5. Discussion

Currently biosystematics is given the rightful importance among biological disciplines, more because of the increasing need for a correct determination of generic and specific complexes, in view of the occurrence of large-scale diversities within species which make bioecological, physiological as well as related interdisciplinary approaches obligatory for a better understanding of these complexes. Understanding of the differences in population distribution and success, differences in adaptation to changing environment, and allometric growth are now emerging as important parameters for differentiating closely related species. An attempt has been made in this thesis to characterize *Typha angustifolia*, a member of the cattail family Typhaceae, using a biosystematics approach.

Cattails are one of the most common plants in wetland ecosystem and under right conditions they can grow and spread vigorously. They have a tendency to grow in thick, nearly impenetrable stands, blocking the view of open water and raising the concern that they will take over and cover a pond. Cattails are competitively superior under stable water conditions. The plant can occur in a variety of natural communities and form extensive monocultures rapidly through vegetative reproduction, thereby reducing plant bio-diversity. The dense foliage and debris from old growth makes it very difficult for competing plant species to grow. Cattail can become a problem in irrigated agricultural lands and managed aquatic systems. The plant invades farm ponds, irrigation canals, and drainage ditches which can result in impeded water flow and increased silt formation. *Typha* are often among the first wetland plants to colonize areas of newly exposed wet mud, with their abundant wind dispersed seeds.
Buried seeds can survive in the soil for long periods of time (van der Valk and Davis, 1976). They germinate best with sunlight and fluctuating temperatures, which is typical of many wetland plants that regenerate on mud flats. The plants also spread by rhizomes, forming large, interconnected stands.

*Typha*, with 9–11 species globally distributed, is characterized by dense, elongate, cylindrical spike like inflorescences, and female flowers having numerous capillary bristles (Kronfeld, 1889; Graebner, 1900; Fedchenko, 1934; Takhtajan, 1997), but the taxonomy of *Typha* remains confusing (Cook et al., 1974), because of the variability in its reproductive and vegetative characters (Smith, 1967; Grace and Wetzel, 1982), and its frequent hybridization (Smith, 1987; Kuehn and White, 1999; Kuehn et al., 1999). Recently, microscopic characters (e.g. stigma width) have been found to be reliable indicators of species identity, due to the absence of environmental selective pressures on these characters (Kuehn and White, 1999). However, these characters cannot be used in the case of immature or senescent plants, so the identification of sterile *Typha* plants can be uncertain (Sharitz et al., 1980). This has necessitated regional taxonomic revisions.

The problem of identification of *Typha* is further complicated by the development of morphological and physiological ecotypes. Geographical variation has been observed in physiological responses to temperature and salinity (McNaughton, 1966), in photosynthetic activity (McNaughton, 1967, 1973; McNaughton et al., 1974), in enzyme kinetics (McNaughton, 1965,1969) and in morphology and phenology (Bohmfalk, 1971; Lee, 1975; Suda, 1976). Most of these differences are not only apparent in *Typha* populations distributed across latitudinal
and altitudinal gradients, but they persist in plants transplanted to a common garden and exposed to the same environmental conditions.

The use of DNA sequence, morphological characters, and ecology to construct phylogenies for taxa of interest, and the use of this information to understand the processes of evolution is gaining momentum in recent years. By building DNA and morphology based phylogenies, it is possible to identify new species and uncover hidden relationships and patterns between species. The primary goal of this thesis was to characterize *Typha angustifolia* prevalent in and around the wetlands of Chennai, based on gross morphology, anatomy, biochemical constituents as well as by using modern molecular taxonomic tools namely the chloroplast DNA biomarkers. Biosystematic study also involves evaluating the plant species in terms of some biological attributes and towards this goal the allelopathic and insecticidal properties of *T. angustifolia* are also investigated.

5.1 Morphological and anatomical characteristics

*T. angustifolia* is a marsh herb with perennial creeping rhizome. The leaves are linear, erect or floating with sheathing leaf-base. The flowers are monoecious and occur on dense cylindrical spike. The male flowers are at the base of the spike and female and neutral flowers at upper portion. Anthers are four celled, and the one celled ovary is borne on a slender gynophore. Fruits are minute and fusiform.

A significant feature in the anatomy of the leaf of *T. angustifolia* is the presence of wide rectangular or squarish air-chambers in transverse row. The air-chambers are divided by thick vertical pillar of portions. The air-chambers apparently
look empty, but thin plate of stellate parenchyma cells have several arms spreading in all planes and the arms are interlinked with those of neighbouring cells, so that small air-spaces are formed within the parenchyma plate.

McManus et al., (2002) reported that the leaf lamina of *T. latifolia* and *T. glauca* has enlarged epidermal cells and a thickened cuticle above the subepidermal vascular bundles. *T. angustifolia* lacked these characteristics. Leaf sheaths were similar among the *Typha* species and all lacked the epidermal thickenings found in the lamina. The fertile stems had typical scattered vascular bundles with a band of fibres and this feature is most prominent in *T. glauca*.

