

# **CYTOLOGICAL INVESTIGATION OF THE ALLELOPATHIC DOMINANCE OF THE WEEDS OVER THE CROP PLANTS IN PUDUCHERRY**

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# CONTENTS

|                                    |     |    |
|------------------------------------|-----|----|
| INTRODUCTION                       | ... | 1  |
| REVIEW OF LITERATURE               | ... | 4  |
| ALLELOPATHY AND CYTOTOXICITY       | ... | 6  |
| <i>PARTHENIUM HYSTEROPHORUS</i> L. | ... | 21 |
| <i>LANTANA CAMARA</i> L.           | ... | 28 |
| MATERIALS AND METHODS              | ... | 36 |
| OBSERVATIONS                       | ... | 42 |
| RESULTS AT A GLANCE                | ... | 52 |
| DISCUSSION                         | ... | 55 |
| SUMMARY                            | ... | 64 |
| REFERENCES                         | ... | 67 |
| PUBLICATIONS                       | ... | 83 |

# INTRODUCTION

The interference in the growth of one plant by another can result either from competition which involves the removal of some factors (nutrient, water and light) from the environment, habitat or through chemicals released from one plant (donor) that affect the other (receiver) sharing the habitat. The phenomenon known as “allelopathy” is now considered as important as competition for influencing plant growth both in natural and agricultural ecosystem. In natural or man managed agro-ecosystems, neighbouring plants may interact with the growth and development of other species. The term allelopathy signifies the interaction or inhibition of growth (Molish 1937) of both crop and weed species, by the release of chemicals from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes. These interactions are widely known in different groups of plants such as algae, lichens, crops, as well as annual and perennial weeds (Rice 1984, Putnam 1985, Horseley 1991, Lawrey 1993, Inderjit and Dakshini 1994 a, b; Rajendiran 2000a, Rajendiran 2004, Bhakat *et al.* 2005, Inderjit 2005).

There is much evidence that allelochemicals liberated from certain weeds into the soil reduce crop growth (Rice 1979, Putnam and Weston 1986, Bhakat *et al.* 2002, Bhakat *et al.* 2005a, Bhakat *et al.* 2005b, Bhakat *et al.* 2006, Bhakat *et al.* 2007, Bhattacharjee *et al.* 2003, Kanp *et al.* 2004). Approximately 6,700 species, out of about 3,000,000 species of the flowering plants are recorded as weeds in agro-ecosystems of the world (Holm *et al.* 1979). Of these, 76 weed species are categorized as “the World’s Worst Weeds” (Holm *et al.* 1977). Only 15 species of the crops which supply 90% of the world’s food occupy 75% of the world’s tilled land (Harlam 1975). Most of the food species belong to five families *viz*, Poaceae, Solanaceae, Convolvulaceae, Euphorbiaceae and Fabaceae and these families also include most of the common weeds. In crop subsystems of agro-ecosystem, crop species (often exotic) are selectively cultivated but weeds grow themselves in crop fields and interact with the crop species in various ways, including reduction in crop yields.

Allelopathy is also an expression of the ecological phenomena which are normal constituents of the environment of the terrestrial plants (Rice 1984). There are some common indices for assessing allelopathic action of plants or plant parts. These include, germination behaviour and other physio-biochemical responses of test species (Bhattacharjee *et al.* 2001, Bhattacharjee *et al.* 2003, Bhakat *et al.* 2002, Bhakat *et al.* 2005, Bhakat *et al.* 2006, Bhakat *et al.* 2007). The dangerous part of allelopathic action is the cytotoxicity of the weed allelochemicals on the surrounding plant species (Rajendiran 1996, Rajendiran 1998a, Rajendiran 1998b, Rajendiran 1999a, Rajendiran 1999b, Rajendiran 1999c, Rajendiran, K. 2000b, Rajendiran 2000c).

*Parthenium hysterophorus* L. (Asteraceae) and *Lantana camara* L. (Verbenaceae) are noxious exotic weeds, spreading rapidly through the country. Its rapid growth has been attributed mainly due to its ability to germinate fast and to inhibit growth of other associated plant species. Moreover, the presence of inhibitory chemicals (allelochemicals) in these weeds has contributed towards their acquisition of dominant status even among crop plants in various areas in Pondicherry. Keeping in view of the extraordinary spread, dominance and their naturalisation in Pondicherry in a short time, these weeds were taken for this study to obtain qualitative as well as quantitative data of the cytotoxic effects of aqueous extracts of the root, stem, leaf and

inflorescence of *Parthenium hysterophorus* L. on the root tip cells of an important food crop rice (*Oryza sativa* L. var. ASD-16), a vegetable crop cucumber (*Cucumis sativus* L. var. Sambar) and an ornamental type of sunflower (*Helianthus annuus* L. var. Tall); and root, stem, leaf, flower and fruit extracts of *Lantana camara* L. on the root tip cells of a large grain cereal maize (*Zea mays* L. var. Cauvery-244), a tropical dual-purpose legume cowpea (*Vigna unguiculata* (L) Walp. cv. BCP-25) and an important grain legume blackgram (*Vigna mungo* L. var. Vamban-16).



# REVIEW OF LITERATURE

Sometimes an individual plant can have a depressive effect on its neighbours. It is therefore more common that a neighbouring plant will interact in a negative manner, where the emergence or growth of one or both is inhibited. Muller (1969) described the adverse effect of a neighbouring plant in association with others and defined it as interference. According to Szczepanski (1977) the potential causes of interference include:

- **Allelopathy** (competition)  
The depletion of one or more resources required for growth.
- **Allelopathy**  
The addition of chemical toxins by one or more species.
- **Allelomediation**  
The selective harbouring of a herbivore that might selectively feed on one species, thus lending to the advantage of another.

Interference refers, therefore, to the overall effect of one plant upon another and encompasses both allelopathy and competition. Competition involves the removal or diminution of a shared resource, while allelopathy involves the addition of a chemical compound to the environment through different processes (Rice 1984, Putnam 1985). Confusion has occurred because some consider allelopathy to be part of competition. In addition, competition has been misused by many to describe interference. It is a specific mechanism for interference, but not the end result.

In a historical overview, Willis (1985) pointed out that allelopathy is not a new concept. Theophrastus in 300 BC first noticed the deleterious effect of cabbage on vine and suggested that it is due to odours. A common problem in both Greek and Roman times was the so-called soil sickness, the declining yields of fields. They did not understand that the condition could be caused by various factors such as mineral deficiencies, toxin accumulation, pathogens and the imbalance of micro-organisms. In the seventeenth and eighteenth centuries, botanists relied strongly on a comparative approach. They compared both plant form and function, particularly in relation to nutrition. The Dutchman, Boerhoove suggested that root exudation may play a role in plants. Stephen Hales believed that root exudates facilitated excretion of used compounds. The theory of root excretions was a basis for the concept of allelopathy. Swiss Botanist Auguste Pyrame de Candolle developed the “plant interaction theory” via root excretions. He was influenced by the increasing information on phytochemistry and the effects of diverse compounds on plant growth. Interest in the concept of allelopathy was rekindled at the close of the nineteenth century, principally for two reasons. The first was that careful agricultural experiments yielded results that could not adequately be explained by the exhaustion of soil nutrients. Secondly, improved techniques in chemistry allowed organic toxins to be identified from unproductive soils.

# ALLELOPATHY AND CYTOTOXICITY

## **Allelopathy**

The allelopathic effect of one plant upon another is so striking that competition for a common resource does not seem adequate to explain the observation. In organism communities, many species appear to regulate one another through the production and release of chemical attractants, stimulators or inhibitors (Putnam and Tang 1986).

## **Definition**

Allelopathy is derived from the Greek words *allelon* “of each other” and *pathos* “to suffer” (Rizvi *et al.* 1992). It therefore translates literally as “mutual suffering”. Allelopathy is described as the beneficial and deleterious biochemical interaction between plants and micro-organisms. Rice (1974) defines allelopathy as any direct or indirect effect by one plant, including micro-organisms, on another through the production of chemical compounds that escape into the environment and

subsequently influence the growth and development of neighbouring plants. It includes both inhibitory and stimulative reciprocal biochemical interactions. The use of the term "allelopathy" may therefore be somewhat controversial. Chemicals found to inhibit the growth of a species at a certain concentration may stimulate the growth of the same species or another at a lower concentration (Rice 1984, Putnam and Tang, 1986). Aldrich (1984) describes two types of allelopathy:

- **True type**

The release into the environment of compounds that are toxic in the form in which they are produced.

- **Functional type**

The release into the environment of a substance that is toxic as the result of transformation by micro-organisms.

### **Proof of allelopathy**

Many field studies implicate allelopathy, but isolation and identification of the chemical agents require a rigorous laboratory effort (Putnam and Tang 1986). It is extremely difficult to prove that any deleterious effect is due to allelopathy rather than to competition for essential products. Numerous studies have provided evidence, but seldom has a specific protocol been followed to achieve convincing proof (Putnam and Tang 1986). These authors pointed out that the shortcomings of the discipline make it hard to differentiate between allelopathy and competition. These include:

- A general lack of nomenclature to adequately describe the plant responses that occur in this manner.
- A dearth of techniques to separate allelopathic interactions from competition.
- A failure to prove the existence of direct compared with indirect influences via other organisms / micro-environmental modification.

A considerable body of information has accumulated implicating allelopathy as an important form of plant interference. According to Willis (1985), Putnam and Tang (1986) and Cheng (1992), the methodology dictates certain points for allelopathic research to be established to suggest that it is operative:

- A pattern of inhibition of one species by another must be shown using suitable controls, describing the symptoms and quantitative growth reduction.

- The putative aggressor plant must produce a toxin.
- There must be a mode of toxin release from the plant to the environment and thus the target plant.
- Mode of toxin transport or accumulation in the environment must be evident.
- The afflicted plant must have some means of toxin uptake, be exposed to the chemical in sufficient quantities and time to cause damage, and to notice similar symptoms.
- The observed pattern of inhibition should not be explained solely by physical factors or other biotic factors, especially competition.

It is important to stress that the above points do not prove that allelopathy is operative, only that it offers the most reasonable explanation for the observed pattern. According to Cheng (1992), once the chemical enters the environment, a number of interacting processes will take place. These processes have been identified as:

- **Retention**

The retarded movement of the chemical from one location to another, through soil, water and air.

- **Transformation**

The change in form or structure of the chemical, leading to partial change or total decomposition of the molecule.

- **Transport**

Defines how the chemicals move in the environment.

Cheng (1992) pointed out that these processes are influenced by the nature of the chemical, the organisms present, the properties of the soil, and environmental conditions. The fate of the chemicals depends on the kinetics and interactions of individual processes with time, at a particular site under a particular set of conditions.

### **Allelochemicals**

According to Putnam and Tang (1986) all alleged cases of allelopathy that have been studied appear to involve a complex of chemicals. No single phytotoxin was solely responsible for or produced as a result of interference by a neighbouring plant. Rizvi *et al.* (1992) pointed out that the subject not only deals with the gross

biochemical interactions and their effects on the physiological processes but also with the mechanism of action of allelochemicals at specific sites of action at the molecular level. Few studies on allelopathy concentrate on the mechanisms and processes involved in the production of allelochemicals. Einhellig (1987) and Putnam and Tang (1986) raised the question whether alleged biochemical agents were in sufficient concentrations and with enough persistence in the environment to affect a neighbouring or succeeding plant. These chemicals could be transformed during the course of extraction. According to Cheng (1992), allelopathic symptoms may not be manifested at the time or site where plant damage has actually occurred.

### **Sources of allelochemicals**

Radosevich and Holt (1984) stated that the primary effect of allelopathy seems to result from an association with plant litter in or on the soil. Rice (1984) and Putnam (1985) reported that allelochemicals are present in virtually all plant tissue, i.e. leaves, fruit, stems, and roots. These allelochemicals are released by such processes as volatilization, root exudation, leaching and decomposition of plant residues. Leaves may be the most consistent source, while roots are considered to contain fewer and less potent toxins. According to Aldrich (1984), allelochemicals must be concentrated in the leaves, stem or roots rather than in the fruit or flowers. If it is concentrated in these organs it is unlikely that it could be available in time to interfere with neighbouring plants. According to Rice (1984) and Putnam (1985), there are four ways in which the chemicals are released (Plate 1):

- **Volatilization**

Release into the atmosphere. It is only significant under arid or semi-arid conditions. The compounds may be absorbed in vapour by surrounding plants, be absorbed from condensate in dew or may reach the soil and be taken up by the roots.

- **Leaching**

Rainfall, dew or irrigation may leach the chemicals from the aerial parts of plants that are subsequently deposited on other plants or on the soil. Leaching may also occur through plant residues. Their solubility will affect their mobility in soil water.

- **Root exudation**

From plant roots into the soil environment. These compounds are actively exuded, leaked or arise from dead cells sloughing off the roots.

- **Decomposition of plant residues**

It is difficult to determine whether toxic substances are contained in residues and simply released upon decomposition, or produced instead by micro-organisms utilizing the residues.

### **Natural products identified as allelopathic agents**

Alleged allelochemicals represent a myriad of chemical compounds from simple hydrocarbons and aliphatic acids to complex poly-cyclic structures. The secondary products could be classified in the following categories but it is impossible to enumerate each and every chemical identified as an allelochemical. Whittekar and Feeney (1971), Rice (1984) and Putnam and Tang (1986) divided allelochemicals into various major chemical groups:

- Simple water-soluble organic acids
- Simple unsaturated lactones
- Long-chain fatty acids and polyacetylenes
- Naphthoquinone, anthroquinones and complex quinones
- Simple phenols
- Benzoic acid and derivatives
- Cinnamic acid and derivatives
- Flavonoids
- Tannins
- Terpenoids and steroids
- Amino acids and polypeptides
- Alkaloids and cyanohydrins
- Sulphides and glucosides
- Purines and nucleotides
- Coumarins
- Thiocyanates
- Lactones
- Actogenins

## **Mode of action of allelochemicals**

Most of the allelochemicals are secondary metabolites and are produced as by-products of primary metabolic pathways (Rice 1984, Putnam and Tang 1986 and Rizvi *et al.* 1992). Secondary compounds have no physiological function essential for the maintenance of life (Aldrich 1984). Reports most frequently identified effects which are readily observed in the field or under controlled conditions. Delayed or inhibited germination and the stimulation or inhibition of root and shoot growth are often reported (Rizvi *et al.* 1992). The major difficulty is to separate secondary effects from primary causes. An important question that always remains is whether the inhibitor reaches the site in the plant in sufficient concentration to specifically influence that reaction and whether other processes may be affected more quickly. The mode of action of a chemical can broadly be divided into a direct and an indirect action (Rizvi *et al.* 1992). Effects through the alternation of soil properties, nutritional status and an altered population or activity of micro-organisms and nematodes represent the indirect action. The direct action involves the biochemical / physiological effects of allelochemicals on various important processes of plant growth and metabolism. Processes influenced by allelochemicals involve:

- **Mineral uptake**

Allelochemicals can alter the rate at which ions are absorbed by plants. A reduction in both macro and micronutrients are encountered in the presence of phenolic acids (Rice 1974).

