Original Research Article

Exploring the biocompatibility and antifungal activity of different biopolymeric derivatives against foliar pathogens

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ABSTRACT

Aims: This study aims to investigate the compatibility of biopolymeric derivatives with the biocontrol agents and its antifungal activity against fungi plant pathogens
Study design: *In vitro* studies has been carried out by using completely randomized design
Place and Duration of Study: Plant Pathology Division, ICAR-Indian Institute of Oilseeds Research, Hyderabad, India, during March to April 2023.
Methodology: Different combinations of treatments were prepared using lignin derivative (LD). Compatibility of LD with *Trichoderma harzianum* Th4d was tested using poison food technique and data was recoded at 4 and 7 days after inoculation. Antifungal activity of lignin derivatives against foliar pathogens namely *Phaeoisariopsis personata* causing leaf spot of groundnut and *Golovinomyces cichoracearum* causing powdery mildew of sunflower was assessed using spore germination technique. Data was recorded at 24 and 48 hours after incubation.
Results: Significant difference was observed among the treatments with respect to mycelial growth of *Trichoderma harzianum* Th4d. LD+chitosan exhibited 100% compatibility with full growth of *T. harzianum*. Further, LD+chitosan+Th4d at the concentration of 0.10% significantly inhibited the spore germination of *P. personata* and *G. cichoracearum* with per cent germination inhibition of 87.53 and 93.27%, respectively.
Conclusion: Results suggests that lignin derivative integration with other biopolymers and biocontrol agents can be effectively used for the management of foliar plant pathogens.

Keywords: Sodium lignosulphonate, Compatibility, Trichoderma harzianum Th4d, antifungal activity, Phaeoisariopsis personata, Golovinomyces cichoracearum

1. INTRODUCTION

Foliar diseases can cause significant yield losses in crops, ranging from 10 to 50% depending on the crop and the severity of the disease. Diseases like late leaf spot of groundnut [1,2] and powdery mildew of sunflower [3,4] can cause yield loss up to 50%, without fungicide application losses may increase up to 70% [5]. These diseases can be effectively managed by the application of chemical fungicides, however growing awareness on the insurmountable impact on the environment due to over use of agro-chemicals, demanding for a cleaner and greener alternatives for agro-chemicals for the sustainable management of crop diseases. One such unexplored solution is; lignin derived from plant residues [6]. Lignin in the form of sodium lignosulphonate is obtained as a major byproduct

in paper and pulp industries [7]. It is a sodium salt of lignosulphonic acid, a natural polymer found in plant cell walls. It can regulate the metabolic activities of the plants and enhances the plant growth and natural resistance of plants against fungi, bacteria and viruses [5, 8, 9,10].

Lignin derivatives are rich in chemical groups such as sulfonic, carboxyl and phenolic hydroxyl groups and exhibits characteristics of integration, slow release and non-polluting through modifications which can be used in agricultural production in a variety of forms [11,12]. Antibacterial nature of lignin is proven by many researchers [13, 14]. But its potentiality against fungal plant pathogens is unexplored. Hence, the present study was conducted to explore the antifungal activity of lignin derivatives against foliar pathogens namely *Phaeoisariopsis personata* (Berk. & Curt.), which causes late leaf spot of groundnut and *Golovinomyces cichoracearum* (DC) V.P. Heluta, which causes powdery mildew of sunflower. Further its compatibility with biocontrol agent, *Trichoderma harzianum* Th4d is also assessed, in order to investigate the integrability of lignin derived from the plants in the biocontrol of plant diseases.

2. MATERIAL AND METHODS

2.1 Compatibility test

Lignin derivative (LD) and its combinations with chitosan were evaluated for their compatibility with biocontrol agent *T. harzianum*, using poison food technique. Lignin derivatives (LD) *viz*., A, B and LD + chitosan were added separately in to the Petri plates before the addition of potato dextrose agar media and then plates were turned clock wise and anti-clock wise for even distribution of the media. After solidification of the media, 5 mm disc of *T. harzianum* was inoculated at the center of the Petri plates and incubated at 25 ± 2 ℃. For each treatment five replications were maintained. Per cent mycelial growth was calculated at 4 and 7 days after inoculation.

