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Chemical Fungicides and Antagonists as Potential Treatments for *Ganoderma lucidum*-Associated Basal Stem Rot in Mandarin Trees

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Authors' contributions

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

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ABSTRACT

Ganoderma lucidum causes basal stem rot disease in mandarin trees, resulting in a variety of symptoms including dieback, foliage discoloration, an unhealthy appearance and eventual tree declines or death. Prior to field application, it is imperative to assess the efficacy of newer

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molecules and possible potential antagonists through *In vitro* testing. In this study, ten chemical fungicides and four antagonists were evaluated for their effectiveness against *G. lucidum* using the poison food method and dual culture technique, respectively. Among the chemical fungicides tested, Copper oxychloride 50% WP (@3g/l), Fluxapyroxad 167 G/L + Pyraclostrobin 333 G/L SC (@0.6 g/l), and Metiram 55% + Pyroclostribin 5% WG (@1g/l) demonstrated the highest effectiveness, entirely suppressing (100%) pathogen growth. In the dual culture technique, the fungal antagonists *Trichoderma harzianum* (79.63%) and *Trichoderma asperellum* (77.57%) exhibited superior effectiveness compared to *Pseudomonas fluorescence* and *Bacillus subtilis* against *G. lucidum*. These findings highlight the efficacy of chemical fungicides and antagonists in treating *G. lucidum*-induced basal stem rot in mandarin trees.

Keywords: Antagonist; fungicides; Ganoderma lucidum; mandarin.

1. INTRODUCTION

The root parasite *Ganoderma lucidum* (Leys) causes basal stem rot disease, which affects a variety of hard wood-fruit, ornamental, social forest and forest trees [1]. It causes white spongy root rot, also known as trunk rot, root rot, basal rot and Ganoderma root rot. Affected trees show varying degrees and combinations of dieback, foliage colour loss and generally unhealthy appearance. The tree eventually declines or dies. The fungi can spread through the air via basidiospores or by root grafting from infected to healthy tree roots. When *Ganoderma* spp. infect main or lateral roots, a whitish mycelium mat forms beneath the bark, gradually transforming into a brownhue. This white mycelium progresses upward from crown roots to the trunk, forming strands or ribbons known as rhizomorphs [2]. Near or at the base of tree, these structures produce fan-shaped fruiting bodies, commonly referred to as brackets or semi-circular conks. These brackets may be either stalked or sessile. Over time, infected roots and trunk wood become spongy, decompose and disintegrate [3].

Citrus encompasses several economically significant species, with only a few being commercially cultivated in India, including mandarins, lemons, limes, sweet oranges, and pomelos. In India, citrus cultivation spans approximately10.91 lakh hectares, yielding production of 141.1 lakh metric tons. Maharashtra state contributes to this total with citrus cultivation covering 2.10 lakh hectares and producing 18.76 MT. Specifically, Nagpur mandarin (*Citrus reticulata*) is cultivated on 1.48 lakh ha in the Vidarbha region of Maharashtra, generating an annual yield of 13.56 lakh tons [4]. The successful cultivation of citrus faces challenges from various diseases, including fungal, viral and bacterial pathogens. Citrus is susceptible to more than 150 diseases and disorders, and the current concern among growers is the basal rot or trunk rot caused by *Ganoderma lucidum*. This particular disease, also known as Ganoderma rot, affects various crops and is caused by different Ganoderma species, posing a significant threat to plantation crop production and productivity [5]. Knorr [6] identified *Ganoderma sessilis*, now known as *G. lucidum,* as a fungal agent capable of invading healthy citrus wood and it was also isolated from trees with heart rot in Florida. Additionally, [7,8,9] reported cases in south Texas where 'Marrs' early orange trees on sour orange rootstock were infected with *G. lucidum.*

There is currently no effective method to control root rot in the field. Since existing control systems are inadequate, research into new molecules or biomaterials becomes crucial. In vitro testing of these newer biopesticides and molecules prior to field use is essential. In vitro evaluation of bioagents or newer fungicide molecules allows their possible inclusion as an alternative to current recommendations under natural conditions. In addition, the use of both effective biological agents and chemical solutions can help minimize the development of resistance. In addition, the use of both effective biological agents and chemical solutions can help minimize the development of resistance.

