



Effect of Eggplant Root Extracts on Growth of Storage Fungi Infesting Wheat Grains in Stores of Babylon

Iman Jawad Kadhim and Selan Hussan Segar

Biological Control Techniques Department, Technical College,
Al-Musayib, Al-Furat Al-Awsat Technical University, Babylon, Iraq
E-mail: com.emn3@atu.edu.iq

Abstract: The fungi associated with seeds negatively affect the germination of seeds and the vitality of the seedlings. The study aims to reveal the effect of aqueous and alcoholic extracts of the eggplant roots *Solanum melongena* L in the radial growth of fungi isolated from local wheat grains stored in the old Hilla silo and the new Hilla silo in Babylon during 2018-2019 according to the technique of poisoned food. The ability of fungal species isolated to produce aflatoxins was determined. The aqueous extract of eggplant roots contained flavonoids and alcoholic extract contained flavonoids and alkaloids. Several species of fungi associated stored wheat fungi were isolated and identified as *Aspergillus niger*, *Penicillium notatum*, and *Fusarium oxysporum*. The aqueous and alcoholic extracts had a significant inhibitory effect on the growth of the tested fungi on the Potato Dextrose Agar medium compared with the control. The alcoholic extract was more effective on the fungi than the aqueous extract. The percentages of inhibition of the radial growth of percent at a concentration of 10 mg / ml were 91.11, 88.88 and 91.11 percent respectively and at aqueous extract, the inhibition averages was 86.66, 86.66 and 83.33 percent, respectively.

Keywords: *Aspergillus niger*, *Penicillium notatum*, *Fusarium oxysporum*, Aflatoxins, Flavonoids, *Triticum aestivum*, Eggplant

Triticum aestivum L is one of the basic foods in human life and the need increases with the increase in population, and studies indicate that the world population will need to one billion tons of wheat compared to the current production that does not excel 600 million tons in 2025 (Jama et al 2018). The wheat crop is highly cultivated in most regions in Iraq (Muhammad and Hamid 2016) and wheat grains are infected during harvesting, transportation or storage with many species of fungi belonging to genera *Alternaria*, *Aspergillus*, *Penicillium*, *Fusarium*, *Rhizopus*, and *Trichoderma* (Thenmozhi et al 2013, Seth et al 2014). These fungi cause significant economic losses due to their effect on the vitality of grains and reducing germination as well as the ability of some fungi species to produce mycotoxins. Among the most important and most dangerous toxins for human health are aflatoxins (Keerthiga and Anand 2014, Kekuda and Raghavendra 2017). Among the most common fungi producing aflatoxins are some species of the genus *Aspergillus* (Shamsi et al 2017). Aflatoxins can also be produced by some species of both genus *Alternaria* and *Penicillium* (Satish et al 2007, Keerthiga and Anand 2014). So it is necessary to reduce the damage caused by toxins by using chemical pesticides. The use of plant extracts instead of chemical pesticides to control fungi is another safe alternative (Talapatra et al 2017, Ravi et al 2019). One of the important vegetable plants, *Solanum melongena* L eggplant contains many active substances such as alkaloid,

flavonoids and saponins and are present in all parts of the plant such as grains, roots, leaves, stems, and fruits (Ghosh et al 2015, Singh et al 2016).

MATERIAL AND METHODS

Collection of seed samples: The local wheat grain used in this study was collected from the old Hilla silo and the new Hilla silo in the Babylon city in November 2019 and three random samples were collected at an average of 1 kg per site of the silo. Samples were placed in polyethylene bags and sealed with ventilation holes to avoid fetal death, then transferred to the laboratory for study.

