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"UmTricho" a liquid bioformulation of indigenous strain of *Trichoderma harzianum* effectively managed the tikka disease (*Cercospora* spp.) of groundnut, *Arachis hypogea* L. under the agroecological condition of Meghalaya

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ABSTRACT: Groundnut (*Arachis hypogea* L., Fam: Fabaceae), an important annual leguminous and oilseed crop grown in an area of 7.78 m ha with an average production and productivity of 7.79 m t and 990 kg ha^{-1} respectively. The crop is susceptible to many fungal diseases such as tikka, rust, charcoal rot and crown rot, of which tikka disease caused by *Cercospora* spp. is the most destructive disease claiming **10-50 % yield losses affecting pod yield and quality. The state of Meghalaya is considered as organic state by default. But there no recommendation on organic management practices of the tikka disease of groundnut which is urgent need for the state. In the present study, a field trial was conducted to evaluate the efficacy of UmTricho, a bioformulation developed from native strain of *Trichoderma harzianum* of Meghalaya against tikka leaf spot disease of groundnut. Efficacy of UmTricho was compared in six different treatment combinations *viz.*, T_1- Biopriming of seeds (10 ml UmTricho in 1000 ml water for 1 Kg seed), T_2- Soil application with UmTricho enriched compost (1 Kg of compost + 40 ml on UmTricho in 1 litre water incubate for 7 days), apply twice first at the time of seed sowing and second at canopy closure stage, T_3- Foliar spray of cow dung slurry enriched with UmTricho (100 ml in 10000 litres of water) from 15 days after germination, thrice at 15 days interval, T_4- T_1 + T_2 + T_3, T_5- Chemical check (Bavistin @ 0.1% as foliar spray) and T_6- Uninoculated control. Combined application (T_4) with UmTricho@ 10 ml in 1 litre as seed treatment + 3 foliar sprays impregnated with enriched compost at 15 days interval starting from appearance of symptom reduced 30-40% disease incidence as compared to control. In addition, enhanced physiological parameters *viz.*, plant biomass, pod number, pod length, root nodulation and yield were observed as compared to control (T_5).**

Keywords: Groundnut, Tikka disease, UmTricho, Plant growth promotion.

INTRODUCTION

Groundnut (*Arachis hypogea* L., Fam: Fabaceae), also known as wonder legume, originated from Brazil, Peru and South America, is the 3rd major oilseed crop next to soybean and cotton, accounting for 432 million tonnes, with China (17.30 million tonnes) being largest producer of groundnut followed by India (6.70 million tonnes) accounting for 37% and 14% of the total world production of 46.01 million tonnes during 2018-19 (Anon., 2020). In India, groundnut ranks second largest oilseed crop accounting for 5.30 million hectares area with production of 5.50 million tonnes and 1.04 tonnes/hectare productivity annually with Gujarat, Andhra Pradesh, Rajasthan, Tamil Nadu, Karnataka, Madhya Pradesh and Maharashtra as major producing states (Source: Directorate of Economics and Statistics, DAS & FW, 2016-17). Etymologically, it is known as peanut, monkeynut, pindar, goobenut (English), chinya-badam (Bengali), mungphali (Gujrati), vilaytimung (Hindi), bhuimug (Marathi), nelakadalai (Tamil) and nelasena-galu (Telgu) in different states of India. Being annual crop, it is grown in semi-arid tropics and tropics with soil pH of 6.0-6.5 for edible seeds containing 48.32% oil, 22-25% protein, 20% carbohydrate, 5% fiber, ash, 30-35% vit. A and E, oil content and fodder (foliage and haulm).

Groundnut production in northeast region (NER) of India is non-traditional, yet, picking up faster in states *viz.*, Assam (Jorhat, Nogaon, North Lakhimpur, North Cachar Hills, lower altitudes), Arunachal Pradesh (East Siang), Meghalaya (Ri Bhoi, Umroi, West Garo Hills, East Garo Hills), Nagaland (Kohima), Manipur (Thoubal, Ukhrul, Senapati), Tripura (South, North, West) and Mizoram (Kolasib, Aizawl) (Singh *et al.*, 2006). The cropping pattern followed in the NER include rice-groundnut, rice-potato-groundnut, rice-mustard-groundnut, maize-groundnut and groundnut-maize/pigeon pea/chilli/pineapple/citrus during kharif (April), rabi (September-October) and Summer (January-February). Major varieties adopted in NER include ICGS 76, Girnar 1, ICGS 11, CSMG 84-1, ICGV 86590, TKG 19A (3200 Kg/ha), BAU 13ICGS (3000 Kg pod yield/ha), ICGS 44 (3970 Kg/ha) and ICGV 88365 (3760 Kg/ha). Among various biotic and abiotic stresses, diseases *viz.*, tikka leaf spot, fusarium wilt, Alternaria leaf spot, stem rot, rust and crown rot have been reported as key constraint in groundnut cultivation in different states of NER (Prasad *et al.*, 1999).

