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CHITINOLYTIC ACTIVITY OF THE INDIGENOUS *TRICHODERMA SPP*. FROM THE NORTH WEST OF PAKISTAN AGAINST THE FUNGAL PHYTOPATHOGENS

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Abstract

Chitinases are the enzymes considered as an important biocontrol mechanism for the putative antifungal activity. Here we report the chitinolytic activity of the indigenous strains of *Trichoderma* previously isolated from the North Western regions of Pakistan. The different strains of *T. longibrachiatum* TMK5, TMK6, TMK9, TMK20 and TMK21, and *T. virens* TMK8 isolated from the Malakand region, and *T. longibrachiatum* TKK3, TKK4 and TKK6 isolated from the Karak areas were included in this study. The strains were screened for their activity on the specific chitin synthetic medium *in vitro* at pH 4.7. The formation of purple colored zones depicted the chitinase activity by the fungi and the intensity of the colored zones was used as the criteria for the assessment. The highest activity was shown by *T. longibrachiatum* strain TKK4 followed by the strains TKK3, TMK5, TKK6, TMK20, and TMK21. The enzymes were then extracted in the modified Richards's medium. The strain TMK21 showed the highest *N*-acetyl-*β*-D-glucosaminidase activity of 0.017 umol/ml/min. Endochitinase activity was noticed by the extent of reduction of colloidal chitin suspension by the tested strains. The highest endochitinase activity was shown by the strain TMK9. The strain TMK20 produced the highest enzyme unit (0.503 EU), while on the other hand, the strain TMK9 had the lowest value (0.033 EU). The crude enzymes were then applied against the phytopathogenic fungal isolates originated from the local diseased vegetables (Green Chili, Onion, Ridge Gourd, Okra, Bottle Gourd, Eggplant, Bitter Gourd and Spinach) including *Fusarium spp., Alterneria spp. and Aspergillus spp.* The partially purified enzymes extracted from *T. longibrachiatum* strains TMK20 and TMK21 consistently inhibited the different phytopathogenic fungi (up to 100%). This study demonstrated the inhibitory role of the chitinolytic enzyme extracts against the fungal pathogens.

Key words: Chitinolysis; Antifungal; Biocontrol; Partially purified enzymes; Khyber Pakhtunkhwa

Introduction

The fungal pathogens cause considerable yield losses in many important crops and vegetables. A number of phytopathogenic fungi including *Fusarium*, *Botrytis*, *Rhizoctonia*, *Phytophthora*, and *Phythium* attack plants and spread through inter and intra seasonal mechanisms in the economically important crops and vegetables. Additionally, the fungi attack the stored fruits also causing considerable losses (Chet, 1997).

The only way for controlling these devastating pathogens is by using higher quantities of fungicides that may result in environmental hazards to humans. It may also result in the build up of resistance in the target populations (El-Katatny *et al*., 2000). The biological control of the plant pathogens may provide with an alternative means for the control of the plant disease avoiding all the drawbacks of pesticide use (Ruana-Rosa *et al*., 2017).

Trichoderma spp. are well known for their biocontrol potential (Anees *et al.,* 2018). They are integral constituents of many commercially marketed products including biofertilizers, biopesticides, and soil amendments (Harman *et al*., 2004). More than 50 different products based on *Trichoderma*-inoculum are registered in different countries, and are used for protection and improvement of yields of fruit, vegetable, and ornaments (Lorito, 2005). A number of *Trichoderma* strains have been reported antagonistic against the diverse phytopathogenic fungi. The fungicidal activity of *Trichoderma* may involve the production lytic enzymes capable of degrading cell walls i.e. the cell wall degrading enzymes (CWDEs; Woo *et al.,* 2006). The other antifungal mechanisms may include the antibiotic production, nutrient and niche competition (Hjeljord & Tronsmo, 1998).

It is assumed that the hydrolytic enzymes produced by *Trichoderma* spp. constitutively detect the presence of other fungus sensing the molecules generated through enzyme degradation by the host (Woo & Lorito, 2007). The chitinolytic enzymes are quite known among the CWDEs produced by *Trichoderma spp*. Additional interest in these enzymes has developed due to the fact that this genus has an established biocontorol ability against plant diseases (Anees *et al*., 2010).

Virtually, CWDEs retain their biological activity at room temperature. There are reports of their fungicidal activity producing comparable results to commercial

fungicides when used against diseases in the greenhouse or in the post-harvest storage stage (Lorito *et al.,* 2001). The enzymes were tested elsewhere in purified forms and showed strong antifungal potential against the diverse fungi including *Rhizoctonia, Colletotrichum, Alternaria, Phytophthora,* and *Botrytis* (Lorito *et al.,* 1994). The direct application of antifungal compounds secreted by the fungal biocontrol agents rather than using the whole living organisms may have a number of advantages in agriculture and industry being more controlled. The active compounds may be selected for production by providing the specific growth conditions including the composition, temperature of incubation and pH of culture medium etc. (Woo & Lorito, 2007).

