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Role of Volatile Metabolites from *T. citrinoviride* in Biocontrol of Phytopathogens

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Abstract: *Biological control of phytopathogens is an ecological, environmental and economical way of disease control. Six isolates of Trichoderma were isolated from rhizosphere soil samples collected and screened for their biocontrol potential. In vitro activities like siderophore, cellulase, chitinase, β-1,3-glucanase, IAA, phosphate solubilization, HCN, ammonia, organic acids, and phytase production were estimated in the cell free filtrate. Among them one potent isolate was selected on the basis of it being able to antagonize two fungal phytopathogens M. phaseolina and S. sclerotiorum showing 92.54 % and 90.94% inhibition by dual culture method and denoted as MBAAT. Identification and profiling of volatile metabolites produced by MBAAT showed the presence of a compound (3-methylbutanal and 2-methyl, 2-butane) at 1.84 and 16.32 mins of retention time respectively. The VOC's of this fungus demonstrated growth inhibitory activity against phytopathogens. An attempt was made to evaluate and compare the isolate with commercial T. viride (CTC) product against the disease under pot trials. The disease incidence with M. phaseolina positive control was 82.35% and a drastic decline in disease to 15.25% was observed in Vigna radiata plants treated with M.phaseolina + MBAAT.*

Keywords: Biological control, Phytopathogens, *T. citrinoviride*, Volatile Metabolites

Introduction

Many chemicals have been developed and used as pesticides to reduce food shortages throughout the world [1]. Their continued use, however, often results into resistance to these chemicals to pathogens, which can develop molecular mechanisms that allow them to survive levels of these chemicals that would previously kill them [2]. The development of fungicide resistance becomes common and the mechanisms of resistance to fungal pathogens may rely on the insensitivity of target sites to fungicides and enhanced detoxification by a number of metabolizing enzymes [3] . Therefore, the application of biocontrol agents and the metabolites produced by them to control diseases of plants is a potentially viable alternative. This strategy is an effective and ecofriendly control mechanism is of great importance. Biological control agents are considered very promising mechanism for plant protection [4].

Trichoderma have been successfully applied for control of plant pathogenic fungi in a variety of important crops [5]. The mechanisms underlying their antagonism for plant disease control involve mycoparasitism, antibiosis, competition with other microorganism, promotion of root and plant development, induction of plant disease resistance, inactivation of the pathogen's enzymes [6].

Glycine max is considered as one of the most popular and widely used crops in form of oilseeds, pulses etc. This is probably due to the fact that even under minimum agricultural inputs and management practices it fetches profitable returns to its growers. In recent years, *Glycine max* has assumed as important crop in India, as it is included in *kharif* crops and produce cost effective yield in agriculture. *Vigna radiata* an important group of grain legumes is a good source of proteins and vitamins. It is also a very important source of energy and vitamins. However, the reduction in yield of *Vigna radiata* is observed because of the diseases caused by plant pathogenic fungi. Root infecting fungi like *M. phaseolina* are reported to produce charcoal rot, seedling blight, root rot, stem rot, pod rot and decline in productivity on *Glycine max* and *Vigna radiata* [7]. *R. solani* is another soil borne pathogen known to produce seed rot, damping off of seedling, wilt and root rot [8], where as, *Fusarium solani f. sp. glycines* infect the root rot of seedling and causes necrosis, chlorosis, defoliation of leaves [9].

Strains of *Trichoderma* like *T. harzianum, T. hamatum, T. asperellum* and *T. atroviride* are applied for the control of phytopathogens and also as plant growth promoters in agriculture [10]. Volatile and non volatile secondary metabolites have been reported to play an

important role in the biocontrol mechanism of *Trichoderma* and disease management [11]. Production of many volatiles like pyrones, sesquiterpenes and non-volatile secondary metabolites like peptaibols have been reported the potential mechanism of *Trichoderma spp*. [12]. Microbial volatile metabolites may be intermediate or end products of metabolic pathways and have been identified as mono- and sesquiterpenes, alcohols, ketones, lactones, esters or C8 compounds [13]. These metabolites play a key role in biocontrol mechanism and can be studied using mass spectrometry (MS) [14] by which individual volatile metabolite can be identified from complex mixture. Diversity of MVOC profiles of *Trichoderma* have been recorded which are influenced by culture media and depicted the structure and function of volatile metabolites of this fungus [15].

The objectives of this research paper are to evaluate the potency of natural isolate as a biocontrol agent in controlling the soil-borne phytopathogen *in vitro* as well as *in vivo* using *Glycine max* and *Vigna radiata* and the role and type of VOC profile are studied.