Tikka (Ascomycota: Dothideomycetes, Capnodiales, Mycosphaerellaceae) disease of groundnut is the most destructive disease attacking 1-2 months old plants causing a yield loss by spots up to 30-50% and pod yield 29% (Siddaramaiah *et al.*, 1977; Faisal and Tiwari, 2015). Under field condition, disease is diagnosed as early leaf spot (*Cercospora arachidicola* Hon., Tel: *Mycosphaerella arachidicola* Jenkins) with subcircular-irregular, 1-10 mm diam. reddish, dark brown to black colored lesion with yellow halo on upper and lower surface of leaves. Whereas, late leaf spot (*Phaeoisariopsis personata* {*C. personata* Berl. & Curt.} Ellis, Tel: *M. berkeleyii* Jenkins) is diagnosed as smaller, 1-6 mm diam. dark grey/black spots on lower leaf surface. In Meghalaya, early leaf spot disease of groundnut is more predominant in mid-altitudes due to prolonged 20-30ºC, long periods of relative humidity and heavy rains. Conventional management strategies include cultural practices *viz.*, removal of crop debris, volunteer crops, crop rotation, early planting, chemical management *viz.*, protectants (Dithane Z-78 @ 0.2%, Cupramar @ 0.2%), systemic fungicides (Benlate, Bavistin, Topsin-M) at 30 DAS @ 10-15 days interval and resistant cultivars *viz.*, T-64, C-501, MH-4, TMV-6, TMV-10 etc (Chandra *et al.*, 2014; Kumar *et al.*, 2017; Sharma *et al.*, 2020). Though chemical fungicides are available for the management of the tikka disease of groundnut but the negative effect of the chemical pesticides can't be ignored. Moreover, the region is considered as organic by default. So, there is an urgent need for organic package of practices involving integrated disease management in order to suit higher production in agro-climatic condition of North east India.

MATERIALS AND METHODOLOGY

UmTricho- liquid based bioformulation of *Trichoderma harzianum*: UmTricho, a liquid based bioformulation derived from *Trichoderma harzianum*, a local strain isolated from native regions of Meghalaya. The bioproduct has already been recommended for sheath blight of rice, leaf spot of turmeric, Alternaria leaf spot of tomato and Phomopsis blight of brinjal. UmTricho spray solution was prepared by diluting 1000 ml of UmTricho in 100 liters of water amended with 0.02% (v/v) tween-80 with thorough stirring.

Field experiment: The experiment was conducted in CPGSAS experimental farm (100 m^2) at Pyllun, Meghalaya (Coordinates: 90°55′15 to 91°16′E, 25°40′ to 25°21′N at an altitude of 1010 m above mean sea level (msl)) prepared by thorough ploughing into 24 plots (2 × 1.5 m^2) and farm yard manure (FYM) @ 10 tonnes/hectare was applied (Fig. 1). The organic package of practices for groundnut cultivation followed from recommended module issued by ICAR NEH-RC, Umiam, Meghalaya. Intercultural operations were done *via.*, hoeing at 25 days after sowing (DAS) and hand weeding at 35 DAS, while irrigation was given at pegging stage.

