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SCREENING, ISOLATION AND CHARACTERIZATION OF HEAT STRESS TOLERANT *TRICHODERMA* ISOLATES: SUSTAINABLE ALTERNATIVE TO CLIMATE CHANGE

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ABSTRACT

To provide food security with quality crops for exponentially growing population brought intense pressure on the limited land and natural resources among developing countries. Abiotic stresses such as continuously rising temperature as consequences of global warming is adding pressure to existing problems by adversely affecting crop productivity through physiological changes in plants. Hence there is need of qualitatively potential vegetable crops that can withstand changing environmental conditions such as Okra (*Abelmoschus* spp.) holding high level of nutrients along with economic importance. But requirement of high temperature and humidity for its cultivation make the plant prone to several phytopathogens that ultimately leads to severe qualitative and quantitative losses depending upon the plant growth stage getting affected. Due to the unenviable problems of chemical fertilizers, biocontrol agents were applied as auxiliary treatments either single or in combination that possess fewer consequences on the environment. But due to increasing environmental and soil temperature, activities of these formulations are getting hindered. Therefore, there is an urgent requirement to procure high temperature stress tolerant strains along with antagonistic and plant growth promoting abilities. In the current study, we mainly focused on isolation of high temperature tolerant *Trichoderma harzianum* (BHU P4) strain with antagonistic abilities against fungal pathogen *Sclerotium rolfsii* causing collar rot disease. The strain was also studied for plant growth promoting attributes in okra plant which resulted in increased fresh weight, dry weight, chlorophyll content and nutrient content in comparison to control and pathogen challenged plants. This study was associated with an improvement in the level of total phenol, SOD, PO and PAL enzymes in order to regulate the host defense mechanism against its confrontation with *S. rolfsii*.

Keywords: Climate change, crop loss, food security, high temperature tolerant *Trichoderma* sp.; *Sclerotium rolfsii*; Okra (*Abelmoschus esculentus*); induced systemic resistance

INTRODUCTION

Crops are getting exposed to numerous biotic and abiotic stresses either simultaneously or consecutively due to changing atmospheric conditions. Gradually rising temperature impart major effects on crop production through adversely effecting morpho-anatomical, physiological, biochemical and genetic alterations (Masipa *et al*., 2017; Raza *et al*., 2019). Because of stunted growth, reduced leaf area, altered plant water relations, hampered morphological development, limited photosynthesis and respiration, noticeable pre- and post- harvest loss and decline in agricultural productivity has been recorded due to elevated temperature (Sade *et al*., 2018; Kasso and Bekele, 2018). Modified protein machinery, altered genetic and metabolic processes, impaired mitochondrial and chloroplast functions, modification of membrane permeability, enhanced production of reactive oxygen species (ROS), damaged cellular organization and even cell death have been observed in plants exposed to prolog high temperature stress (Liu *et al*., 2019; Kim *et al*., 2021). All of these modifications in plants ultimately lead to hindered flowering along with fruit set in both tropical and temperate crops which finally leads towards reduced economic yield (Hsiang *et al.,* 2017; Gull *et al.,* 2019). Various microorganisms regulate their emergence and survival in changing environmental conditions through alteration in their genetic and metabolic pathways that ultimately results into emergence of new diseases (Velásquez *et al*., 2018; Jones and Naidu, 2019). In the same context, okra becomes prone to magnitude of pathogen damages such as Southern Blight caused by *Sclerotium rolfsii* due to required cultivation conditions of high temperature and humidity (Kator *et al*., 2015; Paparu *et al*., 2020). *S. rolfsii*, a corticioid fungus from Atheliaceae family is one of the most devastating necrotrophic, polyphagous, cosmopolitan facultative phytopathogen infecting more than 400 plant species around the whole world especially hot and humid areas of our country (Paparu *et al*., 2020; Sun *et al*., 2020). Moreover, this phytopathogen proliferates aggressively at warm temperatures and high humid conditions on any part of the plant or on the plant surface having close contact with external water causing stem canker, crown blight and most common collar rot (Kator *et al*., 2015; Sahu *et al*., 2019; Sun *et al*., 2020). Due to the disastrous after effects of chemical means,

biological means came into action as auxiliary treatments through application of numerous biocontrol agents either single or in combination that possess less consequences on the environment (Sgobba *et al*., 2015; Bastakoti *et al*., 2017). Among plethora of registered biocontrol agents obtained through rhizospheric microbiome, *Trichoderma* spp. has been well reported for their abilities to mitigate different abiotic stresses, biological control of plant diseases, induction of systemic resistance and plant growth promotion through enhancement in nutrient availability and uptake (Singh *et al*., 2018; Sharma *et al*., 2019). *Trichoderma* spp. uphold sustainable potential to antagonize several phytopathogens through rapid colonization of soil and host plants, competition for space and nutrients, direct or indirect stimulation of plant's defense mechanism, production of cell wall degrading enzymes, metabolites and antibiotics for cell lysis that ultimately inhibit the growth of pathogens (Beneduzi *et al*., 2012; Kaur *et al*., 2020; Saravanakumar and Wang, 2020). Several species of *Trichoderma* spp. has been reported as mutualistic fungi which enhanced the root growth, water holding capacity, nutrient use efficiency and whole plant tolerance against stress conditions (Yildirim *et al.,* 2006, Bisen *et al.,* 2015). *Trichoderma* spp. have been mentioned as sustainable alternative for organic farming with its abilities to mitigate several seed & soil borne phytopathogens and enhance the yield up to multiple folds as they establish and proliferate in soil providing long lasting effects (Ram *et al.,* 2018; Vinci *et al.,* 2018). *Trichoderma* spp. has been reported for their presence at high temperature zones along with enhanced biocontrol and plant growth promoting abilities (Qiu *et al*., 2017; Al-Ani, 2018). Although *Trichoderma* spp. has been established as a potent biocontrol agent, increase in environmental and soil temperature causes hindrance in the efficiency of existing commercial formulations as the strains used are mesophilic. Therefore, there is an urgent requirement to procure *Trichoderma* spp. strains that can withstand the high temperature stressand possess the capability to inhibit phytopathogens along with plant growth promotional attributes (Gangwar and Singh, 2018; Ghazanfar *et al*., 2018). Considering all the above-mentioned limitations, there is an urgent need of sustainable alternative to the whole organism formulation that can survive both biotic and abiotic stresses efficiently along with biocontrol and plant growth promoting abilities. Therefore, in the current present study we focused on the isolation of heat stress tolerant *Trichoderma* strains though the analysis of conidial survivability, mycelial growth and accumulation of intracellular polyols during their exposure to high temperature regimes along with antagonistic ability against *S. rolfsii* and plant defense plus growth promoting abilities towards Okra plant.

MATERIAL AND METHODS

Collection of soil samples from heat stressed agro-ecosystems

A total of twenty soil samples were collected from agricultural and composting sites of heat stressed zone during May – June when the mean temperature was recorded higher. The samples were procured from the depth of 7-10 cm of rhizospheric regions from soil surface near the crops. Similar method was implied to acquire the soil sample from the beneath of composting sites. The sampling process ensured the safety of plant diversity and composting procedure at the site of sample collection. All the collected samples were kept in separate plastic bags for further study. The collection sites were public places so it did not required any specific permission and the involvement of any protected or endangered species were confirmed prior the sample collection.

Isolation of *Trichoderma* spp. from collected soil samples

Isolation of *Trichoderma* spp. was performed by employing serial dilution method of collected soil samples on *Trichoderma* selective media (TSM) (Askew and Laing, 1993). For the process of serial dilution, one gram of collected rhizospheric soil from all forty samples were suspended separately in 10 ml of sterilized distilled water present in glass vials which served as 10^{-1} dilution. For further dilution up to 10^{-7}, 1 ml of suspension from 10^{-1} properly shaken suspension was pipette out and mixed in another glass vial holding 9 ml of sterile distilled water. 100 µlof suspensions were pipetted out from the vials with 10^{-5}, 10^{-6} and 10^{-7} dilution and was spread on petriplates with TSM. Plates were incubated at 28± 2^0C for 4 to 5 days. *Trichoderma* spp. was identified based on their color, size, shape and appearance of the colony on TSM surface. To obtain the pure culture and further identification, single colonies were transferred from the master TSM plates to fresh Potato Dextrose Agar (PDA) plates, labeled accordingly and incubated in BOD at 28± 2^0C for 2-3 days. The single colonies were also transferred to PDA slats for their storage at 4^0C after incubation for further use.

Assessment of survivability of conidial suspension for selection of high temperature tolerant *Trichoderma* spp.

