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RESEARCH ARTICLE

The inhibitory effect of β-D-glucan biopolymer on trichothecenes toxins and its relationship to some vitamins content

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Abstract

The wheat is one of the most strategic crops in Saudi Arabia and in the world which attack with a number of seedborne pathogens like Fusarium fungi that secrete toxins, which in turn contaminate grains. These trials designed to evaluate the β-D-glucan biopolymers as new mycotoxin binder for T-2, HT-2, Nivalenol (NIV) and Deoxynivalenol (DON) toxins. Also, study its effect on water-soluble and/or fat-soluble vitamins of wheat. The fungal isolation was conducted from 3 regions of Saudi Arabia; Riyadh, Jeddah and Qassium. The isolation depended on representative random samples of stored wheat grains. The occurrence of Fusarium isolates in Riyadh, Jeddah and Qassium samples was 13, 17 and 12 isolates, respectively. The most predominate species differed according to isolation locate. The identification was by using BIOLOG technique. The evaluation of biopolymer was carried out using toxigenic different Fusarium isolates isolated from wheat grains. The biopolymer reduced the toxins produced by the most toxigenic isolate Culm-J1 and the maximum detoxification by **β**-D-glucan biopolymer against T-2, HT-2, NIV and DON were 34.1, 40.8, 41.5 and 33.1%, respectively after 45 days. The total trichothecenes inhibited by using biopolymer to 38.7 and 43.2% after 45 days from artificial inoculation with isolates No.Gram-R1 and Culm-J1, respectively. The reduction of trichothecenes types were determined by ELISA. The effect of biopolymer was studied on some vitamins as Thiamine (B1), Niacin (B3), Pantothenic acid (B5), Tocopherols (E) and vitamin (K). All measurement of water –soluble vitamin and fat-soluble vitamin was determined by HPLC. This investigation aims to evaluate a new bio-adsorbent material to reduce some severe mycotoxins, study the applicability utilize on stored wheat grains and their effect on some nutrient value of wheat component as vitamins.

Key word: Bio-adsorbent, Mycotoxins, Thiamine, Niacin, Tocopherols, HPLC, ELISA, Fusarium

Introduction:-

Wheat (*Triticum* spp) is a cereal grain, cultivated worldwide. In 2013, world production of wheat was 713 million tons, making it the third most-produced cereal after maize and rice (745 million tons), (FAO, 2015). This grain is grown on more land area than any other commercial food. World trade in wheat is greater than for all other crops combined. Globally, wheat is the leading source of protein in human food, having higher protein content than other major cereals, maize or rice 6.

Wheat is planted to a limited extent as a forage crop for livestock, although the straw cannot be used as feed. Its straw can be used as a construction material for roofing thatch. The whole grain can be milled to leave just the endosperm for white flour. The by-products of this are bran and germ. The whole grain is a concentrated source of vitamins, minerals, and protein, while the refined grain is mostly starch, (Smith, 1995). In 2012/2013, Saudi Arabia wheat production is declined by 9 percent to one million metric tons (MMT) compared to 1.1 MMT of 2011/2012 level. Saudi Arabia's total 2012/2013 wheat import is reached to 2.5 million MMT due to the increased feed quality wheat imports for animal consumption and Reduction of local wheat cultivation due to a gap in the underground water inventory. Saudi Arabia is planning to import more than 3.0 MMT of wheat annually at 2014 from different countries of world, Mousa H. and T. Al-Saffy, (2012). Wheat grains may be infected by fungi in the field and/or contaminated at any point during handling. Generally, Aspergillus, Fusarium, and Penicillium are considered among the most important fungi in producing mycotoxins detrimental to animals, (El-Naggar and Thabit 2014).

Fungi infect a lot of economic plants and nutrients that benefit the human and animal nutrition. These fungal infections due to a lot of diseases which are vary in deterring and gravity resulting in significant losses. Further increase of the economic cost, whether detection or get rid of it so their impact on the properties and quality of the production (Bryden, 2007). Fungi widespread on various grains, food and feed has a large role in many diseases that infect humans and animals and as a result of what is produced from Mycotoxins as secondary metabolism and assisted by the fungi diversity as well as their ability to grow under different environmental conditions. (Streit, 2013). The seriousness of mycotoxins in their resistance to heat, their relationship with the acute and chronic toxicity which may cause serious diseases such as cancer to humans and their ability to remain for a long time. Mycotoxins have biologically active without any antigenic structure. These toxins are mostly toxic to mammals, while its impact on plant and microorganisms is thoughtful enough (Pestka, 1994; Milicevic, *et al.,* 2010). In general, mycotoxins contaminate human food and animal feed through fungal contamination excrete these toxins during the different stages of production, during transport, by infecting different agricultural crops during the stages of growth and post-harvest or in the storage period.

