ANTIFUNGAL ACTIVITY OF LEAF EXTRACT OF *Cannabis sativa* AGAINST *Aspergillus flavipes*

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ABSTRACT

Cannabis sativa L. is a medicinally important plant, generally grows as a weed in waste lands in Punjab, Pakistan. In the present study, antifungal effect of leaf extract of this weed was assessed against *Aspergillus flavipes.* Methanolic leaf extract of the weed was partitioned into five fractions using organic solvents of variable polarities. A range of concentrations (1.562 to 200 mg mL^{-1}) of each fraction was used in laboratory bioassays. *n*-Butanol fraction showed the highest antifungal activity followed by chloroform and *n*-hexane fractions causing 68–82%, 52–82% and 42–82% decrease in biomass of *A. flavipes.* Ethyl acetate showed a moderate antifungal potential whereas aqueous fraction showed the least antifungal activity causing 47–76% and 38–73% reduction in fungal biomass, respectively. This study concludes that *n*-butanol fraction of leaf extract of *C. sativa* is highly effective in controlling growth of *A. flavipes.*

Keywords: Antifungal, *Aspergillus flavipes, Cannabis sativa,* Natural fungicides.

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INTRODUCTION

Cannabis sativa L. (Cannabaceae), is an annual herbaceous plant being utilized for 12,000 years or more, for the medicinal purposes in Central Asia and South East Asia (Pisanti and Bifulco, 2019). Its production is widespread in Pakistan making it one of the largest cannabis producers in the world (Rehman *et al.,* 2013; Sandeela, 2020). In recent century, Pakistan is looking ahead to export industrial hemp and other derivatives to the international market after the legalization of cannabidiol (Veldman, 2020). It has been used as a source of oil, food and fiber as well as for religious and recreational purposes over the centuries (Bonini *et al.,* 2018). It contains a number of chemically active compounds, including cannabinol, cannabinoids, cannabicyclol, cannabigerol, cannabichromene, cannabidiol, tetrahydrocannabinol, terpenoids, alkaloids, flavonoids and phenolics (El-Sohly *et al.,* 2017; Booth and Bohlmann, 2019). Many indigenous communities depend upon derivatives of *C. sativa* for the procurement of gastrointestinal diseases, pain, vomiting, anorexia, nausea, colitis, snake bite, wound healing, inflammation, anxiety, schizophrenia, epilepsy, sleep disorders and for ethnoveterinary remedies (Zuk-Golaszewska and Golaszewski, 2018; Bernstein *et al.,* 2019).

Agricultural commodities are mainly deteriorated by air-borne fungal pathogens with an annual loss of 30% all around the world (Kazerooni *et al.,* 2020). *Aspergillus flavipes* (Bainier & Sartory) Thom & Church is one of the most common life-threatening filamentous fungus, with the potential to attack on stored grains and cereals (Kermani *et al.,* 2016). It is an air-borne destroyer of foodstuffs, retards their nutritious value and make them unfit for human consumption by the production of mycotoxins (Odetunde *et al.,* 2020). A sizeable population of African and Asian countries is suffering from health problems by consuming mycotoxins contaminated grains (Bhat and Reddy, 2017). The control of *A. flavipes* can be efficiently achieved by the use of synthetic fungicides but their direct application on grains leave residual effects (Mohammed *et al.,* 2018). Thus, there is a strong need to develop some ecofriendly alternative management strategies with no toxic effects and less capital intensive. Products of plant origin have been reported one of the best options to control stored grains pathogens with no impact on the environment and less dangerous to the consumers (Shricharan *et al.,* 2020). Therefore, the present investigation was undertaken to test the antifungal efficacy of *C. sativa* leaf extract against *A. flavipes.*

