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RESEARCH ARTICLE

TRICHODERMA MEDIATED INDUCED SYSTEMIC RESISTANCE IN CUCURBITS AGAINST CUCUMBER MOSAIC VIRUS (CMV)

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ABSTRACT

Trichoderma mediated systemic resistance is often associated with the onset of defense mechanisms by expression of various defense related enzymes such as β-1-3-glucanase, chitins, phenylalanine ammonia lyase (PAL), peroxidase (PO), polyphenol oxidase (PPO) and phenols. The present investigation was undertaken to demonstrate and to exploit the *T.harzianum* isolated from the rhizosphere soil of healthy pumpkin plants for induction of systemic resistance against Cucumber mosaic virus (CMV), the causative agent of cucumber mosaic virus disease in cucurbits. *Trichoderma* treated pumpkin plants challenged with CMV not only showed a pronounced and significant reduction in percent disease incidence (PDI) but also a significant reduction in virus concentration (ELISA) and exhibited maximum activity of PO, PPO and increase in the concentration of proteins, phenols as compared to the plants challenged with CMV alone at 25 days after inoculation (DAI). *Trichoderma* -treated plants were more developed than non treated plants throughout the experiment. Percentage of seed germination, symptom rating (%), plant height and yield measurements were also enhanced in the *Trichoderm* -treated plants inoculated with the virus. Expression of an additional 21.5 kDa protein in PAGE analysis indicates the induction of xylanase has shown to induce ethylene production and plant defense response in pre-treated plants.

INTRODUCTION

Cucumber mosaic cucumovirus (CMV) belong to the genus Cucumovirus (Family Bromoviridae), is one of the five most important viruses affecting production of field-grown vegetables worldwide (Sherf and McNab, 1986; Tomlinson, 1987). CMV is difficult to control because of its extremely broad natural host range in excess of 800 plant species, and the ability to be transmitted in a non-persistent manner by more than 60 species of aphids (Zitter, 1991; Palukaitis *et al*., 1992). Weeds adjacent to vegetable plantings are often symptomless carriers of CMV and have been shown to be a source of infection for subsequent spread of CMV (Tomlinson, 1987). Various strategies, based on the avoidance of sources of infection, control of vectors, modification of cultural practices, use of resistant varieties and transgenic plants have been conventionally employed to minimize the losses caused by CMV. These constitutive defense strategies, however, have not been much effective as inducible defence control measures and hence the potential of the biocontrol agent *Trichoderma harzianum* to trigger plant defense responses was investigated against CMV. Plants defend themselves from pathogen infection through a wide variety of mechanisms that can be local or systemic, constitutive or inducible against pathogens. The induction of host defense through biocontrol agents has been considered as a focus of research only in recent years. The ability of biocontrol agents to induce resistance has varied with strains of antagonists towards bacteria and fungi are known but concentrating towards viral infection had a prominent importance (Van Loon *et al*., 1998). The prior exposure of plants to biocontrol agents could induce the plants to switch on their defense reaction against a broad range of pathogens. Inducible defenses in plants may have selective advantage; hence the present study was undertaken.

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MATERIALS AND METHODS

Screening of *Trichoderma* spp.

Trichoderma harzianum was isolated from rhizosphere of healthy cucumber plants collected from the cucumber growing areas in and around Tirupati using serial dilution technique on *Trichoderma* specific medium (Elad and Chet, 1983) and isolated *T.harzianum* was maintained on the potato dextrose agar medium.

Preparation of *Trichoderma* inoculum

Oatmeal sand medium was prepared in 250 ml conical flasks and inoculated with *T. harzianum* incubated at 28±2°C for 10

days. Soil Sterilized at 121°C for 30 min at 15 lbs pressure for two successive days with 1:1 ratio of sand and soil taken into sterilized 9'' earthenware pots. Surface sterilized cucumber seeds were sown in pots filled with sandy soil containing T. *harzianum* @ 4g/ Kg of soil. Three replicates were maintained throughout the study for all the four treatments T1-Healthy Control, T2- *Trichoderma*, T3- CMV and T4- *T. harzianum*+ CMV

Collection of CMV infected samples

Suspected virus infected plant samples of *Cucurbita moschata, Cucumis sativus,* C*ucumis melo*, *Luffa acutangula, Cucurbita maxima, Lagenaria siceraria, Mimordica charantia, Banincasa hispida* with characteristic mosaic symptoms of light green and dark green mosaic islands and yellowing of leaves were collected from various agricultural fields of different localities and regions of cucurbit growing areas of Andhra Pradesh, Tamilnadu and Karnataka.

