CHAPTER VI

Discussion

6. Discussion

Orchid is the most beautiful flower in God's creation. Taxonomically they represent the most highly evolved family among monocotyledons. Orchid exhibit an incredible range of diversity in size, shape and colours of the flower. They are the most pampered of the plants and occupy top position among all the flowering plants valued for cut flower production and as potted plants. Orchid is an important plant for earning foreign exchange and also plays a significant role in agricultural economy. It is an important plant especially in developing economy of the developing country. Many orchid specialists have developed tissue culture laboratories and green houses for the mass propagation of commercially valuable orchids like *Cymbidium, Cattleya, Vanda* hybrids etc. Small growers buy these clonally propagated elite plantlets and grow them at a small scale for the commercial purpose. Cut flowers and the whole plant are sold in domestic and international markets. *Cymbidium,* the only cut flower among the orchid occupies proud position in global cut flower trade. The North-East Indian hills are richest phytogeographycal habitat for this genus because of the prevailing supporting climate.

In Eastern Himalaya especially in the hills of Darjeeling and its adjoining area, *Cymbidium* have great horticultural value and have been cultivated extensively and plays a vital role, because it is an only source of income of many orchid growers. Major part of orchid growers in this region suffered from huge crop loss due to an epidemic pseudobulb rot since 1995 during every monsoon months. Infected pseudobulb initially turns soft and pulpy followed by oozing out of foul odor liquid. With severity the bulbs and roots lose weights as the internal tissue gradually disintegrates. Finally the bulbs become hollow, fibrous and dry causing death of the plant. The recorded incidence ranged from 60 to100% during the survey period between 2002 to 2005. The severity of the disease is generally associated with high humidity, soil moisture level and often related to poor drainage system and irrigation water. Three organisms were consistently isolated from infected samples collected from various localities. They were

identified as *Erwinia carotovora, Fusarium oxysporum* and *Mucor hiemalis* f. *hiemalis,* predominant at early, middle and later stages of infection. The presences of the organisms were confirmed histopathologically and conventional pathogenecity test. Pathogenicty tests were performed by dipping method in two ways. In the first case the bulbs were dipped separately into fungal as well as bacterial spore /cell suspension (10^6 spores / cells /ml) for 1 min. In another set the sterilized bulbs were dipped first into *E. carotovora,* than into *F. oxysporum* 12 days later and then into *M. hiemalis* f. *hiemalis* 15 days after the second dip. For control set the pseudobulbs were dipped into sterilized water. The samples were incubated aseptically at 20^0C with the relative humidity of 80% and all inoculated bulbs were evaluated for disease 47 days after the first inoculation. When the pathogenicity tests were performed separately *E. carotovora* exhibit maximum (70%) tissue disintegration followed by *F. oxysporum* (30%) and *M. hiemalis* f. *hiemalis* (10%) but none of the individual pathogen caused 100% tissue disintegration. Complete destruction was observed after 47 days of the first inoculation when these three pathogens were inoculated consecutively according to their serial occurrence. Even it was found that when the pathogenicity test was performed in consecutive dipping method symptoms mimicked the natural symptoms found in the field condition. It is an interesting finding on host-pathogen combination as three pathogens act in sequence towards ultimate demolition of the host. This *Cymbidium* pseudobulb rot is a synergistic activity of three pathogens to cause an uncontrolled epidemic disease of *Cymbidium.*

Several chemical control measures have been practiced by the orchid cultivars to control this epidemic disease but till date no significant achievements have been made. So, it was very essential to isolate a biocontrol agent that effectively controls these three pathogens. Literature surveys suggest that bacterial pathogen is unable to control by fungal antagonist. So the objective was to isolate a putative bacterial antagonist that can show the strong inhibitory effect against all three pathogens. Though a range of different bacterial genera and species have been studied but the genus *Pseudomonas* is the

choice of many workers since pseudomonads are not only the dominant bacterial group in the rhizosphere ecosystem but are functionally more versatile, characteristically fast growing, easy to culture and manipulate genetically in laboratory and are able to utilize a range of easily metabolizable organic compounds making them amenable to experimentation.

