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DETECTION OF COMPOUNDS AND EFFICACY OF *N*-BUTANOL STEM EXTRACT OF *CHENOPODIUM MURALE* L. AGAINST *FUSARIUM OXYSPORUM* F.SP. *LYCOPERSICI*

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Abstract

An *in vitro* study was conducted to assess the antifungal efficacy and potential antifungal compounds of *n*-butanol fraction of methanolic stem extract of *Chenopodium murale* L. against *Fusarium oxysporum* f. sp. *lycopersici*. In order to get *n*-butanolic fraction, the methanolic extract was partitioned using *n*-hexane, chloroform and ethyl acetate to separate non-polar and low polarity compounds. Finally, *n*-butanol fraction was separated and its 8 concentrations ranging from 1.562 to 200 mg/ml were assessed for antifungal activity against *F. oxysporum* f. sp. *lycopersici*. There was 1 to 100% reduction in biomass of *F. oxysporum* f. sp. *lycopersici* due to these concentrations. GC-MS analysis of *n*-butanol fraction showed 25 compounds in it. Literature survey showed that among the identified compounds, 10 showed antifungal activities against different fungi. These antifungal compounds included 2-heptanol, 1-hexanol, 2-hexanol, 3-hexanol, 1-nonyne, decane, tridecane, palmitic acid, 3-octanone and β-sitosterol, could be responsible for antifungal activity against *F. oxysporum* f. sp. *lycopersici* in the present study.

Introduction

Fusarium wilt caused by *Fusarium oxysporum* f. sp. *lycopersici* is a highly destructive disease of tomato (Jangir *et al*. 2018). It causes huge economic losses when soil and air temperatures are rather high during most part of the growing season such as in warm climates (Srinivas *et al*. 2019). Currently, many synthetic fungicides are in practice for the control of *F. oxysporum*. However, their effectiveness is limited due to a number of constraints, such as soil-borne nature of the pathogen, resistance development, and toxicity and public concerns about their residual effects (Meena *et al*. 2020). Hence there is a need for identification of new antifungal agents with broad-spectrum antifungal activities that are environmentally safe and economical in use.

Antifungal compounds of family Chenopodiaceae are effective against many phytopathogens such as *Macrophomina phaseolina*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Alternaria solani*, *Ascochyta rabiei*, *Sclerotium rolfsii* and *Sclerotinia sclerotiorum* (Naqvi *et al*. 2019, Alkooranee *et al*. 2020, Javaid *et al*. 2020, Khan and Javaid 2020). *Chenopodium murale* L., commonly known as nettleleaf goosefoot is an important specie of this family. It is an annual weed plant that has spread worldwide, particularly in tropical and subtropical regions (Farhan *et al*. 2019). It is a drought-tolerant plant that grows rapidly along roadsides, dunes, waste places, streamlines, arable lands and on nutritionally poor soils (Bajwa *et al.* 2019). It is enriched with phenols, triterpenes, flavonoids, coumarins, iso-flavonoids, sesquiterpenoids, alkaloids, furano-terpenoids, and their derivatives with strong antifungal activities (Naqvi *et al*. 2019). The present investigation was planned to determine the antifungal efficacy of *n*-butanol stem extract of *Chenopodium murale* L. against *F. oxysporum* f. sp. *lycopersici* and the identification of potential antifungal compounds.

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Materials and Methods

Fresh plants of *Chenopodium murale* L. were collected from, Quaid-i-Azam Campus, Punjab University Lahore, Pakistan. The stems were washed thoroughly under tap water, dried completely under shade and then grinded with the help of a mechanical grinder to form a coarse powder. Dried and coarsely powdered stem material (2 kg) was extracted 3 times with methanol (5 l) for 14 days at room temperature. Crude methanolic stem extract was obtained by filtering through Whatman No. 1 filter papers. The extract was then concentrated at 45°C through vacuum distillation by using a rotary evaporator. After that, it was further concentrated in a dry heating oven at 45°C to have 87 g of the extract in the form of a thick paste. The extract was mixed with 200 ml distilled water and partitioned with 300 ml of *n*-hexane four times in a separating funnel followed by partitioning with chloroform, ethyl acetate and finally with *n*-butanol (300 ml each). Solvent was then evaporated in a rotary evaporator and dried in a dry-heating oven at 45°C to obtain 3 g stem extract. Antifungal bioassay was carried out using *n*-butanol fraction. The experiment was carried out in a completely randomized design with three replications following Banaras *et al.* (2020, 2021).

