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REDUCING GROWTH RATE OF THREE *ASPERGILLUS* SPECIES (MAIZE SEEDS-BORNE FUNGI) BY USING AQUEOUS EXTRACT OF SOME LOCAL PLANTS

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Abstract

Several species of fungi were isolated from maize seeds. The highest frequency was genus *Aspergillus* of three species: *Aspergillus flavus*, *A. niger* and *A. parasiticus*. Poisoned medium method were Applied of five different plant extracts Nigella seeds, pomegranate peels, (Eucalyptus and Euphorbia leaves) and Ginger roots were used to reducing the growth rate of fungi by measured radial growth rate divided by time, the first growth rate (0-2 days), second growth rate (2-4 days) and third growth rate (4-6 days). A decrease in the growth rate of *A. flavus* and *A. niger* was observed by using Nigella and Ginger, while all plant extracts reduced the growth rate of *A. parasiticus*. The growth rate of the three species *A. flavus*, *A. niger* and *A. parasiticus* it was no significant difference among three growing stages of the first growth rate, then decreased sharply at the second growth rate and returned to rise at the third growth rate compared to control.

Key words : Plants extracts, *Aspergillus flavus*, *A. niger*, *A. parasiticus*, Growth rate.

Introduction

Maize seeds were exposed to attack of many pathogenic or saprophytic fungi (Elham, 2015), it is more dangerous when it infected in the field and then transition to the stores (Moshiur, 2016) it effects of amount of the crops due to field injury as well as the damage that leads to destroy and deterioration of the grain in the stores. The risk becomes more severe when these fungi are able to produce secondary metabolites compounds represented as mycotoxins. Mycotoxins are compounds produced by the secondary metabolite of strains of a number of fungi and have harmful effects on human and animal health (Mauricio *et al*., 2017) and their effects extend beyond plants and microorganisms (Flannigan 1991 and Steyn 1995). The most common fungi that produce mycotoxins are *Fusarium* and *Penicillium* and *Aspergillus*, which is widely spread in cereal crops, and the most dangerous groups of mycotoxins in human health are the compounds of aflatoxin, ochratoxin, Fumonisn, zeralenone and triacothsinate. Many seeds are infected by *Aspergillus flavus* at high rates it found increases the damage after they attack the seed embryo and the another parts due to delaying the harvest (Agrios, 1988) *A. niger* is known to be a produce ochratoxin (Célia Soares *et al.,* 2013).

On the other hand, plant extracts are natural organic compounds extracted from plants, which are characterized by a high activity against pests and are non-toxic compounds for plants and easy to break down in the environment, used as natural Antimicrobial Agents for various pathogens at a lower cost and more safety. Medicinal and aromatic plants are an important source of many chemical compounds and essential oils that can be developed to make them pesticides because they are safe compounds for the environment and humans, and the active component involved in the composition of medicinal plants are non-toxic sources or have little toxicity compared to chemical pesticides as well as being rapidly decomposing into the environment Plant extracts used

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as Antimicrobial due to low toxicity on mammals comparing to synthetic chemicals, have multi mechanisms to mode of action and low side effects (Harish *et al*., 2004; Redey *et al.*, 2007; Daniele *et al.*, 2009; Raja, 2014).

Ginger rhizomes (*Zingiber officinale*) has long been used as naturopathy due to its potential antimicrobial activity against different pathogens as dried ginger powder (Pratibha, and Rajendra, 2016).

Punica granatum L. (Punicaceae) Pomegranate is rich in polyphenols and antioxidants (Prasan, 2012) and pomegranate peels have antifungal activity (Endo *et al.*, 2010) and anti-mutagenic (Zahin *et al.*, 2010).

Euphorbia peplus is one of the genera of the Euphorbiaceae family, which is considered one of the large plant families and considered important medicinal plants that grows in the tropics and subtropics and that it has an anti-fungal and bacterial action, a study showed *E. helioscopia* have high antifungal activity against *Fusarium solani* (Muzair, 2009).

The black seed, *Nigella sativa* of the Ranunculaceae family, is a medicinal plant widely used around the world. Several active compounds have been isolated, identified and reported to date on various types of black seeds. The most important active compounds in the seeds are thymoquinone, thymohydroquinone, dithymoquinone, p-cymene, carvacrol, 4-terpineol, t-anethol, sesquiterpene longifolene, α-pinene and thymol. The seeds contain two different types of alkaloids: Isoquinoline alkaloids and pyrazol alkaloids or indazole ring alkaloids that include nigellidine and nigellicine. Moreover, it contains alpha-hederin, pentacyclic triterpene, dissolved in water and anti-cancer Saponin (Aftab *et al.*, 2013).

