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Investigation for Pectinase Pathogenicity & Activity of Mycological disease (Leaf Spot) in *Solanum lycopersicum*

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Abstract: Pathogenic ability of many fungal species qualified for nine of genera were recognized. (8) fungi were optimistic and positively talented to contaminate leaves of tomato seeming contamination indicators for the disease (leaf spot) . *Alternaria citri* *Alternaria alternata* , *Alternaria tenuissima* , *Botrytis cinerea* , *Botrytis squamosa* were extremely infectious and formed this disease in most of infested leaves . Consequences protest this , in unhealthy plant life by *Alternaria citri* the chlorophyll was tremendously communicable presented maximum ominously condensed in (1.16 - 0.18) (mg/g fresh leaves) for chlorophyll(a, b) individually associated in (2 - 1.50) (mg/g fresh leaves) in controller plants. *Alternaria alternate* also march exciting condensed for chlorophyll (a, b) were (1. 89 - 0.98) (mg/g fresh leaves) correspondingly. (6) species were divided to capabilities to products pectinase by (cup-plate) technique. Totally mycological verified remained (pectinase) inventors then through adaptable shades. (3)species displayed in height pectinase inactivity include(*Alternaria citri*, *Alternaria tenuissima* and *Alternaria alternaria*) were(29, 26 and 23 mm) correspondingly, further species were create to be practical pectinase action include(*Mycosphaerella tassiana*, *Botrytis squamosal* , *Botrytis cinerea*). Pectinase formed by *Alternaria citri* increased by increasing of incubation



dated exhibition its supreme afterwards (ten days) of incubation, in (30°C , pH= 6). Among numerous carbon cradles shared definitely in the media , (pectin) shaped resolute pectinase structure through *Alternaria citri* , whereas (peptone)was greatest nitrogen foundation considerable measures to pectinase .

Key words : Tomato, pathogenicity ,Pectinase, *Alternaria citri* , Leaf Spot.

2-Introduction

Tomato (*Solanum lycopersicum* L.) is unique of utmost authoritative vegetable harvests established universal (1) . It is cultivated essentially in wholly nations both in arenas and endangered nations(2). tomato Leaf spot produced by some fungal strains is unique from communal effects reduction tomato's manufacture(3) . Over and done with previous inadequate centuries tomato infections has been fashionable nearly each year, essentially owing to soil-borne survival of mold, native over wintering or over summering of inoculum, farming of susceptible varieties and talented environmental locations. Fungiform herbal pathogenic include collection of fungi exhibition definite countless adaptableness throughout their contamination successions. This flexibility agreements for pollute varied diversity from yields (4,5) . service miscellaneous methods for contaminate in addition to inhabit plant life, similarly create multilayered statement stuck in the middle of fungal types plus armies(6 ,7 , 8). A diverse break is between conceivable crop and harvest understood at agriculturalist's arena, that were mostly since to number of (biotic & abiotic) strains from tomato's harvest was defenseless. The disease leaf spot produced by way of *A. solani* , was unique in greatest communal , harmful tomato's diseases of yield. plug in leaves, abrasion stem besides produce spots were the greatest harmless indications by disease then crop sufferers 80% harm via *Alternaria* species (9, 10, 11, 12). Many houseplant pathogenic elements include bacterial or fungal strains has protracted remained documented for products pectin lytic enzyme , widely recognized production in the enzyme was a chief resources through this microbe inhabit tissue crowd (13) . Contribution in pectin enzyme fashionable squalor from pectin supporters in cell wall besides the middle lamella in herb tissue were designated to varied kinds from infections like (blights , soft rot, wilts ,dry rot and leaf spots)(14).



3-Supplies & Procedures

3-1 Assortment of Tomato samples

(40) diseased leaves examples of Tomato were composed as of dissimilar cultured areas in (Baghdad - Iraq). All taster placed in a sterilized polyethylene carrier bag and transported to fungi. Tasters reserved at (4°C) up until mycological examination.

3-2 Isolation fungi from leaf superficial

Leaf superficial fungal species from Tomato's plant were insulated of unhealthy plant leaves conferring towards procedures designated in (15) proceeding PDA.

3-3 Pathogenicity test

(15) species of fungi composed as of unhealthy Tomato leaves were appraised for pathogenicity of Tomato (16). Tomato were full-grown in a greenhouse through experimentation. Pathogenicity assessment was approved out by disinfected soil placed in (20 cm) pot. Seeds superficial sanitized through from mercuric chloride (0.1) % . washed numerous times by sanitized seawater. (10) tomato's seed spread fashionable every pots. Spores suspension of designated fungi secondhand for injection in plant (20) mL/plant covering (106) cfu/mL . all fungal species cultured in PDA. Pathogenic symptoms of designated fungi were examined after (45) days via leaves scattering, these grownup fashionable greenhouse to (15) days. (3) from replicates that secondhand to every verified isolates , plants were scattered through sanitized distilled water used as control .