2.2 Antifungal activity test

In vitro antifungal activity of Lignin derivative (LD), combination of LD+chitosan and LD+chitosan+Th4d were evaluated against *P. personata* and *G. cichoracearum* using spore germination technique. Double concentration of treatments was prepared using sterile distilled water. Conidial suspension of *P. personata* and *G. cichoracearum* was prepared out of symptomatic leaves collected freshly from the incubated and infected susceptible plants which were maintained in the greenhouse. One drop of each treatment *viz.,* LD, Th4d, LD+chitosan, and LD+chitosan+Th4d of different concentrations (at 0.05 and 0.10 %) was placed in the cavity of glass slides followed by the addition of drop of conidial suspension (4 x 10^3 conidia/ml concentration). Each cavity slide was kept in the Petri dishes lined with wet blotting paper and incubated at 25 ± 1 ºC. Observations were recorded at intervals of 24 and 48 hours after incubation. For each treatment four replications were maintained. The average percentage of germinated conidia was recorded and calculated by number of germinated conidia to the total number of conidia observed. Per cent inhibition over the control was calculated by using the formula given by Vincent [15].

$$I = \frac{C - T}{C} \times 100$$

Where, I = Per cent inhibition, C = Germination of spores in control, T = Germination of spores in treatment

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3. RESULTS AND DISCUSSION

3.1 Compatibility of sodium lignosulphonate with *T. harzianum*

Compatibility of lignin derivative (LD, lignosulphonate) with *T. harzianum*, in combination with chitosan was tested by following poison food technique. Table 1, shows the per cent mycelial growth of *Trichoderma* on PDA plates supplemented with different lignosulphonate treatments. There was a significant difference between the treatments, at 4 days after inoculation in control (only PDA) showed maximum growth of *Trichoderma* (91.18 %) followed by Lignin derivative B. Whereas LD+chitosan showed less mycelial growth compared to others. But, after 7 days of inoculation it showed 100 per cent mycelial growth of *Trichoderma* and was on par with the control (100 % mycelial growth). Others treatments did not show 100 per cent compatibility but they recorded above 90 per cent compatibility (Table 1). Compatibility of chitosan is already proven by many researchers [16, 17, 18, 19], but with respect to the biocompatibility of lignin derivative *i.e.,* lignosulphonate with biocontrol agents, there is no literature available. As of our best knowledge this might be the first report showing compatibility of sodium lignosulphonate with biocontrol agent, *T. harzianum*.

Table 1. Effect of sodium lignosulphonate on mycelial growth of *Trichoderma harzianum* Th4d

Treatments	Mycelial growth (%)	
	4 DAI	7 DAI
Lignin derivative A	73.53 [c]	94.12 [b]
Lignin derivative B	85.29 [b]	97.06 [b]
Lignin deriavtive + chitosan	70.59 [c]	100 [a]
Control	91.18 [a]	100 [a]
S. Em±	1.22	0.57
C. D ($P \leq 0.05$)	4.01	2.74

DAI-Days after inoculation, values represented here are mean of five replications. Means followed by the same letter indicates no significant difference between the treatment according to Duncan's Multiple Range Test ($P = .05$).

3.2 Antifungal activity of sodium lignosulphonate

Effect of lignin derivative (LD), LD+chitosan and LD+chitosan+Trichoderma (Th4d) at different concentrations were evaluated against spore germination of *P. personata* and *G. cichoracearum*. Data obtained is represented in the Table 2. All the treatments significantly inhibited the spore germination of both the pathogens *viz*., *P. personata* and *G. cichoracearum*. Among all the treatments, LD (lignosulphonate) showed highest spore germination inhibition (95.38 %) with least germination per cent (2.78) of *P. personata* followed by LD+chitosan at 0.10 % with spore inhibition per cent of 88.21 and germination per cent of 5.83 at 48 hours after incubation and was on par with LD+chitosan+Th4d at 0.10%. However, at 24 hours of incubation LD+chitosan+Th4d at 0.10 % showed maximum spore inhibition of 89.61 per cent with least germination of 3.33 per cent followed by LD+chitosan+Th4d at 0.05%, which recorded spore inhibition of 88.07 per cent with

