



# Bioefficacy of *Trichoderma* Species Against Javanese Root-Knot Nematode, *Meloidogyne javanica*, in Green Gram

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## Abstract

Root-knot nematodes are mainly controlled by using synthetic nematicides, but their excessive use is prohibited due to associated health hazards which demand for suitable alternatives. The overreliance on nematicides can be curtailed by using biological control agents possessing nematicidal or nematostatic properties. Therefore, in the present study, effectiveness of seven indigenous species of *Trichoderma* were tested for their ability to suppress the population of Javanese root-knot nematode, *Meloidogyne javanica*, and improve growth variables of green gram. All the *Trichoderma* species resulted in an increase in shoot and root lengths and shoot weight while a decrease was observed in root weight. Maximum increase in shoot length (45.5%) was found in case of *T. harzianum* followed by *T. hamatum* and *T. viride* whereas the increase was the minimum where *T. pseudokoningii* and *T. koningii* were applied. Similarly, maximum increase in shoot weight was recorded with *T. viride* (56.1%) followed by *T. harzianum* (55%) and the minimum with *T. pseudokoningii*. As regards root length, it was the maximum in treatments with *T. hamatum* (46.2%) and *T. harzianum* (45.1%) and minimum with those where *T. koningii* and *T. pseudokoningii* were applied. Contrarily, maximum reduction in root weight was observed in treatments where *T. harzianum* (37.8%) and *T. viride* (35.8%) were applied while *T. koningii* and *T. pseudokoningii* resulted in minimum decrease. All the *Trichoderma* species significantly caused reductions in the number of galls and eggs and reproductive factor of the nematode over control. Maximum reduction in numbers of galls and eggs were observed with *T. viride* (49 and 53%) followed by *T. harzianum* (46 and 53%) while the minimum reduction was recorded with *T. pseudokoningii* followed by *T. atroviride*. Likewise, *T. viride* caused the maximum reduction in reproductive factor of *M. javanica* (81%) followed by *T. harzianum* (78%) and *T. asperellum* (75%). On the other hand, the minimum reductions in reproductive factor were observed with *T. pseudokoningii* and *T. koningii*.

**Keywords** Biocontrol · Root-knot nematode · *Trichoderma* species · *Vigna radiata* L · Growth variables · Nematode infestations

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## Bioeffektivität von *Trichoderma*-Arten gegen den Wurzelgallenematoden *Meloidogyne javanica* bei Mungbohnen

### Zusammenfassung

Wurzelgallenematoden werden hauptsächlich mit synthetischen Nematiziden bekämpft, deren übermäßiger Einsatz jedoch aufgrund der damit verbundenen Gesundheitsgefahren verboten ist und nach geeigneten Alternativen verlangt. Der übermäßige Einsatz von Nematiziden kann durch die Verwendung von biologischen Bekämpfungsmitteln, die nematizide oder nematostatische Eigenschaften besitzen, eingedämmt werden. Daher wurde in der vorliegenden Studie die Wirksamkeit von sieben einheimischen *Trichoderma*-Arten auf ihre Fähigkeit getestet, die Population des Wurzelgallenematoden *Meloidogyne javanica* zu unterdrücken und die Wachstumsvariablen von Mungbohnen zu verbessern. Alle *Trichoderma*-Arten führten zu einer Zunahme der Spross- und Wurzellänge sowie des Sprossgewichts, während beim Wurzelgewicht eine Abnahme beobachtet wurde. Die maximale Zunahme der Sprosslänge (45,5 %) wurde bei *T. harzianum* festgestellt, gefolgt von *T. hamatum* und *T. viride*, während die Zunahme bei der Anwendung von *T. pseudokoningii* und *T. koningii* am geringsten war. In ähnlicher Weise wurde die maximale Zunahme des Sprossgewichts mit *T. viride* (56,1 %) verzeichnet, gefolgt von *T. harzianum* (55 %) und dem Minimum mit *T. pseudokoningii*. Die Wurzellänge war bei den Behandlungen mit *T. hamatum* (46,2 %) und *T. harzianum* (45,1 %) am höchsten und bei *T. koningii* und *T. pseudokoningii* am geringsten. Im Gegensatz dazu wurde die maximale Reduktion des Wurzelgewichts bei Behandlungen mit *T. harzianum* (37,8 %) und *T. viride* (35,8 %) beobachtet, während *T. koningii* und *T. pseudokoningii* zu einer minimalen Abnahme führten. Alle *Trichoderma*-Arten bewirkten eine signifikante Verringerung der Anzahl an Gallen und Eiern sowie des Reproduktionsfaktors des Nematoden gegenüber der Kontrolle. Die maximale Reduktion der Anzahl der Gallen und Eier wurde mit *T. viride* (49 und 53 %) beobachtet, gefolgt von *T. harzianum* (46 und 53 %), während die minimale Reduktion mit *T. pseudokoningii*, gefolgt von *T. atroviride*, festgestellt wurde. Ebenso verursachte *T. viride* die maximale Reduzierung des Reproduktionsfaktors von *M. javanica* (81 %), gefolgt von *T. harzianum* (78 %) und *T. asperellum* (75 %). Auf der anderen Seite wurden die geringsten Reduzierungen des Reproduktionsfaktors bei *T. pseudokoningii* und *T. koningii* beobachtet.

