Management of nursery wilt of black pepper (Piper nigrum L.) with antagonistic bacteria

Black pepper is an important agricultural produce of Kerala, that contributes substantially to the foreign exchange earning of India. Black pepper cultivation in India has been under threat due to severe infestation of foot-rot disease incited by Phytophthora capsici, which has been spreading at an alarming rate. Infection in the nursery stage results either in the wilting of the cuttings, i.e. the planting material or rotting of the newly emerging leaves. The disease is often carried to the main field through the infected rooted cutting and the rooting medium.

Biological control, especially that involving Pseudomonas spp., has emerged as an important alternative in managing soil-borne plant diseases in recent years1-3. However, very little work has been done on biological control of diseases in vegetatively propagated crops, except potato4,5. Trichoderma harzianum and Trichoderma viridea have been used as biological control agents against P. capsici attacking black pepper, both in the main field and nursery5. Only limited attempts have been made on the biological control of foot-rot of black pepper using bacterial antagonists4. We report here a rapid screening assay for the selection of efficient bacterial antagonists which can colonize and protect the planting material against P. capsici-induced wilt of black pepper in the nursery.

Fluorescent pseudomonads were isolated on King’s medium B (KMB) from the underground shoot portions of three-month-old healthy rooted cuttings of black pepper cv. Panniyoor-1 raised in the nursery. Virulent black pepper strain of P. capsici was isolated from the infected nursery plants and maintained on either carrot agar (CA) or potato dextrose agar (PDA) slants at 25°C.

All the bacterial isolates were tested against P. capsici on PDA and CA by dual culture technique, and inhibition of fungal growth was noted after five to seven days4. Among the 64 different isolates tested, 11 were found to have antagonism on both CA and PDA with varying degrees, as evidenced by difference in the inhibition zone. A screening on the stem cuttings was done further. This assay was performed on the basis of the interaction of the pathogen, antagonist and host plant, which resembled the field conditions in a better way than the dual culture plate method, where there is no involvement of the host plant. Disease-free, single-node cutting (approximately 8 cm length) obtained from runner shoots of black pepper cv. Panniyoor-1 were surface-sterilized with 0.1% sodium hypochlorite solution for 10 min, rinsed with sterile distilled water and spread in a laminar airflow chamber. Bacterial cells were harvested from 48-h-old KMB plates by drenching 10 ml sterile distilled water and scraping with a glass spreader. Stem cuttings were completely dipped in the bacterial suspension for 30 min and spread in a laminar airflow chamber for another 30 min for drying. A pinprick was given on the middle portion of the cuttings using a sterile needle, and the pricked area inoculated with a small mycelial bit of P. capsici collected from a seven-day-old culture on PDA medium. The inoculated cuttings were kept in 150 mm dia petri dishes. Moist filter paper was kept on the inner side of the lid to provide high humidity, and the plates were incubated at 25°C under darkness. Length of black lesion developed around the inoculated region on the stem cuttings due to the fungal invasion was recorded at 24 h intervals for a period of 96 h (Figure 1). Inoculation with P. capsici alone and pinprick alone served as controls. All the isolates showing antagonism on both CA and PDA were tested for suppression of lesion development.

Bacterial isolates differed in their ability to suppress the lesion development in the assay. Development of lesion was monitored for four days and a conducive environment for the fungal infection, such as high humidity and darkness was provided. However, in a few treatments lesion development was less or was delayed. This could be attributed to the ability of the bacterial isolates to survive on the stem, at least for a limited period in the test conditions, and thereby preventing the disease development. Black pepper being a vegetatively propagated crop, any biocontrol agent that is used for suppressing the soil-borne infection of the planting material by P. capsici should have the ability to survive on the stem cuttings. Four bacterial antagonists, PN-015, PN-026, PN-032 and PN-033, were selected for the in vivo biological control assay, based on their differential antagonism on the dual culture assay and the shoot lesion assay.

