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Murraya koenigii as Potential Substitute of Chemical Fungicides in Controlling Plant Diseases

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ABSTRACT: The need for a better plant disease control approach has always been felt due to the dangerous effects of chemical control measures. Plant extracts have antifungal properties, which could be used to treat plant diseases in a non-chemical, environmentally benign way. In the presented study methanolic leaf extract of Murraya koenigii was screened and evaluated for antifungal activity against six forest nursery pathogens, including A. alternata, F. oxysporum, F. solani, Pestalotiopsis sp., Macrophomina sp., and *Phomopsis* sp. at different concentrations by applying poison food technique. Infected leaf and root samples of Melia dubia (Malabar Neem), Azadirachta indica (Neem) Quercus leucotrichophora (Banj oak) and Saraca indica (Ashoka) were used to isolate these six phytopathogenic fungus. Methanolic leaf extract of Murraya koenigii along with two systemic (Benomyl and Carbendazim) and two non-systemic (Mancozeb and Copper-oxy-chloride) fungicides were evaluated at 0.5 percent, 1 percent, and 1.5 percent concentrations. Methanolic extract was found to have substantial antifungal action against all of the forest diseases tested. Murraya koenigii methanolic extract had the greatest inhibitory impact on Phomopsis sp. (86%), Macrophomina sp. (79.29%), Alternaria alternate (65.07%), Fusarium solani (47.71%), and Fusarium oxysporum (43.93%). This plant being an edible one and abundant in the wild can be utilized as a potential candidate for inhibiting pathogen's growth.

Keywords: Plant disease control, chemical control, antifungal activity, leaf extract, fungicide.

INTRODUCTION

Chemical fungicides are conventionally used in managing plant diseases caused by pathogenic fungi, such as leaf spot, anthracnose, rust, wilt, blight, scab, gall, canker, damping-off, root rot, mildew, and dieback caused by pathogenic fungi (Jain et al., 2019). Though chemical control is a widely used mode of plant disease management, it has been shown to have a harmful influence on the environment, which in turn has an impact on human health and livestock. Furthermore, repeated use of these compounds eventually leads to disease resistance to these chemical fungicides. According to Salhi et al. (2017), 200 species of plant pathogens have evolved resistance against chemical fungicides, necessitating the development of alternative eco-friendly disease management methods (Sales et al., 2016). Botanicals, which are plant extracts, are easily decomposable and an environmentally benign alternative to chemical fungicides. Hence, disease treatment using botanicals is widely accepted. Botanicals are known to possess antimicrobial, anticancer, and antioxidant properties, and are a source

of free radical scavenging agents (Czerwi ska et al., 2015), and antifungal compounds found in plants have been recognised as a key factor for disease pathogenesis (Kurucheve et al., 1997).

Murraya koenigii, popularly known as curry-leaf, is a medicinally important plant that belongs to the Rutaceae family. It is a shrub or small tree that grows up to 6 meters tall on the Indian Subcontinent and is native to South Asian countries such as India, Sri Lanka, Bhutan, and Nepal (Mhaskar, 2000). It has been reported to have a variety of important uses in the traditional system of medicine in Eastern Asia (Jain 2012), including anti-oxidant, anti-diabetic, antipyretic, hepatoprotective, anti-cancerous, anti-bacterial, and anti-fungal properties, as well as being a very rich source of organic compounds (alkaloids, flavonoids, carbohydrates, sterol, and so on) (Tachibana et al. 2001).

In the present study, the efficacy of methanolic extract of Murraya koenigii at different concentrations was tested against various pathogens that have been reported by various researchers from time to time, such

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as Alternaria alternata (Mehrotra and Pandey 1992), Fusarium solani (Pandey et al., 2018), Pestalotiopsis sp. (Ramakrishnan and Subramanian 1952; Dube and Bilgrami1999), Phomopsis sp. (Sahni 1968), Macrophomina sp. (Prakash et al., 2007), that causes leaf blight, leaf spot, and wilt in Quercus leucotrichophora (Oak), Azadirachta indica (Neem), Melia dubia (Malabar neem), and Saraca indica (Ashok).