**Schlüsselwörter** Biokontrolle · Wurzelgallenematode · *Trichoderma*-Arten · *Vigna radiata* L. · Wachstumsvariablen · Nematodenbefall

### Introduction

The utilization of green gram (*Vigna radiata* L.) Wilczek in Pakistan as diet has been expanding for the last few years as a substitute of protein and hence, has been distinguished as high need crop by the farmers. Along these lines, expanding the productivity of green gram has been recognized as a basic need to meet the nation's requirements and guarantee self-sufficiency. Increasing the production of green gram would result in uplifting the income level of farmers and meeting dietary needs of the people of the nation. Green gram is cultivated on an area of 246 thousand hectares with a total production of 178 thousand tons respectively (Anonymous 2018) under a wide range of agro-ecological zones. The normal yield of green gram in Pakistan is far below than its potential yield. Several biotic and abiotic factors affect the yield of green gram (Aslam et al. 2019a; Mukhtar and Hussain 2019). Among biotic factors, diseases are the most destructive. Green gram is attacked by more than 26 diseases throughout the world reducing yield up to 44% (Iqbal and Mukhtar 2020a). Among diseases, root-knot nematodes (*Meloidogyne* spp.) are by far the most destructive and badly impact crop production. Javanese root-knot nematode, *Meloidogyne javanica*, has been found widely

distributed infecting a wide range of crops in the country (Trudgill and Blok 2001; Castagnone-Sereno et al. 2013; Jones et al. 2013; Mukhtar and Kayani 2019, 2020; Tariq-Khan et al. 2020). Root-knot nematodes have been reported to inflict yield losses in pulses to the extent of 26%. Root-knot nematodes are ubiquitously prevalent in tropical and subtropical environments of the country as prevailing climatic conditions are conducive for their pathogenesis and reproduction throughout the year (Hussain and Mukhtar 2019). Furthermore, these nematodes develop disease complexes with soil borne fungal and bacterial pathogens causing vascular wilts and damping off in many crops and result in huge yield losses. Similarly, root-knot nematodes break resistance in cultivars resistant to wilts (Aslam et al. 2019b; Asghar et al. 2020).

For the management of nematodes, various control methods are used which include resistance, chemical, biological, physical, regulatory and cultural etc. (Bhuiyan et al. 2018; d'Errico et al. 2019; Javed et al. 2019a, b; Nazir et al. 2019; Azeem et al. 2021; Gulzar et al. 2020) and each strategy has one or the other limitations. Root-knot nematodes are mainly controlled by using synthetic nematicides, but their excessive use is prohibited due to non-availability of conventional nematicides, growing awareness of global

community against pollution, possible health hazards associated with the use of nematicides, unfavorable cost-benefit ratio, and development of resistance in nematodes etc. which demands for suitable alternatives. The overreliance on nematicides can be curtailed by using biological control agents possessing nematocidal or nematostatic properties.

Several bacterial and fungal biological control agents have been evaluated during recent years for their effectiveness against root-knot nematodes (Huang et al. 2016; Rao et al. 2017; Ghahremani et al. 2019). The main criteria for successful deployment of biocontrol agents in fields are their ability to suppress nematode populations and enhance yields profitably in the presence of nematodes. For their suitability as nematode suppressive agents, the reductions in reproductive and developmental potentials of nematodes by these biocontrol agents must be assessed. Among different groups of fungi, the genus *Trichoderma*, as biocontrol agent of different plant pathogens, has been widely studied. Its species are often very fast growing, colonizing the substrates quickly. The fungus is ubiquitous and has been reported to effectively control different diseases by using a variety of mechanisms (Vinale et al. 2008; Ghazanfar et al. 2018). Aggressiveness among various species and isolates of *Trichoderma* from different regions of the world has been known (Mukhtar 2018; Iqbal and Mukhtar 2020b). This necessitates that indigenous isolates of the fungus should be used for the management of root-knot nematodes. There is meagre cognizance on the effects of indigenous isolates of *Trichoderma* species on the reproductive potential of nematodes and growth variables of green gram. In the present study, effectiveness of seven indigenous species of *Trichoderma* were tested for their ability to suppress population of Javanese root-knot nematode, *M. javanica*, and improve growth variables of green gram.

