# Endophytic *Acremonium kilense* as a potential biocontrol agent against Leaf blotch disease of Clove

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**SHORT COMMUNICATION**

# First report of *Phytophthora cactorum*- a pathogen can be an endophytic biocontrol agent

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*Phytophthora cactorum a* pathogen is found to be associated as endophytic fungus in nutmeg (*Myristica fragrans*) and exhibited its biocontrol potential against various foliar diseases of nutmeg such as Leaf spot and die back caused by *Colletotrichum gloeosporioides*, Seedling blight of nutmeg caused by *Rhizoctonia solani* and Leaf spot of nutmeg caused by *Pestalotia palmarum.* Antifungal activity of the endophytic *Phytophthora cactorum* has also been determined

**Key words:** *Phytophthora cactorum,* endophyte, mutualist, nutmeg

*Phytophthora cactoruma,* pathogen is an important threat to strawberry cultivation and production, it also acts as major hurdle in production of apple by causing collar rot disease. The association of this pathogen with temperate fruit crops like apple and strawberry growing regions in India is a major problem for farmers. But *Phytophthora cactorum* is found to be associated as endophytic fungus in nutmeg (*Myristica fragrans*) and exhibited its biocontrol potential against various foliar disease of nutmeg such as Leaf spot and die back caused by *Colletotrichum gloeosporioides*, Seedling blight of nutmeg caused by *Rhizoctonia solani* and Leaf spot of nutmeg caused by *Pestalotia palmarum*. Many workers have reported the biocontrol efficiency of endophytic microorganisms in various crops. Even pathogen like *Colletotrichum gloeosporioides* reported as endophytic bioagent in tea ecosystem against the Brown blight of Tea caused by *Pestalotiopsis theae* (Aparna *et al.* 2014). But the biocontrol potential of *Phytophthora cactorum* is not reported till yet. However the pathogen converting in to bioagent was in tune with the report of Freeman and Rodrigues (1993).

The experiment was conducted in Department of Plant Pathology College of Horticulture, Vellanikkara to exploite the diversity of endophytes in nutmeg. For isolation of endophytes in nutmeg an experiment was conducted with nutmeg leaf samples collected from different locations of Kerala state. The Mc Inroy and Kloepper (1995) protocol were used for the experiment. For the isolation of endophytes from healthy leaves of nutmeg leaf samples were brought to lab and weighed out to 2g bits followed by washing in three changes of sterile water and blot dried. The leaf bits were then transferred to sterilized mortar containing 8 ml sterile Potassium phosphate buffer (PB 0.1M, pH) washed thoroughly in the buffer. From the final buffer wash, one ml was pipetted out and poured into sterile Petri plate. To this molten and cooled medium was added and this served as a sterility check. If microbial growth was observed in sterility check within four days, the isolates obtained from particular samples were discarded. The surface sterilized leaves of nutmeg were triturated using sterile mortar and pestle with 8 ml of sterile buffer. The triturate was serially diluted in sterile PB up to $10^{7}$. One ml of diluted triturate was pipetted in to sterile Petri plate poured with Potato Dextrose Agar medium (PDA), supplemented with penicillin-G (60mg $L^{-1}$) and streptomycin sulphate (80 mg $L^{-1}$) to inhibit the bacterial contamination. Each sample, plated with media were incubated at room temperature (25°± C approx.) for 4-6 weeks in dark. The plated segments were observed once a day for the growth of endophytic fungi. Hyphal tips growing out on the plates were immediately transferred

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into PDA slants, purified and maintained at 4°C. The fungal isolates were identified based on their morphological and reproductive characters using the standard identification manuals (Barnett and Hunter, 1996; Subramanian, 1983). The fungal cultures that failed to sporulate were categorized as sterile mycelia. All the isolates were maintained in Potato dextrose agar slants. The isolate were tested *in vitro* against the foliar pathogens of nutmeg.

The fungal isolate were evaluated for their antagonistic potential against the pathogens of nutmeg, by dual culture method of Skidmore and Dickson(1976) in comparison with standard culture of *Trichoderma viride*. The organisms were inoculated on dual cultures after giving due consideration of their growth rate. Mycelial disc of the pathogen from seven day old culture grown on PDA was placed on one side of the plate and incubated at room temperature (26 ± 2 ° C) for two days. The mycelial disc, (10 mm) of antagonistic fungi were placed on other side of the plate, four cm away from the pathogen and incubated. Three replications were maintained for each isolate. The pathogen grown as monoculture served as control. The plates were observed daily after 24 h of inoculation of antagonists till the pathogen grew and covered the plate kept as control. The per cent inhibition of the pathogen was calculated using the formula suggested by Vincent (1927).

$$PI = \frac{C-T}{C} \times 100$$

PI = Per cent inhibition, C = Growth of the pathogen in control (mm), T = Growth of the pathogen in dual culture (mm)

Based on the per cent inhibition of mycelial growth of the pathogen, the efficient antagonists were selected for further studies. The isolate *Phytophthora cactorum* showed 60 per cent and above inhibition against the three major foliar pathogens of nutmeg and it showed two types of reaction with pathogens : clear zone formation in case of *Colletotrichum gloeosporioides* and aversion in case of *Rhizoctonia solani* and leaf spot disease of cinnamon caused by *Pestalotia palmarum* with clear zone formation . The isolate was first identified up to genus level in Department of Plant pathology, College Of Horticulture Vellanikkara and later it was sent to National Centre for Fungal Taxonomy (N.C.F.T) New Delhi, and it was identified up to species level as *Phytophthora cactorum* with NCFT. ID.NO. 6762.15. The culture was described as- hyphae irregularly swollen though without characteristic hyphal swellings, sporangiphore regular, sporangia abundant on solid media, broadly and regularly ellipsoid or ovoid to obpyriform, (36-50 x 28-35 µm) apex with a hemi spherical papilla with apical thickening with a deciduous pedicel. Cultures usually slightly radiating with uniform slight aerial mycelium based on CMI descriptions of fungi. Previously there were no reports of *Phytophthora cactorum* as endophyte and about its biocontrol potential. However, the present study brought the antagonistic potential of *Phytophthora cactorum* in to lime light which will be useful to manage the pathogens in an ecofriendly approach.

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