The enzymatic effect of *Fusarium spp.* on wheat plant and its role in pathogenicity as a one of virulence factor.

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Abstract:- Fusarium spp. One of Most important fungal disease that affecting wheat plant, during the period from February 2022 to September 2022, study done by taken 128 isolates, belonging to Fusarium spp. were obtained from wheat plants showing symptoms of crown and root disease taken from different area of waist city including (Al-Numaniyah, Kut, Dabuni, Sheikh Saad).

Aim of our study: (i) studding of Fusarium spp. isolates (obtained from wheat plants for producing various virulence factors such as cellulase and pectinase; and (ii) examine pathogenicity, aggressiveness of Fusarium isolates on wheat, with determine relationships between ability of the isolates to produce virulence factors and their pathogenicity,the result of our study revealed that All Fusarium isolates produced enzymes. Such as cellulase and pectinase which had significant pathogenicity on wheat detected by different diameter zone a appear around colony

Key words: Fusarium spp., virulence, wheat

1-Introduction:

wheat is one of the most important plant in the world as it used as a nutritional food source and as a supply for many products, which is considered one of most commercial plant in the world so detection of its disease &its pathogenic factor is important to keep this kind of plant as big source of nutrition around world (1) Iraq produces about 3 million tons of wheat annually according to FAO GIEWS, 2014, Iraq plants about 1,625 million hectares of wheat according to the Ministry of Agriculture However, Iraq imports 1.7 million tons of wheat each year to cover people need.(2)

Wheat is a grain, which belongs to the grass family and was cultivated from 9,000 years ago in the Euphrates Valley in Iraq. Since then, wheat has been used as a nutritional food source and as a supply for many products. (3)

Wheat Feeding the World Grain-based foods, like those produced with wheat, provide complex carbohydrates, which are the best fuel for our bodies, are low in fat, high in fiber, and provide vitamins, especiallyB vitamins, Thiamin, Riboflavin, Niacin, and Folic Acid, as well as iron. Wheat provides us with a nutritious and delicious supply of breads, pasta, cereals, and many other food products that has wheat as an ingredient (4), Fusarium is one ofwell-known fungi as problematic plant pathogens, Fusarium is a large genus of filamentous fungi, abundant members of the soil microbial community (5)

Fusarium Head Blight is a fungal disease that affects many grains Also referred to as Scab, it can affect wheat, the disease can also affect the germination rate and seedling vigor if the grain is planted again. (6) Fusarium spp. have been isolated from crown and root of wheat & even from soil of wheat growing in different regions in waist city.

Symptoms appear shortly after flowering; disease spikelet's appear bleached on all or part of the head. during favorable weather the fungus produces orange to pink spores at the base of the kernel, these spores will infect adjacent kernels. The infect kernels are shrunken, wrinkled and light in weight, they have a rough scabby appearance and can range in color from light-brown to pink to greyish-white. (7). aggressiveness and virulence of Fusarium spp. is production of extracellular enzymes, which degrade host plant cell walls (8),these cell wall degrading enzymes (CWDEs), such as cellulases and pectinases, are crucial in the processes of colonization and disease establishment(9) involved in softening the cell walls, increasing accessibility of cell wall components for degradation by other enzymes(10),which enables success of further infection steps and spread of fungal mycelia into the inner host plant tissues (11) .

2.Materials and methods

2-1 collection of samples

During the period from February 2022 to September 2022, study done by taken 128 isolates,belonging to Fusarium spp. were obtained from wheat plants showing symptoms of crown and root disease as in figure(1)taken from different area of waist city including (Al-Numaniyah, Kut, Dabuni, Sheikh Saad, during wheat seasons, isolates inoculated in petri dish contain PDA

Figure (1) infected wheat plant by fusarium spp .

