CHAPTER 1

INTRODUCTION

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1.1 Overview

'Green revolution' has led to tremendous increase in the crop yields over the past few years. Consequently this has been responsible for the discovery and utilization of many chemicals (Dayan et al. 2009). Presently agriculture practices around the world involve usage of vast number of chemicals, precisely the agrochemicals. Agro chemicals can be broadly categorized into fertilizers, herbicide/weedicide, insecticides, fungicides, nematicides, growth regulators etc. While these chemicals have been boon to the mankind, they most likely do not behave in the same way to most of the other earthly inhabitants. Humans in the quest of gaining solutions to eradication of the unwanted agricultural guests, make use of the agro chemicals illegitimately. While the human needs are catered, consequences that the agricultural and the adjoining ecosystems face, most often go unconsidered. Through their use, the agro-chemicals enter food chain, sustain there and irreversibly damage the ecosystems and its components. They there by pose potential threats to one or more ecosystems. Growing awareness and increasing knowledge concerning the impact of these chemicals on environment has made possible, the endeavour towards more ecosystem friendly alternatives. Organic farming, eco-agriculture, conservation agriculture, retention forestry and sustainable agriculture are among many such alternatives that are nurturing the idea of environment friendly agriculture or forestry. Organic agriculture is an ecological production management system that promotes and enhances biodiversity, biological cycles and soil biological activity. It is based on minimal use of farm inputs and on management practices that restore, maintain and enhance ecological harmony (Gold 2006). Eco-friendly agriculture describes landscapes that support both agricultural production and biodiversity conservation, working in harmony together to improve the livelihoods of rural communities (Scherr and McNeely 2002). Conservation agriculture (CA) is an agricultural management practice in which there is minimum soil disturbance, retention of residue for soil cover and rotation of major crops (Hobbs et al. 2008). Sustainable agriculture is the one that maintains its resource base, relay minimum on the artificial inputs from outside the farm system, manages pests and diseases through regulating mechanism and is able to recover from disturbances through cultivation and harvest (Gliesmann 1998). Gustafsson et al. (2012) defined retention forestry as an approach to forest management based on the long-term retention of structures and organisms, such as living and dead trees and small areas of intact forests at the time of harvest. The prime aim of these alternative practices are minimal or no use of chemicals and to bring the solution that are energy affordable. Such alternatives endow the farmers with solutions, while nurturing and taking care of their surroundings.

Among all the agricultural concerns, weed eradication is inevitably significant. Pimentel et al. (2001) have reported that in U.S. only, around 12% loss in crop yield is caused by the weeds and their control costs nearly 35 billion US$. The costs of weed control are even more in the developing countries like India. Qasem and Foy (2001) have reported that from evolutionary point of view, weeds are the plants that establish the secondary succession, compete and subsequently benefit themselves utilizing human-made environments. According to definition, weeds are the unwanted plants that are of no use or are ‘unwanted’ in context of the human needs, they are plants that are ‘out of place’. They are found to grow in field, gardens, landscapes, forest floor and generally are of no economic value. Plants that behave as weeds or have tendency to do so form only 1 % or less of the total plant species (Qasem and Foy 2001). Weed plants majorly belong to the plant families like Poaceae, Euphorbiaceae, Solanaceae, Convolvulaceae, Fabaceae, Asteraceae and Amaranthaceae. Astonishing is the fact that world's large part of food supply comes from the very same plant families. Weeds are known to offer competition to the crops and other economically important plant species, primarily for nutrition, water, sunlight and space. Additionally, weeds support certain agricultural pest, serving as food plant while some weeds serve as a host, necessary to complete different stages in life cycle of certain pest, bacteria, virus, fungi, nematode etc. Eradication of the weeds hence becomes obligatory and management of the weeds becomes an important aspect in function and design of the agro-systems (Mamolos and Kalburtji 2001). In the modern times weed suppression or eradication is implemented by employing the use of herbicides, herbicide coupled with manual deweeding or manual deweeding. Manual removal or deweeding of the weeds has become taboo now days as it employs immense labour, is tedious and at times an expensive affair. Herbicide use becomes the most appropriate option available to the farmer.

Dayan et al. (2009) have reported that herbicides account for more than half of the volume of all agricultural pesticides utilized in the developed world and concerns over potential health and environmental impact of these compounds has currently fascinated many public concerns. Herbicides or weedicide are the agrochemicals used for weed mitigation. They are small molecules currently in use, are synthetic in origin, having molecular mass of 700 Dalton or less. They are designed molecules, specially equipped to target only a particular metabolic pathway in weed, disturbing its metabolism and ultimately decline or complete deterrence of growth. Most herbicide target the enzymes involved in the primary metabolic pathways (like photosynthesis and respiration), i.e. they target the processes that are necessary for the growth and development of an organism or certain proteins performing necessary physiological functions. Inhibition of target sites in the secondary metabolism can be less lethal as secondary metabolic processes are not essential to sustain life in duration of herbicide, when its application and working is expected. However herbicidal impact on primary metabolism may subsequently lead to impact on secondary metabolism. Most herbicides are categorised in to different types according to their molecular structure, the pathways that they are meant to attack and the target weed plant. Due to strict target specificity they are capable to hinder or arrest weed growth selectively. Herbicide discovery involves identifying certain, specific metabolic pathway in weed species. Dayan et al. (2012) reported that since first herbicidal discovery to the present times, the action cites of herbicides in the current day use are based only on a few modes of action and from nearly past 20 years no new mode of action has been discovered. This metabolic target specificity of herbicides has a major associated drawback, as the herbicide usage, upon longer exposures tends to develop resistance in the target weed species over the time and the weeds continue to evolve all known modes of action (Powles and Yu 2010; Dayan et al. 2012). Natural products have always been an inspiration for the medicines, pesticides, fungicides discoveries. Compared to pesticides, natural-product-based herbicide discovery has been the least successful (Petroski and Stanley 2009; Huter 2011). There are only few examples of herbicides or weed mitigation tools that are derived from natural products. For all the above enlisted reasons there exist this everlasting need for discovering herbicide, having novel modes of action and describing their novel molecular active sites, importantly having the natural products based origin or a natural product entirely. Use of natural products will allow the eco-friendly and sustainable agriculture practices and will be better substitute for synthetic herbicide. Such practices seek to utilize phenomenons and formulations that are inspired from nature and have better environment compatibility. Allelopathy is one such tool. Allelopathic interaction used by naturally coexisting species can be employed directly or indirectly for the management of weeds (Tesio and Ferrero 2010; Singh et al. 2003, Weston 2005). Certain crop species, trees, weeds etc have been found to possess allelopathic potential. Yang and Tang (1988) reviewed the plants used for pest control as described in Shengnong Ben Tsao Jing (1518-1593 AD) in China, where in they have described 267 plants used against pest and some of them were even showing allelopathy. Reviews on several crop species showing allelopathy have been given by Khanh et al. (2005) and Tesio and Ferrero (2010). Qasem and Foy (2001) studied and found at least 240 weeds species to be allelopathic. Chou (1999) reports, that incorporation of allelopathic substances into agricultural management enables reduction in the use of synthetic agrochemicals such as herbicides, fungicides and insecticides and even lessens environmental deterioration. Utilization of allelopathic potential of plants can possibly aid to the weed management in the modern day sustainable agriculture system (Haig 2001).

1.2. Allelopathy

1.2.1. History and definition: Allelopathy is an ecological phenomenon that refers to chemical interaction or a chemical warfare. It is known to exist since ages though not with the current name that is ‘Allelopathy’. Theophrastus (372-285 B.C.) wrote about such reactions in his botanical works (Albuquerque et al. 2011). He wrote about chickpea plants observed to exhaust the soil and destroying weeds. A Swiss botanist, Augustin Pyramus De Candolle (1832) noted that chemicals released from crop plants cause soil sickness and it was possible to minimize soil sickness by crop rotation. Stickney and Hoy (1881) observed and studied walnut tree inducing harmful effects on plants growing nearby. In the year 1937, Hans Molisch an Austrian plant physiologist coined the term allelopathy while explaining the effect of ethylene on fruit ripening and the term was derived from two separate words ‘allelo’ and ‘pathy’ which means mutual harm. He reported that a plant naturally releases substance that is inhibitory to the growth of the other plants sharing the same habitat. Tukey Jr. (1969) defined allelopathy as, the interactions which involve substances released from one plant influencing another plant particularly an inhibitory influence as allelopathy. Rice (1984) defined allelopathy as the effect (s) of one plant (including microorganisms) on another plant(s) through the release of a chemical compound(s) into the environment. This definition includes both stimulatory and inhibitory effects. Such stimulatory and inhibitory effect has been observed for a growth regulating hormone gibberellic acid. Gibberellins leached from chrysanthemum plants (Kozel and Tukey 1968) have shown to be stimulators to the growth of lettuce seeds and at the same time to be inhibitors to the breakdown of chlorophyll in leaf discs of *Rumex obtusifolius* (Tukey 1969). In 1996, the International Allelopathy Society (IAS) defined allelopathy as “Any process involving secondary metabolites produced by plants, algae, bacteria and fungi that influences the growth and development of agriculture and biological systems” (Hussain and Reigosa 2014). Duke (2015) defines allelopathy as the adverse effects of a plant on other plants by means of chemicals (allelochemicals) that it produces.

1.2.2. Ecological &Evolutionary significance and Evidences of allelopathy

According to the Russian ecologist Rabotnov (1982), “adaptation of plant species to the chemistry of other species plays a crucial role to higher level or ecosystem structures as in the community organization”. Allelopathic processes have an important role in most adaptive strategies of plants and particularly of weed species (Gonzalez et al. 1995). The phenomenon of allelopathy plays a vital role in determining the structure of an ecosystem and is important in some natural or human induced biological warfare's like the exotic plant invasion (Catalan et al. 2013). According to Inderjit and Callaway (2003), the success of invasive plant in an invaded range allows an insight to the understanding of evolutionary level of biological organization in the invaded community. The invasive plant species offers allelopathic inhibition to the native community inhabitants and there by achieve success on new habitat (Hierro and Callaway 2003; Inderjit et al. 2006). Exotic plant invasions being crucial to organization of the community structure are explained in terms of a few hypothesis such as "Novel weapons hypothesis" (Callaway and Ridenour 2004). Tesio and Ferrero (2010) report that success story of non-native weeds to succeed its establishment in new habitats, is by the use of unique chemicals that may offer toxic effect to the plants in the invaded soil. This has an evolutionary implication as these effective chemical has direct selection as a beneficial trait and they increase their competitive advantage in their invaded range. Explaining the evolutionary significance of allelopathy Chou (1999) has reported that the allelopathic compounds are involved in the environmental complex of managed or natural ecosystems and they play significant roles in the determination of plant diversity, dominance, succession and climax of natural vegetation and in the plant productivity of agroecosystems. Some of the popular plant invaders known to have allelopathic potential are *Centaurea* spp. (Fletcher and Renney 1963) and *Parthenium hysterophorus* (Kanchan and Jayachandra 1979, 1980).

