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Full Length Article

In vitro Biocontrol Potential of *Trichoderma pseudokoningii* against *Macrophomina phaseolina*

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Abstract

Macrophomina phaseolina is a saprotrophic pathogen that can cause infections in a wide range of plant species. In order to combat this pathogen, an antagonistic fungus *Trichoderma pseudokoningii* was tested against it by using a dual culture assay. The purity of *T. pseudokoningii* was confirmed on molecular basis by using internal transcribed spacer (ITS) and elongation factor 1-alpha (EF1) primers and submitted to Gene Bank under accession numbers MN721869 and MN736409, respectively. Direct co-culturing with *T. pseudokoningii*, arrested the pathogen growth by 62% over control. To find out the mechanism of action of the biocontrol agent, the fungal DNA was incubated in different concentrations of secondary metabolites of *T. pseudokoningii* for 24 h and 48 h. The findings showed that the secondary metabolites concentration was directly proportional to the DNA cleavage as at higher concentrations it successfully degraded the *M. phaseolina* DNA. For identification of possible fungicidal components in culture filtrates of *T. pseudokoningii*, the filtrates were partitioned with chloroform and ethyl acetate and were subjected to GC-MS analysis. Undecane (36.47%), 9,12-octadecadienoic acid (Z,Z)- (23.48%), decane (23.45%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (20.84%) and 7,8-epoxylanostan-11-ol,3-acetoxy- (10.38%) were recorded as the predominant compounds in culture filtrates which could be involved in arresting the growth of the pathogen. The present study concludes that *T. pseudokoningii* possibly controlled the growth of *M. phaseolina* by disintegration of its DNA.

Keywords: Antagonistic; Biological control; Natural fungicides; Secondary metabolites

Introduction

Macrophomina phaseolina (Tassi) Goid. is a widespread soil-borne phytopathogenic fungus. It is responsible for charcoal rot, basal stem rot and damping off disease in more than 500 species of plant kingdom (Khan *et al.* 2017). It mainly produces pycnidia or either microsclerotia in the soil (Li 1993). It is not only soil-borne but also a seed-borne pathogen and causes infections in plants both at seedling stage and at plant maturity (Purushotham *et al.* 2020). It is very hard to manage this pathogen through agronomic practices because of its diverse nature (Naseri *et al.* 2018). To combat the pathogens, chemical fungicides are the first choice for farmers as are easily available in market (Iqbal and Mukhtar 2020). However, long-term fungicides application has created toxic effects in environment, humans and animals so their usage should be discouraged (Aravind and Brahmbhatt 2018). Chemical fungicides should be replaced with biocontrol organisms because of the public concerns regarding negative impact of the synthetic agrochemicals (Rahman *et al.* 2018). During the past few decades, diverse biocontrol agents have received consideration in disease management programs in addition to their use in commercial enzyme production namely hemicellulases, cellulases, β-1,3-glucanase and proteases (Bischof *et al.* 2016; Deng *et al.* 2018).

Biological control of soil-borne pathogens by microorganisms has been considered as environmentally safe and virtuous supplement to the synthetic fungicides (Sohaliya *et al.* 2019). The widespread application of *Trichoderma* spp. such as *T. asperellum*, *T. atroviride*, *T. gamsii*, *T. hamatum*, *T. harzianum*, *T. polysporum*, *T. virens* and *T. koningii* as biocontrol agents has been exploited and reported against *Phytophthora*, *Pythium*, *Aspergillus*, *Fusarium*, *Rhizoctonia* and *Macrophomina* (Moosa *et al.* 2017; Javaid *et al.* 2018; Sharma and Prasad 2018; Ingale and Patale 2019). Akin to the most fungal biocontrol organisms, genus *Trichoderma* can be efficiently used in the form of conidia or spores which are more tolerant to harsh environmental conditions in comparison to mycelia for field use and product formulations (El-Mougy and Abdel-Kader 2018). The activation mechanism exerts biocontrol weapons either directly or indirectly, by competition for space, nutrients, mycoparasitism and antibiosis (Guzman-Guzman *et al.* 2017). Moreover, *Trichoderma* is a growth promoting fungus that can easily multiply in diverse soil types and

establish persistent colonies for several months. Its commercial success rate depends upon the shelf life, easy and quick multiplication on host, bioefficacy and readily availability in market (Kamal *et al.* 2018; Rini *et al.* 2018). The aim of the present study was to assess *in vitro* antagonistic potential of *T. pseudokoningii* as biological control agents against *M. phaseolina* and to unearth the possible mechanisms involved in antagonism.

Materials and Methods

Molecular characterization of selected isolates

Pure culture of *T. pseudokoningii* was obtained from the First Culture Bank of Pakistan (FCBP), University of the Punjab Lahore, Pakistan. The fungus was multiplied on malt extract agar plates and its DNA was procured through cetyl trimethylammonium bromide (CTAB) method (Doyle and Doyle 1990). The isolated DNA was confirmed on molecular basis by using two sets of primer pairs namely ITS (ITS 1-F and ITS 4-R) and EF1 (EF1-728-F and EF1-986-R). Annealing temperature for all the primers was 60°C. The amplified single band PCR products were got sequenced at 1st Base Sequencing Singapore Co., Ltd. (Fig. 1).