2-2 Preparation of cultured media :

65g from potato Dextrose Agar (PDA) mixed with1000 ml distal water then both sterilized by autoclave at 121 ˚C, and 15 pawned pressures for each inch for 15 min , isolate collected from infected plant cultured on potato dextrose agar (PDA) in Petri dishes were incubated at 25°C After 7days, the lesion length at the point of inoculation on each leaf was determined. The experiment was replicated three times for each isolate,The sporulation pattern group of each culture was examined by microscope Morphological characteristics of the fungus by microscope such as mycelium shape and color; conidium shape,color and size; and conidiophores shape, were used to identify the exact causative agent of Fusarium spp. examination done at × 40 magnification [12]

2-3 Assessment of aggressiveness

Aggressiveness, as another quantitative component of pathogenicity, was investigated for each fungal isolate on detached leaves of wheat plants in laboratory conditions using the methods described by Malihipour et al. (2012) [13] and Pariaud et al. (2009) [14] Analysis of aggressiveness was determined based on hours post inoculation (hpi) for disease symptom appearance After 7 days the lesion length at the point of inoculation on each leaf was determined.

2-4 Detection of extra-cellar enzyme from Fusarium spp.

128 species of Fusarium were evaluated for their ability of producing extracellular enzymes using medium containing substrates such as starch, cellulose**,** ability of producing enzymes is evaluated with various Fusarium species using the plate screening methods with chromogenic substrates ,this method is relatively straightforward and simply applicable tools for specific detection of polysaccharide degrading microorganisms ,The degree of extracellular enzyme activity was evaluated based on the size of formed clear zone [15]

2-4-1 determining cellulose enzyme activity : depending on reese &mandels method (1963) [16] by adding urea &carboxy-methyl cellulose gradually with shaking by magnetic stirrer mixer until dissolved completely then adding the other content of media ,by using steam autoclave at 121 degree for 20 min,,then by adding HCL –iodine solusion which is prepared by adding 100ml (HCL 0.1 concentration) ,500 ml from Iodine 1%&KI 2% [17] (Yeoh ea al,1985) the appearance of faint yellow zone around colony indicating cellulose depredating activity by the fungus the activity of fungus determined by measuring the formed zone by millimeter as shown in figure 3

2-4-2 Amylase detection activity:

depending way of Hankin &Anagnostakis (1975) [18]by adding potassium iodide reagent to media KI (iodide 3 g/L to 5g/L K l) waiting for 5 min then pitting in Petri dish plate waiting for 5min till appearance of yellow zone around the colony ,blue color of rest of media indicating of enzyme activity ,diameter of zone representing strength of fungal activity as shown in figure (4)

2-4-3 Phenol oxidase activity depending on [19]Gessner (1980) Dissolving of tannic acid in 100 ml of distal water then adding the other content of media which is dissolved in 900 ml distal water ,appearance of brown color around the colony indicating of enzyme production ,,sepration of color representing the degree of the enzyme activity .

2-4-4 Pectinase activity detection: using media contain Mineral salt solution 500 ml,1gm Yeast extract, Pectin 5 gm, Agar 20 gm, distal water 1000 ml[20] ,detection of enzymatic effect by adding reagent contain 1%Hexadecyl trimethyl ammonium bromide to colony ,after 5min clear zone will appeared around colony as shown in figure (5)

3-The Result: depending of morphology &PCR the 128 isolate of Fusarium spp divided to 43 isolated of Fusarium culmorum ,28 isolated for F. graminruim ,25 isolate for F.pesudograminruim , 17 isolate of F.solani ,12 for F.equiseta only 3 isolates for F. oxyspruin , percentage of 34% ,22%,20%,13%, 9% and 2 % respectively as shown in table (1)

Table (1): Show numbers &parentage of studied fungi

Fusarium spp.	**Number of isolates**	**Percentage %**
F.culmorum	**43**	**34%**

F.gramenrium	**28**	**22%**
F.pseudograminrum	**25**	**20%**
F.solani	**17**	**13%**
F.equsita	**12**	**9%**
F.oxysprum	**3**	**2%**
Total	**128**	**100%**

Leaf assays of aggressiveness test revealed that the greatest lesion length was produced by *F. solani* isolate, The least disease length was observed for the *F. equiseti* and *F. oxysporum* isolates,other isolates tested fell between them with various levels of virulence on wheat leaf segments (Table 2, Figure 2).