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germination per cent of 5.63. Nevertheless, in case of spore germination of *G. cichoracearum*, treatment LD+chitosan+Th4d at 0.10% concentration showed highest spore inhibition with least germination percent at both 24 and 48 hours of incubation (91.82 and 96.12 % inhibition with 3.75 and 2.38 % germination, respectively) followed by *Trichoderma* (Th4d) (84.24 and 92.63 % inhibition with 7.98 and 4.50 % germination, respectively) (Table 2). Lignin derivative also inhibited spore germination of *G. cichoracearum* significantly, it recorded 91.54 and 88.74 per cent inhibition with 4.58 and 6.90 per cent germination at 24 and 48 hours after incubation, respectively. And it was on par with *Trichoderma* (Th4d) in inhibiting the spore germination of *G. cichoracearum*. Figure 1, shows the mean inhibition of all the treatments against spore germination of *P. personata* and *G. cichoracearum*.

Antifungal activity of lignin derivative (lignosulphonate) is not yet explored against plant pathogens. However, there are some reports showing antimicrobial activity of lignosulphonate against molds and yeast which are isolated from the spoiled forage [20]. Rayes et al. [20] reported 100 per cent inhibition of yeast and molds growth by sodium and manganese lignosulphonates. Kim et al. [13] also reported the antibacterial activity of lignosulphonate embedded with chitosan against *E. coli* and *B. subtilis*. Results indicated that the combination of LD + chitosan + *Trichoderma* (Th4d), showed highest inhibition of spore germination of both the pathogens. Antifungal activity of *Trichoderma* against *P. personata* [21, 22] and *G. cichoracearum* [23, 24] is already proven at laboratory and field level. Ashwini et al. [25] reported that the cultural filtrate of *Trichoderma harzianum* Th-1 recorded 50.15 per cent inhibition of spore germination of *E. cichoracearum* which is the causal organism of bhendi powdery mildew. *Trichoderma* spp. is reported to produce metabolites which hinders the spore germination of many numbers of fungal pathogens [26]. Overall results revealed that the combination of LD+chitosan+Th4d enhanced the antifungal activity of *Trichoderma* against *P. personata* and also against *G. cichoracearum*.

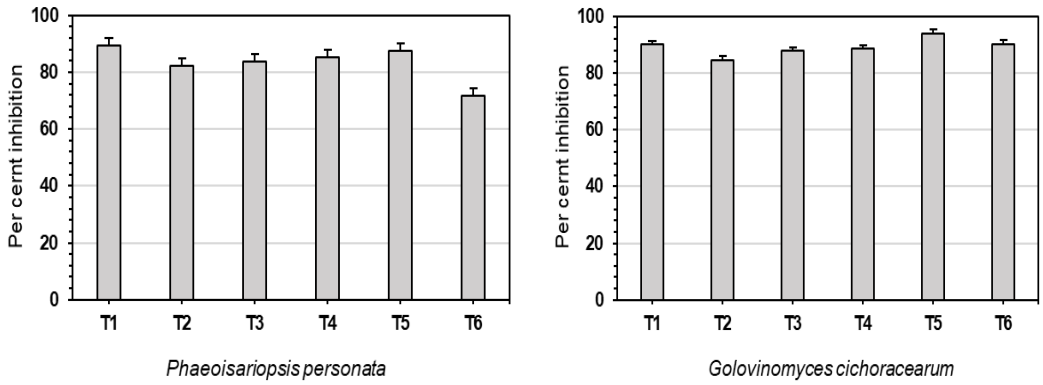

Figure 1. Mean inhibition of *Phaeoisariopsis personata* and *Golovinomyces cichoracearum* spore germination by lignin derivative and combined treatments of LD+chitosan+Th4d [T1- lignin derivative (LD); T2 – LD+ chitosan at 0.05%; T3 – LD+ chitosan at 0.10%; T4 – LD + chitosan + Th4d at 0.05%; T5 – LD + chitosan + Th4d at 0.10%; T6 – *Trichoderma* (Th4d)]

