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RESEARCH PAPER

In-vitro evaluation of antifungal potential of some fungal bio-agents against different *Alternaria* species

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Key Massage: In the present study it has been explored that *Trichoderma viride, Trichoderma koningii* and *Trichoderma hamatum* can be used as promising biological control agents against *Alternaria* species which cause different diseases in various kharif vegetables and other crops.

Abstract: Biological control is always being encouraged to manage several plant pathogens for eco-friendly management. The aim of the current manuscript was to evaluate antifungal potential of some fungal bio-agents (BCAs) against different *Alternaria* species (*Alternaria tenuissima*, *Alternaria solani* and *Alternaria alternata*) isolated from major Kharif vegetables *viz* chilli, tomato and eggplant, respectively. The *in-vitro* antifungal potential of different BCAs revealed significantly ($P < 0.05 = 0.0000$) highest efficacy percent over control against *A. solani* of tomato fruit rot with *T. viride* (54.99%) followed by *Paecilomyces* sp. (53.33%), *T. hamatum* (44.98%) and *T. harzianum* (31.12%), whereas the lowest efficacy percent was observed with *Penicillium* sp. (3.37%) when tested with a dual culture methods. In a volatile method, significantly highest efficacy percent was noticed with *T. viride* (76.667%), whereas lowest was observed with *Paecilomyces* sp. (21.019 %). The efficacy of different BCAs against *A. tenuissima* of chilli fruit rot exhibited significantly highest percent with *T. viride* (53.33%) followed by *T. hamatum* (49.28%), *Paecilomyces* sp. (46.29%) and *T. koningii* (40.927%) through dual culture methods, however in volatile method, significantly highest efficacy percent was noticed with *T. viride* (78.611 %) followed by *T. koningii* (74.259%) and *T. hamatum* (64.63%). Concerning eggplant leaf spot caused by *A. alternata*, highest efficacy percent was recorded with *Paecilomyces* (48.702%) followed by *T. viride* (46.29 %) and *T. hamatum* (43.705%) when tested through dual culture methods, while in volatile method highest efficacy percent was recorded with *T. koningii* (77.778%) followed by *T. viride* (73.148%) and *T. hamatum* (68.704%). In conclusion, *T. viride*, *T. koningii* and *T. hamatum* have maximum antifungal potential against *Alternaria* sp. Thus, these three BCAc should be used as promising BCAs against *Alternaria* sp. in various Kharif vegetables and other crops for sustainable production.

Keywords: *Alternaria* species, Bio-agents, Kharif vegetables, Leaf spot and fruit rot

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Introduction

Alternaria species are remained as an increasing threat to several crops around the globe and causing various diseases. *Alternaria* diseases cause huge yield losses and reduce the economic value of the crop plants in conventional production systems (Gaya Karunasinghe et al., 2020). The blight disease in tomato and *Alternaria* leaf spot in chilli caused by *Alternaria* sp. are economically important diseases. The pathogen has been reported to cause seed, seedling, leaf, fruit diseases as well. Post-harvest decay of fruit and seed has also been reported due to this pathogen (El-Garhy et al., 2020). It is obvious that several *Alternaria* spp. exist to cause infections in the economically important crops. Therefore, it is essential to manage the pathogen effectively using various methods applicable to reduce the intensity. The planting of susceptible varieties in the field should be avoided from the infected residues of a previous crop retained on the surface (Chávez-Ramírez et al., 2020). Apart from this, balanced crop nutrition especially potassium should be provided. The utilization of different herbal extracts and natural products cause no health hazard and pollution, thus are being encouraged. The extract of garlic bulb is effective to restrict the mycelial growth of *A. tenuis* causing brinjal leaf spot. The neem leaf extract showed high efficacy by reducing the radial growth of *A. solani* (Luo et al., 2020; Mulugeta et al., 2020).

Bio-control agents are also being encouraged to control the *Alternaria* sp. The reason of increased application of these bio-agents is their ecology. Primarily antagonists and plant pathogens specific for bio-control can operate several mechanisms synergistically or independently in any microbial interaction (Maeda et al, 2020). In radish, seed-borne, *A. raphani* and *A. brassicicola* are effectively controlled through the use of some antagonists like *C. globosum*, *T. harzianum*, *T. koningii* and *Fusarium* sp. (Youdkes et al., 2020). *Trichoderma* and *B. subtilis* are reported to inhibit the mycelial growth of *A. solani*, the leaf blight causing fungus of tomato. *Trichoderma* sp. are avirulent symbionts, functioning as antagonists of many phytopathogenic fungi, present in plant root systems and soil. *Trichoderma* sp. are widely used as bio-control agents for the management of crop plants against several types of plant pathogens (Zhao et al., 2020). Their bio-control potential has also been explored against several foliar diseases by seed or soil application indicating their effectiveness in inducing disease resistance in the crop plants.