Allelopathy is a significant ecological phenomenon in nature as plants also govern their own population by means of auto-toxicity and can govern presence of other co-occurring macro and micro biota. Earliest record, for occurrences of soil sickness and auto-toxicity in some plants has seeded the whole science of allelopathy.

Some plants after one time growth do not re-grow at the same place for a second time. This behaviour observed in plants is called auto-toxicity, it assign's soil sickness making it difficult for the plant to re-establish at the same place. It occurs as the plant release some allelochemicals in to soil where in the allelopathic or the chemical producing species hinders growth of its own species there by controlling its own population to combat unfavourable conditions (Reigosa et al. 1999; Singh et al. 1999). According to Kruse et al. (2000), prevalence of changes in the distribution pattern of some crops and economic species such as fruit trees facing replanting difficulties or low yield, indicates negative allelopathic activity of these species. These observed difficulties may be due to the auto-toxicity of the plant as observed in members of family Cucurbitaceae, which according to Duke (2015) is sometimes hard to prove, as there may also be an effect of plant pathogens in the soil, soil nutrient depletion, or other effects on the soil unrelated to allelochemicals, especially if the same crop is grown year after year. The plant exhibiting strong auto-toxicity is Alfalfa (Chon et al. 2006). Important crop species such as: Wheat (*Triticum aestivum* L.) (Wu et al. 2000), Barley (*Hordeum vulgare* L.) (Overland 1966), Rice (*Oryza sativa* L.) (Kato-Noguchi et al. 2008) and Sorghum (*Sorghum bicolor* (L.) Moench. (Weston 1996) are observed to possess allelopathic potential. Poor growth of crop species after other species or the same species, has been grown on that field is often attributed to build up of allelochemicals in the soil, when essential resources are not limiting.

Blanco (2007) also reports the importance of accounting allelopathy while studying ecosystem such as forest and has suggested that allelopathy induces certain changes such as altered germination rates, inhibition of seedling growth, microbial functions, litter decomposition and dieback of mature trees which may ultimately affect ecosystem-level process. Allelopathy is also observed in agro-ecosystems where in allelopathic effects like that between, crop to crop, crop to weed and weed to crops has being noticed (Batish et al. 2001; Casini 2004). Many tree species showing strong allelopathic effects on the adjacent ground flora are being studied so far and trees like *Eucalyptus* (Blanco 2007), *Juglans* (Stickney and Hoy 1881) are known to exhibit allelopathy.

In spite of plethora of research being conducted, establishing the evidences of allelopathy and differentiating it from competition is difficult and vaguely explained. According to Duke (2015), allelopathy can be a component of observed plant/plant interference and the other component is competition and separating allelopathy from competition is challenging. Tesio and Ferrero (2010) have suggested that allelopathy and competition could act in synergy because a small disadvantage of allelopathy can alter the final balance when competition is also taking place. Blum et al. (1999) proposed three criteria to establish evidence for allelopathy, these are: 1) the putative aggressor plant or its debris must produce/contain and release chemicals that ultimately will be capable of inhibiting growth or function of another plant, 2) distribution and accumulation of organic complexes (i.e. promoter-inhibitor) dominated by inhibitor in soil must be of sufficient concentration to inhibit nutrient and/or water uptake by roots and/or energy fixation by the sensitive plants and 3) the observed field patterns of plant inhibition cannot be explained solely by physical factors or other biotic factors. According to Inderjit and Weiner (2001), mere production of chemicals by a plant however is not sufficient enough to ensure their allelopathic potential. Certain other factors like abiotic and biotic environmental conditions may be instrumental to determine the allelopathic potential of plant and corresponding chemicals in soil. As can be seen in Figure -1.1, various ecosystem factors, biogeographic variations and co-evolutionary relationships also impacts the production, release and activity of allelochemicals and eventually the allelopathic activity of plant. Allelopathy with enormous number of existing evidences is still considered to be a suspected phenomenon and not all cases of plant predominance in any of the ecosystem are strongly believed to be due to allelopathy. Prevailing challenges in proving the existence of allelopathy cannot disapprove the fact that allelopathy do exist naturally.

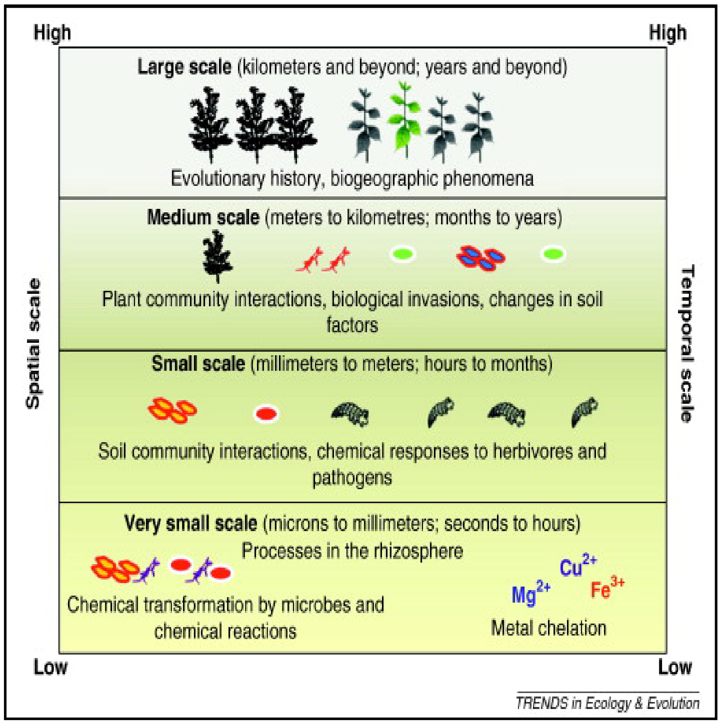


Figure 1.1: The impact of ecosystem factors, biogeographic variations and co-evolutionary relationships on the production, release and activity of allelochemicals along spatial and temporal scales (Figure adapted from Inderjit et al. 2011)

In contrast to the negative role of allelopathy there is this phenomenon called as Allelobiosis which exerts a positive or a neutral effect. Ninkovic et al. (2006) and Glinwood et al. (2011) have explained allelobiosis as an interaction in which exchange of phyto-chemicals has an informative significance to the receiving plant. However for the undertaken research objectives, the study was confined only to the negative or inhibitory effects owing to allelopathy.

1.2.3. Allelochemicals

Whittaker (1968) has suggested the term "allelo-chemics" to describe chemicals from one plant which influence another, however he has not specified nature of the reaction. Whittaker and Feeny (1971) coined the term ‘plant allelochemical’. The plant contributing allelochemicals is recognized as the ‘donor’ plant and the plant being influenced by the released allelochemicals is identified as the ‘target’ or ‘afflicted’ plant (Inderjit and Duke 2003). Thus any plant based chemical compounds or secondary metabolites responsible for allelopathic activity of a plant are termed as allelochemicals. A chemical may act as an allelochemical in one situation but not in another (Inderjit and Duke 2003). Weir (2004) reports that the toxicity of allelochemical compounds may not necessarily be inherent however these compounds induce toxic responses in the receiver. Putnam (1988), Mizutani (1999), Vyvyan (2002), and Haig (2008) have reviewed enormous amount of information available on allelochemicals. According to Putnam (1988) such chemicals with allelopathic potential are present (usually in conjugated form) in virtually all the plants and plant parts like leaves, flowers, fruits, buds, seeds, stems and roots. These compounds may be released into their immediate environment (atmosphere or rhizosphere) under certain conditions in quantities that are sufficient enough to be lethal to a neighbouring or successional plant. Allelopathic effects most often are the result of synergistic activity of many factors that eventually results in suppression or stimulation of growth (Einhellig 1996). Metlen et al. (2009) have reported that allelopathic compounds produced by a plant species may increase fitness of a plant by pre-empting the resources. Interaction of allelochemicals with soil, degradation or modification by microbial action and its half life hinders the measurement of allelochemicals released or absorbed. It is equally difficult to determine the spatial and temporal dynamics of allelochemicals. However considering the natural evidences of allelopathy, Putnam (1988) termed allelochemicals as the nature's own herbicides. Chemically they range from a simple hydrocarbon, ethylene to complex polycyclic compounds with molecular weights of several hundred (Putnam 1988). The major types of compounds having allelopathic tendency are alkaloids, phenolic compounds and their derivatives, mono-, di-, tri-terpenoids, benzoxazinones, cyanogenic compounds , polyacetylenes etc. Reigosa and Pazos-Malvido (2007) investigated phytotoxic potential of twenty one different plant secondary metabolites like sinapinic, syringic, vanillic, ferulic, p-coumaric, chlorogenic, gallic, gentisic, protocatechuic, p-hydroxybenzoic, and trans-cinnamic acids, eucalyptol, quercetin, vanillin, syringaldehyde, rutin, 2-benzoxazolinone, protocatechualdehyde, tyrosol, juglone and l –mimosine, on germination and growth on *Arabidopsis thaliana* L. and they found eleven of the twenty one molecules to be inhibitory to the seed germination and seventeen of them were found to inhibit the root growth.

1.2.3.1. Chemistry of allelochemicals

Alkaloids: This is a large group of secondary metabolites mostly found in plants and in some fungi. Many of the alkaloids are valued as excellent medicinal source. They are usually cyclic or polycyclic and are mostly derived through amino acid like ornithine, lysine, phenylalanine, tyrosine, tryptophan (Daniel 1990). They therefore always contain at least one heterogenous nitrogen atom and often have basicity of an amine. Their chemical structures are very diverse and no core fragment can represent the class (Haig 2008). An excellent example of allelopathic alkaloid is that of, allelopathy exhibited by coffee (*Coffee arabica* L.) and tea plant (*Camellia sinensis* (L.) O. Kuntze). Numerous papers report toxic alkaloids produced by coffee and tea and their possible allelopathic or auto-toxic influences (Rizvi et al. 1981). As caffeine was found to exert differential action on several plant species, Rizvi et al. (1981) proposed that it might be a useful selective herbicide and it was found to inhibit certain weed with no adverse effect on mung bean (*Vigna radiata* (L).Wilczek). Levitt et al. (1984) detected presence of tropane alkaloids like scopolamine and hyoscyamine as the key metabolites from *Datura stramonium* L., which were found in the seeds and foliage aqueous leachates that forms the plant litter and from the farm soil having the weed *Datura stramonium* L. These regions that were inhabited by the weed had poor crop performance and the alkaloidal residues in the soil were considered responsible for the same.

