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FUNGITOXIC POTENTIAL OF *TAGETES ERECTUS* FOR THE MANAGEMENT OF *ALTERNARIA ALTERNATA* STRAINS OF TOMATO

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Abstract

Tomato (*Lycopersicum esculentum* Mill.) is economically the most vital vegetable crops of this world but diseases reduce tomato production to a greater extent worldwide. Plants exudates contribute a lot in fight against pathogens. The current study indicates the pathogenic potential of *Alternaria alternata* FCBP-573 against tomato. RAPD analysis confirmed that *A. alternata* FCBP-573 had variability in its genetic constitution with other two isolates; this disparity in genetic constitution might be a cause to stir up more pathogenicity in this isolate. Therefore, it was selected as the most pathogenic isolate and subjected to biological control through *Tagetes erectus* L. In antifungal bioassays different plant parts of *T. erectus* with 1-4% concentrations of aqueous, and organic extracts of each part were evaluated against *A. alternata* FCBP-573. Results revealed that the growth of *A. alternata* FCBP-573 was greatly inhibited at 4% concentration of methanol extract followed by aqueous and n-hexane extract. Among different plant parts tested, root extract exhibited more promising results by causing 81-92% reduction in biomass. The research concludes that aqueous and organic extracts of ornamentals have potential to obstruct dreadful effect of pathogenic fungi by suppressing their growth. *T. erectus* conferred vital and surprisingly stable compounds having inhibitory potential against *A. alternata* FCBP-573.

Introduction

Tomato is economically imperative and nutritious vegetable crops world over. Tomato is famous for its application in drug, fruit, food products, flowering, ornamental and horticulture sectors (Bakht *et al*., 2014). Total area of tomatoes production in Pakistan is 0.03 million hectares (Hussain, 2005). The average yield of tomato in Pakistan is 10.1 tons/ha (Anony., 2005), which is very low as compared to other leading tomato producing countries in the world. There are about 200 known diseases of tomato, of which 30 are economically important e.g., cankers, leaf spot, wilts, blights and root rots caused by bacteria and fungi (Jones *et al*., 1997). Early blight of tomato, stem canker, black mold rot, leaf spot and black shoulder disease is instigated through the fungus *Alternaria alternata* f sp. *Lycopersici,* so a single pathogen proves economically destructive. It is also recounted to encourage post harvest damages in high frequency. Chemical control method is considered to be the effective way to manage plant maladies and pathogens. Utility of chemicals is effective measure to increase the yield of crops but the major drawback of persistent use of chemicals is the resistance that is induced in the organisms and contaminate the environment with very toxic substances (Carvalho, 2004; Okigbo, 2004). In view of the high cost of chemical pesticides and their hazardous consequences, use of biodegradable materials like effective micro-organisms and fresh plant extracts from different parts gained importance during last three decades for plant disease control (Duke *et al*., 2000; Bajwa *et al*., 2007). Plant derivatives subsidized extensively in fight against pathogens (Vyvyan, 2002; Shafique *et al.,* 2011). Numerous plant families like Acanthaceae, Amranthceae, Apiaceae and Magnoliaceae have been reported to possess mycotoxic and cytotoxic ability (Mansilla & Palenzuela, 1999; Neerman, 2003). Numerous studies conducted in Pakistan revealed a wide spectrum prospects of using extracts of plants for biological control of fungal pathogens (Bajwa *et al*., 2001; Ahmad & Abdelgaliel, 2005; Braga *et al*., 2007; Shafique *et al*., 2011; Shinwari *et al.,* 2013).

On the basis of above mentioned investigations, *Tagetes erectus* (marigold) was selected to investigate antifungal activity of its various parts against *Alternaria alternata*.

Materials and Methods

Procurement and maintenance of cultures: The pure cultures of *Alternaria alternata* f sp. *Lycopersici* with accession no. FCBP- 573, FCBP- 479 and FCBP-349 isolated from tomato plants were obtained from First Fungal Culture Bank of Pakistan (FCBP), Institute of Agricultural Sciences, University of the Punjab, Lahore. These isolates were grown to make pure cultures. They were sub-cultured in fresh Malt Extract Medium (MEA) plates monthly and maintained at 4°C as stock cultures.

Pathogenicity test: Pathogenicity test was performed according to Grogan *et al*., (1975). The soil, at the rate of 2 kg was sterilized at 45°C for 24 h in hot air oven. Sterilization of soil at this temperature eliminates almost all contaminants but preserves natural organic matter of the soil. Conidial suspension of 2.0×10^5 conidia/mL of all strains of *A. alternata* was made using the protocol of Noomrio & Dahot (1992) from 10 days old cultures. Pathogenecity test was performed by spraying one month old tomato plants to runoff with spore suspensions. For the maintenance of moisture content all the plants were enclosed in polythene bags for 48 h for spore germination and development of disease and after that the plants were returned to the greenhouse. Each treatment was replicated thrice.

