PART I

INTRODUCTION
ALLELOCHEMICALS - AN OVERVIEW

In the process of evolution, higher plants have evolved a complex array of biochemical pathways through which a variety of secondary metabolites are synthesized and accumulated which enable them to recognize and respond to signals from the environment. Most of these compounds are released into the environment in appreciable quantities via root exudation and as leachates during litter decomposition and play a significant role in allelopathy as allelochemicals.

The term allelopathy coined by Molisch (1937) generally refers to the direct or indirect detrimental effect of one plant (including micro organisms) on the germination, growth and development of other plants mediated by the chemicals that are released into the soil. Allelopathic interactions among plants have been implicated in the patterning of vegetation (Smith and Martin 1994), weed growth in agricultural systems (Aldrich 1987, Inderjit and Dakshini 1994, Putnam 1985, Rice 1987) and yield of agricultural crop plants (Bansal et al 1992, Chou 1990, Liu and Lovette 1993, Ramamoorthy and Paliwal 1993). In addition to its role in terrestrial ecosystems, allelopathic mechanisms are often well observed in aquatic ecosystems (Gopal and Goel 1993).

In the recent years, allelopathy in agriculture is receiving considerable attention due to the potential of allelochemicals in reducing the agricultural yields (Chou 1982, Chou et al 1981, 1990, Moitra et al 1994) and for their potential as natural herbicides and pesticides (Bernard et al 1990).

1.1.1. Chemical nature of allelochemicals:

The chemistry of various allelochemicals in terrestrial ecosystems shows a wide range of chemical compounds identified from soil (Whitehead 1964) and plants (Glass 1974, Hoagland and Williams 1985, Miles et al 1993) which are implicated in allelopathy. These include compounds ranging from simple
gases and aliphatic compounds to complex multiranged aromatic acids including acetic and butyric acids, long chain fatty acids, quinones, simple phenols, phenolic acids derived from cinnamic and benzoic acids, coumarins, flavonoids, several hydrolyzable and condensed tannins, terpenoids, alkaloids and various nitrogenous compounds (Chou 1982, Mandava 1985, Rice 1987).

1.1.2. Release of allelochemicals into the environment:

Most of the allelochemicals synthesized in the plants are released into the environment by various mechanisms. Mandava (1985) critically reviewed the mechanisms of release of these compounds which include 1. Exudation of volatile compounds from plant parts, 2. Leaching of water soluble compounds from plants due to rain, fog or dew, 3. Exudation of water soluble compounds from the roots (Yu and Matsui 1994), 4. The release of toxic chemicals from the non living plant parts through leaching of toxins from litter.

Based on the route from which allelochemicals are released into the environment, these compounds have been identified as four groups 1. Root exudates, 2. Leaf leachates and decomposition products, 3. Volatile toxicants, 4. Sick soil toxicants includes the accumulation of toxic compounds from the previous crops which inhibit the growth of successive crop (Mandava 1985).

1.1.3. Factors affecting the allelochemical production:

The production or release of allelochemicals vary with plants type, age (Wardle et al 1993) and its microenvironment, which include a number of soil physico-chemical factors like texture, nutrient status, pH, temperature, and rhizospheric microbes (Barz and Koster 1981, Blum et al 1987, 1992, Einhellig 1987, 1989, Hoagland and Williams 1985, Putnam 1985, Rice 1987, Whitehead et al 1981). In addition to these factors, compounds like glucose, methionine and nitrate are also known to modify allelopathic effects of allelochemicals (Blum et al 1993). Moreover the final active concentration of allelochemicals in the soil depends on the relative rates of addition and inactivation (Barz and Koster 1981, Hoagland and Williams 1985) as these chemicals occur in soils as
released products during litter decomposition by the action of soil bacteria (Einhellig 1987, Huang et al 1993) and in aquatic ecosystems the degradation of phenolic compounds by fresh water algae has been observed (Ellis 1977).

