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EFFICACY OF *LACTOBACILLUS ACIDOPHILUS* AND ALCOHOLIC EXTRACT OF MALLOW AND *ALOE VERA* IN REDUCING *ASPERGILLUS NIGER* GROWTH AND PATHOGENICITY ON EGGPLANT FRUITS

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Abstract

This study was conducted to test the effect of the interaction of acidophilus Lactobacillus and baking leaves extract and alcoholic aloe vera in reducing the growth of Aspergillus niger, the prone to rot of eggplant fruits after harvest. The results of the Lactobacillus acidophilus test on eight pathogenic isolates from A.niger showed that the efficacy of bacteria in inhibiting the growth of pathogenic fungi varied according to the fungal isolate. The bacteria had the highest efficiency against A.n8 isolation, which resulted in a percentage of inhibition of 66.67%, while the lowest inhibition rate (42.22%) was recorded in A.n5 isolation compared to the control treatment. The results also showed that either the alcohol extract for baker and aloe vera and for all concentrations under test (20%, 10%, 5%, 1%) resulted in a complete inhibition of the pathogenic growth of the pathogen. The treatment of the interaction of control bacteria and baker and aloe vera extract with a concentration of 5% resulted in complete inhibition of the fungus on the PDA medium after 10 days of incubation, while the lowest percentage of inhibition of the fungus (82.22%) resulted from the treatment of overlapping bacteria and aloe vera extract. Upon testing the above factors in reducing fungal rot on the wounded eggplant fruits and treatment with pathogenic mushrooms, the results showed that the treatment of bacterial interference, baker extract and aloe vera 5% led to the protection and healing of the wounds that were caused and which prevented any symptoms of infection from the fungus and kept the fruits in their healthy appearance and acceptable color even after 10 days of storage in laboratory conditions.

***Key words*:** Plant extract, Eggplant, *Aspergillus niger*, Antagonism, Lactic acid bacteria

Introduction

Most fungi that infect vegetables and fruits during storage are toxins producing fungi. Of the most important are the fungi under *Aspergillus*, which are responsible for the production of the most important and most dangerous toxins: aflatoxins and oxatoxins (Aish *et al.,* 2004; Vargo and Kozakiewicz, 2006). Mycotoxins are secondary metabolite products that are produced by many fungi and are of economic importance to humans. They are either as beneficial as life antibiotics, or harmful as mycotoxins (Eskola, 2002). Poison Ochratoxin OTA is produced by many Aspergillus and Penicillium fungi and has different effects in humans and many animal species. It is primarily Nephrotoxic and affects the liver Heptatoxic and is involved in some Teratogenic abnormalities as it is Carcinogenic and Immunosuppressive, in addition to its relationship to tumors in the urinary system and kidney cancer (Bräse *et al.,* 2009; Hassan *et al.,* 2012; Lates *et al.,* 2012).

Some fungi and mycotoxins have the ability to resist chemical changes and antibiotics as a result of the emergence of resistant strains. This led some studies to the trend towards reducing the use of chemicals and finding alternatives. Among these alternatives is the use of some types of beneficial microorganisms, such as the bacteria producing lactic acid (Lactic acid bacteria). Therefore, these bacteria are important in the vital preservation of food and fodder, due to their production of antifungals organic acids such as lactic acid, acetic acid, hydrogen peroxide and some other compounds including Bacteriocins (Prachyakij *et al.,* 2007; Tropcheva *et al.,* 2014).

On the other hand, many studies have indicated the

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importance of plant extracts in the resistance and inhibition of many fungal and bacterial pathogens (Valverde *et al.,* 2005). Among the important plants in this field is the Aloe vera plant, where many studies have demonstrated the high ability of this plant extract to inhibit fungi, bacteria and viruses in addition to stimulating the immune system (source). The fact that this plant extract contained clikosides, saponins, phenols and alkaloids made it one of the important extracts in the field of controlling pathogens (Tin, 2011 and Mbajiuka *et al.,* 2014).

Several studies reported that aqueous and alcoholic extract of mallow leaves showed inhibitory effects on growth of many fungi, especially toxins producing ones. This is mostly due to the fact that mallow plants contain cyclosides, soaps, alkaloids, flavonoids, phenols, resins and volatile oils, in addition to containing sugars compounds as glucose, galactose, arabinose, ramenose and xylor. (Islam *et al.,* 2010; Ahmad and Ibrahim, 2016). This study aimed to investigate the effect of common mallow extract, aloe vera extract and *Lactobacillus acidophilus* bacteria alone or in combination on the growth and pathogenicity of *Aspergillus niger* in vitro in culture medium and in vivo on eggplant fruits.

