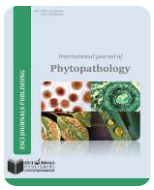

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ALTERNARIA RADICINA CAUSING LEAF SPOT DISEASE OF DATE PALM (*PHOENIX DACTYLIFERA* L.) IN WASIT (MIDDLE OF IRAQ) AND ITS SUSCEPTIBILITY TO BIOASSAY TEST OF TWO FUNGICIDES

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A B S T R A C T

Three *Alternaria radicina* (Meier) Neerg isolates (A1, A2, and A3) were isolated and firstly recorded in date palm, *Phoenix dactylifera* L. leaves in Wasit province (middle of Iraq) and identified as the causative pathogen of black spot disease. Two fungicides (Bavistin and Tachigazole) were used in vitro to measure their ability to control this pathogen. Study results revealed that both chemical fungicides expressed high level of inhibition in fungal radial growth especially at a concentration of 100 ppm and the lowest growth was at the concentration 5 ppm among all three isolates in both two fungicides. Bavistin revealed higher inhibition rate than Tachigazole with lowest LC50 (12.21) at the isolate A3. Tachigazole treatment showed high level of variation at all concentrations.

Keywords: Fungicides, *Alternaria radicina*, Date Palm, black spot.

INTRODUCTION

Date palms, *Phoenix dactylifera* L. are most economically important fruit trees in tropical and subtropical areas and they are growing in a large area in many countries including Iraq. Date palm is one of the most important crops in Iraq with production of about 420,000 tons annually(Erskine *et al.,* 2004). Moreover, Iraq ranked as 7th among the countries that produce date in the world and used to be number one(Erskine *et al.,* 2004). Iraq is aiming to increase its production of date palm in the future to reach 1000,000 ton annually in 10 years' time via a number of programs with USA aimed at increasing the number of date palm trees to 40,000,000.

Alternaria radicina (Meier) Neerg. Is a widespread pathogen in many countries especially Europe and North America causing remarkable damage as reported by (Neergaard 1945). *A. radicina* can cause leaf blight and black rot on carrot under both field and storage conditions (Soteros 1979, Scott and Wenham 1973). Moreover, it can attack plants at all stages causing high yield losses (Coles and Wicks 2003, Farrar *et al.,* 2004, Survilienė and Dambrauskienė 2006). *A. radicina* has a limited host range and carrot is the primarily host; however, it has been reported to cause foliar blight of parsley and a stalk and root rot of celery (Tahvonen 1978, Wearing 1980).

This is the first time for *A. radicina* to be recorded on date palm *Phoenix dactylifera* L. in Wasite province in the middle of Iraq. However, it was reported on date palm in Basrah province in the south of Iraq by (Ahmed 2011).

The main objective of this study is to evaluate antifungal activity of two fungicides with different active ingredients on the radial growth rate of *A. radicina* in vitro.

MATERIALS AND METHODS

Samples Collection: Samples were collected from date palm orchards in Wasit province in the Aziziya site especially at the location where the symptoms emerged. Isolation point was recorded according to Global Positioning System (GPS) with longitude 44°96' East, latitude 32°93' North and Altitude 32.64 in the 18th of November 2013. Symptoms on date palm leaves were noticed as a gray to brown powdery spot lesions. Three isolates from three different locations at the same GPS

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point were obtained with different level of impact on date palm trees of the same variety (A1, A2, and A3) which may indicate a variation of effects depending on isolates virulence.

Fungal Isolation and Purification: Three 2 mm pieces of tissue from leaf were used to get fungal isolates. 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 quarter-strength potato dextrose agar (PDA) plates which contain 100 µg streptomycin sulphate and 10 µg tetracycline hydrochloride mL-1. Plates were incubated at ambient temperature and placed under standard white fluorescent light (35098 F18E/33 General Electric, USA) for 24 hours for 5 -7 days. Spore suspension was made by adding 3-4 drops of sterile distilled water on the fungal colony that were grown around the plated tissues using sterile flame-sterilized loop. This spore suspension was streaked onto 2% water agar media by using a flame-sterilized metal loop and plates were incubated under laboratory conditions for 24 h. A single germinated conidium was transferred onto full-strength PDA media plate and incubated at ambient temperature (Fig. 1) according to Scott and Chakraborty (2010).

