Response of *Fusarium oxysporum* f. sp. *cepae* to shoot extract of *Chenopodium murale*

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Abstract

In vitro study was carried out to assess the antifungal efficacy of methanolic extracts of aerial parts of *Chenopodium murale* L. against *Fusarium oxysporum* f. sp. *cepae* (FOC). This fungus causes basal rot disease in onion. Different concentrations (1.562 to 50 mg mL^{-1}) of leaf, stem and inflorescence of methanolic extracts of this weed were prepared in malt extract broth. Each tube (containing 1 mL growth media) was inoculated with 50 µL of FOC inoculum and incubated at 28 ºC for one week. Extracts of different parts showed variable results. Stem extract was the most effective where all the concentrations significantly suppressed growth of FOC by 25–59%. Leaf extract also showed profound antifungal activity where 6.25 mg mL^{-1} and above concentrations significantly retarded the growth of FOC by 32–60%. The effect of inflorescence extract was insignificant. There was a linear regression between the extract concentration and fungal biomass. This study concludes that methanolic stem extract of *C. murale* is the most effective in controlling growth of FOC.
Keywords: *Chenopodium murale, Fusarium oxysporum* f. sp. *cepae,* Methanolic extract, Shoot extract.

Introduction

Bulbous onion (*Allium cepa* L.) is a significant horticultural crop, with an annual production of 100 million tonnes. Onions are cultivated in about 140 countries on an estimated area of 5.19 million hectares with an average production of 19.2 tons ha^{-1} (FAO, 2019). Its annual production is largely affected by a soil-borne fungal pathogen namely *Fusarium oxysporum* f. sp. *cepae* (FOC), the causal agaent of onion basal rot (Rauf and Javaid, 2013; Akhtar and Javaid, 2018). Onion has little genetic resistance against FOC. Hence, there is difficulty in managing the pathogen. The current application procedures to control FOC with fungicides such as carbendazim and mancozeb are inefficient and costly. These practices often become futile due to emergence ofe resistant pathogen races (Rout *et al.,* 2016).

Alternatives to chemical pesticides are highly preferable because of their chronic effects on human and animal health, soil beneficial mycoflora as well as on the environment (Qin *et al.,* 2021). In this regard, the use of plant derived products is gaining a wider interest due to their proven low toxicity, biodegradability, nature specificity and minimum residual toxicity in the ecosystem (Gahukar and Das, 2020). In nature, plants are a rich source of diverse organic compounds such as isoflavonoids, flavonoids, terpenoids, alkaloids, phenols, coumarins, sesquiterpenoids, isocoumarins and furanoterpenoids that make an excellent lead for the development of fungicides (Kanwal *et al.,* 2010; Tiku, 2018; Othman *et al.,* 2019). Recent literature reported that plant extracts have the ability for control of fungal pathogens (Banaras *et al.,* 2020; Javaid *et al.,* 2020; Khan *et al.,* 2020). *Chenopodium murale* commonly known as nettleleaf goosefoot belongs to family Chenopodiaceae (Ahmed *et al.,* 2017). It is one of the fast-growing annuals and is widespread throughout different type of habitat in Pakistan (Bajwa *et al.,* 2019). Extracts of different parts of *C. murale* have been found antifungal against *Fusarium oxysporum* (Naqvi *et al.,* 2020), and *Macrophomina phaseolina* (Javaid and Amin, 2009). However, information about antifungal activity of this plant against FOC are lacking. Therefore, this study was designed to evaluate antifungal potential of aerial parts of *C. murale* against this fungal pathogen.

Materials and Methods

In vitro bioassays

C. murale plants were collected during January, 2020 and different aerial parts were separated from each other. The plant materials were dried in sun and crushed thoroughly. One hundred grams of each dried plant material *viz.* leaves, stems and inflorescence, were extracted in 500 mL of methanol for 14 days. Thereafter, it was filtered and evaporated on a rotary evaporator. The gummy residues obtained were named as leaf, stem and inflorescence methanolic extracts, which were used in antifungal bioassays against FOC.

For antifungal bioassays, methanolic leaf, infloresence and stem extract different concentrations were prepared. In order to prepare the highest concentration of 50 mg mL^{-1}, 0.30 mg methanolic extract of each of the three selected plant parts were dissolved in 0.25 mL of DMSO and prepared up to 6 mL by adding autoclaved malt extract broth. Half of this growth media was used in

bioassays (1 mL for each replicate in 5-mL volume test tubes), and the remaining 3 mL were used for serial double dilution up to 1.562 mg mL^{-1} concentration. In this way, six concentrations *viz.* 1.562, 3125, 6.25, 12.50, 25 and 50 mg mL^{-1} were prepared. A series of control treatments was also prepared in this way. Each test tube was inoculated with 20 µL of FOC suspension and placed at 28 °C for one week and then filtered to get fungal biomass (Khan and Javaid, 2020).