Isolation and identification of the fungi associated with wheat grains: The fungi associated with wheat grains were isolated, where the grains were divided into two groups. The first included 100 grains superficially sterilized using a solution of sodium hypochlorite at a concentration of 1% for three minutes and then washed with sterile distilled water three times. In the second group, 100 grains were washed with sterile distilled water only, and dried with sterile filter paper and left in the sterile examination site for a period to dry. After that, the seeds were cultivated in a petri dish containing Potato's Dextrose Agar (PDA) medium with five grains in each dish and triplicates per group. The dishes were left in the incubator at a temperature of 25 ° C under conditions of dark and light rotation and after four days the growth of fungi was monitored. The dishes were examined to

for identification of fungi and fungi frequency were calculated as (Kekuda and Raghavendra 2017). Fungi frequency percentage = (number of fungal species colonies / total number of fungal species colonies) x 100

This was followed by purification of fungi isolation on PDA medium. The pure isolates were preserved by cultivated them on the same nutritional medium and incubated for a week at a temperature of 25 °C and then kept in the refrigerator at a temperature of 4 °C until used. Diagnosis of isolated fungi was conducted to the species level, depending on the morphological features of the colony, such as the shape, colour, and colony diameter, and also according to the microscopic features such as the shape, size, colour, and composition of spores, and other structures according to the approved classification foundations) (Hafizi et al 2013, Samson et al 2014, Visagie et al 2014) (Table 1). Then three fungal isolates were selected (*Aspergillus niger*, *Penicillium notatum*, *Fusarium oxysporum*).

Collection the plant parts: The eggplant was obtained and its roots collected from a farm in the Babylon city and were washed with regular water and then distilled water and then allowed to dry at room temperature. After that the ground roots of the eggplant plant were ground with an electric miller and the powder was kept in dry glass containers at room temperature until used.

Preparation of plant extracts: Prepared the aqueous and alcoholic extracts of the roots of the eggplant plant, using the method of Sahana et al (2018). The stock solution was prepared for the extracts used in the study by adding 4 g of each extract in 100 ml of the solvent so that the concentration was 40 mg / ml. It was sterilized by using a 0.22 µm pore membrane filter and stored as a storage solution. The concentrations prepared for both aqueous and alcoholic extracts were 2.5, 5, 10 mg/ml.

Detection of flavonoids: Ten ml of the extract was added to 50 ml of 95% ethyl alcohol and then the solution was filtered and indicated with the letter (A). Then 10 ml of ethyl alcohol

was added with a concentration of 50% to 10 ml of KOH 50% solution and indicated with the letter (B). The equal amounts of solution A and B were mixed, the appearance of yellow indicating the presence of flavonoids (Yadav and Ashish 2011).

Detection of glycosides: One ml of the extract solution was mixed with five-drop benedict reagent, and heated in a water bath at a temperature of 100°C for 5 minutes, then cool the tube, the formation of a red precipitate indicates evidence of the presence of the glycosides compounds.

Detection of resins: Add 5 ml of the extract to 50 ml of ethyl alcohol at 95% concentration, leave the solution in a water bath at 100°C for two minutes, filter the solution and add 100 ml of distilled water acidified with hydrochloric acid at a concentration of 4%, if a cloudy solution appears in the solution evidence of resins (Yadav and Ashish 2011).

Detection of alkaloids: The 10 ml of the extract was boiled and added 50ml of distilled water, acidified with 4% hydrochloric acid, filter the solution after cooling it and put 0.5 ml of solution in an hour bottle and add some drops (2-5 drops) of the dragendroff reagent. The orange precipitate is an indication of the presence of alkaloids (Singh et al 2016).