Disease rating scale was made on the basis of disease incidence and disease severity. Disease severity was calculated by following formula:

$$\text{Disease severity (\%)} = \frac{\text{Infected area of plant}}{\text{Total area of plant}} \times 100$$

Screening of the most pathogenic isolate, on the basis of severe infection in host plant, was carried out on the basis of results of pathogenicity test and subsequent studies were conducted using the most pathogenic isolate.

Molecular analysis of pathogenic strains: The genomic DNA of isolates of *A. alternata* was obtained using CTAB method (Saghai-Maroof *et al*., 1984). The RAPD amplification conditions were optimized by following method described by Williams *et al*., (1990). Five primers RAPD-6 (5'GATGACCGCC3'), RAPD-7 (5'TGTCTGGGTG3'), RAPD-8 (5'GTTGCCAGCC3'), RAPD-9 (5'GAACGGACTC3'), and RAPD-10 (5'TCGCCAGCCA3') were used in RAPD analysis. RAPD amplifications were carried out in Master cycler gradient PCR (TECHNE TC-412) with initial denaturation at 94°C for 5 minutes, followed by 40 cycles of denaturation at 94°C for 1 minute, annealing at 25°C for 1 minute, and primer extension at 72°C for 1 minute. Final extension was set for 5 minutes at 72°C. The reaction was terminated at 4.0°C in 2-3 hours.

Biological control of the most pathogenic isolate: Fresh samples of *T. erectus* were taken from University of the Punjab, Lahore and rinsed carefully under tap water. The shoots, roots and flowers were separated and dried with blotting paper followed by oven drying at 40-45°C for 24 h. The protocol of Bajwa *et al*., (2007) was adopted to prepare stock solution (20% w/v) of aqueous extract of water soluble constituents of respective plant part. It was strained by using muslin cloth and filter paper. This stock was kept at 4ºC.

The method of Alkhail (2005) was monitored to prepare shoot, root and flower extract in methanol and n-hexane. To make control of methanol and n-hexane, 2 mL of each solvent was added separately to sterilize distilled water to prepare 100 mL of each in respective flasks.

The dilutions of 1-4% of aqueous, n-hexane and methanolic shoot, root and flower extracts were prepared using following formula:

$$C_1 V_1 = C_2 V_2$$

Antifungal bioassays: Aqueous and organic solvent extract bioassays were carried out in liquid medium. The basal medium employed to grow fungi was 2% malt extract (ME) medium in 250 mL conical flasks. Disc method was used for inoculation. All the flasks were incubated at 28±3ºC for 7 days. Fungal biomass of each triplicate sample was taken on pre-weighed filter papers after 7 days. Their dry biomass was noticed after 24 h drying at 60ºC according to Bajwa *et al*., (2006). Rate of fungal biomass increase or decrease was determined from the dried biomass to evaluate the relative effects of various treatments. Percentage reduction in fungal biomass was calculated by applying following formula:

$$\text{Growth inhibition (\%)} = \frac{\text{Growth in control} - \text{Growth in treatment}}{\text{Growth in control}} \times 100$$

Statistical analysis: Standard errors of means of all the replicates of each treatment were calculated. All the data was analyzed by analysis of variance (ANOVA), following this, Duncan's Multiple Range (DMR) test (Steel & Torrie, 1980) was applied to separate the treatment means using Computer software COSTAT.

Results

Pathogenicity test: Pathogenecity test was performed by spraying one month old tomato plants to runoff with spore suspension containing approximately 2.0×10^5 conidia/mL and infection was evident in 7-8 days (Plates 1-3). Symptoms observed on tomato plants of the stem canker disease were found to be varied with different isolates. Isolate FCBP-573 of *A. alternata* was proved to be the most pathogenic (Plate 1), as it induced the symptoms of dark brown to black canker with concentric zonation on stems near the soil line or aboveground. Large cankers are often associated with wounds produced by pruning petioles. The cankers became enlarge slowly and by harvest stems were girdled and plants died. Small tan to brown lesions interspersed between the larger lesions were observed. Foliar symptoms were visualized in the form of inward rolling and angular necrotic spotting of lowermost leaflets or in later stages, complete necrosis of leaflets on one or both sides of the midrib was noticed (Plate 1). On the basis of symptoms disease rating scale was developed that is as follows:

Symptoms induced by other two isolates e.g., *A. alternata* FCBP-479 and *A. alternata* FCBP-349 isolated from tomato plant, started with yellowing and browning of the lower leaves and progressed upward under high humid conditions. Symptoms were developed from the leaf tips and along the margins of the leaf petiole. Concentric circles with dark layers of spores were also observed on blighted leaf portions.