Trichothecene group of mycotoxins, which are produced by fungi of the *Fusarium* genus Trichothecenes include many types i.e. T-2, HT-2, deoxynevalenol (DON) and nivalenol (NIV) toxin. These fungi are abundant in various cereal crops and grain based food products. The *Fusarium* species invade and grow on crops, and may produce these toxins under moist and cool conditions. T-2 , HT-2 and nivalenol (NIV) toxin are trichothecene mycotoxins produced mainly by *Fusarium* species which can contaminate a wide range of cereals such as wheat, oats, corn, barley, and derived products. Toxic impact of these mycotoxins is well documented in animals. These trichothecenes is a potent inhibitor of protein, DNA, and RNA synthesis, both *in vitro* and *in vivo*, and has immunosuppressive and hematotoxic effects (Canady *et. al*., 2001; Visconti, 2001; van der Fels-Klerx and Stratakou, 2010). Dermal exposure studies have shown that cause extremely toxic effects on the skin and mucosal surface (Sudakin, 2003). The World Health Organization (WHO) and Scientific Committee for Food of the European Union and by the Joint Food and Agriculture Organization (JFAO) was concluded that the toxic effects of T-2 and HT-2 could not be differentiated and that the a provisional maximum tolerable daily intake(TDI) of 60 ng/kg of body weight per day for combined T-2 and HT-2 was established while, The provisional TDI of 0.7 µg/kg body weight for Nivalenol (Canady *et. al*., 2001; Pascale *et. al.*, 2011).

β-D- glucan biopolymers of yeast cell wall is directly involved in binding process with mycotoxins (Yiannkouris *et al.*, 2004b and Jouany, 2007). It is also known that toxin binding in *saccharomyces cerevisiae* is strain dependent. (Shetty and Jespersen, 2006). It appears that carbohydrate components are common sites for binding, with different toxins. The soluble unit of fungal membrane binding protein that modulates the biosynthesis of β-D-glucan of fungal cell walls and probably has a major role in the regulation of cell wall morphogenesis (Kang and Cabib, 1986). Management of mycotoxins includes many strategies such as prevention, monitoring, avoidance, decontamination, detoxification, and animal treatments (Whittaker, 1991; Jouany, 2007; El-Naggar and Thabit, 2014). The study aims to apply the one of bio-substances as β-glucan biopolymer that may have an impact in reducing and detoxifying Fusarium toxins without any moral impact on the quality of grain, nutritional value and natural components such as vitamins.

Materials and Methods:-

Seed source:-

The wheat grain samples were obtained randomly from of different five regions (Riyadh, Quassium, and Jeddah) of Saudi Arabia In 2014. The samples were hard wheat and their moisture around 13%, while the percentage of protein

about 12.65% as dray base. Five kilogram per region were mixed together to form one representative sample, which was completed work on them.

Fusarium isolated and identification:-
The wheat samples (400 kernels) individually, were surface-disinfected with 1% NaOCl solution for 10 min followed by rinsing twice with sterile water then dried over a filter paper in a sterile laminar flow cabinet. Grains were plated on potato dextrose agar (PDA), ten kernels per plate and incubated at 25±2°C for 7 days in darkness. The replication was carried out three times separately.

The developing fungal colonies were sub-cultured onto PDA and identified based on their macro and microscopic features (Simmons, 1986, 2000; Pitt and Hocking, 1997). Fusarium isolates were purified by single spore method and then cultured on SNA medium in 25±°C in 12 hours light and 12 hours darkness condition for 7 to 14 days. Fusarium isolates were identified by both morphology and characteristic of colonies based on Nelson *et al*., 1983 descriptions. The identification Fusarium isolates was confirmed by BIOLOG technique according to, Grizzle, 2006 and Sing, 2009 and Abdelkareem *et al*., 2014
The Relative Density (Rd) of fungi genera and species were counted by the following formulas (Marasas, 1988):
Rd (%) = (The number of isolated fungi genera or species/ the number of all fungi.) × 100

Bio-polymer material:-
One bio -binder was evaluated in this investigation. This binder consisted of β-D-glucan biopolymer 45.0% as inactivated yeast (*S. cerevisiae*), 43.0% highly adsorbent clay minerals (E562), 2.0% calcium propionate, 7.5% plant derivatives and 2.5 % antioxidant (Ethoxyquin and Citric acid). The recommended applicable dose is 2.5 g/kg or 2.5 g/L for liquid media according manufacturer's recommendations (Innovad, Belgium).

