Plant Archives Volume 20 No. 1, 2020 pp. 1066-1070 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

ISOLATION AND IDENTIFICATION OF THE CAUSES OF DRY ROT DISEASE ON POTATO AND ITS CONTROL BY USING SOME ENVIRONMENT FRIENDLY PLANT EXTRACTS

Kawther F. Alwan[1], Ali H. Al-Asadi[2]*, Muhsen A. Almousawy[3] and Majeed M. Dewan[2]

[1]Department of Biological Control, Technical College-AL Musaib, Babil, Iraq.
[2]Department of Plant Protection, College of Agriculture, Univ. of Kufa, Najaf, Iraq.
[3]Department of Plant Protection, College of Agriculture, Univ. of Kerbala, Karbala, Iraq.

Abstract

This study was conducted in the laboratories of the Faculty of Agriculture - University of Kufa and the Faculty of Medical Sciences - University of Karbala - Iraq for isolating fungi that causes of dry rot disease on potato tuber and its morphological diagnosis. Hot water extracts were used for various parts of turmeric, ginger, oak, clove, chili, lemon, pencil cactus, olive using of five concentrations (0,10,30, 50,70 g / L) to study their effect against *Fusarium solani* isolate in petri dishes. Among these extracts, clove (*Eugenia caryophyllus*) extract was most effective at concentrations of 50 and 70 g / L that inhibited compeletly the growth of *F. solani* followed by the oak (*Quercus robur*) extract which inhibited the fungus by 54% when tested at concentration 70 g / L.

The most pathogenic isolate was selected to study the ability of this isolate to secrete zearalenone toxin, the result of thin layer chromatography (TLC) test showed the ability of this isolate to secret the above toxin in PDA medium as well as in the proper tissue of the infected potato tubers.

Key words: Dry rot disease, *Fusarium solani*, Potato, extract, Clove, Oak, Zearalenone.

Introduction

Dry rot disease is one of the most important diseases affecting on potato crop before or after harvest in all crop growing areas around the world (Du *et al*., 2012 and Stefanczyk *et al*., 2016). The disease causes losses 6-25% for this crop and may increase to 60% if the storage period is long (Heltoft *et al*., 2016). Dry rot disease is mainly caused by several types of *Fusarium* species (Gachango *et al*., 2012). The pathogen enter through wounds and scratches that occur to the tubers as a result of the various agricultural processes (Kirk *et al*., 2013), the disease causes the rotting of the modern sprouts of the plant. The infection appears on the tubers after the harvest in the form of dark areas on the surface of the tuber. The tissue gets dry and becomes dark brown to black, resulting in partial ocular damage to the affected tuber.

Chemical fungicides such as thiabendazole are used as a traditional measure for control of dry rot, but after a period of time the resistance of the pesticide was mentioned by some strains of *Fusarium* causes of disease (Ocamb *et al*., 2007; Gachango *et al*., 2012). The health problems caused by chemical pesticides for humans and animals and polluting the environment has made modern studies are moving towards alternative ways to control plant diseases, studies that some of these extracts can inhibit many plant pathogens, including some of the causes of dry rot disease (Bhardwaj, 2012; Elsherbiny *et al*., 2016). The current study aimed at finding plant extracts that are environmentally friendly and highly effective in inhibition of growth of pathogenic fungi.

***Author for correspondence :** E-mail: alihussainhilal@gmail.com

Materials and Methods

Collect potato samples and isolate pathogenic fungi

Potato tubers with dry rot symptoms were collected from the local markets in the governorate of holy Karbala, Iraq, taking 3-5 pieces of injury area and 0.5 cm in size, sterilized with sodium hypochlorite solution at 10% concentration of commercial solution for 3 minutes.

Distilled sterilized several times was used to wash the infected tubers and eliminated excess water by placing the washed pieces on sterile blotting paper, then planted in Petri dishes containing the PDA In four slices of each dish with three replicates, incubate the dishes in the incubator at 25 ± 2°C for 2-4 days after which the developing growth of fungi were purified on new PDA by taking a small piece of mycelium from the edge of the colony. The dishes incubated at 25 ± 2°C for 7 days, after that the isolates were diagnosed.

