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Combining *Pseudomonas*, *Bacillus* and *Trichoderma* strains with organic amendments and micronutrient to enhance suppression of collar and root rot disease in physic nut

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ABSTRACT

The fungal and bacterial biocontrol agents were tested individually and in combinations with oil cakes, organic manures and micronutrients for their efficacy against collar and root rot pathogen, *Lasiodiplodia theobromae* under *in vitro*, glasshouse and field conditions. Among the fungal (*Trichoderma*) and bacterial (*Pseudomonas* and *Bacillus*) antagonists screened against *L. theobromae* under *in vitro* conditions, *Trichoderma viride* (Tv1), *Pseudomonas fluorescens* (Pf1) and *Bacillus subtilis* (Bs16) isolates exhibited maximum inhibition compared to other isolates. Among the oil cakes, organic manures and micronutrients tested *in vitro* against the pathogen, neem cake, farmyard manure (FYM) and zinc sulphate were most effective in reducing the growth of the pathogen. The compatibility studies revealed the isolate of *T. viride* (Tv1), *P. fluorescens* (Pf1) and *B. subtilis* (Bs16) were compatible with other and also with neem cake and zinc sulphate. Of the biocontrol agents tested individually as well as in mixtures with neem cake, FYM and zinc sulphate against *L. theobromae*, combination of Pf1 + Tv1 + Bs16 + neem cake + FYM + zinc sulphate showed maximum inhibition in mycelial growth of the pathogen. In glasshouse and field experiments also, the mixture of Pf1 + Tv1 + Bs16 + neem cake + FYM + zinc sulphate was found to be superior in reducing the collar and root rot disease incidence compared to other treatments.

1. Introduction

Physic nut (*Jatropha curcas* L.,) is an important commercial biodiesel plant species and is being advocated for development of waste land and dry land. It is one of the prospective oil yielding plants with vast industrial potential in the scene of energy crisis. The bio-diesel is non-toxic, biodegradable, increase the engine life with an advantage of safety in handling and storage. The oil is also useful for illumination without smoke, manufacturing lubricants, soaps, candle, resins, polish, paint, hair oil, liquefied petroleum gas (LPG) and furnace oil (Venkatesh and Lakshmipathaiah, 2008). Among the several constraints in physic nut cultivation, diseases play a major role in yield reduction and it is affected by many fungal and viral diseases. Among the fungal diseases, collar and root rot caused by *Lasiodiplodia theobromae* (Pat.) Griffon & Maubl (Syn: *Botryodiplodia theobromae* (Pat.) is an economically important soil borne disease (Prakasam, 2005).

Control of collar and root rot disease has been almost exclusively based on the application of chemical pesticides. Several effective fungicides have been recommended for use against this pathogen, but they are not considered to be long-term solutions, due to concerns of expense, exposure risks, health and environmental hazards, residue persistence and development of tolerance. There is a vital need for alternative methods of control for collar and root rot. So far, effective and ecologically sound management practices have not been developed for this disease. Therefore, one of the objectives of the current study was to develop a biological control strategy for this disease that is durable and is an alternative to agrochemicals.

Several antagonistic organisms have been successfully used as biocontrol agents for controlling soil borne pathogens (Deacon, 1991). In most of the research, to date, biocontrol agents are applied singly to combat the growth of the pathogens. Although the potential benefits of a single biocontrol agent application has been demonstrated in many studies, it may also partially account for the reported inconsistent performance because a single biocontrol agent is not likely to be active in all kinds of soil environment and all agricultural ecosystems (Raupach and Kloepper, 1998). These have resulted in inadequate colonization, limited tolerance to changes in environmental conditions and fluctuations in production of

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antifungal metabolites (Weller and Thomashow, 1994; Dowling and O'Gara, 1994). Thus, more emphasis was laid on the combined use of two or more strains of biocontrol agents, which turned out to be more successful than either of them alone, as reported by several workers (Nandakumar et al., 2001a; Bharathi et al., 2004; Thilgavathi et al., 2007; Senthilraja et al., 2010a,b). Mixtures of biocontrol agents will also have the advantage of exercising a broad spectrum activity, enhancing the efficacy and reliability of biological control generally and ensuring greater induction of defense enzymes over individual strains (Latha et al., 2009). Hence, the present investigation was undertaken to study the effectiveness of combinations of fungal and bacterial biocontrol agents with organic amendments and micronutrient against collar and root rot of physic nut and to develop ecofriendly management practices to control the disease.

2. Materials and methods

2.1. Isolation of pathogen and biocontrol agents

The collar and root rot pathogen *L. theobromae* was isolated from infected physic nut plants using potato dextrose agar (PDA) as culture medium. The biocontrol agents *Trichoderma*, *Pseudomonas* and *Bacillus* were isolated from rhizosphere soils of physic nut using *Trichoderma* selective medium (TSM) (Elad and Chet, 1983), King's B medium (KMB) (King et al., 1954) and Nutrient Agar (NA) medium (Rangaswami, 1972), respectively. The individual colonies of *Trichoderma* were identified based on the morphological characters (Webster and Lomas, 1964). Similarly, the bacterial isolates were characterized based on standard biochemical tests (Hildebrand et al., 1992). The isolates of *T. viride* (Tv1), *P. fluorescens* (Pf1) and *B. subtilis* (BS16) were obtained from the Culture Collection Section, Department of Plant Pathology, Tamil Nadu Agricultural University (TNAU), Coimbatore, India.