Material and Methods

Isolation and identification of fungal cultures: Six *Trichoderma* isolates were isolated from rhizosphere soil and screened for plant growth promoting and biocontrol activity. They were identified as on the basis of 18S rRNA analysis. *Trichoderma viridae* (CTC) was procured from the commercial product (Monitor) of the Agriland Biotech, Baroda, Gujarat, India and used for sake of comparison. Stock cultures of phytopathogens were maintained on PDA slants and maintained at 4°C. They were procured from the laboratory of corresponding author.

In Vitro plant growth promoting and biocontrol potential of fungal isolates: The isolated fungi were tested for various plant growth promoting and biocontrol activities such as HCN production [16], ammonia production from organic compound using Nessler's reagent [17], IAA production using 1% tryptophan [18], siderophore production using chromeazurol S (CAS) agar plates [19], phosphate solubilization on pikovskya's medium [20], MM9 medium was used for testing organic acid production using phenol red as pH indicator [21], potassium solubilization ability of fungi was detected using insoluble potassium compound (potassium aluminum silicate) and bromophenol blue as pH indicator for monitoring change of colour [21], solubilization of zink was detected using zink compound like zinc phosphate, and zinc carbonate in tris minimal plates and solubilization zone was measured [22], chitinase on using colloidal chitin [23], β-1,3 glucanase using laminarin and cellulase using CMC as a carbon source [24]. The plates inoculated and then incubated at 28^{o}C for 5 days and evaluated for above mentioned activities.

Optimization of growth and in vitro biocontrol activity of MBAAT: Optimumization of the growth of MBAAT was carried out simultaneously using variations in physical conditions and nutrients. Biocontrol activity of MBAAT and *Trichoderma viridae* were tested against two known phytopathogenic fungi *M.phaseolina*, and *S.sclerotiorum* procured from the laboratory of corresponding author by dual culture method in plates and flasks by the method of Skidmore and Dickinson [25].

Percentage inhibition of radial growth (PIRG) was calculated as below:

$$\% \text{ inhibition} = \frac{R1-R2}{R1} \times 100$$

where, PIRG = percentage inhibition of radial growth; R1 = radial growth of pathogen (control); R2 = radial growth of pathogen in the presence of the antagonist.

Interaction of antagonist with phytopathogen: For MBAAT - pathogen interaction, sterile PDA was pipette over the sterile glass slide to make a thin film on the slide. 5 mm discs of actively growing colonies cut from the margin of each pathogen and MBAAT was placed on the other end of the slide 3 cm apart on the PDA surface and incubated 25 ± 1°C for a week. Interaction between *Trichoderma* - *Sclerotinia* was stained with lactophenol and microscopic observation performed for the presence of mycelial penetration and for cell wall disintegration [26].

Antifungal activity of volatile organic compounds: The effect of volatile metabolites from MBAAT was tested in the assemblage described by Dennis and Webster [27]. Two bottoms of petri dishes containing PDA were individually inoculated with a disc of pathogen and antagonist, and bottoms were adjusted and attached by tape. The plate without antagonist acted as a control. Radial growth of fungi was compared with control.

GC-MS analysis: For analysis of volatile metabolites produced, the culture MBAAT was inoculated on PDA and incubated at 28 °C for 7 days. An 8 mm disc from this was later paced on slant of PDA in a 20 m head space vial (HS vial) [28]. The vials were sealed so as to inimize losses of volatile compounds. Negative controls were also used to evaluate results. After three days of incubation at 28 °C the HS vials were equilibrated incubators at 50^{0}C for 10 mins. Gas chromatograph prepared with a gas injection port and coupled to a mass spectrometer (Agilent Technologies) was used for further analysis. Analytes were injected at 240 ºC and separation of VOCs was carried out on a BPX-volatiles capillary column of specified dimensions using helium carrier gas at the rate of 1 ml/ min. For interpretation of results obtained Chem Station (Agilent Technologies) software package Mass spectral library (Data version: NIST 05, Software version 2.0d) was used [28].

Green house experiment

Preparation and application of spore suspensions: *Trichoderma* isolates and phytopathgenic fungi were inoculated to 100 ml PDA containing flask and incubated at 28^{0}C in shaker. After 7days the contents were filtered through sterile muslin cloth, the spores, was collected, and a 5×10^{5} spores/ml was used to treat the seeds of *Glycine*

max and *Vigna radiata*. 10-15 seeds were treated with spore suspension of *Trichoderma* strains for 20 mins and dried in sterile air. Both treated and untreated seeds of *Glycine max* and *Vigna radiata* were again dipped in the spore suspension of 7 days old culture of pathogens for about 20 min. After germination of the seeds, the sterilized pot soil was inoculated with conidial suspension (30 ml) according to respective treatments as shown. The treatment was continued at regular interval of seven days up to harvesting.