Phenolic acid: This group of compounds range from simple structures such as simple aromatic phenols, hydroxy and substituted benzoic acids and aldehydes, hydroxy and substituted cinnamic acids to complex and large flavonoid, quinines, coumarins, tannins etc (Daniel 1990). Phenolic compounds are derived from shikimic acid pathway. They are involved in large number of functions in plants and have special implication in the field of allelopathy. Li et al. (2010) and John and Sarada (2012) have reviewed the literature available on certain plant species exhibiting allelopathic potential owing to number of phenolic compounds they possess. Major allelopathic effect has been observed with respect to decomposing residues of certain plant species rich in phenolics and exhibiting auto-toxicity or soil sickness. Long term cultivation of same plant belonging to same species results in to soil sickness wherein the phenolic compounds present in decomposing plant residues are considered to be source of phytotoxins. Rye seedlings residue that were allowed to decompose were analysed to have seven different phenolic compounds (Wojtkowiak et al. 1990). Min et al. (2000) reports phytotoxicity of *Vulpia sp*. a naturalized annual grass found wide spread in pastures and among cereal crops. The plant accounts for several crop losses, is resistant to wide range of herbicides and was found to offer phytotoxicity to pasture species and wheat. Water soluble extract of this grass showed presence of number of phenolic compounds (Min et al. 2000). Gallic acid and hydroquinone have been identified as the major allelochemicals of the known allelopathic plant *Polygonella myriophylla* (Weidenhamer and Romeo 2004). Growth in the Chinese fir (*Cunninghamia lanceolata* (Lamb.) Hook) seedlings was found to be negatively correlated with total phenolics content in stump-roots that were added to soil (Huang et al. 2000). Sorgoleone, a p-benzoquinone exuded from the roots of several species of *Sorghum* was first isolated by Netzley and Butler (1986). Nimbal et al. (1996) have reported the phytotoxicity of sorgoleone on a number of plant systems and according to Einhellig et al. (1993) and Nimbal et al. (1996) this growth inhibitions offered by sorgoleone is through inhibition of Photosynthesis and Respiration. Streibig et al. (1999) reported that the mode of action for sorgoleone is similar to that of certain herbicides such as s-triazines, phenyl urea, uracils etc. juglone, a 5-hydroxy-1, 4-naphthoquinone found in the members of the walnut family (Juglandaceae) is one of the naturally occurring and most phytotoxic allelochemical (Willis 2000). Some cereal plants possess a series of benzoxazinoid compounds (cyclic Hydroxamic acids) which exhibit a diversity of biological activity, including allelopathy (Friebe 2001).

Flavonoids, chemically are phenolic derivatives, they have the general structure of a 15-carbon skeleton consisting two phenyl rings (A and B) and heterocyclic ring (C) (Daniel 1990). Their carbon structure can be abbreviated C6-C3-C6. They can be classified into, flavonoids or bioflavonoids, isoflavonoids, neoflavonoids. Although the relative role of flavonoids in allelopathy has been less well-characterized than that of other secondary metabolites, Weston and Mathesius (2013) reviews significance of flavonoids, their allelopathic activity and fate in the soil by accounting some examples of pasture, legumes, cereal crops and ferns. Flavonoids from leaves of mango were found to inhibit the invasive weed *Parthenium hysterophorus* L. (Javaid et al. 2010). Red (*Trifolium pratense* L.) or white clover (*Trifolium repens* L.) are widely used as pastures and fodder plants and Carlsen et al. (2012) recovered some 20 flavonoid compounds from both the living and decomposing clover stands and from the soil incorporated with clover as a green manure. Their study addressed rational why the clover plant had weed suppressive potential and replant problems.

Terpenoids: They are synthesised from the biochemical pathway via mevalonic acid and isopentenyl pyrophosphate (a basic C-5 building unit). The isopentenyl pyrophosphate unit combine with itself to form monoterpenes, sesquiterpenes, diterpenes, triterpenes, steroids etc (Daniel 1990). About 24,000 different compounds now known are of a cyclic unsaturated type and they carry oxygen at various functional sites, e.g. aldehydes, ketones, ethers, alcohols, and lactones. Some terpenes exist as stereo-isomers and have different physico-chemical properties from each other. They are diverse in function acting as signal molecules, allelochemicals, phytoalexins, pheromones, pigments, photoprotective agents, membrane constituents, and reproductive hormones. Large number of terpenes are even explored for their medicinal value. In a review, Haig (2008) reports significance of terpenoids as allelopathic compound.

Monoterpenes, the C-10 terpenoids have high volatility and significant odours. They are involved in function like pheromones and allelopathy. Vaughan and Spencer (1993) evaluated and screened 18 volatile monoterpenes for their allelopathic activity against annual weeds found associated with soybean. They found monoterpenes like geraniol and α-terpineol to selectively retard germination of annual weeds like Italian ryegrass, large crabgrass, redroot pigweed, and velvetleaf. Dudai et al. (1999) found that volatile oils from Bible hyssop (*Origanum syriacum* L.), tea hyssop (*Micromeria fruticosa* [L.] Druce) and lemon grass (*Cymbopogon citratus* [Nees] Stapf.) can inhibit the germination of four weed species though effect was depended on the type of soil and has suggested the potential of these compounds to be used as bio-herbicides. Thymol, carvacrol and carvone showed high inhibition even at low concentrations against weed seeds (Azirak and Karaman 2008). Connick et al. (1989) analysed the residues from aerial portions of the plants *Amaranthus retroflexus, Amaranthus hybridus, Amaranthus cruentus, Amaranthus spinosus, Amaranthus hypochondriacus* and *Amaranthus palmeri* and found some volatile organic compounds emitted from the residues. Feo et al. (2002) identified thirty-eight components in the *Ruta graveolens* essential oil and they evaluated allelopathic activity of essential oil and some of its constituents on radish germination and radicle growth and found them to be inhibitory. Muller and Muller (1964) reported presence of terpenes like α-pinene, camphene, β-pinene, cineole, dipentene and camphor, in leaves of *Salvia leucophylla, S. mellifera* and *S. apiana* and in the atmosphere above the macerated leaves of these species. They found camphor and cineole to be most inhibitory and the pinenes as the least inhibitory. Detecting the presence of terpenes in *Salvia* species and inhibition of the same, Muller and Muller (1964) suggested that the growth inhibition of annual grassland species in and around *Salvia* colonies is due to these volatile terpenes.

Sesquiterpenes are a class of terpenes that consist of three isoprene units and may be acyclic or contain rings with many combinations. It has also been implicated in allelopathy. Artemisinin, a sesquiterpene lactone from annual wormwood (*Artemisia annua* L.) also an anti-malarial plant is a potent growth inhibitor and a phytotoxin (Duke et al. 1987; Chen and Leather 1990; Lydon et al. 1997). Macias et al. (1993; 1996; 1998; 1999) analysed allelopathic activity of different varieties of sunflower, subsequently its aqueous leaf extracts were evaluated and found to have different guaianolides and annuolides and these compounds exhibited allelopathic effect on some dicot and monocot species. Ragweed (P*arthenium hysterophorus*) is a noxious weed. Parthenin, (a sesquiterpene lactone) is a major constituent of ragweed and its derivatives exhibit phytotoxicity and some parthenin analogues are more potent phytotoxic than parthenin itself (Batish et al. 1997).

Saponins: They are the glycosides of terpenoids where attachment of a sugar moiety renders detoxification to the aglycon. Alfalfa produces allelopathic saponins such as medicagenic glycosides, the compound was found to be selectively phytotoxic (Anaya 1999). Perez et al. (2014) in a series of experiments conducted using bioassay-guided fractionation have determined the allelopathic potential of about eleven different steroidal saponins from the *Agave offoyana* leaves.

Quassinoids are naturally occurring diterpene lactone compounds found in several plant species belonging to the family Simaroubaceae. Dayan et al. (1999) evaluated the effects of substitution in the oxymethylene ring on the biological activity such as phytotoxicity of several quassinoids. These molecules were found to be highly phytotoxic reducing growth of lettuce (*Lactuca sativa*) root and affecting cell division in onion (*Allium cepa*) root tips.

Glucosinolates, are components of many pungent plants like mustard, cabbage, radish etc. They are pungent and the pungency of these plants is due to glucosinolate. These natural chemicals contribute to plant defence against pests, herbivores etc. Glucosinolates are accompanied by the enzyme myrosinase which cleaves off the glucose group from a glucosinolate in the presence of water. The non sugar moiety is converted to an isothiocyanate, a nitrile or a thiocyanate and these are the active substances that are used for plant defence (Daniel 1990). Glucosinolates can be used as bioherbicides because they are potential seed inhibitors and can be employed in sustainable agricultural practices (Wolf et al. 1984; Brown and Morra 1995; Vaughn and Boydston 1997).

Cyanogenic Glycosides, are composed of an α-hydroxynitrile type aglycone and of a sugar moiety (mostly d-glucose) (Daniel 1990). Plant such as *Sorghum halepense,* has cyanogenic glycosides like dhurrin which after hydrolysis produces hydrogen cyanide, glucose and p-hydroxybenzaldehyde, these glycosides were found to be inhibitory and were suppressive to growth of other plants (Nicollier et al. 1983).

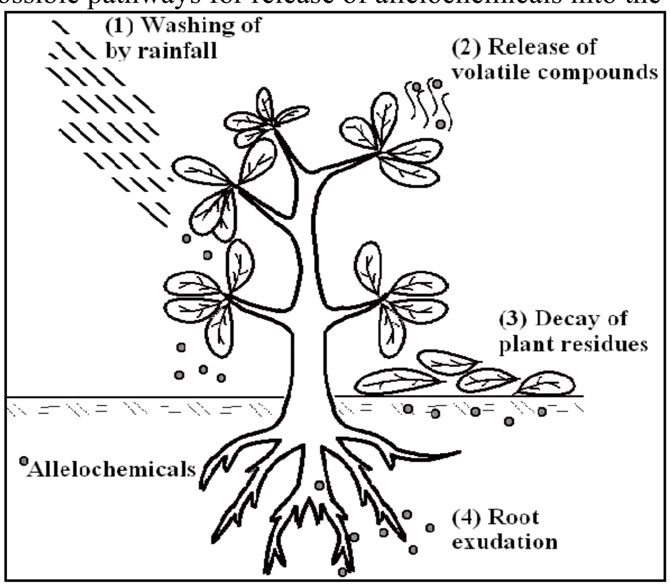
α–Terthienyl (α-T) are sulphur containing compounds with three ring structure. They are found in the plants belonging to the family Asteraceae as in certain *Tagetes* sp. The plant produces a number of metabolites such as α-T compounds and polyacetylenes that are allelopathic in nature. Lambert et al. (1991) suggested that α-T compounds are strongly photoactive, are contact herbicide and are inhibitory to other plants and thus can be exploited for weed management.