Interestingly, similar to *Rhizobacteria*, *Trichoderma* sp. have also been exploited as a growth promoting agent. Thus, it has the potential as a preferred input in Integrated Disease Management (IDM) systems (Terna et al., 2020). Although bio-control agents have tremendously applied in different plants to control different diseases; however, special focus on Kharif crops has never been stated. Keeping these facts in mind, present study was planned to evaluate the potential bio-agents against different *Alternaria* spp. *in-vitro.* Moreover, *Alternaria* spp. were isolated and confirmed from major Kharif vegetable hosts viz; chilli, tomato and eggplant.

Materials and Methods

Study location

The present study was conducted in the laboratories of the Department of Plant Protection, Sindh Agriculture University, Tandojam, Pakistan from February to October, 2017.

Isolation of *Alternaria* spp. from major Kharif vegetables

The samples of major Kharif vegetables *viz* chilli, tomato fruit and eggplant leaves showing typical symptoms of leaf and fruit rot were collected from chilli, eggplant and tomato growing fields and placed in the perforated polythene bags. Samples were brought to the laboratories of the Plant Protection Department for further analysis. The collected samples were thoroughly washed with tap water. Small pieces of infected portion (3 mm in length) were cut at the junction of diseased and healthy tissues with the help of alcohol sterilized sharp blades. These pieces were surface sterilized in 0.1 per cent mercuric chloride solution ($HgCl_2$) for 1 minute followed by three washings with sterilized distilled water in beakers under aseptic conditions using laminar air flow. The pieces were then completely dried by placing on sterilized blotting paper. Five bits were transferred aseptically to the petri-plates containing sterile potato dextrose agar (PDA) medium amended with an antibacterial agent and filled up to quarter strength. The inoculated plates were incubated at 25 ± 2 ^{o}C. All the plates were monitored regularly and growing colonies were subjected to different laboratory codes for further analysis. About 3-5 isolations were made throughout the experiment. The culture, thus, obtained was subjected to purification. A single spore culture technique was used to purify the isolates. Sub-culturing of isolates were made from time to time to maintain the fresh culture for further analysis until the end of experiments.

Identification of pathogens

Slides of fungal isolates from pure cultures were made and observed under a light microscope. Morphological and cultural characteristics of isolated fungi were recorded and compared with standard keys for establishing their identity (Booth & Sutton, 1984; Singh, 1987; Brayford, 1993). In addition, internet databases were also used to compare the morphological characteristics of isolates (Fig. 1).

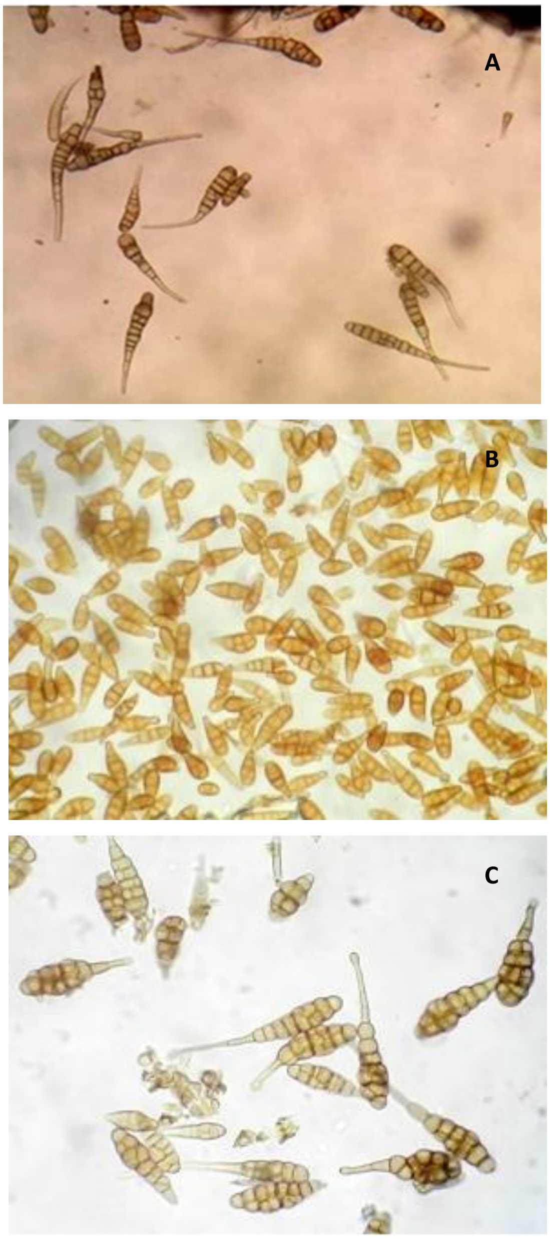

Fig. 1 Morphological characteristics of different *Alternaria* spp. (A) *Alternaria solani* (B) *Alternaria tenuissima* (C) *Alternaria alternata*

In-vitro antifungal evaluation of different biological control agents (BCAs)