MATERIALS AND METHODS

A. Isolation and identification of Phyto-pathogens

Infected leaf and root samples of Azadirachta indica, dubia, Saraca indica, and Melia Quercus leucotrichophora were collected from the Forest Research Institute's central Nursery in Dehradun to isolate the pathogens using moist chamber (Petrini and Fisher, 1986) and surface sterilization technique (Milovanovi et al., 2009). Pathogen cultures were then isolated, purified using the single spore isolation method, and kept on potato dextrose agar slants in a refrigerator at 4°C. Their Characterization was based on macroscopic and microscopic features identified with standard identification keys (Barnett 1972; Booth 1971).

B. Preparation and evaluation of botanicals and fungicide

Murrava koenigii leaves were utilized to make a (methanolic botanical extract) for in-vitro investigations. The leaves were ground into a smooth powder in a mixer grinder for methanolic extraction. This powder was then subjected to hydrodistillation where it was boiled with methanol to produce a crude extract, as described by Nene and Thapliyal (1993). Using the poison food technique (Nene and Thapliyal 1979), the antifungal activity of thus generated botanical was evaluated. Different extract concentrations (0.5 %, 1 % and 1.5 %) were added into PDA growing media. Similarly, at concentrations of 0.5 %, 1 %, and 1.5%, the efficacy of two systemic (Benomyl and Carbendazim) and two non-systemic (Mancozeb Copper-oxy-chloride) fungicides was investigated. An agar plug containing pathogen mycelium from a seven-day-old culture was sliced and aseptically placed in the center of each petri dish using a sterile needle and a sterile cork borer of 5mm. Petri plates with no extract in PDA medium served as control. The Petri plates were then incubated at 25±1°C for 7 days, after which radial growth of mycelium was measured and the Vincent (1947) method was used to compute percent inhibition:

$$I \% = [(C-T)/C)] \times 100$$

Where,

I = inhibition percent of mycelium C= Colony diameter in the control (cm) T = Colony diameter in treated (cm) The result thus obtained is compared with the impact of botanicals on pathogenic fungus growth.

RESULTS AND DISCUSSION

Laboratory evaluation of botanical and fungicides revealed that both the agents caused various inhibitions of forest pathogenic fungi at various concentrations (Table 1 and 2).

In-vitro evaluation of botanicals

Pathogens showed differential growth patterns on different concentrations of botanical. Table 1 depicted that the pattern of growth and inhibition of *Alternaria alternata*, *Fusarium oxysporum*, *Fusarium solani*, *Pestalotiopsis* sp., *Macrophomina* sp., and *Phomopsis* sp., at different botanical concentrations (0.5%, 1%, and 1.5%). It was found that all fungi exhibited some growth only at lower concentrations of botanical fungicides tested, i.e., 0.5% and 1%. While intense growth inhibition was recorded at higher concentrations of botanical fungicides (1.5%) (Fig. 1). Therefore, a decrease in colony growth is directly associated with an increase in the concentration of botanical.

The present study reveals that the methanolic extract of *Murraya koenigii* is highly effective against *Phomopsis* sp., which shows a maximum inhibition of (86.00 %) at 1.5% concentration which can be correlated with the Jakatimath *et al.* (2017) study's results where garlic bulb extract inhibited the mycelial growth of *Phomopsis vexans* by (89.7%) at a concentration of 5% (w/v).

Mycelial growth of Macrpohomina sp. was highly inhibited by methanolic extract of *M. koenigii* by (79.29 %) at 1.5% concentration. Which supports the findings of Abiala et al. (2015) that Methanolic extract of Murraya koenigii showed (79.29 %) inhibitory effect on Macrophomina sp. at a concentration of 0.5% while extract of Piper guineense (62.95%), Xylopla aethiopica (56.90%), and Bambusa vulgaris (20.45%), showed inhibitory effect in Macrophomina sp. at a concentration of 15% which is tenfold higher than the concentration of Murraya koenigii methanolic extract. Deshmukh and Vanitha (2021) reported that cold water extract of curry leaf showed (67.77%) inhibition of mycelial growth of Macrophomina phaseolina at a concentration of 10% (w/v). Girijashankar and Thayumanavan (2005) also reported antifungal activity of Prosopis juliflora leaf extract where mycelial growth of Macrophomina phaseolina was inhibited by (66.6%) and (43.0%) by methanol and cold water extracts respectively at a concentration of 10% (w/v).