Effect of biopolymer on T-2, HT-2 and NIV excretion in vitro:-
The tested Fusarium isolates were inoculated in 250-mL Erlenmeyer flasks containing 100mL of SMKY media (200 g of sucrose, 0.5 g of magnesium sulfate, 3 g of potassium nitrate and 7 g of yeast extract). The tested binders no. 1 and no. 2 were individually added to flasks by 2.5 g/L, while the control flasks did not have any biopolymer. The inoculation was conducted with discs (diameter 1 cm of tested isolates). The discs were harvested from 7-day-old potato dextrose agar cultures of each isolate, individually. The inoculated flasks were incubated for 12 days at25°C according to Jurado *et al*., (2008). The trichothecenes toxins (T-2, HT-2 and Nivalenol) were determined in both treatments and control flasks after filtration with ELISA protocol which described by Sumalan *et al.*, 2013. The method used in this study was enzyme-linked immunosorbent assay (ELISA). Sample preparation and analyzes were conducted according to the instructions outlined in the R-Biopharm kits ELISA. The fungal cultures were blended for 3 minute on high speed and then filtrated. Thirty ml of filtration was mixed and homogenized with 70 ml of methanol HPLC grad (Sigma-Aldric) to form aqous solution methanol: water 70:30 (v/v) for trichothecenes toxins (T-2, Ht-2, NIV and Nivalenol) analysis. The extract was filtered through a Whatman (Maidstone, UK) filter paper (No. 1). The filtered extract was used directly for mycotoxins analysis. A Ridascreen kit for ELISA was provided by R-Biopharm (Darmstadt, Germany). Each kit contains a microtiter plate with 96 wells coated with capture antibodies. Toxin standard solutions (0, 1, 5, 15 and 45 ng/ml), peroxidase-conjugated, antibody, substrate/chromogen, stop solution (1 N sulphuric acid) and washing buffer (contains 0.05% Tween 20). All other chemicals used in the analysis were of analytical grade. ELISA was performed by using ChemWell 2910 (Awareness Technology, USA).

Biopolymer treatment and toxigenic *Fusarium* species inoculation in wheat grains:
Two hundred grams of grains of representative wheat sample was inoculated individually with the most toxigenic *Fusarium* species (two isolates). The treated samples were retreated with β-D-glucan biopolymer (2.5 gm/10^3gm) and incubated for 15 days at 25 ±2°C. The spore suspension of toxigenic Fusarium isolate was adjusted to be containing approximately 10^4 spore ml^{-1}. The positive control was conducted as treatment with the most toxigenic species without β-D-glucan biopolymer while, the negative one was without β-D-glucan biopolymer or fungal inoculation. The dried treated samples of wheat with biopolymers and toxigenic fungi were milled into a coarse powder by using a laboratory mill (D- 6072, Germany) and stored in polyethylene bags at room temperature.

Effect of biopolymer on T-2, HT-2 NIV and DON in wheat grains:
The trichothecenes toxins (T-2, HT-2 and Nivalenol) were determined with the same protocol (ELISA) which described before except the toxins extraction from wheat grain sample as following: Five grams of each sample was

weighed, then 100 mL of 70% methanol was added and the mixture shaken vigorously for three minutes. The extractors were filtrated individually through filter paper and microfilter (22 µm) and then 1 mL of the obtained filtrate was use to complete the analysis protocol.

