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Isolation and Identification of *Trichoderma* spp. for antagonistic activity against Corm Rot of Saffron from Kishtwar Region, Jammu and Kashmir, India

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ABSTRACT

Jammu and Kashmir is the only saffron growing state of India; Saffron is an important crop with high commercial value and low production, various biotic and abiotic factors affected the production of saffron. Corm rot of saffron caused by *Fusarium oxysporum* f sp. *Gladioli* is one of the major diseases of saffron crop of Kishtwar region of Jammu and Kashmir. In order to increase quality of saffron biocontrol agents are isolated from the soil, Thirty soil sample were collected from ten saffron growing areas of which ten Beneficial microbes were isolated from the saffron crop of the Kishtwar Region and were screened for cell wall degrading enzymes, it was found that of 10 isolates the strain T2 (*Trichoderma* spp.) show maximum production of chitinase (23.88 ± 1.161), cellulase (32.0 ± 1.155), protease (20.0 ± 1.155), β-1,3-glucanase (15.0 ± 0.289) against the corm rot of saffron caused by *Fusarium oxysporum* f.sp.*Gladioli. Trichoderma* spp., the efficacy of *Trichoderma* isolate Tri-2 was superior to other isolates with regard to inhibiting the growth of the test pathogen. Thus proved that *Trichoderma* isolate Tri-2 (81.60%) had better biocontrol properties.

Key words: *Saffron, Corm rot, Fusarium oxysporum f sp. Gladioli, Trichoderma spp., Cell wall degrading enzymes*

Introduction

Saffron (*Crocus sativus* L.) an autumn-flowering perennial sterile plant belongs to Iridaceae family of monocots and propagates by corms (Ambardar *et al*., 2014). The long scarlet stigmas are highly valued for flavouring foods and for medicinal values (Winter halter and Straubinger, 2000). The bioactive components in saffron (picrocrocin, crocin and safranal), have a wide range of uses and capabilities in the medical field, their potential in medical applications, particularly for their therapeutic, cytotoxic, anticarcinogenic and antitumor properties, there has been increasing demand for saffron consumptions (Milajerdi *et al*., 2016; Razak *et al*., 2017).

Globally saffron production is around 300 tons per year. Iran, India, Spain and Greece are the major saffron producing countries with Iran occupying the maximum area and contributing about 88 per cent of world's saffron production. India occupies the 2nd largest area with 7 per cent of the total world production. Jammu and Kashmir is the only state in India where saffron is produced. Spain with 600 ha of land is the 3rd largest producer with an average productivity of 8.33 kg/ha which is highest in the world. The total area under saffron cultivation in

J&K is 3715 ha with production and productivity of 16 MT and 3.0 – 4.0 kg/ha, respectively. Saffron in J&K is primarily cultivated in four districts (Pulwama, Budgam, Srinagar, Kishtwar) with 86% saffron farming system in heritage site of Pampore over 3200 hectares. Kishtwar district of J&K contributes about 3 per cent of the total saffron production in India with average productivity of 1.5 kg ha^{-1} (Razdan *et al*., 2018).

There are various factors that are responsible for poor yield of saffron crop in India. Among the various factors, soils are deficient in essential nutrients due to long planting cycle, prevalence of soil borne disease, lack of irrigation during the critical growth stages of crop, frequent drought, lack of quality planting material and increasing temperatures during vegetative phase are the common biotic and abiotic stresses hampering the saffron cultivation responsible for poor yield of saffron crop production when compared with the productivity in Spain, Italy and Iran (Husaini *et al*., 2010).