And the importance of the Leaves extract of *Eucalyptus camaldulensis* leaves which is one of the important medicinal plants belonging to the Myrtaceae family as it contains a group of compounds such as α-pinene, 1,8-cineol, pinocarveol-trans, Isoledene, α-Terpineol, Cymene p, Terpinene gamma, 2- Pentanone, 4-hydroxy-4-methyl-, Eucalyptol, Globulol, Limonene, Pinacarvone, Guaiene and Spathulenol (Kanko *et al.*, 2012 and Sebei *et al.*, 2015).

The study aimed to calculate the growth rate of (*A. flavus, A. niger* and *A. parasiticus*) using Poisoned medium (PDA) method with aqueous extracts (nigella seeds, pomegranate peels, (eucalyptus and Euphorbia leaves) and ginger rhizomes by calculated radial growth rate divided by time to calculate the distance (mm/day) which move it per day and the effect of plant extracts on impeding growth progress.

Materials and Methods

Maize seeds samples were collected randomly from five different places of one of the fields were transferred to the laboratory in paper bags, 20 seeds from each bag have been taken, sterilized superficially with sodium hypochlorite solution (6% of the commercial solution) for two minutes and washed with sterile distilled water and dried on sterile filter paper and then 10 seeds selected randomly were distributed circularly on the potato Dextrose Agar of 10 petri dishes with chloramphenicol antibiotic (250 mg). The dishes were incubated for a period of 7 days at 28.

The diagnosis of the isolated fungi

After the process of isolating the fungi accompanying Maize seeds, the process of diagnosing these fungi was performed to the species level, depending on the form of the fungal colony, the shape and installation of spores and other structures according to the approved classification foundations and by using the classification keys mentioned in the sources which dealt with the classification and study of fungi from the species studied in this research such as(Domsch *et al*., 2003and Moustafa, 1982)

Calculate the Frequency Percentage

The percentage of fungi isolated from Maize seeds was calculated according to the following formula

F% = (Number of samples of occurrence of *Aspergillus* species / Total number of samples) × 100 (Marasas *et al.*, 988).

Preparing aqueous plants extracts

The nigella, pomegranate peels, eucalyptus and ginger's rhizomes (powder) were obtained from the local market and the leaves of the plant (Euphoribia) were collected from the growing plants in gardens of Al-Qasim Green University. All of the nigella, pomegranate peels, eucalyptus and Euphoribia leaves and ginger were collected using tap water then distilled water and left to dry at room temperature, grinded with a blender. Prepare the aqueous extract by dissolving 1 g of dry powder in 20 ml of distilled water at room temperature for 24 hours with continuous stirring. the solution filtered through the filter paper Whitman No.1 Filter and filtrated passed through a 0.22 micron (Millipore).

The effect of aqueous extracts on the Radial growth of *A. flavus, A. niger* and *A. parasiticus,* used the Poisoned medium Technique. 1 ml of sterile filtrate in a Petri dish, 10 ml of PDA were added with gently stirring until the mixture is homogenized, the center of each dishes were inoculated of 5 mm from the edge of the colony for

all isolated fungi at age 7 days and incubated dishes at 28! then measured radial growth at 2, 4 and 6 days after pollination, by calculating average of two orthogonal drops.

Calculate the growth rate of *A. flavus*, *A. niger* and *A. parasiticus* (mm/day).

According to the following formula, first (0-2 days), second (2-4 days) and third (4-6 days) growth rate were calculated:

ALG (mm / day) = $(C_2-C_1) / (T_2-T_1)$

Whereas: Average linear growth (ALG), C_2: colony diameter rate on second reading, C_1: colony diameter rate on first reading, (T_2-T_1): the difference in days between the second reading and first reading according (Elad *et al.,* 1981; Mustafa *et al.,* 2009; Kamaluddin, 2018) with some modification

Statistical analysis

The results were analyzed by (GenStat Discovery Edition 3) According to the model of Factorial experiments with Completely Randomized Design (CRD) model. At Least Significant Difference (L.S.D.) under 0.05 level.

Results and Discussion

Number of genera associated of Maize seeds were Isolated and diagnosis, (*Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria* and another species), *Aspergillus* had a higher frequency which were 56% than the other fungi (22.3, 12.4, 2.8 and 6.3) respectively, the reason is due to its ability to grow with low moisture content that are available pre and postharvest, and to produce a large number of spores (AL-Saadi,2014)

Table 1 showed a significant difference of *Nigella* and *Ginger* Extracts of reducing the growth rate of *A. flavus* its recorded 3.27 and 3.33 mm / day respectively while the control treatment was 4.16 mm / day, and there is a contrast among the three growth rates the first one, recorded 3.22 mm / day, with a significant decrease than the second growth rate which was 2.77 mm / day, and a significant difference from the third growth rate, which was 6.5 mm / day.

