



Sustainable Disease Management: Evaluating Natural Alternatives for Powdery Mildew Control in Cucumbers

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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

Powdery mildew, caused by *Podosphaera xanthii*, is a significant disease impacting cucumber (*Cucumis sativus* L.) crops, leading to major yield losses. The study evaluates the efficacy of botanicals and biostimulants in managing powdery mildew under *in vitro* conditions. Treatments were tested at three concentrations (5, 7.5 and 10%) against *P. xanthii*. Results revealed that the efficacy of treatments increased with concentration, with *Ascophyllum nodosum* (seaweed extract), *Mimosa pudica* (touch-me-not) and *Osmium sanctum* (tulasi) demonstrating the highest inhibition rates at 10%. These findings suggest the potential of eco-friendly alternatives for managing powdery mildew in cucumber.

Keywords: Powdery mildew; cucumber; *Podosphaera xanthii*; botanicals; biostimulants; eco-friendly management.

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1. INTRODUCTION

Powdery mildew is a destructive fungal disease caused by *Podosphaera xanthii* that affects cucumber and other cucurbits, reducing photosynthetic activity and causing significant yield losses upto >60% (Perez-Garcia et al., 2009; El-Naggar et al., 2012; Rur et al., 2018 and Nayak et al., 2023). When resistant sources are unavailable or new virulent pathogen races emerge, fungicides are an alternative method for managing plant diseases. Evaluating new-generation fungicides in both *in vitro* and *in vivo* conditions help to determine their efficacy for field recommendations (Urban & Lebeda 2006). Because of the growing worries about the heavy reliance on chemical fungicides and their long-term effects on crops, contemporary plant protection is focusing more on non-chemical strategies, including plant extracts, to tackle plant diseases such as cucumber powdery mildew. Such studies are very valuable. However, continuous use of the same fungicides can lead to pathogen resistance and negatively impact the ecosystem. Thus, screening plant products (botanicals) and bio-stimulants, for antifungal activity is necessary to reduce fungicide use.

Conventional management strategies often rely on synthetic fungicides, raising concerns about environmental impact and resistance development (Farquhar et al., 2009). Eco-friendly alternatives, such as botanicals and biostimulants, have gained attention as sustainable disease management solutions (Marzani et al., 2021, Ni & Punja, 2021)—the efficacy of various botanicals against powdery mildew of sunflower both *in vitro* and *in vivo* conditions was effective in inhibiting spore germination of the pathogen (Dinesh et al. 2015). The present investigation evaluated ten different botanicals and biostimulants for the possible presence of fungitoxic substance against *P. xanthii* under *in vitro* conditions. This study aimed to investigate the *in vitro* management of cucumber powdery mildew using selected plant extracts and bio-stimulants, emphasizing sustainable and eco-friendly disease management strategies.

2. MATERIALS AND METHODS

2.1 Isolation of *P. xanthii*

P. xanthii isolates were collected from infected cucumber leaves grown under greenhouse conditions at the Zonal Agricultural Research

Station, Bengaluru. The isolation of the powdery mildew fungus, an obligate parasite, was indirectly approached by collecting infected cucumber leaves and preparing spore suspensions for further *in vitro* studies. Botanicals and biostimulants were prepared as aqueous extracts at concentrations of 5, 7.5 and 10% (McGrath, 2001) (Table 1).

2.2 Collection of Pathogen and Processing

Twenty-five grams of leaves of corresponding plant material were rinsed in water and cut into small pieces and macerated using a sterilized pestle and mortar with 25 ml of distilled water. The contents were filtered through a clean double-layered muslin cloth. From this solution 5 ml, 7.5 ml and 10 ml and added to 95 ml, 92.5 and 90 ml of distilled water to get 5%, 7.5% and 10% concentrations respectively. These extracts were centrifuged for five minutes at 3000 rpm to get a clear plant extract. Four drops of plant extract solution were added on to the 2cm cut detached leaf assay with the help of a dropper/camel hair brush. In each treatment three replications were maintained. These Petri plates containing slides were lined with moist blotting paper and were incubated at room temperature ($28 \pm 1^\circ\text{C}$) for 72 hours under dark condition. The observation on per cent leaf area inhibition of powdery mildew was recorded at 72 hours after incubation under Leica microscope with 10X magnification (Sedlakova and Lebeda 2009, Mc Grath and Fox, 2010, Kumar and Chandel, 2018). The average of three replications was calculated and the per cent leaf area inhibition was calculated with the following formula given by Vincent (1927).