## Materials and Methods

### Nematode Inoculum

A population of Javanese root-knot nematode (*Meloidogyne javanica*) originally isolated from cucumber roots, identified on the basis of perineal pattern and maintained on highly susceptible cultivar of tomato (Money Maker) was used in the assessment. The nematode was mass produced on tomato cv. Money Maker and second stage juveniles were extracted from the infected roots for inoculation of plants (Khan et al. 2019).

### Mass Production of *Trichoderma* Species

The seven species of *Trichoderma* evaluated in the present study were obtained from the Institute of Agricultural

Sciences, University of Punjab, Lahore. These species, originally isolated from the country, identified and confirmed were maintained in the Fungal Culture Bank of the university. The species included *Trichoderma harzianum*, *T. hamatum*, *T. koningii*, *T. pseudokoningii*, *T. viride*, *T. atroviride* and *T. asperellum*. These biocontrol fungi were first grown on potato dextrose agar and then mass produced individually on wheat grains using the methodology described by Mukhtar (2018). The concentration of spores per gram of the grains of each *Trichoderma* spp. was counted using haemocytometer after making spore suspensions in distilled water.

### Greenhouse Bioassay for the Effectiveness of *Trichoderma* Species Against *M. javanica*

The relative effectiveness of seven *Trichoderma* species against *M. javanica* was assessed in pots under greenhouse conditions. Each *Trichoderma* spp. colonized on wheat grains was mixed with formalin sterilized soil (60% sand; 19% silt; 20% clay; 1% organic matter and pH 7.6) at the rate of  $10^4$  spores/ml and filled in pots separately. The pots treated with wheat grains only served as controls. Three seeds of green gram (cv. AZRI-06) were sown in pots treated with *Trichoderma* species. Each treatment was replicated five times. The pots were placed in a greenhouse at  $25 \pm 2^\circ\text{C}$  in completely randomized design. After emergence, single healthy seedling was maintained in each pot. Each plant was inoculated ten days after emergence with approximately 2000 freshly hatched second stage juveniles of *M. javanica* contained in 2 ml of water by making three holes around the stem. The pots were kept for seven weeks in the greenhouse at  $25 \pm 2^\circ\text{C}$  and watered as per requirement.

### Data Recording

After seven weeks, the plants were carefully removed from the pots. The shoots were severed from the roots. Shoot and root lengths were measured, and fresh root and shoot weights were determined. The galls and egg masses on each plant root system were counted under a stereomicroscope (NIKON SMZ745T) at 40 $\times$ . For estimation of total nematode populations, eggs were extracted from the roots of individual plants and juveniles were extracted from the soil from each pot (Mukhtar 2018). The total number of eggs and nematodes in soil formed the total population. The reproductive factor was calculated by dividing the final population by the initial one. Percent reductions or increases in plant growth parameters and nematode infestations were calculated over controls as described by Mukhtar (2018).

### Statistical Analysis

Completely randomized design was used in the experiment. All the data were subjected to analysis of variance using GenStat Package 2009 (12th edition) version 12.1.0.3278 ([www.vsni.co.uk](http://www.vsni.co.uk)). Means were compared by Fisher's Protected Least Significant Difference Test at 5%.

### Results

The analysis of variance showed significant results regarding effects of *Trichoderma* species on growth variables and nematode infestations. All the *Trichoderma* species resulted in an increase in shoot and root lengths and shoot weight while a decrease was observed in root weight. Maximum increase in shoot length was found to be maximum in case of *T. harzianum* (45.5%) followed by *T. hamatum* (44.4%) and *T. viride* (42.3%) whereas the increase was the minimum where *T. pseudokoningii* and *T. koningii* were applied (Fig. 1). Similarly, maximum increase in shoot weight was recorded with *T. viride* (56.1%) followed by *T. harzianum* (55%) and the minimum with *T. pseudokoningii* (Fig. 2).

