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Tagetes erectus – A Tool for the Management of *Alternaria alternata* Strains of Tomato

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Abstract

Tomato (*Lycopersicum esculentum* Mill.) is among the most economically valuable vegetable crops in the world. It is estimated that diseases reduce tomato production to a greater extent worldwide. Natural plants derivative compounds 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, *A. alternata* FCBP-573 was selected and subjected to biological control by *Tagetes erectus* L. In antifungal bioassays plant parts of *T. erectus* with 1, 2, 3, & 4% conc. of aqueous, methanol and n-hexane extracts of each part were evaluated against *A. alternata* FCBP-573. Results revealed that growth of *A. alternata* FCBP-573 was greatly inhibited at 4% conc. 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 study 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.

Keywords: Tomato, *Alternaria alternata, Tagetes erectus,* aqueous, organic extracts, antifungal bioassays.

1. Introduction

Tomato is among the economically essential and nutritious vegetable crops in the world. The average yield of tomato in Pakistan is 10.1 tons/ha (Anonymous, 2005). There are about 200 known diseases of tomato, of which 30 are economically important (Jones et al., 1997). Early blight of tomato, stem canker, black mold rot, leaf spot and black shoulder disease is caused by the fungus *Alternaria alternata* f sp. *Lycopersici, so a single pathogen proves economically destructive*. To protect the plants from diseases and pathogens, chemical control methods are exercised. Usage of biodegradable materials as effective micro-organisms and plant extracts from different parts are being used during last few years for plant disease control (Shafique et al., 2011). Numerous studies conducted in Pakistan revealed a wide spectrum prospects of using extracts of plants for biological control of pathogenic fungi (Shafique et al., 2011). On the basis of above mentioned investigations, *Tagetes erectus* (marigold) was selected to investigate antifungal activity of its various parts against *Alternaria alternata*.

2. Materials and Methods

The pure cultures of *A. alternata* FCBP- 573, FCBP- 479 and FCBP-349 isolated from tomato plants were acquired from FCBP, Institute of Agricultural Sciences, University of the Punjab, Lahore.

Pathogenicity test was performed according to Grogan et al. (1975). Conidial suspension of 2.0 × 10^5 conidia/mL of all the isolates was primed as described by Noomrio and Dahot (1992). Disease rating scale was made on the basis of disease incidence and disease severity. Disease severity was calculated by following formula and screening of the most pathogenic isolate was carried out:

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

The genomic DNA of isolates of *A. alternata* was mined 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 (GATGACCGCC), RAPD-7 (TGTCTGGGTG), RAPD-8 (GTTGCCAGCC), RAPD-9 (GAACGGACTC), and RAPD-10 (TCGCCAGCCA) were used in RAPD analysis.

Fresh samples of *T. erectus* were collected from PU Lahore Aqueous extract (20% w/v) was prepared according to Bajwa *et al.* (2007). The protocol of Alkhail (2005) was used to prepare plant extracts in methanol and n-hexane. Aqueous and organic solvent extract bioassays were carried out in liquid 2% malt extract (ME) medium and incubated at 28±3 ºC for 7 days. Their dry weight yield was determined after 24 h oven drying at 60 ºC according to Bajwa et al. (2006). The rate of fungal biomass increase or decrease was determined from the dried biomass. Percentage reduction in fungal biomass was calculated as:

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

All the data was analyzed by analysis of variance (ANOVA), following this, Duncan's Multiple Range (DMR) test (Steel and Torrie, 1980) was applied to separate the treatment means.

3. Results

3.1. Pathogenicity Test

A. alternata FCBP-573 of was proved the most pathogenic (Plate 1), as it induced symptoms of dark brown to black canker with concentric zonation on stems near soil line or aboveground. The cankers became enlarge slowly and plants died. Foliar symptoms were visualized in the form of curling and pointed necrotic lesions on lowest leaflets or in later stages, complete necrosis of leaflets on sides of the midrib was noticed (Plate 1). On the basis of symptoms disease rating scale was developed that is as follows. On the basis of symptoms, *A. alternata* FCBP-573 exhibited maximum pathogenicity towards tomato plants, so was screened out as the most pathogenic strain.

3.2. Molecular Analysis

In RAPD analysis primer RAPD-7 amplified the genome of all isolates while remaining four primers (RAPD-6, RAPD-8, RAPD-9 and RAPD-10) provided no any amplification (Fig. 1). At 225 bp, a unique band was primed by A. alternata FCBP-573 and A. alternata FCBP-479. At 400-500 bp level except isolate A. alternata FCBP-573, in other both isolates amplifications were ob-

served. 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 other two. It was inferred from the analysis of amplicons that this difference in genetic framework could be a reason for more pathogenicity in A. alternata FCBP-573 (Fig. 1). 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

