ISOLATION AND TREATMENT OF PATHOGENS INFECTING GERMPLASM OF CHLOROPHYTUM BORIVILIANUM, SANTA PAU & FERNANDES WITH CHEMOTHERAPEUTIC AGENTS

CHANDER PRABHA, SUNIL KUMAR AND ABHA SINGH
Tissue Culture Lab, Dept. of Botany, Patna University, Patna 800005, Bihar (India)
Correspondence to: cp_ptc_lab@yahoo.com

Chlorophytum borivilianum (Liliaceae), commonly known as Safed musli is a traditional, rare and endangered Indian medicinal herb having many therapeutic applications in Ayurvedic, Unani, Homeopathic and Allopathic systems of medicine. Its storage roots are widely used to cure various sex related diseases because they contain steroidal and triterpenoidal saponins, sapogenins and fructans. It is a rich source of several alkaloids, steroids, phenols, vitamins, proteins, carbohydrate, potassium, calcium, magnesium, resins, and mucilage and also contains high quantity of simple sugars. The present communication deals with the isolation, identification and antimicrobial treatment of those microorganisms which damage the germplasm of medically valuable herb. The planting materials i.e., the fleshy roots bearing shoot buds were stored in perforated polythene bags filled with sand. During storage the tubers were infected with pathogens and almost 20% of germplasm was damaged. The infected fingers became hollow, turned dark brown with thin, wrinkled and disintegrating outer membrane. Isolations were done both for fungi and bacteria on PDA and NA media but colonies appeared only on the PDA plates. Two fungal pathogens, namely species of Fusarium and Aspergillus were isolated and characterized. To evaluate the effectiveness of chemotherapeutic agents on the colony growth, four fungicides namely Bavistin, Captan, Fluconazole and Trichoderma with four different concentrations (5, 10, 15 and 20 mg/l) were used separately and minimum inhibitory concentrations (MICs) were determined. It was observed that all the fungicides exhibited positive response on the growth of the isolated fungal strains, but Bavistin (10 mg/l) and Captan (15 mg/l) appeared to be most effective and should be used to store the germplasm for the next crop.

Key words: Chlorophytum borivilianum, Chemotherapeutic agents, Causative Pathogens, Germplasm, Minimum Inhibitory Concentrations (MICs).

Chlorophytum borivilianum (Liliaceae) is medicinally valuable but endangered commercial crop. The dried roots are rich source of about twenty five alkaloids, saponins, carbohydrate, proteins, vitamins, minerals and also they are globally exported (Parmar et al. 2007). Because of its aphrodisiac properties it is mainly identified as Herbal Viagra. It exhibits various activities like anti-cancerous, anti-arthritic, anti-inflammatory, anti-stresses, anti-oxidant, anti-larvicidal, anti-bacterial, immunomodulatory, hypoglycemaecic and analgesic, etc. (Panda et al. 2007, Kenjale et al. 2007, Deore and Khadabadi 2008, 2009 & 2010, Goyal and Kaushik 2010, Thakur et al. 2010). Efforts are going on to meet the global demands of pharmaceutical and neutraceutical companies by conventional scientific propagation method and also by in-vitro techniques (Haque et al. 2009, Joshi et al. 2009). The herb is (1.0-1.5 ft.) tall with highly condensed underground stem (disc) and tuberous roots (fingers). Leaves radiate from the disc. It perpetuates by two methods: i.e., (i) sexually through seeds and (ii) vegetative through stem discs. The sexual mode of propagation suffers from various problems so, the prevalent method is vegetative. The fleshy tuberous roots bearing shoot buds are stored in perforated polythene or gunny bags filled with sand. During storage, approximately 20% of the germplasms rot due to infection by pathogens. The tubers turn dark brown with wrinkles, thin and membranous disintegrating skin. This is the most common and serious disease encountered by cultivators and so far no information on its etiology is available. Hence, the present investigation was undertaken to isolate, identify and treat the causative pathogens with different chemotherapeutic agents under laboratory conditions to find out the most effective one for final use in the field trial. The work is significant...
for germplasm storage to obtain next crop. The present approach will help in developing a disease management strategy for the endangered and medically important economic crop.