Antifungal activity of extracts: The effectiveness of aqueous and alcoholic extracts of eggplant roots in the radial growth of fungi was estimated (Sahana et al 2018) which are a poisoned food technique (three concentrations were prepared for both aqueous and alcoholic extracts (2.5, 5 and 10) mg / ml). The sterile PDA medium was poured into the dishes and, the control treatment included petri dishes with sterile PDA medium without any addition. After the media was hardened, a half-centimeter piece of pure fungi cultivation was transferred at the age of seven days and placed in the middle of the dish. The dishes were incubated at a temperature of 25 °C and triplicates per treatment and for each fungus and growth rate of each fungus was measured. Using the ruler after the mycelium has reached in the control treatment to the edge of the dish, the percentages of

Table 1. Macroscopic (colonial) and microscopic characteristics of fungi isolates

Macroscopic characteristics	Microscopic characteristics	Fungal isolates
Appeared cottony when young but later turned black completely or dark brown and look powdery.	Hyphae septate and hyaline, smooth walled conidiophores arising from foot cell; vesicles globose, whole vesicle fertile bearing two series of sterigmata; catenate conidia arranged in basipetal manner, unicellular, and globose.	<i>Aspergillus niger</i>
The green colonies.	Blue circular spores are single cells within the chains. Sterigma arises from the branched Conidiophores. The conidiophores were branched to form a brush like conidial head which contains more than one branch of conidiophores. The hyphae appeared branching and septate.	<i>Penicillium notatum</i>
Colonies pale to light pink	Hyphae septate and hyaline. Conidiophores slender and simple, short or branched irregularly or bearing a whorl of phialides; conidia hyaline, variable, principally of two kinds, macroconidia several celled slightly curved or bent at the point ends, microconidia 1-celled, ovoid or oblong, borne singly or in chains.	<i>Fusarium oxysporum</i>

inhibition were calculated using the following equation:

$$\text{Inhibition percentage} = \frac{\text{fungus colony average diameter in control dishes} - \text{the fungus colony average diameter in treatment dishes}}{\text{the fungus colony average diameter in control dishes}} \times 100$$

Detection of aflatoxins production: Petri dishes were prepared containing on the PDA medium after sterilization, then the medium was inoculated with fungal colonies by transferring a portion of the colony with a diameter of 0.5 cm to each plate using a sterile loop. After that, the dishes were incubated for a period of 4-7 days at 25,30,35 ° C, then the dishes were removed from the incubator and turned upside down and then added in the center of each cover of dishes 20% ammonia solution (0.2) ml, then returned to the incubator for 3-7 days at 25,30,35 ° C. The control dishes, they were left without any addition. These dishes were monitored during this period to note the change of the color of their bases. If the colony base changed color to red, indicating that the fungus has the ability to produce aflatoxins according to the intensity of the color, whenever the color is dark, it indicates a high productivity of aflatoxins and vice versa (Nikolic et al 2017).

Statistical analysis: All experiments were performed with triplications for each experiment, the tests were conducted according to a completely randomized design and triplicates for each treatment, and the results of the experiments were expressed in the form of mean \pm standard deviation. The least significant difference was used to for significant differences between the different treatments using the comprehensive statistical program (SPSS) at the probability level ($P \leq 0.05$).

RESULTS AND DISCUSSION

Isolation and identification of the fungi: Types of fungi associated with wheat grains were isolated and identified: were *Aspergillus niger*, *Penicillium notatum* and *Fusarium oxysporum* (Table 2). There were significant differences in

the percentages of fungi frequency from identified surface unsterilized grains and surface sterilized grains. The percentages of fungi-contaminated grains in the surface unsterilized grains were higher compared to the superficially sterilized grains. This is due to the fact that sodium hypochlorite is a sterile substance and effect is directly limited to the fungi carried on the outer shell of the grains and does not affect the fungi that accompany the grains from the inside. Saadoun (2005) confirmed the effect of sodium hypochlorite on the fungi carried externally on the grain shell without affecting the fungi carried inside the grain shell or affecting the embryo. *Aspergillus niger* was had highest percentile for surface unsterilized grains compared to other isolated fungi (54.80%). Sirhan et al (2001) also made similar conservation due to the ability of *A. niger* to grow in weak moisture content, tolerate drought conditions, lack of free water and low temperatures, and these factors are not appropriate for the growth of many other fungi accompanying the grains. Muhammad and Hamid (2016) also indicated that the fungus was present in all studied samples of imported and local wheat grains in Karbala province, Iraq. Talal et al (2009) also concluded that 70% of wheat samples were contaminated with different species of *Aspergillus*. Khaldoun (2012) indicated the *A. niger*, *Penicillium* sp. and *Fusarium* sp. were isolated from surface sterilized and non-sterilized wheat.