Treatments

Experiment consisted of following combinations

T0 = Control (untreated soil and untreated seeds)
T1 = *M. phaseolina*
T2 = *S. sclerotiorum*
T3 = MBAAT + *M. phaseolina*
T4 = MBAAT + *S. sclerotiorum*
T5 = *T. viride* + *M.phaseolina*
T6 = *T. viride* + *S. sclerotiorum*

Vigour index and yield contributing parameters: All treated seeds (10 per pot) were sown separately in each pot filled with treated soil. Untreated seeds containing untreated soil was kept as a positive control. Seed germination percentages were recorded after seven days. Vigour index for each treatment was calculated [29].

$$\text{Germination percentage} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} X\ 100$$

Vigour index = [Mean of root length (cm) + Mean of shoot length (cm)] x percentage of seed germination

Disease incidence was calculated as follow.

$$\text{Disease Incidence} = \frac{\text{No. of infected Plants}}{\text{No. of total Plants}} X\ 100$$

Statistical analysis: Statistical analysis was done using ANOVA software for individual parameters. All hypotheses were tested at the 1% confidence level.

Results and Discussion

Isolation and identification: Six *Trichoderma* isolates were isolated from rhizosphere soil and they were identified as on the basis of 18S rRNA analysis and sequence was submitted in gene bank (Accession numbers awaited). Among them potential antagonist was selected and was denoted as MBAAT (Accession number KF380830) (Figure 1).

Screening of *Trichoderma* for *In vitro* plant growth promoting and biocontrol potential: All the isolates screened for their PGPR and biocontrol potential such as HCN, ammonia, siderophore, IAA, organic acid, chitinase, β-1,3 glucanase, cellulase production, phosphate, zinc and potassium solubilization. Two known phytopathogens were taken from the laboratory of corresponding author and they were *M. phaseolina* and *S. sclerotiorum.* One isolate (MBAAT) formed clear zone around the growth in the plate containing pikovskaya's medium and colour change from blue to yellow indicate the pH reduction because of phosphate solubilization. Only two isolates of *Trichoderma* (MBAAT and MBAAT5) showed IAA production and only one isolate of fungi (MBAAT) produced HCN as evidenced by the change in the colour of filter paper. Two isolates (MBAAT and MBAAT5) showed siderophore production as indicated by formation of orange halos on the CAS agar medium. Only one isolate (MBAAT) showed organic acid production on M9 minimal medium and also produced a visible zone of K solubilization. The isolate MBAAT showed zink solubiization resulting in zone of solubilization in medium. Only isolate MBAAT showed chitinase activity. However, all isolates grew on laminarin amended minimal medium showing β- 1,3 glucanase activity while only MBAAT utilized cellulose indicating cellulase production (Table 1). The production of lytic enzymes by *Trichoderma* species is known as one of the major mechanisms for biocontrol activity against phytopathogenic fungi. involvement of glucanases in control of phytopathogens was reported [30]. Proteases act as cell wall degrading enzyme in biocontrol processes which was showen in the literature [31]. Phosphate solubilizing efficiency of different isolates of *T. harzianum* was observed by Tallapragada and Gudimi [32] on Sperber's medium with modifications. It is also reported the siderophore production by *Trichoderma asperellum Q1* [33]. This is reported the HCN production by fungal isolates SE6, KT28 and BRT11 while ammonia production by *T. harzianum*, KT6, SE6, KT28 and BRT11 [34]. Similarly lytic enzymes like chitinases and β-1,3 glucanases, proteases and cellulases are potential mechanism associated with the ability of *Trichoderma* to control phytopathogens [35].

In vitro biocontrol activity by dual culture technique

Optimum growth condition for MBAAT was determined by optimizing different time, temperature, incubation time, speed of agitation and different nitrogen sources which is shown in Table 2. Inhibition of phytopathogens was evaluated by dual culture technique. The experiment was repeated three times and the results of dual culture showed that the antagonists significantly inhibited the mycelial growth of the pathogen. Specially MBAAT inhibited the growth of *Macrophomina* and *Sclerotinia*. A clear zone of inhibition was observed between MBAAT and pathogenic fungi, Maximum growth inhibition was recorded by MBAAT with *M. phaseolina* that is 92.54% shown in Figure 2. Culture conditions were identified that optimize the growth of the microorganism [36]. This study confirmed that MBAAT grew better with 1% peptone after 72 hours at pH 5.6, at 30°C temp and 200 rpm shown in Table 2. Previous studies also reported that optimum pH for *Trichoderma virens* UKMP-1M was 5.5, optimum temperature was 30°C, optimum agitation speed was 200 rpm and optimum nitrogen source was 1.5% peptone.

