Efficacy of Scabdel70% and GoldenCopper 50% Fungicides on Phytopathogenic Fungi Isolated from Guava Leaves

Abstract

Six fungal species (*Alternaria atra*, *Botryotrichiumverrucosum*,*Cochliobolusspecifer*, *Drechslerahalodes*, *Humicola grisea* and *Stachybotryschartarum*) were isolated from infected guava leaves by direct plate method on PDA medium at 28 °C. Pathogenicity test illustrated only two species were pathogenic to guava leaves and *B. verrucosum* exhibited moderate virulent ability but *C. specifer* was weak virulent. Half of fungal species were pathogenic to apple fruits indicating that species were non host specific to guava plant.Scabdel fungicide completely inhibited the growth of *B. verrucosum*at 50 ppm, but in case *C. specifer*the inhibition ranged 18.8- 33.3 % with doses increase. Golden Copper was stimulatory to the growth of tested fungi at 50-1000 ppm doses.

Key words: Guava, pathogenicity, fungicides.

1. INTRODUCTION

Guava (*Psidium guajava* L.) is an important fruits cultivated in several countriesaround the worlds[1]. It contains carbohydrates, protein and adequate amounts of vitamin C and some minerals [2,3]. Due to its highly nutrition value and low price, guava considers the most popular fruits. In addition, it is used in folk medicine like treatment of coughs, dental pain and diarrhea [4]. Guava trees are susceptible to attack with more than 170 pathogens [5]. Several literatures were documented different diseases on guava trees e.g; damping off and blight of seedling, anthracnose, stem canker, leaf spots and blight, smootymould and rots of fruits [6,7,8]. Among these diseases, leaf spots were consider mainly fungal infection incited by*Alternaria alternata*[9],*Alternaria tenuissima*[10];*Cercosporapsidii*,and *Cercosporasawadae*[11] and *Pestalotiapsidii* [4]; or algal infection incited caused by *Cephaleurosvirescens*[12].

There are different methods used for management of phytopathogenic fungi on cultivated crops including the use of fungicides [13]. The fungicides showed variable effect with different fungal species. Özer et al. [14] reported that seven from nine

fungicides had inhibitive influence on mycelial growth of *Botrytis cinerea* isolates. Also, Equation Pro and Kema Zed showed inhibitory effect on mycelial growth and hydrolytic enzymes produced by Broad bean leaves pathogens [15]. Recently; Abdel-Wahed [16]studied the effect of fungicides (Topsin M 70% and Vitavax 200 75%) and other compounds on the growth of*F.oxysporum,R.solani*and *F. solani*,he concluded that the fungicides were the superior treatment in controlling the pathogens as compared with other compounds.

This work aimed to identity the fungal species from infected guava leaves, evaluated their virulence on guava leaves and apple fruits and controlled it by two fungicides application.

2. MATERIAL METHODS

2.1 Collection of Guava Leaves

Fifteen samples of infected guava leaves were collected from a south valley university farms during May 2023. Each infected sample was putted in plastic bag and transferred to mycological lab and stored at 5 °C until further analysis.

2.2 Isolation of mycobiota from infected Guava leaves.

Direct plate method was employed for fungal species isolation on Potato dextrose Agar (PDA) medium for 5-7 daysat 28°Cas described by Abdel-Fatthah et al. [17]. The developed colonies were purified and stored on PDA slant for further studied. The morphological identification was performed based on macro and microscopic features.

2.3 Pathogenicity Test

The virulence of fungal species (*Alternaria atra*, *Botryotrichiumverrucosum,Cochliobolusspecifer*, *Drechslerahalodes*, *Humicola grisea* and *Stachybotryschartarum*) were tested on two resources.

Firstly on detached guava leavesas described byLi et al. [18] with some modifications. Healthy appearance and freshly guava leaves were washed under tape water then sterilized by 70% ethanol.Leaves were cut into pieces and placed in petri dish containing three filter papers. Leave pieces were wounded using sterile needle and inoculated by fungal disc cut from the margin of 1weekold cultures. The control was inoculated with an agar plug without the fungus. Ten ml of distilled water was added to each plate, and then plates were incubated at 28±1 ºC for seven days[18]. The

developed lesions were determined. Fungal re-isolation from developed lesions was performed according to Koch's postulates.

