J. Plantarum., 7(1): 01-08 ISSN PRINT 2710-4079
ISSN ONLINE 2710-4087

ANTIFUNGAL EFFICACY OF BUTANOLIC ROOT EXTRACT OF *CHENOPODIUM MURALE*

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Received on: 23-11-24; Reviewed on: 21-04-25; Accepted on: 12-05-2025; Published on: 15-06-2025

Abstract

Fusarium oxysporum f. sp. *lycopersici* (FOL) is a very detrimental fungal pathogen that affects tomato crops. This study employed the n-butanol fraction of the root extract of nettle-leaved goosefoot weed (*Chenopodium murale* L.) to control this pathogen. The dried and crushed roots of *C. murale* were extracted using methanol. After removing the soluble components of n-hexane, chloroform, and ethyl acetate, the n-butanol fraction was finally recovered. Laboratory bioassay was conducted using concentrations of this fraction ranging from 1.562 to 200 mg mL^{-1}. The antifungal effect of 12.5 mg mL^{-1} and greater dosages was significant, with a 48–99% reduction in FOL biomass. GC-MS analysis revealed the presence of eighteen phytochemicals in the extract. These included 2-methylnonane (13.20%), 1-hexanol (11.07%), 1-heptanol (10.38%), 3-hexanol (9.55%), γ-sitosterol (6.79%), oleic acid (6.64%), 2-hydroxyoctanoic acid (6.29%), 1-nonyne (6.12%), acetoxyacetone (5.79%), decane (5.39%), palmitic acid (4.95%), 1,1-diethoxypropane (3.54%), acetic acid, hexyl ester (3.11%), methyl oleate (2.37%), stigmasterol (1.87%), β-sitosterol (1.35%), 17-octadecenoic acid (0.93%), and stearic acid (0.56%). A literature survey revealed that the compounds namely 1-hexanol, 1-hepanol, palmitic acid, oleic acid, stearic acid and β-sitosterol are antifungal in nature and may be responsible for FOL control in the current investigation.

Keywords: Antifungal, Butanolic extract, *Chenopodium murale*, Tomato wilt

INTRODUCTION

The tomato, a vital crop in Pakistan, is vulnerable to several diseases that can lead to losses globally. The fungal pathogen *Fusarium oxysporum* f. sp. *lycopersica* (FOL) is the cause of Fusarium wilt, which is thought to be the most harmful of the tomato diseases (Akhter *et al*., 2016). The pathogen typically is soil-borne although stem may show signs of fungal colonization and so the pathogen can be isolated from this plant part as well (Summeral *et al*., 2003). Infected plants often display wilting, yellowing leaves, and stunted growth. The fungus persists in the soil, making crop rotation and soil management practices vital for controlling its spread and maintaining healthy ecosystems. FOL is found worldwide, affecting tomato production in many countries, which makes it a concern for global food security. High rates of economic losses are bound to occur when the pathogen in question is met with higher soil and air temperatures (Srinivas *et al*., 2019)

Usually, fungicides are used to combat wilt diseases (Amini and Dzhalilov, 2010). However, the use of chemicals has their drawbacks as they not only pollute the environment but also are hazardous to humans and animal health while the major concern is that the pathogens can develop resistance against some fungicides (Oruc, 2010). Also, chemical fungicides contaminate soil and water sources, leading to ecological imbalances. Runoff from agricultural fields can carry these substances into local waterways, affecting aquatic ecosystems and biodiversity. Many fungicides pose

risks to human health and animal welfare. Chronic or acute health problems, such as skin irritation, respiratory disorders, and even cancer, can result from prolonged contact. So, a wide array of studies is being carried out to develop disease management methods that are environmentally friendly with less adverse effects by employing the use of natural compounds (Javaid and Naqvi, 2015; Khan and Javaid, 2022a). Numerous essential oils and several allelochemicals have been extracted from various plant parts and have proved to be useful and effective against quite many pathogens (Ferdosi *et al*., 2021; Rafiq *et al*., 2024). These substances are useful substitutes for synthetic fungicides because they can interfere with metabolic processes, break down fungal cell membranes, or prevent spore germination (Nazzaro *et al*., 2017; Zhang *et al*., 2023).

