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SHORT COMMUNICATION

Endophytic *Bacillus* in the biological control of chili anthracnose: An *in vitro* study against *Colletotrichum capsici*

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Chili, a vital economic crop in India, faces significant threats from anthracnose, caused by the fungus *Colletotrichum capsici*, potentially leading to yield losses of up to 80%. This study evaluates the *in vitro* antagonistic efficacy of 13 endophytic *Bacillus* isolates against *C. capsici* as a biocontrol strategy. Ten isolates demonstrated significant antifungal activity, with isolate PNRE4 showing the highest inhibition zone (12.83 mm) and isolate PNRE4 achieving the greatest mycelial growth inhibition (81.25%). Additionally, isolate CFLE3 exhibited a 3.41 mm inhibition zone in culture filtrate assays. These findings suggest that *Bacillus* species, especially in consortiums, could be effectively utilized for managing anthracnose and promoting sustainable chili farming. The effective isolates will be further studied and applied in disease management strategies.

Keywords: Chili, Anthracnose, Endophytic *Bacillus*, *Colletotrichum capsici*

INTRODUCTION

Chili (*Capsicum annum* L.) is a crucial crop in India, the world's largest producer, consumer, and exporter, contributing 40% of global production. Rich in essential nutrients, chili supports health benefits (Grubben and Tahir El, 2004), but faces severe threats from anthracnose, caused by the fungal pathogen *C. capsici*, leading to significant yield losses of 10% to 54% in India (Lakshmesha *et al.* 2005). This pathogen causes pre- and post-harvest fruit damage, characterized by sunken, grey lesions with black margins (Than *et al.* 2008). Traditionally controlled with chemical fungicides, the disease has led to fungicide resistance and ecological concerns.

Biological control using *Bacillus* species, which produce antifungal compounds and enhance plant resistance, offers a sustainable alternative (Sahu *et al*. 2015).This study investigates the antagonistic potential of *Bacillus* species against *C. capsici* to promote sustainable chili cultivation in India.

MATERIALS AND METHODS

Thirteen endophytic *Bacillus* isolates namely PNRE5, PNRE4, NATOO1, AR2, AR1, GR1, GR2, WR2, WR1, WL2, PNSE5, CBRE5 and CFLE3 were sourced from previous research conducted at the Department of Plant Pathology and Microbiology, College of Agriculture, Vellayani, Trivandrum, Kerala for this study. Each isolate was cultured on nutrient agar, while the fungal pathogen *C. capsici* was grown on Potato Dextrose Agar (PDA) plates. The antagonistic activity of the *Bacillus* isolates against *C. capsici* was evaluated using a dual culture plate assay, where mycelial discs of *C. capsici* were placed in the center of PDA plates, with *Bacillus* streaks on opposite edges. Plates were incubated, and the percentage of mycelial growth inhibition and inhibition zones were recorded. Additionally, culture filtrates of *Bacillus* isolates were prepared by incubating them in nutrient broth, followed by

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Table 1. Antagonistic activity of endophytic *Bacillus* against *C. capsici* in dual culture assay and culture filtrate assay

Isolate	Inhibition zone (in mm)* (Dual culture assay)	Inhibition zone (in mm)* (Culture filtrate assay)
PNRE4	12.83 (20.99) ± 1.04^{a}	1.41(6.84) ± 0.14^{cd}
PNSE5	3.67 (11.04) ± 3.40^{de}	0.00
WR1	7.66 (16.07) ± 2.08^{bc}	2.83(9.69) ± 0.62^{ab}
WR2	10.16 (18.59) ± 0.57^{abc}	2.16 (8.47) ± 0.62^{bc}
WL2	11.16 (19.52) ± 1.04^{ab}	2.33 (8.79) ± 0.38^{bc}
CBSE5	9.50 (17.95) ± 2.00^{abc}	0.00
AR2	7.83 (16.25) ± 4.61^{bc}	1.08 (5.97) ± 0.28^{d}
CBRE5	2.83 (9.69) ± 1.04^{e}	1.92 (7.96) ± 0.52^{bcd}
GR1	7.00 (15.34) ± 2.646^{d}	0.00
CFLE3	6.50 (14.77) ± 0.50^{cde}	3.41 (10.65) ± 0.80^{a}
NATOO1	0.00	0.00
PNRE5	0.00	0.00
GR2	0.00	0.00
Control	0.00	0.00
SEm ±	1.31	0.30
CD (5%)	3.88	0.92

* Mean (± SD) of three replications. Values followed by similar superscripts in a column are not significantly different at 5% level (pd ≤ 0.05).