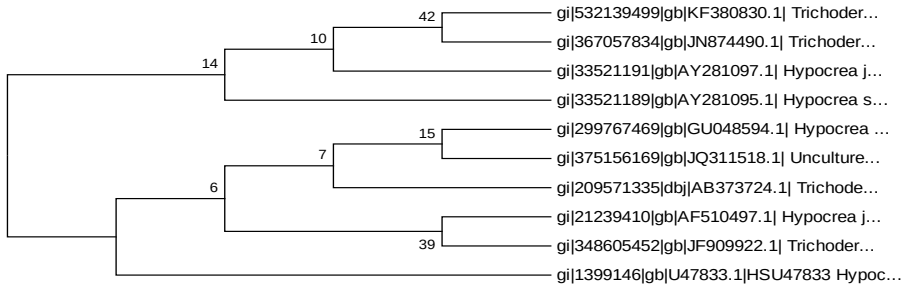


Figure 1: Phylogenetic relationship on the basis of homology index for fungal isolate *Trichoderma citrinoviride* MBAAT

Table 1
Screening of isolates for in vitro Plant Growth Promoting and biocontrol Potential

Isolates		H HCN	NH_3	IAA	Siderophore	PO_4 Solubilization	Organic acid production	Potassium solubilization	Zn solubilization	Chitinase Production	β-1,3 Glucanase production	Cellulase Production
MBAAT	*Trichoderma citrinoviride*	+	+	+	+	+	+	+	+	+	+	+
MBAAT1	*Trichoderma hamatum*	-	-	-	-	-	-	-	-	-	+	-
MBAAT2	*Trichoderma longibrachiatum*	-	-	-	-	-	-	-	-	-	+	-
MBAAT5	*Trichoderma sp.*	+	-	+	+	-	-	-	-	-	+	-
MBAAT8	*Trichoderma longibrachiatum*	-	-	-	-	-	-	-	-	-	+	-
MBAAT10	*Trichoderma hamatum*	-	-	-	-	-	-	+	+	-	-	-

Table 2
Determination of optimal growth of MBAAT by using physical and nutrient parameters.

Parameters	pH	Temperature	Incubation Time	Speed of Agitation	Nitrogen Source
Optimum value	5.6	30^0C	72 hours	200 rpm	1%Peptone
Biomass Production	0.26 g/l	0.24 g/l	0.35 g/l	0.25 g/l	0.96 g/l

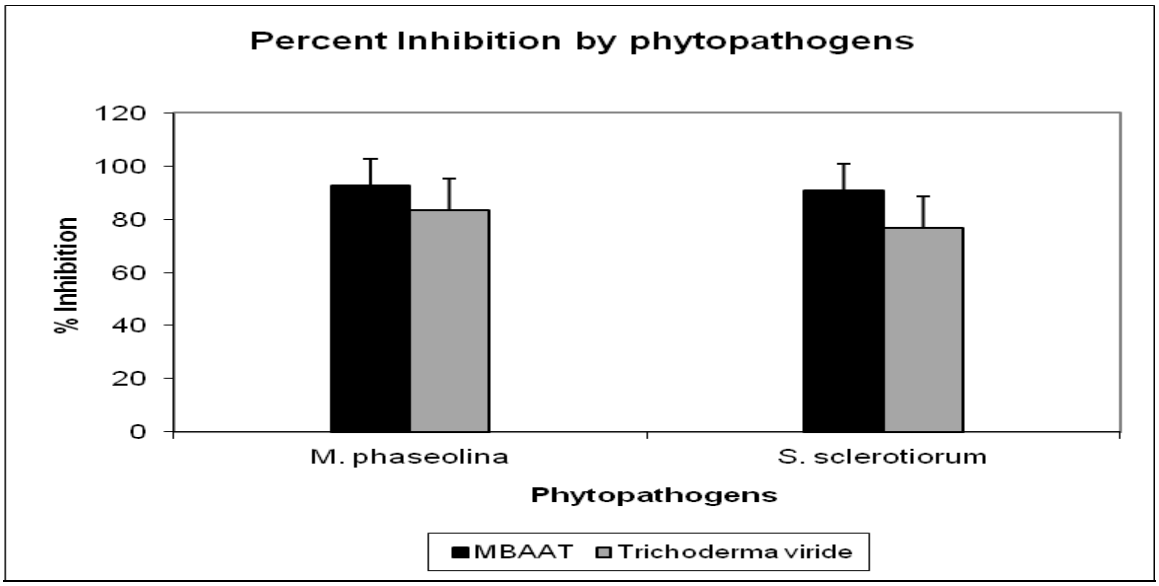
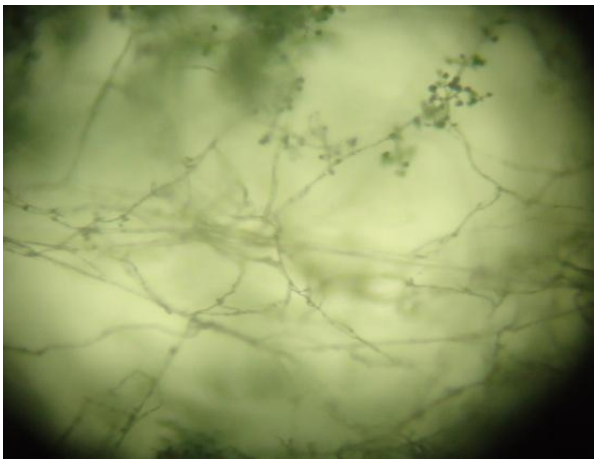
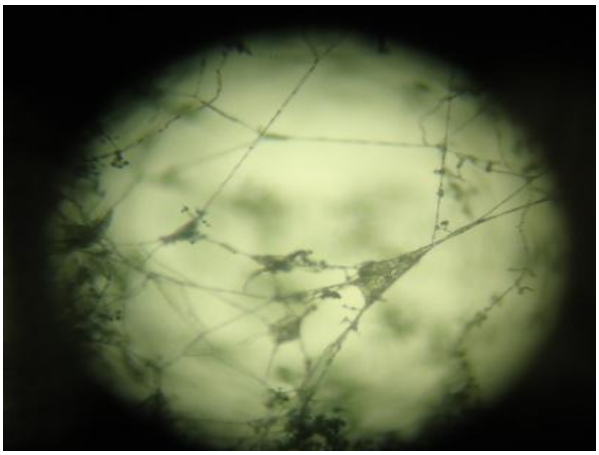