MATERIALS AND METHODS

Preparation of extracts

Fresh *C. sativa* leaves were collected from deserted lands in Gujrat and washed under tap water to remove unwanted soil particles. Leaves were shade dried and grinded into a fine powder by using a mechanical grinder. The powdered leaves (3 kg) were macerated in methanol (7 L) for two weeks. Methanolic extract was passed through a muslin cloth followed by the two layers of Whatman No. 1 filter paper. The obtained material was evaporated on a rotary evaporator at 45 °C under vacuum distillation and the filtrate was then concentrated in an electric oven at 40 °C to get 198 g residues. The gummy methanolic extract was then mixed in 200 mL of autoclaved distilled water and the resultant mixture was partitioned with *n*-hexane (4 × 500 mL), followed by chloroform (400 mL), ethyl acetate (500 mL) and *n*-butanol (300 mL) by using a separating funnel. The obtained solvents were then evaporated on a rotary evaporator and dried completely at 40 °C to get 18.4, 11.7, 9.5 and 10.3 g of each of the fraction, respectively.

Antifungal bioassays

In vitro biological activities of all the fractions were assessed against *A. flavipes.* Each of the fraction (1.2 g) was mixed in dimethyl sulphoxide (1 mL) followed by the addition of 2% (w/v) malt extract broth (5 mL) in order to prepare the highest concentration of 200 mg mL^{-1} that was then serially diluted to get the lower concentrations such as 100, 50, 25,…..1.562 mg mL^{-1}. A control

was also maintained similarly without the addition of plant extracts. Mature colony of *A. flavipes* was scratched in distilled water for the preparation of inoculum. Each treatment was replicated thrice and all the glass tubes were inoculated with 5 µL of fungal suspension under aseptic conditions and kept at 28 °C for 8 days. The fungal mats were filtered through pre-weighed filter papers, dried and weighed (Khan and Javaid, 2020).

Statistical analysis

All the data were analyzed by applying ANOVA followed by LSD test at 5% level of significance using Statistix 8.1.

RESULTS AND DISCUSSION

Different fractions prepared from *C. sativa* leaf extract were effective against *A. flavipes* (Fig. 1 A-E). Among them, *n*-butanol concentrations (1.562 to 200 mg mL^{-1}) showed the highest antifungal activity causing 68–82% growth inhibition in the pathogenic fungal biomass (Fig. 2). Chloroform and *n*-hexane fractions also showed a remarkable reduction of 52–82% and 42–82% in *A. flavipes* growth over control, respectively. Ethyl acetate exhibited a moderate antifungal activity by inhibiting 47–76% and aqueous fraction was found the least effective in comparison to other fractions by arresting 38–73% fungal biomass production. In accordance to the present study, Wanas *et al.* (2016) evaluated the antifungal potential of *n*-hexane fraction of *C. sativa* against a life-threatening human pathogen *Cryptococcus neoformans* which is responsible for lungs infection. The findings revealed that the pathogen growth can be minimized by using the lower concentrations of plant extracts. Singh *et al.* (2018) reported that *C. sativa* is a rich source of bioactive constituents like terpenes, cannabinoids, flavonoids and phenols, which possess strong antifungal properties and might be effective in arresting the growth of *A. flavipes*. Likewise, Bonini *et al.* (2018) reported cannabinoids, a key compound present in *C. sativa,* is extensively used in pharmaceutical studies and for the preparation of medicines to cure human diseases. In a recent study related to our work, *Aspergillus niger, A. flavus* and *A. parasiticus* were isolated from maize seeds. The isolated pathogens were controlled by using different plant extracts, where *Zingiber officinale, Euphorbia peplus* and *Eucalyptus camaldulensis* completely retarded the pathogens growth (Kamaluddin *et al.,* 2020). Nalli *et al.* (2018) isolated cannabinoids from *C. sativa* and screened their antifungal potential against candidiasis disease caused by *C. albicans,* a human fungal pathogen. *C. sativa* essential oils contain caryophyllene, α-humulene and (E)-caryophyllene compounds which significantly reduced the fungal biomass of *Candida* species in comparison of conventionally used compounds (Nafis *et al.,* 2019). Malheiros *et al.* (2019) also worked on ethanolic leaf extracts of *Eugenia dysenterica* against the *Aspergillus* species and obtained remarkable results. Swain *et al.* (2016) prepared gold nanoparticles from leaf extracts of *C. sativa* that were effective against *A. fumigatus, A. flavus,* and species of *Fusarium*, *Penicillium* and *Mucor*. The extracts of *C. sativa* have never been tested earlier against the *A. flavipes*. The present investigation concludes that leaf extract of *C. sativa* has pronounced antifungal potential against *A. flavipes*.