- **Cytology and ultra structure**

A variety of allelochemicals have been shown to inhibit mitosis in plant root tip cells (Rice 1974, Rajendiran 1996, Rajendiran 1998a, Rajendiran 1998b, Rajendiran 1999a, Rajendiran 1999b, Rajendiran 1999c, Rajendiran 2000b, Rajendiran 2000c).

- **Phytohormones and balance**

The plant growth hormones indole acetic acid (IAA) and gibberellins (GA) regulate cell enlargement in plants. IAA is present in both active and inactive forms, and is inactivated by IAA-oxidase. IAA-oxidase is inhibited by various allelochemicals. Other inhibitors block GA-induced extension growth (Rice 1974).



- **Membranes and membrane permeability**

Many biological compounds exert their action through changes in permeability of membranes. Exudation of compounds from roots on root slices have been used as an index of permeability because plant membranes are difficult to study (Harper and Balke 1981).

- **Photosynthesis**

Photosynthetic inhibitors may be electron inhibitors or uncouplers, energy-transfer inhibitors, electron acceptors or a combination of the above (Einhellig and Rasmussen 1979, Patterson 1981).

- **Respiration**

Allelochemicals can stimulate or inhibit respiration, both of which can be harmful to the energy-producing process (Rice 1974).

- **Protein synthesis**

Studies utilizing radio-labelled  $C^{14}$  sugars or amino acids, and traced incorporation of the label into protein, found that allelochemicals inhibit protein synthesis (Rice 1974).

- **Specific enzyme activity**

Rice (1984) reported on a number of allelochemicals that inhibit the function of enzymes in the plant.

Under natural conditions the action of allelochemicals seems to revolve around a fine-tuned regulatory process in which many such compounds may act together on one or more of the above processes (Rizvi *et al.* 1992).

### **Methods for isolation, bioassay and identification**

The concept of allelopathy is still a matter of controversy (Aldrich 1984) and is plagued with methodological problems, particularly those of the distinguishing effects of allelopathy from those of competition (Willis 1985). Only a few investigations have separated the components of interference because of the complexity of the ecological phenomenon (Fuerst and Putnam 1983). The authors reported that evidence must be put forward before any attempt is made to determine the causes of interference. The symptoms will vary from the most obvious germination and mortality responses to the more subtle responses such as a reduction

in size, mass or number of organs. Therefore observations and results are largely descriptive rather than analytical and provide only circumstantial evidence for allelopathy, leaving room for explanations other than allelopathy. Care must be taken to exclude competition as a factor. Competition can be selectively eliminated by adding limiting resources.

The effects of allelopathy are manifested in the soil environment which provides a myriad of physical, chemical and biological processes that may interact with allelochemicals that could influence the study. It is impossible to prove that chemicals released by plants do not affect neighbouring plants. Harper (1977) proposed a rigorous protocol to search for the cause and effect. The cause-and-effect relationship cannot be established merely by observing the appearance of phytotoxic symptoms, on the one hand, and showing the presence of chemicals of demonstrated toxicity in the vicinity of an affected plant, on the other. According to Putnam and Tang (1986), most research activities on allelopathy were concentrated on apparent cases that were conspicuous under field conditions. Under controlled conditions, factors in competition may be segregated. It is possible to prove that chemical interactions are either totally or partially responsible for the interference observed. Since allelochemicals differ in terms of source and type, different methods have been devised for greenhouse and laboratory verification of their presence.

### **Extraction or leaching from plant tissue**

Plant leachates have been collected to support the presence of extracellular bio-active compounds. Isolation of a compound involves collection in an appropriate solvent or adsorbent. According to Putnam (1985), a commonly used extract solvent is water or aqueous methanol in which dried or living plant material is soaked. After extracting the material for varying lengths of time, the exuded material is usually filtered or centrifuged before bioassay. In other cases the material is macerated together with distilled water.

Putnam (1985) also pointed out that under field conditions leaching may be caused by dew, rain or irrigation. Leachates do not include intracellular metabolites released because of physical damage inflicted during sample collection. In many

cases, it is impossible to judge whether or not damage of the living tissue has occurred and the sample in a strict sense would be of doubtful origin.

### **Root exudates**

According to Putnam and Tang (1986), several techniques have been employed. Sand can be used in which both donor and recipient plants are present. The effects on early plant development before competition for growth factors occurs can then be evaluated. Also, donor plants can be grown in sand. The sand can then be leached and the leachate evaluated in terms of influence on recipient plants. Bell and Koeppel (1972) devised a system where donor and recipient plants can be grown together in a system where the pots are altered so that the nutrient solution flows from the donor to the recipient and back to a reservoir, flowing back and forth for varying periods of time.

### **Release from plant litter**

Rice (1984) reported that soils collected in the field were used as sources of allelochemicals. Live or dead material can be placed on or in the soil for a selected period of time before receptor plants are planted directly in the soil for bioassay or the soil can be extracted for allelochemicals.

### **Volatile compounds**

Muller and Haines (1964) germinated seed on filter paper sheets on a cellulose sponge placed in a large container adjacent to beakers containing the donor plants. The only contact between plant material and seed was aerial. Significant inhibition of germination occurred.

### **Bioassays**

Bioassays are an integral part in all studies of allelopathy. They are necessary for evaluating the allelopathic potential of species and following the activity during extraction, purification and identification of bio-active compounds. In their simplest form, bioassays, and the isolation and identification of allelochemical, are regarded by some as techniques for providing initial information only. Both these aspects of

allelopathy research are important and should be used together. Failure to do so would make results inconclusive (Reinhardt *et al.* 1996). Bioassay techniques vary greatly and no researcher follows the same procedure. This is clearly demonstrated in the treatise by Rice (1984). The greatest problem with bioassays is the lack of standardized bioassays. Incomplete information on the allelochemical source, method of extraction, fraction concentrations and the absence of known compounds with demonstrated activity in bioassays are also hampering useful bioassays. Stowe (1979) challenged the validity of bioassays. He concluded that, frequently little agreement between bioassay results and distinctive patterns of vegetation in the field are obtained.

According to Rice (1984) and Putnam and Tang (1985), the most widely used bioassay test is the influence on seed germination. Different types of techniques are used. All, however, include seed placed on substrate saturated with the test solution. Germination is often defined as the emergence of the radicle 2 mm beyond the seed coat and is scored over a period of time. Factors to consider are oxygen availability, osmotic potential of the test solution, pH and temperature. Properly conducted bioassays of this nature have great value. They are simple to conduct and require a small quantity of test solution.

The elongation of the hypocotyl or coleoptile can be used in conjunction with germination percentage. The elongation is, however, tedious to measure and instead dry mass can be used as a measure of growth (Bhowmik and Doll 1984). Growth bioassays are often more sensitive than germination bioassays. When the quantity of test solution poses a problem, agar cultures can be used. Pre-germinated seed can be placed on the surface of the agar containing the allelochemicals.

### **Separation and characterization of chemicals**

Rice (1984) pointed out that chemical separation can be accomplished by partitioning the chemicals on the basis of polarity into a series of solvents. Compounds can also be separated by molecular size, charge or adsorptive characteristics. Various chromatography methods are utilized.

There is little doubt that plants do release significant amounts of substances into the environment. However, their fate remains poorly understood. Limited studies using C<sup>14</sup>-labelled compounds suggest that most simple organic compounds such as phenolic acids are rapidly assimilated by soil micro-organisms or incorporated into humic acids (Willis 1985). It may well be stated that addition of organic compounds to the soil environment is more important in determining the composition of the soil micro-flora and thus the effects of most allelopathic substances are probably indirect.

### **Factors affecting production of allelochemicals**

Plants vary in their production of allelochemicals according to the environmental conditions to which they are exposed. Stress has a marked effect on the production of allelochemicals. According to Aldrich (1984) and Rice (1984), a variety of environmental conditions influence the quantity of chemicals produced:

- **Light**

Some allelochemicals are influenced by the amount, intensity and duration of light. The greatest quantities are produced during exposure to ultraviolet and long-day photoperiods. Thus under-storey plants will produce fewer allelochemicals because over-storey plants filter out the ultraviolet rays. At the peak of plant growing period, it could be expected that more allelochemicals are produced than earlier or later in the growing season.

- **Mineral deficiency**

More allelochemicals are produced under conditions of mineral deficiency.

- **Drought stress**

Under drought conditions, more allelochemicals are produced.

- **Temperature**

In cooler temperatures, greater quantities are produced.

The location within the plant and effects in specific allelochemicals seem to be variable.

There are also numerous other factors influencing the production of allelochemicals. The type and age of plant tissue during extraction is important since compounds are not uniformly distributed in plants. Production differs between species as well as within species.

Aldrich (1984) stated that environmental conditions that restrict growth tend to increase the production of allelochemicals. One could postulate that allelopathy may frequently be an accentuation of competition although not part of competition. If stress from competition increases the quantities of allelochemicals produced, it is conceivable that allelochemicals will inhibit the growth of some species and not others, thereby reducing the ability of the affected species to compete.

Much of the evidence indicates that several chemicals are released together and may exert toxicities in an additive or synergistic manner. Sometimes the allelopathic effect will be obvious and startling, but in the majority of cases the effects are subtle and thus more difficult to assess (Aldrich 1984).

### **Roles of allelopathy in natural and manipulated systems**

There is convincing evidence that allelopathic interactions between plants play a crucial role in natural as well as manipulated ecosystems. According to Rizvi *et al.* (1992), studies of these interactions provided the basic data for the science of allelopathy. The data were applied to understand the problems of plant-plant, plant-microbe and plant-insect interactions and to exploit these in improving the production of manipulated ecosystems.

### **Patterning of vegetation and succession**

Successions of plants occur in nature (Aldrich 1984). Plants modify the environment, thus leading to a predictable succession, with the early colonizers being those species that rely upon large numbers of seeds, and late entrants are those species that rely on their competitive ability. Perennial species concentrate offshoots around a parent and allelopathy could thus be beneficial to the spread of such species. The fact that dense colonies of some perennials frequently occur essentially as pure stands in it implicates allelopathy (Aldrich 1984). The explanation for a specific vegetational pattern has mostly been given to competition. In recent times, evidence is accumulating that point to the fact that, apart from competition, allelopathy does play an important role. According to Rizvi *et al.* (1992), allelopathic plants affect the patterning of vegetation in their immediate vicinity.

## Allelopathy and agriculture

The effect of weeds on crops, crops on weeds and crops on crops have invariably been emphasized. Results obtained so far clearly demonstrate that some of the findings on allelopathic control of weeds, elimination of deleterious allelopathic effects of crops on crops, or exploitation of beneficial interactions in a rotation or mixed cropping system have a direct bearing on crop production (Rizvi *et al.* 1992). According to Aldrich (1984), weeds interfere with crops in two ways:

- Inhibiting germination and seedling establishment.
- Inhibiting the growth of the crop.

*Cyperus esculentus* (yellow nutsedge) is an herbaceous perennial that is considered as one of the world's worst weeds. It is a problem in cropping systems in tropical and temperate climates, where it causes large losses in crop yields. The weed is characterized by prolific vegetative activity which produces a complex underground system of basal bulbs, rhizomes and tubers. Stoller *et al.* (1979) investigated the competition effect of *C. esculentus* on maize (*Zea mays*). They identified a relationship between nutsedge density (shoot/m<sup>2</sup>) and percentage reduction in crop yield. An 8% yield reduction was achieved for every 100 shoots/m<sup>2</sup>. Yield reduction of 41% occurred when no weed control was carried out in a field initially infested with 1200 shoots/m<sup>2</sup>.

*Cyperus esculentus* and *Cyperus rotundus* (purple nutsedge) are known for their allelopathic abilities. Drost and Doll (1980) concluded that extracts and residues of *C. esculentus* have an inhibitory effect on the growth of soyabeans and maize. Tames *et al.* (1973) found compounds in *C. esculentus* tubers that were inhibitory to oat coleoptiles and seed germination of other crops. Horowitz and Friedman (1971) dried *C. esculentus* tubers and mixed with soil. The root and top growth of barley planted in the soil were significantly reduced. Meissner *et al.* (1979) grew *C. rotundus* in sterilised, well-fertilized soil and reported that the growth of barley, cucumber and tomato were considerably reduced.

## **Allelopathy and forestry**

Allelopathic interactions have been demonstrated to play a crucial role in natural and man-made forests. Such interactions are pivotal in determining the composition of the vegetation growing as under-storey vegetation in understanding forest regeneration (Rizvi *et al.* 1992). It can, however, not be used as an universal explanation for regeneration failures or poor stand growth. Rice (1985) described various trials conducted to gain information on the allelopathic effects, not only of woody species, but herbaceous species as well.

### **Allelopathy of woody species**

Kil and Yim (1983) found that toxic substances of *Pinus densiflora* (red pine) inhibited seed germination and growth of the species in the forest. These substances were released in fresh and fallen leaves, roots, pine forest soil and pine pollen rain. Kil (1989) studied the allelopathic potential of five species of Pinaceae, viz. *P. densiflora*, *P. thunbergii*, *P. rigida*, *Larix leptolepis* and *Cedrus deodora*. All five species inhibited germination of test species, but the most severe inhibition was on dry-mass growth of the test species.

