REVIEW OF LITERATURE

Allelopathy

Among different plant-plant interactions, the interactions mediated through chemicals (both positive and negative interactions) have attracted attention of scientists throughout the world. These chemical interactions form the basis of "allelopathy" (term coined by Hans Molisch in 1937) which refers to mechanism of plant interference mediated by the release and addition of plant produced phytotoxins to the plant environment (Rice, 1984). The history of allelopathy is very old and dates back to 300 BC when Theophrastus, the Greek botanist, reported that crops such as chickpea, barley and bitter vetch etc. inhibited the growth of weeds and exhausted the soil and first recognized the allelopathy. Later, several historical references in the literature indicated the inhibitory effect of one plant on the other through the release of chemicals in the environment (Pliny II, 1 AD; Culpeper, 1633; Young, 1804; de Candolle, 1832). However, all these ancient reports on allelopathy were based on simple observations / statements/anecdotes that lacked scientific proofs and experimentations thus could not attract the attention of modern scientific community. Since the time of Molisch, therefore, the progress in the field of allelopathy was slow. Moreover, there was a lack of appropriate and meaningful technology available to design field experiments for chemical based interactions at that time. In the beginning of 19th century, de Candolle (1832) actually performed the experiments and observed that root exudates were responsible for toxicity to the plants and soil sickness, though his work could not
attract the interest of scientists at that time. Later, a series of experiments were conducted by farmers, botanists, agronomists and gardeners to demonstrate allelopathy among plants. However, the term allelopathy actually originated only in the beginning of 20th century (Singh et al., 2001). The interest in allelopathy revived after the work of Muller and his team on the Californian Chaparral in 1960s (Muller et al., 1964; Muller, 1965, 1966; del Moral and Muller, 1969; Chou and Muller, 1972). Later, Rice (1974, 1984 and 1995), Putnam and Tang (1986), Rizvi and Rizvi (1992) and Kohli et al. (2001) revolutionized this field by their excellent research work and exhaustive writings. Availability of suitable bioassay, better technology, methodology, isolation and identifications of chemical involved besides collaborative approach among scientists, allelopathy has gained the momentum during the last two decades.

Allelopathy, now-a-days, is a well established inter-disciplinary science with multi-faceted approach and offers challenges in various fields e.g. botany, chemistry, agriculture, ecology, agronomy and soil sciences. This multi-disciplinary science is being utilized to understand and solve various agricultural and ecological problems including regeneration problems in forest areas, its role in soil sickness, for weed and pest management etc. However, greater emphasis is on the use of allelopathy and allelochemicals (chemicals involved in allelopathy) as an alternative to synthetic herbicides and pesticides in agroecosystems to control weeds and pests. For this, various allelopathic plants are being screened for the purpose of exploring them for selective weed management (Macias, 1995; Macias et al., 1997; Anaya, 1999; Rizvi et al., 1999; Singh et al., 2001).

Allelopathic interactions between plants operate both under natural as well as managed ecosystems such as forests, deserts and agroecosystems. Their role in agroecosystems is very significant. Several authors have reported a number of crops, weeds and agroforestry trees with allelopathic potential and their influence on
crop yield (Batish et al., 2001; Qasem and Foy, 2001; Singh et al., 2001). In agricultural systems, allelopathy is a part of the interference between crops as well as between crops and weeds, which affect the economical outcome of the plant production (Singh et al., 2001). Recently, allelopathy in agroecosystem holds great prospects for enhancing crop productivity, genetic diversity and nutrient cycling, maintaining ecosystem stability, and finding alternative strategies for conservation and management of weed, pests and diseases (Weston, 1996; Kohli et al., 1998a; Anaya, 1999; Chou, 1999).

In natural ecosystems, allelopathy plays a vital role in successful invasion and establishment of alien species in new habitats. In these ecosystems, it has been suggested as an important factor in determining vegetation pattern and dynamics, and composition of plant communities (Kohli et al., 2006). The phytochemical compounds produced by invasive plants serve as defense chemical against native plant pathogens, herbivores and insects. Thus, it increases competitive ability of a plant and hence the survival rate (Ridenour and Callaway, 2001; Hierro and Callaway, 2003; Xie et al., 2010).

Allelochemicals

The phenomenon of allelopathy is operative through production and release of certain chemicals, often referred to as allelochemicals/ phytotoxins/ecochemicals/ allelochemics (Whittaker and Fenny, 1971). Within the plant these are synthesized as secondary metabolites. Contrary to the earlier belief that these are metabolic wastes and functionless, now are considered as compounds of great potential. Besides playing an important role between plants and their environment, allelochemicals also serve as defensive adaptation (Harborne, 1989; Seigler, 1996). These play important ecological functions such as defense against insects, herbivores, pathogens and pests and increase reproductive fitness of the plants (Harborne, 1989; Berenbaum, 1995a,b; Seigler, 1996; Taiz and Zeiger, 1998). Allelochemicals are also now
being considered even for their role in biological life support system for long duration space missions (Stutte, 1999).

Though allelochemicals represent a vast variety of chemical nature, yet these are broadly classified as plant phenolics and terpenoids. These show a great chemical diversity and are involved in a number of metabolic and ecological processes.

Plant allelochemicals/phytotoxins are localized and sequestered in specialized organs which may be glandular or sub-epidermal (Duke et al., 1999). These are released from different parts (leaves, flowers, seeds, stems, roots, fruits, pollens) of living or decomposing plants by four different ways. These are: 1) leachation i.e. release of water soluble inhibitors from living or dead parts through water in the form of rain, dew, snow etc; 2) volatilization i.e. release of volatile compounds particularly terpenes from aerial parts of plants into the atmosphere. It is mostly significant under arid or semi-arid conditions; 3) exudation i.e. release of low molecular weight inhibitors from roots of plants as exudates; 4) decomposition i.e. release of allelochemicals by death and decay of plant parts aided by various biotic and abiotic factors.

Once released into the environment, concentration and nature of allelochemicals is governed by various biotic and abiotic factors. These may be retained in the soil or may leach down to lower layers of the soil or may be chemically transformed and involved in a number of metabolic and physico-chemical processes (Rice, 1984; Einhellig, 1985; Waller et al., 1999; Mizutani, 1999). The biological activity or toxicity of allelochemicals depends upon their concentration at a given time, flux rates, cultivar, age and metabolic stage of the donor plant, and prevailing environmental conditions (Rice, 1984; Einhellig, 1996; Weidenhamer, 1996; Gallet and Pellissier, 1997; Nilsson et al., 1998). Under natural conditions, allelochemicals act synergistically and therefore, inhibit growth of other plants at concentrations well below their individual inhibitory levels (Blum, 1995, 1996).
In soil, their production, transformation and degradation occur simultaneously. However, their bioactive (available) concentration depends upon climatic conditions, soil type and its biotic factors (Kobayashi, 2004). Various biotic (disease, insect damage and interaction of plants with herbivores) and abiotic stress factors (drought conditions, extreme temperatures etc.) enhance their biosynthesis, amount and release (Einhellig, 1996; Xu et al., 1999; Zhou, 1999; Kong et al., 2002). Once released from plant into the environment, these are taken up by other plants and interact with plant insects, herbivores and pests and bring about growth inhibitory effects within plant (Nardi et al., 2000).

Allelochemicals, now have been recognized for their multiple ecological functions. These serve as antifungal, antibacterial, antipathogenic, insecticidal compounds, pollinator attractants, internal regulators of growth and development, seed germination stimulants or inhibitors, and are involved in various plant-plant, plant-animal and plant-microbe interactions (Taiz and Zeigler, 1998; Kong et al., 2002). Of late, owing to their growth inhibitory activity, structural diversity and complexity, allelochemicals are now being explored as bioherbicides particularly for the weeds resistant to commercial herbicides thus proved to be ideal agrochemicals for the sustainable management of pests and weeds in the agro-ecosystems (Duke et al., 2000; Singh et al., 2001, 2003b).

Since it is very difficult to explore each and every plant for their phytotoxic/allelopathic effects against weeds, it is thus imperative to explore plants with known phytotoxic/allelopathic effects. A number of allelochemicals/phytotoxins from weeds and crops are known to suppress the growth of other plants (Singh et al., 2003a,b). Allelopathy can thus help a lot in providing clues about plants with herbicidal potential.