Discs of 5 mm diameter were procured from all the pure cultures of fully sporulated *Trichoderma* isolates after their incubation at 28± 2^0C for seven days. The discs were suspended separately in three glass vials of 1.5 ml capacity as replicates, each containing 1 ml of 0.02 % Tween-80. The vials were placed upright in water-bath at 45^0C for up to 300 min and 100 ml of the samples pipetted from the vials during stress at sixty minutes of interval. The procured spore suspension was smeared separately and evenly onto 9 cm diameter petriplates of the medium (w/v) consisting of 0.2 % trehalose, 0.25 % peptone, 0.25 % yeast extract and 2 % agar. The inoculated plates were further incubated at 28^0C for 36 h. The viability of the

conidia from each plate were examined at 12 h intervals using the counts of germinated and un-germinated conidia in five microscopic fields (>100 conidia per field). Three vials of the same conidial suspension simultaneously kept at 25 ^{0}C (unexposed to the thermal stress) were used as blank controls (Poosapati *et al*., 2014). Thus, all assays of the fungal isolates were repeated three times. At a given sample time, the amount of relative conidial viability in a stressed suspension over that of its blank control was defined as CFU (colony forming unit) count under the thermal stress.

Assessment of colony growth rate of selected high temperature tolerant *Trichoderma* spp.

After the screening of conidial thermos tolerance, *Trichoderma* isolates were further examined for their mycelial growth at different temperature regimes. Mycelial discs of 5 mm diameter from fully grown plate of each isolates were inoculated at center of PDA plates (9 cm dia.) in the replication of three and incubated at 25^{0}C, 30^{0}C, 35^{0}C, 40^{0}C and 45 ^{0}C respectively for six days (Poosapati *et al*., 2014). The colony growth at each temperature was assessed regularly as average linear growth rate by measuring the diameters of each colony with all three replicates. The formula for calculating average linear growth rate (ALG) is:

ALG (mm/day) = (C5-C1)/4

Where;
C5 is colony diameter at 5th day post inoculation
C1is colony diameter at 1st day post inoculation

Quantification of intracellular polyols in selected *Trichoderma* strains

High temperature tolerant isolates were evaluated for their intracellular polyols by the method described by Hallsworth and Magan, 1996 with slight modifications. Selected isolates were inoculated in 100ml of Potato Dextrose Broth and incubated for one week at 28± 2^{0}C. Fully grown cultures were exposed to 50°C maintained in shaking water bath for1 hr, 2 hr and 4 hr at 200 rpm keeping their counterparts as control. Mycelial mat from all samples were harvested and kept in liquid nitrogen to get frozen for being crushed in powdered form. 250 mg of grounded mycelia was mixed with one milliliter of distilled water and was placed in boiling water bath for 5 min. Each suspension was centrifuged at 12,000 rpm for 15 min and supernatant was preserved at −20°C for further use. Quantitative analyses of intracellular polyols were carried out using HPLC (Shimadzu Corp. Japan) with a refractive index detector, an amino form column and acetonitrile: water (80:20) as a mobile phase. Concentration ranging from 0.1 to 1.0% of standards for glucose, trehalose, fructose, sucrose, mannose and raffinose were utilized after the identification of their respective retention times. Twenty-µL of sample and standard were injected and chromatographed at a constant flow rate of 1 ml/min on mobile phase. The concentrations of the sugars in treated samples were compared to their unstressed counterparts and were expressed as µg/mg of hyphae utilized for the analysis (Poosapati *et al*., 2014).

Estimation of growth kinetics and *in-vitro* antagonistic activity of screened *Trichoderma* isolates against *S. rolfsii*

Isolates of *Trichoderma* spp. obtained after high temperature stress bioassay has been inoculated on PDA plates and utilized for further experiments after incubation at 28±2°C for seven days. A mycelial disc of 5mm diameter from each screened isolates were inoculated at the center of each PDA plates and were incubated at 30°C, 35°C, 40°C and 45°Cfor six days. Growth kinetics was measured by increase in radial growth at interval of 24 hr. The screening of high temperature tolerant *Trichoderma* isolates on the basis of their antagonistic potential was performed by dual culture plate assay under *in vitro* conditions. To evaluate the production of antagonistic compounds from screened isolates, 5mm mycelial disc from fresh culture plates of both *Trichoderma* isolates and *S. rolfsii* was inoculated at distance of 5cm from each other and 2 cm from the edges on PDA plates of 90 mm pertiplate. Petriplates inoculated with only *S. rolfsii* at distance of 2 cm from the edge served as control for the bioassay. Both experimental and control plates were incubated at 28± 2^{o}C and 35^{o}C for five days to study the inhibition in growth rate of *S. rolfsii* (Ray *et al.,* 2016). The experiment was conducted three times and the percent inhibition (%) against pathogen was calculated by using the following formula:

I= (C – T) x 100/ C

Where, I = % inhibition in mycelia growth; C = growth of pathogen in control plates; T = growth of pathogen in dual culture plates.

***In-vitro* estimation of plant growth promoting activities and enzymes production by screened *Trichoderma* isolates**

The potential isolates of *Trichoderma* exhibiting antagonism against pathogen were further screened on the basis of their plant growth promoting and enzymatic activities (Kotasthane *et al*., 2015) as mentioned below:

Indole acetic acid (IAA) assay

Production of IAA by the heat tolerant *Trichoderma* isolates was performed according to method suggested by Bric *et al.,* (1991). Screened isolates were inoculated in glass vials holding tryptophan (5mM) amended in sterilized PDB medium and were incubated at 28±2^{0}C,

40^{0}C and 45^{0}C for 72 hr and 120 rpm on shaker incubator. Post incubation, the cultures were centrifuged at 10,000 rpm for 10 min on 4^{o}C. 2 ml of supernatant from each culture was mixed with 20 µl of ortho-phosphoric acid along with 4 ml of Salkowski's reagent comprising 1 ml of 0.5 M ferric chloride and 50 ml of 35% perchloric acid. The prepared mixtures in separate tubes were incubated at room temperature for 30 min and the absorbance was recorded at 530nm.Development of brownish yellow color confirms the positive result and different concentrations of tryptophan (0, 150,and 300 mg ml-1) were utilized for quantitative estimation of IAA produced using the standard curve (Gordon and Weber, 1951).

Phosphate solubilisation assay

The phosphate solubilizing ability of screened *Trichoderma* isolates was performed on NBRIP-BPB agar medium mixed with 0.5 mM glucose and 0.8 mM tricalcium phosphate whereas the quantitative phosphate solubilization was performed in liquid medium. After the inoculation of screened isolates in NBRIP-BPB medium, the broths were incubated at 28± 2^{0}C, 40^{0}C and 45^{0}C for 96 hr on shaker incubator at 120 rpm. The discoloration of medium indicated positive result as the supernatant were subjected to stannous chloride reduction of the molybdo-phosphoric acid after addition of chloromolibidic acid (10 ml) and cholorostannous acid (0.25 ml).Quantitative estimation of solubilized phosphate was recorded from the standard curve of potassium dihydrogen phosphate (KH_2PO_4) prepared by measuring the absorbance at 600 nm against a blank (Onyia and Anyanwu, 2013).

Siderophore production

The method proposed by Ghosh *et al.,* (2017) was applied to screen the high temperature tolerant *Trichoderma* isolates for their siderophore producing ability. The petriplates containing Chromo-Azurol Sulphonate (CAS) agar medium were inoculated with 5mm bit of fully grown culture at the center and further incubated for 72 hr at 28± 2^{0}C, 40^{0}C and 45^{0}C for control and heat stresses conditions. The discoloration of dye present in medium around the cultures confirms the positive result for siderophore production test.

HCN production assay

Screened *Trichoderma* isolates were tested for HCN production ability through the method described by Zhang *et al.,* (2013). Glass vials holding PDB medium amended with 4.4g glycine/l were inoculated with high temperature tolerant isolates fungal and bacterial isolates respectively. Sterilized flags prepared from Whatman filter paper, soaked in 2% sodium carbonate solution containing 0.5% picric acid were placed at the edge of vials and further incubated for 72 hr at 28± 2^{0}C, 40^{0}C and 45^{0}C. Post incubation, the color of flags was examined and change in color from yellow to brown indicated positive result.

Protease production assay

The method described by Liu *et al.,* (2009) with minor changes was used to examine proteolytic activity of the selected *Trichoderma* isolates. The petriplates containing skimmed milk agar medium were inoculated with 5mm disc of fungal isolates at the center. After incubation at28±2^{0}C, 40^{0}C and 45^{0}Cfor 72 hr, appearance of clear zone around the fungal colonies indicated the positive result.