In vitro antagonistic activity of *T. pseudokoningii*

Biological activity of *T. pseudokoningii* was evaluated by direct co-culturing with the tested phytopathogenic fungus *M. phaseolina*. A mycelial plug (0.5 cm diameter) of *T. pseudokoningii* was prepared from the 7-day-old culture and uniformly inoculated in vertical direction at one end of the malt extract agar containing plates opposite to the pathogen mycelial plugs. A control was also prepared by only inoculating the pathogen in the center of malt extract agar (MEA) plates with three replicates of each and let them to grow for five days at 28°C. Percent hyphal growth inhibition of *M. phaseolina* was determined and calculated through a given below growth inhibition formula (Rini and Sulochana 2008).

$$\text{Inhibition (\%)} = \frac{\text{Colony diameter in control} - \text{Colony diameter in dual culture}}{\text{Colony diameter in control}} \times 100$$

For statistical analysis, standard errors of means of five replicates were calculated using MS Excel. To determine significant difference in growth of *M. phaseolina* between control and dual culture treatments up to 5 days growth, LSD test was applied at $P \leq 0.05$ using software Statistix 8.1.

DNA cleavage bioassay

A DNA cleavage experiment was conducted to evaluate the antagonism potential of secondary metabolites produced by *T. pseudokoningii*. For this, malt extract (ME) broth containing flasks were inoculated aseptically with freshly prepared mycelial plugs of *T. pseudokoningii* and kept on an orbital shaker (150 rpm) for two weeks at 28°C. Subsequently, the broth was filtered through Whatman No. 1 filter paper and its concentration was considered as 100%. The resultant filtrate was evaporated in an electric oven at 40°C for the preparation of concentrated broth in order to construct five different concentrations *viz*., 200, 300, 400 and 500%. The isolated *M. phaseolina* ribosomal DNA (5 μL) was mixed in each concentration (5 μL) for 24 and 48 h at 37°C. Experiment was conducted in completely randomized design in triplicates. To determine the extent of pathogenic fungal DNA degradation, all the treatments were loaded on 1% agarose gel with a negative control. The gel was run for 45 min at 100 volts and visualized under UV illuminator (Katrahalli *et al.* 2019).

GC-MS analysis

For identification of bioactive compounds, *T. pseudokoningii* mycelial plugs (5 mm diameter) were added to 250-mL Erlenmeyer flasks containing 100 mL of sterilized ME medium. The flasks were then kept on an orbital shaker (150 rpm) for 15 days at 28°C. The resultant homogenate was passed through a filter paper and extracted with chloroform and ethyl acetate. The organic solvent layers were collected by using a separating glass funnel and were further subjected for GC-MS analysis.

Results

Molecular identification of *T. pseudokoningii*

Molecular characterization of the antagonistic *T. pseudokoningii* DNA was confirmed by sequencing of ITS and EF1 regions. The amplified PCR products with EF1 441 bp and ITS 542 bp showed 100% similarities with the already submitted sequences to NCBI, and deposited under accession No. MN736409 and MN721869, respectively.

Interactions of *T. pseudokoningii* with *M. phaseolina*

Antagonistic effect of *T. pseudokoningii* against the saprotrophic soil-borne phytopathogen *M. phaseolina* is shown in Fig. 2. It was observed that *T. pseudokoningii* successfully restricted the growth of the targeted pathogen. There was up to 62% inhibition in growth of *M. phaseolina* in dual culture treatment with *T. pseudokoningii* as compared to its growth in the control treatment after five days' growth (Fig. 3).

DNA cleavage study

Secondary metabolites produced by the *T. pseudokoningii* rapidly degraded the *M. phaseolina* rDNA as shown in Fig. 4. The findings revealed that after the incubation of 24 h,

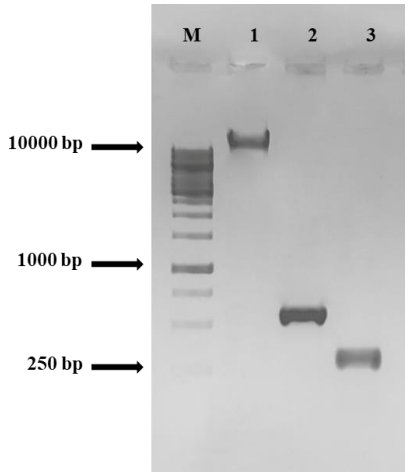

Fig. 1: *Trichoderma pseudokoningii* (M): 1 kb DNA standard marker (1): Genomic DNA, (2): ITS1/ITS4 amplified PCR product (3): $EF1_f/EF1_r$ amplified PCR product.