The leaf lamina margin of *T. latifolia* was reported as oblong, often curved in shape and contains a zone of fibres at the margin with one vascular bundle embedded within the proximal curved zone of fibres (McManus et al., 2002). Along the abaxial and adaxial margins of the leaf, the subepidermal vascular bundles are interspersed with fibre bundles in the chlorophyllous mesophyll. The epidermal cells located above each vascular/fibre bundle are enlarged and thickly cutinized and these give the surface of the leaf a ridged or ribbed effect. However, the lamina margin of *T. angustifolia* is wedge shaped, has a thick zone of fibres at the margin and contains one to four vascular bundles embedded at the proximal edge of the zone. The subepidermal vascular bundles along the abaxial and adaxial margins of the leaf are interspersed with fibre bundles in the chlorophyllous mesophyll. The epidermis located above the vascular bundles lacks enlarged epidermal cells and extra thickened cuticle resulting in the surface being smooth to the touch.
*Typha glauca* has a lamina margin that is narrowly wedge shaped and contains a small zone of fibres at the margin. This zone of fibres contains one or two vascular bundles at the proximal edge (McManus et al., 2002). Along the abaxial and adaxial margins of the leaf the subepidermal vascular bundles are interspersed with fibre bundles and smaller vascular bundles in the chlorophyllous mesophyll that are more numerous than in the other two species.

Definitive anatomical characteristics were found primarily in the leaf lamina of *T. latifolia*, *T. angustifolia* and *T. glauca*. The lack of thickened cuticle and enlarged epidermal cells outside vascular and fibre bundles in *T. angustifolia* clearly distinguishes it from *T. latifolia* and *T. glauca*. Such anatomical differences were not observed by Saccardo (1895), Smith (1961) and Marsh (1962). Indeed, Smith (1961) claimed that there were no definitive anatomical differences in the leaves of these three species. However, there was a depiction of these traits in one of the illustration in Meyer (1933) but not in another for *T. angustifolia*. McManus et al., (2002) suggest that some of Meyer's (1933) specimens may have been the hybrid *T. glauca*. Observations, including the pattern of fibre bundles and marginal fibrous zones, show that the two species and the hybrid can be distinguished anatomically.

The rhizomes of *T. angustifolia* showed a pronounced band of fibres surrounding the central core. The rhizome was also characterized by an outer cortical region with a large multiseriate hypodermis/exodermis and a uniseriate endodermis with Casparian bands, suberin lamellae and secondarily thickened walls. McManus et al., (2002) report that the rhizomes of the *T. angustifolia*, *T. latifolia* and *T. glauca* are similar and consist of a uniseriate epidermis, an outer cortical region and a central
core. The cortical region consists of a multiseriate hypodermis, aerenchymatous
cortex with scattered, small bundles and a uniseriate endodermis. The hypodermis of
all three species has two distinct zones that contain suberin and lignin. There is
evidence of Casparian bands and suberin lamellae throughout the hypodermis, making
this an ‘exodermis’. All cells of the hypodermis have secondarily lignified walls.

The rhizomes of *T. angustifolia*, *T. latifolia* and *T. glauca* are basically similar
except for the characteristic band of fibres located outside the largest vascular bundles
of the central core in *T. angustifolia*. This band of fibres could possibly enhance the
strength of the cortex. The presence of an endodermis in wetland plants such as *Typha*
may not be unusual (Clarkson and Robards, 1975; Mauseth, 1988). While hypodermis
has been illustrated in rhizomes and hypodermal Casparian bands have been
demonstrated in *Typha* (Perumalla *et al.*, 1990), the exodermal nature of the
hypodermis has not been adequately demonstrated. Interestingly, these rhizomes are
very similar to *Typha* roots with a multiseriate exodermis and uniseriate endodermis,
each with Casparian bands, suberin lamellae and secondarily thickened walls
bordering an aerenchymatous cortical region. The thick, lignified hypodermal zones
of the roots (Seago *et al.*, 1999) and rhizomes of *Typha* plants may contribute to their
ability to form the durable mats characteristic of *Typha* marshes (Marsh, 1962).

Spike gap distance is a fairly diagnostic trait in that *T. angustifolia* typically
has a gap between the staminate and pistillate flowers and *T. latifolia* does not (Smith,
2000; Kuehn and White, 1999). The gap in *T. angustifolia* measured about 3.58 cm.
Both cattail species are protogynous, with female flowers opening first on the lower
end of the spike and male flowers releasing pollen after the female flowers begin to senesce.

