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Impact of shifting cultivation on rhizome rot of ginger (*Zinger officinale* Rosc.) and its management by native *Trichoderma* spp. in Manipur

Padamini Rajkumari and Bireswor Sinha

Abstract
Natural incidence of Rhizome rots of ginger at five different districts of Manipur during July and September 2016 showed higher disease incidence in hill districts *viz*., Tamenglong, Churachandpur and Bishnupur where shifting cultivation has been practiced. Among these the highest was recorded in Tamenglong with disease incidence of 16.51% during July and 38.62 % during September 2016. Whereas in plains Imphal East district showed higher disease incidence of 10.79% in July and 29.22% during September as compared to Imphal west district which showed 08.89% during July and 25.39% during September 2016. Isolation of the biocontrol agent was done from the air-dried rhizosphere soil of healthy ginger leaves and rhizome using selective media. The studies resulted in recovery of those organisms which are known to act as biocontrol agents of plant pathogens. The isolated cultures of *Trichoderma* spp. were identified as six isolates of *Trichoderma asperellum*, six isolates of *T. harzianum*, one isolate each of *T. hamatum* and *T. longibrachiatum*. Maximum growth and sporulation of isolates of *Foz* was recorded at a temperature of 30^0C with pH 7. The antagonism test showed that 78% of the Trichoderma isolates strongly invaded *Foz in vitro* (rating =1 or 2). 47% had a rating of 1, 25% had a rating of 2, 15% had a rating of 3, 9% had a rating of 4 and 4% had a rating of 5. Comparison between the population densities of most potent Trichoderma isolate (*T. harzianum*-MH259837) and *Fusarium oxysporum* f.sp. *zingiberi* in ginger field at plains and under jhum cultivation was carried out. The initial population 6.8×10^5 c.f.u /g seed for *T.harzianum*-MH259837 and 2.4×10^6 c.f.u /g soil was recorded. The result showed that among the different substrate tested for mass multiplication of *T. harzianum*-MH259837 in plains at 30 DAS highest population densities was recorded in treatment with rice bran + *T. harzianum*-MH259837 (6.5×10^5 c.f.u /g soil). The success of a biocontrol agent depends much on the establishment of the product, the formulation and delivery system. The production of propagules can be carried out in liquid or solid fermentation. The maximum biomass production is influenced by aeration, agitation, pH, and temperature. It has been reported that the formulation used to introduce *Trichoderma* in soil influences rhizosphere competence. Agricultural wastes that have been reported to be good substrates as a result rice bran was found to be the most suitable substrate and highest spore count was observed for BCA.

Keywords: Ginger rhizome rot, *Trichoderma* spp, *Fusarium oxysporum* f.sp. *zingiberi*, jhum/shifting cultivation, mass multiplication

1. Introduction
India is considered as "The land of spices" and enjoys from time immemorial a unique position in the production and export of spices. Spices are high value and export-oriented commodity crops, which play an important role in agricultural economy of the country. India is the largest producer, consumer and exporter of spice in the world. Spices contributed 1.24 per cent of India's total export earnings. The share of spices in the export earnings from agricultural and allied products is 8.5%. Ginger (*Zinger officinale* Rosc.) is an important commercial spice crop grown in India. Ginger is cultivated for their underground rhizomes, which are used in many ways. Ginger is also used in various forms, as condiments, dried ginger powder is used in making beer, wine, rum, in confectionaries for making cold drinks. Manipur is home to a variety of spices of which ginger is one of the prominent. The climatic condition of Manipur is highly suitable for cultivation of spice such as ginger. Ginger is a main cash crop of North eastern region counting 49% of India's ginger area and 72% of India's ginger production. Ginger is susceptible to infection throughout its growing period. Almost all parts of the plant including sprouts, roots, developing rhizome and collar regions of the pseudo stem are vulnerable to infection. *Pythium* soft rot of ginger is also known as soft rot or rhizome rot of ginger is the major yield reducing malady and important limiting factors of ginger

