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Research Article

Identification of native *Trichoderma* for augmentation of plant growth in geographical indication tagged fragrant Shankarpura jasmine [*Jasminum sambac* (L.) Aiton]

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ABSTRACT: Over the last few years, *Trichoderma* has attained immense practical value as an important input in crop production due to its multifunctional beneficial functions. Our objective was to identify the best-performing native isolate of *Trichoderma* which can adapt quickly to the introduced area and have a beneficial effect on the crop. This research was the first report on the use of biological input in improving the growth of the Geographical Indication (GI) tagged fragrant Shankarpura jasmine crop. From the representative rhizosphere soils of Shankarpura jasmine plants, six representative *Trichoderma* isolates were isolated using a *Trichoderma*-specific medium. Their ability to enhance the germination percentage of green gram seeds was evaluated under *in vitro* conditions. *Trichoderma* isolates WT 15 and WT 14 showed 99.55±0.41 and 96.57±0.91 percent germination, respectively 17 hours after treatment application. The isolate WT15 synthesized maximum IAA and isolate WT 14 produced maximum GA content compared to other isolates which were tested. These isolates also exhibited antagonism against *Fusarium solani*. These isolates also improved the growth of jasmine plants under pot culture condition. The isolate WT -14 was subjected to ITS 1 and 4 sequencing and based on those results it was identified as *Trichoderma lixii*.

KEYWORDS: Gibberellic acid, indole acetic acid, *Jasminum sambac,* rhizosphere, *Trichoderma*

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INTRODUCTION

Flowers play a vibrant role in the socio-cultural milieu of countries like India. They are used in a variety of events, such as religious ceremonies, formal events, family gatherings, and celebrations of all kinds. Widely acclaimed use of flowers has lent it a position as a market commodity leading to a phenomenal growth in commercial cultivation of flowers in the last few decades (Anumala & Kumar, 2021). Jasmine is one of the most important crops among the several flower crops. This evergreen ornamental shrub and climber belongs to the Oleaceae family (Li *et al*., 2020; Zhang *et al*., 2020). Due to the rich fragrance of its flowers, the plant has earned the epithet the 'Princes of aroma'. The genus *Jasminum* is the most prevalent member of the Oleaceae family containing more than 200 species (Zhang *et al*., 2020) and is cultivated commercially as its sweet fragrance flowers are highly sought after for making perfumes, additives, and tea products (Ahmed *et al*., 2016). The present investigation was conducted on Geographical Indication (GI) tagged "Shankarpura jasmine" which belongs to the species *Jasminum sambac* (L.) Aiton. This crop is cultivated by small farmers, especially women in marginal soils of Shankarpura and nearby villages of Udupi district (Barad *et al*.,2017; Handy *et al*.,2011). It is believed that the flower grown in this area has the characteristic property and that has been emphasized with this crop receiving the GI tag. In addition, the flowers have a long shelf life of up to 10-12 h in an unrefrigerated state enabling transport to distant markets (Chaitanya *et al*., 2018). The greater demand for the flowers grown in this region has resulted in a surge in price determined in a unique and daily. To meet this demand as well as to enhance productivity, farmers rely on chemical farming practices involving the use of heavy doses of inorganic inputs such as hormones, chemical fertilizers and pesticides. This practice has resulted in environmental pollution and crop failure due to the susceptibility of the plants to pests and diseases. An alternative to these chemical inputs could be the use of organic and biological sources for enhancing productivity and improving soil fertility. Though there are

many biological inputs available in the market, their survival in the soil seems to be a challenge. Amongst different agents of biological origin used, *Trichoderma* is a better alternative as it can withstand variations in environmental conditions. It also forms chlamydospores and conidia abundantly (Tyśkiewicz *et al*., 2022; Qiao *et al*., 2018) resulting in its easy dissemination. The objective of this study was to isolate and characterize native species of *Trichoderma* associated with Shankarpura jasmine rhizosphere as it can survive better than non-native strains. In addition, their use can decrease the dependence on chemical inputs, prevent the susceptibility of crops to pests and help in conserving beneficial soil flora and fauna which might disappear due to unwarranted application of chemical inputs.

MATERIALS AND METHODS

Collection of samples

Rhizosphere soil samples from the five best jasmine gardens of the Shankarpura region were collected aseptically in a sterilized plastic bag. Samples were brought to the lab and stored at 4°C until further use.