Various researchers worldwide have explored methods to control *Ganoderma* basal rot in crops like oil palm, arecanut and coconut, but limited attention has been given to fruit crops such as mandarin. Notably, there is a research gap in the Vidarbha region regarding fungicides and bio-agents for combating mandarin basal rot. Keeping these considerations in mind, this study was undertaken to identify effective fungicides and antagonists for the control of Ganoderma root rot in Nagpur mandarin.

2. MATERIALS AND METHODS

The basidiocarps were obtained from infected trees of Nagpur mandarin growing in the AICRP on Fruits scheme field. The fruiting bodies were then brought to the laboratories, and subjected for isolation as method proposed by [10] with some modifications. The purified culture was then identified as *G. lucidum* based on morphological and cultural characteristics as described by [11 and 12].

2.1 Evaluation Chemical Fungicides

Ten chemical fungicides were evaluated by employing poisoned food method [13] against Ganoderma basal rot of Nagpur mandarin. Potato dextrose agar (PDA) was prepared and distributed at the rate of 100 ml in 250 ml conical flask; autoclaved at 1.05 kg/cm2 for 15 minutes. Requisite quantity of each of the chemical fungicides as per concentration was added in sterilized melted PDA separately so as to obtain desired concentration. Flask containing poisoned medium was shaken well to have even and uniform distribution of fungicides. About 20ml of poisoned PDA was poured in each pre sterilized Petri plate and allow to solidifying. These Petri plates were inoculated by respective pathogens separately. Six mm discs of ten days old fungus cultures were cut with sterilized cork borer and transferred to the centre of plates in inverted position containing poisoned medium. Suitable controls without fungicides were maintained by placing fungal discs in untreated plates. The whole procedure was carried out under aseptic conditions.

Experiment conducted in Completely Randomized Design (CRD) with four replications. The Petri plates were then incubated in an incubator at 25 ± 2^0C for 7 days. The diameter of the colony of the respective fungal pathogens on medium was measured after 10 days of incubation (DAI) and per cent inhibition was calculated by using the following formula [14].

2.2 Evaluation of Antagonists

The dual culture technique [15] was used to test the antagonist's activity of *Trichoderma harzianum, Trichoderma asperellum, Bacillus subtilis and Pseudomonas fluorescens* received from Center for Organic Research and Training, Dr. PDKV, Akola. The pathogen (*G. lucidum*) and antagonists were grown on PDA (for fungal antagonists) and NA (for bacterial antagonists) media for a week at 25 ± 2^0C. Two culture disc (each 6 mm for fungal antagonists) one each of the test pathogens and test bio-agents were cut out with sterilized cork borer and placed equidistance, exactly opposite to each other on autoclaved and cooled solid surface of PDA medium in Petri plates, and incubated at 25 ± 2^0C. To test bacterial antagonists, a test pathogen mycelium plug was placed in the center of PDA medium on a 90 mm Petri plates. Bacterial suspension loops were streaked on inoculated plates 1 cm away from test pathogen disc. Petri plates were then incubated at 25 ± 2^0C.The experiment was conducted in Completely Randomized Design (CRD) with five treatments and five replications. Linear mycelial growth of test pathogen was tested after 24 hours, continued till untreated control plates were fully covered with mycelial growth of test pathogen and averaged finally. Per-cent inhibition of the test pathogen with test antagonists, over control was calculated by applying formula suggested by [16].

2.3 Statistical Analysis

The data of each character were subjected to statistical test by applying of variance for different treatments. Fisher's protected critical difference (CD) test was used to indicate the difference between the treatments at the probability level of p= 0.01 following the procedure described by [17].

3. RESULTS AND DISCUSSION

3.1 Evaluation of Fungicides

The *In vitro* fungitoxic effects of ten fungicides were assessed against *G. lucidum* at their respective concentrations. The obtained results are outlined in Table 1 and Fig. 1.

