6. Summary

The present study deals with "Studies on postharvest disease of tomato, pineapple and orange and their control by microbial antagonist and botanicals".

After a short introduction to the work a brief review of literature related to the post harvest disease and their control by botanicals and antagonists has been presented. The review mainly deals with the post harvest diseases of orange, tomato and pineapple and their control by leaf extracts (*Xanthium strumarium* and *Datura stramonium)*, fungicides (Bavistin and Roko) and antagonists (*Trichoderma harzianum, Aspergillus flavus, Lysinibacilllus sphaericus* and *Pseudomonas* sp.).

The study consists of: (1) Pathogenicity of selected pathogens. (2) Studies on morphological characteristics of the fungi and bacteria. (3) Studies on biochemical characteristics of the bacterial pathogens and antagonists. (4) Extraction of antifungal compounds from different plants. (5) Elucidation of the chemical structure of the bioactive antimicrobial component by spectroscopic analyses. (6) Studies on efficacy of the plant extracts following *in vitro* and *in vivo* tests. (7) Isolation of antagonistic microorganisms from soil and their identification.

A detailed description of different experimental procedures and techniques used during the present study has been given in different sections of materials and methods.

The work was carried out after thorough survey of different post harvest diseases found in the different markets of North Bengal. During the survey several pathogens were found from the diseased fruits and vegetables. The pathogens were isolated, selected and identified. Three fruits and vegetables (Orange tomato, and pineapple) were taken into consideration for the present work. Altogether six organisms (*Fusarium moniliforme*, *Alternaria alternata* (isolate AaT) and *Alternaria alternata* (isolate AaP), *Xanthomonas* sp., *Pseudomonas syringae*, and *Erwinia* sp. were selected for this study.

Pathogenicities of three fungal pathogens and three bacterial pathogens were determined separately in harvested fruits of different places of North Bengal.

Maintenance of fungal culture and production of fungal spores, in artificial media is very much important for different laboratory and field studies. Hence, growth and sporulation of pathogens and antagonists have been studied on a PDA media. Important physiological and biochemical parameters have also been studied.

Both aqueous and ethanol extracts of fifty different species of plants were screened for their potential antifungal properties against test pathogens following spore germination bio-assay technique. *Xanthium strumarium* and *Datura stramonium* showed promising antifungal activity in spore germination bioassay.

The results were again confirmed by poisoned food technique and agar cup bio-assay. In both the cases *Xanthium strumarium* and *Datura stramonium* showed their antifungal and antibacterial activity against all the test pathogens. On the basis of above results TLC was performed to separate different chemicals from the mixture. Using two different solvent systems [chloroform-methanol (9:1) and hexane-ethylacetate-methanol (60:40:1) respectively]. Two different forms of extracts (aqueous extract and solvent extract) were separated by preparatory TLC plates and subsequently subjected to TLC plate bioassay. The R_f of the bioassay guided antimicrobial zones were recorded and found positive for the *X. strumarium* and *D. stramonium* leaf extracts. Potential plant extracts were also separated by column chromatography.

One of the bioactive compounds have been isolated and tentatively characterized and identified as "Deacetyl xanthumin" with the aid of UV, IR and NMR spectral analyses.

Control of the fungal pathogens by two commercial fungicides was also undertaken for comparison with that of plant extracts. Bioassay of two different fungicides were performed *in vitro* against *F. moniliformi, A. alternata* (isolate AaT) and *A. alternata* (isolate AaP). Minimum inhibitory concentration (MIC) values of the test fungicides were determined following agar cup bioassay.

From the results it was evident that both the fungicides (Bavistin and Roko) were effective but Bavistin was better than the Roko as the minimum inhibitory concentration of Roko was higher than that of Bavistin and if the fungicides are compared with the two plant extracts, it is clear that the leaf extracts (*Xanthium strumarium and Datura stramonium*) are more effective.

In vivo experiments were performed in susceptible fruits. To get susceptible fruits pathogenicity tests of each pathogen were performed in fruits of different places of North Bengal.