Aminoacid derivatives: *Mucuna pruriens* (L.) DC. is an important cover crop, the plant harbours the compound L-DOPA (L-3,4-dihydroxyphenylalanine). In a review Soares et al. (2014) discussed the role of DOPA, it possesses number of biological activity including allelopathy where in it inhibits the seed germination, causes injury to root growth and other meristems and also inhibit seedling growth. Canavanine, a non-protein amino acid, present in jack bean (*Canavalia ensiformis* (L.) DC) was found to be inhibitory to some plants (Nakajima et al. 2001).

1.2.3.2. Mode of allelochemicals release from the plant

Allelopathic substances are released from the plant mainly through four different modes (Figure-1.2). Release of allelochemicals may be: 1) by leaching (by rainwash or by fog drip) over the above ground plant part 2) by volatilization from leaves, 3) by excretion or exudation from roots and 4) by litter decomposition (Whittaker and Feeny 1971). Considerable number of studies have been conducted on each of the above mentioned release modes of allelochemicals.

Figure 1.2: Possible pathways for release of allelochemicals into the environment.



(Note: Figure adapted from Albuquerque et al. 2011)

Leaching: It is the common natural processes observed in plants, where in biological materials are lost to water. Tukey (1966) has given an excellent review on leaching and he reports that a large number of metabolites may be leached from the plant trichomes, natural or induced cracks and oil glands that are found on the surface living leaves. He further suggests that the moisture agent like rain, dew or mist that wets the leaf surfaces can cause leaching and that it depends on many factors like environment, maturity, plant development stage, shape of the leaf, temperature, light, soil and microbial interaction. Kozel and Tukey (1968) in their experiments have demonstrated that the plant growth hormone like gibberellins can be leached from plants in aqueous medium such as rain and dew. In an experiment, Carballeira and Reigosa (1999) analysed the allelopathic effect of different types of leaching from *Acacia dealbata* on the test plant i.e. *Lactuca sativa* L. var. Great Lakes. They studied throughfall (rain passing through the canopies), stemflow (rain flowing over the stems) and soil percolates that was collected from the plantation during one year where in all the three types of samples rendered toxicity to test plant.

Volatilization: It is one of the processes of allelochemical's loss from leaves (for example terpene evaporation). These compounds have ability to combine with leaf wax, surfaces of the same plant and soil particles. Dry soil can absorb the volatile substances and may have impact on ecological functions. Certain volatile compounds such as carbon dioxide, ethylene, and terpenes released by plants, may have diverse effect on the plant growth. Work by Burg and Burg (1965) and Burg (1968) reports compound like ethylene a volatile plant growth hormone to have immense potential and induces process like senescence in the test plants. Heisey and Delwiche (1983) have reported several plant species to release phytotoxic volatile emissions. Lankau (2008) has reported the plant *Brassica nigra* to produce sinigrin in relation to the coexisting species. Inderjit et al. (2009) used plants that were silenced for the suspected volatile allelochemical to study the allelopathy offered by plant owing to its volatile compound. They used ethylene specific transformant of *Nicotiana attenuata* (ir-aco) as a receiver with a wild relative of cultivated tobacco (*Nicotiana tabacum*-WT *)* as an emmiter plant. They found the plant root growth was negatively related to the number of WT plants and the ethylene concentration indicating the allelopathic potential of the compound. Al Harun et al. (2015) in their experiments found that the volatile substance and exudates from *Chrysanthemoides monilifera* plant parts had diverse inhibitory effects on *Lactuca sativa* and *Acacia mearnsii.*

Litter decomposition: The plant parts such as leaf, twigs, fruits, flowers roots when fall on the ground by any natural or induced means, they are subjected to decomposition by weathering and by soil micro flora and as a result number of chemicals are released from the decomposing plant material. The compound may be directly affective or the effect may be by modification. Kimura et al. (2015) observed forest where the red pine (*Pinus densiflora* Sieb. et Zucc.) was growing had very less herbaceous plants growing on its forest floor. They speculated that allelopathy must be a reason for such vegetation growth. They analysed the aqueous methanol extracts of red pine litter on *Lepidium sativum* and *Digitaria sanguinalis* L. and found them to be inhibitory to the weed growth in a concentration dependant manner. The extract were detected to have 9α, 13β-epidioxyabeit-8(14)en-18-oic acid and abscisic acid-β-D glucopyranosyl ester which they suspect to be the compounds synthesized in pine plants and delivered into the forest floor by defoliation of the needles.

Root exudation

Exudates and Rhizosphere: According to Weston et al. (2012), the rooting zone or rhizosphere is a very competitive environment where roots of neighbouring species and microorganisms compete for space, water and nutrients. Bais et al. (2006) suggest that the soil surrounding a plant root is a region where complex biological and ecological processes occur. Bais et al. (2006) and Weston et al. (2012) also reported that when roots are under stress or when they encounter challenges in the rhizosphere, they often respond by releasing low-molecular-mass compounds such as amino acids, organic acids, phenolics and proteins. Woods (1966) reviewed the compounds found in the plant root exudates that are released in to the rhizosphere to have direct or indirect (when acted upon by microorganism) influence other plants. Bertin et al. (2003) has discussed various mechanisms by which plant roots exudation occurs and this include diffusion, vesicle transport and ion channels, where in low-molecular weight compounds like sugars, amino acids, carboxylic acids and phenolics are released across the concentration gradient through passive diffusion from the cytoplasm of root cell into soil solutions. Certain high-molecular weight compounds like polysaccharides, protein, mucilages are released by vesicle transport Battey and Blackbourn (1993). Release of some chemicals like carboxylates occurs in a controlled manner through certain ion channels activated only under specific type of stress (Bertin et al. 2003).

McCully (1999) suggest that biological interactions that occur in terms of root exudates below the soil surface are more complex than those that occur above the soil surface. Yoder (1999) explained significance of such interaction between roots of parasite and its host plant, he suggested that this type of interactions are involved in communication signals between roots of competing plants species. These chemicals have immensely significant role to a plant as they impact on the species surviving in the surrounding (Vivanco et al. 1999).

Studies have been done on plant root exudates and rhizosphere where in the compounds were found to have deleterious effect on the adjacent occurring species. Callaway and Aschehoug (2000) examined effects of the root exudates of *Centaurea diffusa* on bunchgrass species. Addition of activated carbon reduced the deleterious effects of root exudates, implying existence of root exudates mediated allelopathic interaction. Batish et al. (2007) studied the root-mediated allelopathic interference of *Chenopodium murale* on wheat. The outcome of their study was that *C. murale* roots and their exudates imposed allelopathic effects on wheat by releasing water-soluble phenolic acids as putative allelochemicals in soil.

Rhizosphere analysis: Rhizosphere for a plant is a crucial zone which is partly responsible for maintaining good health of the plant and its interaction with its neighbour. Many of the allelopathic interaction are mediated by plant root exudates through the rhizosphere. Significance of rhizosphere for a plant has led to number of the rhizosphere studies. Number of the methods are followed for rhizosphere analysis, such as analysis- of the rhizosphere soil, plant root washings, extracting the plant grown agar, sequestering the live roots by hydroponics and *in situ* rhizosphere analysis using soil probes. Luster et al. (2009) and Oburger et al. (2013) have reviewed different methods pertaining to the rhizosphere analysis. Kalinova et al. (2007) studied the inhibitory root exudates extracted from soil and agar both and were found to possess phenolic compounds. Li et al. (2011) studied the methanol extracts of soil from *Panax ginseng* rhizosphere for its allelopathic effect on its own seedling and it was found inhibitory, analysis of the extracts helped to identify some 30 metabolites in the rhizosphere soil extracts. Cui et al. (2012) analysed the rhizosphere soil from beneath the walnut tree. The tree inhibits growth of plants growing up to some diameter in a region surrounding its trunk and is well known for its allelopathic potential. The rhizosphere soil was extracted with different solvents and the extracts so obtained was analysed for its allelopathic potential against the seed germination and seedling growth of cabbage (*Brassica oleracea* L. var. capitata L). All the extracts inhibited the seed germination and seedling growth in cabbage indicating the strong allelopathic potential of walnut. The extracts were subjected to chemical identification and were found to have certain compounds not present in the soils other then walnut rhizosphere. Hao et al. (2007) in an experiment allowed to grow watermelons in hydroponic culture and analysed the culture medium using the continuous root exudates trapping system (CRETS). He also investigated the allelopathic potential of watermelon and the results indicate allelopathy to play an important role in the phenomenon referred to as 'soil sickness' in case of watermelons.

Allelopathic effect owing to plant litter (Bonanomi et al. 2006) and plant root exudates has been studied for a number of times using both in the *in vivo* and *in vitro* experiments, however less attention has been given to the dynamics of phytotoxicity. The study based on spatial and temporal dynamics of allelochemicals released by plant through whatsoever means, is an avoided area of research in the field of allelopathy. One of the most remarkable studies designed and conducted for the rhizosphere analysis concerning plant allelopathy has been done by Weidenhamer (2005) and his associates through a series of experiments. Rhizosphere of allelopathic plant was analysed using different types of soil probes such as stir bars coated with PDMS (stir bar sorptive extraction), technical grade optical fiber coated with a thin film of PDMS (matrix-solid phase microextraction) and PDMS tubing. In the rhizosphere experiments they utilized the above mentioned soil probes to quantify levels of the photosynthesis inhibitor sorgoleone in the undisturbed rhizosphere of sorghum plants. Amongst all the soil probes used in the experiment, PDMS tubing were found to retain most of the sorgoleone. Weidenhamer et al. (2009) monitored the dynamics of thiophenes exuded by roots of two cultivars of *Tagetus patula* using PDMS-based probes and emphasised the potential of solid-phase root zone extraction method in such analysis. Using the probes they recovered thiophenes 5-(3-buten-1-ynyl) - 2, 2′-bithienyl (BBT) and α-terthienyl per probe per day from the root zone of *Tagetes patula.* Thiophenes were found to be present in the soil in biologically significant concentrations and no bioaccumulation of these compounds was obtained in the surface of 5 cm over the growing season indicating the importance of flux rates to thiophene toxicity. This study provides an insight in to the distribution of thiophenes beneath the plant which was spatially and temporally heterogeneous.

1.2.3.3. Mechanism of allelochemicals action

The plant function that are commonly evaluated for their allelopathic suppression in plant–plant allelopathy include respiration, photosynthesis, water balance and stomatal function, stem conductance of water, xylem element flux, membrane permeability, cell division and development, protein synthesis and enzyme activity alteration (Haig 2008). In an excellent review Weir et al. (2004) describe the possible effects that a target plant may suffer upon its allelopathic inhibition. The review presents some of the practical difficulties and existing lacunae in determining the mechanism of action of allelochemicals. Natural phyto-toxins may share mechanism of action with commercial herbicides. Most often combined efforts are made to assign a mechanism to the allelopathic metabolite where in metabolite is compared with a commercial herbicide having molecular structure similarity. Simultaneously they are analysed *in vitro* and *in vivo* for various biochemical and physiological responses manifested by the target plant as upon the herbicidal attack (Figure-1.3, Wang et al. 2006). Dayan et al. (2010) in the review suggest that the commercial herbicides can be used as probes in the study of mechanism of the allelochemical action. The review also describes important modes of action that the current day herbicides targets upon, as the herbicides inhibits specific molecular target sites within plant's biochemical pathways and/or physiological processes. Such applied studies relating the allelochemical to herbicide and respective molecular targets site can be utilized as the useful tool to dissect the intricacies of plant.

