Int.J.Curr.Microbiol.App.Sci (2021) *10*(09): 152-162

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 10 Number 09 (2021)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2021.1009.018

Evaluation of Botanicals of Invasive Plant Species and Fungicides against Fungal Pathogens of Forest Nursery

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Keywords

Botanical Fungicide; Antifungal Activity; Fungal Pathogens; Invasive Plant Species

Article Info

Accepted:
10 August 2021
Available Online:
10 September 2021

A B S T R A C T

Investigation of plants that possess natural antimicrobial substances for plant protection has been recognized as a promising disease management strategy. *Alternaria alternata*, *Fusarium solani*, *Macrophomina* sp. and *Pestalotiopsis* sp. isolated from diverse hosts such as *Azadirachta indica*, *Melia dubia*, *Saraca indica* and *Quercus leucotrichophora* produce important diseases in forest nurseries such as leaf blight, leaf spot, and wilt. In this study, botanicals from two invasive plant species, *Ageratina adenophora* and *Ageratum conyzoides*, were prepared and tested against plant diseases. Two botanicals (Methanolic extract obtained from the leaves of these two invasive species) and two fungicides were evaluated for their fungal growth inhibitory effects. At 1.5 percent concentration, the methanolic extract of *Ageratina adenophora* was found highly effective, inhibiting the growth of *Macrophomina* sp., (71.94%) followed by *Pestalotiopsis* sp. (70.20%), *Alternaria alternata* (51.92%) *and Fusarium solani* (47.03%). Whereas, Systemic chemical fungicide Thiophanate methylat 1.5% concentration showed maximum mycelial growth inhibition of *Alternaria alternata* (77.20%) and *Macrophomina* sp. (82.43%) and being deadlier to *Pestalotiopsis* sp. (100%) and *Fusarium solani* (100%). Their comparative analysis showed that higher doses of *Ageratina adenophora* caused either more or almost equal pathogen growth inhibition than lower doses of Chlorothalonil for certain fungi. Thus, promoting eco-friendly disease management strategies such as botanical control would be beneficial in reducing the need for pesticides.

Introduction

Developing countries often face heavy yield losses due to lack of capacity to manage diseases, leading to shortage of adequate food supplies (Klauser *et al.*, 2018; Strange and Scott, 2005). Plant diseases are usually caused by pathogens such as fungi, bacteria,

nematodes, and viruses. However, fungi cause the widest range of diseases in various tree hosts, resulting in the biggest impact. The most commonly implied chemical fungicides are hazardous and may have detrimental effect on the surrounding environment and organisms. Some of them, such as halogenated hydrocarbons like methyl bromide, are even known to deplete the ozone layer (Abritton and Watson, 1992). Chemical fungicides' negative impacts on the environment and human health have spurred researchers to look for safer alternatives, which has sparked research and development of bio-pesticides, including botanical fungicides. Generally, bio-pesticides are categorized as natural products (plant-derived, animal-derived, and microorganism-derived) and microorganisms (Yoon *et al.,* 2013). Many plant species contain antifungal compounds (Hostettmann *et al.,* 2000).

Invasive plant species are aggressive colonizers and their successful invasion is an outcome of many morphological, physiological, and ecological features. If an invasive species contains good antifungal activity, it may be a useful source of antifungal compounds or extracts (Mdee *et al.,* 2009) thereby, positively impacting the management of these invasive weed species.

Ageratina adenophora and *Ageratum conyzoides* are the two invasive weed species being studied here for their antifungal activity compared to chemical fungicides. *Ageratina adenophora* (Spreng.) R. M. King and H. Rob. syn. *Eupatorium adenophorum* (Asteraceae), commonly known as Crofton weed, is an endemic plant species of Central America (mainly Mexico). It has invaded more than 30 countries, including India, Thailand, New Zealand, Australia, America and China (Liu *et al.,* 2006; Li and Feng, 2009). *Ageratum conyzoides* (L.) L. (Asteraceae), commonly known as Billy Goat weed, is an aggressive invasive weed of agricultural fields that causes yield reductions of major staple crops in India (Kohli *et al.,* 2006). When it invades rangeland areas, it competes with native grasses, causing scarcity of fodder. Despite its obnoxious nature and bad economic and environmental consequences, *A. adenophora*is reported to have medicinal properties, and its uses in folk medicine as an antimicrobial, analgesic, antipyretic, antiseptic, blood coagulant and enhancer of phenobarbitone induced sleep, etc. have also been reported (Mandal *et al.,* 1981; Ansari *et al.,* 1983; Rai and Sharma 1994). Invasive weeds have a variety of adverse effects on ecological and human health, thus their usage as a source of botanical fungicides has a higher value in terms of management and value addition.

