Efficacy of Scabdeland GoldenCopper Fungicides on PhytopathogenicFungi Isolated from Guava Leaves

Abstract

The objectives of study identify the fungal species from guava leaves symptomatic with leaf spots. Six fungal species (*Alternariaatra*, *Botryotrichiumverrucosum*,*Cochliobolusspecifer*, *Drechslerahalodes*, *Humicolagrisea* and *Stachybotryschartarum*) were isolated from infected guava leaves by thedirect plate method on PDA medium at 28 °C. Pathogenicity test illustrated thatonly two species were pathogenic to guava leaves, and *B. verrucosum* exhibited moderate virulent ability, but *C. specifer* was weakly virulent. Half of thefungal species were pathogenic to apple fruits indicating that the species were non host-specific totheguava plant.Scabdel fungicide completely inhibited the growth of *B. verrucosum*at 50 ppm, but in thecaseof *C. specifer*the inhibition ranged 18.8- 33.3 % with dosesincreases. Golden Copper was stimulatory to the growth of tested fungi at 50-1000 ppm doses.

Key words: Guava leaves, identification, pathogenicity, fungicides.

1. INTRODUCTION

Guava (*Psidium guajava* L.) is an important fruit cultivated in several countriesaround theworld[1]. It contains carbohydrates, protein, and adequate amounts of vitamin C and some minerals [2,3]. Due to its highly nutritional value and low price, guava is considered on of 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 attackby more than 170 pathogens [5]. Several studies were documented different diseases on guava trees e.g; damping off and blight of seedlings, anthracnose, stem canker, leaf spots and blight, smootymold and rots of fruits [6,7,8]. Among these diseases, leaf spots were considered mainly fungal infectionsincited by*Alternariaalternata*[9],*Alternariatenuissima*[10];*Cercosporapsidii*,and *Cercosporasawadae*[11] and *Pestalotiapsidii* [4]; or algal infection incited by*Cephaleurosvirescens*[12].

There are different methods used for themanagement of phytopathogenic fungi on cultivated crops, including the use of fungicides [13]. The fungicides showed variable effect on different fungal species. Özer et al. [14] reported that seven of nine fungicides had an inhibitory influence on themycelial growth of *Botrytis cinerea* isolates. Also, Equation Pro and Kema Zed showed inhibitory effects on mycelial growth and hydrolytic enzymes produced by broad bean leaf 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*,andhe concluded that the fungicides were the superior treatment in controlling the pathogens as compared with other compounds.

This work aimed to identify the fungal species from infected guava leaves, evaluatetheir virulence on guava leaves and apple fruits and control them by two fungicide application.

2. MATERIAL METHODS

2.1 Collection of Guava Leaves

Fifteen samples of infected guava leaves symptomatic with leaf spotswere collected from a South Valley University farms during May 2023. Each infected sample was putted in plastic bag and transferred to themycological lab and stored at 5 °C until further analysis.

2.2 Isolation of mycobiota from infected guava leaves.

The 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 study.The isolated fungi were grown on PDA medium and morphological identification was performed based on macro (colony shape, color, reverse and diameter)and microscopic features(conidial shape, color and size).

2.3 Pathogenicity Test

The virulence of fungal species (*Alternariaatra*, *Botryotrichiumverrucosum*,*Cochliobolusspecifer*, *Drechslerahalodes*, *Humicolagrisea* and *Stachybotryschartarum*) were tested on detached guava leavesas described byLi et al. [18], with some modifications. Healthy appearance and freshly guava leaves were washed under tapwater,then sterilized by 70% ethanol.Leaves were cut into pieces and each two piecesplaced in apetri dish containing three filter papers.

Leave pieces were wounded using sterile needle and inoculated by fungal discs 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 theplates were incubated at 28±1 ºC for seven days[18]. The developed lesions were determined. Fungal re-isolation was performed from developed lesions according to Koch's postulates.

To determine if whether the guava plant is host specific to isolated fungi or not, the aggressiveness of isolated fungi was evaluated on apple fruits as follows; healthy and uniform apple fruits were surface sterilized by sodium hypochlorite (1%) for 3-5 min, then rinsed in sterilized distilled water twice and let dry under sterilized conditions, Active-growing fungal discs wereinserted into holes made in sterilized apple fruits, as described byBaiyewu et al.[19] and Chukwuka et al.[20]. The control wascarried out using asterile PDA. The inoculated fruits were putted in polyethylene bags and incubated for 2 weeks at 28 °C. After theincubation period, thevirulence of thetested fungi was estimated by measuring the diameter of thelesion. All experiments were conducted in triplicate.