Determination of some water-soluble vitamins:
The vitamins were extracted according to a previously described method (AOAC.1990). About 2 gram of wheat powder which prepared before and was placed in 25mL of H_2SO_4 (0.1 N) solution and incubated for 30min at 121°C. Then, the contents were cooled and adjusted to pH 4.5 with 2.5M sodium acetate. The mixture was then filtered through a Whatman No. 4 filter, and the Filtrate was diluted with 50 mL of ultra-pure water and filtered again through a micropore filter (0.45 µm). 20µl of the filtrate was injected into HPLC system (Agilent 1200). The quantification of vitamin B1 (thiamin), B12 (cobalamin) and C (Ascorbic acid) content was accomplished by comparison to vitamins standards.Chromatographic separation was achieved on stationary phase (Agilent ZORBAX Eclipse Plus C18, 150 mm × 4.6 mm, 5 µm) through Gradient linear mobile phase is consisted of A and B mixtures (0 min 100% A/0% B; 6min. 80% A/20% B; 9min. 50% A/50% B and 10min. 100% A/0% B). Mixture A was prepared by dissolve 1.03 g hexane sulfonic acid and 6.8 g potassium dihydrogene phosphate in 1000 mL water. The pH value should be adjusted to pH = 2.3 with phosphoric acid while, the mixture B was Acetonitrile. The flow rate adjusted on 1 mL/min, stop time 10 min and post time 5 min. Ultraviolet (UV) absorbance was recorded at 220 nm at room temperature. The injection volume was adjusted on 30 µL and column temperature at 40°C. The vitamin standers were prepared by dissolve 10 mg of each vitamin individually in water and dilute to 100 mL with the same solvent. Dilute 1 mL of the solution to 20 mL with the mobile phase according to, Detlef Wilhelm, 2010.

Fat-soluble vitamins determination:
A high-performance liquid chromatography method is developed for determination vitamin E and K. Vitamins are separated using dC18 column. The mobile phase is methanol–water (98:2 v/v). Detection is performed with a UV–vis detector at 230 and 265 nm. according to Xue *et al.,* 2008.

Statistical analysis:
A randomized complete block design was used in the present study. Analysis of variance was performed with the MSTAT-C (statistical package, Michigan State University). The least significant difference procedure was used at the 0.05 level of probability (Fisher, 1948).

Results:-

Fungal Isolation and Identification:
Isolation trials were conducted from three regions of Saudi Arabia; Riyadh, Jeddah and Qassium. The isolating procedures depended on representative random samples of wheat grains. The obtained data from Table 1 refer to the common isolation fungi followed 12 genus. The total isolates of Riyadh, Jeddah and Qassium samples were 32, 56 and 39 respectively. The occurrence and relative density of Fusarium isolates were higher than other fungi. The occurrence of Fusarium isolates in Riyadh samples reach to 13 isolates while, in Jeddah and Qassium were 17 and 12 isolates, respectively. The *Fusarium culmorum, F. graminearum and F. verticillioides* were the most predominate of Fusarium isolates in Riyadh samples with 2 isolates per species. *F. graminearum* was the most occurrence In Jeddah samples with 3 isolates followed by *F. avenaceum* and *F. verticillioides* with 2 isolates per species. While, in Qassium samples the most common Fusarium isolates were *F. proliferatum* and *F. pseudograminearum* with 2 isolates per one.

Trichothecenes (T-2, HT-2, NIV and DON) determination *in vitro*:
Trichothecenes toxins consist of many types *i.e.*, T-2, HT-2, NIV and DON. The different tested Fusarium species were examined to trichothecenes execration by using ELISA. The ability of isolates was varied, there are some species hadn't any ability to produces these toxins as *F. avenaceum, F. proliferatum, F. poae, F. oxysporum, F. semitectum.* While, some others species of Fusarium had ability to produce most types of trichothecenes as *F. culmorum, F. graminearum, F. pseudograminearum, F. verticillioides, F. sambucinum* and *F. sporodochia* (Table.2). There was a variation within a species; the isolates No. Pseudo-Q1, Vert-R1 and Vert-J1 didn't have the ability to secrete trichothecenes despite the presence of isolates of the same species have the ability to excrete. Also, the rates of excretion between isolated was varying; the highest production for T-2 and HT-2 were reached to 1423.1 and 77.8 ppb respectively for isolates No. Culm-R1, while the most toxigenic for NIV and DON was isolate No., Gram-R1 (3500 and 865.7, respectively). The total concentration of trichothecenes toxins was 5570.6 ppb for same isolate.

Table (1): Occurrence and% relative density (%Rd) of seed borne fungi isolated from wheat grains in different Saudi regions on PDA media at 25℃ for 7 days