Chemical detection of some active substances in the eggplant roots: The aqueous and alcoholic extracts of the eggplant roots contain flavonoids and alkaloids (Table 3). Torres et al (2001) indicated that the various parts of the eggplant plant contain these active substances. Sharma et al (2011) pointed the effectiveness of extracts of different parts of eggplant against bacteria and fungi because they contain many active substances, the most important of which are flavonoids and saponins.

Effect of aqueous and alcoholic extracts of eggplant roots on the radial growth of fungi: The results of the effect

Table 2. Percentage of fungal frequency in wheat grains

Average of fungal isolates	Old Haila Silo		New Haila Silo		Fungal isolates
	Sterile surface	Non-sterile surface	Sterile surface	Non-sterile surface	
45.12	45.38	54.16	26.16	54.77	<i>Aspergillus niger</i>
35.53	38.45	25.89	49.28	28.48	<i>Penicillium notatum</i>
19.25	16.17	19.92	24.30	16.62	<i>Fusarium oxysporum</i>
	33.33	33.32	33.25	33.29	Average of silo
0.93					L.S.D. 5% (the silo)
0.80					L.S.D. 5% (fungal isolates)
1.62					L.S.D. 5% (Interaction)

of aqueous and alcoholic extracts of eggplant roots on the radial growth of some fungi isolated from wheat grains showed that the extracts had a significant inhibitory effect on the growth of all tested fungi and these fungi are: *Aspergillus niger* (Table 4) and *Penicillium notatum* (Table 5) *Fusarium oxysporum* (Table 6), where the fungi colonies average diameters were inversely proportional to the concentration of the extracts, the diameters rates decrease as the concentration of the extracts increased, in contrast to the percentages of inhibition that were increased with increasing the concentration of the extracts. The results showed that alcoholic extract was superior to aqueous extract to inhibit the radial growth of the fungi (Table 4, 5 and 6). The average

diameter of fungi colonies ranged between 8.33-30.00 mm and the inhibition 66.66-91.11 % in alcoholic extracts compared to control treatments for these fungi (90.00 mm). For the aqueous extract the average diameter of fungi colonies was between 12.33-36.33 mm, with an inhibition of 60-86.66 % compared to control treatments for these fungi (90.00 mm). The variation in the extent of the fungi affected by plant extracts used may be due to the nature of the fungus in terms of composition and thickness of its cell membranes and content of fats and proteins.

Detection of aflatoxins production: The ability of fungi isolated from wheat grains was tested on the production of aflatoxins, as an ammonia solution was used 20% as a reveal of this ability, as once the surface of the fungal colony touches ammonia vapor, the color of its base changes to red and with varying intensity degrees depending on the amount of aflatoxin produced indicating ability fungal on the production of aflatoxins (Talal et al 2009). *Aspergillus niger* and *Penicillium notatum* have ability to produce aflatoxins at a temperature of 35°C as the color became dark red, *Fusarium oxysporum* do not produce aflatoxins. The results agreed with a number of studies that indicated the ability of some

Table 3. Chemical detection of some active substances in aqueous and alcohol extracts of roots eggplant

Active substances	Alcohol extracts	Aqueous extracts
Glycosides	-	-
Flavonoids	+	+
Resins	-	-
Alkaloids	+	-

+, - presence and absence of the active substance

Table 4. Effect of aqueous and alcohol extracts of roots eggplants on the growth of *Aspergillus niger* on PDA medium