production in India. The disease was firstly reported in India by Butler, 1907 [4]. Presently, the disease occurs in ginger growing countries throughout the world (Dohroo, 2005) [6]. Rhizome rot is considered as a complex disease problem. The pathogen responsible can infect host plants at any stage of growth and even during post-harvest storage when growth from latent infections can cause severe losses. Both pre and post emergence rots are noticed due to soft rot. High yield losses of 90% were reported in India (Ranjan and Agnihotri, 1989). In Manipur ginger is mostly grown in sub-tropical hill zones where soil is acidic in nature and cultivation is being practiced on steep slopes under jhum/bun (raised beds) system in rainfed conditions. The deep virgin soils of forest brought under jhum system are giving higher yields in the first and second year of cultivation even under zero nutrient management conditions. In shifting cultivation, the soil had been disturbed with repeated burning, tillage and cropping. This practice has a profound impact on the distribution of rhizosphere microorganism in soil resulting in an altered diversity status as compared to the subsurface layer where impact of fire and other disturbance are less. According to *Jhum* cultivation involves several cultural operations (clearing of forest biomass by slashing, release of nutrients accumulated in vegetation over time by burning of slashed biomass, suppression of weeds, pest and diseases by soil sterilization, etc.) to grow mixed crops on the hill slope for a year or two (cropping phase) followed by abandoning the land as fallow for some years for regeneration of natural vegetation (fallow phase). It is believed that fallowing the land rejuvenate *Jhum* soils and bring back its fertility (Ramakrishnan and Toky, 1981) [12] Besides, the chemical composition of forest floor litters (FFLs) seems to vary greatly depending upon the length of fallow phase (Cornwell *et al*. 2008) [5]. The varied response to soft rot disease in different areas is due to the different cropping pattern, environmental conditions and cultural practices resulting in buildup of varying levels of inoculum. In the absence of desired level of host resistance, the disease is currently managed through excessive application of chemical fungicides, which have drastic effects on the soil biota, pollute the atmosphere and are environmentally harmful. Since it is difficult to achieve control through host resistance or fungicides, biological control may be effective in minimizing the incidence of rhizome rot of ginger. Since, ginger plant is a delicate succulent herb. It is easily succumbing to stresses, both environmental and biological. Therefore, it is essential that disease management practices are integrated within the framework of crop production strategy at all possible stages of crop cycle for reducing the possibility of disease development by adopting suitable cultural practices and biological control agents. Biological control of plant pathogens is considered safe and durable; need to explore the potentialities of biological strategies specific to the environmental conditions of Manipur is therefore imperative.

2. Materials and methods

2.1 Diseased sample collection

The diseased samples of ginger showing typical rhizome rot symptoms were collected in *Kharif* season of 2016 from farmer's field of different ginger growing areas of Manipur *viz*., Imphal East, Imphal West, Tamenglong, Churachandpur and Bishnupur all from local land races. Among the five districts from where samples have been collected, shifting cultivation practice of ginger was practice in Tamenglong, Churachandpur and Bishnupur. The infected parts of the diseased samples were carefully placed in polythene bags, properly tagged and brought to the laboratory and subjected to microscopic examination and tissue isolation.

2.2 Isolation of Pathogen

Isolation from infected rhizome of ginger showing typical rhizome rot symptoms were used to isolate the pathogens by different techniques and pure cultures thus obtained were maintained on potato dextrose agar (PDA) medium for further investigation. Isolations of the pathogen were attempted from all the samples. These infected parts were thoroughly washed in running tap water to remove the adhering soil. These were then cut into small pieces with the help of a sterilized scalpel, washed in sterilized water, surface sterilized by dipping in 0.1 per cent mercuric chloride ($HgCl_2$) for 2 minutes rinsed thrice in sterilized distilled water and transferred on potato dextrose agar (PDA) medium in Petri plates. The plates were incubated at 28 ± 1^0C for growth. Sub cultures were made from the periphery of the mycelial growth which appeared after five to six days of incubation.

2.3 Isolation of biocontrol agent

PDA amended with 25 ppm chloramphenicol and 2 ml of Triton X-100/litre (Budge and Whipps, 1991) was used. Since the amount of rhizosphere soil was limited (1g) soil was used and dilution of 10^5 was proposed. One ml of soil dilution was pipetted in sterilized Petri plates and on this 20 ml of molten almost cool medium was poured and the plates were gently rotated to uniformly spread the propagules in the plates and then allowed to solidify. The plates were incubated at 25^0 C for five days and resultant *Trichoderma* colonies were examined under the stereo-binocular microscope and picked up on fresh poured plates. Hyphal tip pure cultures were made and maintained on PDA slants.

2.4 Morphological variability among the isolates of pathogen and BCA

Three mm disc of the individual isolates of *Foz* and *Trichoderma* spp. removed from the periphery of seven days old culture was aseptically placed in the center of the PDA agar plate, and were incubated at 28 ± 1^0C. The variations in growth pattern and colony growth (diameter) of fungi in all isolates were recorded. Spore production by isolates was determined by removing agar-plugs (3 mm diameter) from three liner spots across the center of the colony, which were suspended in 10 ml sterile water in glass taste tube and agitated twice for about ten seconds each time on a vortex shaker to dislodge conidia. The number of conidia in the resultant suspensions was determined using a haemocytometer, and expressed as number of conidia per mm^2 of medium. For spore (length and width) mounts were prepared in aniline-blue lacto-phenol and measurements were taken by measuring 50 spores of each isolates using stage and ocular micrometer. Temperature studies were conducted with a view to determine the optimum range of temperature for mycelial growth and sporulation of the isolates. The PDA plates were inoculated with 3 mm disc from one-week old pure culture were kept at different temperatures *viz*., 15, 20, 25 and 30^0C maintained in different incubators. The study was carried out to find out the optimum pH levels for the mycelial growth and sporulation of isolates. Potato dextrose agar was adjusted with pH ranges between 4.0 to 7.0 pH with a narrow fraction of 1 pH was adjusted to these levels by using buffers N/10 HCL and N/10 NaOH solutions. The pH

was determined by Pen type digital pH meter. The observations were recorded by measuring the diameter (Radial growth) of the colony of the fungus. The sporulation was measured by taking 1 cm^2 bit from the mycelial growth from each treatment and dissolved in 10 ml sterile distilled water in test tube. The spore load was observed in each treatment under microscope taking ten observations and counting of conidia of *Foz* was done by using a haemocytometer.