Figure 1. a) Leaf spot disease symptoms caused by *A. radicina* b) *A. radicina* (A1, A2, and A3) single spore isolates colony shape c) *A. radicina* spores under 40 × microscope cameras.

Pathogenicity Test: Fungal isolates were grown at 25˚C onto full PDA medium for ten days before preparing spore suspension for the inoculation. The inoculums were prepared by adding 10 ml of sterile distilled water to the 7 days old fungal culture and scrapped by metal scraper. Then the harvested spore solution was poured into a 50ml Falcon tube after filtering the solution through sterile Miracloth. The spores concentration was determined using haemocytometer and concentration adjusted to 1×10^{9}conidia/ml. Two inoculation methods were used via preparing three seedlings for each method each seedling were planted in one pot; then, seedlings of the first inoculation method were inoculated via spraying spore suspension after making wounds by sterile blades and covered by plastic bag, other seedlings of the second inoculation method were inoculated via making 0.5 cm holes in the leaves and 0.5 piece of PDA medium from fungal colony was placed at each hole and covered by a paper tape. The control was prepared by spraying sterile distilled water after wounding leaves for the first inoculation method. The second control treatment was made via making 0.5 holes and placing 0.5 piece of PDA medium for the second inoculation method. The pots were kept in the greenhouse at 24 ±3 ˚C and symptoms were observed after six weeks. The appearance of necrotic lesions was indicator of infection, the pathogen then re-isolated from infected sites to confirm the pathogenicity test.

Fungicides Inhibition Test: Two chemical pesticides (Bavistin with active ingredients carbendazim 50%, systemic fungicide formulation) and (Tachigazole with active ingredients hymexazol 30% SL, systemic fungicide formulation) were used to measure their ability to inhibit *A. radicina* growth on Potato Dextrose Agar medium (PDA) according to (Schmitz 1930). Five concentrations according to the active

ingredient 5, 25, 50, 75, and 100 ppm (1, 5, 10, 15, and 20µl/ml PDA Bavistin) and (1.25, 6.25, 12.5, 18.75, and 25 µl/ ml PDA Tachigazole) were prepared in PDA medium and according to the fungicide active ingredient. PDA medium was sterilized in the autoclave at 121˚C for 25 minutes and cooled down to less than 50˚C before adding the fungicide to avoid heat effects and reach the required concentration. Then 20 ml of the mixture were added to 90mm plastic petri dishes. Control treatment was prepared via adding the same volume of sterile distilled water to the autoclaved PDA medium plates. Three plates for each concentration were used including the control. Full growth fungal colony from thee isolates were grown 7 days before the experiment in the incubator at 25±2 and 70% Rh. By using sterile cork borer 0.5 pieces colony were placed onto the middle of fungicide treated medium plates. Plates were kept in the incubator at 25±2 and 70% Rh for 10 days and radiation growth was measured every two days.