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

The efficacy of two fungicides,Scabdel 70% and Golden Copper 50% were tested against *B. verrucosum* and*C. specifer*growth. Their chemicalnames, active ingredients, manufacturesand agricultural uses are shown in Table (1).For this purpose, different doses of fungicides (50, 100, 500 and 1000 ppm) were incorporated into autoclaved and cooled (about 50 °C) PDA medium, then active growing discs were extracted from 7 day colonies and placed in the center of PDA medium plates containing thefungicide dose. PDA medium without fungicides was used as acontrol. The plates were placed in anincubator 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; *Alternariaatra*, *Botryotrichiumverrucosum*, *Cochliobolusspecifer*,*Drechslerahalodes*,*Humicolagrisea*and*Stachybotryschartarum* were collected from guava diseased leaves on plates of Potato Dextrose Agar medium. From which,*Botryotrichiumverrucosum*, *Cochliobolusspecifer*and*Humicolagrisea* 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 *Alternariaatra*, *Botryotrichiumverrucosum*, *Cochliobolusspecifer*,*Drechslerahalodes*,*Humicolagrisea*and*Stachybotryschartarum* was evaluated on guava leaves and apple fruits. The obtained data indicated that *B. verrucosum* and *C. specifer*were successful in lesion production on guava leaves after 7 days of incubation. *B. verrucosum*showed moderate virulence capacity with lesions less than 50% from leaf area, but in case *C. specifer*, the virulence capacity was low with lesions less than 25% from leaf area.The remaining four species failed to show any detectable disease symptoms on guava leaves (Table 3 and Fig.1).

Exactly 50% of thetested fungi (*B.verrucosum*, *C.specifer* and *D. halodes*) exhibited virulence capacity on apple fruits with different degrees. The highest fruit 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 meansthese species are not host-specific to theguava 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 of 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 concentrations 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 no inhibitory effect on the growthofboth tested fungi, on the contrary the growth of *B. verrucosum*and *C. specifer* were significantly increased by increasing thefungicide dose from 50-1000 ppm (Table 4 and Figs. 3, 4).

4. DISCUSSION

Six fungal species were isolated from infected guava leaves symptomatic of leaf spots and the highest fungal count was achieved by *Botryotrichiumverrucosum*, *Cochliobolusspecifer*and*Humicolagrisea*. Pandey et al.[23] studied the mycoflora of guava from thebud stage to theleaf fall stage in different seasons and they found that *Colletotrichumgloeosporioides*, *Fusariumoxysporum* f. sp. *psidii*, *Pestalotiapsidii* and *Phomapsidi* were the most potent pathogenic species during all seasons.In Egypt, Youssef et al. [24]isolated *Alternariaalternata*, *Lasiodiplodiatheobromae*, *Fusariumsemitectum*and *Pestalotiapsidii* from the dropped guava.

Our results showed that*B. verrucosum*and *C. specifer*successed in producing of disease symptoms on guava leaves and the*B. verrucosum*was more virulent than *C. specifer*. Youssef et al.[24] evaluated disease intensity on petioles and fruits of guava after artificial inoculation with *Alternariaalternata*,*Lasiodiplodiatheobromae*,*Fusariumsemitectum*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 seedlings after wound inoculation with *Alternariatenuissima*.

Scabdel 70% strongly affected the radial growth of*B. verrucosum*and *C. specifer*but in case of Golden Cooper 50%, the radial growth of tested fungi increases with the doses of fungicide. 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 effectsto different degrees. Abdel-Kader et al.[25] illustrated that, Euparen fungicide had noeffect on the growth of *Alternariaalternata*, *Fusariummonilifome*, *Myrotheciumverrucaria* and *Thermoascusaurantiacus* at 4.8, 23.8 and 47.5 ppm concentrations. Also, Vacomilplus 50% (Copper oxychloride +Metalaxyl) at concentrations 50-200 ppmdidnot exhibit any detectable effects on

*Lasiodiplodiatheobromae*and*Fusariumsemitectum* growth [24]. Abdul Wahid [26] studied the effect of two fungicides (Rovral andSumislex) on the growth offive isolates of*Colletotrichumgloeosporioides*causing guava anthracnosein Egypt, he claimed that all tested isolates were strongly affected by the two fungicides used. The efficacies of fungicides toward pathogenic fungi were varied and affected by fungal species and fungicide dose[27]. Saleem et al.[15] found that, the mycelial growth of*A. alternata* was stimulated at lower doses (50 ppm) of Aquation Pro and Kema Zed fungicides and reduced at higher concentrations.

5. CONCLUSION

Third of the isolated fungi were able to infect guava leaves, Scabdelfungicide had inhibitory effect against *Botryotrichiumverrucosum* and *Cochliobolusspecifer*, on contrary Golden Copper showed positive effect on tested species.