2.5 Screening of the biocontrol agent

2.5.1 Preparation of V8 agar medium

200ml V8 tomato juice, 3.0 g $CaCO_3$, 15.0g agar and 800 ml of distilled water were mixed in a 2L Erlenmeyer flask and autoclaved for 15 min at 121^{0}C. *In vitro* comparisons were made by placing 4 mm diameter discs of *Trichoderma* spp. and *Foz* on opposite sides of 9cm diameter Petri dishes containing V8 medium and incubated for five days at 25^{0}C.

After five days the cultures were scored for degree of antagonism using the ratings system of Bell *et al*. (1982) [2]. On a scale of 1-5: Bell *et al*. (1982) [2] considered an isolate of *Trichoderma* spp. to be antagonistic if the mean score was ≤ 2, but not antagonistic if the number was ≥ 3.

Class 1= *Trichoderma* spp. completely overgrew *Foz* and covered the entire medium surface Class 2 = *Trichoderma* spp. overgrew at least two thirds of the medium surface

Class 3= *Trichoderma* spp. and *Foz* each colonized 50% of the medium surface and neither organism appeared to dominate the other

Class 4= *Foz* colonized at least two-thirds of the medium surface and appeared to with stand encroachment by *Trichoderma* spp.

Class 5= *Foz* completely overgrew the entire medium surface.

Observations on colony diameter were recorded up to the complete coverage of control plates, which was inoculated with only pathogen. The linear growth after five days of incubation was recorded and per cent inhibition was calculated

2.6 Mass production of *Trichoderma harzianum*

The mycelial suspension (1 x 10^5/ml conidia) was prepared and confirmed by haemocytometer and inoculated into polypropylene bags containing the substrates (rice bran, paddy chaff, paddy straw, poultry manure, well decomposed FYM and sugarcane bagasse) which was moistened, sterilized, pH adjusted using buffer and incubated at 28 ± 1°C for 15 days with periodical shaking to avoid formation of clump and to enhance uniform growth and sporulation of BCA (*T. harzianum* (MH259837) in the medium. These formulations of *Trichoderma* were mixed with a suitable inert medium like talc and used for seed treatment @ 6g/kg seed in field applications.

2.7 Determination of population [C.F.U (colony forming units)] of the biocontrol agent and pathogen

The population C.F.U. (colony forming units) of *T. harzianum* (MH259837) and *Foz* were determined by dilution plating (Warcup, 1955) on organism specific media. Soil samples were collected from rhizosphere of ginger for determination of population densities of *T. harzianum* (MH259837) and *Foz* at initial stage, 30 and 90 days after sowing. For estimation from soil, 1g soil was suspended in 100ml water, vigorously shaken and further serially diluted. Dilution of 10^5 was used to determine population of *T. harzianum* (MH259837) and 10^6 for *Foz*. For *T. harzianum* (MH259837) the specific modified PDA + Chloramphenicol + Triton X-100 medium (Budge and Whipps, 1991) was used where Chloramphenicol was used as an antibacterial antibiotic instead of Aureomycin. For *Foz*, PDA medium was used. One ml of soil dilution was pipetted in sterilized Petri plates and on it, almost cooled, molten medium was poured and plate was rotated with hand to disperse the propagules present in the soil, and then medium was allowed to solidify. The plates were incubated at 28 ± 1^{0}C for five days and the colonies of the organisms were counted with the help of a Quebec colony counter. The populations were computed to get c.f.u. /g soil.

3. Results and discussions

The cultures of pathogen inciting soft rot in ginger were isolated from the diseased rhizomes collected from farmer's field of Manipur. The isolates of pathogen recovered from different ginger plant samples are used in the present study. The main aim was to explore possibility of existence of different species and/or variables of soft rot pathogen.

Table 1: Details of place of disease samples and soil samples collected during 2016.

S. N	District	Site of collection	Latitude	Longitude
1	Imphal East	Thaiyong -1	N 24°47′78.0″	E 094°04′50.1″
		Moirangkampu	N 24°47′83.2″	E 094°04′57.8″
		Haraorok	N 24°48′48.4″	E 094°04′62.3″
		Porompat-1	N 24°47′80.7″	E 094°04′56.0″
		Porompat-2	N 24°47′67.5″	E 094°04′51.7″
2	Imphal West	Iroisemba	N 24°46′53.9″	E 093°47′66.6″
		New Keithelmanbi -1	N 24°46′60.2″	E 093°47′54.5″
		New Keithelmanbi -2	N 24°46′58.7″	E 093°47′59.6″
		Keithelmanbi Namching -1	N 24°45′54.7″	E 093°47′41.1″
		Keithelmanbi Namching -2	N 24°45′60.2″	E 093°47′55.8″
3	Tamenglong	Khoupum Valley -1	N 24°41′00.7″	E 093°31′10.6″
		Khoupum Valley -2	N 24°41′49.0″	E 093°31′18.1″
		Khoupum Valley -3	N 24°41′35.2″	E 093°31′24.6″
		Khoupum Valley -4	N 24°41′33.5″	E 093°31′28.2″
		Khoupum Valley -5	N 24°41′28.7″	E 093°31′15.9″
4.	Churachandur	Torbung Makha Leikai	N 24°25′86.5″	E 093°43′23.7″
		Torbung Mamang Leikai	N 24°25′84.0″	E 093°43′29.3″
		Vageing Village	N 24°24′04.7″	E 093°42′25.9″
		Khenjang Village -1	N 24°23′40.0″	E 093°42′25.2″
		Khenjang Village -2	N 24°23′28.7″	E 093°42′15.9″
5.	Bishnupur	Leimaram-1	N 24°71′57.2″	E 093°78′54.8″

