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Research Paper

Inhibitory potential of parthenin a sesquiterpene lactone against *Fusarium oxysporum, Aspergillus niger* and *Drechslera hawaiiensis*

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Abstract

Effect of parthenin extracted from the leaves of *Parthenium hysterophorus* and its effect on certain pathogens like *Fusarium oxysporum* (Schlecht.), *Aspergillus niger* (Tiegh.) and *Drechslera hawaiinsis (*M.B. Ellis) at 0%, 25%, 50%, 75% and 100% concentration was studied. The experiment was carried out periodically with 10 days interval upto 20 days. The fungal mycelial colony diameter (in mm) was compared with the control and was contemplated as a measure of phytotoxicity. The efficacy of 50% and 100% fresh aqueous extracts of leaves containing parthenin showed significant inhibition in *D. hawaiiensis* followed by *F. oxysporum* and *A. niger.*

Keywords: Carrot weed*,* Allelochemical, Plant Pathogens, Concentration and Leachates.

Introduction

Famine weed *(Parthenium hysterophorus)* an annual member of the tribe Heliantheae of the family Asteraceae is commonly known as *Parthenium* weed in Australia and congress grass as it is uniquely called in India. *Parthenium* weed contain toxins from the chemical group of C_{15} group [1] .The major component of toxin being parthenin and other phenolic acids such as caffeic, vanillic, ferulic, chlorgenic, p-hydrobenzoic acid, p-cumaric acid and anisic acid are lethal to human beings and animals [2]. *Parthenium* contains 35 lactones of the pseudoguaicinolide and xanthanolide skeletal types. Parthenin a major constituent is a sesquiterpenoid having a pseudoguaianolide structure. It contains an α-methylene γ-butyrolactone moiety (ring C) along with other functionalities and five chiral centers [3]. Arny isolated parthenin from *Parthenium* weed. Parthenin is known to exhibit antifungal properties. Aqueous leaf extracts of *Allium sativum*, *Datura alba* and *Withania somnifera* inhibited the growth of *Alternaria alternata, A. brassicola* and *Myrothecium roridum* [4]. Aqueous extract of *Allium cepa* exhibited antifungal activity against *Helminthosporium turcicum* and *Ascochyta rabiei* and that of *Calotropis procera* against *Alternaria redicina* [5]. The aim of the present study was to evaluate the synergistic effect of parthenin on three pathogenic fungi viz., *Fusarium oxysporum , Aspergillus niger* and *Drechslera hawaiinsis*.

Materials and Methods

Preparation of pure parthenin standard

Preparative high-performance liquid chromatography (HPLC) was used to obtain parthenin as HPLC standards. Fresh leaf material from *P. hysterophorus* plant was dipped for ten seconds in tertbutyl methyl ether (250 mg FM ml-1 TBME). Organic leaf extracts were filtered over anhydrous sodium

sulphate (Na_2S0_4) and the extract concentrated with a rotary evaporator (40°C, 250mbar). The oily, green residue obtained was re-dissolved in 1:1 (v/v) ACN: H_20 and fractionated by preparative HPLC (Varian model chromatograph) with UV detection (Varian UV – VIS detector model 345; detectin wavelength 225/254 nm). A Grom Nucleosil 120 C-4 column [250 mm by 16 rom (5μm), Grom, Germany] was used, and eluted with a gradient of 20% ACN and 80% Na_2HPO_4- buffer (1 mM, pH 3, 10% ACN) for 0-20 min, 100% ACN for 20-26 min then re-equilibrated to starting conditions (6ml min-1 flow rate). Injection volume was 100μl. Parthenin was identified in the fraction ranging from 9.1-10.3 minutes. Standard purity was verified by HPLC-DAD and results confirmed by HPLC-ESI-MS. For the preparation of PDA culture medium, in 1000ml distilled water 200gms peeled potato was taken in 1000ml sterilized conical flask and kept in an autoclave at 15 lb pressure for 1 hour. Extract was taken by filtering through muslin cloth and was made 1000 ml by adding distilled water. 20 gm dextrose and 20 gm agar was added in 1000ml extract. A pinch of chloramphenicol was added in extract to avoid bacterial contamination and kept in an autoclave at 15 lb pressure for 1 hour. The test fungi were were inoculated in freshly prepared 80 ml PDA medium. 20ml of each of 0, 25, 50, 75 and 100%w/v parthenin was added and kept in an incubator at 28°C for 20 days at 10 days interval. All experiments were replicated three times and conclusion was drawn on the basis of two way analysis of variance technique. The calculated values were compared with tabulated value at 5% level of significance.