Electron microscope studies

Infected pumpkin leaf samples collected from fields were prepared by leaf dip method with copper grid and negatively stained with uranyl acetate; samples were examined in the electron microscope for the presence of virus particles.

Transmission of virus

Mechanical/ Vector Transmission

CMV isolate collected from cucurbit growing areas of south India was maintained in the Pumpkin (IIHR, Arka malik) and used for all greenhouse experiments. The primary leaves of each pumpkin plant were lightly dusted with carborandum powder and then inoculated with CMV inoculum consisted of CMV infected pumpkin leaf tissue ground in 0.1 M Phosphate buffer (pH 7.5). Vector transmission studies were performed using two aphid species of *Aphis craccivor, A.gossipi.*

Biological/Immunological screening for the presence of virus

Test and indicator plants

Experiments were conducted on different test plants to select the test and indicator plants. *Cucumis pepo*, *Luffa acutangula,, Benincasa hispida, cucumis moschata, Lycopersicon esculentum*, *Nicotiana tabacum L.*, *Cucurbita pepo* were used as test plants, tested to identify local lesion assay host for single lesion isolation of virus. Seven to eight days old seedlings with two primary leaves of *Vigna unguculata*, *Phaseolus aurensmunga*, *Solanum melangina*, *Vigna mungo*, *Cyamopsis*, *Phaseolus vulgaris, Gomphrena globosa, Chenopodium amaranticolor* and *Chenopodium.qinoa, Vigna unguculata* and *Nicotiana tobacum* were selected as test and indicator plants for local lesions symptoms.

DAC-ELISA

Suspected virus samples were subjected to DAC-ELISA (Hobbs *et al*., 1987) for the detection of virus using cucumber mosaic virus antisera provided by Department of Virology, S.V. University, Tirupati, India. Leaf samples were ground in 50mM carbonate buffer (pH 9.6) and added to microtiter plates at a final dilution of 1: 10 (1gram tissue per 10ml buffer). Plates were incubated overnight at 4 °C and then washed 3 times with PBS-T. Anti-CMV (primary antibody) was added to the plates at a concentration of 1 fg/ml in PBS-T. The plates were again incubated overnight at 4 °C and washed 3 times with PBS-T. Goat anti-rabbit immunoglobulin conjugated to alkaline phosphatase was diluted to 1:7000 in PBS-T and added to the plates. The plates were incubated at 35ºC for 4 hours. Plates were triple-washed with PBS-T, and substrate (p-nitrophenyl phosphate at l mg/ml in 10% diethanolamine, pH 9.8) was added and reactions allowed develop colour reaction at room temperature. Absorbance values were read at 405 nm on a Thermo scientific multi scan Elisa reader.

Green house experiment

Screening of *Trichoderma* for Antiviral properties

The antiviral properties of the *Trichoderma* culture filtrate of the different isolates of were assayed via local acquired resistance using the half leaf assay. Each treatment was performed in duplicate for local lesions and counted after 3-4 days. Antiviral properties were studied by symptom rating formula to evaluate specific isolate of *Trichoderma* for induced resistance against Cucumber mosaic cucumovirus (CMV) in cucurbits. *Trichoderma (A.P Isolate -2)* treated plants shows only 30 to 40% of symptomatic leaves, compared with the plants treated with other strains with the range of 80 to 90% of symptomatic leaves when challenged with CMV. Hence, the A.P isolate-2 selected as a most effective novel strain for induced systemic resistance studies

***Trichoderma* mediated induced systemic resistance (ISR) in cucurbits against CMV**

About ten *Trichoderma* isolates were tested for rhizosphere competence/ antiviral properties in pumpkin plants. Based on the results of initial screening experiments, one of the most effective *Trichoderma* isolates was evaluated for induced systemic resistance. The effective strain of *Trichoderma* was selected and treated to pumpkin seeds sown in pots containing potting medium (sand: farmyard manure @ of 1:1 w/w). Experiments were conducted in completely randomized design with three replications in each treatment. Leaf samples were collected randomly at two different stages of 15, 25 days after challenge inoculation with pathogen to assay the changes in activities of defense related enzymes like peroxidases (PO) and polyphenol oxidases (PPO), proteins and phenol. In response to local infection with necrotizing pathogen, plants display a whole plant enhanced immunity to secondary challenge (systemic acquired resistance or SAR stated by Metraux J.P *et al.,* 2002)