Meterials and Methods

Pathogenic *Aspergillus niger* and bio-controlling Lactobacillus acidophilus

A morphologically and molecularly diagnosed fungal isolate of the pathogenic *Aspergillus niger* was obtained from the Plant Pathology Laboratory for Postgraduate Studies in the Department of Plant Protection/Faculty of Agriculture-University of Kufa. While the bio-controlling *Lactobacillus acidophilus* isolate was obtained from graduate laboratory in the Department of Food Science, College of Agriculture/University of Kufa.

Preparing alcoholic leaf extract of mallow and aloe vera

The extract was prepared using 20g of fresh superficially sterilized leaves of aloe vera or mallow plants. The leaf material was mixed for 30 seconds in electric food processor with 400 ml of 95% ethylene. The mixture was then filtered using sterile filter paper and then passed through 0.20µm Whatman Millipore. The extract was then put in the oven at 45°C for 30 minutes to remove alcohol excess. The resulted extract was considered to be 100% fully effective stock compound for the alcoholic extract of aloe vera or mallow, and was stored in sterile glass closed flasks at 4°C until use.

Effect of alcohol leaf extract of mallow and aloe vera on *Aspergillus niger* growth on PDA medium

Four concentrations (1%, 5%, 10% and 20%) of stoke solution were prepared for both mallow and aloe vera extract in four sterile glass flasks. In this experiment, the poisoned medium technique was used by adding 1 ml of each concentration to 100 ml of sterile PDA and shaking to prepare a culture medium in concentrations of 0.01%, 0.05%, 0.1% and 0.02%, respectively, and poured into sterile Petri dishes. Each dish was then inoculated in the center with a 0.5 cm tablet from 7 days old pathogenic fungal culture and Incubated at 25 ± 2°C for 7 days. Then, the radial growth of the pathogenic fungus was measured and the percentage of inhibition was calculated according to the Abbott equation adapted by Shaban and Al-Mallah (1993).

Efficacy of *Lactobacillus acidophilus* against *Aspergillus niger* in the PDA medium

The acidophilus Lactobacillus isolation was activated in the PDB medium by adding 1 ml of bacterial suspensions to 200 ml of the pre-prepared and incubated PDB medium at 35 ± 2°C for 48 hours. Then, 1 ml of bacterial suspension was added to 200 ml of the PDA medium, poured in 9 cm Petri dishes and stirred until the hardness. Dishes were inoculated with pathogenic fungus as (as formerly described) and the rate of inhibition was calculated after seven days incubation (abott 1999).

The synergistic effect of Lactobacillus acidophilus, mallow extract and aloe vera extract on *Aspergillus niger* growth in the PDA medium

Seven glass flasks were prepared containing 50 ml of sterile PDA medium to test the effect of different treatments on the growth of pathogenic *Aspergillus niger*. Treatments included standard medium PDA without addition (control), PDA treated with bacterial suspension (105 × 1.4), PDA treated with mallow extract 0.5%, aloe vera extract 0.5% PDA, in addition to other treatment combinations. After pouring and the hardening of the medium, the dishes were inoculated with *Aspergillus niger* and incubated. The percentage of inhibition was calculated after 7 days of incubation as previously mentioned.

Effect of Lactobacillus acidophilus, Alcoholic extractof mallow and aloe vera in reducing infection with *Aspergillus niger* on eggplant fruits

Three plastic containers (30cm long, 20cm width and 10cm height) were prepared and filled with 3 liters of sterile distilled water, fungal suspension of *Aspergillus niger* (1.1×10^4) or a mixture of bacterial suspension (1.4×10^5 T. m) and 0.05% alcoholic extract of mallow and Aloe vera. In this experiment, 24 uniform eggplant fruits were used and divided into four groups, two groups

of which were longitudinally wound with four wounds at a depth of 1cm. The first group was soaked for 5 minutes in the *Aspergillus niger* suspension and then air dried on filter papers for 30 minute, and then soaked also for 5 minutes in the combined treatment of bacterial suspension and alcoholic extract of mallow and aloe vera, dried on the filter papers and the fruits were kept in Polyethylene bags under laboratory conditions, while fruits of the control treatment were soaked in distilled water. The second group underwent the same treatments as the first group using unwounded fruits. Whereas, the same first procedure first was performed on the third group with reverse order by soaking the eggplant fruits in the combined treatment mixture (bacterial suspension and alcohol extract for mallow and aloe vera) then in fungal suspension following same steps. The unwounded fruits of the fourth group were subjected to same treamnet of group three. Bags containing eggplant fruits of all treatments were left for 10 days after which the injury level and infection severity were recorded and photographed.