Fungal isolates	Occurrence (Oc) and relative density (Rd%) of seedborne fungi isolated from random representative samples of wheat grains in different regions of Saudi Arabia					
	Riyadh		Jeddah		Qassium	
	Oc	Rd%	Oc	Rd%	Oc	Rd%
Fusarium isolate:						
Fusarium avenaceum	1	3.13	2	3.2	1	2.2
F. culmorum	2	6.25	1	1.6	0	0.0
F. graminearum	2	6.25	3	6.5	1	8.9
F. proliferatum	1	3.13	1	6.5	2	8.9
F. pseudograminearum	0	0.00	1	1.6	2	4.4
F. verticilliodes	2	6.25	2	6.5	1	4.4
F. poae	0	0.00	1	1.6	1	2.2
F. oxysporum	1	3.13	0	0.0	1	2.2
F. sambucinum	0	0.00	1	1.6	0	0.0
F. sporodochia	1	3.13	1	1.6	0	0.0
F. semitectum	1	3.13	1	1.6	1	2.2
Fusarium sp.	2	6.25	3	4.8	2	4.4
Another fungi:						
Alternaria alternata	2	6.25	2	3.2	1	2.2
Alternaria tenuissima	0	0.00	1	1.6	0	0.0
Alternaria sp	1	3.13	2	3.2	2	4.4
Aspergillus candidus	1	3.13	1	1.6	0	0.0
A. flavus	3	9.38	4	6.5	3	6.7
A. fumigatus	2	6.25	2	3.2	1	2.2
A. niger	2	6.25	4	6.5	4	8.9
Aspergillus sp.	1	3.13	3	4.8	3	6.7
Chaetomium globosum	1	3.13	3	4.8	0	0.0
Cladosporium cladosporioides	1	3.13	2	3.2	0	0.0
Curvularia sp	0	0.00	1	1.6	1	2.2
Epicoccum nigrum	0	0.00	2	3.2	1	2.2
Mucor sp	1	3.13	3	4.8	2	4.4
Penicillium sp.	3	9.38	5	8.1	4	8.9
Rhizopus sp.	1	3.13	2	3.2	2	4.4
Trichoderma spp	0	0.00	1	1.6	0	0.0
Trichotecium spp	0	0.00	1	1.6	3	6.7
Total	32		56		39	

The all types of trichothecenes toxins were reduced with all tested isolates due to treated with bio-polymers. The highest reduction for T-2 toxins was 50.6% and recorded with isolate Sporod-J1 while, for HT-2 and NIV toxins was 55.3 and 50.0%, respectively with isolate No. Culm-R2. The most reduction for DON toxin recorded with Samb-J1 isolate after treated with biopolymer and the reduction percentage was 44.2%. The total trichothecenes level was reduced due to treatment with β –D glucan biopolymer as well as with all tested toxigenic isolates of Fusarium and the percentage of this reduction ranged between 38.5 and 50.6% (Table 2 and Fig. 1).

The effect of β –D glucan biopolymer on trichothecenes formation in wheat grains:

The experiment was performed using the most toxigenic Fusarium isolates (Culm-J1 and Gram-R1). The treatment was with β –D glucan biopolymer with ratio 2.5g/kg. The treated samples were stored for different periods as 0, 10, 20 and 45 days. The trichothecenes toxins were determined by using ELISA protocol. The tested isolates were affected with biopolymer and their toxin excretion was reduced in all types. The second isolates were affected than first one (Table 3). The ability of isolate Culm-J1had inhibited with β –D glucan biopolymer. T-2 toxins reduced

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Table (2): The effect of β –D glucan biopolymer on different types of trichothecenes production (ppb) and percentage of toxin reduction (%) on SMKY liquid broth media after 10 days at 25±2°C