Benzoxazolin-2(3H)-one (BOA) is a commonly found compound in many of the monocot species and is a natural plant product that is phytotoxic to certain plant species. It is formed in a two-step degradation process from the O-glucoside of 2,4-

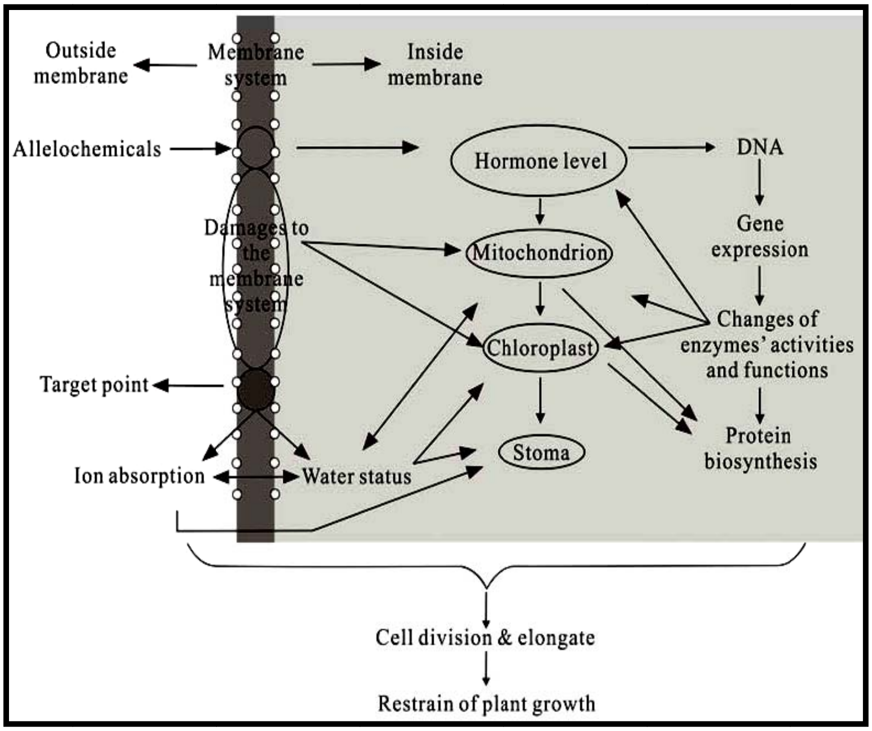


Figure 1.3 Various mechanism of allelochemical action
(Note: Figure adapted from Wang et al. 2006)

dihydroxy-2H-1,4- benzoxazin-3(4H)-one. In a few experiments BOA was found to retard the germination and growth of the target plant owing to its effects on a few mechanisms in it. Upon studies considering mechanism of action, BOA was found to cause oxidative damage in *Lactuca sativa* (Sanchez-Moreiras et al. 2008), delay of cell cycle with selective activity at G2/M checkpoint in a BOA-treated *Lactuca sativa* meristems (Sanchez-Moreiras et al. 2008), inhibits plasma membrane bound H ATPases in roots of *Avena fatua* (Friebe et al. 1997), disrupt water balance in plants, alter protein content and directly destructs photosynthetic reaction centre (PSII) (Hussain et al. 2011).

Phenolic compounds have been analysed to have diverse mechanism of action in the test plants. Compounds such as salicylic acid and ferulic acid could significantly affect mineral absorption by plants in the proper field conditions of pH and related concentration of acids (Harper and Balke 1981). Both the acids inhibited potassium ion (K+) absorption in excised root tissue of *Avena sativa* L. however salicylic acid was most inhibitory. In a study by Ding et al. (2007), cinnamic acid, a phenolic compound found in root exudates of cucumber (*Cucurbita ficifolia* Bouche) was found to increase activities of enzymes such as NADPH oxidase, superoxide dismutase, guaiacol peroxidase, catalase and O_2 production and H_2O_2 content and as a response to the cinnamic acid large amount of reactive oxygen species (ROS) were produced resulting in to increase in membrane peroxidation, decreasing membrane H+-ATPase activity and eventually leading to root death. Babula et al. (2009) has reported the action of naturally occurring naphthoquinones like plumbagin and juglone causes toxicity by generating reactive oxygen species (ROS), chromatine condensation, damaging the DNA resulting in to disturbance in mitosis and such disturbances caused as that by plumbagin and juglone eventually leads to programmed cell death.

One of the best characterized mechanism is that of sorgoleone, a lipophilic benziquinone from *Sorghum bicolour,* that is component of plants root exudates. In studies conducted using intact chloroplasts and thylakoid membranes, sorgoleone acts in a way similar to triazine herbicides such as atrazine, by specifically inhibiting the chloroplast electron-transport chain. It acts on PS II and there by affect photosynthesis and eventually affecting the plants growth (Nimbal et al. 1996; Czarnota et al. 2001). Sorgoleone also inhibits enzyme hydroxyphenyl pyruvate dioxygenase (HPPD) which is capable of disrupting the biosynthesis of carotenoids causing foliar bleaching (Meazza et al. 2002).

(□) - catechin, from *Centaurea maculosa* inhibits the seed germination of the plant itself regulating its own population however its seedlings are resistant to the compound (Weir et al. 2004). Several of phenolic compounds such as vanillic, p-coumeric, p-hydroxybenzoic and protochatechuic acid tested alone and in combinations caused inhibition to certain enzymatic activity (Muscolo et al. 2001). This suggests that the decrease in enzymatic activity is a secondary effect of these compounds, which might be caused by general protein damage leading to decreased enzymatic activity. Monoterpenes like camphor, α-pinene and limonene components of essential oils of several aromatic species, strongly affect the respiratory activity of soybean mitochondria but with different modes of action (Abrahim et al. 2003).

Mechanisms for allelochemicals's action in target plant are way behind its place. Advances in plant analysis techniques over the last decade, as that of development in various "Omic" techniques such as transcriptomics, proteomics, metabolomics and physionomics, has provided considerable insight in to the modes of action for various synthetic and natural phytotoxins (Duke 2013). Use of such techniques aids better understanding of allelopathic effect on biochemical and physiological processes. Some researchers have utilized noval approaches in the field of allelopathy. Babula et al. (2009) used dihydroethidium as fluorescent probe to evaluate mechanism of naphthoquinones action in tobacco BY-2 suspension culture. Roshchina et al. (2011) reported that, use of coloured allelochemicals such as pigmented secretions enriched in red anthocyanins (glucosides of pelargonidin from *Hippeastrum hybridum* petals), blue azulenes (synthetic azulene and azulene extracted from leaves of *Artemisia absinthium)* and yellow alkaloids (rutacridone from *Ruta* *graveolens* roots) may serve as tool to study the mechanisms of cell-cell interactions. Coloured compounds may reach particular molecular sites in the target plant or cells enabling their precise detection.

1.2.4. Allelopathy and stress:

Plants in natural or unnatural ecosystems are exposed to numerous stress conditions. Environmental factors like water deficit, mineral deficiency, light and temperature, pathogen attack, competition with weeds pose stress condition to plants. Plants uniquely react to stress condition and it has an inbuilt mechanism to survive the stress conditions and if not so the plant may die. Allelopathy, by imposing chemical inhibition to the receiver plant, offers stress very much similar to all other kind of stresses (Lovett et al. 1989).

The scale and effect of allelopathic stress may be alleviated upon action by environmental pressures such as temperature, irradiation, nutrients and moisture stress (Einhellig 1996). Schmidt and Lipson (2004) and Orr et al. (2005) have discussed the implication of microbial activity to the nutrient and allelochemicals availability to the plants and they also relate toxicity of allelochemical to availability of nutrient. For example, toxicity of vanillic and *p*-coumaric acids to barley (*Hordeum vulgare*) is higher when the crop seedlings are Nitrogen or Phosphorous deficient (Einhellig 1996) and providing fertilizers can overcome the toxicity.

1.2.5. Factors influencing production of allelochemical by donor plant

For a metabolite that is secreted by the allelopathic plant, from the time of release to the time of uptake and even after that, number of parameters and factors are crucial for a metabolite so as to induce inhibitory action on the receiver plant. Studies conducted *in vitro* bioassays, mainly concentrate on unhindered allelochemical action while in the soil the conditions are much different and complicated. In a review, Kobayashi (2004) suggests that the inhibitory effect exhibited by allelochemical in soil may be affected by soil condition, growth condition of the donor and receiver plants and climatic condition. Further he suggests that for exerting direct effect the allelochemical must have to be present in soil water, so that it is readily available for absorption to the receiver plant. According to Willis (2000) for any allelochemical compound to have effect on the receiver plant there should be some mechanism in the receiver plant for their uptake. Once released, the metabolite's fate in the environment is crucial, where in whether it maintains its chemical state or undergoes degradation due to short residence time or undergoes microbial action. In addition to the direct effects of allelochemicals on receiver plants growth, their indirect effects may be mediated by microbial activity where in degradation product of metabolites may exhibit allelopathy. According to Chou (1999) the putative metabolites may undergo different modifications such as polymerization, mineralization or oxidative ring cleavages to form low molecular weight compounds. Meier and Bowman (2008) in their study found the effect of phenolic compounds on the neighbouring plant to be direct and indirect where they also explained the role of microbial action on the indirect availability of the phenolic compounds. Kaur et al. (2009) showed that allelopathic effect of m-tyrosine was present in the sterilized soil and this activity was nullified in the non sterilized soil suggesting the role of soil micro-biota. The vicinity of donor and receiver plants are equally important for the translocation and manifestation of allelopathic activity as it decides the length of space and time the metabolite will have to travel in order to have any kind of inhibitory effect on receiver plant. Inderjit and Nilsen (2003) suggest that another factor playing significant role in determining toxicity of allelochemicals, include accumulation of allelochemical concentration that is biologically active to offer the allelopathic effect to the receiver plant. Finally allelochemicals in soil may be adsorbed on soil solids where in various soil factors, such as soil texture, organic and inorganic matter, moisture control its concentration and may affect its activity (Kobayashi 2004). The allelochemicals being toxic, the producing plants have self-protection mechanisms, as by means of trichome-specific synthesis (example artemisnin produced in *Artemisia annua* L.), compartmentalization (Duke et al. 2000) or by detoxification of the allelochemical by attaching a sugar or other moiety that enables the phytotoxic compound to transport and localize in cell organelles such as vacuole (Inderjit and Duke 2003). According to Tharayil et al. (2009) concentrations of soil nutrients can also induce certain allelochemicals and they found that mineral deficiencies as that of iron can stimulate highly complex exudation responses that can mobilizes metals and makes them available to the exuding plant for uptake. Allelopathic activity of a metabolite is usually a synergistic action where in the activity is observed due to participation of more than one metabolite rather than a single metabolite acting alone. For example, Lydon et al. (1997) reported that soil amended with annual wormwood leaf extract (which is chemically more complex) was more inhibitory to the growth of redroot pigweed than soil amended with pure artemisinin.