In most of the cases, the allelopathic effects usually are the result of the combined actions of several allelochemicals, often with each below the threshold concentrations for its effect (Barz and Koster 1981). In most of the allelopathic situations, the concentrations of the chemicals ranged from 10-\text{1000ppm} for each compound and often additive and synergistic effects have been demonstrated (Einhellig and Rasmussen 1979, Einhellig et al 1982). Such combined interactions are very important in the field conditions as they determine the final allelopathic effect.

1.1.4. Detoxification of allelochemicals:

Plants have acquired a number of detoxification mechanisms to minimize the levels and toxic effects of allelochemicals (Shimabukuro 1985, Shimabukuro et al 1982). Mandava (1985) recognized three types of detoxification mechanisms which include phase I reactions like oxidation, reduction and / hydrolysis that include the peroxidases, monoxygenases and other oxygenase enzymes which occur commonly in the plants (Shimabukuro 1985).

Another type of detoxification reaction that is most commonly observed in plants is the conjugation reactions which include the conjugation of allelochemicals with an endogenous substrate to form a new compound. The importance of conjugation reactions lies in the greater water solubility of the conjugates (Balke et al 1987). The structural modification of the allelochemical during the conjugation reduces the toxicity and the higher molecular weights of the conjugates impose a limitation for its free movement. The most important conjugation mechanism in plants include glucosylation, which involves the conjugation of allelochemicals with glucose mediated by an enzyme glucosyl transferase. A large number of glucosyl transferases based on the type of
allelochemical being conjugated have been identified and purified (Balke et al 1987, Sun and Hrazdina 1991, Yalpani et al 1992a,b).

In addition to these above discussed mechanisms, an another additional mechanism has been observed in plants which include further conjugation of primary conjugates to other endogenous substrates that results in tertiary conjugates. This mechanism is found to be the most important process which ultimately reduces the toxicity of the allelochemicals to considerable extent. In general these conjugation reactions are thought to be the ultimate processes involved in the detoxification of the allelochemicals to a greater extent from sites of continuous metabolic activity in the organism (Mandava 1985). The final toxicity of the compounds, however is determined as to what extent an organism is able to reduce the accumulation of allelochemicals from site of action.

1.2.1. Phenolic compounds as allelochemicals:

Among the allelochemicals identified today, phenolic compounds constitute an important group which are of great significance in plant soil interactions (Kuiters 1990, Rice 1987, Siqueira et al 1991). Recently Zaprometov (1992) reviewed the functional role of phenolic compounds in plants. Some of the commonly identified phenolic compounds that are found in plants (Harborne 1980, Putnam and Tang 1986, Rice 1984, Waller 1987) are listed in table 1.

Table 1. Some of the phenolic compounds commonly found in plants

<table>
<thead>
<tr>
<th>Category</th>
<th>Name of the phenolic compound</th>
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<tbody>
<tr>
<td>Simple phenols</td>
<td>Phenols, catechol, hydroquinone, phloroglucinol, pyrogallol</td>
</tr>
<tr>
<td>Phenolic and benzoic</td>
<td>p-hydroxy benzoic, catechuic acids: vanillic, gallic, syringic, salicylic</td>
</tr>
<tr>
<td></td>
<td>protocatechuic and gentisic acids</td>
</tr>
<tr>
<td>Cinnamic acids</td>
<td>p-coumaric, <strong>cinnamic</strong>, caffeic, ferulic and sinapic acids</td>
</tr>
<tr>
<td>Flavonols</td>
<td><strong>Kaempeferol</strong>, quercetin and myrcetin</td>
</tr>
</tbody>
</table>
these varied roles, Palm and Sanchez (1991) observed interference of phenolic compounds with nitrogen release from leaves of tropical legumes during decomposition and Hartley (1992) observed the regulation of cell wall biodegradation by phenolic compounds.

Furthermore, these compounds which are released into the soil during decomposition of litter are known to influence variety of metabolic processes in plants. The most important role of phenolic compounds in agricultural ecosystems is their involvement in the autointoxication mechanism which is well observed in Taiwan rice fields where in a considerable reduction in the yield of second crop has been observed when compared to first crop due to allelochemicals (mostly phenolic compounds) released from the leftover residues of the first crop (Chou et al 1981).