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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
Alternariaatra	1	8.3	6.6
Botryotrichiumverrucosum	2	16.6	6.6
Cochliobolusspecifer	2	16.6	6.6
Drechslerahalodes	1	8.3	6.6
Humicolagrisea	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)
Alternariaatra	ND	ND
Botryotrichiumverrucosum	++	30.5
Cochliobolusspecifer	+	20
Drechslerahalodes	ND	12
Humicolagrisea	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 *Botryotrichiumverrucosum* 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)

* *P* value < 0.05 was considered statistically significant from the control.

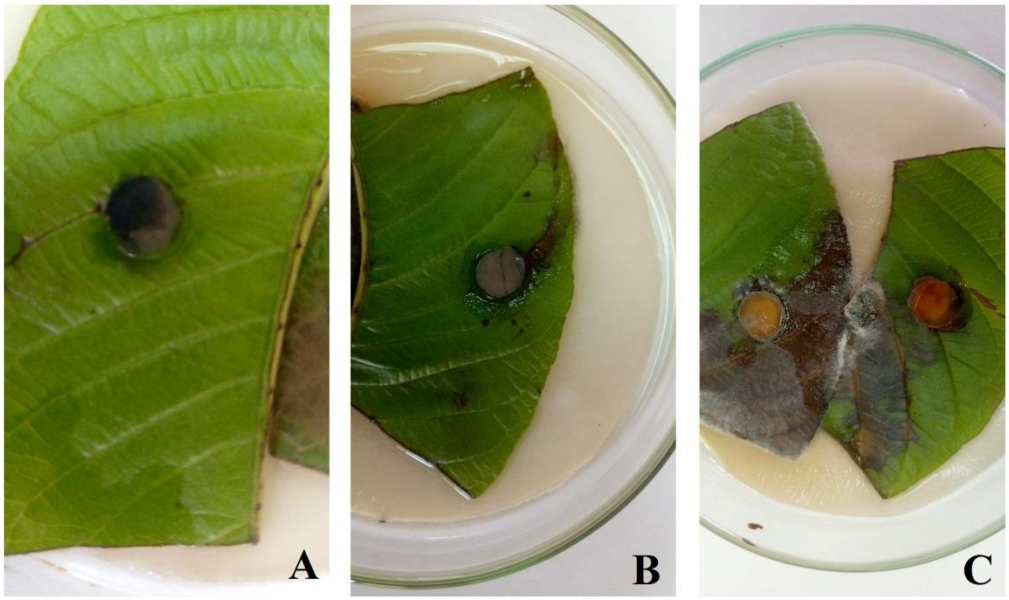

Fig. 1. Disease symptoms on guava leaves after 7 days of incubation; A: control; B: weak virulent (*C.specifer*)and C: moderate virulent (*B.verrucosum*).