Secondly the Aggressiveness of isolated fungi was evaluated on apple fruits as follows; healthy and uniform apple fruits was surface sterilized by Sodium hypochlorite (1%) for 3-5 min, then rinsing in sterilized distilled water for twice and let dry under sterilized condition, Active growing fungal disc inserted into holes made in sterilized apple fruits as described byBaiyewu et al.[19] and Chukwuka et al.[20]. The control wascarried out by using sterile PDA. The inoculated fruits were putted in polyethylene bags and incubated for 2 weeks at 28 °C. After incubation period, virulence of tested fungi was estimated by measuring the diameter of lesion. All experiments were conducted in triplicate.

2.4 Effect of Fungicides on *Botryotrichiumverrucosum* and*Cochliobolusspecifer*Growth.

The efficacy of two fungicidesScabdel 70% and Golden Copper 50% were tested against *B. verrucosum* and*C. specifer*growth. Their chemicalnames, active ingredient, manufacturesand agricultural uses are shown in Table (1).For this purpose, Different doses of fungicides (50, 100, 500 and 1000 ppm) were incorporated to autoclaved and cooled (about 50 °C) PDA medium, Then active growing disk were extracted from 7 day colonies and placed in the center of PDA medium plates containing fungicide dose. PDA medium without fungicides were used as control. The plates were placed in incubator at 28°C[21]. The Radial fungal growth was determined after 7 days of incubation and the percentage of inhibition was determined by Equation % Ix= [Xc-Xi / Xc] x 100 [22].where:% Ix: percentage of radial inhibition., Xc: mean radius (mm) of the control colony and Xi: mean radius (mm) of colonies in media withfungicide.

2.5 Statistical Analysis

Data were analyzed using one way ANOVA and statistically significant values were considered at $P< 0.05$.

3. RESULTS

3.1 Mycobiota recovered from infected guava leaves.

Six fungal species attributed to six fungal genera namely; *Alternaria atra*, *Botryotrichiumverrucosum*, *Cochliobolusspecifer*,*Drechslerahalodes*,*Humicola grisea* and*Stachybotryschartarum* were collected from guava diseased leaves on

plates of Potato Dextrose Agar medium. From which, *Botryotrichiumverrucosum*, *Cochliobolusspecifer*and*Humicola grisea* were recovered from 6.6%, 6.6% and 13.3% of the matching 16.6% of total fungi for each (Table 2).

3.2 The Pathogenicity of Isolated Fungi

The virulence of *Alternaria atra*, *Botryotrichiumverrucosum*, *Cochliobolusspecifer*,*Drechslerahalodes*,*Humicola grisea* and*Stachybotryschartarum* was evaluated on guava leaves and apple fruits. The obtained data indicated that *B. verrucosum* and *C. specifer*were successed in lesion production on guava leaves after 7 days of incubation. *B. verrucosum*showed moderate virulence capacity with lesion less than 50% from leaf area but in case *C. specifer*, the virulence capacity was low with lesion less than 25% from leaf area.The remaining four species failed to give any detectable disease symptoms on guava leaves (Table 3 and Fig.1).

Exactly 50% of tested fungi (*B.verrucosum*, *C. specifer* and *D. halodes*) exhibited virulence capacity on apple fruits with different degrees. The highest fruits infection (30 mm) was observed on *B.verrucosum* followed by *C. specifer*(20 mm) and the lowest necrosis was achieved by *D. halodes*(12 mm).The success of some tested fungi to produce necrosis symptoms on apple fruits meaning these species are not host specific to guava plant (Table 3 and Fig. 2)..