Figure 2: Percent inhibition of phytopathogens by MBAAT and CTC

Microscopic observations of MBAAT and pathogen interaction (slide culture): The mycelia of *Sclerotinia* showed abnormal morphology and distortion gives bead-chain appearance. The mycelia of MBAAT retained it's original structure and showed branching as they approached *Sclerotinia* (Figure 3). Distinct changes were observed in morphology on comparing phytopathogen mycelia in presence of MBAAT in comparison to absence of MBAAT. phytopathogen mycelia mounted from control plate showed normal structure retaining to it's morphology [37]. Dual culture study report of *T. harzianum* shown that it controlled many phytopathogenic fungi of *Phomabetae*, *Rosellinia necatrix*, *Botrytis cinerea* and *Fusarium oxyporum* f. sp. *Dianthia* [38]. Similar reports are found that microscopy of the interaction region between *R.solani* and *Pythium* spp. with *T. harzianum* showed that the mycelia of *T. harzianum* grew on the surface of the pathogens always coiling round their mycelia and later penetrating their cell walls directly without formation of appresorium structures [26].



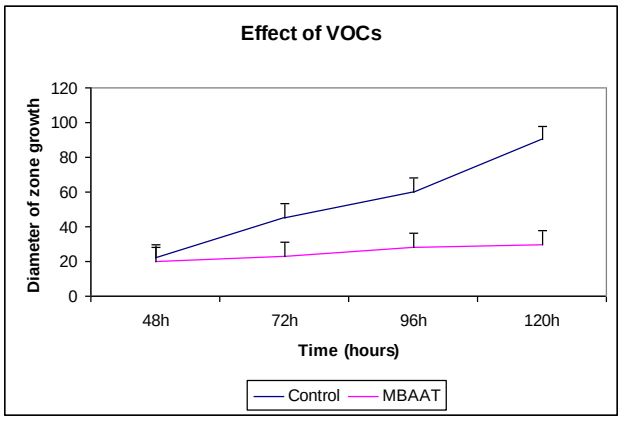
A



b

Figure 3: (a) Control: *S. sclerotiorum* (b) Coiling of pathogen hyphae by MBAAT

Antifungal activity of volatile organic compounds: VOCs produced by MBAAT found to reduce the mycelium growth of both phytopathogens. MBAAT showed the maximum reduction of *S.sclerotiorum* compared of to *M.phaseolina* (Figure 4). After the incubation of five days, the phytopathogen growth appeared to be almost negligible. It is showed that maximum reduction of *R.solani* growth compared to strain Bga ICMP 12322 [39]. On the other hand, it was found that volatile metabolites of *Pseudomonas* spp., *Serratia* spp., *Stenotrophomonas* spp. greately decreased the growth of *R. solani*, while a minimum amount of decline was observed with the volatile metabolites of *B. cepacia and Staphylococcus epidermidis*, respectively [40].



