Variation in *Phomopsis azadirachtae*, the incitant of die-back of neem

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**ABSTRACT:** Six isolates of *Phomopsis azadirachtae* infecting neem collected from different districts of Karnataka State, South India were characterized based on cultural characteristics and their protein profile. Isolates varied significantly in colony colour, growth pattern and sporulation behaviour on potato dextrose agar medium. Mycelial proteins of these isolates exhibited significant variation in their electrophoretic pattern. The isolates from northern Karnataka shared close similarities among themselves in their protein profiles and differed with the isolates from southern Karnataka.

**Key words:** *Phomopsis azadirachtae*, protein profile, variability

Neem (*Azadirachta indica* A. Juss.) the ‘Indian lilac’ or Margosa is one of the most eco-friendly trees of the tropics with immense potential of medicinal and pesticidal value (Anonymous, 1992; Sateesh, 1998).

Neem trees are infected by the fungal pathogen *Phomopsis azadirachtae* Sateesh, Bhat and Devaki causing die-back leading to severe twig blight, inflorescence blight and fruit rot. The disease, when severe, results in total loss of fruit production (Bhat, *et al*., 1998) limiting the availability of neem seeds, a highly valuable source of botanical soft pesticide. Intraspecific variability in *Phomopsis* species is known (Brayford, 1990; Higley and Tachibana, 1987 and Shivas *et al*., 1991).

Variation in cultural characters and protein profile of a set of six isolates of *P. azadirachtae* collected from different agroclimatic regions of Karnataka State, South India was examined.

**MATERIALS AND METHODS**

**Fungal Isolates**

Diseased neem twig samples were collected from Gulbarga, Mysore, Bijapur, Raichur, Belgaum and Davanagere districts of Karnataka State, South India.

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0.1%, 2-mercaptoethanol. The homogenate was centrifuged at 20,000g for 10 minutes. The supernatant was again centrifuged at 20,000g for 10 min. The clear supernatant referred as mycelial extract was separated and immediately used for electrophoresis (Devaki, 1991).

**Polyacrylamide gel electrophoresis:** Protein content of the mycelial extract was quantified (Lowry et al., 1951). The proteins were fractionated by electrophoresis according to the method of Laemmli (1970). A vertical slab gel electrophoresis apparatus (Broviga vertical mini slab gel- Chennai, India) was used. A concentration of 0.1% of ammonium per sulphate was used and it was prepared fresh every time just before use. Each sample containing 20µg of fungal protein was loaded on the gel (SDS-polyacrylamide gel (12% resolving gel containing acrylamide-bis acrylamide (22:0.8g), in the resolving gel buffer, 1.5 M Tris-HCl at pH 8.8). Standard protein marker (Sigma low molecular weight protein marker) was also loaded in one of the wells. The gel was run in Tris-glycine buffer at pH 8.3 at room temperature (26 ± 2°C) at a constant current of 20mA for 4-5 h. After electrophoresis, the gel was stained with 0.25% coomassie brilliant blue R-250 for 4-5 h. Destaining was carried out in a solution containing glacial acetic acid, methanol and distilled water in a concentration of 1:1:8, respectively.

| Isolate Designation | Colony colour | Colony characteristics | Pycnidial Pycnidia Conidia formation per colony (Range) Alpha Beta |
|---------------------|---------------|------------------------|----------------------------------------------------------|---------|---------|
| Pa 02 Bright orange | Bright orange raised felty mycelium with concentric rings at centre and sparse mycelium towards periphery, margin was circular | Distinct in concentric rings | 80-100 | Abundant | Sparse |
| Pa 03 Greyish white | Thin and adpressed greyish mycelium with concentric rings, margin was even | Distinct and scattered | 120-130 | Abundant | Sparse |
| Pa 04 Lilac white | Raised and felty lilac white mycelium, margin was wavy with sparse mycelium towards periphery | Submerged and scattered | 140-155 | Sparse | Abundant |
| Pa 09 Greyish white | Greyish white raised felty mycelium with concentric rings of pycnidia, margin was wavy with sparse mycelium towards periphery | Distinct in concentric rings | 100-110 | Abundant | Sparse |
| Pa 13 Yellowish white | Yellowish white raised wooly mycelium with concentric rings, margin was circular | Submerged in concentric rings | 120-125 | Abundant | Sparse |
| Pa 14 White to pale brown | White to Pale brown thin adpressed mycelium with wavy margin | Distinct and scattered | 130-140 | Abundant | Sparse |
RESULTS AND DISCUSSION

Cultural characters

The isolates of *P. azadirachtae* derived from naturally infected neem shoots exhibited variable colony characteristics when grown on PDA. The isolates varied considerably in their mycelial growth, colour of the colony, sporulation behaviour, growth pattern, texture and formation of pycnidia, production of alpha and beta conidia and length and breadth of conidia produced (Table 1). The isolates also varied significantly for colony growth and sporulation rate. The isolates from northern Karnataka produced bright orange to orange grey colonies. In contrast, the isolates from southern Karnataka produced grey or dull white colonies. There was difference in the size of alpha and beta conidia. Further, almost all the isolates produced more number of alpha conidia than beta conidia except the isolate Pa 04 which produced large number of beta conidia and very few alpha conidia.

Electrophoretic studies

The protein profiles of the six isolates of *P. azadirachtae* subjected to electrophoresis revealed that bands of 52.0 kD, 26.5 kD and 16.0 kD were common to all the isolates. Pa 04 and Pa 09 isolates were distinct in having protein band of 34.5 kD. Isolate Pa 13 was unique in having two distinct bands of 29.5 kD and 21.0 kD. Isolate Pa 14 was quite distinct in having three bands of 32.0 kD, 30.5 kD, and 6.5 kD. Isolate Pa 02 had two bands of...
24.5 kD and 11 kD. The molecular weights are approximate values according to the Rf values, compared with Rf values of standard bands (Table 2).

The variation in the cultural characteristics of different isolates of P. azadirachtae prompted us to examine their mycelial protein profiles. Mycelial protein profile has been used by several authors to differentiate many fungal species (Devaki, 1991; and Hall et al., 1969). The variation in the protein profiles has been exploited as a diagnostic taxonomic tool (Aggarwal et al., 2001).

Intraspecific variations commonly occur among fungal pathogens (Aggarwal et al., 2001; Mathur et al., 2001; Sharma et al., 2002; Singh et al., 1990). Brayford (1990), reported the existence of variation among isolates of P. oblonga collected from different agroclimatic regions of British Isles and Italy. Higley and Tachibana (1987) identified several races among the isolates of Diaporthe phaseolorum the teleomorph of Phomopsis based on the difference in pathogenicity and physiological specialisation. Shivas et al., (1991), demonstrated intraspecific variation in the isolates of P. leptostromiformis using cultural and biochemical characteristics. The present investigations which were aimed at exploring the diversity among the isolates of die-back pathogen revealed marked morphological variation with regard to the traits such as mycelial growth, colour of the colony and sporulation behaviour. The protein profiles of six isolates showed presence of protein bands common to all the isolates and also protein bands specific to each isolate. This reveals the intraspecific variability among the isolates examined. The isolates from northern Karnataka shared close similarities among themselves, while they differed with the isolates from southern Karnataka in their protein profiles showing geographical variation.

ACKNOWLEDGEMENTS
The authors are grateful to the University of Mysore for providing necessary facilities and Syeda Kousar Fathima acknowledges University Grants Commission, New Delhi for granting FIP fellowship.

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Received for publication July 7, 2003