2.2. In vitro screening of fungal antagonists against L. theobromae

Antagonism of *T. viride* against *L. theobromae* was assayed with the dual-culture method of Rajeev and Mukhopadhyay (2001). Discs (8 mm diameter) of the pathogen and the antagonist were cut from the edge of 3-day-old culture and placed on opposite side of Petri dishes containing PDA, 1 cm away from the edge. Three replications were maintained. Petri dishes were incubated for 4 days at $28 \pm 2\,^{\circ}C$ and the mycelial growth of *L. theobromae* was measured. Percent inhibition (PI) of mycelial growth was calculated using the formula suggested by Pandey et al. (2000).

2.3. In vitro screening of bacterial antagonists against L. theobromae

For *in vitro* screening of bacterial antagonists against *L. theobromae,* the bacterial isolates were streaked on one side of a Petri dish (1 cm from the edge of the dish) with PDA medium and a mycelial disc (8 mm diameter) of 3-day-old culture of *L. theobromae* was placed on the opposite side of the Petri dish perpendicular to the bacterial streak (Vidhyasekaran et al., 1997). The dishes were incubated at room temperature ($28 \pm 2\,^{\circ}C$) for 4 days and the mycelial growth of the pathogen was measured.

2.4. Effect of micronutrients on the mycelial growth of L. theobromae

The efficacy of following micronutrients *viz*., calcium sulphate, ferrous sulphate, borax, calcium nitrate, manganese sulphate, zinc sulphate at 0.5% concentration was tested on the mycelial growth of *L. theobromae* by poisoned food technique (Schmitz, 1930). Control dishes were maintained without micronutrients. Four replications were maintained. The difference in colony diameter between poisoned medium and control was used to calculate the per cent inhibition (Paul and Mishra, 1993).

2.5. Effect of organic amendments on the mycelial growth of L. theobromae

Required quantity of oilcake/organic manure was taken and made into powder. It was soaked in sterile distilled water @ 1 g in 1.25 ml of water and kept it for overnight. The material was ground using a pestle and mortar and filtered through a muslin cloth and the filtrate was centrifuged at 10,000 rpm for 15 min. The supernatant served as the standard solution (100%) (Dubey and Patel, 2000).

The efficacy of following oilcakes *viz*., neem (*Azadirachta indica* L.), pungam (*Pongamia glabra* L.), sesamum (*Sesamum indicum* L.), groundnut (*Arachis hypogaea* L.) and castor (*Ricinus communis* L.) and organic manures *viz*., farmyard manure, coir pith, vermicompost, goat and poultry manure at 10% concentration was tested on the mycelial growth of *L. theobromae* by poisoned food technique. Control dishes were maintained without oilcakes and organic manures. Four replications were maintained and the per cent inhibition was calculated.

2.6. Compatibility studies

The individual strains with organic amendments and micronutrient were first assessed for compatibility *in vitro* and then investigated under glasshouse and field conditions. The strains were also screened for the production of antifungal metabolites.

2.6.1. Compatibility of biocontrol agents with each other

The bacterial strains were tested for their compatibility with each other following the method of Fukui et al. (1994). The compatibility of the fungal biocontrol agent with the bacterial strains was tested by their mycelial overgrowth on the bacterial strains without any inhibition zone, using the dual culture technique (Dennis and Webster, 1971).

2.6.2. Compatibility of fungal and bacterial antagonists with zinc sulphate

The compatibility of zinc sulphate with *P. fluorescens* (Pf1), *B. subtilis*16 (Bs16) and *T. viride* (Tv1) was assayed by poisoned food technique (Schmitz, 1930). For preparing the zinc sulphate amended PDA medium, 100 ml of PDA was taken in a sterile conical flask and mixed with zinc sulphate at 0.5% concentration. The amended medium thus prepared was poured into sterile Petri dishes, allowed to solidify and then streaked with Pf1 and Bs16 and inoculated with mycelial disc of Tv1 in the centre of Petri dishes. To study the compatibility of bacterial antagonist, bacterial strains were streaked on the Petri dishes. PDA without zinc sulphate served as control. Five replications were maintained for each treatment. The growth of bacterial and fungal antagonists was recorded on 2nd and 4th day of incubation at room temperature, respectively, and expressed as compatible (+) or incompatible (−).

2.6.3. Compatibility of fungal and bacterial biocontrol agents with neem cake

The compatibility of neem cake with bacterial and fungal antagonists was also tested by poisoned food technique. Ten per cent concentration of neem cake extracts was prepared and tested for their compatibility with Pf1, Bs16 and Tv1. The Petri dishes containing PDA, KB and NA medium were inoculated with 8-mm disc of Tv1 and streaked with Pf1, Bs16, respectively. The growth of bacterial and fungal antagonists was recorded on incubation at