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ANTAGONISTIC ACTIVITY OF PROBIOTIC AND SEA WEED EXTRACT AGAINST VEGETATIVE GROWTH FOR SOME FUNGI AND ZEARALENONE PRODUCTION

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ABSTRACT

The current study was carried out to evaluate the use of Iraqi probiotic and sea weed extract *Ascophyum nodosum* (foreign product) against some phytopathogenic fungi and reduce the production of Zearalenone which it is produced by *Fusarium graminearium* in liquid medium Yeast Extract Sucrose (YES). Using different concentrations from probiotic (0.0, 0.5, 1, 1.5, 2)% w/ v and sea weed extract (0.0, 2, 4, 6, 8) % v/v .It has all the concentrations of probiotic showed high efficiency in inhibiting the growth of fungi, especially at concentrations (1, 1.5) inhibition percentages were 100% against *Fusarium graminearium* and *Pythium aphanidermatum*. While the results of sea weed extract was shown differences in inhibition percentage for all concentration based on the type of fungus except *Penicillium digitatum* and *Pythium aphanidermatum* the inhibition percentages were 100% at higher concentrations (1, 1.5,2). The research presented here mainly concerns the inhibition of fungal growth *Fusarium graminearum* and Zearalenone (ZEN) production in liquid medium using higher concentrations of probiotic (1.5, 2) % w/v and sea weed extract (4,8) % v/v. The dry weight of *Fusarium graminearum* was evaluated (gm) and the results of probiotic treatment was more efficiency in inhibition of fungal growth and inhibition the level of Zearalenone production completely 100 % compared with sea weed extract inhibition percentages of fungal growth were (0.33, 0.47) % and (74.2, 79.4) % the reduction percentage of Zearalenone at (4, 8) % v/v respectively.

KEYWORDS: *Fusarium graminearium, Pythium aphanidermatum,* Zearalenone.

INTRODUCTION

The recent studies in the world is focused on utilization alternative methods for chemical pesticides are safe for the environment one of these methods utilization biological agents as a safety method to reduce plant pathogenic fungi and the effects of mycotoxins through the use of probiotic. The term "probiotic" has been firstly defined by Fuller as "a live microbial feed supplement which beneficially affects the host by improving its intestinal microbial balance" such as *Saccharomyces cerevisiae*, *Lactobacillus acidophilus*, *Bacillus subtlis etc* (Beltran *et al.*, 2004). The mechanisms used include the production of inhibitory substances against pathogens, competition for essential nutrients and adhesion sites, the supply of essential nutrients and enzymes resulting in enhanced nutrition in the host, and the modulation of interactions with the environment and the development of beneficial immune responses (Gomez and Balcázar, 2008) .Numerous studies had revealed the ability of these microorganisms to inhibit the pathogenic fungi , Hamed and others 2011 found that application of a multispecies probiotic inhibited growth of *Fusarium oxyosporium* completely this may be due to the effect of Lactobacillus , indicating that besides acetic and lactic acid , other metabolic products of the LAB contribute to the inhibition and also has the ability to reduce mycotoxins production specially mycotoxins of *Fusairum* species (Al-Nezami *et al.*, 2002 ; - Mahdi et al., 2014). A recent strategies the use of algae extracts in controlling fungal plant diseases like Cyanobacteria (Blue Green Algae) *Scytonema* and *Nostoc* spp. These have been recorded to inhibit growth of fungi are mostly phytopathogenic fungi (Kiviranta *et al.*, 2006). In turn, the extract of the seaweed that brown algae *Ascophyllum nodosum* stimulates induced systemic resistance (ISR; or systemic acquired resistance, SAR) of plants against phytopathogenic fungi which involves phytoalexins and of PR proteins , The *Ascophyllum nodosum* extract is able to effectively reduce the number of colonies in *Alernaria cucumerinum***,** *Fusarium oxysporium*, *Botrytis cinerea* and *Didymella applanata* (Jayaraman et al., 2010) , other study showed use seaweeds extract in biological control against *Fusarium oxyspoium* f. sp.lycopersici caused wilt fusarium in tomato (Al-Hamery) . So seaweeds have developed among other defense mechanisms, the ability to produce biologically active compounds , the majority of these are Terpenes and Polyphenols (BLUNT *et al.*, 2006).

MATERIAL AND METHODS

Fungi

Fungi isolates were obtained from Department of plant protection College of Agriculture – Baghdad university. *Trichoderma harzianum* isolated from soil while *Fusarium graminearium, Aspergillus flavus, Aspergillus terreus, Pythium aphanidermatum* and *Penicillium digitatum* isolated from different plants.

Probiotic and Seaweed Extract

Probiotic and seaweed extract were obtained from Department of plant protection College of Agriculture – Baghdad university . probiotic is Iraqi product each one gram involves numerous microorganisms :- *Lactobacillus acidophilus* 10^8 Colony Forming Unit (CFU), *Bacillus subtilis* 10^9 (CFU) , *Lactobacillus* spp. 10^8 (CFU , *Saccharomyces cerevisiae* 10^9 (CFU). Seaweed extract of brown algae *Ascophyllum nodosum* is obtained as a commercial foreign product from College of Agriculture Baghdad university.