Trichoderma *mediated induced Plant growth promotion*

The % of seed germination was measured by raising seeds in the sterilized soil inoculated with *Trichoderma* culture in 1:1 ratio and calculated by the formula:

% of seed germination = Number of seeds sown X 100
Number of seeds germinated

The shoot length of *Trichoderma* treated and control plants measured at different time intervals after 10-15 days after germination. Root length of *Trichoderma* treated and control plants were measured at 10-15 days after germination. Replicates were maintained for all the treatments. Similar to procedure followed by Palukaitis *et al.* (1997) stated in Replicase-mediated resistance to plant virus diseases.

Disease incidence and Symptoms rating

After 25 days of planting, the number of plants exhibiting CMV symptoms was recorded. Percent disease incidence (PDI) was estimated according the equation:

PDI = Number of symptomatic plants X 100
Total number of plants

The number of symptomatic plants were recorded 2 weeks after challenge inoculation and counted twice a week for an additional 1 week. Disease was rated using the following scale (George S. Raupach *et al.,* 1996):

10 = 100% of leaves showing mosaic symptoms;
8 = 50% of leaves showing mosaic symptoms;
6 =mosaic symptoms just beginning;
4 =50% of leaves on plant appear puckered or curled;
2 = 25%-leaf puckering or curling just beginning,
0 = no symptoms,

Disease severity values were calculated using the formula: (Yang *et al.,* 1996)

Disease severity = total number of plants X 100
(Y) Highest disease grade

Extraction and Quantification of Phenol compounds

Total phenols

Leaf samples of various treatments (T1-Control (without *Trichoderma* and virus), T2-only biocontrol agent (*Trichoderma*), T3-only Pathogen (Cucumber mosaic virus), and T4-*T. harzianum*+virus) were homogenized in 10 ml of 80% methanol and agitated for 15 min at 70^0C. To 1 ml of the extract 5 ml of distilled water and 250 ml of Folin-ciocalteau reagent (1 N) were added and incubated at 25^0C for 3 min. After that 1 ml of 20% sodium carbonate was added and mixed well. Then the tubes were placed in boiling water for 1 min and cooled. The absorbance was read at 750 nm and catechol was used as the standard. The total phenol content was expressed in mg of catechol/g of fresh tissue (Zieslin and Ben Zaken 1993).

Peroxidases (PO)

The procedure adopted for determining the activity of peroxidases was essentially as that of Fehrmann and Diamond (1967). The peroxidases enzyme activity was determined for all the treated as well as control plants. About 0.5 g of freshly harvested leaf material was ground in a pre-chilled mortar with 20 ml of 0.1M ice cold phosphate buffer (pH 7.1) and centrifuged at 2000 rpm for 10min. The supernatant was made up to 25 ml and used for assay. Freshly prepared 0.1 ml of pyrogallol reagent (0.2 M) and 1.0 ml of the enzyme extract, 1.4 ml of 0.1M phosphate buffer (pH–7.1) were mixed in a cuvette and the mixture was immediately adjusted to zero absorbance of a spectrophotometer. Enzyme activity was recorded as the change in absorbance per minute (ÄA / min/ä) at 430nm.

Poly phenol oxidases (PPO)

0.5 ml of enzyme extract and 2.3 ml of 0.1M phosphate buffer (pH–6.1) were mixed together in a cuvette and adjusted to zero absorbance of spectrophotometer (Mahadevan and Sridhar, 1982). 0.2ml of 0.1 M catechol solution was added to the above mixture and the reactants were quickly mixed. The enzyme activity was measured as the change in absorbance per minute (ÄA/min) at 400 nm, immediately after the addition of 0.2 ml of 0.1M catechol solution which initiated the reaction.