### **Cytotoxic activities of Allelochemicals**

Presence of hyoscyamine and hyoscyne alkaloids in *Datura stramonium* fruit was confirmed by Cooper and Johnson (1984) and Frohne and Pflander (1984). These two alkaloids were highly mitodepressive in action on root tip cells of sunflower (Rajendiran 1996) and onion (Kaushik *et al.* 1983). The allelochemicals in the form of alkaloids occurred maximum in *Catharanthus roseus* roots followed by leaves (Bose *et al.* 1959). This correlated with the report of Rajendiran (1998b) that mitotic inhibition and chromosomal aberrations were induced more in sunflower by root extracts than the leaf. Four major triterpenes such as azadirachtin, salannin, melinatriol and nimbidin were confirmed by Santhakumari and Stephan (1981) in neem leaves. Kaushik (1997) in *Vicia faba* and Rajendiran (1998a) in *Helianthus annuus* believed that these allelochemicals induced chromosomal and nuclear abnormalities. Sharma (1985) classified antimitotics into two groups: clastogens and mitotic poisons. Clastogens are agents causing chromosome break also damaging the genetic apparatus. Mitotic poisons are those altering the chromosome number mainly



affecting the spindle. The toxic principles viz. Lantadane-A and Lantadane-B in the leaf extract of *Lantana camara* are clastogenic as well as spindle poisoning in *Helianthus annuus* root tip cells (Rajendiran 1999a). The damage in chromosome structure was revealed in the form of chromosome breakage, stickiness, laggards and micronuclei, while occurrence of polyploid cell and precocious movement of chromosomes indicated spindle disturbance (Rajendiran 1999a). The allelochemicals such as Ricinine and N-Demethylricinine present in *Ricinus communis* leaf extract are capable of causing chromosome stickiness and forming a potential source of bringing chromosomal translocation and consequent changes in nuclear set up in *Helianthus annuus* (Rajendiran 1999b). The seven alkaloids viz. Vasicine, Vasicinone, Vasicol, Vasicoline, Vasicolinone, Adhatodine and Anisotine in the leaves of *Adhatoda vasica* (Joshi *et al.* 1994) were severely clastogenic as they brought about extensive chromosomal structural damages in *Helianthus annuus* (Rajendiran 1999c). Likewise Punarnavine alkaloid in the root extracts of *Boerhaavia diffusa* (Singh *et al.* 1983) were potent enough to cause chromosomal structural damage and polyploidy in *Helianthus annuus* (Rajendiran 2000b). Kanchan (1975) reported Parthenin, Caffeic acid and p-Coumaric acid in the plant extracts of *Parthenium hysterophorus* which were clastogenic as well as spindle poisoning in *Helianthus annuus* (Rajendiran 2000c), *Vigna radiata* (Rajendiran 2005) and in *Cucumis sativus* L. var. CO 1 (Hridya and Rajendiran 2013).

# ***PARTHENIUM***

## ***HYSTEROPHORUS L.***

### **ORIGIN AND SPREAD**

The genus *Parthenium* has about twenty species belonging to western hemisphere. Included in the family Asteraceae, *P. hysterothorus* L. (Plate 2, Fig. 1 to 3) is supposed to have originated as a result of natural hybridization between *P. confertum* and *P. bipinnatifidum* (Nath 1988). The diploid chromosome number for the Indian species has been reported to be 18 (Hakoo 1963). Two types of population of *P. hysterothorus* L. makes a complex. The plants show two distinct phases in life, juvenile, rosette or the vegetative stage and adult, mature or the reproductive stage. *P. hysterothorus* L. considered as a serious weed in several tropical and subtropical countries across the world has originated in North East Mexico (Dale 1981, Haseler 1976, Mc Clay 1984) and during the last hundred years has found its way into Africa, Australia and Asia. It has been reported from United States, Central America, South America, West Indies, Lesser Antilles of the new world and from India, Nepal, Africa, China, Vietnam and Australia of the old world (Aneja *et al.* 1991). It is a weed of roadsides, vacant lots and non cropped areas along non disturbed habitat (Singh *et*

*al.* 1993) and has also been recorded as a minor pest of cultivation in United States, Brazil, Argentina (Muenscher 1955) and India (Krishnamurthy *et al.* 1993, Dhawan and Dhawan 1994). The weed was first brought to India as an ornamental plant in 1910 but it failed to catch up. Again it invaded India and Australia in fifties as a contamination of wheat and pasture seeds, imported from the United States of America (Rao 1956; Haseler 1976). In India it was first reported as a weed from North India (Sharma and Tyagi 1979). As a species facing little environmental resistance the weed has become a menace in wastelands and non cropped areas (Plate 2, Fig. 4). Nitrogenous wastes of humans and livestock promotes its growth and this is the reason for its extensive growth close to cities and other human settlements (Singh *et al.* 1993). Under suitable soil and moisture conditions it becomes dominant species and results in the exclusion of beneficial plants (Jayachandra 1971, Kanchan 1975, Krishnamurthy *et al.* 1975, Dale 1981). So it has become one of the seven most dangerous weeds of the world.

## PHYTOCHEMISTRY

### Whole plant

Two types of sesquiterpene lactones, hysterin and dihydroisoparthenin have been isolated from *P. hysterothorus* (Picman *et al.* 1982). Histamine (0.58%) is present in aerial parts of the plant (Kamal and Mathur 1991) while Syringaresinol has also been isolated from this weed (Das *et al.* 1999). Three ambrosanoides;  $\alpha$ -epoxymethylacrylyloxy parthenin, its 11 $\alpha$ 13-dihydro derivatives and 8 $\alpha$ -eposeymethylacrylylos-ambrosin; have been isolated from chloroform extract of the aerial parts of the *P. hysterothorus* (Chhabra *et al.* 1999). A normal sesquiterpenoid, charminarone (the first seco-pseudoguaianolide) has been isolated from the whole plant (Venkataiah *et al.* 2003). Relative compositions of these lactones vary in different species of *Parthenium*. Parthenin is characteristic of *P. hysterothorus* while hysterin, hymenin and ambrosin of *P. kipinnatifidum*. Hysterin is the major sesquiterpene of *P. glomeratum*. Quercetagenin 3,7-dimethyl ether is a major flavanol present in this plant (Shen *et al.*, 1976). Small amount of 6-hydroxykaempferol, 3,7-dimethyl ether and the glucosides, quercetin 3-O-glucoside, kaempferol 3-O-glucoside and kaempferol 3-O-arabino glucoside have also been isolated from *P. hysterothorus*

(Rodriguez 1975). Phenolic glucosides show fluctuations in number and amount depending on the collection of site. Accumulation of fumeric acid in stem and leaves and ferulic acids in all parts of the plant except pollens, have also been reported. These phenolic acids are said to be responsible for allelopathic impact of the weed on other plants (Kanchan 1975).

### **Leaves**

Parthenin, hexacosanol, myricyl alcohol,  $\beta$ -sitosterol, campesterol, stigmasterol, betulin, ursolic acid,  $\beta$ -D-glucoside of  $\beta$ -sitosterol and saponin have been isolated from leaves of *P. hysterophorus*. The saponin on hydrolysis yield oleanolic acid and glucose. The aqueous extract of *P. hysterophorus* contains free amino acids, glucose, galactose and potassium chloride (4.8%) (Gupta *et al.* 1977). Methoxypseudoguaianolides viz. 13-ethoxy-dihydroambrosin, 13-methoxydihydro parthenin and 2 $\beta$ ,13 $\beta$ -dimethoxy-dihydroparthenin have been isolated from leaves of this plant (Bhullar *et al.* 1997). The leaves also contain parthenin, caffeic, chlorogenic, p-hydroxybenzoic, vanilic, salicylic, gentisic, neo-chlorogenic and proto-catechuic acids (Rainhardt *et al.* 2005).

### **Flower**

Methanolic extract of flower of *P. hysterophorus* contains several constituents such as 2 $\beta$ -hydroxycoronopilin, 8 $\beta$ -hydroxycoronopilin, 11-H,13- hydroxyparthenin, parthenin and coronopilin. Parthenin up to 8% is present in capitulum (Das *et al.* 2005). A new highly oxygenated pseudoguaianolides (8- $\beta$ -acetoxyhysterone C), parthenin, coronopilin and hysterone C have also been isolated from the flowers (Ramesh *et al.* 2003, Das *et al.* 2007).

### **Root**

Histamine (0.35%) is present in the roots of *P. hysterophorus* (Kamal and Mathur 1991). The roots contain parthenin, caffeic acid, chlorogenic, p-hydroxybenzoic, p-anisic, vanilic, salicylic, gentisic, neochlorogenic and proto-catechuic acids (Kamal and Mathur 1991).

## BIOLOGICAL ACTIVITIES

Sesquiterpene lactones exhibit a wide spectrum of biological activities, which include cytotoxic, antitumour, allergen, antimicrobial, antifeedant, phytotoxic and insecticidal properties (Rodriguez *et al.* 1976). The sesquiterpene lactone parthenin is the main secondary metabolite of *P. hysterophorus* L. and possess all the properties mentioned above. Parthenin the major sesquiterpene lactone becomes useful when properly processed. The early reports suggest that parthenin can be used in pest and pathogen control, either by itself or as a lead compound for the development of active and more selective analogues (De la Fuente *et al.* 2000, Datta and Saxena 2001, Fazal *et al.* 2011).

### Insecticidal

Parthenin the active compound present in *Parthenium hysterophorus* is known to show activity against termites, cockroaches (Tilak 1977) as well as migratory grasshoppers, *Melanoplus sanguinipes* (Picman *et al.* 1981, Fagoonee 1983). Whole plant extract of *Parthenium hysterophorus* showed insect growth regulatory activity against the cotton stainer, *Dysdercus angulatus* (Kareem 1984), fifth instar larvae of *Spodoptera litura* (Ranjandran and Gopalan 1979, Balasubramanian 1982) and toxic effect on cabbage leaf webber (*Crocidolomia binolalis* Zell), and pulse beetle (*Callosobruchus maculatus*) infesting cowpea seeds (Bhaduri *et al.* 1985) and mites (Gupta 1968). The naturally occurring resin material of the weed has been demonstrated to protect wood against termite, molluscan borer and fungal attacks (Bultman *et al.* 1998). Petroleum ether extract of leaves, stem and inflorescence of *P. hysterophorus* shows toxic effect on mean life span and progeny production of adults of the mustard aphid, *Lipaphis erysimi* (Sohal *et al.* 2002). The environmental biologists have identified its cholinesterase antagonistic properties which can be used in control of insects and worms (Dhawan and Dhawan 1995).

### Antifeedant

*Parthenium* has been shown to act as a feeding deterrent to the adult of *Dysdercus koenigii*, *Tribolium castaneum* Hbst, *Phthorimaea operculella* (Zell), *Callosobruchus chinensis* L. (Sharma and Joshi 1977) and sixth instar larvae of *Spodoptera litura* (Datta and Saxena 1997).

### **Nematicidal**

Extract of *P. hysterothorus* show toxicity against root knot nematodes *Meloidogyne incognita*, Chitwood, *Helicotylendus dihysslera* (Cobb) Sher (Hasan and Jain 1984). Crushed leaves admixed into the soil are used to reduce root galling in papaya caused by *M. incognita* (De la Fuente *et al.* 2000).

### **Herbicidal**

Pure parthenin as well as extract of different parts of *P. hysterothorus* show phytotoxic effects on many aquatic (Pandey 1994, Pandey 1995, Pandey 1996) as well as terrestrial weeds (Khosla *et al.* 1980, Khosla and Sobti 1981, Kumari 1990, Singh *et al.* 1992, Batish *et al.* 1997, Acharya and Rahman 1997). The sesquiterpene lactone parthenin has received most attention regarding allelopathy or potential herbicidal properties of the plant (Duke *et al.* 2007).

### **Antifungal**

Antifungal potential of different extracts of *P. hysterothorus* against human pathogenic fungi were investigated by Rai and Upadhyay (1990) and Rai (1993, 1994, 1995). The dermatophytes and other fungal pathogens have been found to be sensitive to sesquiterpene lactones which are present as active agent in this weed (Rai *et al.* 2003).

### **Antibacterial**

The volatile oil which contains sesquiterpene and flavanoids were found to be highly effective against gram positive and gram negative bacteria (Chopra 1960) and various species of dermatophytes (Dikshit and Dixit 1982).

### **CYTOTOXIC ACTIVITIES**

The methanolic extract of *P. hysterothorus* has been found to have antitumour effect in host mice bearing transplantable lymphocytic leukemia. The active compound leads to slow development of tumour and increases the survival of mice bearing lymphocytic leukemia (Mukherjee and Chatterjee 1993). Studies conducted at the Cancer Research Institute, Bombay and *in vitro* cytotoxicity against human cancer

cells has shown that *P. hysterophorus* possess anticancerous properties (Haq *et al.* 2011, Ramamurthy *et al.* 2011). Parthenin exhibits cytotoxicity with chromosomal aberrations in peripheral blood lymphocytes when administered to mice. A single intra-peritoneal dose of 4 to 31 mg / kg body weight of animal of parthenin increases the frequency of micro-nucleated reticulocytes in mice (Ramos *et al.* 2002).

The aqueous extracts of the root, stem, leaf and inflorescence of the weed induced different types of chromosomal aberrations in dividing cells of various test plants, which increased with increasing concentration and the maximum was recorded at the highest concentration (Rajendiran 2000c, Hridya and Rajendiran 2013). However, the extracts of leaves and inflorescence caused severe inhibition and greater number of chromosomal abnormalities than the stem and root extracts, the least being with root extract (Rajendiran 2000c, Hridya and Rajendiran 2013).

