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Efficacy of Fungicides for *In vitro* Control of Date Palm Black Scorch Disease Agent (*Thielaviopsis paradoxa*)

Awadalla I. A. Irabi[1], Mohamed Y. A. Abubaker[1*] and Siddig M. Elhassan[2]

[1]Department of Pests and Plant Health, College of Agriculture, University of Bahri, Alkadro, Sudan.
[2]Department of Crop Protection, Faculty of Agriculture University of Khartoum, Shambat, Sudan.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Seven fungicides (Bayleton, Benlate, Tilt, Vitavax, Antracol, Copper-oxychloride and Soufrel) were evaluated for the *in vitro* chemical control of *Thielaviopsis paradoxa* (de Seynes) Von Höhn. isolated from symptomatic date palm fronds showing typical symptoms of black scorch disease. The pathogen growth was assessed by inoculating plates containing potato dextrose agar (PDA)-fungicide mix at 10, 50 and 100 ppm a.i. of the respective fungicides with plugs from 4-day-old fungus culture. All tested fungicides caused significant reductions in the mycelial growth of the pathogen with significant variations in their efficacies. The systemic fungicides were found to be superior to the non-systemic ones and their inhibitive effective, with the exception of Bayleton, was profoundly stable. The ED_{50} values indicated that Benlate, Tilt and Vitavax ranked on the top (ED_{50} = 4 and 5 ppm), Bayleton and Antracol were intermediate (ED_{50} = 14 and 20 ppm respectively) while Copper- oxychloride was the least efficacious fungicide in this respect (ED_{50} = 100 ppm). However, the antifungal activity of all tested fungicides appeared to be fungistatic since the treated fungus presumed growth in few to several days of incubation in clean PDA plates.

**Corresponding author: E-mail: moyousif10@hotmail.com, abubaker.mohamed@gmail.com;*

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1. INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is one of the oldest and most important fruit trees known in the world and one of the first crops domesticated [1,2]. The exact date in which the date palm was introduced to Sudan is not known [3]. Its cultivation is generally of a traditional type, characterized by almost complete absence of operations such as pollination, male selection, propagation, thinning, fertilization, irrigation and pest control. In Sudan, up-to-date literature about date palm diseases situation is meagre if not lacking. Some of the diseases observed in previous surveys include disease such as Abushaiba (suspected phytoplasma) and black scorch in Northern regions, Graphiola disease in the wetter parts of the country and some other minor diseases [4,5].

Black scorch disease is caused by the fungus *Thielaviopsis paradoxa* (De Seynes) Höhn or *T. punctulata* (Hennebert), A. E. Paulin, T. C. Harr. and Mc New [6] These two species of fungi are soil- borne wound pathogens that can attack the different parts (leaves, inflorescence, heart, trunk or bud) of the date trees causing them to rot [7,8]. The Fool's disease or Medjnoon stage of black scorch has been somewhat frightening to date palm growers and agriculturists alike in several scattered parts of the Northern States of Sudan, where the main growing areas of date palm occur. It shows, in case of terminal bud and heart attack, a characteristic bend in the region of infection (this is, why it is called the 'Medjnoon' or 'fool's' disease). The disease is an important constraint facing the date palm industry, with losses of >50% in newly planted offshoots and fruit [9,10].

Taking in mind the date palm disease situation in Sudan, and the great importance of date palm, the present study was undertaken to test *in vitro* the efficacy of several fungicides, in controlling the pathogen.

2. MATERIALS AND METHODS

2.1 The Pathogen

The fungus *Thielaviopsis paradoxa* (de Seynes) Von Hohn. was isolated from infected date palm fronds that showing typical symptoms of black scorch disease. Its identity was confirmed based on its cultural characteristics and the microscopic examination of its two spore forms, microconidia and macroconidia in addition to pathogenicity test on sugarcane [11]. Pure culture of the pathogen was maintained on potato dextrose agar (PDA) plates incubated at 26°C.

2.2 Pathogenicity Test

Healthy, treated sugarcane seed pieces (supplied by Kenana Sugar Company, Sudan) were randomly pricked with sterile needle, dipped for two hours in an inoculum already prepared by blending one Petri Plate of 7 days old pure culture of date palm-isolate. Setts after being removed from the inoculum were immediately planted. Another healthy seed pieces were also pricked and planted without dipping them into the inoculum to serve as control. Severity of infection was classified as follows: lack of bud germination was considered a highly severe; die back of shoots as severe infection; retarded growth of shoots as moderately severe; and healthy-looking growth shoots considered as disease escape setts.