5.2 Morphometric scaling relationships between different morphological characters

Different parts of a plant grow in relation to the whole, an aspect known as relative growth. The subject of allometry is variation in morphometric variables or other features of organisms associated with variation in size. Such variation can be produced by several biological phenomena that could be influenced by environmental factors as well as nutritional factors. Size of the leaf has been the primary basis for taxonomic distinction between different species of *Typha*. Therefore it becomes imperative to value size variation between individual plants collected from different localities and assess whether they belong to the same species or different species. As far as the *Typha* species is concerned the matter appears more complicated as hybrids are also available that are born as a result of cross pollination between two different species. Eleven morphological traits of *T.angustifolia* were considered of which six traits are vegetative characters and five reproductive characters. Analysis of the means of measurements of samples taken from four locations indicate that overall there were no morphological differences between the samples from the four locations with regard to these vegetative and reproductive traits. Therefore it could be construed that the plants in the four locations belong to the same species. The scaling of the relationship between the various traits of the plant species and the Pearson's correlation coefficient between trait pairs were determined to assess the relative growth of the different plant parts. The Number of leaves (NL) was significantly
correlated with width of leaf (WL), width of male inflorescence (WMI), and length of female inflorescence (LFI). Similarly, WL was significantly correlated with WMI and LFI; and WMI was significantly correlated with LFI. A hierarchical clustering routine analysis was conducted combining the significant morphological traits in order to visualize the difference in the morphological characters among the plants collected from the four locations. The sample collected from Ramavaram alone was slightly different from the other three locations. Principal component analyses (PCA) was performed to elucidate the components accounting for as much as possible of the variance in the multivariate data. Accordingly, the width of the leaf (WL) was the most important variable, followed by WMI, NL and LFI. This result is supported by the observations of Ohwi (1978) and Kadono (1996) who have reported that the species of the genus *Typha* can be more readily identified by the width of the leaf, length of the gap between male and female inflorescences, and length of female inflorescence. Further, the presence/absence of bracteoles, and the length of bracteole and female flowers were less important in delimiting the species (Kronfeld, 1889; Graebner, 1900). Kim et al., (2003) used principal components analysis (PCA) and UPGMA cluster analysis to determine taxonomically definable limits and to estimate the phenetic relationships among four *Typha* species using 25 quantitative characters. The clusters corresponded to the four currently recognized species. *Typha latifolia* was readily distinguished from other species by having wide leaves and female inflorescences. *Typha angustifolia* was distinguished from *T. orientalis* and *T. laxmanni* by the long male inflorescences and large gap between male and female inflorescences. *Typha laxmanni* was distinguished from *T. orientalis* by a higher ratio of male and female inflorescence lengths than others. The present study also indicated
that *T. angustifolia* could be characterized by the large gap between the male and female inflorescence. Plants of *T. orientalis* are similar to *T. angustifolia* in appearance except for the gap between male and female inflorescences. Individuals of *T. orientalis* were distinguished from those of *T. angustifolia* by the triangular shape in the cross-sectional leaf and by ebracteolate flowers as observed by Kim et al., (2003).

5.3 Molecular characterization using matK gene

Historically, plants have been identified based on flower, fruit, leaf and stem morphology covering only one third of the known plant species. Many plant groups, especially the grasses, are difficult to identify because they can be most readily distinguished only by the reproductive structures that are available for a short period each year. Species of *Typha* form the most dominant plant among the order Poales in the marsh land ecosystem. Currently about eleven species of *Typha* have been recorded worldwide. The identification of these species require highly skilled personnel and time-consuming procedures such as cellular characterization and enormous chemical tests. Under such circumstances, the future of plant identification lies in the development of DNA-based diagnostic systems for which the life stage or source of tissue is irrelevant. The slow mutational tempo and horizontal gene transfer in plants make mitochondrial barcodes less attractive for barcoding plants. The nuclear ITS regions suffer from practical difficulties associated with the existence of multiple paralogous copies in many plant taxa (Alvarez et al., 2003) and therefore of limited utility. The chloroplast genome has a slow tempo of evolution (Cho et al., 2005) and is a promising candidate for barcoding plants. The chloroplast coding regions namely,
ndhF, matK and rbcL and the non-coding regions namely, trnD-trnT and rpoB-trnC are presently being vouched as promising candidates (Shaw et al., 2005, 2007). Givnish et al., (2007) used the ndhF sequence to analyse the phylogenetic relationship of the members of the family Bromeliaceae. The matK gene of chloroplast is 1500 bp long, located within the intron of the trnK and codes for maturase like protein, which is involved in Group II intron splicing. The two exons of the trnK gene that flank the matK is lost, leaving the gene intact in the event of splicing. The gene contains high substitution rates within the species and is emerging as potential candidate to study plant systematics and evolution (Notredame et al., 2000; Takundwa et al., 2012). A homology search for this gene indicates that the 102 AA at the carboxyl terminus are structurally related to some regions of maturase-like polypeptide and this might be involved in splicing of group II introns (Sugita et al., 1985; Neuhaus et al., 1987; Mohr et al., 1993; Ems et al., 1995). It is another emerging gene with potential contribution to plant molecular systematics and evolution (Khidir and Hongping, 1997). The matK gene has ideal size, high rate of substitution, large proportion of variation at nucleic acid level at first and second codon position, low transition/transversion ratio and the presence of mutationally conserved sectors (Selvaraj et al., 2008). These features of matK gene are exploited to resolve family and species level relationships. Here an attempt has been made to study the matK gene sequence as molecular marker for identifying Typha angustifolia L., and using this gene for reconstructing its phylogeny.
5.3.1 RFLP analysis

The matK gene was extracted from the leaf of *T. angustifolia* from the four locations and subjected to digestion by BamHI restriction endonuclease. BamHI (from *Bacillus amyloli*) is a type II restriction endonuclease, having the capacity for recognizing short sequences (6 bp.) of DNA and specifically cleaving them at a target site. BamHI binds at the recognition sequence 5'-GGATCC-3', and cleaves these sequences just after the 5'-guanine on each strand. This cleavage results in "sticky ends" which are 4 bp long.

Four bands were observed for plants collected from Pulicat, Maduranthagam and Pallikaranai, but only three bands were observed for the plants from Ramavaram. The sequence alignment indicated that there was considerable similarity among the four sequence data with a high percentage of conserved regions. A cluster analysis using the Neighbor joining tree method showed that plants from three of the four locations were grouped together. There was slight variations in the sequence of the plants collected from Ramavaram, although the total base pairs of the matK gene was the same as that collected from the other three locations. Mutations in certain base pairs in the Ramavaram plants probably resulted in the BamHI not being able to cleave the DNA fragment.

5.3.2 matK partial gene sequence analysis

The partial nucleotide sequence for the matK gene of *T. angustifolia* had a total of 879 nucleotides. The base statistics for this sequence indicate the sequence had the maximum number of T nucleotides followed by the A nucleotide. Also the
percentage of A+T was more than double that of G+C. Guisinger et al., (2010) reported that the complete *T. latifolia* plastid genome has a 33.8% GC content. In the current study, for *T. angustifolia*, a GC content of 32.08% was recorded with reference to the *mat*K gene.

