

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 potato^{4,5}. *Trichoderma harzianum* and *Trichoderma viride* have been used as biological control agents against *P. capsici* attacking black pepper, both in the main field and nursery⁶. Only limited attempts have been made on the biological control of foot-rot of black pepper using bacterial antagonists⁷. 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 days⁷. 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 scrapping 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



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

| Treatment | Percentage wilt of black pepper cuttings ^{a,b} | |
|--------------------|---|---------------|
| | 60 DAP | 90 DAP |
| PN-026 | 40.60 (39.44) | 45.00 (41.92) |
| Pathogen control | 95.83 (79.78) | 97.92 (83.99) |
| Healthy control | 6.25 (14.00) | 6.25 (14.00) |
| 0.2% COC drenching | 27.08 (30.83) | 27.08 (30.83) |
| CD ($P = 0.05$) | 6.15 | 6.02 |

^aMean of three replications having fifteen cuttings each; ^bFigures in parenthesis are arc-sine transformed values.

cuttings. The isolate PN-026, which showed maximum suppression of lesion development in the screening (8.22 mm compared to 59.22 mm in the pathogen control after 96 h of incubation), reduced the incidence of the disease significantly compared to other bacterial antagonists (Table 1). In general, isolates giving greater suppression of lesion development in the shoot assay, irrespective of their antagonism on the dual culture plate assay, showed higher degree of biocontrol efficiency. Our study suggests that the screening procedure described in the present investigation can be used as a rapid and more accurate technique for selecting bacterial antagonists against soil-

borne infection by *P. capsici* in black pepper nursery, as it gives a direct relationship between the lesion suppression and *in vivo* biological control activity.

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Characterization of polyhydroxy alkanates – Biodegradable plastics from marine bacteria

A wide variety of bacteria accumulate polyhydroxy alkanates (PHAs) as intracellular storage material^{1–4}. 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 semi-crystalline elastomers with a low melting point (T_m), low tensile strength and high elongation to break^{6,7}, and can be used as biodegradable rubber. Polyhydroxy butyrate (PHB), smallest known PHA displays a similar degree of crystallinity and T_m as polystyrene⁸, and is stiffer and more brittle than polypropylene^{8,9}; but its copolymerization with hydroxy valerate (HV) monomer units reduces its stiffness and increases its toughness, giving a pro-

duct with desirable properties for commercial applications¹. 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 PHA¹¹. 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 $\text{NaNH}_4\text{HPO}_4 \cdot 4\text{H}_2\text{O}$, 3.5 g; $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, 7.5 g; KH_2PO_4 , 3.7 g per litre; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.17 g, and microelements stock solution, 1 ml containing $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 2.78 mg; $\text{MnCl}_4 \cdot 4\text{H}_2\text{O}$, 1.98 mg; $\text{CoSO}_4 \cdot 7\text{H}_2\text{O}$, 2.81 mg; $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 1.47 mg; $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, 0.17 mg, and $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 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