The indigenous strains of *Trichoderma* spp. were isolated from the North Western regions of Pakistan and were found antagonistic against the phytopathogenic fungi (Anees *et al*., 2018). The main objective of the present study was to evaluate chitinolytic activity of the selected strains hypothesizing their probable role in the antifungal behavior as observed in our previous report (Anees *et al*., 2018). The chitinases were extracted from the strains and tested against the different fungal phytopathogens using *in vitro* assays. The phytopathogens were locally isolated from the different fields of diseased vegetables. Moreover, *N*-acetyl-*β*-D-glucosaminidases and endochitinases were also extracted in the present study.

Materials and Methods

Isolation of fungal phytopathogens: The infected plant parts of the vegetables were sampled from the different regions of Kohat and used to isolate the pathogenic fungi on Sabouraud dextrose agar (SDA) plates. The diseased parts were surface sterilized by dipping in ethanol (70%) for 1 min and then placed on SDA plates for isolation. The Petri plates were incubated for 24 hours at 25-30ºC before sub culturing for further identification based on morphological characteristics.

***Trichoderma* spp. for chitinase production:** The strains of *Trichoderma longibrachiatum* TMK5, TMK6, TMK9, TMK20 and TMK21, and *T. virens* TMK8 from the Malakand region, and *T. longibrachiatum* TKK3, TKK4 and TKK6 from the Karak areas previously isolated by Anees *et al*., (2018) were included in the present investigation. Potato dextrose agar (PDA) and malt agar (MA) media were used for culture and proliferation of these strains.

Screening the *Trichoderma* isolates for chitinolytic activity: The method of Roberts & Selitrennikoff (1988) was used to prepare colloidal chitin with some modifications and was included as a sole carbon source in the chitin synthetic medium (CSM). For this purpose, the commercial chitin was acid hydrolyzed by adding chitin powder (5g) gradually into the concentrated HCl (50ml) and then shaking vigorously overnight at 4°C. Ethanol (250ml; 100%) was added while stirring at 4°C and then kept at room temperature (25°C) overnight. The solution was then centrifuged at 4°C for 20 min at 4000 rpm. The precipitated colloidal chitin was washed several times with sterile distilled water to remove the alcoholic smell. The chitin was then removed using spatula and stored at 4°C.

The CSM was prepared comprising of colloidal chitin (4.5 g), $MgSO_4.7H_2O$ (0.3 g), $(NH_4)_2\ SO_4$ (3.0 g), citric acid monohydrate (1.0 g), KH_2PO_4 (2.0 g), bromocresol purple (0.15 g) and 200 µl of Tween-80, 15 g of agar (all amounts are per liter); pH adjusted to 4.7 and autoclaved (121°C; 15 min). *Trichoderma spp.* were then cultured onto the CSM plates using fresh culture plugs to assess the chitinase activity followed by incubation for 2-3 days at 25±2°C. The colored zone formation was observed. Based on formation of purple zone in the assayed plates, the chitinase activity was identified. Extent of the activity was assessed by observing the intensity of the purple colored zone after incubation for 3 days. The *Trichoderma* strains were grouped based on an ordinal variable depending on the diameter of the purple zone: N) no activity L) low activity M) medium activity H) high activity and H+) the highest activity. The color developed in the media because of the pH indicator dye bromocresol purple which was supplemented in the media and transformed the yellowish orange color at pH 4.7 of the media into purple by increasing pH. The increase in pH of media was because of the breakdown of chitin into monomers i.e. N-acetyl glucosamine by *Trichoderma* shifting the pH to alkaline.

Enzyme extraction: *Trichoderma* spp. were cultured on the modified Richards medium that contained KNO_3 (10 g), KH_2PO_4 (5 g), $MgSO_4.7H_2O$ (2.5 g), $FeCl_3$ (2 mg), colloidal chitin (5 g), V8 juice1 (50 ml), polyvinyl pyrrolidine (10 g) and 850 ml H_20. The pH was adjusted at 6.0. The media (100 ml) were inoculated in 250ml Erlenmeyer flasks with a suspension of fungal spores at a final concentration of 5 x 10^6 conidia per ml and incubated in a rotary shaker at 25±2°C at 150 rpm for 4-5 days. The liquid culture was centrifuged for 10 min at 8000 g to separate the enzymes from the biomass. The supernatant was then filtered through Whatman filter paper with a pore size of 0.45 µm.