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Table 2. Effect of lignin derivative and its combination with chitosan and *Trichoderma* (Th4d) against spore germination of foliar pathogens

Treatments	*Phaeoisariopsis personata*				*Golovinomyces cichoracearum*			
	24 hours		48 hours		24 hours		48 hours	
	X (%)	Y (%)	X (%)	Y (%)	X (%)	Y (%)	X (%)	Y (%)
Lignin derivative (LD) at 10 %	7.30 (15.68) ^{c}	83.55 (66.07) ^{bc}	2.78 (9.59) ^{e}	95.38 (77.58) ^{a}	4.58 (12.35) ^{de}	91.54 (73.09) ^{ab}	6.90 (15.23) ^{b}	88.74 (70.39) ^{bc}
LD+ Chitosan at 0.05 %	6.70 (15.00) ^{c}	81.33 (64.40) ^{c}	8.40 (16.85) ^{c}	83.40 (65.95) ^{bc}	8.75 (17.21) ^{b}	82.48 (65.26) ^{c}	7.83 (16.24) ^{b}	86.76 (68.66) ^{c}
LD + chitosan at 0.10 %	9.20 (17.66) ^{b}	79.32 (62.95) ^{cd}	5.83 (13.97) ^{d}	88.21 (69.92) ^{b}	6.68 (14.97) ^{c}	87.22 (69.05) ^{bc}	7.33 (15.70) ^{b}	88.27 (69.97) ^{bc}
LD+chitosan+Th4d at 0.05 %	5.63 (13.72) ^{d}	88.07 (69.79) ^{ab}	7.75 (16.16) ^{c}	82.71 (65.43) ^{c}	7.98 (16.40) ^{b}	84.24 (66.61) ^{c}	4.50 (12.25) ^{c}	92.63 (74.25) ^{ab}
LD+chitosan+Th4d at 0.10 %	3.33 (10.51) ^{e}	89.61 (71.20) ^{a}	7.70 (16.11) ^{c}	85.44 (67.57) ^{bc}	3.75 (11.17) ^{e}	91.82 (73.38) ^{a}	2.38 (8.87) ^{d}	96.12 (78.63) ^{a}
Trichoderma (Th4d)	9.45 (17.90) ^{b}	75.41 (60.27) ^{d}	15.43 (23.13) ^{b}	68.04 (55.58) ^{d}	5.00 (12.92) ^{d}	90.15 (71.71) ^{ab}	5.43 (13.47) ^{c}	90.29 (71.85) ^{bc}
Control	41.93 (40.35) ^{a}		52.50 (46.43) ^{a}		55.18 (47.97) ^{a}		60.63 (51.13) ^{a}	
S. Em ±	0.44	1.29	0.53	1.46	0.48	1.57	0.51	1.56
CD	1.23	3.99	1.39	4.08	1.35	4.24	1.39	4.38

X = Per cent germination, Y= Per cent inhibition. Values mentioned in parenthesis are arc sine converted mean values. Means followed by same letter indicates no significant difference between the treatment according to Duncan's Multiple Range Test (*P* = .05)

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4. CONCLUSION

In summary, results unveiled the antifungal activity of lignin derivative (LD) against foliar pathogens and good compatibility with the biocontrol agent, *Trichoderma harzianum.* Initially, it resulted in slow growth of *Trichoderma* up to 4 days but after 7 days of incubation 90 to 100 per cent growth of *Trichoderma* was observed. Further, the combination of lignin derivative (LD, lignosulphonate) with chitosan significantly enhanced the antifungal activity of *Trichoderma*, which noted 87.53 and 93.27 per cent mean inhibition of spore germination of *P. personata* and *G. cichoracearum*, respectively. In conclusion, lignin derivative (lignosulphonate) alone or in integration with other natural polymers and biocontrol agents, can be potentially utilized for the development sustainable disease management strategies.

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