The present study investigates antifungal activities of botanicals derived from invasive species compared to traditionally used systemic and non-systemic fungicides against foliar and root rot pathogens such as *Alternaria alternata* (Mehrotra and Pandey 1992), *Fusarium solani* (Pandey *et al.*, 2018), *Pestalotiopsis* sp. (Ramakrishnan and Subramanian 1952; Dube and Bilgrami, 1999)., *Macrophomina* sp. (Prakash *et al.*, 2007), and *Phomopsis* sp. (Chowdhury 1966; Sahni 1968) that cause leaf blight, leaf spot, and wilt in *Quercus leucotrichophora* (Oak), *Azadirachta indica* (Neem), *Melia dubia* (Malabar neem) and *Saraca indica* (Ashok). This study will be useful for researchers and farmers to employ the selected effective botanical fungicides in the field condition to effectively manage the disease by including eco-friendly treatments such as bio-agents.

Materials and Methods

The methodology for screening different botanicals and chemical fungicides for the selected fungal pathogens was finalized referring to different techniques by Dhingra and Sinclair (1995). The details of the

materials and methods employed were as below:

Isolation and identification of phyto-pathogens

Infected leaf, stem, and root samples of Melia, Oak, and Neem were collected from the Forest Research Institute's Central Nursery in Dehradun to isolate pathogens using a moist chamber (Petrini and Fisher, 1886) and a surface sterilization technique (Milovanović *et al.*, 2009). Pathogen cultures were then isolated, purified using the single spore isolation method, and maintained on potato dextrose agar slants and stored in a refrigerator at 4℃. Their Characterization was based on macroscopic and microscopic characters, viz. *Alternaria alternata*, *Fusarium solani*, *Pestalotiopsis* sp., and *Macrophomina* sp. using standard identification keys (Barnett 1972; Booth 1971).

Preparation of botanicals

Leaves from two invasive plant species, *Ageratina adenophora* and *Ageratum conyzoides*, were used to make two distinct botanicals (methanolic extract) for in-vitro investigations. Formethanolic extraction, the leaves were ground in a mixer grinder to get a smooth powder. This powder was then subjected to sonication as given by Nene and Thapliyal (1993).

In vitro evaluation of botanicals

The poisoned food technique (Nene and Thapliyal 1979) was used to investigate the antifungal efficacy of plant extracts. PDA growing medium was combined with different concentrations of extracts (0.5%, 1%, and 1.5%). With a sterile cork borer of 5mm, an agar plug containing pathogen mycelium from a seven-day old culture was sliced and aseptically put in the center of each petri dish using a sterile needle. The Petri plates without extracts in the PDA medium served as control. Then, the Petri plates were incubated at 25±1°C for7 days, after which radial growth of mycelium was measured and percent inhibition was calculated by the Vincent (1947) formula:

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

Where,

I = inhibition percent of mycelium

C= Colony diameter in the control(cm)

T = Colony diameter in treated(cm)

In-vitro evaluation of fungicide

The efficacy of systemic (Thiophanate methyl) and non-systemic (Chlorothalonil) was tested at concentrations of 0.5 %, 0.75 %, and 1%.

The active ingredient concentration was calculated to evaluate the fungicide, which was then compared to the impact of botanicals on pathogenic fungus. The above mentioned Vincent, 1947 formula was used to evaluate inhibition percentage.

Results and Discussion

Effect of different botanicals and fungicides on colony growth of fungal pathogens.