3.3 Effect of Fungicides on *Botryotrichiumverrucosum* and*Cochliobolusspecifer*Growth.

Four doses (50, 100, 500 and 1000 ppm, active ingredients) from two fungicides (Scabdel70% and Golden copper 50%) were used to estimate the effect of fungicides on *B. verrucosum*and*C.specifer*growth.

Scabdel 70% exhibited strong inhibition activity on *B. verrucosum* growth; the fungicide at concentration (50 ppm) completely stopped the fungal growth. The growth of*C. specifer* greatly inhibited by Scabdel doses and the inhibition rate increased with increasing fungicide concentration from 50-1000 ppm.*B. verrucosum*greatly influenced by scabdel than *C. specifer*(Table 4 and Figs. 3, 4)

The data illustrated that golden copper 50% has non inhibitory effect on the growth both tested fungi, on the contrary the growth of *B. verrucosum*and *C. specifer* were significantly increased by increasing fungicide dose from 50-1000 ppm (Table 4 and Figs. 3, 4).

4. DISCUSSION

Six fungal species were isolated from infected guava leaves symptomatic with leaf spots and the highest fungal count was achieved by *Botryotrichium verrucosum*, *Cochliobolusspecifer*and*Humicola grisea*. Pandey et al.[23] studied the mycoflora of guava from bud stage to leaf fall stage in different seasons and he conducted that *Colletotrichum gloeosporioides*, *Fusariumoxysporum* f. sp. *psidii*, *Pestalotiapsidii* and *Phomapsidi* were the most potent pathogenic species during all seasons.In Egypt, Youssef et al. [24]isolated *Alternaria alternata*, *Lasiodiplodiatheobromae*, *Fusarium semitectum*and *Pestalotiapsidii* from the dropped guava.

Our results conducted that*B. verrucosum*and *C. specifer*successed in production of disease symptoms on guava leaves and the*B. verrucosum*was the most virulent than *C. specifer*. Youssef et al.[24] evaluated disease intensity on petioles and fruits of guava after artificial inoculation with *Alternaria alternata*,*Lasiodiplodiatheobromae*, *Fusarium semitectum*and *Pestalotiapsidii*, they concluded that the highest disease incidence on both petioles and fruits was achieved by *L. theobromae*. The inoculation of guava leaves with *Pestalotiapsidii*resulted in typical leaf spot symptoms [4]. Song et al. [10] noticed that wilt and necrosis symptoms appeared on guava seedling after wound inoculation with *Alternaria tenuissima*.

Scabdel 70% strongly affected the radial growth of*B. verrucosum*and *C. specifer*but on the hand Golden Cooper 50% has positive altitude. This finding is fully agreement with Özer et al.[14] he evaluated the effect of nine fungicides on the mycelial growth of *Botrytiscinerea*isolates and they concluded that all tested fungicides except Triadimentol and Tebuconazol showed inhibitory effect with different degrees. Abdel-Kader et al.[25] illustrated that, Euparen fungicide had nil effect on the growth of *Alternaria alternata*, *Fusarium monilifome*, *Myrothecium verrucaria* and *Thermoascusaurantiacus* at 4.8, 23.8 and 47.5 ppm concentrations. Also, Vacomilplus 50% (Copper oxychloride +Metalaxyl) at concentrations 50-200 ppm not exhibited any detectable effects on *Lasiodiplodiatheobromae*and *Fusarium semitectum* growth [24]. Abdul Wahid [26] study the effect of two fungicides (Rovral and Sumislex) on the growth offive isolates of*Colletotrichum gloeosporioides*causing guava anthracnosein Egypt, he claimed that all tested isolates were strongly affected with two fungicides used. The efficacies of fungicides toward pathogenic fungi were varied and affected with fungal species and fungicide dose[27]. Saleem et al.[15] conducted that, the mycelial growth *A. alternata* was stimulated at lower dose (50 ppm) of Aquation Pro and Kema Zed fungicides and reduced at higher concentrations.

5. CONCLUSION

Scabdel 70% was the most active fungicide against *Botryotrichium verrucosum* and *Cochliobolusspecifer* infected guava leaves.