Out-break of soil borne diseases due to various pathogens such as, *Fusarium oxysporum, Rhizoctonia corcorum*, *Phomacrocophila, Macrophomina phaseolina* (Thakur *et al.,* 1992), *F. pallidoroseum*, *F. equiseti* (Ahmad and Sagar, 2007) and *Sclerotium rolfssi* (Kalha *et al*., 2007) *Fusarium oxysporum* R1 (Gupta and Vakhlu, 2015) are the major factor in reducing the production and productivity of saffron crop. Incidence of corm rot of saffron (6.70-98%) in Kashmir region (Rekhi *et al.,* 1990; Husaini *et al*., 2010) and 6.60 to 33.70 per cent in Kishtwar district (Gupta and Vakhlu, 2015) caused major encumbrance in saffron crop for sustainable farming. *Fusarium oxysporum* being soil plant pathogen caused infection in the injured corms, resulting in symptoms such as dark brown sunken patches below corm scales and die back of foliage and in severe case the entire corms turns into black powdery mass (Hussaini *et al*., 2010)

Rhizosphere being biologically active zone inhabitates huge population of microbes with diverse metabolic activity (Hiltner,1904). The rhizosphere is the largest ecosystem on earth with enormous energy flux and abundant microorganism such as bacteria and fungi (Barriuso *et al*., 2008). Rhizosphere has been the focus of agricultural research for many years, due to its importance in crop productivity, soil health and sustainable agriculture (Ordookhani *et al*., 2011). Plant growth promoting bacteria (PGPB) and plant growth promoting fungi (PGPF) are predominately rhizosphere inhabitants living in close proximity with root system. Trichoderma exhibit antagonistic behaviour against several phytopathogenic organisms, including bacteria, nematodes and especially fungi, by inhibiting their growth either by direct interactions by hyperparasitism, competition for nutrient and space, and antibiosis) Zhang *et al*., 2017. The Trichoderma species shows antagonistic effect by production of secondary metabolites and cell wall degrading enzymes b-1,3-glucanase, NAGAse, chitinase, acid phosphatase, acid proteases and alginate lyase (Qualhato *et al.,* 2013). Biocontrol can be achieved by introduction of biocontrol agents and native microorganism having potential to suppress the pathogen, and found to be promising strategy for the management of the soil born disease. Understanding the mechanisms of these microbes with saffron crop is worthwhile to achieve the biotechnological potential of proficient PGPF and PGPB affiliation for a range of application so that the production of saffron is enhanced. Keeping in view, the dynamics of beneficial microbes for disease suppression and growth promotion, the putative and indigenous beneficial microbes (PGPF and PGPB) from saffron crop of Kishtwar location shall be characterized and explored for their functional diversity and management potential of soil borne diseases in increasing the productivity of saffron.

Materials and Methods

The present study was conducted in the Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Jammu, to evaluate bio-control potential of beneficial fungal isolates collected from saffron crop. Materials and methods used for conducting experiments are elucidated as under:

Isolation, purification, identification and mass multiplication of beneficial microbes obtained from the rhizosphere of saffron crop

Soil samples were collected from the rhizosphere of healthy saffron plants and air-dried. The soil collected was processed for isolation of beneficial fungal isolates by serial dilution method (Dhingra and Sinclair, 1995). Serial dilutions were made using sterilized distilled water in which one gram of dried soil was suspended in 9 ml of sterilized water blank and mixed by rolling the tube back and forth between the hands to obtain uniform soil suspension.

From this dilution, 1 ml of the suspension was transferred to the second dilution blank with a pipette, diluting the original suspension and marking it as 10^{-2}. From the 10^{-2} suspension, 1ml of suspension was transferred to third dilution blank with a pipette. This process was repeated till the original sample was diluted up to 10^{-7} times. From 10^{-7} dilution, 1 ml was placed at the centre of petriplates containing *Trichoderma* Selective Agar Base medium. The inoculated plates were incubated in inverted position for 48 hours in BOD incubator at 27±2 ^{0}C. The isolates of *Trichoderma* were picked up and purified by single hyphal tip method (Sekhar *et al*., 2017).

Identification of *Trichoderma* isolates

Ten isolates of beneficial fungi obtained from the rhizosphere of healthy saffron corms, were used in the present study, Identification of *Trichoderma* species was based on morphology (Colony colour, Reverse colour), growth pattern, shape size of conidia and conidiophores, and their growth on *Trichoderma* selective agar based medium.

Characterization of beneficial microbes for the production of cell wall degrading enzymes

Quantitative production of different cell wall degrading enzymes such as cellulase, chitinase, protease and β-1,3-glucanase by the beneficial microbes was estimated by adopting the following methodologies.