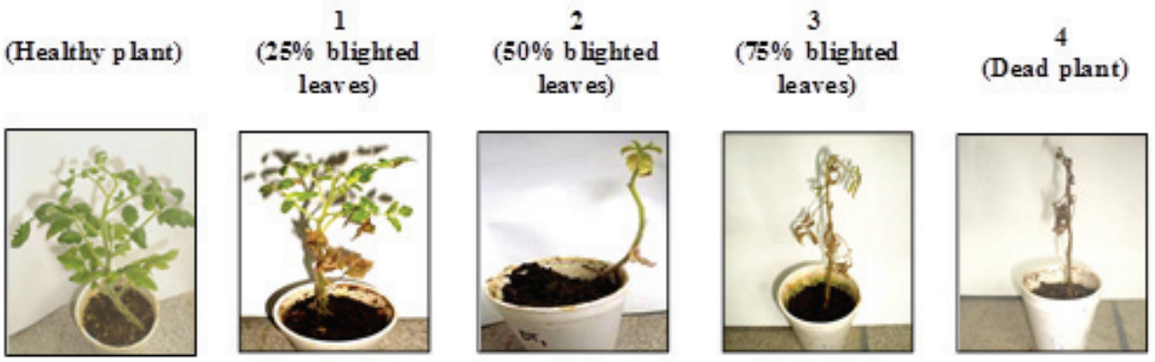

Plate 1: Disease rating scale for *A. alternata* FCBP-573.

highest similarity was found to be ~ 94.11% among *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 maximum infection in host plant and exhibited about 79% genetic disparity from other two strains so this most pathogenic isolate was selected for subsequent biocontrol through *T. erectus.*

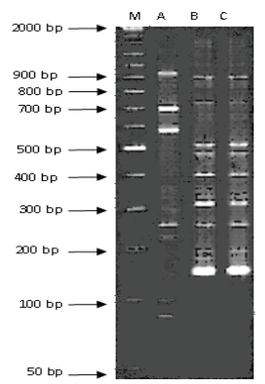

Fig 1. RAPD DNA fragment amplification with primer RAPD-7 showing genetic constitution of *A. alternata i*solates. Lane M indicates DNA marker, A: *A. alternata* FCBP-573, B: *A. alternata* FCBP-479 and C: *A. alternata* FCBP-349.

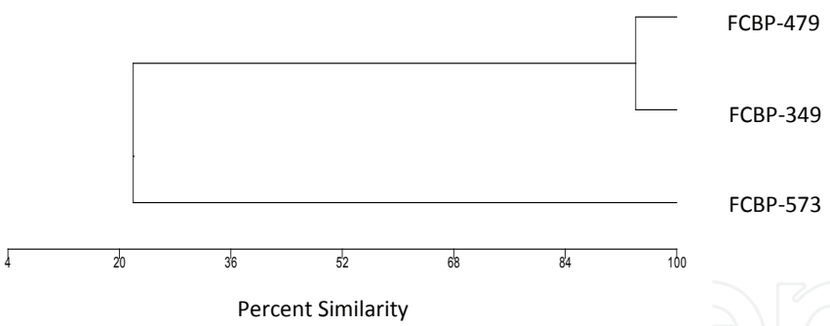

Fig 2. Dendrogram showing similarities among different isolates of *A. alternata.*

3.3. Biological Control of A. alternata FCBP-573 through Aqueous Extracts

Aqueous fractions of root extracts of *T. erectus* exhibited the most promising results in suppressing the fungal growth. A marked decrease of 90% in biomass production was recorded at 4% conc. Aqueous fractions of shoot extracts of *T. erectus* exhibited significant but less inhibitory potential in suppressing the fungal growth than aqueous root extract. However, 4% conc. caused significant reduction of about 44% in mycelial growth. The antifungal activities of aqueous flower extracts of

T. erectus in terms of growth inhibition potential were recorded highest at 3% concentration. This suppression in biomass production was in the range of 40-57%.The results of aqueous fractions conclude that root extracts of *T. erectus* exhibited more promising results 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% biomass production, respectively.

3.4. Biological Control of A. alternata FCBP-573 through Methanolic Extracts

The maximum antifungal stress was induced by 4% root and shoot extract conc. causing 92% and 86% decline in dry biomass., respectively. 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. The reduction in biomass was ranged from 28 to 82%.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.

3.5. Biological Control of A. alternata FCBP-573 through n-Hexane Extracts

The n-hexane extract of root showed the highest antifungal activity against A. alternata FCBP-573. At 4% concentration extract exhibited the strongest antifungal upshot by expressing 81.5% growth inhibition. 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 up to 64%. The data of n-hexane flower extracts revealed a steep and significant reduction in growth 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.