Allelopathic / Phytotoxic effects of weeds on other plants

Allelopathic effect of plants on weeds have been viewed by various
workers (Qasem and Foy, 2001; Singh et al., 2001, 2003a,b). Reports in this study are being done from 2002 onwards.

Table 1: Important allelopathic plants and their effect on other plants.

<table>
<thead>
<tr>
<th>Weed</th>
<th>Allelopathic part</th>
<th>Target plants</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acroptilon repens (L.) D.C</td>
<td>roots</td>
<td>Bouteloua gracilis (Willd. ex Kunth) Lag. ex Griffiths, Koelaria cristata (Pers.) and Agropyron smithii Rydb.</td>
<td>Grant et al., 2003</td>
</tr>
<tr>
<td>Ageratum coryzoides L.</td>
<td>leaf, below- and above-ground parts</td>
<td>Triticum aestivum L., Oryza sativa L., Cicer arietinum L., Glycine max [L.] Merr.</td>
<td>Singh et al., 2003a; Batish et al., 2006a, 2009a,b; Verma and Rao, 2006; Kaur et al., 2012</td>
</tr>
<tr>
<td>Alliaria petiolata (Bieb.) Cavara &amp; Grande</td>
<td>roots</td>
<td>Geum urbanum L., G. gialinatum Murr.</td>
<td>Prati and Bossdorf, 2004</td>
</tr>
<tr>
<td>Alliaria petiolata (Bieb.) Cavara &amp; Grande, Lonicera maackii (Rupr.) Herder, Ranunculus ficaria L.</td>
<td>whole plant</td>
<td>Arabidopsis thaliana (L.) Heynh., Brassica oleracea L., Lactuca sativa L., Ocimum basilicum L., Ranunculus ficaria L.</td>
<td>Kendra et al., 2012</td>
</tr>
<tr>
<td>Amaranthus retroflexus L., Chenopodium album L., Erigeron canadensis L. and Solanum nigrum L.</td>
<td>whole plant</td>
<td>Glycine max L., Pisum sativum L. and Vicia sativa L.</td>
<td>Marinov-Serafimov, 2010</td>
</tr>
<tr>
<td>Amaranthus retroflexus L.</td>
<td>leaf, stem and roots</td>
<td>Hordeum vulgare L.</td>
<td>Shahrokhi et al., 2011</td>
</tr>
<tr>
<td>Anisomeles indica (L.) Kuntze</td>
<td>leaf and root</td>
<td>Phalaris minor Retz.</td>
<td>Batish et al., 2007b</td>
</tr>
<tr>
<td>Artemisia siebery Bess A. auchary Boiss. and A. scoparia Waldst. &amp; Kit.</td>
<td>leaf</td>
<td>Amaranthus retroflexus L.</td>
<td>Samedani and Baghestani, 2005</td>
</tr>
<tr>
<td>Artemisia scoparia Waldst. &amp; Kit.</td>
<td>leaf</td>
<td>Avena fatua L., Cyperus rotundus L. and Phalaris minor Retz., Achyranthes aspera L., Cassia occidentalis L., Parthenium hysterophorus L.</td>
<td>Singh et al., 2009; Kaur et al., 2010</td>
</tr>
<tr>
<td>Species</td>
<td>Part(s)</td>
<td>Plants</td>
<td>Authors</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>----------------------------------</td>
<td>---------------------------------</td>
<td>-------------------------------</td>
</tr>
<tr>
<td>Echinochloa crus-galli (L.) P. Beauv.</td>
<td>leaf, whole plant</td>
<td>Echinochloa crus-galli (L.) P. Beauv.</td>
<td>Deba et al., 2007</td>
</tr>
<tr>
<td>Beta vulgaris L.</td>
<td>whole plant</td>
<td>Triticum aestivum L.</td>
<td>Hegab et al. (2008)</td>
</tr>
<tr>
<td>Cardaria draba (L.) Desv.</td>
<td>roots</td>
<td>Medicago sativa L.</td>
<td>Kiemnec and Mcinnis, 2002</td>
</tr>
<tr>
<td>Ceratiola ericoides Michx.</td>
<td>roots and leaves</td>
<td>Lechea deckertii L., Palafoxia feayi A. Gray., Hypericum cumulicola (Small) P. Adams., Lechea cernua Small, and Polygonella basiramia (Small) G.L.</td>
<td>Hewitt and Menges, 2008</td>
</tr>
<tr>
<td>Chenopodium ambrosioides L.</td>
<td>whole plant</td>
<td>Lycopersicon esculentum Mill., Beta vulgaris var. rapa, Mellilotus indicus (L.) All., Sonchus oleraceus L.</td>
<td>Hegazy and Farrag, 2007</td>
</tr>
<tr>
<td>Chenopodium murale L.</td>
<td>roots, leaves and whole plant</td>
<td>Cicer arietinum L., Pisum sativum L., Cassia occidentalis L.</td>
<td>Batish et al., (2007c,d)</td>
</tr>
<tr>
<td>Dicanthium annulatum Stapf., Cenchrus pennisetiformis Hochest. and Sorghum halepense Pers.</td>
<td>root and shoot</td>
<td>Parthenium hysterophorus L.</td>
<td>Javaid and Anjum, 2006</td>
</tr>
<tr>
<td>Fagopyrum esculentum Moench.</td>
<td>roots</td>
<td>Brassica juncea (L.) Czem., Trifolium repens L., Echinochloa crus-galli (L.) P. Beauv., Digitaria ciliaris (Retz.) Koeler, Lactuca sativa L. Amaranthus palmeri S. Watson</td>
<td>Iqbal et al., 2002</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Type of Plant/Part Used</td>
<td>Species/Strain</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------------------</td>
<td>-------------------------</td>
<td>---------------------------------------------------</td>
<td>------------------------------------------</td>
</tr>
<tr>
<td>Hypericum myrianthum</td>
<td>whole plant</td>
<td>Lactuca sativa L.</td>
<td>Fritz et al., 2007</td>
</tr>
<tr>
<td>Cham. &amp; Schlecht. and H. polyanthemum</td>
<td>whole plant</td>
<td>Echinochloa crus-galli (L.) P. Beauv. and Medicago sativa L.</td>
<td>Chon et al., 2003</td>
</tr>
<tr>
<td>Lactuca sativa L., Xanthium occidentale</td>
<td>whole plant</td>
<td>Eichhornia crassipes (Mart.) Solms., Physcomitrion japonicum (Hedw.) Mitt.</td>
<td>Sexena, 2000; Ambika et al., 2003; Chaudhary and Bhansali, 2006</td>
</tr>
<tr>
<td>Lantana camara L.</td>
<td>whole plant</td>
<td>Echinochloa crus-galli (L.) Beauv., Cyperus difformis L. and Monochoria vaginalis Presi.</td>
<td>Lin et al., 2004</td>
</tr>
<tr>
<td>Ophiopogon japonicus</td>
<td>whole plant</td>
<td>Avena sativa L. and Lemna minor L.</td>
<td>Economou et al., 2007</td>
</tr>
<tr>
<td>Tagetes minuta L. and Eupatorium rugosum</td>
<td>whole plant</td>
<td>Aster scaber Thunb., Bidens bipinnata L. and Lotus corniculatus L. var. japonicas</td>
<td>Kil and Shim, 2006</td>
</tr>
<tr>
<td>Thespesia populnea (L.) Sol. ex Correa.</td>
<td>whole plant</td>
<td>Amaranthus tricolor L.</td>
<td>Hemaiswarya and Raja, 2008</td>
</tr>
<tr>
<td>Trianthema portulacastrum L., Dactyloctenium aegyptium L. and Eleusine indica L.</td>
<td>root, shoot, leaf, seed and whole plant</td>
<td>Oryza sativa L.</td>
<td>Mubeen et al., 2011</td>
</tr>
</tbody>
</table>
Allelopathy and weed management

1) Use of mulch for weed management

The direct application of residues / fresh parts of a number of plants as mulch or as a amendment in soil not only reduce weed growth but also enhance nutrient levels, soil moisture and their allelochemicals work synergistically to inhibit weed growth and thus act as green manure. A number of plants have been demonstrated to control agricultural weeds when used as mulch under field conditions without affecting growth and yield of the crops (Chung and Miller, 1995; Hong et al., 2003, 2004; Singh et al., 2003b; Xuan et al., 2003, 2005; Khanh et al., 2006).