The *in-vitro* antifungal assay of different BCAs against different *Alternaria* spp. (*A. tenuissima*, *A. solani* and *A. alternata*) isolated from major Kharif vegetables *viz* chilli, tomato and eggplant, respectively, was conducted in order to determine the antifungal potential of BCAs. Isolated cultures were identified using morphological characteristics under the light microscope. A total of 6 different BCAs were assessed *in-vitro* through dual culture and volatile methods. The experiment was conducted in complete randomized design (CRD) with six replications. Petri dishes containing PDA medium were inoculated with a 5 mm disk of freshly prepared culture of both fungi, bio-agent and control fungus (*Alternaria* spp.) under aseptic conditions. The PDA plates inoculated with only controlled fungus (*Alternaria* spp.) were treated as control. The details of both techniques are summarized here:

Dual culture and volatile methods

A total of six BCAs were used against three *Alternaria* spp. (*A. tenuissima*, *A. solani* and *A. alternata*) through dual culture methods. The PDA plates in dual culture methods were inoculated by placing 5 mm freshly cultured BCAs disk placed 2 cm away from the edge of the petri plate one side. Similarly, 5 mm of test fungi were placed 2 cm away from the edge of the petri dish on the opposite side with BCAs. Thus, PDA plates contained one side of bio-agent and opposite side of pathogen. Six replications were maintained for each pathogen along with bio-agent and six plates were kept as control containing test fungi without BCAs. The data on radial mycelial colony growth in both treated and control was recorded on a daily basis until the colony growth of control plates became full. The percent inhibition of radial mycelial growth over control was calculated using the below given formula:

$$I = \frac{(C - T)}{C} \text{X100}$$

Where I = Percent inhibition, C = Radial colony growth in control, T = Radial colony growth in treatment.

In the volatile method, similar six BCAs were used against three *Alternaria* spp. (*A. tenuissima*, *A. solani* and *A. alternata*). Six replications were maintained for each treatment including control under aseptic conditions. PDA plates of each treatment were separately inoculated with 5mm disk at the center plates for both bio-agent and test fungi. Thereafter, on the next day the lids of both plates were took off and plates were assembled in such a way that in one opposed bio-agent disc was placed and in another opposed test fungi was placed. All assembled plates were then carefully wrapped with tape to avoid contamination. The control plates were maintained without BCAs that only contained test fungi, *Alternaria* spp. (*A. tenuissima*, *A. solani* and *A. alternata*). The data on radial mycelial growth in both treated and control was recorded on a daily basis until the colony growth of control plates became full. The percent inhibition of radial mycelial colony growth over control was calculated by using the formula as described earlier.

Source of bio-control agents

Two strains of *Trichoderma* sp. were kindly provided by Plant Pathology Section, ARC, Tandojam. However, the other four were obtained from the laboratory of the Plant Protection Department, Sindh Agriculture University, Tandojam, Pakistan (Table 1).

Table 1 List of biological control agents used in the current study

S. No.	Bio-agents	Source
1	*Trichoderma viride* Pers.	Plant Protection Department
2	*Trichoderma hamatum*	Plant Pathology Section, ARC
3	*Trichoderma harzianum* Rifai	Plant Pathology Section, ARC
4	*Trichoderma koningii* Oudern	Plant Pathology Section, ARC
5	*Paecilomyces* sp.	Plant Protection Department
6	*Penicillium* sp.	Plant Protection Department

Statistical analysis

The data obtained were statistically analyzed using the computer software Statistix 8.1 (Analytical Software, 2005). Standard procedures for analysis of variance, ANOVA (linear model), and mean separation (least significant difference, LSD) of all parameters including frequency (%), inhibition (%) (After calculating with corresponding formulae) were used. All differences described in the text were considered significant at the 5% level of probability.

Results

Antifungal potential of BCAs through dual culture methods

The antifungal potential of different BCAs for the inhibition of radial mycelial colony growth of different *Alternaria* spp. of major Kharif vegetables *viz* tomato, chilli and eggplant showed significant ($P<0.05$) influence, respectively. Similarly, the efficacy percent of different BCAs also revealed the significant variation among different treatments of BCAs and different *Alternaria* spp. of major Kharif vegetables *viz* tomato, chilli and eggplant. Significantly lowest radial mycelial colony growth of *A. solani* of tomato leaf spot was recorded with *T. viride* (20.25 mm) followed by *Paecilomyces* sp. (21 mm), *T. hamatum* (24.75 mm) and *T. koningii* (28.67 mm). However, the highest radial colony growth was recorded in control (44.5mm) followed by *Penicillium* sp. (43 mm) and *T. harzianum* (31 mm) when tested through dual culture method (Fig. 1 and 3). The efficacy percent of different BCAs over control against *A. solani* of tomato leaf spot showed significantly highest percent with *T. viride* (54.99%) followed *Paecilomyces* (53.33%), *T. hamatum* 44.98%), *T. koningii* (36.29%) and *T. harzianum* (31.12%). Whereas the lowest efficacy percent was observed with *Penicillium* sp. (3.37%) and against *A. solani* when tested with dual culture method (Fig. 2 and 3).