Many members of Chenopodiaceae such as *Chenopodium album, C. ambrosioides* and *Kochia indica* are known to exhibit antifungal properties against *Sclerotium rolfsii* and *Macrophomina phaseolina* (Javaid and Amin, 2009; Javed *et al*., 2018; Ali *et al*., 2020). There aren't many reports, nevertheless, about their antifungal activity against *Fusarium oxysporum*. To suppress *F. oxysporum*, Naqvi *et al*. (2019) found that *C. murale* leaf extract has a variety of antifungal components. The objective of the present study was to investigate the antifungal characteristics of the n-butanol fraction of the root extract of *C. murale* against FOL and to identify possible antifungal components in this root extract fraction.

MATERIALS AND METHODS

Preparation of extract

Five liters of methanol were used to extract two kilograms of dry powdered root material. The root extract was separated from the debris by filtering through Whatman No. 1 filter sheets. The solvent was evaporated at 45 °C using a rotary evaporator. When a small amount of methanol was left in the rotary flask, the mixture was transferred to a beaker and placed in a dry heating oven at 45 °C for complete evaporation of the methanol. Finally, 87 g of a thick paste was obtained that was named as crude methanolic extract. In a separating funnel, the crude methanolic stem extract was vigorously mixed with 200 mL of distilled water, and then it was partitioned four times using 300 mL of *n*-hexane. Subsequently, chloroform, ethyl acetate, and n-butanol (300 mL each) were mixed with the remaining mixture and partitioned in a separating funnel. The last n-butanol was collected for further experimentation. This fraction was selected to identify the compounds in roots of *C. murale* which were soluble in the highly polar solvent. Phytochemical composition of a less polar solvent ethyl acetate fraction has already been reported in a previous study (Javaid *et al*., 2021). Initially, the solvent was evaporated in a rotary evaporator. Finally, three grams of crude extract were obtained by drying the solvent in a dry-heating oven at 45 °C (Rafiq *et al*., 2024).

Antifungal bioassay

To prepare a range of concentrations of the n-butanol fraction for antifungal bioassay, Khan and Javaid (2020) devised a serial double dilution approach. This was achieved by making 6 mL of a stock solution with a 200 mg mL^{-1} concentration by dissolving 1.2 g of the extract in 0.5 mL of dimethyl sulfoxide (DMSO) and mixing it with autoclaved malt extract broth. This solution was serially double diluted seven times to get concentrations ranging from 100 to 1.562 mg mL^{-1}. To maintain the same level of DMSO in the

corresponding control treatments, the same amount of DMSO and malt extract were mixed and serially double diluted. For antifungal bioassays, 20 µL of the pathogen inoculum was added to 10 mL test tubes containing 1 mL of each treatment mixture, and the tubes were then incubated at 30 °C for a week. Fungal biomass was then dried, filtered, and weighed. Four replications in a completely randomized design were used to carry out the experiment.

GC-MS analysis

The GC-MS analysis of n-butanol fraction was carried out on GC-MS QP-2010 following the procedure of Naqvi *et al*. (2019).

Statistical analysis

In vitro study was conducted in a completely randomized design using four replicates of each treatment. Means were used to prepare the graph and the replicates were used to calculate standard errors of the means and to apply statistics. Using the Statistix 8.1 software, the data were analyzed using a one-way ANOVA and then the LSD test at the 5% level of significance.