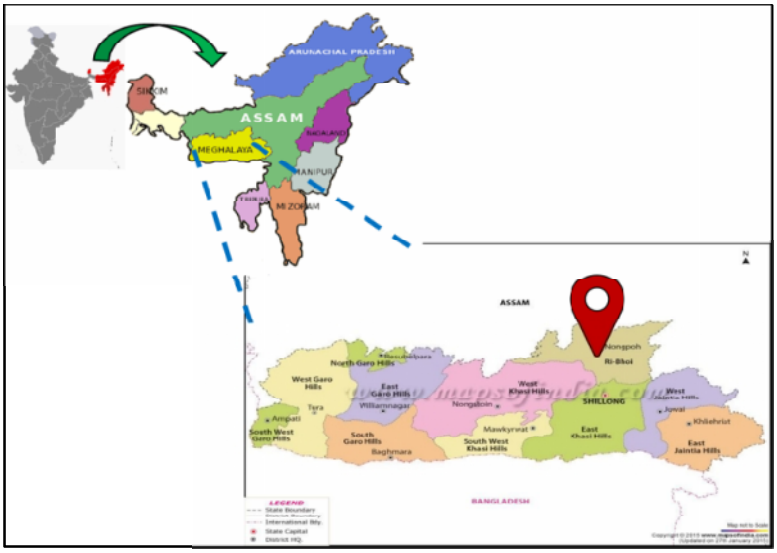

Fig. 1. Site of experiment.

Application of UmTricho in field condition. Seed treatment was done by inoculating 1 Kg viable groundnut seeds (local variety, seed rate: 100-120 Kg/ha) in 1000 ml UmTricho spray solution, incubated at room temperature (25±2 ºC) for 60 minutes, whereas un-inoculated seeds were maintained in equal volumes of double distilled water (DDW). UmTricho enriched compost prepared by mixing 1 Kg of fine, sieved compost with 40 ml of UmTricho, incubated for seven (7) days at room temperature with adequate moisture and applied between the rows @ 1Kg/m^2 during planting time and at canopy closure stage. Three (3) foliar sprays of fresh cow dung slurry (1 Kg) enriched with UmTricho (40 ml), incubated for 48 hours at room temperature and applied by diluting 100 ml in 10000 litres of water with the help of knapsack sprayer from 15 days after germination at 15 days interval. The treatment combinations used in the study are as follows:

T_1- Biopriming of seeds (10 ml UmTricho in 1000 ml water for 1 Kg seed)

T_2- Soil application with UmTricho enriched compost (1 Kg of compost + 40 ml on UmTricho in 1 litre water incubate for 7 days), apply twice first at the time of seed sowing and second at canopy closure stage

T_3- Foliar spray of cow dung slurry enriched with UmTricho (100 ml in 10000 litres of water) from 15 days after germination, thrice at 15 days interval

T_4- $T_1 + T_2 + T_3$

T_5- Chemical check (Bavistin @ 0.1% as foliar spray)

T_6- Uninoculated control

Plate 1: (A) UmTricho- a liquid based bioformulation of *Trichoderma harzianum* used in the study, (B) groundnut seed treatement with UmTricho, (C) enriched compost prepared with UmTricho and (D) foliar spray with UmTricho.

Observations: Typical symptom of tikka leaf spot disease of groundnut were observed and occurrence of disease was recorded at matured crop stage by calculating per cent disease incidence (PDI %).

Per cent disease incidence (PDI %)

$$= \left\{ \frac{\text{No. of infected plants}}{\text{Total number of plants assessed}} \right\} \times 100$$

Per cent disease severity index of tikka disease was scored and graded as per standard disease scoring (0-9 scale) (Plate 3) given by (Mayee and Datar, 1986).

Table Disease scoring (0-9 scale) for tikka disease of groundnut (Mayee and Datar, 1986).

0	No symptoms on leaf
1	Few small necrotic spots covering 1% or less of leaf area
3	Few small necrotic spots covering 1-5% or less of leaf area
5	Spots coalescing enlarging 6-20% of the leaf area
7	Spots enlarging, coalescing to cover 21-50% of the compound of leaf area
9	Spots enlarging, coalescing to cover 51% or more of the leaf area

Yield attributes studied:

- No. of branches, trifoliate leaves, shoot length (cm), root length (cm), fresh weight (g) and dry weight (g)
- Total no. of rootlets, no. of roots bearing nodules, total no. of nodules/plant
- No. of nuts/plant, length of nuts (cm), no. of seeds/nuts, total seeds/plant, test weight (for 100 seeds), total pod yield (t/ha) and total seed yield (t/ha)

Statistical analysis: The statistical design used in the present study was randomized block design (RBD) using ANOVA. The significant difference, if any, among the treatment means were compared by using critical difference (CD) at $P=0.05$ (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

The bio-efficacy of liquid-based bioformulation UmTricho was evaluated under different treatments *viz.*, seed treatment, soil application and foliar spray either alone or in combination with each others, for their ability to reduce tikka disease of groundnut under field condition (Table 1, Fig. 2). Peculiar early tikka leaf spot symptoms of circular to irregular black spots with yellow halo on upper surface of leaves were observed at matured crop stage and per cent disease incidence and severity (%) were recorded. Different treatment combinations have significantly influence plant growth promoting (PGP) and yield attributes.