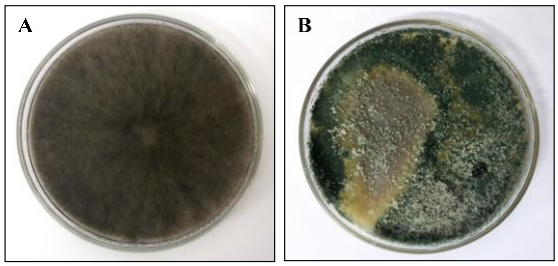

Fig. 2: Interaction of *Macrophomina phaseolina* with *Trichoderma pseudokoningii*
A)- Pure culture of *Macrophomina phaseolina* (MP); **B)-** MP co-culture with *T. pseudokoningii.*

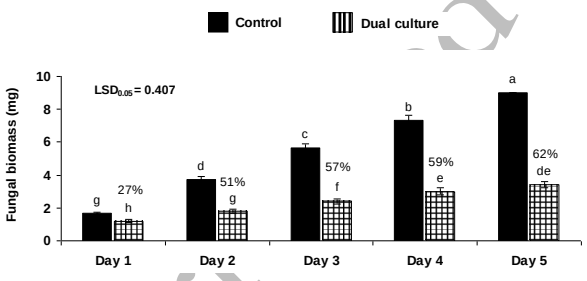

Fig. 3: Effect of *Trichoderma pseudokoningii* on growth of *Macrophomina phaseolina* in dual culture interaction. Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference ($P \leq 0.05$) as determined by LSD Test. Values (%) given on bars of dual culture treatment show decrease in growth of *M. phaseolina* in dual culture over corresponding control treatment.

pathogenic fungal DNA bands appeared in the form of smears at lower concentrations of 100%, 200% and 300%, whereas, at the higher concentrations of 400% and 500% the genomic DNA was fully degraded. At higher incubation time of 48 h, it was noted that all the concentrations successfully degraded the *M. phaseolina* DNA bands.

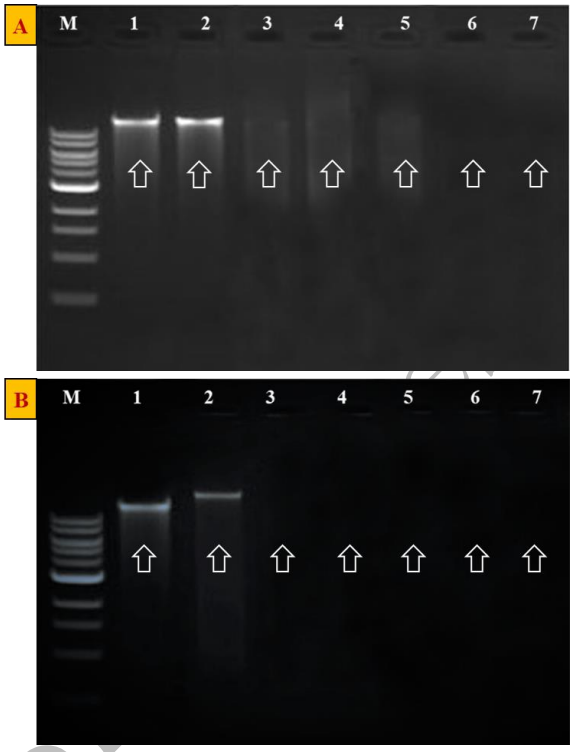

Fig. 4: DNA cleavage. **A)-** After 24 hours, **B)-** After 48 hours.
(M): 1 kb DNA standard marker **(1):** Genomic DNA of *M. phaseolina* **(2):** Negative control (Genomic DNA of *M. phaseolina* + Malt extract broth) **(3):** 100% concentration (Genomic DNA of *M. phaseolina* + *T. pseudokoningii* fungal metabolite) **(4):** 200% concentration (Genomic DNA of *M. phaseolina* + *T. pseudokoningii* fungal metabolite) **(5):** 300% concentration (Genomic DNA of *M. phaseolina* + *T. pseudokoningii* fungal metabolite) **(6):** 400% concentration (Genomic DNA of *M. phaseolina* + *T. pseudokoningii* fungal metabolite) **(7):** 500% concentration (Genomic DNA of *M. phaseolina* + *T. pseudokoningii* fungal metabolite). Arrows indicate the presence or absence of DNA

GC-MS analysis

The GC-MS analysis of chloroform and ethyl acetate fractions of *T. pseudokoningii* metabolites is given in Fig. 5, which presented 9 peaks in each of the two chromatograms. The compounds identified in chloroform fraction with their percent abundance and retention time are given in Table 1 and their structures are illustrated in Fig. 6. The compounds present in higher concentrations were undecane (36.47%) and decane (23.45%). On the other hand, moderately abundant compounds were benzene, 1,2,3-trimethyl (9.24%), 1-hentetracontanol (8.78%), *n*-dodecylpyridinium chloride (6.14%), octadecanoic acid, 9,10-dihydroxy-, methyl ester (5.74%), pentatriacontane (5.44%), tetracontane (4.43%) and benzene, 1-ethyl-2,3-dimethyl- (3.03%).