## **PHARMACOLOGY**

Oral administration of ethanolic extract of this plant leads to various behavioural as well as physiological changes like decrease in body weight, erythrocyte count, haemoglobin and lymphocyte percentage and increase in relative liver weight, neutrophil and leukocyte count in rat (Kushwaha and Maurya 2010, Maurya and Kushwaha 2010). 10% cold aqueous extracts of flowers successfully elicit a hypotensive response in dogs. Methanolic extract of the plant is reported to cause significant relaxant activity on skeletal muscles and depressant on central nervous system (Jha *et al.* 2011a, Jha *et al.* 2011b). *P. hysterophorus* L. whole plant is used as allergen (Dwivedi *et al.* 2008). It also shows hypoglycemic effect in normal and diabetic rats (Patel *et al.* 2008). The flower extract has apasmogenic action in isolated rabbit duodenum. The flower extract possess cardiac depressant effect as concluded from experiments on perfused frog heart (Patel *et al.* 2008). Aqueous extract of flowers and leaves exert lethal effect on frog tadpoles (Patel *et al.* 2008). Phytoconstituents particularly phytotoxins present in extracts have been reported to be responsible for this action. The plant is also reported to be effective in neuralgia, dysentery and as stimulant to menstrual functions (Dhawan and Dhawan 1995).

## **AS FOOD**

The leaf protein from this plant is reported to be better than cereal and legume proteins. It is used as spices in many parts of the world. The leaf protein concentrate contains 48 to 54% protein and 6 to 8% ash (Khan *et al.* 2011). Dried fibres of the weed after removing Parthenin contains 1.6 to 2.4% of nitrogen which can be used as cattle feed (Narasimhan *et al.* 1993).



# *LANTANA CAMARA* L.

*Lantana camara* L. is a flowering ornamental plant belonging to family Verbenaceae. *L. camara* is also known as Lantana, Wild Sage, Surinam Tea Plant, Spanish flag and West Indian lantana. *L. camara* is a well known medicinal plant in traditional medicinal system and recent scientific studies have emphasized the possible use of *L. camara* in modern medicine

## **TAXONOMY**

|          |   |                             |
|----------|---|-----------------------------|
| Kingdom  | : | Plantae                     |
| Division | : | Magnoliophyta               |
| Class    | : | Magnoliopsida               |
| Order    | : | Lamiales                    |
| Family   | : | Verbenaceae                 |
| Genus    | : | Lantana                     |
| Species  | : | <i>Lantana camara</i> Linn. |

## PLANT DESCRIPTION

*L. camara* is a low erect or subscandent vigorous shrub with tetragonal stem, stout recurved pickles and a strong odour of black currents. Plant grows up to 1 to 3 meters and it can spread to 2.5 meter in width. Leaves are ovate or ovate oblong, acute or sub acute, crenate serrate, rugose above, scabrid on both sides. The leaves are 3-8 cm long by 3-6 cm wide and green in colour. Leaves and stem are covered with rough hairs. Small flower held in clusters (called umbels). Colour usually orange, sometime varying from white to red in various shades and the flower usually change colours as they ages. Flowers are having a yellow throat, in axillary head almost throughout the year. The calyx is small, corolla tube slender, the limb spreading 6 to 7 mm wide and divided in to unequal lobes. Stemen four in two pairs, included and ovary two celled, two ovuled. Inflorescences are produced in pairs in the axils of opposite leaves. Inflorescences are compact, dome shaped 2-3 cm across and contain 20-40 sessile flowers. Root system is very strong and it gives out new fresh shoots even after repeated cuttings (Sastri 1962) (Plate 2).

## GEOGRAPHICAL DISTRIBUTION

*L. camara* is a tropical origin plant and native to Central and Northern South America and Caribbean. *L. camara* is now spreaded to nearly 60 countries viz, New Zealand, Mexico, Florida, Trinidad, Jamaica and Brazil. It is reported in many African countries including Kenya, Uganda, Tanzania and South Africa. In India, *L. camara* was probably introduced before 19<sup>th</sup> century. Currently *L. camara* is distributed throughout India. *L. camara* is known by different name in various different languages in India viz, Raimuniya (Hindi), Chaturangi and Vanacehdi (Sanskrit), Arippe and Unnichedi (Tamil), Aripoo, Poochedi, Konginipoo and Nattachedi (Malayalam), Thirei, Samballei and Nongballei (Manipuri), Tantani and Ghaneri (Marathi), Pulikampa (Telugu), Kakke and Natahu (Kanada). Ghana, infusion of the whole plant is used to cure bronchitis and the powdered root in milk was given to children for stomach-ache and as a vermifuge. Lantana oil is used in the treatment of skin, itches, as an anticeptic for wounds. In leprosy and scabies decoctions were applied externally (Khare 2007, Kirtikar and Basu 2006, Chopra *et al.* 1956)

## PHYTOCHEMICAL COMPOSITION

Phytochemical composition of the *L. camara* has been extensively studied in last few decades. Different parts of *L. camara* are reported to possess essential oils, phenolic compounds, flavonoids, carbohydrates, proteins, alkaloids, glycosides, iridoid glycosides, phenyl ethanoid, oligosaccharides, quinine, saponins, steroids, triterpens, sesquiterpenoides and tannin as major phytochemical groups (Venkatachalam 2011a, Kensa 2011, Kalita 2011, Bhakta and Ganjewala 2009).

## PHARMACOLOGICAL STUDIES

*L. camara* is an important medicinal plant of the family Verbenaceae. In recent history this plant is reported for various medicinal properties.

### Antibacterial activity

Different varieties of *L. camara* plants' leaves and flowers were reported for antibacterial activity. Three different solvent extract of leaves and flowers of four different varieties of *L. camara* exhibited significant antibacterial activity *E. coli*, *Bacillus subtilis* and *P. aeruginosa* whereas poor antibacterial activity against *Staphylococcus aureus* (Ganjewala *et al.* 2009). Ethanolic extracts of *L. camara* leaves and roots were reported for antibacterial activity. The *in vitro* antibacterial activity was performed by microdilution method. The extracts exhibited antimicrobial activity against *Staphylococcus aureus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Vibrio cholerae*, *Escherichia coli* and two multiresistant strains *E. coli* and *S. aureus*. (Barreto 2010). Methanolic extracts of different parts of *L. camara* were screened for antimicrobial activity against 10 bacteria and 5 fungi by disk diffusion method and broth microdilution method. The leaves extract of *L. camara* showed highest activity against Gram positive *Bacillus cereus* and Gram negative *Salmonella typhi* (Badakhshan 2009).

### Antifungal activity

Antifungal potential of *L. camara* was screened against *Alternaria* sp. which causes different plant diseases especially in vegetable plants. The antifungal activity was performed by food poison plate method at three different concentrations of

extract viz, 10 mg/ml, 15 mg/ml and 20 mg/ml. At 20mg/ml dose *L. camara* exhibited significant antifungal activity against *Alternaria* sp. (Srivastava and, Singh 2011). Antifungal activity of ethanol and hot water extract of *L. camara* was screened against wood destroying white and brown rot fungi. Both extracts exhibited efficient antifungal activity against white and brown rot fungi, however ethanol extract was highly potential at very low concentration (0.01%) (Tripathi 2009).

### **Antiulcerogenic activity**

Antiulcerogenic activity of the methanol extract of leaves of *L. camara* was reported on aspirin, ethanol and cold resistant stress induced gastric lesions in rats. Pre-treatment of the effected rats with the extract (200 and 400 mg/kg body weight) showed significant protective effect in aspirin induced, ethanol induced and cold restraint stress induced ulcers in rats. The extract resulted in dose dependent antiulcerogenic activity in all models (Thamotharan 2010).

### **Hemolytic activity**

The hemolytic activity of *L. camara* aqueous extract and its solvent fractions was performed by modified spectroscopic method at four different concentrations (125, 250, 500, 1000 µg/ml). The aqueous extract and its solvent fractions exhibited very low hemolytic activity towards the human erythrocytes. The hemolytic activity of the different extracts was found in the following order: chloroform fraction > hexane and ethyl acetate fraction (50:50) > aqueous extract > ethanol fraction > methanol fraction (Kalita 2011).

### **Antihyperglycemic activity**

Antihyperglycemic activity of methanol extract of leaves *L. camara* was reported in alloxan induced diabetic rats. Oral administration of the methanol extract of *L. camara* (400 mg/kg body weight) leaves resulted in decrease in blood glucose level to 121.94 mg/dl in alloxan induced diabetic rats (Ganesh 2010). Hypoglycemic activity of methanol extract of *L. camara* Linn fruits was screened in streptozotocin induced diabetic rats (Wistar albino rats). Extract treatment at doses of 100 and 200 mg/kg body weight resulted in dose dependent decrease in serum glucose level in streptozotocin induced diabetic rats. Extract treatment also showed improvement in

body weight, HbA1c profile as well as regeneration of liver cells (Venkatachalam 2011b).

### **Wound healing activity**

Wound healing property of aqueous extract of leaf of *L. camara* was reported in rats. Topical application of the extract on the wound (100 mg/kg/day) significantly enhanced the rate of wound contraction (98%), synthesis of collagen and decreased wound healing time (Nayak 2009). Ethanol extract of leaf of *L. camara* was reported for wound healing activity in adult male Wister rats. Topical application of the extract over the wound significantly increased the wound healing activity. Histological analyses of healed wounds confirmed the role of extract in healing. (Abdulla 2009).

### **Antimotility activity**

Methanol extract of *L. camara* leaves was reported to possess antimotility activity in mice. Intestinal motility was assayed by charcoal meal test in mice. At a dose of 1 g/kg body weight, the extract completely inhibited the transit of charcoal in normal mice. Intraperitoneal administration of 125 and 250 mg/kg body weight the extracts significantly reduced the fecal output in castor oil induced diarrhoea in mice. (Sagar *et al.* 2005).

### **Mosquito controlling activity**

Essential oil from the leaves of *L. camara* was reported to possess adulticidal activity against *Aedes aegypti*, *Culex quinquefasciatus*, *Anopheles culicifacies*, *An. fluviatilis* and *An. stephensi* mosquitoes with LD50 values 0.06, 0.05, 0.05, 0.05 and 0.06 mg/cm(2) while LD90 values were 0.10, 0.10, 0.09, 0.09 and 0.10 mg/cm(2) against *Ae. aegypti*, *Cx. quinquefasciatus*, *An. culicifacies*, *An. fluviatilis* and *An. stephensi* respectively (Dua *et al.* 2010). Mosquito larvicidal activity of methanol and ethanol extracts of leaves and flowers of *L. camara* were reported against 3rd and 4th instar larvae of *Ae. aegypti* and *Cx. quinquefasciatus* mosquito. Both extracts exhibited significant larvicidal activity against both species of mosquitoes, however, at low concentrations (1mg/ml) extracts were highly active against *Ae. aegypti* than that of *Cx. quinquefasciatus* (Kumar 2008).

### **Antifilarial activity**

Antifilarial activity of crude extract of *L. camara* stem was reported. The extract and its chloroform fraction resulted in the death of adult *Brugia malayi* and sterilised most of the surviving female worms in the rodent model *Mastomys coucha* (Misra 2006).

### **Antiinflammatory activity**

Aqueous extract of *L. camara* was reported for anti-inflammatory activity in albino rats. Extract treatment (500mg/kg body weight) significantly decreased paw volume in carrageenan induced paw oedema test in rats (Gidwani 2009).

### **Anti fertility activity (Embryo toxicity)**

Effects of hydroalcoholic extract of *L. camara* leaves was studied on fertility, general reproductive performance and teratology in female albino Wistar rats. The extract interfered in the frequency of fetal skeleton anomalies from dams treated with the extract and induced embryotoxicity as indicated by post-implantation loss, without any signs of maternal toxicity (De Mello 2003).

### **Antiuro lithiatic activity**

Ethanollic extract of the leaves of *L. camara* was reported for antiuro lithiatic activity against ethylene glycol and ammonium chloride induced calcium oxalate urolithiasis in male albino rats. Extract treatment significantly reduced the deposition of calcium, oxalate and also reduced urinary excretion of calcium, oxalate and creatinine (Mayee and Thosar 2011).

### **Anticancer and antiproliferative activity**

Oleanonic acid isolated from *L. camara* was screened for anticancer activity against a murine tumour (Ehrlich ascites carcinoma), and three human cancer cell lines, namely A375 (malignant skin melanoma), Hep2 (epidermoid laryngeal carcinoma) and U937 (lymphoma). Oleanonic acid exhibited promising cytotoxicity against A375 cells (Ghosh and Das Sarma 2010). Leaves of *L. camara* were reported to exhibit cytotoxicity effect on Vero cell line. *In vitro* cytotoxicity test was

performed by MTT assay. The methanol extract (500 µg/ml) concentration inhibited the growth of cells 2.5 times less than did Triton 100 × 1% (Pour *et al.* 2011). Leaves of *L. camara* were reported for antiproliferative activity against HEP-2 (laryngeal cancer) and NCI-H292 (lung cancer) cell lines. In vitro antiproliferative test was performed by MTT assay. Methanol extract of *L. camara* leaves exhibited antiproliferative activity against NCI-H292 cells (% living cells= 25.8±0.19) (Gomes 2010).

### **Anti mutagenic activity**

22-acetoxylantic acid and 22-dimethylacryloyloxy lantanolic acid from *L. camara* showed antimutagenic activity. The antimutagenicity test was performed by micronucleus test in Swiss mice. Both compounds exhibited high antimutagenic activity in Mitomycin C induced mutagenesis in mice (Barre 1997).

### **Antioxidant activity**

Ethanol extract of *L. camara* exhibited significant antioxidant activity in *in vivo* studies. The extract treatment decreased the extent of lipid peroxidation in the kidneys of urolithic rats. *In vitro* studies were carried out by DPPH radical scavenging assay and Nitric oxide free radical scavenging assay. Extract exhibited high antioxidant properties in both the assays (Mayee and Thosar 2011). Antioxidant activity of the leaves of *L. camara* was reported by reducing power activity and 1, 1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assay. Leaves extracts exhibited high antioxidant effect, however younger leaves exhibited strong antioxidant activity than the older or matured leaves (Bhakta and Ganjewala 2009).