Concentration (mg/ml)	Alcohol extracts		Aqueous extracts		Average of concentration
	Inhibition (%)	Diameter (mm)	Inhibition (%)	Diameter (mm)	
2.5	66.27	0.99 ± 30.00	60.96	0.96 ± 35.66	63.61
5	76.13	0.32± 22.33	67.23	0.75 ± 29.00	71.68
10	91.15	0.66 ± 8.33	83.70	0.32 ± 15.66	87.41
control	0.00	90.00± 0.00	0.00	90.00± 0.00	0.00
Average extracts		58.38		52.97	
L.S.D.5% (Concentration)					0.55
L.S.D. 5% (Extracts)					0.39
L.S.D. 5% (Interaction)					0.77

Table 5. Effect of aqueous and alcohol extracts of roots eggplants in the growth of *Penicillium notatum* on PDA medium

Concentration (mg/ml)	Alcohol extracts		Aqueous extracts		Average of concentration
	Inhibition (%)	Diameter (mm)	Inhibition (%)	Diameter (mm)	
2.5	73.77	0.51 ± 24.00	60.67	0.96 ± 36.33	67.22
5	82.68	0.66 ± 16.33	74.74	15± 23.00	78.71
10	88.66	0.73 ± 10.00	86.88	0.72 ± 12.33	87.77
Control	0.00	90.00± 0.00	0.00	90.00± 0.00	0.00
Average extracts		61.28		55.57	
L.S.D.5% (Concentration)					0.64
L.S.D. 5% (Extracts)					0.45
L.S.D. 5% (Interaction)					0.91

The results shown in the table represent the average of triplicates ± standard error

Table 6. Effect of aqueous and alcohol extracts of roots eggplants in the growth of *Fusarium oxysporum* on PDA medium

Concentration (mg/ml)	Alcohol extracts		Aqueous extracts		Average of concentration
	Inhibition (%)	Diameter (mm)	Inhibition (%)	Diameter (mm)	
2.5	81.74	0.44 ± 17.66	71.64	0.51 ± 26.0	76.69
5	84.86	0.51 ± 14.00	77.39	0.87 ± 20.6	81.13
10	91.01	0.32 ± 8.66	86.93	0.37 ± 12.0	88.97
Control	0.00	90.00 ± 0.00	0.00	90.00 ± 0.00	0.00
Average extracts	64.40		58.99		
L.S.D.5% (Concentration)			0.64		
L.S.D. 5% (Extracts)			0.45		
L.S.D. 5% (Interaction)			0.90		

Mean ± Standard error

Table 7. Detection of the ability of fungi isolated from wheat grains to the production of aflatoxins

Fungal species	Temperatures		
	35	30	25
<i>Aspergillus niger</i>	+	-	-
<i>Penicillium notatum</i>	+	+	-
<i>Fusarium oxysporum</i>	-	-	-

(+) ability to produce aflatoxin, (-) Inability to produce aflatoxin

isolates *A. niger* on the production of aflatoxins. Papagianni (2007) indicated the ability of isolates of *A. niger* isolated from some agricultural soils to the production of aflatoxins. Ounleye and Olaiya (2015) also concluded that the isolates of *A. niger* have the ability to produce aflatoxin. Saleh et al (2009) studied effect temperature variations on the ability of fungi to the production of aflatoxins and observed that most of the fungi had ability to produce aflatoxins. Hadi and Taimooz, (2018) mentioned that 35° C is the best temperature in which the color of the colonial bases changes clearly. The isolates that have shown a negative result in the production of aflatoxins, the reason may be due to the effect of temperature variation or due to their inability to produce aflatoxins or they are produced in very small quantities, so more sensitive methods should be used than this technique, which is a fast qualitative method (Abass 2004). Frisvad et al (2007) showed that some isolates of *A. niger* have the ability to produce fumonisin B₂ and ochratoxin, which are considered to be carcinogenic mycotoxins. These studies indicate the risk of *A. niger* in wheat grains, as this fungus has the ability to produce many carcinogenic toxins.

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