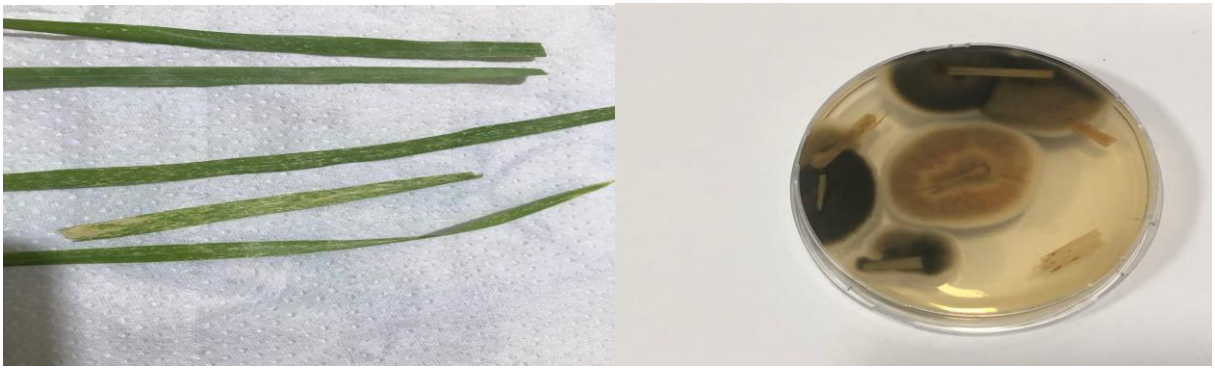

Figure 2 show disease symptoms caused by *Fusarium isolates* on wheat leaves and corelated with colony appearance around involved leaves in plate

Table (2) results of the aggressiveness test on detached leaves showed more rapid development of disease symptoms by *F. solani* isolate

Fusarium spp.	Leaf segment length (mm)	Growth around leaf segments hpi (h)
F. solani	25	12
F.pseudograminrum	6	120
F.equiseti	5	120
F.oxysporum	5	120
F.culmorum	6	120
F.graminrum	6	120

Enzymatic activity measurement divided to three group depending on diameter of clear zone formed around colony as shown in table 3

Table 3 represent degree of activity depending on zone size

Activity	Diameter of clear zone by cm	Sample
Strong	2.5~4 cm	S
Medium	1~2.4 cm	M
Lowor no activity	less than 1 cm	N

Good cellulase activities detected by *Fusarium spp.* with yellow zone appeared around the colony as shown in figure 3

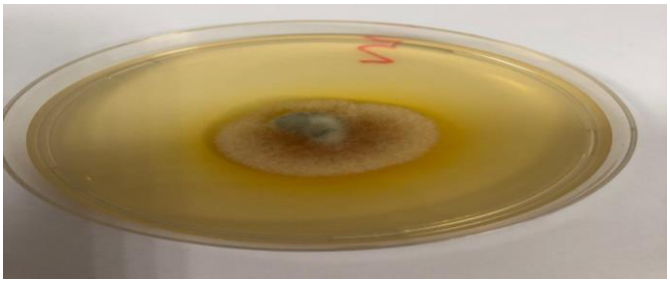

Figure (3)cellulase activities shown by *Fusarium spp*. With yellow zone around colony

While amylase activity detected by appearance of yellow zone around colony with blue color of rest of media as shown in figure 4