3-4 Photosynthetic pigments extraction

chlorophyll a ,chlorophyll b resolute by (spectrophotometric technique). This pigments removed by recognized heaviness renewed leaves at (85)% aqueous acetone. Excerpt centrifuged at 7000 (rpm) to 15(min) , supernatant



poured , finished toward certain capacity with aqueous acetone(85)% to convert appropriate to spectrophotometric dimensions. Absorption of pigment segments Chlorophyll a , Chlorophyll b unhurried to (663)and (648) nm (17).

3-5Transmission fungal species designed for manufacture of pectinase:

(6) types of fungi partitioned to capabilities for products pectinase . Fungi cultivated on (PDA) , protected to 28 °C to five days .By sterilized plug bit (10)mm disk censored to immunize (50) ml disinfected liquefied medium (18). Afterwards (7) days of , cultures filtered , filtrates secondhand to perceive activity of pectinase (19), (0.1) ml filtrate of culture were pipetted in (10) mm hollows completed in plates comprising solid medium. Afterward (1) day of cultivation in (28)°C, plates swamped by iodine. Clear zone around the holes designated pectinase activity .

3-6Influences affecting pectinase creation

A. citri were initiate greatest pectinase creators. ,working to education influence of dissimilar environmental and nutritious influences on pectinase manufacture .

3-6-1incubation times

Containers comprising by using Hankin medium (50 ml) pH =5 , immunized by *A. citri* , hatched in (28)°C to (5,7,10 ,14) days. 3 duplicates secondhand for every treatment. Centrifuged the filtrates to (15) min. to (15000)rpm ,pure supernatants assessed to pectinase (20).

3-6-2Temperature

(50) ml of liquefied medium injected by (*A. citri*) , protected to (20, 25, 30, 35) °C to(7)days. 3 duplicates were prepared to every management. At the finale of cultivation time, cultures filtered, centrifuged to (15) min. to (15000) rpm , pure supernatants were assessed to activity of pectinase.



3-6-3pH values

Containers covering (50) ml from medium attuned to dissimilar pH values (2, 4,6 , 8) ,inoculated with (*A.citri*). in (28) °C to (7)days. 3 duplicates were prepared to every management. At the finale of cultivation time, cultures filtered, centrifuged to (15) min. to (15000) rpm , pure supernatants were assessed to activity of pectinase.

3-6-4Carbon sources

Culture media complemented by 0.5 % from (glucose. pectin, CMC, , maltose). immunized by way of *A.citri* . in (28) °C to (7)days. 3 duplicates were prepared to every management. At the finale of cultivation time, cultures filtered, centrifuged to (15) min. to (15000) rpm , pure supernatants were assessed to activity of pectinase.

3-6-5Nitrogen sources:

Medium was complemented by using (peptone ,ammonium nitrate, sodium nitrite ,potassium nitrate) in 0.2% , in adding using ammonium sulphate as per controller , in (28) °C to (7)days. 3 duplicates were prepared to every management. At the finale of cultivation time, cultures filtered, centrifuged to (15) min. to (15000) rpm , pure supernatants were assessed to activity of pectinase .

4-Consequences & Conversation

4-1Isolated fungi

Fungal growing occurred on all revealing leaves , Initially (15) mycological species remained supposed that include (*Curvularia lunata*, *Stachybotrys chartarum*, *Mycosphaerella tassiana*, *Cladosporium cladosporioides*, *Cladosporium sphaerspermum* , *Drechslera papendorfii* *Fusarium oxysporum*, *Fusarium moniliforme*, *Fusarium solani*, *Alternaria tenuissima* , *Alternaria alternate*, *Alternaria citri* , *Rhizoctonia solani* , *Botrytis cinerea*, *Botrytis squamosa*).



as of (19) examples of tomato collected as of different dividends isolated (41) species of fungi money-making to 22 genera. The greatest communal fungi that *F. moniliforme*, *F. solani*, *A. alternata* *A. flavus* (21).