In Hungary, ~430 plant species have been suggested to be directly used in agricultural fields against various weeds such as Chenopodium album L. (common lambsquarter), Amaranthus retroflexus L. (redroot pigweed), Setaria viridis (L.) Beauv. (green foxtail), Sinapis alba L. (white mustard), Echinochloa crus-galli (L.) P. Beauv. (barnyard grass), Hordeum distichon L. (barley) and Trifolium repens L. (white clover) (Solymosi, 1994). Xuan and Tsuzuki (2001) used pellets of Medicago sativa L. residues for control of obnoxious weeds Dopatrium junceum (Roxb.) Buch.-Ham. ex Benth., Lindernia pyxidaria sensu Pennell, and Elatine triandra Schkuhr. Reduction in weed growth in response to plant mulch may be due to that nutrient release from the plant debris, preservation of soil moisture content, and synergistic effect of allelochemicals coupled with pesticidal (against nematodes, aphids etc.) activity of plant.

Root residues of Piper methysticum G. Forst (kava) showed a strong inhibition against harmful weeds of paddy field i.e. Echinochloa crus-galli (L.) P. Beauv., Monochoria vaginalis [Burm. f.] C. Presl ex Kunth and Paspalum distichum L. (Xuan et al., 2003). A complete inhibition was observed in emergence of M. vaginalis and E. crus-galli by P. methysticum in O. sativa fields. In addition, P. methysticum also
inhibited pathogenic fungi *Fusarium solani* Snyder and Hansen, *Pyricularia grisea* Sacc., *Rhizopus stolonifer* Vuill., *Taphrina deformans* Tul. and *Thanatephorus cucumeris* Donk. The authors thus suggested kava as a promising natural herbicide and fungicide as an alternative to synthetic ones.

Under laboratory conditions, aqueous extracts (1, 2, 4, 8%) of underground parts of *Ophiopogon japonicus* Ker-Gawl adversely suppressed seed germination and seedling growth of weeds of *Oryza sativa* fields i.e. *Echinochloa crus-galli* (L.) P. Beauv., *Monochoria vaginalis* [Burm. f.] C. Presl ex Kunth and *Cyperus difformis* L. (Lin et al., 2004). Under glasshouse experiments, treatment with dried powder of underground parts @ 50, 100, 150 g m\(^{-2}\)) severely inhibited seed emergence and dry weight of paddy weeds without affecting rice crop thus suggesting its use as a natural rice field herbicide. Aqueous extracts of dried powder of *Houttuynia cordata* Thunb. suppressed germination and initial growth of *Lactuca sativa* L. and two major weeds *E. crus-galli* and *M. vaginalis* in *O. sativa* fields of Japan, indicating its bioherbicidal potential (Lin et al., 2006).

Aromatic plants incorporated as green manure into the soil or left on the top of soil as mulches play an important role in weed suppression in sustainable agroecosystem. Batish et al. (2007a) explored the herbicidal activity of *Tagetes minuta* L. leaf powder (@1, 2 or 4 t ha\(^{-1}\)) against two weeds of *O. sativa* i.e. *Echinochloa crus-galli* (L.) P. Beauv. and *Cyperus rotundus* L. Leaf powder mulch significantly reduced the emergence and growth of both weeds without affecting rice. Rather, yield of rice was more when mulch was applied @ 1 and 2 t ha\(^{-1}\) thus could be used as natural herbicide. Later, Batish et al. (2007b) demonstrated that *Anisomeles indica* (L.) Kuntze., a medicinal plant holds good promise for use as a natural herbicide for control of *Phalaris minor* Retz. and other weeds of wheat crop. Leaf and root powder of *A. indica* when applied as mulch @ 1 and 2 t ha\(^{-1}\) significantly reduced emergence and growth of *P. minor* and other

Wakjira et al. (2009) studied the inhibitory effects of fresh *Parthenium hysterophorus* L., composting *P. hysterophorus* alone and compositing *P. hysterophorus* with other plants, on germination rate and radicle and plumule length of *Lactuca sativa* L. The fresh *P. hysterophorus* reduced germination rate, and growth of *L. sativa* up to 97% while compositing *P. hysterophorus* had lesser inhibitory activity (up to 43%). The authors suggested composting parthenium with locally available plant materials as a means to reduce its allelopathic effect and as a potential way to manage the noxious weed (Wakjira et al., 2009).

Straw mulches (1 kg m$^{-2}$) of *Oryza sativa* L., *Hordeum vulgare* L., *Zea mays* L. harvest residues, *Artemisia absinthium* L. etc. changed the composition of weed flora in *Lycopersicon esculentum* Mill. fields over the years of treatment (Anzalone et al., 2010). These mulches also effectively reduced the weed density of *Chenopodium album* L., *Portulaca oleracea* L., *Digitaria sanguinalis* L. Scop., *Cyperus rotundus* L. The increase in growth and yield of crops upon treatment of mulch could be attributed to increased fertility of the soil and improvement of soil structure due to nutrient release from the plant
debris coupled with pesticidal activity of plant mulch (Anzalone et al., 2010).

Laosinwattana et al. (2010) determined allelopathic effects of aqueous extracts and dried leaf powder of *Suregada multiflorum* Baill. to explore herbicidal effects of its granule on weed species *Echinochloa crus-galli* (L.) P. Beauv. and *Amaranthus viridis* L. At 100 g L\(^{-1}\) leaf extracts of *S. multiflorum*, a strong inhibition in germination and growth of weeds was observed. Dried leaf granule as well as dried leaf powder exhibited stronger inhibitory effects thus hold potential to be used as organic herbicide for controlling *A. viridis*.

Karkanis et al. (2012) demonstrated that mulching with *Hordeum vulgare* L. straw effectively controlled the weed density and biomass of broadleaved weeds *Amaranthus retroflexus* L., *Convolvulus arvensis* L., *Datura stramonium* L., *Hibiscum trionum* L., *Portulaca oleracea* L. and *Solanum nigrum* L., and the grass weed *Echinochloa crus-galli* (L.) P. Beauv. in *Allium porrum* L. fields. The authors concluded that use of plant mulch could reduce the reliance on herbicides and is a good option for weed management in organic *A. porrum* crop.

2) **Direct use of allelochemicals for weed management**

i) **Use of volatile allelochemicals**

Essential oils are the allelochemicals with natural fragrances, flavors and provide characteristic odor to the donor (Mukhopadhyay, 2000). These are secreted into various glands and stored in specialized organs such as cavities, ducts, glands and hair. Due to quick breakdown, these are classified as “generally regarded as safe”. Besides their traditional use as flavoring agents, pharmaceuticals and cosmetic industries, essential oils are also involved in allelopathic interactions. The essential oils and their constituent monoterpenes and sesquiterpenes suppress seed germination and growth of other plants and affect vegetational patterning in communities.
Volatile oils from lemon grass (*Cymbopogon citrates* [Nees] Stapf.), bible hyssop (*Origanum syriacum* L., and tea hyssop (*Micromeria fruticosa* [L.] Druce) inhibited germination and growth of *Amaranthus palmeri* S. Wats), *Brassica nigra* L. and a number of other weeds (Dudai *et al.*, 1999). The workers concluded that these essential oils can be used as herbicides for management of weeds. Tworkoski (2002) tested herbicidal potential of 25 plant species and observed cinnamon (*Cinnamomum zeylanicum* Blume) essential oil to be the most active against weeds. The cinnamon oil as well as its major constituent monoterpene eugenol were suggested to be useful as natural product herbicide for organic farming systems. Angelini *et al.* (2003) reported that oils from some members of family Lamicaeae e.g. rosemary (*Rosmarinus officinalis* L.), thyme (*Thymus vulgaris* L.) and savory (*Satureja montana* L.) were inhibitory towards germination and establishment of weeds (*Chenopodium album* L., *Portulaca oleracea* L. and *Echinochloa crus-galli* (L.) P. Beauv.) and crops (*Raphanus sativus* L., *Capsicum annuum* L. and *Lactuca sativa* L.). More phytotoxic effects was observed in weeds while crops showed insignificant inhibition thus strongly suggesting the potential of oils for weed control without affecting crops.

and *Digitaria sanguinalis* (L.) Scop. (Barney et al., 2005). The presence of bioactive terpenoids suggested their potential role in imparting phytotoxic properties to *A. vulgaris* and for establishment and proliferation in introduced habitat. Seven-days old seedlings of *Bidens pilosa* L. exposed to 5, 10 and 20 ppm oil of *Minthostachys mollis* (Kunth) Griseb. inhibited elongation of shoot and root and caused necrosis in the hypocotyls (Alonso-Amelot et al., 2006).