Isolation of *Trichoderma*

The *Trichoderma* from the representative jasmine rhizosphere soil samples were isolated by serial dilution and spread plating method. Trichoderma Specific (TS) Medium (Papavizas & Lumsden, 1982) was used for isolation purposes. After plating, the inoculated plates were maintained for 4 days in an incubator set at 28±2°C. The representative colonies appearing morphologically distinct on the Petri plates were subjected to purification using Potato Dextrose (PD) Agar. These isolates were preserved in a refrigerator until further use.

Green gram seed germination bioassay

The Plant Growth Prompting effect of isolated *Trichoderma* was evaluated by conducting a green gram seed germination bioassay using a modified method of Kumar *et al*. (2017). The representative isolates of *Trichoderma* were grown using PD Broth in culture tubes for one week. The broth cultures were then centrifuged at 10,000 rpm for 20 min. The supernatants of each culture were transferred to separate Petri plates. To these, 30 water-soaked green grams seeds were transferred and kept for 1 h. After seed treatment, treated seeds were aseptically transferred into a sterile moist filter paper-lined Petri plate. The germination of green gram seeds after 17 h of incubation was determined and the germination percentage was calculated by the following formula:

$$\text{Germination Percentage} = \frac{Number\ of\ Seeds\ Germinated}{Total\ Number\ of\ Seeds\ Incubated} x100$$

Indole Acetic Acid (IAA) producing ability

IAA producing ability of the selected isolates was determined by following the Salkowski method as described by Gang *et al*. (2019). The selected isolates were grown using TS broth medium added with 0.5 per cent (w/v) of L -tryptophan. These broth cultures were incubated at 28±2°C with continuous agitation of 120 rpm for 4 days (Kumar *et al*., 2017). These liquid cultures were centrifuged and each culture supernatant was mixed in a ratio of 1:2 (v/v) with Salkowski's reagent. This mixture was maintained at room temperature for 20 min which later gave rise to pink colour. The IAA produced by the culture was quantified by measuring the intensity of the pink colour developed at 530 nm with a double-beam Systronics UV-visible spectroscope. The experiment was conducted in triplicate.

Evaluation of Gibberellic Acid (GA) production

GA producing capacity of *Trichoderma* was evaluated as per the procedure outlined by Berryos *et al.* (2014). The selected isolates of *Trichoderma* were inoculated into PD broth and incubated at 30±2°C for seven days. The gibberellic acid production was determined by the spectrophotometric method. The liquid cultures were subjected to centrifugation at 10,000 rpm for 20 min. The pH of the resulting supernatant was adjusted to 2.5 using the liquid-liquid extraction method and the ethyl acetate $NaHCO_3$ extraction method. The gibberellic acid was measured using a UV-visible spectrophotometer at 254 nm.

Antagonistic property of isolates

The dual culture technique was used to evaluate the antagonistic ability of representative *Trichoderma* isolates (Morton & Stroube, 1955). The target organism used was *Fusarium solani* obtained from the Culture collection center of the Microbiology Department of Alva's College, Moodubidire. Pathogenic *F. solani* and selected isolates of *Trichoderma* were grown separately using PDA medium for seven days at 30±2°C. From the periphery of the culture, round discs (measuring 5 mm) of the *F. solani* were cut and aseptically placed on a PDA plate. *Trichoderma* isolate's agar disc (measuring the same size) was placed aseptically on the same plate just opposite *F. solani*. These plates were maintained at room temperature for 7 days. Three replications were maintained. Growth inhibition of *F. solani* in terms of percentage was calculated by using the following formula.

$$I = \frac{C - T}{C} \times 100$$

where, C is the mean diameter of *F. solani* in the control plate (*i.e.*, plate without *Trichoderma* isolate under study),

and T is the mean diameter of *F. solani* in Petri plates inoculated with *Trichoderma* isolate under study.

Pot culture experiment

This experiment evaluated plant growth promotional efficiency of representative strains of *Trichoderma* isolated from the jasmine rhizosphere. This experiment was conducted using Completely Randomized Design (CRD). The treatments followed in this experiment were as follows:

T1, Control (uninoculated and treated with sterilized PD Broth); T2, *Trichoderma* isolate NT9, T3, *Trichoderma* isolate NT13; T4, *Trichoderma* isolate RT41; T5, *Trichoderma* isolate RT42; T6, *Trichoderma* isolate WT14 and T7, *Trichoderma* isolate WT15. Three replications were maintained for each treatment.