## **TOXICOLOGY**

*L. camara* is one among the most toxic plants known so far, possibly with in top ten. Reports of *L. camara* toxicity have been reported from Australia, India, New Zealand, South Africa and America. However, the toxicity occurs only on the consumption of high amount of plants material. It is reported that sheep, cattle and goats are susceptible to lantadenes A, B, D and icterogenic acid toxicity, whereas horses, rats, neonatal calves and lambs are not susceptible to lantadene A. The prominent clinical sign of poisoning includes photosensitisation and jaundice. Loss of

appetite in poisoned animals occurs within 24 hours and decrease in appetite also observed. The most severely poisoned animals die within 2 days of poisoning but usually death occurs after 1 -3 weeks after poisoning. The kidneys are swollen and pale in colour, the gall bladder is grossly distended and the liver is enlarged. The oral toxic dose of lantadene A for sheep is 60 mg/kg is toxic and 1–3 mg/kg by intravenous route (Sharma 1981, Sharma 1988).

## **CYTOTOXIC ACTIVITIES**

The leaf extract of *Lantana camara* contain toxic principles viz. Lantadane-A and Lantadane-B which are clastogenic as well as spindle poisoning in *Helianthus annuus* root tip cells (Rajendiran 1999a). The damage in chromosome structure was revealed in the form of chromosome breakage, chromosome stickiness, anaphasic and telophasic laggards and micronuclei (Rajendiran 1999a). The occurrence of polyploid cell and precocious movement of chromosomes after treatment with the weed extracts indicated spindle disturbance (Rajendiran 1999a).



# MATERIALS AND METHODS

## PLANT MATERIALS

The certified seeds of *Oryza sativa* L. var, ASD-16, *Cucumis sativus* L. var. Sambar, *Helianthus annuus* L. var. Tall, *Zea mays* L. var. Cauvery-244 *Vigna unguiculata* (L) Walp. cv. BCP-25 and *Vigna mungo* L. var. Vamban-16 were obtained from Department of Vegetable crops, Tamil Nadu Agricultural University, Coimbatore. For preparing extracts *Parthenium hysterophorus* L. and *Lantana camara* L. weeds were collected in and around Tagore Arts College Campus, Lawspet and near agricultural lands near Pondicherry (Plate 2, 3).

## PREPARATION OF EXTRACTS

The fresh roots, stem, leaves and inflorescence of *Parthenium hysterophorus* L. were washed and ground separately in an electric grinder and the extracts were prepared in each case by boiling 10 gm of ground plant material in 100 ml of distilled water at 100°C for 25 minutes. After filtration with Whatman No.1 filter paper, stock solutions were prepared (Plate 4, 5).

The fresh roots, stem, leaves and inflorescence of *Lantana camara* L. were washed and ground separately in an electric grinder (Plate 3) and the extracts were prepared in each case by boiling 10 gm of ground plant material in 100 ml of distilled water at 100°C for 25 minutes. After filtration with Whatman No.1 filter paper, stock solutions were prepared (Plate 6 to 8).

## **DETERMINATION OF LD<sub>50</sub> CONCENTRATIONS**

LD stands for "Lethal Dose". LD<sub>50</sub> is the amount of a material, given all at once, which causes the death of 50% (one half) of a group of test animals. The LD<sub>50</sub> is one way to measure the short-term poisoning potential (acute toxicity) of a material. LC stands for "Lethal Concentration". LC values usually refer to the concentration of a chemical in air but in environmental studies it can also mean the concentration of a chemical in water. For inhalation experiments, the concentration of the chemical in air that kills 50% of the test animals in a given time (usually four hours) is the LC<sub>50</sub> value. Toxicologists can use many kinds of animals but most often testing is done with rats and mice. It is usually expressed as the amount of chemical administered (e.g., milligrams) per 100 grams (for smaller animals) or per kilogram (for bigger test subjects) of the body weight of the test animal. The LD<sub>50</sub> can be found for any route of entry or administration but dermal (applied to the skin) and oral (given by mouth) administration methods are the most common.

For determining the LD<sub>50</sub> concentration of the extracts three separate sets of experiments each with triplicates were conducted.

## **LD<sub>50</sub> FOR *PARTHENIUM HYSTEROPHORUS* L. EXTRACTS**

### **Experiment Set 1**

In the first set, various concentrations of root, stem, leaf, and inflorescence extracts (25, 50, 75, and 100%) of *Parthenium hysterophorus* L. were made in distilled water. Viable seeds of *Oryza sativa* L. var. ASD-16, *Cucumis sativus* L. var. Sambar and *Helianthus annuus* L. var. Tall soaked in distilled water for 6 hours were allowed to germinate separately in petri plates lined with moist Whatman No.1 filter paper. Seven days old seedlings with healthy roots were treated with 5 ml of each

concentration of the extracts for three days. Seedlings watered with distilled water served as control.

### **Experiment Set 2**

The second treatment of different concentrations of the weed extracts (25, 30, 35, 40, 45, 50% concentrations) was given to fresh set of seven day old seedlings grown in petri plates.

### **Experiment Set 3**

The third set of treatment consisted of 25, 26, 27, 28, 29, and 30% concentrations of the extracts to a new set of seven day old seedlings.

Through the three sets of experiments conducted using three test plants, the extract concentrations for cytological activity were fixed as 5, 10, 15, 20, 25 and 30% based on the LD<sub>50</sub> values obtained.

## **LD<sub>50</sub> FOR *PARTHENIUM HYSTEROPHORUS* L. EXTRACTS**

### **Experiment Set 1**

In the first set, various concentrations of root, stem, leaf, flower and fruit extracts (25, 50, 75, and 100%) of *Lantana camara* L. were made in distilled water. Viable seeds of *Zea mays* L. var. Cauvery-244, *Vigna unguiculata* (L) Walp. cv. BCP-25 and *Vigna mungo* L. var. Vamban-16 soaked in distilled water for 6 hours were allowed to germinate separately in petri plates lined with moist Whatman No.1 filter paper. Seven days old seedlings with healthy roots were treated with 5 ml of each concentration of the extracts for three days. Seedlings watered with distilled water served as control.

### **Experiment Set 2**

The second treatment of different concentrations of the weed extracts (25, 30, 35, 40, 45, 50% concentrations) was given to fresh set of seven day old seedlings of *Zea mays* L. var. Cauvery-244 grown in petri plates.

### Experiment Set 3

The third set of treatment consisted of 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 and 35% concentrations of the extracts to a new set of seven day old seedlings of *Zea mays* L. var. Cauvery-244.

Through the three sets of experiments conducted using the test plant, the extract concentrations for cytological activity were fixed as 5, 10, 15 and 20% based on the LD<sub>50</sub> values obtained and assessment of scar development in the treated root tips.

### CYTOTOXIC ACTIVITY

Viable seeds of *Oryza sativa* L. ASD-16, *Cucumis sativus* L. var. Sambar and *Helianthus annuus* L. var. Tall, soaked in distilled water for 6 hours were allowed to germinate in petri plates lined with moist Whatman No.1 filter paper separately. Seven days old seedlings with healthy roots were treated with 5 ml of 5, 10, 15, 20, 25 and 30% concentrations of the four extracts of *Parthenium hysterophorus* L. separately. Similarly viable seeds of *Zea mays* L. var. Cauvery-244, *Vigna unguiculata* (L) Walp. cv. BCP-25 and *Vigna mungo* L. var. Vamban-16, soaked in distilled water for 6 hours were allowed to germinate in petri plates lined with moist Whatman No.1 filter paper separately. Seven days old seedlings with healthy roots were treated with 5 ml of 5, 10, 15, 20, 25 and 30% concentrations of the five extracts of *Lantana camara* L. separately.

The root tips were excised from the ten day old seedlings of the three test plants from the control and treated seedlings (5, 10, 15, 20, 25 and 30% concentrations of the four extracts). The excised root tips were thoroughly washed in distilled water and pre-treated in 0.2% 8-hydroxyquinoline at 4°C for 3 hours. After thoroughly washing with distilled water the root tips were fixed in Carnoy's fixative (1:3 acetic alcohol) for 3 hours. Rapid root tip squash technique of Rajendiran (2005) was followed. Squashes were made in one root tip per slide and sealed with sealing medium. The mitotic index in the root tip cells of control and treated test plants were calculated. The prepared slides were thoroughly examined for the presence of different types of chromosomal aberrations, the important stages photographed at

1000x magnification in oil immersion microscope and the data presented in the form of tables.

### **Schedule for Rapid Root Tip Squash Technique (Rajendiran 2005)**

Root tips were washed thoroughly, trimmed and placed on a clean slide. They were hydrolysed in few drops of 1N HCl at 60°C on a hot plate or by flaming in a spirit lamp for 1 min. A root tip was rinsed in distilled water and transferred to a fresh slide. A few drop of 4% iron alum was added as a mordant 60°C for 2 min. It was against rinsed in distilled water and stained in a few drops of 4% haematoxylin at 60°C for 2 min. The root tip was sliced longitudinally to facilitate cell spreading and mounted on a drop of 45% acetic acid. A cover slip was carefully placed without any air bubble and pressed gently with blunt end of a needle to enable the cells to spread uniformly. The excess of acetic acid was removed using a blotting paper and a thermocol seal was applied along the margin of the cover slip. This seal can be removed by immersion in absolute xylol as and when required while making it permanent. As the entire procedure was carried out over a hot plate, care should be taken to avoid complete drying of root tips. The prepared slides were examined for the presence of somatic metaphase and the chromosome numbers and chromosomal abnormalities were counted and important stages photographed at 1000x magnification in oil immersion microscope.

### **Preparation of 0.2% 8-hydroxyquinoline:**

1 gram of 8-hydroxyquinoline crystal was dissolved in 50 ml of distilled water and stored at 4°C.

### **Preparation of 1N HCl:**

3.64 grams of concentrated hydrochloric acid was mixed in 100 ml of distilled water.

### **Preparation of Iron Alum:**

2 grams of Iron Alum (Ferric Ammonium Sulphate) was dissolved in 50 ml of distilled water and the solution was filtered.

**Preparation of 4% Haematoxylin:**

2 grams of Haematoxylin powder was dissolved in 50 ml of 95% ethyl alcohol and the solution was allowed to ripen fully for 2 weeks. The well ripened stain would be cherry red in colour.

**Preparation of Sealing Medium for semi-permanent slides:**

Dissolve 50 g of Thermocol in 25 ml of absolute xylol and store in an air tight container.

**STATISTICAL ANALYSIS**

Percentage values for the data obtained were calculated and the results used for comparing mitosis, mitotic reductions, chromosomal abnormalities and the number of types of aberrations in all the treated and control seedlings. At least three replicates were maintained for all treatments and control.

# OBSERVATIONS

## ALLELOPATHIC EFFECT OF *PARTHENIUM* EXTRACT

### LD<sub>50</sub> CONCENTRATION

The root, stem, leaf and inflorescence extracts of *Parthenium hysterophorus* L. affected the process of seedling growth in *Oryza sativa* L. var. ASD-16. All the seedlings treated with 50, 75, and 100% concentrations in the first set died, while the lethality ranged from 41.66 to 46.33% in 25% concentration of all the extracts (Table 1). In the second set the whole lot of seedlings treated with 35, 40, 45, 50% concentrations of the four extracts died, while in 25 and 30% concentrations the lethality was 41.66 to 68.7% respectively. In the third set of experiments the LD<sub>50</sub> concentration for the leaf and inflorescence extracts was recorded as 26%, while 27% concentrations of root and stem extracts proved to be LD<sub>50</sub> (Table 1). The maximum inhibition of seedling growth was recorded at the highest concentration of leaf extract treatment (Plate 9, Fig. 1 to 4).

In *Cucumis sativus* L. var. Sambar the root, stem, leaf and inflorescence extracts of *Parthenium hysterophorus* L. affected the process of seedling growth. The entire lot of the seedlings treated with 50, 75, and 100% concentrations in the first set

died (Table 2). However the seedlings in 25% concentration of the extracts survived as the lethality was between 40 to 46.7% only. In the second set the seedlings treated with 35, 40, 45, 50% concentrations of the four extracts died completely, while in 25 and 30% concentrations the lethality ranged from 40 to 46.7% and 55 to 68.3% respectively. In the third set of experiments the LD<sub>50</sub> concentration for the leaf and inflorescence extracts was recorded as 26%, while 28% concentrations of both root and stem extracts proved to be LD<sub>50</sub> (Table 2). The inhibition of seedling growth was recorded maximum at the highest concentration of leaf extract treatment (Plate 10, Fig. 1 to 4).

The growth of *Helianthus annuus* L. var. Tall seedlings was also affected by the root, stem, leaf and inflorescence extracts of *Parthenium hysterophorus* L. The seedlings recorded 100% lethality in 50, 75, and 100% concentrations in the first set (Table 3). The seedlings treated with 25% concentration of the extracts survived as the lethality was between 41 to 47.3%. In the second set the full lot of seedlings treated with 35, 40, 45, 50% concentrations of the four extracts died, while in 25 and 30% concentrations the lethality was 41 to 69% respectively. In the third set of experiments the LD<sub>50</sub> concentration for the leaf and inflorescence extracts was recorded as 27%, while 28% concentrations of both root and stem extracts proved to be LD<sub>50</sub> (Table 3). Severe inhibition of seedling growth was recorded at the highest concentration of leaf extract treatment (Plate 11, Fig. 1 to 4).

## **ALLELOPATHIC EFFECT OF *LANTANA* EXTRACT**

### **LD<sub>50</sub> CONCENTRATION**

The root, stem, leaf, flower and fruit extracts of *Lantana camara* L. affected the process of seedling growth in *Zea mays* L. var. Cauvery-244 (Plate 12, Fig. 1 to 3). All the seedlings treated with 50, 75, and 100% concentrations in the first set died, while the lethality ranged from 21.66 to 46.66% in 25% concentration of all the extracts (Table 4). In the second set the whole lot of seedlings treated with 35, 40, 45, 50% concentrations of the root and leaf extracts died, while all the seedlings treated with 40, 45, 50% concentrations of the stem, flower and fruit extracts died. In 25, 30 and 35% concentrations the lethality ranged from 21.66 to 88.33% (Table 4). In the third set of experiments the LD<sub>50</sub> concentration for the root and leaf extracts was



recorded as 26%, for flower extract it was 31%, while 32% concentrations of stem and fruit extracts proved to be LD<sub>50</sub> (Table 4). The maximum inhibition of seedling growth was recorded at the highest concentration of root extract treatment (Plate 12, Fig. 1 to 3).