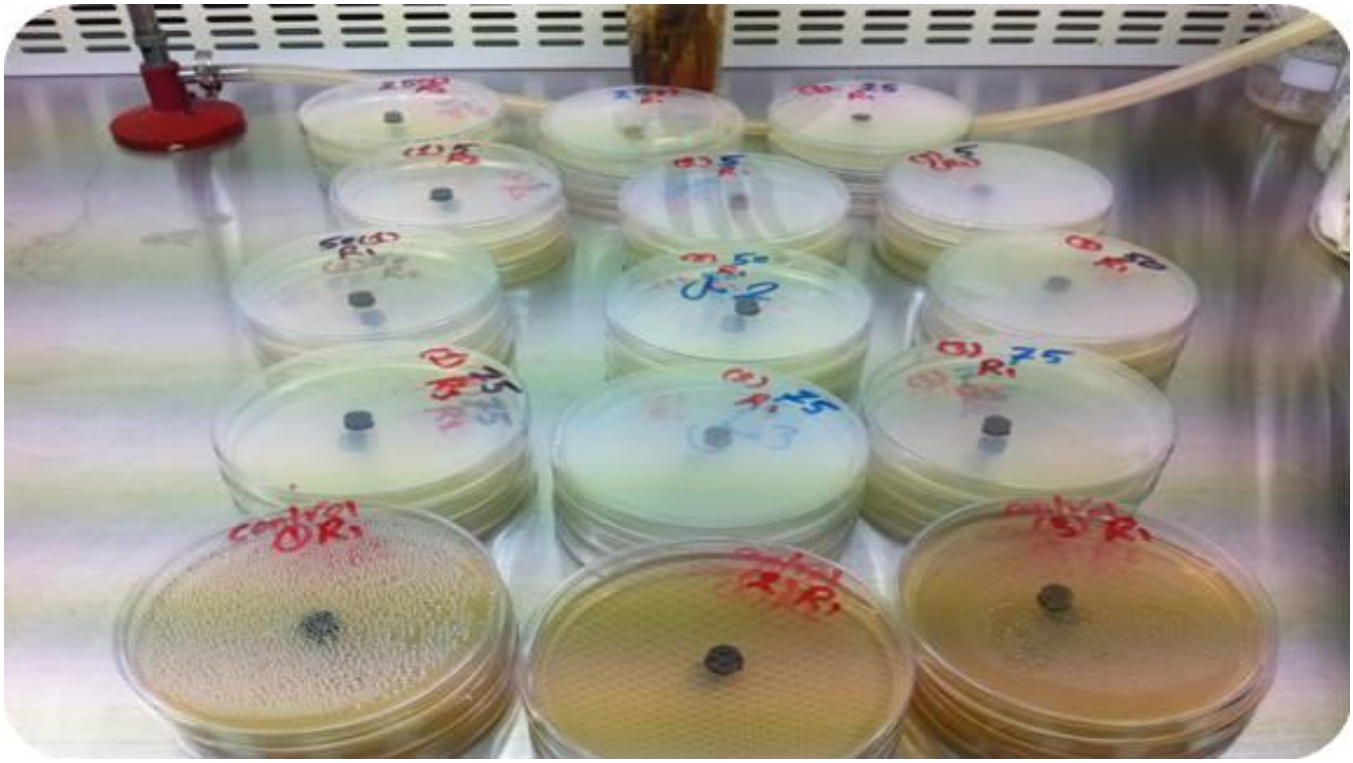

Figure 2. Fungicides inhibition test in the laminar air flow using sterile cork borer 0.5 pieces colony.

Statistical Analyses: The experiment was designed using Complete Random design (CRD). The percentage of inhibition growth was measured according to (Abbott 1925). Data were analyzed using SPSS 20 statistical analysis software:

$$\text{Inhibition } \% = \frac{\text{Average radial growth in control} - \text{Average radial growth in treatment}}{\text{Average radial growth in control}} \times 100$$

RESULTS

Pathogenicity test results showed that the pathogen is capable of making infection. This can be seen via the increasing length of lesions in the used replicates reaching about 3 cm after six weeks of inoculation. Black colored lesions were noticed in all inoculated replicates with the two methods; while, these symptoms did not appear at the control treatments. Moreover, black spots were noticed on leaves long distance from the inoculated area on leaves' both sides.