In the current study, the maximum mycelial growth inhibition for *Altrernaria alternata* was recorded as 65.07% at 1.5% curry leaf extract concentration which closely resembles with the report of Thaware *et al.* (2010) where bulb extract of garlic (*Allium sativum*) inhibited the mycelial growth of *Alternaria alternata* by 63.33% at a concentration of 10% (w/v).

Mohana and Raveesha (2007) reported that rhizome extract of Decalepis hamiltonii showed 100% inhibition of *Alternaria alternata* at 50% concentration while it inhibited 63.50% mycelial growth when tested at a concentration of 20% which is close to above presented study but their tested concentration is 13.33 times more than our maximum tested concentration i.e. 1.5%.

For Fusarium oxysporum and F. solani, maximum growth inhibition was recorded as 43.93% and 47.71% respectively at 1.5% concentration which is in close resemblance with the report of Singh et al. (2021) where the leaf extract of Terminalia arjuna showed maximum inhibition of Fusarium oxysporum f. sp. ciceri by 47.39% at a concentration of 15% (w/v). Abiala et al. (2015) reported that the extract of Piper guineense (91.15%) Xylopla aethiopica (60.95%) and Bambusa vulgaris (61.10%) showed inhibitory effect at 15% which is tenfold higher than the concentration of Murraya koenigii. Mohana and Raveesha (2007) reported that Decalepis hamiltonii rhizome extract showed 100% inhibition of F. oxysporum at 40% concentration but when tested at 10% concentration, it inhibited 44.68% mycelial growth which is close to our result but the concentration is 6.66 times higher than our maximum tested concentration. In the case of

Fusarium solani the rhizome extract of *Decalepis hamiltonii* showed 48.20 % inhibition at 10% concentration which is near to our result but the concentration is 6.66 times greater than highest tested concentration of leaf extract from *Murraya koenigii*.

Murraya koenigii leaf extract at 1.5% concentration inhibited the mycelial growth of *Pestalotiopsis* sp., to 37.31%. Saha and Dasgupta (2005) confirmed that aqueous and ethanol extract of curry leaf (*Murraya*) inhibited spore germination of *Pestalotiopsis theae* by 12.0% and 43.7% respectivelywhile the aqueous and ethanol extract of *Lantana camara* showed 36.6% and 39.4% inhibition respectively. Aqueous and ethanol extract of *Azadirachta indica* showed 100% and 44.7% inhibition of spore germination respectively but their tested concentration was found to be 33.33 times more than maximum tested concentration of botanical being studied i.e. 1.5%.

Literature study and our result showed that methanolic extract of *Murraya koenigii* showed good efficacy in controlling all pathogen's growth in comparison to the above references as they are inhibiting the pathogen at a tenfold higher concentration than *Murraya koenigii* extract hence, *Murraya koenigii* can be used as a potential candidate for inhibiting pathogen's growth.

Table 1: Effect of different concentrations of Murraya koenigii leaf extracts on vegetative g	rowth of
pathogens.	

Isolates	Botanicals concentration (%)/Average Mycelia Growth (mm) Murraya koenigii			Mean			lls concent elia Growtl <i>Murraya</i>		on (%)	Mean	
	Control	0.50%	1%	1.50%			Control	0.50%	1%	1.50%	
A. alternata	42	24.33	21.67	14.67	20.22	1	0.00	42.07	48.40	65.07	51.85
F. oxysporum	44	37.33	29.00	24.67	30.33	1	0.00	15.16	34.09	43.93	31.06
F. solani	51	32.33	30.00	26.67	29.67		0.00	36.61	41.18	47.71	41.83
Pestalotiopsis sp.,	42	30.33	28.33	26.33	28.33		0.00	27.79	32.55	37.31	32.55
Macrophomina sp.,	66	20.33	18.67	13.67	17.56		0.00	69.20	71.71	79.29	73.40
Phomopsis sp.,	50	10.33	8.33	7.00	8.55		0.00	79.34	83.34	86.00	82.89
Mean		25.83	22.66	18.83				45.02	51.87	59.88	