The details of identified compounds in ethyl acetate fraction with retention time and percent abundance is presented in Table 2 and their structures are given in Fig. 7. The most prevailing compounds were 9,12-octadecadienoic acid (Z,Z)- (23.48%), 9,12-octadecadienoic acid (Z,Z)-, methyl ester (20.84%), cyclohexanecarboxylic acid, 4-pentyl-, 4-ethoxyphenyl ester (11.45%), 7,8-epoxylanostan-

Table 1: Compounds identified from chloroform fraction of culture filtrate of *Trichoderma pseudokoningii* through GC-MS analysis

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Decane	$C_{10}H_{22}$	142	2.374	23.45
2	Benzene, 1,2,3-trimethyl	C_9H_{12}	120	2.425	9.24
3	Undecane	$C_{11}H_{24}$	156	2.800	36.47
4	*n*-Dodecylpyridinium chloride	$C_{17}H_{30}ClN$	283	2.875	6.14
5	Benzene, 1-ethyl-2,3-dimethyl-	$C_{10}H_{14}$	134	3.155	3.03
6	1-Hentetracontanol	$C_{41}H_{84}O$	592	8.015	8.78
7	Pentatriacontane	$C_{35}H_{72}$	492	8.462	5.44
8	Octadecanoic acid, 9,10-dihydroxy-, methyl ester	$C_{19}H_{38}O_4$	330	9.414	5.74
9	Tetracontane	$C_{40}H_{82}$	562	9.492	4.43

Table 2: Compounds identified from ethyl acetate fraction of culture filtrate of *Trichoderma pseudokoningii* through GC-MS analysis

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	Benzene, nitro-	$C_6H_5NO_2$	123	2.858	7.03
2	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	6.909	3.15
3	1-(+)-Ascorbic acid 2,6-dihexadecanoate	$C_{38}H_{68}O_8$	652	7.070	5.43
4	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	294	7.627	20.84
5	9,12-Octadecadienoic acid (Z,Z)-	$C_{18}H_{32}O_2$	280	7.798	23.48
6	7,8-Epoxylanostan-11-ol, 3-acetoxy-	$C_{32}H_{54}O_4$	502	8.016	10.38
7	Cyclohexanecarboxylic acid, 4-Pentyl-, 4-ethoxyphenyl ester	$C_{20}H_{30}O_3$	318	8.927	11.45
8	gamma-Sitosterol	$C_{29}H_{50}O$	414	9.378	7.66
9	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester	$C_{19}H_{38}O_4$	330	9.630	10.36

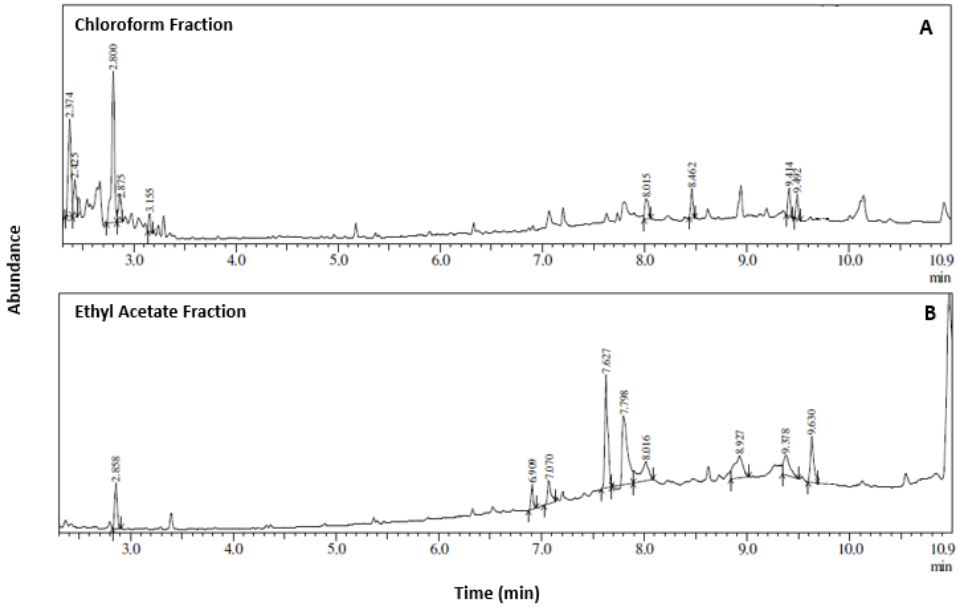

Fig. 5: GC-MS chromatograms of chloroform and ethyl acetate fractions of culture filtrate of *Trichoderma pseudokoningii*.

11-ol, 3-acetoxy- (10.38%) and hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester (10.36%). The compounds in less abundance were gamma-sitosterol (7.66%), benzene, nitro- (7.03%), 1-(+)-ascorbic acid 2,6-dihexadecanoate (5.43%) and hexadecanoic acid, methyl ester (3.15%).

Discussion

The morphological characters of *Trichoderma* spp., can give misleading results so the accurate identification on molecular basis is necessary (Mokhtari *et al*. 2017). The amplification and sequencing of ITS and EF1 regions is common and highly trusted to differentiate among the species (Li *et al.* 2020). Therefore, in the present study, molecular characterization of *T. pseudokoningii* was carried out with ITS and EF1 that gave accurate identification. Consequently, accession numbers were deposited in GeneBank.