Morphological diagnosis of *Fusarium* isolates

Fusarium isolates were identified depended on their growth, colors and other parameters as well as using the composite microscope and based on the taxonomic characteristics of the fungi (Lesli and Summerell, 2006).

Test the pathogenicity of isolated fungi on potato tubers

Potato tubers were almost equal in size and free of scratches brought from the local markets. These tubers were washed with regular washing powder and then washed thoroughly with water several times, after that the tuber sterilized with Sodium hypochlorite (NaOCl 1%) for 3 minutes and removed the free water by sterile paper blotting. Each tuber was cut from one side using a sterile scalpel and then a portion of the new fungal growth was grown in a 7-day in Petri dish, taking into account the use of three replicates for each fungus. Three tubers of infected potatoes were left without inoculation as comparative treatments. Each tuber was placed in a poly Ethylene bag sterilized and incubated at 25 ± 2°C, measurements of the extent of infection on the tubers were taken after 4 weeks using a transparent ruler to determine the development of the disease.

Detection of the ability of *Fusarium solani* fungus to produce the Zearalenone toxin in the PDA medium

Petri dishes containing 15 ml of the PDA medium were Forgiven with *F. solani* (Fda10) isolate was the most pathogenic to potato tubers. The dishes were incubated at 25°C for three weeks. The medium with fungal growth dishes were removed and was cut off to small pieces and were placed in the blender. A quantity of distilled water was added to the dish by 25 ml per dish. The mixture was thoroughly crushed and then filtered by a piece of cloth and sterile filter paper,after that placed in the electric oven to get rid of the chloroform and get the pure dry extract of the fungus to carry out toxicology tests.

Detection of the ability of *F. solani* to the secretion of zearalenone in potato tubers

The inoculated tubers with *F. solani* were incubated for 5 weeks at 25°C. 20 g of healthy tissue was taken from infected tubers and placed in the electric blender. The extract was prepared as in the previous method.

Detection of Zearalenone toxin by the using TLC plates

Thin layer chromatography (TLC) was used 20 × 20 cm and 0.25 mm thick and the plates were activated in an electric oven at 110°C for 1 hour. A straight line was made on the plate 2 cm from the bottom and the top and 1.5 cm from both sides. The standard toxin was then used to compare the fluorescence intensity and the rate of flow (RF) with the extract, 10 microliters of the standard toxin were placed on the straight line below the plate. Then add the samples in the same amount of the standard toxin and on the same line below the plate and left the plate until to dry samples on it and then placed in the basin of the former glass chapter and the container system Separate gasoline - acetone (90-10) ml and leave the plate in the basin until the mix of the separation system to a distance of 2 cm before the edge of the upper plate, The plate was then removed and left to dry and examined under ultraviolet radiation at a wavelength of 365 nanometers. The color of the fluorescence and the relay coefficient of the tested models was compared with the standard toxin model.

Effect of some plant extraction in growth inhibition of *F. solani*

Plant extracts derived from various plant parts were used: turmeric rhizoms, chilli fruits, ginger rhizomes, olive leaves, oak peels, clove flower buds, pencil cactus branchs and lemon peels. The purpose of the study was to test the efficacy of the hot water extract of these derived in the growth of *F.solani* by adding dry powder for these plants to the P.S.A. medium in concentrations 0, 10, 30, 50 and 70 g / L. as well as the control treatment without adding the extract and then sterilized by auotoclave. Chloramphenicol added at 250 mg/L to the medium with or without extract and pour into petri dishes according to their transactions and concentrations.

The prepared dishes were inoculated by *F.solani* isolate (Fda10) by taking 0.5 cm tablet from the edge of the fungus colony using a cork borer and placing it in the center of the petri dish with three replicates for each treatment and PSA medium only without a plant extract as control treatment, all dishes were placed in the incubator at a temperature of 25 + 2°C. This experiment was carried out according to the full CRD design. Results were recorded when the fungal colony diameter of the comparative analysis reached the edge of the dish by measuring two orthogonal diameter of the colony. For the following equation:

Percentage inhibition = C-T/C × 100

Where, C = colony diameter of the control

T = colony diameter of the test plate.