The sequence similarity search in the genbank database resulted in 17 hits for *Typha* represented by 4 species, namely *T. angustifolia, T. latifolia, T. domingensis, and T. capensis*. The alignment of the *mat*K sequences of *T. angustifolia* showed that among the 2768 base pairs (bp), 31.79% were conserved, and 0.002% was variable. However, Liang and Hilu (1996) studied the entire *mat*K sequence of eleven plant species and reported that among the 1581 base pairs (bp), 1086 (69%) were variable and 803 (51%) were phylogenetically informative. They observed several small indels, 3-15 bp along the entire length of the coding region that are mostly found in multiples of three nucleotides and opined that in most cases it does not result in a frame shift. The *mat*K sequence of *T. angustifolia* had a very low percentage of variable sites. The section of the sequence devoid of indels seems to be functionally important. This section of the gene corresponds to what is called “domain X”; a section that *mat*K genes share with group II intron maturases and is believed to reflect an essential function in binding of the intron RNA during reverse transcription and RNA splicing (Mohr et al., 1993; Ems et al., 1995). Again, this type of comparison between different species shows that the gene does not represent a homogenous unit in terms of amount of nucleotide variation.

In the present study, the nucleotide sequences were also translated into amino acids to compare the patterns of amino acid variation with those of the nucleotide
substitutions and to further evaluate the functional constraints on the gene. The 2768 nucleotides were translated to 922 amino acids and 267 (28.95%) were conserved and only 0.006% were variable. The percentage conserved sites at the amino acid level is slightly lower than that at the nucleotide level (28.95% Vs 31.79%), while no significant differences were observed for the variable sites. In functionally constrained genes, such as rbcL, nucleotide sequences are translated into lower amino acid variation. Johnson and Soltis (1995) reported in their study of the Saxifragaceae 5% amino acid variation for the rbcL. In the present study the low variation percentage observed indicate that the matK gene could be functionally constrained as the rbcL gene.

5.3.3 Relative synonymous codon usage

For any given protein, two sources of bias in the codon usage are present: 1) amino acid bias, which is due to the non-uniform distribution of amino acids in protein and 2) synonymous codon usage bias, which is the uneven distribution of synonymous codons, i.e., various synonymous codons are not equally used to represent a given amino acid. Within the standard genetic codes, all amino acids except Met and Trp are coded by more than one codon. DNA sequence data from diverse organisms clearly show that synonymous codons for any amino acid are not used with equal frequency, even though choices among these codons are equivalent in terms of protein sequences (Grantham et al., 1980; Aota and Ikemura, 1986; Murray et al., 1989; Sharp et al., 1988; Shields et al., 1988; D'Onofrio et al., 1991). The relative frequency of synonymous codons varies with both the genes and the organisms.
Due to the degeneracy of genetic code, most amino acids are coded by more than one codon (synonymous codon). Studies on the synonymous codon usage can reveal information about the molecular evolution of individual genes. RSCU values are the number of times a particular codon is observed, relative to the number of times that the codon would be observed in the absence of any codon usage bias. In the absence of any codon usage bias, the RSCU value would be 1.00. A codon that is used less frequently than expected will have a value of less than 1.00 and vice versa for a codon that is used more frequently than expected (McInerney 1998). Details of the RSCU for the \textit{matK} gene among the species of \textit{Typha} studied here indicate the average number of codons observed here is 332. The codons TTA (Leucine), CCA (Proline) and AGA (Arginine) had high usage bias in the sequence. Different factors have been proposed to be related to codon usage bias, including gene expression level (reflecting selection for optimizing translation process by tRNA abundance), %G+C composition (reflecting horizontal gene transfer or mutational bias), GC skew (reflecting strand-specific mutational bias), amino acid conservation, protein hydropathy, transcriptional selection, RNA stability, optimal growth temperature and hypersaline adaptation (Ermolaeva, 2011; Lynn \textit{et al}., 2002; Paul \textit{et al}., 2008). Molecular evolutionary investigations suggest that codon usage bias is widespread across genomes and may contribute to genome evolution in a profound manner (Sharp and Matassi, 1994). In the post-genomic era, investigations are now directed towards analyzing the codon bias pattern in a holistic manner throughout the genome rather than looking at specific genes or sets of genes to understand patterns of codon bias better within and among genomes (Plotkin and Kudla, 2011).
### 5.3.4 Nucleotide substitutions per site