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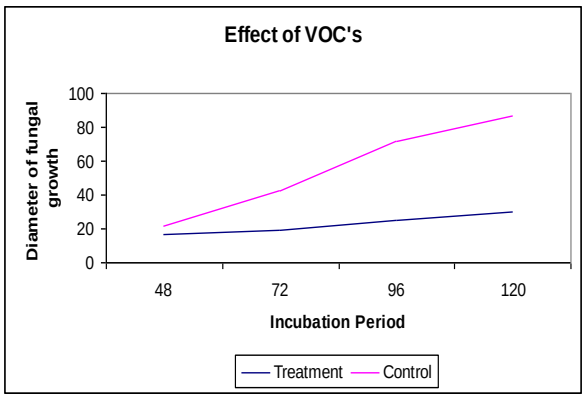
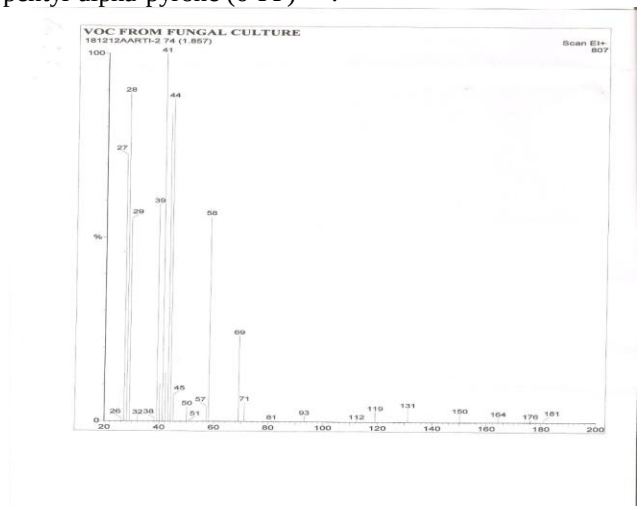


b

Figure 4: (a) Effect of volatile metabolites of MBAAT on the growth rate of *S. sclerotiorum* (b) Effect of volatile metabolites of MBAAT on the growth rate of *M. phaseolina*

Determination of VOCs by GC-MS analysis

GC/MS analysis of the VOCs produced by MBAAT after 5 days incubation at 28 ºC on headspace vial PDA medium as described in the methods section. The analysis of metabolites produced by MBAAT showed the presence of 3-methylbutanal and 2-methyl, 2-butane which were detected at retention time 1.86 and 16.32 min (Figure 5) and having molecular weight described in the Table 3. Figure shows a typical chromatogram recorded from a *T. citrinoviride* MBAAT culture after incubation for 5 days. An library spectra was used, containing mass spectra and LTPRI of putative volatile metabolites produced by *Trichoderma spp*. The GC-MS data were deconvoluted using the NIST 05, version 2.0d software and the obtained mass spectra were compared. The volatile metabolites were identified on the basis of the match factor for sample spectra ≥90% with reference to the in-house sub-library. These compounds could be responsible for the biocontrol activity of MBAAT against studied phytopathogenic fungi. Volatile metabolites have significant role in biocontrol of phytopathogens. Volatile compounds such as 1-methyl-and cyclopropane pentene were present including butanal as well as some alkanes derivatives, and ketones. Similar reports were found that the volatile compound β-Phelandrene and 6-Pentyl-2H-pyran-2-one produced by *T. atroviride* O.Y. 3807 and was found to be 85 % of all the produced volatile metabolites it is reasonable as a potential active antifungal compound produced by *T.atroviride* [28]. It is reported that the compound in the greatest abundance, or relative percentage, from days 9–21 was 3- octanone [41]. It was reported that 11 volatile compounds from *Trichoderma atroviride* and one volatile metabolite identified was 6-pentyl-alpha-pyrone (6-PP) [42].



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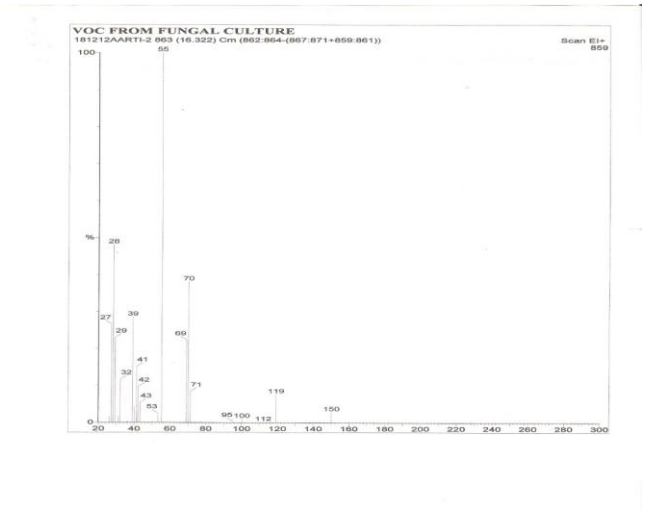


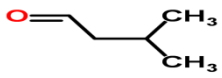
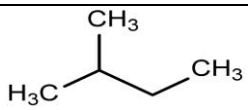
b

Figure 5: Mass spectrum of (a) 3-methylbutanal at retention time 1.86 min (b) 2-methyl, 2-butane at retention time 16.32 min

Green house experiment : Plants treated with MBAAT were showed vital biocontrol potential against *M. phaseolina* and *S. sclerotiorum* (Table 4). Whereas, the maximum disease reduction was achieved with *S. sclerotiorum* and followed by *M. phaseolina*. Effect of phytopathogen can affect the germination of *Glycine max* and *Vigna radiata* plant as the pathogens affect the root system. Control seeds (untreated) had higher germination rate was observed then pathogen infested control.

