EFFECT OF HERBICIDES, GROWTH CONDITIONS AND CARRIERS ON PHYSIOLOGICAL, BIOCHEMICAL AND ANATOMICAL CHANGES IN EUPATORIUM (*Chromolaena odorata* K & R)

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BY
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C E R T I F I C A T E

This is to certify that the thesis entitled **"EFFECT OF HERBICIDES, GROWTH CONDITIONS AND CARRIERS ON PHYSIOLOGICAL, BIOCHEMICAL AND ANATOMICAL CHANGES IN EUPATORIUM (*Chromolaena odorata* K & R.)"** submitted by **Mr. UMESH V. MUMMIGATTI** for the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **Crop Physiology of the University of Agricultural Sciences, Dharwad,** is a record of research work done by him during the period of his study in this University under my guidance and supervision, and the thesis has not previously formed the basis of the award of any degree, diploma, associateship, fellowship or other similar titles.

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Affectionately
Dedicated
To
Beloved
Well Wisher's

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To my knowledge, it is the unique section in the thesis which goes unedited and uncensored, by the Advisory Committee and is left as a previledge and perogative of the author and hence I am here to express free and frank opinion based on my experience. For the deeds, the credit goes to my esteemed "Advisory Committee, Well-wisher's, Beloveds, Nearests and Dearests, and the MISDEEDS TO ME ALONE".

I regret for such short and brief acknowledgement and if it has hurt the feelings of any one, I once again apologise, since the feelings are so much so imprinted in my heart which agitated to depart at the time of writing this section.

Dharwad
Date : 17-10-1994

(U.V. Mummigatti)

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I. INTRODUCTION

I INTRODUCTION

From time immemorial, weeds have claimed their own share in soil fertility and productivity of food and other crops. Despite several advances in weed control technology in the past 40 years, weeds still remain as major problem to agricultural production and often agriculture has been defined as confrontation with weeds. Erratic rainfall, unabated soil erosion and decline in fertility in addition to weed menace threaten the very establishment and sustenance of forests, plantations and agricultural ecosystem.

The invasion of Eupatorium (*Chromolaena odorata* K.& R.~ *Eupatorium odoratum* L.) from neotropical world into tropical regions of old world, like India and getting established as noxious weed, has resulted in complete suppression of plantations, regeneration of plant species has posed a serious threat for establishing aforested areas and grazing lands. This weed, a few years back, was not considered to be of much importance, has now become a menace due to its alarmingly increasing intensity day by day.

Eupatorium a member of Asteraceae, is a composite, native to West Indies and continental tropical America. It is thought to be introduced to India in the early part of this century through navigators. Due to its aggressive invasive

nature, hardiness and wide adaptability to various agro-climatic conditions, it is encroaching fast to new areas and has become ubiquitous in relatively short period, posing a serious threat for its effective control. In India, eight species of Eupatorium are reported and of which, only two species viz., *Chromolaena odorata* K. & R. (Siam weed) and *Eupatorium adenophorum* L. (Craften weed) have attained the obnoxious status in Western Ghats and Eastern Himalyan ranges, respectively. Other species include *E. capillifolium, E. chinense, E. lingutrinum, E. nodiflorum, E. riparium* and *E. triplinerve* (Malhotra and Jain, 1978).

Eupatorium is a fast growing, woody and much branched shrub attaining a height of three to eight meters. In India, it has become a serious threat to aforestation programmes especially in the states of Assam, Nagaland, West Bengal, Orissa, Tamil Nadu, Kerala, Karnataka and Goa. The rapid spread of Eupatorium is attributed to its capacity to produce numerous seeds that can travel to long distance through wind (Mogali, 1991).

Eupatorium can suppress young seedlings that are slow in growth and deprive them of a major share of nutrients, moisture and space due to its efficient tap root system. Hence it tends to smother practically all other plants and grasses causing interference in plantation development and regeneration of tree coppices in forests. Recently, its

invasion in aerable lands causing nuisance in farming community has been reported by Mumbareddy (1989). The inhibitory nature of this weed on other crops/plants is attributed to its allelopathic effect (Doddamani, 1992). In addition, it has posed a threat to tobacco industry in Sumatra by harbouring *Psuedomonas* (Burnet and Rao, 1968). It has eclipsed the natural grass lands to a great extent causing acute shortage of fodder. It presents difficulties in harvesting coffee, cashew and cassava (Akobundu, 1979). Because of its poisonous nature, the natural species of Eupatorium cause trembles disease in cattle, horse and sheep and milk sickness in human beings (Chopra *et al.*, 1965). Dry stalks of Eupatorium cause fire hazards in forests during summer. Looking into all these problems created by this weed, there is an urgent need to take deterrent steps to control this obnoxious weed, so as to restore balance among interacting ecosystems.

Conventional methods like hand cutting or burning which are widely adapted for controlling Eupatorium have their own limitations, owing to laboriousness, time consuming, expensive, ineffective in checking the regeneration and spread of Eupatorium. Constantly increasing cost of labour and the compulsions of modern agriculture are helping herbicides to come to the fore front and are now catching imagination of the farming community. Although, biological

method of control seems to be feasible, but it is precluded by problems like pest identification, host specificity, pest multiplication and its survival under varying environmental conditions. Under such circumstances, only chemical method offers most practical and feasible solution to control this perennial weed. Infact, several herbicides are capable of controlling broad spectrum of weeds but the selection of best individual herbicide from the economic point of view is most important.

Eupatorium can not survive under dense and already established forest and plantations due to heavy shading (Mumbareddy, 1989). Response of this weed to varying light regimes, with respect to anatomical and physiological adaptations helps to explain its competitive ability and to evolve effective control measures.

Agriculture is most intimately connected with plant chemistry. The relationship between chemical constituents of plants and their physiological activity has been the subject of intensive study and speculation. In this context, changes in biochemical and physiological parameters of plants resulting from the herbicide spray, help to explain how weeds can be killed by spraying herbicides. Due to the presence of cuticle, herbicide penetration into the plant system can be a serious problem while using the foliar applied herbicides,

which leads to decreased efficacy. Cuticular penetration also depends on the environment which infringes on the plant at the time of spray. A more constructive approach is to incorporate carriers with spray solutions in order to maximise herbicide penetration and translocation into the plant system.

The problem of effective control of Eupatorium has become an urgent concern, particularly because most of the human beings and animals are allergic to it. Several herbicides have been tried to control this weed, but the available information on the effective concentration, time of application and use of best carriers is very meagre. Keeping all these views in mind, present investigation was undertaken on Eupatorium with the following objectives,

1. To find out the effective herbicide and its concentration to control naturally grown Eupatorium,
2. To find out the critical time of application during the weed growth,
3. To find out the physiological, biochemical and anatomical changes in Eupatorium plants grown under light and shade and their response to herbicide spray,
4. To find out the most effective carrier so as to increase herbicide efficacy and reduce the cost and
5. To find out the possibility of reducing viable seed production capacity of Eupatorium by using herbicides.

II. REVIEW OF LITERATURE

II REVIEW OF LITERATURE

Since time immemorial, weeds have been causing great losses in agriculture production. There are several reports, indicating the physiological and biochemical changes brought about by spraying different herbicides and altering the growth conditions for effective control of weeds. Much information is available on chemical weed control in several field crops which are specific to the most common and a particular weed incidence in a particular crop. Recently an obnoxious weed, Eupatorium which has been introduced from neotropical world has became the centre of attraction in forest plantations and other horticulture gardens due to its aggressive and fast growth rate. The information available on the control of this weed and the influence of several herbicides at different concentrations and the growth conditions favourable for its effective growth is very meagre. An attempt has been made to gather all the relevant information on the physiological, biochemical, morphological and anotomical changes due to the application of herbicides in different concentration in Eupatorium and other weed species and is presented in this chapter.

2.1 Chemical control of Eupatorium

Agricultural technology has recently made considerable progress in many ways, especially in chemical weed control. Although, the use of herbicides in agriculture started only 40 years ago, its intensive practice these days has provoked one of the most radical changes that agriculture has ever experienced. Herbicides have been of great help in controlling weeds with certain limitations like residual toxicity in the soil, which is deleterious to several crops and presents planting for several months. Broad spectrum herbicides without residual toxicity would be of added advantage. Any herbicide proposed control Eupatorium should be such that it should be translocated into plants, kill the entire plants including the below ground rhizome like roots. Economic feasibility of the use of herbicide(s) should also be taken into account. It should be safe for soil microbes and animals. The herbicide that satisfies these requirements could be suggested for practical use. Hence, efforts are being made by several workers find out such of these chemical and an attempt has been made to review the available literature on control of Eupatorium in this chapter.

George (1968) reported that 0.1 per cent paraquat and 1.5 per cent 2,4-D were lethal to Eupatorium after three days under laboratory conditions. Whereas, in the field, good results were obtained with 1.5 kg/ha paraquat or 2,4-D spraying

at 17.5 kg/ha. Further he stated that spraying should be taken before the flowering of Eupatorium. Edberink (1969) suggested that the application of dimethyl urea at 20 kg/ha is effective in controlling Eupatorium.

Results of the field experiment conducted by Nair (1973) on shade and open grown Eupatorium with 0.01 to 0.3 per cent paraquat indicated that, partial killing of the weed was noticed with 0.15 per cent in shade whereas, in open condition, even the highest concentration failed to kill as the woody portions of the weed remained alive after treatment and new sprouts appeared after some time. He opined that manual control is effective in terms of economy as compared to paraquat.

Ivens (1974) reported that foliar spray of attrazine and diuron @ 5.0 kg/ha gave a high degree of root kill of Eupatorium plants when treated at early stage of growth. Similarly, Olaoye (1974) reported that diuron at 5.6 kg/ha, gave superior control of Eupatorium seedlings.

The foliage of Eupatorium is very susceptible to growth regulator type herbicides, but the control of whole plant does not appear to be easy (Ivens, 1974). 2,4-D ester or amine or 2,4,5-T @ 2 kg/ha, caused severe dieback of plants of 80 cm height and killed smaller plants. Nevertheless, on aged plants, phenoxy herbicides are less effective and stronger concentrations of the chemical will be required.

The most effective combination of herbicides for the control of Eupatorium is picloram and 2,4-D (Ivens, 1974, Olaoye, 1974; Dufer and Quencez, 1978). Ivens (1974) observed that the weed was eliminated by picloram at 0.4 kg + 2,4-D amine at 1.6 kg per ha after six months of application. The herbicides were much more effective on plants that were allowed to resprout after slashing than on unslashed thickets. Picolram persisted in the soil for seven months when applied @ 20 kg/ha while 2,4-D is less persistent.

The pot experiment conducted by Risidino (1975), indicated that the application of Tordon 101 (picloram 10% + 2,4-D 40%), tordon 105 (picloram 5% + 2,4,5-T 20%) and tordon 155 (picloram 12%+ 2,4,5-T 48%) at 1.0 kg/ha was very effective to control *E. odoratum* and was not toxic to *Brachiaria brizantha*, an introduced pasture grass in Indonesia. Picloram at the rate of 1.0 kg/ha was also effective after the 12th week but 2,4-D at 2 kg and 2,4,5-T at 1.0 kg/ha were less effective.

Studies made by Rai (1976) in rubber plantations and semi-evergreen forests indicated that control of Eupatorium could be achieved by spraying the combination of paraquat and 2,4-D three to four times a year. Further, he stated that the chemical control is prohibitively expensive and a cover crop like *Pueraria phoseoloides*(tropical kudzu) should be used in

combination with slashing or uprooting. According to Mathew *et al.*, (1977) spraying a mixture of praquat 1.15 l/ha and fernoxone 0.75 kg/ha was ideal in rubber plantations infested with Eupatorium.

Delabarre and Lhoste (1978) reported that treatment of woody Eupatorium plants of 1.5 m tall with 3,6-dichloropicolinic acid at 0.3 kg/ha or 3,6-dichloropicolinic acid at 0.15 kg + MCPA amine at 0.7 kg/ha gave effective control. They also reported that on the land from which Eupatorium has been removed, the germination of seeds left in the soil can be inhibited by the application of diuron, monolinuron, linuron, atrazine or simazine all at 2 kg/ha and these treatments may safely be made in banana and pineapple plantations.

Best results were obtained by Dufer and Quencez (1978) in oil palms for the control of Eupatorium with metribuzin and glyphosate each at 6.0 kg/ha but was not found practicable due to the danger of erosion by metribuzin and high cost of glyphosate. Further trials by them with Tordon 101 showed that it was selective in oil palm since it did not reach the leaves. While slashing of Eupatorium two weeks before the treatment in young trees gave good control.

According to Cadeno *et al.*, (1986) sequential application of 0.54 kg/ha of glyphosate followed by 1.6 kg/ha

diuron controlled the growth of weeds like *Xanthium, Jacquinia, Eupatorium and Ipomea* spp. in plantations. Control of these weeds lead to increase in tree height upto 2.04 m and trunk diameter by 4.02 cm.

Application of imazethapyr (2(4,5, dihydro, 4-methyl (1-methyl) -5-oxo-1H-imidozolyl) 5-ethyl, 3-pyridine carboxylic acid) at the rate of 0.375 to 0.5 kg/ha controlled the growth of *Chromoleana odorata* even under rainy season more consistently than glyphosate in rubber plantation of Ivory coast (Keli, 1986).

In a preliminary screening trial of herbicides on Eupatorium Mumbareddy (1989) found that 2,4-D and glyphosate were more effective in controlling the weed. Paraquat, 2,4-D and glyphosate did not allow the plants to attain more height even at lower concentration. In another trial of herbicidal combinations, he found that the application of 2,4-D + glyphosate + paraquat was more effective in checking the weed growth.

Singh *et al.,* (1992) reported that *Eupatorium adenophorum* could be effectively controlled by hand cutting and spraying of herbicides on the resprout foliage. Herbicides DOWCO 433 (Gallant) @ 0.25 kg/ha, paraquat @ 1.0 kg/ha and glyphosate @1.0 kg/ha were found to be most effective in checking this weed in Himachal Pradesh. Although 2,4-D

suppressed the weed growth, but failed to control completely.

Doddamani (1992) reported that Eupatorium can be effectively controlled by spraying glyphosate and 2,4-D both at 7.5 ml/l. However, the lower concentrations of these herbicides were effective only at early growth stages and did not control the growth and regrowth of this weed at later stages. It was also pointed out that glyphosate is environmentally safe with no mammalian toxicity as compared to 2,4-D and hence it was advocated to use glyphosate for controlling Eupatorium.

2.2 Mode of action of herbicides

Mode of action of herbicides comprises the sum total of anatomical, physiological and biochemical responses that make up the total phytotoxic effect of a chemical in the plant (Ashton and Crafts, 1973).

Generally, most of the herbicides have both primary and secondary mechanism of action. Primary mechanism of action are mainly related to the processes in which herbicides interfere biochemically or biophysically with molecules or structure of cells such as proteins, DNA, RNA, membranes or enzymes. Inhibition of photosynthesis at a specific site in the electron transport chain (ETC), formation of toxic radicals which inturn oxidize pigments and lipids, inhibition of enzymes synthesizing carotenoids or chlorophyll or interference with

membranes influencing the ion transport are some of the instances of primary mechanism of action.

Secondary mechanism of action of herbicides include the sequence of reactions following the primary response leading to the physiological alterations of plant metabolism and cell structure and finally to the death of the plant. These include the interference with photosynthetic reactions, destruction of chlorophyll, lipid peroxidation, loss of membrane functions, suppression of cell division and disorganization of the ultrastructure of plant cell and cell organelles (Pfister and Urabch, 1983).

The mode of action of herbicides included in the present investigation are given here under.

2.2.1 2,4-D

2,4-D (2,4-Dichlorophenoxy acetic acid) is a selective systemic post emergent herbicide used to control many annual broad leaf weeds in cereal crops, sugarcane, plantations and non cropped areas. It is available in salt and ester formulations. The esters of 2,4-D have low toxicity to both humans and warm blooded animals (LD_{50} for rats is 490-1500 mg/kg) (Gruzdyev *et al.*, 1980). 2,4-D is absorbed by leaves, stem and roots, when applied to the foliage, it enters the plant tissue through the cuticle by diffusion and moves inside

the plant both apoplastically and symplastically, causing characteristic phytotoxic symptoms such as epinasty, swelling, twisting and bending of the the treated parts and also distant parts. The plants stop growing, meristematic cells stop dividing, young leaves stop expanding and the mesophyll tissue stops developing. Roots may loose the ability to absorb water and mineral nutrients and leaves may loose photosynthetic ability.

Chrispeels and Hanson (1962) reported that 2,4-D doubled the RNA content in soybean hypocotyl 48 hours after spraying with over half of the increase appearing in the microsomal fraction and one quarter in the soluble fraction. They suggested that the primary site of action of 2,4-D is nucleus.

O'Brein *et al.*, (1968) found that 2,4-D promoted the synthesis of RNA polymerise in soybean hypocotyls and suggested that this could lead to the read out of a part of the template not read by indigenous polymerise or alternatively there may be a change in DNA sites available for trnascription. Thus, 2,4-D exerts its influence at the gene level. Selective phytotoxicity of 2,4-D is based on the differential alterations of RNA species and interference with protein synthesis (Chen *et al*, 1971).

2.2.2 Paraquat (Gramaxone)

Paraquat (1,1 dimethyl, 4,4-bipyridinium ion) is a bipyridinium potent contact herbicide. It is readily soluble in water, stable at physiological pH ranges and rapidly absorbed through foliage. Translocation of paraquat is apoplastic through xylem (Wood and Gosnell, 1965), as the destruction of pheloem does not stop the translocation to the rest of the plant (Baldwin, 1963).

Paraquat has a redox potential of -0.446 V and exists as dication which can accept an electron from ferredoxin (-0.42V) in photosystem I to form a stable free radical. This radical is reoxidized by molecular oxygen to produce active oxygen species and the paraquat dication is then free to accept another electron from freedoxin. Thus, paraquat interferes with electron flow at photosystem I. This cycle operates futile whereby, di-cation is constantly regenerated in the light and active oxygen species kill the plant.

Paraquat is not metabolised in plants. Its mode of action in green plants appears to be mediated by generation of phytotoxic hydrogen peroxide and super oxide radical ($\bar{O}_2$). The phytotoxic action of paraquat requires light, chlorophyll and oxygen. Reactive single oxygen, super oxide radical, hydrogen peroxide and hydroxyl radical are generated through normal photochemical and biochemical reactions in

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photosynthesis. However, green plants posses natural protective mechanisms that prevent or minimise damage by the reactive froms of oxygen. The reactive or toxic forms of oxygen become significant herbicidally when their formation becomes excessive and the natural protective mechanisms are unable to quench, neutralise or detoxify them to prevent irreversible damage; paraquat enhances the generation of the reactive forms of oxygen and cause the natural defence to be swamped, leading to the eventual death of plants (Halliwell, 1982).

It is reduced photochemically by electrons from photosystem I to yield the paraquat radical, which undergoes rapid autoxidation by transfer of single electron to molecules oxygen to yield the superioxide radical. Hydrogen peroxide may react with $\bar{O}_2$ to yield hydroxyl radical. Hydroxyl radical are the most reactive oxygen species known and will damage nearly all molecules in a living cell (Halliwell, 1982).

2.2.3 Glyphosate

Glyphosate (N-phosphonomethyl gylcine) is a broad spectrum, post emergent, non-selective herbicide usually formulated as mono-isopropylamine salt. It has low soil residual activity combined with great systemic action in plants. Foliar applied glyphosate is absorbed and translocated through phloem (Fletcher, 1983). It is environmentally safe, with low mammalian toxicity. The acute oral LD_{50} for rat is

4320 mg/kg, tolerance to fish and wild life is high and has half life of less than 60 days.

The mode of action of glyphosate has been the subject of much research and it appears to affect a number of biochemical processes within the plant. It has been reported to inhibit the aromatic amino acid biosynthetic pathway (Jaworski 1972) induce chromosomal aberractions (Boyle and Evans, 1974), affect respiration (Sprinkle *et al,* 1975), ion absorption (Breeke and Duke, 1978), modify the chloroplast ultrastructure (Hoagland *et al,* 1979) and alter the metabolism of phenolic compounds by inducing the synthesis of ammonia lyase (PAL) activity (Duke and Hoagland, 1978 and Hoagland *et al,* 1979). Results of experiments by Cole *et al,* (1979) on *Agropyron repens* indicate that the rate of protein synthesis is limited by reduced level of phenyl alinine.

The target site of action of glyphosate in plants is the inhibition of 5-enol-pyruvyl shikimic acid 3-phosphate (EPSP) synthase activity. This enzyme is involved in the biosynthesis of aromatic amino acids, tryptophan, phenylalanine and tyrosine and also leads to the synthesis of numerous secondary plant products. Approximately 20 per cent of the carbon fixed by green plants is routed through shikimate pathway with an impressive number of significant end products including vitamins, lignins, alkaloids and wide array of phenolic compounds such as flavonoids. A common pathway exists

from erythrose 4-phosphate provided from photosynthetic carbon reduction in the chloroplast stroma to chorismic acid and hence inhibition at EPSP synthesis by glyphosate is at a strategic location (Ashton and Crafts, 1973).

Glyphosate treated plants may take upto three weeks to die and the precise sequence of events is by no means clear. This slow action in the field probably reflects the time taken for the depletion of aromatic amino acid pool size to cause decreased rates of protein synthesis.

2.3 Use of carriers for increasing the efficacy of herbicides

Successful control of perennial weeds with foliar applied herbicides depends on the rapid absorption and the basipetal translocation of the biologically active compounds into the underground storage organs in sufficient quantities to kill the entire plant before the plant metabolism can degrade the compounds.

The activity of foliar applied herbicides is influenced by many factors including surface retention, cuticle penetration, translocation from the region of penetration, metabolism, adsorption at metabolically non-active sites together with its inherent effect on biochemical processes. Enormous amount of work has been done in trying to find ways of improving the efficiency of herbicides. In this respect,one of

the most popular ways has been to investigate the effect of different carriers and additives on the efficacy of the chosen herbicide. The chemical chosen has to be effective against weed and should be translocated preferably in the phloem so as to reach metabolically active sites (Richardson, 1977).

2.3.1 2,4-D

Addition of nutrient elements to 2,4-D sprays to enhance its efficacy was tried by Sexsmith (1953) on Russian thistle and he found enhanced 2,4-D activity with ammonium phosphate, ammonium sulfate, ammonium nitrate and potassium phosphate. He also found that nitrogen additives were more effective than phosphorus additives and ammonium nitrogen being better than organic nitrogen and combined nitrogen, phosphorus and potassium was not as effective as ammonium nitrogen alone.

Membrane penetration of 2,4-D was more at lower pH (3.5) than at higher pH (5.5) (Szabo and Buckholtz, 1961). They also examined the effect of ionic additives and pH on the penetration of living and non-living membranes by 2,4-D. Penetration of 2,4-D through a collodion membrane was enhanced by the additives tried, with ammonium and phosphate ions giving the greatest effect. When the experiment was repeated using *Sedum* sp. epidermis, the ammonium and phosphate ions still gave maximum penetration. Penetration into sunflower and beans cotyledon surfaces was measured by the same authors and

found that the ammonium and phosphate ions gave maximum penetration at pH 3.0 and concluded that in organic additives, especially ammonium and phosphate ions enhance foliar penetration. Greenham (1962) showed that when phosphate was applied to the leaf blade and 2,4-D to the petioles of skeleton weed (*Chondrilla juncea*) leaves, the translocation was increased by 50 per cent over the controls.

Brady(1970a) examined the effect of ammonium nitrates and phosphoric acid on the uptake of 2,4,5-T by four woody species. He found that addition of 0.1 per cent ammonium nitrate (pH 4.0) was more effective and doubled the absorption of herbicide by sweetgum (*Liquidamber styraciflua*), post oak (*Quercus stellata*) and red maple (*Acer rubrum*) and gave a 77 per cent increase in loblolly pine (*Pinus tueda*). Phosphoric acid was more effective than ammonium nitrate in increasing 2,4,5-T uptake in post oak, similarly in loblolly pine and less effective in the other two species. A mixture of the two was less effective than ammonium nitrate alone and less effective than phosphoric acid alone except on red maple.