Laboratory evaluation of botanicals 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 in different botanical concentrations. Table 1 depicted the pattern of growth and inhibition

of *Alternaria alternata*, *Fusarium solani*, *Pestalotiopsis* sp., and *Macrophomina* sp. at different botanical concentrations (0.5%, 1% and 1.5%). It was found that all fungi exhibited good growth only at lower concentrations of botanical fungicides tested, i.e., 0.5% and 1%. While complete or intense growth inhibition was recorded at higher concentrations of botanical fungicides (1.5%) (Figure.1). Therefore, a decrease in colony growth is directly associated with an increase in the concentration of botanicals.

In the case of *Alternaria alternata*, *Ageratina adenophora*(1.5%) caused maximum inhibition (51.92%), followed by *Ageratum conyzoides* 1.5 %(30.8%), *Ageratina adenophora*1% (22.6%) and *Ageratum conyzoides* 1% (16.6%) etc. *Ageratina adenophora* was found to be more effective against *Alternaria alternata* as compared to *Ageratum conyzoides*.

Botanical fungicides from *Ageratina adenophora*1.5% caused maximum inhibition of *Fusarium solani* (47.03%), followed by *Ageratina adenophora* 1% (43.44%), *Ageratina adenophora* 0.5% (40.39%) and *Ageratum conyzoides* 1.5% (30.69%) etc. *Ageratina adenophora* was found to be more effective against *Fusarium solani* as compared to *Ageratum conyzoides*. *Ageratina adenophora* 1.5% caused maximum inhibition against *Macrophomina* sp. (71.94%), followed by *Ageratina adenophora*1%(66%), *Ageratina adenophora* 0.5% (60.21%) and *Ageratum conyzoides* 1.5% (46.49%) etc. *Ageratina adenophora* was found to be more effective against *Macrophomina* sp. as compared to *Ageratum conyzoides*.

Pestalotiopsis sp. was maximum inhibited by *Ageratina adenophora*1.5% (70.2%), followed by *Ageratum conyzoides* 1.5% (60%), *Ageratina adenophora* 1% (51.21%) and *Ageratum conyzoides* 1% (42%) etc. *Ageratina adenophora* was found to be more effective against *Pestalotiopsis* sp. as compared to *Ageratum conyzoides*.

Although Methanolic extract of *Ageratina adenophora* showed greater efficacy in controlling all pathogens growth, *Ageratina adenophora* and *Ageratum conyzoides* both can be used as potential candidates for inhibiting pathogen's growth.

In-vitro evaluation of fungicides

Systemic (Thiophanate methyl) and non-systemic (Chlorothalonil) fungicides were tested at three different concentrations in the laboratory for their efficacy against different pathogens and used for comparative study against botanicals' effect on pathogens.

Table 2 states that all the pathogens tend to exhibit growth inhibition at lower concentrations of chemical fungicides tested.

It was noticed that *Fusarium solani* and *Pestalotiopsis* sp. showed no growth even at slightly higher concentrations of fungicides i.e. 1% and 1.5% (Figure 2).

Lower doses of thiophanate methyl are equally effective as higher doses of chlorothalonil. Therefore, its higher doses can be lethal to plant growth-promoting microbes in the soil.

In the case of *Alternaria alternata*, Thiophanate methyl 1.5% caused maximum growth inhibition i.e (77.20%) followed by Thiophanate methyl1% (75.12%), Thiophanate methyl 0.5% (73.05%), Chlorothalonil 1.5% (58.03%) etc. *Fusarium solani* showed complete growth inhibition (100%) through Thiophanate methyl 1.5% and 1% followed by Thiophanate methyl 0.5% (75.58%), Chlorothalonil 1.5% (54.65%) etc.