Poly acrylamide gel electrophoresis (PAGE) analysis

Leaf samples collected on the 15th day were subjected to PAGE analysis to find out the expression of PO and PPO isoforms. Samples were homogenized with 1 ml of 0.1 M sodium phosphate buffer pH 7.0 and centrifuged at 10,000 rpm for 20 min at 48^0C. The protein content of the sample was determined by the Bradford (1976) method. Samples (50 mg protein) were loaded onto 8% poly acrylamide gel. After electrophoresis, the gel was stained in 0.2 M acetate buffer at pH 4.2 containing 0.05% benzidine for 30 min in the dark. Then drops of H_2O_2 (0.03%) were added slowly with constant shaking to visualize the Poly phenol isoforms. After staining the gel was washed with distilled water (Nadlony and Sequerira, 1980) [14]. For PPO, the gel was immersed in P-phenylene diamine (0.1%) in 0.1 M potassium phosphate buffer at pH 7.0 for 30 min. Later 10 mM catechol was added and kept in a shaker with gentle shaking. The appearance of dark brown protein bands was noticed after some time.

RESULTS

Screening of *Trichoderma* spp.

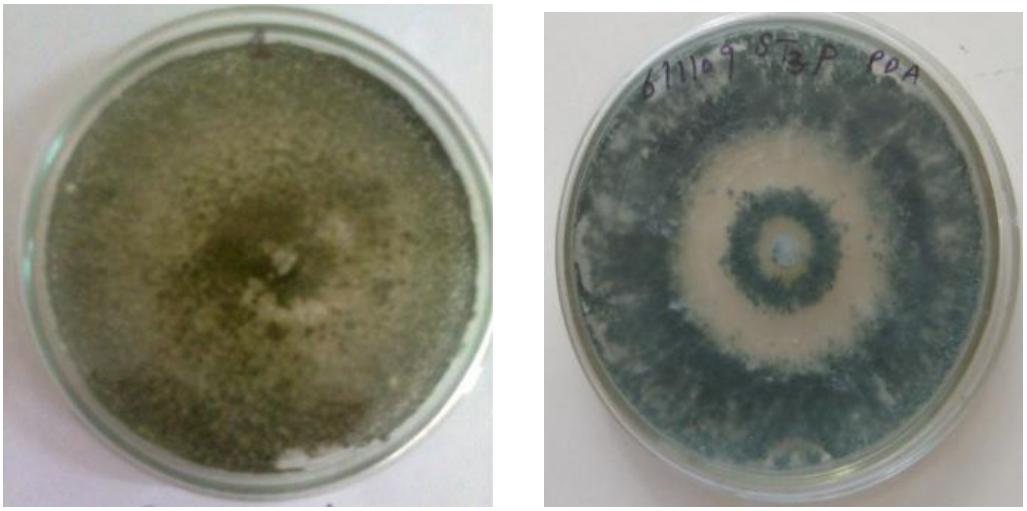
Different strains of *Trichoderma* isolated from the rhizosphere and roots of cucurbits from various regions were maintained in the lab on PDA slants to study the rhizosphere competence and antiviral properties to select a novel isolate for induced systemic resistance against CMV in cucurbits (Figure 1).

Collection of CMV infected samples

The virus infected leaves of pumpkin, Cucumber, Ash gourd, Ridge gourd showing severe mosaic symptoms includes yellow and light green islands in leaf lamina were collected and transmission studies were carried out (Figure 2).