***N*-acetyl-*β*-D-glucosaminidase enzyme activity:** The activity of the enzyme, *N*-acetyl-*β*-glucosaminidase, was assessed by quantifying p-nitro-phenol released from the breakdown of *p*-nitrophenyl-*N*-acetyl-*β*-D-glucosaminide. Test samples (30 µl) were added into wells in a microtiter plate. The substrate (50 µl) was added into the wells of plate containing *p*-nitrophenyl-*N*-acetyl-*β*-D-glucosaminide (300 µg/ml) dissolved in potassium phosphate (50 mM; pH 6.7). This was followed by incubation of plates at 50ºC on Styrofoam beads in a water bath. The enzyme reactions were stopped by adding 50 µl 0.4M Na_2CO_3. The absorbance was measured at 405 nm using a microplate reader and the values in the range 0.05 to 1.00 were considered. The enzyme activity was defined as 1 unit if it released "1 µmol of *p*-nitrophenol per milliliter enzyme per minute under the specified condition".

Endochitinase enzyme activity: The endochitinase activity was assessed by measuring decrease in turbidity of colloidal chitin. The enzyme solution (0.5 ml/sample) was mixed with the 0.5 ml of colloidal chitin that consisted of the moist purified chitin (1% w/v) mixed in potassium phosphate (50 mM) at pH 6.7 and 0.02% NaN_3. The reaction mixture was incubated for 24 h at 25ºC. The mixture was further diluted with water (5 ml) and the absorbance was determined at the wavelength of 510 nm. The percentage reduction in turbidity relative to that of control that contained water rather than enzyme solution was defined as the enzyme activity and 1 enzyme unit equaled the amount of enzyme needed to decrease by 5% the turbidity of the chitin suspension. The data were statistically analyzed by using analysis of variance (ANOVA).

Purification of the extracted enzyme: The extracted enzyme solution was precipitated in the culture filtrate by adding 80% ammonium sulphate in equal volume and kept for 30 min at 4°C in falcon tubes. As a result, a pellet developed upon centrifugation for 20 min at 12000 rpm which was re-suspended in distilled water. The resulting filtrate was dialyzed in 50 mM potassium phosphate buffer at 4°C and pH 6.7 stirring overnight**.** The dialysis tubes were transferred into 4% polyethylene glycol at room temperature until 25-fold reduction in volume. To store the filtrate for more than a week, 0.02 % NaN_3 was added as preservative and kept at 4°C until used.

Use of purified enzyme for antagonistic activity against phytopathogens: The extracted *Trichoderma* enzymes were tested for antifungal ability against the phytopathogens and the percentage inhibition was calculated. The enzyme solution (500 µl) was diluted with the sterile distilled water (500ul), added to the 200 ml PDA and the media were poured into Petri plates. The plates were then inoculated with culture plugs of the fungal phytopathogens placed in center. The control plates consisted of only the medium without enzymes. The plates were completely randomized and each of the test was performed in triplicate. The percentage inhibition as compared to control was noted after three days. The data was analyzed statistically using ANOVA and the means were compared using Fisher's least significant difference (LSD) test.

Results

Fungal plant pathogens isolated from the infected vegetables: The plant pathogenic fungi were isolated from the vegetable fields infected with fungal plant pathogens in the Kohat District of Khyber Pakhtunkhwa Pakistan. The different strains of *Alterneria spp.* namely B9-1F, B9-2F, B6L were obtained from infected plant parts of spinach and egg plant. The different strains of *Fusarium spp.* including J1L, J3L, D3L, D5-1L, D5L2 were picked from the vegetables fields of Ladyguard, chilli and okra. A strain of *Aspergillus spp.* B4 was obtained from the infected onion bulbs. The fungi were identified by the disease symptoms from where they were isolated and the colony morphology.

Screening *Trichoderma spp.* for production of chitinases: *Trichoderma* strains showed chitinolytic activity on chitin CSM (Table 1). The highest chitinase activity was shown by *T. longibrachiatum* TKK4 followed by *T. longibrachiatum* strains TMK5, TMK20, TMK21, TKK3 and TKK6. The strains *T. longibrachiatum* TMK6, TMK9 and *T. virens* TMK8 had moderate activities.

Table 1. *Trichoderma spp* with chitinase activity where 'M' denotes the medium activity 'H' denotes the high activity and 'H+' denotes the highest activity.

Trichoderma species	Strains	Extent of chitinase activity
T. longibrachiatum	TKK4	H+
T. longibrachiatum	TKK3	H
T. longibrachiatum	TKK6	H
T. longibrachiatum	TMK5	H
T. longibrachiatum	TMK20	H
T. longibrachiatum	TMK21	H
T. longibrachiatum	TMK6	M
T. virens	TMK8	M
T. longibrachiatum	TMK9	M

***N*-acetyl-*β*-D-glucosaminidase activity:** *T. longibrachiatum* TMK21 showed the highest enzyme activity (0.017 umol/ml/min). *Trichoderma virens* TMK8 showed the least enzyme activity (0.0014 umol/ml/min). The enzyme activities of 0.003, 0.0071, 0.011, 0.014, 0.016, 0.015 and 0.004 µmol/ml/min were shown by the *T. longibrachiatum* strains TMK5, TMK6, TMK9, TMK20, TKK3, TKK4 and TKK6 respectively.