In-vitro evaluation of fungicides

Table 2 shows that all pathogens have varied growth suppression patterns when exposed to different fungicides and at different concentrations. carbendazim 1.5 % (90.81 %) induced the most growth inhibition in *Alternaria alternata*, followed by Benomyl 1.5 % (89.66 %), Copper-oxy-chloride 1.5 % (85.64 %), and Mancozeb (84.48 %) at 1.5 %. Farooq *et al.*, (2019) found that carbendazim inhibited *Alternaria solani* growth by 90% at 500ppm, which is consistent with the current finding that carbendazim suppressed *Alternaria alternata* growth by 90%.

Mancozeb 1.5 %, 1 %, and 0.5 %, as well as Benomyl at concentrations of 1.5 % and 1 %, inhibited *Fusarium oxysporum* development completely (100 %), followed by Carbendazim 1.5 % (91.78 %), and Copper-oxychloride 1.5 % (64.74 %), and so on. Mancozeb was determined to be the most effective even at very low

fungicide concentrations in this study. Benomyl and copper-oxy-chloride inhibited Fusarium solani growth completely at 1.5% and 1% concentrations, respectively, followed by carbendazim 1.5 % (92.65 %) and mancozeb 1.5% (84.31%). Here, even at very low fungicide concentrations, benomyl and copper-oxychloride were shown to be the most effective. In the case of Pestalotiopsis sp., Benomyl induced the most inhibition (100 %) at all three concentrations (0.5 %, 1 %, and 1.5 %), followed by carbendazim 1.5 % (100 %), mancozeb 1.5 % (100 %), and copper-oxy-chloride 1.5 % (89.62 %), and so on. The majority of fungicides are found to be highly efficient against it at different concentrations. Similarly, Saju et al., (2011) found carbendazim significantly effective against Pestalotiopsis sp. at all concentrations tested (0.05%-0.15%).

Isolates		Murraya	Murraya koenigii							
	Control	0.50%	1%	1.5%						
A. alternata										
F. oxysporum										
F. solani										
Pestalotiopsis sp.										
Macrophomina sp.										
Phomopsis sp.										

Fig. 1. Effect of different concentrations of methanolic extract of Murraya koenigii on the growth of pathogens.

