions. Growth parameters and biochemical studies were recorded in *T. harzianum* treated *N. sativa* along with untreated plants. The results indicated that there was a significant increase in plant height (24.07 cm), number of branches (19.33), fresh and dry weight of the plants (1.296 and 0.219 g/plant, respectively) inoculated with strain SDT isolated from zone 6 as compared to uninoculated control plants (13.03 cm, 8.00, 0.307 and 0.055 g/plant, respectively). However among the ten strains inoculated, *T. harzianum* isolates from the zone 6 (SDT) and zone 5 (EDT) recorded the highest biochemical parameters and least were recorded in uninoculated control plants.

KEY WORDS: *Trichoderma harzianum,* agroclimatic zones*, Nigella sativa*

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INTRODUCTION

The geographical area of Karnataka is classified into ten agroclimatic zones, *viz*., north eastern transition zone (NET), north eastern dry zone (NED), northern dry zone (ND), central dry zone (CD), eastern dry zone (ED), southern dry zone (SD), southern transition zone (ST), northern transition zone (NT), hilly zone (H) and coastal zone (C). Each zone has its own characteristic feature in relation to climatic condition, soil type, vegetation etc., which has influence on the establishment of diversified flora and fauna (Table 1). *Trichoderma* is widely distributed all over the world and occur in nearly all soils and natural habitats, especially in those containing organic matter.

Trichoderma is a genus of asexually reproducing fungi that are often the most frequently isolated soil fungi. *Trichoderma* spp. is free-living fungi that are common in soil and root ecosystems. *Trichoderma* isolates grow quickly on many substrates, produce metabolites with demonstrable antibiotic activity, and may be mycoparasitic against a wide range of pathogens (Grondona *et al*., 1997). They release a variety of compounds that induce localized or systemic resistance responses, and this explains their lack of pathogenicity to plants. *Trichoderma* species are favored by the presence of high levels of plant roots, which they colonize readily. These root–microorganism associations cause substantial changes to the plant proteome and metabolism. Root colonization by *Trichoderma* spp. also frequently enhances root growth and development, crop productivity, resistance to abiotic stresses and the uptake and use of nutrients (Harman *et al*., 2004). India's biodiversity coupled with its vast natural resources like natural flora and fauna make our country ideal for growing medicinal plants for realizing in the world market. Varieties of medicinal plant resources are assuming importance because of growing demand for use of natural sources of drugs. *Nigella sativa* is a medicinal plant and is important in Ayurveda, Unani and Siddha system of medicine and is a spice cum medicinal plant, belonging to family Ranunculaceae. It is commonly called as black cumin, *Kalonji*, *Kalajira*, *Neelajeeiakaira*, *Karunjiragam*, *Karejirage,* etc. *N. sativa* seeds (Kalonji) have been widely used in traditional medicine as diuretic, anti-hypertensive liver tonic, digestive, antidiarrheal, and appetite stimulant, and emmenagogue, analgesic, anthelmintic, antibacterial and useful in skin disorders. Essential oil of *N. sativa* exhibited a strong antimicrobial activity against *Salmonella typhi*, *Pseudomonas aeruginosa, Shigella shigae*, *Pythium vexans*, *Rhizoctonia solani* and *Colletotrichum capsici.* Carvone present in the oil was suggested to be potent antimicrobial agent (Rathee *et al.*, 1982). The seeds of black cumin are beneficial for the digestive system, soothing stomach pains and spasms and easing wind, bloating and colic (Chevallier, 1996). Consequently, Kalonji has been extensively studied for its biological activities and has been shown to be antidiabetic, anticancer and immunomodulator, analgesic, antimicrobial, anti-inflammatory, spasmolytic, bronchodilator, hepatoprotective, antihypertensive, renal protective and antioxidant properties (Gilani *et al*., 2004).

However the information on the compatibility of *Trichoderma* spp. in localized soil conditions in promoting growth of plants and biochemical characterization of plant growth promoting fungi isolated from different soil types of different zones is very much limited. The present studies were, hence, undertaken to study the diversity of *T. harzianum* in different agroclimatic zones of Karnataka and further selecting the best strain which enhances growth and biomass of the medicinal plant *N. sativa* L.