Chitinolytic Activity

Trichoderma isolates were examined for the ability of chitinase production through the method proposed by Agarwal and Kotasthane (2012).Commercial chitin (Hi media) was utilized for the preparation and storage of colloidal chitin as sole carbon source in chitinase assay medium(Fadhil *et al*., 2014). Sterilized chitinase detection medium (4.5g of colloidal chitin, 0.30g of $MgSO_4.7H_2O$, 3.00g of NH_4SO_4, 2.00g of KH_2PO_4, 1.00g of citric acid monohydrate, 15g of agar, 0.15g of Bromo Cresol Purple, 0.20ml of Tween-80 per liter, pH 4.7) was inoculated with 5mm bit of actively growing *Trichoderma* cultures and incubated at 28±2^{0}C, 40^{0}C and 45^{0}C for 96 hr. The formation of purple colored zone around the fungal isolates was examined as positive bioassay after the addition of bromocresol green reagent.

Chitinase and β-1, 3-glucanase assay

250 µL of crude sample and 1% (w/v) substrate in 250 µL of 50 mM potassium phosphate buffer (pH 7.0) were incubated in water bath at 50°C. Reduced sugar was released in mixture and was calculated by recording the absorbance at 575 nm for chitinase in comparison to control prepared from colloidal chitin. One unit of enzyme activity (U) was defined as releasing 1 µmol of N-acetyl-D-glucosamine from the substrate per min. Similarly β-1,3-glucanase activity was measured using laminarin as the substrate for control. 50 mM acetate buffer (pH 5.5) with 250 µL of crude sample was also incubated for 30 min at 50°C in water bath. Reducing sugar released in mixtures was determined by recording the absorbance at 550 nm for β-1,3- glucanase. One unit of β-1,3-glucanase activity was defined as releasing 1 µmol of glucose from the laminarin per min (Chairin and Petcharat, 2017).

Molecular identification of selected *Trichoderma* isolates

Trichoderma isolate selected for better performance in biocontrol, plant growth promoting and enzyme producing activities was further subjected for molecular identification. Flask containing PDB medium was inoculated with 5 mm mycelial plug from the margins of freshly grown isolate

and was further incubated at 27 °C for 5 days without shaking. Post incubation, mycelium was harvested and genomic DNA was isolated using HiPurA™ SP Fungal DNA Mini Kit (Himedia Laboratories Pvt., Ltd., Mumbai, India) and 1.2% agarose gel in 1X TAE buffer was utilized to study the sharp and distinct bands that indicate the good quality of isolated genomic DNA. Identification of *T. viride* BHU P4 was carried out by ITS sequencing of rDNA and amplification of the gene using universal primers, ITS1-F(5'-TCCGTAGGTGAACCTGCGG-3') and ITS4-R (5'-TCCTCCGCTTATTGATAT-3') by PCR reaction. The thermocycler was programmed (Techne, UK) with initial denaturation at 94 ◦C for 1 min followed by 35 cycles of 4 min, annealing at 56 ◦C for 1 min, elongation period at 72 ◦C for 50 s and a final extension step of 7 min at 72 ◦C. The PCR product ranged from 560 to 600 bps was analyzed on 1.5 % (w/v) agarose gel electrophoresis with ethidium bromide (0.5µg/ml) and was further subjected for sequencing to Operon Technologies, Bangaluru, India. The gene sequence was subjected to BLAST for species identification and making phylogenetic tree.

Seed biopriming of okra seeds by spore suspension of potential *Trichoderma* isolates

Spores from selected *Trichoderma* strains were collected in phosphate buffer saline (PBS; 20mM sodium phosphate, 150mM NaCl, pH 7.4) which were further filtered through utilization of sterilized muslin cloth to prepare stock spore suspension. Prepared stock solution was evaluated for its spore count through assistance of hemocytometer and recording OD at 600nm for mixture of stock suspension (1 ml) and saline solution (9 ml) (Singh *et al*., 2016). The supernatant collected after centrifugation 10000 rpm for 10 min was replaced by equal amount of sterilized CMC (carboxymethyl cellulose) solution (1.0%). Okra seeds were dipped in the homogeneous spore solution obtained after vortexing to procure the seed priming and further seeds were incubated at 28±2˚C with maintain 98% relative humidity for 24 hrs (Singh *et al*., 2013; Jain *et al*., 2015).

Evaluation of plant growth and defense induction in okra plants primed with *Trichoderma* isolate

All the pot trial experiments were conducted in glass-house conditions at Department of Mycology and Plant Pathology, Banaras Hindu University, Varanasi, India (25˚15' 59" N, 82˚59'30"E) to evaluate the antagonistic, plant growth and defense provoking efficiency of *Trichoderma* spore suspension on primedokra plants. Four sets of treatments were prepared i.e.T1- Control (without any treatment), T2- *S. rolfsii* inoculation, T3- BHU P4 treatment and T4- BHU P4 + *S. rolfsii* inoculation. Three unprimed and bioprimed seeds were sown in the pots holding sterilized sand and soil (1:1 ratio) potting mixture. Soil of treatment T2 and T4 were mixed with homogenized inoculum of *S. rolfsii* containing both active fungal mycelium and sclerotia at the rate of 20g per pot before the sowing of seeds. Pots were kept at 30/22°C day/night temperatures with 16/8 h light/dark cycle photoperiod and ~70% relative humidity in the glass house. Plant growth parameters were measured after 25 days of seed germination.

Assessment of plant growth parameters and total chlorophyll content

Plant samples from all the treatments were harvested and analyzed for measuring plant growth parameters including shoot length (SL), root length (RL), lateral roots, leaf area (LA) and fresh weight (FW) and dry weight (DW) of shoot and root tissues. The shoot length was measured from the base of the plant (ground level) to the tip of the plant main axis whereas root length was measured from the base of the plant (ground level) to the tip of the main root after stretching the plant with the help of meter scale. Shoot and root length were recorded in centimeter on 35 days after sowing (DAS). Number of leaves and roots were counted per replication for each treatment by randomly selecting one plant. To calculate fresh weight, the plants were uprooted, washed with tap water, dried on blotting paper and weighed on electronic balance (Sartorius BT-224S). Further, the plant samples were kept in pre-heated hot air oven at 60°C for drying for 6 hours and weighed on electronic balance to calculate the dry weight. The fresh weight and total biomass (DW) of samples from each treatment was measured was calculated in grams (Ray *et al*., 2018).

Total chlorophyll content was measured by centrifuging 1 g of leaf tissue crushed into 20 ml of 80% acetone at 500 rpm for 5 min followed by incubation of supernatant under dark conditions for 24 hours. The amount of pigment released from the leaf tissue was determined by spectrophotometric analysis at 645 nm and 663 nm against the solvent blank 80% acetone. The result was expressed as mg chlorophyll/g FW (Singh *et al*., 2017).

Estimation of enzyme activities

Three plants were randomly selected from each treatment at 7, 14 and 21 days after seed germination and leaves were collected from the third nodes to sixth nodes for biochemical analysis. Leaves were homogenized in 2 ml of ice cold potassium phosphate buffer (0.1 M; pH 7) and centrifuged at 12000 g for 10 min at 4°C to use supernatant as crude enzyme extract.

Phenylalanine ammonia lyase (PAL) activity

Leaf sample (0.1 g) was mixed in 2 ml of 0.1 mol l^{-1} sodium borate buffer (pH 7.0) at 4°C containing 1.4 mmol l^{-1} mercaptoethanol which was further centrifuged at 16,000g at 4°C for 15 min. The reaction mixture with 200 µl of enzyme extract, 500 µl of 0.2 mol l^{-1} borate buffers

(pH 8.7) and 1.3 ml of water was incubated at 32°C for 30 min after addition of 1 ml of 0.1 mol l^{-1} L-phenylalanine (pH-8.7). The reaction was ended by addition of 0.5 ml trichloroacetic acid (TCA, 1 mol l^{-1}). PAL (EC 4.1.3.5) enzyme activity expressed in terms of µmol l^{-1} TCA per g fresh weight (FW) was measured by formation of transcinnamic acid at 290nm and (Singh *et al*., 2014).

Peroxidase (PO) activity

0.1 g of leaf sample from each treatment was homogenized in 2 ml of 0.1 mol l^{-1} phosphate buffer (pH 7.0) at 4°C and was centrifuged at 16000 g at 4°C for 15 min. The reaction mixture comprised of 1.5 ml pyrogallol (0.05 mol l^{-1}), 0.5 ml H_2O_2 (1% v/v) and 0.05 ml supernatant as enzyme extract. Change in the absorbance was measured at 420 nm for 3 min at every 30 sec intervals in comparison to control and enzyme activity was expressed as change in the Unit $min^{-1}g^{-1}$FW (Ray *et al*., 2016).