In dual culture plate assay the inhibition in two interacting organisms' radial growth has been attributed to the inhibitory substances produced by one or both of the organisms as a result of competition, hyper-parasitism, mycoparasitism or secretion of antibiotics (Deng *et al.*

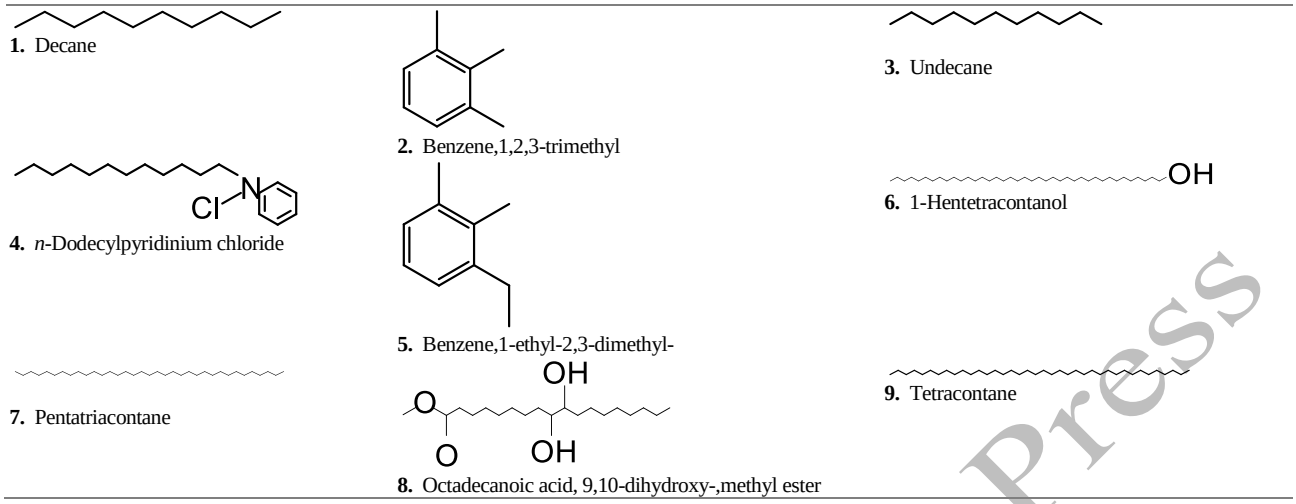

Fig. 6: Structures of compounds identified in chloroform fraction of culture filtrate of *Trichoderma pseudokoningii* through GC-MS analysis

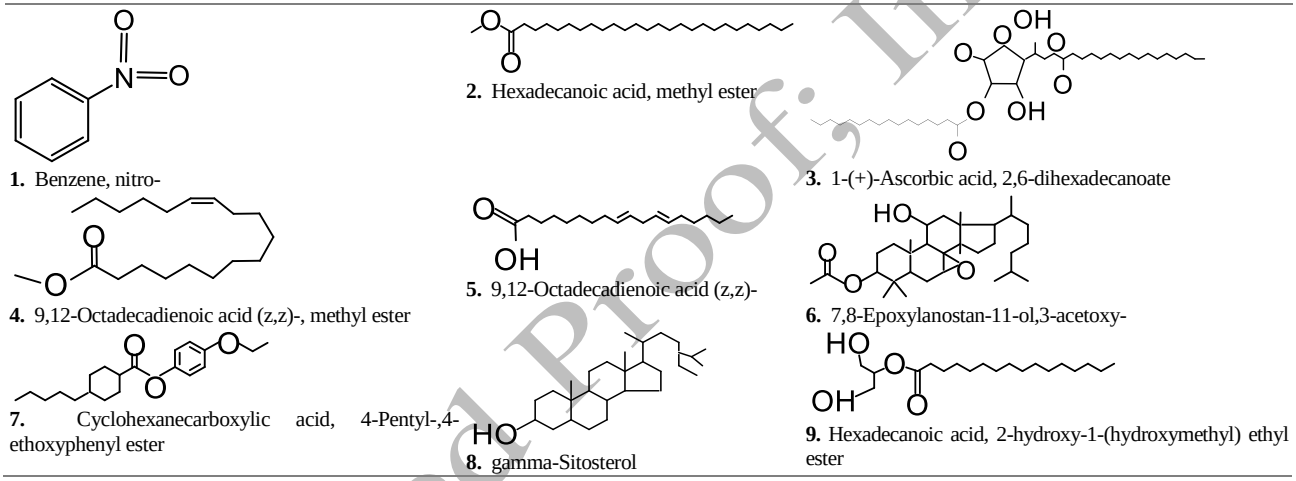

Fig. 7: Structures of compounds identified in ethyl acetate fraction of culture filtrate of *Trichoderma pseudokoningii* through GC-MS analysis