Results and Discussion

Collect of fungi causes dry rot disease from local potato tubers

Pathogen from infected potato tubers with dry rot disease to obtain 10 fungal isolates, all of which belong to the *Fusarium* species. The isolates were treated with numbers from 1-10 according to the priority of isolates FdA1, FdA2, FdA3, FdA4, FdA5, FdA6, 7FdA, FdA8, FdA9, FdA10. The isolates differed in their phenotypic characteristics in terms of color and growth speed. The color of the colonies varied between white and pink. This was consistent with Monuj *et al.*, (2017) of differences in the color of the different isolates of *Fusarium* on the same nutrient medium.

Identification of *Fusarium* isolates which causes dry rot disease in this study

The *Fusarium* isolates were identified depending on the external appearance of the fungus colony, as well as the microscopic diagnosis using the taxonomic keys of the fungus (Lesli and Summerell, 2006). The fungal isolates were *Fusarium solani*, *F. hostae*, *F. crookwellence*, *F. sambucinum*.

The pathogenicity test of *Fusarium* isolates on potato tubers

The results showed that there were significant differences between *Fusarium* isolates in their susceptibility to dry rot disease and the severity of the disease. Fda10 isolates were the most pathogenic to the tubers, with the highest diameter infection of 1.26 cm and a depth of 1.2 cm, followed by Fda2 and Fda8 with a diameter of 0.93 cm each with a depth of 0.76 and 0.8 cm respectively, while Fda6 did not achieve any tuber injury, that is, it was a non-pathogenic isolate. These results were consistent with Theron and Holz, (1990) that mentioned to difference in the pathogenicity of the *Fusarium* isolates causes dry rot as well as the different among isolates in the same species. Aydin and Inal, (2018) indicated the difference in pathogenicity of some *Fusarium* isolates.

Table 1: Pathogenicity of *Fusarium* species on potato tubers.

Depth of infection	Diameter of infection (cm)	*Fusarium* isolates	*Fusarium* species
0	0	Control	
0	0	FdA6	*F.crookwellence*
0.2	0.47	FdA9	
0.2	0.46	FdA4	*F. hostae*
0.3	0.69	FdA3	*F. sambucinum*
0.36	0.79	FdA5	
0.5	0.78	FdA1	*F.solani*
0.76	0.93	FdA2	
0.1	0.40	FdA7	
0.8	0.93	FdA8	
1.2	1.26	FdA10	
0.041	0.063	L.S.D.(0.05)	

The ability of *F.solani* isolates to produce the zearalenone toxin in the PDA medium

The results of thin layer chromatography (TLC) test showed that the tested *F.solani* isolate produced the zearalenone toxin on PDA medium, based on the Rf value of the sample tested compared to the spot for the standard, which was the same value (Wyllie and Morhouse, 1977) *F.solani* isolated from some parts of the plant in northern Kaloraina as it was found to have the ability to produce this toxin (Afriyie-Gyawu *et al.*, 1985). Several studies have shown that some *Fusarium* fungi, which cause dry rot disease, have the ability to produce Zearalenone, such as *F.culmorum* (Latus-Ziętkiewicz *et al.*, 1987) and *F. crookwellense* (Golinski *et al.*, 1988).

The ability of *F.solani* isolates to produce zearalenone toxin in the proper tissue of infected potato tubers

The results of this study showed that the isolate of *F.solani* pathogen susceptibility to secretion of zearalenone in the healthy tissues of potato tubers infected with the disease. This study may be the first to have found the possibility of producing *F.solani* to Zearalenone

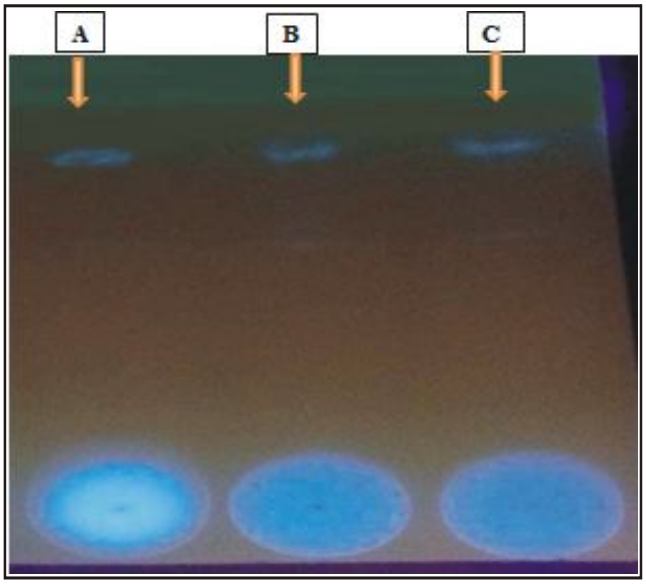

Fig. 1: T.L.C plate shows (A) standard zearalenone spot (B) spot for sample of *Fusarium solani* isolate growing on the PDA medium (C) spot for sample of proper tissue of potato tuber infected with dry rot .