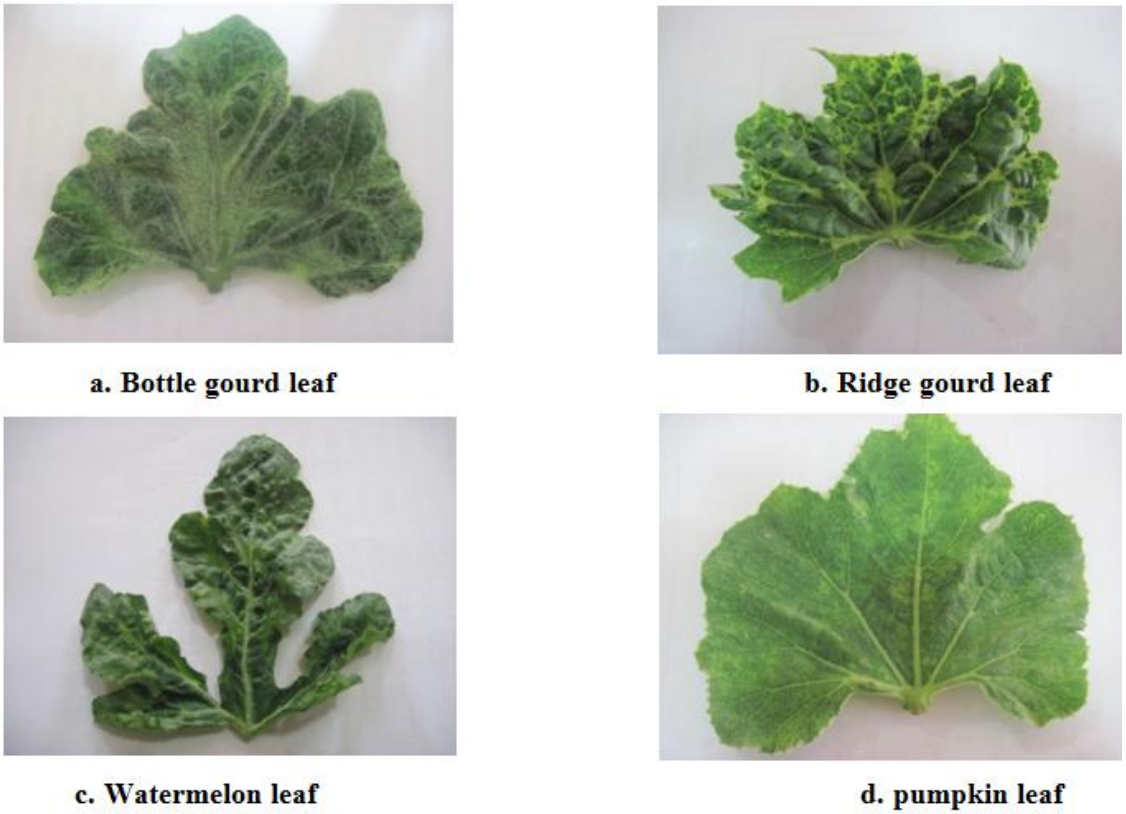
Detection of virus by Electron microscope

Leaves of pumpkin plants infected with Cucumber Mosaic Virus (CMV) were sectioned and examined by Electron Microscopy, shows distinguish small isometric virus particles in groups confirmed the presence of CMV particles (Figure 3).



a. Isolate-1 b. Isolate-2

Fig. 1. Different *Trichoderma* isolates screened



a. Bottle gourd leaf b. Ridge gourd leaf

c. Watermelon leaf d. pumpkin leaf

Fig. 2. Cucurbits leaves showing mosaic symptoms

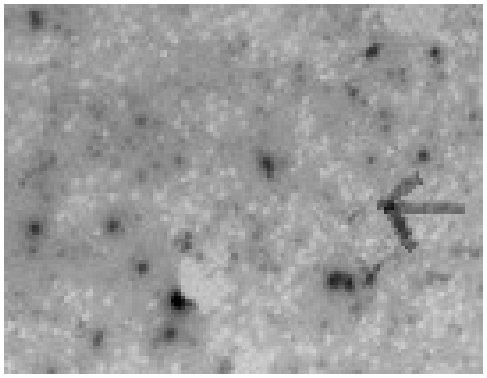


Fig.3. Electron microscopic image of Isometric particles of cucumber mosaic virus (CMV)

Collection and Indexing of virus infected samples by DAC-ELISA

The collected test samples from various regions were subjected to DAC-ELISA with CMV and Potyvirus antisera (to check the mixed infections of Zucchini Yellow Mosaic Virus (ZYMV) with CMV in the same common hosts). Most of the samples reacted positively with CMV antisera and few samples reacted with Potyvirus antisera (Table 1and Figure 6).

Transmission of Virus

Mechanical /Aphid transmission of CMV

Pumpkin plants expressed the symptoms after 30 days of mechanical inoculation. The symptoms like severe mosaic and leaf deformations were observed in inoculated plants, which were confirmed through DAC-ELISA. Among *Myzus percicae and Aphis craccivora, M.percicae* efficiently transmitted the virus in a non-persistent manner within 20 min of acquisition period and inoculation period of 10 to 15 min and the symptoms were observed after 30 days of inoculation and hence, confirmed that the CMV is both mechanical and aphid transmissible (Figure 4).

a. Mechanical transmission

b. Aphid transmission

Fig. 4. Transmission of CMV on to pumpkin plants

Biological screening of virus by single lesion isolation on indicator hosts

The CMV positively tested samples in DAC-ELISA were inoculated on to the *Vigna unguculata*, *Nicotiana glutinosa, N.tobaccum, Chenopodium* seedlings for local lesion assay. After 3 days of inoculation, chlorotic lesions were observed later progressed to necrotic lesions on cowpea- c152 variety and on *Nicotiana tabaccum* plants (Figure 5).

a.

b.