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Table1. Fungicides used, their chemical names, active ingredients, manufacturers and agricultural uses.

	Scabdel 70%	Golden copper 50%
Chemical name	Dimethyl 4,4-(o-phenylene) bis (3 thioallophanate)	Copper oxychloride
Active ingredient	Thiophanate Methyl 70%	Copper oxychloride 50%, Cymoxanil 4%.
Manufactures	Wuxi Xinan, China	Aristo, India
Agriculture uses	Systemic fungicide used to control many plant diseases including: Powderly mildews, scab, leaf spots and rot of fruits and roots.	Systemic fungicide used to control many plantdiseases including: Early, late blight, downy mildews, Scab rot and fall of fruits.

Table 2. Total counts (TC), percentage count (%C) and percentage frequency of isolated fungi on Potato Dextrose Agar medium at 28 °C.

Fungal species	TC	% C	%F
Alternaria atra	1	8.3	6.6
Botryotrichium verrucosum	2	16.6	6.6
Cochliobolusspecifer	2	16.6	6.6
Drechslerahalodes	1	8.3	6.6
Humicola grisea	2	16.6	13.3
Stachybotryschartarum	1	8.3	6.6
Sterile mycelia	3	25	
Total count	12		

Table 3. Pathogenicity level of tested fungi on detached guava leaves and apple fruits.

Fungal species	Pathogenicity rate	
	Guava leaves	Apple fruits (in mm)
Alternaria atra	ND	ND
Botryotrichium verrucosum	++	30.5
Cochliobolusspecifer	+	20
Drechslerahalodes	ND	12
Humicola grisea	ND	ND
Stachybotryschartarum	ND	ND

+: Weak virulent (lesion less than 25% of leaves area); ++: moderate virulent (lesion 25-50% of leaves area)

Table 4. Effect of Scaddel70% and Golden Cooper 50% fungicides on the growth (in mm) of *Botryotrichium verrucosum* and *Cochliobolusspecifer*.

Fungicide dose	Scabdel 70%		Golden copper 50%	
	B. verrucosum	***C. specifer***	***B. verrucosum***	***C. specifer***
Control	43	45	43	45
50	0*	36.5*	52*	46
100	0*	35*	57*	65*
500	0*	31.5*	65*	70*
1000	0*	30*	71*	73.5*
Inhibition (%)	100	18.8 - 33.3	-20.9-(-65.1)	-2.2-(-63.3)

*: Significant difference from the control.

