ABSTRACT

Microbial diversity serves as potential source for the isolation of bioactive secondary metabolites. Fungi are the most versatile and diversified organisms, exploited for the isolation of therapeutics and agriculturally important metabolites from decades. Indigenous and unexploited fungi serve as a source to isolate new metabolites or known metabolites with new biological activities. In the present thesis, EtoAc extracts of thirty species of fungi indigenously available were evaluated against Gram-positive and negative bacteria for antibacterial activity and larvicidal activity against *Spodoptera litura* 4th instar larvae. Preliminary screening results reveals that *Aspergillus funiculosus*, *Aspergillus gorakhpurensis* and *Curvularia oryzae* exhibited significantly higher biological activities and warranted for further extensive studies. Chemical structures of the metabolites produced from these fungi were elucidated by $^1$H, $^{13}$C NMR and mass spectral data. Mode of action of the isolated bio active metabolites was established against the bacteria, fungi and *S. litura* larvae.

Kojic acid, 3-phenylpropionic acid and butanoic acid were isolated from *A. funiculosus*. Antibacterial and antifungal activity of the kojic acid and 3-phenylpropionic acid demonstrated moderate activity against the test bacteria and fungi. Minimum inhibitory concentration (MIC), minimum bactericidal activity (MBC) and time-kill kinetic study results revealed the bacteriostatic nature of the metabolites. Scanning
electron microscopic observations of the bacteria treated with metabolites reveals that the metabolites were not induced morphological changes. Moderate antifeedent (DC$_{50}$ = 180.64 and 113.26 µg/cm$^2$ for kojic acid and 3-phenylpropionic acid respectively) and larvicidal activity (LD$_{50}$ = 693.52 and 381.72 µg/ml respectively) were ascertained against 4$^{th}$ instar S. litura larvae. Sublethal larvicidal activity results demonstrate the effect of metabolites against S. litura larvae such as decrease in the mean body weight & length, delay in the time to reach pupation, decrease in the pupal weight, decrease in the percentage of adult emergence and delay in the case of adult emergence. The metabolites were examined for integumentary damage and cytotoxicity against Sf-9 cells, revealed no effect (IC$_{50}$ = >150 µg/ml). Mouse fibroblast cells were also resistant to the metabolite (IC$_{50}$ = >150 µg/ml). In turn the metabolites failed to induce comet formation and DNA fragmentation in Sf-9 cells. From the bioactivity results mode of action of the metabolites was identified as moderate feeding deterrency and insect growth regulatory activity.

Aspergillus gorakhpurensis was exploited for the production of bioactive metabolites and resulted in the isolation of 4-(N-methyl-N-phenylamino) butan-2-one and itaconic acid. In both the metabolites tested for bioactivity only 4-(N-methyl-N-phenylamino) butan-2-one was active. The metabolite exhibited moderate antibacterial activity. The MIC, MBC and time-kill kinetic study of the bacteria treated with
the metabolite clearly indicates the bacteriostatic ability at lower concentrations. Whereas it acts as bactericidal in a dose dependent manner. Moderate antifungal activity was observed in the antifungal assay using the metabolite. *S. litura* larvae was more deterrent ($DC_{50} = 98.55 \mu g/cm^2$) to the metabolite. Larvicidal result ($LD_{50} = 330.24 \mu g/ml$) demonstrates the strong toxicity of the metabolites towards 4th instar *S. litura* larvae. The result indicates contact toxicity of the metabolite on insect larvae. The sublethal toxicity of the metabolite at lower concentrations manifested abnormalities in the growth and development of the larvae. Topical application of larvae with the metabolite leads to decrease in the mean larval weight and length, compared to control. Sublethal toxicity of the metabolite was further evidenced with increase in the time to reach pupation, decrease in the pupal weight, decrease in the number of larvae which reached pupation and increase in the time for adult emergence. The metabolite delayed the growth and development of the larvae indicating growth regulatory activity of the metabolite. The metabolite was tested for AChE inhibition and no such activity was observed. To further investigate the exact mode of action of the metabolite, treated larvae was examined for histopathological changes, cytotoxicity and DNA damage. Results of histopathological studies revealed the toxic effect of the metabolite on the *S. litura* larvae by damaging the integument. The metabolite was considerably toxic to Sf-9 cells ($IC_{50} = 30.31 \mu g/ml$)
and the results substantiate its contact toxicity. DNA damage was observed in the treated Sf-9 cells. The control cells i.e. mouse fibroblast cells were less responsive (IC\textsubscript{50} = 93.41 µg/ml) to the metabolite indicating selective toxicity of the metabolite. From the results it is concluded that the metabolite was acting as feeding deterrent and toxic to the larvae at higher concentrations and growth inhibitor at lower concentrations.

Screening of Curvularia oryzae afforded 11-α-methoxycurvularin and (S)-5-Ethyl-8, 8-dimethyl nonanal as metabolites. 11-α-methoxycurvularin showed substantial bioactivity against bacteria, fungi and S. litura larvae. The metabolite was found to be moderately antibacterial in the zone of inhibition studies. Bacteriostatic effect of the metabolite was established by the MIC, MBC and time-kill kinetic studies. Bactericidal ability was observed in dose dependent MBC experiment. Scanning electron microscopy pictures are substantive evidences for the morphological alterations induced by the metabolite. The metabolite was moderately antifungal. The metabolite induced strong feeding deterrency (DC\textsubscript{50} = 69.89 µg/cm\textsuperscript{2}) when applied to leaves. The 4\textsuperscript{th} instar S. litura larvae was extremely sensitive (LD\textsubscript{50} = 204.87 µg/ml) to the metabolite. When the metabolite was employed topically the larvae showed high rate of mortality. Sublethal toxicity of the metabolite was evident by its anomalous development. Insect growth regulatory activity was evident in sublethal toxicity assay by
diminution in larval weight and length, lag in the time to reach pupation, decrease in the number of larvae which reached pupation and increased time for adult emergence. The AChE inhibition assay results demonstrate that enzyme inhibition was not the cause for acute toxicity. Using histopathological studies integumentary damage was observed in larvae which received the metabolite topically. The metabolite was strongly cytotoxic (IC$_{50}$ = 21.33 µg/ml) to Sf-9 cells. DNA damage was also detected in the Sf-9 cells using comet assay and DNA fragmentation studies. Comparative cytotoxicity (IC$_{50}$ = 89.40 µg/ml) and DNA damage was not observed in the control mouse fibroblast cells. Mode of action of the metabolite is corroborating to be feeding deterreny, strong acute toxicity and IGRA. This is the first report of isolation of the metabolites from the respective fungal species and mode of action the metabolites against bacteria, fungi and agriculture pest *S. litura* was established.