In the study of molecular evolution, it is important to know the number of nucleotide substitutions per site (d) between DNA sequences. Two important factors that are considered in the estimation of d are the inequality of the rates of transitional and transversional nucleotide substitution (transition-transversion bias) and the deviation of the G+C content from 0.5 (G+C-content bias) (Sanjayan and Rama, 2013). The nucleotide pair frequencies computed for the data of the different *Typha* species showed an average of 796 identical pairs out of a total average of 798.33 of which there were 302 TT pairs, 245 AA pairs and 115 GG pairs. The ratio of the Transitional pairs versus Transversional pairs was 1.25. When two DNA sequences are derived from a common ancestral sequence, the descendant sequences gradually diverge by nucleotide substitution. A simple measure of the extent of sequence divergence is the proportion of nucleotide sites at which the two sequences are different. This is estimated as the p-distance for nucleotide sequence. It is useful to know the frequencies of different nucleotide pairs between the two sequences. Since there are four nucleotides, there are 16 different types of nucleotide pairs. There are four pairs of identical nucleotides (AA, TT, CC, GG represented as O), four transition-type pairs (AG, GA, TC, CT represented as P) and remaining 8 transversion-type pairs (represented as Q). The p distance for nucleotide sequence, given by the relationship p = P + Q was calculated to be 3 (ie. 2+1). If nucleotide substitution occurs at random, Q is expected to be about two times higher than P when p is small. This was not the case in the present investigation. In general, transition usually occurs more frequently than transversions. Therefore P may be greater than Q. When the extent of divergence is low, the ratio (R) of transitions to transversions can be estimated from the observed
values of P and Q. R is usually 0.2 2 in many nuclear genes, but in mitochondrial DNA it can be as high at 15 (Vigilant et al 1991). In the present study the value of R was 1.25. The analysis of the p value indicate that no synonymous substitution occur in the first three codons (p for 1st codon is 0.01, 2nd codon could not be calculated, and 3rd codon is 0.01). Transitions are generally more frequent than transversions (Quicke, 1993). Transversions are considered the more reliable type of mutations in constructing phylogenies (Quicke, 1993). Consequently, some workers have assigned more weight to transversions in phylogenetic analyses, or based the analyses on transversions alone, resulting in what is called transversion parsimony (Quicke, 1993; Lake, 1987). Transition/ transversion ratios have been observed to be 2.0 for relatively recently diverged sequences and exceed 0.4 for highly substitution saturated sequences (Holmquist, 1983). The ratio of transition to transversion (nr/nv) ranged from 0.39 between the two pine species to 1.35 between Pinus contorta and Sullavantia sullivantii (Saxifragaceae). The rate of transversion substitutions must be influenced by other factors, possibly intrinsic genetic or external environmental. The likelihood of transversions was also found to be influenced by the GC content (Mortan, 1995; Liang and Hilu, 1996). If this is true, then transversions, and subsequently nr/nv values, might not represent particularly conserved characters in phylogenetic studies, but are rather a product of an intrinsic nucleotide composition pattern that characterizes a lineage. This was true for some lineages of the grass family (Johnson and Soltis, 1995).
5.4 Phylogenetic reconstruction

All methods of phylogenetic inference depend on their underlying models. The maximum Likelihood of 24 different nucleotide substitution models were computed and since the T92+G had the least BIC score it was considered the best model for the present data set. Another way of selecting the most appropriate model for a data set is to use the Akaike information criterion (AIC) (Akaike, 1974), which can be thought of as the amount of information lost when a particular model is used to approximate reality. The AIC implements best-fit model selection by calculating the likelihood of proposed models, and imposing a penalty based on the number of model parameters. Parameter-rich models incur a larger penalty than more simple models so that fitting an excessively complex model is not likely. The best fitting model is the one with the smallest AIC value. The T92+G had the smallest AIC value for the present data set.

Evolutionary analyses conducted in MEGA5 reveals a low estimate of evolutionary divergence between the sequences among the 13 species of plants studied. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura 3- parameter model. \emph{T.capensis}, \emph{T.aungustifolia} and \emph{T.latifolia} share a common ancestor among themselves with \emph{T. domingensis} being a closely related species. Again, all the species of \emph{Sparganium} were grouped together in terms of their similar number of substitutions per site values and all the other remaining plant species formed a different clade. In the phylogenetic hierarchy, the genera \emph{Typha} and \emph{Sparganium} were clustered at a further distance to the Bromeliaceae member \emph{Portea leptantha}. Graham \textit{et al.} (2006) performed a phylogenetic analysis of 17 plastid protein-coding genes and included taxa from
Poaceae, Typhaceae, and eight additional Poales families. They observed that the branch lengths for most members of the Poales were long except for the three earliest diverging families, including Typhaceae. The Sparganiaceae are closely related to the Typhaceae and the APG III system (2009) includes Sparganium in that family. It has been determined from phylogenetic analysis to be the closest living relative of the genus Typha. Sparganium is a genus of about 18 species largely distributed in the temperate and cool regions with a couple of species extending to the tropical regions. Sparganium confertum is of great phylgoenteic importance because, apart from its habit, it has certain characters, such as spike-like inflorescence, pellucid and membranous scales with lobed margins, stalked ovary and sterile female flowers on pistillate heads, which suggest closeness with Typha, especially, T. orientalis. According to Chen Yao-dong (1981), the discovery of S. confertum as a connecting link between Sparganium and Typha makes it unacceptable that they are treated as two separate families.

The utility of matK at deep-level phylogenetics was established in a collaborative study using a large data set of 374 genera representing all angiosperm orders and 12 gymnosperm genera (Hilu et al., 2003). Although the data set included only two thirds of the gene, the resolution and support obtained by partial matK was quite impressive. The relationship discerned from the partial sequences of the matK gene matched those that used sequences from three to 11 genes combined. In phylogenetic reconstructions the matK is so informative either because of the large number of variable sites or the signal per site in matK that renders the gene to be superior to other genes used in deep-level phylogenetics. In a study conducted in which sequences of the slowly evolving rbcl, the rapidly evolving trnL-trnF spacer
regions, and matK (all plastid genomic regions) from the same 42 species representing mostly early diverging flowering plant lineages (Muller et al., 2006) were analysed, the matK provided about 3 times as many parsimony informative sites as rbcL (287 vs 862), which correspond to 24 vs 52% of the variable characters found in the two genomic regions. The phylogenetic trees based on the individual genomic region matK and trnL-trnF sequences provided far higher resolution and support compared with the one based on rbcL (Muller et al., 2006). The matK not only provides more parsimony informative characters, but also the information per site is statistically stronger than that obtained from rbcL. Hence the usage of matK to draw a phylogenetic tree of T.angustifolia is justified.

