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Trichoderma harzianum elicits defense response in *Brassica juncea*plantlets

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Abstract

Indian mustard (Brassica Juncea) is a well-known oil producer with its composition of linoleic Acid, oleic Acid and linolenic Acid. The qualitative and quantitative changes in these fatty acids can be due to infection of pathogen. Presented work summarizes the main findings concerning the Trichoderma–plant interaction and its beneficial effect on plant. 30 days old plants were selected for treatment of Trichoderma powder formulation. Quantitative increase in total phenol, total protein and major three fatty acids was found after treatment. Trichoderma also observe to induce resistance capacity of plants. Mustard plant showed induction of jasmonic acid (JA) after treatment. JA was quantified by HPTLC method. The overall plant beneficial effect of Trichoderma as bio-control agent and inducer of defense response were discussed in detail.

Keywords: *Brassica juncea*, defense response, JA, *Trichoderma.*

Introduction

Members of the genus *Trichoderma* (where known, the teleomorphs belong to *Hypocrea*) are Sordariomycetes, like the model filamentous fungus *Neurosporacrassa*. Trichoderma's variations on the *Neurospora* themes of morphogenesis and photobiology have been studied for several decades, pointing to those molecular details that are conserved and those that vary between species. A compelling reason to study this group is that several species are promising agents for the biocontrol of plant pathogens. In addition, the non-biocontrol agent *Trichoderma reesei* is a biotechnological factory for the production of secreted cellulases, and a model for basic studies on protein secretion[1,2].

Trichoderma harzianum is a fungal genus found in many ecosystems. Some strains have the ability to reduce the severity of plant diseases by inhibiting plant pathogens, mainly in the soil or on plant roots, through their high antagonistic and mycoparasitic potential[3]. The recent comparative genome sequence analysis of two recognized biocontrol species – *Trichoderma atroviride* and *Trichoderma virens* – has afforded us a better understanding of how mycoparasitism arose in a common *Trichoderma* ancestor as a lifestyle of the genus[4]. The presence of fungal prey and the availability of root-derived nutrients may have been major attractors for the ancestors of *Trichoderma* to establish themselves in the rhizosphere and to facilitate the evolution of positive interactions with plants[5]. The control of a broad range of plant pathogens, including fungi, oomycetes, bacteria and viral diseases, through elicitation by *Trichoderma* of ISR or localized resistance has been reported[6]. Some *Trichoderma* rhizosphere- competent strains have been shown to have direct effects on plants, increasing their growth potential and nutrient uptake, fertilizer use efficiency, percentage and rate of seed germination, and stimulation of plant defences against biotic and abiotic damage[7]. In recent years, an increasing number of studies have contributed to unravelling the molecular basis of the plant–*Trichoderma* dialogue and the beneficial effects of *Trichoderma* to plants.

Here in this paper we present in detailed about overall plant beneficial effect of *Trichoderma* as bio-control agent and inducer of defense response.

Material and Methods

Isolation of *Trichoderma* from soil sample: Soil samples were collected from different agriculture soil and rhizosphere of plantation crops located in local region of Patan district of North Gujarat region. Soil samples were kept for shed drying and serial dilution method was applied for isolation of different fungi. *Trichoderma* species were isolated in specific selective medium. TSMC which contained (gm/lit); $MgSO_4 \cdot 7H_2$ O-0.2; KH_2PO_4-0.9; KCl-0.15; NH_4NO_3 -1.0; glucose-3.0, chloramphenicol-0.25, fenaminosulf-0.3, rose Bengal-0.15, pentachloronitrobenzene-0.2, captan-0.02 (post autoclaving), agar-20 as well as in modified TSM containing (gm/lit): $Ca(NO_3)_2$ -1.0, KNO_3 0.26, $MgSO_4 \cdot 7H_2O$-0.26, KH_2PO_4 -0.12, $CaCl_2$, $2H_2O$-1.0, citric acid-0.05, sucrose-2.0, agar-20.0, chlortetracycline-0.05, captan (50% wettable powder)-0.04[8].

Identification of *Trichoderma* species: Another method for identification of *Trichodermaharzianum*species includes DNA isolation and PCR amplification by using species specific primer (OLIGO 631).

Fungal DNA isolation: Isolation of fungal genomic DNA was done by growing the fungi for 3-4 days. The mycelia were

incubated with lysis buffer containing 250 mMTris-HCl (pH 8.0), 50 mM EDTA (pH 8.0), 100 mM NaCl and 2% SDS, for 1 hr at 60°C followed by centrifugation at 12,000 rpm for 15 min. The supernatant was then extracted with equal volume of water saturated phenol and further centrifuged at 12,000 rpm for 10 min; the aqueous phase was further extracted with equal volume of phenol: chloroform: isoamyl alcohol (25:24:1) and centrifuge at 12,000 rpm for 15 min; the aqueous phase was then transferred in a fresh tube and the DNA was precipitated with chilled ethanol (100%). DNA was pelleted by centrifuging at 12000 rpm for 15 min and washed in 70% ethanol by centrifugation. The pellets were air dried and suspended in TE buffer (pH 8.0).