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

Where, I = Per cent inhibition of spore germination

C = Germination of conidia in control

T = Germination of conidia in treatment

Experimental design: The experiment was conducted in a completely randomized design (CRD) with 10 treatments and three replications and control. Disease severity was assessed using a 0-9 scale (Jenkins and Wehner 1983) and converted to Percentage Disease Index (PDI) proposed by Wheeler (1969) (Table 2). Pathogen inhibition was calculated based on disease severity reduction relative to an untreated control and within treatment.

Table 1. List of botanicals and biostimulants used for *in vitro* evaluation against *P. xanthii*

Sl. No.	Treatment code	Treatments
1	T1	<i>Simarouba glauca</i> (Simarouba)
2	T2	<i>Cymbopogon ambiguous</i> (Lemon grass)
3	T3	<i>Azadirachta indica</i> (Neem)
4	T4	<i>Bougainvillea spectabilis</i> (Bougainvillea)
5	T5	<i>Pongamia pinnata</i> (Pongamia)
6	T6	<i>Eucalyptus obliqua</i> (Eucalyptus)
7	T7	<i>Osmium sanctum</i> (Tulsi)
8	T8	<i>Mimosa pudica</i> (Touch-me-not)
9	T9	<i>Ascophyllum nodosum</i> (Seaweed extract)
10	T10	<i>Sargassum sp.</i> (Seaweed extract)
11	T11	Propiconazole (Positive control)

$$PDI (\%) = \frac{\text{Sum of individual disease ratings}}{\text{Total no. of leaves observed} \times \text{Maximum disease grade}} \times 100$$

Table 2. Disease Severity Scale for Powdery Mildew

Score	% Disease severity (PDI)	Description
0	0	No disease
1	0–3	Few small leaf lesions
2	3–6	Few lesions on few leaves with no stem lesions
3	6–12	Few lesions on few leaves or with superficial stem lesions
4	12–25	Few well-formed leaf lesions or superficial stem lesions
5	25–50	Few well-formed leaf lesions or enlarging stem lesions
6	50–75	Many large leaf lesions or deep stem lesions with abundant sporulation, or plant more than 50 per cent defoliated
7	75–87	Many large coalescing leaf or stem lesions, over 75 per cent of plant area affected or defoliated
8	87–100	Plants largely defoliated, leaf or stem with abundant sporulating lesions
9	100	Plants dead (Jenkins and Wehner, 1983)

2.3 Statistical Analysis

Data were analyzed using ANOVA, and significant differences were determined using LSD at $P \leq 0.01$ significance level using OPSTAT software. Arc-sine transformations were applied to percent values for normalization.

3. RESULTS AND DISCUSSION

3.1 *In Vitro* Evaluation of Botanicals and Biostimulants

3.1.1 Effect of botanicals and biostimulants at 5% concentration

Among the treatments, *Ascophyllum nodosum* (T9) and *Osmium sanctum* (T7) recorded the lowest PDI of 27.06 and 29.63 per cent, with inhibition rates of 51.21 and 54.25 per cent,

respectively. *Eucalyptus obliqua* (T6) showed a PDI of 30.82 per cent with an inhibition rate of 52.41 per cent. Moderate efficacy was observed for *Pongamia pinnata* (T5) and *Azadirachta indica* (T3), with inhibition rates of 44.83 and 41.75 per cent (Table 3).

3.1.2 Effect at 7.5% concentration

At 7.5%, disease suppression was highest with *Mimosa pudica* (T8) and *Ascophyllum nodosum* (T9), achieving inhibition rates of 58.21 and 58.41 per cent. *Osmium sanctum* (T7) and *Eucalyptus obliqua* (T6) showed high effectiveness, with inhibition rates of 55.42 and 54.29 per cent, respectively. Moderate reductions were noted for *Azadirachta indica* (T3) and *Bougainvillea spectabilis* (T4) (Table 3) (Fig. 1a).

