CHAPTER 5

CONCLUSIONS AND RECOMMENDATIONS FOR FUTURE RESEARCH

Conclusions .

The increasing cost of herbicides stimulate the author to investigate the use of allelopathy for weed suppression. These studies were undertaken to investigate; (1) the germination and growth inhibitory activity of extracts from green leaves, fallen leaves and bark of E. camaldulensis against M. pigra (2) the identities of inhibitors from the green leaves by simultaneous chromatography with five chemicals known to be allelochemicals in E. camaldulensis leaf litter : gallic, ferulic, caffeic, p-coumaric and chlorogenic acids and (3) the inhibitory activity of 13 phenolic compounds known to be allelochemicals in E. camaldulensis and some other plants on the growth of M. pigra and O. sativa seedlings. The results summarized in this paper indicated several interesting observations and speculations.

1. Germination and Growth Inhibitory Activity of the Extracts .

The results indicated that methanolic extracts of green leaves, fallen leaves and bark of E. camaldulensis contained physiologically important phytotoxins. Both methanolic extracts of the green and fallen leaves demonstrated the presence of inhibitory substances at both germination and seedling growth state of M. pigra. While germination was not inhibited, M. pigra seedling growth bioassay with methanolic extract from bark of E. camaldulensis demonstrate the presence of phytotoxins at this state. Germination of O. sativa

seeds were much less sensitive to methanolic extract of green leaves than that of M. pigra seeds.

2. Isolation and Identification of Inhibitors from the Green Leaves.

M. pigra seedlings were used for bioassay in this experiment. Of the three fractions (n-hexane, ethyl acetate and aqueous fractions) extracted from green leaf methanolic extract, the aqueous fraction was easily the most inhibitory. The growth inhibitory effect of the aqueous extract was nearly as high as that of the original methanolic extract. This suggested that they were in fact polar substances.

Aqueous extract of bark was the most inhibitory too. However, the ethyl acetate fraction being slightly less inhibitory than the aqueous fraction was more inhibitory than that of green leaf methanolic extract.

Trying to isolate toxic substances from one extract is easier than from more than one extract. Since all of the three fractions fractionated from the methanolic extract of green leaves contained inhibitors, the methanolic extract was used for isolation and identification of the inhibitors.

Two chromatographic methods were used for isolating the inhibitors from the methanolic extract of green leaves. Rough isolation was carried out by using of charcoal-celite column chromatography. Of the six fractions eluted from the column, the first fraction eluted by the highest polar eluent (50% acetone in water)

gave the highest inhibitory activity. Decreasing polarity of the eluent successively decreased the toxicity of the eluate. Kieselgel thin-layer chromatographic analysis of the 50% acetone fraction revealed two inhibitors one of which was identified as chlorogenic acid. Although the author was unable to identified the other inhibitor through comparisons of chromatography with well known inhibitors, gallic, ferulic, p-coumaric and caffeic acids were eliminated as possibilities. This unidentified inhibitor seemed to be more inhibitory to M. pigra seedling growth than the identified inhibitors. Both inhibitors were more inhibitory to seedling growth of M. pigra than that of O. sativa.

3. Inhibitory Activity of 13 Phenolic Compounds.

The effects of benzoic acid, salicylic acid, gentisic acid, protocatechuic acid, gallic acid, vanillin, thymol, t-cinnamic acid, p-coumaric acid, caffeic acid, ferulic acid, methyl cinnamate and chlorogenic acid on seedling growth of M. pigra and O. sativa grown in cellulose powder moistened with distilled water were determined. The growth inhibitory activity of these compounds varied with the plant species, concentration, and the presence, position and number of -OH and $-OCH_3$ substituents attaching to their aromatic rings.

M. pigra seedlings were much more susceptible than O. sativa seedlings to all of the phenolic compounds tested. Generally, increasing concentration of the compounds resulted in an increase of the inhibitory activity. However, increasing in a certain range of concentration of benzoic acid, salicylic acid,

vanillin, methyl cinnamate and chlorogenic acid, no increase in inhibitory activity against M. pigra seedlings was observed. The concentration ranges of some phenolic compounds exhibiting inhibitory effect towards M. pigra fall well below the concentration of the compounds that have been reported in various soil types.

Increasing -OH group of the aromatic ring tended to decrease the growth inhibitory activity of the compounds towards M. pigra seedlings. The presence of one $-OCH_3$ group in ferulic acid also enhanced its growth inhibitory activity towards M. pigra seedlings.

Recommendations for Future Research.

The results of the present study provide only preliminary information for future work on the role of E. camaldulensis tissue extracts and some phenolic compounds in weed management. Albeit growth and germination of M. pigra was significantly reduced by E. camaldulensis tissue extracts and some phenolic compounds tested this does not conclusively prove that they are important in field condition. It does, however, offer a possibility and suggests that the importance of the leachate and phenolic compounds is well worth further investigation.

Before attempts are made to control weeds by standing E. camaldulensis or phenolic compound means the following work should be carried out:

1. Identification of possible synergisms and/or antagonisms among phenolic acids. Several phenolic acids are leached

from E. camaldulensis tissues at one time, a mixture of two or more may have a correspondingly greater or lessor effect than a single acid.

2. Growth inhibitory effect of the soil under E. camaldulensis in which all phenolic acids have been washed out is compared with that of unwashed soils.

3. Evaluation of the alleviation effect of artificial fertilization in soils containing phenolic acids.

4. Evaluation of the difference in amount of phenolic acids in the soils under E. camaldulensis and other soils.

It is hoped that this report may have drawn attention to the importance of allelopathy, not only to the plant ecologist but also to others working in diverse field of plant biology.