2018). The present findings revealed that *T. pseudokoningii* significantly retarded the growth of *M. phaseolina* and is in confirmation of the recent work performed by Li *et al.* (2019) who reported that three antagonistic strains of *Trichoderma* namely *T. pseudokoningii*, *T. harzianum and T. asperellum* were very effective against the *Fusarium oxysporum*, the pathogen of Fusarium wilt in various economically important host plants. Previously, Nirwan *et al.* (2016) also evaluated the antagonistic potential of *T. pseudokoningii* against the root rot pathogen *Ganoderma lucidum* with promising results. Similarly, Akinbode *et al.* (2018) managed *Fusarium verticillioides*, a seed-borne fungal pathogen by using *T. pseudokoningii* and benomyl a synthetic chemical under field conditions. The results showed that *T. pseudokoningii* was found to be more efficient against the targeted pathogen in comparison with the fungicide. Ibrahim and Abdel-Azeem (2015) also effectively controlled the *M. phaseolina* by using *T. pseudokoningii* under field conditions.

Trichoderma species are used worldwide as lucrative potent biocontrol agents against a diverse range of phytopathogens due to their prominent antimicrobial activities under *in vitro* and *in vivo* conditions (Vinale *et al.* 2008). Undoubtedly, it has been documented that novel secondary metabolites produced by the genus *Trichoderma* are the efficient source of bioactivities (Khan *et al.* 2019). To understand the mechanism of antagonism, a DNA cleavage experiment was carried out to evaluate the potential of *T. pseudokoningii* towards the *M. phaseolina.* This study clearly demonstrated that disintegration of the pathogen's DNA is possibly the key mechanism of *T. pseudokoningii* as biocontrol agent against *M. phaseolina.* Previously, DNA cleavage studies were generally carried out to study the mechanism of nanoparticles (Jadhav *et al.* 2018). Application of silver nanoparticles fully deteriorated the gram-positive *Staphylococcus warneri* bacterial DNA

(Dong *et al*. 2017). Likewise, plant extracts of *Euphorbia prostrata* showed excellent results against the bacterial DNA structure of *E. coli*. Furthermore, the nanoparticles prepared from *E. prostrata* plant extracts when used alone and in combinations actively degraded the DNA (Dashamiri *et al.* 2018). Similarly, Katrahalli *et al.* (2019) tested a naturally occurring compound benzothiazol-2-yl-malonaldehyde against a gram-negative bacterium *Pseudomonas aeruginosa* DNA with promising results.

GC-MS analysis of chloroform and ethyl acetate fractions of *T. pseudokoningii* secondary extrolites was undertaken to evaluate the bioactive compounds. Among the identified constituents, undecane was a predominant compound in chloroform fraction and was previously isolated from a medicinal plant *Equisetum arvense* with potent antibiotic, antioxidant, viricidal and anti-inflammatory properties (Altameme *et al.* 2015). Moreover, Moya *et al.* (2018) also identified this compound from the secondary metabolites produced by *T. harzianum* and found it effective against a pathogenic fungal strain *Pyrenophora teres*. 9,12-Octadecadienoic acid (Z,Z)- was another predominant compound present in ethyl acetate fraction. Bruno *et al.* (2019) recently identified this compound as an antimicrobial agent by testing it against bacterial pathogens *Staphylococcus aureus* and *Bacillus subtilis* as well as against a fungal pathogen *F. oxysporum* with promising results. Likewise, an important compound 7,8-epoxylanostan-11-ol,3-acetoxy- identified in the present study was previously found in ethanolic extracts of *Rhus muelleri* with strong antimicrobial potential (Rodriguez *et al.* 2015). Similarly, Soni *et al.* (2014) reported the antimicrobial, pesticide and pharmaceutical properties of gamma-sitosterol. Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl) ethyl ester was also identified from the methanolic extracts of *Melia azedarach* with anthelmintic, anti-inflammatory and antioxidant properties (Al-Marzoqi *et al.* 2015). Arora and Kumar (2018) identified 9,12-octadecadienoic acid (Z,Z)-, methyl ester having hypocholesterolemic, anti-histaminic, aepatoprotective and antieczemic activities. Likewise, decane and benzene, 1,2,3-trimethyl are known for their antifungal and antibacterial activities (Mohammed *et al.* 2015; Adomi 2017). 1-Hentetracontanol also possess antimicrobial properties (Ravi *et al*. 2018).

Conclusion

Findings of this study revealed that *T. pseudokoningii* is an effective biocontrol agent that can efficiently arrest the growth of *M. phaseolina*. *T. pseudokoningii* extracted secondary extrolites can effectively cleave the pathogenic fungal DNA. The literature survey showed that chloroform and ethyl acetate fractions are rich in antifungal compounds such as undecane, 9,12-octadecadienoic acid (z,z)- decane and gamma-sitosterol, which could be responsible of control of pathogen growth.

Author's contribution

Iqra Haider Khan did experimental work and wrote a part of paper. Arshad Javaid supervised the work and also contributed in paper writing.