5.5 Biochemical and Pharmacognistic characters

In the field of taxonomy, cytotaxonomical research has proved to be often successful. It may be an invaluable aid for an unambiguous identification of distinct entities and in many instances it has offered even a clue for a better understanding of the past history of such puzzlingly complex aggregates. Frequently past history gave rise to slightly differing metabolic patterns in members of a species aggregate. The study of their chemical constituents may therefore bring to light new characteristics helpful in identification. It is evident that each botanical study concerning present-day distribution, ecological preferences and past history of members of an aggregate species or of several closely related species of a genus depends on the unambiguous identification of each available specimen. Unfortunately morphological characters are often rather vague and cytological work is restricted to living plants. Moreover clear cut distinctive morphological characters may be restricted to organs many often
lacking in the available plant specimens. *Nasturtium officinale* R.Br. and *Nasturtium microphyllum* (Boenningh.)Rchb., for instance, can only be identified with certainty if mature fruits and seeds are present and the three sub-species of *Sparganium erectum* L. are identifiable by their fruits only. A thorough study of the chemistry of each member of such aggregates and the elaboration of analytical methods can be useful to plant taxonomy.

The study of chemical characters at infraspecific and specific levels are very fascinating. Besides being helpful with the identification of plant specimens it informs us about patterns of chemical variation within genera and aggregate species and it may ultimately demonstrate how one pattern of plant constituents evolved from a preceding one. Moreover, joint botanical and phytochemical studies may provide us with a better understanding of the biological and ecological meaning of distinct spectra of primary and secondary plant metabolites. A thorough knowledge in these fields is essential for a judgement of the overall taxonomic implications of the overwhelming multitude of phytochemical patterns. A preliminary investigation was therefore made on the biochemical and pharmacognistic characteristics of *T. anugstifolia*.

Proximate analysis facilitates in evaluating the plant as a potential source of not only a medicinal drug but also as an insecticide. The total ash content of the plant material mainly is a measure of the presence of inorganic compounds. A larger value indicates that the plant material contains more of inorganic compounds. The leaf had the highest percentage of total ash followed by the rhizome and the inflorescence and a similar trend was seen for the acid insoluble ash. The high value of Acid insoluble
ash content indicates the presence of less number of calcium oxalate crystals in the plant material. Water soluble ash content gives the crude estimate of the water soluble extractable matter present in the ash. The inflorescence had the maximum percentage of water soluble ash followed by the rhizome and the least in the leaf. The relatively high total ash values observed in the present study indicate a high content of physiological ash.

A preliminary screening for the presence of various secondary metabolites in *T. angustifolia* was carried out. The chemical tests revealed the presence of major secondary metabolites such as alkaloids, flavonoids, sterols, reducing sugar, tannins, phenols etc in the extracts. Sterols were found to be present in the extracts of all the three plant parts tested, while the presence of glycosides were not evident.

Alkaloids function in the defence of plants against herbivores and pathogens, and are widely exploited as pharmaceuticals, stimulants, narcotics, and poisons due to their potent biological activities. In nature, the alkaloids exist in large proportions in the seeds, and roots of plants and often in combination with vegetable acids. Alkaloids have pharmacological applications as anesthetics and CNS stimulants (Madziga *et al.*, 2010). Verma *et al.*, (1986) reported that alkaloids present in plants inhibited feeding of *Spodoptera litura*. Feeding behavioural experiment showed that alkaloids acted as potent feeding deterrents in lepidopteran larvae (González-Coloma *et al.*, 2004; Kathuria and Kaushik, 2005).

Saponins are regarded as high molecular weight compounds in which, a sugar molecule is combined with triterpene or steroid aglycone. Saponins are soluble in water and insoluble in ether, and like glycosides on hydrolysis, they give aglycones.
Saponins are extremely poisonous, as they cause hemolysis of blood and are known to cause cattle poisoning (Kar, 2007). They possess a bitter and acrid taste, besides causing irritation to mucous membranes. Saponins are also important therapeutically as they are shown to have hypolipidemic and anticancer activity. Saponins are also necessary for activity of cardiac glycosides. Barbosa et al., (1990) proved that saponins have antifeeding activity as is the case of saponins extracted from *Ix aproceae* which inhibit the food uptake of *Lymantria dispa* L.. Antifeeding activity of saponnin on mite species (*Oligonychus illicis* (McGregor) and two caterpillar’s species (*Hyphantria cunea* and *Malacosoma americanum* (Fab.)) were explained by Kreuger and Potter (1994) and Oleszek et al., (1999).

Tannins are widely distributed in plant flora. They are phenolic compounds of high molecular weight. They form complexes with proteins, carbohydrates, gelatin and alkaloids. Tannins are used as antiseptic and this activity is due to presence of the phenolic group. In Ayurveda, formulations based on tannin-rich plants have been used for the treatment of diseases like leucorrhoea, rhinorrhoea and diarrhea.

Flavonoids are important group of polyphenols widely distributed among the plant flora. Over four thousand flavonoids are known to exist and some of them are pigments in higher plants. Quercetin, kaempferol and quercitrin are common flavonoids present in nearly 70% of plants. Other group of flavonoids include flavones, dihydroflavons, flavans, flavonols, anthocyanidins proanthocyanidins, calchones and catechin and leucoanthocyanidins.