The findings indicated that ten different chemical fungicides significantly impede the radial growth of *G. lucidum*. Notably, Copper oxychloride 50% WP (@3g/l), Fluxapyroxad 167 G/L + Pyraclostrobin 333 G/L SC (@0.6 g/l), and Metiram 55% + Pyroclostrobin 5% WG (@1 g/l) emerged as the most effective, completely inhibiting the growth of the test fungus at their recommended concentrations.For Kasugamycin 5% + Copper oxychloride 45% WP (@ 2 g/l), Metalaxyl 8% + Mancozeb 64% WP (@ 2.5 g/l), Mandipropamid 23.4% SC (@ 0.8 ml/l), Propineb 70% WP (@ 3 g/l), Zineb 75% WP, (@ 2 g/l)and Cymoxanil 8% + Mancozeb 64% WP (@ 2.5 g/l),

the results showed radial mycelial growth of 1.32, 1.66, 1.77, 2.64, 2.78 and 2.97 cm, respectively, accompanied by growth inhibition percentages of 86.64%, 81.47%, 80.24%, 70.63%, 69.06%, 67.36%. Fosetyl-AL 80% WP (@2.5g/l) exhibited a radial mycelial growth of 6.66 cm with least growth inhibition (26.55%). The control plate recorded the highest radial mycelial growth at 9.0 cm.

Researchers have effectively utilized chemical fungicides to control *Ganoderma,* as demonstrated by studies conducted by [18,19,20,21,22,23,24,25].

This finding aligns with the observations made by [22 and 1], who similarly noted the inhibitory effectiveness of copper oxychloride 50% WP against *Ganoderma lucidum* growth. Additionally, [21] documented that metalaxyl + mancozeb and carbendazim + mancozeb were also successful in impeding the growth of *G. lucidum*.

In the present investigation, Fluxapyroxad 167 G/L + Pyraclostrobin 333 G/L SC and Metiram 55% + Pyroclostrobin 5% WG emerged as the most potent fungicides in suppressing the mycelial growth of *G. lucidum* in laboratory conditions. A review of existing literature suggests that these results may contribute novel information, as the effectiveness of Fluxapyroxad 167 G/L + Pyraclostrobin 333 G/L SC and Metiram 55% + Pyroclostrobin 5% WG against *G. lucidum* might be reported here for the first time. These findings could prove valuable for citrus growers grappling with Ganoderma basal rot management. However, a further investigation is needed to determine the safety and efficacy of these fungicides in field conditions.

Table 1. Efficacy of chemical fungicides against *G. lucidum* (10 DAI)

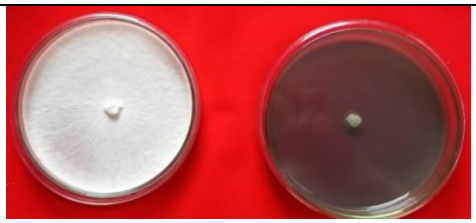
SN	Treatments	Formulation	Conc. used (%)	Radial mycelium growth (cm)	% Growth inhibition
T_1	Propineb 70%	Wettable powder	3 g/l	2.64	70.63
T_2	Zineb 75%	Wettable powder	2 g/l	2.78	69.06
T_3	Copper oxychloride 50%	Wettable powder	3 g/l	0.00	100.00
T_4	Fosetyl-AL 80%	Wettable powder	2.5 g/l	6.66	26.55
T_5	Cymoxanil 8% + Mancozeb 64%	Wettable powder	2.5 g/l	2.97	67.36
T_6	Metalaxyl 8% + Mancozeb 64%	Wettable powder	2.5 g/l	1.66	81.47
T_7	Fluxapyroxad 167 G/L + Pyraclostrobin 333 G/L	Suspension concentrates	0.6 g/l	0.00	100.00
T_8	Metiram 55% + Pyroclostrobin 5%	Wettable granules	1 g/l	0.00	100.00
T_9	Kasugamycin 5% + Copper oxychloride 45%	Wettable powder	2 g/l	1.32	86.64
T_{10}	Mandipropamid 23.4%	Suspension concentrates	0.8 ml/l	1.77	80.24
T_{11}	Control		-	9.00	
	SE(m)±		**-**	**0.57**	**-**
	CD(P=0.01)		**-**	**2.28**	**-**