RESULTS AND DISCUSSION

Antifungal activity of extract

Three lower dosages (1.562 to 6.25 mg mL^{-1}) had no significant antifungal effect against FOL. All the dosages above 6.25 mg mL^{-1}, however, considerably decreased the fungal biomass to varying degrees. A progressive decrease in fungal biomass from 45% to 99% was observed, with the effect of doses ranging from 12.5 to 200 mg mL^{-1} being concentration dependent (Fig. 1 and 2). Earlier studies were carried out using leaf and stem extracts of *C. murale* where significant antifungal activities were reported against FOL, which support our findings (Naqvi *et al*., 2019, 2022). There are also reports that extracts of other species of the genus *Chenopodium* such as *C. quinoa, C. album* and *C. ambrosioides* exhibited antifungal activities against *Macrophomina phaseolina* and *Sclerotium rolfsii* (Shah and Khan, 2017; Khan and Javaid, 2020; Javaid *et al*., 2023). Antifungal activity of plants in Chenopodiaceae family may be attributed to the presence of various fungal metabolites including phenolic, saponins, terpenoids, fatty acid methyl esters and flavonoids (Stuardo and Martin, 2008; Naqvi *et al*., 2022).

GC-MS analysis

GC-MS chromatogram is shown on Fig. 3 and Table 1. There were 18 compounds in n-butanol fraction as presented in Table 1. The most abundant compounds were 2-methylnonane (13.20%), 1-hexanol (11.07%), 1-heptanol (10.38%), and 3-hexanol (9.55%). 2-Methylnonane is a volatile aroma compound, reported in some plant species such as Prunus mahaleb and Phoenix rupicola. However, its bioactivity is not known (Yilmaz and Karatas, 2023). 1-Hexanol, 3-hexanol and 1-heptanol are primary alcohols found in plant essential oils. The former one is known to possess antibacterial activity against Gram-negative bacteria (AbouZeid *et al*., 2022; Kyoui *et al*., 2023). 1-Hexanol and 1-heptanol, emitted by bacteria, displayed antifungal activity against Aspergillus flavus (Zhang *et al*., 2024).

Moderately abundant compounds included γ-sitosterol (6.79%), oleic acid (6.64%), 2-hydroxyoctanoic acid (6.29%), 1-nonyne (6.12%), acetoxyacetone (5.79%), decane (5.39%), palmitic acid (4.95%), 1,1-diethoxypropane (3.54%), and acetic acid, hexyl ester (3.11%) as shown in Table 1. Among these, γ-sitosterol has been reported in many plant species including *Beaumontia grandiflora* (Ferdosi *et al*., 2022) and *Chenopodium quinoa* (Khan and Javaid, 2022b).

So far, there is not any report of antifungal activity of this compound. However, it is known for its anticancer (Sundarraj *et al*., 2012) and antidiabetic (Sirikhansaeng *et al*., 2017) activities. Oleic acid possesses antifungal activity. *Pythium ultimum* and *Crinipellis perniciosa* both had their mycelial growth considerably inhibited by its 100 and 1000 µM concentrations, respectively (Walters *et al*., 2004). The virulence factors of the fungus *Candida tropicalis* were also suppressed by palmitic acid (Prasath *et al*., 2020).

Methyl oleate (2.37%), stigmasterol (1.87%), and β-sitosterol (1.35%) were ranked as less abundant compounds (Table 1). All these compounds are known for their antifungal properties against different fungal species. Methyl oleate belongs to fatty acid methyl esters groups whose members are well-known for their antifungal activities (Pinto *et al*., 2017). Stigmasterol isolated from red betel leaves exhibited *in vitro* antifungal activity against *Candida albicans* (Lestari *et al*., 2024). β-sitosterol extracted from *Senecio lyratus* showed antifungal activity against Fusarium spp. (Kiprono *et al*., 2014). Two compounds, namely 17-octadecenoic acid (0.93%), and stearic acid (0.56%) were termed as the least abundant ones due because of their occurrence below 1% (Table 1).

CONCLUSION

It concludes that n-butanol extract of *C. murale* root can decrease FOL growth by 96% and 99%, respectively, at concentrations of 100 and 200 mg mL^{-1}. The antifungal activity of this extract may be due to compounds such fatty acids, fatty acid methyl esters, β-sitosterol, stigmasterol, 1-hexanol, and 1-heptanol as also reported in previous literature.