Isolates	Fungicides		e concenti		Average	Mean			ration (%)/		Mean
		mycelia (Growth (m				myc	elia growtl	n Inhibitior	1 (%)	
		Control	0.50%	1%	1.5%		Control	0.50%	1%	1.5%	
Alternaria	Carbendazim	58	7.33	6.33	5.33	6.33	0.00	87.36	89.09	90.81	89.09
alternata	Benomyl		11.00	8.67	6.00	8.56	0.00	81.03	85.05	89.66	85.25
	Copper-oxy- chloride		14.67	13.00	8.33	12.00	0.00	74.71	77.59	85.64	79.31
	Mancozeb		13.67	11.00	9.00	11.22	0.00	76.43	81.03	84.48	80.65
Fusarium	Carbendazim	69	10.00	6.67	5.67	7.45	0.00	85.51	90.33	91.78	89.21
oxysporum	Benomyl		5.00	0.00	0.00	1.67	0.00	92.75	100.0	100.0	97.58
<i>2</i> 1	Copper-oxy- chloride		38.33	36.33	24.33	33.00	0.00	44.45	47.35	64.74	52.18
	Mancozeb		0.00	0.00	0.00	0.00	0.00	100	100	100	100
Fusarium solani	Carbendazim	68	10.67	5.00	5.00	6.89	0.00	84.31	92.65	92.65	89.87
	Benomyl		5.00	0.00	0.00	1.67	0.00	92.75	100.0	100.0	97.55
	Copper-oxy- chloride		5.00	0.00	0.00	1.67	0.00	92.65	100.0	100.0	97.55
	Mancozeb		24.33	16.33	10.67	17.11	0	64.22	75.99	84.31	74.84
Pestalotiopsis sp.,	Carbendazim	61	5.00	5.00	0.00	3.33	0.00	91.80	91.80	100	94.54
	Benomyl		0.00	0.00	0.00	0.00	0.00	100.0	100.0	100.0	100.0
	Copper-oxy- chloride		9.67	7.00	6.33	7.67	0.00	84.15	88.52	89.62	87.43
	Mancozeb		9.00	7.33	0.00	5.44	0.00	85.25	87.98	100.00	91.08
Macrophomina sp.,	Carbendazim	70	12.67	11.00	9.67	11.11	0.00	81.90	84.29	86.19	84.12
	Benomyl		10.33	8.33	6.67	8.44	0.00	85.24	88.10	90.47	87.94
	Copper-oxy- chloride		5.00	0.00	0.00	1.67	0.00	92.86	100.00	100.00	97.62
	Mancozeb		5.00	0.00	0.00	1.67	0.00	92.86	100.00	100.00	97.62
Phomopsis sp.,	Carbendazim	65	5.00	5.00	5.00	5.00	0.00	92.31	92.31	92.31	92.31
/	Benomyl		5.33	0.00	0.00	1.78	0.00	91.80	100.00	100.00	97.27
	Copper-oxy- chloride		5.67	5.00	5.00	5.22	0.00	91.28	92.31	92.31	91.96
	Mancozeb		5.00	5.00	0.00	3.33	0.00	92.31	92.31	100.00	94.87
Mean	Carbendazim		8.44	6.50	5.11			87.19	90.07	92.29	
	Benomyl		6.11	2.83	2.11			90.47	95.51	96.68	
	Copper-oxy- chloride		13.05	10.22	7.33			79.92	84.29	88.71	
	Mancozeb		9.50	6.61	3.27			85.17	89.55	94.79	

Table 2: Effect of different concentrations of Carbendazim, Benomyl, copper-oxy-chloride, and Mancozeb on vegetative growth of pathogens.

In *Macrophomina* sp., copper-oxy-chloride and mancozeb causeed the maximum growth inhibition (100%) at 1.5 % and 1 % concentrations, respectively, followed by benomyl 1.5 % (90.47 %) and carbendazim 1.5 % (86.19 %), and so on. Even at very low concentrations of the fungicide, mancozeb was found to be the most effective. Carbendazim demonstrated 89.01 % mycelia inhibition in *M. phaseolina* at 500ppm concentration, according to Parmar *et al.*, (2017), which validates the findings of the present study. For *Phomopsis* sp., benomyl at 1.5 % and 1 %

concentrations caused the most inhibition (100%), followed by mancozeb 1.5% (100%), carbendazim 0.5%, 1%, and 1.5% (92.31%) and copper-oxy-chloride 1.5% and 1% (92.31%). According to Jakatimath *et al.* (2017), carbendazim, tebuconazole, and hexaconazole all showed 100% inhibition at 0.5% concentration, followed by propiconazole (93.67%) and difenoconazole (88.00%).

The overall estimate shows that benomyl was the most efficient fungicide for suppressing pathogens growth even at very low fungicide concentrations.

Isolates		Carbe	ndazim	
	Control	0.50%	0.75%	1%
A. alternata				
F. oxysporum				
F. solani				
Pestalotiopsis sp.		•	•	•
Macrophomina sp.				
Phomopsis sp.				

Fig. 2. Effect of different concentrations of Carbendazim (systemic fungicide) on the growth of pathogens.