Table 2. Effect of antagonists on radial mycelial growth of *G. lucidum*

SN	Treatments	Radial mycelium growth (cm)	% Growth inhibition
T_1	*Trichoderma harzianum*	1.83	79.63
T_2	*Trichoderma asperellum*	2.01	77.57
T_3	*Bacillus subtilis*	6.28	30.15
T_4	*Pseudomonas fluorescens*	4.67	48.08
T_5	Control	9.00	-
	SE(m)±	**0.39**	**-**
	CD(P=0.01)	**1.58**	**-**

T1: Propineb 70% WP

T2: Zineb 75% WP



T3: Copper oxychloride 50% WP

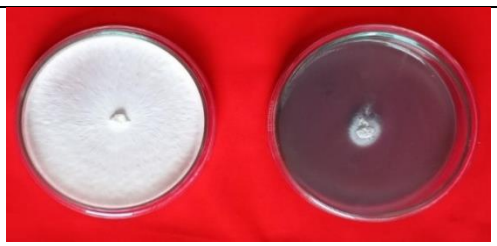
T4: Fosetyl Al 80% WP

T5: Cymoxanil 8% +Mancozeb 64% WP

T6: Metalaxyl 8% + Mancozeb 64% WP

T7: Fluxapyroxad 167 G/L+ Pyraclostrobin 333 G/L SC

T8: Metiram 55% + Pyroclostribin 5% WG



T9: Kasugamycin 5%+Copper oxychloride 45% WP

T10: Mandipropamid 23.4% SC

Fig. 1. *In vitro* efficacy of chemical fungicides against *G. lucidum* (10 DAI)

Copper oxychloride 50% WP, Fluxapyroxad 167 G/L + Pyraclostrobin 333 G/L SC, and Metiram 55% + Pyroclostrobin 5% WG were shown to be fungitoxic to *G. lucidum*, according to the study findings. Copper oxychloride is a broad-spectrum copper-based fungicide that is effective against a variety of fungus. It is commonly used in agriculture to protect plants from fungal infections. Copper oxychloride's fungicidal impact results from its capacity to liberate copper ions, which disrupt fungi's enzymatic processes. This disease hinders fungal growth and reproduction. This disruption inhibits fungal growth and reproduction. Fluxapyroxad and Pyraclostrobin are pre-formulated combination fungicides commercially available in a single application which behave in both preventive and curative modes against different fungi. Fluxapyroxad belongs to the SDHI class while Pyraclostrobin concerns the strobilurin fungicides. Unique MOAs for fluxapyroxad and pyraclostrobin, which have the ability to control the test pathogen by acting on the inhibition of energy synthesis in mitochondria and disruption of electron transport chain and energy metabolism in fungi. Another new highly effective pre-mixed fungicide was Metiram 55% + Pyraclostrobin 5% WG and could be used in controlling multiple fungal diseases. Metiram, an ethylene bis-dithiocarbamate (EBDC) fungicide, is a protectant fungicide that prevents spore germination and mycelial growth on plant surfaces. Simultaneously, the blockage of the mitochondrial electron-transport chain with the exotoxic element Pyraclostrobin hampers the fungal respiration and hence the extent with which the active agent is efficient against fungal diseases. These concentrations of these chemicals were found to be effective in inhibiting the radial growth of *G. lucidum* and could therefore serve as antifungal agents against this fungus. The action mechanism of fungitoxicity may be related to the inhibition of some metabolic processes, or even other essential functions of the fungus, leading to the observed growth inhibition [23].

3.2 Evaluation of Antagonists

In present study, four antagonists were subjected to *In vitro* evaluation against *G. lucidum* using the dual culture technique, and percent inhibition of the respective pathogens over control was calculated. The results indicated that all the evaluated antagonists exhibited fungistatic activity, and significantly inhibited the mycelial growth of *G. lucidum* (Table 2). Of the four bio-agents tested, *T. harzianum* and *T. asperellum* exhibited the highest effectiveness, recorded minimal mycelial growth of 1.83 cm and 2.01 cm, respectively. This corresponded to the highest mycelial growth inhibition, with values of 79.63% and 77.57%, respectively, compared to the control (9.0 cm). *Pseudomonas fluorescens* and *Bacillus subtilis* demonstrated comparatively lower effectiveness, with radial mycelial growth of 4.67 cm and 6.28 cm along with inhibition percentage of 48.08% and 30.15% against the test pathogen, respectively. The control plate exhibited the maximum growth of *G. lucidum*, measuring 9.00 cm.