PCR Amplification by using OLIGO 631 primer: All isolates of *Trichoderma* were taken up for PCR amplification by using OLIGO-631 primer. Sequence of primer was 5'-ATCCGTACGC -3'. Genomic DNA was amplified by mixing the template DNA (50 ng), with the polymerase reaction buffer, dNTP mix, OLIGO-631 primer and *Taq* polymerase. Polymerase Chain Reaction was performed in a total volume of 100 µl, containing 78 µl deionized water, 10 µl 10 X *Taq* pol buffer, 1 µl of 1 U *Taq* polymerase enzyme, 6 µl 2 mMdNTPs, 1.5 µl of 100 mM primer and 1 µl of 50 ng template DNA. PCR was programmed with an initial denaturing at 94°C for 5 min. followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 30 sec and extension at 70°C for 2 min and the final extension at 72°C for 7 min in a Primus 96 advanced gradient Thermocycler. PCR product (20 µl) was mixed with loading buffer (8 µ1) containing 0.25% bromophenol blue, 40 % w/v sucrose in water and then loaded in 2% Agarose gel with 0.1 % ethidium bromide for examination with horizontal electrophoresis.

Morphological study: Morphological observations were made to the isolates used for study to confirm the species, which included colony growth rate, colony characters, color of the surface and reverse of the colony, texture of conidiation, conidiophore branching and texture.

Mass production of Trichoderma and formation of powder formulation: Isolated *Trichodermaharzianum*was grown in autoclaved sorghum grains followed by incubation at 28°C f6r 5-7 days in dark. Outer layer of sorghum grain were covered by fungal spore. Mix this grain in 1 kg of powder thoroughly and count total CFU from powder. 1 kg powder which now containing *Trichoderma* spore were packed in commercial box. This powder was further use for *Trichoderma* – plant interaction study.

Experimental plants: For *Trichoderma* – plant interaction, *Brassica juncea* plants were used. Mustard plants were grown in plastic pots. Pots divided in four sets and each set contain five pots with 10 plants. Each set were treated with specific treatment as shown in table 1.

Table-1
Experimental design showing distribution of plants for different treatment

Set	No. of pots	Treatment
Set-1	5	Control
Set-2	5	Infection
Set-3	5	Infection + powder
Set-4	5	Powder

Infection was made by using spore and mycelium suspension of *Sclerotium rolfsii*, a causative agent of southern blight in mustard. 5 grams of *Trichoderma* powder was used for treatment.

Analysis of different parameter in treated plant: Different parameter was analyzed in plant after treatment with *Trichoderma* and pathogen. Parameter include, total phenol, protein quantification of profiling by native and SDS PAGE, Lipid quantification, JA measurement and lipoxigenase enzyme activity.

Estimation of total phenol: Estimation of total phenols and phenolics Plant phenols were extracted and purified by the method of Andersen and Pedersen[9]. The extract was suspended in acetone and made up to 10 ml with distilled water. Total phenols were estimated using the method of Malickand Singh[10] and expressed as mg phenols (in terms of catechol) per 1 g fresh tissue.

Estimation of total protein and protein profiling: Tris- HCl (0.05 m, pH 7.4) was added at 5 ml per gram of leaves for extraction. The homogenate was centrifuged at 8000 g for 20 min at 48°C (Sigma 3K30 centrifuge). The clear supernatant was collected for total protein estimation by folin-lawry method[11] and polyacrylamide gel electrophoresis (PAGE). Native and SDS PAGE with 10% resolving gel was carried out at 30 mA current for 2-3 h until the dye front moved to the bottom of the gel. After turning off the power supply, the gel was carefully taken out and stained using Commasive Brilient Blue (CBB). Immediately after resolution of bands, gel was recorded photographically.

Estimation of total lipid: 1.0 g of fresh tissue was weighted and grinded in a mortar with about 10ml of solvent mixture [Ethanol: Ethyl ether (1:3, v/v)]. Then it was centrifuged at 2000 rpm for 10min and clean extract was transferred into a new tube. 2ml of 0.05M KCL solution was added to the extract and mixed. Two layers were separated and then carefully below layer was taken out at which lipid was present. Thin Layer Chromatography of lipids was performed with the solvent system made up of Hexane: Ethyl ether: glacial acetic acid (80:20:1 V/V) and the results were observed after spraying the 50% H_2SO_4 and heating at the 110°C temperature for 10 minutes. Preparative TLC was performed for lipid profiling.

Jasmonic acid measurement: Methanolic extract of 1.0 gram of chopped leaves of treated mustard plant was prepared and

concentrated extract was applied on HPTLC plate by using automatic applicator (Linomate 5). After loading sample, plate was allowed to run in mobile phase. Plate was observed under UV and Rf value were calculated for each separated bands and JA band was compared with standard JA run along with samples. JA quantification was made by densitometry analysis by camag analyzer. Band density was compared with standard band density[12].

Results and Discussion

Identification of *Trichoderma harzianum* and its powder formulation: Trichoderma harzianum was isolated from agricultural soil and identified by species specific primer amplification. OLIGO-631 primer used for PCR amplification and it showed 1.6 kb size band amplification in only one strain. Amplification of 1.6 kb band confirm for Trichoderma harzianum. In other DNA sample, 1.6 kb band was not amplified but small fragments were amplified as shown in figure 1.