Growth stage of target plant has marked influence on the absorption of herbicides. Agbakoba and Goddin (1969) measured the abroption of 2,4-D over 48 hours by leaves of different day old seedlings of field bindweed (*Convolvlus arvensis*). They found that five week old plants absorbed and translocated 2,4-D significantly less than the older plants. On the

contrary, Sargeant and Blackman (1972) reported faster and more absorption of 2,4-D by immature leaves than mature leaves of *Phaseolus vulgaris*.

Similar study on poplar (*Populus tremuloides*) with 2,4-D over different periods (June to September) by Sharma and Vanden Born (1970) indicated that maximum 2,4-D was absorbed in July. They reasoned out that the maximum absorption during this period was due to cracks and punctures in the cuticle which would favour absorption of polar compounds by aqueous route.

Effect of the addition of isoparaffinic oil carrier to 2,4-D on penetration of purple nutsedge (*Cyperus rotundus*) was examined by Burr and Warren (1972) and found that after three days, 2,4-D was absorbed significantly more with oil (43%) than with water (18%). Similar results were reported by Prendeville and Warren (1975) on beans (*Phaseolus vulgaris*) leaves. Penetration of 2,4-D was greater through the lower leaf surface when applied in a water-surfactant carrier. This suggested stomatal entry was involved for aqueous solutions. However, penetration of 2,4-D in oil carriers was essentially the same on both surface which suggests stomatal entry is not an important factor with oils.

Que and Sutherlad (1973) reported that aqueous emulsion of amino salt of 2,4-D penetrated faster into sunflower (*Helianthus annus*) leaves than diethylamine salt. the

initial absorption (70 per cent in 13 hours) was independent of light but the following much lower absorption was strongly light dependent. The amine salt sprayed in diesel oil as a carrier had 90 per cent in four hours. They concluded that 2,4-D amine salt should be sprayed with diesel oil as a carrier to facilitate better penetration into leaves.

Devendra *et al.*, (1986) reported that lowering of pH, addition of Triton (anionic phosphate surfactant) and sucrose to the spray solution enhanced the rate of entry and amount of 2,4-D translocated to the underground roots of *Solanum elaegnifolium*.

2.3.2 Glyphosate

Glyphosate, a broad spectrum post emergent herbicide is absorbed and translocated in the phloem in both annual and perennial weeds (Bird *et al.*, 1974). Many workers have reported increased efficacy of glyphosate when sprayed with additives and the effect is detrimental at various stages of weed growth.

Sprinkle *et al.*, (1975) were of the opinion that the adjuvant used with glyphosate was important in determining its phytotoaxicity in quackgrass (*Agropyron repens*). In canada thistle (*Cirsium arvense*), bentazon (3-isopropyl-1 H-2,1,3 benzothiadiazin -(4) 3H-one 2,2-dioxide) at 2.24 kg/ha applied prior to treatment with ^{14}C-glyphosate reduced ^{14}C

translocation. Iron or nitrilotriacetic acid (NTA) did not appear to affect glyphosate activity. Addition of nonionic surfactant MON 0818 at 0.8% to the spray solution increased glyphosate absorption rate in quack grass.

Turner and Loader (1978) found increased effect of glyphosate with several acids that act as complexing agents when tried on *Agropyron repens* and *Stellaria media*. These acids include orthophosporic, citric, tartaric, lactic and glycolic acids. Sodium or ammonium salts acted like parent acids. It was suggested that activation of glyphosate was due to interactions with calcium or other metallic ions, which would otherwise immobilize the herbicide.

Almost 50 years ago, ammonium sulphate was used in USA to activate salt formulations of dintrophenol herbicide,DNOC (Herris and Hyslop, 1942; Crafts and Rieber, 1945). Since then, enhancement of herbicidal activity by ammonium sulfate or nitrate has been demonstrated with water soluble leaf applied herbicides (Tischler *et al.*, 1951; Simon, 1953 and Sexsmith, 1953).

Ammonium sulphate increased herbicidal activity of picloram, phenoxy acid salts and glyphosate sprays against woody species (Turner and Loader, 1978). Blair (1975) also reported enhancement of glyphosate and aminotriazole activity against field grown *Agropyron repens*. Similar effects into

other perennial weeds were observed by Lutman and Richardson (1978).

Turner and Loader (1978) reported increased glyphosate toxicity with addition of ammonium sulphate (1-10%) on *Agropyron repens*. Higher concentration of ammonium sulphate were some time antagonistic. Additions of ammonium sulphate without surfactant generally had less effect on phytotoxicity. Lipophilic surfactant with ammonium sulphate were found to be best combination with ghyphosate herbicide in control of *Agropyron repens*.

McWhorter *et al.*, (1980) observed increased glyphosate absorption and translocation in soybeans when applied with surfactant and KH_2PO_4, but the addition of surfactant alone reduced the translocation. While, the addition of KH_2PO_4, alone increased translocation.

2.3.3 Paraquat

Although, paraquat is primarily a contact herbicide, is translocated under certain situations and in some plant species (Klingman, 1954). Nitrogenous carriers found to be effective with paraquat. Thangaraj and Shakaram Rao (1973a) reported that potassium nitrate as an effective carrier for paraquat as it reduced significantly the sugars and starch content of nutgrass (*Cyperus rotundus*) tubers compared to paraquat spray alone. Urea also found to be good carrier but

not as effective as potassium nitrate. Activity of respiratory enzymes viz., peroxidase and catalase were increased significantly when paraquat was sprayed with potassium nitrate or urea (1973b) and total nitrogen and protein content of tubers reduced by these carriers (1973 c).

2.4 Influence of herbicides on seed production and seed viability

One of the reasons for fast spread of weed species to new lands is their tremendous potential to produce numerous seeds. Eupatorium produces numerous pappus bearing small seeds (achenes) that spread to long distance through wind during the month of March. The seeds remain viable and germinate with the receipt of monsoon. Attempts to reduce the weed seed production by selective herbicides has been very unsuccessful. The top growth is damaged but the plants recover later and produce large number of normal seeds. Decreased seed production and seed viability through the use of herbicides have been reported for foxtail (*Setaria glauca*), goose grass (*Eleusine indica*) and crow foot grass. (*Dactyloctenium aegyptium*) by Taylorson (1966), for red root pig weed (*Amaranthus retroflexus*) by Rojas-Garciduenas and Kommedahl (1958).

Effect of 2,4-D spray on seed production and embryo development in curly dock (*Rumex crispus*) was studied by Maun and Cavers (1969). They reported complete prevention of viable

seed formation by 1000 ppm of 2,4-D spray at 12 days before anthesis. Although 91 per cent of seeds had embryos when sprayed at seven days before anthesis, only five per cent seeds were germinated and the weight was significantly reduced by the herbicide spray. Spraying the inflorescence 34 days after anthesis did not affect the number of seeds produced or seed viability. In a similar experiment, Fawcett and Slife (1978) reported that the application of 2,4-D (1.0 kg/ha) at near flowering to different weed species reduced the seed production by 99 per cent in *Chenopodium album* and 77 per cent in *Amaranthus retroflexus*. Whereas, the seed production was increased to 300 per cent in *Setaria faberi*. Further, seeds of *Amaranthus* and *Chenopodium* from herbicide treated plants found to be more dormant than the control while *Seteria* seeds were less dormant. However, viability of treated and untreated seeds did not differ greatly.

Eterjere (1980) studied the loss of viability of Euipatorium seeds treated with herbicides and stored under different temperatures. He found that 75 to 80 per cent of the seeds were affected by temperature of the storage environment. The loss of viability increased with longer period of storage. Seeds stored under -10°C or 8°C retained their viability even after 26 months, while those stored under 27 or 37°C, lost their viability during this period. The rate of loss of viability of herticide treated seeds was greater under 27 and

37°C and eventually led to complete loss after a short storage period of 13 months. Among different herbicides tested, EPTC and molinate had the greatest effect on seed viability.

Application of chlorosulfuron, chloroflurenol or glyphosate during early flowering of *Abutilon theophrasti* and early heading of *Seteria faber* reduced seed production more than later period applications when some seeds are present (Biniak and Aldrich, 1986). With the early application of glyphosate, 99 per cent prevention of *Abutilon* and 96 per cent of *Seteria* seed production were attained. Glyphosate reduced the seed germination by 50 per cent in *Abutilon* and 95 per cent in *Setaria*.

Isaacs *et al.*, (1989) reported that the application of chlorimuron and imazaquin @ 0.28 kg/ha to sicklepod (*Cassia obtusifolia*) at early bloom and early fruit stage, almost eliminated seed production. Glyphosate applied (0.28 kg/ha) at early bloom decreased the seed production by 84 per cent, but did not affect seedling emergence. Application of 2,4-DB @ 0.28 kg/ha and 2,4-D @ 0.56 kg/ha at early bloom did not affect seed production or emergence. They concluded that herbicide application at late fruit stage was generally less effective than earlier application in reducing the seed production and emergence.

Laboratory studies conducted by Erasmus and Vanstaden (1986) to investigate factors affecting Eupatorium seed germination indicated that alternate temperatures of 15 and 30°C were optimum. The seeds were positively photoblastic and an imbibition period prior to exposure was required to sensitize seeds to light. The red light exposure promoted seed germination which could be reversed by subsequent exposure to far-red light and the inhibitory effect of far-red light and the inhibitory effect of far-red light could be nullified by a subsequent red lights indicating typical phytochrome mediated germination response. The seeds germinated over wide range of pH (6 to 10) with highest germination at pH 8.0 (Macdonald *et al.*, 1992).

Mogali *et al.*, (1991) tested the influence of aging and seed maturity on the Eupatorium seed germination. They found that the germination percentage was low (15%) immediately following seed harvest and declined to 2.81 per cent at 14 months. Mature seed germination was superior (82 and 64% at third and fifth months respectively) over immature seeds. Germination per cent of immature seeds was maximum at 11 month of harvest.

Effect of 2,4-D on seed production in leaf spurge (*Euphorobia escula*) was reported by AL-Henaid *et al.* (1993). Viable seed production was reduced when 2,4-D @ 2.2 kg/ha sprayed at flower bud formation to seed production period.

However, herbicide should be applied prior to flower bud development to prevent seed production.

Histological studies on the effect of glyphosate on wild oat (*Avena fetua*) during seed development indicated abnormal embryo formation (Shuma and Raju, 1993). The herbicide did not stop the normal process of double fertilization in the florets. The zygote divided but did not proceed beyond proembryonic stage. There was no proper development of embryo parts such as scutellum, epiblast, shoot and root apices. Embryonic tissue showed considerable obliteration and shrinkage, the cells appeared lacunose. The embryo and endosperm in caryopsis treated with glyphosate nine day after anthesis or later were morphologically and structurally very similar to those of nontreated controls of comparable stage. They concluded that greater the delay of glyphosate application after anthesis, the less was the effect on seed development.

2.5 Plant adaptation to light and shade

Ability of plants to adapt to changes in their light environment allows them to succeed in habitats ranging from the arid sun soaked deserts to the deeply shaded floors of dense tropical forests. Plants must cope with contrasting light environment that vary by three orders of magnitude in irradiance. The irradiance available varies seasonally,

diurnally and spatially not only to the level of plant canopy but even at leaf level. Shade plants in the forest receive less than 0.5 per cent of the irradiance experienced above plant canopy. The importance of light for photosynthesis and phtoperiodism is well known and differences among plants with respect to tolerance of shading is important to ecology. However, light or more broadly radiation has important effects on plant growth in addition to its role as a source of energy that drives photosynthesis and transpiration. Light also controls the temperature that influences the rate of most physiological processes. It produces formative effects that are seen in variations in leaf size, stem growth and sturdiness, root to shoot ratio and photo-periodic control of flowering. Infact the radiation environment has a wider variety of effects on plant physiology and morphology than any other abiotic environmental factor to which plants are exposed. Light could be considered as a critical natural resource and essentially controls the morphogenesis and production in plants. Plants harvest solar radiation with their complex biological machinery to produce biomass.

2.5.1 Photosynthetic characters

It is well established that plants growing in open sunny habitat have much greater photosynthetic capacities which saturate at higher irradiance than those of plants growing in

shade habitat. However, shade plants use low irradiance more effectively for net photosynthetic CO_2 uptake than do sun plants due to the lower dark respiration rates of shade plants.

The capacity of plants to adapt to differing light conditions has long been recognized. Under contrasting light regimes, leaves have different anatomical, morphological and biochemical properties leading to differences in apparent photosynthetic rates. Small thick leaves develop in the sun and large thin leaves in the shade of many plants. Sun leaves tend to have more highly developed palisade and spongy mesophyll regions and higher photosynthetic rates at light saturation. Leaves of shade-adapted species generally contain more chlorophyll on weight basis but less on area basis because they are usually thinner. The richer content of chlorophyll in chloroplast of shade-adapted leaves combined with the reduction leaf thickness may allow for more efficient light utilization. The chlorophyll a :b ratio decreases in shade grown leaves compared to light grown leaves. Studies of Nobel *et al.*, (1975), indicated that in *Plectranthus parviflorus* increase in internal leaf area for the sun leaves lowers their liquid phase resistance to CO_2 diffusion and was responsible for their higher photosynthetic rates. Further, they concluded that changes in leaf morphology will influence more than just CO_2 resistance. Large leaves can have a temperature quite different from that of the air and the temperature affects photosynthesis and transpiration.

2.5.2 Leaf characters

Nobel (1976) reported variation in leaf characters of desert shrub, *Hyptis emoryi* grown in sun and shade conditions. Full sunlight (40 MJ m^{-2}) grown plants had less than one cm length leaves while shaded (3 MJ m^{-2}) plants had more than seven cm leaves. Sun leaves possessed three fold high chlorophyll per unit area, mesophyll thickness and internal to external leaf ratio than shaded leaves. Higher internal to external leaf area ratio caused sun leaves to have three fold lower CO_2 liquid phase resistance than did shade leaves. Large thin shade leaves captured photosynthetically active radiation (PAR) effectively (less than 7% passed through) but were not adapted to full sunlight.

Effect of different irradiance levels on valvet leaf (*Abutilon theophrusti*) and cotton (*Gossypium hirsutum*) indicated that in both species photosynthetic rate decreased with decrease in irradiance (Patterson *et al.*, 1978). Reduction of air O_2 concentration from normal 21% to 1% increased the photosynthesis rate by 1.5 to 2.3 times. The stomatal conductance was not significantly affected by growth irradiance and the differences in maximum photosynthetic rate were found to be associated with differences in mesophylll conductance. Mesophyll conductance increased with growth irradiance and correlated positively with mosophyll thickness, chlorophyll content and photosynthetic unit density per unit leaf area.

Patterson (1979) demonstrated the inhibitory effect of shading on dry matter production by itchgrass (*Rottboelia exaltata*). Plants grown at 2, 25 and 60 per cent of full sunlight produced only 0.3, 16 and 55 per cent as much dry matter, respectively as plants grown in full sunlight.

Leaf anatomy and ultrastracture can be greatly altered by the irradiance under which a plant grows (Paul and Patterson, 1980). Itchgrass plants grown under 100, 60, 25 and 2 per cent sunlight (2000 u $Em^{-2}S^{-1}$) showed that shading caused reduction in cell wall thickness, shrinkage of plastids, reduction in starch deposits and vacuolization of the cytoplasm. In general, plastids and mitochondria retained their membrane integrity but underwent stomatal deterioration. Shading effects on cytoplasm were similar for mesophyll and bundle sheath cells. Mesophyll chloroplasts lost starch grains and the peripheral reticulaum tended to decrease with greater shading. Chloroplast size decreased with shading although grana were well developed at all irradiance levels. Conspicuous movement of mesophyll chloroplasts away from bundle sheath mesophyll borders was noticed at 60 per cent sunlight and was progressively greater at the two irradiance. These observations suggested that the structural relationship could be necessary for intercellular transfer of C_4 acids and the functioning of the C_4 photosynthetic pathway were disrupted by shading.

2.5.3 Stomatal frequency

The rate of bioproductivity per unit land area is dependent on the total leaf area and the amount of CO_2 entering and fixed by the leaf tissue. The diffusion of CO_2 to the site of carbon fixation is dependent on the amount of CO_2 entering the leaf which is controlled by the number of stomata per unit leaf area and stomatal pore size. Since stomata regulate both trasnspiration and phtosynthesis, a high stomatal frequency lead to higher photosynthetic rate. However, this may also lead to higher transpiration.

Teare *et al.* (1970) suggested that stomatal frequency would be an important criterion for producing high yield in wheat. They also observed that stomatal frequency decreased in relation to leaf position on the canopy. The younger leaves had higher stomatal frequency and decreased progressively towards the lower leaves. Miskin and Rasumsson (1970) observed that in barley flag leaf had approximately twice the number of stomata per cm^2 of the basal leaves. They also noted increased light intensity resulted in higher stomatal frequency and transpiration rate, the photosynthetic rate remained almost constant.

Kneent and O'Leary (1972)compared the stomatal frequency in the third trifoliate leaf of *Phaseolus vulgaris* grown at different light intensity with the photosynthetic

rate. Stomatal frequency was significantly greater for plants grown at higher light intensity. But the total stomatal number per trifoliate leaf did not vary significantly due to light treatment.

Ciha and Brun (1975) found that stomatal frequency significantly decreased with temperature only on the adaxial surface. However, stomatal frequency increased with light intensity on both the surfaces in soyabean. They also pointed that resistance to diffusion of water vapour and CO_2 between leaf and atomosphere is a function of stomatal frequency and degree of opening.

A positive correlation between stomatal frequency and photosynthetic rate was observed in barley (Yoshida, 1972). Genotypes with higher stomatal frequency showed higher transpiration rates and carbon exchange. Similarly, Wilson (1975) observed that low stomatal frequency was always shown to be associated with less leaf conductance and thus low transpiration per unit leaf area.

2.5.4 Physiological and biochemical changes

A physiological process such as photosynthesis, respiration or transpiration actually is an aggregation of chemical and physical processes. In order to understand the mechanism of physiological process it is necessary to resolve it into its physical and chemical components and plant

physiology depends more and more on methods of biochemistry in its efforts to accomplish this. The biochemical approach has been very fruitful as shown by the progress made toward better understanding of such complicated processes as photosynthesis and respiration. It is difficult to say whether changes in plant biochemistry alter the physiological process or changes in physiological process alter the biochemical constituents. In order to understand the relationship between physiological and biochemical systems it is necessary to classify plant metabolism into basic, intermediate and secondary metabolism. Basic metabolism includes the production of organic compounds by photosynthesis, generation of high energy chemical bonds through respiration and oxidative phosphorylation and synthesis of basic cellular polymers such as starch, cellulose and proteins. Intermediate metabolism acts as via media between degrading and synthetic process, interconversion and/or incorporation of generated organic molecules into new building blocks. Secondary metabolism deals with synthesis of different specific plant constituents such as alkaloids, lignins, pectins, coumarins, phenylpropanes, anthocyanins, growth hormones, tannins and others.

Herbicides may interfere with any one or many of these pathways which contribute to the metabolism and growth of plants. It has been shown that metabolic pathways specific for plant tissues / organelles possess most of the known sites of action.

Reduction of nitrate to nitrite by nitrate reductase is the first step in nitrogen assimilation in plants. Activity of nitrate reductase precedes the activity of nitrite reductase that further reduces nitrite to ammonia which could be incorporated into organic acids to from amino acids. These two enzymes in leaves, differ in their cellular location, electron donor cofactor and energy generation system (Ritenour *et al.* 1967). Nitrate reductase is located in cytoplasm, uses NADH as electron donor and derives its source of NADH primarily from the oxidation of 3-Phospho glyceraldehyde or malate. Whereas, nitrite reductase is located in chloroplast, utilizes reduced ferredoxin as its electron donor and derives its reductive energy from photosynthetic electron flow.

Among the various biochemical factors limiting growth, nitrate reductase is a rate limiting step in nitrate assimilation (Hageman *et al.*,, 1967). This observation was made based on the fact that under the conditions influencing growth adversely, such as low light intensity, there was accumulation of nitrate in maize leaves. This could however, result from low photosynthetic rates which limited the supply of energy and reducing potential or from inhibition of nitrate reductase activity, because plants with low nitrate reductase activity accumulated more nitrate.

Schrader *et al.*, (1968) characterised the nitrate reductase (NRA'se) in corn seedlings. They reported that NRA'se has half life of approximately four hours. It has a single moiety with the ability to utilize either NADH or $FMNH_2$ required for optimal activity suggests that *in vivo* NADH is the electron donor and that NR'ase in higher plants should be designated as NADH-NR'ase. With respect to cofactor requirements NADH and $FMNH_2$ were equally effective as electron donors for NR'ase obtained from leaves of maize and spinach when cofactors were supplied in optimal concentrations. The concentration of $FMNH_2$ required to obtain half maximal activity was from 40 to 100 fold higher than for NADH. For maximal activity with the corn enzyme 0-8 mm $FMNH_2$ was required. In contrast NADPH was functional only when supplied with ANDP reductase and exogenous FMN.

Nitrate reduction is inhibited by photosynthesis inhibitor herbicides with little or no immediate effects upon nitrate reduction. In contrast, certain non-photosynthesis inhibitor herbicides stimulate nitrate reduction and permit it to occur in darkness with little or no immediate effect upon photosynthetic nitrate reduction. Thus, several class of herbicides have been shown to cause free nitrite to accumulate in treated plant tissues; the photosynthetic inhibitors by inhibiting nitrite reduction in light while, nitrate reduction continues certain other herbicides by

permitting nitrate reduction to occur in the absence of photosynthetic nitrite reduction.

Klepper (1979) postulated that herbicides those which inhibit photosynthesis, act by causing nitrate accumulation within the cell. The accumulation of nitrite is proposed to be due to inhibition by the herbicide of nitrite reduction without a concurrent inhibition of nitrate reduction. Such a differential effect could be expected since nitrite reductase is localized within chloroplast and utilizes ferredoxin as an electron source, whereas nitrate reductase is localized in the cytoplasm and utilizes NADH as an electron donor. Nitrite is toxic to many plant species and causes symptoms such as leaf yellowing, chlorosis, necrosis, distortion and eventually death.

Finke *et al.*, (1977) studied the effect of various herbicides on *in vivo* nitrate and nitrite reduction by vacuum infiltrating leaf sections of barley (*Hordeum vulgare*) and bean (*Phaseolus vulgaris*) with solutions containing nitrate and herbicides. They found that among different herbicides, only dinozeb and potassium azide significantly reduced nitrate reduction in both barley and bean. All the herbicides which inhibit photosynthesis inhibited nitrite reduction but had no significant effect on nitrate reduction in barley and bean. Triazines also inhibited significantly nitrite reductase in both species.

In sorghum application of 2,4-D and atrazine resulted in increased activity of nitrate reductase (Sairam *et al.*, 1988). It also increased protein content and nitrogen uptake by the plants.

III. MATERIAL AND METHODS

III MATERIAL AND METHODS

Chromolaena odorata K & R (earlier known as *Eupatorium odoratam* L) weed has become a major threat in forests as a fire hazard. Its rapid invasion in aerable, waste lands, conservation areas and pastures has decreased the livestock carrying capacity. It is also known to suppress the regeneration of plant species in forests and plantations. In the recent past, it is also invading agricultural land. An attempt was made to study the spread, establishment and chemical control of Eupatorium. Field and pot experiments were conducted to find out the physiological, biochemical and anatomical changes brought out by various herbicides along with carriers to increase the efficiency of herbicides. The details of the materials used and methods adopted are given in this chapter.

The investigation consisted of four experiments.