		Leimaram-2	N 24°71′47.1″	E 093°78′13.0″
		Sabual village	N 24°71′48.6″	E 093°78′19.8″
		Yumnam Khunou-1	N 24°71′41.0″	E 093°78′94.7″
		Yumnam Khunoou-2	N 24°71′42.5″	E 093°78′91.5″

Natural incidence of Rhizome rots of ginger at five different districts of Manipur during July and September 2016 showed higher disease incidence in hill districts *viz*., Tamenglong, Churachandpur and Bishnupur where shifting cultivation has been practiced. Among these the highest was recorded in Tamenglong with disease incidence of 16.51% during July and 38.62 % during September 2016. This was followed by Churachandpur district with 13.79% during July and 32.78% during September. Followed by Bishnupur district with a close margin of 13.58% disease incidence during July and 32.07% during September. Whereas in plains Imphal East district showed higher disease incidence of 10.79% in July and 29.22% during September as compared to Imphal west district which showed 08.89% during July and 25.39% during September 2016.

Table 2: Natural incidence of Rhizome rots of ginger at five different districts of Manipur during July and September (2016)

S. N	District	Site of collection	Disease Incidence (%) July	September
1.	Imphal East	Thaiyong -1	12.01	27.03
		Moirangkampu	09.07	30.50
		Haraorok	10.50	28.06
		Porompat-1	10.80	31.20
		Porompat-2	11.60	29.30
		Mean	10.79	29.22
2.	Imphal West	Iroisemba	09.30	25.80
		New Keithelmanbi -1	08.01	23.34
		New Keithelmanbi -2	10.20	28.05
		Keithelmanbi Namching -1	09.89	26.65
		Keithelmanbi Namching -2	07.23	23.15
		Mean	08.89	25.39
3.	Tamenglong	Khoupum Valley -1	16.30	39.01
		Khoupum Valley -2	14.70	35.80
		Khoupum Valley -3	14.12	36.20
		Khoupum Valley -4	19.60	42.01
		Khoupum Valley -5	17.80	40.07
		Mean	16.51	38.62
4.	Churachandpur	Torbung Makha Leikai	12.60	30.01
		Torbung Mamang Leikai	11.09	29.70
		Vageing Village	15.70	33.78
		Khenjang Village -1	13.44	31.20
		Khenjang Village -2	15.08	35.65
		Mean	13.79	32.78
5.	Bishnupur	Leimaram -1	15.40	34.06
		Leimaram -2	12.08	30.90
		Sabual village	14.50	31.20
		Yumnam khunou-1	15.10	38.01
		Yumnam khunou-2	11.90	29.70
		Mean	13.58	32.07

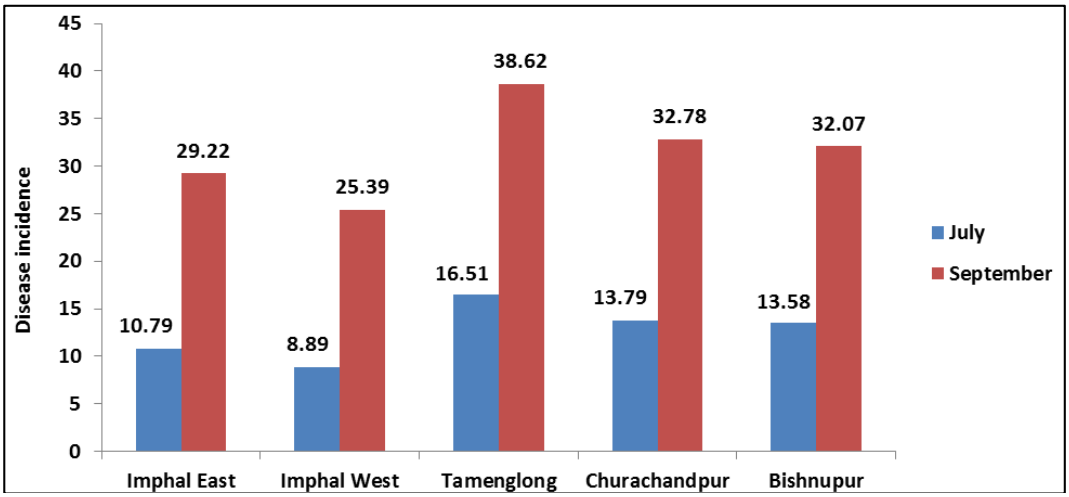

Fig 1: Disease Incidence of Rhizome rots of ginger at five different districts of Manipur during July and September (2016)

To purify the cultures of *Fusarium oxysporum* f.sp. *zingiberi* single spore was picked under a stereo-binocular microscope and transferred onto fresh PDA plates. The pure cultures were maintained on PDA slants at 4°C for further studies. The morphological characters, like cultural characteristics, shape and size of spores of *Foz* were studied and compared with the standard descriptions of Booth (1971) [3].