The radial colony growth of *A. tenuissima* of chilli leaf spot also showed significant variation for different BCAs. Significantly lowest radial mycelial colony growth was recorded with *T. viride* (21 mm) followed by *T. hamatum* (22.83 mm), *Paecilomyces* (24.167 mm) and *T. koningii* (26.583 mm). Whereas, the highest radial colony growth *A. tenuissima* was recorded with control (44.333 mm) followed by *Penicillium* sp. (44.17 mm) and *T. harzianum* (34.167 mm) when assessed through dual culture methods (Fig. 1). The efficacy of different BCAs revealed a great potential against *A. tenuissima* of chilli leaf spot. Significantly highest efficacy percent over control was noticed when treated with *T. viride* (53.33%) followed by *T. hamatum* (49.28%), *Paecilomyces* sp. (46.29%) and *T. koningii* (40.927%) through *in-vitro* dual culture methods. It was observed lowest when treated with *Penicillium* sp. (2.035%) and *T. harzianum* (24.075 %) through *in-vitro* dual culture methods (Fig. 2 and 3). The effect of different BCAs against *A. alternata* of eggplant leaf spot showed great variation in the reduction of radial mycelial colony growth. Here the radial mycelial colony growth of *A. alternata* was significantly inhibited with *Paecilomyces* sp. (23.083 mm) followed by *T. viride* (24.167 mm), *T. hamatum* (25.33 mm) and *T. koningii* (29.417 mm). The higher radial mycelial colony growth of *A. alternata* was observed in control (44.833 mm) followed by *Penicillium* sp. (42 mm) and *T. harzianum* (33.417 mm) when tested through dual culture methods (Fig. 1). The efficacy of different BCAs over control against *A. alternata* of eggplant leaf spot showed great antifungal potential. The greatest efficacy percent over control was recorded with *Paecilomyces* (48.702%) followed by *T. viride* (46.29%), *T. hamatum* (43.705%) and *T. koningii* (34.63%) when tested against *A. alternata* through dual culture methods. It was lowest with *Penicillium* sp. (6.66%) and *T. harzianum* (25.74%) against *A. alternata* (Fig. 3 and 4).

On overall basis the mean of all three *Alternaria* spp. revealed significantly lowest radial mycelial colony growth with *T. viride* (21.806 mm) followed by *Paecilomyces* (22.75 mm), *T. hamatum* (24.306 mm) and *T. koningii* (28.222 mm). It was highest in control (44.56 mm) followed by *Penicillium* sp. (43.306 mm) and *T. harzianum* (32.861 mm) when tested through dual culture methods. Significantly greatest efficacy percent over control on overall basis for all three *Alternaria* spp. was recorded with *T. viride* (51.543%) followed by *Paecilomyces* sp. (49.44%), *T. hamatum* (45.98%) and *T. koningii* (37.285%). It was observed poorest with *Penicillium* spp. (4.0106%) and *T. harzianum* (26.976%) when tested through dual culture methods against all three *Alternaria* spp. (Fig. 3 and 4).

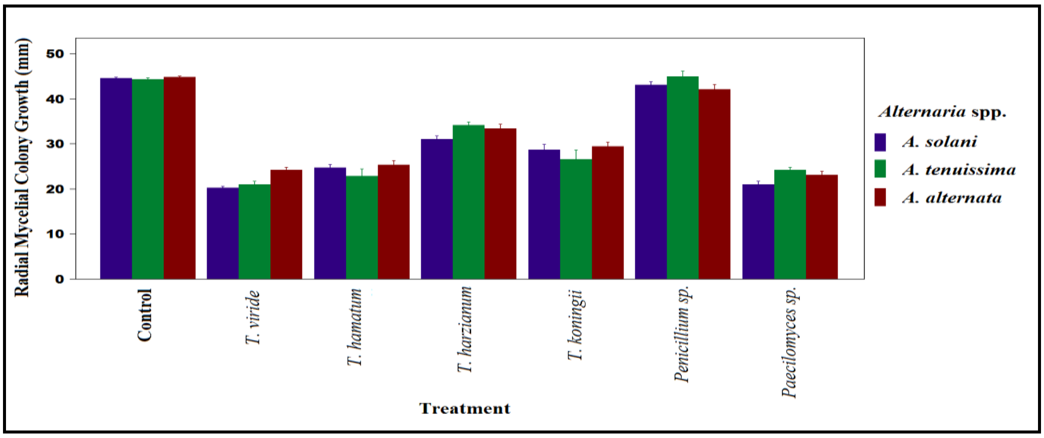

Fig. 2 *In-vitro* effect of different bio-agents assessed through dual culture methods against radial mycelial colony growth of different *Alternaria* spp.