Trichoderma isolates were inoculated to PD broth and then incubated for ten days at 35±2^{0}C. The cultures thus developed were used for inoculating jasmine plants. The jasmine plants used in the present study were developed as follows.

Stem cuttings of Shankarpura jasmine plant of equal length were rooted on sand and soil (@1:2 proportion) medium in plastic pots of 14.2 cm diameter and 15 cm height. The cuttings were maintained in the greenhouse for 2 months. The sterile sand-soil mixture of a 2:1 ratio was used as a growing medium. Each sterile pots were filled with 200 g sterile sand soil mixture. From the rooted cuttings, 21 (7 treatments x 3 replications = 21 plants) cuttings each (having 5 leaves and almost similar growth) were selected and transplanted separately in 21 sterile plastic pots of 14.2 cm diameter and 15 cm height containing sterile growth medium. The liquid cultures of different isolates of *Trichoderma* were aseptically added using a sterile measuring cylinder at 20ml/200 g potting medium as per the treatments described earlier. Plants were watered with sterile water regularly to avoid moisture stress. The number of leaves, number of internodes and plant height were recorded after 180 days of transplating.

Molecular identification

An isolate of *Trichoderma*, exhibiting the best performance with respect to the promotion of plant growth and inhibition of *F. solani* was selected and subjected to identification based on sequencing of the housekeeping Internal Transcribed Spacer (ITS) region.

Using the Blast program (https://blast.ncbi.nlm.nih.gov/Blast.cgi) the sequence obtained was analyzed. The top six sequences of taxa belonging to the *Trichoderma* species, compatible with the isolate of this study, were obtained to construct the phylogenetic tree. Multiple sequence alignment of six sequences was established using Clustal W. Mega software was also used to construct a phylogenetic tree through the Neighbor-Joining method and statistical significance was evaluated with a bootstrapping of 500 repetitions.

Statistical analysis

Statistical analysis was performed using GRAPES 1.0.0, an online agricultural statistics tool (Gopinath *et al*., 2021). Data were subjected to one-way ANOVA with a significance threshold set at $p \leq 0.05$ followed by Tukey's test.

RESULTS

Isolation of *Trichoderma*

In the present study, six *Trichoderma* isolates were obtained from the shankarpura jasmine rhizosphere using TS medium. The colony morphology of the isolates is presented in Table 1.

All six isolates produced conidia which were subglobose to obovoid and their production begins at the centre of the colony and progresses towards the periphery. Conidiophores exhibited high regular branching and were verticillate while phialides were ampuliform.

Effect of *Trichoderma* inoculation on green gram seed germination

The culture supernatants of all the isolates were characterised for the promotion of green gram seed germination. The results of this experiment are presented in Figure 1.

The treatments involving the inoculation of different Trichoderma isolates to green gram seeds namely WT 15, NT13, WT 14 and RT 42 exhibited germination percentages of 99.55±0.41, 96.57±0.91,93.24±1.80 and 93.24± 2.96, respectively. The germination percentage values recorded by these treatments were significantly higher than control (89.91±0.65%). Isolates RT41 and NT9 recorded germination percentages of 86.58±1.01% and 83.25±2.33% which was significantly lower than all the other treatments including control.

IAA and GA production by different *Trichoderma* isolates

The amount of IAA and GA produced by six isolates of *Trichoderma* isolated from rhizosphere soils of Shankarpura jasmine are presented in Table 2. Among the *Trichoderma* isolates tested, WT15 and WT14 recorded significantly higher IAA (49.24±0.58 µg/ml and 39.76±0.31 µg/ml, respectively). In gibberellic acid production too WT14 and WT15 showed higher efficiency compared to other isolates with GA productions of 239.40±0.53 µg/ml and 194.50±0.54

Table 1. Colony morphology of *Trichoderma* isolates isolated from jasmine rhizosphere

***Trichoderma* Isolate no.**	**Location of sampling**	**Colony morphology**
NT9	13°15'32.9"N 74°46'18.0"E	Initially white to green-coloured mycelia appeared. Later at the centre, a dark green colour was gradually formed.
NT 13	13°15'32.7"N 74°46'18.2"E	White to pale green mycelial growth was formed and later turned to dark green.
RT41	13°15'33.1"N 74°46'18.9"E	Initially, the colony was whitish to light green with the formation of water droplets in the centre. Subsequently, the colony became deep grass green in colour.
RT42	13°15'32.7"N 74°46'18.2"E	Initially colony showed a light green colour in the centre. Gradually it became dark green.
WT14	13°15'32.9"N 74°46'18.0"E	Fast growing compared to all the isolates. The colony was white to light green initially and the colour became very dark after 13 days.
WT15	13°15'42.4"N 74°46'02.9"E	The next best isolate with respect to growth after WT14. White in growth initially and becomes light green in 4 days and dark green after 5th Day.