Salamci et al. (2007) studied that *Tanacetum aucheranum* (D.C.) Sch. Bip. and *T. chiliophyllum* (Fisch. and Mey) Sch. Bip. var. *chiliophyllum* oils completely inhibited germination and establishment of *Amaranthus retroflexus* L., *Chenopodium album* L. and *Rumex crispus* L. and concluded that these could be directly used for control of these weeds. Kordali et al. (2007) recommended use of oxygenated monoterpenes β-citronellol, nerol and terinen-4-ol as potential bioherbicides as these monoterpenes completely inhibited germination and growth of weeds *viz.* *Amaranthus retroflexus* L., *Chenopodium album* L. and *Rumex crispus* L. and the inhibitory effect was stronger than that of commercial herbicide 2,4-D. A number of studies indicated that lemon scented eucalypt oil (from leaves of *Eucalyptus citriodora* Hook.) possess growth inhibitory activity against a number of noxious weeds *Parthenium hysterophorus* L., *Cassia occidentalis* L., *Amaranthus viridis* L., *Echinochloa crus-galli* (L.) P. Beauv., *Phalaris minor* retz. (Singh et al., 2005b; Batish et al., 2004, 2006c,d, 2007e, 2008a).

Under natural conditions, oil (0 – 10%) caused visible injury such as chlorosis, necrosis, wilting and even plant death, severely inhibited seedling growth, photosynthetic machinery, respiratory metabolism and enhanced the electrolyte leakage from cellular membranes. The authors concluded that volatile oils from *E. citriodora* offers a good option as lead molecules for control of noxious weeds and could be used as a potential bioherbicide for integrated weed management programmes (Singh et al., 2005b; Batish et al., 2004, 2006a,b, 2007e, 2008a).
Azirak and Karaman (2008) reported that essential oil from some aromatic plants viz. *Carum carvi* L., *Coriandrum sativum* L., *Foeniculum vulgare* Mill., *Lavandula stoechas* L., *Mentha spicata* L., *Origanum onites* L., *Pimpinella anisum* L., *Rosmarinus officinalis* L., *Salvia officinalis* L. and *Thymbra spicata* L. exhibited growth inhibitory activity against some common weeds *Alcea pallida* Waldst. and Kit., *Amaranthus retroflexus* L., *Centaurea salsolitialis* L., *Raphanus raphanistrum* L., *Rumex nepalensis* Spreng., *Sinapis arvensis* L. and *Sonchus oleraceus* L. The major constituents of these oils i.e. carvacrol, thymol, carvone and limonene were also highly toxic towards weeds even at lower concentrations. Kordali et al. (2009) isolated essential oils from aerial parts of *Achillea gypsicola* Hub-Mor., *Achillea biebersteinii* Afan. and n-hexane extracts obtained from their flowers and found that oils and hexane extracts were toxic towards pathogenic fungi *Fusarium equisetii* and *F. graminearum* and weeds of cultivated areas i.e. *Amaranthus retroflexus* L., *Chenopodium album* L., *Cirsium arvense* L. (Scop.), *Lactuca serriola* L. and *Rumex crispus* L. The antifungal activity and herbicidal activity of oils was more compared to hexane extracts due to relatively high content of oxygenated monoterpenes (Kordali et al, 2009).

Montana L., Santolina chamaecyparissus L., and Thymus vulgaris L. were demonstrated to be promising alternatives to the synthetic herbicides agrocide and prowl as these were highly toxic towards two weeds (Portulaca oleracea L. and Vicia sativa L.) but least to the crops Zea mays L., Triticum durum L., Pisum sativum L., and Lactuca sativa L. (Grosso et al., 2010). Volatile essential oils from Artemisia scoparia and its monoterpenes, β-myrcene and p-cymene significantly impaired chlorophyll content and respiratory activity of wasteland and agricultural weeds Achyranthes aspera L., Cassia occidentalis L., Parthenium hysterophorus L., Echinochloa crus-galli (L.) P. Beauv., and Ageratum conyzoides L. (Singh et al., 2008; Kaur et al., 2010).

Li et al. (2011) demonstrated that the saturated aqueous solution of the volatile oils from Descurainia sophia (L.) Webb ex Prantl possesses strong phytotoxic potential and could cause substantial germination reduction and seedling growth inhibition of eight different wheat cultivars. Verdeguer et al. (2011) showed that essential oils (0.125–1 μL mL−1) of Peumus boldus Molina and Drimys winterii J.R. Forst. G. Forst. inhibited seed germination and seedling growth of Amaranthus hybridus L. and Portulaca oleracea L. and suggested their possible use as a natural herbicide. Ismail et al. (2011) evaluated that essential oils from leaves of Juniperus oxycedrus L. subsp. macrocarpa completely inhibited seed germination and seedling growth of three weeds i.e. Phalaris paradoxa L., Trifolium campestre Schreber. and Lolium rigidum Gaud. thus suggesting a possible use of these essential oils as natural herbicides. The oxygenated compound rich essential oil of Cistus ladanifer L. shrub were found to be highly useful for the control of growth of Amaranthus hybridus L., Portulaca oleracea L., Chenopodium album L., Conyza canadensis L. and Parietaria judaica L. (Verdeguer et al., 2011). Recently, Kaur et al. (2011b) investigated phytotoxic potential of essential oil from E. tereticornis against growth and establishment of Amaranthus viridis L. in a view to explore its herbicidal potential.
The oil and its two major monoterpenes α-pinene and 1,8-cineole adversely affected its seedling vigor, energy metabolism and photosynthetic pigments, chlorophylls a, b and carotenoids thus holds potential to control weeds.

**ii) Use of non-volatile allelochemicals**

Phenolic acids, the water soluble allelochemicals, have been widely implicated in allelopathy. Due to their phytotoxic nature towards weeds, these have also shown the promising results in sustainable weed management programs.

Batish *et al.* (2002) explored the herbicidal potential of parthenin, a sesquiterpene lactone from *Parthenium hysterophorus* L., against two weedy species i.e. *Avena fatua* L. and *Bidens pilosa* L. The germination, growth, chlorophyll content and respiration were inhibited in weeds exposed to different concentrations of parthenin. The study revealed that due to its growth inhibitory action, parthenin could be explored as herbicide for future weed management programs.

Chon *et al.* (2003) investigated herbicidal potential of aqueous extracts from leaves of 16 weeds of Compositae. Of these, *Lactuca sativa* L., *Xanthium occidentale* Bertol. and *Cirsium japonicum* DC. showed significant inhibition against root growth of *Medicago sativa* L. and *Echinochloa crus-galli* (L.) P. Beauv. Upon HPLC (high pressure liquid chromatography) analysis, coumarin, trans-cinnamic acid, o-coumaric acid and p-coumaric acid were found to be putative phytotoxins. The study revealed that weeds of Compositae possess herbicidal potential, however, activities, types and amount of active compounds vary with plant species (Chon *et al.*, 2003). Extracts of *Hordeum vulgare* L., *Avena fatua* L., *Oryza sativa* L. and *Triticum aestivum* L. significantly reduced root growth of *Medicago sativa* L., *Echinochloa crus-galli* (L.) P. Beauv., *Eclipta prostrata* L., *Cyperus iria* L. and *Dactyloctenium aegyptium* (L.) Willd (Chon and Kim, 2004). Iqbal *et al.* (2004) demonstrated that methanolic extracts of roots of *Ophiopogon*
japonicus Ker-Gawl and its constituent allelochemical salicylic acid and hydroxybenzoic acid inhibited root and hypocotyls growth of lettuce by 50%.