Experiment I : Use of different herbicides and time of application for control of Eupatorium

Experiment II : Use of herbicides for control of seed production in Eupatorium

Experiment III : Effect of herbicides and growth conditions on physiological, biochemical and anatomical changes in Eupatorium

Experiment IV : Use of carriers to increase the efficacy of herbicides under different growth conditions

3.1 Experiment I : Use of different herbicides and time of application for control of Eupatorium

It was intended to find out different concentrations of various herbicides by spraying at monthly time intervals during the grand growth period (August, September and October). The details of the experiments are mentioned below.

3.1.1 Selection of experimental site

The experiment was conducted during 1989-90 at two locations of varying agro-climatic conditions viz., Forest Research Stations, Prabhunagar (Dharwad district) and Yellapur Forest (Uttar Kannada district). Prabhunagr is located 18 km West of Dharwad and is characterised by mixed deciduous forests with teak predominance. Yellapur Forest is 120 km West of Dharwad, situated in the middle of dry deciduous forests in Western Ghat areas of Karnataka.

3.1.2 Weather parameters

Monthly mean weather data of the experimental period of both the locations are given in Table 1. The data indicated that total rainfall for the experimental year was maximum (2206.5 mm) at Yellapur forest as compared to

Table 1 : Monthly meterological data during the experimental period (1989-90) at Prabhunagar and Yellapur

Month/ Year	Prabhunagar			Yellapur		
	Rainfall (mm)	Mean air temperature Minimum °C	Maximum °C	Rainfall (mm)	Mean air temperature Minimum °C	Maximum °C
May, 89	31.8	21.2	31.9	62.7	30.3	38.1
June, 89	182.6	22.4	26.8	555.4	20.1	28.6
July, 89	328.4	21.6	27.1	559.2	20.8	24.5
August, 89	251.8	28.8	25.5	650.2	20.6	24.3
September, 89	109.2	19.4	27.7	199.6	20.3	26.8
October, 89	7.3	20.2	33.1	121.2	24.8	30.1
November, 89	0.0	18.6	32.4	0.0	20.1	30.3
December, 89	0.0	16.4	33.3	0.0	22.4	32.6
January, 90	0.0	17.4	33.5	0.0	19.3	32.9
February, 90	0.0	17.4	32.5	0.0	19.6	33.2
March, 90	26.6	18.1	32.0	0.0	28.5	36.1
April, 90	0.0	20.4	37.3	58.2	28.9	37.8
Total	937.7	---	---	2206.5	---	---

Prabhunagar (937.7 mm). The maximum rainfall decreased during August at Yellapur while, it was maximum during July at Prabhunagar. However, the pattern of rainfall was similar i.e., rainfall occurred from May to October at both the sites. The maximum temperature varied from 38.1 (May) to 24.3°C (August) and the minimum ranged from 19.3 (January) to 30.3°C (May) at Yellapur forest. It was noticed that the maximum temperature was slightly less at Prabhunagar as compared to Yellapur and it ranged from 37.3 to 25.5°C. With respect to minimum temperature, it was lowest (16.4°C) during December at Prabhunagar. It is clear from the weather data that the maximum temperature was more at Yellapur and Prabhunagar had low minimum temperature as compared to Yellapur.

3.1.3 Design and layout

The experiment was laid out in a randomized block design with three replications in naturally grown Eupatorium at both the sites. The plot size was 2.0 X 2.0 m for each treatment. The plan of layout of experiment is given in figure 1.

3.1.4 Imposition of treatments

The following herbicides at different concentrations were sprayed at monthly intervals from August to October and the control treatment was sprayed with water. The details of the herbicides used in the experiment are presented in Table 2.

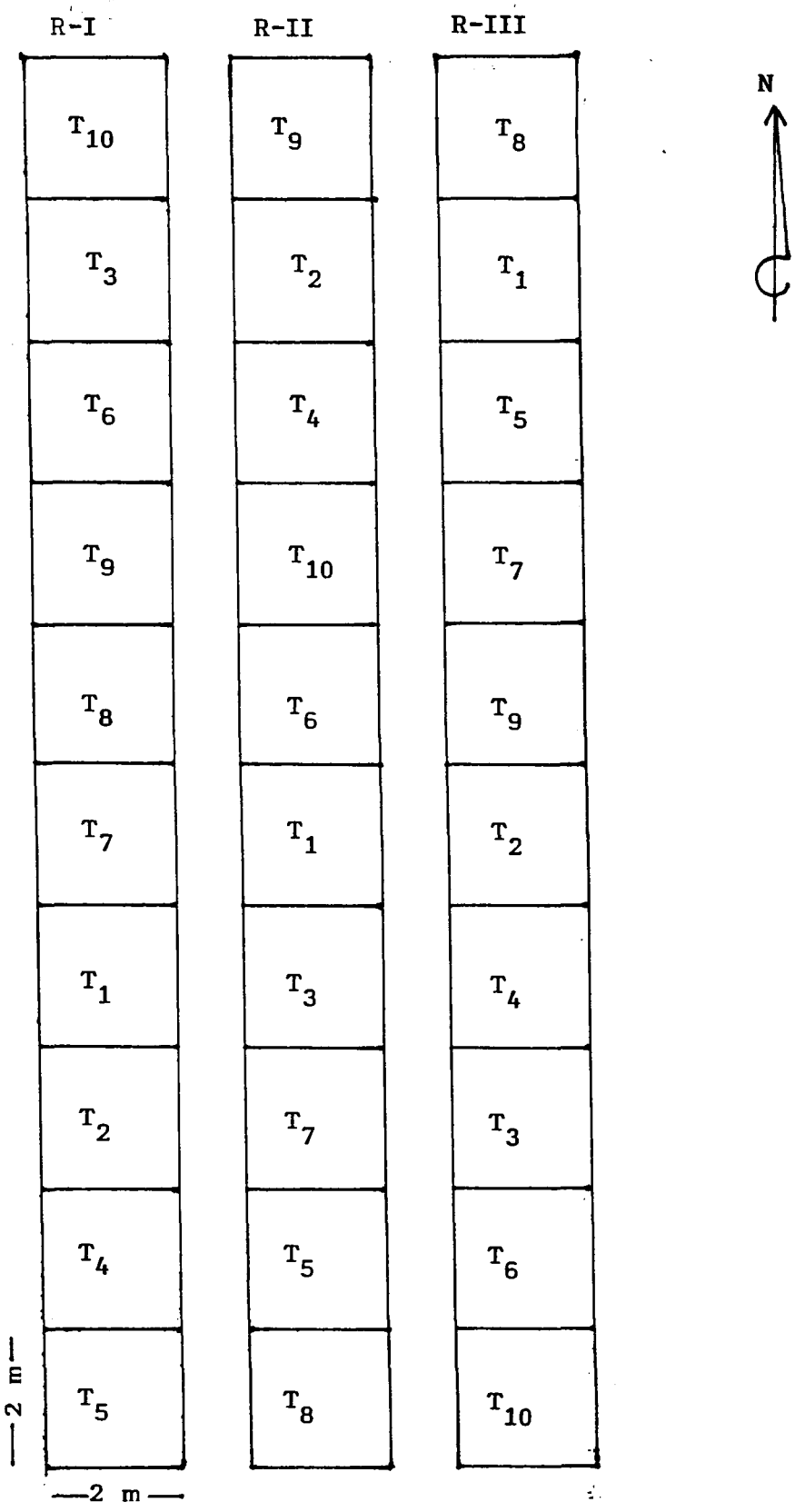
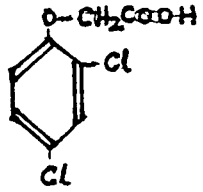
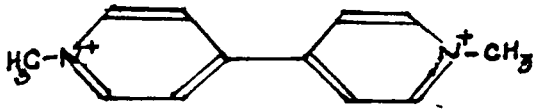
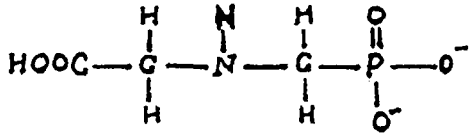


Figure 1: Plan of layout of the experiment I

Table 2 : Details of herbicides used in the experiment

Sl. No.	Common name	Chemical name	Active ingradient	Structural formula	Nature of action	Manufacturing organization
1.	2,4-D	2,4-dichlorophenoxy acetic acid	34 %		Selective, systemic, pre and post emergent	Agromore Ltd. Bangalore
2.	Paraquat (Gramaxone)	1,1-dimethyl, 4,4-bipyridinium ion	24 %		Non-selective contact, post emergent	ICI Ltd. Culcutta
3.	Glyphosate (Weedoff)	N-phosphonomethyl glycine	41 %		Non-selective, systemic, post emergent	NOCIL Ltd. Bombay

Treatments

T_1 - Control (Unsprayed)

T_2 - 2,4-D (2500 ppm)

T_3 - 2,4-D (5000 ppm)

T_4 - 2,4-D (7500 ppm)

T_5 - Paraquat (2500 ppm)

T_6 - Paraquat (5000 ppm)

T_7 - Paraquat (7500 ppm)

T_8 - Glyphosate (2500 ppm)

T_9 - Glyphosate (5000 ppm)

T_{10} - Glyphosate (7500 ppm)

3.1.5 Collection of experimental data

Five plants from each treatment were tagged randomly for recording various morphological observations before the imposition of treatment. For dry matter production, five plants from each treatment were uprooted randomly at different time intervals and were separated into leaf and stem.

3.1.5.1 Plant height

Five uniform plants were tagged at the time of herbicidal spray. Plant height was measured on main shoot of these plants from base to the growing tip at 15 days interval up to 90 days after treatment. Mean plant height (cm) was computed.

3.1.5.2 Dry matter production and its partitioning

Five plants were selected randomly and separated into leaf and stem portions at 15 days interval after the imposition of treatments and continued up to 90 days. These samples were dried in hot air oven at 80°C until constant weight was achieved. The dry weight of leaf, stem and total dry matter was expressed on per plant basis.

Experiment II : Use of herbicides for control of seed production in Eupatorium

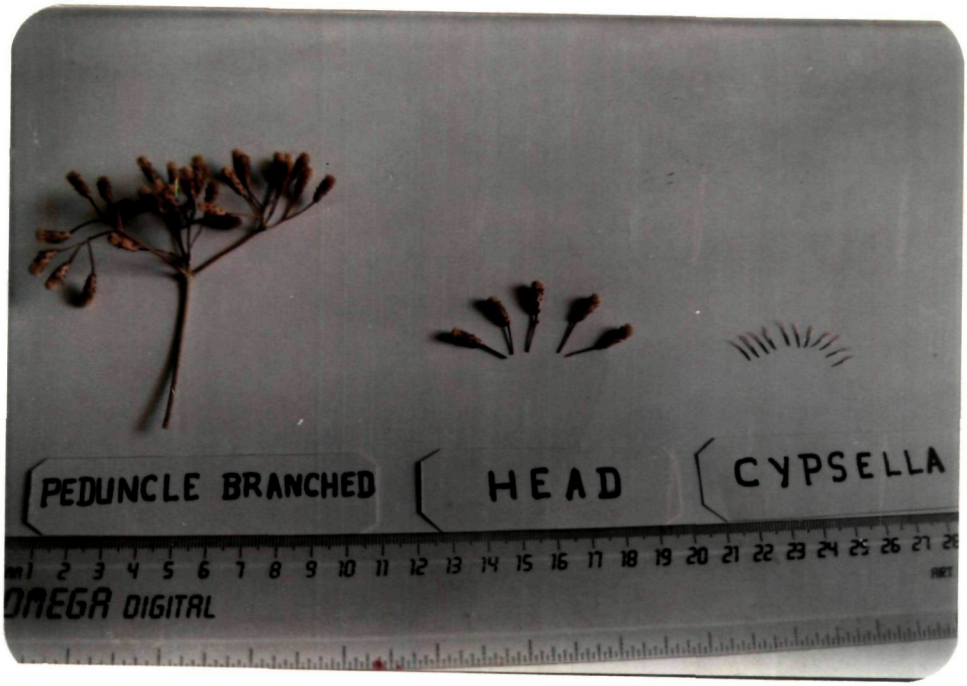
The fast spreading nature of Eupatorium to aerable lands is mainly attributed to its abundant capacity to produce million of small seeds that float in air by virtue of pappus nature and travel long distance. The details of matured inflorescence, peduncle, head and cypsella are give in plates 1. In view of this, an experiment was conducted to know the possibility and extent of reducing the seed production capacity by spraying various herbicides at different concentrations.

3.2.1 Selection of experimental site

Newly infested waste land with Eupatorium at University of Agricultural Sciences, Dharwad campus was selected for this experiment.



a. Matured Inflorescence



b. Branched Peduncle, Head and Cypsella

PLATE 1. REPRODUCTIVE STRUCTURES IN EUPATORIUM

3.2.2 Weather parameters

University of Agricultural Sciences, Dharwad is located in the transitional tract at 15°C 26' N latitude, 76° 17' E longitude and at an elevation of 667 m from mean sea level. The mean annual rainfall during 1990 was 730.5 mm, which was well distributed from June to November with two peaks, one in July and the other in October. The mean maximum temperature ranged from 25.9°C (August) to 37.6°C (April). the mean minimum temperature varied from 13.0°C in January to 20.4° in June. The relative humidity was highest (92%) during August and lowest (62%) during April. The details of the whether parameters recorded are presented in Figure 2.

3.2.3 Design and lay out

The experiment was laid out in randomised block design with three replications. The plot size for each treatment was 2.0 X 2.0 m. The plan of lay out of the experiment is given in figure 3.

3.2.4 Treatment imposition

The following treatments were imposed at bud formation stage (First week of November, 1990) and full bloom stage (Third week of December, 1990).

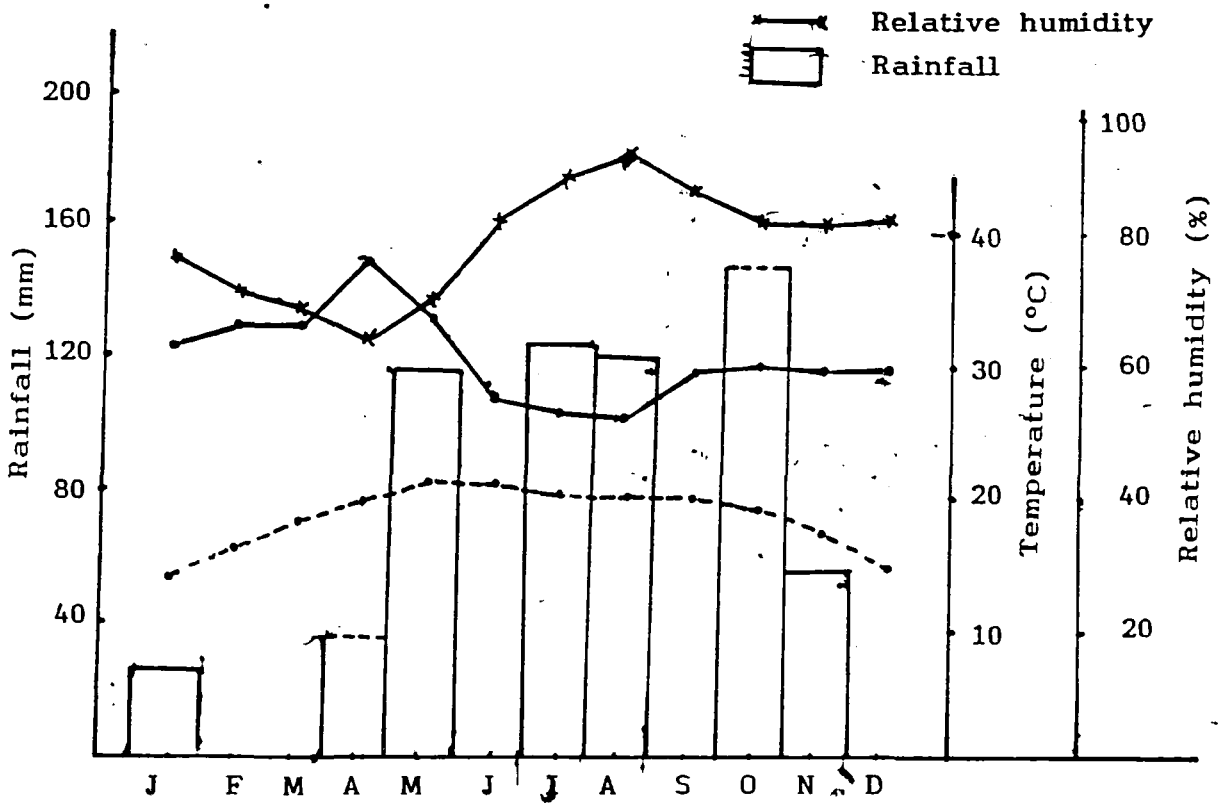


Figure 2. Monthly meteorological data of Agricultural College Farm Dharwad for the year 1990

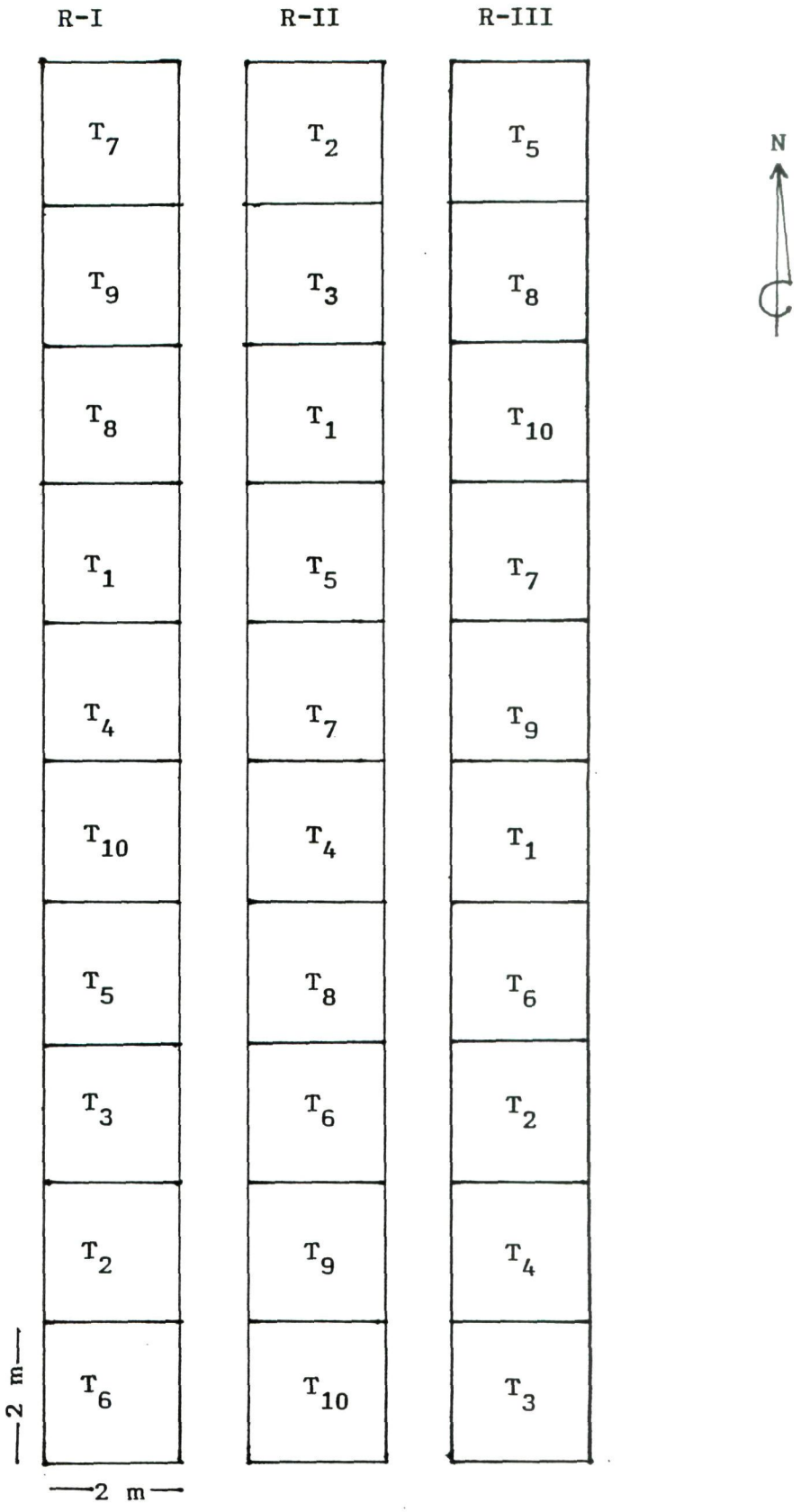


Figure 3. Plan of layout of the experiment II

Treatments

T_1 - Control (unsprayed)

T_2 - 2,4-D (2000 ppm)

T_3 - 2,4-D (3000 ppm)

T_4 - 2,4-D (4000 ppm)

T_5 - Paraquat (2000 ppm)

T_6 - Paraquat (3000 ppm)

T_7 - Paraquat (4000 ppm)

T_8 - Glyphosate (2000 ppm)

T_9 - Glyphosate (3000 ppm)

T_{10} - Glyphosate (4000 ppm)

3.2.5 Collection of experimental data

Various observations on inflorescence and the germination of seeds were collected after the end of reproductive phase and the details are given here under.

3.2.5.1 Number of Capitula per shoot

The main shoot of the labelled plants in each treatment was collected just before the dispersal of seed (April, 1991). The number of capitula per shoot was counted and the average of five shoots was recorded.

3.2.5.2 Thousand capitula weight (g)

Dry weight of one thousand capitula in each treatment was taken with the help of electronic balance and recorded in g.

3.2.5.3 Number of cypsella per capitula

The cypsella from ten capitula were separated and counted. Average number of cypsella per capitulum was recorded.

3.2.5.4 Thousand cypsella weight (mg)

Weight of one thousand cypsella along with the pappus were weighed accurately on electronic balance and recorded in mg.

3.2.5.5 Seed germination per cent

Matured seeds (Cypsella) collected from each treatment were subjected to germination test in petridish lined with moist filter paper. Each treatment was replicated thrice. Filter paper was regularly moistened with distilled water. Seed germination count was recorded on fifth and tenth day of germination test and expressed in per cent.

3.3 Experiment III :Effect of herbicides and growth conditions on physiological, biochemical and anatomical changes in Eupatorium

It was intended to find out the effect of various herbicides at different concentrations under different growth conditions viz., open light and shade, on various physiological, biochemical and anatomical changes in Eupatorium seedlings.

3.3.1 Experimental details

Fresh seedlings of uniform height (25-30 cm) were transplanted in earthern pots of 15" diameter and 30" height. For each treatment, five pots were kept and arranged in randomised block design. The experiment was conducted at college of Agriculture, Dharwad Farm during 1991 (August to October). The entire pots were divided into two groups (Plate 2), one of which was kept in artificially created shade condition at which the light intensity was 200 $\mu E\ m^{-2}S^{-1}$. The other group of pots were allowed to grow in natural sun light during which the light intensity varied from 1500 $\mu E\ m^{-2}S^{-1}$ (August) to 1800 $\mu E\ m^{-2}S^{-1}$ (October). The plants kept under both growth conditions were allowed to acclimate to the changed growth conditions for two months before the imposition of treatments.



1. Shaded Condition
2. Open Light

PLATE 2. GENERAL VIEW OF THE EXPERIMENT CONDUCTED UNDER OPEN LIGHT AND SHADE CONDITIONS

3.3.2 Treatment imposition

The following treatments were imposed separately for light and shade grown plants.

Treatments

T_1 - Control (unsprayed)

T_2 - 2,4-D (2000 ppm)

T_3 - 2,4-D (3000 ppm)

T_4 - 2,4-D (4000 ppm)

T_5 - Paraquat (2000 ppm)

T_6 - Paraquat (3000 ppm)

T_7 - Paraquat (4000 ppm)

T_8 - Glyphosate (2000 ppm)

T_9 - Glyphosate (3000 ppm)

T_{10} - Glyphosate (4000 ppm)

3.3.3 Collection of experimental data

The data on various physiological, biochemical and anatomical changes brought about by the treatments under different growth conditions were recorded at 2,4, 6 and 8 days after the imposition of the treatments. The details of the methods employed are described below.