Table 3: Radial growth and cultural characters of the isolates of *Fusarium oxysporum* f. sp. *zingiberi* on PDA

S. N.	Site of collection	Isolates	Colony diameter (mm*)	Growth characters and colony color
1	Thaiyong -1	TH-1	82.0	Aerial felty, white regular margins
2	Moirangkampu	MK-1	85.0	Velvety, cottony, suppressed growth
3	Haraorok	HR-1	90.0	White irregular margins
4	Porompat-1	PO-1	87.0	Zonations present, white regular margins
5	Porompat-2	PO-2	90.0	Suppressed growth, white irregular margins
6	Iroisemba	IR-1	90.0	Cottony, suppressed, white, margins irregular
7	New Keithelmanbi -1	NK-1	80.0	Cottony, white aerial growth
8	New Keithelmanbi -2	NK-2	85.0	Cottony, white with regular margins
9	Keithelmanbi Namching -1	KN-1	87.0	White suppressed mycelium growth
10	Keithelmanbi Namching -2	KN-2	90.0	White mycelium, regular margins
11	Khoupum Valley -1	KV-1	90.0	Velvety, cottony pinkish mycelium
12	Khoupum Valley -2	KV-2	90.0	Velvety, white cottony suppressed growth
13	Khoupum Valley -3	KV-3	88.0	White regular margins
14	Khoupum Valley -4	KV-4	85.0	White irregular margins
15	Khoupum Valley -5	KV-5	86.0	Cottony, white aerial growth
16	Torbung Makha Leikai	TM-1	90.0	Velvety, cottony, suppressed growth
17	Torbung Mamang Leikai	TM-2	84.0	White suppressed mycelium growth
18	Vageing Village	VV-1	82.0	Velvety, pinkish mycelium
19	Khenjang Village -1	KH-1	86.0	Cottony, white aerial growth
20	Khenjang Village -2	KH-2	88.0	Cottony, white aerial growth
21	Leimaram-1	LM-1	80.0	Cottony, white aerial growth
22	Leimaram-2	LM-2	82.0	Suppressed growth, white irregular margins
23	Sabual village	SV-1	85.0	Cottony, white with regular margins
24	Yumnam Khunou-1	YK-1	85.0	Suppressed growth, white irregular margins
25	Yumnam Khunoou-2	YK-2	88.0	Zonations present, white regular margins
	SEm ±		1.8	
	CD at 5%		5.5	
	CV (%)		3.5	

The initial symptoms of the disease in the form of fast developing rot occurs on rhizome that prevents germination were visible on 15th day after sowing. The shoots are very weak and plants become stunted, yellowing of lower leaves which dry out was observed 45 days after sowing. From the diseased rhizomes, re-isolation of the pathogen was made which yielded the typical cultures of *Fusarium oxysporum* f.sp. *zingiberi* identical with the original ones that was inoculated. Isolation of the biocontrol agent was done from the air-dried rhizosphere soil of healthy ginger leaves and rhizome using selective media. The studies resulted in recovery of those organisms which are known to act as biocontrol agents of plant pathogens. The isolated cultures of *Trichoderma* spp. were identified as six isolates of *Trichoderma asperellum,* six isolates of *T. harzianum,* one isolate each of *T. hamatum* and *T. longibrachiatum* which were used for further *in vitro* screening.

All the isolates of *Foz* differed in colony characters on seven day of incubation under uniform environments and medium. The isolates showed growth range of 80.0 mm to 90.0 mm diameter and colony color of white to slight pinkish**.** Growth of different isolates of *Fusarium oxysporum* f.sp. *zingiberi* were recorded as described in experimental methods adopted, to know the optimum temperature for mycelial growth and sporulation of *Fusarium oxysporum* f.sp. *zingiberi* at 15,20,25 and 30^{0}C *Foz* isolates showed maximum growth and sporulation at 30^{0}C followed by 25^{0}C and minimum growth and sporulation were recorded at 15^{0}C temperature. The relationship of pH to the mycelial growth and sporulation of *Foz* was determined at different pH levels *viz*. 4.0 to 7.0 at 20°C for seven days which is described in experimental methods. The result showed maximum growth and sporulation of isolates of *Foz* at pH 7 followed by that at pH 6, whereas significantly less growth and sporulation were observed at pH 4. The soil samples collected belonged to shifting cultivation fields that had been burnt, cultivated and then left fallow after the crop harvest for two consecutive years. Soil pH ranges from 5-6 (Ralte *et al*., 2005; Ghosh *et al*.,2009) [11, 7], and this acidic nature is a consequence of its richness in aluminum and iron deposits, which can be stressors for existing life-forms and microbial population is uneven throughout the soil (Aishiki *et al*., 2017) [1].