Endochitinase activity: The highest percentage reduction of turbidity was observed in case of *T. longibrachiatum* TMK9 (72%) while the strain TMK20 exhibited the least reduction (5%; Fig. 1). The rest of *Trichoderma* strains also showed activities i.e. TMK8 (67%), TKK6 (65%), TMK21 (35%), TKK3 (21%), TKK4 (21%), TMK5 (13%) and TMK6 (9%).

T. longibrachiatum TMK20 showed the highest endochitinase activity with 0.503 EU while the lowest was found in TMK9 with 0.033 EU (Fig. 2). The EU of other strains were also recorded i.e. TMK6 (0.423), TMK5 (0.217), TKK4 (0.130), TKK3 (0.123), TMK21 (0.077), TKK6 (0.040) and TMK8 (0.037).

Antagonistic Activity of purified enzyme against phytopathogens: The enzymes extracted from the *Trichoderma spp.* showed antifungal potential against the tested plant pathogens. *T. longibrachiatum* strain TMK21 significantly inhibited *Fusarium spp.* B3 after three days by 67% while the least inhibition was recorded by *T. longibrachiatum* TKK6 (7%; Fig. 3). The %age inhibition of chitinolytic enzymes by other strains were also recorded i.e. TMK20 (63), TKK4 (60), TMK9 (51), TKK3 (39), TMK5 (33), TMK8 (33) and TMK6 (28) against the strain B3.

The highest inhibition of 65% by the strain TMK 21 followed by 60, 58, 44, 30, 30, 18 and 17 % by the *Trichoderma* strains TKK4, TMK20, TMK9, TMK5, TKK3, TMK6 and TMK8 respectively against the *Fusarium spp.* strain D5-1 was observed (Fig. 4).

Enzymes extracted from the strains TKK4 and TMK9 showed the highest inhibition (80%) against *Alterneria spp*. strain B6. On the other hand, the least inhibition was observed in TMK6 (11.75%; Fig. 5). The %age inhibition of 78, 71, 71, 55, 51 and 37 against *Alterneria spp.* B6 was observed in case of the strains TMK21, TMK20, TKK3, TMK5, TKK6 and TMK8 respectively.

The chitinolytic enzymes produced by *Trichoderma* strains TKK4 and TMK20 produced the highest inhibition (58%) against *Alterneria spp.* strain B9 followed by the TMK21 (54%), TKK3 (44%), TMK8 (44%), TMK9 (44%), TKK6 (42%) and TMK5 (38%) (Fig. 6). *Trichoderma* strain TMK6 was the least inhibitory (26%) against this pathogenic fungus.

Aspergillus spp. strain B4 was completely inhibited by the enzymes produced by different *Trichoderma* strains including TMK5, TMK6, TMK21 and TKK6 (Fig. 7). The enzymes from the strains TKK3, TKK4 and TMK20 were inhibitory too against B4 (63%). The strain TMK9 was comparatively less inhibitory (55 %).

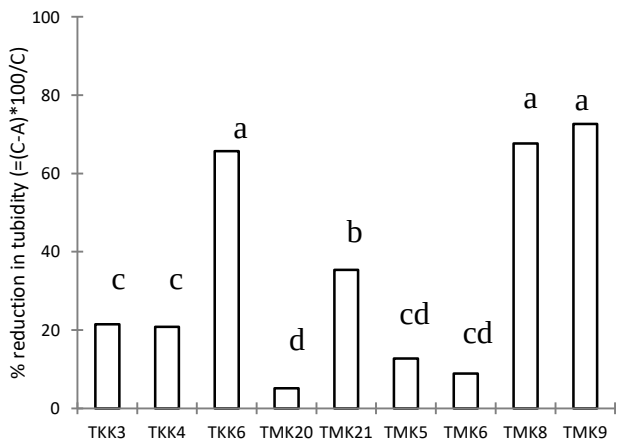

Fig. 1. Percentage reduction in turbidity by endochitinases extracted from the different *Trichoderma* strains at 510 nm where 'C' and 'A' denote the absorbance of the control and the sample respectively. The different bars represent the average of 3 replicates. The data were analyzed by analysis of variance (ANOVA) and the different small letters on the top of each bar indicate the statistically different data (least significant difference at $p<0.05$).