2.3 Preparation of Fungicide-PDA Medium Mix

Using sterilized distilled water, stock concentrations of each of the seven fungicides listed in Table 1 were prepared. Aliquots of each concentration were added to warm potato-dextrose agar (PDA) to have final concentrations of 10, 50 and 100 ppm a.i., respectively. About 15 ml of the mix per plate were used for each concentration to evaluate *in vitro* the efficacy of the fungicides in inhibiting mycelial growth of *T. paradoxa* on PDA. When solidified, a plug of 4-day-old culture of the pathogen was removed by a cork borer (4mm in diameter) and placed at the centre of each plate. Fungicides-free plates (only PDA) were also inoculated with similar pathogen plugs to serve as controls. Three replications per treatment were used, incubated at 26°C and measurements of the mycelium radial growth were taken daily for a period of 5 days (mycelial growth in the control plates had covered the entire agar surface).

Table 1. Fungicides evaluated *In vitrro* against *Thielaviopsis paradoxa* on potato-dextrose agar (PDA)

Trade name	Common name	Chemical name
Systemic fungicides		
Bayleton	Triadimefon	1-(4-chlorophenoxy)-3,3-dimethyl-1(1*H*-1,2, 4-triazol-1-yl) butanone
Benlate	Benomyl	Methyl 1-(Butyllcarbamyl 1)-2-benzimidazole carbamate
Tilt	Propiconazole	-1-[2-(, 4-dichlorophenyl)-4propyl-1, 3-dioxolan-2ylmethyl]-*H*=1, 2, 4-triazole
Vitavax	Carboxin	5, 6-dihydro-2-methyl-1, 4-oxathin-3-carboxanilide
Non-systemic fungicides		
Antracol	propineb	Polymericzinc propylenebis
Copper-oxychloride	Copper-oxychloride	Dicopperchloride trihydroxide
Soufrel	Sulphur	sulphur

Source: [12]

2.4 Evaluation of Efficacy of Different Doses of the Test Fungicides

a) The Percent inhibition data of the mycelium growth in response to fungicide treatment were calculated each day during the experiment using the formula: 1 = (A–B/A) × 100

Where,

1 = percent inhibition of mycelial growth
A = radial mycelial growth of the control
B = radial mycelial growth of the treatment

The results were analyzed statistically using Duncan's Multiple Range Test. They were also illustrated in time growth inhibition curves using 10 ppm of systemic and non-systemic test fungicides.

The stability during the experiment of the inhibitive effect of the different fungicides can be inferred from the calculation of the percent drop in the inhibitive effect of the fungicide under test, as follows:

% drop in inhibitive effect of = (%inhibition at day1-% inhibition at day5×100/ the fungicide (DI) %inhibition at day1)

b) ED_{50}: Probit mycelial inhibition data were estimated from the graphical probit line of the different fungicides under test. These values represent the fungicides doses required to inhibit 50% mycelial growth of the pathogen.

2.5 Assessment of Antifungal Activity of the Test Fungicides

In order to determine whether the antifungal activities of the seven test fungicides fungistatic or fungicidal, mycelial plugs which showed no growth at the end of 72 h incubation in PDA fungicide mix were transferred to fungicide-free PDA plates and left to grow for further 5 days at 26°C. These plates were then examined for mycelium growth, and accordingly the test fungicides were classified as fungistatic (plugs resumed growth) or fungicidal (plugs couldn't resume growth).

Regarding the effect of the concentration on the stability of the fungicide, over time, two groups can be distinguished: In the first group, the performance of the fungicides was stable over the period of test irrespective of the concentration. This group includes the systemic fungicides Benlate, Tilt and Vitavax. The second group which showed reduction in stability over time includes the systemic fungicides Bayleton and all the non-systemic fungicides.

3. RESULTS

Sugarcane seed pieces previously inoculated with date palm-isolate showed, when up Rooted, the typical symptoms of pineapple disease in sugarcane, which is also caused by the fungus *Thielaviopsis paradoxa*. The symptoms consisted of broken parenchyma, hollow blackened interior and a mass of black cottony fungal growth on the sett ends (Plate 1A). Not less than 85% of the inoculated setts contracted the disease; of which, 64.3% were very severely infected, 14.3% with severe infection and about 7.1% of the setts showed stunted or retarded growth. About 14.3% were apparently healthy (Plate 1B).