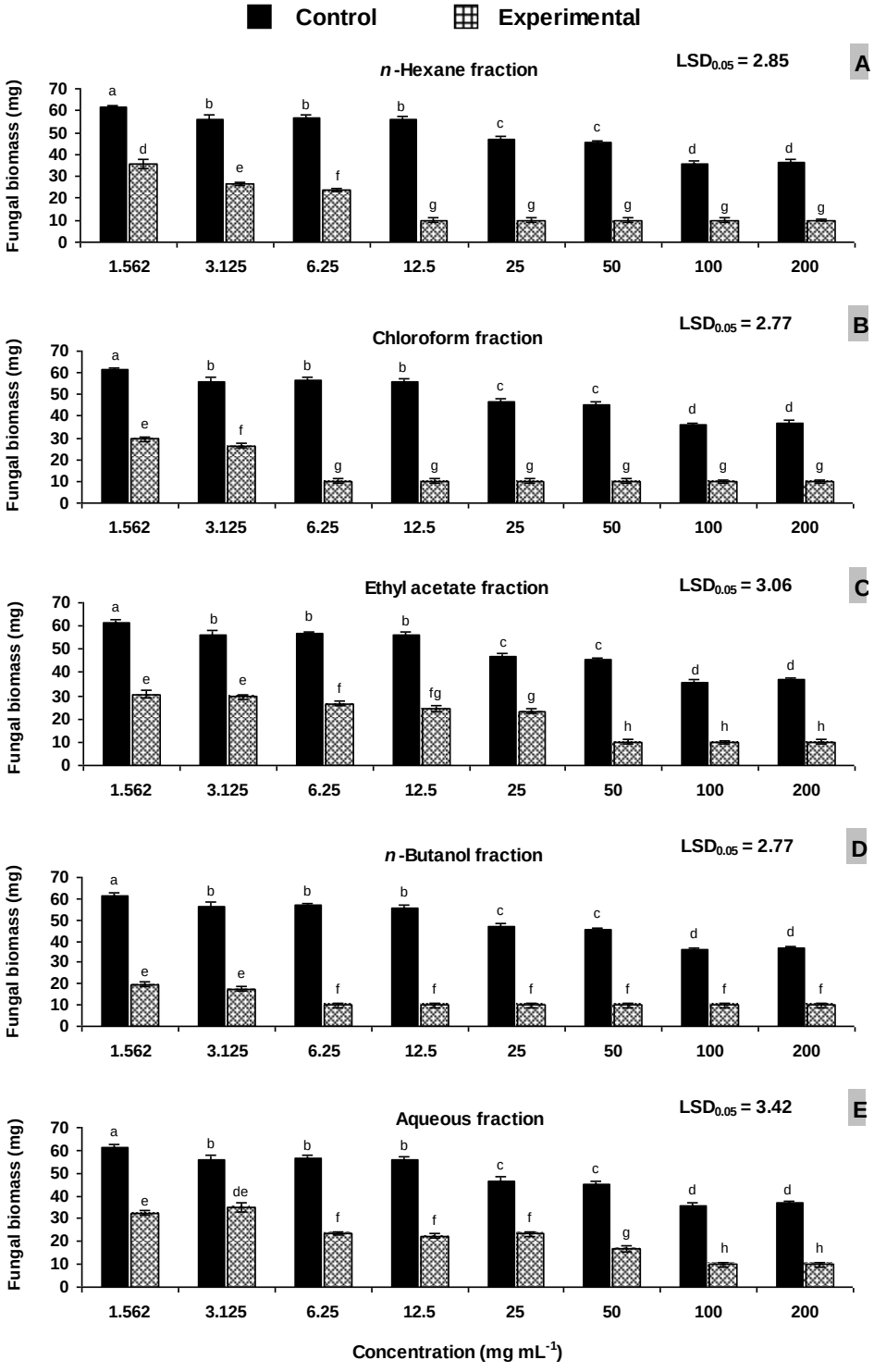

Fig. 1 (A-E): Effect of different concentrations of fractions of methanolic leaf extract of *Cannabis sativa* on growth of *Aspergillus flavipes*.

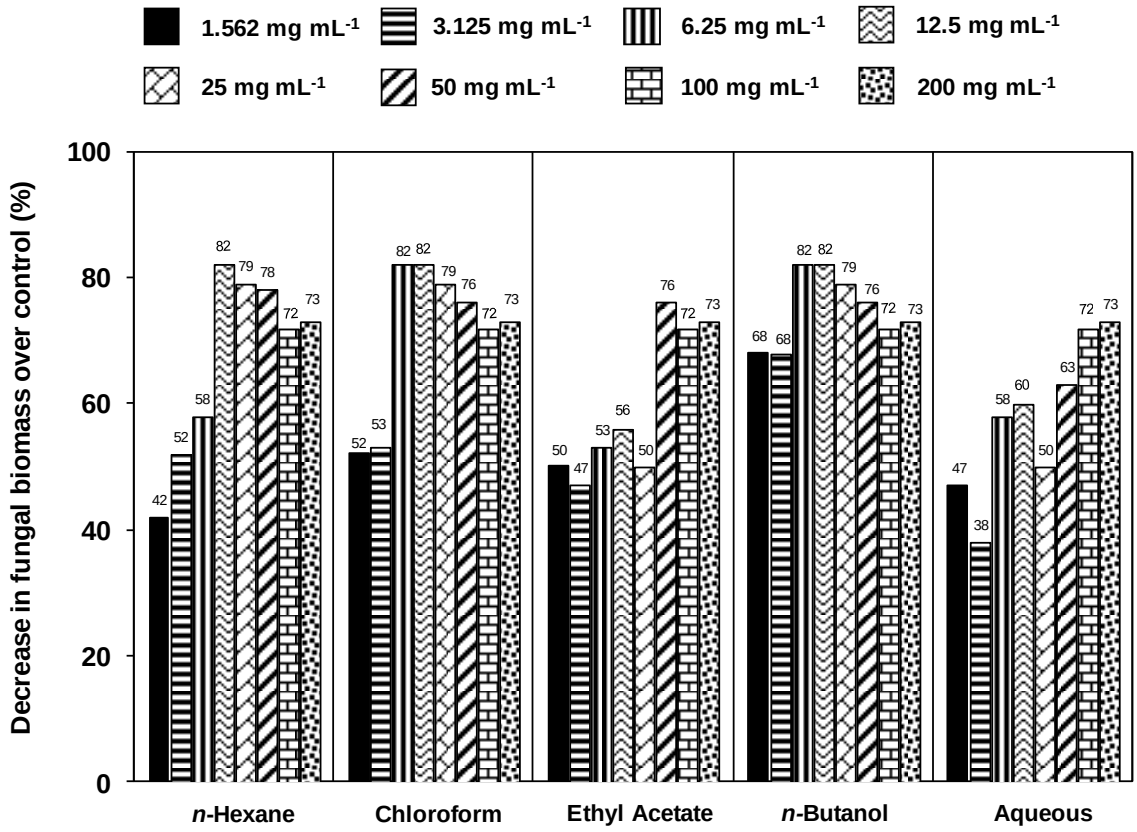

Fig. 2: Percentage decrease in biomass of *Aspergillus flavipes* due to different fractions of methanol leaf extract of *Cannabis sativa* over control.

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