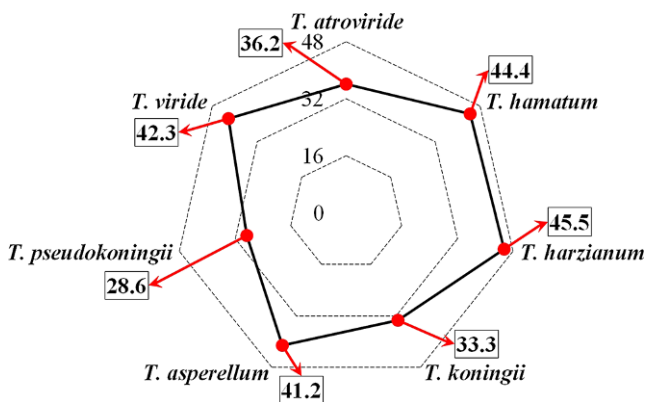


Fig. 1 Effect of *Trichoderma* species on percent increase in shoot length of green gram

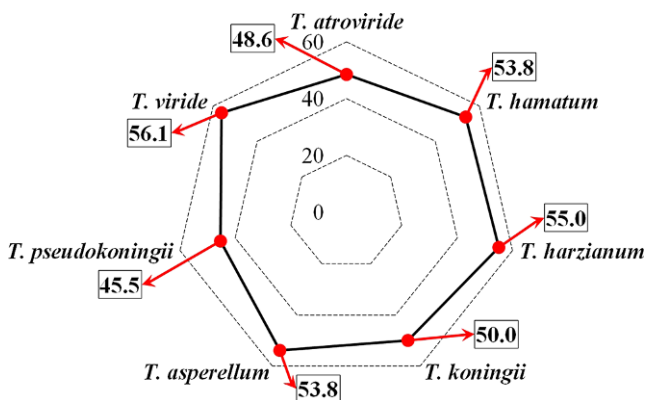


Fig. 2 Effect of *Trichoderma* species on percent increase in shoot weight of green gram

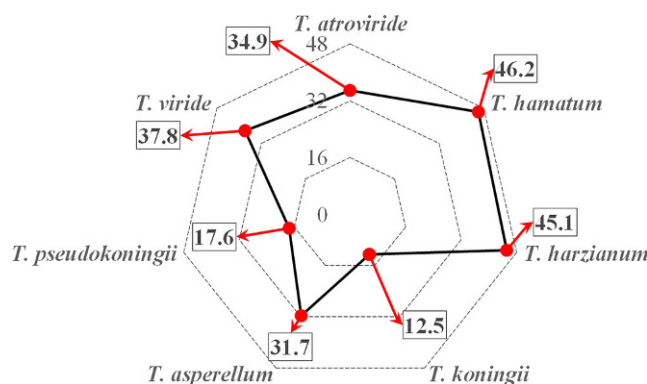


Fig. 3 Effect of *Trichoderma* species on percent increase in root length of green gram

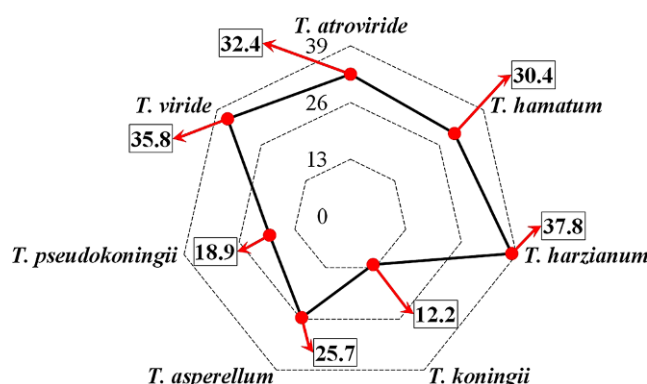
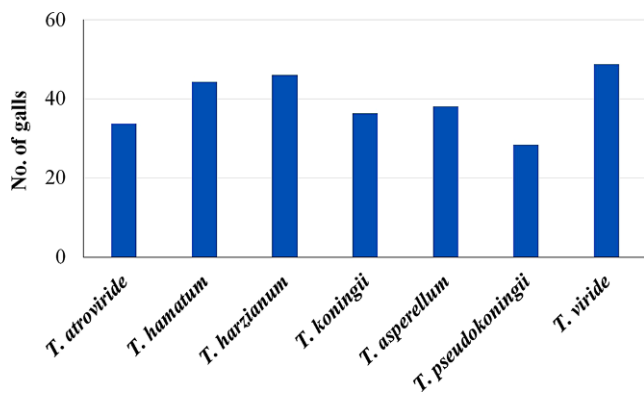
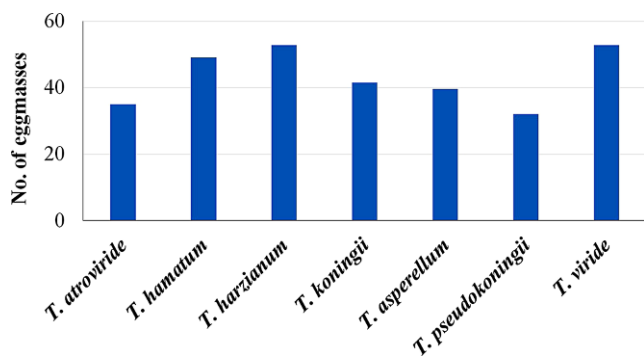