Statistical analysis

Each treatment had three replications. Means were used for preparations of graphs. MS Excel program was used to calculate the standard errors of three replicates. Data regarding fungal biomass production in different treatments were subjected to one-way ANOVA along with LSD test application at $P \leq 0.05$ to separate the means of all treatments by using Statistix 8.1 computer software.

Results and Discussion

The tested extracts of *C. murale* exhibited variable antifungal activities against FOC. Among the three aerial parts, stem extract showed the highest activity. All of its concentrations suppressed growth of the fungus by 25–59%, over control (Fig. 1B and 2). The regression analysis showed a linear relationship between concentration of leaf extract and biomass of the fungus with $R^2 = 0.8838$. Methanolic stem extract of this weed was also effective against *M. phaseolina* where it reduced fungal biomass by 73–90% (Javaid and Amin, 2009). Many phenolic acids such as syringic, *p*-coumaric and ferulic acid have been identified in different *C. murale* plant parts which might cause control of FOC in the present study (Batish *et al.,* 2003). Recently, Naqvi *et al.* (2020) exlopred many antifungal compounds including phytol, oleic acid, methyl oleate, palmitic acid, β-sitosterol and stigmasterol from the stem extract of this weed plant.

Leaf extract exhibited remarkable activity against FOC. However, its activity was less marked than the activity of stem extract. The lowest concentrations (1.562 and 3.125 mg mL^{-1}) of leaf extract had nonsignificant effect. On the other hand, all other concentrations significantly suppresed biomass of FOC by 32–60%, as compared to control (Fig. 1A and 2). The relationship between concentration of leaf extract and biomass of FOC was linear with $R^2 = 0.9456$ (Fig. 3A). Earlier, *C. murale* leaf methanolic extract was found very effective against *in vitro* growth of *M. phaseolina* causing 87–90% reduction in its growth (Javaid and Amin, 2009). Similarly, Naqvi *et al.* (2019) checked antifungal potential of methanolic leaf extract of this weed and reported 14–45% reduction in biomass of *F. oxysporum* due to 1–5% extract concentrations. They also reported presence of antifungal compounds namely hexadecanoic acid, methyl linolenate, stigmasterol, palmitic acid and hexadecanoic acid, methyl ester.

None of the concentration of inflorescence extract showed significant antifungal activity (Fig. 1C). The highest antifungal activity was exhibited by 25 mg mL^{-1} concentration that declined fungal biomass by just 14%, over control (Fig. 2). In contrast to the present study, in a previous study by Javaid and Amin (2009), methanolic inflorescence extract of this weed was found very effective against *M. phaseolina* resulting in 62–77% reduction in its biomass. It indicates that inflorescence extract of this plant has variable activity against different fungal species.

Conclusion

This study demonstrated the highest antifungal efficacy of stem extract of *C. murale* followed by leaf extract against *F. oxysporum* f. sp. *cepae*.

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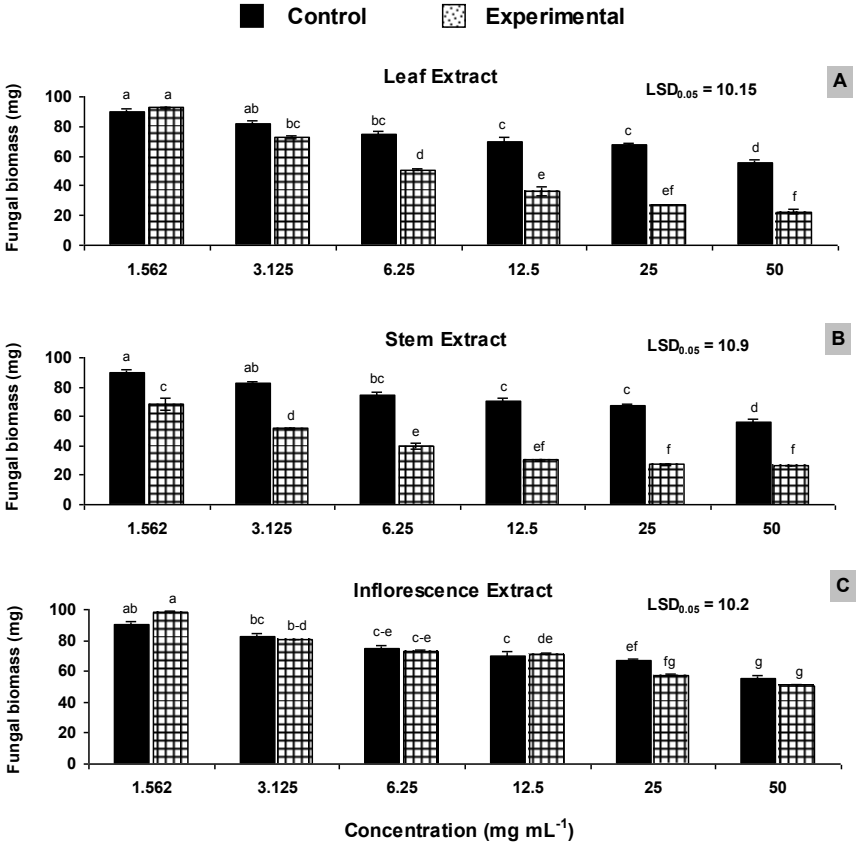