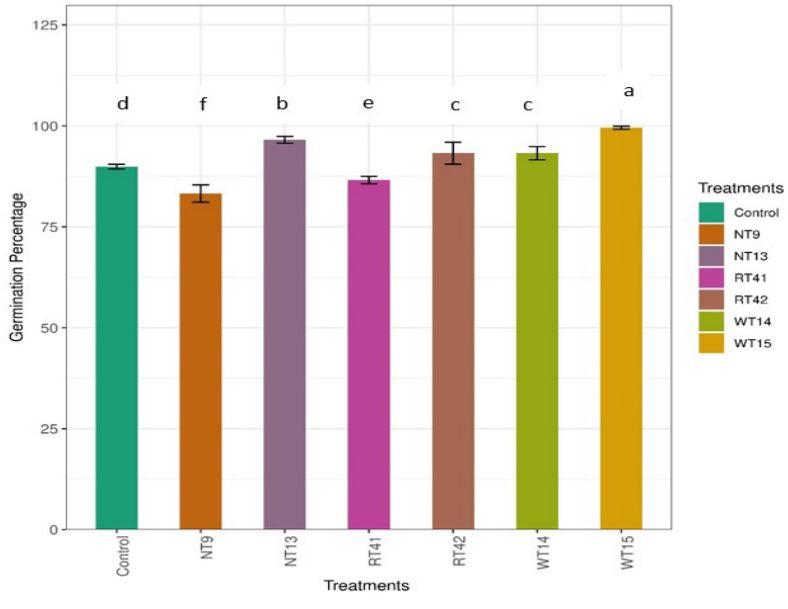


Figure 1. Germination of green gram seeds in the presence of *Trichoderma* isolates. Alphabets indicate significant differences among the mean values ($p \leq 0.05$) analyzed by Tukey's test.

µg/ml, respectively. Among all isolates, NT 13 recorded the lowest concentration of IAA (24.62±0.65 µg/ml) while the lowest concentration of GA was recorded by isolate NT9 (82.30±0.48 µg/ml) (Table 2).

Inhibition of *Fusarium solani* by different *Trichoderma* isolates

The ability of *Trichoderma* isolates to inhibit the growth of *F. solani* was tested by dual culture technique (Fig. 2). The isolates WT14 and WT15 were on par with each other concerning per cent inhibition of *F. solani* recording inhibition percentages values of 48.84 ±1.15% and 44.02±2.98%, respectively (Fig. 3). The inhibition percentage values recorded by these two *Trichoderma* isolates were significantly higher than the remaining four *Trichoderma* isolates.

Pot culture experiment

Data about the effect of different *Trichoderma* isolates on plant growth parameters obtained during a pot culture experiment conducted on Shankarpura jasmine is presented in Table 3. Among isolates, WT14 performed the best followed by WT15 concerning all the growth parameters tested in the pot culture experiment. Concerning the number of internodes, isolate WT14 was statistically superior (27.33±3.42) compared to other treatments.

Molecular identification of the best-performing isolate

As *Trichoderma* isolate WT -14 emerged as the best-performing isolate compared to others, it was subjected to ITS1 and ITS4 sequence analysis. A phylogenetic tree was

Table 2. IAA and GA production by different isolates of *Trichoderma* isolated from Shankarpura jasmine rhizosphere

Treatments	IAA (µg/ml)	GA (µg/ml)
NT9	24.62±0.65^{c}	82.30±0.48^{e}
NT 13	13.25±1.15^{d}	133.40±1.29^{d}
RT41	1.89±0.29^{e}	185.60±2.49^{c}
RT42	17.23±1.26^{d}	195.08±1.20^{b}
WT14	39.76±0.31^{b}	239.40±0.53^{a}
WT15	49.24±0.58^{a}	194.50±0.54^{b}

*Different letters indicate significant differences among values ($p \leq 0.05$) by Tukey's test. Treatments with the same letters are not significantly different. Values are means±SD (n=3).