Table 2: Effect of Some Plant Extracts Added with P.S.A. medium against *F. solani* causes dry rot in Petri dishes.

% Inhibition in 70 g/L of extracts	Extracts average	*F.solani* growth (cm) Concentrations G/L					Type of extract
		70	50	30	10	0	
41	6.6	5.3	5.4	5.8	7.6	9	Turmeric rhizomes
50	6	4.5	4.8	5.2	6.4	9	Lemon peels
42	6.7	5.2	6	6.2	7	9	Ginger rhizomes
0	9	9	9	9	9	9	Olive leaves
54	5.7	4.1	4.3	5.2	6.1	9	Oak peels
0	9	9	9	9	9	9	pencil cactus branches
100	2.2	0	0	0.3	1.9	9	Clove flower buds
3.3	8.7	8.2	8.5	9	9	9	Chilli fruits
		5.6	5.8	6.2	7	9	Concentration average
for interaction = 0.092	for concentrations = 0.032			for extracts = 0.041			L.S.D. (0.05)

in the potato tubers. Some studies have shown that several types of *Fusarium* can produce toxin on some food crops. *F. oxysporium, F. tricinctum* and *F. roseum* have been found to produce Zearalenone in animal diets and barley grains (Wyllie and Morehouse, 1977).

The effect of some plant extracts to inhibit the growth of *F.solani* which causes dry rot disease on potatoes

The results in the table 2, showed that there was a significant difference among various plant extracts and their concentrations used on the growth of *F.solsni* causes dry rot disease on the PDA medium. The results of this study showed that the extract of clove flower buds was the most effective against *F.solani*, which inhibit growth of pathogen by 100 % at concentrations 50 and 70 g / L. compared with control treatment as shown in fig. 2, followed by Oak peels extract, which inhibit *F. solani* growth by 3.3% at the former concentration. While the extracts of olive leaves and pencil cactus branches don't give any inhibitory effect against *F. solani*.

The results of this study are consistent with those of Thobunluepop *et al.*, (2008) that some plants contain active compounds that have the ability to inhibit certain microorganisms, the researcher explained that these compounds differ in their chemical structures and thus vary in inhibitory activity in the growth and survival of fungi. As well as that there is a difference in *F. solani* sensitivity for various plant extracts (Bhardwaj, 2012).

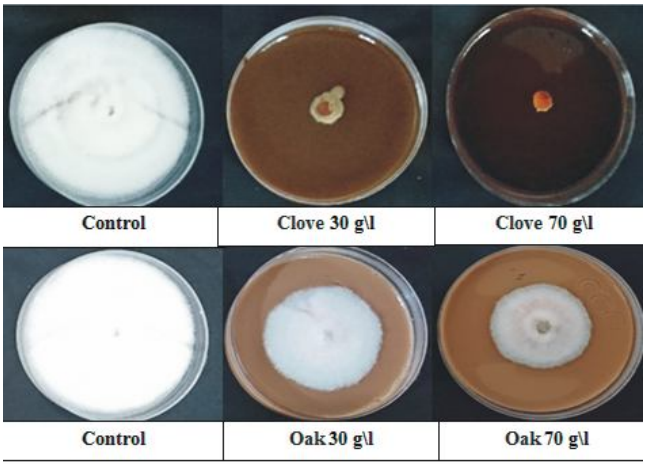

Fig. 2: Inhibitory effect of clove and oak extracts against *Fusarium solani* causes dry rot disease.

The effect of the hot water extract of cloves in the growth of *F.solani* may be attributed to the containment of tannins and alkaloids that have a disinfectant such as vanillin and bain (Bowers *et al*., 2000). Kuppusamy *et al*., (2016) noted that the oak peels extract contains effective phenolic compounds in inhibiting microorganisms.