The *n*-butanol fraction was analyzed for the estimation of potential volatile antifungal constituents using GC-MS analysis. GC-MS analysis was carried out on GCMS QP2010 apparatus using helium as a carrier gas. Total running time was 30 min. The percentage composition of active volatile compounds was computed from the peak areas of chromatograms. Various volatile compounds were identified by comparing the data with the NIST library (Naqvi *et al.* 2019). Data were analyzed by applying ANOVA followed by application of LSD test at $p \leq 0.05$ using software Statistix 8.1.

Results and Discussion

The *n*-butanol fraction of methanolic extract of *Chenopodium murale* was found highly effective in retarding the growth of *F. oxysporum* f. sp. *lycopersici* (Fig. 1). The effect of the two lowermost concentrations (1.562 and 3.125 mg/ml) was insignificant where just 1–3% reduction in fungal biomass was recorded. By contrast, higher concentrations (6.25 mg/ml and above) of this extract significantly decreased the fungal biomass by 54–100%. These findings clearly indicate that *n*-butanol fraction contains potential antifungal constituents to control the growth of *F. oxysporum* f. sp. *lycopersici*. Recently, Khan and Javaid (2020a) reported that *n*-hexane and chloroform fractions of methanolic stem extract of *Chenopodium quinoa* completely inhibited growth of *Macrophomina phaseolina*. Soil infected with *Sclerotium rolfsii* was amended with 3% concentration of dry plant biomass of *C. album* that significantly controlled the collar rot disease in chickpea (Ali *et al.* 2020). Many members of Chenopodiaceae have physiologically active phytoconstituents that possess strong antifungal properties (Akopian *et al.* 2020).

GC-MS analysis of *n*-butanol fraction contained 25 constituents belonging to a diverse range of natural organic compounds. The phytochemical composition, retention time and peak areas of the compounds are summarized in Table 1. Out of the identified phytoconstituents, the most abundant compound was 2-heptanol (11.77%) followed by 3-hydroxyhexanoic acid (11.71%), 1-hexanol (9.97%), 2-butoxypentane (8.28%), pentafluoropropionic acid (8.19%), 2-hexanol (7.34%), 1,1-dipropoxyethane (7.24%) and 3-hexanol (6.52%). The compounds present in moderate concentrations were 1-butoxy-1-ethoxyethane (4.58%), 1-iodoheptane (4.41%), decane (3.68%), 1,2-decanediol (2.36%), 1-nonyne (2.35%), palmitic acid (2.20%) and 1,9-Nonanediol nonanediol (1.82%). The remaining 10 compounds were recorded as less abundant ones with their peak areas ranging from 0.31 to 1.61%.

Many of the identified compounds possess strong antifungal properties. Among them, 2-heptanol was recently isolated from the extracts of a lactic acid producing bacterium namely *Lactobacillus plantarum*, that exhibited strong antifungal properties and found to be highly useful in increasing shelf life of wheat bread (Sun *et al*. 2020). Similarly, 1-nonyne and 3-octanone showed significant potential against *Candida albicans* (Jaradat *et al.* 2017, Nainangu *et al*. 2020). Likewise, 2-hexanol seemed to be very effective against opportunistic fungal pathogens namely *Cryptococcus neoformans*, *C. albicans* and *Aspergillus niger* (Lawson *et al*. 2019). Decane also showed the maximum inhibitory potential against a wide range of soil- and seed-borne fungi namely *Sclerotinia sclerotiorum, Rhizoctonia solani, Verticillium dahlia, Alternaria solani* and *F. oxysporum* (Bayan 2016). Souza *et al.* (2015) reported the antifungal activities of palmitic acid against *Candida glabrata, C. parapsilosis, C. krusei* and *C. albicans*. β-Sitosterol was identified from the *n*-hexane bark extract of *Picea abies* with potent antifungal potential against *A. alternata* (Burcova *et al*. 2018). Choi *et al.* (2017) also reported this compound from the root extracts of *Dipsacus asper* and it was thought to be responsible in suppressing the growth of late blight of tomato, gray mold tomato and rice blast diseases caused by *Phytophthora infestans, Botrytis cinerea* and *Magnaporthe grisea*, respectively. Similarly, 3-hexanol and tridecane significantly retarded growths of *Ganoderma boninense* (Angel *et al*. 2016) and *F. oxysporum* f. sp. *niveum* (Raza *et al*. 2015), respectively.