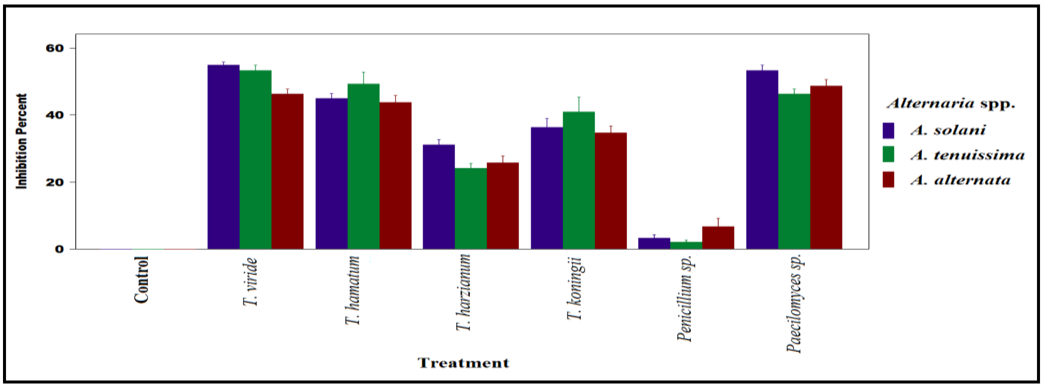

Fig. 3 *In-vitro* antifungal efficacy of different bio-agent assessed through dual culture methods against different *Alternaria* spp. from major Kharif vegetables

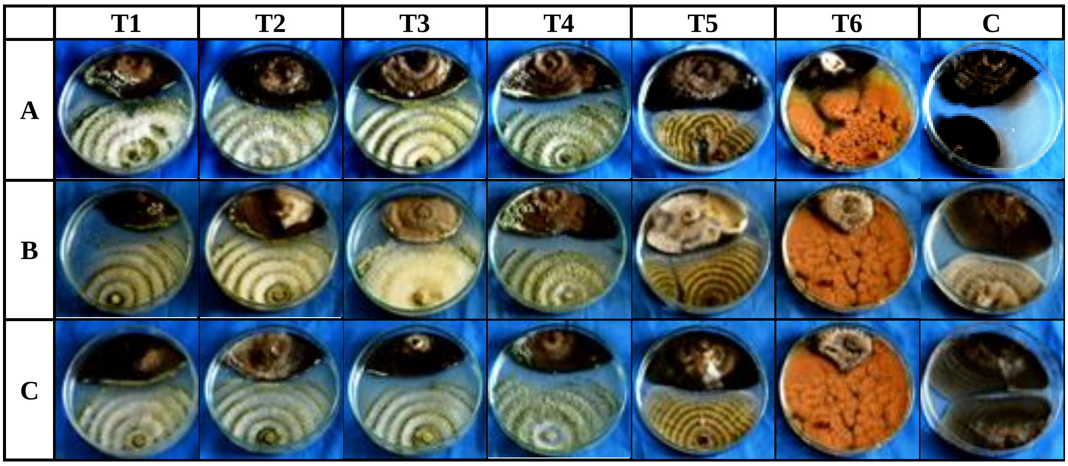

Fig. 4 Effect of different BCAs on three *Alternaria* spp. through dual culture methods
A = *Alternaria solani;* B = *Alternaria tenuissima;* C = *Alternaria alternata;* D = Combined

Antifungal potential of different BCAs through volatile method

The antifungal potential of different BCAs through volatile method for the inhibition of radial mycelial colony growth of different *Alternaria* spp. of major Kharif vegetables *viz* tomato, chilli and eggplant showed significant (P<0.05) impact, respectively. Similarly the efficacy percent of different BCAs through volatile method also revealed the significant variation among different treatments of BCAs and different *Alternaria* spp. of major Kharif vegetables viz; tomato, chilli and eggplant. Significantly lowest radial mycelial colony growth of *A. solani* of tomato leaf spot was recorded with *T. viride* (21 mm) followed by *T. hamatum* (27.167 mm), *T. koningii* (29.25 mm) and *T. harzianum* (31.5 mm). However, the highest radial colony growth was recorded in control (89 mm) followed by *Penicillium* sp. (71.083 mm) and *Paecilomyces* (60.58 mm) when tested through volatile method (Fig. 5). The efficacy percent of different BCAs over control against *A. solani* of tomato leaf spot showed significantly highest percent with *T. viride* (76.667%) followed by *T. hamatum* (69.815%), *T. koningii* (67.5%) and *T. harzianum* (65%). Whereas the lowest efficacy percent was observed with *Paecilomyces* (21.019 %) and *Penicillium* sp. (32.685%) against *A. solani* when tested through volatile method (Fig. 6).

The radial colony growth of *A. tenuissima* of chilli leaf spot also showed significant variation for different BCAs. Significantly lowest radial mycelial colony growth was recorded with *T. viride* (19.25 mm) followed by *T. koningii* (23.167 mm), *T. hamatum* (31.833 mm) and *T. harzianum* (34.917 mm). Whereas, the highest radial colony growth *A. tenuissima* was recorded with control (88.67 mm) followed by *Paecilomyces* (46.167 mm) and *Penicillium* sp. (74.167 mm) when assessed through volatile method (Fig. 5 and 6). The efficacy of different BCAs revealed a great potential against *A. tenuissima* of chilli leaf spot. Significantly highest efficacy percent over control was noticed when treated with *T. viride* (78.611%) followed by *T. koningii* (74.259%), *T. hamatum* (64.63 %) and *T. harzianum* (61.204%) through *in-vitro* volatile method, however it was observed lowest when treated with *Paecilomyces* (17.593%) and *Penicillium* sp. (48.704%) (Fig. 5).