Cellulase production

In order to have the quantitative assay of cellulase, carboxy methyl cellulase (CMCase) activity was assayed Wherein, 0.5 ml of culture supernatant was added to 0.5ml of 1 per cent CMC prepared in 50 mM sodium citrate buffer (pH 4.8) in a test tube and incubated at 60±1°C for 30 min. The reaction was terminated by adding 4.0 ml of dinitrosalicylic acid (DNS) and subsequently placed the reaction tubes in a water bath at 100 °C for 15 minutes. One ml of Rochelle salt solution (45 g Rochelle salt in 100 ml distilled water) was then added to stabilize the colour. The absorbance was recorded at 575 nm wave length against a blank of 50 mM sodium citrate buffer. One unit of CMCase activity was defined as the amount of enzyme that liberated 1 ìmol of reducing sugar (glucose) in 1 min at 37 °C and pH 7 (Khatiwada *et al.,* 2016).

Chitinase production

Quantitative chitinase production assay, the beneficial isolates were centrifuged at 12,000g for 20 min at 4ºC and the supernatant was used as enzyme source. An aliquot of the supernatant (1ml) was pipetted out and put into a test tube contained the reaction mixture prepared by mixing 500µl of 1 M phosphate buffer with pH 7.0 and 500µl of colloidal chitin. The colloidal chitin was prepared from crab shell chitin. The reaction in the test tube was then stopped by the addition of 2ml of dinitrosalicyclic acid and then heated the reaction mixture for 5 minute on boiling water bath. The reaction mixture was vortex and then measured absorbance at 575 nm. The chitinase activities was determined by the amount of enzyme that produced reducing sugar corresponding to 1 µmol of *N*-acetyl-D-glucosamine and glucose equivalent from colloidal chitin per minute (Sahoo *et al*., 1999).

Protease production

Quantitative production of protease enzymatic activity was estimated by taking the culture supernatant, the culture broth of isolates were centrifuged at 12000 *g* for 15 minute. 0.2 ml of culture supernatant pipetted out and put into test tube and added with 0.5 ml of 5 per cent casein, 1.25 ml of tris buffer (pH 8.0) and formed a reaction mixture. The reaction mixture was then incubated for 30 minute at 37±2 ^{0}C in water bath.3 ml of trichloroacetic acid was added to the reaction mixture at 40 ^{0}C for 10 minute to form precipitate and then again centrifuged at 12000 *g* for 15 minutes and 0.5 ml of the supernatant was collected. The supernatant was used to measure protease activity based on the basis of color development. For color development, 5 ml of 0.4 M sodium carbonate solution was added to 1 ml of the supernatant and kept for 10 min. To this, 1:1 diluted Folin's phenol reagent was added and kept in the dark for 30 min, and the optical density was recorded at 630 nm using UV-visible spectrophotometer. One unit of protease activity was equivalent to the amount of enzymes required to release 1µg tryosine/ml under standard assay conditions. (Akcan and Uray, 2011).

β-1,3-glucanaseproduction

Culture isolates were grown at 27±2 °C for 96 h on a rotary shaker in 250 ml conical flasks containing 50 ml of peptone medium containing laminarin. The

cultures were then centrifuged at 12,000g for 20 min at 4°C and the supernatant was used as enzyme source. A reaction mixture was prepared in a test tube by adding 500µl of culture supernatant, 500µl of 1Mcitrate buffer (pH 5.0) and 500µl of 4 per cent laminarin. The reaction mixture was then incubated in water bath at 40R C for 30 minute and the reaction was terminated by the adding 2 ml of dinitrosalicyclic acid and heated for 5 minute on boiling water bath till the development of colour of end product. The reaction mixture was then vortex and its absorbance was measured at 500nm under UV-visible spectrophotometer (Cattelan *et al.,* 1999).

Dual culture technique

The fungal isolates were screened for antagonistic activity against *Fusarium oxysporum* f. sp. *gladioli,* causing corm rot of saffron, by dual culture technique (Dennis and Webster, 1971). Per cent inhibition of pathogen growth was calculated by using the given below formula (Vincent, 1947).