Fig. 4 Effect of *Trichoderma* species on percent decrease in root weight of green gram

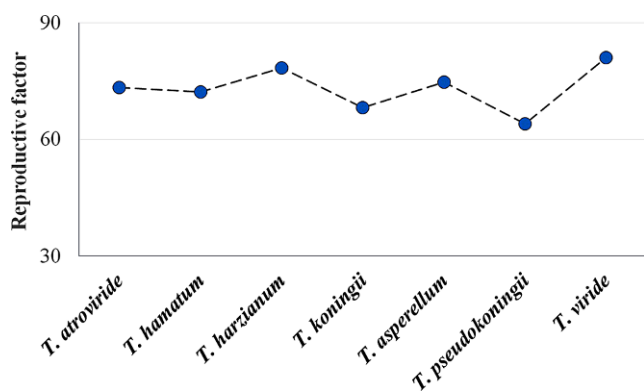
As regards root length, it was the maximum in treatments with *T. hamatum* (46.2%) and *T. harzianum* (45.1%) and minimum with those where *T. koningii* and *T. pseudokoningii* were applied (Fig. 3). Contrarily, maximum reduction in root weight was observed in treatments where *T. harzianum* (37.8%) and *T. viride* (35.8%) were applied while *T. koningii* and *T. pseudokoningii* resulted in the minimum decrease (Fig. 4). All the *Trichoderma* species significantly caused reductions in number of galls and eggmasses and reproductive factor of the nematode over control. Maximum reduction in number of galls and eggmasses was observed with *T. viride* (49 and 53%) followed by *T. harzianum* (46 and 53%) and *T. hamatum* (49%) while the minimum reduction was recorded with *T. pseudokoningii* followed by *T. atroviride* (Figs. 5 and 6). Likewise, *T. viride* caused the maximum reduction in reproductive factor of *M. javanica* (81%) followed by *T. harzianum* (78%) and *T. asperellum* (75%). On the other hand, the minimum reduction in reproductive factor was observed with *T. pseudokoningii* and *T. koningii* (Fig. 7).



**Fig. 5** Effect of *Trichoderma* species on percent decrease in number of galls on roots of green gram



**Fig. 6** Effect of *Trichoderma* species on percent decrease in number of eggmasses on roots of green gram



**Fig. 7** Effect of *Trichoderma* species on percent decrease in reproductive factor of *Meloidogyne javanica*

## Discussion

*Trichoderma* species are free-living soil borne fungi and are commonly found in root ecosystems. These opportunistic fungi develop symbiotic relationships with different kinds of plants and fungi. Some species of *Trichoderma* have the ability to colonize root surfaces and enter the epidermis resultantly enhancing development and growth of roots, increasing production, imparting resistance to abiotic stresses

and uptake of nutrients is greatly improved. In the present study, effectiveness of seven indigenous species of *Trichoderma* was tested for their ability to suppress population of Javanese root-knot nematode and improve growth variables of green gram. All the *Trichoderma* species resulted in increase in plant growth and caused reductions in nematode infestations over control.