The effect of different BCAs against *A. alternata* of eggplant leaf spot showed great variation in the reduction of radial mycelial colony growth. Here the radial mycelial colony growth of *A. alternata* was significantly inhibited with *T. koningii* (20 mm) followed by *T. viride* (24.167 mm), *T. hamatum* (28.167 mm) and *T. harzianum* (32 mm) when tested through volatile method. The higher radial mycelial colony growth of *A. alternata* was observed in control (89.167 mm) followed by *Paecilomyces* (46.83 mm) and *Penicillium* sp. (68.167 mm) when tested through a volatile method (Fig. 6 and 7). The efficacy of different BCAs over control against *A. alternata* of eggplant leaf spot showed great antifungal potential. The greatest efficacy percent over control was recorded with *T. koningii* (77.778%) followed by *T. viride* (73.148%), *T. hamatum* (68.704%) and *T. harzianum* (64.44%) when tested against *A. alternata* through volatile method. It was lowest with *Paecilomyces* (24.259%) and *Penicillium* sp. (47.96%) against *A. alternata*. On overall basis the means of all three *Alternaria* spp. revealed significantly lower radial mycelial colony growth with *T. viride* (21.472 mm) followed by *T. koningii* (24.139 mm), *T. hamatum* (29.056 mm) and *T. harzianum* (32.806 mm). It was highest in control (88.94 mm) followed by *Paecilomyces* (51.194 mm) and *Penicillium* sp. (71.139 mm) when tested through a volatile method. Significantly greatest efficacy percent over control on overall basis for all three *Alternaria* spp. was recorded with *T. viride* (76.142%) followed by *T. koningii* (73.179%), *T. hamatum* (67.716%) and *T. harzianum* (63.549%). It was observed poorest with *Paecilomyces* (20.957%) and *Penicillium* spp. (43.117%) when tested through a volatile method against all three *Alternaria* spp. (Fig. 5 and 6).

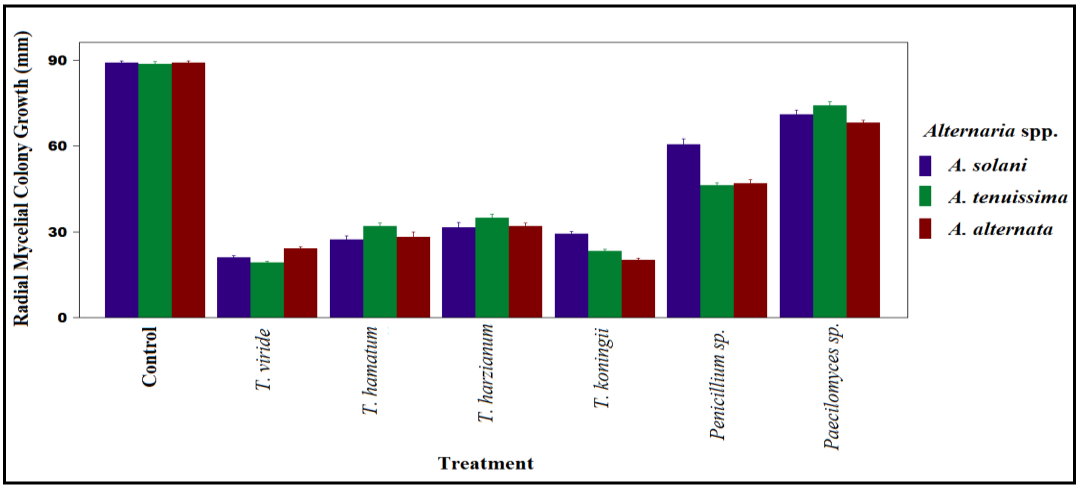

Fig. 5 *In-vitro* effect of different bio-agent assessed through volatile method against radial mycelial colony growth of different *Alternaria* spp. from major Kharif vegetables