After confirmation by PCR amplification, this fungus were further analyzed by it morphological observations made by using microscope. Mycelia were smooth surfaced, watery white and sparse but soon developed aerial hyphae on their surface. The color of the colony changed from whitish green with the development of conidia. The reverse of the colony remained uncolored (figure 2).The conidiophores were much branched, formed loose tufts, the main branches were mostly in groups of 2-3 and stood at right angle to the bearer and their length increased with the distance from the tip of the main branch which gave a conical or pyramidal appearance (figure 2). Sorghum grains now covered with spores of *Trichoderma* were mixed with talk and powder was thoroughly mixed. Total CFU count in newly formed powder was approximate 1 X 10^{-4} CFU / gram, which confirmed by plate method. Each 1 kg powder contains 0.5 % of *Trichoderma* spores and remaining was powder. 5 gram of this powder was used as further treatment to mustard plants.

Analysis of different parameters: Total phenol induction: Total phenol was measured in treated mustard plants as well as control plants. When plants were treated with pathogen and pathogen + powder, phenol level was increasing up to 1 week, afterward level become decrease gradually. Plants treated only with powder also showed same pattern of inductionup to 8 µg/ml. Our findings showed that, when plants were exposed to only pathogen level of phenol was very high at initial level i.e. 7 dyas, but plants treated with pathogen + powder level of phenol remain enough high up to 3 weeks after treatment and level was 5-13 µg/ml. These results suggest long term effect of powder treatment (figure 3).

Total protein and its profiling: Like phenol, protein was also increasing in whole course of study. Plants treated with pathogen showed initial increase in total protein level up to 100 µg/ml and then decrease and reach up to control level of 80 µg/ml. Plants treated with powder and pathogen + powder showed higher induction of proteins up to 168 µg/ml after 14 days and it remain increase up to 2 weeks after treatment. Control plant showed protein level from 78 to 84 µg/ml. only powder treatment to plant also showed induced level from 115 to 185 µg/ml during 3 weeks (figure 4).

Native PAGE showed higher molecular weight proteins in all treated samples. Density of protein bands was higher in pathogen + powder treated plants compared to pathogen treated plant. Powder treated plant showed similar profile than that of pathogen treated plant after 14 days compared to 7 days.In pathogen + powder treated plants after 14 days protein bands ranged from 30 kd to 100 kd were highly induced compared to 7 days sample as shown in figure 5.

SDS PAGE showed higher as well as lower molecular weight proteins in all treated samples. Density of protein bands was higher in pathogen + powder treated plants compared to pathogen treated plant. Powder treated plant showed similar profile than that of pathogen treated plant after 14 days compared to 7 days. In pathogen + powder treated plants after 14 days protein bands ranged from 10 kd to 100 kd were highly induced compared to 7 days sample as shown in figure 6.

Lipid content and profiling: When mustard plats treated with pathogen total lipid level slightly increase but when plant treated with pathogen and pathogen + powder higher increasing in lipid level was observed. Powder alone not significantly increases lipid level but along with pathogen it significantly induces lipid level. Control plant showed 10 to 12 mg/GFW lipid level. Pathogen and powder treated plants showed induced level of lipid from 20 to 24 mg/GFW, which is two time higher than control plants. When plants treated with pathogen + powder it showed very high induction of lipid from 30 to 40 gm/GFW up to 3 weeks (table 2).

Table-2
Lipid quantification in all treated mustard plants, Value is expressed in milligram per gram of fresh tissue

Days	Treatment			
	control	Pathogen treated	Powder treated	Powder + Pathogen treated
7 days	10.34 ± 0.2	20.37 ± 0.3	20.42 ± 0.2	30.27 ± 0.4
14 days	11.45 ±0.06	24.62 ± 0.2	21.40 ± 0.3	38.46 ± 0.2
21 days	10.36 ± 0.1	22.67 ± 0.3	22.50 ± 0.4	40.26 ± 0.5
28 days	11.35 ± 0.3	23.36 ± 0.1	21.12 ± 0.5	35.25 ± 0.2