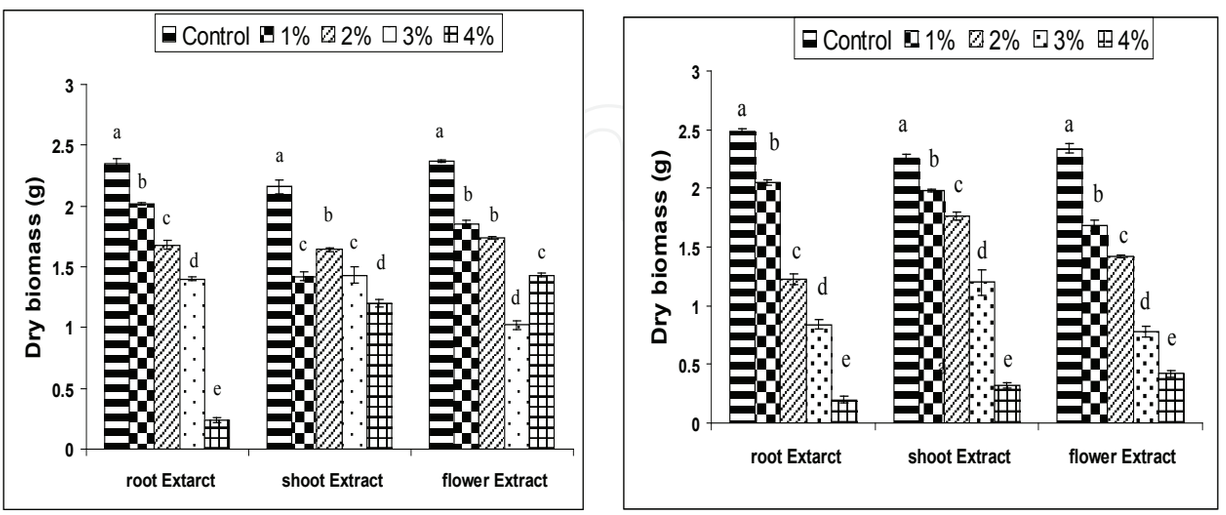

Fig 3. Effect of various conc.s of different parts of aqueous extracts of T. erectus on the biomass production of A. alternata FCBP-573.

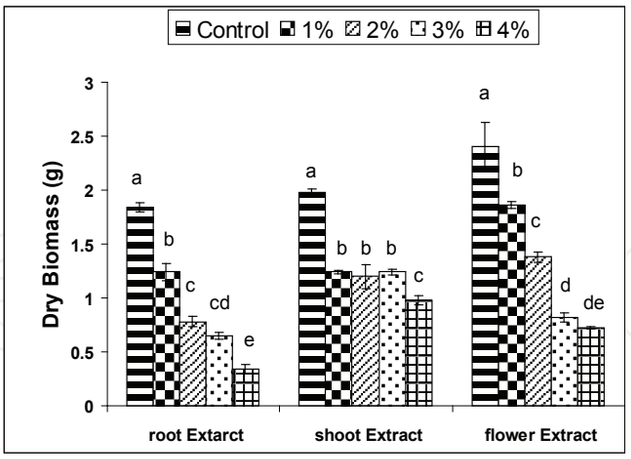

Fig 4. Effect of various conc.s of different parts of methanolic extracts of T. erectus on the biomass of A. alternata FCBP-573.

4. Discussion

In Pathogenecity test *A. alternata* FCBP-573 induced maximum characteristic symptoms. These results were found in agreement with the work conducted by Gilchrist and Grogan (1974) who reported same trend of disease development in tomato by *A. alternata*. Presently, RAPD analysis was carried out to evaluate the isolates of *A. alternata* for their genetic diversity. In dendrogram analysis, isolate *A. alternata* FCBP-479 and *A. alternata* FCBP-349 showed 79% disparity with *A. alternata* FCBP- 573. In previous study, Roberts et al. (2000) also reported a high genetic variation in *A. alternata* populations at molecular level.

Pathogenicity test and RAPD analysis confirmed the strong pathogenic potential of *A. alternata* FCBP-573 so subsequently in present study, root, shoot and flower extracts of *T. erectus* in different solvents were examined. 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% conc. of aqueous root extracts because at higher concentration, thiophenes concentration also increased. It is similar to the work done by Riaz et al. (2008) who observed 54-79% suppression in biomass by employing various concentrations of aqueous fractions of *T. erectus.* In present study, Methanol extracts showed maximum antifungal stress at 4% conc. of root, shoot and flower extracts causing a decline of about 92, 86 and 82%, respectively, in biomass. Working on parallel line Bajwa et al. (2008) reported maximum antifungal activity of methanol extract of rice varieties on *M. phaseolina* and *A. rabiei*. The n-hexane root extract showed the highest antifungal activity against *A. alternata* FCBP-573. A gradual decrease in biomass production was observed with increase in extract conc. At 4% conc. root, shoot and flower extracts exhibited the strongest antifungal effect 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*.

This study concludes that aqueous and methanolic extracts of *T. erectus* possess potential antifungal compounds against *A. alternata*, which hold strong antimycotic activity and can be used as a perfect approach for future plant disease management programs eliminating fungal spread.

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