Table 3

compounds identified after GC-MS at retention time 1.86 min and 16.32 min

Hit	Retention Time	Name of the compound	Molecular weight	Structure	Molecular Formula
865	1.86 mins	3-methylbutanal	86		C5H100
864	16.32 mins	2-methyl, 2-butane	70		C5H10

The maximum diseased incidence was found in *Sclerotinia* treated plants and infection rate reached up to 97.45% in the absence of antagonist. Plants treated with MBAAT + pathogen showed increase in all biometric parameters of plant and chlorophyll content of plant significantly ($p<0.05$) as compared to negative (+disease) and positive (-disease) controls. Moreover, MBAAT and CTC show a significant increase in plant growth in the absence of pathogen. However, antagonistic strain CTC was the less successful in disease control as compared to strain MBAAT against both phytopathogens. Likewise, phytopathogens also caused decrease of chlorophyll content of *Glycine max* and *Vigna radiata* leaves However, MBAAT significantly enhanced chlorophyll content of *Glycine max* (7.538 mg/g) in the presence or absence of *M. phaseolina* as compared to CTC (5.214 mg/g). In comparison least chlorophyll content (1.352 mg/g) was observed with *M. phaseolina* infected plants. Under pot trials, MBAAT and CTC treated plant showed significant decline in disease symptoms in *Glycine max* and *Vigna radiata* as compared to pathogen infested plants. The maximum diseased incidence was found in *Sclerotinia* treated *Glycine max* plants and infection rate reached up to 97.45% in the absence of antagonist. Seedling treatment with MBAAT reduce the disease incidence up to 35.32% and plants treated with CTC showed 56% disease incidance. While in case of *Vigna radiata* plants treated with *Macrophomina* showed higher disease incidence (82.3%) and the percent disease was reduced in plants treated with MBAAT (33.22%) and CTC (41.36%). The results showed that our isolate MBAAT was more effective to control the diseases of *Glycine max* and *Vigna radiata* then the commercial isolate CTC. All the yield parameters were more appreciable in *Vigna radiata* with MBAAT then the CTC. However, disease could be controlled or reduced by MBAAT application which was better than the CTC commercial grade application (Table 4).

Table 4

(a) Biometric Parameters with different treatments after 40 days in *Glycine max*

Treatment	Root length (cm)	Shoot length (cm)	% Seed Germi-nation	Vigour Index	% Disease Incidence	No.of leaves	No. of lateral roots	No. of fruits	Total Chlorophyll Content (mg/g)	Plant Fresh weight	Plant Dry weight
T0	$9\pm1.2^{*}$	$10\pm1.2^{*}$	$67\pm1.6^{*}$	1273	NIL	9 ± 1.2^{ns}	$29\pm1.8^{*}$	$4\pm1.4^{*}$	$6.32\pm1.3^{*}$	$2.36\pm0.4^{*}$	0.98 ± 1.1^{ns}
T1	7 ± 2.5^{ns}	$9.5\pm0.8^{*}$	26 ± 1.2^{ns}	759	$87\pm1.8^{*}$	$8\pm0.6^{**}$	17 ± 0.7^{ns}	$0\pm0.4^{*}$	$1.352\pm0.9^{*}$	0.89 ± 0.5^{ns}	$0.35\pm1.2^{*}$
T2	$10\pm1.2^{*}$	9.5 ± 1.1^{ns}	16 ± 10.5^{ns}	312	97.45 ± 0.5^{ns}	6 ± 1.1^{ns}	23 ± 0.5^{ns}	1 ± 1.5^{ns}	$2.35\pm1.8^{*}$	$1.97\pm1.3^{*}$	$0.10\pm2.3^{*}$
T3	$10\pm1.2^{*}$	9.5 ± 1.1^{ns}	66 ± 10.5^{ns}	1287	35.12 ± 0.5^{ns}	11 ± 1.1^{ns}	23 ± 0.5^{ns}	4 ± 1.5^{ns}	$7.538\pm1.8^{*}$	$2.97\pm1.3^{*}$	$1.10\pm2.3^{*}$
T4	6.7 ± 1.2^{ns}	$8.6\pm1.7^{*}$	$22\pm1.4^{*}$	550.8	35.32 ± 0.8^{ns}	11 ± 2.5^{ns}	23 ± 2.1^{ns}	$4\pm1.6^{*}$	7.538 ± 1.06^{ns}	2.65 ± 1.2^{ns}	$1.13\pm0.3^{*}$
T5	14 ± 1.1^{ns}	10.2 ± 1.0^{ns}	$41\pm0.9^{*}$	992.2	$45.44\pm1.2.13^{ns}$	11 ± 1.3^{ns}	35 ± 1.6^{ns}	$3\pm0.08^{**}$	$5.214\pm1.05^{*}$	2.21 ± 0.9^{ns}	$1.12\pm1.6^{**}$
T6	11.3 ± 1.5^{ns}	$13.0\pm1.1^{**}$	22 ± 1.0^{ns}	534.6	56 ± 0.8^{ns}	$13\pm0.7^{*}$	$37\pm1.7^{*}$	2 ± 2.2^{ns}	$6.238\pm1.05^{*}$	$2.03\pm1.2^{*}$	$0.99\pm1.02^{*}$