Table.1 Effect of different concentration of *Ageratina adenophora* and Ageratum conyzoides leaf extracts on vegetative growth of pathogens

Isolates	Botanicals	Botanical concentration%/ Average mycelia Growth (mm)				Mean	Botanical concentration (%)/ Average mycelia growth Inhibition (%)				Mean
		Control	0.50%	1%	1.5%		Control	0.50%	1%	1.5%	
Alternaria alternata	*Ageratina adenophora*	44.2	38.5	34.2	21.25	31.32	0.00	12.89	22.6	51.92	29.14
	Ageratum conyzoides	49.5	44	41.25	34.25	39.89	0.00	11.11	16.6	30.8	19.25
Fusarium solani	*Ageratina adenophora*	55.7	33.2	31.5	29.5	31.4	0.00	40.39	43.44	47.03	43.62
	Ageratum conyzoides	50.5	44	43.75	35.25	41	0.00	12.87	13.36	30.69	18.97
***Macrophomina* sp.**	*Ageratina adenophora*	59.7	23.75	20	16.75	20.17	0.00	60.21	66	71.94	66.05
	Ageratum conyzoides	54.25	41.75	40.75	29	37.16	0.00	23.06	24.9	46.49	31.48
***Pestalotiopsis* sp.**	*Ageratina adenophora*	53.7	32.5	26.2	16	24.9	0.00	39.47	51.21	70.20	53.63
	Ageratum conyzoides	50	47.25	29	20	32.08	0.00	12	42	60	38
Mean	*Ageratina adenophora*		31.99	27.9	20.87			38.24	45.81	60.27	
	Ageratum conyzoides		44.25	38.69	29.62			14.76	24.23	41.99	

Table.2 Effect of different concentrations of thiophanate methyl and chlorothalonil on vegetative growth of pathogens

Isolates	Botanicals	Botanical concentration%/ Average mycelia Growth (mm)				Mean	Botanical concentration (%)/ Average mycelia growth Inhibition (%)				Mean
		Control	0.50%	1%	1.5%		Control	0.50%	1%	1.5%	
Alternaria alternata	*Thiophanate methyl*	48.25	13.1	12	11	12.03	0.00	73.05	75.12	77.20	75.12
	Chlorothalonil		22	21.25	20.25	21.17	0.00	54.4	55.95	58.03	56.13
Fusarium solani	*Thiophanate methyl*	43	10.5	0.00	0.00	3.5	0.00	75.58	100	100	91.86
	Chlorothalonil		23.5	21	19.5	21.33	0.00	45.34	51.16	54.65	50.38
***Macrophomina* sp.**	*Thiophanate methyl*	63.75	12	11.5	11.25	11.58	0.00	81.17	81.96	82.43	81.85
	Chlorothalonil		33.25	26	24.5	27.92	0.00	47.84	59.21	61.56	56.20
***Pestalotiopsis* sp.**	*Thiophanate methyl*	43	0.00	0.00	0.00	0.00	0.00	100	100	100	100
	Chlorothalonil		20	18.5	15.5	18	0.00	53.48	56.97	63.95	58.13
Mean	*Thiophanate methyl*		8.9	5.87	5.56			82.45	89.27	89.91	
	Chlorothalonil		24.69	21.69	19.94			50.26	55.75	59.55	

Fig.1 Effect of different concentrations of botanicals of *Ageratina adenophora* and *Ageratum conyzoides* on growth of pathogens