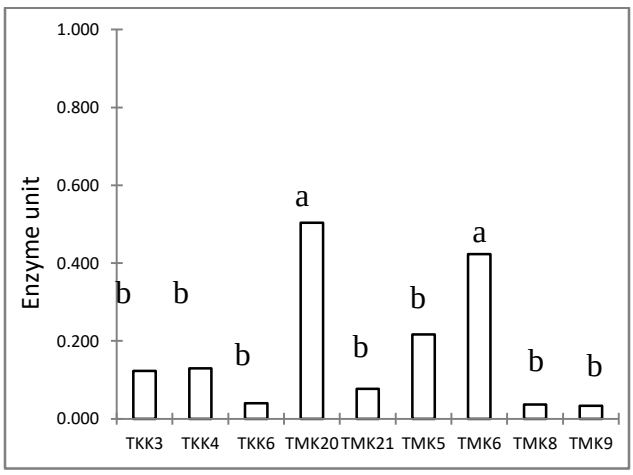

Fig. 2. Enzyme Units of endochitinases extracted from the different *Trichoderma* strains. The different bars represent the average of 3 replicates. The data were analyzed by the analysis of variance (ANOVA) and the different small letters on the top of each bar indicate the statistically different data (least significant difference at $p<0.05$).

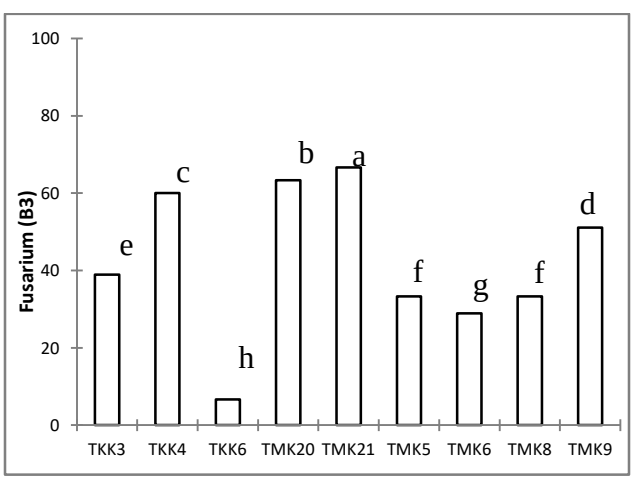

Fig. 3. The %age inhibitory effect of the chitinolytic enzymes of different strains of *Trichoderma spp*. against *Fusarium spp*. B3. The different bars in the graph represent the average of 3 replicates. The data were analyzed by the analysis of variance (ANOVA). The different letters on the top of each bar indicates the statistically different data (least significant difference at $p<0.05$).

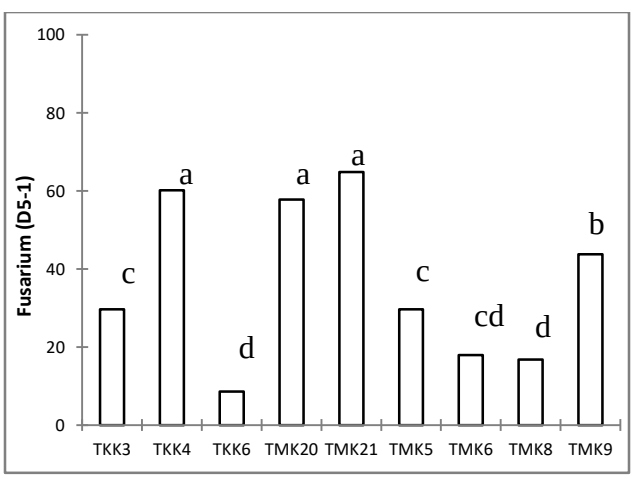

Fig. 4. The %age inhibitory effect of the chitinolytic enzymes of different strains of *Trichoderma spp.* against *Fusarium spp*. D5-1. The different bars in the graph represent the mean of 3 replicates. The data were analyzed by the analysis of variance (ANOVA). The different letters on the top of each bar indicates the statistically different data (least significant difference at $p<0.05$).

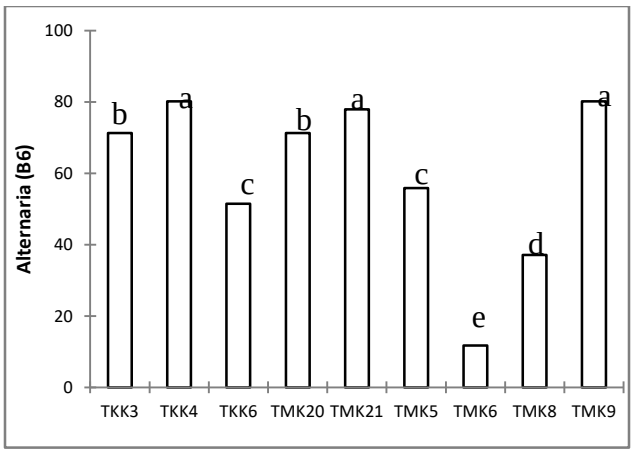

Fig. 5. The %age inhibitory effect of the chitinolytic enzymes of different strains of *Trichoderma spp*. against *Alterneria spp*. B6. The different bars in the graph represent the average of 3 replicates. The data were analyzed by the analysis of variance (ANOVA). The different letters on the top of each bar indicates the statistically different data (least significant difference at $p<0.05$).