Table 4

(b) Biometric Parameters with different treatments after 40 days in *Vigna radiate*

Treatment	Root length (cm)	Shoot length (cm)	% Seed Germi-nation	Vigour Index	% Disease Incidence	No.of leaves	No. of lateral roots	No. of fruits	Total Chlorophyll Content (mg/g)	Plant Fresh weight	Plant Dry weight
T0	9±1.2**	10±1.2*	67±1.6*	1273	NIL	9±1.2^{ns}	29±1.8*	4±1.4*	6.32±1.3*	2.36±0.4*	0.98±1.1^{ns}
T1	7±2.5^{ns}	9.5±0.8*	26±1.2^{ns}	759	87±1.8*	8±0.6**	17±0.7^{ns}	0±0.4*	1.352±0.9*	0.89±0.5^{ns}	0.35±1.2*
T2	10±1.2*	9.5±1.1^{ns}	16±10.5^{ns}	312	97.45±0.5^{ns}	6±1.1^{ns}	23±0.5^{ns}	1±1.5^{ns}	2.35±1.8*	1.97±1.3*	0.10±2.3*
T3	10±1.2*	9.5±1.1^{ns}	66±10.5^{ns}	1287	35.12±0.5^{ns}	11±1.1^{ns}	23±0.5^{ns}	4±1.5^{ns}	7.538±1.8*	2.97±1.3*	1.10±2.3*
T4	6.7±1.2^{ns}	8.6±1.7*	22±1.4*	550.8	35.32±0.8^{ns}	11±2.5^{ns}	23±2.1^{ns}	4±1.6*	7.538±1.06^{ns}	2.65±1.2^{ns}	1.13±0.3*
T5	14±1.1^{ns}	10.2±1.0^{ns}	41±0.9*	992.2	45.44±1.2.13^{ns}	11±1.3^{ns}	35±1.6^{ns}	3±0.08**	5.214±1.05*	2.21±0.9^{ns}	1.12±1.6**
T6	11.3±1.5^{ns}	13.0±1.1**	22±1.0^{ns}	534.6	56±0.8^{ns}	13±0.7*	37±1.7*	2±2.2^{ns}	6.238±1.05*	2.03±1.2*	0.99±1.02*

Ns = non significant, ∗ = significant, ∗∗ = highly significant. Significant level ≤ 0.05. Values are the means of three replications.

Both laboratory and green house condition showed identical results. MBAAT provided significant biological control against disease induced by phytopathogens under green house conditions. Indigenous T105 isolates was proved as a potential biocontrol agent in controlling the damping-off disease of beans caused by the *P. aphanidermatum.* The results reported agree with our results for the control of *M.phaseolina* and *S.sclerotiorum* [43]. It was observed the strains WH77/1, WH68 and WH48/1A were the most effective to control blight of walnut in the seedling test, yielding 55%, 42%, and 82% disease reduction, respectively, in two consecutive years [44].

Conclusion

The current study highlights the efficiency of *T.citrinoviride* as biocontrol agents against phytopathogens. *T.citrinoviride* effectively controlled the pathogen of *Glycine max* plant in addition to their plant growth promoting attributes under *In vitro* conditions. Its mechanism of biocontrol appears to be volatile metabolites it is producing. The isolated indigenous strain of *T.citrinoviride* had potential to be used as a biocontrol agent for the management of diseases of *Glycine max* and *Vigna radiata* as indicated by the reduced percent disease incidence. Besides biocontrol properties, the antagonist suspension applied also showed best performance in yield, yield attributes, physiological and biochemical parameters indicating its Plant growth promoting potential. However, the effective biocontrol agent can be applied under field conditions or further commercialized.

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