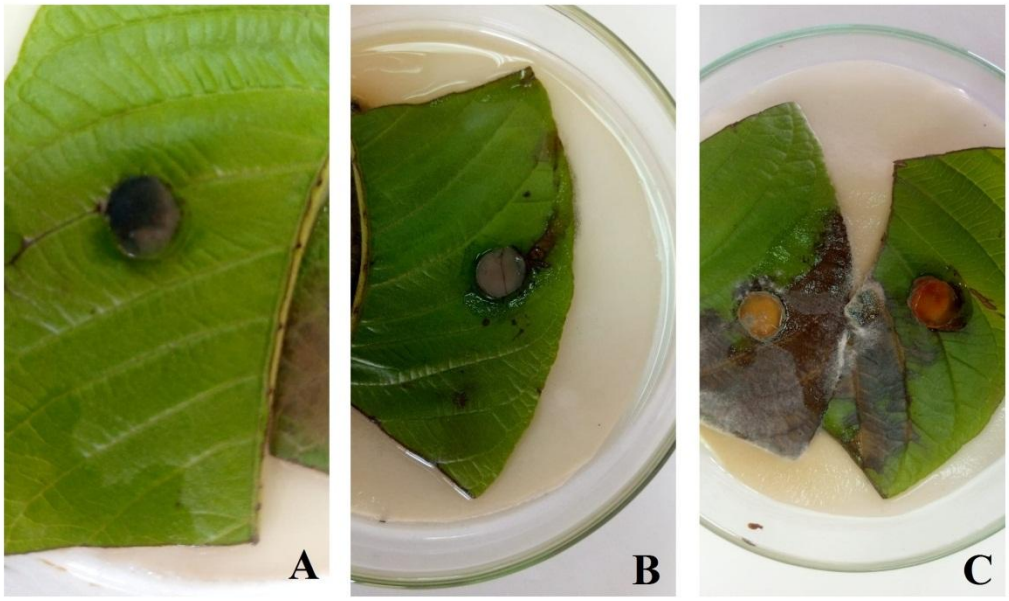

Fig. 1. Disease symptoms on guava leaves after 7 days of incubation; A: control; B: weak virulent (*C.specifer*)and C: moderate virulent (*B.verrucosum*).

Fig. 2. External symptoms of infection resulted from tested fungi on apple fruits after 15 days of incubation, A: control, B: *A. atra* (nonpathogenic), C: *D. halodes*, D: *C. specifer*and E: *B.verrucosum*