On the basis of symptoms, *A. alternata* FCBP-573 exhibited maximum pathogenicity towards tomato plants, so was screened out as the most pathogenic strain.

Molecular analysis: In order to study the genetic diversity among different isolates of *A. alternata* RAPD analysis was carried out. For this purpose, the intact DNA was isolated from three different isolates of *A. alternata,* and subjected to RAPD analysis. The primer RAPD-7 was used to screen the genome of these *A. alternata* isolates while remaining four primers (RAPD-6, RAPD-8, RAPD-9 and RAPD-10) provided no any amplification.

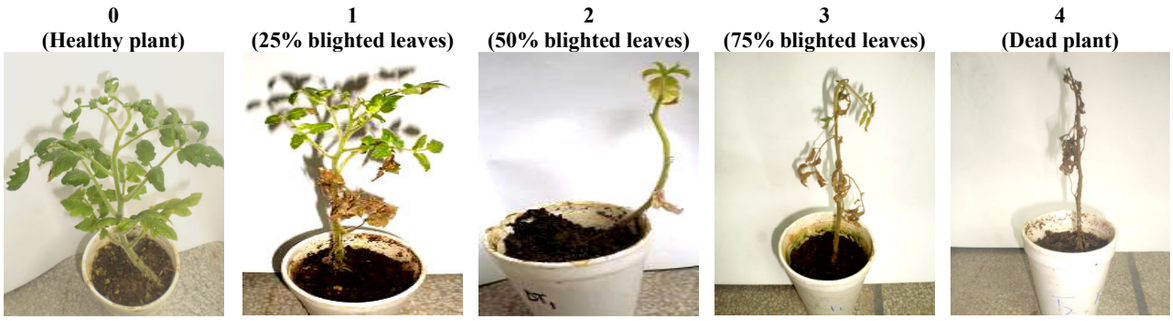

Plate 1. Disease rating scale for *A. alternata* FCBP-573.

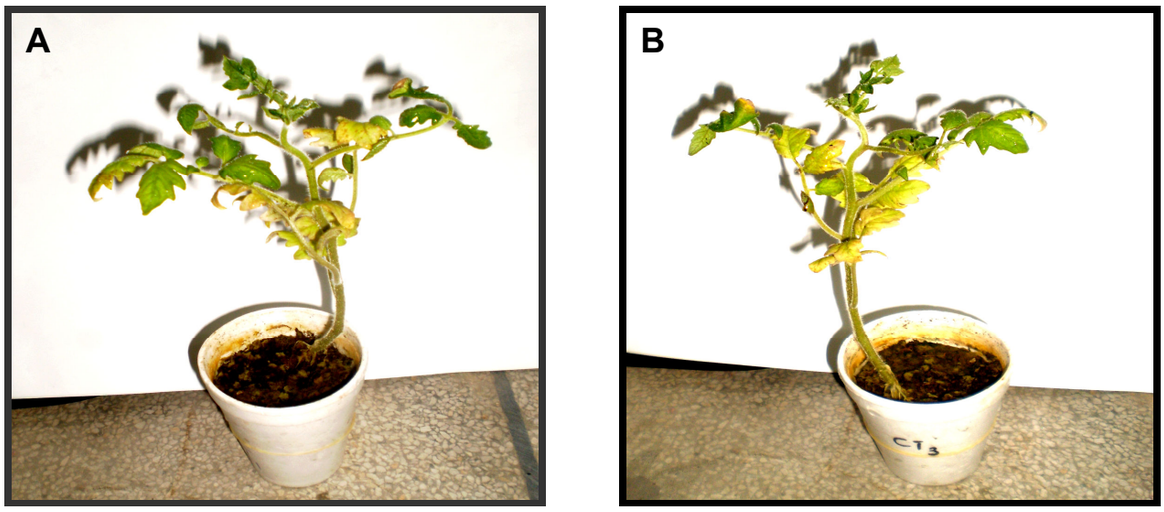

Plate 2. Symptoms induced by *A. alternata* FCBP-479. A: Control. B: Diseased.

