The enzymatic effect of *Alternaria alternata* on wheat plant and its role in pathogenicity as a virulence factor

Asia Naji Obaid AL-Hachami [1]

Biology Department, College of Education for Pure Science,University of Thi Qar , Iraq.

asia@uowasit.edu.iq

Yass, K. Abbass[2]

Biology Department,College of Education for Pure Science,University of Thi Qar , Iraq

Meethaq Sattar Abood [3]

Biology Department, College of Education for Pure Science,University of Thi Qar , Iraq

Abstract:

wheat is one of the most important plant in the world as it used as a nutritional food source can avvacted by many fungal disease like leaf blight disease &black mold disease which caused by *A.alternata* ,during the period from February 2022 to September 2022, study done by taken 180 samples from infected wheat plant lesions collected in regions where A.alternaria leaf blight and black mold has been reported were studied from different area in wasit city including (Al-Numaniyah, Kut, Dabuni, Sheikh Saad,

Aim of our study: Studding the characteristic morphological &microscopical appearance of A. alternate isolates as one of important fungal disease in wheat plant &explaining their enzymatic effect as virulence factor in disease occurrence, our research shows that all isolates give positive enzymatic activity in produced cellulose , Lipase , Amylase and pectinase,phenole oxidase enzymes with different diameter zone a appear around colony representing its activity

Key words: *Alternaria spp* , wheat plant disease

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& 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, especially B 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), one of most fungal disease that affecting wheat plant its Alternaria related leaf blight disease and black mold disease,which represent one of possible health-endangering problem as it widespread group of fungi contaminating plants including wheat, during growth as well as during storage ,Symptoms appear shortly after flowering of wheat (5)

The pathogenicity of *Alternaria* isolated from wheat plant disease collected in different regions from Al -kut city where isolates obtained from different plant parts (leaf, stem, and spike)involved by leaf blight and black mold disease of wheat (6,7)

The ability of a pathogen to produce enzymes determines the degree of cell wall degradation during pathogenesis (8,9)

2-Materials and methods

2-1 Collection of sample and cultured in media ::during the period from February 2022 to September 2022, study done by taken 180 samples from infected wheat plant lesions collected in regions where *A.alternaria* leaf blight and black mold has been reported were studied from different area in wasit city including (Al-Numaniyah, Kut, Dabuni, Sheikh Saad, during wheat seasons, 3-5 mm pieces of tissue from each stem, leaf and spike were subjected to fungal isolation. The pieces were surface sterilized in bleach (1% available chlorine) for 5 minutes, and washed twice in sterile water for 5 minutes. Then, the pieces were dried by placing them on sterile paper towel. Subsequently, tissue pieces were transferred onto potato dextrose agar (PDA) plates which contain 100 µg/m chloramphenicol were incubated at 28 temperature (10) according to Scott and Chakraborty(2010)after 7days colones appear,part of colony taken again Cultured in PDA media at incubator with 25 degree for 7 days,the sporulation pattern group of each culture was examined by microscope depending on appearance in involved plant and black to brown colored colony on PDA medium reaching 80 mm after five days at28 ˚C and 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 Alternaria alternate ,, examination done at ×40 magnification resulted in sporulation groupings as obtained by **Simmons &Roberts (1993**) and **Andersen *et al*.(2002**).(11)

2-2 determining of enzymatic activity

2-2-1 cellulose enzyme activity : depending on reese &mandels method (1963) (12) by adding urea &carboxy-methyel cellulose gradually with shaking by magnetic stirrer mixer until disloved 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% (13) (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

2-2-2 AMYLASE detection activity : depending way of Hankin &Anagnostakis (1975) (14) by adding potasum 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

2-2-3 Phenol oxidase activity depending on (15)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

2-2-4 Lipase activity detection: according to Sierra (1957) (16)After adding of sterilized tween 80 o the other media content ,, appearance of visible white crystal around the colony or precipitating under the colony representing the enzyme activity