Polyphenol oxidase (PPO) enzyme assay

0.1g of leaf sample homogenized in 2 ml of 0.1 mol l^{-1} ice cold phosphate buffer (pH 6.5) was centrifuged at 16,000 g for 30 min at 4°C. The absorbance of reaction mixture comprising of 0.4 ml enzyme extract along with 0.4 ml catechol (1mmol l^{-1}) in 3 ml of 0.05 mol l^{-1} sodium phosphate buffer was recorded for 3 min at 405 nm at the interval of 30 sec. PPO enzyme activity was expressed as change in OD $min^{-1}g^{-1}$FW (Singh *et al*., 2014).

Estimation of Total Phenol Content (TPC) Assay

Leaf samples (0.1 g) were placed in glass vials holding 5ml of 95% ethanol and were stored at 0˚C for 48 hours. Supernatant was collected from the sample centrifuged at 15,000 rpm for 10 min. To 1 ml of the supernatant 1 ml of 95% ethanol and 5 ml of SDW were mixed along with 0.5 ml of 1 N Folin- Ciocalteau reagent. The vials were incubated at room temperature for 60 min after adding 1 ml of 5% Na_2CO_3 at the gap of 5 min. Standard curve was prepared through various concentration of gallic acid (GA) in 95% ethanol and the absorbance was recorded at 725 nm which were further converted to mM Gallic acid equivalent (GAE) g per fresh weight (Zheng and Shetty, 2000).

Assessment of nitrogen, phosphorus, sodium and potassium contents in okra plants

0.2 g of dried shoot tissues from each treatment were soaked overnight in 15 ml of di-acid mixture (H_2SO_4: $HClO_3$:: 9:1) further digested on a sand bath hot plate till the deep brown fumes were subsided and the contents turned transparent. The final volume was maintained to 50 ml with distilled water after cooling down of samples. Total *in vivo* nitrogen content was estimated by recording the absorbance at 400nm of 25 ml reaction mixture subjected to constant shaking holding 0.2ml sample mixed in 2-3 ml of distilled water, 2 ml 4M sodium hydroxide and 1 ml 10% sodium silicate along with 0.2ml Nessler's reagent. Standard value was recorded by using dehydrated ammonium chloride. To analyze the total *in vitro* phosphorus amount, 4.5 ml of sample was added to 0.5 ml of Barton's reagent [25g of ammonium molybdate dissolved in 400 ml distilled water (solution A) + 1.25g ammonium metavendate dissolved in 300 ml of distilled water along with 250 ml conc. nitric acid (HNO_3) (solution B); final volume (solution A +solution B) maintained to 1000 ml with distilled water]. The absorbance of samples was recorded at 470nm post incubation for 10 min at room temperature. The standard curve was prepared with 50-600 mg L^{-1} KH_2PO_4 and P content was calculated through the following formula: K (mg/g) = OD/ standard value; where standard value is 0.0031.To calculate the in vitro potassium, 5ml of sample was diluted up to 50 ml with distilled water to make 10X dilution of the stock. 50-600 mg L^{-1} concentrations of potassium chloride (KCl) were used to make standard curve. Flame photometer was used to evaluate sample through following formula: K (mg/g) = (Rx dilution faction x 0.2)/ 1000; where, R is flame photometer reading. Similar protocol has been used for the estimation of in vivo sodium level with the modification in standard i.e. sodium chloride and Na content is also measured by using same formula utilized by K estimation (Ray *et al*., 2016).

Statistical analysis

All experiments were designed and repeated in Randomized Block Design (RBD) with three replications. Data were subjected in one-way ANOVA analysis through SPSS package version 20. Means were compared by Duncan's multiple range tests at $p \leq 0.05$ significant. Results were discussed in terms of percentage and fold changes with respect to control plants.

RESULTS AND DISCUSSION

Isolation of *Trichoderma* spp.

Thirty eight isolates were procured from twenty soil samples from twelve different thermal stressed sites including agricultural and composting regions across four states in India. The isolates recovered from TSM plates according to the method given by Askew and Laing (1993) were further characterized as *Trichoderma* spp. on the basis of their colony color, appearance and morphology based on the species concept given by Rifai (1969). The pure isolates were maintained on PDA slants at 4°C and utilized for further experiments. Among several biocontrol agents approved for field application, *Trichoderma* spp. has established itself as most potent biocontrol and plant growth promoting agent in commercial agriculture (Singh *et al*., 2018; Sharma *et al*., 2019). As the existing bioformulations have not been

screened for their antagonistic and plant growth promoting efficiency at rising regimes of temperatures, there is an urgent need of high temperature tolerant strains of the biocontrol agents to protect host plant against abiotic and biotic stresses(Gangwar and Singh, 2018; Ghazanfar *et al*., 2018). The samples were procured from agricultural fields and composting sites because of higher probability to obtain plant beneficial and high temperature tolerant isolates as the temperature of these locations have been reported to be higher (Steel *et al*., 2018; Smith and Aber, 2018). Numerous *Trichoderma* spp were isolated whose selectivity was confirmed by the addition of rose bengal and chloramphenicol in TSM medium as selective and fungal inhibitor respectively (Gil *et al*., 2009; Siddiquee, 2017).Similar medium was utilized by Mukherjee *et al*., 2014 to isolate two *T. harzianum* and one *T. atroviride* from wild mushroom and tree bark whereas Liu *et al.,* 2020 and Saravana kumar and Wang, 2020 isolated *Trichoderma* spp. from the soil samples.

Assessment of conidial survival in presence of high temperature stress and mycelial growth of selected isolates at different temperature range

Difference in conidial tolerance was observed in spore suspension of twenty eight selected isolates at thermal stress of 45^0C in the time range of 60 min to 300 min. Survival ratios of most of the spores declined either to zero or near 120 min whereas spores of only five isolates *viz* BHU P1, BHU P3, BHU P4, BHU P9 and BHU P12 persisted their survival up to 300 min with varying viability of one isolate to other in given time slot during thermal stress. The spore suspensions of all the selected isolates were surviving with germination rates of 98% in the optimum conditions as control. Inverted sigmoid, reverse hyperbola and straight line curves were procured in the graph for conidial survival ratio showing BHU P4 with highest CFU count value followed by BHU P1 and BHU P3 respectively at thermal stress of 45^0C(Fig. 1). The relative survivability of conidial suspension declined with the progressing time period. *Trichoderma* isolates screened after the conidial survivability were studied for the mycelial growth of five selected isolates in the temperature regime from 25^0C to 45^0C (Table 1). Difference in the growth pattern with increasing temperature appeared among all the five isolates. Optimal growth was observed in all cultures up to 30^0C which declined as the temperature increased. Slow growth rate of 1.05 mm/day was recorded for BHU P2 and BHU P5 during the temperature range of 35^0C to 40^0C whereas and remaining three isolates viz. BHU P4, BHU P1 and BHU P3 were capable to grow up to 45^0C with growth rate of 3.92mm/day, 3.11mm/day and 2.75 mm/day respectively. Maximum temperature tolerance was observed in BHU P4with average linear growth of 13.92±0.58mm followed byBHUP1 and BHU P3 with growth rate of 8.41±1.12mm and 5.08±1.09mm respectively. Colony growth rates differed significantly among all the isolates at a given temperature or within each isolate grown at different temperatures. Number of studies has been performed to study the influence of several abiotic stresses on growth of *Trichoderma* spp. (Zaidi *et al*., 2014; Vithya *et al.,* 2018; Tandon *et al*., 2020). Some of the studies have reported the survival of ascomycetes fungi up to 30°C-35°C but the current study has isolated some of the heat tolerant *Trichoderma* strains that have shown conidial survival till 45°C (Poosapati *et al*., 2014; Anwer *et al*., 2020; de Rezende *et al*., 2020). Assessment of conidial tolerance to high temperature was studied by checking the viability of spores at regular interval of sixty minutes after exposing the spore suspension to 45°C for 300 min. Conidia of isolates shown their survivability for more than 120 min with high CFU count. The isolates BHU P4, BHU P1 and BHU P3 possessed high spore count with values of 73.65±1.09, 58.17±0.92 and 46.29±1.36 CFU count with the power of 10^5 respectively at 45°C. Gradual decrease was observed in the spore viability with increase in temperature above 50°C. Conidial germination and survival of *Trichoderma* isolates at high temperature for given time confirm their tolerance to heat stress environment (Qiu *et al*., 2017; Li *et al*., 2019; Hewedy *et al.,* 2020). Significant variability in colony growth rate has been observed among all the isolates at given temperature. The mycelial growth of all five strains was recorded higher at optimal temperature i.e. 30°C but the growth declined after 35°C. However, there was no significant correlation was found between geographical location of sample and their survival at high temperature.