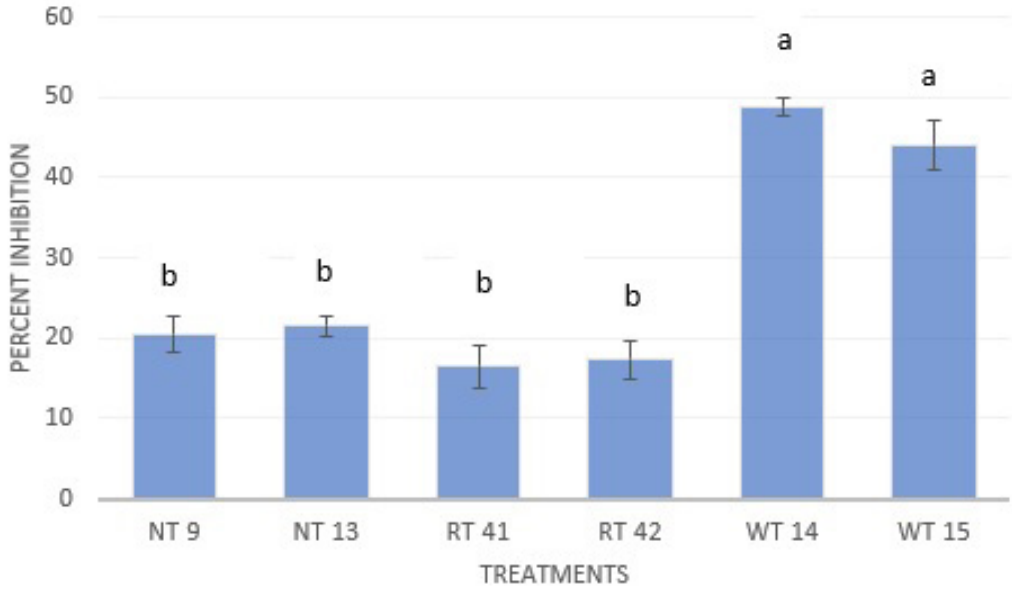


Figure 2. Percent inhibition of *F. solani* by different isolates of *Trichoderma* isolated from jasmine rhizosphere. Different letters represent significant differences among the mean value ($p \leq 0.05$) by Tukey's test.

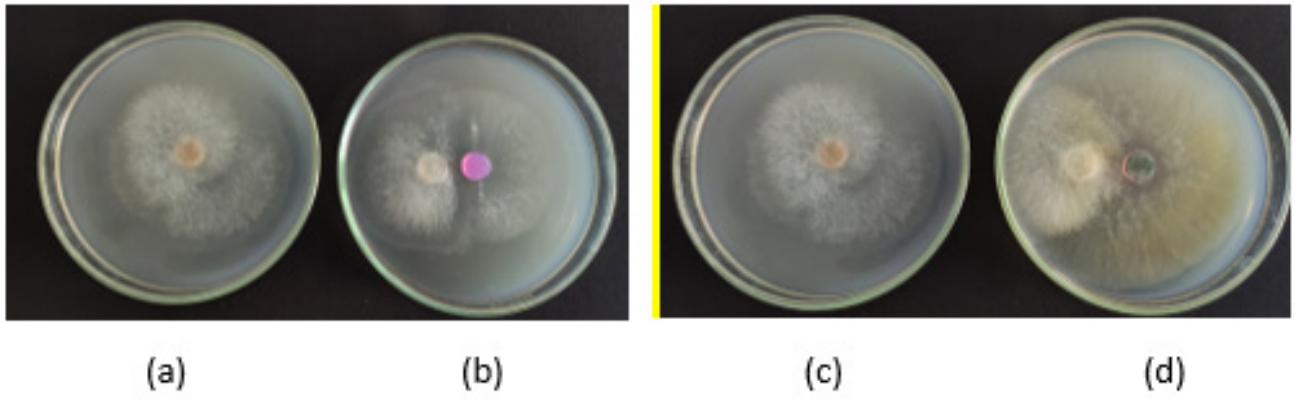


Figure 3. Interaction between *Trichoderma* isolates and *Fusarium;* **(a).** Fusarium alone; **(b).** *Fusarium* and *Trichoderma* WT 15 dual culture; **(c).** *Fusarium* alone; **(d).** *Fusarium* and *Trichoderma* WT 14 dual culture.