Per cent inhibition (I) = C-T/C× 100

C= mycelial growth of the pathogen in control

T= mycelial growth of the pathogen in treatment

Table 1. Isolates of beneficial microbes collected from the rhizosphere of saffron crop in District Kishtwar

S. No.	Village	Location	No. of soil samples	Beneficial microbes isolated *Trichoderma* spp.
1	Lower Pochhal	3	3	1
2	Upper Pochhal	3	3	1
3	Hutta	3	3	1
4	Matta	3	3	1
5	Cherhar	3	3	1
6	Hullar	3	3	1
7	Bher-Bhatta	3	3	1
8	Tund	3	3	1
9	Naghani	3	3	1
10	Laynal	3	3	1
	Total	30	30	10

Results

Isolation, purification and identification of beneficial microbes obtained from the rhizosphere of saffron crop

In the present study, beneficial fungi having potential biological control were collected from the rhizosphere of saffron crop from different villages of District Kishtwar. Major saffron growing villages such as Lower Pochhal, Upper Pochhal, Hutta, Matta, Cherhar, Hullar, Bhera-Bhatta, Tund, Naghani and Laynal were selected for the purpose (Table 1). Three locations (saffron fields) were selected randomly at each village for the collection of soil samples from the rhizosphere of saffron crops. Total of thirty soil samples were collected from which ten *Trichoderma* spp. were isolated. Isolations were made to grow on specific selective media such as, *Trichoderma* selective medium (TSM), pure culture of each isolate was maintained for further studies.

Morphological characterisation of *Trichoderma* isolates obtained from the rhizosphere of saffron crop in District Kishtwar

All the ten isolates of *Trichoderma* spp. (Tri-1 to Tri-10), obtained from the rhizosphere of the saffron crops of Kishtwar district were characterised based on their respective colony colour, reverse colony colour, mycelial growth pattern, and shape and size of conidia (Table 2). Isolates Tri-1, Tri-8 and Tri-9 showed light green colony colour, whereas, Tri-2, Tri-6, Tri-7 and Tri-10 isolates formed green coloured colonies and isolates Tri-3, Tri-4 and Tri-5 formed dark green coloured colonies. Reverse colony colour of all the isolates was white. Filamentous and aerial mycelium was observed in Tri-1, Tri-3, Tri-7 and Tri-9 isolates, whereas, in Tri-2, Tri-4, Tri-5, Tri-6 Tri-8 and Tri-10 the mycelial growth was floccose to arachnoid. Conidial shape and average size also varied in the *Trichoderma* isolates. While the conidia were round and smooth in isolates Tri-1, Tri-5 and Tri-8, they were obovoid and smooth in Tri-2, Tri-6 and Tri-7. Conidia of Tri-3 and Tri-4 were subglobose to ovoid and smooth, in case of Tri-9 and Tri-10 conidia were round and obovoid, respectively. The average conidial size in the ten *Trichoderma* isolates varied from 2.5x2.5 to 3.5x3.5 µm.

Characterization of *Trichoderma* isolates collected from saffron crop for the production of cell wall degrading enzymes

Cellulase production

The data presented in the Table 3 depict the cellulase production activity, by the collected *Trichoderma* isolates. It was observed that *Trichoderma* isolate Tri-2 exhibited maximum (23.88±1.161 U/ml) cellulase production activity, followed by Tri-3 (18.25±0.144 U/ml) and Tri-6 (14.10±1.159 U/ml). Five isolates

(Tri-4, Tri-10, Tri-9, Tri-1 and Tri-7) showed cellulase production in the range of 9.57±0.051 to 11.11±1.789 U/ml, whereas, Tri-5 and Tri-8 showed cellulase activity of 6.17±0.167 and 6.33±0.015U/ml, respectively. Minimum enzymatic activity was recorded in Tri-8 (6.17±0.167U/ml).