Fusarium isolates	Toxigenic isolates code	Trichothecenes determination (ppb) on SMKY liquid broth media after 10 days at 25±2°C														
		without β-D-glucan biopolymer (Control)					after treated with biopolymer (2.5g/L)					% of toxin reduction				
		T-2	HT-2	NIV	DON	Total	T-2	HT-2	NIV	DON	Total	T-2	HT-2	NIV	DON	Total
F. culmorum	*culm-R1*	1423.1	77.8	1400	425.2	3326.1	882.1	41.8	705.0	277.3	1906.2	38.0	46.3	49.6	34.8	42.8
	culm-R2	987.2	23.5	1800	515.3	3326	601.9	10.5	900	315.0	1827.4	39.0	55.3	50.0	38.9	45.1
	culm-J1	1089.6	72.6	2600	782.1	4544.3	665.4	39.8	1400	495.8	2601.0	38.9	45.2	46.2	36.6	42.8
F. graminearum	*gram-R1*	1123.3	81.6	3500	865.7	5570.6	686.3	44.0	1800	549.1	3079.4	38.9	46.1	48.6	36.6	44.7
	gram-R2	856.9	56.1	2000	731.9	3644.9	521.1	31.3	1100	462.1	2114.5	39.2	44.2	45.0	36.9	42.0
	gram-J1	1274.6	0.0	900	245.3	2419.9	780.1	0.0	500	149.1	1429.2	38.8	0.0	44.4	39.2	40.9
	gram-J2	1241.0	0.0	2400	771.4	4412.4	769.2	0.0	1300	492.2	2561.4	38.0	0.0	45.8	36.2	41.9
	gram-J3	829.6	48.6	1100	345.8	2324	504.2	24.0	600	213.1	1341.3	39.2	50.6	45.5	38.4	42.3
	gram-Q1	0.0	0.0	0.0	161.6	161.6	0.0	0.0	0.0	95.9	95.9	0.0	0.0	0.0	40.7	40.7
F. pseudograminearum	*Pseudo-J1*	112.6	55.4	0.0	0.0	168	59.8	28.9	0.0	0.0	88.7	46.9	47.8	0.0	0.0	47.2
	Pseudo-Q1	128.4	0.0	700	289.7	1118.1	71.6	0.0	400	172.8	644.4	44.2	0.0	42.9	40.4	42.4
	PseudoQ2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0	0.0
F. verticilliodes	*Vert-R1*	110.8	0.0	0.0	0.0	110.8	62.7	0.0	0.0	0.0	62.7	43.4	0.0	0.0	0.0	43.4
	Vert-R2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Vert-J1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Vert-J2	123.6	0.0	0.0	198.6	322.2	66.6	0.0	0.0	114.5	181.1	46.1	0.0	0.0	42.3	43.8
	Vert-Q1	0.0	0.0	0.0	325.4	325.4	0.0	0.0	0.0	200.1	200.1	0.0	0.0	0.0	38.5	38.5
F. sambucinum	*Samb-J1*	0.0	0.0	0.0	128.3	128.3	0.0	0.0	0.0	71.6	71.6	0.0	0.0	0.0	44.2	44.2
F. sporodochia	*Sporod-R1*	143.7	0.0	90.0	319.9	553.6	79.1	0.0	50	198.5	327.6	45.0	0.0	44.4	37.9	40.8
	Sporod-J1	159.2	0.0	0.0	0.0	159.2	78.7	0.0	0.0	0.0	78.7	50.6	0.0	0.0	0.0	50.6
LSD at 0.05%		3.256	4.235	3.789	2.968	3.352	2.571	1.598	5.214	3.873	6.548	3.689	2.875	1.987	1.145	2.015

and its concentration ranged between 35.2 to 36.9 ppb after 10 and 45 days respectively. Also, HT-2 and DON concentration produced by this isolates reduced due to treated with biopolymer after 45 days to 44.9 and 33.7 ppb,

respectively. Generally, the reduction of trichothecenes produced by isolates (Culm-J1) reached to 43.2% after 45 days from artificial inoculation. Also, the biopolymer reduced the toxins produced by second isolates Gram-R1. The detoxification of T-2, HT-2, NIV and DON were 34.1, 40.8, 41.5 and 33.1% respectively after 45 days. Generally the trichothecenes inhibited by using biopolymer with this toxigenic isolates to 38.7% after 45 days from artificial inoculation.

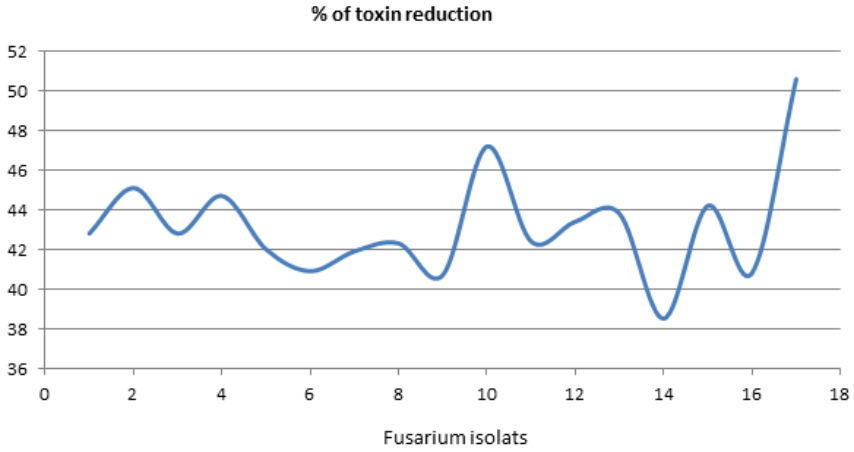

Fig (1): The reduction range (%) of trichothecenes toxins produced by toxigenic Fusarium isolates after treated with β –D glucan biopolymer (2.5 mg/L) and incubated for 10 days at 25 ± 2° C