4-2 Pathogenicity of isolated fungi

Pathogenicity of 15 species accredited for 9 Genes were documented. 8 species of fungi were affirmative, effectively capable to contaminate plant leaves looking pollution indications of leaf plug. *A. citri* *A. alternata*, *A. tenuissima*, *B. cinerea*, *B. squamosa* were extremely infectious and shaped the disease symptoms on extra 75 % from diseased leaves. (*M. tassiana*) was reasonable capability towards contaminate vegetal leaves, cut protection (50 – 75)% of diseased leaves. *R. solani*, *C. lunata*, *F. moniliforme*, *F. solani* and *C. sphaerspermum* showed fewer infectious. Its grazes were experiential on fewer from (50)% of diseased leaves, although residual fungi had undesirable pathogenicity consequence and powerless to contaminate leaves of tomato deteriorating to yield every leaf spot indications on immunized leaves. These fungi include *D. papendorfii*, *F. oxysporum*, *C. cladosporioides* *S. chartarum*, Table (1).

Table (1):- Pathogenicity of fungi

Ser.	Fungal isolates	Pathogenicity
1	<i>A. alternata</i>	75%
2	<i>A. tenuissima</i>	75%
3	<i>A. citri</i>	75%
4	<i>B. squamosa</i>	75%
5	<i>B. cinerea</i>	75%
6	<i>M. tassiana</i>	50%
7	<i>F. solani</i>	25%
8	<i>F. moniliforme</i>	25%
9	<i>R. solani</i>	25%
10	<i>C. lunata</i>	25%
11	<i>C. sphaerspermum</i>	25%
12	<i>F. oxysporum</i>	0.0%
13	<i>S. chartarum</i>	0.0%



14	D. papendorffii	0.0%
15	C. cladosporioides	0.0%

The fungal *Alternaria* is dishonest pathogenic element, particulars contagions to several fruits and vegetable (22). Over previous insufficient centuries plant diseases chic nearly every year, fundamentally payable for soil borne existence of fungi, (2). *Alternaria*, decorous in place of passive assailant rather than principal pathogen, (23) detailed this, *A. tenuissima* that chief (leaf spot) significant pathogen. In the (eggplant), *Alternaria* testified by way of insignificant, sphere-shaped, chocolate necrotic spots altogether over plant (24). (25) originate *Alternaria* distant after tomato performance indications in leaf spot infection cultivated below glasshouse environments.

4-3 Result of fungal contagion on photosynthetic pigments

Photosynthesis degrees of are exceptional of greatest important symbols on functional evaluation in plants that associated with chlorophyll content. Consequences protest infected plant by *A. citri*, chlorophyll a, b tremendously communicable presented uppermost threateningly condensed by (1.16), (0.18) (mg/g fresh leaves) correspondingly compared with (2), (1.50) (mg/g fresh leaves) in controller plants. *A. alternate* also protest animated abridged for (chlorophyll a and b) were (1.89), (0.98) (mg/g fresh leaves) correspondingly, established fungi not protest evocatively abridged to (chlorophyll a, b). Chlorophyll reduction from plant leaves for the reason that *Alternaria* infection can be a position of fungal importance on statement of overjoyed toxins important to deliverance of subtle oxygen sp. instigating instinctive cell death (26).

Table(2) : Influence of fungal contagion on (chlorophyll a and b) in leaves

Managements	Chl.a (mg/g fresh leaves)	Chl.b (mg/g fresh leaves)
Controller	2	1.50



A. citri	1.16	0.18
A . alternata	1.89	0.98
A.tenuissima	1.56	0.85
M. tassiana	1.90	0.88
B. squamosa	1.87	0.83
B. cinerea	1.86	0.92

(27) originate that in (broad bean) Chl.a , Chl. b besides the Carotenoids in leaves evocatively reduced owing for contagion of *A.alternata* that was highly transmissible presented uppermost evocatively inhibitive importance on formation of photosynthetic pigments likened with control plant. (3) specified that diseased tomato seedlings by *A.solani* obtainable tremendously significant discount in contents of Chl.a , Chl. b besides the Carotenoids associated with controller.

4-4pectinase activity

(6) fungal species that divided intended for capabilities for harvest the enzyme bythe methods of cup-plate technique. Totally experienced fungi were pectinase originators then with adaptable degrees. (3) fungi presented great pectinase activity include (*A. citri*, *A.tenuissima* , *A. alternaria*) (28, 25 , 24) mm correspondingly, additional fungi that create for remain sensible pectinase activity include (*M. tassiana*, *B.squamosal* , *B. cinerea*) were (18,15, 14) respectively Boccas et al., (1994) create that, from (248) isolates of fungi enhanced as of chocolate plant besides earth examples divided to the ability for create enzyme, 119 isolate gifted for products pectinase . (28) create that from (5) fungal isolates experienced intended for capability to creating pectinase , many species of *A. oryzae* besides *A. niger* show highest manufacturers. (29) found that from(39) isolates of fungi confirmed for dimensions of creating pectinase in media, single strain individual connected to *E. nidulans* made this enzyme.