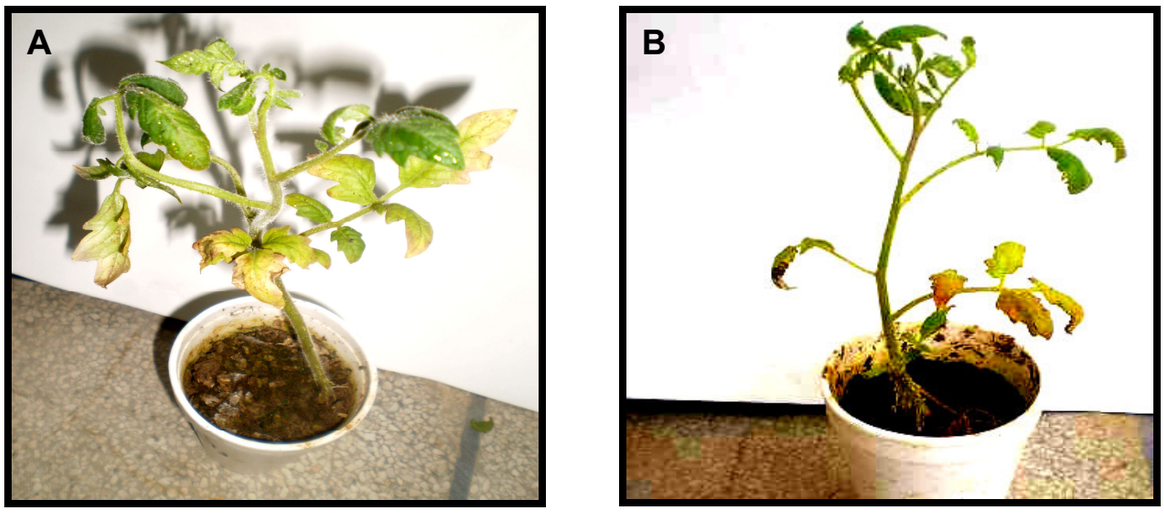

Plate 3. Symptoms induced by *A. alternata* FCBP-349. A: Control. B: Diseased.

The RAPD amplifications of different sizes by using primer RAPD-7 were observed on 1% Agarose Gel (Fig. 1 and Table 1). In all isolates except isolate "*A. alternata* FCBP-573" very prominent amplifications at 150 bp was observed. However, at 225 bp, a unique band was primed by "*A. alternata* FCBP-573" and "*A. alternata* FCBP-479". In isolates "*A. alternata* FCBP-479" and "*A. alternata* FCBP-349" sharp band at 325 bp was observed while in "*A. alternata* FCBP-573" this band was not detected. At 400-500 bp level except isolate "*A. alternata* FCBP-573", in other both isolates amplifications were observed. Similarly at 725 bp size in

case of isolates "*A. alternata* FCBP-573" no amplification was observed while in remaining isolates prominent band at this level were quite clear. A gene at 870 bp level was amplified by isolates "*A. alternata* FCBP-479" and "*A. alternata* FCBP-349" while in sample "*A. alternata* FCBP-573" no such amplifications were observed. Thus, "*A. alternata* FCBP-573" was distinguished by the presence of two DNA fragments with an approximate size of 700 and 900 bp produced by the same primer which was not evident in "*A. alternata* FCBP-479" and "*A. alternata* FCBP-349". It was inferred from the analysis of RAPD amplicons that this difference in genetic framework could be a reason for more pathogenicity in "*A. alternata* FCBP-573" (Fig. 1 and Table 1).

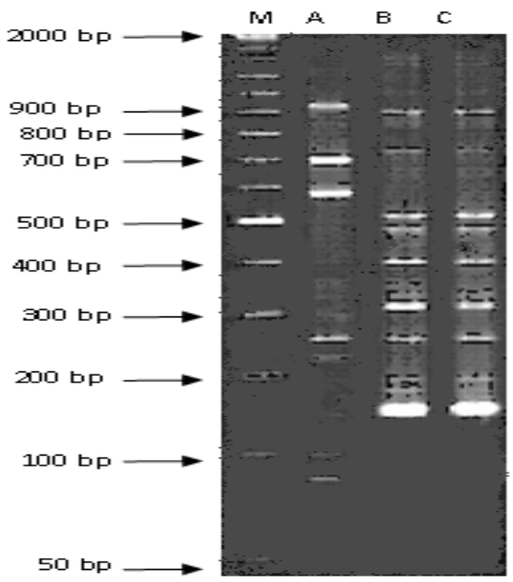

Fig. 1. RAPD DNA fragment amplification with primer RAPD-7 showing genetic constitution of *A. alternata* isolates. Lane M indicates DNA marker, Lane A: *A. alternata* FCBP-573, Lane B: *A. alternata* FCBP-479 and Lane C: *A. alternata* FCBP-349.

Table 1. Sizes of amplified fragments in RAPD Analysis obtained with decamer RAPD-7.