3.3.3.1 Physiological parameters

Observations on leaf chlorophyll content, relative water content, leaf leachate analysis, nitrate reductase activity were estimated at 2,4,6 and 8 days after the spray. Whereas, the transpiration rate, stomatal conductance and leaf temperature were recorded at fifth and tenth day of the imposition of treatments.

3.3.3.1.1 Leaf chlorophyll contents

Total chlorophyll, chlorophyll-a and chlorophyll-b contents were estimated in fresh leaf samples by adopting the procedure of Arnon (1949).

Fresh leaf discs of known surface area from each treatment were cut in to small pieces and homogenised in a morter with 80 per cent acetone. The homogenate was filtered through Whatman filter paper No.42. After repeated washing, the filtrate was made up to 25 ml with 80 per cent acetone. The absorbance of the filtrate was measured at 645, 652, and 663 nm, using 80 per cent acetone as blank. Total chlorophyll and chlorophyll contents were calculated by using the formula given below and expressed in mg cm^{-2} leaf area.

$$\text{Total chloropyll } (\text{mg cm}^{-2} \text{ leaf area}) = \frac{27.8 \times OD_{652}}{a \times A} \times V$$

$$\text{Chlorophyll-a } (\text{mg cm}^{-2} \text{ leaf area}) = \frac{12.7 \times OD_{663} - 2.69 \times OD_{645}}{a \times A} \times V$$

Where, OD = Optical density at respective wave lengths

a = Path length of light in absorbing medium (usually 1.0 cm)

V = Volume of acetone extract

A = Leaf area in cm^2

chlorophyll-b was determined by substracting chlorophyll-a from total chlorophyll content.

3.3.3.1.2 Relative water content

Relative water content (RWC) was estimated according to the method of Barrs and Weatherly (1968). Leaf samples were collected at 2,4,6 and 8 days after herbicide spray.

Ten fresh leaf discs (2.0 cm^2) were collected from the plants and weighed accurately up to fourth decimal in an electronic balance. This weight was taken as initial weight. The weighed discs were floated on distilled water for four hours after which, the leaf discs were taken out, blotted

gently to remove the water present on the surface of discs and were weighed. This was referred as the turgid weight. Then the leaf discs were dried in oven at 80°C for 48 hours and the dry weight was recorded. From these weights, RWC was calculated by using the formula,

$$\text{RWC (\%)} = \frac{\text{Initial weight - Dry weight}}{\text{Turgid weight - Dry weight}} \times 100$$

3.3.3.1.3 **Leaf leachate analysis**

Loss in membrane integrity due to herbicidal treatments at different times after spraying was studied by leaf leachate analysis. This also gives as an index of herbicide penetration in leaf tissue and leaf injury (Vanstone and Stobbe, 1977). Ten fresh leaf discs were taken and washed two to three times with distilled water to remove the dust and sap of the cut edges. These discs were soaked in distilled water of known volume for three hours at room temperature. After soaking, the solution was decanted and filtered to collect the leaf leachate. These leaf leachates were further utilized for measuring electrical conductivity (EC) and optical density (OD) at 525 nm (anthocyanin) and OD at 275 nm (soluble protein).

Electrical conductivity

Electrical conductivity (EC) of leaf leachate was measured by using conductivity bridge at room temperature and expressed as ds/m/dm^2 of leaf area.

Absorbance at 525 nm (Anthocyanin)

The leaf leachate was diluted appropriately with distilled water and absorbance was measured at 525 nm. This is an index for leaching of anthocyanin pigment from leaf tissue. It was expressed as OD at 525 nm per dm^2 leaf area.

3.3.3.1.4 Nitrate reductase in leaf

Nitrate reductase activity in each fresh leaf was assyed *in vivo* at 2,4, 6 and 8 days of herbicide treatment following the procedure given by Sardhambal *et al.* (1978). Leaves were cut into small discs of known area and suspended in 25 ml flasks containing 0.1 M phosphate buffer (pH 7.6), 0.02 M KNO_3, propanol (5%) and two drops of chloramphenicol (0.5 mg/ml). The flasks were incubated at 30°C for 20 minutes. After incubation period, the reaction was stopped by adding 0.1 ml of 1.0 M zinc acetate and 1.9 ml of ethanol (70%). The contents were centrifuged at 3000 rpm for 10 minutes and nitrite formed was determined in an aliquat of the supernatant by adding 1 ml of sulfanilamide (1%) in 1.0 M HCl and 1.0 ml of N-1 naphtylethylene diamine dihydrochloride (0.02%).

Absorption was measured at 540 nm in spectrophotometer (Systronics, UV=VIS-108) after 20 minutes. The activity of the nitrate reductase was determined from the standard curve of KNO_2 and expressed as μ moles NO_2 formed per minute per cm^2 leaf area.

3.3.3.1.5 Stomatal resistance, transpiration rate and leaf temperature

Transpiration rate, stomatal resistance and leaf temperature were measured by using steady state pormeter (LICOR, Model LI 1600, USA). These measurements were made between 10.00 AM and 12.00 Noon on fifth and tenth day after spray.

The transpiration rate ($\mu g\ H_2O\ m^{-2}S^{-1}$) was measured directly from the same leaf used for measuring stomatal resistance (sec cm^{-1}) by the porometer. The leaf temperature (°C) was recorded using the built in thermocouple of the steady state porometer pressed against the abaxial surface of the leaf. The leaves used for measuring stomatal resistance and transpiration rate were used for recording the leaf temperature.

3.3.3.2 Biochemical parameters

3.3.3.2.1 Preparation of acetone powder

Leaf samples were collected from the field in an ice bath for preparing acetone powder. These leaf samples were cut into small pieces and ground in chilled acetone. The mixture was filtered in Buckner funnel through Whatman No.1 filter paper under suction. The powder on the filter paper was again ground with chilled acetone and filtered to remove most of the pigments. Then the powder was air dried and stored in air tight container under deep freezer till the enzyme analysis.

3.3.3.2.2 Phenylalanine ammonia lyase (PAL) assay

Assay and determination of PAL was carried out by adopting the procedure of Mahadevan and Shridhar (1986).

About 50-100 mg of acetone powder was extracted in 10 ml chilled 0.05 M borate buffer (pH 8.8). The incubation mixture consisting of 1.0 ml enzyme source, 1.0 ml of 0.05 M borate buffer (pH 8.8) and 0.5 ml of 0.05 M phenylalanine was incubated at 40°C in a water bath for exactly one hour. The reaction was stopped by adding 1.0 ml of 5 M HCl and cooled. the contents of the tubes were extracted in diethyl ether with the help of separating funnel and the ether fraction was evaporated under a stream of air. The residue containing trans-cinnamic acid, formed in the reaction was dissolved in

0.05 N NaOH and the absorbance was measured at 290 nm in UV-Spectrophotometer (Systronics UV-VIS-108). The quantity of cinnamic acid formed was calculated by using a standard curve of cinnamic acid. The protein content of the enzyme source was estimated by the procedure of Lowry *et al.* (1951). The specific enzyme activity was expressed as µmoles of transcinnamic acid formed per mg protein per hour.

3.3.2.3 Tyrosine ammonia lyase (TAL) assay

The method used to estimate the activity of PAL was adopted for the determination of TAL also, except that the substrate L-phenylalanine was replaced by L-tyrosine and the P-coumaric acid formed due to TAL activity was determined spectrophotometrically by using P-coumaric acid standard curve and expressed as p-coumaric acid formed per mg of protein per hour.

3.3.3.2.4 Peroxidase activity

Peroxidase activity from leaf acetone powder was determined by adopting the procedure given by Mahadevan and Sridhar (1986).

About 100 mg of acetone powder was extracted with 10 ml of chilled 0.1 M phosphate buffer (pH 6.0) in a morter with pestle. The mixture was centrifuged at 3000 rpm and the supernatant was used as an enzyme source.

Three ml of 0.05 M guiacol in 0.1 M phosphate buffer (pH 6.0) and 0.1 ml of enzyme extract were pipetted into a cuvette. The absorbance was adjusted to zero at 420 nm in a spectrophotometer (Systronics, UV-VIS-108). The change in absorbance was noted at an interval of 20 seconds after adding 0.5 ml of 2 per cent H_2O_2 and inverting the cuvette. The protein content of the enzyme extract was determined following the method of Lowry *et al.* (1951). The enzyme activity was expressed as change in optical density (OD) per mg protein per minute.

3.3.3.2.5 Estimation of total soluble sugars

The sugars were estimated as per the procedure given by Mahadevan and Sridhar (1986).

Alcohol extract

A known quantity of dry leaf sample was suspended in 10 to 15 ml of 80 per cent alcohol. The mixture was kept on hot water bath for few minutes. The supernatant was decanted and collected. This was repeated two to three times to ensure complete extraction. This supernatant was filtered through Whatman No.1 filter paper and the filtrate was made upto a known volume. The alcohol extract was clarified by adding neutral lead acetate. The mixture was shaken thoroughly and saturated disodium hydrogen phosphate was added. The mixture

was filtered and the filtrate was stored in refrigerator for the estimation of sugars, phenols and free amino acids.

A known amount of alcohol extract was taken in a test tube, evaporated in a hot water bath and cooled. To this, 1.0 ml of 1 N H_2SO_4 was added and the mixture was kept at 50°C in water bath for 30 minutes. Then the tubes were cooled and the mixture was neutralized by 1.0 N NaOH using phenopthalene indicator. The contents in the tubes were made up to a known volume with distilled water. This formed an aliquot for the estimation of sugars.

A known amount of aliquot was drawn in a test tube and the volume was made up to 2.0 ml with distilled water. One ml of alkaline copper reagent was added and the tubes were kept in a boiling water bath for exactly 20 minutes. The tubes were cooled and 1.0 ml of arsenomolybdate reagent was added. The contents were mixed thoroughly and the volume was made up to 10.0 ml with distilled water. The absorbance of blue colour developed was read in Spetrophotometer (Systronics, UV-VIS-108) at 540 nm. The quantity of sugars was calculated by using the glucose standard curve and expressed as mg per gram dry weight.

3.3.3.2.6 Estimation of total soluble free amino acids

The free amino acids were determined by adopting the procedure given by Moore and Stein (1948).

A known quantity (1.0 ml) of alcohol extract was drawn to which, one ml of ninhydrin reagent was added. The contents of the tubes were mixed thoroughly and kept for 20 minutes in a boiling water bath. After cooling, volume was made up to 10.0 ml with 50 per cent n-propanol. The intensity of violet colour produced was read at 570 nm in spectrophotometer (Systromics Uv-VIS-108). The quantity of free amino acids was calculated by using glycine standard curve and expressed as mg per gram dry weight.

3.3.3.2.7 Estimation of total free phenols

Total free phenols were estimated by employing the procedure of Bray and Thorpe (1954).

A known quantity of aliquot was drawn in test tubes to which two ml of distilled water followed by one ml of 1.0 N Folin-Ciocalteau reagent and two ml of two per cent sodium carbonate solution were added. The mixture was stirred and placed in a boiling water bath for exactly one minute. Then, the tubes were cooled under running tap. The blue colour developed was diluted to 25 ml with distilled water and absorbance was measured at 650 nm in spectrophotometer (Systronimcs, UV-VIS-108). the total phenols in the sample was determined by using catechol standard curve and expressed as mg per gram dry weight.

3.3.3.2.8 Estimation of crude protein content

Crude protein content was computed by using the conversion factor (6.25) for nitrogen content and expressed on per cent basis.

3.3.3.2.9 Estimation of leaf Nitrogen content

Total nitrogen was estimated by adopting the micro Kjeldahl method (Jackson, 1967).

A known weight (100 mg) of dry powdered leaf sample was taken in a Kjeldahl digestion flask to which five ml of concentrated H_2SO_4 and a pinch of digestion mixture were added. The mixture was digested until a clear pale green colour solution was obtained. The digested mixture was transferred to a nitrogen distillation apparatus. To this, 25 ml of 40 per cent NaOH was added and liberated ammonia was collected in 20 ml of four per cent boric acid containing mixed indicator. The indicator was red in boric acid and turned pale green when ammonia was dissolved. This solution was titrated against standard 0.1N HCl. A blank was carried out without the sample. The nitrogen present in the sample was calculated and expressed on per cent basis.

3.4 Anatomical studies

3.4.1 Fixation

The plant samples collected were fixed in the

standard fixative, FAA (Formaline : Acetic acid : alcohol in the ratio of 1 : 1 : 18) prepared fresh. The samples were allowed to remain in the fixative for 48 hours.

3.4.2 Dehydration

The fixed material was thoroughly washed in 70 per cent alcohol and further dehydrated by passing through 80 and 90 per cent absolute alcohol. Further, dehydration was carried out using n-butanol in combination with ethanol. The material was treated for a fixed period of three hours in each of the grades. Details of the grades and the series are given below.

Ethanol-n-Butanol grades employed in the process of de-hydration

S.No.	Water content (%)	Ethanol (%)	n-butanol
1	30	70	-
2	20	80	-
3	10	90	-
4	-	100	-
5	-	75	25
6	-	50	50
7	-	25	75
8	-	-	100

3.4.3 Paraffin infiltration

For the process of infiltration, paraffin wax with melting point of 58-60°C was used. Small chips of paraffin were added to specimen tubes containing dehydrated plant material in n-butanol to get the medium saturated under room temperature. Later, specimen tubes were kept under a table lamp for 24 hours. Then the specimen tubes were transferred to the hot-air oven maintained constantly at 60°C. Subsequent changes of fresh molten paraffin wax was given at the interval of four hours to remove the last traces of butanol.

3.4.4 Embedding

After the material was free from n-butanol, it was embedded in paraffin wax and smered with glycerin. The material with molten wax was poured to the boats, immediately followed by preboiled molten wax. The material was arranged in paper boats in convenient manner for easy cutting of blocks.

3.4.5 Microtoming/sectioning

The paraffin block containing plant material was cut into proper size and fixed on the holder. Later, the block was hardened using cold water. Thin sections of 7 µm thickness were obtained by rotary microtome.

3.4.6 Affixing the sections

Gelatin was used as an adhesive to fix the sections to slides. One per cent gelatin was used, prepared in warm water. Small amount of potassium dichromate crystals were added to it, the solution was later filtered.

The paraffin ribbon at convenient length was spread carefully on the clean slides using sufficient amount of gelatin. The slides were warmed on hot plate maintained at 45°C to stretch the sections. The excess gelatin on the slides was removed and slides were again warmed to 45°C on a hot plate. The sections were further dried under room temperature for 72 hours and stored in a clean slide box.

3.4.7 Deparaffinizing and hydrating the sections

Sections were deparafinzed using xylene. Further, were hydrated using the alcohol series. Based on the requirement, hydration of the sections were carried out following the steps listed below.

		Durations of the treatment
Xylene	:	5 minutes
Xylene + Ethanol 1:1	:	5 "
Absolute ethanol	:	5 "
90 per cent ethanol	:	5 "
70 per cent ethanol	:	5 "
50 per cent ethanol	:	5 "
Water	:	5 "

3.4.8 Staining for anatomical studies

For the anatomical observations and photographs the tissues were stained using the combination of safranin-o and fast green FCF.

3.4.9 Preparation of safranin stain

Safranin dye was prepared by dissolving 4 g of safranin-o in 200 ml of methyl cellosolve. When the solution was complete, 100 ml each of 95 per cent alcohol and distilled water were added, followed by 4 g sodium acetate and 8 ml formalin. Acetate was used for intensifying the stain and formalin was the mordant (Johansen, 1940).

3.4.10 Preparation of fast green stain

Fast green stain was prepared by making nearly saturated solution of fast green FCF in equal parts of methyl cellosolve and absolute alcohol. Enough of this solution was added to a mixture of 25 parts absolute alcohol and 75 parts clove oil to give the desired intensity. The stain was stored in a dropping bottle.

3.4.11 Staining schedule

-- Slides were deparaffinized and hydrated as detailed earlier.

-- Stained in safranin for six hours.

-- Excess stain was washed in running water.

-- Gradually dehydrated passing through quickly by using 50, 70, 90 per cent and absolute alcohol.

-- Counter stained with fast green for a brief period of fifteen seconds.

-- Excess stain was removed by using clove oil.

-- Slides were cleared in a mixture of 50 parts of clove oil, 25 parts of absolute alcohol and 25 parts of xylene.

-- Cleared again in xylene.

-- Two changes of xylene was given and finally mounted using DPX mount.

3.3.3.3.11 **Micrometry**

Observations on the size of different tissues of the plant samples, number of cells per unit area and thickness of the material were measured using a calibrated occular micrometer standardized with the help of stage micrometer.

3.5 Experiment IV : Use of carriers to increase the efficacy of herbicides under different conditions

It is known the absorption efficiency of herbicides can be increased when these herbicides are sprayed with carriers like potassium nitrate (KNO_3), Ammonimum sulfate ($(NH_4)_2 SO_4$), Urea, ($CO(NH_2)_2$), Teepol and other detergents. In order to arrive at appropriate combination of herbicide concentrations along with different carriers, this experiment was designed. It was also intended to see that by using carriers, is it possible to reduce the concentration of herbicides which in turn would greatly minimize the cost involved in controlling Eupatorium.

The details of the experiment are given below.

3.5.1 Experimental site

The experiment was conducted at College of Agriculture Dharwad farm under pot culture during 1991.

3.5.2 Experimental details

Same as described in 3.3.1 of experiment III.

3.5.3 Treatment imposition

The following treatments were imposed separately for light and shade grown plants and before the imposition of treatments plants were acclimatized for light and shade conditions for two months.

Treatments

T_1	-	Control	(unsprayed)	
T_2	-	2,4-D	(2,000 ppm) +	Ammonium sulfate (1%)
T_3	-	2,4-D	(2,000 ppm) +	Potassium nitrate (1%)
T_4	-	2,4-D	(2,000 ppm) +	Urea (1%)
T_5	-	Paraquat	(2,000 ppm) +	Ammonium sulfate (1%)
T_6	-	Paraquat	(2,000 ppm) +	Potassium nitrate (1%)
T_7	-	Paraquat	(2,000 ppm) +	Urea (1%)
T_8	-	Glyphosate	(2,000 ppm) +	Ammonium sulfate (1%)
T_9	-	Glyphosate	(2,000 ppm) +	Potassium nitrate (1%)
T_{10}	-	Glyphosate	(2,000 ppm) +	Urea (1%)

3.5.3.1 Physiological parameters

3.5.3.1.1 Relative water content

3.5.3.1.2 Leaf leachate analysis

3.5.3.2 Biochemical parameters

3.5.3.2.1 Total soluble sugars

3.5.3.2.2 Total phenols

The procedures adopted for estimating the above listed physiological and biochemical parameters are same as detailed in experiment III from 3.3.3.1.1 to 3.3.3.2.9.

The results obtained in the course of investigation were subjected to statistical analysis as per the procedur egiven by Panse and Sukhatme (1967).

IV. EXPERIMENTAL RESULTS

IV EXPERIMENTAL RESULTS

The rapid infestation of Eupatorium in forests, plantations and in plains has created a great concern due to its fast regeneration capacity and allelopathic effect on several field crops, forest species and plantations. There have been efforts to control and suppress the regeneration ability of Eupatorium by several conventional methods but have proved futile. There are also some reports of using herbicides to control Eupatorium but no systematic work has been done to check its spread and regeneration.

Keeping these aspects in mind, a study was conducted to control the growth and development of Eupatorium by using different herbicides in different concentrations. It was also intended to find out how best the efficacy of different herbicides can be increased by using various carriers and their impact in modifying various physiological, biochemical, morphological and anatomical character in Eupatorium. Four experiments were conducted to achieve the objectives and the results are presented separately experimentwise in this chapter.

4.1 Experiment I : Use of different herbicides and time of application for control of Eupatorium at two different locations

4.1.1 Effect of herbicides on plant height at Yellapur

Herbicides were sprayed during August, September and October months and the results are presented in Tables 3 to 5.

4.1.1.1 August Spray

Significant differences in plant height were observed in all the days after spray (DAS) except at zero DAS (Table 3). the plant height increased rapidly from 0 to 90 DAS in control and the increase was marginal in all the herbicide treated plants. Among the herbicides the lowest plant height (77.9 cm) was recorded in glyphosate at 7500 ppm and the maximum plant height of 95.5 cm was recorded in paraquat (2500 ppm) at 90 DAS. No significant differences were observed between 2500 and 5000 ppm concentrations of 2,4-D and paraquat. However, significant differences were observed between 2500 and 7500 ppm of all the herbicides used.

4.1.1.2 September Spray

The result of herbicides sprayed during September month on plant height presented in Table 4 indicated that they had significant influence in reducing the plant height.

Table 3 : Effect of herbicides spray during August on plant height (cm) in Eupatorium at Yellapur

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	73.4	90.6	112.5	148.3	163.6	179.5	187.6
2,4-D	2500	72.4	75.8 (16.3)	76.8 (31.7)	79.6 (44.0)	84.5 (48.3)	88.1 (50.9)	89.3 (52.4)
2,4-D	5000	73.9	74.8 (17.4)	75.5 (32.9)	77.2 (45.7)	81.8 (50.0)	84.0 (53.2)	86.3 (54.0)
2,4-D	7500	72.9	73.6 (18.8)	75.1 (33.2)	75.7 (46.8)	78.0 (52.3)	80.9 (54.9)	81.8 (56.4)
Paraquat	2500	74.9	79.7 (13.1)	79.2 (29.6)	84.6 (40.5)	89.6 (45.2)	93.7 (47.8)	95.5 (49.1)
Paraquat	5000	74.3	76.3 (15.8)	77.4 (31.2)	80.4 (43.5)	84.3 (48.5)	87.9 (51.0)	89.2 (52.5)
Paraquat	7500	74.6	76.0 (16.1)	76.9 (31.6)	78.2 (45.0)	81.4 (50.2)	83.3 (53.6)	86.1 (54.1)
Glyphosate	2500	73.9	75.7 (16.4)	78.3 (30.4)	79.2 (44.3)	80.9 (50.6)	82.3 (54.2)	84.6 (54.9)
Glyphosate	5000	73.8	74.2 (18.1)	75.0 (33.3)	76.8 (46.0)	78.6 (52.0)	79.9 (55.5)	80.2 (57.2)
Glyphosate	7500	73.1	73.8 (18.5)	174.2 (34.0)	75.1 (47.2)	76.4 (53.3)	77.5 (56.8)	77.9 (58.5)
S Em±		1.39	0.95	1.23	1.30	1.19	1.44	1.28
C D at 5%		NS	2.76	3.62	3.88	3.55	4.18	3.83

Figures in the parenthesis indicate per cent decrease over control

Table 4 : Effect of herbicides spray during September on plant height (cm) in Eupatorium at Yellapur

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	115.3	141.8	164.5	177.6	189.4	195.4	197.3
2,4-D	2500	118.7	125.8 (11.3)	133.1 (19.1)	136.6 (23.1)	139.2 (26.5)	141.6 (27.5)	143.2 (27.4)
2,4-D	5000	115.4	123.2 (13.1)	126.0 (23.4)	130.5 (26.5)	132.6 (30.0)	135.6 (30.6)	136.3 (30.9)
2,4-D	7500	116.8	120.5 (15.0)	122.3 (25.7)	125.7 (29.2)	127.1 (32.9)	129.1 (33.9)	130.4 (33.9)
Paraquat	2500	114.5	126.6 (10.7)	134.4 (18.3)	138.0 (22.9)	140.9 (25.6)	143.3 (26.7)	146.6 (25.7)
Paraquat	5000	116.3	124.4 (12.3)	129.6 (21.2)	133.1 (25.1)	135.2 (28.6)	137.8 (29.5)	138.2 (30.0)
Paraquat	7500	115.7	123.6 (12.8)	127.0 (22.8)	130.6 (26.5)	131.1 (30.8)	133.2 (31.8)	134.6 (31.8)
Glyphosate	2500	116.6	122.5 (13.6)	123.6 (24.9)	124.0 (30.2)	126.2 (33.7)	127.7 (34.6)	128.9 (34.7)
Glyphosate	5000	118.9	120.7 (14.9)	122.3 (25.7)	123.1 (30.7)	123.6 (34.7)	124.6 (36.2)	124.9 (36.7)
Glyphosate	7500	115.4	117.3 (17.3)	119.0 (27.7)	120.5 (32.2)	120.6 (36.3)	121.5 (37.8)	122.0 (38.2)
S Em±		2.86	1.16	0.90	1.16	1.29	1.79	1.49
C D at 5%		NS	3.47	2.62	3.41	3.82	5.16	4.38

Figures in the parenthesis indicate per cent decrease over control

However, at 0 DAS no significant differences were observed between the treatments. The plant height increased continuously from 0 to 90 DAS in all the treatments, but the rate of increase was more in control as compared to herbicide treatments. The data indicated that the plant height increased by 81.6 cm from 0 to 90 DAS in control, whereas it ranged from 32.1 cm (paraquat 2500 ppm) to 6.0 cm (glyphosate 5000 ppm). It was further observed that the rate of increase in plant height was minimum in glyphosate treatments at all the concentrations as compared to paraquat and 2,4-D. Except control the increase in plant height from 0 to 90 DAS was more in plants treated with paraquat. The lowest plant height (122.0 cm) was recorded in glyphosate at 7500 ppm and the maximum plant height (146.6 cm) was recorded in paraquat at 2500 ppm. It was also observed that there were no significant differences between 2500 and 5000 ppm; 5000 and 7500 ppm concentrations of paraquat. Whereas, significant differences were observed between 2500 and 7500 ppm concentrations. Similarly, no significant differences were observed between the two consecutive concentrations in 2,4-D and glyphosate upto 45 DAS and significant differences were observed at later stages.