Table 3. Effect of *Trichoderma* isolates on the growth of Shankarpura jasmine

Treatments	Plant height	Number of internodes	Number of leaves
Uninoculated control	8.33±2.03^{d}	7.00±00^{b}	6.66±3.78^{c}
NT9	20.00±2.28^{bcd}	14.00±3.60^{b}	13.33±3.69^{abc}
NT 13	24.33±1.15^{bc}	11.66±3.12^{b}	11.33±4.72^{bc}
RT41	13.00±1.00^{cd}	13.00±4.36^{b}	6.33±±0.50^{c}
RT42	14.33±3.21^{cd}	8.00±4.35^{b}	7.66±3.2^{c}
WT14	138.00±3.60^{a}	27.33±3.42^{a}	25.66±2.65^{ab}
WT15	28.00±3.00^{b}	15.333.65^{ab}	29±2.01^{a}

* Different letters indicate significant differences among values ($p \leq 0.05$) by Tukey's test. Treatment with the same letters is not significantly different. Values are means±SD (n=3).



Figure 4. Effect of *Trichoderma* isolates on growth parameters of Shankarpura jasmine.

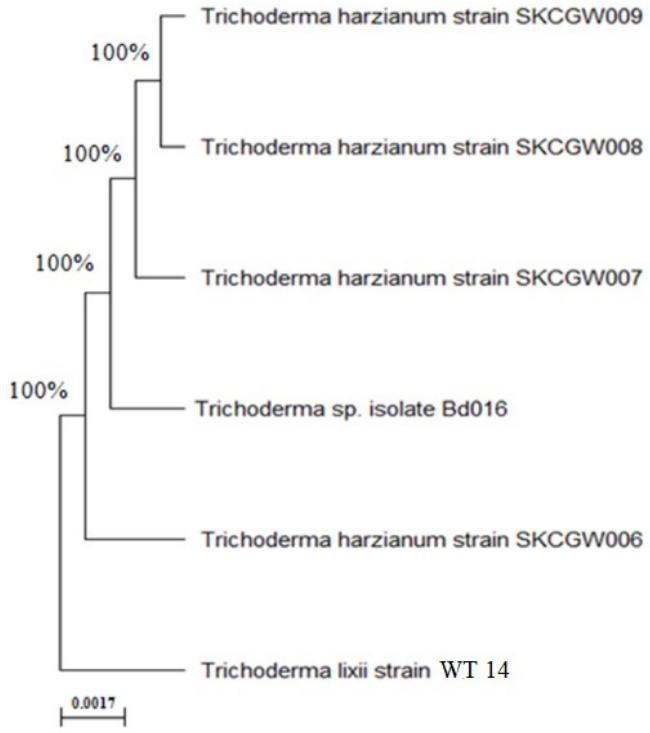


Figure 5. *T. lixii* isolate against reference *Trichoderma* strains. The phylogenetic tree was constructed using neighbour-joining parsimony analysis. Next to the branches is the percentage of replicate trees in which the relevant taxa clustered together. The Sum of Branch Length (SBL) was 0.0017.

constructed for isolate WT 14 based on comparing its ITS sequence with the ITS regions of six *Trichoderma* isolates obtained from GenBank (http:/ncbi.nih.gov) as a reference. The result of the ITS sequence-dependent phylogenetic tree is shown in Figure 5. Based on BLASTN search and phylogeny analysis isolate WT14 was identified as *T. lixii*.

DISCUSSION

In agriculture, to boost crop yield, there is a practice of chemical farming involving the use of chemical inputs like pesticides and fertilizers. Indiscriminate use of chemical inputs like pesticides and fertilizers has a destructive effect on the environment and human health (Zin & Badaluddin, 2020; Pathak *et al*., 2022). One of the possible alternatives to chemical inputs is the agricultural bio-inputs. They are products of biological origin and are envisioned for the biological control of plant pests, meeting plant nutrition or ameliorating biotic or abiotic stresses (Medina *et al.*, 2024). The most significant benefits of these inputs are their cost-effectiveness and eco-friendly nature. Despite these benefits,

some bio inputs especially bio-inoculants and bio-control agents suffer from a few challenges like lower efficacy compared to chemical inputs, slower adaptability to the environment, greater susceptibility to changing environments, *etc*. These conditions necessitate the development of efficient bio-inoculants or bio-control agents with better adaptability and beneficial effects to be more attractive than chemical inputs.