Chitinase production

The Table 8 further reveals that maximum chitinase production was observed in *Trichoderma* isolate Tri-2 (32.0±1.155 U/ml) followed by Tri-3 (22.2±0.601 U/ml) and Tri-6 (20.0±1.155 U/ml). Five isolates (Tri-4, Tri-10, Tri-9, Tri-1 and Tri-7) showed cellulase activity in the range of 10.0±0.577 to 18.0±0.577 U/ml. Tri-5 and Tri-8 showed cellulase activity of 8.3±0.033 and 8.3±0.058 U/ml. Minimum enzymatic activity recorded in Tri-5 (8.3±0.033U/ml).

Protease production

Quantitatively maximum protease activity was observed in *Trichoderma* isolate by Tri-2 (20.0±1.155 U/ml), followed by isolate Tri-3 (17.2±0.551 U/ml) and Tri-6 (4.06±0.028 U/ml). However in six isolates (Tri- 8, Tri-4, Tri-10, Tri-9, Tri-1 and Tri-7) cellulase activity was in the range of 8.1±0.058 to 14.7±0.441 U/ml with the Minimum enzymatic activity was recorded in Tri-5 (7.2±0.058 U/ml).

β-1, 3 glucanase production

β-1, 3 glucanase activity calculated in terms of nmol sec^{-1}, was maximum in the case of Tri-2 (15.0±0.289 U/ml), followed by isolate Tri-3 (12.9±0.745 U/ml), Tri-6 (10.2±0.960 U/ml). Five isolates (Tri-10, Tri-4, Tri-9, Tri-1 and Tri-7) showed β-1, 3 glucanase activity ranging from 7.3±0.436 to 9.4±0.219 U/ml. Mini-

Table 3. Characterization of *Trichoderma* isolates, collected from the rhizosphere of saffron crop in District Kishtwar, for the production of cell wall degrading enzymes

Isolate	Quantitative production (U/ml) of enzymes			
	Cellulase	Chitinase	Protease	â-1,3-glucanase
Tri-1	10.00 ± 1.155	14.0 ± 0.577	13.1 ± 0.058	09.2 ± 0.145
Tri-2	23.88 ± 1.161	32.0 ± 1.155	20.0 ± 1.155	15.0 ± 0.289
Tri-3	18.25 ± 0.144	22.2 ± 0.601	17.2 ± 0.551	12.9 ± 0.745
Tri-4	08.71 ± 1.459	10.0 ± 0.577	08.9 ± 0.058	07.5 ± 0.033
Tri-5	06.33 ± 0.015	08.3 ± 0.033	07.2 ± 0.058	06.5 ± 0.153
Tri-6	14.10 ± 1.159	20.0 ± 1.155	16.3 ± 0.088	10.2 ± 0.960
Tri-7	11.67 ± 0.882	18.0 ± 0.577	14.7 ± 0.441	09.4 ± 0.219
Tri-8	06.17 ± 0.167	08.3 ± 0.058	08.1 ± 0.058	06.4 ± 0.033
Tri-9	11.11 ± 1.789	12.3 ± 0.176	10.7 ± 0.441	08.2 ± 0.033
Tri-10	09.57 ± 0.051	12.1 ± 0.088	09.7 ± 0.882	07.3 ± 0.436
CD(P=0.05)	2.997	1.896	1.579	1.276
SE±(m)	1.009	0.638	0.531	0.429

Table 2. Morphological characterization of *Trichoderma* spp. isolated from the rhizosphere of saffron crop in District Kishtwar

Isolate	Colony colour	Reverse colony colour	Mycelium growth	Conidia Shape and size
Tri-1	Light green	White	Filamentous and aerial mycelium	Round, smooth; 3.2 x3.3 µm
Tri-2	Green	White	Floccose to arachnoid	Obovoid, smooth; 3.0x1.8 µm
Tri-3	Dark green	White	Filamentous and aerial mycelium	Sub-globose to ovoid, smooth; 3.5x 2.5µm
Tri-4	Dark green,	White	Floccose to arachnoid	Sub-globose to ovoid, smooth; 3.5x 2.5µm
Tri-5	Dark green	White	Floccose to arachnoid	Round, smooth; 3.5x3.5 µm
Tri-6	Green	White	Floccose to arachnoid	Obovoid, Smooth; 3.0x1.8 µm
Tri-7	Green	White	Filamentous and aerial mycelium	Obovoid, Smooth; 3.0x1.8 µm
Tri-8	Light green	White	Floccose to arachnoid	Round, smooth; 3.2x3.3 µm
Tri-9	Light green	White	Filamentous and aerial mycelium	Round; 2.5x2.5 µm
Tri-10	Green	White	Floccose to arachnoid	Obovoid; 2.9x2.4 µm

