Plant Archives Vol. 19, Supplement 2, 2019 pp. 1275-1278 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

EFFECT OF *CALOTROPIS PROCERA* EXTRACT ON FUNGI ACCOMPANIED TO WHEAT GRAINS IN THE STORAGES OF BABYLON PROVINCE

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Abstract

This study included testing the effect of the *Calotropis procera* extract with three concentrations (0.5, 1, 1.5 mg.ml^{-1}) on the used local wheat seeds from the main grain silo in Babylon province and storage station in El-Mouradia where the effect of the extract on the percentage of germination for wheat seeds was tested. The results showed that there were significant differences in the percentage of germination and the concentration of (1.5 mg.ml^{-1}) was the most influential, which amounted to (93.3%) compared to the control treatment which amounted to (73.3%). It was found that the extract had a significant inhibitory effect on the tested fungi on the solid media (Potato Dextrose Agar) compared to the control treatment. These fungi were *Penicillium notatum* and *Aspergillus flavus*. The concentration of (1.5 mg.ml-1) was the best effect for *Penicillium notatum* and *Aspergillus flavus*, where amounted to (81.4, 59.2%), respectively, compared to the control treatment which amounted to 0%. As for the effect of the extract on the percentage of dry weight inhibition, where the concentration (1.5 mg.ml^{-1}) was the best effect where amounted to (70, 59.2%) compared to the control treatment which amounted to 0% for the two fungi mentioned above, respectively.

Keyword: *Calotropis procera, Aspergillus flavus*, wheat grains.

Introduction

Wheat crop (*Triticum aestivum* L.) is considered among the staple foods in human life, it is increasingly needed as population increases. Studies indicate that in 2020 the world population will need 1 billion tons of wheat (Yusuf *et al.*, 2001). It does not need only the availability of food, but also estimated the percentage of food losses. Pollution and corruption of fungi is about 25-30% (Al-Othmani, 1997). Wheat seeds are infected during harvesting, Transporting or storage in many fungal species, including *Aspergillus* and *Penicillium*. These fungi grow on many nutrients such as grains, seeds of pistachio and oils, Thus affecting the local production and the global economy, as well as their ability to produce millions of spores that are rapidly released into the air, which affects the marketing and consumption rates for this yield (Mazzola *et al.*, 1992). It also affects the vitality of the seeds and reduces their percentage of germination, which leads to the lack of agricultural production when using such seeds in the cultivation, as well as the ability of some fungi species on producing Mycotoxins and the most dangerous toxins are aflatoxin (Turcksess *et al*., 1997). These genera were chosen because of their potential to live in a variety of different environments (Council, 2003). Studies have indicated that 25% of global food crops are polluted annually with variable levels of Mycotoxins (Institute of Food Science and Technology IFST). This percentage agrees with estimates of the Food and Agriculture Organization (FAO) in the percentage of the contaminated world crop with Mycotoxins, leading to a significant economic impact on crops. At present, there is no contamination-free zone with Mycotoxins and their adverse effects on human health (Devegowa *et al.*, 2005). Therefore, it was necessary to protect the human and animal from the damage caused by these toxins in several methods to reduce the growth of these contaminated fungi, it is often using some chemical pesticides and considering that the majority of pesticides have significant damage to the health and the environment, Being a contaminant of the environment, it is toxic and carcinogenic to humans and animals in the case of using the seeds as a food source (Cowan, 1999). It is also accumulated in their soil and leads to the death of many of the microorganisms in which they live. It also seeps into water sources, affecting aquatic organisms and moving them through the food chain to other living organisms (Al-Saadi, 2002). In order to obtain products free from the residual effect of chemicals and environment and to maintain environmental balance, the studies aimed to find safe methods and alternatives to pesticides for the purpose of controlling agricultural pests, including the associated fungi with seeds. The researchers found the possibility of using plant extracts that are highly effective against these pests, As well as being non-toxic compounds of the plant and easy to breakdown in the environment, therefore, *Calotropis procera* plants were selected as safe medical plants and to know its efficiency in protecting the wheat seeds from fungal infections and being the first time used to resist the pathogens, The following study was conducted as a starting point for plant use in pest control. The following were conducted:

- Isolating and diagnosis of some of the growing fungi on wheat grain and the selection of the most existing in it
- Studying the effect of alcoholic extracts for the leaves of *Calotropis procera* in inhibiting the growth of some the isolated fungi from wheat grain laboratory.

Materials and Methods

Collecting wheat seeds

The used local wheat seeds were collected from the main grain silo in Babylon province and storage station in El-Mouradia. The wheat plant was selected as a host plant for many fungi. These grains are free from impurities and dust, where are used for nutrition and cultivation. Random samples were collected with 1 kg per location in storages.