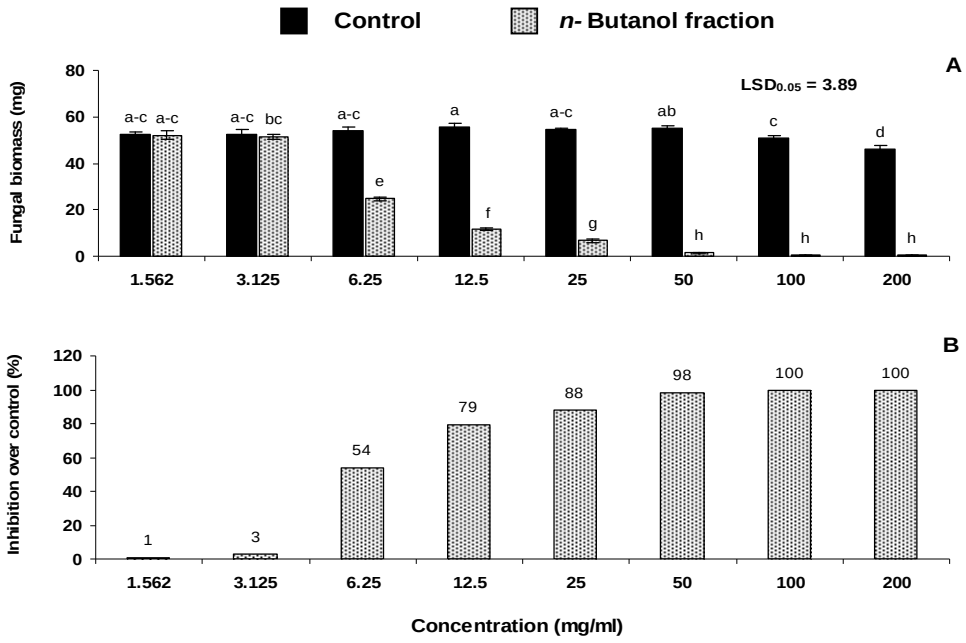

Fig. 1. Antifungal activity of different concentrations of *n*-butanol fraction of methanolic stem extract of *Chenopodium murale* against the biomass (A) and inhibition (B) of *Fusarium oxysporum* f. sp. *lycopersici*.

From the present study, it may be concluded that *n*-butanol fraction of *C. murale* stem extract has the ability to retard the growth of *F. oxysporum* f. sp. *lycopersici.* The various antifungal phytoconstituents such as 2-heptanol, 1-hexanol, 2-hexanol, 3-hexanol, 1-nonyne, decane,

tridecane, palmitic acid, 3-octanone and β-sitosterol, might be responsible for suppressing the growth of targeted pathogen.

Table 1. Compounds identified from *n*-butanol fraction of methanolic stem extract of *Chenopodium murale* through GC-MS analysis.

Sl. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	2-Heptanol	$C_7H_{16}O$	116	5.133	11.77
2	Pentafluoropropionic acid	$C_9H_{13}F_5O_2$	248	5.250	8.19
3	1-Hexanol	$C_6H_{14}O$	102	5.442	9.97
4	2-Hexanol	$C_6H_{14}O$	102	5.575	7.34
5	3-Hexanol	$C_6H_{14}O$	102	5.892	6.52
6	3-Hydroxyhexanoic acid	$C_6H_{12}O_3$	132	6.058	11.71
7	1,1-Dipropoxyethane	$C_8H_{18}O_2$	146	6.267	7.24
8	1-Iodoheptane	$C_7H_{15}I$	226	6.392	4.41
9	1-Butoxy-1-ethoxyethane	$C_8H_{18}O_2$	146	6.458	4.58
10	2-Butoxypentane	$C_9H_{20}O$	144	6.733	8.28
11	Butylpentyl ether	$C_9H_{20}O$	144	8.142	1.61
12	Acetic acid, hexyl ester	$C_8H_{16}O_2$	144	8.192	1.49
13	1-Nonyne	C_9H_{16}	124	8.342	2.35
14	Decane	$C_{10}H_{22}$	142	8.575	3.68
15	Hecogenin	$C_{27}H_{42}O_4$	430	9.817	0.47
16	Tridecane	$C_{13}H_{28}$	184	10.017	0.31
17	1,1-Dipropoxypropane	$C_9H_{20}O_2$	160	10.183	0.49
18	1,2-Decanediol	$C_{10}H_{22}O_2$	174	10.442	2.36
19	1,9-Nonanediol	$C_9H_{20}O_2$	160	11.750	1.82
20	1,1-Dibutoxyethane	$C_{10}H_{22}O_2$	174	12.033	0.35
21	2-Heptenoic acid	$C_7H_{12}O_2$	128	13.017	0.93
22	Palmitic acid	$C_{16}H_{32}O_2$	256	21.208	2.20
23	Methyl oleate	$C_{19}H_{36}O_2$	296	22.500	0.33
24	3-Octanone	$C_8H_{16}O$	128	26.017	1.60
25	β-Sitosterol	$C_{29}H_{50}O$	414	31.667	1.00

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