*Trichoderma* species have been found effective against several phytopathogenic nematodes and fungi. Several species of *Trichoderma* including *T. harzianum*, *T. viride*, *T. atroviride*, *T. citrinoviride* and *T. asperellum* have provided excellent control of root-knot nematodes in previous studies (Sharon et al. 2007; Affokpon et al. 2011; Saikia et al. 2013; Martínez-Medina et al. 2017; Sonkar et al. 2018). In one study, *T. harzianum* and *T. viride* reduced root galling by *M. incognita* and *M. javanica* by 31% in tomato (Sharon et al. 2001; Dababat et al. 2006). Bokhari (2009) showed that *T. harzianum*, *T. hamatum*, *T. koningii*, *T. viride* and *T. reesei* culture filtrates significantly decreased females and eggs of reniform and root-knot nematodes. Jindapunapat et al. (2013) found that inoculation of root zones of guava plants with *T. harzianum* reduced nematode numbers in soil and roots and arrested the development of juveniles. Similarly, another species *T. longibrachiatum* significantly controlled *M. incognita* on cucumber (Zhang et al. 2015). Al-Hazmi and Javeed (2016) reported that *T. harzianum* and *T. viride* suppressed nematode multiplication and root galling and increased growth of tomato plants. Moreover, many soil borne pathogens including *Rhizoctonia solani*, *Pythium ultimum*, *Fusarium moniliforme*, *Macrophomina phaseolina* and *Sclerotium rolfsii* have also been controlled by different *Trichoderma* species (Bae et al. 2016; Giurgiu et al. 2018; Ghazanfar et al. 2018; Iqbal and Mukhtar 2020b).

Several mechanisms have been reported to be involved in the biocontrol activity of *Trichoderma* against fungal plant pathogens. The important mechanisms are antibiosis, competition, enzymatic hydrolysis, parasitism and systemic induced resistance (Benitez et al. 2004; Harman et al. 2004; Ragozzino and d'Errico 2011; Hhmau et al. 2015; Lombardi et al. 2018). Many biocontrol compounds and activators such as Trichokonins, trichodermin and trypsin-like protease obtained from *Trichoderma* spp. have been proven to be nematicidal (Suarez et al. 2004; Xiao-Yan et al. 2006; Reino et al. 2008; Chen et al. 2009; Yang et al. 2010, 2012). The highly branched conidiophores of *Trichoderma* produce conidia that can attach to different nematode stages. Conidial attachment and parasitism varies among fungal species and strains (Sharon et al. 2007). This process was often associated with the formation of fungal coiling and appressorium-like structures. *T. harzianum* colonizes isolated eggs and J2s of *M. javanica* (Sharon et al. 2007). Successful parasitism of the nematode by *Trichoderma* re-

quires mechanisms to facilitate penetration of the nematode cuticles or eggshells. The involvement of lytic enzymes has long been suggested and demonstrated in *Meloidogyne* parasitism (Spiegel et al. 2005). Besides direct antagonism, other mechanisms involved in *Meloidogyne* control by *Trichoderma* spp. include production of fungal metabolites and induced resistance (Bokhari 2009). Reduction in nematode galls and eggs might be due to high rhizosphere competency of bio-agents as they can easily colonize roots and may reduce feeding sites for nematodes. The reduction in root galling might be due to failure of majority of the juveniles to enter the host roots. In general, *Trichoderma* should be applied before planting to achieve maximum nematode control as good establishment of the fungus in plant rhizospheres seems to be important for nematode control.

In the present study, *Trichoderma* species significantly enhanced growth parameters of green gram over control. Plant growth enhancement by *Trichoderma* isolates can be attributed to different mechanisms which include enhanced nutrient uptake, solubilization of phosphates, sequestration of inorganic nutrients, secretion of exogenous enzymes, siderophores and enhanced root hair development (Harman 2006; Lorito et al. 2010). *Trichoderma* was found helpful in increasing plant hormones which help to increase root hairs and growth and make them more efficient in absorbing nitrogen, phosphorus, potassium and micronutrient (Mastouri et al. 2010). Besides enhanced growth and yields, *Trichoderma* species also increased chlorophyll, starch, nucleic acids contents, total protein and phytohormones in maize plants (Akladios and Abbas 2014). In the present study, *Trichoderma* species showed variations in their effectiveness as growth promotor of green gram and in suppressing nematode. This might be due to variations in genetics, pathogenic potential and the origins of different *Trichoderma* species and isolates.

## Conclusion

All the seven *Trichoderma* species showed nematicidal potential against *Meloidogyne javanica* and caused significant reductions in nematode infestations resulting in enhancement of growth variables of green gram. Although *Trichoderma* species varied in their effectiveness against the nematode but *Trichoderma viride*, *T. harzianum*, *T. asperellum* and *T. hamatum* proved more effective and can be potential alternatives to synthetic nematicides. As these *Trichoderma* species are commonly found in the country they can be effectively exploited for the management of root-knot nematodes.

**Conflict of interest** T. Mukhtar, M. Tariq-Khan and M.N. Aslam declare that they have no competing interests.

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