Table 2. Inhibitive effect of different fungicides assessed at different concentrations (10, 50, 100 ppm) on mycelium radial growth of *Thielaviopsis paradoxa* on PDA

Fungicides		Conc. (ppm)	% inhibition of mycelium radial growth day				
			1	2	3	4	5
Systemic fungicides	**Bayleton**	10	61.0e	57.0e	45.0e	36.0de	29.0e
		50	87.0b	70.0dc	67.0c	61.0cd	56.0bc
		100	98.0a	77.0c	77.0b	72.0bc	58.0bc
	Benlate	10	100a	100a	100a	100a	100a
		50	100a	100a	100a	100a	100a
		100	100a	100a	100a	100a	100a
	Tilt	10	100a	99a	92a	90a	88a
		50	100a	100a	100a	100a	100a
		100	100a	100a	100a	100a	100a
	Vitavax	10	100a	100a	95a	92a	86a
		50	100a	100a	100a	100a	100a
		100	100a	100a	100a	100a	100a
		10	42d	38f	34f	30de	19ef
		50	99a	86b	67c	56cde	47cd
		100	100a	91ab	80a	72bc	67b
	Copper-oxychloride	10	29e	25g	16h	8f	4gh
		50	33ed	27g	16h	13f	11fgh
		100	33ed	27g	23gh	18ef	15fg
	Soufrel	10	33ed	25g	20h	10f	0.0h
		50	62c	41f	30gf	22ef	15fg
		100	83b	65ed	58d	50de	42d

Means followed by similar letter (s) within the same column are not significantly different from each other at 0.05 level of probability, according to Duncan's Multiple Range Test

In comparison with the control, all the tested fungicides even at 10 ppm significantly reduced the mycelium radial growth of *Thielaviopsis paradoxa* (de Seynes) Von Hohn. (Table 2, Fig. 1 and Plate 1).

With the exception of Bayleton, the inhibitive effect of the systemic fungicides namely, Benlate, Vitavax and Tilt on the pathogen growth was significantly greater than that of the non-systemic ones, namely Antracol, Soufrel and Copper-oxychloride (Table 2, Fig. 1 and Plate 1). Among the latter group, Antracol was significantly more effective than Soufrel and Copper-oxychloride, while Bayleton was significantly the least effective compared to the tested systemic fungicides. No significant differences were found between concentrations of the three superior systemic fungicides; but it was demonstrated for Bayleton, Antracol and Soufrel (Table 2).

The inhibitive effect (IE) of the systemic fungicides, except for Bayleton, was profoundly stable during the experiment period, particularly at higher concentrations. The most stable inhibition was produced by Benlate which showed no drop in the IE with time. The percent drop in inhibitive effect of the remaining two systemic fungicides remained rather low at 10 ppm (12 – 14%) and declined to zero at 50 ppm. However, the drop in IE was remarkably high (52%-100%) for the non-systemic fungicides, being least in Antracol (Table 3). The variation in the time-growth inhibition relationships were significant while the inhibition started extremely high and remained so far systemic fungicides with the exception of Bayleton, it was comparatively rather low at the beginning and declined progressively for non-systemic ones (Fig. 1).

The efficacy of these fungicides measured by the ED_{50} values (concentrations required to inhibit 50% mycelial growth) indicated that Benlate (4 ppm) followed by Tilt and Vitavax (5 ppm) were the most efficacious, while Bayleton and Antracol (14 ppm and 20 ppm, respectively) were intermediate, and Copper-oxychloride was the least effective (100 ppm) (Table 3 and Fig. 2).

The antifungal activity of all tested fungicides appeared to be fungistatic. For all fungicides tested, the treated fungus presumed growth

3 days after its transfer to PDA free from the corresponding fungicide except for Benlate treatment which presumed growth after 5 days.

Plate 1A. Sett with blackened, hollow, broken parenchyma cells due to infection with *Thielaviopsis paradoxa* 4 weeks post inoculation (middle sett) the upper and lower sets are control

Plate 1B. Pathogenicity of *Thielaviopsis paradoxa*-date palm isolate on sugarcane. Left: severely infected sett indicated by failure of germination. Middle: non-treated sett showing vigorous shoot and root growth. Right: infected sett showing very weak growth