Later, Xuan et al. (2006) extracted phytotoxins responsible for growth inhibitory activities of kava roots. As many as six lactones viz. desmethoxyyagonin, kavain, 7,8-dihydrokavain, yagonin, methysticin and dihydromethysticin were identified. Application of these lactone @ 1–10 ppm and 10–50 ppm significantly inhibited growth of weeds (Lactuca sativa L. and E. crus-galli) and pathogenic fungi (Colletotrichum gloeosporioides, Fusarium solani, Fusarium oxysporum and Trichoderma viride), respectively. The study concluded that kava lactones exhibit herbicidal and antifungal activities thus might be useful for development of bioactive herbicides and fungicides (Xuan et al., 2006). Kong et al. (2006) showed that extracts of leaves of Lantana camara L. reduced biomass of aquatic weed Eichhornia crassipes (Mart.) Solms-Laub. and the alga Microcystis aeruginosa (Kützing) Kützing under laboratory as well as field conditions. The fractions of extracts were isolated and identified as pentacyclic triterpenoids, lantadene A and lantadene B. Both these triterpenoids were also phytotoxic towards E. crassipes and M. aeruginosa growth. The study suggests that allelochemicals of L. camara could potentially be used to improve the management of weeds and algae in aquatic systems (Kong et al., 2006).

Phenolic acids—chlorogenic acid, ferulic acid, p-hydroxybenzoic acid, p-coumaric acid, protocatechuic acid, salicylic acid, trans-cinnamic acid and vanillic acid (frequently occur in plant biomass used for mulches) reduced the growth and chlorophyll a and b, carotenoids contents in Echinochloa crus-galli (L.) P. Beuav., Setaria viridis L., Chenopodium album L. and Galinsoga parviflora Cav. (Stupnicka-Rodzynkiewicz et al., 2006). Topal et al. (2006) evaluated herbicidal effect of catechol, a phenolic allelochemical, for control of weeds Papaver rhoeas L., Cirsium arvense (L.) Scop., Lamium amplexicaule
L. and *Sinapis arvensis* L.. The workers found that under field conditions, 13.6 mM catechol showed inhibited germination, shoot and root elongation, fresh weight in all test weeds and possesses a strong herbicidal effect which was comparable to that of 2,4-D.

Maharjan *et al.* (2007) investigated that aqueous extracts (2–10%) of *Parthenium hysterophorus* leaves suppressed germination and early growth of three cereal crops (*Oryza sativa* L., *Zea mays* L. and *Triticum aestivum* L.), three cultivated crucifers (*Raphanus sativus* L., *Brassica campestris* L. and *Brassica oleracea* L.) and two wild species of family Asteraceae (*Artemisia dubia* Wall ex. Besser and *Ageratina adenophora* (Spreng) King and HE Robins. The authors concluded that leaves of *Parthenium hysterophorus* L. may be a source of natural weedicide against *Ageratina adenophora* R.M.King & H.Rob. which help to control invasive plants.

Cheema *et al.* (2010) conducted a field trial to assess allelopathic effects of aqueous extracts of *Sorghum bicolor* (L.) Moench. + *Helianthus annuus* L., and *Oryza sativa* L. husk for suppressing potential of rice weeds. Residue mixture of *S. bicolor* + *H. annuus* and *O. sativa* husk (@ 18 L ha⁻¹) suppressed density (up to 46-50%) and dry weight (up to 49%) of *O. sativa* weeds. Compared to aqueous extracts only, mixture of extracts with half dose of herbicide was as effective as full dose of herbicide. The study suggests that herbicide dose can be minimized by combining it with extracts of allelopathic plants (Cheema *et al.*, 2010).

Mahmood *et al.* (2010) investigated growth suppressing potential of aqueous extracts of *Sorghum bicolor* (L.) Moench. and *Helianthus annuus* L. in combination against *Trianthema portulacastrum* L. Under laboratory and pot conditions, *S. bicolor* + *H. annuus* extract completely suppressed germination and growth traits (shoot and root length) of *T. portulacastrum* and reduced shoot dry weight by 66%. In crux, combination of aqueous extracts of *S. bicolor* + *H. annuus* may be used as natural herbicide to control *T. portulacastrum*. Al-Tawaha
and Odat (2010) showed that extracts from different parts of *Sorghum bicolor* and *Zea mays* alone and in combination significantly reduced germination and radicle and hypocotyl length of *Hordeum vulgare* L. subsp. spontaneum (wild barley). The workers thus concluded that effective allelochemicals from these crops i.e. *S. bicolor* and *Z. mays* can be used to develop herbicides for the control of one of the world’s most aggressive weeds.

El-Rokiek *et al.* (2010a) demonstrated use of mango leaves as a safety tool to suppress growth of *Cyperus rotundus* L. Under greenhouse condition, mango leaf extracts (5, 10, 15 and 25% applied weekly for one month) and leaf powder (20, 40, 60, 80 and 100g/kg soil) mixed in soil inhibited growth of *C. rotundus* after 30 and 75 days of treatment. The number of mother shoot, number of daughter shoots as well as dry weight of underground organs reduced significantly and this inhibition was reported to be due to presence of phenolic acids *viz.* caffeic acid, ferulic acid, coumaric acid, benzoic acid, vanillic, chlorogenic, gallic; hydroxybenzoic and cinnamic acid. In fact, allelochemicals such as coumarin, o-coumaric acid, p-coumaric acid, benzoic acid, p-hydroxybenzoic acid, ferulic acid and cinnamic acid have been known to possess herbicidal potential by many authors (Singh *et al.*, 2003b; Chon and Kim, 2004). El-Rokiek *et al.* (2010b) evaluated allelopathic potential of foliage and tubers of *C. rotundus* against *Chorchorus olitorius* L. (broad leaved weed), and *Echinochloa crus-galli* (L.) P. Beauv. (grassy weed) associated with *Glycine max* (L.) Merr. Treatment of foliage and tuber residues @ 20, 40, 60 and 80 g kg⁻¹ significantly reduced growth and dry weight of both weeds up to 85% accompanied by enhancement in growth and yield of *G. max*. Javaid *et al.* (2010) investigated phytotoxic effects of aqueous leaf and bark extracts of *Alstonia scholaris* (L.) R. Br. against abnoxious weed- *Parthenium hysterophorus* L. The seedling growth and fresh biomass were significantly reduced in response to 2-10%
extracts and foliar spray (50-100%) thus possess herbicidal potential for management of *P. hysterophorus*.

Recently, Lemerle *et al.* (2010) and Asaduzzaman *et al.* (2011) revealed competitiveness of *Brassica napus* L., one of the leading crops in world against most common weed-*Lolium multiflorum* Lam. A significant reduction (60-100%) was observed in weed density in presence of canola and attributed to its allelopathic property which can be exploited for management of *L. multiflorum*. Vasilakoglou *et al.* (2011) incorporated biomass of four biotypes of *Origanum vulgare* L. as green manure and observed that emergence and growth of *Echinochloa crus-galli* (L.) P. Beauv., *Setaria verticillata* (L.) P. Beauv., *Portulaca oleracea* L., *Gossypium hirsutum* L. and *Zea mays* L. Compared to control (oregano green manure-free treatments), oregano green manure treatments suppressed the emergence of *P. oleracea*, *E. crus-galli*, and *S. verticillata* up to 52–86%. In addition, *G. hirsutum* lint and *Z. mays* yields were also enhanced upon oregano treatments. The workers concluded that use of *O. vulgare* biotypes as green manure not only suppress weeds in *G. hirsutum* but also minimized herbicide usage.

Later, El-Rokiek *et al.* (2011) observed that aqueous extracts of stems and leaves of *Codiameum variegatum pactum* L. significantly reduced germination and seedling growth of *Portulaca oleracea* L., broadleaved weed and *Echinochloa colonum* (L.) Link., a grassy weed. *C. variegatum pactum* leaf residues when applied @ 50-300 g m⁻² inhibited emergence and density of broadleaved and grassy weeds 60 days after treatment thus, hold potential to be used as bioherbicide to control weeds. Nikneshan *et al.* (2011) evaluated allelopathic potential of *Helianthus annuus* L. cultivars on several crops and associated weeds (*Amaranthus retroflexus* L., *Portulaca oleracea* L., *Lolium rigidum* Guad., *Hordeum spontaneum* C. Koch., *Triticum aestivum* L. and *Carthamus tinctorius* L. Aqueous extracts of *H. annuus* cultivars (25, 50 and 100%) significantly inhibited germination of weeds while it
stimulated wheat and *Portulaca oleracea* L. germination. Kapoor (2011) revealed *Hyptis suaveolens* L. residues inhibited growth and altered physiology of *Parthenium hysterophorus* L. and may be used as potent bioherbicide to control the spread of *P. hysterophorus*. Dry leaf residues of *H. suaveolens* inhibited size and number of leaves, height, branches, capitula and seeds/plant. Further, amount of chlorophyll, sugar, lipids and protein decreased while amount of organic acids was increased. The accumulation of organic acids may be an adaptation strategy of *P. hysterophorus* to avoid environmental stress by allelochemicals. Shafique et al. (2011) evaluated herbicidal effects of aqueous extracts (2, 4 and 6%; w/v) of three *Chenopodium* species i.e. *C. album* L., *C. murale* L. and *C. ambrosioides* L. on *Avena fatua* L. Aqueous extracts of all three *Chenopodium* species significantly reduced germination, seedling growth and biomass of *A. fatua*. The study revealed that aqueous extracts of *Chenopodium* species contain substantial amount of inhibitors and can therefore be effectively manipulated for management of one of problematic grassy weed of wheat.