1.2.2. Biosynthesis of phenolic compounds:

Phenolic compounds which accumulate in plants are synthesized in a multibranched metabolism represented primarily by a phenylpropanoid pathway and flavonoid/chalcone pathway and most of them are 6 to 10 carbon skeleton compounds (Gross 1981, Hahlbrock and Scheel 1989). Some of the key enzymes involved in the synthesis of these compounds include phenylalanine ammonialyase, chalcone synthase and enzymes of cinnamic acid pathway which are partially self regulated and known to be influenced by both biotic and abiotic stresses (Bolwell et al 1986, 1988, Liang et al 1989). The general biosynthetic pathway of phenolic compounds is shown in figure 1. The concentrations of these compounds in the tissue vary according to the rate of their biosynthesis, storage, degradation and are affected by internal balance of plant growth regulators (Bell and Charlwood 1980, Harborne 1980, Stumpf and Conn 1981, Tokhver and Palm 1991).

1.2.3. Accumulation of phenolic compounds in plants and soil:

Plants contribute to a large extent in the accumulation of phenolic compounds in the soil. The major route of entry for these compounds into the
Fig. 1. The biosynthetic pathways of phenolics.
environment include root exudates and litter decomposition (Yu and Matsui 1994). In addition to their release into the soil, phenolic compounds undergo continuous cycles of deposition, decomposition, plant uptake, leaching and chemical immobilization as shown in figure 2 (Siqueira et al 1991). Whether the available concentration of inhibitors in the soil is adequate to inhibit seedling growth, finally depends on the above said factors (Fig. 2).

1.3.1. Allelopathic potential of ferulic acid:


FA has been identified as a structural component in the cell walls of both monocot and dicot plants (Kamisaka et al 1990, Locher et al 1994, Tan et al 1991, 1992). Cyclodimers of FA as substitutes in the cell wall polysaccharides in graminaceous plants has been observed (Hartley and Jones 1976, Smith and Hartley 1983). Ohashi et al (1987) observed 5- hydroxy ferulic acid in maize and barley and Locher et al (1994) noticed isomers of FA in growing maize roots. Esters of FA have been observed in the stem bark of Pavetta (Blade et al 1991). FA linked to arabinoxylan in the cell walls has been identified in cell cultures of Festucaarundinacea (Myton and Fry 1994). Weidner et al (1992) reported the occurrence of FA in barley seeds as a dormancy factor. In addition to its association mostly with cell walls, FA is also identified in its bound form in the aleurone layers of barley (Gubler and Ashford 1985, Gubler et al 1985) and as N-acyl terminal group in a protein of barley seeds (Vansumere et al 1973) linked directly to glycine and phenylalanine and has been detected as a
FIGURE 2. Major processes regulating phenolics in plant-soil systems
lipid conjugant (Chatterjee et al 1977). Ehmann (1974) reported N-ferulyl tryptamine in kernals of Zea mays and as germination inhibitor (Vansumere et al 1972). In addition to its existence mostly in bound form, often free forms of FA are observed in some plants such as sugar beet and cereals (Hartley and Jones 1976). Apart from FA, several of the metabolic products of FA released during microbial metabolism, such as vanillic, caffeic, and protocatechuic acids are also known to be toxic either equally and in some cases even more toxic than FA (Turner and Rice 1975)

1.3.2. Biosynthesis of ferulic acid:

It is well documented that most of the phenolic compounds including FA are derived from the shikimic acid pathway and the precursor being the phenylalanine derived from the shikimic acid (Gross 1981, Hahlbrock and Scheel 1989). Earlier it has been demonstrated that the parent compound cinnamic acid undergoes ring substitutions in a series of hydroxylation and methylation steps yielding various p-hydroxylated cinnamic acids (Fig. 2).(Gross 1981).