Table 4: Effect of different temperatures on growth and sporulation of the isolates of *Fusarium oxysporum* f. sp. *zingiberi* on PDA

S. N	Isolates	Temperatures (^{0}C)							
		15		20		25		30	
		Growth (mm*)	Sporulation× 10^6 conidia/ mm^2	Growth (mm*)	Sporulation× 10^6 conidia/ mm^2	Growth (mm*)	Sporulation× 10^6 conidia/ mm^2	Growth (mm*)	Sporulation× 10^6 conidia/ mm^2
1	TH-1	53.0	2.5	60.0	2.6	69.0	2.5	80.0	2.6
2	MK-1	35.0	2.3	45.0	2.5	60.0	2.7	78.0	2.8
3	HR-1	72.4	2.2	78.0	2.3	82.0	2.4	90.0	2.5
4	PO-1	68.0	2.4	71.0	2.7	78.0	2.7	85.0	2.8
5	PO-2	70.0	2.1	75.0	2.2	80.0	2.3	88.0	2.3
6	IR-1	72.0	2.5	77.0	2.8	82.0	2.8	90.0	2.8
7	NK-1	65.2	3.0	71.0	3.1	79.0	3.1	86.0	3.1
8	NK-2	60.0	2.5	65.0	2.8	75.0	3.0	83.0	3.0
9	KN-1	56.0	2.2	62.0	2.3	70.0	2.5	80.0	2.5
10	KN-2	61.0	2.3	65.0	2.4	72.0	2.5	85.0	2.6
11	KV-1	69.0	2.0	73.0	2.1	78.0	2.3	89.0	2.4
12	KV-2	62.0	2.4	66.0	2.5	75.0	2.6	86.0	2.6
13	KV-3	60.0	2.6	65.0	2.6	75.0	2.8	88.0	3.0
14	KV-4	58.0	2.5	66.0	2.7	77.0	2.8	85.0	2.8
15	KV-5	61.0	2.2	69.0	2.4	78.0	2.5	86.0	2.5
16	TM-1	63.0	2.1	68.0	2.2	75.0	2.3	85.0	2.5
17	TM-2	59.0	2.0	65.0	2.2	73.0	2.3	88.0	2.4
18	VV-1	56.0	2.3	60.0	2.5	75.0	2.6	83.0	2.6
19	KH-1	60.0	2.2	69.0	2.3	80.0	2.4	90.0	2.4
20	KH-2	59.0	2.0	65.0	2.2	75.0	2.3	85.0	2.4
21	LM-1	55.0	2.5	61.0	2.5	79.0	2.5	89.0	2.5
22	LM-2	62.0	2.3	67.0	2.4	76.0	2.5	84.0	2.5
23	SV-1	60.0	2.6	65.0	2.6	78.0	2.6	89.0	2.6
24	YK-1	55.0	2.1	60.0	2.4	69.0	2.5	78.0	2.5
25	YK-2	57.0	2.2	63.0	2.3	70.0	2.4	85.0	2.5
S. Em ±		1.2	0.5	1.4	0.6	1.7	0.6	1.7	0.7
CD at 5%		1.5	0.1	2.8	0.9	3.2	0.2	2.4	0.2
CV (%)		3.9	1.2	4.5	1.5	5.2	2.9	5.2	1.2

Table 5: Effect of different pH levels on growth and sporulation of the isolates of *Fusarium oxysporum* f. sp. *zingiberi* on PDA

S. N	Isolates	pH ranges							
		4		5		6		7	
		Growth (mm*)	Sporulation× 10^6 conidia/ mm^2	Growth (mm*)	Sporulation× 10^6 conidia/ mm^2	Growth (mm*)	Sporulation× 10^6 conidia/ mm^2	Growth (mm*)	Sporulation× 10^6 conidia/ mm^2
1	TH-1	55.0	1.5	65.0	1.8	75.0	2.0	86.0	2.2
2	MK-1	42.0	1.8	58.0	2.0	66.0	2.0	73.0	2.0
3	HR-1	70.0	2.0	75.0	2.0	82.0	2.2	90.0	2.3
4	PO-1	63.0	2.0	70.0	2.0	80.0	2.0	90.0	2.0
5	PO-2	67.0	1.8	72.0	2.0	79.0	2.0	90.0	2.0
6	IR-1	70.0	2.0	78.0	2.0	85.0	2.2	88.0	2.4
7	NK-1	63.0	2.2	70.0	2.4	80.0	2.4	84.0	2.4
8	NK-2	65.0	1.8	70.0	2.0	78.0	2.1	82.0	2.2
9	KN-1	66.0	2.0	72.0	2.3	80.0	2.5	85.0	2.5
10	KN-2	65.0	1.8	70.0	2.0	80.0	2.0	90.0	2.0
11	KV-1	70.0	2.0	73.0	2.1	82.0	2.3	90.0	2.3
12	KV-2	65.0	2.1	70.0	2.0	78.0	2.0	85.0	2.1
13	KV-3	64.0	2.2	75.0	2.3	75.0	2.5	82.0	2.5
14	KV-4	60.0	2.0	68.0	2.0	75.0	2.0	88.0	2.0
15	KV-5	67.0	2.2	70.0	2.4	74.0	2.5	80.0	2.5
16	TM-1	62.0	2.0	65.0	2.2	70.0	2.3	80.0	2.3
17	TM-2	60.0	2.0	65.0	2.2	75.0	2.3	85.0	2.4
18	VV-1	55.0	1.8	62.0	2.0	78.0	2.0	90.0	2.0
19	KH-1	61.0	2.0	65.0	2.0	78.0	2.2	85.0	2.4
20	KH-2	60.0	2.0	65.0	2.2	72.0	2.3	80.0	2.4
21	LM-1	65.0	2.2	71.0	2.5	80.0	2.5	90.0	2.5
22	LM-2	62.0	2.1	70.0	2.2	80.0	2.2	90.0	2.2
23	SV-1	60.0	2.4	68.0	2.4	75.0	2.5	85.0	2.5
24	YK-1	58.0	2.2	63.0	2.5	70.0	2.5	82.0	2.8
25	YK-2	60.0	1.8	68.0	1.8	77.0	2.0	82.0	2.5
S.Em ±		1.7	0.3	1.6	0.5	1.5	0.6	1.7	0.7
CD at 5%		3.1	0.9	5.4	0.5	4.3	0.8	5.4	0.2
CV %		3.5	3.6	3.7	3.7	3.9	3.9	3.6	3.8