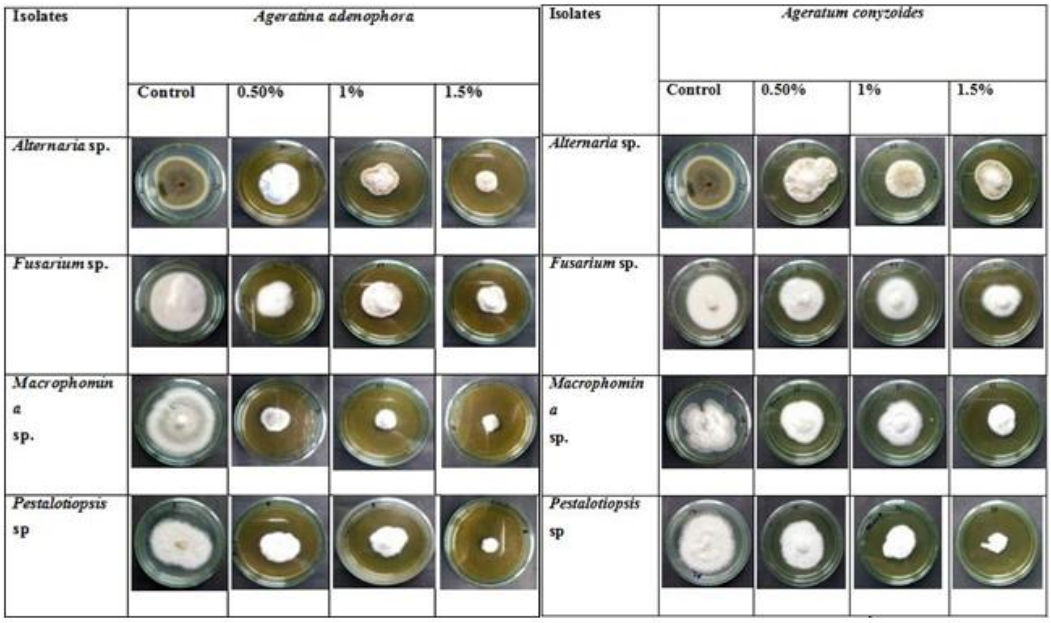


Fig.2 Effect of different concentrations of Thiophanate methyl and Chlorothalonil fungicides on the growth of pathogens