Deamination of phenylalanine is catalyzed by phenylalanine ammonialyase which results in cinnamic acid or p-coumaric acid (Jones 1984, Jorrin et al 1990). Among the hydroxylase enzymes involved in the cinnamic pathway i.e. cinnamic 4-hydroxylase and p-coumarate 3-hydroxylase catalyzing the sequence cinnamic acid > p-coumaric acid > caffeic acid, have been isolated and characterized as membrane associated mixed function oxygenases (Billett and Smith 1980, Vaughan et al 1975). The methyl group of FA is formed by methylation of the metahydroxyls of its precursor 5-hydroxy FA by the action of o-methyltransferase (Pellegrini et al 1993)

1.3.3. Effects of exogenous ferulic acid:

Vast information is available on the toxicity of FA since its identification as an allelochemical. A number of physiological and biochemical
processes are known to be altered by FA either alone or in combination with other phenolic acids acting synergistically or antagonistically.

**a. Germination:**

FA has been reported to inhibit the germination of sorghum, cucumber, wheat and many other crop plants either alone or in combination with other phenolic acids in the soil (Blum and Dalton 1985, Blum and Rebbeck 1989, Leather and Einhellig 1985, Rasmussen and Einhellig 1979, Williams and Hoagland 1982). Khan and Ungar (1986) observed an inhibition up to 80% in the germination of *Atriplex triangularis* seeds with FA. It is also known to retard the germination of isolated barley embryos (Weidner *et al.* 1992). In addition to crop plants, FA is also known to inhibit the germination of fungal spores and conidia (Alfenas *et al.* 1982, Kasenberg and Traquair 1988). Similar to FA, some of its derivatives are known to impose self inhibitory effect on spore germination of rust fungi (Allen 1972, Faudin and Macko 1974).

**b. Growth:**

In addition to germination, FA is reported to alter the growth of the seedlings by reducing the shoot and root growth. However, the primary effect being observed to be on roots. Earlier studies observed a reduction in the growth of primary root and decrease in the number of secondary roots which turn to brown frequently with deformed root tips (Blum and Dalton 1985, Blum and Rebbeck 1989, Blum *et al.* 1984). Reduction in growth (Blum and Dalton 1985, Blum and Rebbeck 1989, Holappa and Blum 1991), fresh weight and dry weight of shoots and roots have been observed in cucumber, tomato, maize and soybean (Blum and Dalton 1985, Blum and Rebbeck 1989, Devi and Prasad 1992, Holappa and Blum 1991, Patterson 1981). Furthermore, inhibition in leaf expansion and wilting of plants is observed in a variety of crop and vegetable plants even after a short period of application (Blum and Dalton 1985, Blum *et al.* 1984, Einhellig *et al.* 1985, Holappa and Blum 1991, Klein and Blum 1990, Waters and Blum 1987). Interference in the vegetative and reproductive growth
of *Phaseolus* has been observed by Waters and Blum (1987). Reduction in the growth of *Oryza sativa* coleoptiles due to a decrease in the cell wall extensibility by FA is well observed (Kamisaka et al 1990, Tan et al 1991, 1992).

c. Stomatal functions:

FA, together with many other phenolic acids are known to interfere with stomatal function. Several studies observed a decrease in stomatal conductance of the leaves (Balke 1985, Blum and Dalton 1985, Einhellig and Kuan 1971, Einhellig and Stille 1979, Einhellig et al 1985, Patterson 1981). In addition to stomatal conductance and stomatal closure, reduction in transpiration rates of leaves has been observed for a number of plants (Einhellig and Kuan 1971, Einhellig and Stille 1979).

d. Water potentials:

The uptake of water which is essential to maintain turgor in the cell is adversely affected by FA. A significant reduction in leaf water potentials are observed in soybean, cucumber, sorghum and tomato (Patterson 1981) and water utilization (Blum and Dalton 1985, Blum et al 1985a,b) with exogenous FA. Further, Einhellig et al (1985) observed interference of FA with water metabolism of grain sorghum. However, Blum et al (1985b), and Klein and Blum (1990) did not observe any change in the water potential of bean plants, indicating differential sensitivity of the plants to FA. The decrease in the stomatal conductance, transpiration and leaf expansion have been ascribed to FA induced reduction in water potentials (Einhellig et al 1985) and ascribed this reduction in water potentials due to reduction in osmotic potential and turgor pressure (Einhellig et al 1985).