Fig. 5. local lesions on a. Cowpea (c152) and b. *Nicotiana tobaccum*

Quantification of virus in *Trichoderma* treated and control plants

At different time intervals the concentration of virus in the treated plants was measured. Experiments indicated that titer of CMV was significantly lower in the *Trichoderma* treatments than in the disease control (Figure 6) and there is gradual decrease in the virus titer as the age of the plant increased in *Trichoderma* treated plants in contrast to the increase in the virus titer with the age of the plant in disease control. The response of the *Trichoderma* treated pumpkin plants to CMV was quantitatively analyzed through ELISA (Table 2). *Trichoderma* treated plants significantly reduced the virus concentration, the titer of the virus in T4 plants, 0.921 $\pm$1.601 and it is 1.465 $\pm$1.503 respectively at stage 1 and 2 while the T3 plants exhibited 2.840 $\pm$2.907 and 4.095 $\pm$4.508 at stage 1 and 2.

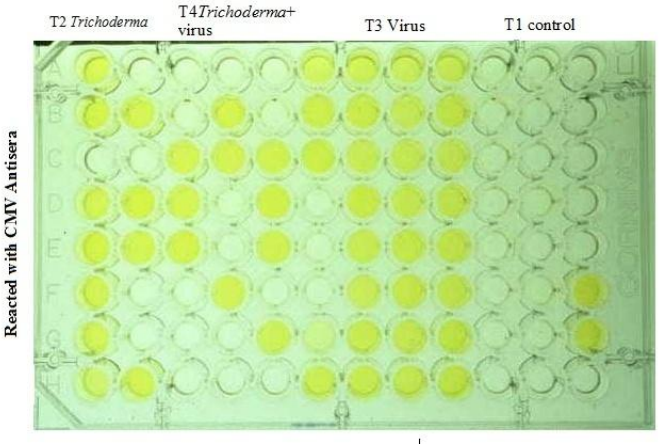


Fig. 6. Indexing of virus titer in *Trichoderma* treated CMV infected samples by ELISA

Table 1. Field survey and collection of virus infected samples from various location

S.No	Places	Host	Number of samples	CMV antisera	Potyvirus antisera
1	Kuppam	Pumpkin	1	+	-
			2	+	-
		Ridge gourd	3	+	-
			4	+	-
2	Mulbagal	cucumber	5	+	-
			6 a	+	-
		Pumpkin	6 b	-	+
3	Guntur	Ash gourd	7	+	-
		Pumpkin	8	+	-
		Bottle gourd	9	-	+
4	Chennai	Pumpkin	10	+	-
		Ridge gourd	11	+	-
		Pumpkin	12	+	-
5	Hyderabad	Ash guard	13	+	-
		cucumber	14	+	-

Table 2. Post inoculation Effect of *Trichoderma* on CMV titer

Treatments	Absorbance at 405nms Stage 1	Absorbance at 405nms Stage 2
T1 control	0.751 ±0.83	0.875 ±0.904
T2 *Trichoderma*	0.655 ±8.054	0.779 ±0.804
T3 Virus	2.840 ±2.907	4.095 ±4.508
T4 *Trichoderma* +virus	0.921 ±1.601	1.465 ±1.503

Each value is an average of 3 replicate samples. In a column, means followed by a common letter are not significantly differ at P = 0.05 by DMRT (Duncan's Multiple Range Test)

Green house experiment

Trichoderma mediated induced Plant growth promotion

The number of plants exhibiting CMV symptoms was less in *Trichoderma* trated plants, compared with the untreated challenged control. The percentage of symptomatic plants with *Trichoderma* treatment ranged from 20 to 55% percent where as in the non-treated and challenged plants it is 60 to 80%. The percentage of seed germination ranges from 80 to 100% in the *Trichoderma* treated plants and it is 60% in control plants (Table 3).