Results and Discussion

Effect of alcohol leaf extract of mallow and aloe vera on *Aspergillus niger* growth on PDA medium

The results showed that the alcoholic extract of mallow and aloe vera at all the concentrations resulted in 100% inhibition of fungus growth after 7 days of incubation compared to the control treatment in which the fungus appeared at 100% growth rate and without any inhibition. This is consistent with previous results (Islam *et al.,* 2010; Al-Tikriti, 2012) that the active substances present in the plant extract, including saponins, resins, clicosides, tannins, alkaloids, ficomarines, carbohydrates, and flavonoids, have a role in inhibiting the growth of fungi, especially the alcoholic extract which is more effective.

Table 1: Efficacy of *Lactobacillus acidophilus* against *Aspergillus niger* isolates in PDA medium.

***Aspegillus niger* isolate**	**% inhibition due to *Lactobacillus acidophilus* treated PDA medium**
A.n1	64.88
A.n2	58.88
A.n3	57.44
A.n4	46.33
A.n5	42.22
A.n6	51.88
A.n7	60.77
A.n8	66.66
A.n9	48.55
A.n10	51.55
A.n11	53.77
A.n12	63.0
A.n13	56.0
A.n14	55.22
Control	0.00
L.S.D. $_{(\leq 0.05)}$	0.5625

Efficacy of *Lactobacillus acidophilus* against *Aspergillus niger* isolates in PDA medium

The results showed (Table 1) that all *Lactobacillus acidophilus* isolates inhibited the growth of *Aspergillus niger* in different proportions on the PDA medium. The highest percentage of inhibition (66.66%) was recorded in the A.n8 isolate while the lowest percentage of inhibition was 42.22% with A.n5, compared with the control treatment at 7 days of incubation.

The reason for inhibiting the growth of pathogenic fungi when bacteria are present in the same medium is because the bacteria possess several mechanisms, including.

Competition for location and food, antagonistic effects against the pathogen and secretion of many analytical enzymes that work to inhibit the growth of microorganisms, especially fungi. Organic acids such as lactic acid and acetic acid, as well as hydrogen peroxide, are among the most important antagonistic compounds that these bacteria produce against fungi (Rolf, 2000; Ross, 2002 and Prachyakij *et al.,* 2007).

The synergistic effect of Lactobacillus acidophilus, mallow extract and aloe vera extract on *Aspergillus niger* growth in the PDA medium

The results of testing the synergistic effect of different treatments on the growth of pathogenic fungi (Table 2) showed that the highest efficiency of bacteria, baker extracts and aloe vera (alcoholic) in inhibiting the growth of the fungus Aspergillus niger, as other factors have shown a reduction in the radial (country) growth of the fungus in Fungal colonies with significant differences, as the highest percentage of inhibition (100%) in the interaction treatment of bacteria and mallow and aloe vera extract, while the lowest inhibition rate in combination of bacteria and aloe vera extract was 82.22% compared to the control treatment which recorded fungi growth of

Table 2: Synergistic effect of Lactobacillus acidophilus, mallow extract and aloe vera extract on *Aspergillus niger* growth in the PDA medium.

Treatments	**% inhibition of *A. niger* on PDA medium**
Aloe vera extract+ *L. acidophilus*	82.22
Mallow extract+ *L. acidophilus*	87.11
Aloe vera extract+ Mallow extract	
+ *L. acidophilus*	100
Aloe vera extract+ Mallow extract	97.77
Control	0.00
L.S.D. $_{(\leq 0.05)}$	0.5103