Results and Discussion

Colony diameter (in mm) of *D. hawaiinsis* after 10 DAI and 20 DAI

The treatment of parthenin extracted from *P. hysterophorus* exhibited marked significant variation in inhibiting mycelial colony of *D. hawaiinsis*. Maximum significant inhibition was observed in 100% concentration after 20 DAI in which 2 mm mycelial colony diameter was observed followed by 75%, and 50% in which no significant effect was observed i.e 2.7mm. Lowest inhibition was recorded in 25% in which 3.5mm diameter was observed. At intermediate growth level of 10 DAI maximum inhibitions was observed in 100% concentration in which 2.5mm colony diameter was observed followed by 75% and 50% in which 3.9mm diameter was observed and was found to be significant. However, lowest inhibition was recorded in 25% in which 4.5mm colony diameter was observed. No inhibition was observed in control (Figure 1).

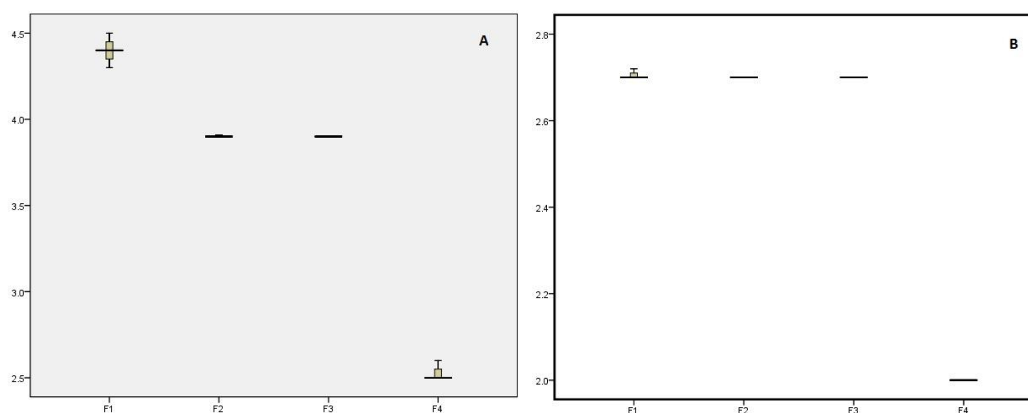


Figure 1: Effect of Parthenin on mycelial colony diameter of *D. Hawaiinsis*
(A) After 10 DAI and (B) After 20 DAI

Colony diameter (in mm) of *F. oxysporum* after 10 DAI and 20 DAI

Maximum significant inhibition was recorded after 20 DAI in *F. oxysporum* i.e. 2.8mm of colony diameter however in 10 DAI 3.1mm of colony diameter was observed. In 75% concentration 3.2mm of colony diameter was observed after 20 DAI which was 0.7mm lesser then 10 DAI. The effect of 50%, and 25% concentration after 20 DAI and 10 DAI was found to be 4.8mm and 5.2mm, respectively and was found to be non- significant. Control received distilled water and was not affected (Figure 2).