Table 2 Using Aqueous extracts of *Nigella* and *Ginger* gave significant reduction the growth rate of *A. niger* it was 4.38 and 4.33 mm / day respectively compared to the control treatment which was 6.05 mm / day, and there is a contrast appeared among first, second and third growth rate it was 3.91, 3.33 and 8.16 mm / day respectively**.**

The results in table 3 indicated a significant reduction in growth rate of *A.parasiticus* by using of aqueous extracts of *Nigella, pomegranate* peels, *Eucalyptus, Euphorbia* and *Ginger* as they were 3.33, 3.5, 3.27, 3.38 and 3.5 mm / day, respectively, compared to a treatment control was 4.27 mm / day, and a difference was found between the first, second and third growth rate they were 3.55, 2.13 and 4.94 mm / day respectively.

The growth rate of the three species *A. flavus, A. niger* and *A. parasiticus* it was appeared to be symmetric of three growing stages as shown in Figs (1, 3 and 5) respectively. In the first growth rate was revealed a natural path way, then decreased sharply at the second growth rate and returned to rise at the third growth rate compared

Table 1: Growth rate (mm / day) of *Aspergillus flavus* in Poisoned medium PDA method by plants extracts.

Plants extract	Growth rate mm/day of *A. flavus*			
	0-2/days	2-4/days	4-6/days	
Nigella	2.83	3.33	3.66	3.27
Pomegranate peels	4.16	4.16	7	5.11
Eucalyptus	2.5	1.5	9	4.33
Euphorbia	3.83	3	7.5	4.77
Ginger	2.5	0.66	6.83	3.33
Control	3.5	4	5	4.16
	3.22	2.77	6.5	

l.s.d.:0.786 of Extract, l.s.d.:0.556 of Growth rate

Table 2: Growth rate (mm / day) of *Aspergillus niger* in Poisoned medium PDA method by plants extracts.

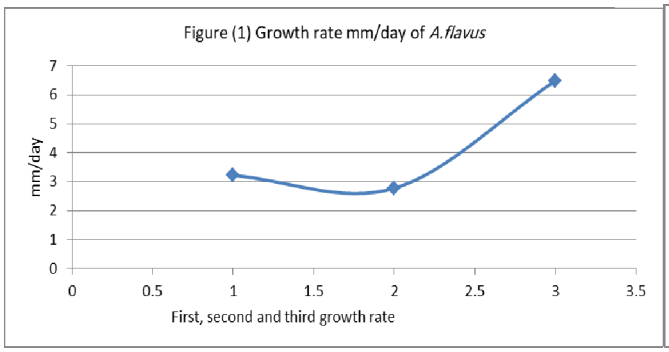
Plants extract	Growth rate mm/day of *A. niger*			
	0-2/days	2-4/days	4-6/days	
Nigella	3.5	3.66	6	4.38
Pomegranate peels	4.33	5	8.16	5.83
Eucalyptus	4.16	1.66	9.83	5.22
Euphorbia	4.33	2.83	7.83	5
Ginger	2.66	2.33	8	4.33
Control	4.5	4.5	9.16	6.05
	3.91	3.33	8.16	

l.s.d.:1.173 of Extract, l.s.d.:0.829 of Growth rate

Table 3: Growth rate (mm / day) of *Aspergillus parasiticus* in Poisoned medium PDA method by plants extracts.

Plants extract	Growth rate mm/day of *A.parasiticus*			
	0-2/days	2-4/days	4-6/days	
Nigella	3.16	2.33	4.5	3.33
Pomegranate peels	3.66	1.33	5.5	3.5
Eucalyptus	3	1.83	5	3.27
Euphorbia	3.5	1.66	5	3.38
Ginger	3.33	3	4.16	3.5
Control	4.66	2.66	5.5	4.27
	3.55	2.13	4.94	

l.s.d.:0.499 of Extract, l.s.d.:0.353 of Growth rate



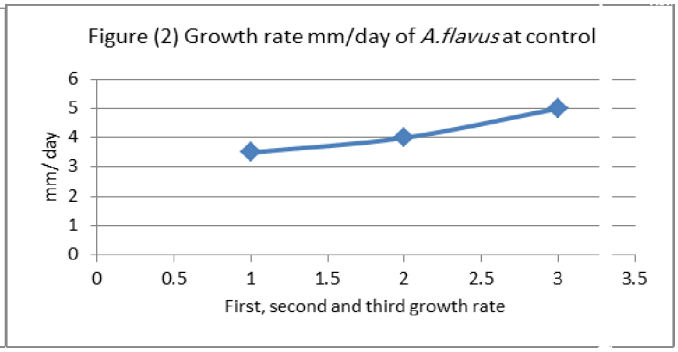
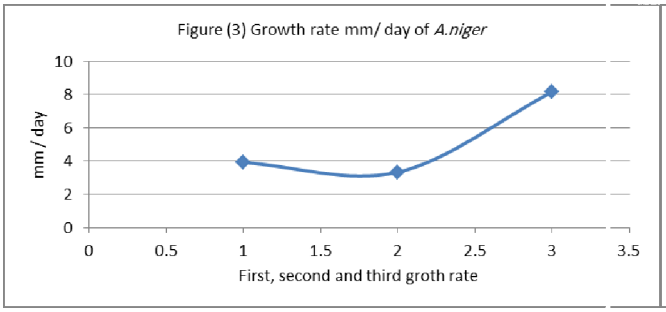


Fig. 1: left and 2 right: first, second and third growth rate (mm/day) of *A. flavus* on Poisoned medium PDA method by plants extracts.