MATERIAL AND METHODS

Isolation of *T. harzianum*

Trichoderma harzianum was isolated by serial dilution from soil samples collected from ten different agroclimatic zones of Karnataka (Table 1). Isolation and maintenance of each of the isolate was done on potato dextrose agar medium. Each isolate was identified as *T. harzianum* by conducting the test according to specific characters described in 'The manual of soil fungi' (Gilman, 1961).

Pot culture experiment

Ten *T. harzianum* strains isolated from different agroclimatic zones of Karnataka were grown separately, in a 250 ml flask containing 100ml potato dextrose broth for 2 days. The grown cultures were homogenized and 15 ml of each of the solution was inoculated to each pot. Two week old seedlings were transplanted to pots containing sterile sand: soil mixture (1:1 w/w). One plant per pot was maintained with three replications per each

Table 1. Characteristic features of agro-climatic zones of Karnataka

Zone	Name	Soil type	Annual Rainfall (mm)	Temperature (°C)	Humidity %	Sand %	Silt %	Clay %	pH %	CEC Centi-mols
1	North Eastern Transition Zone	Laterite soil	860	31.1–20.7	65.0	55.7	10.1	34.2	8.07	30.00
2	North Eastern Dry Zone	Sandy clay loam	526–754	22.3–33.5	63.0	14.3	24.7	61.0	8.5	63.00
3	Northern Dry Zone	Clay loam	585	32.4–21.9	67.0	17.86	17.98	64.16	9.17	44.68
4	Central Dry Zone to Black soil	Red sandy loam	456–717	30.8–20.7	73.0	34.66	28.96	36.38	9.54	47.82
5	Eastern Dry Zone	Red sandy soil	674–889	29.2–18.6	71.0	72.97	4.93	22.1 6.21	5.31–	16.5
6	Southern Dry Zone	Red sandy soil	670.6–888.6	29.1 (max)	61.6	71.45	4.62	23.93	6.72	15.5
7	Southern Transition Zone	Red sandy loam	619–1300	30–19.2	81.0	76.17	5.86	17.94	5.8	–
8	Northern Transition Zone	Black soil	780	30.1–18.0	76.0	16.35	36.38	57.27 7.85	6.72-	64.0
9	Hilly Zone Sandy loam	Red loam to Red	904–3695	25.2–16.6	89.0	69.7	20.5	9.79	5.3	20.5
10	Coastal Zone	Lateritic	4000	30.5-23.5	96.5	48.46	12.76	38.78	5.2	25.56

*Source: Department of Soil Science, GKVK, UAS, Bangalore treatment. All the treatments were inoculated with respective isolates along with one uninoculated control. The plants were grown for 90 days and observations with respect to germination, plant height, and number of branches, fresh and dry weights of seedlings was recorded at different intervals. Observations on biochemical parameters which include chlorophyll content, nitrogen, phosphorous, total sugars, reducing sugars and total soluble proteins were recorded at 90 days after transplanting (DAT).

Treatments

T0 – uninoculated control, T1 – north eastern transition zone *T. harzianum* (NETT), T2 – north eastern dry zone *T. harzianum* (NEDT), T3 – northern dry zone *T. harzianum* (NDT), T4 – central dry zone *T. harzianum* (CDT), T5 – eastern dry zone *T. harzianum* (EDT), T6 – southern dry zone *T. harzianum* (SDT), T7 – southern transition zone *T. harzianum* (STT), T8 – northern transition zone *T. harzianum* (NTT),T9 – hilly zone *T. harzianum* (HT), T10 – coastal zone *T. harzianum* (CT).

Plant growth and biochemical parameters

The germination test was conducted by inoculating each *T. harzianum* strain to the *N. sativa* seeds placed on sterile blotting paper in petridish and incubated for 7 days in dark. Observations were recorded on radicle and plumule lengths. From pot culture experiment, at 90 DAT the height of seedlings was measured. Fresh weight was recorded, dry weight of the seedlings were determined after drying them in an oven at 65°C for 72 hours. The chlorophyll content was estimated by employing dimethyl sulfoxide method (Hiscox and Israelstam, 1979). *N. sativa* seedlings were dried at 70°C and made into fine powder. This powder was used for analyzing other biochemical parameters. Analysis of total nitrogen was done by Microjeldhal method (AOAC, 1980), phosphorus by Vanado-molybdo-phosphoric yellow color method (Jackson, 1973), total sugars by phenol sulphuric acid method (Dubios *et al*., 1951) and total reducing sugars by Nelson and Somogyi (1944 and 1952). The total soluble protein estimation was done using fresh plant sample as described by Lowry *et al*. (1951).