Amongst, several bio-based inputs, the use of *Trichoderma* in crop production is most promising as it has biological control as well as a plant growth-promoting potential (Joo & Hussein, 2022). In addition, they are saprophytic and fast-growing fungi with strong adaptability to the environment (Yao *et al*., 2023). In the case of *Trichoderma,* crop specificity with respect to exerting plant growth promotional effect was also reported by Dwivedi and Tewari (2017). Taking these things into consideration the present study involving isolation and characterization of *Trichoderma* from rhizosphere soils of Shankarpura jasmine was undertaken. From the representative soils, a total of six *Trichoderma* isolates were isolated by using a Trichoderma Trichoderma-specific medium. These representative isolates were more prominently found and produced conidia which were subglobose to obovoid. Conidiophores exhibited high regular branching and were verticillate while phialides were ampuliform. The morphology of the isolates obtained in the present study resembled the morphology of *Trichoderma* spp. which was earlier reported by Abdenaceur *et al*. (2022). Based on these observations the representative isolates obtained in the present study were identified as *Trichoderma*.

The ability of all the *Trichoderma* culture supernatants was evaluated to improve the germination percentage of green gram seeds. Amongst all the tested isolates, WT -15 and WT-14 recorded the highest germination percentages of 99.55±0.41 and 96.57±0.91 respectively. All the isolates were also evaluated for production of phytohormones *viz*., IAA and GA. In this evaluation, WT15 (49.24±0.48 µg/ml) and WT14 (39.76±0.31µg/ml) produced maximum IAA compared to other isolates while the WT-14 and WT15 isolates recorded maximum concentration of GA (239.4 and 239 µg/ml, respectively). Saleemi *et al.* (2017) reported that treatment of wheat seeds with IAA and Gibberellic acid-producing plant growth-promoting microbes increased seed germination and root /shoot growth similar to exogenous treatment of wheat seeds with IAA and GA. In another study, it has also been reported that some selected strains of *Trichoderma* were capable of producing plant hormones like auxin and auxin-like compounds (Contreras-Cornejo *et al.,* 2009; Chang *et al*., 1986). According to Stewart and Hill (2014), a few species of *Trichoderma* also produce gibberellic acid. Carrillo *et al*. (2002) and Windham (1986) reported that IAA promotes root development and nutrient uptake. Duarah *et al*. (2011) reported that inoculation of plant growth-promoting microbes increases the amylase activity during germination in legumes. This leads to the hydrolysis of starch into easily metabolizable sugars which are required as a source of energy for the formation of radicles and plumules during germination (Akazawa & Nishimura, 1985).

The results from the dual culture technique revealed that isolates 14 and 15 exhibited significantly high per cent inhibition of 48.84±1.15% and 44.02±2.98% respectively against *F. solani* compared to other isolates. There was no significant difference between these two isolates with respect to the per cent inhibition of *F. solani*. The isolates RT-42 and 41 showed minimum per cent inhibition of *Fusarium*. *Trichoderma* is mycoparasitic and it can kill pathogenic fungi by parasitism (Kubicek *et al*., 2011; Druzhinina *et al*., 2011). It is also known to secrete hydrolytic enzymes like chitinases and glucanases which are cell wall degrading enzymes. In addition, *Trichoderma* is also known to secrete some antifungal antibiotics (Katoch *et al.*, 2019). Overall, isolate WT-14 showed better results in this experiment, it was subjected to molecular identification and based on this study the isolate was identified as *T. lixii.* Katoch *et al*. (2019) studied the antifungal activity of *T. lixii* and attributed this property is due to its ability to produce an antifungal peptide antibiotic called “peptaibols”. Even in pot culture studies, WT14 and WT15 recorded maximum plant height and number of leaves of Shankarpura jasmine justifying their ability to promote plant growth.

CONCLUSION

This work is a new report on the isolation and characterization of *Trichoderma* strains isolated from the popular GI tagged Shankarpura jasmine. We attempted to evaluate the plant growth promotional properties as well as antagonistic potential against *F. solani,* a prominent wilt-causing pathogen of jasmine. The result of the pot culture experiment indicated that an isolate WT 14 identified as *T. lixii* enhanced the jasmine plant growth and inhibited the growth of *F. solani*. There is a scope for evaluation of this isolate for the promotion of plant growth and control of jasmine wilt under field conditions.

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