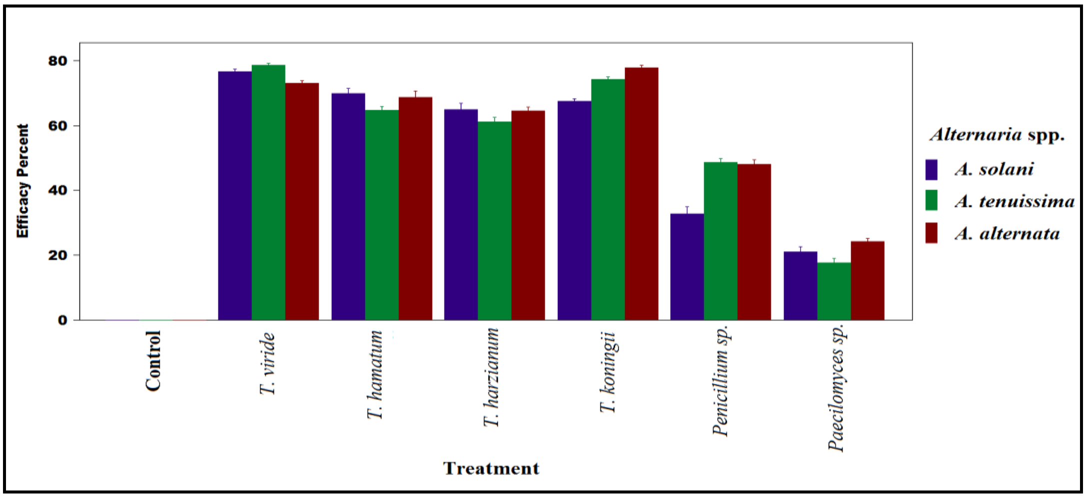

Fig. 6 *In-vitro* antifungal efficacy of different bio-agent assessed through volatile method against different *Alternaria* spp. from major Kharif vegetables

Discussion

The pathogen evolution is a continuous process and has been accelerated by modern plant trade. Nurseries are now prime grounds for species mixing and exposure to new hosts. On the other hand, climate change may have mixed effects on disease establishment. Therefore, it is necessary to evaluate the potential control measures, especially bio-agents from time to time as proper management of existing and new species of *Alternaria* genus can be achieved successfully. Several studies are in agreement with the current research results, however, the specific strains of *Trichoderma* varied and that is due to environmental conditions and natural evolution in pathogens as well as in bio-agents. It has been mentioned that *Trichoderma* sp. are among the most frequently isolated soil fungi and present in the plant root system. These fungi are opportunistic avirulent symbionts and function as antagonists of many phytopathogenic fungi. Similar to our findings, Goudjal et al. (2016); Shenashen et al. (2017); Li et al. (2018); Malandrakis et al. (2018); Rguez et al. (2018); Testen & Miller, (2018); Yang et al. (2019); Koka et al. (2020); Tyagi et al. (2020) also reported the supportive results. Further, *Trichoderma* spp. as bio-control agents are widely used in management of fungal diseases of crop plants exhibiting mycoparasitism against a wide range of plant pathogens. Their bio-control potential has also been explored against several foliar diseases by seed or soil application indicating their effectiveness in inducing disease resistance in the crop plants. These findings are in line with (Li et al., 2018; Zhang et al., 2018; Jing-Yu et al., 2020).

In the current study, in addition to *Paecilomyces* and *Penicillium* sp. all *Trichoderma* sp. were explored against foliar pathogens. Some species of *Trichoderma viz T. harzianum, T. viride, T. virens* and *T. koningii* are well known antagonists and are being utilized to control plant pathogens under field conditions as reported by (Hamid et al., 2014; Abdeljalil et al., 2016; Elshahawy et al., 2018; Jangir et al., 2018; Kashyap et al., 2020). The reported species of earlier studies such as *T. harzianum, T. viride, T. virens* and *T. koningii* though are used under field conditions; however, in current study these strains significantly produced greatest efficacy against *Alternaria* spp. Karthika et al. (2020) also studied the biological control of *Alternaria* fruit rot of chilli by *Trichoderma* sp. under field conditions. Application of *T. harzianum* significantly suppressed the disease compared to *A. tenuis* (T2) treatment. Our study was also in agreement with Liu et al. (2019) who evaluated ten *Trichoderma* spp. against sensitive and resistant isolates of *A. tenuissima* in dual culture methods and recorded up to 80% antagonistic activity by *T. viride, T. koningii* and *T. pseudokoningii*. Moreover, our studies are consistent with Shao et al. (2018) who reported that *Trichoderma* sp. are omnipresent and very popular as effective means of plant disease management. In their study, *T. viride* and *T. harzianum* were screened against *A. alternata* and other fungi by dual culture techniques and the efficacy of volatile and non-volatile metabolites released by them were evaluated by inverted plate method and poisoned food technique. Both antagonists exerted inhibitory effects on the growth of selected seed borne phytopathogens to a varied extent.

Our findings are in accordance with Wang et al. (2019) who revealed that *Trichoderma* sp. are well-known biological agents and have significant antagonistic activity against several plant pathogenic fungi. In their study, *Trichoderma* sp. were tested *in-vitro* for their antagonistic activity against different sp. of *Alternaria viz A. alternata*, *A. brassicae* and *A. solani* by dual plate assays and by the production of volatile and non-volatile compounds. The results obtained revealed that *T. harzianum* and *T. viride* effectively inhibited the growth and spore production of different sp. of *Alternaria*. The highest growth inhibition was found in *A. alternata* (62.50% and 60.00%) by non-volatile compounds of *T. harzianum* and *T. viride*, respectively. Similarly, the volatile compounds inhibited the maximum growth of *A. alternata* (40% and 35%) by *T. harzianum* and *T. viride*, respectively. The results of the present study also confirmed that *T. viride, T. koningii* and *T. hamatum* can be used as promising biological control agents against *Alternaria* spp. that cause diseases in various vegetables crops.