1.2.6. Applications of allelopathy

Many crop species have been identified to have allelopathic potential. Khanh (2005) has reviewed the applicability of crop allelopathy in achieving sustainable agriculture. He suggested that exploiting the crop allelopathy may help biological control of pests and weeds, decomposing crop residues can enhance soil quality by adding number of nutrient to the soil, incorporating allelopathic crops in the crop rotation can increase diversity of crops under cultivation which also minimizes growth of the dependant pests and weeds and may lead to development of biological herbicides from crop plants using isolated allelochemicals. Incorporation of 1–2 tonnes hacter $^{\square 1}$ of allelopathic crops to a field can reduce weed by an average of 70 % and increased rice yield by 20 % (Xuan et al. 2005). Khanh et al. (2005) giving an example discusses importance of alfalfa (*Medicago sativa* L.) as an allelopathic crop, wherein he has reported that an alfalfa variety (Rasen, selected from common alfalfa cultivars) was capable of inhibiting total weed biomass by 80 % and improving rice yield by 80.6 % in comparison to the control (without any weed and fertilizer management). In contrast, herbicide treatment could suppress about 75 % paddy weeds but favoured increase in the rice yield by only 10 %, whereas the same in hand weeding were about 70 and 25 % respectively (Khanh et al. 2005). The review suggests that alfalfa stands as a good example of ecofriendly weed manager as well as a nutrient supplier.

Crops having allelopathic potential can be employed as cover crop/ green manures and smother crops, crop residues or mulch, as rotational crop, intercropping and for genetic breeding with best allelopathic varieties (Singh et al. 2003; Khanh et al. 2005; Albuquerque et al. 2011). However selection of crop is important in management strategies concerning weed and pest control. These allelopathic plants can be a lead to discovery of herbicide molecule.

1.2.6.1. Cover crop/ Green manure and Smother crops

Cover crops are defined as crops cultivated with regular cropping for soil and moisture conservation, enhancement of nutrient recycling, biomass production, temperature lowering, weed suppression and forage supply (Swanton and Murphy 1996; Caamal-Maldonado et al. 2001; Price and Norsworthy 2013). Cover crops are alternatively referred as green manure owing to their tendency of supplementing nutrition to soil. Some crop species that function as the cover crop are barley

(*Hordeum vulgare*), sorghum (*Sorghum* spp.), corn (*Zea mays*), wheat (*Triticum aestivum*), rye, buckwheat (*Fagopyrum esculentum*), velvetbean (*Mucuna pruriens*), crimson clover (*Trifolium incarnatum*), subterranean clover (*Trifolium. subterraneum*), hairy vetch (*Vicia vilosa*), *Ipomoea batatas* and *Ipomoea tricolor*. Use of cover crops like cereals and pulse, suppress weeds and increase nitrogen content of soil. While selecting a cover crop the sowing times of these crops as are different, should be taken care of (Tesio and Ferrero 2010). Cover crops when grown with an additional intention of choking the weeds by occupying space and shading it, than it is called as smother crop. Cover crops are also used as smother crops.

1.2.6.2. Crop residues or mulch

Crop residues are defined as crop or its parts left in field for decomposition after it has been thrashed or harvested (Kumar and Goh 2000). Earlier the crop residues were removed out of field but in recent practices they are kept in the field after harvesting. According to Wilson and Foy (1990) the crop residue improves the soil, increases water filtration in soil, reduces labour and suppresses weeds under some situations by affecting the weed flora. The presence of crop residues on the soil surface as mulch suppresses weeds by the action of allelopathy (in part) and thus they may reduce reliance on herbicides (White et al. 1989). Utilizing the crop residues may require use of additional weed mitigation tools. Some crop residues are also known to enhance the efficiency of herbicides (Teasdale et al. 1991). Number of *in vivo* or *in vitro* studies, concerning the use of leaf mulch or whole plant mulch against common weeds has been conducted till date. Singh et al. (2003) found residue of different crop species like wheat, rye, sorghum etc to be effective against common field weeds. In a study by Batish et al. (2007), conducted for exploring herbicidal potential, leaf powder of *Tagetes minuta* was used against weeds found in rice fields. *Tagetes* *minuta* leaf powder applied (at the rate of 1, 2, and 4 tonne per hector) to the rice field soil was found to significantly reduce emergence and growth of two invasive weeds *Echinochloa crus-galli* and *Cyperus rotundus* (commonly found in rice field) in rice field plots and in pots under greenhouse.

1.2.6.3. Crop rotation

Crop rotation or polyculture is a powerful cultural technique utilized by farmers for effective weed management and it involves growing different crops in systematic and recurring sequence on the same land, as compared to monoculture, in which a particular crop is planted repeatedly in the same field (Liebman and Dyck 1993). According to Mamolos and Kalburtji (2001) problems of monoculture, auto-toxicity and plant mulch toxicity may occur due to allelochemicals released in to soil. Overland (1966) postulated that many allelopathic species act through a combination of competition and allelochemical interaction in living or decomposing crops. In comparison to monoculture, crop rotation systems implemented by growing multiple types of crops in sequence results in to higher crop yields (Liebman and Dyck 1993). According to Mamolos and Kalburtji (2001), selection of certain plant sequences under standard environmental conditions may suppress weeds, insects and diseases and thus avoid the resulting yield decline. A review on several crop species showing allelopathy has been given by Tesio and Ferrero 2010 and Khanh et al. 2005). Incorporating allelopathic crop species in crop rotation can prove to be a natural eco-friendly tool for weed and pest mitigation (Khanh et al. 2005).

1.2.6.4. Intercropping

Intercropping involves growing the additional or a companion crop along with the main agronomic crop or plant. When allelopathic crop or plant is employed as an inter-crop with main crop, effective weed management can be achieved. Han et al.

(2008) studied effect of aqueous extract of ginger rhizome, stem and leaf to have allelopathic effect on the two species often intercropped with ginger, are soybean and chive. Thus the allelopathic effect of ginger must be considered while selecting it for intercropping or mixed cropping system. Chou (1999) has discussed various combinations that have been practiced in intercropping such as: Pasture and forest intercropping and cover grass and orchard trees.

1.2.6.5. Breeding the crops for allelopathic traits

Crop plants showing allelopathic potential should be screened for the same, across different cultivars if possible. As such cultivated plant species vary in their allelopathic traits among different cultivars and such traits may be seen genetically correlated, for example alfalfa (*Medicago sativa* L.) (Khanh et al. 2005). The cultivars showing best and maximum allelopathic potential should be selected for their economic application. Hoult and Lovett (1993) have reported several wild accessions of the modern day crop plant such as barley (*Hordeum vulgare*) to possess the allelopathic traits that can inhibit both weeds and pests.

An excellent example is that of rice allelopathy. Olofsdotter et al. (2002) in an extensive review have reported the possibilities of utilizing the allelopathic rice (*Oryza sativa*) cultivar to improve its competitive ability against weeds. In the field experiments they found allelopathy to account for 34% of overall competitive ability in rice and found allelopathy as a dominant factor that determining the same. Based on the rice cultivars screening results, recombinant inbreed line populations were developed for identification of quantitative trait loci (QTL) controlling the allelopathy in rice. Recombinant inbred lines (RILs) population was derived from single-seed descent from crosses between varieties with contrasting behaviour and eventually QTL controlling the allelopathy were identified. Their findings suggest that it is possible to improve allelopathy in rice using marker-assisted selection. Kato-Noguchi (2003, 2004, 2005, 2010) briefly discusses progress in research that has been conducted for the selection of allelopathic rice strains and the identification of allelochemicals like momilactone B in rice. Number of rice varieties, have been screened for their allelopathic potential and many of them have been found to be allelopathic to several plant species. Analysis of rational for allelopathic potential of rice led to a result that, the plant root releases a chemical in to its surrounding that is lethal to the neighbouring plants. Structural analysis of the chemical led to it identification as momilactone B. Its release in rice root exudates is sufficient enough to impose inhibitory action on other neighbouring plants. Later he screened allelopathic potential of eight varieties of rice on co-cultured *Echinochloa crusgalli* in a bioassay medium. They found all rice varieties to inhibit the shoot and root growth in *Echinochloa crusgalli* and found the compounds Momilactone A and B in the bioassay medium indicating the secretion of the same from all rice varieties and inhibitory action of the same. He also reports that the allelopathic activity of rice varieties depend mainly on the secretion level of momilactone B and the compound plays an important role in the rice allelopathy.

According to Weston (1996) the germplasm of the allelopathic traits of a species can be valued for application as a weed-suppressive cultivars or such a cultivar can be included as a rotational crop, intercrop or cover crop so as to achieve effective weed suppression. Further the cultivars with superior allelopathic traits should be selected for further analysis. Such a cultivar should be analysed for the molecular and associated genetic trait conferring benefits of allelopathic potential to the selected cultivar. Using certain genetically advanced tools such as polymerase chain reaction, random amplified polymorphic DNA, restriction fragment length polymorphism, near-isogenic lines or cloning of genes the genetic investigation of allelopathic cultivar can be achieved (Tesio and Ferrero 2010). Once the detailed genetic study on allelopathic trait in the species is achieved the trait should be attempted for breeding into cultivars lacking the particular trait.

1.2.6.6. Natural Herbicide

Every component of the nature has its own mannerism to encounter the coming challenges. Plants too, have their own unique way to do so. They face varied type and level of stress imparted by some abiotic factors like light, water, nutrients or those imparted by the biotic factors like pest, bacteria, virus, fungi, nematodes and most importantly the competing plant species. Plants sometimes manifest exclusive tendencies or adaptations to combat different stress. Considering the biotic stress, plants deal with the pest, bacteria, virus, fungi, nematodes or the competing plant species, using unique defence systems. Allelochemicals are among such chemical weapon that the plants use to fight, suppress or exclude the other competing plant species. These allelochemicals are secondary metabolites naturally produced or induced in an allelopathic plant. Allelochemicals or the plant defence chemicals are the natural products that may provide clues to the discovery of commercial herbicides as the mode of action of some of the allelochemicals is similar to the synthetic herbicides (Soltys et al. 2013).