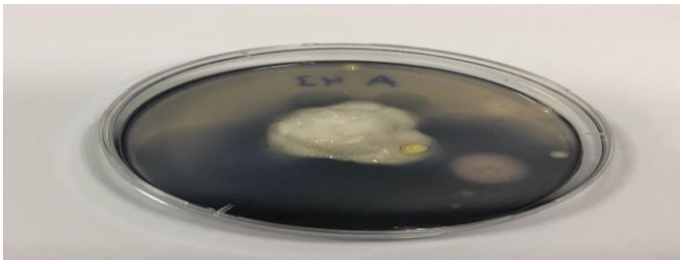

Figure (4) show amylase activities with yellow zone around colony,blue color of rest of media

pectinase activities shown on media with clear zone around colony as in figure 5

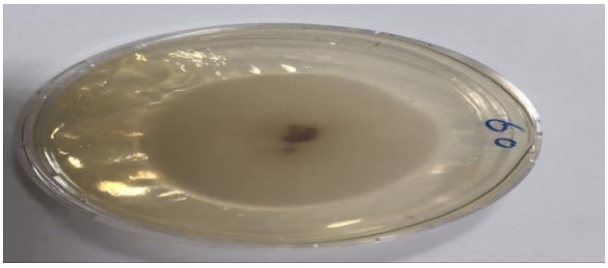

Figure (5) pectinase activities with clear zone around colony

production and release of extracellular enzymes which degrade plant cell walls which is crucial in the process of pathogen colonization ,establishment of disease, isolates to wheat leaf tissues evaluated ,the production of this enzyme looks popular in Fusarium. An example of this enzyme detection is among these 128 species, shows strong activity nearly in all isolate was much apparent in *F.solani ,F. graminearum*, were amylase , phenoloxydase showed strong activity with strongest activity detected in *F. oxysporum*. ,,less activity in *F.peudograminrum* ,,Amylase is the enzyme which degrades amylose in plant cell wall, Strong activity of pectinase also popular ,these enzyme activity summarized in Table 4. The strongest pectinase activity was also apparent with highest vale in *F.solani* flowed by *F. oxysporum and F.graminrum* ,High cellulase activity seen nearly in all *Fusarum spp.* with highest activity seen by *F. solani* least activity seen by *F. oxysporum* with strong pectinase activity ,quantitative results of CWDE assays agreed with the qualitative results,,based on the size of clear culture medium zones for enzymatic activity.

Table 4 summarized strength of enzymatic activity of studied *Fusarium spp.*

Species name	cellulase	Amylase	Phenoloxydase	Pectinase
F. culmorum	S	S	S	M
F. equiseti	S	S	S	M
F. graminearum	S	S	S	S
F.pseudograminearum	S	N	M	N
F. oxysporum	M	S	S	S
F. solani	S	S	S	S

4-Discussion

Fungal pathogens are able to produce a variety of enzymes degrading the plant cell wall, and these enzymes help the pathogens in penetration and colonization of their host plants,our research studding wheat plant involvement by Fusarium head blight disease which is considered

one of the most common wheat fungal disease in Iraq,this similarly with Oadi et al.(2019)who study *F.culmorum* as one causal agent of wheat crown rot disease involvement in Iraq .(21)

In our study nearly all *Fusarium* isolate show effective enzymatic activity which one of most important virulence factor in pathogenicity of *Fusarium spp* in wheat plant disease were *F. solani* isolate showed the greatest enzymatic activities and leaf length involvement correlation analysis revealed high levels of direct association between the capability of *Fusarium spp*. in producing cell wall enzymatic activity and their virulence on the wheat leaves matching with Khaledi et al. (2016) demonstrated the association of aggressiveness and virulence of *Fusarium spp.* isolates causing head blight of wheat with the levels of CWDE activity.(22) and with Albayrak *et al.*(2016) in studied the relationship between *Fusarium* spp. isolates and its virulence effect (23)

Our study also compatible withYoon et al.(2007)successfully detected high cellulase in *F.solani* using same plate assay methods based on chromogenic media (24) .

5-Conclusion

The findings of the present study shows that *Fusarium spp*.can release extracellular enzymes that degrade wheat plant cell walls which is crucial in the processes of pathogen colonization and establishment of disease as one of virulence factors with pathogenicity of *Fusarium spp* on wheat leaf segments this need suitable ways to decreased and control this virulence factor

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