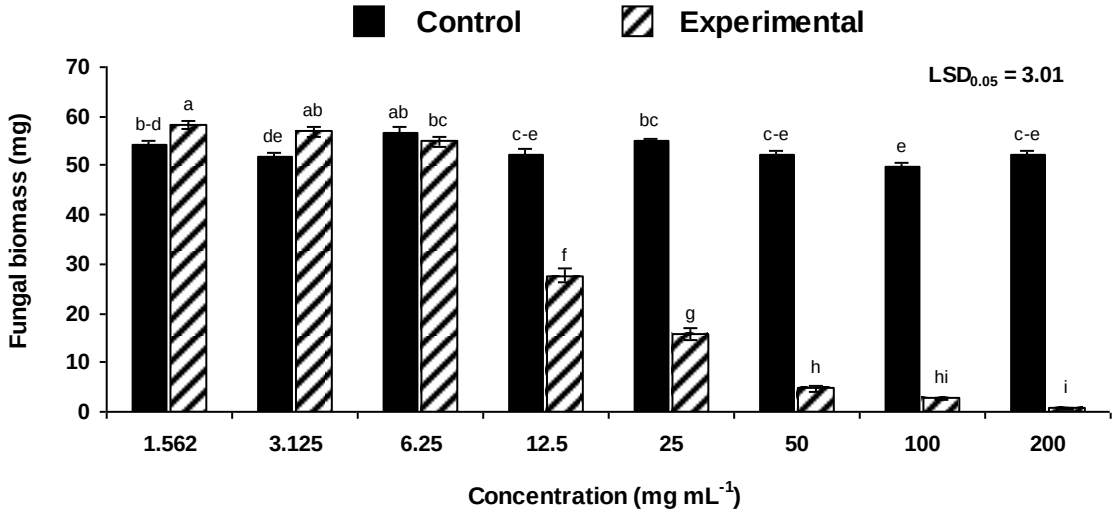

Fig. 1: Effect of *n*-butanol fraction of methanolic root extract of *Chenopodium murale* on biomass of *Fusarium oxysporum* f. sp. *lycopersici.* Vertical bars show standard errors of means of four replicates. Values with different letters at their top show significant difference (P≤0.05) as determined by LSD Test.

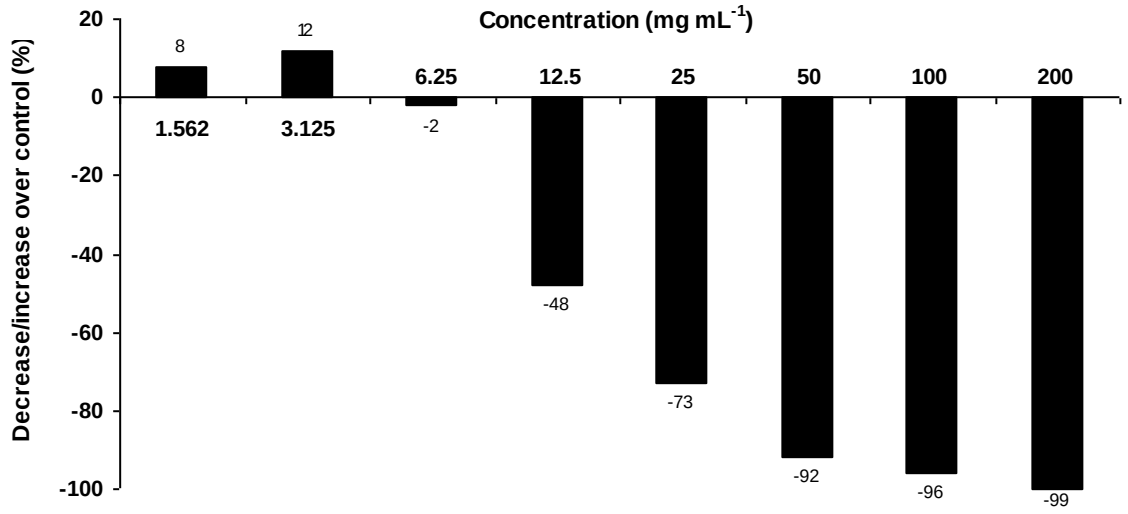

Fig. 2: Percentage increase/decrease in biomass of *Fusarium oxysporum* f. sp. *lycopersici* due to different concentrations of *n*-butanol fraction of methanolic root extract of *Chenopodium murale*