Biopriming of groundnut seeds with UmTricho showed reduced primary and secondary seed-borne infections as compared to uninoculated seeds at seven (7) days post inoculation (Plate 2).

Table 1: Disease incidence (%) and disease severity (%) of *Cercospora arachidicola* causing tikka disease of groundnut along with yield attributes under different treatment combinations of UmTricho in field condition.

Treatments	*Disease incidence (%)	*Disease severity (%)	Shoot length (cm)	Root length (cm)	Fresh weight (g)	Dry weight (g)	No. of branches	No. of trifoliate leaves	Total No. of roots	No. of roots bearing nodules	Total No. of nodules	No. of nuts/ plant	Length of nuts (cm)	No. of seeds/ nut	Total seeds/ plant	Test weight (g)	Total pod yield (Kg/ ha)	Total seed yield (Kg/ ha)
T_1	38.00(38.03)	45.00 42.11)	44.00	12.00	14.70	10.63	5	25	10	11	102	7	2	2	15	48	866.6	395.5
T_2	36.00(36.85)	42.00(40.37)	45.00	12.20	14.80	12.00	5	26	12	10	101	13	2	2	18	52	890.0	446.6
T_3	32.00(34.42)	39.00(38.62)	47.00	12.20	14.90	11.27	5	27	11	12	105	11	2	2	21	53	927.7	467.7
T_4	23.00(28.63)	34.00(35.64)	49.00	12.20	15.88	12.69	6	28	13	12	112	17	3	2	27	53	956.6	554.9
T_5	12.00(20.21)	27.00(31.28)	51.00	14.00	17.00	13.00	8	29	12	15	125	19	3	2	28	53	1408.8	726.6
T_6	89.00(70.65)	91.00(72.58)	26.00	12.68	7.92	4.17	4	15	6	6	47	6	2	1	9	39	663.7	367.4
SEm (±)	0.173	0.195	0.577	0.077	0.004	0.437	0.001	0.017	0.731	0.004	1.326	0.136	0.021	NS	0.029	0.009	0.010	0.004
$CD_{0.05}$	0.551	0.623	1.799	0.247	0.013	1.395	0.004	0.053	2.334	0.013	4.323	0.435	0.068	--	0.091	0.027	0.033	0.011

T_1: Seed treatment with UmTricho, T_2: Soil application with UmTricho enriched compost, T_3: Foliar spray of cow dung slurry enriched with UmTricho at 15 days interval, T_4: Combined application of seed treatment + soil application + Foliar spray with UmTricho, T_5: Chemical check (Bavistin @ 0.1%) as foliar spray and T_6: Uninoculated control
Figures in data are mean of three (3) replications, *Data in parentheses are angular transformed values.