mum enzymatic activity was recorded in Tri-8 and Tri-5 (6.4±0.033 and 6.5±0.153 U/ml respectively).

Evaluation of *Trichoderma* isolates against the growth of *Fusarium oxysporum* f.sp. *gladioli in-vitro*

A perusal of the data presented in Table 11 revealed that ten isolates of *Trichoderma* spp. were evaluated *in vitro*, to test their biocontrol potential against *Fusarium oxysporum* f. sp. *gladioli,* by using dual culture technique**.** All the isolates (Tri-1 to Tri-10) significantly inhibited the mycelial growth of *Fusarium oxysporum* f.sp. *gladioli* in dual culture method with inhibition ranging from 64.00 to 81.60 on potato dextrose agar**.** Out of the ten isolates of *Trichoderma* spp., the efficacy of *Trichoderma* isolate Tri-2 was superior to other isolates with regard to inhibiting the growth of the test pathogen. Thus proved that *Trichoderma* isolate Tri-2 (81.60%) had better biocontrol properties. It was observed that there was significant reduction in the radial growth of the test pathogen i.e., *F.oxysporum* f. sp. *gladioli.* Minimum radial growth of 13.8 mm was recorded there by effecting 81.60 per cent reduction in radial growth over control.

As per the effectivity of the *Trichoderma* isolate**,** Tri-2 was followed by isolate Tri-3. Isolate Tri-3 influenced radial growth of 18.2 mm resulting in growth inhibition of 75.73 per cent over control in *F. oxysporum* f. sp *gladioli*, the isolate Tri-6 was found to be at par with Tri-3 with redial growth of 19.5 mm resulting in the growth inhibition of 74.00 percent. Maximum mycelia growth (27.0 mm) was observed with Tri-10 having inhibition of 64.00 per cent on PDA as compared to control.

Table 4. In *vitro* evaluation of *Trichoderma* isolates, collected from the rhizosphere of saffron crop, against the growth of *Fusarium oxysporum* f. sp. *gladioli,* causing corm rot of saffron

Isolate	Radial growth (mm) of *F. oxysporum* f. sp. *gladioli*	Inhibition over control (%)
Tri-1	21.8	70.93
Tri-2	13.8	81.60
Tri-3	18.2	75.73
Tri-4	24.0	68.00
Tri-5	27.0	64.00
Tri-6	19.5	74.00
Tri-7	21.2	71.73
Tri-8	25.2	66.40
Tri-9	23.3	68.93
Tri1-10	23.6	68.53
Control	75	
CD(P=0.05)	1.040	
SE±(m)	0.352	