Preparing the alcoholic extract for *Calotropis procera* plant

The plant leaves were collected, washed, dried, then grinded with an electric grinder and then a mixture of 20%

methanol and 80% distilled water was prepared, the powder of plant leaves mixed with the prepared mixture by 1 g of the powder of plant leaves with 3 g of the mixture and mix in the blender for 30 minutes at laboratory temperature. It was then filtered with a soft cloth and then put in suitable dishes in the oven at 45 °C to dry and then keep the dry extract in a sealed container in the refrigerator until use (Sato *et al.*).

Isolating the accompanied fungi with wheat seeds

The fungi were isolated from the used wheat seeds in the experiment. The seeds were sterilized with sodium hypochlorite solution at a concentration of 2% for 3 min, then washed with sterilized distilled water, then dried and cultured in Petri dishes containing on the Potato Dextrose Agar media, with Five seeds in each dish, with three replicates. The dishes were incubated in the Incubator at 25 °C and after four days, the dishes were examined to identify the growing fungi, where they were diagnosed and purified for subsequent experiments. A tablet from each colony was transferred to a new dish containing PDA media, The process was repeated several times until a pure fungus farm was obtained (Diwan, 1984). After its diagnosis, the fungus *Aspergillus flavus* and *Penicillium notatum* were selected for this study based on frequency and appearance of fungi in the used samples in the research, where the percentage of fungus frequency was determined according to the following equation:

The percentage of fungus frequency (%) =

$$\frac{\text{Number of fungus isolates in samples}}{\text{Total number of isolates in samples}} \times 100$$

The percentage for the emergence of fungal colonies in dishes for each fungus was calculated from the following equation:

The percentage of germination (%)

$$= \frac{\text{Number of germinated seeds}}{\text{Total number of seeds}} \times 100\%$$

Testing the effect of the concentrations of *Calotropis procera* plant in some physiological traits for the two fungus *Aspergillus flavus* and *Penicillium notatum*

(a) In the solid media (the growth of the radiation for fungi)

The PDA media was prepared in 250 mL flasks. The extract was then added to the nutrient media in 9 cm diameter Petri dishes, with three replicates for each concentration of the used concentrations (0.5, 1, 1.5 $mg.ml^{-1}$), except the control treatment, They were left with no addition. Each dish was inoculated with a piece of the used fungus culture in the experiment with a diameter of (0.5 cm). The incubator was then incubated at a temperature of 25 °C and after the diameter of the fungal culture reach for the control treatment to the edge of the dish (9 cm). The diameter of the fungus growth was measured in the dishes of the treatments. The results were taken by calculating the average of two perpendicular diameters of each colony, the percentage of inhibition was calculated according to the equation mentioned by (Shaaban and Al-malah).

The percentage of inhibition (%) =

$$\frac{\text{Total average of fungus growth in the control treatment} - \text{The average of fungus growth in the treatment}}{\text{The average of fungus growth in the treatment}} \times 100\%$$

(b) In liquid media (dry growth)

To test the effect of the extract on the growth of fungi in the liquid media, a 250 mL conical flasks were used, place in it the liquid media (P.D. Broth) and the different concentrations for the used extract in the experiment were added to each fungus individually. The flasks were then inoculation with a 5 mm diameter disc from the culture for both *Aspergillus flavus* and *Penicillium notatum* and at 7 days age. After seven days of incubation at a temperature of 25 °C, the fungal growth for fungus was filtered on sterile filter paper and dried at 60 °C for 24 hours with three replicates per concentration (Shaaban and Al-Malah, 1993).

(c) Formation of fungus spores

A disc with the diameter of (0.5 cm) was taken from the PDA media with fungus in the radial growth experiment, Each disc was placed in a tube containing 4.5 ml of distilled sterilized water. The number of germs was calculated using the haemocytometer and the percentages were also calculated to inhibit the formation of the spores.

Statistical analysis

All the results of the experiments were analyzed according to the Completely Randomized Design (C.R.D) as a factorial experiment. The averages were compared according to the least significant difference (L.S.D) test, with a probability level of 0.05 (Al-Rawi and Khalaf Allah, 1980).

Results and Discussion

Isolating the accompanied fungi to wheat seeds

Table (1) indicates that there are many fungi that have been accompanied by local wheat grains such as *Alternaria alternate*, *Aspergillus flavus*, *A. niger*, *Penicillium notatum*, *Fusarium oxysporum*. *Aspergillus flavus* was the most frequent and occurrence, where their percentage of frequency amounted to (54.8%) followed by *Penicillium notatum* (51.8%) compared to the rest of the other fungi. The percentage of appearance was 100% for *Penicillium notatum* and *Aspergillus flavus* compared with other fungi. The reason for the dominance of *A. flavus* genus is due to its simple nutrients requirements as well as its ability to withstand the critical environmental conditions and the possession of an enzyme system enabled him to exploit the various nutrients sources.