Phenolics, phenols or polyphenolics (or polyphenol extracts) are chemical components that occur ubiquitously as natural colour pigments responsible for the
colour of fruits of plants. Phenolics in plants are mostly synthesized from phenylalanine via the action of phenylalanine ammonia lyase (PAL). They are very important to plants and have multiple functions. The most important role may be in plant defence against pathogens and herbivore predators, and thus are applied in the control of human pathogenic infections.

Plant steroids (or steroid glycosides) also referred to as ‘cardiac glycosides’ are one of the most naturally occurring plant phytoconstituents that have found therapeutic applications as arrow poisons or cardiac drugs (Firn, 2010). The cardiac glycosides are basically steroids with an inherent ability to afford a very specific and powerful action mainly on the cardiac muscle when administered through injection into man.


GC-MS studies facilitated identifying the chemical present in the various extracts of the leaf, rhizome, and inflorescence of *T. angustifolia*. *T. angustifolia* is characterized by the presence of at least 26 different chemicals at various parts of the plant. Some of the compounds significantly identified were Hydroxymethyl colchicines; Docosahexaenoic acid; 2(3H)-Furanone, duhydro-5-tetradecyl; Dasycarpidan-1-methanol; Ascorbic acid 2,6-dihexadecanoate; and Propanoic acid.
Compounds present in the ethylacetate extract of *T. angustifolia* appeared to be the most biological potent in the present study.

### 5.6 Allelopathic properties of *T. angustifolia*

Investigations of allelopathic activity have often been initialised by field observations mainly related to changes in agricultural, horticultural or silvicultural productivity or to changes in vegetation patterns in natural habitats. Occurrence of monocultural strands of *T. angustifolia* is a common feature and this points to the fact that the plant is allelopathic. In cases where the success of a plant, typically a weed, can not be explained by the competitive ability, allelopathy has been suspected to play a role. Investigations of such observations have established or strongly indicated an allelopathic activity of weeds, e.g. *Avena fatua* (wild oat), *E. repens* (quackgrass), *Cirsium arvense* (Canada thistle) and *Stellaria media* (common chickweed) (Putnam and Weston 1986, Seigler 1996, Inderjit and Dakshini 1998).

Several researchers have suggested that allelopathy holds great prospects for finding alternative strategies for weed management. Thereby, the reliance on traditional herbicides in crop production can be reduced (An *et al.*, 1998; Inderjit and Keating, 1999, Macias, 1995; Macias *et al.*, 1997; Olofsdotter, 1999; Wu *et al.*, 1999).

In several bioassays, seed germination and seedling development is measured after the exposure to alleged allelochemicals because seed and seedlings development is generally considered to be the most susceptible stages (Leather and Einhellig, 1986; Putnam and Tang, 1986; Inderjit and Olofsdotter, 1998) and therefore used as an effective parameter for demonstrating allelopathy. In this study, the black gram, *Vigna*
mungo (L)Hepper was used to evaluate the allelopathy of the aqueous extracts of the cattail. The aqueous extract of all parts of T. angustifolia tested inhibited germination of the seeds of V.mungo. The percentage inhibition was also dose dependant. Extracts of the Inflorescence showed the highest inhibitory potential (48%) followed by the leaf (29%) and the rhizome (20%). A similar hierarchy in the inhibitory property of the extracts of various plant parts was reported by Neelamegam, (2011) who tested the extracts of Ixora coccinea Linn. on seed germination and early seedling growth of paddy, Oryza sativa L. Femina et al., (2012) also reported on the allelopathic effect of the weed, Tridaxprocumbens L, on seed germination and seedling growth of some leguminous plants. Utilizing aqueous extracts have always found favour among agricultural farmers rather than using organic solvents. In this context, the results obtained here with the aqueous extracts of T. angustifolia has greater acceptance. However, in allelopathic studies while using seed germination as a test parameter, other bioassay conditions such as test species, light conditions, osmotic potential and interactions between these factors strongly influence the result as pointed out by Haugland and Brandsaeter, 1996). Factors such as seed size, seed dormancy and the length of the after-ripening period to which the seed has been subjected can influence on the concentration of allelopathic compound necessary to produce an effect on seed germination (Pérez, 1990). Therefore higher percentage of inhibition of seed germination using the aqueous extracts of T.angustifolia can be achieved by increasing the concentration of the test extract. Thicumporn Yongvanich, (1997) attributed the inhibitory effect of T. angustifolia on the seed germination of Mimosa pigra to be due to the condensed tannins.
Aqueous extracts of the rhizome and inflorescence of *T. angustifolia* at 100% concentration showed the maximum reduction (76.70% and 85.60%) in the growth of the roots while the extracts of the leaves were significantly lesser. Similar results were observed for the shoot length with the extracts of the inflorescence and rhizome being more allelopathic than the leaf. Meghann and Cook (2009) found that *T. angustifolia* had a strong allelopathic effect on *Batasio fluviatilis*, reducing the root and shoot growth. Some recent studies indicate the reduction of root and shoot length to be due to phytotoxic/allelopathic effect of plants such as *Tectona* (Rincy et al., 2012) and *Parthenium hysterophorus* (Batish et al., 2002; Singh et al., 2003).

Allelopathic property of *T. angustifolia* was also assessed in terms of reduced biomass of the shoots and roots due to treatments. Studies by Eyini et al., (1996) revealed that the leaf extracts of *Tephrosia purpurea, Albizia amara* and *Delonix regia* caused a decrease in the biomass of maize.