Discussion

Thirty soil samples were collected from the rhizosphere of saffron crop from ten villages of Kishtwar District of Jammu province *viz*., Lower Pochhal, Upper Pochhal, Hutta, Matta, Cherhar, Hullar, Bera-Bhatta, Tund, Naghani and Laynal, for isolation and identification of beneficial microbes, and screening them for biocontrol potential and plant growth promoting traits. Ten isolates obtained from the 30 soil samples, on the basis of growth on selective media belonged to *Trichoderma* spp. Isolates of *Trichoderma* spp. (Tri-1, Tri-8 and Tri-9) produced light green colony having round smooth conidia, whereas, Tri-3,Tri-4 and Tri-5 isolates formed dark green colonies, sub-globose conidia and Tri-2,Tri-6, Tri-7 and Tri-10 isolates formed green colour colonies with obovoid smooth conidia. Kumar *et al*. (2012b) in their studies had identified the isolates of *Trichoderma viride*, *Trichoderma koningii*, *Trichoderma* spp., *Trichoderma reeseii*, *Trichoderma harzianum* and *Trichoderma aureoviride* based on colony colour, conidia and colony texture. In the present study, production of cell wall degrading enzyme cellulase, in the range of 6.17±0.167 to 23.88±1.161 U/ml was observed by the isolates of *Trichoderma* species. Isolate Tri-2 exhibited maximum cellulase activity (23.88±1.161 U/ml). Kotasthane *et al.* (2014) evaluated *Trichoderma* spp. for cellulase production and found that *T. harzianum* developed maximum (56.50±0.71mm) colony diameter. Gajera and Vakharia (2010) reported that *T. harzianum* showed higher specific activity of enzyme cellulase (2.26 µM glucose $min^{-1}ml^{-1}$) during antagonism of *Trichoderma* isolate with the pathogen (*T. harzianum* × *Aspergillus niger*). All isolates of *Trichoderma* spp., showed production of chitinase enzyme in the range of 8.3±0.033 to 32.0±1.155U/ml. However, maximum chitinase production was observed in *Trichoderma* isolate Tri-2 (32.0±1.155 U/ml). Rao *et al*. (2015) reported that native *Trichoderma* isolate ThJt1, isolated from the rhizosphere of tobacco plant, showed maximum chitinase activity of 62.12±3.15 p kat ml^{-1}.Kumar *et al*. (2012b) recorded highest chitinase enzyme activity with *T. viride* (42.0U), followed by *T. harzianum* (40.3 U). Janifer *et al*. (2014) isolate *T.viride* (N9) from

the forest soil and chitinase was purified by ammonium sulphate precipitation which was effectively used against phytopathogenic fungi. Gajera and Vakharia (2010) also found higher concentration of chitinase production (1.07µM N acetylglucosamine $min^{-1}ml^{-1}$) in *Trichoderma* isolates. All the isolates of *Trichoderma* also showed the production of protease in the range of 7.2±0.058 to 20.0±1.155 U/ml and maximum protease activity was observed by Tri-2(20.0±1.155 U/ml). Radjacommare *et al*. (2010) reported that the secretion of lytic enzymes such as glucanase, protease and chitinase by *Trichoderma* played a very important role in the degradation of cell wall of the pathogenic fungi. They observed that the increased levels of these hydrolytic enzymes proved to inhibit the growth of the vanilla pathogens (*Fusarium oxysporum* f.sp. *vanillae*, *P. meadii* and *Colletotrichum vanilla*). The maximum protease production was observed in Tricho12 (4.70±0.37µmol $^{-1}min^{-1}$ mg^{1} protien). Gajera and Vakharia (2010) reported maximum protease production (5.63 µg free ammonia acids min^{-1} ml^{-1}) in the interaction (*T.harzianum* ×*Aspergillus niger*). *Trichoderma* isolates showed β-1, 3 glucanase productions in the range of 6.4±0.033 to 15.0±0.289 U/ml, the maximum units were shown by isolate Tri-2 (15.0 ± 0.289 U/ml), followed by isolate Tri-3 (12.9±0.745 U/ml). Rao *et al.* (2015) reported that isolate TvHt2 showed maximum β-1, 3 glucanase production (9.94±0.19 $nmolsec^{-}1$). They further found that *T.harzianum* showed maximum β-1, 3 glucanase production on Czapek Dox Broth (22.6 $nmolsec^{-1}$). Radjacommare *et al*. (2010) recorded that maximum glucanase activity was shown by isolate Tricho-10 (108.5.91nmol $glucosemin^{-1}mg^{-1}$protein) and also found that the isolate caused 49.65±0.40 per cent growth inhibition of the pathogen (*Fusarium oxysporum* f.sp. *vanilla*). Srinivas *et al*. (2017) reported that *T. viride* V-19 and *T.harzianum* H-10 showed maximum β-1, 3 glucanase productions of 18.19 $IUmg^{-1}$ and 13.16IU mg^{-1}, respectively.

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