Isolates of *A. alternata*	FCBP-573	FCBP-479	FCBP-349
Sizes of the amplified fragments	---	---	---
	900 bp	---	---
	---	870 bp	870 bp
	---	---	---
	---	725 bp	725 bp
	700 bp	---	---
	590 bp	---	---
	---	500 bp	500 bp
	---	480 bp	480 bp
	---	400 bp	400 bp
	---	325 bp	325 bp
	---	---	---
	250 bp	250 bp	250 bp
	225 bp	225 bp	---
	---	150 bp	150 bp

The dendrogram was constructed on the basis of pattern of amplifications in RAPD analysis by using software MVSP32 3.12 version (Fig. 2). In dendrogram analysis the highest similarity was found to be ~ 94.11% among isolates "*A. alternata* FCBP-479B" and "*A. alternata* FCBP-349". While isolate "*A. alternata* FCBP-573A" showed 21% similarity with both the other isolates. The studies reveal that *A. alternata* FCBP-573 induced the maximum infection in the host plant and also exhibited about 79% genetic disparity from other two strains so this most pathogenic isolate was selected for subsequent biocontrol assays through *T. erectus.*

Biological control of *A. alternata* FCBP-573 through aqueous extracts

Response of *A. alternata* FCBP-573 to aqueous root extracts of *T. erectus*: The *In vitro* antifungal potential of *T. erectus* root extracts was recorded against *A. alternata* FCBP-573 and the results are presented in Fig. 3. The results revealed a significant inhibitory activity of employed extract. Aqueous fractions of root extracts of *T. erectus* exhibited promising results in suppressing the fungal growth than aqueous shoot and flower extracts. However, differences in growth rate were evident with respect to the concentrations employed. The assays revealed a gradual and significant reduction in fungal growth inhibition in all concentrations. A marked decrease of 90% in biomass production was recorded at 4% concentration. However, the percentage growth inhibition of *A. alternata* FCBP-573 in aqueous root extracts at 1, 2 and 3% concentration of *T. erectus* was 14, 28.5 and 40.4%, respectively.

Response of *A. alternata* FCBP-573 to aqueous shoot extracts of *T. erectus*: The *In vitro* antifungal potential of aqueous shoot extracts of *T. erectus* was recorded against *A. alternata* FCBP-573. The data regarding fungal growth, exposed to various concentrations of aqueous shoot extracts of *T. erectus* is portrayed in Fig. 3. Aqueous fractions of shoot extracts of *T. erectus* exhibited significant but less inhibitory potential in suppressing the fungal growth than aqueous root extract. However, the highest concentration of 4% caused significant reduction of about 44% in mycelial growth. The lowest concentration (1%) was more effective than 2 and 3% in suppressing the growth of *A. alternata* FCBP-573 as it caused a reduction of 34% followed by 24 and 34% reduction by 2 and 3%, respectively.

Response of *A. alternata* FCBP-573 to aqueous flower extracts of *T. erectus*: The antifungal activities of aqueous flower extracts of *T. erectus* in terms of growth inhibition potential are also summarized in Fig. 3. Data obtained from antifungal assays revealed that aqueous extract of flower also depicted the significant and variable antifungal activity against *A. alternata* FCBP-573 at all the concentrations**.** Maximum inhibitory effect was recorded at 3% concentration. A marked increase in biomass production was recorded at 1, 2 and 4% concentrations as compared to 3% but it was still significantly lower than control. This suppression in biomass production was in the range of 40-57%.

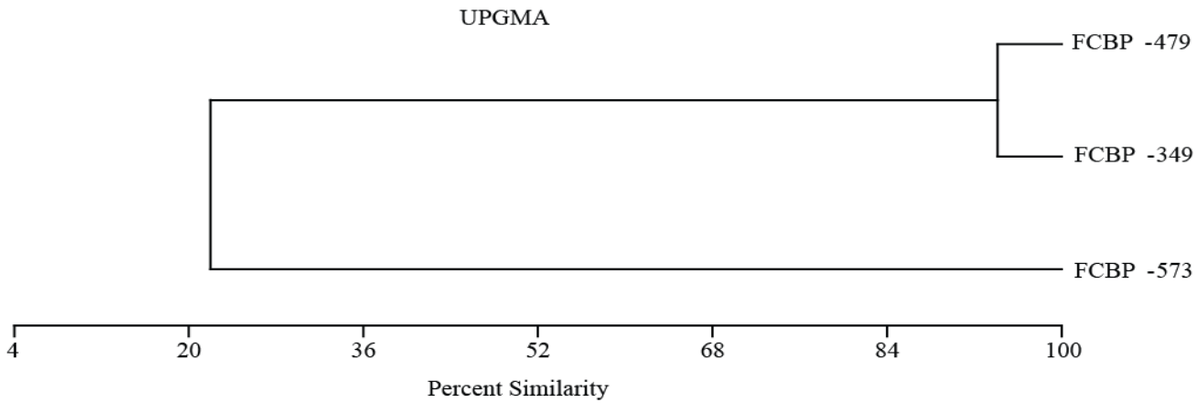

Fig. 2. Dendrogram showing similarities among different isolates of *A. alternata.*

The results of aqueous fractions conclude that root extracts of *T. erectus* exhibited more promising results in suppressing the fungal growth as compared to aqueous shoot and flower extract as it suppressed the growth of *A. alternata* FCBP-573 up to 90% while shoot and flower extract induced only 44% and 57% in fungal biomass production, respectively.