Estimation of intracellular polyols under heat stressed condition

All the three high temperature tolerant isolates were exposed to 45°C for 1 h, 2 h and 4 h, respectively to study the production of stress protectant intracellular polyols through the help of HPLC. Cyclic increase in the amount of mannose, trehalose and raffinose were recorded in the cell free extracts of isolates during the heat stress condition in comparison to control set. Moreover, maximum accumulation of trehalose, mannose and raffinose were observed in BHU P4 isolate followed by BHU P1 and BHU P3 respectively, during the heat stress indicating their possible role in high temperature tolerance (Fig 2). The attribute of high temperature tolerance found in three isolates were also supported by the quantitative analysis of stress protectant intracellular polyols. Various evidences from extensive experiments have established the role of sugar molecules as osmolytes which assist the host to mitigate the stress conditions and protect the cellular membrane and proteins. In addition, low molecular weight molecules induce several biological mechanism including stress signaling, balanced cellular redox system and also acts as chaperons in response to environmental cues (Bhattacharya and Kundu, 2020). In the same context, higher accumulation of non-reducing disaccharides of glucose i.e. trehalose has been reported

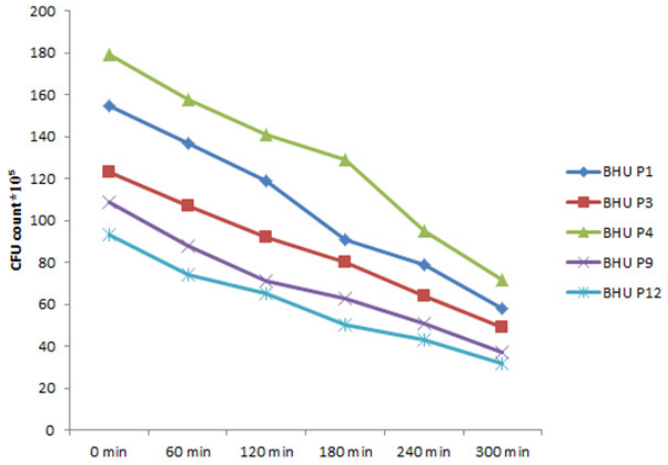


Fig. 1: Difference in conidial tolerance of selected *Trichoderma* isolates at thermal stress of 45°C in the time range of 60 min to 300 min

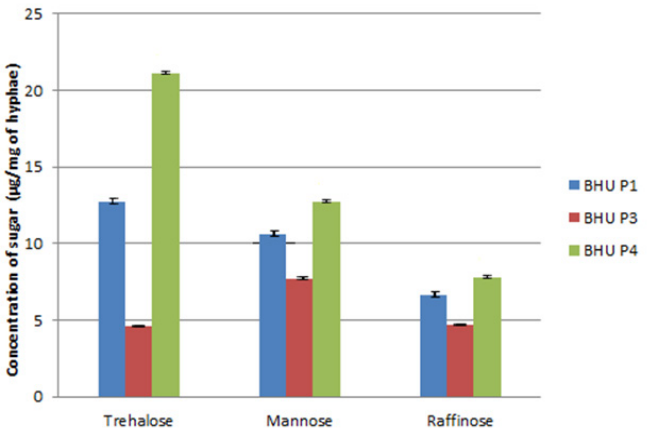
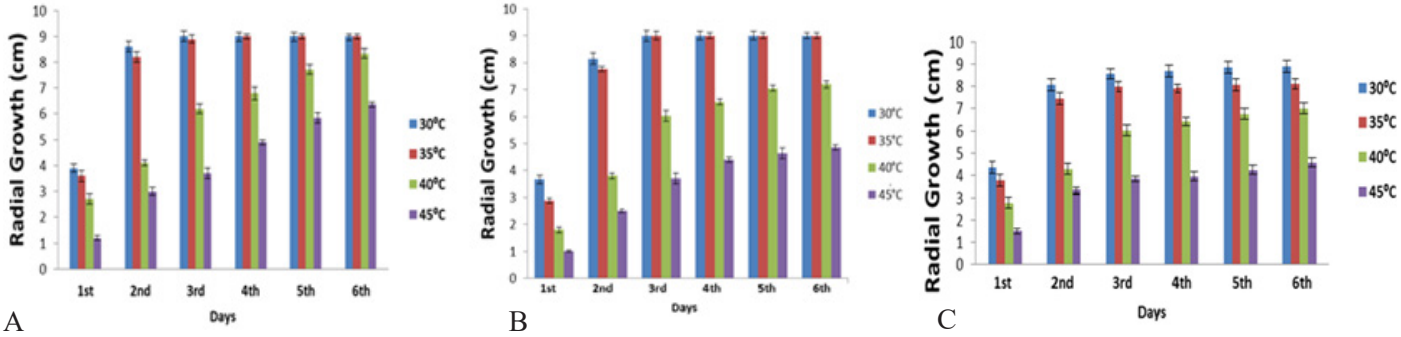
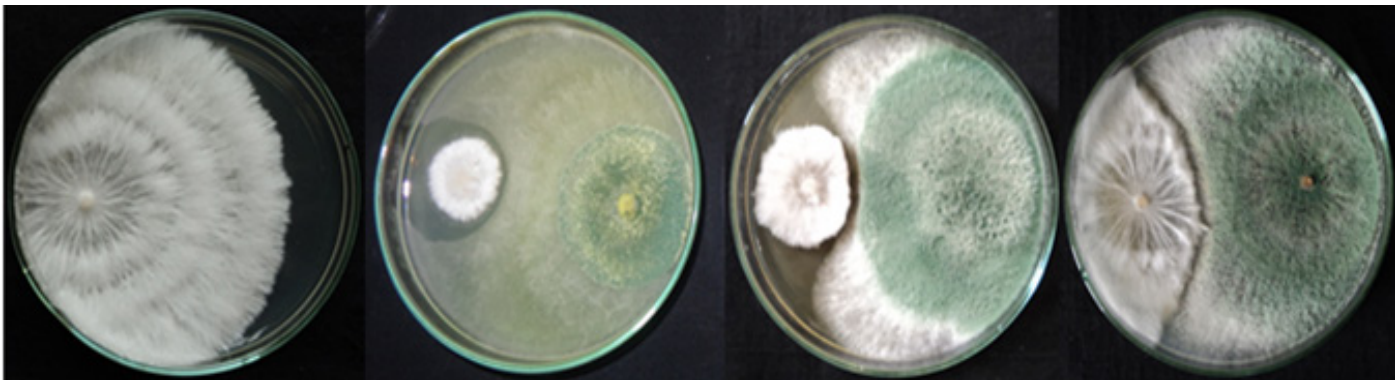


Fig. 2: Biochemical estimation of intracellular polyols in selected heat stress tolerant *Trichoderma* isolates exposed to high temperature



A B C

Fig. 3: Growth kinetics of selected *Trichoderma* isolates at different temperatures (A=BHU P4; B=BHU P1 and C=BHU P3)



Control BHU-P4 BHU-P1 BHU-P3

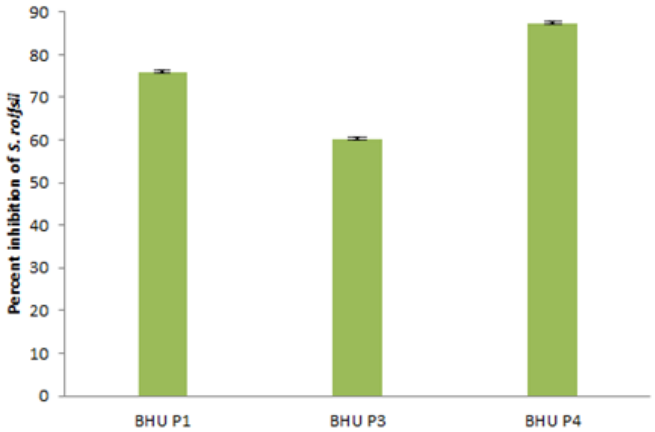


Fig. 4: Dual plate culture assay and percentage inhibition of S. rolfsii by high temperature tolerant *Trichoderma* isolates

in various microrganisms during heat stress (Lei *et al*., 2019; Liu *et al*., 2019; Kosar *et al*., 2019). In our study, the amount of trehalose, raffinose and mannose was found highest in BHU P4 followed by BHU P1 and BHU P3 respectively during their exposure to high temperature stress. Cell structure, protein stabilizing and fungal stress survival abilities of mannose and trehalose under heat stress conditions has been deeply studied (Poosapati *et al.,* 2014; Zhao *et al.,* 2019; Zhang *et al.,* 2020).