Esmaeili et al. (2012) studied that phenolic acids namely ferulic acid, *m*-coumaric acid, *p*-coumaric acid, vanillic acid, *p*-hydroxybenzoic acid and cinnamic acid at $10^{-3}$, $10^{-4}$ and $10^{-5}$ M showed significant inhibitory activity in terms of germination, root and shoot length, total fresh weight and dry weight of *Echinochloa crus-galli* (L.) P. Beauv. Recently, Manikandan and Jayakumar (2011) studied that post emergence treatment of aqueous leaf and bark extracts of *Ficus benghalensis* markedly showed mortality (80%) against *Ipomoea pentaphylla* L. due to presence of water soluble allelochemicals, phenolics, and revealed its herbicidal potential against *I. pentaphylla*.

Application of extracts of combination of two or more allelopathic plants have been recommended to control weeds more efficaciously due to synergistic mode of action of allelochemicals (Duke et al., 2000; Jamil et al., 2009; Jabran et al., 2010). Cheema et al. (2002b) reported
that compared to *Sorghum bicolor* (L.) Moench. aqueous extracts alone, mixtures of *S. bicolor*, *Helianthus annuus* L. and *Eucalyptus* sp. water extracts suppressed weeds up to 70% thus served a better bioherbicide. Later, Rehman et al. (2010) demonstrated use of allelopathic aqueous extracts of *Sorghum bicolor* (L.) Moench., *Helianthus annuus* L. and *Oryza sativa* L. in combination for control of weeds in *O. sativa* fields. Application of mixture of extracts (@ 15 L\(^{-1}\)) along with reduced rates (½ and ½ of recommended rate; 15–600 g ha\(^{-1}\)) of three pre-emergence herbicides (butachlor, pretilachlor and ethoxysulfuronethyl) reduced density and dry weight of *Echinochloa crus-galli* (L.) P. Beauv., *Cyperus iria* L. and *Dactyloctenum aegyptium* (L.) Willd. density (by 66-75%). Further, compared to extracts alone, extract mixtures with ½ of the label rates of pre-emergence herbicides improved yield of *O. sativa* by 41–61% thus suggesting use of combined allelopathic water extracts for weed management (Rehman et al., 2010). Further, application of such mixtures in combination with low doses of herbicides reduce reliance upon synthetic farm chemicals and have shown promising results towards low-cost weed management (Cheema et al., 2005; Hussain et al., 2007; Iqbal and Cheema, 2007; Jabran et al., 2010).

**Mechanism of action of allelochemicals**

Allelochemicals exhibit a wide range of mechanism of action on the recipient plants. The readily visible morphological effects of allelochemicals include inhibition of seed germination, reduced root or radicle and shoot or coleoptile elongation, necrosis or swelling of root tips, curling of root axis, distortion of root hairs, increased number of lateral roots, retardation of plant biomass. However, these effects may be as a result of a variety of other effects at cellular, biochemical or molecular level. Interpretations of mechanisms of action are complicated by the fact that individual compounds can have multiple phytotoxic effects (Einhellig, 2002). In order to explore the herbicidal potential of an allelochemical, understanding and determining its mode of action is one of the challenging aspects in allelopathic studies.
**Effect on ion and water uptake**

The ion uptake and growth are the most energy consuming processes in plant cells (Van der Werf et al., 1988). It is logical to study the effect of allelochemicals on ion or water uptake, since the plant root is the first tissue to come into contact with allelochemicals in the rhizosphere. Inhibition of seedling growth upon allelopathic stress may, therefore, be as a result of decreased ion uptake. Cinnamic acid from root exudates of *Cucumis sativus* inhibited uptake of mineral ions like nitrates ($\text{NO}_3^-$), dihydrogen phosphates ($\text{H}_2\text{PO}_4^-$), sulphates ($\text{SO}_4^{2-}$), potassium ($\text{K}^+$), calcium ($\text{Ca}^{2+}$), and magnesium ($\text{Mg}^{2+}$) by intact seedlings of cucumber (Yu and Matsui, 1997).

Exposure to stem and root extracts of tropical weeds *Ageratum conyzoides* L., *Melilotus indicus* (L.) All. and *Parthenium hysterophorus* L. decreased ion uptake of phosphorus ($^{32}\text{P}$) and zinc ($^{65}\text{Zn}$) in three *Triticum aestivum* L. varieties (Saxena et al., 2003). Abenavoli et al. (2003) reported that cells from *Daucus carota* L. suspension culture treated with coumarins took up ammonium ions preferentially to nitrate ions and attributed this to lower energy cost associated with ammonium assimilation. Inhibition in respiration and insufficient amount of ATP synthesis may be one of the causes of alteration or inhibition in ion uptake in response to allelochemicals (Gniazdowska and Bogatek, 2005).

$\text{H}^+$-ATPase, a membrane bound enzyme responsible for generation of proton electrochemical gradient provides driving force for the uptake and efflux of ions and metabolites across the plasma membrane (Palmgren, 2001). Inhibition in $\text{H}^+$-ATPase activity results in reduction in mineral and water uptake by plant roots, consequently, limits the essential plant functions such as photosynthesis, respiration or protein synthesis, leading to growth reduction (Gniazdowska and Bogatek, 2005). Treatment of *Lactuca sativa* L. seedlings with allelochemicals from *Polygonum hydropiper* L. also resulted in inhibition of $\text{H}^+$-ATPase activity (Fujita and Kubo, 2003).
Barkosky et al. (1999, 2000) opined that allelochemicals interfere with normal membrane functions and disrupt active transport resulting in either impairment of plant water balance, and mineral deficiency or indirectly change the water relation. Volatile essential oils from *Prinsepia utilis* L. inhibited stomatal opening and reduced the stomatal conductance by inhibiting K⁺ influx into the guard cells (Rai et al., 2003). Exposure to allelochemicals resulted in reduced stomatal conductance in addition to loss of leaf turgor in *Cucumis sativus* L. seedlings (Yu et al., 2003). Any alteration in H⁺-ATPase together with ion and water uptake by roots leads to stomatal closure, reduced cell turgor of leaves and indirectly affect photosynthesis (Zhou and Yu, 2006).

**Effect on cell structure and proliferation**

Inhibition of mitosis and disruption of various organelles e.g. nucleus, mitochondria etc. has been proposed as one of the important causes of inhibition of plant growth upon exposure to allelochemical stress (Gniazdowska and Bogatek, 2005).

Cells of apical root meristem of *Allium cepa* L. showed condensed mitochondria with electron dense matrix and extended intracristal spaces with the treatment of coumarins (Kupidłowska *et al.*, 1994a,b). In some organelles, protrusions containing membrane fragments due to hypertrophy of mitochondrial membrane were also observed. Based on these, authors suggested that coumarins react with membrane lipids or proteins, resulting in changes of membrane permeability and thus modifying the energy production (Kupidłowska *et al.*, 1994a,b). Treatment of aqueous leachates of *Sicyous deppei* G. Don resulted in compression of cells around quiescent center in root tips of *Phaseolus vulgaris* L. (Cruz-Ortega *et al.*, 1998). In addition, the cell structure was disorganized, lacked evident symptoms of cell differentiation, and root tip cells showed amorphous and inactivated (nondividing) nuclei (Cruz-Ortega *et al.*, 1998). Galindo *et al.* (1999) demonstrated that cotyledons of *Cucumis sativus* L. showed symptoms of separation of plasma membrane from cells in presence of dehydrozaluzianin (DHZ).
Burgos et al. (2004) reported that 2(3H)-benzoxazolinone (BOA) and 2,4-dihydroxy-1,4-benzoxazin-3(4H)-one (DIBOA) reduced the regeneration of *C. sativus* root cap cells and increased the width of cortical cells resulting in increased root diameter. Further, these allelochemicals increased cytoplasmic vacuolization, reduced ribosome and dictyosome density, and decreased the number of mitochondria. Mitotic activity in root-tip cells of *A. cepa* was completely arrested in response to BOA treatment, and the cells exhibited abnormality in shape and size (Singh et al., 2005a). Several volatile terpenoids from *Salvia leucophylla* L. inhibited cell proliferation in root apical meristem by inhibiting cell-nuclear and organelle DNA synthesis (Nishida et al., 2005). Monoterpenes camphor (100 mg/10 L) and menthol (50 mg/10 L) induced stomatal opening accompanied by extreme swelling and a final break down of the protoplasts in *Arabidopsis thaliana* (L.) Heynh. (Schulz et al., 2007).