Effect of Probiotic and Seaweed Extract on Growth of Fungi

Antifungal activity assay was used to determine antifungal activity of the probiotic and algae extracts against fungi isolates after mixing probiotic and seaweed extract with Potato Dextrose Agar (PDA) medium to obtain different concentrations of probiotic (0.0, 0.5, 1, 1.5, 2) % w/v and seaweed extract were (0.0., 2, 4, 6, 8) v/v. An agar plug (5 mm) was removed from culture of each fungus (*Trichoderma harzianum, Fusarium graminearium, Aspergillus flavus, Aspergillus terreus, Pythium aphanidermatum* and *Penicillium digitatum*) then placed in the center PDA plates, the plates were incubated at 25°C until the fungal growth in the control plates reached at least the edge of the plate. Then, the radius (mm) of the growth in the treatments and the control were measured. The antifungal activity was calculated according to the following formula.

% FI = (Rc – Rt /Rc) x 100

Where: %FI = % fungal inhibition.

Rc= radius of growth zone in the control.

Rt = radius of growth zone in the treatment.

The Efficacy of Probiotic and Seaweed Extract in Removing of Zearalenone Toxicity

This study was conducted the ability of higher concentrations of probiotic (0.0, 1.5 , 2) % w/v and seaweed extract (0.0, 4, 8) % v/v in removing or destruction ZEN by using produced isolate *Fusarium graminearum* (20.047) ng/ ml after growing on a specific medium

for ZEN production YES (Okazaki and Saito , 1992) , which was taken in a 100 ml Erlenmeyer conical flask and sterilized at 121°C, 15 lb/inch² pressure for 15 minutes and allowed to cool. The flasks were inoculated with 7 mm diameter mycelia disc of *Fusarium graminearum* taken from 7 days old culture, After incubation at 25 ±2 ºC for 14 days , the content of the each flask were extracted on the 14th day of culture according to the method described earlier with some modifications (Liao *et al.*, 2009) by using 50 ml of extraction solution (acetonitile / water = 90 / 10, v/v) then added 1.5 ml from fungal filteration with 7.5 ml extraction solution , the mixture was centrifuged for 30 min. at 3000 rpm . The extract was filter through milipore filter (0.22 µm) in diameter of pore. Then estimated the concentration of Zearalenone using by High performance liquid Chromatography (HPLC) according to the following formula.

Concentration of sample = concentration of standard soultion × Area of sample / Area of standard solution (ECC).

High performance liquid Chromatography (HPLC) / Analysis

The HPLC system consisted of a Shimadzu HPLC system model LC-2010 AHT, the colume Hyper Clone 5µCN 120A (250 ×4.6mm) Phenomenex. Injection volume was 0.2 µl. The detector wavelength was UV. 218 nm. The mobile phase, acetonitrile / water (50/50) v/v, was pumped at a flow rate of 0.5 ml/min., according to ECC, 1992 calculated the concentration of ZEN by comparing curve area for samples with standard solution of ZEN (ECC). Tested percent of ZEN, then The Percent Reduction of Zearalenone was calculated using the formula.

Percentage of Reduction = concentration of control – concentration of sample / concentration of control X 100.

RESULTS AND DISCUSSION

Determine the concentrations affecting probiotic and seaweed extract on growth of fungi

Probiotic is considered the growth inducer agents ,produced by different microorganisms ,the word 'probiotic' is also derived from the same Greek term 'biotikos' which may be literally translated as 'for life' and is the opposite of antibiotic word which means anti-life (Beltran *et al.*, 2004). Also, probiotic is antimicrobial effects (Plummer *et al.*, 2005). The results of Table – 1 showed the ability of probiotic in inhibition of growth phytopathogenic

fungi specially at higher concentrations (1.5 , 2) % w/v in most isolates except *Trichoderma harzianum* . the statistical analysis results showed significant differences at ($p < 0.01$) between means of diameters of colonies of all treatments compare with control (without probiotic) while two concentrations (1 , 1.5) % w/ v showed completly inhibition 100 % in growth of *Fusarium gramenarium* and *Pythium aphanidermatum* . The mechanism of action of probiotics with anti-microbial properties is maybe due to the role of combination probiotic and its contents from bacteria and yeasts Caused increasing in the percentage of inhibition by production of bacteriocins such as nicin or lowering the pH by producing acidic compounds like lactic acid (Verschuere *et al.,* 2000) . The reason of inhibition may be due to the producing bacteriocins and acidic compounds such as lactic acid and thereby reduce the pH in medium (Ashraf *et al.,* 2009) . These results were in agreement with (Ali *et al.,* 2013) that revealed the antifungal agent of probiotic (consists from 14 bacterial isolates isolated from cheeses and milk) against phytopathogenic fungi *F.oxysporim* ، *Ristoctonia solani***.**

Table -1- Mean inhibition percentage of different fungi at different concentrations of probiotic.