Biological control of *A. alternata* FCBP-573 through methanolic extracts

Response of *A. alternata* FCBP-573 to methanolic root extracts of *T. erectus*: The data regarding fungal growth inhibition, exposed to various concentrations of methanolic root extracts of *T. erectus* is presented in Fig. 4. Methanolic fractions exhibited more promising results in suppressing the fungal growth than aqueous and n-hexane fractions (Figs. 3-5). Differences in growth rate were exhibited with respect to the concentrations employed. The results pertaining in Fig 4 displayed a gradual and significant decrease in fungal dry biomass production with an increase in extract concentration. The maximum antifungal stress was induced by 4% concentration causing a decline of about 92% in dry biomass production of *A. alternata* FCBP-573 while all the other concentrations (1, 2 and 3%) tempted 17, 51 and 66% reduction, respectively.

Response of *A. alternata* FCBP-573 to methanolic shoot extracts of *T. erectus*: The results obtained from biocontrol assays of *A. alternata* FCBP-573 in various concentrations of methanolic shoot extract of *T. erectus* showed significant antifungal activity in all the concentrations in comparison to control (Fig. 4). However, a similar pattern of the effect of various concentrations was recorded against target fungal pathogenic species as was exhibited by methanol root and flower extract. A marked decrease in dry biomass production was observed at 4% concentration with about 86% reduction whereas 1, 2 and 3% concentrations evidenced less inhibitory but significant antifungal potential, in comparison to 4% concentration, by bringing about 12, 22 and 47% biomass inhibition in fungal growth, respectively.

Response of *A. alternata* FCBP-573 to methanolic flower extracts of *T. erectus*: Antifungal activity of methanolic flower extracts was assayed and its effect on the growth of *A. alternata* FCBP-573 is presented in Fig. 4. The data analysis revealed significant reduction in growth of *A. alternata* FCBP-573 with flower extracts of *T. erectus* but the extract fractions showed significant differences in their efficacy. All the concentrations invariably and significantly depressed the fungal growth. The reduction in biomass was ranged from 28 to 82%. The maximum antimycotic activity (82%) was observed by 4% flower extract.

It is indicated from the results that methanolic extracts of root of *T. erectus* exhibited the best potential in suppressing the fungal growth than methanolic shoot and flower extract. Methanolic extracts of root inhibited the growth of *A. alternata* FCBP-573 up to 92% while shoot and flower extract induced 86 and 82% suppression in fungal growth, respectively (Fig. 4).

Biological control of *A. alternata* FCBP-573 through n-hexane extracts

Response of *A. alternata* FCBP-573 to n-hexane root extracts of *T. erectus*: The antifungal activities of n-hexane extracts of *T. erectus* root in terms of percentage growth inhibition are summarized in Fig. 5. The n-hexane extract of root showed the highest antifungal activity against *A. alternata* FCBP-573 as compared to n-hexane shoot and flower extracts. A gradual decrease in dry biomass production was observed with an increase in concentration of extract. At 4% concentration extract exhibited the strongest antifungal upshot against *A. alternata* FCBP-573 by expressing 81.5% growth inhibition.

Response of *A. alternata* FCBP-573 to n-hexane shoot extracts of *T. erectus*: In case of n-hexane shoot extract a variable pattern of antimycotic activity was observed but 4% concentration of shoot extract was the most effective in suppressing the growth of *A. alternata* FCBP-573 as it inhibited the growth up to 64% whereas 1, 2 & 3% concentrations were less depressive (Fig. 5). Shoot extract caused about 37–64% arrest in growth rate of *A. alternata* FCBP-573. Variation in growth inhibition of *A. alternata* FCBP-573 by n-hexane extracts at 1, 2 and 3% concentrations was insignificantly different among them while significantly different with respect to control.