The effect of β –D glucan biopolymer on some vitamins content:

The effect of biopolymers on some vitamins adsorption studied on some water soluble and some fat soluble vitamins (Table 4). The first group includes B1, B3 and B5 and the concentration was determined after treated with biopolymer 2.5 mg/kg and inoculated artificially with most toxigenic isolates Culm-J1and Gram-R1. These vitamins reduced with first isolates Culm-J1 to 0.33, 5.12 and 0.92 mg comparing to control 0.39, 5.47 and 0.96 mg respectively. The reduction percentage of vitamins content comparing to control 5.13, 3.66 and 7.29% and it appeared non-significant after treated with biopolymer. The inoculation with second isolate had the same trend although the reduction ratios were different. The vitamins B1, B3, B5 reduced by 3.85, 2.19 and 5.21% comparing to control.

The fat- soluble vitamins; E and K were determined also after treated with bio-adsorbent. Vitamin E reduced from 1.04 mg in control to 0.95 mg with biopolymer and isolate Culm-J1 while with second isolate the reduction arrived to 0.94 mg. the percentage of reduction was 5.77 and 4.81, respectively. Vitamin K had same trend; it reduced from 2.11µg with biopolymer and Fusarium isolate (Culm-J1), while was 1.96 µg with Gram-R1isolate and after treated with biopolymer. The reduction % with biopolymer was 3.79 and 6.16% respectively.

Discussion:-

Wheat is strategic crops grown around the world countries. It have many varieties differed in, color, harness and productivity. The wheat grain is good source for human food as well animal feed. The nutrition value of wheat flour is rich with protein (10-18%), carbohydrate (60-75%), lipid (1.54%), dietary fiber (12.2%), different kind mineral and vitamins (Šramková *et al*., 2009). The forecast of wheat productivity in 2015 may reach to723 million tones (FAO, 2015). Wheat plant and grains were attacked with several species of fungi through the growth and field stages as damping off, smut, rust and leaf spot as well as through storage period (Al-Defiery and Merjan, 2015). The harm of some fungi is extending to secrete several toxins which effect on human and animal health and cause chronic and/or acute toxicity.

The results of isolation trials refer that, many of fungal genera and species associated with wheat grains which collected from 3 different regions as Riyadh, Jeddah and Qassium. The occurrence of isolated fungi was 32, 56 and 39, respectively. The differentiation of occurrence and relative density may be return to location of isolation and

Table (3): The effect of biopolymer on trichothecenes concentration in wheat grains after inoculated with most toxigenic Fusarium isolates and incubated for many periods at 25±2°C

Incubation periods	Toxin type	The effect of biopolymer on trichothecenes concentration in wheat grains after inoculated with most toxigenic Fusarium isolates						
		- Control	+ Control	Isolate No. *Culm-J1*	% of reduction	+ Control	Isolate No. *Gram-R1*	% of reduction
0 days	T-2	50.4	50.3	50.3	0.0	50.4	50.4	0.0
	HT-2	4.3	4.4	4.4	0.0	4.4	4.4	0.0
	NIV	5.2	5.0	5.0	0.0	5.1	5.1	0.0
	DON	9.9	9.8	9.8	0.0	9.9	9.9	0.0
	Total	69.8	69.5	69.5	0.0	69.7	69.7	0.0
10 days	T-2	50.7	1158.3	750.6	35.2	1098.6	730.6	33.5
	HT-2	4.5	81.6	46.6	42.9	85.4	51.2	40.0
	NIV	5.1	2498.6	1341.7	46.3	3350.2	1996.7	40.4
	DON	9.8	856.1	580.4	32.2	851.9	588.7	30.9
	Total	70.1	4594.6	2719.4	40.8	5386.1	3436.9	36.2
20 days	T-2	50.3	979.6	620.1	36.7	998.1	662.7	33.6
	HT-2	4	75.9	42.8	43.6	79.8	47.7	40.2
	NIV	5.3	2381.2	1228.7	48.4	3243.5	1920.2	40.8
	DON	9.4	734.5	495.1	32.6	839.6	571.8	31.9
	Total	69	4171.2	2386.6	42.8	5161	3202.4	38.0
45 days	T-2	50.2	879.4	554.9	36.9	981.6	646.9	34.1
	HT-2	4.2	73.6	40.6	44.9	71.6	42.4	40.8
	NIV	5.3	2314.9	1199.1	48.2	3183.4	1862.3	41.5
	DON	10	665.4	441.2	33.7	805.4	538.8	33.1
	Total	69.7	3933.3	2235.7	43.2	5042	3090.4	38.7