2-2-5 protease activity enzyme :using media containing gelatin which described by sociality of American bacteriologist (1951) (17)for detect production of protease enzyme which consist of :nutrient agar 950 ml & gelatin 0.4% ,Sterile Gelatin prepared adding to nuteriant agar as 5ml/100 ml for media by Frazier's reagent depending on Bisson &Cabelli (1979) (18)

5g HgC_2 &20 g con. Hcl &100 ml distal water, appearance of faint zone around colony after adding of reagent indicating production of protease enzyme by the fungus ,diameter measuring the activity level

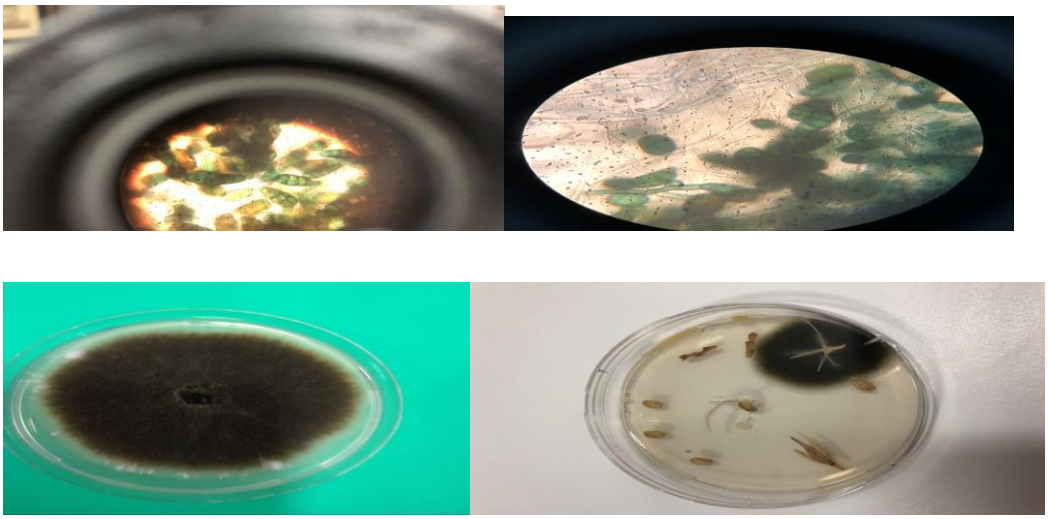
Detection of enzymatic activity done by using of aqueous solution containing reagent consistent of 1% trimethyl ammonium bromide added to colony after 5minetes there is clear zone

In each assay, Petri plates each inoculated with a PDA plug without fungus were used as negative controls

2-2-6 Pectinase activity detection :using media contain Mineral salt solution 500 ml with 1gm Yeast extract, Pectin 5 gm, Agar 20gm ,distal water 1000 ml(19),detection of enzymatic effect by adding reagent contain 1%Hexadecyl trimethyl ammonium bromide to colony ,after 5min clear zone will appeared around

3-The Result:

Morphological identification. Of total of 180 isolates of A.alternata have been isolated from infected plant lesions where alternaria leaf blight & black mold has been reported confirmed on the basis of on appearance in plant disease,, black to brown colored colony on PDA &morphological appearance by microscope as mentioned in relevant scientifc literatures describing key morphological characteristics available for Alternaria species were identified by employing compound microscope at 40X magnification by **Simmons & Roberts (1993**) and **Andersen *et al*. (2002**). Isolates were identified as belonging to Alternaria genera . all *A.alternata* isolates showed a long primary conidiophore ,alternation of aerial and submerged mycelium growth rings on PDA was also very characteristic for A.alternata as in figure (1)



Figure(1)show shape,color of *Alternaria alternate* colony by microscopical appearance and its growth on PDA agar ,around wheat seed

Table 1 .depending on al –sadoon test (1989) (20)for enzyme activity measurement as following

Degree of enzyme activity	Diameter (mm)	Details
Negative -	Zero	No enzyme activity
Weak positive +	1-3	Weak
Positive +	3-5	Medium
Positive ++	5-8	Good
Positive +++	8-11	Active
Positive ++++	More than 11	Very active