The root, stem, leaf, flower and fruit extracts of *Lantana camara* L. affected the process of seedling growth in *Vigna unguiculata* (L) Walp. cv. BCP-25 (Plate 13, Fig. 1 to 3). All the seedlings treated with 50, 75, and 100% concentrations in the first set died, while the lethality ranged from 23.33 to 43.33% in 25% concentration of all the extracts (Table 5). In the second set the whole lot of seedlings treated with 35, 40, 45, 50% concentrations of the root and leaf extracts died, while all the seedlings treated with 40, 45, 50% concentrations of the stem, flower and fruit extracts died. In 25, 30 and 35% concentrations the lethality ranged from 23.33 to 83.33% (Table 5). In the third set of experiments the LD<sub>50</sub> concentration for the root and leaf extracts was recorded as 26%, for flower extract it was 31%, while 32% concentrations of stem and fruit extracts proved to be LD<sub>50</sub> (Table 5). The maximum inhibition of seedling growth was recorded at the highest concentration of root extract treatment (Plate 13, Fig. 1 to 3).

The root, stem, leaf, flower and fruit extracts of *Lantana camara* L. affected the process of seedling growth in *Vigna mungo* L. var. Vamban-16 (Plate 14, Fig. 1 to 3). All the seedlings treated with 50, 75, and 100% concentrations in the first set died, while the lethality ranged from 21.66 to 43.33% in 25% concentration of all the extracts (Table 6). In the second set the whole lot of seedlings treated with 35, 40, 45, 50% concentrations of the root and leaf extracts died, while all the seedlings treated with 40, 45, 50% concentrations of the stem, flower and fruit extracts died. In 25, 30 and 35% concentrations the lethality ranged from 21.66 to 81.66% (Table 6). In the third set of experiments the LD<sub>50</sub> concentration for the root and leaf extracts was recorded as 26%, for flower extract it was 31%, while 32% concentrations of stem and fruit extracts proved to be LD<sub>50</sub> (Table 6). The maximum inhibition of seedling growth was recorded at the highest concentration of root extract treatment (Plate 14, Fig. 1 to 3).

## **CYTOTOXIC ACTIVITY OF *PARTHENIUM* EXTRACT**

The root tips of 10 day old seedlings were highly injured after treatment with 30% concentrations of the weed extracts. Even though few seedlings survived, their root tips were unhealthy for preparing root tip squash. Hence the cytological studies with three test plants were restricted to 5, 10, 15, 20 and 25% concentrations of the weed extracts.

### **Rice**

In control condition, the root tips of *Oryza sativa* L. var. ASD-16 showed normal cell division (Plate 15, 16). Mitotic index of *Oryza sativa* L. var. ASD-16 showed a steady decrease with increasing concentrations of all the extracts (Table 7). The percentage value of mitotic index in control was 34.44% and after treatment with root, stem, leaf and inflorescence extracts it declined rapidly with the increase in concentrations. The least values of 15.23%, 13.87%, 8.71% and 10.84% were recorded after treatment with root, stem, leaf and inflorescence extracts respectively in 25% concentration (Table 7).

All the extracts of the weed induced seven different types of chromosomal aberrations in dividing cells, which increased with increasing concentration and the maximum was recorded at the highest concentration (Table 7). However, the extracts of leaves and inflorescence caused severe inhibition and greater number of chromosomal abnormalities (25.14 and 22.42% respectively) than the stem and root extracts (17.41% and 15.11% respectively), the least being with root extract (Table 7; Plate 16). Application of extracts of the weed changed the normal cycle of events of mitosis in *Oryza sativa* L. var. ASD-16 root tip cells producing chromosome fragments, stickiness of chromosome ends, chromosome bridges during anaphase, micronuclei, laggard formation, precocious movement of chromosomes and polyploidy (Plate 16).

### **Cucumber**

The root tips of *Cucumis sativus* L. var. Sambar in control condition showed normal cell division (Plate 15). Mitotic index of *Cucumis sativus* L. var. Sambar followed a steady decrease with increasing concentrations of all the extract treatments

(Table 8). The percentage value of mitotic index in control was 41.33% and it declined rapidly to 21.33% in root and stem extracts, reaching the minimum of 13.66% and 20.66% after treatment with leaf and inflorescence extracts respectively in 25% concentration (Table 8).

Various chromosomal abnormalities accounting to seven types were induced by all the extracts of the weed in the dividing cells, which increased with increasing concentration and the largest values were recorded at the highest concentration (Table 8). The extracts of the leaves and inflorescence caused severe inhibition with more number of chromosomal aberrations (17.65 and 11.98% respectively) than the stem and root extracts (8.33 and 10.99% respectively), the least being with root extract (Table 8). Irrigation of the weed extracts to *Cucumis sativus* L. var. Sambar seedlings altered the events of somatic cell division and caused fragmentation of chromosome, chromosome stickiness, ring chromosomes, chromosome bridges during anaphase, laggard formation during anaphase movement, micronuclei and precocious movement of chromosomes (Plate 16).

### **Sunflower**

The cell division in the root tips of *Helianthus annuus* L. var. Tall control plants were normal (Plate 15, 17). Mitotic index of *Helianthus annuus* L. var. Tall showed a steady decrease with increasing concentrations of all the extracts (Table 9). The percentage value of mitotic index in control was 36% and after treatment with root, stem, leaf and inflorescence extracts it declined rapidly to the minimum of 23.66%, 23%, 20.66% and 19.66% respectively in 25% concentration (Table 9).

All the extracts of the weed induced different types of chromosomal aberrations in dividing cells, which increased with increasing concentration and the maximum was recorded at the highest concentration (Table 9). The extracts of leaves and inflorescence caused severe inhibition and greater number of chromosomal abnormalities than the stem and root extracts (Table 9). Application of the weed extracts to the test plants caused changes in the somatic cell divisions, producing chromosome fragments, stickiness of chromosome ends, chromosome bridges during

telophase, laggard formation during anaphase and favouring micronuclei formation and polyploidy (Plate 17)

### **Comparative Study**

Over all, the cytotoxic effects of *Parthenium hysterophorus* L. leaves were found to be severe in all the three test plants. Inflorescence extract stood next to leaf extract as it caused the second highest mitotic reduction inducing seven types of abnormalities. Stem extracts performed third with root extracts being least effective. Out of the three plants taken for testing the cytotoxic activity of *Parthenium hysterophorus* L. plant extracts, *Oryza sativa* L. var. ASD-16 was found to be more susceptible to the weed extracts. At the highest concentration of leaf extract *Oryza sativa* L. var. ASD-16 recorded a reduction of 35% in mitosis involving 25.14% aberrations. The cell cycle of *Oryza sativa* L. var. ASD-16 was also disturbed by the inflorescence extract of the weed which followed leaf extracts causing 27% mitotic reduction and 23.42% chromosomal abnormalities. *Cucumis sativus* L. var. Sambar also showed susceptibility to the leaf extract of the weed with 23.33% mitotic suppression with 17.65% abnormalities in chromosomes followed by the inflorescence extracts of the weed. On the other hand *Helianthus annuus* L. var. Tall exhibited much resistance than the other two test plants in all the extracts of *Parthenium hysterophorus* L. However the same trend of more mitotic reductions and increased chromosomal abnormalities with leaf extract of the weed continued in this ornamental plant also (Table 7 to 9).

### **CYTOTOXIC ACTIVITY OF LANTANA EXTRACT**

The root tips of 10 day old seedlings were highly injured after the 3 days treatment with 25 to 35% concentrations of the weed extracts. Even though few seedlings survived their root tips were unhealthy for preparing root tip squash as they developed scars in response to the injuries caused by the extract treatments. Hence the cytological studies with three test plants were restricted to 5, 10, 15 and 20% concentrations of the weed extracts.

## Maize

In control condition, the root tips of *Zea mays* L. var. Cauvery-244 showed normal cell division (Plate 15). Mitotic index of *Zea mays* L. var. Cauvery-244 showed a steady decrease with increasing concentrations of all the extracts (Table 10). The percentage value of mitotic index in control was 39.54% and after treatment with root, stem, leaf and inflorescence extracts it declined rapidly with the increase in concentrations. The least values of 10.27%, 16.98%, 13.75%, 16.22% and 17.26% were recorded after treatment with root, stem, leaf, flower and fruit extracts respectively in 20% concentration (Table 10).

All the extracts of the weed induced seven different types of chromosomal aberrations in dividing cells, which increased with increasing concentration and the maximum was recorded at the highest concentration (Table 10). However, the extracts of root and leaf caused severe inhibition and greater number of chromosomal abnormalities (24.75% and 17.55% respectively) than the flower, stem and fruit extracts (13.70%, 9.93% and 10.56% respectively), the least being with fruit extract (Table 10). Application of extracts of the weed changed the normal cycle of events of mitosis in *Zea mays* L. var. Cauvery-244 root tip cells producing chromosome fragments, stickiness of chromosome ends, chromosome bridges during anaphase, micronuclei, laggard formation, ring chromosomes and polyploidy (Plate 18).

## Cowpea

In control condition, the root tips of *Vigna unguiculata* (L) Walp. cv. BCP-25 showed normal cell division (Plate 15). Mitotic index of *Vigna unguiculata* (L) Walp. cv. BCP-25 showed a steady decrease with increasing concentrations of all the extracts (Table 11). The percentage value of mitotic index in control was 33.33% and after treatment with root, stem, leaf and inflorescence extracts it declined rapidly with the increase in concentrations. The least values of 8.71%, 16.77%, 11.57%, 14.44% and 15.46% were recorded after treatment with root, stem, leaf, flower and fruit extracts respectively in 20% concentration (Table 11).

All the extracts of the weed induced seven different types of chromosomal aberrations in dividing cells, which increased with increasing concentration and the

maximum was recorded at the highest concentration (Table 11). However, the extracts of root and leaf caused severe inhibition and greater number of chromosomal abnormalities (25.14 and 16.47% respectively) than the flower, stem and fruit extracts (11.69%, 10.12% and 9.12% respectively), the least being with fruit extract (Table 11). Application of extracts of the weed changed the normal cycle of events of mitosis in *Vigna unguiculata* (L) Walp. cv. BCP-25 root tip cells producing chromosome fragments, stickiness of chromosome ends, ring chromosomes, chromosome bridges, laggard formation, micronuclei and polyploidy (Plate 18).

### **Black gram**

In control condition, the root tips of *Vigna mungo* L. var. Vamban-16 showed normal cell division (Plate 15). Mitotic index of *Vigna mungo* L. var. Vamban-16 showed a steady decrease with increasing concentrations of all the extracts (Table 12). The percentage value of mitotic index in control was 42.46% and after treatment with root, stem, leaf and inflorescence extracts it declined rapidly with the increase in concentrations. The least values of 11.71%, 19.88%, 14.57%, 17.44% and 18.46% were recorded after treatment with root, stem, leaf, flower and fruit extracts respectively in 20% concentration (Table 12).

All the extracts of the weed induced seven different types of chromosomal aberrations in dividing cells, which increased with increasing concentration and the maximum was recorded at the highest concentration (Table 12). However, the extracts of root and leaf caused severe inhibition and greater number of chromosomal abnormalities (25.93 and 17.85% respectively) than the flower, stem and fruit extracts (14.13%, 11.11% and 10.22% respectively), the least being with fruit extract (Table 12). Application of extracts of the weed changed the normal cycle of events of mitosis in *Vigna mungo* L. var. Vamban-16 root tip cells producing chromosome fragments, stickiness of chromosome ends, ring chromosomes, chromosome bridges, laggard formation, micronuclei and polyploidy (Plate 18).

### **Comparative Study**

Over all, the cytotoxic effects of *Lantana camara* L. roots were found to be severe over *Zea mays* L. var. Cauvery-244. Leaf extract stood next to root extract as it

caused the second highest mitotic reduction inducing seven types of abnormalities. Flower extracts performed third, stem extracts fourth with fruit being least effective. The cytotoxic effects of *Lantana camara* L. roots were found to be severe over *Vigna mungo* L. var. Vamban-16. Leaf extract stood next to root extract as it caused the second highest mitotic reduction inducing seven types of abnormalities. Flower extracts performed third, stem extracts fourth with fruit being least effective. In *Vigna unguiculata* (L) Walp. cv. BCP-25, the roots of *Lantana camara* L. were found to be severe. Leaf extract stood next to root extract as it caused the second highest mitotic reduction inducing seven types of abnormalities. Flower extracts performed third, stem extracts fourth with fruit being least effective.

Out of the three plants taken for testing the cytotoxic activity of *Lantana camara* L. plant extracts, *Zea mays* L. var. Cauvery-244 was found to be more susceptible to the weed extracts. At the highest concentration of root extract *Zea mays* L. var. Cauvery-244 recorded a reduction of 74.03% in mitosis involving 24.75% aberrations. *Vigna unguiculata* (L) Walp. cv. BCP-25 also showed susceptibility to the root extract of the weed with 73.86% mitotic suppression with 25.14% abnormalities in chromosomes. *Vigna mungo* L. var. Vamban-16 exhibited susceptibility next to the other two crops in all the extracts of *Lantana camara* L. The same trend of more mitotic reductions (72.42%) and increased chromosomal abnormalities (24.93%) with root extract of the weed continued in this plant also (Table 10 to 12).

## **BIOCHEMICAL ANALYSIS OF EXTRACTS**

### **Thin Layer Chromatography (TLC)**

All the samples resolved into two major components corresponding to R<sub>f</sub> 0.86 and 0.43 which represented Caffeic acid and Parthenin respectively. The spot at R<sub>f</sub> 0.43 representing Parthenin was highly intense in leaf and inflorescence samples and less intense in stem. The spot appeared as evanescent in root sample. Three minor spots at R<sub>f</sub> 0.93, 0.75, and 0.18 were identified as Ambrosin, 2-β-Hydroxycoronopilin and Dihydroxyparthenin. Ambrosin and Dihydroxyparthenin appeared in stem while 2-β-Hydroxycoronopilin and Dihydroxyparthenin resolved in leaf sample (Plate 19).