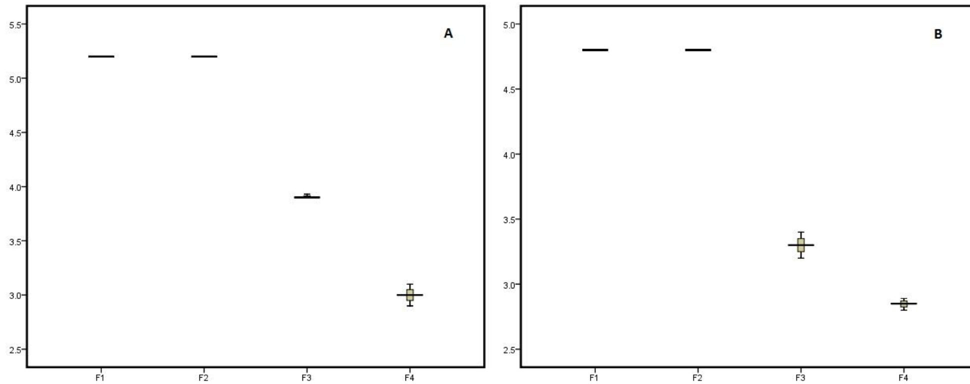
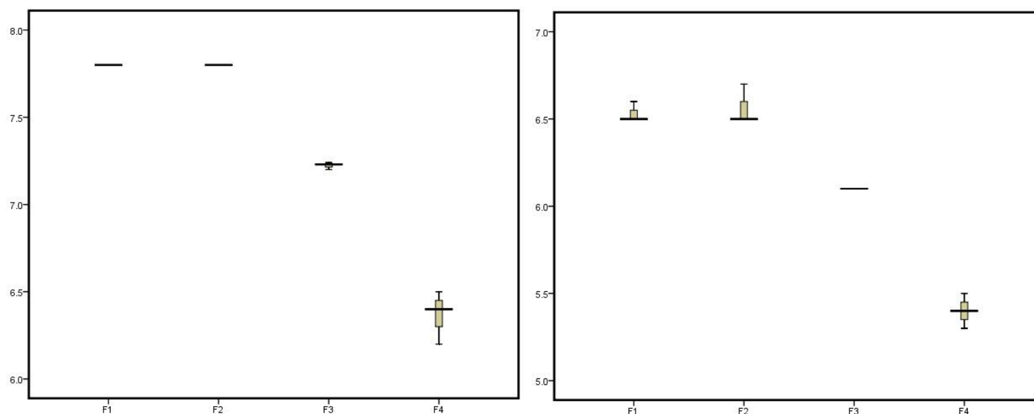


Figure 2: Effect of Parthenin on mycelial colony diameter of *F. Oxysporum* (A)After 10 DAI and (B) After 20 DAI

Colony diameter (in mm) of *A. niger* after 10 DAI and 20 DAI

Periodic treatment of parthenin in various treatments clearly revealed a slightly different pattern in colony diameter of *A. niger*. It is evident from the data shown in the figure that 100% concentration was found to be statistically significant from 75%, 50% and 25% concentration of parthenin . At lower concentrations inhibition was found to be static. Effect of parthenin after 20 DAI revealed that parthenin has a positive impact in inhibiting the growth of mycelia of *A. niger* as compared after 10 DAI (Figure 3).



- Where, F1= 25%, F2= 50%, F3= 75% and F4= 100%
- Control received distilled water

Figure 3: Effect of Parthenin on mycelial colony diameter of *A. Niger* (A)After 10 DAI and (B) After 20 DAI

Allelopathic potential of *Parthenium hysterophorus* L. against three pathogenic fungal species viz. *Drechslera hawaiiensis* (M. B. Ellis), *Alternaria alternate* (Fr.) Keissl and *Fusarium moniliforme* Sheld was studied. These species were subjected to various concentrations of aqueous extracts of aerial parts of *P. hysterophorus* in liquid malt extract medium. The growth of all the three test pathogenic species was generally inhibited by lower concentrations viz. 10, 20, 30 and 50% of the *Parthenium* extracts while aqueous extracts of higher concentration (60 and 70%) stimulated biomass production of test fungal species [6]. The treatment of parthenin extracted from *P. hysterophorus* exhibited marked significant variation in inhibiting mycelial colony of test fungi i.e. *F. oxysporum, A. solani* and *C.lunata*. Maximum significant inhibition was observed in *F. oxysporum* in which the mycelial colony (diameter) was found to be 2.5 mm at 100% concentration of parthenin, followed by *A.solani* in which 2.8 mm mycelial colony was observed at 100 % concentration. Mycelial colony diameter was recorded 4.3 mm in *C. lunata* at 100% concentration of parthenin [7]. It was reported that 10% concentration of neem oil

gave 100% inhibition of mycelial growth in *Aspergillus niger, Drechslera rostrata* and *M. Phaseolina* [8]. Similarly, leaf extract of *Chenopodium murale* and *Cannabis sativa* reduced mycelial growth of *Ascochyta rabiei* significantly [5] .

Conclusion

It is amply indicative from the observations recorded that parthenin does have some role to play by way of biomolecular interaction in suppressing these test fungi. Thus, it provides an efficient and environment-friendly alternative to other time-consuming, costly, toxic, physical, and chemical methods.

Acknowledgement

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