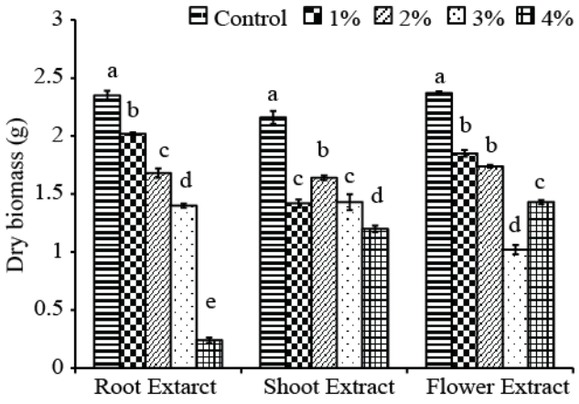

Fig. 3. Effect of different concentrations of different parts of aqueous extracts of *T. erectus* on the biomass production of *A. alternata* FCBP-573.
Vertical bars indicate standard error of means of three replicates. Values with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's multiple range (DMR) test.

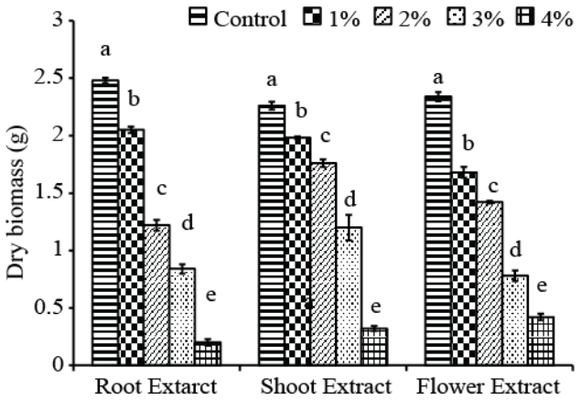

Fig. 4. Effect of different concentrations of different parts of methanolic extracts of *T. erectus* on the biomass of *A. alternata* FCBP-573.
Vertical bars indicate standard error of means of three replicates. Values with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's multiple range (DMR) test.

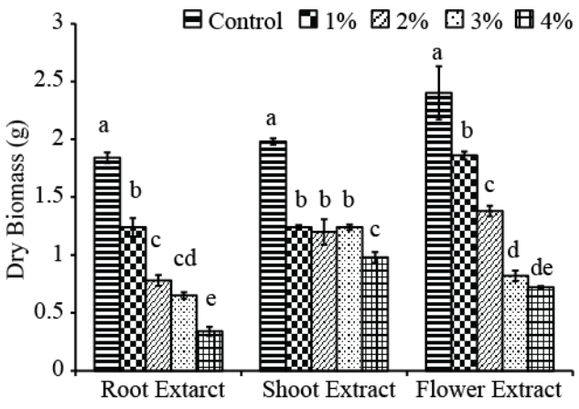

Fig. 5. Effect of different concentrations of different parts of n-hexane extracts of *T. erectus* on the biomass of *A. alternata* FCBP-573.
Vertical bars indicate standard error of means of three replicates. Values with different letters show significant difference ($p \leq 0.05$) as determined by Duncan's multiple range (DMR) test.

Response of *A. alternata* FCBP-573 to n-hexane flower extracts of *T. erectus*: Antifungal activity of n-hexane flower extracts was assayed and the results obtained of the effect of plant extracts on the growth of *A. alternata* FCBP-573 is assembled in Fig. 5. The data revealed a steep and significant reduction in growth of *A. alternata* FCBP-573 by 1 to 4% concentrations in comparison to control. It is indicated from the results that there was insignificant reduction in growth at 3 and 4% concentrations. Maximum arrest in biomass production was evident at 4% concentration as it induced about 57% capture at this dose.

The antifungal activities of n-hexane extracts of *T. erectus* root exhibited more promising results in suppressing the fungal growth than n-hexane shoot and flower extract as it suppressed the growth of *A. alternata* FCBP-573 up to 82% while shoot and flower extract caused only 44% and 57% inhibition, respectively (Fig. 5).

Discussion

In Pakistan, *A. alternata* is known as a leaf spot as well as saprophytic pathogen of tomato causing post harvest losses. Control of these diseases caused by pathogenic fungi has become a requisite. The practice of chemical treatment aids in yields increment but the constant use of chemicals induce resistance in target organisms and contaminate the environment with very toxic substances. Biological control is an approach that provides safe fungal management program, and corresponds to a substitute for reliance on chemical treatment methods. Numerous studies conducted in Pakistan revealed a wide spectrum prospects of using extracts of plants for biological control of pathogenic fungi (Ahmad & Abdelgaliel, 2005; Braga *et al*., 2007). With the importance of biological control method to manage fungus, present study has been designed to verify the antifungal activity of *T. erectus* against phytopathogenic fungus *A. alternata* FCBP-573.