Present investigation demonstrated the allelopathic effects of *T. angustifolia* by studying the percentage germination of seeds of *V. mungo* and assessing the growth of the roots and shoots and their biomass on exposure to different aqueous concentrations of the extracts of rhizome, leaf and inflorescence. Although all these plant parts had allelopathic effect, the inflorescence appeared to be the most potent part of the plant with allelopathic properties. Meghann and Jarchow. (2009) reported that *T. angustifolia* competes with native plant species and becomes invasive by producing different phenolic compounds. It is now construed that a mixture of allelochemicals would have a greater effect than a single compound. Furthermore, some experiments have indicated that mixtures of some allelochemicals such as
phenolics and organic compounds such as carbohydrates, and aminoacids can possess allelopathic activity even though concentrations of individual compounds are significantly below their inhibitory levels.

5.7 Insecticidal properties of *T. angustifolia*

Plants produce a wide spectrum of chemicals; many of which have not been explored for their physiological properties (Norduland and Sauls, 1981). Phytochemicals are known to specifically inhibit growth, morphogenesis, metamorphosis and reproduction (Ahmad, 2007). Currently there is resurgence of interest in plant derived compounds for developing them commercially as eco-friendly insecticides. Tropical plants are more promising for the development of new insecticides (Jacobson and Crosby, 1971). Despite, the fact that hundreds of tropical plants are reported to possess insecticidal property, only few compounds have been commercialized (Chopra et al., 1994).

Toxicity of a chemical is usually expressed in relative toxicity. All chemicals, even those generally considered nontoxic, can become toxic depending on the dosage given to an organism. In experiments based on quantal response, the data needed are the proportions of each batch responding to the compound in a particular way. The purpose is to estimate the dose level that is just sufficient to produce death within the given proportion of insects and to use the estimate to make comparisons. It is generally easiest to estimate the median (50%) response level of the population. LC\textsubscript{50} is used for a 50% lethal dose. The higher the LC\textsubscript{50} value, the lower the toxicity. The usual way to estimate LC\textsubscript{50} is from a regression line relating log dose to a transformed percentage response (Busvine, 1971) and the usual transformation used is probits.
A preliminary assay using aqueous extracts of the rhizome, leaf and inflorescence was undertaken to evaluate the concentration required to bring about mortality of the larvae. Topical application of the extract was adopted and the mortality recorded after 24hrs of application. A regression line fitted between the log dose and mortality for the aqueous extracts indicate that topical application of the extracts did not bring about significant mortality of the larvae. Therefore feeding bioassays were conducted to determine the LC50 value. All the extracts tested brought about mortality of the larvae and these values were significantly different from the control group except in the aqueous extracts. The ethyl acetate extract of the leaf (LC50 = 2.023%) and the methanol extract of the inflorescence (LC50 = 2.033%) appeared to be the most potent extract bring about high mortality at lower concentrations. Comparatively the extracts from the rhizome brought about lesser mortality of the larvae. Baskar et al., (2010) also reported that the ethyl acetate extract of Couroupita guianensis (Aubl.) showed promising antifeedant activity of 66.68% and 69.70% against H. armigera at 2.5% and 5% concentrations, respectively.

Simmonds et al., (1990) has reported high antifeedancy for pure compounds isolated from different plants against the larvae of H. armigera. Janarthanan et al., (1999) showed that 0.2 and 0.5% petroleum ether extracts of Parthenium histerophorus L. exhibited 100% feeding deterrence in H. armigera. Similarly, aqueous extracts of Calotropis procera and Daturastromonium have been shown to display about 90% feeding protection against H.armigera (Dodia et al., 1998). All the three solvent extracts of T.angustifolia appeared to deter H. armigera from feeding in the present study.
Jaglan et al., (1997) evaluated the effect of *Azadirachta indica* extracts against *H. armigera* and reported that Chloroform : methanol (9:1) extracts of Neem seed kernels and leaves showed better insecticidal properties than methanol extracts. Similar observations were made by Murugan and Babu (1998) on growth and feeding physiology of *H. armigera* larva on extracts of *Ricinus communis L.*, *Glycosmis pentaphylla* (Retz.), *Vitex negundo* L. and *Nerium oleander* L. Murugan et al., (1998) reported that larvae fed on 0.3% ethyl acetate fraction of *Glycosmis pentaphylla*, *Vitex negundo* and *Nerium oleander* showed significant effect on weight gain of *H. armigera* larva. The aqueous leaf extracts of *Gnidia glauca* (Fresen.) showed more than 50% larval mortality at 0.8-1.0% and 86.1% mortality observed at 1.0% on *Toddalia asiatica* L. extract against the sixth-instar larvae of *H. armigera* (Sundararajan and Kumuthakalavalli, 2001).

The ability of an organism to convert nutrients, especially protein, will positively influence its growth and development (Sogbesan and Ugwumba, 2008). Differences in allelo-chemical concentrations can affect an insect’s performance as larva (Martin and Pulin, 2004). In the present study the CR, GR and ECI decreased after feeding the larvae with the extracts. Among the nutritional indices, ECI may vary with the digestibility of food and the proportional amount of the digestible portion of food which is converted to body mass and metabolized for energy needed for vital activity (Abdel Rahman and Al-Mozini, 2007). ECI is an overall measure of an insect's ability to utilize the food ingested for growth and development. The present study indicates that the inflorescence of *T. angustifolia* has potent insecticidal properties.