Growth kinetics and *in-vitro* antagonistic activity of screened *Trichoderma* isolates against *Sclerotium rolfsii*

Prolonged conidial survival, higher mycelial growth and enhanced polyols accumulation in the three isolates *viz.*

Table 1: Average linear growth (ALG) of screened high temperature tolerant *Trichoderma* isolates measured by mycelium growth (mm/day) at different time intervals

***Trichoderma* isolates**	**25°C**	**30°C**	**35°C**	**40°C**	**45°C**
BHU P1	13.41±1.07	14.83±1.36	12.09±0.86	10.41±0.62	8.41±1.12
BHU P3	10.45±0.67	11.33±1.04	9.47±0.89	8.41±1.21	5.08±1.09
BHU P4	18.23±0.42	19.56±0.85	18.79±0.68	15.93±0.34	13.92±0.58
BHU P9	9.31±0.58	10.44±0.79	9.12±0.37	7.03±0.99	4.69±0.33

Table 2: Qualitative assays to estimate growth promotion traits and enzymatic activities exhibited by selected *Trichoderma* isolates at different temperatures

Isolates	**28±2⁰C**				**40⁰C**				**45⁰C**			
	SP	**HCN**	**PA**	**CA**	**SP**	**HCN**	**PA**	**CA**	**SP**	**HCN**	**PA**	**CA**
BHU P1	++	++	+++	+	++	+	++	+	++	+	+	+
BHU P3	++	+	++	++	+	++	+	+	+	+	-	-
BHU P4	+++	++	+++	+++	++	++	++	++	++	+	++	+

SP: Siderophore production; HCN: Hydrogen cyanide; PA: Proteolytic activity; CA: Chitinolytic activity (+++ = High; ++ = Moderate; + = Low and; - = Absence)

Table 3: Effect of *T. harzianum*BHU P4 on okra plant growth parameters under different treatment conditions

Treat-ments	**Shoot length (cm)**	**Root length (cm)**	**Fresh weight (g)**	**Dry weight (g)**	**No. of leaves (number)**	**Leaf area (cm^2)**	**No. of lat-eral roots (number)**	**Total Chl content (mg chloro-phyll/g FW)**
T1	21.34±0.64	6.14±0.53	3.82±0.35	1.77±0.16	5.89±0.57	23.11±0.72	32.51±1.31	4.74±0.23
T2	15.03±0.51	4.47±0.67	2.09±0.17	0.77±0.29	3.89±0.61	13.21±1.01	22.41±1.71	2.51±0.47
T3	28.39±0.82	10.66±0.78	4.89±0.75	2.71±0.81	8.91±0.23	29.79±0.83	63.59±1.05	6.73±0.59
T4	20.78±0.81	6.34±0.89	3.78±0.23	1.28±0.24	4.76±0.71	19.35±0.97	54.39±2.09	4.73±0.92

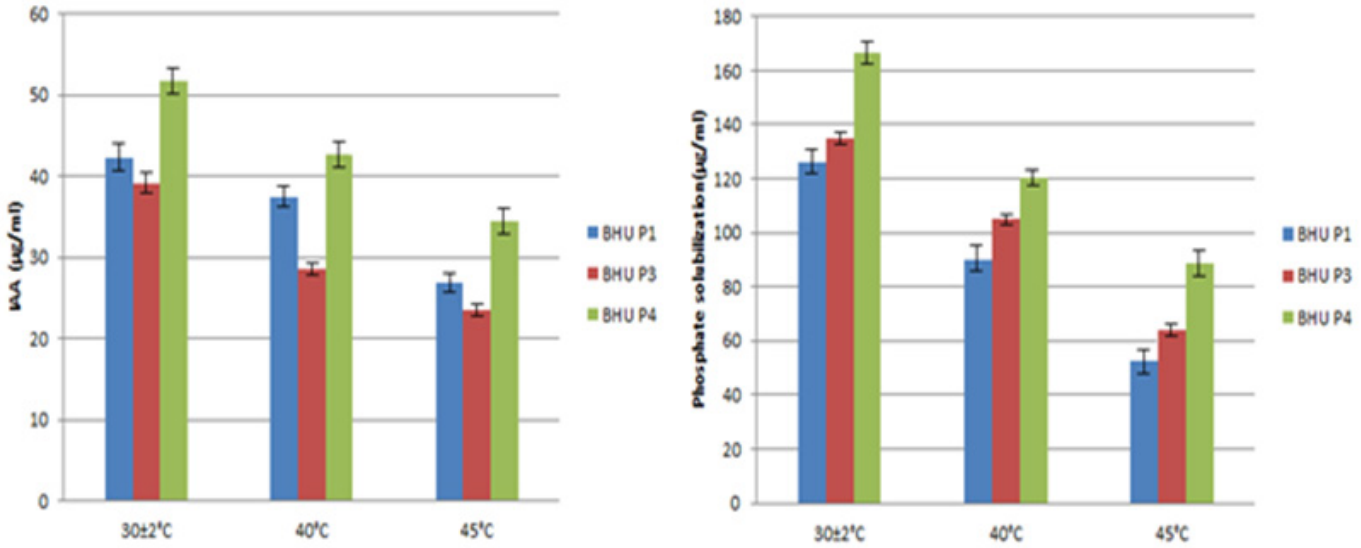


Fig. 5: Quantitative estimation of IAA production and phosphate solubilization by selected *Trichoderma* isolates at different temperatures

BHU P4, BHU P1 and BHU P3 during high temperature stress condition screen them for further studies. Growth kinetics of all the isolates were measured at 30°C, 35°C, 40°C and 45°C for six days at the interval of 24 hr by calculating the radial growth. *Trichoderma* isolate BHU P4 shown highest value of radial growth (8.33±0.28cm) at 30°C for continuous six days whereas the value of radial growth at 40°C (8.16±0.19cm) and 45°C (6.95±0.37cm) was slighter. Growth of BHU P1 and BHU P3 isolates were also higher at 30°C (7.33±0.17cm and 6.47±0.91cm) but the values reduced as the temperature increased (Fig. 3). All the three isolates selected after high temperature tolerance assay were screened for their antagonistic abilities. Results reported significant success in biocontrol under *in vitro* conditions with highest inhibition of 85.7% by BHU P4 followed by BHU P1 and BHU P3 with 78.6% and 61.4% respectively through antibiosis and mycoparasitism.(Fig. 4) Moreover, this phytopathogen proliferates aggressively at warm and high humid conditions on any part of the plant or on the plant surface in close contact with moisture which causes various plant diseases such as stem canker, crown blight and most common collar rot (Sahu *et al*., 2019; Sun *et al*., 2020). To obtain solution for the prevailing conditions, all the three high temperature tolerant *viz.* BHU P4, BHU P1 and BHU P3 strains were taken forward to examine their growth kinetics at higher temperature and antagonistic abilities against *S. rolfsii* on 28±2^{0}C and 35^{0}C as the hyphal survivability of *S. rolfsii* has been recorded up to 35^{0}C (Kumar *et al*., 2017; Sekhar *et al*., 2020). Similar inhibition has been reported in *S. rolfsii* in presence of *Trichoderma* isolates during *in vivo* experimental setup (Singh *et al*., 2016; Hirpara *et al.,* 2017*;* Kushwaha *et al*., 2018).