Statistical analysis

The data obtained in the pot experiments were subjected to one way analysis of variance using MSTAT-C software.

RESULTS AND DISCUSSION

Isolation and identification

Isolation of *T. harzianum* was done from soil collected from ten agro-climatic zones of Karnataka by growing in potato dextrose agar media by serial dilution plate method. For isolation, preliminary identification was carried out by morphological observations of the fungal colonies such as color, mycelial growth pattern, color of the spores, etc. All the check isolates and the standard strains formed white fungal colonies initially and turned to complete dark green color after sporulation due to color of the spores. All the isolates exhibited the typical spore arrangement on the conidial head as that of the standard reference strain, in which the spores are arranged linearly on the conidial head and also spores. These tests were positive for the species belonging to the *T. harzianum* as described in'The Manual of Soil Fungi' (Gilman, 1961).

Plant growth and biochemical parameters

The *T. harzianum* strains representing each agroclimatic zone of Karnataka along with uninoculated control were screened for their efficiency in improving the growth of medicinal plant *N. sativa*. The data in Table 2 reveal that in germination studies, *N. sativa* inoculated with the isolate of zone 1 (NETT) recorded maximum plumule length of 1.13cm, whereas the maximum radicle length of 9.67 cm was seen in isolate of zone 9 (HT). This suggested that the zone 1 (NETT) and zone 9 (HT) has the ability to enhance the early growth of *N. sativa*. This may be attributed to the increase in cell elongation and multiplication due to increased uptake of water since no photosynthesis occurs at this stage and the production of plant growth promoting substances and enzymes. These results draw considerable support from reports of Harman (2000), Harman (2001), Harman and Donzelli (2001), who treated maize seeds with T-22 strain of *Trichoderma* and observed maximum yield and found increase in root lengths.

During harvest time five plants from each replication was taken randomly to assess the growth parameters and results are presented in Table 2 and 3. The results were recorded at 30, 60 and 90 DAT (Days after transplanting) and the maximum plant height of 5.73 cm, 19.80 cm and 24.07 cm respectively were recorded in zone 6 (SDT) isolate treated plants, which was followed by zone 5 (EDT) of 5.43 cm, 17.20 cm and 23.27 cm, respectively, whereas the lowest plant height of 1.63 cm, 7.37 cm and 13.03 cm, respectively were recorded in control plant.

The highest number of branches of 5.0, 14.33 and 19.33 respectively at 30th, 60th and 90th DAT were recorded in zone 6 (SDT) isolate treated plants, which was followed by zone 5 (EDT) isolate (4.67, 14.0 and 18.33, respectively). The lowest number of branches was recorded in control (1.33, 6.0 and 8.0, respectively). This indicated that zone 6 (SDT) isolate was more efficient in enhancement of plant height and number of branches as

Table 2. Effect of inoculation of *Trichoderma harzianum* isolates on seed germination, plant height and number of branches of *Nigella sativa*