Further, allelochemicals induced different chromosomal abnormalities such as stickiness, scattering, precocious movement, secondary associations, laggards, bridges, non-synchronous disjunction and disturbed polarity thus caused chromotoxicity in *Helianthus annuus* L. (Kumar and Gautam, 2008).

**Alterations in physiological and biochemical processes**

Studies providing information at physiological and biochemical modes of action such as various metabolic processes and enzymatic activities provide useful information about the modes of action of natural compounds.

Alkaloids (e.g. colchicine and vinblastine) and terpenoids (e.g. taxol) interfere with cell division and possess similar mode of actions like synthetic herbicides (Vaughan and Vaughan, 1988). Monoterpene α-pinene, reduced the growth of *Glycine max* (L.) Merr. cotyledons by interfering with rates of electron transport, ATP generation thus energy metabolism (Penuelas et al., 1996). Cinnamic acid, an active
allelochemical of *Agropyron repens* (L.) P. Beauv. is also known to alter uptake and transport of ions and reduce chlorophyll content of *G. max* root (Baziramakenga *et al*., 1997).

α-Amylase regulates starch breakdown for supplying substrates to respiratory metabolism thus play a key role in seed germination of cereals. Leaf leachates of *Eucalyptus globulus* Labill. decreased α-amylases activity in seeds of *Eleusine coracanta* resulting in suppression in seed germination (Padhy *et al*., 2000).

Monoterpenes, 1,4- and 1,8-cineole showed strong growth inhibition towards sicklepod (*Cassia obtusifolia* L.) and barnyard grass (*Echinochloa crus-galli* [L.] P. Beauv.) by completely inhibiting mitotic activity (Romagni *et al*., 2000). Abrahim *et al.* (2000) studied that monoterpenes camphor and eucalyptol (hydrophobic) and α-pinene and limonene (lipophilic) inhibited germination as well as primary root growth by inhibiting mitochondrial respiration in *Zea mays* L. Sorgoleone, a p-benzazquinone, when applied @ 0.6 kg ha⁻¹ inhibited growth of broadleaved weeds by impairment of photosynthetic machinery and its mode of action was comparable to those of synthetic herbicides—diuron and triazines (Weston and Czarnota, 2001).

Phenolics present in soils covered by *Fagus sylvatica* L. and *Pinus laricio* inhibited germination of pine seeds by lowering the activity of glucose-6-phosphate dehydrogenase, glucosephosphate isomerase and aldolases, the enzymes of glycolysis and oxidative pentose phosphate pathway (Muscolo *et al.* 2001). This indicates that phenolics interfere with the supply of sufficient level of reducing power, ATP and carbon skeletons for biosynthesis in pine. Muscolo *et al.* (2001) suggested that inhibition of enzymatic activity is related to protein degradation as the secondary effect of allelochemicals stress. These studies show that allelopathic stress-caused suppression in seed germination which is mediated through disruption of normal cellular metabolism. In other words, mobilization of reserves which
under normal conditions takes place rapidly in early stages of seed germination seems to be delayed or decreased upon exposure to allelochemicals (Gniazdowska and Bogatek, 2005).

Batish et al. (2002) revealed that parthenin, a potent phytotoxin from *Parthenium hysterophorus* L., inhibits growth and establishment of weeds (*Avena fatua* L. and *Bidens pilosa* L.) by inhibiting synthesis of chlorophyll, by inhibiting respiration or by causing water loss in weeds. In response to leaf allelochemicals of *Helianthus annuus* L., a significant decrease in ATP/ADP level and energy was observed in *Sinapis alba* L. (Bogatek et al., 2002). Volatile oil from cinnamon (*Cinnamomum zeylanicum* Blume) and red thyme (*Thymus vulgaris* L.) adversely affected growth of potato (*Solanum tuberosum* L.) sprout by killing meristematic cells, due to leakage of ions thus resulting in loss of membrane permeability (Tworkoski, 2002).

During early stages of germination in fat storing seeds, activity of enzymes of glyoxylate cycle e.g. isocitrate lyase (ICL) increases due to enhanced lipid metabolism in the storage tissue (McLaughlin and Smith, 1994). A significant inhibition was observed in lipid mobilization upon exposure to ferulic and p-coumaric acids in germinating seeds of *Brassica napus* L. (Baleroni et al., 2000). The inhibition of lipid mobilization accompanied by decreased ICL activity was also observed in *Sinapis alba* L. in response to *Helianthus annuus* L. leaf extracts (Kupidtowska and Bogatek, 2003, Bogatek and Stepień, 2003). These observations suggest that upon stress imposed by allelopathy, decreased activity of ICL result in inhibition or delay in seed germination (Gniazdowska and Bogatek, 2005).

Scrivanti et al. (2003) revealed that volatile oil from *Tagetes minuta* L. and *Schinus areira* L. inhibited root growth of *Zea mays* L. by enhancing peroxidation of membrane lipids thus degrading their structure and composition. It is well known that essential oils and their monoterpenes affect chlorophyll content, hill reaction and activities of various enzymes (Kong et al., 1999; Morardshahi et al., 2003). Rai et

*Eucalyptus citriodora* Hook. essential oil showed inhibitory activity towards noxious weeds *Bidens pilosa* L., *Amaranthus viridis* L., *Rumex nepalensis* Spreng. and *Leucaena leucocephala* (Lam.) de Wit, *Phalaris minor* Retz. *Parthenium hysterophorus* L. by adversely affecting photosynthetic machinery, respiratory activity and membrane integrity (Singh et al., 2005b; Batish et al., 2007; Setia et al., 2007).

Sánchez-Moreiras et al. (2009) studied that BOA induces salt sensitivity (markedly high putrescine content, increased lipid peroxidation etc.) in plants showing tolerance to salt stress in terms of reduced stomatal density, increased proline content, and high K^+ concentration. Lara-Núñez et al. (2009) demonstrated that *Sicyos deppei* G. Don exhibits phytotoxicity by causing an allelochemical stress in *Lycopersicon esculentum* Mill. Weed leachates delayed seed germination and inhibited growth because of sum total of metabolic changes such as delay in starch degradation and sucrose hydrolysis, lowering the activity of sucrose synthetase, cell wall invertase and α-
amylase, and also lowering the activity of endo β-mannanase, β-1,3 glucanase, α-galactosidase and exo-polygalacturonase, high content of abscisic acid etc.

BOA (2,3-H-benzoxazolinone), a hydroxamic acid from graminaceous crops, has been reported to be used as a natural herbicide, especially for small weedy species like *Echinochloa crus-galli* (L.) P. Beauv. (Burgos and Talbert, 2000). It interferes with auxins (Anai et al., 1996), inhibits plasma membrane bound H⁺ ATPases and disrupts membrane integrity in roots of *Avena sativa* L. (Friebe et al., 1997), causes ultrastructural changes in the roots of *Cucumis sativus* L. (Burgos and Talbert, 2000), inhibits α-amylase activity in roots of *Lactuca sativa* L. (Kato-Noguchi and Macías, 2005). Singh et al. (2005a) demonstrated that BOA reduces germination, early growth and development and rhizogenesis by interfering with cell division, photosynthetic machinery and energy metabolism in *Phaseolus aureus* L. Later, Batish et al. (2006e) explored the effect of BOA, on *P. aureus*. BOA reduced radicle and plumule elongation of *P. aureus* and altered the macromolecular content, activities of various enzymes viz. amylases, proteases, peroxidases and polyphenol oxidases and induced generation of reactive oxygen species leading to oxidative damage in *P. aureus*.