In the quantitative assays, the amounts of (CWDE)cell wall degradation enzyme activity among isolates varied .,all isolate give positive enzymatic activity as shown in table 2 ,with high results of CWDE and enzymatic activity to produce cellulose enzyme as in figure 2 with 8mm diameter zone a appear around colony in 5-6 PH ,28 degree temperature after 6-8 days from incubation ,adding glucose &cellulose to media as source of carbon ,,&urea as source of nitrogen increase the activity (21)

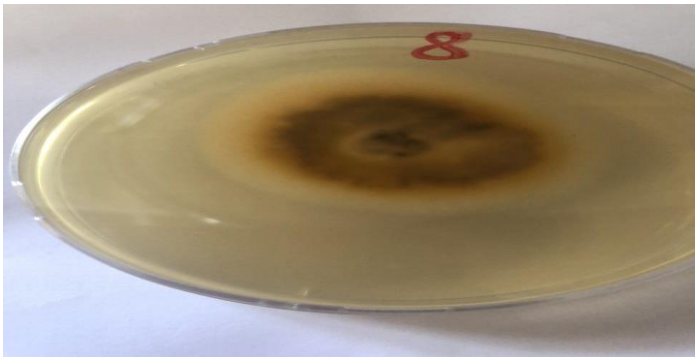


Figure 2 showing yellow zone around colony representing cellulase activity effect

Amylase give positive result with 9 mm diameter zone as shown in figure(3)

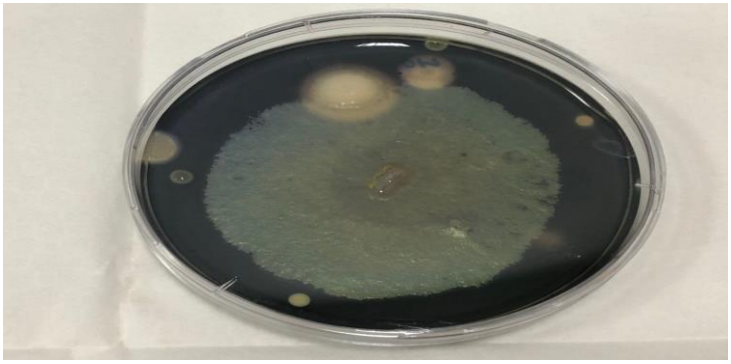


Figure 3 showing Amylase activity around colony with yellow zone around the colony ,blue color of rest of media indicating of enzyme activity

enzymatic activity in *A. alternata* for lipase enzyme occur only after 14 days (22) with 4.8mm diameter zone , as shown in figure (4)

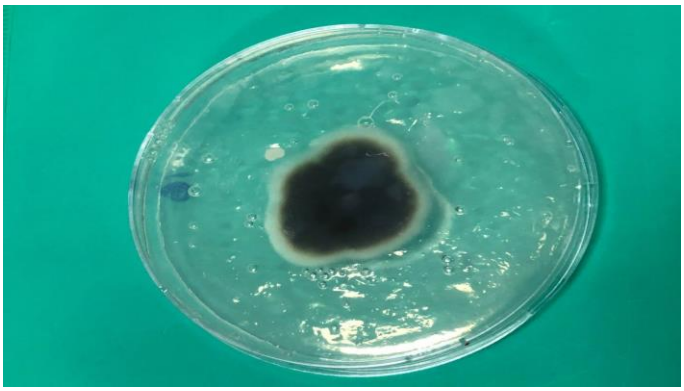


Figure 4 showing white crystal around the colony and precipitating within the media representing lipase enzyme activity

while other enzyme give positive result at 6-8 days highest for protease as 15 mm diameter zone as in figure 5 adding fructose to media enhance its enzymatic production (23)