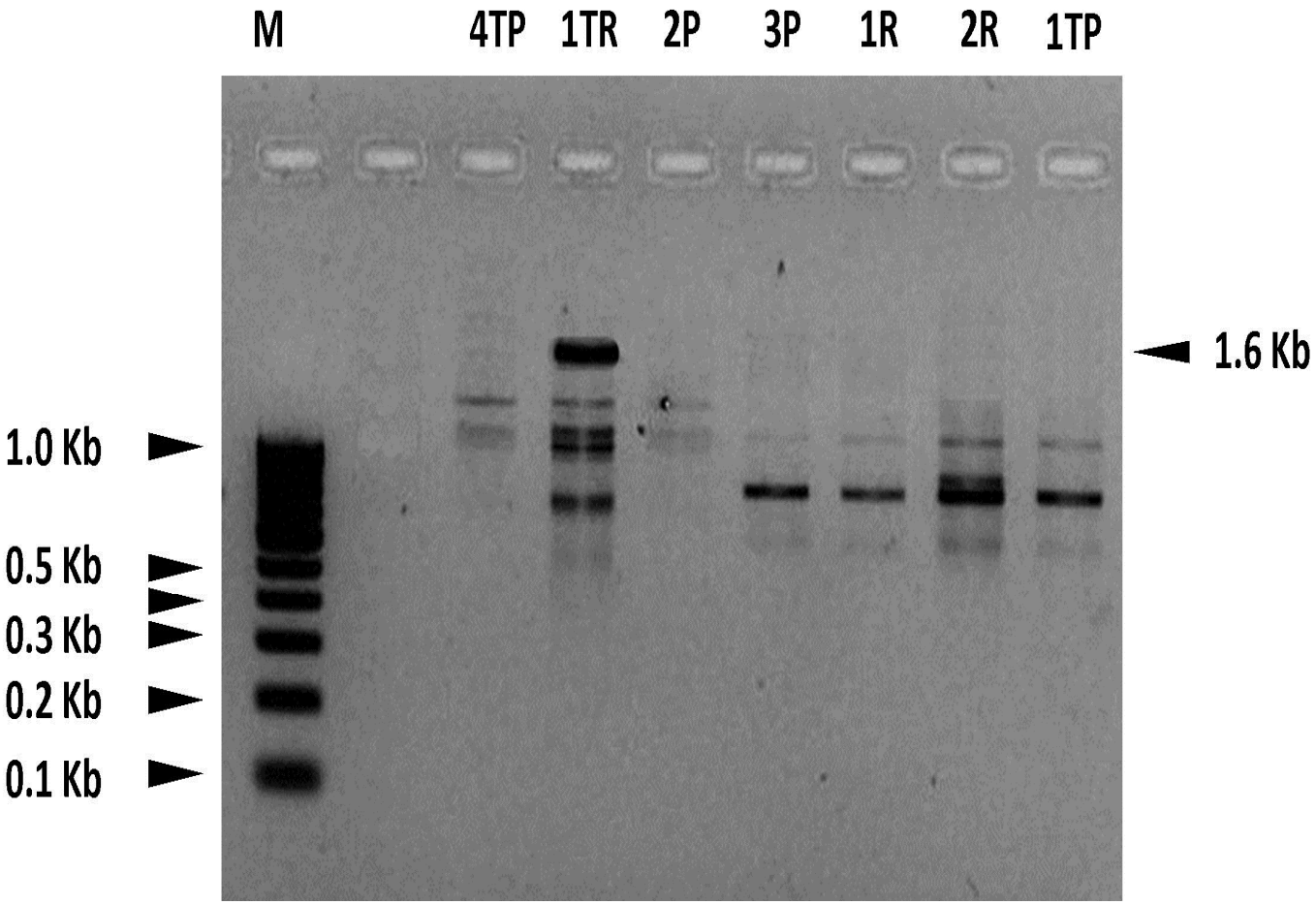

Figure-1
PCR amplification of DNA of seven different Trichoderma isolates by using OLIGO-631 primer, Only 1TR isolate showed 1.6 kb fragment amplification and confirmed as *T. harzianum*. M: 1.0 Kb DNA ladder, other are different isolates

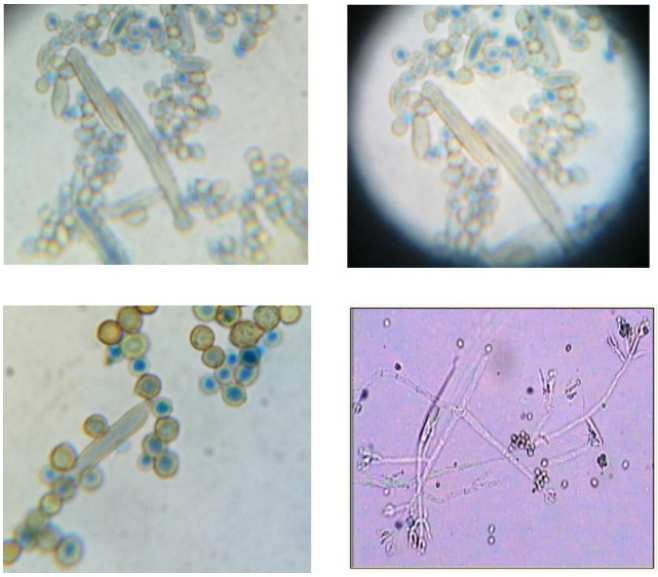

Figure-2
Conidiophore structure in different isolates, All observation was done under compound microscope by using 100 X lense