Treatments	Agroclimatic Zones	Isolate	Germination			Plant height (cm)			Number of branches		
			Plumule length (cm)	Radicle length (cm)	Total length (cm)	30 DAT	60 DAT	90 DAT	30 DAT	60 DAT	90 DAT
Control (T0)	–	–	0.40	0.77	1.17	1.63	7.37	13.03	1.33	6.00	8.00
T1	Zone–1	NETT	1.13	2.52	3.65	3.33	11.97	16.00	3.00	8.33	13.00
T2	Zone–2	NEDT	0.75	7.20	7.95	4.27	13.80	18.07	3.00	11.00	14.00
T3	Zone–3	NDT	0.94	7.13	8.07	4.43	15.37	19.80	3.67	11.00	17.00
T4	Zone–4	CDT	1.10	6.00	7.10	3.47	15.10	21.40	4.00	12.00	17.67
T5	Zone–5	EDT	0.93	5.90	6.83	5.43	17.20	23.27	4.67	14.00	18.33
T6	Zone–6	SDT	0.91	6.27	7.18	5.73	19.80	24.07	5.00	14.33	19.33
T7	Zone–7	STT	0.91	5.00	5.91	2.53	10.17	18.03	3.00	8.33	14.33
T8	Zone–8	NTT	0.78	5.67	6.45	5.10	17.83	22.30	3.00	8.00	10.00
T9	Zone–9	HT	1.03	9.67	10.70	3.57	10.33	17.37	3.00	9.00	13.33
T10	Zone–10	CT	0.77	8.90	9.67	2.83	12.53	16.87	3.67	10.00	14.00
SEM±			0.103	0.353	0.399	0.412	0.857	0.183	0.560	0.651	0.689
CD (P = 0.05)			0.303	1.036	1.170	1.207	2.513	0.538	1.641	1.910	2.021

DAT = days after transplanting

Table 3. Effect of inoculation of *Trichoderma harzianum* isolates on biomass of *Nigella sativa* seedlings

Treatments	Agroclimatic Zones	Isolate	Fresh weight		Dry weight		Total weight	
			Shoot (g/plant)	Root (g/plant)	Shoot (g/plant)	Root (g/plant)	Fresh wt (g/plant)	Dry wt (g/plant)
Control (T0)	–	–	0.277	0.030	0.043	0.012	0.307	0.055
T1	Zone–1	NETT	0.456	0.052	0.084	0.027	0.508	0.111
T2	Zone–2	NEDT	0.568	0.074	0.106	0.030	0.642	0.136
T3	Zone–3	NDT	0.635	0.115	0.110	0.042	0.750	0.152
T4	Zone–4	CDT	0.691	0.195	0.135	0.044	0.886	0.179
T5	Zone–5	EDT	0.696	0.207	0.137	0.049	0.903	0.186
T6	Zone–6	SDT	0.977	0.319	0.145	0.074	1.296	0.219
T7	Zone–7	STT	0.635	0.142	0.110	0.041	0.777	0.151
T8	Zone–8	NTT	0.424	0.050	0.082	0.025	0.474	0.107
T9	Zone–9	HT	0.385	0.047	0.088	0.028	0.432	0.116
T10	Zone–10	CT	0.539	0.066	0.095	0.030	0.605	0.125
SEM±			0.031	0.023	0.083	0.0034	0.049	0.011
CD (P = 0.05)			0.090	0.069	0.024	0.010	0.145	0.032

Table 4. Effect of inoculation of *Trichoderma harzianum* isolates on chlorophyll, nitrogen and phosphorus content of *Nigella sativa* seedlings

Treatments	Agroclimatic zones	Isolate	Chlorophyll (mg/g fw)	Chlorophyll (mg/g fw)	Total chlorophyll (mg/g fw)	Shoot Nitrogen (mg/plant)	Root Nitrogen (mg/plant)	Total Nitrogen (mg/plant)	Shoot Phosphorous (mg/plant)	Root Phosphorous (mg/plant)	Total phosphorous (mg/plant)
Control (T0)	–	–	0.693	0.413	1.106	0.292	0.166	0.458	0.007	0.002	0.009
T1	Zone–1	NETT	0.934	0.573	1.507	1.268	1.198	2.466	0.093	0.030	0.123
T2	Zone–2	NEDT	1.029	0.579	1.608	1.379	0.881	2.260	0.045	0.016	0.061
T3	Zone–3	NDT	0.770	0.454	1.224	2.998	1.886	4.884	0.085	0.027	0.112
T4	Zone–4	CDT	0.767	0.505	1.272	3.163	3.973	7.136*	0.025	0.007	0.032
T5	Zone–5	EDT	1.075	0.755	1.830	1.834	1.181	3.015	0.036	0.012	0.048
T6	Zone–6	SDT	1.048	0.583	1.631	2.101	1.694	3.795	0.097	0.050	0.147
T7	Zone–7	STT	0.796	0.437	1.233	1.046	0.657	1.703	0.025	0.008	0.033
T8	Zone–8	NTT	0.835	0.574	1.409	1.387	0.829	2.216	0.017	0.005	0.022
T9	Zone–9	HT	0.821	0.535	1.356	1.928	0.839	2.767	0.053	0.020	0.073
T10	Zone–10	CT	0.897	0.563	1.460	1.626	0.889	2.515	0.030	0.011	0.041
SEM±			0.017	0.006	0.021	0.132	0.136	0.245	0.004	0.002	0.006
CD (P = 0.05)			0.049	0.017	0.060	0.387	0.400	0.720	0.011	0.007	0.016