The antifungal potential of different BCAs for the inhibition of radial mycelial colony growth of different *Alternaria* spp. of major Kharif vegetables *viz* tomato, chilli and eggplant showed significant influence, respectively. Specifically with reference to *A. solani* causing tomato leaf spot, the efficacy percent of different BCAs over control showed significantly highest percent with *T. viride* (54.99%) followed by *Paecilomyces* (53.33%), *T. hamatum* 44.98%) and *T. koningii* (36.29%). The efficacy of different BCAs against *A. tenuissima* of chilli leaf spot also showed significantly higher efficacy percent over control when treated with *T. viride* (53.33%) followed by *T. hamatum* (49.28%), *Paecilomyces* sp. (46.29%) and *T. koningii* (40.927%) through *in-vitro* dual culture methods. When *A. alternata* of eggplant leaf spot was treated with these BCAs, showed great antifungal potential specially *Paecilomyces* (48.702%) followed by *T. viride* (46.29%), *T. hamatum* (43.705%) and *T. koningii* (34.63%). Through a volatile method, significantly greater efficacy of BCAs against all three *Alternaria* spp. was recorded with *T. viride* (76.142%) followed by *T. koningii* (73.179%), *T. hamatum* (67.716%) and *T. harzianum* (63.549%). Our results are in agreement with those of Xu et al. (2014); Awan et al. (2018); Debbi et al. (2018); Deng et al. (2020); El-Katatny & Emam (2020); Kamou et al. (2020); Karthik et al. (2020); Ling et al. (2020).

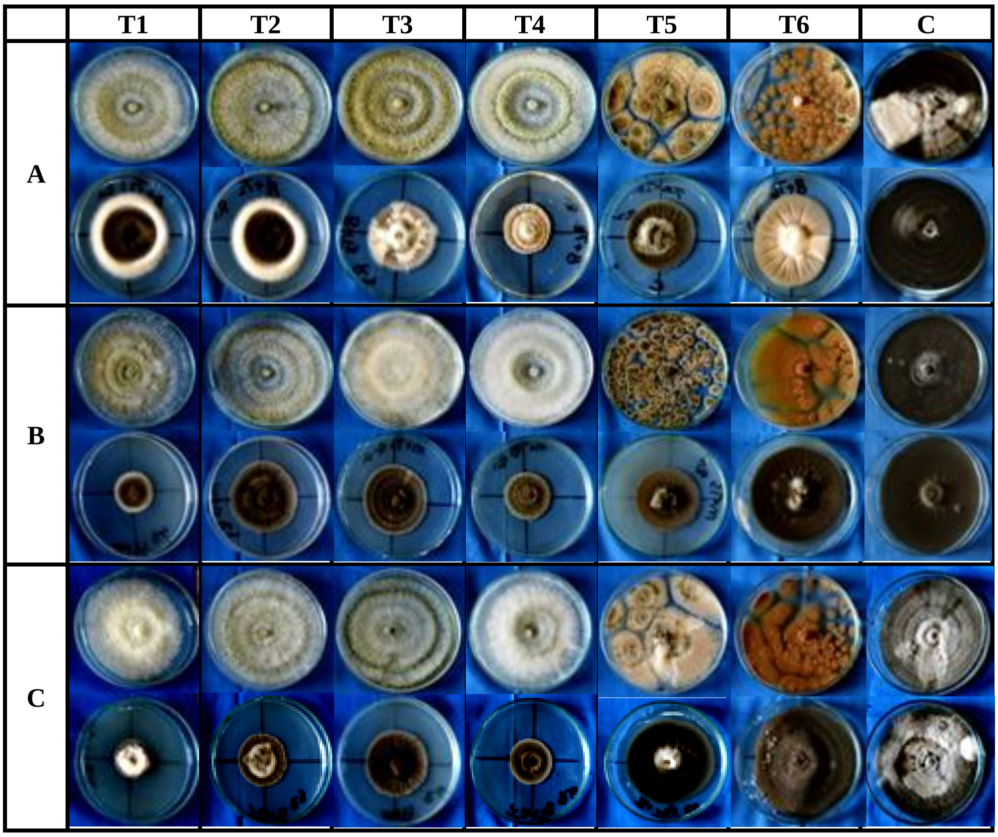

Fig. 7 Effect of different BCAs on three *Alternaria* spp. through volatile method
A = *Alternaria solani;* B = *Alternaria tenuissima;* C = *Alternaria alternata*

Conclusion

Present study concludes that *T. viride*, *T. koningii* and *T. hamatum* are very potent bio-agents against *Alternaria* spp. These bio-agents are recommended to be used as a promising biological control strategy against various diseases of vegetables crops caused by *Alternaria* spp.

Author Contribution Statement: Safia Nizamani executed the research and performed research experiments, Muhammad Ibrahim Khaskheli conceived the idea and supervised the research, Abdul Mubeen Lodhi analysed the data, Imtiaz Ahmed Nizamani contributed reagents, Asad Ali Khaskheli wrote the manuscript, Allah Jurio Khaskheli and Gul Bahar Poussio provided research facility, Sajad Ali Khaskheli helped in survey.

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