Isolates		Ben	omyl	
	Control	0.50%	0.75%	1%
A. alternata				
F. oxysporum				
F. solani				
Pestalotiopsis sp.				
<i>Macrophomina</i> sp.				
Phomopsis sp. (P1)				

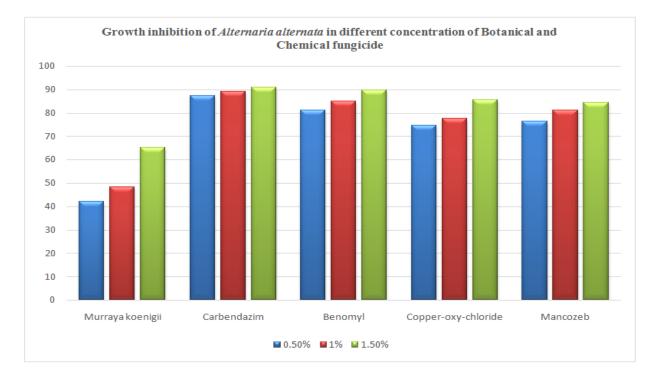
Fig. 3. Effect of different concentrations of Benomyl (systemic fungicide) on the growth of pathogens.

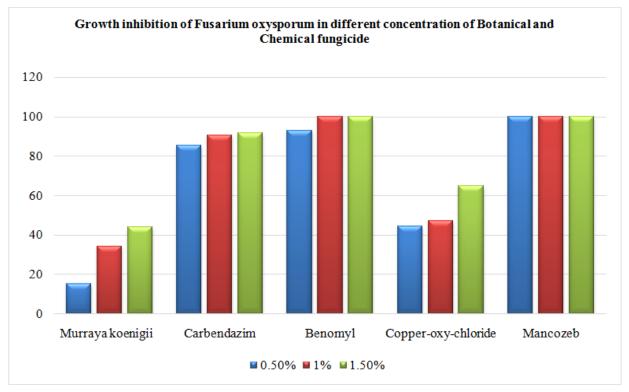
Isolates		Copper ox	xy chloride	
	Control	0.50%	0.75%	1%
A. alternata				
F. oxysporum				
F. solani				
Pestalotiopsis sp.		8		-
Macrophomina sp.				
Phomopsis sp.				

Fig. 4. Effect of different concentrations of Copper-oxy-chloride (non-systemic fungicide) on the growth of pathogens.

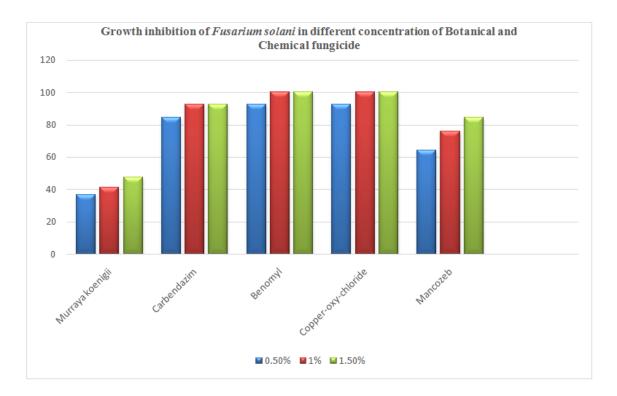
Isolates	Mancozeb							
	Control	0.50%	0.75%	1%				
A. alternata								
F. oxysporum								
F. solani								
Pestalotiopsis sp.								
<i>Macrophomina</i> sp.								
Phomopsis sp.								

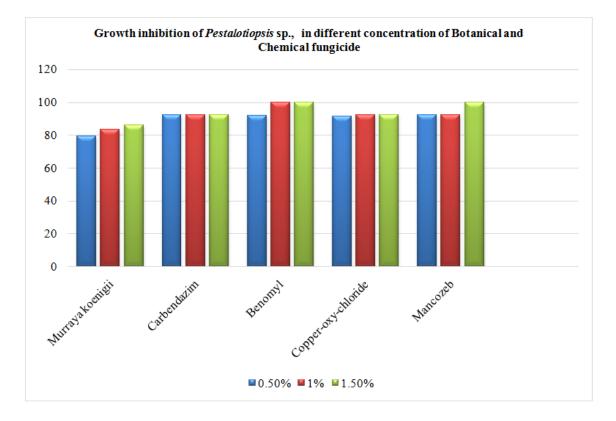
Fig. 5. Effect of different concentrations of Mancozeb (non-systemic fungicide) on the growth of pathogens.