### Gas Chromatography - Mass Spectrometry (GC-MS)

The GC-MS pattern indicated the presence of ten allelochemicals *viz.* Caffeic acid, p-Coumaric acid, Ambrosin, Hymanin, Hysterin, 8- $\beta$ -Hydroxyparthenin, 2- $\beta$ -Hydroxycoronopilin, Dihydroxyparthenin, Anhydroxyparthenin and Parthenin in all the four extract samples of *Parthenium hysterophorus* L. Caffeic and p-Coumaric acids are Phenolic acids while Ambrosin, Hymanin, Hysterin, 8- $\beta$ -Hydroxyparthenin, 2- $\beta$ -Hydroxycoronopilin, Dihydroxyparthenin, Anhydroxyparthenin and Parthenin are Pseudoguaianolides. The occurrence of the number of compounds varied in each samples. All the ten compounds appeared in the leaf extract sample of the weed while only lesser number of compounds appeared in inflorescence, stem and root extract samples. The inflorescence stood next to leaf sample recording six compounds *viz.* Caffeic acid, Ambrosin, 8- $\beta$ -Hydroxyparthenin, 2- $\beta$ -Hydroxycoronopilin, Dihydroxyparthenin and Parthenin, followed by stem which showed five compounds *viz.* Caffeic acid, Ambrosin, 2- $\beta$ -Hydroxycoronopilin, Dihydroxyparthenin and Parthenin. Only four compounds *viz.* Caffeic acid, Ambrosin, 2- $\beta$ -Hydroxycoronopilin and Parthenin appeared in the root sample. These four allelochemicals were present in the samples from all the parts of *Parthenium hysterophorus* L. (Plate 20).



# RESULTS AT A GLANCE

## 1. CYTOTOXICITY OF *PARTHENIUM* LEACHATES

- All the extracts at all concentrations **reduced mitosis and increased chromosomal abnormalities**.
- Mitotic reduction, chromosomal abnormalities and number of types of aberrations **increased with increasing concentrations**.
- **25% concentration** of all the extracts induced **maximum effects**.
- **Leaf extracts** of the weed proved to be **most effective** which was closely followed by **inflorescence**.
- **Stem extracts** were **less effective** while the **root extracts** performed the **least**.
- TLC of **leaf and inflorescence** resolved **four components**, while **stem and root** extracts resolved **only two** each.
- GC-MS reported **ten allelochemicals** in leaf, **six** in inflorescence, **five** in stem and **only four** in root extracts.

- **Cytotoxic studies could not be carried out at 30% concentrations**, as the root tips of all test plants **developed scars**.
- **25% concentration of leaf and inflorescence extracts** showed **drastic cytotoxic activities against rice**.
- Root tip cells of **cucumber suffered less** when compared with rice.
- **Sunflower** recorded only **minimum damages**.
- Test plants were categorised **based on their responses** to the weed extracts:

| TEST PLANT       | BINOMIAL                    | VARIETY | CYTOTOXICITY  |
|------------------|-----------------------------|---------|---------------|
| <b>Rice</b>      | <i>Oryza sativa</i> L.      | ASD-16  | <b>High</b>   |
| <b>Cucumber</b>  | <i>Cucumis sativus</i> L.   | Sambar  | <b>Medium</b> |
| <b>Sunflower</b> | <i>Helianthus annuus</i> L. | Tall    | <b>Low</b>    |

## 2. CYTOTOXICITY OF *LANTANA CAMARA* L. LEACHATES

- All the extracts at all concentrations **reduced mitosis and increased chromosomal abnormalities**.
- Mitotic reduction, chromosomal abnormalities and number of types of aberrations **increased with increasing concentrations**.
- **20% concentration** of all the extracts induced **maximum effects**.
- **Root extracts** of the weed proved to be **most effective** which was closely followed by **leaf**.
- **Flower and stem extracts** were **less effective** while the **fruit extracts** performed the **least**.

- **Cytotoxic studies could not be carried out at above 20% concentrations**, as the root tips of all test plants **developed scars**.
- **20% concentration of root and leaf extracts showed drastic cytotoxic activities against maize, cow pea and black gram seedlings.**
- Test plants were categorised **based on their responses** to the weed extracts:

| TEST PLANT        | BINOMIAL                               | VARIETY     | CYTOTOXICITY |
|-------------------|--|-------------|--------------|
| <b>Maize</b>      | <i>Zea mays</i> L.                     | Cauvery-244 | <b>High</b>  |
| <b>Cow pea</b>    | <i>Vigna unguiculata</i><br>(L.) Walp. | BCP-25      | <b>High</b>  |
| <b>Black gram</b> | <i>Vigna mungo</i> L.                  | Vamban-16   | <b>High</b>  |

# DISCUSSION

## ***PARTHENIUM HYSTEROPHORUS* L. EXTRACTS**

### **LD<sub>50</sub> CONCENTRATION**

The root, stem, leaf and inflorescence extracts of *Parthenium hysterophorus* L. affected the process of seedling growth in *Oryza sativa* L. var. ASD-16, *Cucumis sativus* L. var. Sambar, *Helianthus annuus* L. var. Tall. The seedlings recorded 100% lethality in 50, 75 and 100% concentrations in the first set. The seedlings treated with 25% concentration of the extracts survived with lethality well below 50%. In the second set the entire seedlings of the three test plants died after treatment with 35, 40, 45 and 50% concentrations of all extracts. However both in 25 and 30% concentrations the lethality was 40 to 70%. In the third set of experiments, the LD<sub>50</sub> concentration for the leaf and inflorescence extracts was recorded as 26%, while 27% concentrations of root and stem extracts proved to be LD<sub>50</sub> in *Oryza sativa* L. var. ASD-16 (Table 1). In *Cucumis sativus* L. var. Sambar, the LD<sub>50</sub> concentration for the

leaf and inflorescence extracts was 26%, while 28% concentrations of both root and stem extracts proved to be LD<sub>50</sub> (Table 2). In *Helianthus annuus* L. var. Tall the LD<sub>50</sub> concentration for the leaf and inflorescence extracts was 27%, while 28% concentrations of both root and stem extracts were recorded as LD<sub>50</sub> (Table 3). However severe inhibition of seedling growth was recorded at the highest concentration of the leaf extract treatment in all the plants. The inhibition of growth of seedlings by *Parthenium hysterophorus* L. was earlier reported by Putnam and Weston (1986), Bhakat *et al.* (2002), Bhakat *et al.* (2005a), Bhakat *et al.* (2005b), Bhakat *et al.* (2006), Bhakat *et al.* (2007), Bhattacharjee *et al.* (2003), Kanp *et al.* 2004, Rajendiran (2000a), Rajendiran (2004) and Hridya and Rajendiran (2013).

Based on these three sets of experiments with the test plants, the weed extract concentrations for cytological activity were fixed at 5, 10, 15, 20, 25 and 30%, as 54 to 69% of the seedlings died above 30% concentrations of the extracts and 100% lethality was recorded in 35% concentration (Plate 9 to 11; Table 1 to 3).

## CYTOTOXIC ACTIVITY

Even though few seedlings of the 30% concentration treatment survived, their root tips were not suitable for preparing root tip squash due to injuries they sustained from extracts. Hence the cytological studies with three test plants were restricted to 5, 10, 15, 20 and 25% concentrations of the weed extracts.

Application of extracts of the weed disturbed the cycle of events of mitosis in all the three test plants causing chromosomal changes. Root extracts from *Parthenium hysterophorus* L. suppressed the mitotic divisions in all the test plants, the maximum being in *Oryza sativa* L. var. ASD-16, followed by *Cucumis sativus* L. var. Sambar and the least being in *Helianthus annuus* L. var. Tall (Table 7 to 9). Mukherjee and Chatterjee (1993) reported that methanolic extract of *P. hysterophorus* leaves was found to have antitumour effect in host mice bearing transplantable lymphocytic leukemia. The active compounds lead to slow development of tumour and increased the survival of mice bearing lymphocytic leukemia. Reduction in mitotic divisions was also supported by Haq *et al.* (2011), Ramamurthy *et al.* (2011) who demonstrated

that the weed possessed anticancerous properties when treated against the human cancer cells.

In control conditions the root tips of *Oryza sativa* L. var. ASD-16, *Cucumis sativus* L. var. Sambar and *Helianthus annuus* L. var. Tall carried out normal cell divisions (Plate 15 to 17). All the extracts of the weed induced different types of chromosomal aberrations in dividing cells of test plants, which increased with increasing concentration and the maximum was recorded at 25% concentration (Table 7 to 9; Plate 16, 17). Similar observations were reported with *Ammi majus* (Adam and Rashad 1984), *Datura stramonium* (Rajendiran 1996), *Azadirachta indica* (Rajendiran 1998a), *Catharanthus roseus* (Rajendiran 1998b), *Lantana camara* (Rajendiran 1999a), *Ricinus communis* (Rajendiran 1999b), *Adhatoda vasica* (Rajendiran 1999c), *Boerhaavia diffusa* (Rajendiran 2000b) and with *Parthenium hysterophorus* extracts (Rajendiran 2000c, Hridya and Rajendiran 2013). However, the extracts of leaves and inflorescence caused severe inhibition and greater number of chromosomal abnormalities than the stem and root extracts, the least being with root extract. Similar results were reported by Rajendiran (2000a) in *Helianthus annuus* L. var. EC 68414 and Hridya and Rajendiran (2013) in *Cucumis sativus* L. var. CO 1 seedlings.

Similar results were reported in experiments with animals as test species. Parthenin exhibited cytotoxicity with chromosomal aberrations in peripheral blood lymphocytes when administered to mice (Ramos *et al.* 2002). A single intra-peritoneal dose of 4 to 31 mg / kg body weight of animal of parthenin increased the frequency of micro-nucleated reticulocytes in mice (Ramos *et al.* 2002).

Seven types of chromosomal abnormalities in *Oryza sativa* L. var. ASD-16, five types in *Cucumis sativus* L. var. Sambar and in *Helianthus annuus* L. var. Tall respectively were recorded (Table 7 to 9). Even though similar trend was followed by the root, stem, leaf and inflorescence extracts, the leaf extract reduced mitotic activity severely in *Oryza sativa* L. var. ASD-16 followed by *Cucumis sativus* L. var. Sambar and *Helianthus annuus* L. var. Tall. Over all, the cytotoxic effects of *Parthenium hysterophorus* L. leaves were found to be severe in all the three test plants (Table 7 to 9; Plate 16, 17). Inflorescence extract stood next to leaf extract as it caused the second highest mitotic reduction inducing seven types of chromosomal abnormalities. Stem

extracts performed third with root extracts being the least effective. Similar observations were reported by Rajendiran (2000a) in *Helianthus annuus* L. var. EC 68414 and Hridya and Rajendiran (2013) in *Cucumis sativus* L. var. CO 1.

The two phenolic acids viz. Caffeic and p-Coumaric acids and eight sesquiterpene lactones (Pseudoguaianolides) viz. Parthenin, Ambrosin, Hymanin, Hysterin, 8- $\beta$ -Hydroxyparthenin, 2- $\beta$ -Hydroxycoronopilin, Dihydroxyparthenin, Anhydroxyparthenin from the weeds induced changes in macromolecules, proteins, nucleic acids and lipids which manifested in massive damage to cellular membranes and loss of enzyme activity (Picman *et al.* 1981). Sesquiterpene lactones reacted with cysteine, glutathione and many proteins, while some of them are novel uncouplers of oxidative phosphorylation (Pandey 2009). Due to non-availability of the required enzymes to support DNA replication and protein deficiency reducing the production of histones, abnormal cell divisions with aberrated chromosomes were formed (Rajendiran 2000c),

The chromosomal aberrations induced by the extract of the weed include breakage of chromosomes, stickiness, chromosomal laggards, anaphasic and telophasic bridges, polyploidy, micronuclei and ring chromosomes (Table 7 to 9; Plate 16, 17).

*Oryza sativa* L. var. ASD-16 was found to be more susceptible to the weed extracts activity out of the three plants taken for testing the cytotoxic activity of *Parthenium hysterophorus* L. plant extracts. Similarly *Cucumis sativus* L. var. Sambar also showed susceptibility to the leaf extract of the weed with mitotic suppression and abnormalities in the chromosomes followed by the inflorescence extracts of the weed. On the other hand *Helianthus annuus* L. var. Tall exhibited much resistance than the other two test plants in all the extracts of *Parthenium hysterophorus* L. However the same trend of more mitotic reductions and increased chromosomal abnormalities with leaf extract of the weed continued in this ornamental plant also. As evident from the tabulated data the leaves and inflorescence of the weed showed intensive inhibitory effects and were severely clastogenic and spindle poisoning as compared to stem and root extracts (Table 7 to 9; Plate 16, 17).

The TLC resolved into two major components and three minor components (Plate 19). Further GC-MS confirmed the presence of ten allelochemicals in the leaf extract sample of the weed while six compounds appeared in inflorescence, five in stem and only four compounds in root extract (Plate 20). This result correlated with the report of Kanchan (1975) and Pandey (2009) that the toxins *viz.* parthenin and phenolic acids such as caffeic acid, vanillic acid, anisic acid, chlorogenic acid, parahydroxy benzoic acid, p-anisic acid and p-coumaric acid were maximum in the leaves of *Parthenium hysterophorus* L. followed by inflorescence, stem and roots. While the presence of four chemicals *viz.* Caffeic acid, Ambrosin, 2- $\beta$ -Hydroxycoronopilin and Parthenin in all the extracts of *Parthenium hysterophorus* L. accounted for the mitotic disturbance and induction of chromosomal aberrations, occurrence of additional six allelochemicals *viz.* p-Coumaric acid, Hymanin, Hysterin, 8- $\beta$ -Hydroxyparthenin, Dihydroxyparthenin and Anhydroxyparthenin in the leaf extract vigoured its cytotoxic activity.

The severe mitodepressive action and the induction of chromosomal abnormalities by *Parthenium hysterophorus* L. leaf and inflorescence extracts proved that the weed utilised its leaf and inflorescence leachates as potent tools to suppress the growth of other plants growing in the vicinity by suppressing cell division and inducing chromosomal aberrations in them. The GC-MS report that allelochemicals occurred more in the aqueous extracts of leaf and inflorescence than in the stem and root also supported this view. Further it was evident that the cereal crop and the vegetable crop were more susceptible to cytotoxic activity of weed leachates, while the ornamental variety of sunflower could tide over the effects with meagre suppression.