MATERIALS AND METHODS

Isolation of Pathogens: Both healthy (control) and rotten fingers (Figs. 1, 2) were washed thoroughly with tap water to remove all the soil particles before surface sterilization. They were sterilized by 10% Chlorox (3 min.), 1% sodium hypochlorite (3 min.) and 70% ethanol (5-10 min.), and then washed thoroughly with ddw. The sterilized samples were cut into small pieces, again washed with sterilized ddw and blotted on dry, clean and sterile blotting paper to remove the sterilant and excess water, and then the pieces were placed on potato dextrose agar (PDA) and Nutrients Agar (NA) plates aseptically. Usually 2-3 pieces of roots per plate were incubated in inverted position at 33± 2°C for 3-5 days.

Purification of isolates: Isolates were purified by serial dilution and streak plate methods.

Identification: The isolates were subjected to:
(i) Morphological (Elevation, Colour, Growth rate of the colony and shape of conidia/spores) and (ii) Physiological (Temp. and pH) screening and on that basis the preliminary identification was done referring to the manual of Barnett and Hunter (1972). The isolates were designated as fungi 1 (F1) and fungi 2 (F2).

Minimum Inhibitory Concentrations (MICs): In vitro evaluation of four fungicides namely Bavistin (Bast India Ltd.), Captan (Hindustan Pulversing Mills), Fluconazole (Pharma Force Lab.) and Trichoderma (Arihant Naturecrop Pvt. Ltd.) to check the colony growth of Fusarium and Aspergillus was done through poisoned food technique (Borum and Sinclair 1968) on PDA medium. All the four fungicides were tested separately with four doses i.e., 5, 10, 15 and 20 mg/l. Colony growths of both the fungi were determined by measuring the colony diameter (mm) on medium pre-amended with selected fungicidal concentrations.

RESULTS

Almost 20% of planting materials (roots) were found to be infected (Figs. 2). Microbes were isolated from rotten roots on both PDA and NA plates. No microbial colony appeared either on PDA or on NA media from healthy roots whereas from infected roots, two colonies developed on PDA plates only, which were further purified. The isolate F1 had white, woolly surface, with multicellular crescent shaped spores, while isolate F2 was white to greenish with powdery surface but spores were in chain on sterigmata. Both the isolates were fast growing with tangled surface. They grew vigorously at the temperature range between 35°C - 45°C, while very poor growth was recorded at 25°C. Both the isolates did not show any growth below 15°C and above 55°C. Vigorous growth was observed at pH 6, moderate at pH 5 and 7, but zero growth below 3 and above 8. On the basis of morphological, physiological parameters and referring the manual of Barnett and Hunter, the fungal isolates were identified as Fusarium sp. (Fig. 3) and Aspergillus sp. (Fig. 4). All the used fungicides exhibited positive response in inhibiting the colony growth of the two isolates. Fluconazole and Trichoderma were effective at higher concentrations i.e., >15 mg/l where as Bavistin and Captan at lower concentrations. The MICs of Bavistin and Captan recorded are 10mg/l and 15 mg/l respectively giving 100% suppression of the colony growth (Figs. 5, 6). The present findings indicate that two fungicides i.e. Fluconazole and Trichoderma were less effective then Bavistin and Captan inhibiting the growth of pathogens under in vitro conditions.

DISCUSSION

The endangered medicinal herb, C. borivilianum has been frequently investigated for its commercial scientific cultivation, in-vitro propagation using various explants, acclamatization with the use of various
Board. The crop suffers due to: low seed setting, viability, germination and long dormancy. Moreover, the seed raised plants produce small and large tubers in the subsequent 1st and 2nd seasons of growth (Mathur et al. 2008, Haque et al. 2009), so it is vegetatively propagated through shoot buds. The planting materials (disc with finger) are stored for the next crop by various techniques: (i) cold storage, (ii) burial in soil (15 cm below the ground level) under shade, (iii) bonny bags filled with equal amount of sand and (iv) perforated plastic bags filled with sand. But due to microbial infections huge loss of planting material occurs. Unfortunately, not much of systematic work has been done on this aspect.