Type of fungus	Concentrations			
	0.5%	**1.0%**	**1.5%**	**2.0%**
Aspergillus terreus	72.9	70.5	69.4	74.1
Trichoderma harzianum	23.5	25.2	29.4	17.6
Penicillium digitatum	78.8	74.1	88.2	88.2
Pythium aphanidermatum	76.4	70.5	100	100
Fusarium graminearum	84.7	78.8	100	100
Aspergillus flavus	78.8	75.8	83.5	82.3

The anti fungal activity of seaweed extract are shown in Table – 2, the percentage of inhibition between fourth concentrations (2, 4, 6, 8) % v/v of seaweed extract were varied at most fungal isolates compared with control except *Penicillium digitatum*and *Pythium aphanidermatum* the percentage of inhibition was 100% at higher concentrations (4, 6, 8) % v/v. the variation of antifungal activity of seaweed extract might be due to the presence of antifungal substances , which varied from species to species and the type of chemical components that are secondary metabolites like Terpens, Alkaloids and Polyphenols, numerous studies are found the inhibition activity of seaweed extract against pathogenic bacteria (Wang *et al.,* 2009 ; Peres *et al.,* 2012) and phytopathogenic fungi *Pythium ultimum* and *Rhizotonia solani* using of Cladophora glomerata (Mohammed *et al.,* 2013) and *Fusarium oxysporium* f.*sp*.lycopersii caused wilt fusarium of tomato using of seaweed extract *Ascophyum nodosum* (Al-Hamery).

Table -2- Mean inhibition percentage of different fungi at different concentrations of seaweed extracts.

Type of fungus	Concentrations			
	2%	4%	6%	8%
Aspergillus terreus	64.7	68.2	70.5	71.7
Trichoderma harzianum	47.0	61.1	70.5	70.5
Penicillium digitatum	88.2	100	100	100
Pythium aphanidermatum	63.3	70.5	100	100
Fusarium graminearum	70.2	71.7	74.1	88.2
Aspergillus flavus	61.1	72.9	76.4	76.4

The Efficacy of Probiotic and Seaweed Extract in Removing of Zearalenone Toxicity

The results of treatments of *Fusarium graminearum* which is produced Zearalenone (20.047) ng / ml in liquid medium Yeast Extract Sucrose (YES) with two concentration (1.5, 2) %w/v of probiotic and (4, 8) % v/v of seaweed extract exhibited variations in percentage of reduction of Zearalenone production Table-3- at probiotic concentrations (1.5 , 2) %w/v showed a great potential in completely inhibition of fungal growth 100 % therefore the percentage of reduction for both concentrations recorded 100% compared with control . The reason may be due to the secretions of microorganisms embedded in the probiotic of antifungal substances such as lactic acid or antibiotic production Bacterioins (Reuterin that produced by *L.reuteri* (El-Ziney *et al.*, 999) that is responsible to reduce of mycotoxins specially toxigenic species of Fusarium (Al- Nezami *et al*., 2002 ; Mahdi *et al.*, 2014) . whereas the results of treatments of of *Fusarium graminearum* which is produced Zearalenone with concentrations of seaweed extract (4,8) % v/v showed variation in the percentage of reduction Zearalenone production and fungal growth (dry weight) the percentage of reduction were (74.02 , 79.04) % and (0.33 , 0.47) gm of dry weight at concentrations (4, 8) % v/v respectively . Several studies have shown that seaweeds or its extracts have different biological activities in addition to antimicrobial in general and specially anti-phytopathogenic fungi and have effects on mycotoxins because of bioactive compounds in study of (Zineb *et al.*, 2003) the use of methanolic extract of *Cystoseira tamaricifolia* exhibited antifungal activity against the growth of Aspergillus flavus with reduction of Aflatoxins production. Also *Nostoc commune* extract has a great potential to inhibit the growth and sporogenesis of *Fusarium oxysporium* f.*sp*.lycopersii (Kim And Kim , 2008).

Based on these results have been interpreted distinction of probiotic Iraqi local product on seaweed extract *Ascophyum nodosum* commercial foreign product to the diversity of

microorganisms comprising the probiotic in addition to the concentrations of microbiology ,whenever there is diversity in microbiology and even within the same species will lead this to bring out more of the role of probiotic and there recent studies have pointed to the importance of the use of probiotic as bio-agents to remove or destroy the mycotoxins in general (Biernasiak *et al*., 2006).

Table- 3- Effect of different concentrations of probiotic and seaweed extract on Zearalenone production

Probiotic		
Concentrations (%)	**Zearalenone conc. (ng/ml)**	**Reduction percentage(%)**
1.5	0.00	100
2	0.00	100
Seaweed extract		
4	5.208	74.02
8	4.200	79.04
Control	20.047	0.00

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