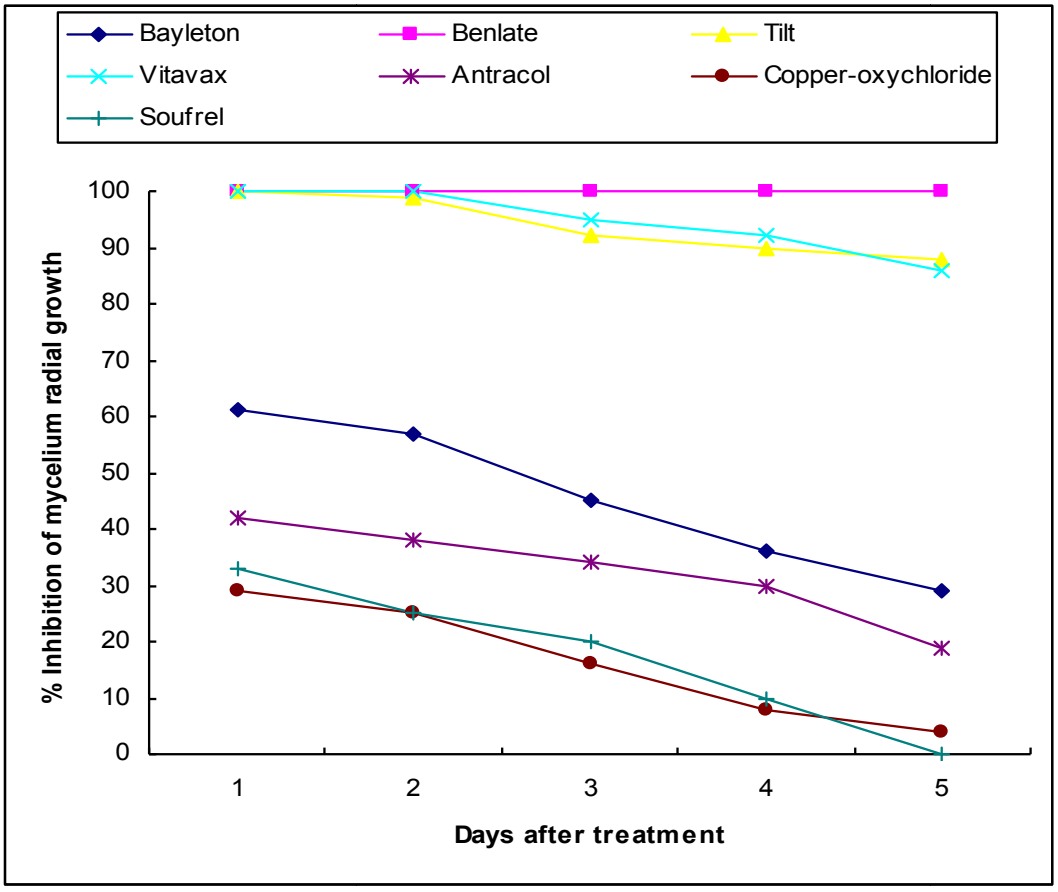

Fig. 1. Time-growth inhibition relationships of systemic and non-systemic Fungicides (10 ppm) applied against *T. paradoxa* (de Seynes) Von Hohn

4. DISCUSSION

The *in vitro* chemical control data revealed that, Benlate, Vitavax and Tilt (systemic fungicides) were potent fungicides that almost completely inhibit the mycelial growth of *Thielaviopsis paradoxa* (de Seynes) Von Hohn.– date palm isolate, while Bayleton (systemic), Antracol, Soufrel and Copper-oxychloride (non-systemic fungicides) were significantly less efficacious at the same concentrations. Other investigators [13,14,15] have reported similar results with the systemic fungicides (Benlate, Vitavax and Tilt). The stability of the inhibitive effect (IE) data and the ED_{50} values clearly indicated the potency of the first group. For instance, the ED_{50} ranged between 4 to 14 ppm for systemic fungicides and up to more than 100 ppm for contact fungicides.

These *in vitro* results have been substantiated in vivo by the encouraging results obtained from a crash experiment in which some symptomatic trees severely affected by the disease in Khartoum city were sprayed with Tilt (10 ppm, 3 applications). The treated trees produced healthy inflorescence and beard normal fruits in the next year.

Table 3. Grouping according to ED_{50} values of 7 fungicides applied against *Thielaviopsis paradoxa*-date palm isolate

Fungicide	ED_{50} (ppm)[1]
Systemic fungicides	
Benlate	4.0
Tilt	5.0
Vitavax	5.0
Bayleton	14.0
Non-systemic fungicides	
Antracol	20.0
Soufrel	79.0
Copper-oxychloride	›100.0