e. Ion uptake

The uptake of water and ions by the roots is usually facilitated by the transport of these across the cell membranes (Tyerman 1992). FA like many
other phenolic compounds has been reported to interfere with ion uptake. Bergmark et al (1992) observed an inhibition in the uptake of nitrate, ammonium and potassium by maize roots and inhibition in the uptake of potassium has been noticed by Booker et al (1992). Inhibition in the uptake of phosphate by barley and Glycine max roots (Glass 1975a, Mc Clure et al 1978) and potassium absorption by roots of Hordeum vulgare (Glass 1974) and Avena sativa (Harper and Balke 1981) with exogenous FA is observed. The inhibition in the uptake is found to be either non-competitive (Mc Clure et al 1978) or uncompetitive (Glass 1973). Even low concentrations of FA (100μM) are reported to inhibit the rubidium absorption in Pauls scarlet rose within 10min (Danks et al 1975). Lyu and Blum (1990) noted an inhibition in the capacity of roots to absorb phosphorus and potassium due to FA.

g. Photosynthesis:

A significant reduction in the photosynthetic rates with allelochemicals have been documented earlier. FA is known to inhibit the photosynthetic functions in a number of crop plants. Patterson (1981) observed significant reduction in net photosynthetic rates and chlorophyll content in soybean with FA along with many other phenolic compounds. Lodhi and Nickell (1973) observed similar reduction in the net CO₂ exchange rates in three grasses. Reduction in the chlorophyll content in soybean has been observed with FA (Einhellig and Rasmussen 1979). Blum and Rebbeck (1989) observed significant reduction in the proportion of carbon allocated to roots in addition to reduction in the photosynthetic rates and chlorophyll content. In addition to its effect at whole plant level, FA is found to inhibit photosynthetic functions at the cellular level. Moreland and Novitzky (1987) reported an inhibition in the electron transport rates and ATPase activities of chloroplasts incubated with FA. Mersie and Singh (1993) observed an inhibition in the photosynthetic rates of isolated leaf cells of velvet leaf by FA and other phenolic compounds.

g. Interaction with plant growth regulators:
Many of the researchers are of the opinion that phenolic acids exert their effect by altering the levels of endogenous growth regulators or by blocking their sites of action. Tayal and Sharma (1981, 1985) observed changes in IAA levels on FA treatment. Tomaszewski and Thimann (1966) observed a synergistic interaction of IAA and phenols in rooting of oat and pea seedlings. FA, when applied alone at concentrations below 0.1mM increased root length in *Phaseolus vulgaris* hypocotyl cuttings (Waters and Blum 1987). However, a decrease in root length was observed in the presence of FA+IAA indicating the antagonistic nature of FA towards IAA (Tayal and Sharma 1985). Recently Kathiresan *et al* (1990) observed an interactive effect of auxin-phenol determining the rooting of mangrove and Ray and Laloraya (1983) observed interaction of phenolic compounds with GA and ABA as examined earlier (Nutbeam and Briggs 1982). Rasmussen and Einhellig (1979) observed an inhibition of GA3 stimulated germination in sorghum by FA together with p-coumaric acid and vanillic acid. In addition to its action on the functions mediated by these growth regulators, FA also alters the levels of growth regulators in the plants. FA induced increase in the levels of ABA is observed in cucumber seedlings (Holappa and Blum 1991). Li *et al* (1993) observed a similar increase in the endogenous ABA levels of lettuce with FA and other phenolic compounds. Kamisaka *et al* (1990) observed an inhibition in the auxin stimulated elongation of cells in rice with FA application. Ishi and Saka (1992) also observed a similar effect of FA in rice lamina joints and reported the involvement of feruloylated arabinoxylans in regulation of the IAA induced growth.

h. Interaction with enzymes:

Many of the phenolic compounds are known to modify the levels and activity of enzymes in both *in vitro* and *in vivo*. Interference of FA with activities of a variety of enzymes that are involved in various metabolic processes has been observed in a number of situations. Hoover *et al* (1977)
observed an inhibition in glucose 6-phosphate dehydrogenase activity and isozymes with FA and many other phenolic compounds. Several nitrogen fixing enzymes are inhibited by FA (Dhir et al 1992). In majority of the cases, FA is known to modify the IAA levels by regulating the activities of IAA oxidase and peroxidase which are involved in the IAA oxidation and the FA effect found to be concentration dependent (Lee et al 1982). An increase in the peroxidase activity has been observed with FA treatment (Shann and Blum 1987b, Van Huystee and Zheng 1993).

1.4.1. Problem and prospects of allelopathic research:

As the impact of allelochemicals on the plant growth are increasing, the need to understand the mechanism of action of these chemicals has become inevitable for the proper management of weeds and crops in agroecosystems, and to develope natural herbicides and pesticides for better yields (Aldrich 1987)

Recently Lovette (1991) critically reviewed the utilization of these allelopathic mechanisms in biological control of weeds. For proper management of weeds and crops, these compounds are to be tested in various combinations to find out the threshold concentrations for their activity (Rippin et al 1994). Crop varieties are to be screened or new varieties need to be developed to exploit the potential of allelochemicals for controlling weeds. Similarly, if crop varieties allelopathic to pests are developed, their residues can be used for pest control (Wiseman et al 1992).

In agroforestry, agricultural crops are grown together with forest plants to increase the yields (Chou et al 1987). However, the major limitation of this system is, the allelochemicals released during the litter decomposition of forest plants that are toxic to crop plants. Therefore, the suitable management systems to select the complimentary tree species and crop plants for better yields in agroforestry is possible only with thorough understanding of allelopathy and mechanism of allelochemical's action.
Synthesis of the bio-pesticides and growth promoters is emerging as an important area in crop improvement. The understanding of the mechanism of action of these **allelochemicals** is useful which can be exploited in developing natural pesticides that can replace the synthetics (Bernard *et al* 1990, Miles *et al* 1993)

Further the beneficial effects of allelochemicals can be extended to use them as growth promoters as many of them are known to promote growth at very low concentration. Developments in crop management based on allelopathic studies would not only increase production but also reduce the expenditure on farm labour, and reduction in use of synthetic agrochemicals. It is even possible that allelochemical production can be induced by genetic manipulation into cultivars, to provide an inexpensive safe and permanent means of biological pest control.

**Objectives of the present work:**

Though earlier studies reported the inhibitory nature of FA on various metabolic processes, the possible sites and mechanism of action particularly, the physiological and biochemical aspects through which it exerts deleterious effects on the plants are not critically investigated. Further more, most of these studies were aimed in observing the FA effects on a particular process in a plant. Information is rather scanty on the FA effect on various physiological processes in a single experimental system. It is thus important to understand how FA affects these interactive physiological processes in a single experimental system starting from seed germination to seedling development which would provide better understanding of FA effects and physiological basis of allelopathy. Hence, the present work has been carried out to study the FA action on growth of maize seedlings with respect to the following:

1. Percentage of seed germination; length, fresh weight and dry weight of shoots and roots.
2. Activities of hydrolytic enzymes viz., amylase, maltase, invertase, acid phosphatase protease and interaction with gibberelic acid.

3. Activities of oxidative enzymes viz., peroxidase, catalase, IAA oxidase, and polyphenol oxidase.

4. The activities of key phenylpropanoid enzyme viz. phenylalanine ammonialyase and cinnamylalcohol dehydrogenase.

5. Accumulation of hydroxyphenolic compounds and lignin.

6. Photosynthetic functions viz., net CO₂ assimilation rate, stomatal conductance electron transport rates, fluorescence emission, cyclic and non-cyclic photophosphorylation, Mg²⁺ and Ca²⁺ ATPase activities.

7. The uptake of phosphate.