References

Afriyie-Gyawu, E., M.C. Wiles, H.J. Huebner, M.B. Richardson, C. Fickey and T.D. Phillips (2005). Prevention of zearalenone-induced hyperestrogenism in prepubertal mice. *Journal of Toxicology and Environmental Health, Part A.*, **68(5):** 353-368.

Aydin, M.H. and B. Inal (2018). Comparative susceptibility of some commercial potato cultivars to *Fusarium sambucinum* and F. solani isolates causing tuber dry rot. *Applied Ecology and Environmental Research.*, **16(4):** 4879-4892.

Bhardwaj, S.K. (2012). Evaluation of Plant Extracts as Antifungal Agents Against *Fusarium solani* (Mart.) Sacc. World *Journal of Agricultural Sciences.*, **8(4):** 385-388.

Bowers, J.H. and C.L. James (2000). Effect of botanical extract on the population density of *Fusarium oxysporum* in soil and control of *Fusarium* wilt in the green house *Journal of the American oil chemists Society*., **84(3):** 300-305.

Du, M., X. Ren, Q. Sun, Y. Wang and R. Zhang (2012). Characterization of *Fusarium* spp. causing potato dry rot in China and susceptibility evaluation of Chinese potato germplasm to the pathogen. *Potato Research.*, **55(2):** 175-184.

Elsherbiny, E.A., B.H. Amin and Z.A. Baka (2016). Efficiency of pomegranate (*Punica granatum* L.) peels extract as a high potential natural tool towards *Fusarium* dry rot on potato tubers. *Postharvest Biology and Technology.*, **111:** 256-263.

Gachango, E., L.E. Hanson, A. Rojas, J.J. Hao and W.W. Kirk (2012). *Fusarium* spp. causing dry rot of seed potato tubers

in Michigan and their sensitivity to fungicides. *Plant Disease.,* **96(12):** 1767-1774.

Golinski, P., R.F. Vesonder, D. Latus-Zietkiewicz and J. Perkowski (1988). Formation of fusarenone X, nivalenol, zearalenone, alpha-trans-zearalenol, beta-trans-zearalenol and fusarin C by *Fusarium crookwellense*. *Appl. Environ. Microbiol.,* **54(8):** 2147-2148.

Heltoft, P., M.B. Brurberg, M. Skogen, V.H. Le, J. Razzaghian and A. Hermansen (2016). *Fusarium* spp. causing Dry Rot on potatoes in Norway and development of a Real-Time PCR method for detection of Fusarium coeruleum. *Potato research.,* **59(1):** 67-80.

Kirk, W.W., E. Gachango, R. Schafer and P.S. Wharton (2013). Effects of in-season crop-protection combined with postharvest applied fungicide on suppression of potato storage diseases caused by *Fusarium* pathogens. Crop protection, **51:** 77-84.

Kuppusamy, S., P. Thavamani, M. Megharaj, R. Nirola, Y.B. Lee and R. Naidu (2016). Assessment of antioxidant activity, minerals, phenols and flavonoid contents of common plant/tree waste extracts. *Industrial Crops and Products.,* **83:** 630-634.

Latus-Ziętkiewicz, D., J. Perkowski and J. Chełkowski (1987). *Fusarium* species as pathogens of potato tubers during storage and their ability to produce mycotoxins. *Mycotoxin research.,* **3(1):** 99-104.

Stefañczyk, E., S. Sobkowiak, M. Bryliñska and J. Śliwka (2016). Diversity of Fusarium spp. associated with dry rot of potato tubers in Poland. *European Journal of Plant Pathology.,* **145(4):** 871-884.

Theron, D.J. and G. Holz (1990). Effect of temperature on dry rot development of potato tubers inoculated with different Fusarium spp. *Potato research.,* **33(1):** 109-117.

Thobunluepop, P., C. Jatisatienr, E. Pawelzik and S. Vearasilp (2008, August). *In vitro* screening of the antifungal activity of plant extracts as fungicides against rice seed borne fungi. *In Asia Pacific Symposium on Assuring Quality and Safety of Agri-Foods.,* **837:** 223-228.

Wyllie, T.D. and L.G. Morehouse (1977). Mycotoxic fungi, mycotoxins, mycotoxicoses. M. Dekker.