[1] *ED_{50} values were obtained graphically using probit line*

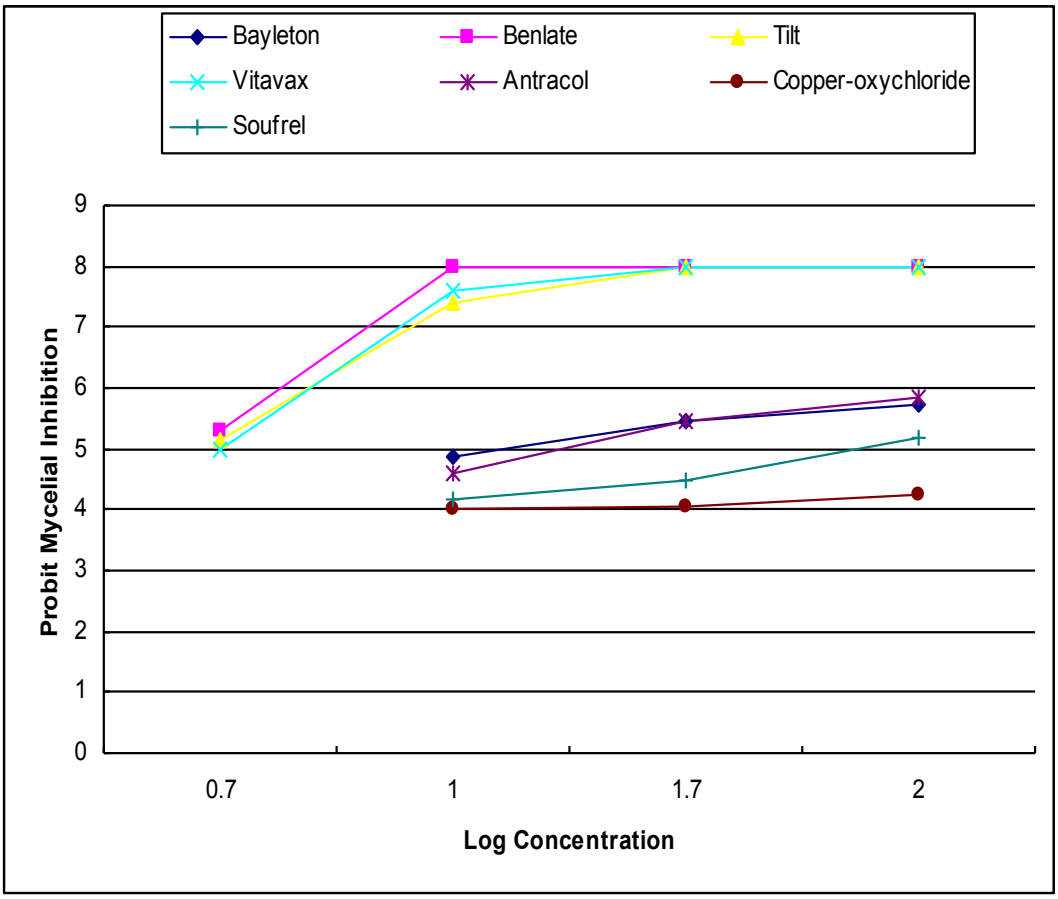

Fig. 2. ED_{50} (concentrations required to inhibit 50% mycelial growth values for systemic and non-systemic fungicides tested *in vitro* against *Thielaviopsi paradoxa* obtained graphically using probit line

Plate 2. Inhibitive effect of seven fungicides on mycelial growth of *Thielaviopsis paradoxa* (de Seynes) Von Hohn. using 10 ppm a) and b) 100 ppm of the fungicides under test: Upper row: from left to right: Control, Benlate, Vitavax and Tilt. Lower row: From left to right: Copper-oxychloride, Soufrel, Antracol and Bayleton

The present study also demonstrated the fungistatic antifungal activity of the fungicides under test. This was indicated by the ability of the fungus inoculum to resume growth in clean PDA plates. Similarly, [16] working in the control of potato black scurf disease found that Benlate and Carboxin (Vitavax) have fungistatic activity against *Rhizoctonia solani.* However, as almost all systemic fungicides are site-specific [17], many target fungi through simple mutation become resistant to each frequently used systemic fungicide within a few years of introduction of the compound. To avoid abandonment of a systemic fungicide after appearance of a pathogen strain resistant to it, the fungicide must be used in combination with another broad-spectrum contact fungicide. Thus, it may be advisable to try using Antracol under various schemes of application with the above mentioned efficacious systemic fungicides to suppress or curtail the spreading epidemic of black scorch singly or combined with other destructive diseases of date palm such as Ganoderma disease. However, based on the fungistatic nature of the fungicide-pathogen interaction, the treatment needs to be repeated and frequency of application to be optimized.

5. CONCLUSION

The systemic fungicides Benlate, Vitavax, and Tilt were most inhibitive to in vitro fungal growth. The isolate obtained from the date palm seemed to be severely pathogenic 0n to sugarcane. The

significant implication of this is that date palms which grow near the sugarcane schemes in Sudan may constitute a serious threat to sugarcane industry by harboring the pathogen inoculum.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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