Table 3. *In vitro* efficacy of botanicals/ biostimulants against *P. xanthi* infecting cucumber

Treatment	Botanicals/ Biostimulants	Per cent inhibition over control				Efficacy within treatment			
		5%	7.5%	10%	Mean	5%	7.5%	10%	Mean
T1	<i>Simarouba glauca</i>	27.03 (31.39)*	29.10 (32.63)	32.03 (34.45)	29.38 (32.08)	11.03 (19.39)	13.89 (21.88)	15.85 (23.45)	13.59 (21.57)
T2	<i>Cymbopogon ambiguous</i>	28.82 (32.45)	36.83 (37.35)	38.88 (38.56)	34.84 (36.12)	11.48 (19.80)	16.83 (24.21)	20.99 (27.26)	16.44 (23.76)
T3	<i>Azadirachta indica</i> A. Juss	41.75 (40.24)	41.75 (43.65)	47.67 (49.39)	49.03 (44.42)	32.42 (34.69)	35.90 (36.79)	37.67 (37.85)	35.33 (36.44)
T4	<i>Bougainvillea spectabilis</i>	35.89 (36.79)	43.11 (41.03)	60.16 (50.84)	46.39 (42.88)	34.89 (36.19)	39.03 (38.65)	49.82 (44.88)	41.25 (39.91)
T5	<i>Pongamia pinnata</i> L	44.83 (42.02)	46.44 (42.94)	47.78 (43.71)	46.35 (42.89)	14.17 (22.10)	23.28 (28.84)	27.78 (31.79)	21.74 (27.58)
T6	<i>Eucalyptus obliqua</i>	52.41 (46.36)	54.29 (47.44)	55.86 (48.34)	54.18 (47.38)	23.68 (29.10)	35.62 (36.63)	46.52 (42.99)	35.27 (36.24)
T7	<i>Osmium sanctum</i>	54.25 (47.42)	55.42 (48.09)	56.89 (48.94)	55.52 (48.15)	34.25 (35.25)	33.33 (35.25)	48.06 (44.35)	38.82 (38.47)
T8	<i>Mimosa pudica</i>	48.15 (43.92)	58.21 (49.71)	58.93 (50.12)	55.10 (47.92)	28.15 (32.02)	34.75 (36.11)	38.93 (38.59)	33.94 (35.57)
T9	<i>Ascophyllum nodosum</i>	51.21 (45.67)	58.41 (49.82)	63.37 (52.73)	57.66 (49.41)	23.87 (29.24)	37.41 (37.69)	43.79 (41.42)	35.02 (36.11)
T10	<i>Sargassum</i> sp.	43.32 (41.14)	54.49 (47.56)	58.24 (49.72)	52.02 (46.14)	21.56 (27.65)	25.39 (30.24)	38.39 (38.27)	28.45 (32.06)
T11	Propiconazole (Positive control)	30.53 (33.53)	26.60 (31.03)	23.17 (28.76)	26.77 (31.14)	52.85 (46.61)	58.93 (50.12)	64.22 (53.24)	58.40 (49.81)
T12	Control	64.76 (53.56)	-	-	-	-	-	-	-
* Arc sin transformed values									
		S. Em ±				C. D. at 1%			
Botanicals (B)		0.36				1.39			
Concentration (C)		0.27				1.08			
B x C		0.62				2.41			