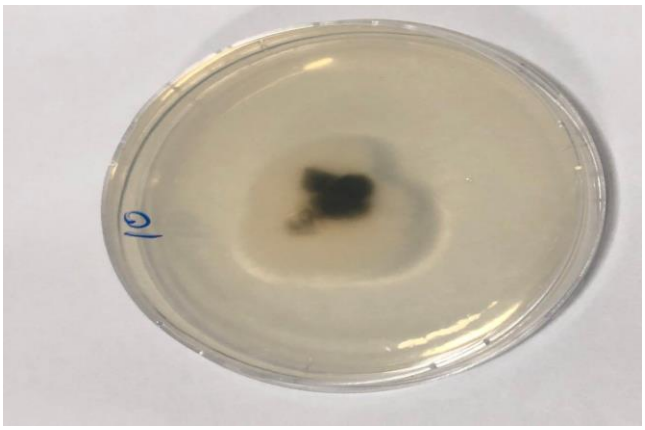


Figure 5 showing appearance of faint zone around colony representing protease enzyme activity

pectinase show high enzymatic result with 14mm zone diameter ,pectinase play very important roll cell wall degradation and disease occurrence

also phenol oxidase show positive result with 4.2mm diameter zone as shown in figure 6

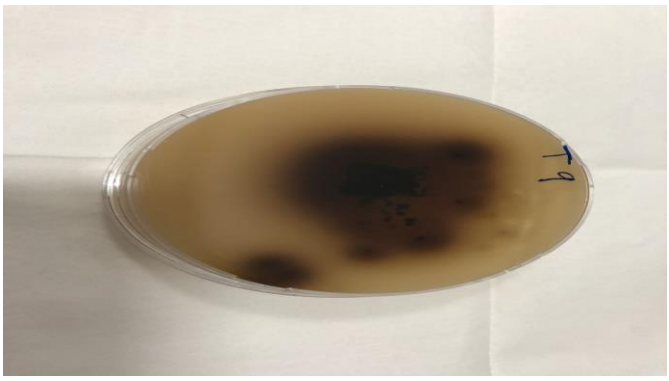


Figure 6 representing Phenole oxidase enzyme activity around the colony by appearance of brown color zone around the colony indicating of enzyme production

Table 2 enzymatic activity in *Alternaria alternata* for each enzyme with its diameter zone

Enzyme	Enzyme activity in A.alternata	Diameter of zone(mm)
Protease	+++	15
Cellulose	+++	8
Lipase	_ at 7 days +(after 14 days	4.8
Pectinase	+++	14
Amylase	+++	9
Phenole oxidase	+	4.2

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 combatable with study by (Yang et al. 2005). Were show that the pathogen A. alternata produced both cellulolytic enzymes and pectinolytic enzyme (24)

Cellulose is a major structural constituent of the cell wall of host plants, All these cell wall splitting enzymes are mostly adaptive, secreted by the pathogen in the presence of appropriate substrates. Pectinolytic enzymes were produced only in the presence of pectin in the medium. Cellulolytic enzymes were produced only in cellulose containing media

The results obtained in the present study, indicated that activity of these enzymes increased with the increase in the age of the culture as lipase enzyme show positive result after 14 days ,this matching with (Marimuthu et al. 1974; Muthulakshmi 1990)That said activity of enzymes increased with the increase in the age of the culture (25)

The virulent isolates of *A. alternata* produced cellulolytic enzymes Similarly, Anand et al.(2008) reported high cellulase activity in culture filtrate of A. *alternata* Anand et al. (2008) (26)also reported that *A. alternata* produced extra cellular enzymes which degraded CMC and cellulose.

Jha and Gupta (1988) reported, that the combination of glucose and pectin induced secretion of these enzyme in *A. triticina* infecting wheat, the virulent isolates of produced more macerating enzymes the maceration increased with the increase in age of the culture in vitro this corroborated with the observation of Muthulakshmi (1990) and Anand et al. (2008) in *A. alternata*, causing fruit disease, The production and activity of enzymes detected in vitro suggest their active role in disease development (27) .

5-Conclusion

The findings of the present study shows virulence of *Alternara altrnata* isolates on wheat plant showed that all isolates were pathogenic to wheat and need suitable ways to control this virulence factor

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