Fig. 1: Effect of methanolic extracts of *Chenopodium murale* on biomass of *Fusarium oxysporum* f. sp. *cepae* (FOC). Vertical bars show standard errors of means of three replicates. Values with different letters at their top show significant difference as determined by LSD test at $P \leq 0.05$.

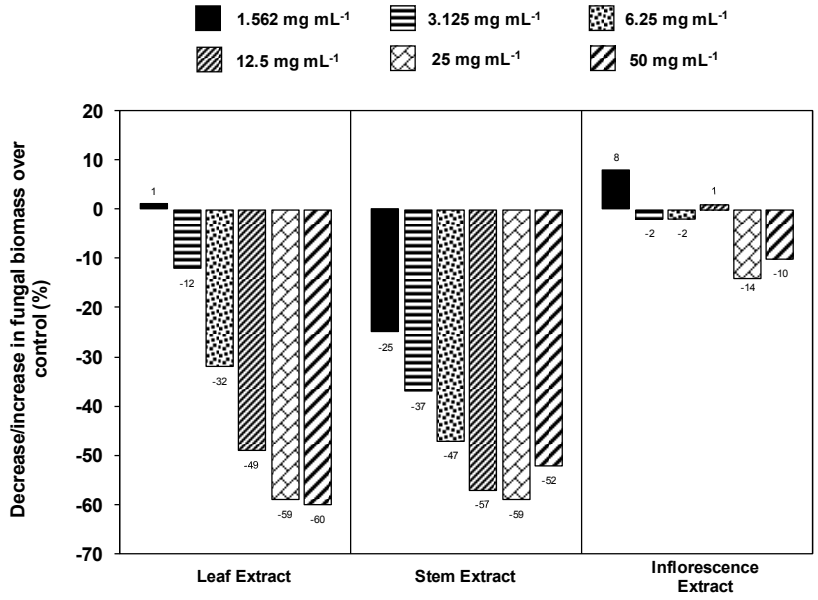

Fig. 2: Percentage decrease in biomass of *Fusarium oxysporum* f. sp. *cepae* (FOC) due to methanolic extracts of different parts of *Chenopodium murale*.

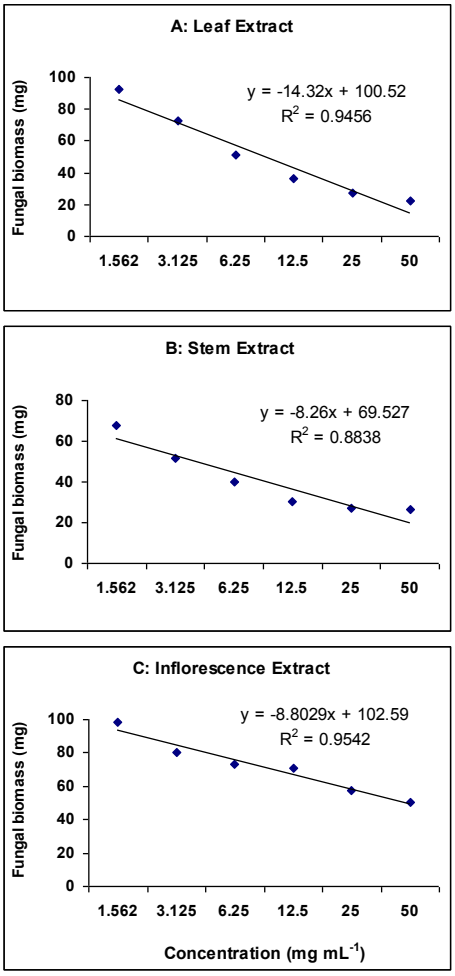

Fig. 3: Linear regression of the effect of different concentrations of methanolic extracts of *Chenopodium murale* on biomass of *Fusarium oxysporum* f. sp. *cepae* (FOC).

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