So, the objective of the present communication was to isolate, characterize, identify and ascertain the MIC of the most effective chemotherapeutic agent. Two fungal strains (Figs. 3, 4) were isolated from the infected fingers on PDA media but not a single bacterial colony appeared on NA plates. These observations are very important because it suggests that the microorganisms infecting roots are fungi and not bacteria. Studies on antimicrobial activity of *C. borivilianum* roots have been done by various workers (Chakraborthy and Aeri 2009, Sundaram et al. 2011) on several species of bacteria like *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aureginosa*, *Bacillus cereus*, *B. subtilis*, *Proteus vulgaris*, *Shigella sonnei* and fungi e.g., *Aspergillus fumigatus*, *A. niger*, *Candida albicans*, *Trichophyton rubrum*. Five endophytic bacterial species have been isolated and screened for enzymes of biotechnological importance from its roots namely *Bacillus pumilus*, *B. subtilis*, *B. megaterium*, *Pseudomonas mendocina* and *Staphylococcus pasteuri* (Panchal and Ingle 2011). According to Sundaram et al. (2011) when safed musli roots are consumed orally, the sugars (mannose and glucose) makes a mucilaginous layer around the urinogenital, gastrointestinal and respiratory tracts which traps the microbial flora, making them unable to invade the system. All the studies indicate that the roots of safed musli has very potent antibacterial agent which may be the probable reason for non appearance of bacteria on NA plates. The first report of tuber rot caused by *Fusarium solani* appeared by Raghavendra et al. (2005). Generally safed musali is free from pests and diseases, but species of *Fusarium*, *Aspergillus*, *Rhizoctina* and *Collectotrichum capsici* have been reported by Chandra and Tandon, (1965), Sattar et al. (2005). The identification of isolated fungal strains as species of *Fusarium* and *Aspergillus* were done by comparison of cultural and morphological characteristics to literature and reference isolates. The use of fungicides in the laboratory *vis-à-vis* field depends on their *in vitro* efficacy at minimal, economically acceptable doses along with their efficient and rapid transport to the infection site. The MICs of the four chemotherapeutic chemicals were recorded for selecting the best fungicides as experts suggest judicious use of fungicides otherwise the pathogenic fungus becomes resistant. Two fungicides namely Bavistin and Captan at dose levels of (10mg/l) and (15mg/l) proved to be most suitable for *Fusarium* and *Aspergillus* respectively. Gaur and Chakrabarti (2009) have reported Captan to be most effective in arresting the mycelial growth of *Fusarium mangifera*. In order to avoid rotting of roots during storage, use of Thiram and Captan (4g/kg) weight of roots and drying of fleshy roots for 2-3 weeks under shade to lose some moisture have been recommended. Fungus are air borne, capable of growing anywhere indoor where there is moisture and food source because they are wide spread plant pathogen and saprophytic soil living beings. In the present study, as the roots were not partially dried, resulted into pathogenecity by species of *Aspergillus* and *Fusarium*, but the main offender is *Aspergillus* followed by *Fusarium*.

**Conclusion**

The observations made during this study suggests that: (i) Two fungus species i.e., *Aspergillus* and *Fusarium* cause root rot disease during storage of germplasm in *C. borivilianum*
and the loss is up to 20%, (ii) Bavistin (10 mg/l) and Captan (15 mg/l) is suitable for inhibiting the growth of *Fusarium* and *Aspergillus* respectively, (iii) *Aspergillus* is more resistant than *Fusarium*, (iv) Bacteria do not infect the storage roots and (v) before storage, proper drying of tubers is recommended. This study can be used as reference in the establishment of modern Hazard Analysis and Critical Control Point (HACCP) schemes.

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