4.1.1.3 October spray

The data indicated that the plant height was maximum in control at all the stages (Table 5). There was a significant

Table 5 : Effect of herbicides spray during October on plant height (cm) in Eupatorium at Yellapur

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	165.8	181.4	192.6	198.7	202.5	204.3	207.8
2,4-D	2500	163.3	170.1 (6.2)	176.8 (8.2)	179.6 (9.6)	182.9 (9.8)	184.6 (9.6)	186.5 (10.3)
2,4-D	5000	162.3	168.3 (7.2)	170.4 (11.5)	173.9 (12.5)	175.8 (13.2)	179.0 (12.4)	180.3 (13.2)
2,4-D	7500	163.3	167.3 (7.8)	168.3 (12.6)	190.6 (14.1)	173.8 (14.2)	175.9 (13.9)	176.9 (14.9)
Paraquat	2500	163.9	171.6 (5.4)	178.4 (7.4)	180.9 (9.0)	184.5 (8.9)	187.1 (8.4)	189.3 (8.9)
Paraquat	5000	162.2	166.9 (8.3)	169.8 (11.8)	172.3 (13.3)	174.4 (13.9)	184.0 (9.9)	185.1 (10.9)
Paraquat	7500	163.2	164.1 (9.5)	167.8 (12.9)	171.4 (13.7)	175.6 (13.3)	177.0 (13.5)	179.9 (13.4)
Glyphosate	2500	161.7	164.3 (9.4)	166.6 (13.5)	168.3 (15.3)	164.3 (18.9)	171.8 (15.9)	173.2 (16.7)
Glyphosate	5000	163.3	163.8 (9.7)	164.5 (14.6)	164.9 (17.0)	166.3 (17.9)	169.2 (17.2)	169.8 (18.3)
Glyphosate	7500	162.3	162.8 (10.3)	163.9 (14.9)	164.0 (17.5)	165.1 (18.5)	166.6 (18.5)	167.3 (19.5)
S Em±		1.66	0.51	0.44	0.30	0.93	0.86	1.30
C D at 5%		NS	1.47	1.36	0.87	2.70	2.51	2.98

Figures in the parenthesis indicate per cent decrease over control

reduction in plant height due to the application of different herbicides and the results differed significantly at all the DAS except at 0 DAS. AT 15 DAS, no significant differences were observed between 5000 and 7500 ppm of 2,4-D; 2500 and 5000 ppm, 5000 and 7500 ppm of glyphosate. At 30 DAS except between 5000 and 7500 ppm of glyphosate, significant differences were observed between all the treatments. A similar trend was observed with respect to glyphosate at 60 and 90 DAS. At 45 DAS, all the treatments differed significantly with each other and glyphosate at 7500 ppm registering the minimum plant height (164.0 cm). The rate of increase in plant height was very low in higher concentrations of glyphosate, paraquat and 2,4-D and the maximum was in control. Among the herbicide treated plants the lowest plant height (167.3 cm) was recorded in glyphosate (7500 ppm) and the maximum (189.3 cm) in 2500 ppm of paraquat.

4.1.2 Effect of herbicides on plant height at Prabhunagar

The data on the effect of herbicides spray during different months on plant height at Prabhunagar are presented in Tables 6 to 9.

4.1.2.1 August spray

Except at 0 DAS plant height differed significantly at all the stages in herbicide trèatments and control (Table 6).

Table 6 : Effect of herbicides spray during August on plant height (cm) in Eupatorium at Prabhunagar

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	65.7	77.6	96.8	126.4	145.6	159.8	168.7
2,4-D	2500	66.3	71.4 (8.0)	80.6 (16.7)	86.4 (31.6)	90.5 (37.8)	93.2 (41.7)	96.6 (42.7)
2,4-D	5000	66.7	68.3 (12.0)	75.4 (22.1)	78.6 (37.8)	83.2 (42.9)	85.6 (46.4)	87.7 (48.0)
2,4-D	7500	65.0	66.6 (14.2)	72.1 (25.5)	74.2 (41.3)	75.6 (48.1)	79.4 (50.3)	81.3 (51.8)
Paraquat	2500	66.5	73.4 (5.4)	82.2 (15.1)	89.4 (29.3)	93.1 (36.1)	95.5 (40.2)	99.3 (41.1)
Paraquat	5000	67.2	69.3 (10.7)	78.4 (19.0)	80.9 (36.0)	84.4 (42.0)	89.3 (44.1)	93.4 (44.6)
Paraquat	7500	66.4	68.6 (11.6)	74.5 (23.0)	76.6 (39.4)	79.8 (45.2)	88.3 (44.7)	89.9 (46.7)
Glyphosate	2500	66.8	68.3 (12.0)	71.6 (26.0)	75.4 (40.3)	79.4 (45.3)	83.4 (47.8)	84.9 (49.7)
Glyphosate	5000	66.3	67.9 (12.5)	69.0 (28.7)	71.3 (43.6)	74.6 (48.8)	77.2 (51.7)	78.3 (53.6)
Glyphosate	7500	65.0	66.1 (14.8)	67.4 (30.4)	68.9 (45.5)	69.4 (52.3)	69.5 (56.5)	69.7 (58.7)
S Em±		1.33	0.15	0.37	0.66	0.95	2.67	1.45
C D at 5%		NS	0.46	1.09	1.94	2.76	7.96	4.26

Figures in the parenthesis indicate per cent decrease over control

the plant height increased sharply from 0 to 90 DAS in control and marginally in herbicide treated plants. The rate of increase was very low in 7500 ppm of glyphosate at all the DAS followed by 7500 ppm of 2,4-D. Among the herbicide treatments the rate of increase was more in paraquat at all the concentrations. At 15 DAS the lowest plant height (66.1 cm) was recorded in 7500 ppm of glyphosate and the maximum (73.4 cm) in 2500 ppm of paraquat among the herbicides treatments. A similar trend was recorded at all the DAS. At 90 DAS, the maximum plant height among herbicides was recorded in 2500 ppm of paraquat (99.3 cm) followed by 2500 ppm of 2,4-D (96.6 cm) and 5000 ppm of paraquat (93.4 cm). Whereas, the lowest plant height was recorded in 7500 ppm of glyphosate (69.7 cm). It was further observed that no significant differences were recorded between 5000 ppm of 2,4-D, 7500 ppm paraquat and 2500 ppm glyphosate at 15 DAS. Similarly at 30 DAS no significant differences were observed between 5000 and 7500 ppm of 2,4-D and paraquat, respectively; 7500 ppm of 2,4-D and 2500 ppm of glyphosate. A similar trend of non significant differences between 7500 ppm paraquat and 2500 ppm glyphosate; 5000 ppm 2,4-D, 5000 and 7500 ppm paraquat and 2500 ppm glyphosate at 60 and 75 DAS.

4.1.2.2 September spray

Effect of herbicides sprayed during September month indicated significant differences on plant height between

herbicide treatments and control at all the DAS except at 0 DAS (Table 7). The maximum plant height was recorded in control from 15 DAS (127.3 cm) to 90 DAS (183.4 cm). If we compare the rate of increase in plant height, it was more during early stages as compared to later stages. The rate of increase in plant height was lowest (7.0 cm) in 7500 ppm glyphosate and was maximum (85.9 cm) in control. Similarly, the rate of increase in plant height was low at higher concentrations (7500 ppm) of all the herbicides as compared to lower concentrations (2500 and 5000 ppm). At 15 DAS most of the herbicides did not differ significantly with each other except 7500 ppm of 2,4-D and 2500 ppm of paraquat. But in all the cases plant height decreased significantly over control.

At 30 DAS significant differences were observed between all the herbicide treatments except between 7500 ppm paraquat and 5000 ppm glyphosate; 2500 ppm of 2,4-D and glyphosate; 5000 ppm of paraquat and 2500 ppm glyphosate; 7500 ppm of 2,4-D and glyphosate. At 45 DAS, all the herbicide treatments differed significantly except 5000 ppm 2,4-D and 2500 ppm glyphosate; 7500 ppm 2,4-D and 5000 ppm glyphosate. A similar trend was maintained at 60 and 75 DAS also. At 90 DAS significantly higher plant height (139.6 cm) was observed in paraquat at 2500 ppm and significantly lower plant height (106.8 cm) was observed at 7500 ppm glyphosate.

Table 7 : Effect of herbicides spray during September on plant height (cm) in Eupatorium at Prabhunagar

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	97.5	127.3	147.7	162.8	172.5	175.9	183.4
2,4-D	2500	98.1	104.3 (18.1)	108.1 (26.8)	111.3 (30.4)	114.6 (33.6)	119.1 (32.3)	121.4 (33.8)
2,4-D	5000	97.6	103.3 (18.9)	106.2 (28.1)	109.4 (32.8)	111.6 (35.3)	114.2 (35.1)	117.7 (35.8)
2,4-D	7500	98.2	102.9 (19.2)	104.3 (29.4)	106.6 (34.5)	108.1 (37.3)	110.3 (37.3)	112.0 (38.9)
Paraquat	2500	99.3	106.8 (16.1)	114.4 (22.5)	118.9 (27.0)	123.3 (28.5)	134.6 (23.5)	139.6 (23.9)
Paraquat	5000	98.9	104.4 (18.0)	108.0 (26.9)	113.4 (30.3)	117.1 (32.1)	120.5 (31.5)	121.6 (33.7)
Paraquat	7500	98.2	103.6 (18.6)	105.9 (28.3)	110.0 (32.4)	112.4 (34.8)	115.1 (34.6)	116.6 (36.4)
Glyphosate	2500	100.1	104.1 (18.2)	107.5 (27.2)	109.4 (32.5)	111.0 (35.7)	113.9 (35.2)	115.2 (37.2)
Glyphosate	5000	99.3	103.9 (18.4)	105.3 (28.7)	106.7 (34.5)	108.1 (37.3)	110.4 (37.2)	112.0 (38.9)
Glyphosate	7500	99.8	103.3 (18.9)	104.8 (29.0)	105.7 (35.1)	106.0 (38.6)	106.2 (39.6)	106.8 (41.8)
S Em±		0.92	1.13	0.26	0.18	0.56	0.75	1.37
C D at 5%		NS	3.34	0.77	0.53	1.67	2.21	4.08

Figures in the parenthesis indicate per cent decrease over control

4.1.2.3 October spray

The data on the effect of herbicides sprayed during October month indicted that the plant height decreased significantly in the herbicide treated plants over control at all the stages except at 0 DAS (Table 8).

Plant height increased continuously from 0 DAS to 90 DAS in all the treatments. The maximum plant height at 90 DAS was in control (186.3 cm) and least (153.4 cm) in 7500 ppm of glyphosate. At 15 DAS no significant differences were observed between herbicide treatments except between 2500 ppm 2,4-D and 7500 ppm paraquat and at all the concentrations of glyphosate. However, the control had significantly higher plant height over all the herbicide treated plants. A similar trend was observed at 30 and 45 DAS with 2,4-D 2500 ppm having the maximum plant height and 7500 ppm glyphosate having the minimum plant height.

At 60 DAS, no significant differences were observed between different concentrations of herbicides except glyphosate. Whereas, at 75 DAS significant differences were observed between 2500 ppm and 5000 ppm of 2,4-D, paraquat and glyphosate. Similarly, significant differences were observed between 5000 ppm and 7500 ppm of all the herbicides. The same trend was continued even at 90 DAS. However, no significant differences were observed between 5000 ppm 2,4-D, 7500 ppm paraquat and 5000 ppm of glyphosate.

Table 8 : Effect of herbicides spray during October on plant height (cm) in Eupatorium at Prabhunagar

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	146.5	157.6	169.7	174.3	180.3	183.2	186.3
2,4-D	2500	146.4	155.6 (1.3)	162.4 (4.3)	166.5 (4.5)	169.7 (5.9)	171.8 (6.5)	174.5 (6.3)
2,4-D	5000	147.6	154.6 (1.9)	157.7 (7.1)	159.2 (8.7)	162.3 (10.0)	162.4 (11.6)	162.6 (12.7)
2,4-D	7500	147.8	154.7 (1.8)	155.9 (8.1)	157.1 (9.9)	158.3 (12.2)	158.4 (13.8)	158.2 (15.1)
Paraquat	2500	146.6	154.8 (1.8)	159.8 (5.8)	163.3 (6.3)	166.9 (7.4)	169.4 (7.8)	171.5 (7.9)
Paraquat	5000	147.4	154.3 (2.1)	156.4 (7.8)	158.4 (9.1)	160.0 (11.2)	163.1 (11.2)	165.5 (11.2)
Paraquat	7500	146.0	153.5 (2.6)	154.2 (9.1)	156.7 (10.1)	157.4 (12.7)	159.5 (13.2)	161.0 (13.6)
Glyphosate	2500	147.5	153.2 (2.8)	158.4 (6.7)	159.6 (8.4)	161.2 (10.6)	164.1 (10.7)	164.9 (11.5)
Glyphosate	5000	146.3	152.9 (3.0)	155.8 (8.2)	156.7 (10.1)	157.2 (12.8)	158.9 (13.5)	160.4 (13.9)
Glyphosate	7500	146.7	150.3 (4.6)	152.6 (10.1)	152.9 (12.3)	153.3 (15.0)	153.3 (16.5)	153.4 (17.7)
S Em±		1.49	0.49	1.49	1.31	1.66	1.44	0.99
C D at 5%		NS	1.45	4.38	3.80	4.86	4.28	2.94

Figures in the parenthesis indicate per cent decrease over control

4.1.3 Effect of herbicides on stem dry weight (g plant-1) at Yellapur

The data on the effect of herbicides sprayed during different months on stem dry weight at Yellapur are presented in Table 9 to 11.

4.1.3.1 August Spray

Herbicides sprayed during August indicated significant differences in stem dry weight among various herbicides and control at all the stages except at 0 DAS (Table 9). The stem dry weight increased from 0 to 90 DAS in all the treatments and the increase was maximum in control. The maximum weight (56.84 g) was recorded in control and the minimum (21.58 g) in 7500 ppm of glyphosate at 90 DAS. It was further observed from the data that glyphosate at 7500 ppm had the lowest stem dry weight at all the stages followed by 7500 ppm of 2,4-D. It is clear from the data that the stem dry weight did not differ significantly between any of the concentrations within 2,4-D and paraquat. Whereas, the stem dry weight at 7500 ppm had significantly lower values over 2500 ppm and 5000 ppm of glyphosate and over all the concentrations of 2,4-D and paraquat.

4.1.3.2 September spray

Data on stem dry weight as influenced by various herbicides treatment during September month are given in

Table 9 : Effect of herbicides spray during August on stem dry weight (g plant-1) in Eupatorium at Yellapur

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	18.6	24.9	30.1	36.2	42.7	49.1	56.8
2,4-D	2500	19.3	23.0 (7.5)	27.1 (9.9)	32.7 (9.8)	37.8 (11.5)	43.5 (11.4)	49.2 (13.4)
2,4-D	5000	18.7	22.6 (9.1)	26.1 (13.4)	31.8 (12.2)	35.5 (16.7)	41.9 (14.6)	48.4 (14.7)
2,4-D	7500	18.2	20.7 (16.8)	21.4 (28.9)	21.9 (39.4)	22.2 (47.9)	22.2 (54.7)	22.2 (60.8)
Paraquat	2500	19.1	23.1 (7.2)	27.9 (8.5)	34.2 (5.6)	39.6 (7.1)	46.8 (4.6)	53.4 (6.0)
Paraquat	5000	19.7	22.8 (8.4)	27.2 (9.6)	33.1 (8.8)	37.9 (11.2)	43.5 (11.3)	50.9 (10.3)
Paraquat	7500	19.2	21.6 (13.0)	25.4 (15.7)	32.0 (11.8)	36.1 (15.3)	41.9 (14.7)	48.1 (15.3)
Glyphosate	2500	18.1	22.9 (7.9)	25.7 (14.7)	31.6 (12.7)	36.4 (14.6)	42.7 (13.1)	48.7 (14.2)
Glyphosate	5000	18.1	22.1 (11.1)	23.0 (23.0)	30.2 (16.7)	33.9 (20.6)	40.7 (17.0)	46.3 (18.4)
Glyphosate	7500	18.9	19.7 (20.7)	20.6 (31.4)	21.1 (41.7)	21.4 (49.8)	21.5 (56.1)	21.5 (62.0)
S Em±		0.40	0.61	1.01	2.69	2.18	4.13	3.82
C D at 5%		NS	1.79	2.99	7.88	6.47	12.18	11.19

Figures in the parenthesis indicate per cent

Table 10. There was significant differences between herbicides treatments and control only after 30 DAS up to 90 DAS. the increase in stem dry weight between two consecutive stages was sharp in control and in lower concentrations (2500 ppm and 5000 ppm) of any herbicide but it was marginal in higher concentration (7500 ppm) of glyphosate and 2,4-D herbicides. Among the herbicides glyphosate and 2,4-D only at 7500 ppm recorded lowest stem dry weight at all the stages of growth.

At 30 DAS, except paraquat at 2500 ppm, all other treatments had significant reduction in stem dry weight over control. The lowest value (31.34 g) was in 7500 ppm glyphosate fallowed by same concentration of 2,4-D (31.90 g). Similar trend was observed at all the stages. At 90 DAS, control pants recorded 69.79 g plant^{-1} dry weight. There was maximum reduction in stem dry weight (53.8 %) over control in 7500 ppm of glyphosate closely followed by 2,4-D (52.5%). Among the herbicides, paraquat recorded higher stem dry weight at any given concentration.

4.1.3.3 October spray

Significant differences in stem dry weight due to herbicide spray in October month were recorded (Table 11) at all the stages except 0 and 15 DAS. All the three herbicides reduced the stem dry weight compared to control and the degree of reduction increased with the increase in concentration of

Table 10 : Effect of herbicides spray during September on stem dry weight (g plant-1) in Eupatorium at Yellapur

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	29.3	36.7	41.6	48.5	55.9	63.2	69.7
2,4-D	2500	30.1	34.8 5.2	38.5 (7.4)	44.9 (7.4)	52.9 (5.5)	60.1 (4.6)	65.2 (6.5)
2,4-D	5000	30.9	32.7 (10.9)	35.9 (13.7)	41.8 (13.9)	50.1 (10.4)	56.9 (9.6)	61.5 (11.8)
2,4-D	7500	29.2	31.4 (14.4)	31.9 (23.5)	32.2 (33.6)	32.6 (41.1)	33.1 (47.6)	33.1 (52.5)
Paraquat	2500	29.3	35.1 (4.2)	39.9 (4.2)	46.7 (3.8)	53.2 (4.8)	61.7 (2.2)	66.8 (4.2)
Paraquat	5000	30.6	33.9 (7.5)	37.2 (10.6)	43.9 (9.5)	51.7 (7.5)	59.2 (6.3)	63.9 (8.4)
Paraquat	7500	30.2	32.3 (11.9)	35.9 (13.8)	42.2 (13.0)	49.9 (10.7)	57.1 (9.6)	60.7 (12.9)
Glyphosate	2500	30.7	34.6 (5.6)	38.1 (8.6)	44.2 (8.8)	52.1 (6.8)	59.7 (5.5)	64.5 (7.5)
Glyphosate	5000	29.6	31.9 (13.0)	34.2 (17.8)	41.3 (14.8)	49.4 (11.6)	55.2 (12.5)	59.1 (15.2)
Glyphosate	7500	29.4	30.8 (16.0)	31.3 (25.3)	31.9 (34.2)	32.1 (42.6)	32.2 (49.0)	32.2 (53.8)
S Em±		0.61	0.74	0.79	1.01	0.79	0.96	2.39
C D at 5%		NS	2.14	2.29	2.99	2.33	2.86	7.00

Figures in the parenthesis indicate per cent decrease over control

Table 11 : Effect of herbicides spray during October on stem dry weight (g plant^{-1}) in Eupatorium at Yellapur

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	42.54	49.45	56.18	62.75	70.10	76.87	83.45
2,4-D	2500	41.96	46.84 (5.30)	52.13 (7.20)	57.97 (7.60)	66.19 (5.60)	71.54 (6.90)	77.38 (7.30)
2,4-D	5000	41.64	46.18 (6.60)	51.73 (7.90)	56.84 (9.40)	96.97 (8.70)	69.25 (9.90)	74.96 (10.20)
2,4-D	7500	41.28	41.94 (15.20)	42.58 (24.20)	43.16 (31.20)	43.25 (38.30)	43.29 (43.70)	43.29 (48.10)
Paraquat	2500	41.73	47.16 (4.60)	23.96 (40.00)	60.15 (4.10)	68.24 (2.60)	73.24 (4.60)	80.57 (3.40)
Paraquat	5000	42.38	46.25 (6.60)	51.39 (8.50)	58.75 (6.40)	65.58 (6.40)	70.29 (8.50)	78.16 (6.30)
Paraquat	7500	42.46	44.93 (9.10)	50.16 (10.70)	56.25 (10.40)	63.84 (8.90)	67.79 (11.80)	75.68 (9.30)
Glyphosate	2500	42.83	46.15 (6.70)	51.24 (8.70)	57.18 (8.90)	65.46 (6.60)	70.38 (8.40)	76.79 (8.00)
Glyphosate	5000	41.16	44.83 (9.30)	48.96 (12.90)	53.25 (15.10)	57.79 (17.50)	62.46 (18.70)	67.12 (19.60)
Glyphosate	7500	42.75	43.24 (12.60)	43.78 (22.10)	43.97 (29.90)	44.12 (37.10)	44.25 (42.40)	44.24 (47.00)
S Em±		0.61	1.01	0.89	1.37	3.17	2.45	2.88
C D at 5%		NS	2.92	2.64	4.06	9.29	7.28	8.35

Figures in the parenthesis indicate per cent decrease over control

any herbicide at given stage. Significant reduction in stem dry weight was recorded by higher concentration (7500 ppm) of 2,4-D and glyphosate over the control. Among the different concentrations, significant differences were noticed only between lowest (2500 ppm) and highest concentrations (7500 ppm) of glyphosate and 2,4-D. Whereas, all the concentrations of paraquat reduced the stem dry weight significantly over the control but were on par with each other. control plants recorded a continuous sharp increase in stem dry weight from 0 to 90 DAS. Whereas it was marginal in 2,4-D and glyphosate treatments. At 90 DAS control plants recorded 83.45 g plant^{-1} stem dry weight. It was lowest (43.29 g) in 7500 ppm of 2,4-D closely followed by 7500 ppm of glyphosate (44.24 g). Paraquat treatment did not reduce the stem dry weight significantly even at higher concentration tested.