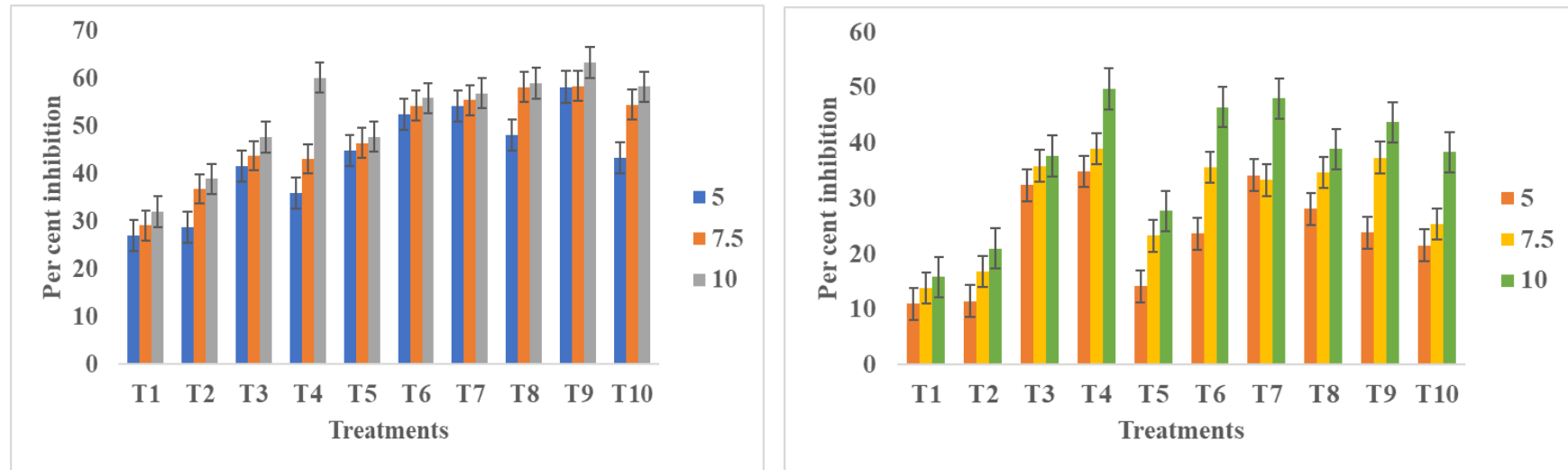


Fig. 1. Per cent powdery mildew leaf area inhibition a) botanicals/biostimulants over control; b) botanicals/biostimulants within treatment

3.1.3 Effect at 10% concentration

At 10%, *Ascophyllum nodosum* (T9) demonstrated the highest inhibition rate of 63.37 per cent, followed by *Mimosa pudica* (T8) and *Osmium sanctum* (T7) with 58.93 and 56.89 per cent, respectively. *Eucalyptus obliqua* (T6) continued to perform well, with a PDI of 17.40 per cent and an inhibition rate of 55.86 per cent (Table 3). Moderate efficacy was observed for *Pongamia pinnata* (T5) and *Sargassum* sp. (T10). *In vitro* evaluation of botanicals and biostimulants revealed that as the concentration of the extracts increased the effectiveness was also increased. The results are in agreement with several workers Dinesh et al. 2015.

3.1.4 Treatment efficacy of botanicals and biostimulants for managing powdery mildew of cucumber under *in vitro* conditions

The efficacy of different botanicals and biostimulants in inhibiting *P. xanthi* at three concentrations: 5, 7.5 and 10% within treatment. Among the treatments, *B. spectabilis* showed the highest disease inhibition, particularly at 10% concentration, reducing disease severity by 49.82 per cent with an overall mean inhibition of 41.25 per cent. Similarly, *E. obliqua* was highly effective, with 46.52 per cent inhibition at 10%, demonstrating a notable reduction in disease severity from 39.42 to 25.52 per cent. *A. indica* and *O. sanctum* also performed well, with mean inhibitions of 35.33 and 38.82 per cent, respectively. Neem reduced the disease severity from 52.01 to 33.63 per cent, and Tulasi exhibited its highest inhibition (48.06%) at 10% (Table 3) (Fig. 1b).

Moderate efficacy was observed with *C. ambiguous*, *S. glauca* and the seaweed extracts *A. nodosum* and *Sargassum* sp. Lemon grass had a mean inhibition of 16.44 per cent, with the highest inhibition of 20.99 per cent at 10%. Simarouba had a mean inhibition of 13.59 per cent, with its peak inhibition at 15.85 per cent at 10%. The seaweed extracts had lower mean inhibitions 35.02 per cent for *Ascophyllum* and 28.45 per cent for *Sargassum*, although their effectiveness increased at higher concentrations. *P. pinnata* and *M. pudica* exhibited moderate disease inhibition, reducing the severity by up to 27.78 and 38.93 per cent at 10%, respectively. In contrast, the control treatment showed no inhibition, maintaining a constant disease severity of 64.76 per cent.

Overall, higher concentrations (10%) resulted in the most significant disease suppression. *Ascophyllum nodosum*, *Osmium sanctum*, and *Eucalyptus obliqua* were consistently effective across all concentrations. These findings align with studies by Marzani et al. (2021) and Kumar and Chandel (2018), which demonstrated the potential of plant-based extracts for managing powdery mildew.

4. CONCLUSION

The study highlights the potential of botanicals and biostimulants as eco-friendly alternatives to synthetic fungicides for managing powdery mildew in cucumber. Among the tested treatments, *Ascophyllum nodosum*, *Mimosa pudica* and *Osmium sanctum* emerged as the most effective, particularly at higher concentrations. Future research should focus on field-scale validation and the development of integrated disease management strategies.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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