For biological control of the diseases, antagonistic potentialities of some unknown bio-control agents were tested against the pathogens of the present study. Four biocontrol agents found out of this study are *Trichoderma harzian um* (SF1), *Aspergillus flavus* (SF7), SB1 (*Lysinibacillus sphaericus*) and SB2 (*Pseudomonas* sp.). Two fungal (*Trichoderma harzian um, Aspergillus flavus*) isolates and two bacterial (*Lysinibacillus sphaericus, Pseudomonas* sp.) isolates were used for their efficacy against all the test pathogen.

Results of dual cultures involving either SB1 or SB2 as one of the cultures against the pathogens (separately) clearly indicated that SB2 is the best biocontrol agent. SB2 inhibited (88.57%) growth of *Xanthomonas* sp.. SB1 inhibited growth of *Xanthomonas* sp. (77.14%) [Plate: 22, fig. a]. SB1 also inhibited growth of *Alternaria alternata* (AaP) (61.11%) [Plate: 20, fig.a]. *Alternaria alternata* (AaT) was least inhibited by SF1 (Plate: 25, fig. c). *Trichoderma harzianum* showed highest inhibition (90.00%) over *Alternaria alternata* (AaP), [Plate: 25, fig. e]. Results of the dual culture involving the four antagonists clearly indicated that SF1 is the best biocontrol agent. From the results it was evident that *Trichoderma harzianum* showed maximum inhibition of growth of the pathogen whereas minimum growth inhibition showed by *Lysinibacillus sphaericus* between the two bacterial biocontrol agents. Cent percent inhibition of growth of the pathogen was observed when cell free culture filtrate of the *Trichoderma harzianum, Aspergillus flavus* species were tested in PDA plates (culture filtrate supplemented).

One of the bacterial isolates SB1 (*Lysinibacillus sphaericus*) was subjected to molecular analysis. 16S rDNA were amplified by using universal primer:

16S-Forward primer:5'**AGAGTTTGATCATGGCTCAG** 3'

16S-Reverse primer:5'**GGTTACCTTGTTACGACTT**3'

The amplified product was sent to Bangalore Genei for sequencing. The sequence was compared with several others sequences of the gene bank by using MEGA4.0. The sequence of the present study matched with *Lysinibacillus sphaericus* (99%).

In this study cell free culture filtrates were subjected to six biochemical tests (Pectinase, phosphatase, cellulase, chitinase, siderophore production and DNase activity) to know their mechanism of antimicrobial activity. *Aspergillus flavus*, *Trichoderma harzianum, Pseudomonas* sp. and *Lysinibacillus sphaericus* did not show pectinase and phosphatase activity. All the four organisms mentioned above exhibited cellulase activity. *Aspergillus flavus*, *Lysinibacillus sphaericus* and *Pseudomonas* sp. produced siderophore too. Out of four organisms only *Trichoderma harzianum* did not produce siderophore. Chitinase activity was shown by lone *Trichoderma harzianum* among the four organisms tested. Lipase activity test was performed in case of the two antagonistic bacteria only and both the bacteria produced lipase.

Finally, SEM analysis of pathogen and antagonistic bacteria and fungi were performed. The spores of antagonistic fungi (*A. flavus*) attached with the pathogenic hyphae and degraded the hyphae by releasing certain chemicals. On the other hand the antagonistic bacteria (*Pseudomonas* sp.) colonized the hyphae of some of the pathogens tested indicating their affinity towards the hyphae of the said pathogen. In densely colonized (by the antagonistic bacteria) pathogenic hyphae scars of damage were prominent. This study indicated that the bacteria may have produced certain fungal cell wall degrading enzyme which in turn degraded the hyphae. SEM analysis also showed that bacteria were occupying the degraded hyphae, as degraded portion showed cell aggregates of the antagonistic bacteria.

Implications of the results have also been discussed in the discussion section. The results were encouraging since several potential plant extracts and biocontrol

agents came out of this study against the post harvest pathogens of the fruits. Further these may be integrated with other biocontrol agents and may be used in fields as part of integrated disease management system.

In summary, the present investigations have established designing of the suitable control measures of the post-harvest diseases of three economically important fruits/vegetables in North Bengal. Furthermore, attempts have been made to establish the chemical entity along with structural information of the specific fractions of the bioactive antifungal agent through various chromogenic and spectroscopic methods.