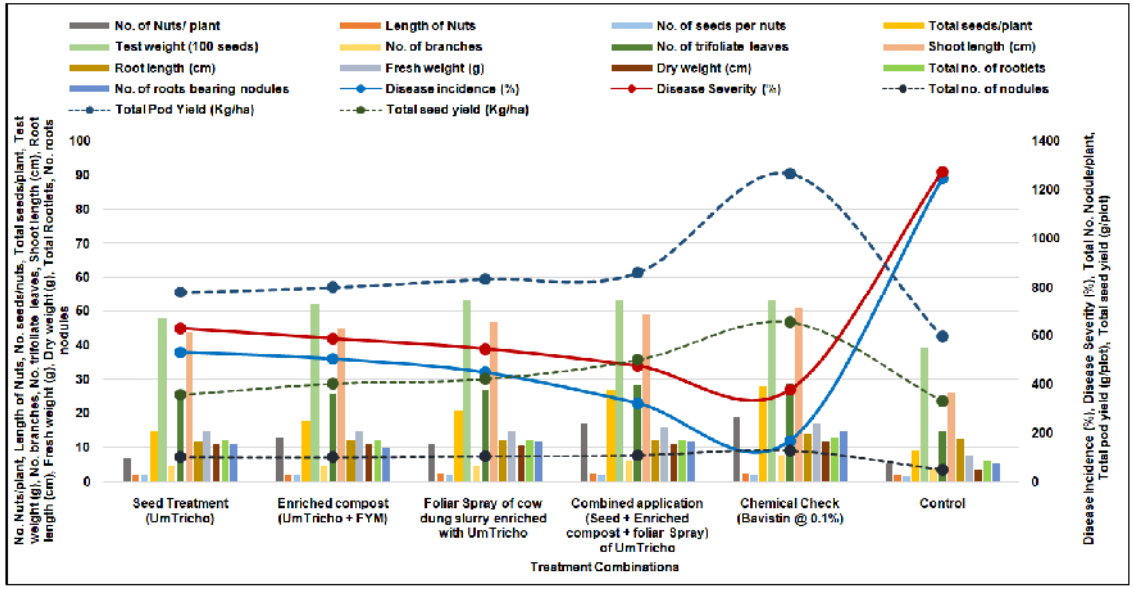

Fig. 2. Disease incidence (%) and disease severity (%) of *Cercospora arachidicola* causing tikka disease of groundnut along with yield attributes under different treatment combinations of UmTricho in field condition.

Under field condition, combined application of UmTricho performed better as compared to single treatment and control and recorded 34.00% and 23.00% reduced disease incidence and severity as compared to control (Plate 4). Lowest per cent tikka disease incidence of 23.00% was recorded in case of combined application of UmTricho (T_4) followed by 32.00% and 36.00% in case of foliar spray of UmTricho enriched cow dung slurry (T_3) and soil application with Umtricho enriched compost respectively (T_2) as compared to control (89.00%) and chemical check (12.00%). However, lowest disease severity of 34.00% was recorded in case of T_4 followed by 39.00% (T_3) and 42.00% (T_2) as compared to control (91.00%) and chemical check (27.00%). The results were found similar with (Kishore *et al.*, 2001; Ihejirika *et al.*, 2010; Ramesh and Zacharia, 2017), reported application of *T. viride* reduced tikka disease incidence up to 11.31%, 18.69% and 31.50% on 30 DAS, 60 DAS and 90 DAS respectively with increased yield of 7.78 q/ha as compared to control (4.85 q/ha). In similar studies, Hasan *et al.*, (2014) reported *Trichoderma harzianum* based BAU-bio fungicide as seed + foliar spray reduced groundnut leaf spot by 53.61% with enhanced length, biomass and yield as compared to control. Earlier, report showed that *Trichoderma harzianum* as one of the effective biocontrol agents as for seed treatment and soil application for the management of soil bore plant pathogens like *Rhizoctonia solani*, *Sclerotinia Sclerotinia sclerotiorum rolfsii* causing stem rot of soybean (Dutta and Das, 1999a; Dutta and Das, 1999b; Dutta *et al*., 2000; Das *et al*., 2006), sheath light of rice (Das, *et al.*, 1997) , white mold of French bean, collar rot of tomato (Dutta and Das, 2002), respectively.

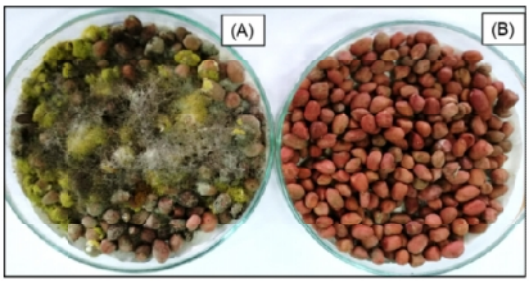

Plate 2: Untreated groundnut seeds with seed-borne infection (A) and bio primed seeds with UmTricho without seed infection (B).

Plate 3: Disease scoring of tikka leaf spot of groundnut; 0 (No symptoms), 1 (<1%), 3 (1-5%), 5 (6-20%), 7 (21-50%) and 9 (>50%).