BOA and cinnamic acid (CA) exhibited different morphological and physiological changes in 1-month old C₃ plant species (*Dactylis glomerata* L., *Lolium perenne* L. and *Rumex acetosa* L.) (Hussain and Reigosa, 2011). Within 24 h of treatment (1.5 mM), both allelochemicals reduced leaf osmotic potential, decreased F₅/F₆, FPSII, photon energy absorbed by PSII, and leaf protein content. Understanding such modes of action of allelochemicals provide a rational and scientific basis for the design of safe and effective herbicides. Gangasuresh et al. (2011) demonstrated that treatment of cotton seeds with leaf exudates of *Parthenium hysterophorus* L. and *Limbrosia* species showed morphological variations accompanied by
increase in proteins and phenol content due to release of protease inhibitors. The plants with higher content of phenols were observed to show pest resistance compared to controlled plants. Further, activity of nitrate reductase and peroxidases increased while the activity of catalases decreased. Kapoor (2011) explored herbicidal potential of *Hyptis suaveolens* L. residues against *Parthenium hysterophorus*. Dry leaf residues of *H. suaveolens* inhibited growth of *P. hysterophorus* by adversely affecting contents of chlorophyll, sugar, lipids and proteins in response to environmental stress by allelochemicals.

**Oxidative stress and hormonal imbalance**

Recently, oxidative stress has been proposed as one of the mechanisms by which allelopathic plants exert toxic effects on other plants. Allelochemical stress is caused by altering or modifying diverse metabolic processes, having many molecular targets in the receptor plants. In general, at lower concentrations, ROS have been implicated as second messengers in hormone mediated intracellular signaling cascades that mediate several plant responses in plant cells including response to allelochemical stress.

The exposure of cucumber (*Cucumis sativus* L.) roots to ferulic acid and *p*-coumaric acids resulted in increase in hydrogen peroxide (H$_2$O$_2$) content and activities of peroxidases (POD), catalases (CAT) and superoxide dismutases (SOD) (Yu *et al.*, 2003; Politycka *et al.*, 2004). Recently, it has been proposed that allelochemicals cause production and accumulation of reactive oxygen species (ROS) accompanied by activation of cellular antioxidant system in the target tissue (Weir *et al.*, 2004). It has been demonstrated that (-)-catechin result in root tissue damage of *Centaurea diffusa* Lam. and *Arabidopsis thaliana* (L.) Heynh. via ROS-triggered Ca$^{2+}$-signaling cascade leading to drop in cellular pH and allelochemical-induced genome wide changes in gene expression pattern (Bais *et al.*, 2003; Callaway and Vivanco, 2005).

BOA, a well known allelochemical with herbicidal activity, induced
oxidative stress in roots of *Phaseolus aureus* L. as indicated by increased lipid peroxidation, electrolyte leakage, hydrogen peroxide level, and proline content, and upregulation of the activities of ROS-scavenging enzymes viz. superoxide dismutase (SOD), ascorbate peroxidase (APX), guaiacol peroxidase (GPX), catalase (CAT) and glutathione reductase (GR) (Batish *et al.*, 2006e).

Allelochemicals from *Sicyos deppei* G. Don caused an imbalance in oxidative status of cells of *Lycopersicon esculentum* Mill. roots leading to alteration in activities of CAT, APX, SOD, GR and the plasma membrane NADPH oxidase, as well as in the levels of H$_2$O$_2$ and O$_2^\cdot$ (Lara-Nuñez *et al.*, 2006). Singh *et al.* (2006a) opined that exposure to monoterpenic α-pinene induce oxidative stress through generation of ROS, as indicated by membrane damage, enhanced lipid peroxidation levels, proline accumulation and by activation of antioxidant enzyme systems in *Cassia occidentalis* L. roots.

It has been suggested that loss of seed viability in response to phytotoxins from *Helianthus annuus* L. involved induction of oxidative stress accompanied by restriction in reserve mobilization in germinating seeds of *Sinapis alba* L. (Oracz *et al.*, 2007). Extracts of *H. annuus* caused oxidative burst expressed in terms of enhanced levels of lipid peroxidation, H$_2$O$_2$ content and electrolyte leakage in germination of mustard seeds (Bogatek *et al.*, 2006; Oracz *et al.*, 2007). Bogatek and Gniazdowska (2007) reported that allelochemicals from *H. annuus* enhanced the level of abscisic acid while suppressed emission of ethylene from seeds and blocked the metabolic activity of embryo thus leading to hormonal imbalance between two stress hormones in germinating *S. alba* seeds.

Later, Singh *et al.* (2009) determined that volatile essential oil from *Artemisia scoparia* Waldst. & Kit. and constituent monoterpenic β-myrcene inhibited growth of other plants through generation of ROS-induced oxidative stress associated with increased lipid peroxidation, accumulation of H$_2$O$_2$ and up-regulation of scavenging enzymatic
machinery in *Cyperus rotundus* L. roots. Recently, it was found that an oxygenated monoterpenic citronellol (50–250μM) disrupts membrane integrity through ROS production as indicated by increased lipid peroxidation, enhanced solute leakage from root membrane, decreased conjugated dienes, and enhanced lipoxygenase activity in *Triticum aestivum* L. roots (Kaur *et al.*, 2011a).

Mutlu *et al.* (2011) demonstrated that oil from *Nepeta meyeri* Benth. applied @ 0.01% inhibited growth of weeds (*Amaranthus retroflexus* L., *Bromus danthoniae* Trin., *Bromus intermedius* Guss., *Chenopodium album* L., *Cynodon dactylon* L., *Lactuca serriola* L., and *Portulaca oleracea* L.) by exhausting antioxidant system by generation of reactive oxygen species. The oils enhanced activity of CAT (catalases) but reduced SOD (superoxide dismutases) activity in all test weeds. The essential oils increased level of lipid peroxidation and hydrogen peroxide content. The workers attributed this activity due to presence of monoterpenoids especially the oxygenated ones in the oil. Recently, Matsumoto (2011) revealed that oxidative damage caused by ROS (generated from melanin synthesis pathway) induced lipid peroxidation in response to non-protein amino acids L-DOPA (L-3, 4-dihydroxyphenylalanine) and m-tyrosine (L-3-hydroxyphenylalanine) in *Lactuca sativa* L. and *Echinochloa crus-galli* (L.) P. Beauv.

Disruption of membrane by essential oils and their constituent monoterpenoids have been suggested as one of the mechanisms for their phytotoxic and herbicidal effects. In fact monoterpenes, being lipophilic in nature increase membrane fluidity due to expansion, damage membrane structure through generation of reactive oxygen species and inhibit membrane enzymes (Oracz *et al.*, 2007; Singh *et al.*, 2009; Kaur *et al.*, 2011a).

**Effect at molecular level**

Western blott analysis showed that aqueous extracts of *Helianthus annuus* L. enhanced the synthesis of CAT 5 isoforms in *Sinapis alba*
Aqueous leachates of *Callicarpa acuminata* modified the expression of 21 proteins in the roots of *Zea mays* L. *Lycopersicon esculentum* Mill. and *Phaseolus vulgaris* L. (Cruz-Ortega *et al.*, 2002). Using a microarray technique to study the early changes in DNA expression pattern, more than 200 genes were observed to be up- or down-regulated as a result of BOA action (Reigosa *et al.* 2004). Baerson *et al.* (2005) demonstrated that BOA retard growth of other plants by alteration in expression of genes involved in detoxification and stress responses.

Treatment of juglone, a naphthaquinone found in many species of Juglanaceae, induced oxidative stress on glutathione transferase (*Gstl*) gene expression patterns in *Zea mays* L. seedlings. Compared to control, the transcriptional activity of *Gstl* was stimulated in *Z. mays* seedlings in response to juglone exposure (Sytykiewicz, 2011). Kekec *et al.* (2012) investigated that volatile oils from the aerial parts of *Nepeta meyeri* Benth. inhibited germination and growth of *Bromus danthonia* Trin. and *Lactuca serriola* L. and caused a change in random amplified polymorphic DNA (RAPD) profiles in terms of variation in band intensity, loss of bands, and appearance of new bands thus, possess genotoxic potential.