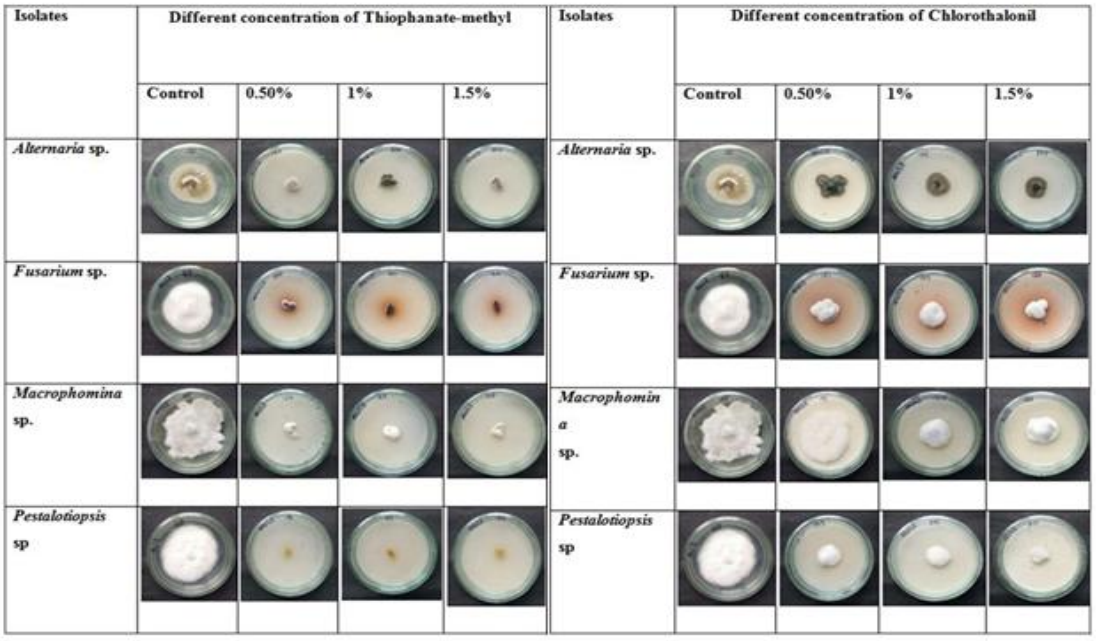
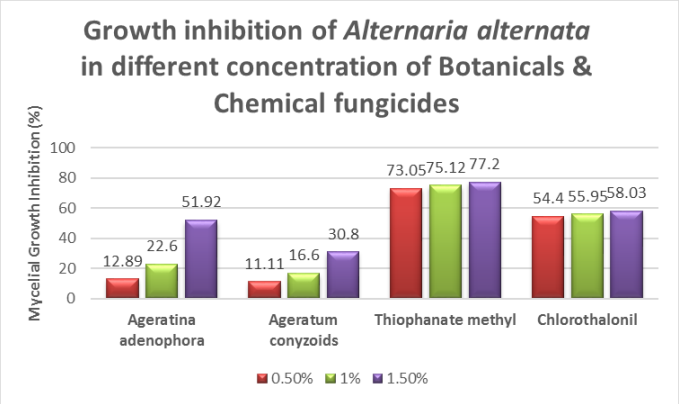
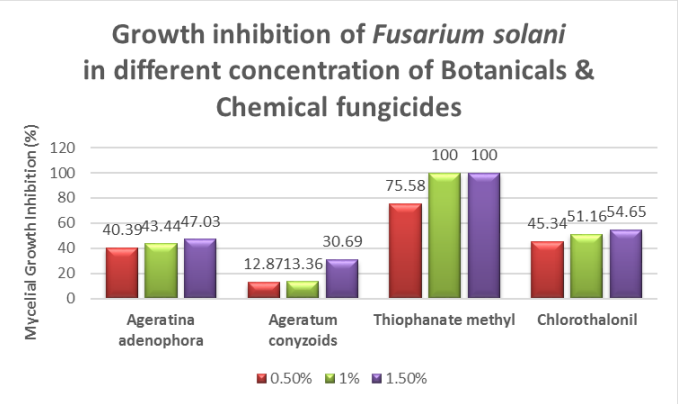
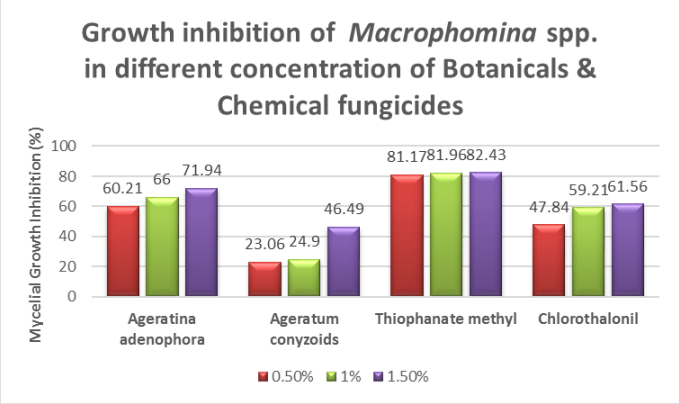
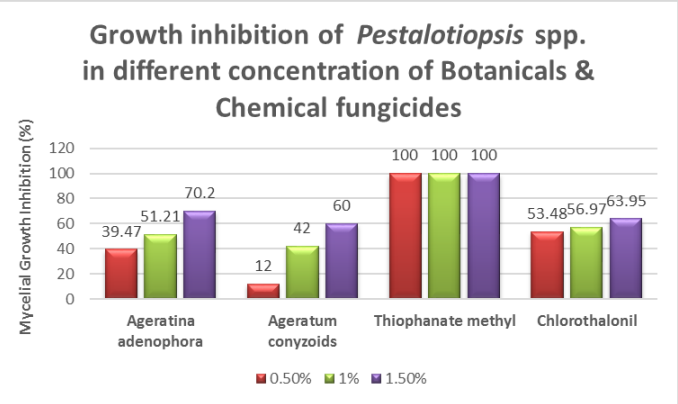


Fig.3









Here, Thiophanate methyl was found to be the most effective, even at a very low concentration of the fungicide. Growth inhibition pattern in *Macrophomina* sp. Thiophanate methyl 1.5% (82.43%), Thiophanate methyl 1% (81.96%), Thiophanate methyl 0.5% (81.17%), Chlorothalonil 1.5% (61.56%) etc. For *Pestalotiopsis* sp., Thiophanate methyl 1.5% caused maximum inhibition (100%) followed by Thiophanate methyl1%(100%), Thiophanate methyl 0.5% (100%), Chlorothalonil 1.5% (63.95%) etc. The overall estimate reveals that Thiophanate methyl was the most effective fungicide for controlling the growth of selected pathogens.