Pathogenecity test was performed to determine the pathogenic potential of *A. alternata* FCBP-573, *A. alternata* FCBP-479 and *A. alternata* FCBP-349 isolated from tomato plant. *A. alternata* FCBP-573 induced the maximum characteristic symptoms. These results were found in agreement with the work conducted by Gilchrist and Grogan (1974) who reported the same trend of disease development in tomato by *A. alternata*.

Presently, RAPD analysis was carried out to evaluate the different isolates of *A. alternata* for their genetic diversity. It was found that primer RAPD-7 provided good amplification results with all isolates while all other primers showed no any amplification. In dendrogram analysis, isolate *A. alternata* FCBP-479 and *A. alternata* FCBP-349 showed 79% disparity with *A. alternata* FCBP- 573. In previous studies, Roberts *et al*., (2000) and Bock *et al*., (2002) also reported a high genetic disparity in *A. alternata* strains. Morris *et al*., (2000) also identified 69 isolates of *A. alternata* from tomato fruit with characteristic sunken black lesions in California using RAPD markers.

Pathogenicity test and RAPD analysis confirmed the strong pathogenic potential of *A. alternata* FCBP-573 so subsequent studies were under taken using this isolate to evaluate the mycotoxic potential of *Tagetes erectus*. *T. erectus* is known to possess antifungal properties due to the presence of thiophenes in its tissues (Montes & García, 1997; Gomez *et al*., 2003). Peng (2008) prepared 3 pesticide formulations from *Tagetes* root extract against *Fusarium* wilt and provided scientific basis for developing new type bio-pesticide through utilizing *Tagetes* root. In present study, root, shoot and flower extracts of *T. erectus* in different solvents were examined for their antimycotic activity against *A. alternata* FCBP-573. The results predicted a dosage dependent response as a gradual and significant arrest in fungal growth with an increase in concentration of extract in all solvent types. The diverse chemical nature of different solvents renders divergent antifungal potential of extracts. This might be due to dissolution capacity of different chemicals in different solvents which cause variable response of extracts of same plant in different solvents. Many studies in literature are known to support these outcomes. As Bajwa *et al*., (2007) worked on antifungal potential of aqueous and n-hexane shoot extracts of *Aloe vera* against few pathogenic species viz., *Alternaria alternata*, *A. citri* and *A. tenuissima* and described substantial reserve in fungal growth.

Presently, the results indicated that aqueous fractions of root extracts of *T. erectus* effectively inhibited the growth of *A. alternata* FCBP-573. A marked decrease in biomass production (90%) was recorded at 4% concentration of aqueous root extracts because at higher concentration, thiophenes concentration also increased. These verdicts are in accordance with the research carried out by Riaz *et al*., (2008), they observed 54-79% suppression in fungal biomass production because of different concentrations of aqueous fractions of *T. erectus*. This work is supported by the findings of previous study in which aqueous extract of allelopathic trees viz., *A. indica* and *M. indica* showed maximum inhibition against storage fungi of wheat grains (Shafique *et al.,* 2007).

In present study, Methanol extracts also showed significant antifungal activity. The supreme antifungal compression was induced by 4% concentration of root, shoot and flower extracts causing a decline of about 92, 86 and 82%, respectively, in biomass production of *A. alternata* FCBP-573. Working on parallel line Bajwa *et al*., (2008) reported maximum antifungal activity of methanol extract of rice varieties on *Macrophomia phaseolina* and *Ascochyta rabiei*. These findings are also in accordance with the work of Fardos (2009) who reported that among 5 different plants tested, lemon grass was the most effective against different pathogenic fungi including *Microsporum canis, M. gypseum,* and *T. mentagrophytes.*

The antifungal activities of n-hexane extracts of *T. erectus* root, shoot and flower extracts in terms of percentage growth inhibition were also assessed. The n-hexane root extract showed the highest antifungal activity against *A. alternata* FCBP-573 as compared to shoot and flower extracts. A gradual decrease in biomass production was observed with increase in concentration of extract. At 4% concentration root, shoot and flower extracts exhibited the strongest antifungal effect against *A. alternata* FCBP-573 by expressing 81, 64 and 70% growth inhibition. Earlier Daoud *et al*., (1990) have reported good antifungal activity of *M. azedarach* against *Alternaria, Aspergillus* and *Penicillium spp*. Similarly Iqbal *et al*., (2002) have accounted that extract of *M. azedarach* was effective against *Fusarium chlamydosporum, A. niger* and *Hyloflora ramosa*.

This study concludes that aqueous and methanolic extracts of *T. erectus* possess potential antifungal compounds against *A. alternata*, which may hold strong antifungal activity and can be exploited as an ideal strategy for future plant disease management programs eliminating fungal spread.

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