climate factors as temperature, relative humidity and these findings was in a harmony with (Grigoryan and Hakobyan, 2015). The effect of β –D glucan biopolymer was measured on all isolated Fusarium isolates *in vitro*. Some Fusarium isolates hadn't any ability to produce any type of trichothecenes toxins (T-2, HT-2, NIV and DON) as *F. avenaceum, F. proliferatum, F. poae, F. oxysporum* and *F. semitectum.* Also, there was a variation between the tested isolates in toxigenic potential and the type of produced toxins. This may explain the absence of genotype or gene that secrete of these toxins and the results was in parallel with (Hymery *et al.,* 2014) and may be un-parallel with (Nelson *et al.,* 1993) who state that may return to physiological systems. The detoxify effect of biopolymer against total trichothecenes toxins ranged between 38.5 to 50.6%.

The effect of β –D glucan biopolymer on trichothecenes in wheat grains was measured by using the most toxigenic Fusarium isolates (Culm-J1 and Gram-R1). All types of these toxins were decreased due to adsorption by molecule of this binder but the efficiency of this material had limit. The maximum effect ranged between 43.2 and 38.7 for tested isolates. The variation of toxins adsorption may return to potential of toxin, efficiency of binder material and the natural and chemical specification of toxin type and these findings was in same trend with (Kolossova *et al.,* 2011) and (El-Naggar and Thabit, 2014).

All adsorbent material or binder hadn't selective action because all time depending on charge and physical properties. B-D glucan biopolymer often extracted from fungi itself but had same specification of adsorbent material. The trial conducted to know how much the effect of this material award some nutrient substances as vitamins; water-soluble and fat-soluble. The obtained results referred that there the tested binder adsorbed some amount of tested vitamins but the percentage is very little if compared with tested trichothecenes toxins. That's meaning the absorbance of trichothecenes is more than tested vitamins and there are significant advantage.

Table (4): The effect of biopolymer on some vitamin content in wheat grains after inoculated with most toxigenic Fusarium isolates and incubated for 45 days at 25±2°C

Vitamin type		Biopolymer effect on some vitamin content in wheat grains after inoculated with most toxigenic Fusarium isolates and incubated for 45 days at 25±2°C						
		Control [a]	Isolate Culm-J1 without biopolymer	Isolate Culm-J1 with biopolymer	% reduction	Isolate Gram-R1 without biopolymer	Isolate Gram-R1 with biopolymer	% reduction
Water - soluble	B1	0.39	0.33	0.37	5.13	0.35	0.38	3.85
	B3	5.47	5.12	5.27	3.66	5.39	5.35	2.19
	B5	0.96	0.92	0.89	7.29	0.89	0.91	5.21
Fat - soluble	E	1.04	0.95	0.98	5.77	0.94	0.99	4.81
	K	2.11	1.93	2.03	3.79	1.96	1.98	6.16
LSD at 0.05%		0.36	0.055	0.14		0.48	0.32	

Control [a]: Random representative sample without any treatment or any artificial inoculation

The reduction percentage of vitamins B1, B3 and B5 were 5.13, 3.66 and 7.29% respectively after treated with biopolymer and inoculated with isolate Culm-J1 while, was 3.85,2.19and 5.21% with Gram-R1. The variation in this reduction may be return back to the efficiency of bio-adsorbent material, the quantity of secondary metabolite produced by testes Fusarium isolates (Whitlow *et al.*, 1998) and (Whitlow and Hagler, 2005). This problem can be solved by compensation and add some vitamins artificially such as enrichment materials of vitamins in case using biopolymers material.

Conclusion:

Biopolymers utilization to adsorb toxins produced by fungi is one of promising trends that aim to get rid of mycotoxins. This technique reduces huge amount of agricultural commodities which were designed in waste as well as protect the human, animal and the environment from dangerous risk. The using bio-binder is considering one of available technologies that can be applied but may be need to further studies to codify the doses and how to use with human products. Also, it is considered safe alternative materials to pesticide that may harm the environment, human and animal health. *In vitro* investigation and/or on grains there was significant findings to eliminate the trichothecene toxins by this material found and its adsorbent effect of other substances as vitamins are not significant when compared to the amount of toxins that have been disposed.

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