4.1.4 Effect of herbicides on stem dry weight (g plant^{-1}) at Prabhunagar

The effect of herbicides sprayed during different months on stem dry weight at ARS Prabhunagr is presented in Tables 12 to 14.

4.1.4.1. August Spray

Herbicides sprayed during August month indicated that the stem dry weight differed significantly in sprayed and

control treatments (Table 12) after one month of time. Although there was an increase in stem dry weight from 0 to 90 DAS in all the treatments, control plants registered a sharp increase than herbicide treatments. Among the herbicides tested glyphosate and 2,4-D reduced the stem dry weight to a greater extent compared to paraquat at all stage of growth. there was significant difference between lowest (2500 ppm) and higher (7500 ppm) concentrations of 2,4-D and glyphosate but not in the case of paraquat.

At 90 DAS control plants registered a maximum gain of 35.21 g in stem dry weight over 0 DAS, while, it was lowest in 7500 ppm of 2,4-D (1.05 g) followed by glyphosate (1.16 g).

4.1.4.2 September Spray

The data on stem dry matter as influenced by spraying of herbicides during September month showed significant differences due to herbicides (Table 13). However, no significant differences were observed between the treatments at 0 and 15 DAS. At 30 DAS the treatments 5000 ppm of 2,4-D and glyphosate and 7500 ppm paraquat did not differ significantly with each other. Similarly 2,4-D and glyphosate both at 2500 ppm were insignificant. The treatment, glyphosate at 7500 ppm recorded significantly lower stem dry weight (26.93 g) followed by 7500 ppm of 2,4-D

Table 12 : Effect of herbicides spray August on stem dry weight (g plant^{-1}) in Eupatorium at Prabhunagar

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	15.62	20.61	26.93	32.77	38.35	45.15	50.83
2,4-D	2500	15.11	17.89 (13.20)	19.65 (27.00)	22.93 (30.00)	26.89 (29.90)	31.52 (30.20)	36.71 (27.80)
2,4-D	5000	14.65	16.42 (20.30)	18.38 (31.70)	21.61 (34.10)	23.56 (38.60)	26.18 (42.00)	29.79 (41.40)
2,4-D	7500	15.34	16.16 (21.60)	16.24 (31.70)	16.31 (50.20)	16.36 (27.30)	16.38 (63.70)	16.39 (67.80)
Paraquat	2500	16.41	18.44 (10.50)	21.27 (21.00)	24.81 (24.30)	29.07 (24.20)	34.04 (24.60)	38.65 (23.90)
Paraquat	5000	14.90	17.86 (13.30)	19.93 (26.00)	22.39 (31.70)	26.09 (32.00)	31.46 (30.30)	35.93 (29.30)
Paraquat	7500	15.84	17.67 (14.30)	19.32 (28.30)	22.20 (32.30)	25.57 (33.30)	30.23 (33.10)	34.74 (31.60)
Glyphosate	2500	16.10	18.04 (12.50)	20.54 (23.70)	22.62 (31.00)	25.79 (32.70)	28.46 (37.00)	33.89 (33.30)
Glyphosate	5000	14.86	16.93 (17.90)	19.21 (28.70)	20.38 (37.80)	22.61 (41.00)	25.18 (44.20)	27.96 (45.00)
Glyphosate	7500	15.18	16.14 (21.70)	16.29 (39.50)	16.39 (50.00)	16.34 (57.40)	16.39 (63.70)	16.34 (67.80)
S Em±		0.68	1.72	0.49	1.08	1.40	1.28	1.48
C D at 5%		NS	NS	1.45	3.21	4.01	3.70	4.34

Figures in the parenthesis indicate per cent decrease over control

Table 13 : Effect of herbicides spray during September on stem dry weight (g plant^{-1}) in Eupatorium at Prabhunagar

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	24.46	30.84	37.46	43.84	49.56	55.10	65.89
2,4-D	2500	25.34	27.81 (9.80)	30.16 (19.50)	34.25 (21.90)	38.87 (21.60)	41.28 (25.10)	45.53 (30.90)
2,4-D	5000	24.93	27.54 (10.70)	28.24 (24.60)	31.83 (27.40)	34.62 (30.10)	37.25 (32.40)	39.46 (40.10)
2,4-D	7500	25.26	26.85 (12.90)	27.59 (26.30)	28.34 (35.40)	28.53 (42.40)	28.74 (47.80)	28.92 (56.10)
Paraquat	2500	24.54	28.16 (5.70)	31.78 (15.20)	35.46 (19.10)	39.52 (20.30)	43.70 (20.70)	47.74 (27.50)
Paraquat	5000	25.56	27.34 (11.30)	29.26 (21.90)	33.38 (23.90)	36.35 (26.70)	39.49 (28.30)	41.73 (36.70)
Paraquat	7500	25.61	26.58 (13.80)	28.06 (25.10)	30.75 (29.90)	34.04 (31.30)	36.28 (34.20)	37.42 (43.20)
Glyphosate	2500	26.08	28.64 (7.10)	30.17 (19.50)	33.19 (24.30)	37.67 (24.00)	39.25 (28.80)	42.63 (35.30)
Glyphosate	5000	25.74	27.36 (11.30)	28.12 (24.90)	30.54 (30.30)	32.28 (34.90)	33.86 (38.50)	34.57 (47.50)
Glyphosate	7500	25.47	26.42 (14.30)	26.93 (28.00)	27.16 (38.10)	27.73 (44.10)	28.52 (48.20)	28.61 (56.60)
S Em±		0.61	1.59	0.44	0.78	0.83	1.04	1.36
C D at 5%		NS	NS	1.29	2.31	2.43	3.09	4.01

Figures in the parenthesis indicate per cent decrease over control

(27.59 g) which were significantly inferior over all other treatments. A similar trend of the lowest stem dry weight were recorded in these two treatments at all other stages. The rate of increase in stem dry weight amongst the herbicides was more at 2500 ppm of all the herbicides. the rate of increase in stem dry weight was the least at higher concentration (7500 ppm) in all the herbicides.

4.1.4.3 October Spray

Significant differences in the stem dry weight were observed due to the effect of herbicides sprayed during the month of October at all the stages except at 0 and 15 DAS (Table 14). the maximum stem dry weight was recorded in control at all the stages and the lowest in 7500 ppm of glyphosate. At 30 DAS, the treatments 5000 ppm of 2,4-D, 2500 ppm and 5000 ppm of glyphosate and 5000 ppm of paraquat did not differ significantly with each other. Similarly, 2,4-D, paraquat and glyphosate at 7500 ppm were on par with each other. It was also observed that no significant differences between 2500 ppm and 5000 ppm; 5000 ppm and 7500 ppm of all the herbicides. A similar trend was observed at 45 DAS with no significant difference between the lower concentrations or the higher concentrations of different herbicides. It was further observed that the rate of increase in stem dry weight was maximum at all the concentrations of paraquat as compared to 2,4-D and glyphosate.

Table 14 : Effect of herbicides spray during October on stem dry weight (g plant^{-1}) in Eupatorium at Prabhunagar

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	38.10	43.86	48.92	56.24	65.38	73.69	80.58
2,4-D	2500	37.69	40.41 (7.90)	43.14 (11.80)	47.51 (15.50)	52.25 (20.10)	57.82 (21.50)	64.43 (20.00)
2,4-D	5000	37.42	39.25 (10.50)	42.93 (12.20)	46.13 (17.90)	51.39 (21.40)	54.84 (25.60)	62.35 (22.60)
2,4-D	7500	37.28	38.13 (13.10)	39.72 (18.80)	43.37 (22.90)	46.36 (29.10)	48.73 (33.90)	50.44 (37.40)
Paraquat	2500	38.26	41.31 (8.10)	44.63 (8.80)	48.53 (13.70)	53.49 (21.20)	56.42 (23.40)	63.82 (20.80)
Paraquat	5000	38.75	40.05 (8.70)	42.34 (12.40)	46.02 (18.20)	51.88 (20.60)	54.13 (26.50)	59.10 (26.70)
Paraquat	7500	37.18	37.96 (13.50)	39.54 (19.20)	41.48 (26.20)	43.77 (33.10)	45.37 (38.40)	51.52 (36.10)
Glyphosate	2500	36.93	39.30 (10.40)	42.63 (12.90)	47.15 (16.20)	51.38 (21.40)	54.28 (26.30)	58.47 (27.40)
Glyphosate	5000	36.84	38.94 (11.20)	41.92 (14.30)	45.84 (18.50)	48.54 (25.80)	51.33 (30.30)	53.25 (33.90)
Glyphosate	7500	38.00	39.17 (10.70)	39.81 (18.60)	41.75 (25.80)	44.05 (32.60)	46.25 (37.20)	49.27 (38.90)
S Em±		0.88	1.61	0.66	0.78	0.91	0.75	1.19
C D at 5%		NS	NS	1.92	2.32	2.68	2.20	3.53

Figures in the parenthesis indicate per cent decrease over control

At 90 DAS, no significant differences between 7500 ppm concentrations of all the herbicides. Similarly the stem dry weight in 2500 ppm and 5000 ppm of 2,4-D and 2500 ppm of paraquat were on parwith each other.

4.1.5 Effect of herbicides on leaf dry weight (g plant^{-1}) at Yellapur

The data on effect of herbicides on leaf dry weight at Yellapur sprayed during different months are presented in Tables 15 to 17.

4.1.5.1 August Spray

The data on the effect of herbicides sprayed during August on leaf dry weight presented in Table 15, indicated that treatments differed significantly at all the stages except at 0 DAS and the rate of increase between the treatments varied. The maximum leaf dry weight was observed in control and the minimum in 7500 ppm at all the stages. At 30 DAS, no significant differences were observed between the herbicide treatments but the control had significantly higher leaf dry weight over all other treatments. Whereas at 30 DAS, 2,4-D at 7500 ppm had the lowest leaf dry weight followed by glyphosate at 7500 ppm. The treatments 5000 ppm of 2,4-D, all the concentrations of paraquat and 5000 ppm and 7500 ppm of glyphosate were on par with each other. A similar trend was

Table 15 : Effect of herbicides spray during August on leaf dry weight (g plant^{-1}) in Eupatorium at Yellapur

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	5.51	7.49	8.87	10.78	12.66	13.97	15.59
2,4-D	2500	5.48	5.93 (20.80)	6.84 (22.90)	7.49 (30.50)	8.13 (35.80)	8.94 (36.00)	9.58 (38.60)
2,4-D	5000	5.64	6.00 (20.00)	6.28 (29.20)	6.78 (37.10)	7.24 (42.80)	7.86 (43.70)	8.61 (44.80)
2,4-D	7500	5.46	5.75 (23.20)	5.96 (32.80)	6.30 (41.50)	6.63 (47.60)	6.97 (50.10)	7.24 (53.60)
Paraquat	2500	5.58	6.05 (19.20)	6.30 (29.00)	6.63 (38.5)	7.34 (42.0)	8.03 (42.50)	8.92 (42.80)
Paraquat	5000	5.49	5.89 (21.40)	6.21 (29.00)	6.38 (40.80)	6.71 (47.00)	7.23 (48.20)	7.78 (50.10)
Paraquat	7500	5.60	5.93 (20.80)	6.13 (30.90)	6.21 (42.40)	6.42 (49.30)	6.49 (53.50)	6.95 (50.40)
Glyphosate	2500	5.41	5.70 (23.90)	6.48 (26.90)	6.96 (35.40)	7.53 (40.50)	7.89 (43.50)	8.41 (46.10)
Glyphosate	5000	5.56	5.83 (22.20)	6.21 (29.00)	6.48 (40.00)	6.75 (46.70)	7.13 (49.00)	7.45 (52.50)
Glyphosate	7500	5.54	5.74 (23.40)	6.06 (31.70)	6.18 (42.70)	6.05 (52.20)	6.39 (54.30)	6.44 (58.70)
S Em±		0.23	0.39	0.09	0.26	0.41	0.39	0.60
C D at 5%		NS	1.14	0.26	0.74	1.17	1.09	1.77

Figures in the parenthesis indicate per cent decrease over control

continued even at 45 and 60 DAS. At 75 DAS 5000 ppm of 2,4-D, 2500 ppm of glyphosate and 5000 ppm of paraquat were on parwith each other. In the same way 2500 ppm of 2,4-D and paraquat did not differ significantly with each other. A similar trend was observed at 90 DAS with 7500 ppm glyphosate recording the lowest (6.44 g) and control highest (15.59 g) leaf dry weight.

4.1.5.2 September spray

Influence of herbicides spray during September month on leaf dry weight is presented in Table 16. the data indicate that the treatments differed significantly at all the stages except at 0 DAS. the leaf dry weight increased continuously from 0 to 90 DAS in all the treatments and the increase was rapid in control compared to herbicide treatments. Among the herbicides, paraquat affected least for leaf dry weight accumulation at all the stages.

At 30 DAS, all the concentrations of any herbicide tested recorded significantly lower leaf dry matter than control. However, among the concentrations 2500 ppm and 5000 ppm of 2,4-D and glyphosate; 5000 ppm and 7500 ppm of paraquat did not differ significantly with each other. Similar trend continued at 45 and 60 DAS. However, at 75 DAS except paraquat 2500 ppm and 7500 ppm concentrations, all other herbicidal treatments differed significantly with each other,

Table 16 : Effect of herbicides spray during September on leaf dry weight (g plant^{-1}) in Eupatorium at Yellapur

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	8.58	10.72	12.49	13.85	15.42	16.98	18.56
2,4-D	2500	8.64	9.16 (14.10)	9.87 (21.00)	10.59 (23.50)	11.32 (26.60)	12.43 (26.90)	13.28 (28.40)
2,4-D	5000	8.50	8.86 (17.40)	9.43 (24.50)	10.36 (25.20)	10.78 (30.10)	11.54 (32.00)	12.13 (34.60)
2,4-D	7500	8.55	8.59 (19.70)	8.86 (29.10)	9.15 (33.90)	9.29 (39.80)	9.31 (45.20)	9.31 (49.80)
Paraquat	2500	8.68	8.83 (17.60)	10.15 (18.70)	10.92 (21.10)	11.74 (23.90)	12.89 (24.10)	13.98 (24.70)
Paraquat	5000	8.68	8.73 (18.70)	9.65 (22.50)	10.12 (26.70)	10.84 (29.70)	11.79 (31.10)	12.28 (33.80)
Paraquat	7500	8.67	8.69 (18.90)	9.41 (24.70)	9.93 (28.30)	10.49 (32.00)	11.12 (34.50)	11.78 (36.50)
Glyphosate	2500	8.60	9.12 (14.90)	9.68 (22.50)	10.10 (27.10)	10.74 (30.40)	11.35 (33.20)	11.98 (35.40)
Glyphosate	5000	8.56	9.03 (15.80)	9.41 (24.70)	9.83 (29.00)	10.46 (32.20)	10.95 (35.50)	11.61 (37.40)
Glyphosate	7500	8.56	8.41 (21.50)	8.58 (31.30)	8.73 (37.00)	8.79 (43.00)	8.81 (48.10)	8.81 (52.30)
S Em±		0.31	0.52	0.29	0.37	0.53	0.60	0.83
C D at 5%		NS	1.49	0.85	1.09	1.54	1.80	2.46

Figures in the parenthesis indicate per cent decrease over control

recording lowest (8.81) leaf dry weight in 7500 ppm glyphosate.

At 90 DAS also, glyphosate at 7500 ppm recorded lowest leaf dry weight followed by 2,4-D. Different concentrations of paraquat were on par with each other.

4.1.5.3 October Spray

Leaf dry weight as influenced by herbicides sprayed during October indicated no significant difference between the treatments at 0 and 15 DAS (Table 17). The leaf dry weight increased continuously from 0 to 90 DAS in all the treatments with control recording the maximum at all the stages. At 30 DAS the treatments 2500 ppm of 2,4-D and glyphosate and 5000 ppm of paraquat; 5000 ppm of 2,4-D, 7500 ppm of paraquat; 5000 ppm and 7500 ppm of glyphosate did not differ significantly with each other. The lowest leaf dry weight (11.31 g) was recorded in 7500 ppm of glyphosate. At 45 DAS significantly lower leaf dry weight of 11.33 g plant^{-1} was recorded in 7500 ppm of 2,4-D followed by 5000 ppm of 2,4-D. However no significant differences were observed between 7500 ppm of paraquat, 5000 ppm and 7500 ppm of glyphosate. At 60, 75 and 90 DAS 7500 ppm of glyphosate recorded significantly lower leaf dry weight. the treatments having the same concentration of all the herbicides viz., 2500 ppm, 5000 ppm and 7500 ppm did not differ significantly with each

Table 17 : Effect of herbicides spray during October on leaf dry weight (g plant^{-1}) in Eupatorium at Yellapur

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	11.10	12.65	14.19	15.53	17.10	18.49	19.68
2,4-D	2500	11.16	11.61 (8.20)	12.46 (12.20)	13.28 (14.50)	14.39 (15.80)	15.25 (17.50)	15.96 (18.90)
2,4-D	5000	11.24	11.48 (9.20)	12.16 (14.30)	12.93 (16.70)	13.79 (19.40)	14.68 (20.60)	15.21 (22.70)
2,4-D	7500	11.18	11.33 (10.40)	11.75 (17.20)	12.09 (22.20)	12.16 (28.90)	12.18 (34.10)	12.18 (38.10)
Paraquat	2500	11.25	11.81 (6.60)	12.73 (10.30)	13.69 (11.80)	14.71 (14.00)	15.83 (14.40)	16.74 (14.90)
Paraquat	5000	11.20	11.69 (7.60)	12.54 (11.60)	13.29 (14.40)	14.16 (17.20)	15.00 (18.90)	15.35 (22.00)
Paraquat	7500	11.27	11.38 (10.80)	11.96 (15.70)	12.68 (18.30)	13.29 (22.30)	14.53 (21.40)	15.68 (20.30)
Glyphosate	2500	11.15	11.52 (8.90)	12.28 (13.50)	12.96 (16.50)	13.64 (20.30)	14.21 (23.10)	15.10 (23.30)
Glyphosate	5000	11.22	11.31 (10.60)	12.00 (15.40)	12.28 (20.90)	13.05 (23.70)	13.54 (26.80)	14.00 (28.70)
Glyphosate	7500	11.13	11.26 (11.00)	11.31 (20.40)	11.33 (27.00)	11.46 (33.00)	11.47 (38.00)	14.47 (26.50)
S Em±		0.38	1.44	0.08	0.18	0.46	0.62	0.13
C D at 5%		NS	NS	0.23	0.54	1.36	1.82	0.37

Figures in the parenthesis indicate per cent decrease over control

other. At 90 DAS also a similar trend of insignificant differences between the same differences of different herbicides was observed.

4.1.6 Effect of herbicides on leaf dry weight (g plant^{-1}) in Eupatorium at Prabhunagar

The data on effect of herbicides on leaf dry weight at Prabhunager sprayed during different months are presented in Tables 18 to 20.

4.1.6.1. October Spray

Significant differences in the leaf dry weight were observed due to the effect of herbicides sprayed during the month of October at all the stages except at 0 and 15 DAS (Table 18). the maximum leaf dry weight was recorded in control at all the stages and the lowest in 7500 ppm of glyphosate. At 30 DAS, the treatments 5000 ppm of 2,4-D, 2500 ppm and 5000 ppm of glyphosate and 5000 ppm of paraquat did not differ significantly with each other. Similarly, 2,4-D, paraquat and glyphosate at 7500 ppm were on parwith each other. It was also observed that no significant differences between 2500 ppm and 5000 ppm; 5000 ppm and 7500 ppm of all the herbicides. A similar trend was observed at 45 DAS with no significant difference between the lower concentrations or the higher concentration of different herbicides. It was further observed that the rate of

Table 18 : Effect of herbicides spray during August on leaf dry weight (g plant^{-1}) in Eupatorium at Prabhunagar

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	4.60	6.26	7.37	9.03	10.87	11.58	14.38
2,4-D	2500	4.56	4.96 (20.80)	5.38 (27.00)	5.89 (34.80)	6.94 (36.10)	7.81 (32.60)	8.46 (41.20)
2,4-D	5000	4.73	4.89 (21.90)	4.93 (33.10)	5.41 (40.10)	5.92 (45.50)	6.24 (46.10)	6.98 (51.50)
2,4-D	7500	4.49	4.54 (27.50)	4.64 (37.00)	4.89 (45.90)	5.16 (52.50)	5.21 (55.00)	5.20 (63.80)
Paraquat	2500	4.68	4.86 (22.40)	5.64 (23.50)	6.63 (26.60)	7.18 (33.90)	7.93 (31.50)	8.64 (39.90)
Paraquat	5000	4.51	4.69 (25.10)	5.10 (30.80)	5.73 (36.50)	6.10 (43.90)	6.74 (41.80)	7.10 (50.60)
Paraquat	7500	4.64	4.75 (24.10)	4.96 (32.70)	5.38 (40.40)	5.96 (45.20)	6.28 (45.80)	6.93 (51.80)
Glyphosate	2500	4.70	4.87 (22.20)	5.21 (29.30)	5.74 (36.40)	6.29 (42.10)	6.93 (40.20)	7.86 (45.30)
Glyphosate	5000	4.45	4.58 (26.80)	4.84 (34.30)	5.29 (41.40)	5.78 (46.80)	6.13 (47.10)	6.74 (53.10)
Glyphosate	7500	4.52	4.62 (26.20)	4.69 (36.40)	4.72 (47.70)	4.76 (56.20)	4.80 (58.50)	4.85 (66.30)
S Em±		0.46	0.89	0.14	0.19	0.28	0.59	0.48
C D at 5%		NS	NS	0.40	0.55	0.83	1.76	1.42

Figures in the parenthesis indicate per cent decrease over control

increase in leaf dry weight was maximum at all the concentrations of paraquat as compared to 2,4-D and glyphosate.

At 90 DAS, 7500 ppm of glyphosate recorded significantly lowest leaf dry weight (4.85 g) followed by the same concentration of 2,4-D. The control recorded the highest (14.38 g) leaf dry weight.

4.1.6.2 **August Spray**

The data on the effect of herbicides sprayed during August on leaf dry weight presented in Table 19, indicated that treatments differed significantly at all the stages except at 0 and 15 DAS. The leaf dry weight increased continuously from 0 to 90 DAS in all the treatments and the rate of increase between the treatments varied. The maximum leaf dry weight was observed in control and the minimum 7500 ppm of glyphosate at all the stages. At 30 DAS, no significant differences were observed between the herbicide treatments but control had significantly higher leaf dry weight (10.31 g) over all other treatments. Whereas, 2,4-D of 7500 ppm had the lowest leaf dry weight (7.43 g) followed by glyphosate at 7500 ppm (7.47 g). The treatments 5000 ppm of 2,4-D, all the concentrations of paraquat and 5000 ppm and 7500 ppm of glyphosate were on par with each other. A similar trend was continued even at 45 and 60 DAS.