References

Adomi PO (2017). Investigation of bioactive phytochemical compounds from aqueous ethanol extracts of leaves of *Phyllanthus amarus* Schum and Thonn by gas chromatography-mass spectrometry (GC-MS). *Pac J Sci Technol* 18:284–291

Akinbode OA, KT Kareem, OF Oduwaye, AI Obafemi (2018). Influence of eco-friendly control strategies on the germination of mycotoxin secreted *Fusarium verticillioides* infested maize. *J Appl Sci Environ Manage* 22:1657–1660

Al-Marzoqi AH, IH Hameed, SA Idan (2015). Analysis of bioactive chemical components of two medicinal plants (*Coriandrum sativum* and *Melia azedarach*) leaves using gas chromatography-mass spectrometry (GC-MS). *Afr J Biotechnol* 14:2812–2830

Altameme HJ, IH Hameed, NA Abu-Serag (2015). Analysis of bioactive phytochemical compounds of two medicinal plants, *Equisetum arvense* and *Alchemila valgaris* seed using gas chromatographymass spectrometry and fourier-transform infrared spectroscopy. *Malays Appl Biol* 44:47–58

Aravind T, AB Brahmbhatt (2018). Management of root and collar rot (*Macrophomina phaseolina* (Tassi) Goid.) of okra (*Abelmoschus esculentus* (L.) Moench) through bioagents, oil cakes and fungicides. *J Pharm Phytochem* 7:631–635

Arora S, G Kumar (2018). Phytochemical screening of root, stem and leaves of *Cenchrus biflorus* Roxb. *J Pharm Phytochem* 7:1445–1450

Bischof RH, J Ramoni, B Seiboth (2016). Cellulases and beyond: the first 70 years of the enzyme producer *Trichoderma reesei*. *Microb Cell Factories* 15:1–13

Bruno M, A Modica, G Catinella, C Canlı, T Arasoglu, S Çelik (2019). Chemical composition of the essential oils of *Centaurea tomentella* Hand.-Mazz. and *C. haussknechtii* Boiss. (Asteraceae) collected wild in Turkey and their activity on microorganisms affecting historical art craft. *Nat Prod Res* 33:1092–1100

Dashamiri S, M Ghaedi, R Naghiha, A Salehi, R Jannesar (2018). Antibacterial, anti-fungal and *E. coli* DNA cleavage of *Euphorbia prostrata* and *Pelargonium graveolens* extract and their combination with novel nanoparticles. *Braz J Pharm Sci* 54:1–8

Deng JJ, WQ Huang, ZW Li, DL Lu, Y Zhang, XC Luo (2018). Biocontrol activity of recombinant aspartic protease from *Trichoderma harzianum* against pathogenic fungi. *Enzyme Microb Technol* 112:35–42

Dong ZY, M Prabhu, N Rao, M Prabhu, M Xiao, HF Wang, WN Hozzein, W Chen, WJ Li (2017). Antibacterial activity of silver nanoparticles against *Staphylococcus warneri* synthesized using endophytic bacteria by photo-irradiation. *Front Microbiol* 8; Article 1090

Doyle JJ, JL Doyle (1990). Isolation of plant DNA from fresh tissue. *Focus* 12:39–40

El-Mougy NS, MM Abdel-Kader (2018). '*Trichoderma harzianum*' and some antioxidants for suppressing faba bean chocolate spot incidence under natural field infection. *Aust J Crop Sci* 12:794–799

Guzman-Guzman P, MI Aleman-Duarte, L Delaye, A Herrera-Estrella, V Olmedo-Monfil (2017). Identification of effector-like proteins in *Trichoderma* spp. and role of a hydrophobin in the plant-fungus interaction and mycoparasitism. *BMC Genet* 18; Article 16

Ibrahim ME, AM Abdel-Azeem (2015). Management of sesame (*Sesamum indicum* L.) charcoal rot caused by *Macrophomina phaseolina* (Tassi) Goid. through the application of different control measure. *J Pure Appl Microbiol* 9:1–10

Ingale ST, SS Patale (2019). Antagonistic potential of *trichoderma* spp. against fungal pathogens of pulses. *J Life Sci* 16:251–252

Iqbal U, T Mukhtar (2020). Inhibitory effects of some fungicides against *Macrophomina phaseolina* causing charcoal rot. *Pak J Zool* 52:709–715

Jadhav MS, S Kulkarni, P Raikar, DA Barretto, SK Vootla, US Raikar (2018). Green biosynthesis of CuO & Ag–CuO nanoparticles from *Malus domestica* leaf extract and evaluation of antibacterial, antioxidant and DNA cleavage activities. *J Chem* 42:204–213

Javaid A, IH Khan, A Shoaib (2018). Management of charcoal rot of mungbean by two *Trichoderma* species and dry biomass of *Coronopus didymus*. *Plant Danin* 36:1-8

Kamal RK, V Athisayam, YS Gusain, V Kumar (2018). *Trichoderma*: A most common biofertilizer with multiple roles in agriculture. *Biomed J Sci Technol Res* 4:4136–4137

Katrahalli U, BC Yallur, DH Manjunatha PM Krishna (2019). BSA interaction and DNA cleavage studies of anti-bacterial benzothiazol-2-yl-malonaldehyde. *J Mol Str* 1196:96–104

