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Mycotoxic effect of Abrus precatorius and Rauvolfia tetraphylla root extracts on the growth of Colletotrichum capsici

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Ethanolic root extracts of *Abrus precatorius* and *Rauvolfia tetraphylla* and the chemical fungicide Mancozeb were tested for their mycotoxicity on the mycelial growth (biomass), total protein and nucleic acid content of *Colletotrichum capsici*. The extracts of *Abrus precatorius* showed significant inhibition on mycelial biomass and synthesis of total protein, DNA and RNA. The mycotoxicity might be due to the presence of antifungal compounds like proteins, alkaloids, phenolics and other secondary metabolites in root extracts.

Key words: root extracts, antifungal activity, mycelial biomass, protein, nucleic acid

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Kořenové extrakty z *Abrus precatorius* a *Rauvolfia tetraphylla* a fungicid Mancozeb byly testovány s ohledem na jejich mykotoxicitu na růst mycelia (biomasy) a celkový obsah proteinů a nuklelových kyselin houby *Colletotrichum capsici*. Extrakty z *Abrus precatorius* významně potlačovaly biomasu mycelia a syntézu proteinů, DNA a RNA. Toxicita je zřejmě zapříčiněna přítomností antifungálních látek jako proteinů, alkaloidů, fenolů a jiných sekundárních metabolitů v kořenových extraktech.

INTRODUCTION

Antimicrobial compounds of plant origin are much preferred to synthetic compounds in vogue, since they are environmentally safe, easily degradable and leave no harmful and hazardous residues (Mahadevan 1982). Application of extracts of higher plants for the control of various fungal diseases have been reported earlier (Gilliver 1947, Tiwari et al. 1990, Ganesan 2000, Gomathi and Kannabiran 2000). Root extracts of *Abrus precatorius* (*Fabaceae*) and *Rauvolfia tetraphylla* (*Apocynaceae*) were found to show a higher percentage of inhibition on conidial germination and mycelial radial growth of *Colletotrichum capsici* (Syd.) Butler et Bisby (coelomycete), causing anthracnose in Chilli (Kumaran et al. 2003). The present study attempts to find out the mycotoxic effects of root extracts of

Abrus precatorius and *Rauvolfia tetraphylla* and the chemical fungicide Mancozeb on the mycelial biomass and protein and nucleic acid contents of *Colletotrichum capsici* under in vitro conditions.

MATERIAL AND METHODS

Colletotrichum capsici causing fruit rot in *Capsicum annuum* was isolated from infected fruit tissues and brought into pure culture. The culture was deposited at the Microbial Type Culture Collection Centre, Institute of Microbial Technology, Chandigarh, India (MTCC No. 3414).

Ethanolic extracts were prepared from fresh roots of *Abrus precatorius* and *Rauvolfia tetraphylla* growing wildly in the Pondicherry region (South India). The roots were surface-sterilised with a 0.2 % mercuric chloride solution. The roots were chopped and softened using a sterile wooden mortar and pestle. These were soaked in 80 % ethanol for seven days and then the ethanolic extract was evaporated in a desiccator with KOH pellets in vacuum. The dry extract was dissolved in the ratio of 1:1 w/v (weight roots/volume of distilled water) in sterile distilled water and centrifuged for 10 min. at 5000 rpm (28 ± 2 °C). The supernatant was collected and it was considered a 100 % extract. From that, 5 ml was supplemented with 45 ml of growth media, so that the final concentration of plant extract in the growth medium was 10 %. The chemical fungicide Mancozeb (Dithane M-45) was also prepared in sterile distilled water and tested at 320 ppm, as above (Josef et al. 1984).

Two discs with actively growing mycelial mats of 9 mm diameter of a 7 day old culture of *Colletotrichum capsici* were inoculated in a 250 ml Erlenmeyer flask containing 50 ml of Czapek's Dox liquid medium. Medium devoid of extracts and Mancozeb served as control. These flasks were incubated for a period of 7 days at 28 ± 2 °C. On the 8^{th} day, the mycelial mats were harvested and the fresh and dry weight estimated. The fresh mycelia were used for the extraction (Schneider 1945) and estimation of DNA (Burton 1956), RNA (Rawal et al. 1977) and protein (Furlong et al. 1973).

Data were subjected to statistical analysis. Each parameter was analysed separately by using one way of variance (ANOVA) with the student's SPSS package.

RESULTS AND DISCUSSION

The fresh and dry weight of the mycelial mats treated with the root extracts of *Abrus precatorius* and *Rauvolfia tetraphylla* were found to be very low (3.38 & 0.32 g; 4.04 & 0.36 g) when compared to that of Mancozeb and control (Fig. 1). The reduction in the fresh and dry weight of the treated mycelial biomass