Induced biochemical changes

Total phenols

The total phenol activity increased at different stages of disease development, significant differences were found among two stages and represented in Table 4. The total phenol content markedly increased in T4 plants (*T.harzianum*+virus) (1.45mg g-1 fr.wt.) than in T3 plants (virus alone) (0.84 mg g-1 fr.wt) at stage 2 compared with the stage 1.

Peroxidase and poly phenoloxidase activity

The induction of defense enzymes, PO and PPO in Pumpkin was studied at different stages of infection after challenge inoculation with CMV and *T. harzianum*. The enzyme activity was increased up to 15 days and maximum induction was observed at stage 1. In T4 plants (*T. harzianum* + CMV) there is an increase in peroxidase activity (0.359 OD min–1 g–1) than in T3 plants (Virus alone) (0.242 OD min–1 g–1) at stage 1 and the enzyme activity started declining drastically at stage 2 (Table 5). The polyphenol oxidase enzyme activity was increased in 25 days and maximum enzyme activity was observed at stage 2 in T4 plants (0.391 OD min–1 g–1 of sample) in comparison with T3 plants (0.298 OD min–1 g–1 of sample), T2 plants (*T. harzianum),* T1 control plants (Table 5).

Poly acrylamide gel electrophoresis (PAGE) analysis

The PAGE analysis revealed that the additional 21.5 KDa protein was observed in the T4 treatment (*Trichoderma* + Virus) in lane 1 compared to the other treatments.

Table 3. *Trichoderma* induced changes on plants

Treatment	%of seed Germination			Symptom Rating (%)			Yield measurement (No. of leaves)			Height of the plant (cms)		
	10 DAI	20 DAI	30 DAI	10 DAI	20 DAI	30 DAI	10 DAI	20 DAI	30 DAI	10 DAI	20 DAI	30 DAI
T1 *Trichoderma* alone	100	0	0	0	0	0	8	13	20	36	80	115
T2 Virus alone	60	0	0	100	100	100	4	9	14	22	38	54
T3 *Trichoderma* + Virus	80	0	0	55	35	20	6	11	16	34	78	109
T4 Healthy control	90	0	0	0	0	0	9	15	24	40	84	122

Table 4. Effect of *Trichoderma harzianum* and Virus applied either alone or in combination on total phenols content of Pumpkin plants

Treatments	Total phenol (mg g–1 fresh weight) 1st Stage	Total phenol (mg g–1 fresh weight) 2nd Stage
T1 control	0.51^{d} ±0.03	0.75^{d} ±0.04
T2 *Trichoderma*	0.64^{c} ±0.05	0.79^{c} ±0.04
T3 Virus	0.84^{b} ±0.07	0.95^{b} ±0.08
T4 *Trichoderma* +virus	1.39^{a} ±0.01	1.45^{a} ±0.03

Each value is an average of 3 replicate samples. In a column, means followed by a common letter are not significantly differ at P = 0.05 by DMRT (Duncan's Multiple Range Test)

Table 5. Peroxidase and Polyphenol oxidase activities in pumpkin plants

Treatments	Peroxidase (changes in OD min^{-1} g^{-1} of sample)		Poly phenolxidase (changes in ODmin^{-1} g^{-1} of sample)	
	Stage-1	Stage-2	Stage-1	Stage-2
T1 control	0.280^{b} ±0.007	0.301^{b}±0.008	0.268^{c} ±0.006	0.274c+0.007
T2 *Trichoderma*	0.237^{d} ±0.004	0.245^{d}.±0.005	0.266^{d} ±0.004	0.276^{d} ±0.005
T3 Virus	0.242^{c} ±0.006	0.256^{c}±0. 007	0.298^{b}±0.005	0.329^{b}±0.006
T4 *Trichoderma* +virus	0.359^{a} ±0.08	0.460^{a}.±0.005	0.391^{a} ±0.007	0.402^{a} ±0.009

Each value is an average of 3 replicate samples. In a column, means followed by a common letter are not significantly differ at P = 0.05 by DMRT (Duncan's Multiple Range Test)

Fig. 7. *Trichoderma* mediated ISR Treatments T1 -Control, T2- *Trichoderma* , T3-Cucumber mosaic virus, and T4- *T. harzianum*+virus