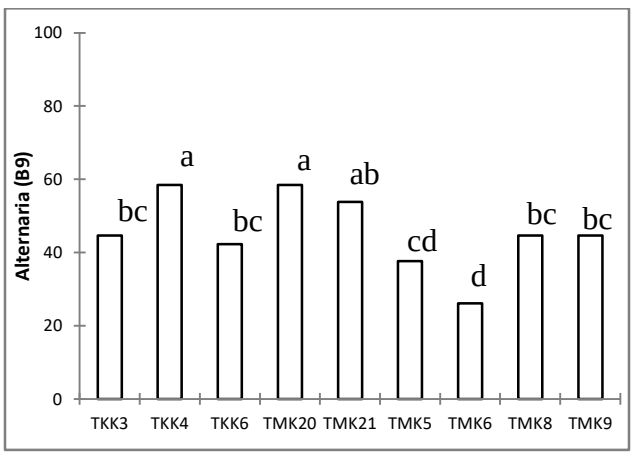

Fig. 6. The %age inhibitory effect of the chitinolytic enzymes of different strains of *Trichoderma spp*. against *Alterneria spp*. B9. The different bars in the graph represent the average of 3 replicates. The data were analyzed by the analysis of variance (ANOVA). The different letters on the top of each bar indicates the statistically different data (least significant difference at $p < 0.05$).

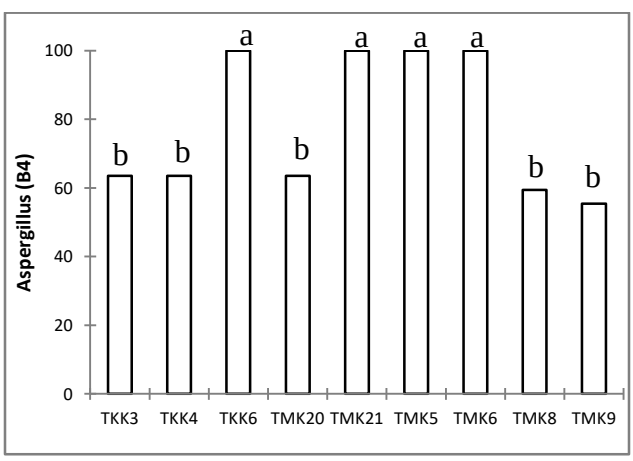

Fig. 7. The %age inhibitory effect of the chitinolytic enzymes of different strains of *Trichoderma spp*. against *Aspergillus spp*. B4. The different bars in the graph represent the average of 3 replicates. The data were analyzed by the analysis of variance (ANOVA). The different letters on the top of each bar indicates the statistically different data (least significant difference at $p < 0.05$).

Discussion

Trichoderma spp. are frequently reported as biological control agent against a variety of plant diseases caused by various microorganisms. Traditionally, the plant diseases are controlled by application of chemical pesticides and fungicides, however, it leads to a number of environmental hazards. The biological control offers an environment-friendly alternative to avoid these chemical hazards (Li *et al.,* 2018; Anees *et al.,* 2010, 2018). The microorganisms known as biocontrol agents are generally used for this purpose. There are a number of biocontrol agents reported so far including *Bacillus* spp., *Paenibacillus* spp., *Pseudomonas fluorescens*, *Lysobacter spp.*, *Trichoderma* spp (Naing *et al.,* 2014; Anees *et al.,* 2018; Li *et al.,* 2018). The advantages of using biocontrol agents have a number of advantages over the use of synthetic fungicides being environment friendly, and more efficient against the plant pathogens resistant to fungicides (Tsror *et al*., 2001; Lee *et al.,* 2013).

Production of CWDEs is a well reported mechanism in biological control activity of *Trichoderma* spp. The CWDEs include chitinases used as a tool by the biocontrol agents of fungal phytopathogens. Fifty-four samples were taken from different plant parts from highly disease-affected fields of vegetables. The different isolated pathogens from the infested vegetable parts included ascomyceteous fungi such as *Alternaria*, *Fusarium*, and *Aspergillus*. We selected 9 strains of *Trichoderma* previously isolated from the Northern and Western areas of Khyber Pakhtunkhwa Pakistan (Anees *et al.,* 2018). They were screened for their chitinolytic activities. *Trichoderma longibrachiatum* TKK4 was found as the most efficient chitinase producer on plates. This study is supported by a number of previous reports such as El-Katatny *et al.,* (2000) reported that *T. harzianum* exhibited the chitinase activity and could hydrolyze the mycelia of the phytopathogenic *Sclerotium rolfsii*. The fungal pathogen, *S. rolfsii* was inhibited by 62 % using the enzyme preparations secreted by *T. harzianum.*

In this study we observed that *T. longibrachiatum* TMK9 was the highest and TMK20 was the lowest producer of endochitinase activities. There are frequent reports about the production of endochitinases by *Trichoderma* spp. (Anand & Reddy, 2009). The higher activities were previously related to the efficiency of *Trichoderma* isolates inducing the acquired systemic resistance in plants (Saksirirat *et al.,* 2009).