Khan AN, F Shair, K Malik, Z Hayat, MA Khan, FY Hafeez, MN Hassan (2017). Molecular identification and genetic characterization of *Macrophomina phaseolina* strains causing pathogenicity on sunflower and chickpea. *Front Microbiol* 8; Article 1309

Khan SU, B Ahmad, I Khan, S Rauf, S Azam, I Ullah (2019). Optimization of growth conditions for the maximum production of secondary metabolites from *Trichoderma harzianum* and their biological activities. *Pharmacology* 15:351–360

Li L (1993). Progress in research on sesame diseases in the world. *Oil Crop Sci* 2:75–77

Li M, GS Ma, H Lian, XL Su, Y Tian, WK Hunag, XL Jiang (2019). The effects of *Trichoderma* on preventing cucumber *Fusarium* wilt and regulating cucumber physiology. *J Integr Agric* 18:607–617

Li S, Y Deng, Z Wang, Z Zhang, X Kong, W Zhou, Y Qu (2020). Exploring the accuracy of amplicon-based internal transcribed spacer (ITS) markers for fungal community. *Mol Ecol Resour* 20:170–184

Mohammed SB, NH Azhari, YM Mashitah, NH Abdurahman, AS Mazza (2015). *In vitro* antimicrobial activity and GC-MS analysis of medicinal plant *Swietenia macrophylla* King. *J Chem Pharm Res* 7:519–524

Mokhtari W, N Chtaina, E Halmschlager, H Volgmayr, C Stauffer, W Jaklitsch (2017). Potential antagonism of some Trichoderma strains isolated from Moroccan soil against three phytopathogenic fungi of great economic importance. *Rev Maroc Sci Agron Vet* 5:248–254

Moosa A, ST Sahi, IU Haq, A Farzand, SA Khan, K Javaid (2017). Antagonistic potential of Trichoderma isolates and manures against Fusarium wilt of tomato. *Intl J Veg Sci* 23:207–218

Moya P, JR Girotti, AV Toledo, MN Sisterna (2018). Antifungal activity of trichoderma VOCs against *Pyrenophora teres*, the causal agent of barley net blotch. *J Plant Prot Res* 58:45–53

Naseri B, M Veisi, N Khaledi (2018). Towards a better understanding of agronomic and soil basis for possible charcoal root rot control and production improvement in bean. *Arch Phytopathol Plant Prot* 51:349–358

Nirwan B, S Choudhary, K Sharma, S Singh (2016). *In vitro* studies on management of root rot disease caused by *Ganoderma lucidum* in *Prosopis cineraria*. *Curr Life Sci* 2:118–126

Purushotham N, A Jones, B Poudel, J Nasim, D Adorada, AH Sparks, N Vaghefi (2020). Draft genome resource for *Macrophomina phaseolina* associated with charcoal rot in sorghum. *Mol Plant Microb Interact* 33:724–726

Rahman SFSA, E Singh, CM Pieterse, PM Schenk (2018). Emerging microbial biocontrol strategies for plant pathogens. *Plant Sci* 267:102–111

Ravi R, NSH Zulkrnin, NN Rozhan, NRN Yusoff, MSM Rasat, MI Ahmad, Z Hamzah, IH Ishak, MFM Amin (2018). Evaluation of two different solvents for *Azolla pinnata* extracts on chemical composition and larvicidal activity against *Aedes albopictus* (Diptera: Culicidae). *J Chem* 2018; Article 7453816

Rini CR, KK Sulochana (2008). Usefulness of *Trichoderma* and *Pseudomonas* against *Rhizoctonia solani* and *Fusarium oxysporum* infecting tomato. *J Trop Agric* 45:21–28

Rini CR, J Ramya, G Jayakumar, VR Shajan (2018). Low cost carrier material for mass production of Trichoderma inoculants. *J Trop Agric* 56:63–68

Rodriguez DJ, FAT Gonzalez, R Rodriguez-Garcia, MLV Diaz-Jimenez, A Saenz-Galindo, FD Hernandez-Castillo, JA Villarreal-Quintanilla, FM Pena-Ramos (2015). Antifungal activity in vitro of *Rhus muelleri* and *Fusarium oxysporum* f. spp. *lycopersici*. *Ind Crop Prod* 75:150–158

Sharma KS, L Prasad (2018). Bioactivity of *Trichoderma asperellum* against *Colletotrichum asianum* and *Sclerotinia sclerotiorum*. *Pest Res J* 30:251–255

Sohaliya NN, DM Pathak, JR Pandya (2019). In vitro antagonistic activity of *Trichoderma* species against important soil borne pathogens. *Intl J Plant Prot* 12:127–131

Soni N, S Mehta, G Satpathy, RK Gupta (2014). Estimation of nutritional, phytochemical, antioxidant and antibacterial activity of dried fig (*Ficus carica*). *J Pharm Phytochem* 3:158–165

Vinale F, K Sivasithamparam, EL Ghisalberti, R Marra, MJ Barbetti, H Li, M Lorito (2008). A novel role for *Trichoderma* secondary metabolites in the interactions with plants. *Physiol Mol Plant Pathol* 72:80–86