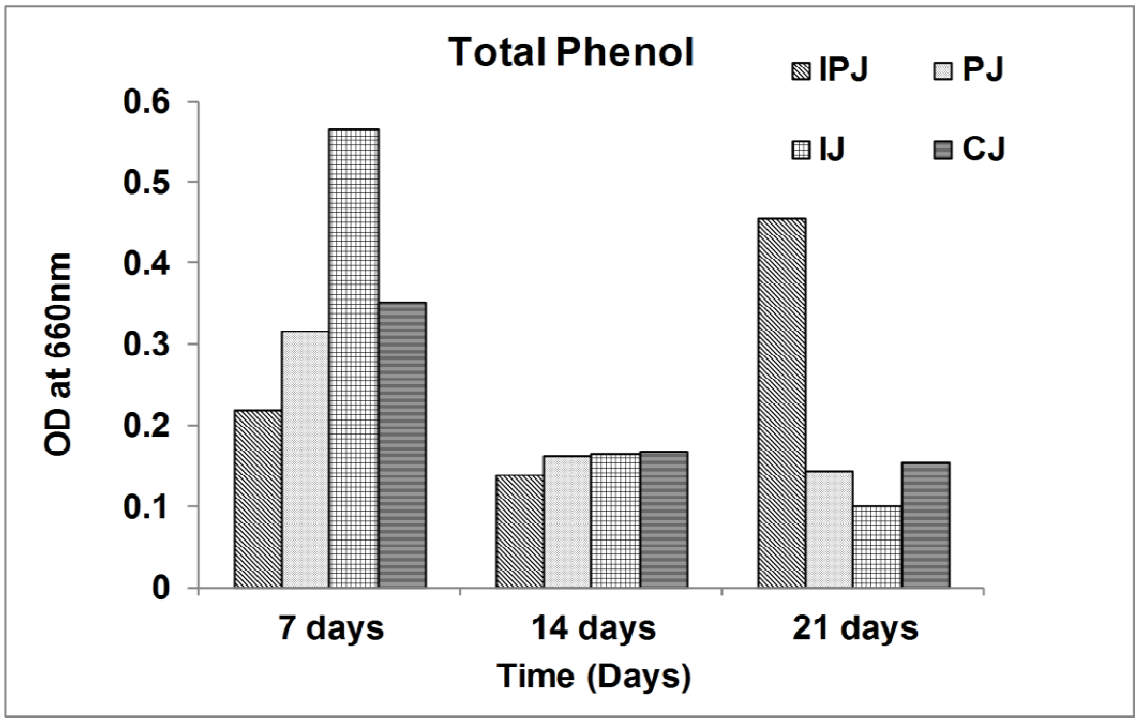

Figure-3
Total phenol induction in treated as well as control platns. Phenol level is expressed in terms of µg/ml. catachole used as standard. IPJ: pathogen + powder treatment ; PJ: powder treatment ; IJ: pathogen treatment and CJ: control plant

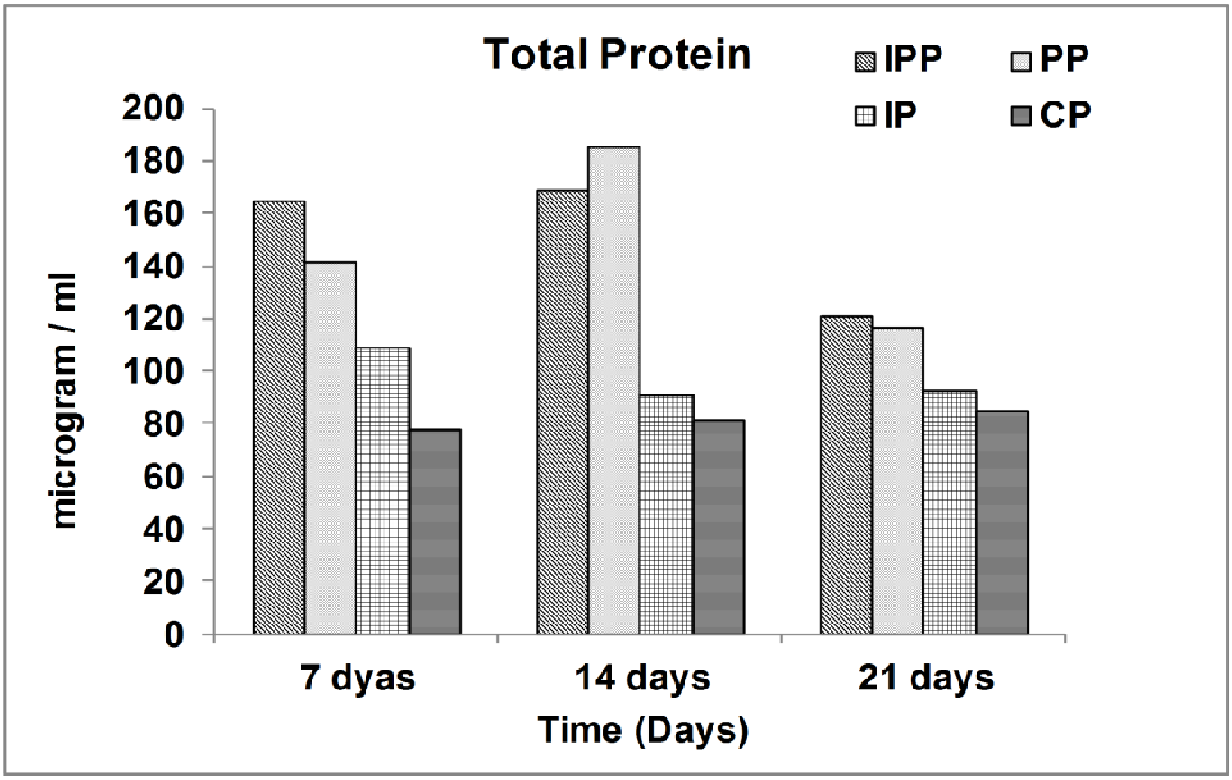

Figure-4
Total protein induction in treated as well as control platns. Phenol level is expressed in terms of µg/ml. catachole used as standard. IPP: pathogen + powder treatment ; PP: powder treatment ; IP: pathogen treatment and CP: control plant