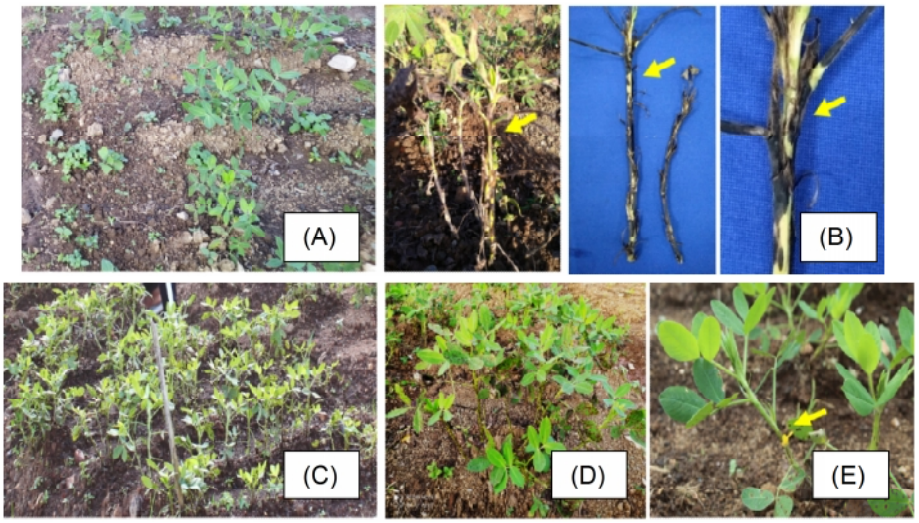

Plate 4: Uneven, sparse growth in control plots (A), Leaf spots and stem infections at severe stage in control plots (B), Dense crop growth in UmTricho treated plots (C), Lowest disease severity of tikka in plots treated with UmTricho (D) and early flowering in treated plants (E).

Plate 5: Healthy pods with higher root nodulation in UmTricho treated plant (A) poor growth with less nodulation in control plant (B) and Peg infection at different growth stages due to fungal infection (*Cercospora* sp.) (C).

Plate 6: Unhealthy, small-sized, dark-coloured pods from control plots (A) and healthy pods from UmTricho treated plots (B), pod yield from control plots (C) and pods yield from UmTricho treated plots (D), deformed, rotten, small-sized, dark-coloured seeds due to fungal infection from control plots (E) and healthy, well-shaped seeds from UmTricho treated plots (F).

Further, in the present study, combined application of UmTricho (T_4) showed enhanced plant growth parameters *viz.*, shoot length (49 cm), root length (12.20 cm), fresh weight (15.88 g), dry weight (13.00 g), total no. of roots (12), roots bearing nodules (12) and no. of nodules (110) as compared to control (Plate 5). In addition, combined application of UmTricho also showed enhanced pod (956.67 Kg/ha) and seed yield (554.93 Kg/ha) as compared to control with 663.77 Kg/ha pod and 367.42 Kg/ha seed yield respectively (Plate 6). In UmTricho treated plots, harvested pods were healthy, uniform shape with higher root nodulation was observed, whereas, in case of untreated control, peg infection was observed at all growth stages of peg formation leading to malformation of pegs (Plate 5). In contrast, pegs from untreated control were unhealthy, small-sized, dark-coloured with deformed, rotten, small-sized seeds as compared to healthy, uniform-shaped nuts and seeds obtained from UmTricho treated plots of more economic value. Increased plant growth parameter with reduced per cent disease incidence of white mold of French bean caused by *Sclerotinia sclerotiorum* was reported when *Trichoderma harzianum* based bioformulation was applied as seed and soil treating agents (Dutta *et al*., 2008; Das *et al.*, 2012). In soybean, similar results were also obtained by (Mane, 2012; Hossain and Hossain, 2013; Kumar *et al.*, 2017) reported *T. viride* based bio fungicide reduced tikka leaf spot of groundnut up to 22.19% @ 90 DAS with enhanced plant growth parameters *viz.*, plant height (34.16 cm), leaves/plant (184.36), pod weight (25.90 g), pods/ plant (36.12) and yield (27.07 q/ha) at 60 DAS having 1: 1.90 cost: benefit ratio as compared to control.

CONCLUSION AND FUTURE SCOPE

The present study showed encouraging results of UmTricho against tikka disease of groundnut and can be used as a component of plant protection in organic package and practices for cultivation of groundnut in agro-ecological zone of Meghalaya. UmTricho as seed treatment (10 ml in 1 litre of water for per Kg seed) + soil application with enriched compost (1 Kg compost + 40 ml UmTricho) + 3 foliar sprays of enriched cow dung slurry (@ 100 ml in 10000 litres of water from 15 days after germination at 15 days interval) can reduce

tikka disease incidence. The devised technology can be further studied in pilot field trials in farmer's field, if found effective can be transferred to farmers. Further work on interaction of the biocontrol agents with other botanical pesticides may be done for the synergistic effect of both the components for better management of plant diseases with enhanced plant growth and biochemical parameters.

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Conflict of Interest. There is no conflict of interests among the authors.

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