Five concentrations from each fungicide were used separately of each isolate. Study result revealed that there was a co-relationship between increasing fungicide concentration and increasing inhibition percentage (Table 1). The highest inhibition percentages were scored at 100ppm concentration in the Bavistin treatment and the lowest were at 5ppm over all examined isolates. The concentration 100 ppm (20 µl/ml) expressed significantly highest inhibition rate in comparison with all other used concentrations for all three isolates (A1, A2, and A3). There were not significant differences among the concentrations 25, 50,

and 75 ppm and the fungus still grow over the PDA medium although the highest inhibition rate (Table 1). There was a significant decrease in the effects of Bavistin fungicide over time reaching the lowest inhibition rate after 10 days of the treatment at all concentrations and for the three isolates. The isolate A1 revealed the highest sensitivity level in comparison with other A2 and A3 isolates (Table 1).

Table 1. Radiation growth of *Alternaria radicina* three isolates in 10 days' time after treating them with the chemical pesticide Bavistin with five concentrations.

Date	% inhibition with Concentration µl /ml PDA (Active ingredient)				
	1µl/ml PDA	5 µl/ml PDA	10 µl /ml PDA	15 µl/ml PDA	20 µl /ml PDA
(A1)	(5 ppm)	(25 ppm)	(50ppm)	(75 ppm)	(100ppm)
2 days	15.6085 a	37.3016 a	37.3016 a	29.8942 a	100 b
4 days	40.6148 a	58.3084 b	61.9848 b	62.5408 b	90.224 c
6 days	40.3704 a	64.1481 b	62.6667 b	66.2222 b	90.4074 c
8 days	26.1425 a	63.8575 b	59.5954 b	65.265 b	89.8974 c
10 days	30 a	51.8519 b	59.2593 b	62.963 b	89.8148 c
(A2)					
2 days	25 a	38.0556 a	40.4167 a	38.1944 a	100 b
4 days	8.5101	41.5657	40.0758	47.2222	87.75
6 days	9.6342	33.9285	47.4825	49.2747	80.9464
8 days	25.1389	49.4775	56.8122	57.8571	83.2672
10 days	21.6021 a	45.5728 b	44.5306 b	57.8295 b	81.6753 c
(A3)					
2 days	17.2078 a	34.9928 a	21.9336 a	31.8543 a	100 b
4 days	26.7035 a	44.1909 a	38.8227 a	51.3227 a	94.0656 b
6 days	39.1532 a	54.8402 ab	53.2418 ab	62.5523 bc	80.6613 c
8 days	41.455 ab	62.5529 b	56.1905 ab	63.1614 b	83.2672 c
10 days	42.7821 a	57.2093 a	54.2636 a	62.7649 a	82.3652 b

The fungicide Tachigazole behaves almost the same as Bavistin; however, its effect was lower than Bavistin treatment. The highest inhibition rate of *Alternaria radicina* was scored at the concentration 100 ppm (25 µl/ ml PDA) and the lowest was at 5ppm (1.25 µl/ ml PDA) (Table 2).

Table 2. Radiation growth of *Alternaria radicina* three isolates in 10 days time after treating them with the chemical pesticide Tachigazole with five concentrations.

Date	% inhibition with Concentration µl /ml PDA (Active ingredient)				
	1.25 µl/ ml PDA	6.25 µl/ ml PDA	12.5 µl/ ml PDA	18.75 µl/ ml PDA	25 µl/ ml PDA
(A1)	(5 ppm)	(25 ppm)	(50 ppm)	(75 ppm)	(100 ppm)
2 days	15.6085 a	37.3016 a	37.3016 a	37.3016 a	100 b
4 days	40.6148 a	58.3084 b	61.9848 b	62.5408 b	87.626 c
6 days	40.3704 a	64.1481 b	62.6667 b	66.2222 b	81.8148 c
8 days	34.0741 a	61.1111 b	61.4815 b	64.8148 b	82.963 c
10 days	31.4815 a	60.3704 b	60.1852 b	64.0741 b	81.7407 c
(A2)					
2 days	35.7888 a	50.6238 ab	54.4372 abc	64.4269 bc	71.5037 c
4 days	38.4524 a	42.5053 a	52.8766 a	55.4644 a	80.1847 b
6 days	34.7277 a	39.6324 ab	44.9483 ab	54.872 b	76.2745 c
8 days	32.2222 a	39.6296 ab	47.037 bc	56.6667 c	77.4074 d
10 days	31.1111 a	37.7778 ab	47.037 bc	55.9259 c	76.5556 d
(A3)					
2 days	42.931 a	49.1879 ab	74.3817 bc	61.7017 c	97.656 d
4 days	40.1635 a	45.5111 a	67.8298 b	67.2783 b	77.5292 b
6 days	43.9499 a	41.1766 ab	58.1425 bc	71.7254 cd	77.0596 d
8 days	41.8519 a	41.8519 a	60 b	72.5926 c	78.5185 c
10 days	38.1481 a	40 a	58.7037 b	70.5556 c	77.963 c