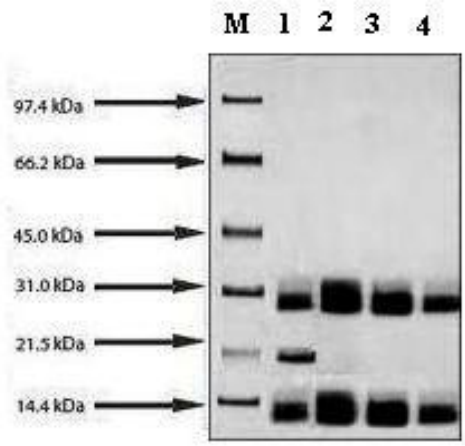


Fig. 8. SDS-PAGE analysis of polyphenol oxidases and peroxidases (*Trichoderma* induced systemic resistance)

Lane1- T4 treatment (virus+*Trichoderma*),
Lane2- T1 treatment (Control)
Lane3- T2 treatment (*Trichoderma*),
Lane4- T3 treatment (only virus)

The pathogen related protein (PR) protein raised against the cucumber mosaic virus was triggered by biocontrol agent in the T4 treatment in lane 4 correlated with the medium range protein marker (MX-0213) (Figure 8).

DISCUSSION

Induced systemic resistance (ISR) in pumpkin plants raised by inoculation of *Trichoderma* in releasing of elicitors and activates the expression of genes involved in the plant defense response system against CMV, this may promote the growth of the plant system and nutrient availability. The results of the experiment revealed that soil application of *T. harzianum* was found more effective in enhancing the growth and suppressing mosaic disease incidence caused by CMV in cucurbit plants. From this study it has been concluded that cucurbit plants treated with native bioagents of *T.harzianum* followed by challenge inoculation of cucumber mosaic virus (CMV) enhances induction of defense related enzymes such as PO, and PPO and these were very effective in the control of mosaic disease of cucurbit plants. In the present study the accumulation of phenolics was observed in all the treatments. Induced PO and PPO activity in pumpkin plants enhances the antiviral properties and plant phenolics and their oxidation products offer resistance against a wide range of pathogens (Vidhyasekaran, 1988). Synthesis of phenol depends on PAL activity. Enhanced PAL activity in response to pathogen infection led to the accumulation of more phenols (Nuckles, Hammerschmidt 1982; Charitha Devi *et al*., 2012).

Plants which received the challenging pathogen after exposure to the biocontrol agent showed higher peroxidase and poly phenol oxidases levels than those from other treatments in agreement with the other reports (Murphy *et al*., 2003) which demonstrates oxidative burst (Martinez *et al.,* 1998) leads to ISR against pathogen. The application of extracts either enabled the plants to resist the nematode invasion or activated directly the defense mechanisms of plants. There are reports of induction of resistance and/or defense reactions in host plants against plant pathogens by compounds produced by biocontrol agents and chemicals contained in extracts of antagonistic plants (Yedida *et al.,* 1999). In conclusion, the *Trichoderma* isolated from pumpkin roots seems to be promising inducer of systemic resistance in cucurbits (*C*. moschata) to a challenge CMV infection under greenhouse conditions. This result could

be very important practically since it may offer a simple, environmentally safe and economically accepted mean to protect Cucurbit plants from CMV infection. However, additional studies are needed to confirm these results under field conditions.

Conclusion

The present investigation was to understand the Induced systemic resistance of cucumber plants with the native, highly potent *Trichoderma* isolates possessing rhizosphere competence and antiviral properties against cucumber mosaic virus (CMV) at different stages of growth. After pre and Post inoculation studies the cucurbit plants responded well to select *Trichoderma* isolate and induced some resistance systemically against CMV infection and significant changes in the host plant in terms of total Proteins, Phenols, Peroxidases and polyphonel oxidases. All the physiological aspects were also studied by comparing control and inoculated plants like root length, shoot length, % of seed germination, % of disease incidence and yield parameters. Due to increase in plant defense enzymatic activity, and decrease in disease incidence, the *Trichoderma inoculum* can also applied in the fields in order to enhance the yield up to 40% compared to control plants. Hence, this study shows clear evidence that the native isolates of *Trichoderma* inoculum can not only enrich the soil fertility and yield of the crop but also induces the disease resistance and helps to increase the production and cope up with market demand.

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