4-5 Influence of changed incubation times on enzyme action produced from *A. citri*



Pectinase formed from (*A. citri*) was increased with growing of incubation times performance its all-out after (10) days of incubation, afterward (14) days Pectinase activity was concentrated (Table 3).

Table(3): Effect of incubation times on pectinase activity

incubation times(days)	pectinase activity
5	1.6
7	2.1
10	3.1
14	2.9

(30) labeled that, extreme products of pectinase through *A. phoenicis* and *A. flavus* were increased in 6 days , granting now *A. wentii* and *A. niger* were in 8 days from growth. (31) create that, extreme the enzyme action in *A. niger* recognized afterward 7 days of growth.

4-6 Influence from changed temperature going on enzyme action formed from *A. citri*

Full enzyme formed from *A. citri* reached in 30°C . Considerable amounts from pectinase remained as well increased in (20°C ,25°C , 35°C) nonetheless this amounts remained usually shortened. Lowest enzyme action reported in(35) °C (Table 4).

Table(4): Effect of Temperature on pectinase activity

Temperature(°C)	pectinase activity
20	0.7
25	1.9
30	2.8
35	1.5

These consequences analogous to results extended by (32, 33 ,34). They labeled that, optimal pectinase activity from the fungi include *A. japonicus*, *A.*



niger, *A. giganteus* was at (30) °C . (35) labeled that finest temperature to pectinase manufacture from (*A. falvus*) was at (35°C). (36) empirical that pectinase manufacture from (*P. chrysogenum*)was progressive at (35°C).

4-7Influence of changed pH values going on enzyme action created from *A. citri*

Full amount of enzyme might be synthetic once culture medium of (*A. citri*) initially familiar toward pH= 6 . Substantial quantities of pectinase also reported in pH =2 ,4 , 8, while little amounts of pectinase were experimental in cultures using extra acidity or alkalinity (Table 5).

Table(5): Influence of pH standards in enzyme action

pH values	pectinase activity
2	0.3
4	1.7
6	3.1
8	1.4

(30) found that the greatest pH for pectinase formation by (*A.flavus*, *A. niger*, *A. phoenicis* , *A. wentii*) were (8 , 7 , 5 , 7) correspondingly. (37) found that extreme pectinase activity by (*A. fumigatus*) become at (pH 4.0). (38)originate that polygalacturonase showed highest pectinase action in pH =5 . Furthermore, pH= 6.5 was highest appropriate to pectinase formation from (*P. chrysogenum*), (39).

4-8 Influence of changed Carbon foundations going on enzyme activity produced from (*A. citri*)

Among four sources of carbon united definitely in culture medium to pectinase manufacture, pectin created resolute pectinase creation from (*A. citri*) . Substantial quantities from pectinase talented fashionable attendance CMC, maltose by way of carbon sources ,whereas glucose was least inducible source for carbon to pectinase creation (Table 6).



Table(6): Effect of carbon sources on pectinase activity

carbon sources	pectinase activity
pectin	3.3
CMC	2.1
glucose	0.5
maltose	2.6

(33) labeled that, extreme formation of polygalacturonase was highest by using pectin as carbon source. (32) untried that highest formation of polygalacturnase (*A. niger*) was reached by apple fleshy tissue and apple pectin as carbon source.

4-9 Influence of changed nitrogen sources going on enzyme action formed from (*A. citri*)

Full produces of pectinase formed from *A. citri* concluded fashionable incidence of peptone by way of foundation for nitrogen followed via sodium nitrite . Considerable amounts from this enzyme that likewise industrial fashionable presence of ammonium nitrate as source for nitrogen. Potassium nitrate was fewer nitrogen sources talented for the pectinase manufacture, (Table 7).

Table(7): Effect of nitrogen sources on pectinase activity

nitrogen sources	pectinase activity(U/mL)
ammonium nitrate	1.1
Potassium nitrate	0.6
sodium nitrite	2.8
peptone	3.1

(40) labeled that pectinase manufacture from *P. chrysogenum* industrialized by ammonium sulphate trailed in ammonium sulphate by way of nitrogen foundation. (41)originate that pectinase action larger in incidence of



nitrogen foundations like ammonium sulphate 65U/mL , peptone displays 37.5U/mL then years extract showed 31.6U/mL .

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