Screening of *Trichoderma* spp. against *Fusarium oxysporum* f.sp. *zingiberi* was conducted according to Bell *et al*.1982 [2]. The antagonism test showed that 78% of the Trichoderma isolates strongly invaded *Foz in vitro* (rating =1 or 2). 47% had a rating of 1, 25% had a rating of 2, 15% had a rating of 3, 9% had a rating of 4 and 4% had a rating of 5.The biocontrol agent (*T.harzianum*-MH259837) could successfully establish in the ginger rhizosphere and multiplied to reach high densities counts (c.f.u) of *Trichoderma* seed treated. Comparison between the population densities of most potent Trichoderma isolate (*T. harzianum*-MH259837) and *Fusarium oxysporum* f.sp. *zingiberi* in ginger field at plains and under jhum cultivation was carried out. The initial population 6.8 × 10^5 c.f.u /g seed for *T.harzianum*-MH259837 and 2.4× 10^6 c.f.u /g soil was recorded. The result showed that among the different substrate tested for mass multiplication of *T.harzianum*-MH259837 in plains at 30 DAS highest population densities was recorded in treatment with rice bran + *T.harzianum*-MH259837 (6.5 × 10^5 c.f.u /g soil) which was followed by well decomposed FYM + *T.harzianum*-MH259837 (6.2 × 10^5 c.f.u /g soil), poultry manure + *T.harzianum*-MH259837 (6.1 × 10^5 c.f.u /g soil), paddy chaff + *T.harzianum*-MH259837 (5.8 × 10^5 c.f.u /g soil), paddy straw + *T.harzianum*-MH259837 (5.3 × 10^5 c.f.u /g soil), sugarcane bagasse + *T.harzianum*-MH259837 (5.0 × 10^5 c.f.u /g soil) respectively. Also, population densities of *Fusarium oxysporum* f.sp. *zingiberi* at 30 DAS showed the highest in treatment with sugarcane bagasse + *T.harzianum*-MH259837 (2.2 × 10^6 c.f.u /g soil) as compared to untreated control (2.5 × 10^6 c.f.u /g soil) followed by paddy straw + *T.harzianum*-MH259837 (1.8 × 10^6 c.f.u /g soil), poultry manure + *T.harzianum*-MH259837 (1.5 × 10^6 c.f.u /g soil), well decomposed FYM + *T.harzianum*-MH259837 (1.2 × 10^6 c.f.u /g soil), paddy chaff + *T.harzianum*-MH259837 (1.1 × 10^6 c.f.u /g soil), rice bran + *T.harzianum*-MH259837 (1.0 × 10^6 c.f.u /g soil) respectively. Result of population densities of *T.harzianum*-MH259837 at 60 DAS showed the highest with rice bran + *T.harzianum*-MH259837 (5.1 × 10^5 c.f.u /g soil), followed by well decomposed FYM + *T.harzianum*-MH259837 (4.0 × 10^5 c.f.u /g soil), poultry manure + *T.harzianum*-MH259837 (3.7 × 10^5 c.f.u /g soil), paddy chaff + *T.harzianum*-MH259837 (3.5 × 10^5 c.f.u /g soil), paddy straw + *T.harzianum*-MH259837 (2.9 × 10^5 c.f.u /g soil), sugarcane bagasse + *T.harzianum*-MH259837 (2.6 × 10^5 c.f.u /g soil) respectively. Whereas the population densities of *T.harzianum*-MH259837 and *Fusarium oxysporum* f.sp. *zingiberi* in ginger field under jhum cultivation showed the highest population densities of *T.harzianum*-MH259837 at 30 DAS with rice bran + *T.harzianum*-MH259837 (6.0 × 10^5 c.f.u /g soil) which was followed by well decomposed FYM + *T.harzianum*-MH259837 (5.8 × 10^5 c.f.u /g soil), paddy straw + *T.harzianum*-MH259837 (5.6 × 10^5 c.f.u /g soil), poultry manure + *T.harzianum*-MH259837 (5.2 × 10^5 c.f.u /g soil), paddy chaff + *T.harzianum*-MH259837 (5.0 × 10^5 c.f.u /g soil), sugarcane bagasse + *T.harzianum*-MH259837 (5.0 × 10^5 c.f.u /g soil) respectively. And the population densities of *Fusarium oxysporum* f.sp. *zingiberi* was recorded the highest with sugarcane bagasse + *T. harzianum*-MH259837 (2.8 × 10^6 c.f.u /g soil) as compared to untreated control (3.0 × 10^6 c.f.u /g soil). Followed by paddy chaff + *T.harzianum*-MH259837 (2.5 × 10^6 c.f.u /g soil), poultry manure + *T.harzianum*-MH259837 (2.4 × 10^6 c.f.u /g soil), paddy straw + *T.harzianum*-MH259837 (1.8 × 10^6 c.f.u /g soil), well decomposed FYM + *T.harzianum*-MH259837 (1.6 × 10^6 c.f.u /g soil), rice bran + *T.harzianum*-MH259837 (1.0 × 10^6 c.f.u /g soil) respectively. Greater microbial populations in FYM treated plots as compared to chemically amended plots were reported by Venkateswarlu (2000) [14] and Sharma *et al*. (1983) [13]. Application of farm yard manure to agricultural fields can be viewed as an excellent way to recycle nutrients, maintain soil quality and in harbouring higher fungal populations.