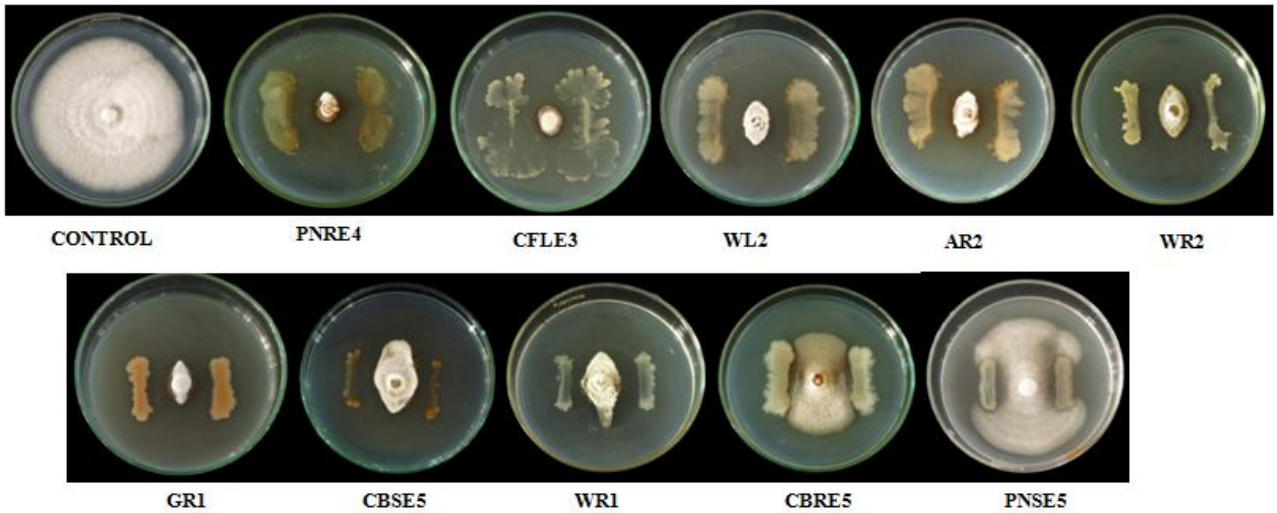

Fig.1 : Mycelial growth inhibition of *C.capsici* in dual culture plate assay

centrifugation and filtration. The filtrates were then tested for antagonistic activity using the agar well diffusion method, with inhibition zones measured after 48 hours of incubation.

RESULTS AND DISCUSSION

Of the 13 *Bacillus* isolates tested, 10 showed significant inhibition of *C. capsici* in dual culture assays, with isolate PNRE4 displaying the largest inhibition zone (12.83 mm). Other isolates like WL2, WR2, and CBSE5 also showed strong antifungal activity, while CBRE5 had the smallest zone (2.83 mm), indicating variability in antifungal compound production. These results align with findings by Srikhong *et al*. (2018) on the consistent antifungal mechanisms of *Bacillus*. Percent mycelial growth inhibition further supported the biocontrol potential, with PNRE4 achieving the highest inhibition (81.25%), followed by CFLE3, WL2, and AR2, all exceeding 80%. These findings were consistent with Kumar *et al*. (2021), who reported that *Bacillus* spp. inhibit phytopathogens by over 61%. However, PNSE5 showed the lowest inhibition (36.25%), suggesting a need to investigate its lower efficacy to improve biocontrol agent selection.

Culture filtrate assays revealed differences in antifungal activity, with CFLE3 showing the greatest inhibition (3.41 mm), followed by WR1 (2.83 mm). These results suggest that metabolites in the culture filtrates play a significant role, but direct bacterial interaction may be more critical in inhibiting *C. capsici*. The lowest inhibition in the filtrate assay by AR2 (1.08 mm) highlights the complexity of antifungal activity among isolates. Isolates such as PNRE4, GR1, CFLE3, and WL2, which performed well in both assays, show promise as biocontrol agents, likely due to antifungal compounds like lipopeptides, as noted by Bhakat *et al.* (2023). Future research could explore combining effective strains to enhance biocontrol efficacy and further investigate the genetic diversity of less effective isolates like PNSE5 to refine selection for large-scale agricultural applications. Results are presented in Table 1 and Fig.1.

This study underscores the potential of these *Bacillus* isolates as sustainable alternatives to chemical fungicides for managing chili anthracnose and supports the development of integrated disease management strategies for chili cultivation.

DECLARATION

Conflict of Interest. The authors declare that they have no conflict of interest.

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