Table 1: Frequency and appearance of fungi accompanied by wheat seeds

fungi	Frequency (%)	Appearance (%)
Alternaria alternate	14.81	50
Aspergillus flavus	54.8	100
A. niger	14.81	50
Fusarium oxysporum	11.11	50
Penicillium notatum	51.8	100

Effect of *Calotropis procera* extract in germination of wheat seeds

Table (2) shows significant differences in the percentage of wheat germination for different treatments compared to the control treatment at level of 5% where amounted to (73.3, 80 and 93.3%) for concentrations of (0.5, 1, 1.5 mL) compared to the control treatment which amounted to (73.3%). The 1.5 ml concentration is the most effective. It

was found that the percentage of germination increases with the increase of the used concentrations in the experiment because increasing the concentration increases the effect of active substances in the extracts against the growth of accompanied fungus with wheat seeds, thus germination of as many seeds as possible. The decrease in the percentage of germination in the control treatments may be due to the effect of fungus on the internal tissues for seeds and their negative effect on the fetus or attacking the fungus for seeds during the germination process, which reduces the percentage of germination. Also, the competition of fungi with seeds on the amount of oxygen in the growth media is related to the average of germination (Said, 1986).

Table 2 : Effect of *Calotropis procera* extract in germination of wheat seeds

The concentration of extract (mg.ml^{-1})	The percentage of germination (%)
0.5	73.3
1	80
1.5	93.3
control	73.3
L.S.D(0.05)	**18.83**

Test the effect of the interaction type of fungus and concentrations of the *Calotropis procera* extract in some of the physiological traits for the two fungi the *Aspergillus flavus* and *Penicillium notatum*

The results of the statistical analysis showed significant differences in the percentage of inhibition the radial growth for *Aspergillus flavus* amounted to (19.6, 47.3, 81.4%) compared to the control treatment which amounted to (0%), whereas for *Penicillium notatum* amounted to (8.8, 29.6, 59.2%), compared to the control treatment (0%). It was found that the higher the concentration, the greater the percentage of inhibition. The reasons for the effect of the extract on the fungus were varied because it was first used in the field of fungal control, and its effect may be attributed to the fact that it contains many chemical compounds, including glycosides, flavonoids, methanol compounds, resins, Anthocyanin and Proteinases enzymes (shaker, *et al.*, 2010). It should be noted that the difference in the extent of the effect of fungi with plant extract may be due to the nature of the fungus in terms of composition and thickness of its cell membranes, it's content of fats and proteins and its relationship to the mechanism of action for the effective compounds for the extract, where the effect of the extract on fungi may be the result of deformities in its membranes and internal structures. The results of the study showed that the extract of the *Calotropis procera* plant had an effective effect on the percentage of inhibition for dry growth. As for *Aspergillus flavus*, the percentage of dry growth inhibiting at concentrations (0.5, 1, 1.5) amounted to (32.0, 43.3, 70%) compared to the control treatment which amounted to 0%. As for *Penicillium notatum*, the percentage of inhibition amounted to (16.6, 37.0, 59.2) at concentrations (0.5, 1, 1.5%), respectively, compared to the control treatment which amounted to 0%. As for the effect of the extract on the percentage of inhibition for spores formation, there were significant differences between the treatments. As for *Aspergillus flavus*, the percentage of spores formation at a concentration (0.5, 1, 1.5) amounted to (32.3, 46.7, 67.2%). For *Penicillium notatum*, the percentage of inhibition for spores formation at concentrations (0.05, 1, 1.5) amounted to (50.8, 53.4, 70.1%) compared to the control treatment which amounted to 0%

Table 3: Testing the effect of the interaction type of fungus and concentrations of the *Calotropis procera* extract in some of the physiological traits for the two fungi the *Aspergillus flavus* and *Penicillium notatum*

Fungi	Concentration of extract (mg.ml^{-1})	Inhibition of radial growth (%)	Inhibition of dry weight (%)	Inhibition of spores formation (%)
Aspergillus flavus	0.5	19.6	32.0	32.3
	1	47.3	43.3	46.7
	1.5	81.4	70	67.2
	Control	0	0	0
Penicillium notatum	0.5	8.8	16.6	50.8
	1	29.6	37.0	53.4
	1.5	59.2	59.2	70.1
	Control	0	0	0
L.S.D(0.05)		**5.99**	**19.5**	**7.45**

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