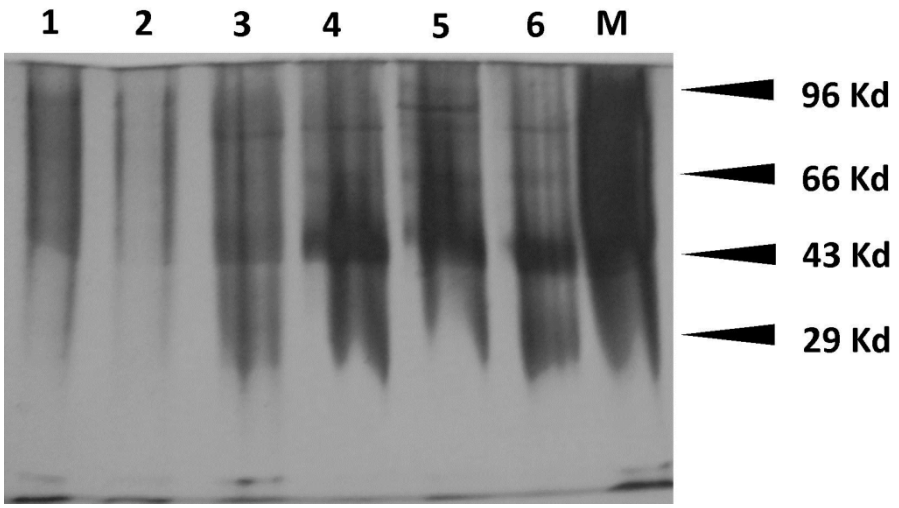

Figure-5

Native gel electrophoresis of proteins from treated mustard plants. Mainly higher molecular weight proteins were observed. 1: Pathogen infected plant (7 days); 2: Powder treated plant (7 days); 3: pathogen + powder treated plants (7 days); 4: Pathogen infected plant (14 days); 5: Powder treated plant (14 days); 6: pathogen + powder treated plants (14 days); M: Molecular weight marker

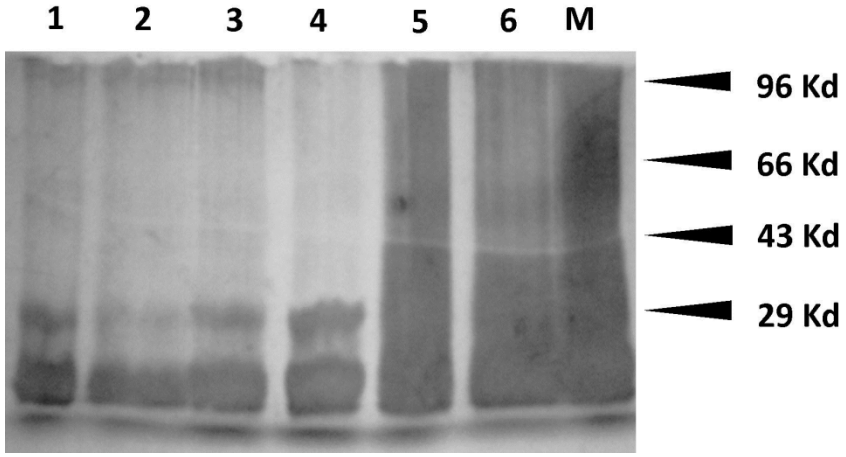

Figure-6

SDS gel electrophoresis of proteins from treated mustard plants. Mainly higher molecular weight proteins were observed. 1: Pathogen infected plant (7 days); 2: Powder treated plant (7 days); 3: pathogen + powder treated plants (7 days); 4: Pathogen infected plant (14 days); 5: Powder treated plant (14 days); 6: pathogen + powder treated plants (14 days); M: Molecular weight marker

Lipid profile was done by using preparative TLC method. After separation it was observed that several different types of lipid components were separated according to their respective R_f value. Two fatty acids, linoleic acid and oleic acid, were identified in all samples. Plant treated with pathogen did not show induction of linoleic and oleic acid. Powder treated and pathogen + powder treated plants showed induction of both fatty acid and other lipids also as shown in figure. Induction of these two fatty acids was almost similar at 7 days and 14 days. Control plant showed very less induction of lipids or fatty acids (figure 7).

Jasmonic acid estimation: Jasmonic acid (JA) is very important signaling molecules in plant defense mechanism. In powder treated sample JA level no significant induction was found. Plants treated with pathogen + powder showed very high induction of JA and was very significance lasting up to 3 weeks. Control plant showed 48 ηg /GFW of JA level. Treatment of plants with powder resulted in induction of JA up to 180 ηg /GFW. Plant treated with pathogen showed almost similar induction as showed by powder treatment. Pathogen + powder treatment prove very significant in JA induction up to 300 ηg /GFW during 2-3 weeks after treatment (figure 8).