In contrast to the available herbicides being based on only a few mechanism of action the natural compound may have a new mechanism of action with an affinity towards a novel target site (Koch et al. 2005). Thus natural product, lead the discovery of herbicides that has new target sites. The natural compounds based herbicide may have an added advantage owing to its molecular structure. The chemical structure of natural chemicals is more environmentally friendly than synthetic ones (Soltys et al. 2013). According to Henkel et al. (1999) natural products are oxygen and nitrogen rich molecules that possess more stereogenic centers and sp3-hybridized carbons than commercially available synthetic compounds. They may also contain sulphate or phosphate groups where halogenation is uncommon. Dayan (2012) in the review, suggest that the significant advantage of natural product derived herbicide is, absence of unnatural ring structure and low or no amount of heavy metals. He further adds that such chemistry is common in nature and is easily accessible for degradation and decomposition by various biotic and abiotic factors. However the advantages of herbicides derived from natural products allow their uncontroversial development as a successful herbicide.

If the plant metabolites acting as an allelochemicals or natural herbicide are intended for their commercialization, they may be directly employed for weed control or used after incorporating certain modifications in them. However instance of a plant metabolite directly extracted from plant and utilized as commercial herbicide or its precursor do not exist. Instead there exist examples of herbicides precursor that is inspired from natural metabolite. Agronomic or commercial utilization of the natural metabolite as herbicide or herbicide precursor depends on economy of its production and that is also a reason why till the date no natural plant product has been directly commercialized as herbicide. Many natural products are under investigation for their direct or indirect utility as a commercial herbicide (Putnam 1988; Vyvyan 2002; Dayan Et al. 2010; Dayan et al. 2012). Rizvi et al. (1987) reports an allelochemical from seeds of *Coffea arabica*, that is 1,3,7–trimethylxanthine to be allelopathic and its utility as herbicide, Vaughan and Spencer (1993) reviewed importance and potential of volatile monoterpenes as a lead to new herbicides, Brown and Morra (1995) reports the use of plant tissues containing glucosinolate as bioherbicides and Batish et al.

(2007) have reported the use of dried powder from *Tagetes minuta* as a natural herbicide.

An example of a commercial herbicide containing a natural product moiety is cinmethylin. The portion of the herbicide molecule is cineole which is a terpene found in high concentrations in certain plants especially those found in desert. In future, If extraction of such compound from the plant itself is economic, then this will greatly revolutionize production of herbicides and the plants may be genetically manipulated for such traits for higher yields (Putnam 1988).

Moreover for the effective mitigation of weed in different eco-friendly agriculture system all the above discussed applications of allelopathy can be employed singly or in combination of two or more.

1.3. Medicinal plants: Allelopathy and cultivation

Medicinal plants are treasure houses of variety of chemical compounds and of special interest are their secondary metabolites. Secondary metabolites in a plant play a major role in the survival of the plant in its environment and may be institutional in attraction of pollinators, renders defence against pest, herbivores, diseases etc (Verpoorte et al. 2002). Plants produce secondary metabolites with a purpose. Allelopathy as is an ecological interaction specially mediated by the plant secondary metabolites and the medicinal plant being rich sources of secondary metabolites, screening allelopathic plants from the medicinally important plants is fairly promising (Patel and Pandya 2013). Some medicinal plants with particular secondary metabolites may offer phytotoxic inhibition to other plants and should be exploited for their allelopathic potential.

Importantly, large number of the medicinal plants have pharmaceutical value. The pharmaceutical demand of medicinal plant supply is met through different sources. Ved and Goraya (2007) have given a report on the sources of medicinal plant supply and the contribution of each source. According to the report there are about 960 medicinal plant species being under trade, out of which about 178 species are consumed in volumes exceeding 100 metric tonne per year and this 178 species (consolidated consumption) account for about 80% of the total industrial demand of all botanicals in the country. Information on major sources of supply of these 178 species depicts that 21 species (12%) are obtained from temperate forests, 70 species (40%) are obtained from tropical forests, 36 species (20%) are obtained largely or wholly from cultivations/plantations, 46 species (25%) are obtained largely from road sides other degraded land use elements and remaining 5 species (3%) are imported from other countries. This data given by Ved and Goraya (2007) clearly reveal the pressure of medicinal plant supply on natural resources in contrast to demands fulfilled through cultivation or plantation. This observed pressure on natural resources is owing to lesser cultivation of medicinal plants due to the low economic gains and small or no accessible market at all. Thus increasing the gains of medicinal plants cultivation may lure their cultivation. If the medicinal plant cultivation also helps weed eradication from the agriculturel, the practice of such cultivation may provide dual benefits i.e. medicinal plant crop and weed mitigation. This may be in a way possible if the medicinal plant in addition to medicinal values also has allelopathic potential. Proven allelopathic, medicinal plant may be analysed for the allelopathic metabolite which through extensive research may lead a new herbicide discovery possibly with new or existing target sites. Allelopathic potential of medicinal plant may thus promote their commercial large scale cultivation, provide an additive advantage in terms of weed mitigation and may lead to discovery of a new herbicide with a novel mode of action.

Little information is available on allelopathic potential of the medicinal plants and only few studies satisfying the motive exist till the date. In a series of experiments, Fujii et al. (1990, 1991, 2003) have screened some around 387 medicinal plants occurring in Japan for their allelopathic potential. They conducted the allelopathic bioassay studies of medicinal plants in laboratory conditions using water and methanol based extracts. They have also introduced an agar based bioassay method termed as Sandwich method. Their results indicate many of these medicinal plants to possess allelopathic potential and they suggest that these plants are promising for further research in the area. Xuan et al. (2004) have conducted allelopathic studies on neem (*Azadirachta indica*. A. Juss) and found it, to strongly inhibit germination and growth of several specific crops such as alfalfa (*Medicago sativa* L.), bean (*Vigna angularis*), carrot (*Daucus carota* L.), radish (*Raphanus sativus* L.), rice (*Oryza sativa* L.), sesame (*Sesamum indicum* L.) and weeds such as *Echinochloa crus-galli*, *Monochoria vaginalis*, and *Aeschynomene indica* L. in a bioassay and in soil. The neem bark was more, inhibitory than the leaves and six phenolic compounds were isolated and identified in both neem bark and leaves. In a preliminary survey, (Khanh et al. 2005) analysed four common medicinal and two leguminous plant species from Southeast Asia for their potential use in paddy weed control. All the plant species show allelopathic potential, *Nerium oleander* being the strongest of all. Batish et al. (2007) analysed the allelopathic effect of leaf and root powder mulch, of aromatic medicinal plant *Anisomeles indica* against weeds found in wheat crop. The applied mulch reduced emergence of the weeds found among wheat crop similar to herbicides without any effect on the wheat. They also observed enhancement of grain yield in wheat upon the mulch treatment. Nazir et al. (2007) studied allelopathic behaviour of aqueous extracts of three medicinal herbs viz.

Rheum emodi, Saussaurea lappa and Potentilla fulgens on some traditional food crops. Khan et al. (2009) studied the allelopathic potential of medicinal plants from the Himalaya and Hindukush ranges of Pakistan. The leaf leachet's from *Inula falconeri, Inula koelzii, Lactuca dissecta* and *Anthemis nobilis* were analysed using sandwich and homogenated sandwich methods and were found to be inhibitory. Sodaeizadeha et al. (2010) studied the effect of *Peganum harmala* L. residues on the seedling growth of *Avena fatua* L. and *Convolvulus arvensis* L. The results from their study suggest that the plant can be used for natural weed mitigation. Gulzar and Siddiqui (2013) evaluated allelopathic effect of the aqueous extract of medicinally important bark of *Terminalia arjuna* on a weed *Cassia sophera* under laboratory conditions and found it allelopathic. Upadhyay et al. (2011) reviews importance of two medicinal plants i.e. *Asparagus racemosus* Willd. and *Andrographis paniculata* (Burm.f.) Nees for the weed mitigation through integrated weed management. Chandra et al. (2012) analysed the aqueous alcoholic extracts of *Withania somnifera* on germination and radical growth of *Cicer arietinum* and *Triticum aestivum* seeds. The extract affected the germination and radicle elongation of both *C. arietinum* and *T. aestivum* in a concentration dependent manner. The allelopathic effect of ashwagandha was attributed to the alkaloid and withanolide present in the plant. Mominul Islam and Kato-Noguchi (2012) analysed the aqueous methanol extracts of *Leucas aspera* (Willd.) Linn., against seven test plant species, namely *Lepidum sativum* L., *Medicago sativa* L., *Lactuca sativa* L., *Lolium multiflorum* Lam., *Echinochloa crusgalli* L., *Echinochloa colonum* L. Link and *Phleum pratense* L. The aqueous methanol extract were found to inhibit the seedlings growth of all test plant species and the inhibitory activity was found to be concentration dependent. They suggest the plant to be potential candidate for isolation and identification of allelochemicals as the herbicidal lead. Pukclai and Kato-Noguchi (2012) studied the aqueous methanol extracts of the medicinal plant, *Tinospora tuberculata* Beumee and found it to inhibited the growth of roots and shoots of *Lepidum sativum* L., *Lactuca sativa* L., *Medicago sativa* L., *Phleum pratense* L., *Lolium multiflorum* Lam., *Lolium rigidum* Gaud., *Digitaria sanguinalis* L., *Eriogonum compositum* Douglas ex Benth., *Leptochloa chinensis* [L.] Nees., *Echinochloa colona* [L.] Link., *Echinochloa crus-galli* [L.] Beauv. and *Festuca myuros* L. Mandal et al. (2013) studied the allelopathic effect of *Rauwolfia tetraphylla* L. on germination, exaggeration of seedling and biochemical actions in *Cicer arietinum* L. seed. They found medium concentrations of plant roots aqueous extract to impart stimulatory effect on the parameters analysed such as seed germination, growth and biochemical constituents (total sugar, protein, amino acid and DNA and RNA concentrations) of gram with higher concentration extracts showing more or less stagnant effect. Seedling parameters in treatments with all the root extracts showed variation, wherein it was better than control. They suggest the root aqueous extracts to be potential enough to be stimulatory and propose its consideration for an invigoration treatment under seed/crop production programme. Mominul Islam and Kato-Noguchi (2013) conducted allelopathic study using five medicinal plants i.e. *Leucas aspera* L., *Leonurus sibiricus* L., *Ocimum tenuiflorum* L., *Mentha sylvestris* L. and *Hyptis suaveolens* L. against barnyard grass. Of all the five studied plants, *L. aspera* and *H. suaveolens* were found to possess strong allelopathic potential and they suggested use of these plants for natural control of barnyard grass. Kakati and Baruah (2013) studied allelopathic effect of aqueous leaf extract of medicinal plant species namely *Azadirachta indica* A. Juss, *Murraya koenigii* (Linn.) Spreng and *Paederia foetida* Linn. on seed germination and seedling growth of *Vigna radiata* (L.) Wilczek. They found the extracts to be inhibitory in concentration dependant manner. Fanaei et al. (2013) evaluated the allelopathic effects of *Ocimum basilicum* extract and its essence on chlorophyll content of *Abutilon theopharasti, Chenopodium album* and *Centaurea depressa* and the plant was found to affect the chlorophyll content. Mominul Islam and Kato-Noguchi1 (2013) conducted the study to explore the allelopathic potential of the aqueous methanol extracts from the medicinal plant *Hyptis suaveolens* Poit, on the seedling growth of eight test plant species *Lepidum sativum* L., *Lactuca sativa* L., *Medicago sativa* L., *Brassica napus* L., *Phleum pratense* L., *Digitaria sanguinalis* L. scop., *Echinochloa crus-galli* L. and *Lolium multiflorum* Lam. and on the germination of *Lepidum sativum* L. and *Lolium multiflorum* Lam. The extracts from plant inhibited growth of all the test plants. Patel and Pandya (2013) studied the phytotoxic effect of aqueous extract from medicinal plants, such as *Boerhaavia diffusa* L., *Aerva lanata* (Linn.) Juss. ex Schult, *Acalypha indica* L., *Synedrella nodiflora* (L.) *Gaertn.* on the growth of radish (*Raphanus sativus* L.). *Boerhaavia diffusa* L., *Acalypha indica* L. and *Synedrella nodiflora* (L.) Gaertn offered varied level of inhibition on growth of radish. Bidarnamani et al. (2015) have analysed the allelopathic effect of essential oils from the medicinal plants *Artemisia annua* L., *Rosmarinus officinalis* L. and *Lavandula vera* L. on the weed *Cynodon dactylon* L. The essential oils were found to be inhibitory to the growth of weed and suggest to be used as herbicide. Thus, from the researches and associated results concerning the medicinal plant allelopathy it is clearly indicative that the medicinal plants are worth for the future allelopathic exploration.