Table 5. Effect of inoculation of *Trichoderma harzianum* isolates on total sugars, reducing sugars and soluble protein content of *Nigella sativa* seedlings

Treatments	Agro-climatic Zones	Isolate	Total sugar (mg/plant)			Reducing sugar (mg/plant)			Total soluble protein (mg/plant)		
			Shoot	Root	Total	Shoot	Root	Total	Shoot	Root	Total
Control (T0)	–	–	0.390	0.022	0.412	0.321	0.034	0.355	3.883	0.062	3.945
T1	Zone–1	NETT	1.393	0.295	1.688	0.906	0.141	1.047	9.560	0.344	9.904
T2	Zone–2	NEDT	1.208	0.287	1.495	1.161	0.259	1.420	12.552	1.433	13.985
T3	Zone–3	NDT	1.668	0.370	2.038	1.281	0.194	1.475	13.360	1.286	14.646
T4	Zone–4	CDT	1.010	0.208	1.218	0.856	0.117	0.973	11.343	0.517	11.860
T5	Zone–5	EDT	1.923	0.225	2.148	1.639	0.184	1.823	18.20	4.932	23.132*
T6	Zone–6	SDT	2.536	0.351	2.887*	1.301	0.545	1.846*	13.944	1.170	15.114
T7	Zone–7	STT	0.900	0.212	1.112	0.815	0.128	0.943	9.492	0.320	9.812
T8	Zone–8	NTT	0.894	0.171	1.065	0.893	0.147	1.040	8.151	0.224	8.375
T9	Zone–9	HT	1.264	0.117	1.381	1.167	0.204	1.371	18.06	1.060	19.12
T10	Zone–10	CT	1.164	0.277	1.441	1.039	0.192	1.231	6.836	0.205	7.041
SEM±			0.084	0.021	0.099	0.082	0.026	0.101	0.650	0.199	0.799
CD (P = 0.05)			0.246	0.061	0.289	0.024	0.075	0.297	1.910	0.583	2.344

compared to others. Increase in the plant height and number of branches of crop plants due to inoculation of *T. harzianum* and other plant growth promoters has been reported by several workers (Yedidia *et al*., 1999; Earanna *et al*., 2001; Guojing *et al*., 2001; Sailo and Bagyaraj, 2006; Sharma and Haseeb, 2006). Increased cell elongation and multiplication due to the enhanced uptake of mineral nutrients (Yedidia *et al*., 2001), production of plant growth substances and enzymes by *T. harzianum* may be the cause for increased plant height and number of branches.

Among different treatments, the zone 6 (SDT) isolate treatment recorded significantly maximum total biomass of 1.296 and 0.219 g/plant fresh and dry weights respectively at 90 DAT, which was followed by zone 5th (EDT) (0.903 and 0.186 g/plant fresh and dry weight, respectively) (Table 3). The lowest total biomass was recorded in control (0.307 and 0.055 g/plant fresh and dry weight, respectively). Increased biomass may be due to enhanced plant growth, number of leaves and branches, increase in root growth which was influenced probably by greater availability of mineral nutrients, plant growth promoting substances and enzymes. Similar result on medicinal plant was reported by Earanna *et al.* (2001), who studied the effect of *T. harzianum* singly and in combination of other PGPR (plant growth promoting rhizomicroorganisms). They reported that there was significant increase in number of leaves, branches as well as total biomass in *Coleus aromaticus*. Arpana (2000) studied influence of *Glomus mosseae* and *T. harzianum* on medicinal plant Kalmegh. The results showed maximum biomass of treated plants as compared to control.