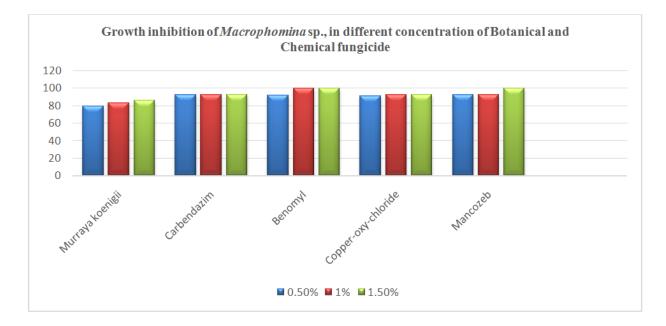


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Disease inhibition potential among fungicides and botanicals

The growth inhibition results were compared to find out the potential of botanicals for disease control against chemical fungicides. The results revealed that all the fungicides had very high efficacy in controlling many fungal pathogens, but a higher concentration of M. Koenigii 1.5% methanolic extract showed nearly similar growth inhibition (86.00%) of Phomopsis sp., as in Carbendazim (92.31%) and Copper-oxy-chloride (92.31%) at 1.5% concentration. In the case of Macrophomina sp., carbendazim showed inhibition (86.19%) at maximum 1.5% concentration, whereas M. koenigii 1.5% showed inhibition of 79.29%. In the case of Alternaria alternata, all four fungicides showed much higher inhibition (84.48-90.81%) at 1.5% concentration than M. koenigii 1.5% methanolic extract. Being the most effective fungicide, Benomyl caused up to 100% growth inhibition in Pestalotiopsis sp., at the lowest 0.5% concentration and 100% growth inhibition in Fusarium oxysporum, Fusarium solani, and Phomopsis sp., at 1% and 1.5% concentrations. Although both systemic and non-systemic fungicides are chemical in nature, they will inhibit the tested pathogens to a greater extent compared to botanical tests in the same ratio. However, it is possible that botanicals will produce effective results when tested at a higher concentration.

CONCLUSION

The use of chemical fungicides for management strategies is always subjected to critiquing as being chemical in nature the fungicide may help to fight the pathogen, but there have been reports of them targeting non-targeted microorganisms in soil and disturbing the microbiota of the soil ecosystem. The present study reveals that the methanolic extract of *Murraya koenigii*

is highly effective against plant pathogens of forest nurseries and holds potential as an economic, affordable, and easily available botanical fungicide, being abundant throughout the Indian subcontinent. Comparative data has shown that *Murraya koenigii* is a very potent candidate for disease management as compared to other botanicals as its leaf extract at a lower concentration of 1.5% showed promising results compared to other botanicals used in previous investigations, which gave analogous results at a much higher concentration (5%, 10%, or 15%).

All of the diseases studied had varied reactions to the Murrava koenigii botanical. Lower concentrations of botanicals inhibit the growth of some pathogens to some extent, but they are all fungi static to all the pathogens. However, at greater concentrations, it has a devastating effect on most pathogens' mycelial growth. The mycelial inhibition caused by methanolic extract of Murrava koenigii was as efficient as many chemical fungicides tested and sometimes greater. The growth inhibition in Phomopsis sp. (86.00%) at 1.5% concentration shows its potency. Botanicals can be effective in field trials and can be used in relatively high concentrations as compared to chemical fungicides as there is no chance of it being harmful to the plant and soil, which might be a concern with other chemical agents that can develop resistance in fungal pathogens. It is clear from the study that botanical fungicides are considerably effective and can be used as an alternative to traditionally used fungicides in controlling the growth of fungal plant pathogens. Hence, to control all the pathogens, a bioformulation involving extracts from more than one plant may be necessary for effective application in the field of developing new, safer, and effective fungicides and can be used as an alternative to traditionally used fungicides in controlling the growth of fungal plant pathogens.

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