Screening of the *Trichoderma* isolates on the basis of their *in-vitro* plant growth promoting and enzymatic activities

The potential *Trichoderma* isolates screened after high temperature tolerant assay (BHU P4, BHU P1 and BHU P3) with significant antagonistic ability against *S.rolfsii* were further examined for their plant growth promotion and production of hydrolytic enzymes at 28±2^{0}C for control condition and 40^{0}C and 45^{0}C for heat stressed conditions. Among all the three isolates, BHU P4exhibitedhigh level of all the tested PGP and enzymatic activities at both control and stressed conditions which reduced during the increase in temperature. Under controlled condition, 51.69 ± 2.73 µg/ ml, 42.27± 1.55 µg/ ml and 39.16± 2.34 µg/ ml IAA was produced by BHU P4, BHU P1 and BHU P3 respectively which reduced up to 34.49± 2.24 µg/ ml, 28.39± 2.09 µg/ ml and 23.48± 2.91 µg/ ml at 45^{0}C. Similarly, high level of phosphate solubilization viz. 165.79± 1.56 µg/ ml and 82.61± 1.48 µg/ ml has been recorded in BHU P4 in comparison to BHU P1 and BHU P3 at both control and high temperature stress condition (Fig 5). The decreased level of phosphate solubilization has been observed in all the three isolates during high temperature condition. Maximum discoloration or change in color has been observed on the plates inoculated by BHU P4 holding media used for analysis of siderophore, protease and chitinase production. Change in the color from yellow to light brown of Whatman strips dipped in picric acid placed at the edge of glass vials inoculated with *Trichoderma* isolates confirms the production of HCN. In the same context, maximum color changes in flags were observed in vials inoculated by BHU P4 followed by BHU P1 and BHU P3 respectively. All the three strains were also tested positive for their proteolytic and chitinolytic activities at both controlled and stress condition but maximum production of both the enzymes viz. protease and chitinase was recorded in presence of BHU P4 at both 28±2^{0}C and 45^{0}C (Table 2).Among all the three isolates, BHU P4 performed well in antagonistic bioasssay, plant growth promotion and enzymatic activities even at high temperature representing itself as potential isolate. Various studies have reported the augmented host plant growth in presence of *Trichoderma* isolates under stress conditions (Lei and Zhang, 2015; Hidangmayum and Dwivedi, 2018; Tseng *et al*., 2020). During the quantitative estimation of IAA production and phosphate solublization, BHU P4 possesses higher value in both activities at both conditions in comparison to BHU P1 and BHU P3 (Kumar *et al*., 2017; Vithya *et al*., 2018; Singh *et al*., 2019; Zhang*et al*., 2019; Bader*et al*., 2020). Production of IAA promotes the root development in host plants which leads to the formation of numeral lateral roots and also increases the nutrient and water uptake by plants (Mike-Anosike *et al*., 2018; Verma *et al*., 2018; Mehmood *et al.,* 2019). Similarly solublization of inorganic phosphate present in soil becomes available to the plants which in turn enhance the growth in host plants (Junior *et al*., 2019; Bononi *et al*., 2020; Tandon *et al.,* 2020). Similarly siderophore, HCN, chitinase and protease production were observed higher on petriplates and vials inoculated with BHU P4 at both room temperature and stressed conditions. Various studies have reported the production of these hydrolytic enzymes by biocontrol agents having crucial role in plant growth promotion and defense mechanism (Aamir *et al*., 2019; Baiyee *et al*., 2019; Swain *et al*., 2021). Siderophore production enhances the uptake of micronutrients such as Fe, Zn, Cu, etc. from soil as iron chelators and also depletes the available iron needed by pathogenic microorganisms for their survival (Srivastava *et al*., 2018; Zhao *et al*., 2020). Production of HCN confer antagonistic potential to the isolate as it inhibit cytochrome C oxidase enzyme that in turn hinder the electron transport chain of pathogen (Vithya *et al*., 2018; Biam *et al*., 2019; Singh *et al*., 2019; Banerjee *et al.,* 2020).

Molecular identification of selected *Trichoderma* isolates

BHU P4 possessed highest antagonistic, plant growth promoting and enzymatic activity among all the three

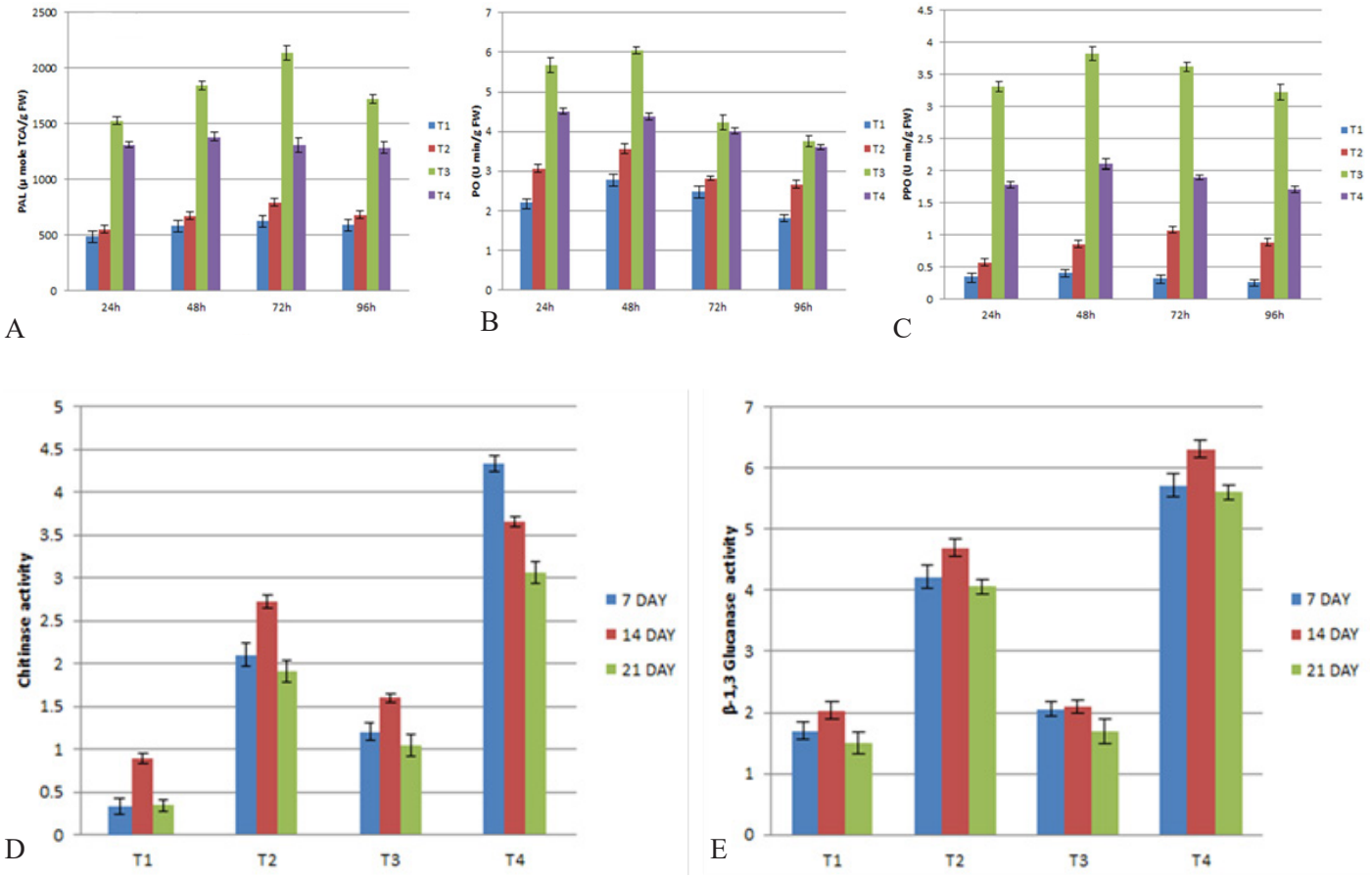
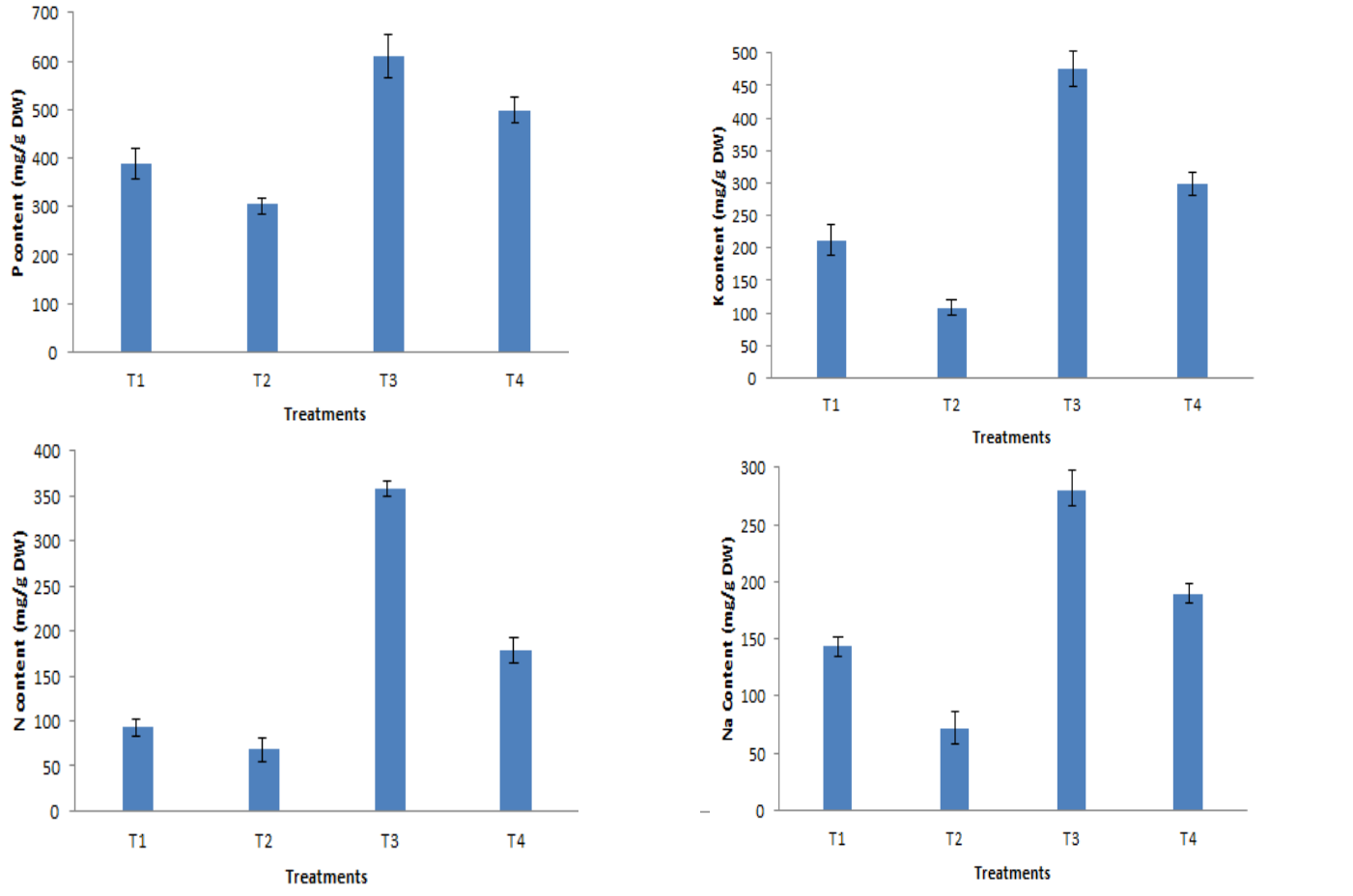