The data on biochemical parameters is presented in Table 4. Significant increase in chlorophyll a, chlorophyll b and total chlorophyll content was recorded in plants inoculated with zone 5 (EDT) which recorded 1.075, 0.755 and 1.830 mg/g fw (fresh weight) of leaves respectively and lowest was recorded in control (0.693, 0.413 and 1.106 mg/g fw of leaves, respectively). This may be due to the increased uptake of nutrients, which resulted in higher chlorophyll content in treated plants as compared to control. Similar result was reported by Inbar *et al.* (1994) who studied effect of *T. harzianum* applied to cucumber and pepper seedling and found higher chlorophyll content in treated plants as compared to uninoculated plants.

Table 4 shows that *N. sativa* plants inoculated with zone 5 (CDT) isolate recorded the highest nitrogen content of 7.136 mg/plant, which was followed by zone 3 (NDT) and zone 6 (SDT) inoculated plants which gave 4.884 and 3.795 mg/plant respectively. The lowest nitrogen content of 0.458 mg/plant was noticed in untreated control. Highest phosphorous content of 0.147 mg/plant was recorded in *N. sativa* plants inoculated with zone 6 (SDT) isolate, which was followed by zone 1 (NETT) (0.123 mg/plant) and zone 3 (NDT) (0.112 mg/plant). The lowest phosphorous was recorded in untreated control (0.009 mg/plant), which was significantly different between control and treated plants. The increase in nitrogen and phosphorus may be attributed to increase in uptake of nutrients and plant growth promoting substances. These results could be due to increased root proliferation and increase in surface area for absorption of phosphorus from soil solution and transporting it to roots (Yedidia *et al.*, 2001). Similar observation was noticed by Abbot and Robson (1982) by inoculation of AM fungi on plant nutrient uptake.

The data on total soluble sugars, reducing sugars and total soluble proteins is presented in Table 5. The highest total soluble sugar content was recorded in zone 6 (SDT) inoculated plants of 2.887 mg/plant, which was followed by zone 5 (EDT) and zone 3 (NDT) inoculated plants which gave 2.148 mg/plant and 2.038 mg/plant respectively. The control plants recorded only 0.412 mg/plant among all other treatments. The highest reducing sugars (1.846 mg/plant) was recorded in plants inoculated with zone 6 (SDT), followed by zone 5 (EDT) and zone 3 (NDT) isolates which recorded 1.823 mg/plant and 1.475 mg/plant respectively. The lowest reducing sugar (0.355 mg/plant) was recorded in control. This may be attributed to increased number of leaves and chlorophyll content of leaves which leads to increase in photosynthetic rates of inoculated plants. Similar results were observed in Eucalyptus, inoculated with mycorrhizal fungi (Govindasamy, 2003). In tomato, inoculated with mycorrhizal fungus (Suresh and Bagyaraj, 1984; Shivakumara, 2002) similar results were found.

N. sativa inoculated with zone 5 (EDT) isolate showed highest total soluble proteins of 23.132 mg/plant (Table 5), which was followed by plants inoculated with zone 9 (HT) and zone 6 (SDT) isolates (19.12 mg/plant and 15.114 mg/plant, respectively). The uninoculated plants showed least amount of total soluble proteins (3.945 mg/plant). The reason may be attributed to increase in more nutrient uptake and more chlorophyll content of leaves, which leads to increase in photosynthetic rates. Similar results were observed by Gupta *et al.* (1995) who reported increased total soluble proteins (34 to 54μg ml^{-1}) in *Ocimum carnosum* applied with Triacontanol, a microbial inoculant of *Trichoderma* as foliar spray which was associated to promotory effect of Triacontanol on this crop and by stimulation of the enzyme activity associated with protein metabolism. Similarly Vinutha (2005) noticed increased total soluble protein content as influenced by triple inoculation of *G. fasciculatum* + *Azatobacter chrococcum* + *Aspergillus awamori* as compared to control.

The findings of the present studies indicate *T. harzianum* isolated from zone 1 (NETT) and zone 9 (HT) has the ability to enhance the early growth of *N. sativa*. However, at later stage zone 6 (SDT) has the potential to enhance plant growth and along with zone 5 (EDT) it can even enhance biochemical parameters. Thus it can be concluded that all the above isolates can be used in combination to enhance overall growth and development of the medicinal plant *N. sativa*.

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