

Title of the Ph.D.Thesis

“ Chemical Investigation and Modification of Compound(s) Isolated from *Pongamia glabra* as Potential Nitrification Inhibitors.”

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ABSTRACT

The present thesis comprises of four chapters. The first chapter "Introduction" deals with survey of the literature on work done in the area of synthetic and botanical nitrification inhibitors and their use to increase nitrogen use efficiency of nitrogenous fertilizers. It also defines the objectives of the investigation. The second chapter "Results and Discussion" deals with isolation and spectral characterization of karanjin, its conversion to various semi-synthetic derivatives and their evaluation as nitrification inhibitors to increase nitrogen use efficiency. Third chapter deals with "Materials and Methods" that include information on the experimental details about isolation of karanjin, karanj oil, synthesis of test compounds, their spectral characterization, nitrification inhibitory activity and antifungal activity. "Conclusion" part of the thesis has been given in the fourth chapter followed by Bibliography at the end. The salient findings of the research work are highlighted below.

- The methanolic extract of *Pongamia glabra* has been analysed for its karanjin and other flavonoid and hydrocarbon content by electrospray ionisation (ESI) mass spectroscopy. It showed the presence of six flavonoids namely karanjin, desmethoxykaranjin, pongachalcone, pongapin, glabrachromene I, glabrachromene II and six hydrocarbons namely octadecane, undecane, decane, octane, undecanol and tetradecanol.
- Karanjin, the major furanoflavonoid was isolated from karanj oil extracted from timely powdered karanj seeds with n-hexane. The purity of the compound was checked by HPLC (Rt 48.007 min), and structure confirmed by NMR (^1H and ^{13}C) and mass spectroscopy (M^+ , 292).
- Karanjin was hydrogenated in the presence of palladised charcoal to obtain dihydrokaranjin. The structure was confirmed by its NMR spectral data. ESI-MS of dihydrokaranjin exhibited characteristic protonated molecule ion peak at m/z 295.5 alongwith four MS-MS fragment ion peaks at m/z 280, 196, 146 and 95.
- Karanjin was reacted with alcoholic KOH to obtain karanjic acid. Besides $^1\text{HNMR}$ spectral data, its structure was also confirmed by ESI-mass spectroscopy which exhibited characteristic molecular ion peak at m/z 180. Karanjic acid was

- then reacted with acid chloride namely furoyl chloride, benzoyl chloride and chloroacetyl chloride to obtain corresponding three ester derivatives. Their structure were also confirmed by NMR and ESI-mass spectroscopy.
- Karanj ketone was obtained by chemical modification of karanjin following its reaction with potassium hydroxide in absolute ethanol. Karanj ketone was then reacted with hydroxylamine hydrochloride to obtain karanj ketone oxime. Structure of karanj ketone ($MH^+207.3$, $MNa^+ 229.4$) and karanj ketone oxime ($MH^+ 222.5$) were confirmed by their ESI-mass spectroscopy. The compounds also exhibited 1H and ^{13}C NMR spectral data characteristic of the molecules.
 - Oxime ether derivatives of karanj ketone (N-O-alkyl karanj ketone oxime) have been synthesized following reaction of karanj ketone oxime with alkyl bromides (ethyl bromide, isopropyl bromide, allyl bromide and n-butyl bromide) (Scheme 2). In these reactions, oxime group was preferentially alkylated rather than the sterically hindered phenolic - OH group. Structure of oxime ether derivatives have also been confirmed by their NMR and ESI-mass spectroscopy.
 - All the karanj based products namely karanjin, karanjic acid, karanj ketone and karanj oil showed potential activity as nitrification inhibitors but dihydrokaranjin, in general showed poor activity. This is attributed to the conversion of bioactive furan moiety in karanjin to inactive dihydrofuran moiety in dihydrokaranjin molecule.
 - Among the karanj ketone oxime ethers, N-O-butyl karanj ketone oxime was the most active followed by N-O-ethyl karanj ketone oxime. The activity pattern for ethyl karanj ketone oxime varied only on 28th day for all the doses otherwise it was quite similar to N-O-butyl karanj ketone oxime. All the four ethers remained active upto the 42nd day, after which the activity fell rapidly.
 - The karanj based products exhibited outstanding antifungal activity against the soil-borne phytophagous fungus *Sclerotium rolfsii* (Sacc.). Karanj oil in the karanjin was more active than karanj oil without karanjin. Karanjin, however, exhibited moderate antifungal activity. Karanjic acid and their three esters exhibited significant antifungal activity. Karanjic acid showed the highest

antifungal activity (EC_{50} 52.97 ppm) followed by chloroacetyl ester (EC_{50} 50.19 ppm). Karanj ketone and its oxime derivatives also exhibited outstanding antifungal activity. The activity was enhanced when karanj ketone was converted to its oxime derivatives.

- Among the karanj ketone oxime ethers N-O-ethyl karanj ketone oxime and N-O-butyl karanj ketone oxime exhibited excellent antifungal activity against *S. rolfsii*. At lowest test concentration of 31.25 ppm, these compounds exhibited 100 percent inhibition of the fungal growth.

In view of the excellent nitrification inhibitory and antifungal properties, N-O-alkyl karanj ketone oximes namely N-O-butyl and N-O-ethyl karanj ketone oxime hold promise and deserve further attention. Detailed field studies are required to explore their potential to increase nitrogen use efficiency and as antifungal agent in crop protection.