Table 19 : Effect of herbicides spray during September on leaf dry weight (g plant^{-1}) in Eupatorium at Prabhunagar

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	7.21	9.33	10.31	12.09	13.69	14.68	15.85
2,4-D	2500	6.91	7.84 (15.90)	8.71 (15.50)	9.67 (20.00)	10.73 (21.60)	11.61 (20.90)	12.46 (21.40)
2,4-D	5000	7.38	7.69 (17.60)	7.98 (22.60)	8.64 (28.50)	9.14 (33.20)	9.83 (33.00)	10.34 (34.80)
2,4-D	7500	6.98	7.27 (22.10)	7.43 (27.90)	7.74 (35.90)	7.95 (41.90)	8.27 (43.90)	8.44 (46.80)
Paraquat	2500	7.13	7.91 (15.20)	8.83 (14.40)	9.79 (19.00)	10.83 (20.90)	11.73 (20.10)	12.54 (20.90)
Paraquat	5000	6.99	7.69 (17.60)	8.24 (20.10)	8.93 (26.20)	9.24 (32.50)	9.96 (32.20)	10.48 (33.90)
Paraquat	7500	7.24	7.58 (18.80)	7.75 (24.80)	7.93 (34.40)	8.25 (39.70)	8.49 (42.20)	8.67 (45.30)
Glyphosate	2500	7.16	7.71 (17.40)	8.58 (16.80)	8.96 (25.90)	9.73 (28.90)	10.68 (27.20)	11.93 (24.70)
Glyphosate	5000	7.31	7.69 (17.60)	7.90 (23.40)	8.46 (30.00)	8.97 (34.50)	9.61 (34.50)	10.13 (36.10)
Glyphosate	7500	7.20	7.32 (21.50)	7.42 (27.50)	7.71 (36.20)	7.95 (41.90)	8.02 (45.30)	8.04 (49.30)
S Em±		0.33	0.91	0.13	0.21	0.29	0.49	0.38
C D at 5%		NS	NS	0.36	0.62	0.85	1.44	1.12

Figures in the parenthesis indicate per cent decrease over control

At 75 DAS 5000 ppm of 2,4-D, 2500 ppm and 5000 ppm of glyphosate and 5000 ppm of paraquat were on par with each other. In the same way 2500 ppm of 2,4-D and paraquat did not differ significantly with each other. A similar trend was observed at 90 DAS with 7500 ppm glyphosate recording the lowest (8.04 g) and control higher (15.85 g) leaf dry weight.

4.1.6.3 September Spray

Influence of herbicides spray during September month on leaf dry weight is presented in Table 20. The data indicate that the treatments differed significantly at all the stages except at 0 and 15 DAS. The leaf dry weight increased continuously from 0 to 90 DAS in all the treatments and the increase was rapid in control compared to herbicide treatments. Among the herbicides, paraquat affected least in leaf dry weight accumulation at all the stages.

At 30 DAS all the concentrations of any herbicide tested recorded significantly lower leaf dry weight than control. However, among the concentrations 2500 ppm and 5000 ppm of 2,4-D and glyphosate, 5000 ppm and 7500 ppm of paraquat did not differ significantly with each other. Similar trend continued at 45, and 60 DAS. However at 75 DAS except paraquat of 2500 ppm and 7500 ppm concentrations all other herbicidal treatments differed significantly with each other,

Table 20 : Effect of herbicides spray during October on leaf dry weight (g plant^{-1}) in Eupatorium at Prabhunagar

Treatments	Concentration (ppm)	Days after spray (DAS)						
		0	15	30	45	60	75	90
Control	---	10.94	12.28	13.86	14.93	16.06	16.93	17.38
2,4-D	2500	10.71	11.18 (8.90)	11.93 (13.90)	12.79 (14.30)	12.81 (14.00)	14.69 (13.20)	15.37 (11.60)
2,4-D	5000	10.52	10.93 (11.00)	16.64 (16.00)	12.10 (18.90)	12.93 (19.50)	13.81 (18.40)	14.38 (17.30)
2,4-D	7500	10.43	10.72 (12.70)	11.33 (18.30)	11.64 (22.00)	11.93 (25.70)	12.46 (26.40)	12.79 (26.40)
Paraquat	2500	10.85	11.28 (8.10)	12.03 (13.20)	12.93 (13.40)	13.98 (13.00)	14.76 (12.80)	15.79 (9.10)
Paraquat	5000	10.79	11.10 (9.60)	11.73 (15.40)	12.31 (17.50)	13.06 (18.70)	13.93 (17.70)	14.41 (17.10)
Paraquat	7500	10.84	11.07 (9.80)	11.58 (16.50)	11.96 (19.90)	12.61 (21.50)	13.48 (20.40)	13.83 (20.40)
Glyphosate	2500	10.82	11.26 (8.30)	11.74 (15.30)	12.60 (15.60)	13.58 (15.40)	14.18 (16.20)	14.96 (13.90)
Glyphosate	5000	10.79	11.04 (10.10)	11.46 (17.30)	11.92 (20.10)	12.54 (21.90)	12.96 (23.40)	13.84 (20.40)
Glyphosate	7500	10.88	11.05 (10.00)	11.40 (17.70)	11.82 (20.80)	11.89 (26.00)	12.11 (28.50)	12.59 (27.60)
S Em±		0.26	0.47	0.09	0.06	0.18	0.22	0.46
C D at 5%		NS	NS	0.27	0.17	0.53	0.65	1.36

Figures in the parenthesis indicate per cent decrease over control

recording lowest (11.89 g) leaf dry weight in glyphosate at 7500 ppm.

At 90 DAS also glyphosate at 7500 ppm recorded lowest leaf dry weight followed by 2,4-D. Different concentrations of paraquat were on par with each other.

4.2 Experiment II : Use of herbicides for control of seed production in Eupatorium

The data pertaining to the spray of herbicides to control seed production are presented in Tables 21 to 22.

4.2.1 Seed parameters in Eupatorium (Flower bud stage spray)

The data on the various seed parameters in Eupatorium as influenced by the spray of herbicides at flower bud stage are presented in Table 21.

4.2.1.1 Number of capitula per shoot

Significant differences due to the application of various herbicides at different concentrations were noticed. The maximum number of capitula per shoot (2347) was recorded in control (Fig. 4a). Among the herbicides, lower concentration of all the herbicides (2500 ppm) had the maximum number of capitula. It was further observed that with an increase in concentration of herbicides, there was a decrease in the number of capitula. Among the herbicides the least number was

Table 21. Effect of herbicides spray at flower bud stage on seed parameters in Eupatorium

Treatments	Concentrations (ppm)	No. of capitula per shoot	1000 captula weight (g)	No. of cypsella per capitula	1000 cypsella weight (mg)	Seed germination (%)	
						5th Day	10th Day
Control	----	2347	8.26	30.6	234.6	7.5 (55.6)	9.9 (80.3)
2,4-D	2000	1854	3.84	26.5	135.2	4.1 (16.3)	4.6 (20.7)
2,4-D	3000	1536	3.05	23.3	112.3	3.7 (12.7)	3.8 (13.9)
2,4-D	4000	1072	2.47	19.28	93.4	3.2 (9.2)	3.5 (11.3)
Paraquat	2000	1926	3.16	22.4	96.3	2.8 (6.8)	3.0 (8.3)
Paraquat	3000	1451	2.58	20.21	64.3	1.9 (2.6)	1.0 (0.0)
Paraquat	4000	961	1.76	16.6	53.2	1.0 (0.0)	1.0 (0.0)
Glyphosate	2000	1937	4.28	24.4	120.3	4.4 (18.5)	4.7 (21.4)
Glyphosate	3000	1468	3.37	21.5	98.7	3.8 (14.1)	4.1 (16.3)
Glyphosate	4000	1036	2.26	17.4	76.3	3.0 (8.2)	3.1 (9.0)
S Em±		95	0.31	1.6	5.3	0.3	0.42
C D at 5%		287	0.92	4.9	17.0	0.92	1.17

* - Figures in the parenthesis are original values

Treatments

1. Control (unsprayed)
2. 2,4-D (2000 ppm)
3. 2,4-D (3000 ppm)
4. 2,4-D (4000 ppm)
5. Paraquat (2000 ppm)
6. Paraquat (3000 ppm)
7. Paraquat (4000 ppm)
8. Glyphosate (2000 ppm)
9. Glyphosate (3000 ppm)
10. Glyphosate (4000 ppm)

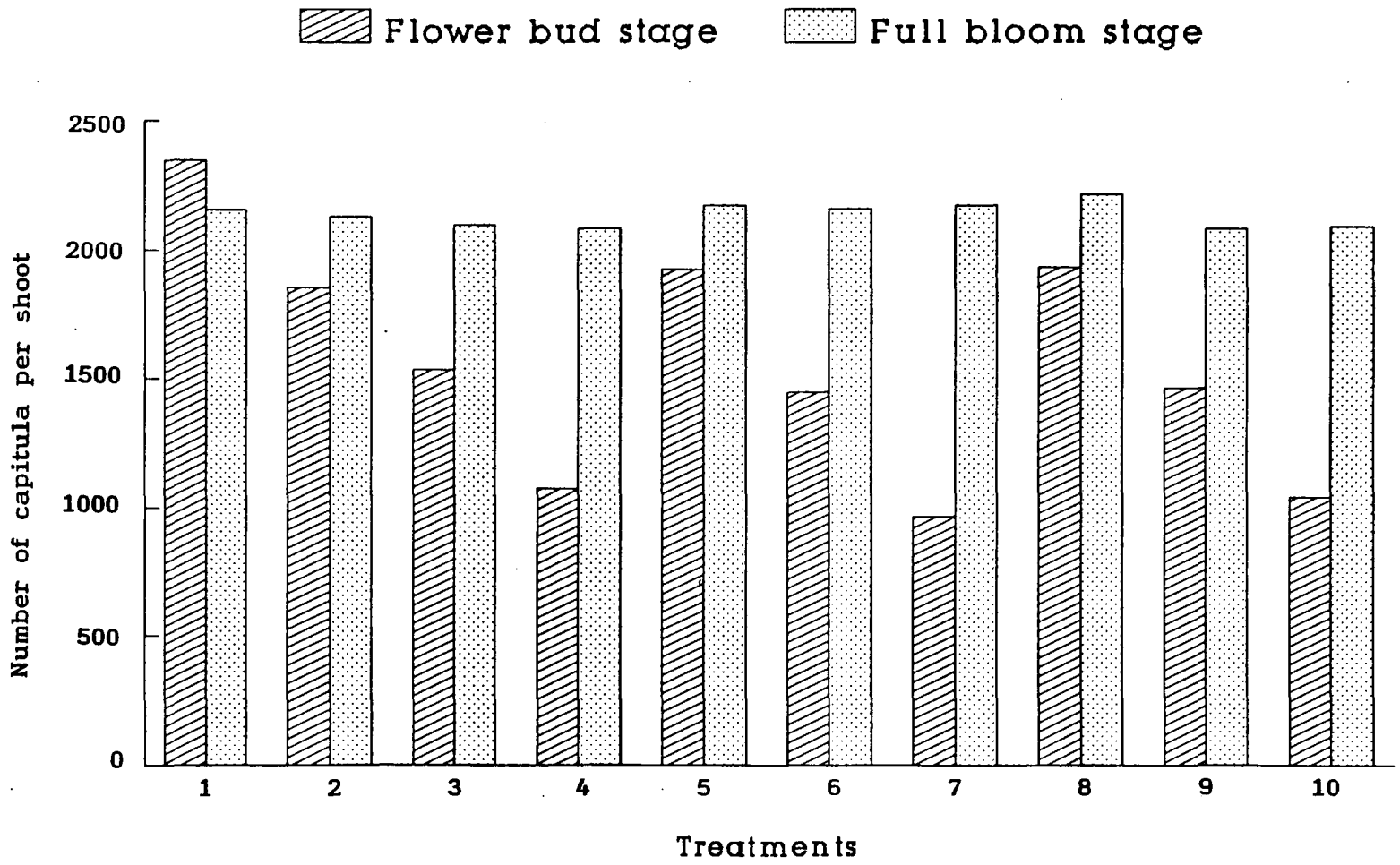


Figure 4a. Effect of herbicides spray on number of capitula per shoot.

recorded with paraquat 3000 and 4000 ppm. Whereas, at lower concentrations it was with 2,4-D.

4.2.1.2 1000 - capitula weight (g)

It was observed that with an increase in the concentration of herbicides 1000 capitula weight decreased in all the herbicides with 4000 ppm of paraquat recording the lowest value (1.76 g). Significant differences were observed only between 2000 and 4000 ppm concentrations of all the herbicides. However, no significant differences were observed between 2000 and 3000 ppm; 3000 and 4000 ppm concentrations of any of the herbicides.

4.2.1.3 Number of cypsella per capitula

Significant differences were observed with respect to number of cypsella per capitula between treatments and control (Fig. 4b). Maximum number of cypsella (30.6) were observed in control and the minimum (16.6) in 4000 ppm of paraquat. It was also observed that no significant difference were observed between any of the two consecutive concentrations of herbicides. With an increase in concentrations of herbicides the number of cypsella decreased with significantly lower values at 4000 ppm of all the herbicides.

Treatments

1. Control (unsprayed)
2. 2,4-D (2000 ppm)
3. 2,4-D (3000 ppm)
4. 2,4-D (4000 ppm)
5. Paraquat (2000 ppm)
6. Paraquat (3000 ppm)
7. Paraquat (4000 ppm)
8. Glyphosate (2000 ppm)
9. Glyphosate (3000 ppm)
10. Glyphosate (4000 ppm)

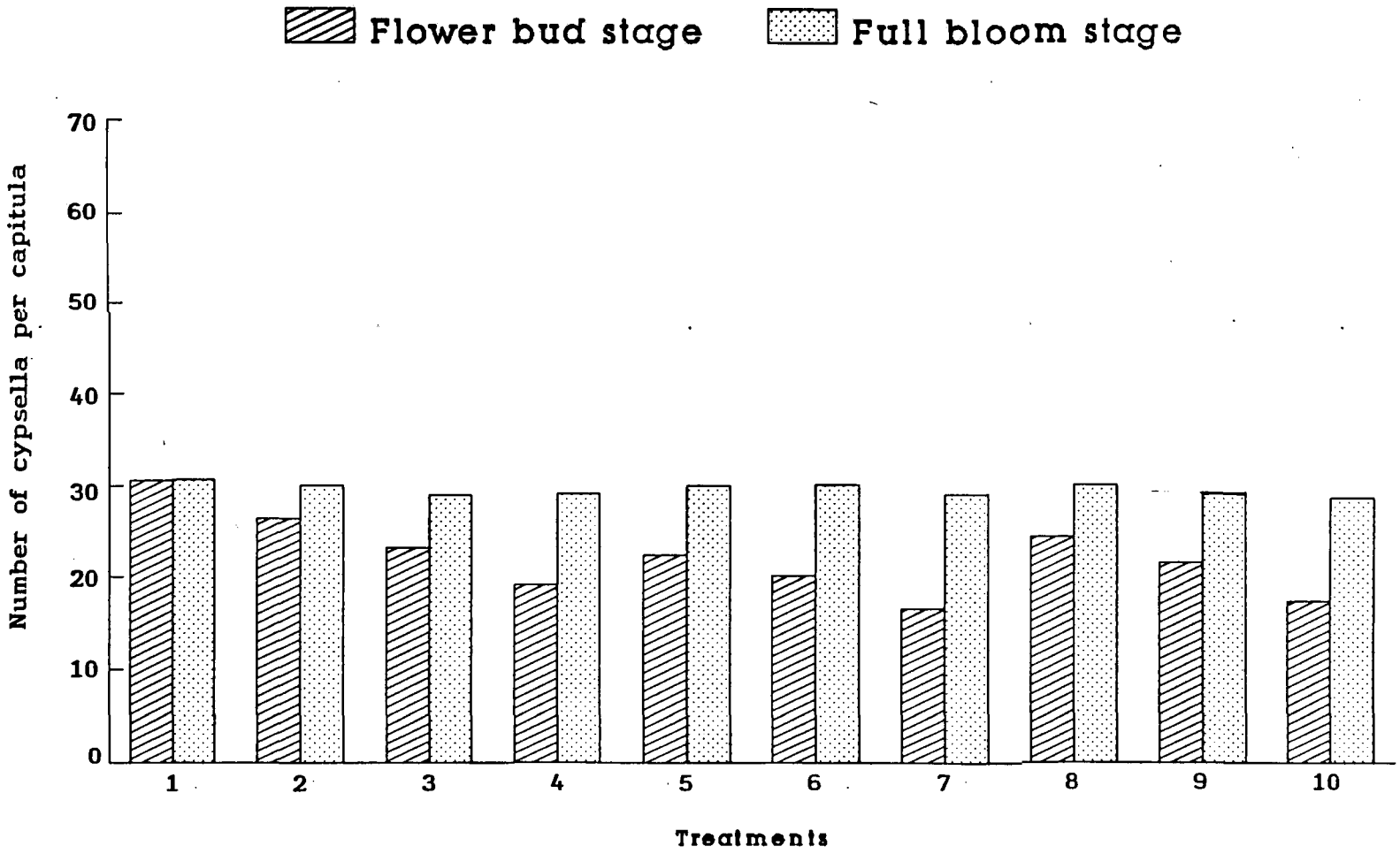


Figure 4b. Effect of herbicides spray on number of cypsella per capitula

4.2.1.4 1000 - cypsella weight (mg)

1000-cypsella weight ranged from 234.6 mg in control to 53.2 mg in 4000 ppm of paraquat. Amongst various herbicides tested in different concentrations paraquat at all the concentrations had the lower 1000-cypsells weight compared to 2,4-D and glyphosate. Of the three herbicides, 2,4-D at all the concentrations had the least influence in reducing 1000-cypsella weight.

4.2.1.5 Seed germination (%)

Seed germination tests of matured cypsella was done and the germination counts were taken on fifth and tenth day. The data indicated the significant differences in seed germination between the treatments both at 5th and 10th day. In general, it was observed that the seed germination increased from 5th to 10th (Fig. 4c) day in all the treatments but the per cent increase was more in control (55.6 to 80.3) and the least in paraquat (0 per cent at both days). It is clear from the data that paraquat at all the concentrations had significant influence in reducing the seed germination at all the concentrations. However, the seed germination per cent was maximum at lower concentration (2000 ppm) of glyphosate and higher concentration (4000 ppm) of 2,4-D at both the stages.

Treatments

1. Control (unsprayed)
2. 2,4-D (2000 ppm)
3. 2,4-D (3000 ppm)
4. 2,4-D (4000 ppm)
5. Paraquat (2000 ppm)
6. Paraquat (3000 ppm)
7. Paraquat (4000 ppm)
8. Glyphosate (2000 ppm)
9. Glyphosate (3000 ppm)
10. Glyphosate (4000 ppm)

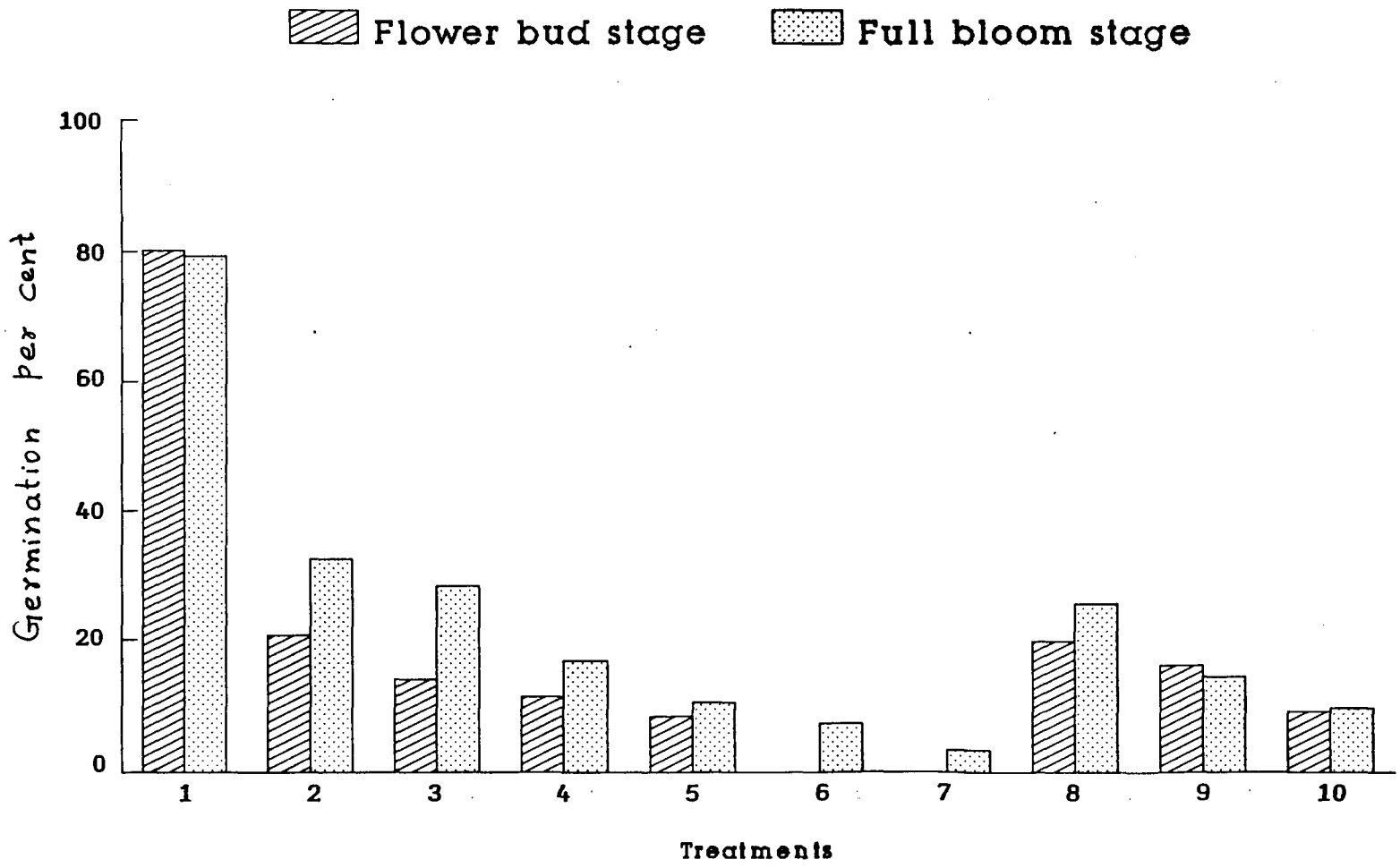


Figure 4c. Effect of herbicides spray on seed germination at tenth day

4.2.2 Seed parameters in Eupatorium (full bloom stage spray)

4.2.2.1 Number of capitula per shoot

The data indicated non significant differences between the treatments in number of capitula per shoot due to the application of herbicides at full bloom stage (Table 22 and Fig. 4a). However, the lowest number of capitula per shoot was observed with respect to 3000 ppm of glyphosate (2086).

4.2.2.2 1000 - capitula weight (g)

The treatments differed significantly with each other with control having the maximum (7.54 g) weight. Among the herbicides paraquat at all the concentrations had the lower capitula weight compared to 2,4-D and glyphosate. Significantly the lowest capitula weight (2.43 g) was recorded in 4000 ppm of paraquat over all other treatments. Among the herbicides, glyphosate at all the concentrations recorded the maximum capitula weight indicating the lowest influence of glyphosate even at higher concentrations in comparison to other two herbicides tested.