Furthermore, *N*-acetyl-*β*-D-glucosaminidase activity for the different *Trichoderma* strains was also investigated in the present study. *Trichoderma longibrachiatum* strain TMK21 showed the highest enzyme activity (0.017 umol/ml/min) while *T. virens* strain TMK8 produced the lowest enzyme activity (0.0014 umol/ml/min) in the present study. Tronsmo & Harman (2004) also observed *N*-acetyl-β-glucosaminidase activity in *Trichoderma* strains. These enzymes were able to break *p*-nitrophenyl- *N*-acetyl-*β*-D-glucosaminide and release p-nitro-phenol. This enzyme plays an important role in the fungal cell wall degradation and helps thereby in killing it. This may be why, *T. longibrachiatum* TMK21 showed higher %age inhibitions against the different fungal phytopathogens. The chitinolytic enzymes produced by the strain TMK21 inhibited *Fusarium* spp. strains B3 and D5,

Alternaria spp. strains B6 and B9, and *Aspergillus* spp. strain B4 by 67, 65, 78, 54, and 100 % respectively in our *in vitro* results. This strain produced the most significant and consistent inhibitory effect comparing with the other strains of *Trichoderma* used in this study. Overall, the *Trichoderma* strains in the present study produced rich mixture of antifungal enzymes in the present study that directly inhibited the phytopathogenic fungi. These results indicated that the chitinolytic mixtures of enzymes possessed integrated modes of action that may be required for maximum output and, furthermore, their correct combinations may increase the antifungal activity (Ruana-Rosa *et al.*, 2017).

Different strains of *T. longibrachiatum* produced chitinolytic enzymes and showed inhibition of variable extents in the present study. Lorito *et al.*, (1993) also showed 95% inhibition of *B. cinerea* a combination of enzymes was used. The variability of *Trichoderma* spp. in their biocontrol ability was also evident at intra specific strains level (Anees *et al.*, 2010). The similar results were reported previously by Matroudi *et al.*, (2009) who showed variability in inhibition of two plant pathogenic isolates of *Sclerotinia sclerotiorum*, and *T. atroviride* showed the maximum inhibitory effect, reducing growth by 85-93%. In another report by Nashwa *et al.*, (2008), the different isolates of *Trichoderma* showed different inhibitory effects against *Rhizoctonia* and *Fusarium*.

In the present study, we are reporting direct antifungal activity of the chitinolytic enzymes produced by indigenous strains of *Trichoderma* that might have resulted in significant antifungal potential of the strains against the plant pathogens (Anees *et al.*, 2018). Further studies are, however, required to investigate the production of the antifungal secondary metabolites by these strains (Naing *et al.*, 2015).

References

Anand, S. and R. Jayarama. 2009. Biocontrol potential of *Trichoderma* Sp. against plant pathogens. *Int. J. Agri. Sci.*, 1(2): 30-39.

Anees, M., R. Azim, S. Ur Rehman, M. Jamil, S. El Hendawy and N.A. Al Suhaiban. 2018. Antifungal potential of *Trichoderma* strains originated from North Western regions of Pakistan against the plant pathogens. *Pak. J. Bot.*, 50(5): 2031-2040.

Anees, M., A. Tronsmo, V. Edel-Hermann, L.G. Hjeljord, C. Héraud and C. Steinberg. 2010. Characterization of field isolates of *Trichoderma* antagonistic against *Rhizoctonia solani*. *Fung. Biol.*, 114: 691-701.

Chet, I., J. Inbar and I. Hadar. 1997. Fungal antagonists and mycoparasites. *In:* Wicklow, D.T. and B. Soderstrom (eds.), *The Mycota IV: Environmental and microbial relationships. Springer-Verlag*, Berlin. pp 165-184.

El-Katatny, M.H., W. Somitsch, K.H. Robra, M.S. El-Katatny and G.M. Gubitz. 2000. Production of chitinase and b-1, 3-glucanase by *Trichoderma harzianum* for control of the phytopathogenic fungus *Sclerotium rolfsii*. *Food Tech. Biol.*, 38: 173-180.

Harman, G.E., C.R. Howell, A. Viterbo, I. Chet and M. Lorito. 2004. *Trichoderma* species-opportunistic, avirulent plant symbionts. *Nat. Rev. Micro*., 2: 43-56.

Hjeljord, L. and A. Tronsmo. 1998. *Trichoderma* and *Gliocladium* in biological control: an overview. *In:* Harman, G. E. and C. Kubicek, *Trichoderma and Gliocladium: enzymes, biological control and commercial applications. Vol. 2*. London, United Kingdom: Taylor and Francis, Ltd. pp. 131-151.