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*

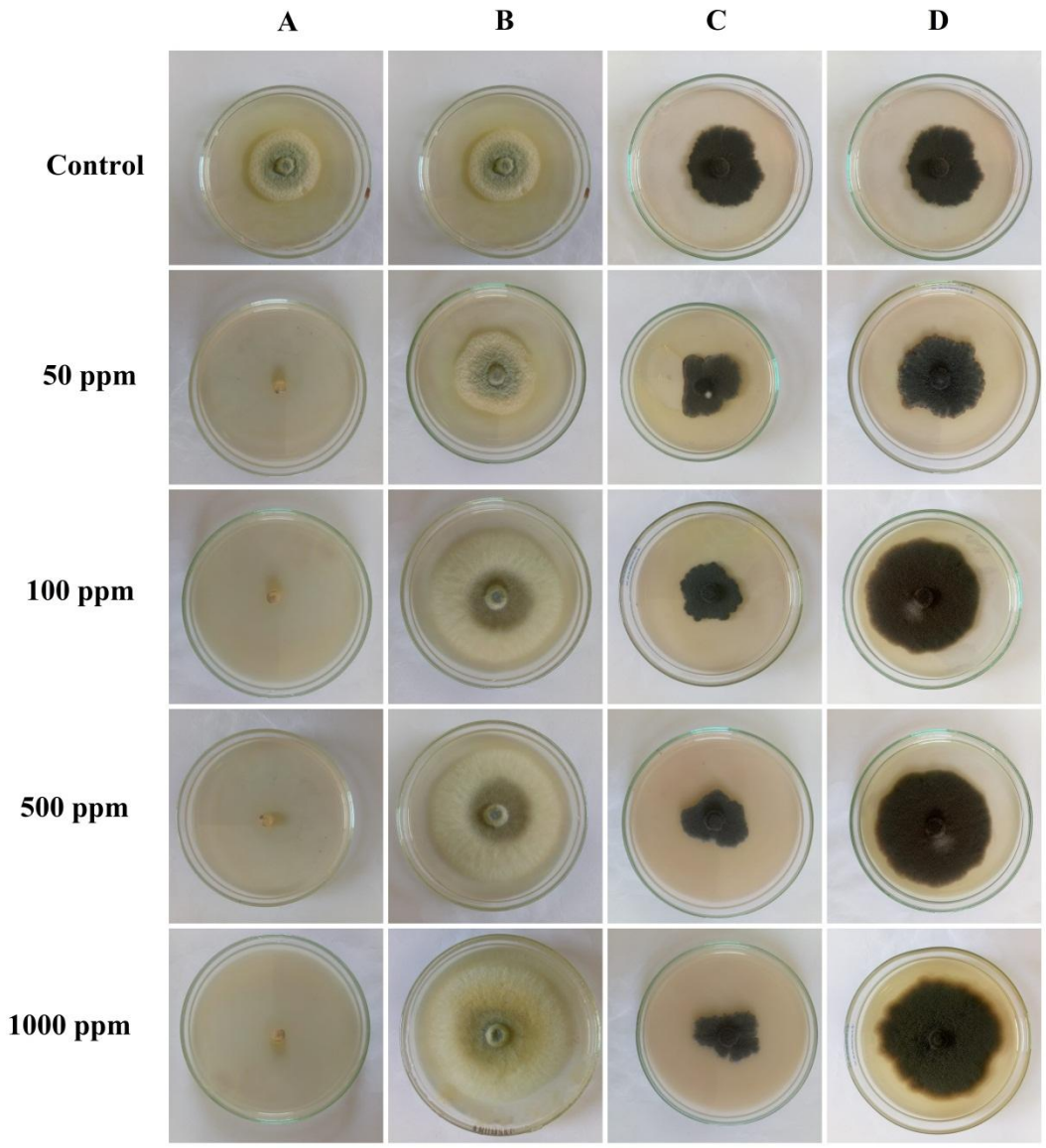

Fig. 3. Colony diameter of *B.verrucosum*and*C.specifer* on different concentration of fungicides, A: *B.verrucosum* on Scabdel, B: *B.verrucosum*on Golden Copper, C: *C.specifer* on Scabdel and D: *C.specifer*on Golden Copper.

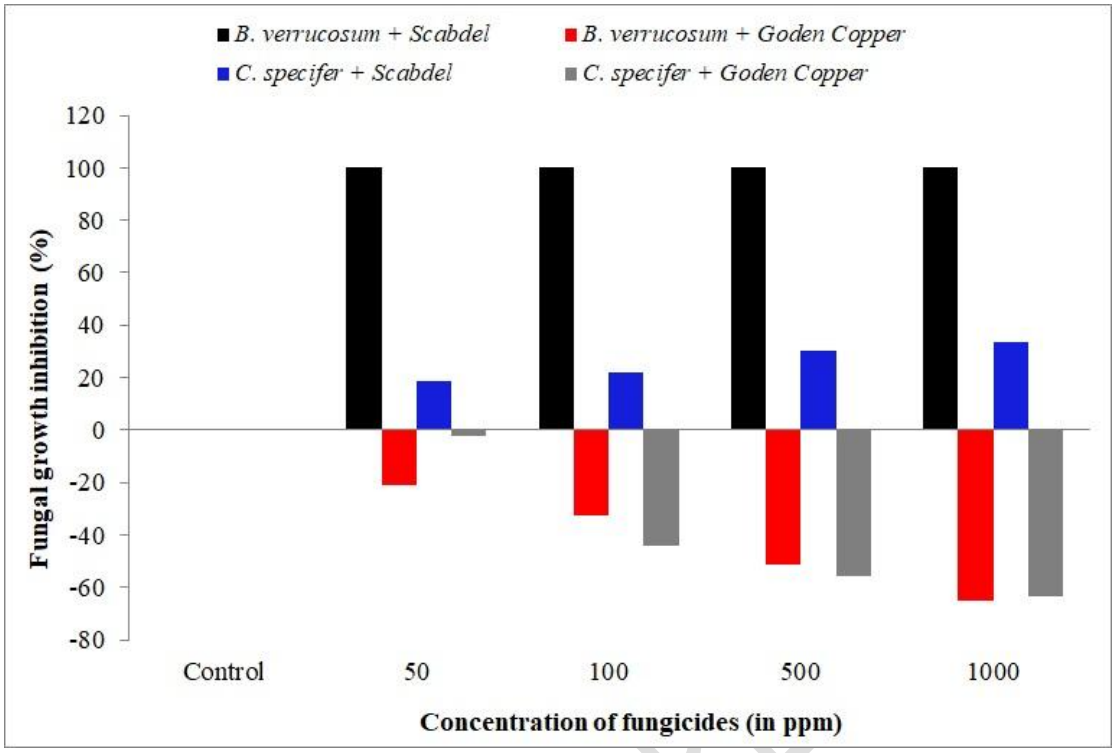

Fig. 4. Effect of different concentration of Scabdel and Golden Copper fungicides on *B. verrucosum*and*C. specifer* growth.