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 Thiophanate methyl and Chlorothalonil had very high efficacy in controlling *Alternaria alternata*, but a higher concentration of *Ageratina adenophora* 1.5% showed almost similar growth inhibition (51.92%) as in Chlorothalonil (54.4%). *Fusarium solani* showed maximum inhibition (upto 100%) by the systemic fungicide Thiophanate methyl. Whereas, in the case of Chlorothalonil and *Ageratina adenophora* growth pattern was 54.65% and 47.03% (1.5%), 51.16% and 43.44% (1%) and 45.34 and 40.39 (0.5%) respectively. *Macrophomina sp.* showed better inhibition (71.94%) in *Ageratina adenophora* 1.5% than in Chlorothalonil 1.5% (61.56%). Similarly, *Pestalotiopsis* sp. showed better inhibition (70.20%) in *Ageratina adenophora* 1.5% than in Chlorothalonil 1.5% (63.95%). However,

thiophanate methyl was still the most effective, causing up to 82.43% in *Macrophomina* sp. and 100% in *Pestalotiopsis* sp. growth inhibition.

Many plants and plant products have been reported to have antimicrobial activities against plant pathogenic fungi (Bashar and Rai, 1992). Invasive species can be utilized for plant disease control as they are rapid colonizers of an ecosystem and have no natural enemies. Their disease resistance capacity may be attributed to their bioactive compounds that hinder pathogen growth. The disease resistance potential of botanicals from some invasive species has also been studied by several workers (Pal *et al.,* 2013; Srivastava and Singh, 2011).

The present study reveals that the methanolic extract of *Ageratina adenophora* is highly effective against *Macrophomina* sp., followed by *Pestalotiopsis* sp., *Alternaria alternata and Fusarium solani*. Similar trends were observed in earlier studies for inhibiting plant pathogenic fungi growth by botanicals derived from different species (Khatun and Shamsi, 2016; Rai *et al.,* 2000; Deshmukh *et al.,* 2021). Whereas, in the case of fungicides, thiophanate methyl was found to be the most effective, inhibiting maximum growth in *Alternaria alternata and Macrophomina* sp. and being deadlier to *Pestalotiopsis* sp. and *Fusarium solani.* Chase *et al.,* 1993 also observed that thiophanate methylcan control the leaf spot of *Dracaena marginata* caused by *Fusarium*. A comparative analysis showed that higher doses of *Ageratina adenophora* caused either more or almost equal pathogen growth inhibition than lower doses of Chlorothalonil for certain fungi. It is a well-known fact that chemical fungicides are best suitable for pathogen treatments, but their environmental implications are irreparable. The accumulation of fungicides in the soil negatively affects organisms present in this ecosystem as well as biological processes (Devashree *et al.,* 2014). Chlorothalonil applied to sandy loam and loamy sand soils causes changes in the biological homeostasis of the soil and disturbs the soil microbiota (Baćmaga *et al.,* 2018). Therefore, the use of plant-derived fungicides isa better alternative that is both eco-friendly and economical.

If we compare the effect of chemical fungicides with botanicals on pathogen growth suppression, we find that botanicals are better against the low concentration of some chemical fungicides. Botanicals can be effective in field trials in high concentrations as there is no chance of them being harmful to the plant and soil. That might be a concern with other bio-control agents that can be opportunistic pathogenic. 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. Thus, the promotion of eco-friendly strategies such as botanical control via invasive weed species would minimize the use of chemicals in disease management and would also be a valuable addition to invasive weed species.

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How to cite this article:

Ranjana Juwantha, Akshit K. Lohan, Mohit Singh Bisht, Jalaj Saxena and Pooja Kapoor. 2021. Evaluation of Botanicals of Invasive Plant Species and Fungicides against Fungal Pathogens of Forest Nursery. *Int.J.Curr.Microbiol.App.Sci.* 10(09): 152-162.
doi: https://doi.org/10.20546/ijcmas.2021.1009.018