Planting medium consisting of sieved, autoclaved sand and soil (pH 6.2) in the ratio of 1:1 was filled in plastic pots (11 cm dia) and two-node runner shoot cuttings collected from a garden at the Regional Agricultural Research Station (RARS), Wayanad were planted after surface sterilization, as described earlier. Antagonists were applied to the cuttings by dipping the 5 cm basal portion in bacterial suspension for 30 min. The fungal pathogen P. capsici was artificially inoculated into the planting hole prior to planting, by applying 2 ml zoospore suspension prepared in sterile distilled water (approximately 10⁵ propagules/ml) from ten-day-old culture grown on CA plates. Three replications with 15 plants each were maintained in a glasshouse (25 ± 2°C). Irrigation was given daily with sterile distilled water. P. capsici-inoculated control, uninoculated healthy control and chemical control (0.2% copper oxy chloride (COC) drenching at 15 days interval) were maintained for comparison.

Disease incidence in the in vivo condition was recorded by counting the wilted https://www.currentscience.ernet.in/pdf/2022/05/561.pdf

Figure 1. Suppression of lesion on black pepper shoots during screening of bacterial antagonists P. capsici after 48 h of inoculation. PN-026, PN-015, PN-033, PN-032, P. capsici control and pinprick control.
**Table 1.** Percentage wilt of black pepper cuttings in the nursery

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Percentage wilt of black pepper cuttings*</th>
<th>60 DAP</th>
<th>90 DAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>PN-026</td>
<td>40.60 (39.44)</td>
<td>45.00</td>
<td>(41.92)</td>
</tr>
<tr>
<td>Pathogen control</td>
<td>95.83 (79.78)</td>
<td>97.92</td>
<td>(83.99)</td>
</tr>
<tr>
<td>Healthy control</td>
<td>6.25 (14.00)</td>
<td>6.25</td>
<td>(14.00)</td>
</tr>
<tr>
<td>0.2% COC drenching</td>
<td>27.08 (30.83)</td>
<td>27.08</td>
<td>(30.83)</td>
</tr>
<tr>
<td>CD (P = 0.05)</td>
<td>6.15</td>
<td>6.02</td>
<td></td>
</tr>
</tbody>
</table>

*Mean of three replications having fifteen cuttings each; †Figures in parenthesis are arc-sine transformed values.


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**Characterization of polyhydroxy alcanoates – Biodegradable plastics from marine bacteria**

A wide variety of bacteria accumulate polyhydroxy alcanoates (PHAs) as intracellular storage material1–4. Because of their physical and structural properties and amenability to biodegradation, PHAs are considered potential substitutes for petrochemical plastics. PHAs vary in their mechanical properties depending on the composition of the monomeric units5. The medium chain-length PHAs are semicrystalline elastomers with a low melting point (Tm), low tensile strength and high elongation to break6,7, and can be used as biodegradable rubber. Polyhydroxy butyrate (PHB), smallest known PHA displays a similar degree of crystallinity and Tm as polystyrene8, and is stiffer and more brittle than polypropylene9,10, but its copolymerization with hydroxy valerate (HV) monomer units reduces its stiffness and increases its toughness, giving a product with desirable properties for commercial applications11. Polymers may fail in specific applications, simply because they do not possess the necessary strength to carry the designed load or occasional overload10. Hence, it is important to study the mechanical and physical properties of such commercially important polymers before their use in the industry.

Tropical mangrove and marine ecosystems from the mid-west coast of India were screened for promising bacteria, with capability of accumulating high amounts of PHA12. The isolates designated as 61/4, 64/4, 87/4, 182/5, 12/BL, 85/6 and 86/6, which accumulated more than one gram PHA per litre culture broth were studied for physico-chemical factors influencing quantitative yield of PHA (unpublished results). The physical and mechanical properties of the PHA produced by these organisms are presented here.

The selected isolates were grown routinely in 50 ml of E2 mineral medium12 consisting of NaNO3, 4H2O, 3.5 g; K2HPO4, 3H2O, 7.5 g; KH2PO4, 3.7 g per litre; MgSO4·7H2O, 0.17 g, and microelements stock solution, 1 ml containing FeSO4·7H2O, 2.78 mg; MnCl2·4H2O, 1.98 mg; CoSO4·7H2O, 2.81 mg; CaCl2·2H2O, 1.47 mg; CuCl2·2H2O, 0.17 mg, and ZnSO4·7H2O, 0.29 mg supplemented with yeast extract 0.04% (w/v), glucose 2% (w/v), for 48 h on an Orbitek shaker at 28°C and 150 rpm. The cells were washed with saline by centrifugation. The PHA extracted from the cell pellet by the hypochlorite method13, was washed with methanol and acetone consecutively and centrifuged at 8000 rpm for 20 min. The polymers were then dissolved in hot