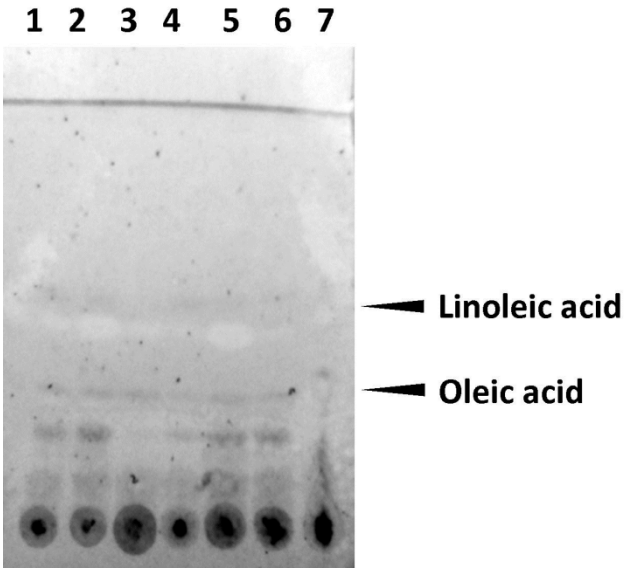

Figure-7

Lipid profiling of all treated as well as control mustard plants of 7 days and 14 days after treatment. Two fatty acids were identified as shown by arrow. 1: powder treated plant (7 days); 2: powder treated plant (14 days); 3: pathogen treated plants (7 days); 4: Pathogen treated plant (14 days); 5: Pathogen + powder treated plant (7 days); 6: Pathogen + powder treated plants (14 days); 7: Untreated plants (control)

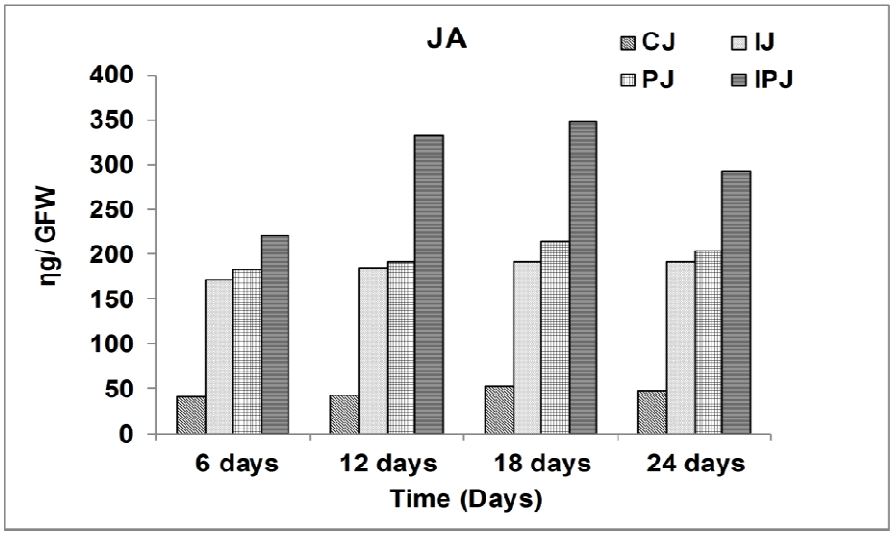

Figure-8

Jasmonic acid quantification in all treated mustard plants by using HPTLC. Quantity of JA is expressed as ηg / GFW

Discussion: *Trichoderma* species are fast growing filamentous deuteromycetes widely distributed around the world[13]. They are found in most soils and are frequently the dominant component of the soil microflora. Among the different orders of the Deuteromycotina, *Trichoderma* species belong to the moniliales. A revised classification of the genus *Trichoderma* was proposed by Bissett[14] in 1991 based on key morphological characteristics.

Several papers were reported worldwide and all address the *Trichoderma*–plant interaction[15,16]. The work of 30 years of strain improvement, as part of the history of utilization of *Trichoderma* in cellulose degradation for this purpose and for the production of heterologous proteins[1, 2]. A series of studies has reported the use of high-throughput sequencing in gene expression analysis leading to the discovery of genes controlling asexual reproduction in response to different environmental cuesand of genes involved in the *Trichoderma*–host interaction. Lysis of the prey's cell wall is one of the key steps during mycoparasitism. The genome analysis of two mycoparasitic*Trichoderma* species, *T. atroviride* and *T. virens*, revealed an expanded arsenal of genes encoding enzymes potentially involved in cell wall hydrolysis. It has always been

puzzling how *Trichoderma* differentiates between self and non-self fungal cell wall degradation. Mukherjee et al.[17] review the genes which determine the secondary metabolite repertoire of *Trichoderma*, including both useful and toxic compounds, known and novel. Polyketide synthases and non-ribosomal peptide synthases (NRPSs) define two major classes of secondary metabolites[18,19]. Peptaibols (made by NRPSs) belong to the antifungal arsenal of *Trichoderma*, and have now been found to trigger apoptotic death of the host.

Here in this paper we prepared a one powder formulation by very economic method and use this powder for treatment to mustard plants. Different parameters were measured after treatment of powder. We found that trichioderma powder induce defense repose in mustard plants up to 3 weeks.

Treatment of *Trichoderma* induces phenolic level in mustard plants. Pathogen treated and powder + pathogen treated plants also showed induction of phenol. Initially level of phenol was high on 7 days but after that it gradually decrease. In many reports it was observed that induction of phenol in plant is a primary level of defense and it reach up to its optimum level within 2 days[20]. Here we collect leaves for analysis was after 7 days, 14 days, so may be chances of decrease in phenol level after treatment. In case of pathogen + powder treatment phenol level was remain high up to 3 weeks.

