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A PTEROCARPAN COMPOUND FROM *FUSARIUM OXYSPORUM* F.SP. *CICERIS* INVOLVED IN CHICKPEA WILT.

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Abstract

Fusarium oxysporum f.sp. *ciceris* was found to produce at least six phytotoxic compounds in the culture filtrate. One of the active compounds was purified by chromatographic techniques that produced wilting to chickpea seedlings. Mass spectrometry UV, ^{13}C /^{1}H NMR revealed that the structure was of a pterocarpan compound having a molecular formula of $C_{16}H_{16}O_5$. Chickpea leaves from two cultivars CM88 and AUG 424 when treated with aqueous solution of toxins, the loss of phosphates, total phenols, protein, carbohydrates, K^+ and Ca^{+2} were significantly higher in toxin treated tissues than in control of both the cultivars. The susceptible cultivars released higher amount of phosphates, carbohydrates, K^+, Ca^{+2} and Na^+ ions from the toxin treated tissues than the resistant cultivars. This data confirms that electrolytic leakages against phytotoxins provide a reliable method for screening of resistant materials in chickpea to wilt disease.

Introduction

Chickpea is an important grain legume crop in Pakistan and a rich source of protein for the poor man diets. The crop is vulnerable to many diseases among which blight and wilt are most important for crop losses.

Toxins are known to be one of the significant casual factors in the development of a number of destructive diseases of plants (Schaffer, 1983, and Alam *et al.*, 1989). Most of them are low molecular weight compounds, which may cause necrosis, chlorosis, wilting or combination of these symptoms. The strawberry pathotype of Alternaria alternata produces AF toxin, which consist of three related compounds (Akamatsu *et al.*, 1997) in all cases, the sensitivity of the host plant to the toxin is correlated with the pathogenicity of the isolates. These three toxins showed similar biological effect of leaf necrosis and K^+ leakage.

Materials and methods

Isolation of Phytotoxin: *Fusarium oxysporum* f.sp. *ciceris* was grown on Czapek dox liquid medium containing hot water chickpea root extract for 21 days at 25 ^{0}C. The culture filtrate was partitioned into ethyl acetate at pH 3. The concentrated ethyl acetate phase was chromatographed by flash column and TLC (Alam & Iftikhar, 1996).

Electrolytic Leakages assay: Chickpea leaflets (0.1g) were removed from 15-20 days old plants and taken into scintillation vials. Toxic compounds extracted from culture filtrate in ethyl acetate were dried and dissolved into distilled water. The aqueous solution of toxin was added to the scintillation vials while distilled water was added in control and then agitated on shaker for 1 h. The toxin or the water was

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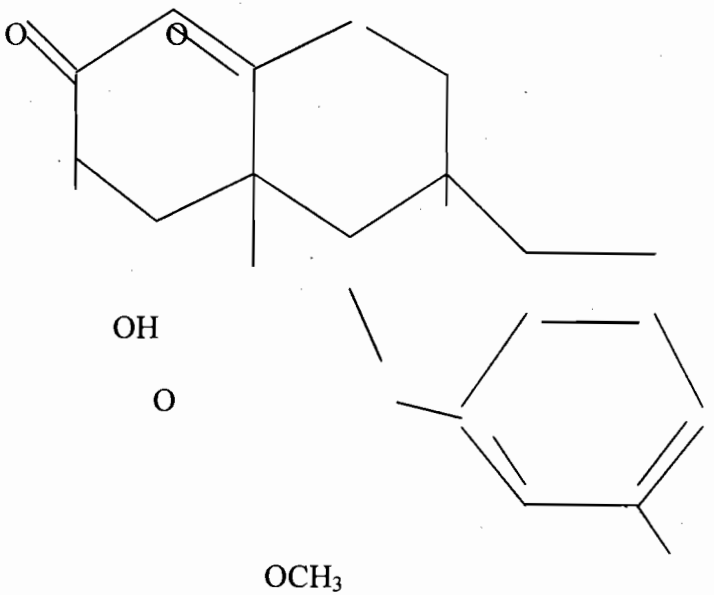
decanted from the vials and leaflets were rinsed for five times in distilled water. Five ml of distilled water was added and agitated on a shaker at room temperature. The conductivity (μScm^{-1}) was measured at 24 h using EC meter. Analyses of the materials leached from tissue were conducted by the following methods: total carbohydrates by the Anthrone test; total phosphates by the method of Fiske and Subbarow (1925); protein by the 280/260 spectrophotometeric method and total phenols by Folin ciocalteu reagent. Flame photometric methods were used to determine sodium, potassium and calcium electrolytes leakages.

Results and discussion

Six toxic compounds were identified by TLC separation. One of the purified compound analyzed by Mass spectrometry, UV and $^{13}C/^{1}H$ NMR revealed that it was pterocarpan with Molecular formulae of $C_{16}H_{16}0_5$ with following structure.



Leachers such as phosphates, total phenols, protein, carbohydrates, K^{+} and Ca^{++} were significantly higher in toxin treated tissues in both the varieties (Fig.1). The phosphates showed the highest leakages (100%) in Aug 424 from its control followed by phenols (99%), protein (96%), carbohydrates (88%) and electrolytic leakages of potassium and Ca^{++}(88%) with 64% of Na^{+} ion. The toxin-induced leachers in Aug424 were greater than CM88. Phosphates were released in higher amount in both the varieties when treated with toxins. The release of potassium, carbohydrate and calcium was almost the next highest leachers (36-39%) in the Aug424. Phenols, which was the next higher leathers against control in both the cultivars showed 27% increased leaches against CM88. Proteins and sodium had 20% and 70% leakages. Field screening method is usually employed for the identification of resistance which is time consuming and frequently unrepeatable. In

plant breeding the efficiency of the selection depends on the speed and accuracy of the screening method. The above data suggested that the electrolytes leakage method is rapid, convenient and provides a quantitative data of resistance in chickpea against wilt disease.

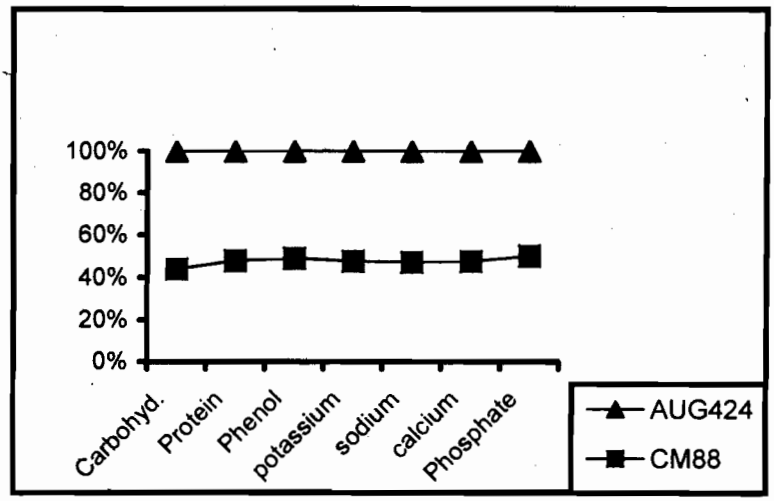


Figure 1. Leachers from resistant (CM88) and susceptible (Aug424) cutivars

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