1.4. Current trends in allelopathic research and associated challenges or lacunae

According to Inderjit et al. (2011), the allelopathic interaction takes place under most natural conditions and quantifying various aspects of how ecosystem factors influence allelopathy is the key to a better understanding of how plants interact with each other

Contemporary researches in the field of allelopathy are aimed at identifying the allelopathic plant, screening it for allelopathic potential, isolating, identifying and quantifying the responsible active allelochemical, evaluating its mechanism of action and to very little extent the real time static concentrations and dynamics of the potent allelochemicals required for inhibition of the said plant. Hence for better understanding the allelopathic studies require in-depth knowledge of one or more of the plant science related disciplines such as ecology, biochemistry, chemistry (natural product isolation and synthesis), plant physiology (including mode-of-action studies), technical agriculture, forestry, genetic breeding, soil studies, metabolomics proteomics and genomics (Macias et al. 2007).

In spite of the enormous allelopathic research that is being currently taken-up, understanding and acceptance of the fact that phenomenon of allelopathy do exist, is still controversial. The available information on allelopathy is not sufficient enough to rationalize role and importance of allelopathy in certain observed instances as in case of success stories of exotic plant invasions.

The success of exotic invasion by a foreign plant is partly attributed to the allelopathic potential and the chemical weapons that the plants utilize against species in the invaded habitat (Callaway and Ridenour 2004). The allelochemicals released by the invading plant in to the invaded habitat soil, have been isolated and identified. Allelopathic activity of such isolated allelochemical have been studied individually but this do not imply that the same compound, in the same form is present in soil in the concentration that is enough to be absorbed and that it is even being absorbed by the plant. Though many researchers have attempted, the information on exact allelopathic metabolite from an entire pull of metabolites released by any allelopathic plant, its structural stability, biologically important structure of that metabolite, biological effective concentrations, absorption and translocation in another plant and exact mechanism of allelopathic action inside the absorbing plant is not being much worked-out or is vaguely explained.

Another is allelopathic potential of agronomically important or forest tree species. It is wrongly demonstrated as a sole causal interference observed for certain crop cultivars as many of the processes such as resource competition, nutrient immobilization, microbial influence etc which may operate parallel to allelopathy are ignored (Bhowmik and Inderjit 2003). Only few researches pertaining to in-field allelopathic potential of a crop cultivar has been conducted so far, which have also accounted the resource competition that is offered by an allelopathic crop. Thus there is a need for separating allelopathy from other mechanism like competition, microbial interaction, edaphic characteristics and environmental effect in order to establish allelopathy of crops.

Discussing problems related to establishing the phenomenon of allelopathy Inderjit and Nilsen (2003) reported that allelochemical quantification, selection of the concentration of allelochemicals in bioassays, interaction between allelochemicals and other substances and *in situ* allelochemical bioassays to be of major importance are yet to be addressed. According to them the main concern of most researches is the difficulty in showing the causal-effect correspondence between the reputed allelopathic agents and any ecological effect related with plant distribution and/or predominance. Allelopathic interaction as are mediated through soil they are liable to the alterations owing to the biotic and abiotic factors existing in the natural soils the establishment of so called conditionality. Inderjit and Callaway (2003) suggest that the term ‘allelopathic plant’ hence is misleading unless this conditionality is being addressed.

Difficulty in screening the allelopathic metabolite or allelochemicals for its potential use as a commercial herbicide, is that in the unnatural conditions (as that in laboratory or pots) an isolated metabolite from allelopathic plant may appear toxic to the studied test plant however its effect may not be same in natural condition and using it as a herbicide may not be very feasible. Thus there are certain concerns for the applicability of a natural plant allelochemical as herbicide. High cost, limited activity and selectivity restrict the use of natural herbicides (Bhowmik and Inderjit 2003). Natural herbicide may also be toxic to non-target organisms and this toxicity to non-target species which mostly is crop, is one of the main reasons which limit the use of natural compounds as herbicides (Bhowmik and Inderjit 2003). Very few studies have been done where attention is given to find out a dose response, i.e. suppressive to weed growth but ineffective to the crop growth. Purohit and Pandya (2013) analysed the allelopathic effect of *Ocimum sanctum* L. and *Tephrosia purpurea* (L.) Pers. They studied the allelopathic effect of aqueous medicinal plant leaf extracts on the legumes crops like *Phaseolus radiata* (L.)Wilczek), *Phaseolus unguiculata* (L.)Walp), *Cajanus cajan* L., *Cicer arietinum* L., *Phaseolus mungo* (L.) Heeper, *Phaseolus aconitifolius* Jacq. and on a weeds i.e. *Dichanthium annulatum* L., *Chloris barbata* L., *Acalypha indica* L .and *Amaranthus spinosus* L. *T purpurea* was found more inhibitory to the weeds. Results were dose responsive where certain concentration was inhibitory to the weed but not to the crop. Bhowmik and Inderjit (2003) review the concentration of natural product with that of herbicide that is required for its herbicidal activity. He suggest that the concentrations used to test the allelochemicals activity range from 10^{-4} to 10^{-7} M and considering the environmental safety a natural herbicide to be a good herbicide, should have activity between 10^{-5} and 10^{-7} M. He reports that natural secondary metabolite such as phenolic compounds, alkaloids and quinones have a stipulated activity range such as $10^{-2} – 10^{-5}$ M and thus in natural conditions they are poor candidates to be used as natural herbicides. Thus there is a need to identify plant allelochemical metabolites which are effective in even the stipulate concentration range equivalent to or similar to any of the herbicides. Bhowmik and Inderjit (2003) suggest that limited environmental stability of a natural product, molecular complexity in addition to above covered points limit the scope of any developmental process by industries.

Adding to the above mentioned challenges and important lacunae is the petite research on extending the laboratory studies to the field. Most studies evaluate the bioassay studies in petri-plates or pots in the laboratory, green houses or not so natural conditions. However, for the preliminary analysis laboratory bioassays provide a best means to study allelopathy of a plant, as the selected parameters important for the study are controlled for experimental purpose. Although the interaction that occur in nature outside the laboratory or a green house are more complex and vivid. Thus the preliminary experiments conducted in unnatural conditions should be extended to natural conditions. This kind of research will avail better understanding of allelopathy in nature and will be of great application value. Advancement in the development of sophisticated high accuracy techniques addressing all the aspects of allelopathy has revolutionized the approach of allelopathic analysis.

1.5. Research objectives:

Owing to the literature review and realizing the potential of medicinal plants as source of natural herbicides, the present study was designed. To fill the lacunae of isolated researches, an integrated approach was adapted, where the study was designed by incorporating wide range of parameters and aspects needed to understand the mechanism of allelopathy as much as possible. The study includes array of objectives including a wide screening to elaborative cellular studies. The objectives are:

- Screening and identifying medicinal plants with allelopathic potential through field observations and through bioassay.
    - Segregating the plants for further analysis, based on the potential part (leaves / stem / root)
    - Isolation, Screening and confirmation of pharmacologically active fractions of the potent plant parts (leaves/ stem/ root) extract for their allelopathic potential, through bioassay.
- Conducting the rhizospheric soil analysis of the plant species for studying dissipation of allelochemicals, in the case where the roots are potential part.
- Studying some indicative cytological and biochemical parameters as a response of the receiver plants.

1.6. Rationale: Allelopathic potential of plants is a promising natural phenomenon that can be explored for eco-friendly management of the weeds. Medicinal plants are treasure houses of pharmacologically active secondary metabolites and so are likely to stand out as potential source of allelopathic molecules. With small scale cultivation of the medicinal plants and increasing pressure on collection of the same from the wild, identifying allelopathic potential of the medicinal plant will impart value addition to cultivation and encourage a large scale cultivation of the medicinal plants. Allelopathic potential of medicinal plant can be utilized by cultivating it as a cover crop, rotation crop or as inter crop with the main crop. Allelopathic plant material can also be used in form of mulch or extracts alone or as an addition to the herbicides, reducing the requirement of environmentally lethal doses of herbicides. Proven allelopathic, possible chemical fraction from the identified medicinal plants can be evaluated for its allelopathic potential which may direct a lead to discovery of an ecofriendly herbicidal alternative. Analysing the biochemical and cytological parameters in the test plant that are being affected by the medicinal plant treatment may help identifying the possible mechanism of action for that allelopathic plant. Also natural release of allelochemicals occurs by a variety of mechanisms such as volatile emission, leaching from leaves, decomposition of the plant parts and exudation from roots. Thus for utilization of allelopathic potential of medicinal plant, natural dynamics of allelochemicals if any, is a challenging task to be explored. Evaluation of allelochemicals from root exudates in natural conditions can be made possible by conducting rhizosphere analysis of allelopathic plant which would aid deeper understanding of allelopathic applications. Eighteen medicinal plants were selected for evaluation of their allelopathic potential at a primary level.

A brief introduction of the selected medicinal plants and test plants has been given in the appendix I. The medicinal plants have been abbreviated for the ease of reference and the short forms of the same are given in appendix II.