## **LANTANA CAMARA L. EXTRACTS**

### **LD<sub>50</sub> CONCENTRATION**

All the seedlings of *Zea mays* L. var. Cauvery-244, *Vigna mungo* L. var. Vamban-16 and *Vigna unguiculata* (L) Walp. cv. BCP-25 treated with 50, 75, and 100% concentrations in the first set died. The lethality in maize, black gram and cow pea ranged from 21.66 to 46.66%, 21.66 to 43.33% and 23.33 to 43.33% respectively at 25% concentration of all the extracts (Table 4 to 6). In the second set the whole lot



of seedlings of three test plants treated with 35, 40, 45, 50% concentrations of the root and leaf extracts died, while all the seedlings treated with 40, 45, 50% concentrations of the stem, flower and fruit extracts died. In 25, 30 and 35% concentrations the lethality ranged from 21.66 to 88.33% (Table 4 to 6). In the third set of experiments the LD<sub>50</sub> concentration for the root and leaf extracts was recorded as 26%, for flower extract it was 31%, while 32% concentrations of stem and fruit extracts proved to be LD<sub>50</sub>. The maximum inhibition of seedling growth was recorded at the highest concentration of root extract treatment (Table 4 to 6; Plate 12 to 14, Fig. 1 to 3). Similar results for LD<sub>50</sub> were observed by Badakhshan and Sreenivasan (2011) in shrimp treated with *Lantana camara* L. extracts, in which root extract was the most toxic part followed by leaves, flower, fruits and stem in descending order. The inhibition of growth of seedlings by *Lantana camara* L. was also reported by Romel *et al.* (2007) and Arpana (2013) in several types of agricultural crops.

## CYTOTOXIC ACTIVITY

The root tips of 10 day old seedlings were highly injured after the 3 days treatment with 25 to 35% concentrations of the weed extracts. Even though few seedlings survived their root tips were unhealthy for preparing root tip squash as they developed scars in response to the injuries caused by the extract treatments. Hence the cytological studies with three test plants were restricted to 5, 10, 15 and 20% concentrations of the weed extracts.

Mitotic index of *Zea mays* L. var. Cauvery-244, *Vigna unguiculata* (L) Walp. cv. BCP-25 and *Vigna mungo* L. var. Vamban-16 showed a steady decrease with increasing concentrations of all the extracts (Table 10 to 12). The percentage value of mitotic index in the three control plants were 39.54%, 42.46% and 33.33%. After treatment with root, stem, leaf and inflorescence extracts the values declined rapidly with the increase in concentrations. The least were recorded after treatment with root, stem, leaf, flower and fruit extracts respectively in 20% concentration (Table 10 to 12). Rajendiran (1999a) reported that aqueous extract of *Lantana camara* L. leaves was found to have mitotic poisoning effect on the root tip cells of *Helianthus annuus* L. According to Badakhshan and Sreenivasan (2011) all the tested extracts exhibited

very low cell division in brine shrimp larva and the root extract was the most toxic part of *Lantana camara* L. which may have the potential as anticancer agent.

All the extracts of the weed induced seven different types of chromosomal aberrations in dividing cells of the three crops, which increased with increasing concentration and the maximum was recorded at the highest concentration (Table 10 to 12; Plate 18). However, the extracts of root and leaf caused severe inhibition and greater number of chromosomal abnormalities than the flower, stem and fruit extracts, the least being with fruit extract. Application of extracts of the weed changed the normal cycle of events of mitosis in *Zea mays* L. var. Cauvery-244, *Vigna unguiculata* (L) Walp. cv. BCP-25 and *Vigna mungo* L. var. Vamban-16 root tip cells producing chromosome fragments, stickiness of chromosome ends, chromosome bridges during anaphase, micronuclei, laggard formation, ring chromosomes and polyploidy (Plate 18). Similar observations were reported with *Ammi majus* (Adam and Rashad 1984), *Datura stramonium* (Rajendiran 1996), *Azadirachta indica* (Rajendiran 1998a), *Catharanthus roseus* (Rajendiran 1998b), *Lantana camara* (Rajendiran 1999a), *Ricinus communis* (Rajendiran 1999b), *Adhatoda vasica* (Rajendiran 1999c), *Boerhaavia diffusa* (Rajendiran 2000b) and with *Parthenium hysterophorus* extracts (Rajendiran 2000c, Hridya and Rajendiran 2013a, Hridya and Rajendiran 2013b, Hridya and Rajendiran 2013c and Hridya and Rajendiran 2014). However, the extracts of roots and leaves caused severe inhibition and greater number of chromosomal abnormalities than the stem, flower and fruit extracts, the least being with root extract. Similar results were reported by Rajendiran (2000a) in *Helianthus annuus* L. var. EC 68414 and Hridya and Rajendiran (2013a) in *Cucumis sativus* L. var. CO 1 seedlings.

Over all, the cytotoxic effects of *Lantana camara* L. roots were found to be severe over *Zea mays* L. var. Cauvery-244. Leaf extract stood next to root extract as it caused the second highest mitotic reduction inducing seven types of abnormalities. Flower extracts performed third, stem extracts fourth with fruit being least effective. The cytotoxic effects of *Lantana camara* L. roots were found to be severe over *Vigna mungo* L. var. Vamban-16. Leaf extract stood next to root extract as it caused the second highest mitotic reduction inducing seven types of abnormalities. Flower extracts performed third, stem extracts fourth with fruit being least effective. In *Vigna*

*unguiculata* (L) Walp. cv. BCP-25, the roots of *Lantana camara* L. were found to be severe. Leaf extract stood next to root extract as it caused the second highest mitotic reduction inducing seven types of abnormalities. Flower extracts performed third, stem extracts fourth with fruit being least effective. Similar results were recorded by Rajendiran (1999a) in *Helianthus annuus* L. var. EC 68414 and Badakhshan and Sreenivasan (2011) in shrimp after treatment with *Lantana camara* L. extracts.

Out of the three plants taken for testing the cytotoxic activity of *Lantana camara* L. plant extracts, *Zea mays* L. var. Cauvery-244 was found to be more susceptible to the weed extracts. At the highest concentration of root extract *Zea mays* L. var. Cauvery-244 recorded a reduction of 74.03% in mitosis involving 24.75% aberrations. *Vigna unguiculata* (L) Walp. cv. BCP-25 also showed susceptibility to the root extract of the weed with 73.86% mitotic suppression with 25.14% abnormalities in chromosomes. *Vigna mungo* L. var. Vamban-16 exhibited susceptibility next to the other two crops in all the extracts of *Lantana camara* L. The same trend of more mitotic reductions (72.42%) and increased chromosomal abnormalities (24.93%) with root extract of the weed continued in this plant also (Table 10 to 12).

The two toxic principles viz. Lantadane-A and Lantadane-B from the weed induced changes in macromolecules, proteins, nucleic acids and lipids which manifested in massive damage to cellular membranes and loss of enzyme activity (Singh *et al.* 1983a, b). Lantadane-A reacted with many proteins, while Lantadane-B is the novel uncouplers of oxidative phosphorylation (Singh *et al.* 1983a, b). Due to non-availability of the required enzymes to support DNA replication and protein deficiency reducing the production of histones, abnormal cell divisions with aberrated chromosomes were formed (Rajendiran 2000c).

The chromosomal aberrations induced by the extract of the weed include breakage of chromosomes, stickiness, chromosomal laggards, precocious movement of the chromosomes to the poles, anaphasic and telophasic bridges, polyploidy, micronuclei and ring chromosomes (Table 10 to 12; Plate 18). The broken portion of the chromosomes created stickiness of the ends (Rajendiran 1996), facilitating fusion of sticky ends of two different chromosomes forming dicentric chromosome which

ultimately created chromosome bridges during telophase (Rajendiran 1998a). These bridges formed by the allelochemicals favoured laggard formation from the stress of anaphase movement (Rajendiran 1998b). These laggards and chromosome fragments became surrounded by nuclear membrane forming micronucleus (Rajendiran 1999c). According to Rajendiran (2000b), disturbance of the spindle apparatus by the allelochemicals created early movement of the chromosomes towards the poles, while inhibition of the spindle mechanism by the extracts resulted in doubling of chromosome numbers (Rajendiran 1999c).

As evident from the tabulated data the extracts from roots and leaves of the weed showed intensive inhibitory effects in seedling growth as well as in cell division and were severely clastogenic and spindle poisoning as compared to flower, stem and fruit extracts (Table 10 to 12; Plate 18). This result correlated with the report of Singh *et al.* (1983b) that the active principles *viz.* Lantadane-A and Lantadane-B occur in maximum quantity in root and leaf of *Lantana camara* L. followed by other parts.

The severe mitodepressive action and the induction of chromosomal abnormalities by *Lantana camara* L. root and leaf extracts proved that the weed utilised its root and leaf leachates as potent tools to suppress the growth of other plants growing by the side by reducing cell division and inducing chromosomal aberrations in them. Hence it was evident that eradicating this weed from the vicinity of the crop field is necessary as the weed extracts have proved to be clastogenic which are capable of eroding the genotype of all food crops.

# SUMMARY

*Parthenium hysterophorus* L. and *Lantana camara* L. are notorious weeds that have caused more concern than any other weeds in the recent past, in India, to both the common man and the weed scientist. The common man abhors any contact with this weed as it is a proven health hazard to sensitized humans and *cattle*. The conventional methods have failed to control the growth and prevent their unchecked spread. Infestations by these weeds degrade natural ecosystems. They aggressively colonize disturbed sites and reduce pasture growth and depress forage production. The germination and growth of indigenous plants are inhibited by their allelopathic effects. This effect, coupled with the absence of natural enemies like insects and diseases, is responsible for their rapid spread in their introduced ranges. Growth inhibitors are released from these plants into the soil through leaching, exudation of roots and decay of residues which suppresses the growth and yield of native plants.

In this context, it was felt necessary to make an assessment of cytotoxicity of the *Parthenium hysterophorus* L. extracts to explore the degree to which the allelochemicals possessed the destructive action on root tip cells of an important food

crop rice (*Oryza sativa* L. var. ASD-16), a vegetable crop cucumber (*Cucumis sativus* L. var. Sambar) and an ornamental type of sunflower (*Helianthus annuus* L. var. Tall); and root, stem, leaf, flower and fruit extracts of *Lantana camara* L. on the root tip cells of a large grain cereal maize (*Zea mays* L. var. Cauvery-244), a tropical dual-purpose legume cowpea (*Vigna unguiculata* (L) Walp. cv. BCP-25) and an important grain legume blackgram (*Vigna mungo* L. var. Vamban-16).

The mitotic chromosome behaviour in control plants was normal. Mitotic index of the test plants treated with the two weed extracts separately, showed regular decrease with increasing concentrations of the extracts. All the extracts of the weeds induced different types of chromosomal aberrations in dividing cells of the six test plants, which increased with increasing concentration and the maximum was recorded at the highest concentration. However, the extracts of leaves and inflorescence of *Parthenium hysterophorus* L. and root and leaves of *Lantana camara* L. caused severe inhibition and greater number of chromosomal abnormalities than the extracts from other parts. Even though the trend was similar in all the test plants, the chromosomes of *Oryza sativa* L. var. ASD-16 suffered heavily than the other two plants and the least was by *Helianthus annuus* L. var. Tall under *Parthenium hysterophorus* L. treatment. *Zea mays* L. var. Cauvery-244, *Vigna unguiculata* (L) Walp. cv. BCP-25 and *Vigna mungo* L. var. Vamban-16 suffered equally under *Lantana camara* L. treatment. The extracts of both the weeds caused fragmentations leading to stickiness of chromosomes, ring formation, laggards, anaphasic and telophasic bridges, polyploidy and micronuclei.

The result that the leaves and inflorescence of *Parthenium hysterophorus* L. caused intensive inhibitory effects and were severely clastogenic and spindle poisoning as compared to stem and root extracts was supported by the TLC report that resolved into two major components: Caffeic acid and Parthenin and three minor components: Ambrosin, 2- $\beta$ -Hydroxycoronopilin and Dihydroxyparthenin. The GC-MS report gave additional support that ten allelochemicals viz. Caffeic acid and p-Coumaric acid (two Phenolic acids) and Ambrosin, Hymanin, Hysterin, 8- $\beta$ -Hydroxyparthenin, 2- $\beta$ -Hydroxycoronopilin, Dihydroxyparthenin, Anhydroxyparthenin and Parthenin (eight Pseudoguaianolides) in the leaf extract sample of the weed while only six compounds appeared in inflorescence, five in stem

and only four compounds in root extract. Caffeic acid, Ambrosin, 2- $\beta$ -Hydroxycoronopilin and Parthenin present in all the weed extracts disturbed the mitotic cycle inducing chromosomal aberrations and together with p-Coumaric acid, Hymanin, Hysterin, 8- $\beta$ -Hydroxyparthenin, Dihydroxyparthenin and Anhydroxyparthenin present in the leaf extract increased the cytotoxic effects.

The result that the root and leaves of the *Lantana camara* L. caused severe inhibitory effects and spindle poisoning as compared to stem, flower and fruit extracts was supported by the findings of Singh *et al.* (1983b), that the active principles viz. Lantadane-A and Lantadane-B occur in maximum quantity in root and leaf of *Lantana camara* L.

The present study revealed that the allelochemicals in the leaves and inflorescence of *Parthenium hysterophorus* L. and the root and leaves of *Lantana camara* L. were more potent mitodepressive agents and they played a vital role in maintaining the dominance of the weed by suppressing the growth of associated plant species. An immediate measure is necessary to check the population of these weeds as the leachates of *Parthenium hysterophorus* L. and *Lantana camara* L. have proved to be more vulnerable in eroding the chromosomes of the crops and ornamental plants. As no commercial cultivars of cereals, vegetables and ornamental plants carrying allelopathic resistant properties are available, the knowledge of the behaviour of the test plant chromosomes in response to allelochemicals of *Parthenium hysterophorus* L. and *Lantana camara* L. obtained from this study will serve as a basement for the possibility of breeding new crops with allelopathic resistant property in near future.

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