Thus, all the antagonists' agents tested *In vitro* demonstrated fungistatic activity against *G. lucidum*, with the fungal antagonists proving more effective than bacterial antagonists in inhibiting the tested pathogen. These findings are in consistent with those documented by previous researchers such as [26,27 and 28]. Their studies reported that *Trichoderma* antagonists significantly suppressed the growth of *G. lucidum* in comparison to *Pseudomonas fluorescens* and *Bacillus subtilis*. According to [1], the research findings revealed significant growth reduction of *Trichoderma* compared to the control *in vitro* when exposed to *G. lucidum*. Among the *Trichoderma* species tested, *T. harzianum* exhibited the maximum reduction in radial growth of the test fungus (68.5%), followed by *T. viride* (65.7%) and *T. pseudokoningii* (61.11%), in comparison to the control.

Siddiquee [26] explored the antagonistic characteristics of48 local isolates of *T. harzianum* against *Ganoderma boninense* (strain PER 71) using dual culture techniques. The findings revealed that every *T. harzianum* isolate inhibited the growth of PER 71, exhibited percentage values for the inhibition radial growth ranging from 47.86 to 72.06%. Various antagonists employ distinct mechanisms to combat pathogens. These mechanisms can include direct parasitism and lysis of the pathogen, competition with the pathogen for space and nutrients, enzymatic degradation, and the production of inhibitory compounds, like antibiotic compounds that are toxic to the pathogen, collectively contributing to the inhibition or suppression of *G. lucidum* growth [29].

Trichoderma species are recognized as myco-parasites that target various fungi, displaying an active hyphal parasitic nature [30]. The efficacy of *Trichoderma* as a biocontrol agent is attributed to several mechanisms, including competition for space and nutrients, the release of chitinolytic

enzymes, myco-parasitism, and the production of an inhibitory compound [31 and 32]. The dual culture experiments likewise demonstrated that the presence of *Pseudomonas fluorescens* inhibited the growth of *G. lucidum* mycelium (48.08%), but to a lesser extent than the fungal antagonist. Pseudomonades have been shown to produce a diverse range of chemicals with antimicrobial activity against phyto-pathogenic fungi and bacteria [33]. Pseudomonades strains produce many antimicrobial compound, including various phenazines, phenazine-1-carboxylic acid and 2, 4-diacethylploroglucinol, pyoluteorin and pyrrolnitrin [34], which may contribute to the antifungal activity against *G. lucidum* in our study. New quinone and hydroquinone antibiotics generated by *Pseudomonas* spp. have been shown to be effective against Gram positive bacteria, certain fungi, and yeasts [8]. These antibiotics' antifungal efficacy was defined by their particular inhibition of fungal cell wall production. Lim [9] found that *Pseudomonas aeruginosa* B6 isolate had the highest percent growth inhibition (75.76%) against *Ganoderma boninense,* with a minimum inhibitory dose of 0.04 mg/ml. The compound that may contribute to this biocontrol function was identified as 3-demethylubiquinone-9 utilizing LC-MS.

4. CONCLUSION

Hence, the following conclusions can be drawn from the obtained results across various aspects. Among the chemicals examined, Copper oxychloride 50% WP, Fluxapyroxad 167 G/L + Pyraclostrobin 333 G/L SC and Metiram 55% + Pyroclostribin 5% WG demonstrated greater effectiveness in inhibiting the growth of *G. lucidum*. In terms of bio-agents, the fungal antagonist *T. harzianum* and *T. Asperellum* outperformed the bacterial antagonist's *P. fluorescens* and *B. subtilis* in suppressing the growth of *G. lucidum*.

5. FUTURE SCOPE

According to the findings of the current laboratory investigation, fungicides such as Copper oxychloride 50% WP or Fluxapyroxad 167 G/L + Pyraclostrobin 333 G/L SC or Metiram 55% + Pyroclostribin 5% WG, as well as antagonists *T. harzianum* and *T. asperellum*, can be used in the field to manage *G. lucidum*.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology.

Details of the AI usage are given below:

1.Chat GPT 4
2.Quill Bot

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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