Fig. 6: Effect of T. *harzianum* BHU P4isolate on defense enzymes activity in okra plants under different treatments. (A) Phenyl ammonia lyase activity (PAL); (B) Peroxidase activity (PO); (C) Polyphenol oxidase activity (PPO); (D) Chitinase activity and (E) β 1-3 Glucanase activity.



plants treated with *T. harzianum* BHU P4

screened isolates hence taken further for molecular identification by ITS gene sequencing of 600–700 bp PCR products from 18S rDNA of the isolate. The analysis of BHU P4 ITS sequence showed homology with *Trichoderma harzianum*. The sequence was submitted to GenBank, NCBI database with accession number as MH730446. After screening through antagonistic, plant growth promoting, enzymatic bioassay and metabolic efficiency, *T. harzianum* BHU P4 can be considered as potent and sustainable alternative to other biocontrol agents with limited survivability at higher temperatures (Anwer *et al*., 2020; Sarsaiya *et al*., 2020).

Okra seed priming by *Trichoderma harzianum* BHU P4augmented plant growth and defense enzyme activities

Plant growth promotion parameters were higher in treatments T3 and T4 as compared their respective control. *S. rolfsii* inoculation into soil was found to reduce okra plant growth in terms of length, lateral root formation, fresh weight and biomass content. However, treated plants (T4) were exhibited significantly higher shoot length (23.77%), root length (25.84%), lateral root number (117.38%), fresh weight (39.91%) and biomass content (45.63%) as compared to respective control plants (T2). Total chlorophyll content was also reduced under pathogen inoculation condition. Treated plants (T4) were retained (36%) higher chlorophyll content as compared to T2 treatment plants (Table 3). All defense enzymes activities were induced in okra plants during *S. rolfsii* inoculation. However, Treated plants (T4) exhibited higher activity as compared to respective control plants (T2). PAL and PPO activities were found higher (0.81 & 0.37 fold respectively) in T4 treated plants as compared to T2 plants at 14th day (Fig. 6A & 6C). PO activity was exhibited higher at 7th day in T4 plants (0.35 fold) as compared to T2 plants (Fig. 6B). Activities of cell wall degrading enzymes chitinase and β-1, 3-glucanase were also measured in okra plants upon *S. rolfsii* pathogen inoculation. Chitinase enzyme was found highest at 7th day in T4 plants (0.92 fold) as compared to T2 plants (Fig. 6D).β-1, 3-glucanase was observed highest at 14th day in T4 plants (0.53 fold) as compared to T2 plants (Fig. 6E). Although *S. rolfsii* inoculation resulted in intense wilting and finally resulted in reduced growth of control plants, plants treated with *T. harzianum* BHU P4 remained tolerant and modified root morphology of okra plants along with plant height, number and size of leaves, plant biomass and chlorophyll content. Other studies also confirmed the induction in plant growth, biomass and chlorophyll content during the presence of *Trichoderma* isolates which suggest their effect on phytohormone pathways (Mei *et al*., 2019; Mendes *et* al., 2020; Ali *et al*., 2020).Phenol content in host plants is studied to gain the insight of defense mechanism and integrity of cell structure during their exposure to biotic stress which was found related (Ray *et al.,* 2016; Shen *et al*., 2019). In the current study, *T. harzianum* BHU P4 systematically stimulated the defense mechanisms of okra plant during plant-pathogen interaction. Reactive oxygen species are produced by the plants under stress which damage plant cells and lead to oxidative stress. *Trichoderma* isolates have been affirmed for their potential in induction of resistance in plants against soil-borne pathogens by activating defense mechanism (Contreras-Cornejo *et al*., 2018; Jogaiah *et al*., 2018; Poveda *et al*., 2020). The enhanced production of defense enzymes such as PAL, PO and PPO regulate the production of peroxides at site of infection. The induced systemic defense system has been associated with increased activity of fungal cell wall degrading enzymes and up-regulation of defense enzymatic activities. The enhanced activity of PAL enzyme up-regulate the biosynthetic pathways of phenolics which also provide requisite substrate for oxidative reaction conducted in the presence of PPO enzyme. These phenomena give rise to fungitoxic compounds and inhibit the growth of phytopathogens (Bhardwaj and Kumar, 2017; Nawrocka *et al*., 2018; Landa *et al.,* 2019).

Total nutritional content in plants treated with *Trichoderma harzianum* BHU P4

Plants treated by *T. harzianum* BHU P4 accumulated maximum amount of nutrients i.e. nitrogen (353.78 mg/g DW), phosphorus (586.91 mg/g DW), sodium (263.84 mg/g DW) and potassium (472.63mg/g DW) in comparison to other treatments. The amount of nutrient declined (nitrogen- 68.91mg/g DW, phosphorus-358.15 mg/g DW, sodium-89.32 mg/g DW and potassium-110.79mg/g DW) in the plants infected with *S.rolfsii* whereas the infected plants treated with *T. viride* BHU P4 supported elevated nutrient contents (nitrogen- 182.77 mg/g DW, phosphorus-477.38 mg/g DW, sodium-291.64 mg/g DW and potassium-182.43mg/g DW) (Fig.7).The plants treated with *T. harzianum* BHU P4 were observed with higher accumulation of nutrient contents which included sodium, potassium, calcium and phosphorus. Previous studies have confirmed the role of potassium ions as the key element in the process of photosynthesis, protein synthesis, enzymatic activities and cellular metabolism whereas sodium plays an important role as vacuolar osmotic. Similarly, elevated phosphorus imparts a pivotal role in photosynthesis, carbohydrate metabolism and energy regulation (Habtamu *et al.,* 2018; Gupta *et al*., 2019; Saheed, 2020).

CONCLUSION

To provide food security for enormously growing population, there is a requisite to take our orientation towards the crops that can withstand the rising temperature. Biocontrol agents have been utilized to antagonize the pathogen and enhance the plant growth but the alterations in climatic conditions bring about certain setbacks for whole organism formulations. Therefore, the current

study provides an impetus to utilize *T. harzianum* BHU P4 in the form of suitable bio-formulations for expansion of sustainable agriculture to reduce the adverse effect of higher temperature up to a tolerable level and also increase the crop yield. Extensive study and field level investigation on the mechanisms and interaction of *T. harzianum* BHU P4 would help in developing strategies for improving the efficacy of *Trichoderma* in field conditions in the presence of abiotic and biotic stress.

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