Induction of protein was also observed after *Trichoderma* treatment in mustard plants. Powder along with pathogen treatment showed high induction of total protein. Induction of protein after infection and elicitor treatment was reported by many researchers worldwide[20,21,22]. Powder treatment to mustard plant proves significant for induction of protein and native PAGE and SDS PAGE also carried out for further confirmation. Result of PAGE showed different protein expression in treated plants and it include PR protein expression after treatment. The nearby same result reported by Sriram et al.[23]while studying the potential use of elicitors from *Trichoderma* in induced systemic resistance for the management of *Phytophthora capsici* in red pepper.

Lipid is a most important constituent in mustard plant and it affects the economic value of oil seeds. Induction of lipid upon infection was reported by many other researchers worldwide. On our result it was observed that powder treatment and powder + pathogen treated plant showed induced level of lipid in mustard plants. Lipid profiling suggested that two fatty acids induced greatly after treatment. Induction of linoleic and oleic acid leads to JA induction and this was reported by Dhandhukia and Thakkar[12].

Jasmonic acid (JA) is very important signaling molecules in plant defense mechanism. *Trichoderma* is able to trigger a long-lasting up-regulation of SA gene markers in plants unchallenged by pathogens, although when plants are infected by a pathogen such as *B. cinerea*, the pretreatment with *Trichoderma* may modulate the SA-dependent gene expression and, soon after infection, the expression of defense genes induced through the JA signal transduction pathway occurs, causing ISR to increase over time. In this paper we also found that upon treatment JA was induced in mustard plants. High induction of JA was observed in powder + pathogen treatment.

It was observed that *Trichoderma* in form of powder induced plant defense in terms of induction of phenol, protein, lipids and signaling molecule JA. *Trichoderma* had received little attention as a potential inducer of plant resistance until the publication of studies describing that bean root colonization by *T. harzianum* was effective in inducing defence responses and that penetration of *T. asperellum* in the root system triggered ISR in cucumber seedlings[24]. As a consequence of *Trichoderma* root colonization and MAMP interaction the proteome and transcriptome of plant leaves are systemically affected[7]. The ISR triggered by *Trichoderma* occurs through the JA/ET signalling pathway similarly to PGPRISR[25], as confirmed by several authors: i. cerato-platanin Sm1 is required for *T. virens*-mediated ISR against *Colletotrichumgraminicola* in maize[26], ii. *Trichoderma* treatment of JA/ET-deficient *Arabidopsis* genotypes leads to enhanced susceptibility to *Botrytis cinerea*, iii. ISR triggered by PGPR and *Trichoderma* converges upstream from MYB72, an early key component of the onset of ISR. However, other studies have shown that in the *T. asperellum*–cucumber interaction the induction of plant responses is a time- and concentration-dependent phenomenon, and in the first hours of contact a SARlike response is observed, with an increase in SA and peroxidase activity. In fact, a systemic increase in SA and JA levels was observed after inoculation of high densities of *Trichoderma*. Gallou*et al.*[27] also observed that the defence response of *T. harzianum*challenged potato to *Rhizoctoniasolani* was dependent on JA/ET and SA.

Finally *Trichoderma* genomes have revealed mycotrophy and mycoparasitism as ancestral lifestyles of species of this genus. Some *Trichoderma* strains have become established in the plant rhizosphere and evolved as intercellular root colonizers. As a result, they stimulate plant growth and defenses against pathogens. Like other beneficial microbes, *Trichoderma* elicits ISR by JA/ET-dependent pathways and triggers priming responses in the plant. However, the *Trichoderma*–plant cross-talk is dynamic and the expression of defense-related genes and its product of the JA/ET and/or SA pathways may overlap, depending on the *Trichoderma* strains and the concentrations used, the plant material, the developmental stage of the plant, and the timing of the interaction. The expression of *Trichoderma* genes in plants has beneficial results, mainly in the control of plant diseases and resistance to adverse environmental conditions. The experimental evidence reviewed here indicates that *Trichoderma*–plant interactions have features in common with other beneficial microbe associations but that they also display their own characteristics due to *Trichoderma*'s particular lifestyle.

Nevertheless, there is a need for more studies aimed at gaining insight into the signaling transduction pathways, related to defense and development, resulting from *Trichoderma*–plant interactions in the presence of pathogens and/or different types of abiotic stress.

Conclusion

Trichoderma genomes have revealed mycotrophy and mycoparasitism as ancestral lifestyles of species of this genus. Some *Trichoderma* strains have become established in the plant rhizosphere and evolved as intercellular root colonizers. As a result, they stimulate plant growth and defenses against pathogens. Like other beneficial microbes, *Trichoderma* elicits ISR by JA/ET-dependent pathways and triggers priming responses in the plant. However, the *Trichoderma*–plant cross-talk is dynamic and the expression of defense-related genes and its product of the JA/ET and/or SA pathways may overlap, depending on the *Trichoderma* strains and the concentrations used, the plant material, the developmental stage of the plant, and the timing of the interaction. The expression of *Trichoderma* genes in plants has beneficial results, mainly in the control of plant diseases and resistance to adverse environmental conditions. The experimental evidence reviewed here indicates that *Trichoderma*–plant interactions have features in common with other beneficial microbe associations but that they also display their own characteristics due to *Trichoderma*'s particular lifestyle.

Nevertheless, there is a need for more studies aimed at gaining insight into the signaling transduction pathways, related to defense and development, resulting from *Trichoderma*–plant interactions in the presence of pathogens and/or different types of abiotic stress.

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