The isolate A1 revealed the highest level of sensitivity to the fungicide in comparison with A2 and A3 as well; however, Tachigazole expressed high level of variations in terms of inhibition rate among the isolates A1 and A2. The concentration 75ppm expressed high level of inhibition rate especially among the isolates A1 and A2 (Table 2). At some extend the fungicide had an accumulative effect exemplified by increasing inhibition rate over time which was differ from Bavistin treatment.

The lowest IC50 was at A3 isolate with 12.21ppm in the Bavistin treatment and the highest was at A2 isolate with 32.99ppm (Table 3). The lowest IC50 in Tachigazole treatment was at A1 isolate with 17ppm and the highest was at A2 as well with 30ppm expressing fussy behavior by A2 isolate. The fungicide Bavisitin scored the lowest IC50 especially at A3 isolate which expressed higher effect to control *A. radicina* in vitro.

Table 3. The lowest concentration scores 50% inhibition rate among the three used isolates A1, A2 and A3.

Isolate	IC50- Tachigazole	CL- Tachigazole	IC50-Bavistin	CL- Bavistin
A1	17	(10.3-25)	19.67	(10.1-30.611)
A2	30	(19-45)	32.99	(19.37-53.73)
A3	17.52	(8.48-27.54)	12.21	(1.3-25)

DISCUSSION

The study results revealed that both used fungicides were effectively inhibited radial growth of *A. radicina* colonies in vitro. However, Bavistin fungicide expressed marginally higher effect than Tachigazole. Kumar et al. (2013) found that Bavistin scored the highest inhibition rate controlling *Alternaria* leaf spot on chili in vitro. Bavistin showed high controlling rate of *Alternaria* spp via treating seeds especially seed borne ones (Islam *et al.,* 2007). Moreover, Van Nghiep and Gaur (2005) mentioned that treating rice seeds with Bavistin can improve seeds quality and inhibit seeds borne fungi. Rai and Mamatha (2005) also reported that Bavistin expressed high level of fungal growth inhibition both in vitro and vivo especially on seedlings of some forests trees.

Tachigazole also showed high level of inhibition rate of *A. radicina* colonies in vitro especially at higher concentration. Al-Taae and Al_Taae (2010) found that Tachigazole can manage *Verticillium dahliae* Kleb on olive inhibiting fungal growth remarkably. Alwan (2011) also reported that Tachigazole inhibits Fusarium oxysporum f.and *Rhizoctonia solani* growth in vitro.

This is the first record of *A. radicina* the causative pathogen of black rot on carrot and some other crops on date palm trees especially leaves in Wasit province in the middle of Iraq; however, It was recorded in Basrah by (Ahmed 2011). Emerging this pathogen on date palm leaves can be explained in many different ways: one way, it was imported to Iraq via date palm seedlings. Another, explanation is gene development by *A. radicina* that is already excited in Iraq but did not find its specific host. Moreover, changing climate in Iraq and the region especially in the last five years exemplified by the increase of rains, temperature, and CO_2 level might induce the pathogen to find another suitable host.

Further studies should focus on screening all Iraqi provinces that planting date palm trees to investigate the incidence of *A. radicina* and estimating its economically importance on date palms. Moreover, establishing plant physiological study studying the relationship between *A. radicina* preferable host (carrot) and date palms.

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