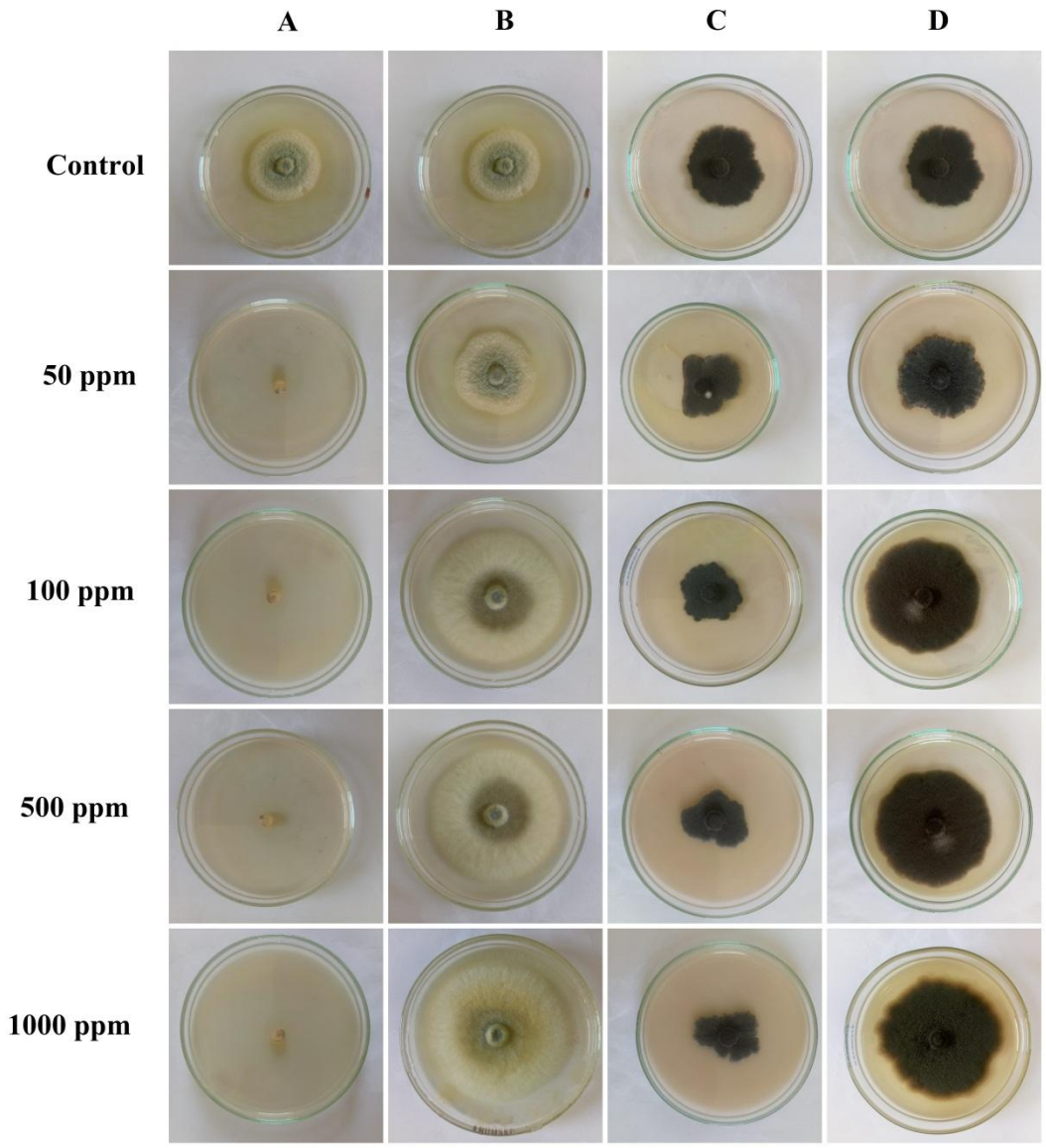

Fig. 3. Colony diameter of *B.verrucosum*and*C.specifer* on different concentration of fungicides, A: *B.verrucosum* on Scabdel, B: *B.verrucosum*on Golden Copper, C: *C.specifer* on Scabdel and D: *C.specifer*on Golden Copper.

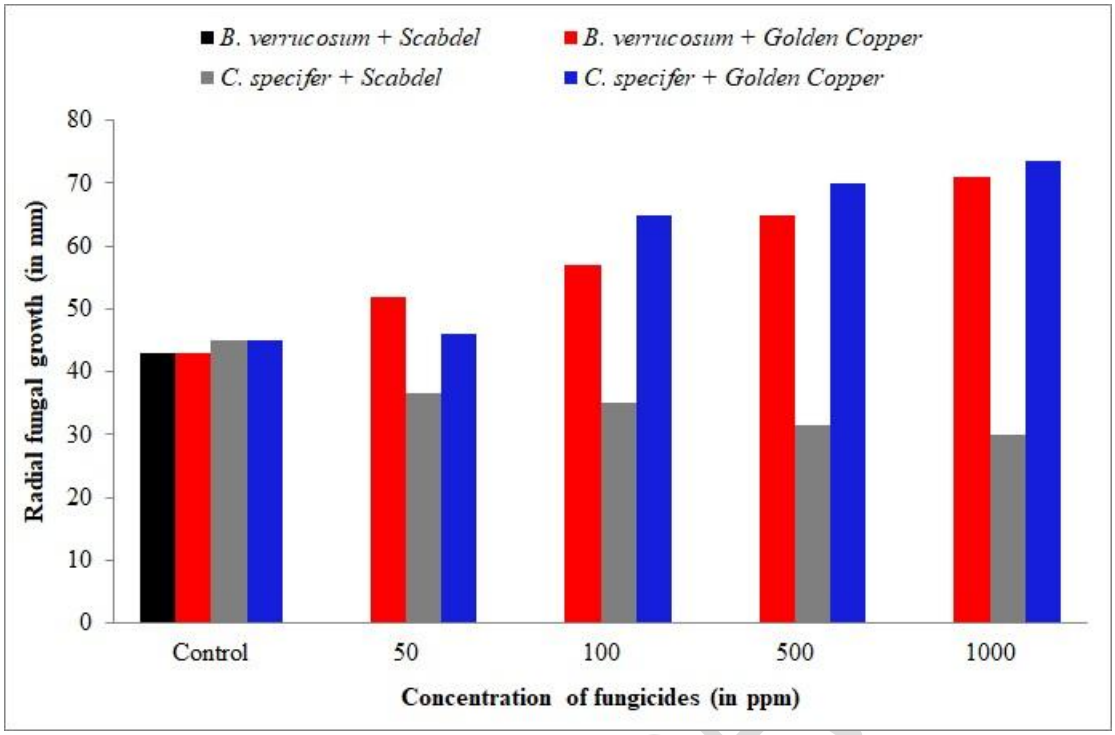

Fig. 4. Effect of different concentration of Scabdel and Golden Copper fungicides on *B. verrucosum*and*C. specifer* growth.