Lee, Y.S., S.P. Yun, M. Anees, Y.C. Kim, H.K. Yong and K.Y. Kim. 2013. Nematicidal activity of *Lysobacter capsici* YS1215 and the role of gelatinolytic proteins against root-knot nematodes. *Biocon. Sci. Technol., 23*(12): 1427-1441.

Li, Z., T. Wang, X. Luo, X. Li, C. Xia and Yuqiang Zhao, *et al*., 2018. Biocontrol potential of *Myxococcus* sp. strain BS against bacterial soft rot of calla lily caused by *Pectobacterium carotovorum*. *Biolog. Cont.*, 126: 36-44.

Lorito, M. 2005. Molecular Biology of the Interactions between *Trichoderma*, Phytopathogenic Fungi and Plants: Opportunities for Developing Novel Disease Control Methods. Second Global Conference. *Plant Health-Global Wealth*. 162-163.

Lorito, M., C.K. Hayes, A. Di Pietro, S.L. Woo and G.E. Harman. 1994. Purification, characterization and synergistic activity of a glucan 1,3-β-glucosidase and an *N*-acetyl-β- glucosaminidase from *Trichoderma harzianum*. *Phytopathol.*, 84: 398-405.

Lorito, M., F. Scala, A. Zoina and S.L. Woo. 2001. Enhancing biocontrol of fungal pests by exploiting the *Trichoderma* genome, in *Enhancing Biocontrol Agents and Handling Risks*, *IOS Press*, Amsterdam. pp. 248-259.

Lorito, M., G.E. Harman, C.K. Hayes, R.M. Broadway, A. Tronsmo, S.L. Woo and A. Di Pietro. 1993. Chitinolytic enzymes produced by *Trichoderma harzianum*: antifungal activity of purified endochitinase and chitobiosidase. *Phytopathol.*, 83: 302-307.

Matroudi, S., M.R. Zamani and M. Motallebi. 2009. Antagonistic effects of three species of *Trichoderma* sp. on *Sclerotinia sclerotiorum*, the causal agent of canola stem rot. *Egypt. J. Biol.*, 11: 37-44.

Naing, K.W., M. Anees, J.K. Sang, N. Yi, Y.C. Kim and K.Y. Kim. 2014. Characterization of antifungal activity of *Paenibacillus ehimensis*, KWN38 against soilborne phytopathogenic fungi belonging to various taxonomic groups. *Ann. Microbiol.*, 64(1): 55-63.

Naing, K.W., Y.S. Lee, X.H. Nguyen, M.H. Jeong, M. Anees, B.S. Oh, J.H. Moon and K.Y. Kim. 2015. Isolation and characterization of an antimicrobial lipopeptide produced by paenibacillus ehimensis ma2012. *J. Basic Microbiol., 55*(7): 857-868.

Nashwa, M.A., K.A.M. Sallam, Abo-Elyousr and M.A.E. Hassan. 2008. Evaluation of *Trichoderma* Species as biocontrol Agents for Damping-Off and Wilt Diseases of *Phaseolus vulgaris* L. and Efficacy of Suggested Formula. *Egypt. J. Phytopath.*, 36(1-2): 81-93.

Roberts, W.K. and C.F. Selitrennikoff. 1988. Plant and bacterial chitinases differ in antifungal activity. *J. Gen. Micro.*, 134: 169-176.

Ruano-Rosa, D., I. Arjona-Girona and C.J. López-Herrera. 2017. Integrated control of avocado white root rot combining low concentrations of fluazinam and *Trichoderma* spp. *Crop Protec.*, 112: 363-370.

Saksirirat, W., P. Chareerak and W. Bunyatrachata. 2009. Induced systemic resistance of biocontrol fungus, *Trichoderma* spp.against bacterial and gray leaf spot in tomatoes. *Asian. J. Food & Agro-Ind.*, pp. 99-104.

Tronsmo, A. and G.E. Harman. 2004. Coproduction of chitinolytic enzymes and biomass for biological control by *Trichoderma harzianum* on media containing chitin. *Bio. Cont.*, 2(4): 272-277.

Tsror, L., R. Barak and B. Sneh. 2001. Biological control of black scurf on potato under organic management. *Crop Protec.*, 20(2): 145-150.

Woo, S.L. and M. Lorito. 2007. Exploiting the interactions between fungal antagonists, pathogens and the plant for biocontrol. *In:* Vurro, M. and J. Gressel (Eds.), *Novel Biotechnologies for Biocontrol Agent Enhancement and Management. IOS*, Springer Press, Amsterdam, the Netherlands. pp. 107-130.

Woo, S.L., F. Scala, M. Ruocco and M. Lorito. 2006. The molecular biology of the interactions between *Trichoderma* spp., phytopathogenic fungi, and plants. *Phytopathol.*, 96: 181-185.

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