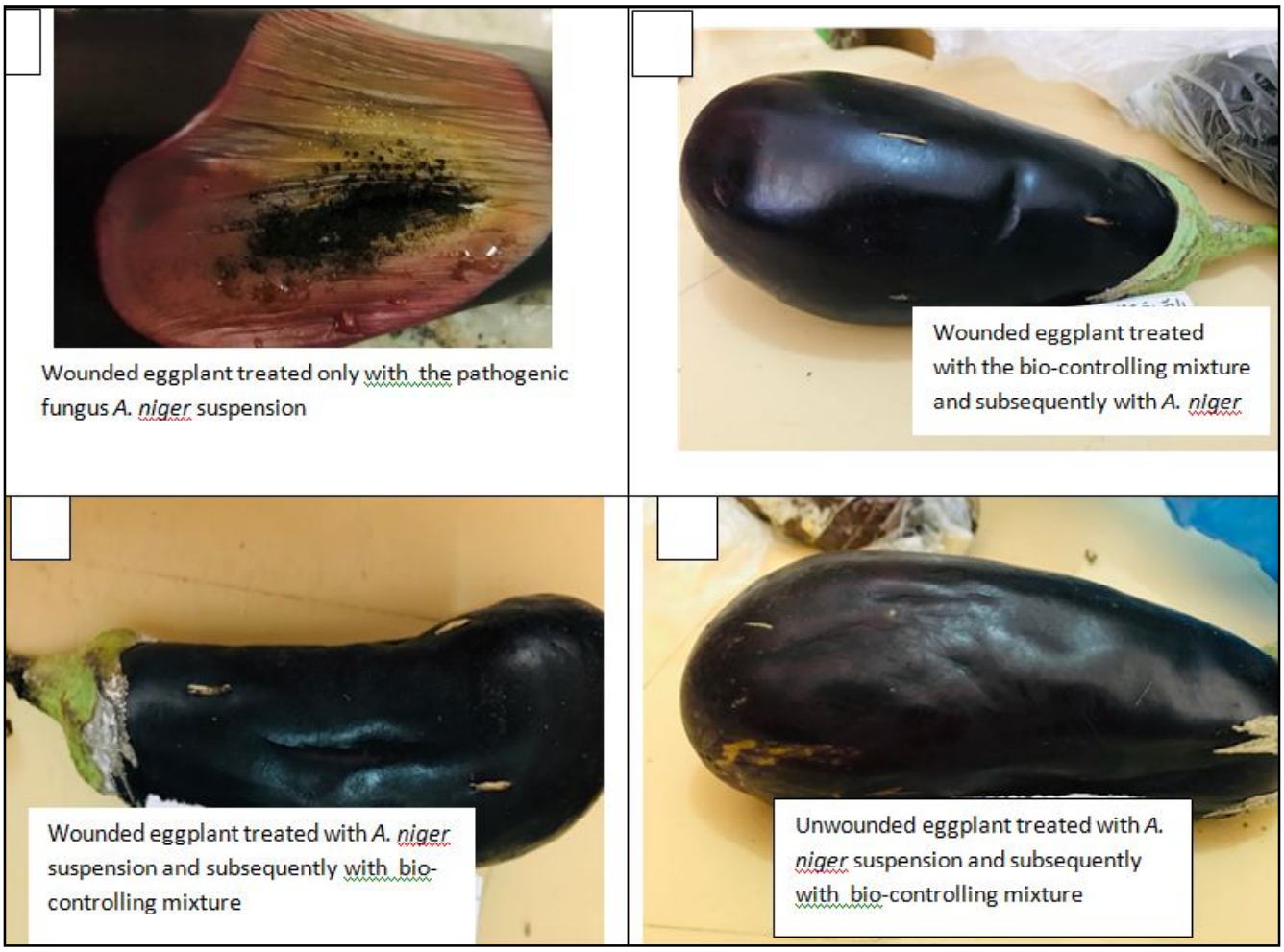

Fig. 1: Effect of Lactobacillus acidophilus, Alcoholic extractof mallow and aloe vera in reducing infection with *Aspergillus niger* on eggplant fruits.

100% after 10 days of incubation.

The reason for this is due to the synergistic effect of both bacteria and alcoholic extracts of baker and aloe vera, so that the active substances present in baking extracts and aloe vera are highly soluble in organic solvents such as ethyl alcohol and not soluble in water, so the alcoholic extract is more effective in inhibiting fungi compared to the aqueous extract This is consistent with (Islam *et al.,* 2010; Nabigol and Asghari, 2013 and, Tamilarasi *et al.,* 2014), and that bacteria contain many antifungal agents. This led to an increased rate of inhibition and this is consistent with Tropcheva *et al.,* 2014).

The results of examining the fruits of eggplant after 10 days of storage in polyethylene bags in laboratory conditions showed that treatments sequence (soaking in the biological control mixture and then in the fungal suspension of the pathogenic fungus or vice versa) did not increase the fungus pathogenicity nor reduced efficacy of the controlling mixture against the pathogenic fungus (Figure 1 B, C, and D). Fruits of eggplant (wounded or unwounded) treated with a mixture of bakery extract and 5% aloe vera and Lactobacillus acidophillus, which were subsequently treated with Aspergillus niger, did not show symptoms of rotting with no damage and the treated

fruits appeared healthy and in natural color, unlike the positive control of fruits treated only with the pathogenic fungus suspension only, which lost its natural color and showed symptoms of tissue degeneration in the sites of infected wounds that were brown surrounded by a red color to the tissue surrounding the site of the injury, which eventually led to small size fruits with shrinking and deformation (Fig. 1A).

The symptoms of infection and rotting did not appear in the fruits treated with the extract of aloe vera and bakeries, despite the presence of the pathogen. This is often due to the plant extracts used containing many essential minerals and compounds (Nagata *et al.,* Ebizuka, 2002; Balasubramanian, and Narayanan, 2013), which stimulate growth hormone, increase zinc levels and some amino acids that play an important role in stimulating this hormone. Thus, this will increase in cell division and

tissue size leading to repairing wounds and cuts in the fruit (Hassan, 2001).

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