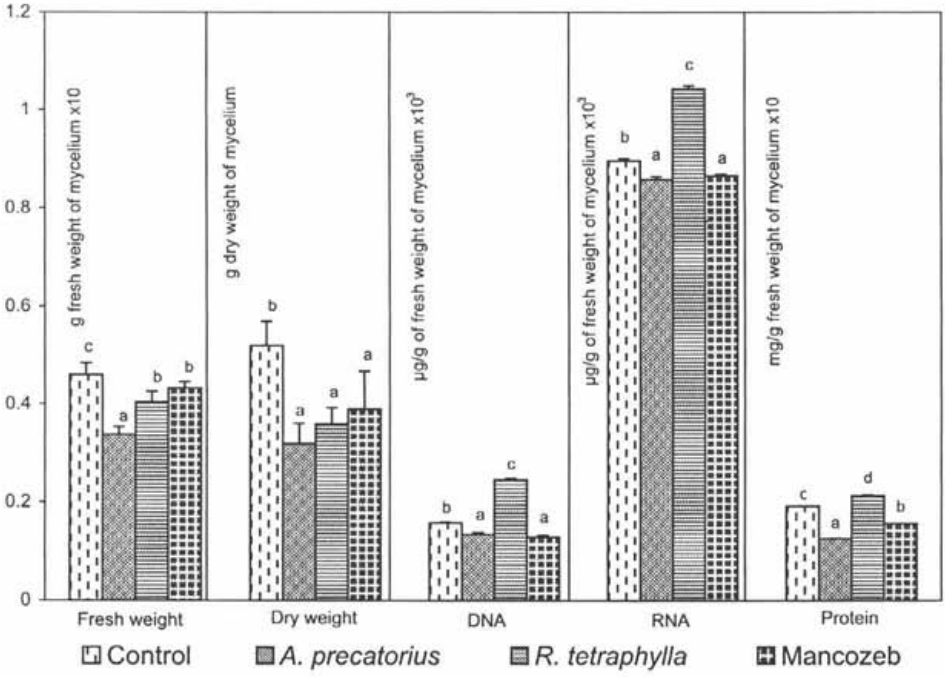

Fig. 1. Effect of root extracts (*Abrus precatorius* and *Rauvolfia tetraphylla*) and Mancozeb on the mycelial biomass and total DNA, RNA and protein content of *Colletotrichum capsici*. The letter at the tops of the error bars indicate statistical significance; means with different letters are significantly different (where P = 0.05).

might be due to the inhibition of membrane energy metabolism and biosynthesis of essential enzymes of the pathogen, as stated by Kalaichelvan and Sumathi (1994). It might also be due to the mycotoxic compound(s) of the plant extracts by causing swelling or thickening of the growing tip of the hyphae (Sariah 1994). Statistically, no difference between fresh weight of the mycelial mat treated with the extracts of *Rauvolfia tetraphylla* and Mancozeb was observed, whereas the extract of *Abrus precatorius* showed significant difference with other treatments. Dry weight of the fungal mat treated with the extracts of *Abrus precatorius, Rauvolfia tetraphylla* and Mancozeb showed significant difference with control.

The effects of ethanolic root extracts on the protein and nucleic acid content of *Colletotrichum capsici* are presented in Fig. 1. The results show that there is significant difference in total protein content among different treatments. The total DNA and RNA content of mycelial mats treated with *Abrus precatorius* and

Mancozeb was more or less equal but they were significantly different from that of control and *Rauvolfia tetraphylla*.

The results indicate a reduction of total protein (1.2689 mg), DNA (129.6 μg) and RNA (858.1 μg) content in the mycelial tissue treated with the extract of *A. precatorius* in comparison with control and Mancozeb. The inhibition might be due to the reduced rate of cell division and inhibition of respiration, as suggested by Natarajan and Lalithakumari (1987). They found reduction of DNA, RNA and protein content in *Drechslera oryzae* due to treatment with leaf extracts of *Lawsonia inermis*. The present study finds support in the studies of Ragsdale and Sisler (1970), where respiration inhibitor carboxin was proved to interfere with the synthesis of protein, DNA and RNA in rapidly metabolising cells of all organisms.

On the contrary, mycelial tissue treated with root extracts of *Rauvolfia tetraphylla* showed higher protein (2.14 mg), DNA (246.31 μg) and RNA (1043.44 μg) content than that of control. This can be attributed to the triggering of stress-induced DNA and RNA synthesis promoted by the plant extract. Mycelial growth and nucleic acid and protein content were found to be directly proportional.

Inhibition of DNA (135 μg), RNA (865.72 μg) and protein (1.575 mg) content of the mycelial mat treated with Mancozeb was found to be lower than that of *Abrus precatorius*. This might be due to resistance developed by the isolate against Mancozeb, which was sprayed routinely in the fields where *Colletotrichum capsici* was isolated. This was supported by Griffee (1973), who found *Colletotrichum musae* isolated from the bananas, which had received pre-harvest benomyl sprays, proved to be resistant to benomyl and related fungicides under in vitro conditions.

Hedge and Podder (1997) showed that cytotoxic lectin (abrin) are the proteins (active principle) found in *Abrus precatorius*. The proteinaceous nature of antifungal compounds has also been reported in *Beta vulgaris* (Nielson et al. 1997), *Aegle marmelos* and *Prosopis juliflora* (Senthilnathan and Narasimhan 1993). Chukuo et al. (1995) have isolated five isoflavanquinones from the root of *Abrus precatorius*, called abruquinones (A, B, C, D, E and F). Schmidt and Stoeckigt (1995) studied the biosynthesis of sarpagine and ajmaline types of alkaloids in *Rauvolfia tetraphylla*. The active compounds of *Abrus precatorius* inhibit mycelial growth, total protein, DNA and RNA content of *Colletotrichum capsici* but the constituents of *Rauvolfia tetraphylla* stimulate mycelial growth and total protein, DNA and RNA content of *Colletotrichum capsici*.

In the present investigation, ethanolic root extracts of *Abrus precatorius* showed significant inhibitory effects on growth, biomass and the total protein, DNA and RNA content of *Colletotrichum capsici*. Further in vivo study will show whether root extracts of *A. precatorius* can be used as an alternative biofungicide in an ecofriendly environment.

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