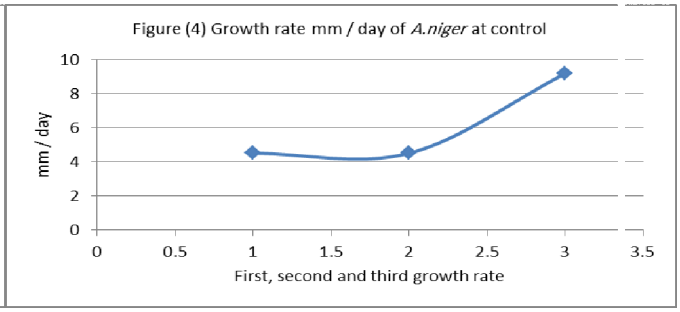
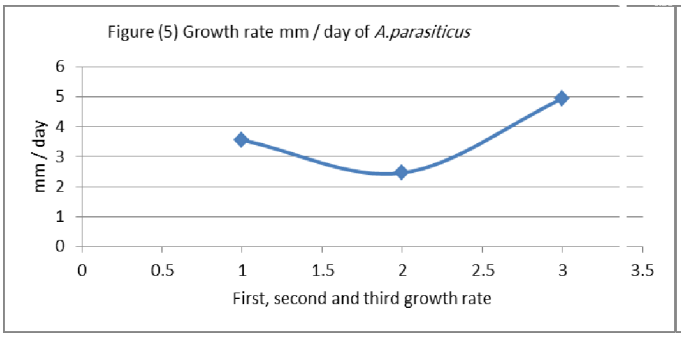


Fig. 3 left and 4 right: first, second and third growth rate (mm/day) of *A. niger* on Poisoned medium PDA method by plants extracts.



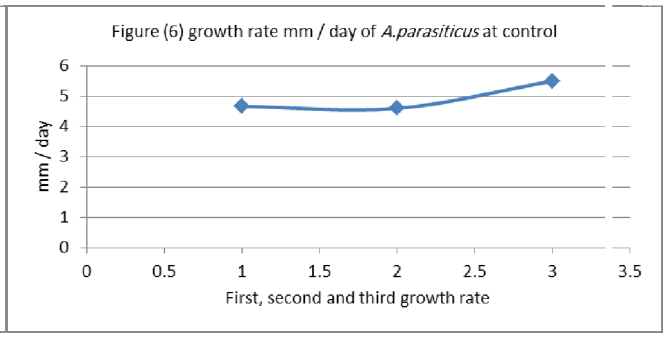


Fig. 5 left and 6 right: first, second and third growth rate (mm/day) of *A. parasiticus* on Poisoned medium PDA method by plants extracts.

to the natural growth rate shown in Figs (2, 4 and 6) respectively.

Appearance of the genus *Aspergillus* in food and feedstuffs is attributed that its species have the ability to secrete a large number of enzymes that analyze the food that are used in nutrition and growth, as well as increase its spreading capacity, while there some of its species can grow in a low moisture content furthermore the production of high density of the spores.

Inhibition the radial growth of *Aspergillus* due to the phytochemical compounds of medicinal plants contain cyclosides, flavonoids, alkaloids, tannins, saponins and other active substances with an antifungal effect that obstruct the function of the fungal cell membrane (Cowan, 1999). The results are agreed with study of (perumal *et al.*, 2004). And it had been found that Phenols in the plant extracts also inhibit the action of enzymes because they are oxidative compounds and may interact with sulfahydryl compounds or interfere with non-specific interactions with proteins (Mason and Wasserman, 1987) as they play a role in changing proteins and damage the membranes through their binding with Effective sites of cellular enzymes with the hydroxyl group by forming hydrogen bonds with those sites without binding the substrate which their action on this Enzyme, so they inhibit one or more of the necessary metabolic reactions that controlled those enzymes (Pelezar *et al.*, 1986).

It should be noted that the variation that occurred

from the influence of the growth rates of the fungi studied with plant extracts may be due to the nature of the fungal characteristic, components, thickness of its cell membranes, diversity of enzymes, its content of fats and proteins, and the extent of its sensitivity affected by the effective compounds of plant extracts (Honda and Tabata, 1982).

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