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Effect of garlic bulb extract on the growth and enzymatic activities of rhizosphere and rhizoplane fungi

Tawfik M. Muhsin, Sami R. Al-Zubaidy & Eman T. Ali
Department of Biology, College of Education, University of Basrah, Basrah, Iraq

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Abstract

Eighteen fungal species were isolated from rhizospheric soil and rhizoplane samples of three plant crops in southern Iraq. The fungal isolates were examined for the activities of four enzymes (amylase, cellulase, phenoloxidase, and protease), as well as their growth, against crude garlic extract added to the culture agar medium. A high reduction or inhibition of enzymatic activities was observed for the fungi treated with garlic extract compared with untreated fungal cultures. However, most of the species showed inhibition of enzymes due to the effect of garlic extract. The growth of the fungal species was also remarkably reduced by the garlic extract.

Key words: *Allium sativum*, enzyme activity, fungal growth, rhizosphere and rhizoplane fungi

Introduction

Garlic (*Allium sativum* L.) extract is known to possess antibacterial and antifungal activities [1–3] and such action has been attributed to the presence of volatile components specifically diallyldisulphid. Most of the studies were conducted on the effect of garlic extract on growth and spore germination of certain fungal species [2–5]. Although, hypotheses concerning the modes of action of garlic extract volatiles on fungal growth inhibition are still contraversial. Evidence on the action of allicin as a major component of garlic on fungal cell metabolism has been proposed [6]. This study aimed to examine the effect of garlic bulb extract on the enzymatic activities and growth of some rhizosphere and rhizoplane fungi isolated from three economically important plant crops (okra, tomato and wheat) in southern Iraq.

Materials and methods

Garlic extract preparation

Fresh garlic bulbs (local cultivar source) were dehulled, weighed and ground in a blender. Ten grams of garlic in 100 ml sterile distilled water were homogenized. The crude extract was vacuum filtered and the filtrate was centrifuged at 10.000 rpm for 3 min. The supernatant was then immediately used.

Fungal isolates

Eighteen fungal species were isolated from rhizospheric soil and root samples (10 samples of soil and roots per plant) of three plant crops; okra (*Hibiscus esculentum* L.), tomato (*Lycoperscum esculentum* L.) and wheat (*Triticum vulgari* L.) cultivated in southern Iraq during June–August, 1999. A dilution plating technique [7] was used for the isolation of fungi from the rhizospheric soil samples, while a washing method [8] was applied for isolation of fungi from root samples after being cut into 1 cm long fragments and then placed on Czapeks agar medium in Petri-dishes. Chloamphinicol (250 μg/L) was added to prevent bacterial growth. The inoculated Petri-dishes were incubated at 25 °C for 7 days and surveyed for fungal growth. A pure culture of each fungal species was made for the enzyme assays.

Enzymatic assay

Amylase

To determine the amylase activity of the isolated fungi, a described method [9] was used. A solid medium

containing, 2 g peptone, 15 g yeast extract and 18 g agar dissolved in 1 L of distilled water, was prepared and autoclaved. Five ml of fresh prepared garlic extract were added into the Petri-dish and left to solidify. An inoculum disc (0.5 mm diam.) of 7 day old fungal culture was removed with a sterilized cork borer and placed on the surface of the agar medium, Triplicate plates were made for each fungal isolate and incubated at 25 °C for 5 days. Potassium iodide (KI) was added to the growing colonies, if a clear zone (CZ) appeared around the colony, it indicated the production of amylase. Amylase activity was measured in terms of CZ diameter. Control cultures, without garlic extract, were made for comparison.

Cellulase

Cellulase activity was determined as previously described on an agar medium [10]. Urea was added to the autoclaved medium. Five ml of garlic extract was added to each plate. Fungal inocula were placed on the agar medium's surface and incubated at 25 °C for 7 days. Hydrochloric acid/iodine was added to the growing colonies for 10 min. Development of a yellow zone that appeared around a colony indicated cellulase production.

Protease

A specific agar medium was used [11] to determine protease activity. The medium consisted of 950 ml nutrient agar and 0.4% gelatin. Each Petri-dish was amended with 5 ml garlic extract. Fungal inocula were placed on the agar medium's surface and incubated at 25 °C for 7 days. If a clear zone appeared around the colonies, it indicated protease production.

Phenoloxidase

To determine the production of phenoloxidase by the fungal isolates, an agar medium previously described [9] was prepared. The medium consisted of; 15 g malt extract, 20 g agar, 0.8 g tannic acid in 1 L distilled water. Similarly, garlic extract was added to the culture medium, fungal inocula were placed on the agar's surface and incubated at 25 °C for 5 days. If a brown zone appeared on the colony's reverse, it indicated production of this enzyme. For all the enzyme assays, fungal growth was measured in terms of colony diameters (CD).

Results

Eighteen fungal species were isolated from the soil and root samples (Table 1). Among the species, *Alternaria raphani* and *A. sonchi* were isolated from okra roots, *Fusarium flocciferum, F. moniliforme* and *F. oxysporum* were isolated from the tomato roots while *F. graminearum* and *Rhizoctonia solani* were isolated from wheat roots. Other fungal species were isolated from the soil samples. The enzymatic activities and growth rates of the examined fungi are presented in Table 1. Untreated fungal cultures (without garlic extract) showed higher production of amylase, cellulase, phenoloxidase and protease than the fungal cultures treated with the garlic extract. Among the tested enzymes, amylase was produced by all of the examined fungi, except *Fusarium flocciferum, F. graminearum* and *Rhizoctonia solani*. The highest activity of this enzyme was revealed by *Alternaria raphani, F. moniliforme* followed by *A. sonchi* (Table 1). Other fungal species showed lower amylase activity (<15 mm clear zone diam.). The addition of garlic extract to the culture medium reduced the production of amylase about two-four folds than non-treated fungal cultures (Table 1). However, complete inhibition of this enzyme by garlic extract was observed in some fungal isolates. Cellulase assay revealed that *Aspergillus flavus* and *A. niger* did not possess this enzyme while *Drechslera hawaiinesis* showed the highest activity of cellulase followed by *F. oxysporum* (Table 1). Inhibition of cellulase by garlic extract was observed for most of the examined fungi. Phenoloxidase activity was highest in *R. solani* and *Scytalidium lignicola*. However, *A. niger, F. oxysporum* and a *Penicillium* sp. did not produce this enzyme. A reduction or inhibition of phenoloxidase by garlic extract was detected for all the examined fungal species (Table 1). On the other hand, protease activity did not vary among the fungal species but all of them showed no production of this enzyme when treated with garlic extract (Table 1). The fungal growth in garlic-free extract medium was greater than in the garlic extract medium. The reduction in fungal growth was about two-four fold for the fungi treated with garlic extract. No correlation was noticed between enzymatic activity and the growth rates of the examined fungi.