Fifty-four fluorescent pseudomonad strains were isolated from rhizosphere of potato and *Cymbidium*. Morphological, physiological, cultural and biochemical parameters were evaluated for characterization of these native isolates. All the rhizospheric fluorescent pseudomonads were preliminary screened for antagonistic activities against both fungal as well as bacterial pathogens. During preliminary screening of potential antagonist it has been found that six isolates showed antagonism against all three pathogens. *In vitro* assay of these six screened antagonists were evaluated against *Cymbidium* rot pathogens by dual plate and dual liquid culture technique. In dual culture, growth inhibitions of the fungal pathogens were variable inhibited by all the six strains which were evident with clear inhibition of colony diameter. Among these six isolates and when comparing their effect of antagonism it was evident that the strain fluorescent *Pseudomonas* BRL-1 was found to be the most potent one. Maximum reduction of mycelial dry weight was furnished by strain BRL-1 which was 68% and 61% in case of *Fusarium oxysporum* and *Mucor hiemalis* f. *hiemalis* respectively. Growth inhibition of *E. carotovora* was seen after 48 hours of incubation. All the six isolates showed positive result to check the growth the pathogen, but it was more prominent in case of fluorescent *Pseudomonas* BRL-1 which showed no growth in the interacting zone and abundant growth away from the interaction.

Microscopic observation of the mycelium from the interacting zone showed hyphal shriveling, mycelial deformities, swelling, fragmentation, short branching and granulation of cytoplasm ultimately resulting into lysis. Morphological abnormalities in hyphae of fungal pathogen, was clearly observed under microscope. Such abnormalities occurred due to secondary metabolites and diffusible lytic substances produced by the

antagonist. Further study was made to screen for the production of different secondary metabolites including siderophore, hydrogencyanide, non-volatile and volatile compound, hydrolytic enzymes, IAA investigated for their effect on fungal as well as bacterial pathogens. It was well understood that the fluorescent *Pseudomonas* BRL-1 isolate was showing significant antagonistic property through combined and / or individual effect of siderophore, production of proteolytic enzyme, IAA and chitinolytic activity.

To introduce a potential antagonist form laboratory to the trial field and its survival, proliferation, growth activity and establishment to a new environment needs proper formulation after cost effective biomass production, if it is considered from the commercial view point. Fluorescent *Pseudomonas* BRL-1 has been shown to act as an effective biocontrol agent as well as having the ability to produce significant amount of IAA. Considering these phenomenons, it was aimed to develop a cost effective media, its powder formulation, survivility of the organism in the inert carrier and finally *in vivo* application of inert carrier based formulated organism. After standardization of various growth factors, cost effective carbon and nitrogen sources were investigated. Different carbon sources gave various degree of biomass yield in the fermentation broth. However, considering the cost effectiveness and the biomass production, molasses has been chosen as best carbon sources for commercial production of the strain. Different nitrogen sources, including both inorganic and organic compounds were examined. It has been found that organic N_2 sources like peptone, and fishmeal were superior to inorganic N_2 sources and other organic nitrogen sources in giving higher cellular yield. Of the various organic N_2 sources tested, fishmeal gave maximum yield and from the commercial production point of view fishmeal was the best option so it has been selected as the cheapest nitrogen source. Finally, from the experimental result a new, cost effective media was formulated with the composition of 4% molasses, 2% fishmeal, $MgSO_4$ – 0.15% and K_2HPO_4- 0.15% with pH 7.5. The very next step was the development of low cost inert carrier which should be easy to preparation and

application as well as provide stability and adequate self life. Among the different formulated carrier for fluorescent *Pseudomonas* BRL-1, talc based formulation was the superior one. Upto 90 days, the organism survived without any drastic decline from the initial population. After 6 months of storage only 4% reduction in total population was detected in talc based formulation. This inert carrier based formulation was used for *in vivo* application in a polyhouse at Darjeeling. The *Sets* of different treatments were monitored for disease incidence after 1½ years growth. It was very evident that application of the powdered formulation of the antagonist not only protects the plant from the disease but also showed healthy and vigorous growth of the plant. If this result is applied to the grower's field then it will help the horticulturalists to protect this economically important plant and may ultimately boost up the economy of this area.