Table 6: Population densities of potent isolate *T. harzianum* (MH259837) and *Fusarium oxysporum* f. sp. *zingiberi* in rhizosphere of ginger at 30 and 90 days after sowing in the field under plains and jhum cultivation during *Kharif* 2017

Rhizosphere population of *Trichoderma* and *Fusarium oxysporum* f. sp. *Zingiberi*									
S. N	**Treatments**	**Population under Plain**				**Population under Jhum cultivation**			
		30 DAS		**90 DAS**		**30 DAS**		**90 DAS**	
		***Tricho derma* × 10^5 / g soil**	***Foz* × 10^6/ g soil**	***Tricho derma* × 10^5 / g soil**	***Foz* × 10^6/ g soil**	***Tricho derma* × 10^5 / g soil**	***Foz* × 10^6/ g soil**	***Tricho derma* × 10^5 / g soil**	***Foz* × 10^6/ g soil**
1	Rice bran + *T. harzianum* (MH259837)	6.5	1.0	5.1	0.8	6.0	1.4	5.0	1.1
2	Paddy chaff + *T. harzianum* (MH259837)	5.8	1.1	3.5	1.0	5.0	2.5	2.9	0.5
3	Paddy straw + *T. harzianum* (MH259837)	5.3	1.8	2.9	1.4	5.6	1.8	3.6	0.7
4	Sugarcane bagasse + *T. harzianum* (MH259837)	5.0	2.2	2.6	1.8	4.5	2.8	2.8	0.7
5	Poultry manure + *T. harzianum* (MH259837)	6.1	1.5	3.7	1.1	5.2	2.4	3.1	0.5
6	Well decomposed FYM + *T. harzianum* (MH259837)	6.2	1.2	4.0	0.9	5.8	1.6	4.2	1.0
7	Untreated control	-	2.5	-	3.8	-	3.0	-	4.2
SEm±		0.3	0.0	0.0	0.8	0.1	0.5	0.1	0.1
CD at 5%		0.9	0.2	0.1	0.5	0.4	0.4	0.2	0.2
CV (%)		2.2	1.5	1.8	1.2	2.0	1.6	1.6	1.2

* Initial population of *T. harzianum* (MH259837) 6.8 × 10^5 /g seed in all the treatments.
Initial population of *Fusarium oxysporum* f. sp. *Zingiberi*. In soil 2.4 × 10^6 c.f.u /g soil

The success of a biocontrol agent depends much on the establishment of the product, the formulation and delivery system. The production of propagules can be carried out in liquid or solid fermentation. The maximum biomass production is influenced by aeration, agitation, pH, and temperature. It has been reported that the formulation used to

introduce *Trichoderma* in soil influences rhizosphere competence. Agricultural wastes that have been reported to be good substrates as a result rice bran was found to be the most suitable substrate and highest spore count was observed for BCA. According to the studies conducted by Henry *et al*., (2016) [8], the possible causes of negative impact of burning on soil enzyme activities are: sudden reduction in soil biota population and their activities, depletion of hydrolytic enzyme pools due to breakdown of above- and below-ground community linkages, nutrient enrichment in soils after burning reduce the dependency of crop plants on enzyme activities. The decrease in microbial biomass is an indication of lower abundance of microbial population as observed in burnt soils as compared to unburnt soils. Miah *et al*., (2010) [9] reported that shifting cultivation lands show a considerable decrease in the microbial community as well as a loss of certain species of both bacteria and fungi, as compared to native forest lands. Such factors associated with jhum create a stressful environment for micro-organisms to thrive, thus, only a small fraction of the microbial isolates was able to exhibit PGP abilities when tested for these traits (Panday *et al*., 2011) [10].

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