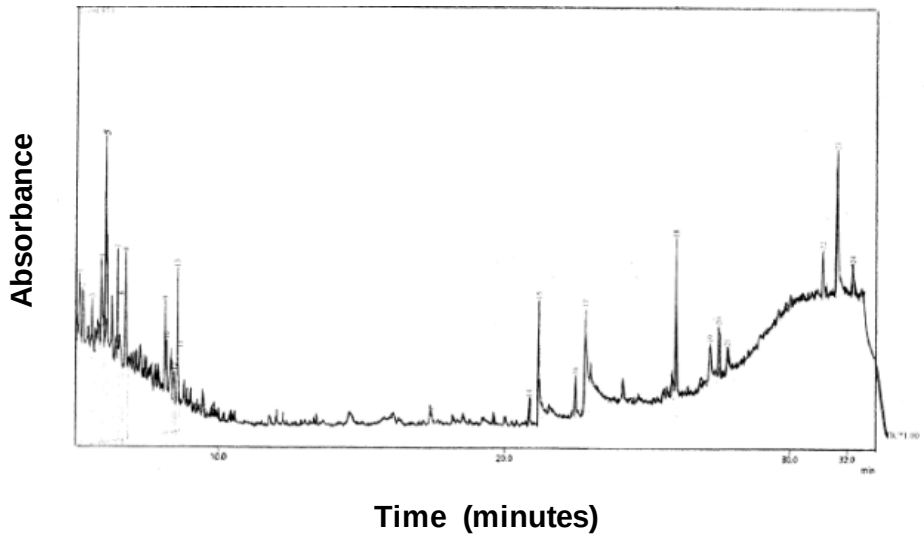

Fig. 3: GC-MS chromatograms of *n*-buttanol sub-fraction of methanolic root extract of *Chenopodium murale*

Table 1: GCMS analysis *n*-buttanol sub-fraction of methanolic root extract of *Chenopodium murale.*

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	1-Heptanol	$C_7H_{16}O$	116	5.133	10.38
2	1-Hexanol	$C_6H_{14}O$	102	5.233	11.07
3	2-Hydroxyoctanoic acid	$C_8H_{16}O_3$	160	5.575	6.29
4	3-Hexanol	$C_6H_{14}O$	102	5.892	9.55
5	2-Methylnonane	$C_{10}H_{22}$	142	6.742	13.20
6	Acetoxyacetone	$C_5H_8O_3$	116	8.142	5.79
7	Acetic acid, hexyl ester	$C_8H_{16}O_2$	144	8.192	3.11
8	1-Nonyne	C_9H_{16}	124	8.342	6.12
9	1,1-Diethoxypropane	$C_7H_{16}O_2$	132	8.450	3.54
10	Decane	$C_{10}H_{22}$	142	8.575	5.39
11	Palmitic acid	$C_{16}H_{32}O_2$	256	21.208	4.95
12	17-Octadecenoic acid	$C1_9H_{36}O_2$	296	22.500	0.93
13	Oleic acid	$C_{18}H_{34}O_2$	282	22.842	6.64
14	Methyl oleate	$C_{19}H_{36}O_2$	296	27.217	2.37
15	Stearic acid	$C_{18}H_{36}O_2$	284	27.800	0.56
16	Stigmasterol	$C_{29}H_{48}O$	412	31.150	1.87
17	γ-Sitosterol	$C_{29}H_{50}O$	414	31.650	6.79
18	β-Sitosterol	$C_{29}H_{50}O$	414	32.208	1.35

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