4.2.2.3 Number of cypsella per capitula

The data indicated no significant differences due to the application of herbicides at different concentrations on number of cypsella. However, the maximum cypsella number

Table 22 : Effect of herbicides spray at full bloom stage on seed parameters in Eupatorium

Treatments	Concentrations (ppm)	No. of capitula per shoot	1000 captula weight (g)	No. of cypsella per capitula	1000 cypsella weight (mg)	Seed germination (%)	
						5th Day	10th Day
Control	----	2158	7.54	30.7	223.6	7.3 (52.3)	8.4 (79.3)
2,4-D	2000	2130	5.49	30.1	186.8	4.6 (20.2)	5.8 (32.5)
2,4-D	3000	2098	4.16	29.2	152.3	4.0 (15.3)	5.4 (28.4)
2,4-D	4000	2085	3.78	29.3	104.3	3.5 (11.5)	4.2 (16.8)
Paraquat	2000	2175	3.98	30.1	101.5	2.9 (8.5)	3.4 (10.4)
Paraquat	3000	2160	3.15	30.3	77.6	2.3 (5.4)	2.9 (7.3)
Paraquat	4000	2176	2.43	29.2	53.2	1.7 (2.8)	2.0 (3.2)
Glyphosate	2000	2218	5.64	30.2	154.5	3.9 (15.5)	5.1 (25.5)
Glyphosate	3000	2086	5.22	29.3	127.4	3.2 (10.3)	3.9 (14.4)
Glyphosate	4000	2095	4.33	28.7	88.4	2.6 (6.8)	3.3 (9.6)
S Em±		44	0.22	0.74	6.2	1.6	1.9
C D at 5%		NS	0.63	NS	18.1	4.7	5.8

* - Figures in the parenthesis are original values

(30.7) and the minimum (28.7) were recorded in control and 4000 ppm of glyphosate, respectively (Fig. 4b).

4.2.2.4 1000-cypsella weight (mg)

The maximum 1000-cypsella weight (223.6 mg) was recorded in control. Among the herbicides the lower concentrations of all the herbicides had the maximum cypsella weight and there was a continuous decrease in 1000 - cypsella with an increase in the concentrations of herbicides. It was further observed that there were significant differences between 2000 and 3000; 3000 and 4000 ppm concentrations in all the herbicides. Significantly lower cypsella weight was recorded at all the concentrations of paraquat compared to 2,4-D and glyphosate.

4.2.2.5 Seed germination (%)

The data indicated that seed germination per cent increased from 5th day to 10 th day in all the treatments. In control, it ranged from 52.3 (5th day) to 79.3 (10th day). the rate of increase was higher at lower concentration of all the herbicides and was lower at higher concentration (Fig. 4c). Among the herbicides the lowest germination was recorded in paraquat at all the concentrations and there was a marginal increase i.e.,, from 2.8 to 3.2 per cent from 5th day to 10th day at 4000 ppm of paraquat. Within the herbicides,

significant differences were observed between the concentrations in glyphosate and 2,4-D.

4.3 Experiment III : Effect of herbicides and growth conditions on physiological, biochemical and anatomical changes in Eupatorium.

The data pertaining to the effect of growth conditions on plant morphology and anatomy and the effect of herbicides under different growth conditions (open light and shade) are presented in Table 23 to 41.

4.3.1 Morphological and anatomical characters

Visual morphological and anatomical differences in open light and shade grown Eupatorium plants are listed in Table 23.

4.3.1.1. Morphological characters

Distinct morphological differences were observed between open light and shade grown plants. shade grown plants appeared dark green (Plate 3a) with tender, errect and tall stem. Plants grown in open light were pale green, branched and short in height (Plate 3b). The apical bud of light grown plants appeared to be brown due to the accumulation of anthocyanin pigment which was conspicuously absent in shade grown plants. It was interesting to note that the light grown plants flowered during the usual month of November but shade

Table 23 : Effect of different growth conditions on anatomical characters in Eupatorium

Leaf characters	Growth conditions	
	Open light	Shade
Stomatal number per mm^2 leaf area	320	242
Leaf thickness (mm)	0.184	0.019
Petiole diameter (mm)	2.13	1.49
Thickness of epidermis		
(i) abaxial	35.4	26.8
(ii) adaxial	24.6	19.3
Degree of pubesence (No. of hairs mm^{-2} leaf area)		
(i) abaxial	51	36
(ii) adaxial	436	349
Mesophyll thickness (mm)	0.091	0.072
No. of mesophyll cells per mm^2 leaf area	62.7	48.3

Observations are the means of five readings.



a. At Seedling Stage



b. At Full Bloom Stage

PLATE 3. MORPHOLOGICAL DIFFERENCES BETWEEN OPEN LIGHT AND SHADE GROWN EUPATORIUM PLANTS

grown plants did not flower but continued the vegetative growth.

4.3.1.2 Anatomical characters

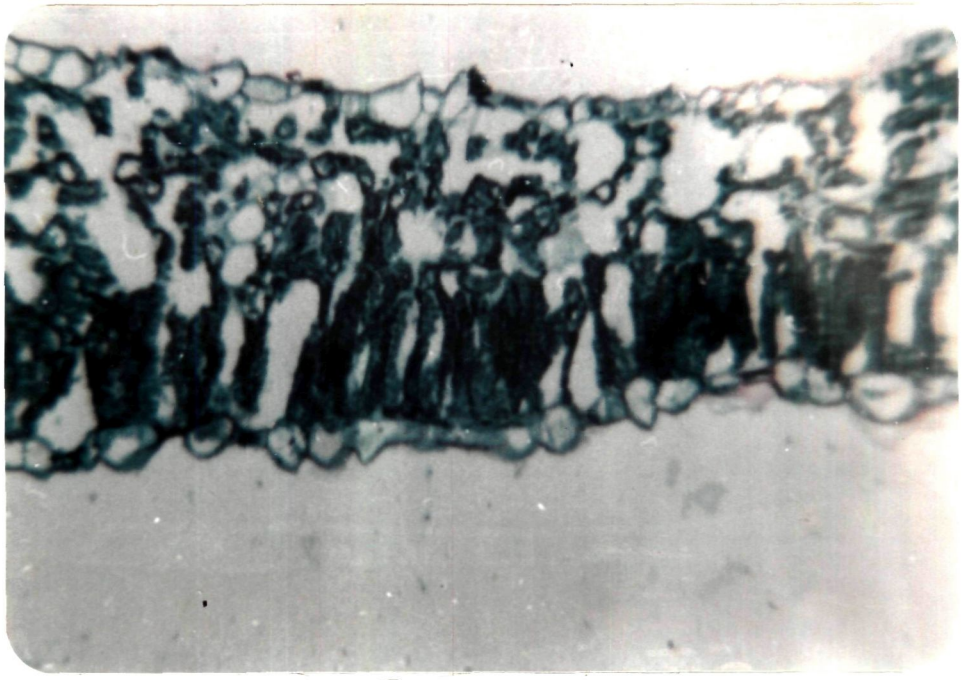
Observations made on various leaf anatomical characters indicated that there was decrease in the stomatal number, leaf thickness, petiole diameter, thickness of epidermis and mesophyll tissue with decrease in light intensity in shaded condition. The degree of pubsescence was more in plants grown in open light compared to shade grown plants.

Leaf thickness

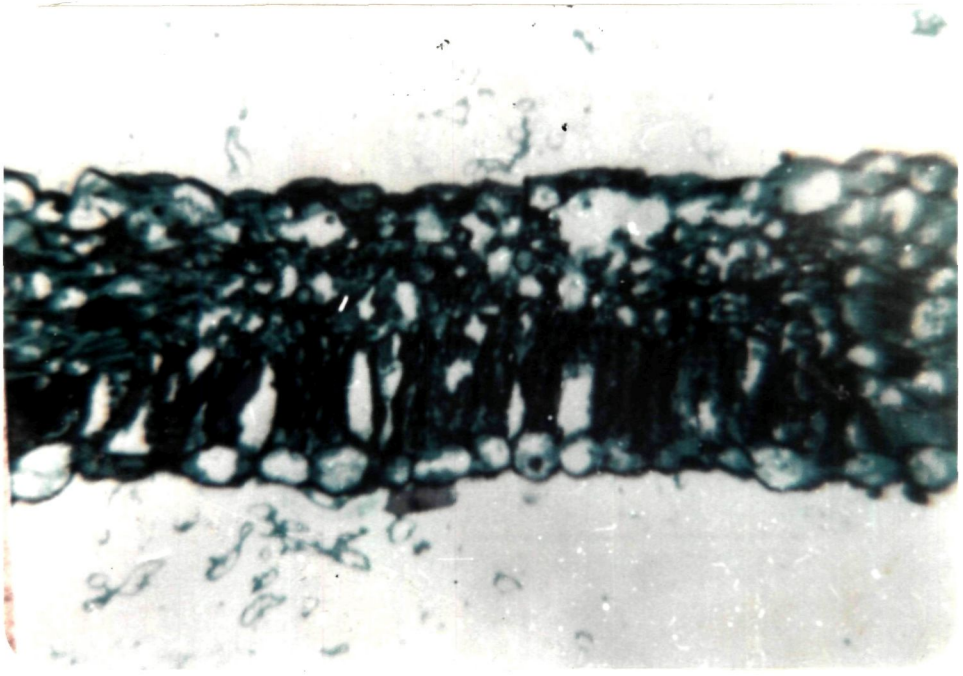
It was observed that leaf thickness significantly differed between the treatments at all the DAS both under open light and shaded conditions (Table 24). It was observed that the leaf thickness was more in the plants grown under open light (Plate 4a) as compared to shade grown plants (Plate 4b). Leaf thickness decreased continuously from 2 to 8 DAS in all the treatments except control, where it increased from 168.0 to 183.6 um in open light and 118.5 to 124.9 um under shaded condition. among the herbicides treatments the maximum leaf thickness was recorded in 2000 ppm paraquat at all the stages under both the conditions. It was further observed that the differences between 2000 and 3000 ppm or between 3000 and 4000

Table 24 : Effect of herbicides on leaf thickness ($mmX10^{-3}$) in Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	168.0	172.3	174.1	183.6	118.5	120.1	123.4	124.9
2,4-D	2000	110.5	107.5	104.0	100.6	116.3	114.8	111.0	108.7
2,4-D	3000	97.3	94.1	89.6	86.9	111.6	108.3	104.2	103.2
2,4-D	4000	92.0	88.3	82.7	80.6	105.8	100.7	96.5	95.1
Paraquat	2000	132.6	129.1	126.5	124.2	116.9	115.0	113.4	111.3
Paraquat	3000	128.3	124.5	119.4	115.3	113.8	110.5	107.6	105.7
Paraquat	4000	125.0	120.4	114.3	109.0	109.4	104.1	99.8	97.2
Glyphosate	2000	98.3	94.1	91.7	90.2	115.2	113.5	110.7	106.4
Glyphosate	3000	92.6	87.4	83.0	81.5	108.4	105.8	100.3	98.2
Glyphosate	4000	85.3	79.2	74.3	71.4	98.3	93.4	88.5	82.5
S Em±		2.1	2.4	3.3	4.5	3.4	4.4	6.3	5.1
C D at 5%		5.9	6.8	9.6	12.3	9.9	12.8	18.2	14.3



a. Open Light Grown



b. Shade Grown

PLATE 4. CROSS SECTION OF EUPATORIUM LEAVES GROWN UNDER DIFFERENT GROWTH CONDITIONS

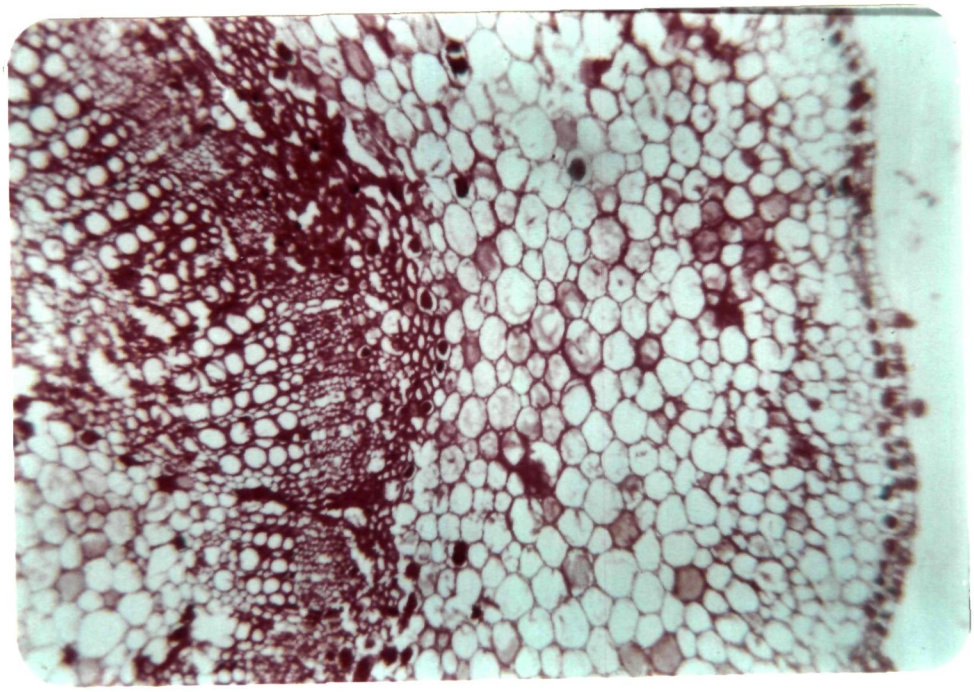
ppm concentrations of 2,4-D, paraquat, glyphosate were non significant at all the DAS under both the conditions. However, the differences between 2000 and 4000 ppm of all the herbicides was significant under open light. Whereas, under shaded condition it was significant only between 2000 and 4000 ppm of 2,4-D at 2 DAS and glyphosate at all the stages. All the concentrations of paraquat treatments have no significant differences at any stages. It was further observed that no significant difference was found between the same concentrations (lower or higher) of different herbicides. Under open light the leaf thickness ranged from 132.6 µm (2000 ppm of paraquat of 2 DAS) to 71.4 µm (4000 ppm of glyphosate at 8 DAS). Whereas under shaded condition it ranged from 116.9 µm to 82.5 µm respectively, in the same herbicide concentration and DAS as has been noticed under open light.

Petiole diameter

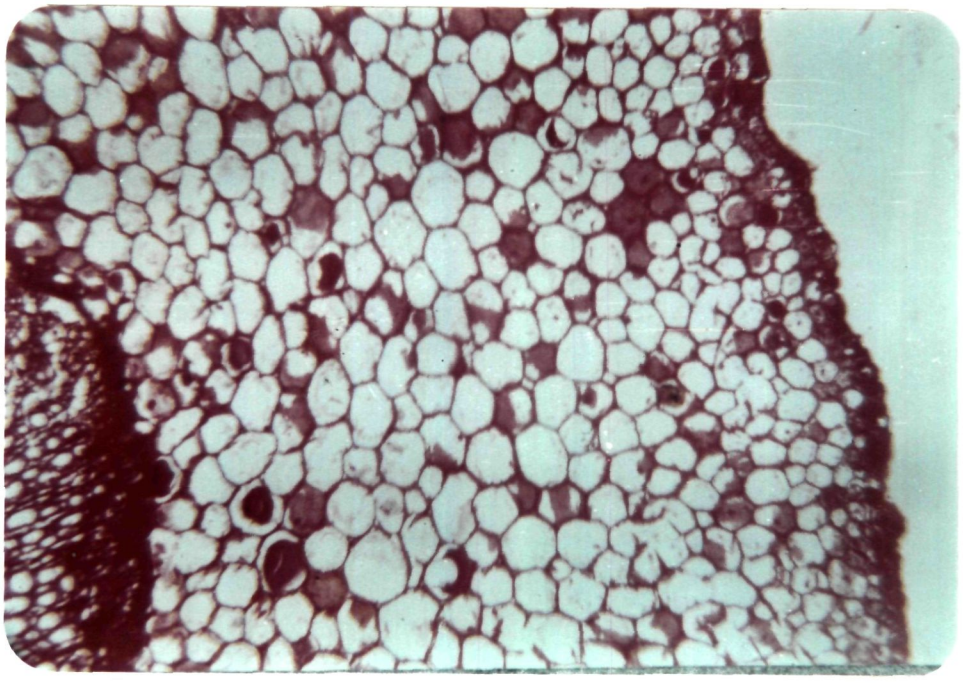
The data on the petiole diameter as influenced by different herbicide treatments at different DAS under open light and shaded conditions indicated that the cells were compactly arranged in light grown plants (Plate 5a). Whereas, under shade grown plants, the number of cells per unit area were less, large and loosely arranged (Plate 5b). The results revealed that petiole diameter of control plants increased continuously from 2 to 8 DAS under both the conditions (Table 25). The extent of increase was more under open light as

Table 25 : Effect of herbicides on petiole diameter (mm) in Eupatorium grown in open light and shade condition

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	1.98	2.04	2.10	2.13	1.49	1.53	1.50	1.51
2,4-D	2000	1.71	1.68	1.64	1.61	1.41	1.38	1.34	1.29
2,4-D	3000	1.69	1.54	1.47	1.36	1.37	1.34	1.26	1.22
2,4-D	4000	1.64	1.43	1.31	1.13	1.33	1.29	1.21	1.16
Paraquat	2000	1.84	1.81	1.76	1.73	1.45	1.43	1.40	1.37
Paraquat	3000	1.79	1.67	1.61	1.59	1.41	1.39	1.36	1.32
Paraquat	4000	1.72	1.58	1.49	1.36	1.37	1.32	1.29	1.23
Glyphosate	2000	1.36	1.32	1.28	1.23	1.38	1.35	1.29	1.22
Glyphosate	3000	1.26	1.21	1.15	1.08	1.34	1.30	1.23	1.15
Glyphosate	4000	1.18	1.06	0.98	0.85	1.30	1.24	1.16	1.06
S Em±		0.31	0.24	0.19	0.23	0.22	0.25	0.08	0.09
C D at 5%		NS	0.71	0.55	0.64	NS	NS	0.25	0.28



a. Open Light Grown



b. Shade Grown

PLATE 5. CROSS SECTION OF PETIOLES OF EUPATORIUM GROWN UNDER DIFFERENT GROWTH CONDITIONS

compared to shaded condition. In control however, under shaded condition there was marginal decrease in petiole diameter from 4 to 8 DAS. Treatments differed significantly at all the stages. Among different herbicides, paraquat at all the concentrations recorded higher petiole diameter at all the stages in comparision to the respective concentrations in different herbicides under both the conditions. The lowest petiole diameter was observed in glyphosate at all the concentrations corresponding to their respective concentrations in respective herbicide. Among the herbicide treatments the maximum petiole diameter of 1.84 mm was recorded in 2000 ppm of paraquat at 2 DAS and the lowest of 0.85 mm in 4000 ppm glyphosate at 8 DAS in open light. Similarly under shaded condition the maximum of 1.45 mm and the minimum of 1.06 mm petiole diameter was recorded respectively, in 2000 ppm paraquat and 4000 ppm glyphosate. In general it was observed that petiole diameter was more under open light as compared to shaded condition at all the concentrations in all the herbicides and at all the stages.

4.3.2 Relative water content (RWC%)

The data indicated that herbicides had significant influence on RWC under both open light and shaded conditions (Table 26 and Fig. 5). at different days after spraying (DAS). It was observed that in general, RWC decreased from 2 DAS to 8

Table 26 : Effect of herbicides on leaf relative water content (RWC %) in Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	83.2	84.6	83.9	84.1	86.3	85.9	85.5	84.5
2,4-D	2000	76.1	70.4	61.8	50.2	83.7	82.6	72.4	68.3
2,4-D	3000	73.8	67.3	58.1	46.4	82.4	81.3	68.6	64.6
2,4-D	4000	72.4	65.5	55.4	43.3	80.5	80.2	63.4	59.1
Paraquat	2000	74.5	53.6	48.6	44.5	82.6	78.4	69.6	65.9
Paraquat	3000	72.8	50.3	46.3	44.1	81.2	75.1	66.7	62.2
Paraquat	4000	71.6	49.4	40.2	35.7	80.3	72.8	60.3	57.5
Glyphosate	2000	75.3	69.1	60.9	49.4	82.7	83.2	76.3	69.8
Glyphosate	3000	72.9	65.6	56.1	44.5	83.5	82.3	74.5	66.3
Glyphosate	4000	71.2	63.3	51.5	39.1	81.2	78.6	70.8	61.6
S Em±		0.8	0.7	0.7	0.8	1.1	0.9	1.0	0.9
C D at 5%		2.3	2.1	2.2	2.4	3.4	2.6	2.9	2.7

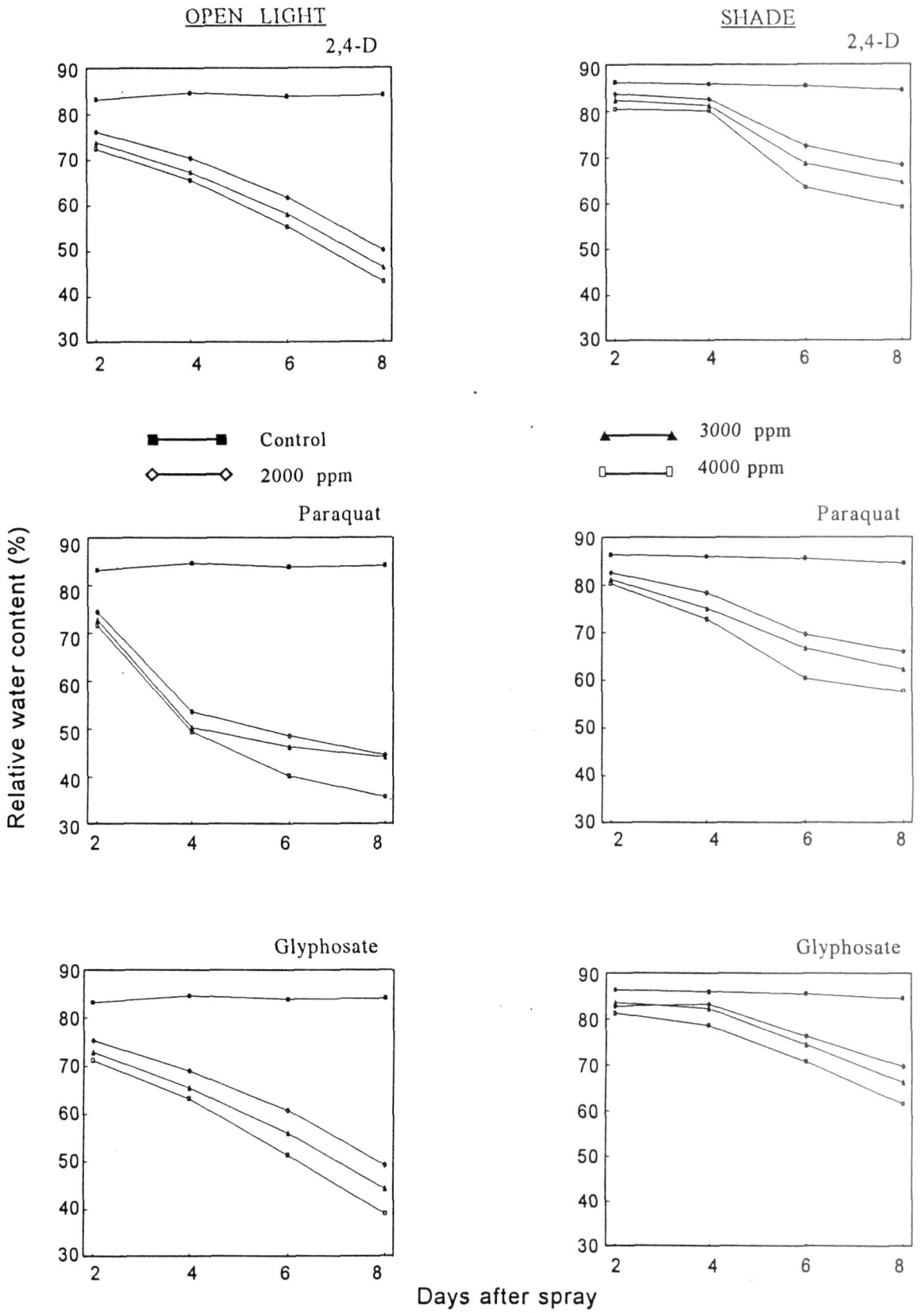


Figure 5. Effect of herbicides on leaf relative water content in Eupatorium grown in open light and shade conditions

DAS under both open light and shaded conditions. Amongst the treatment the maximum RWC was recorded in control and the minimum RWC was recorded at 4000 ppm of paraquat at all the stages. The RWC varied from 86.3 per cent to 35.7 per cent. It was observed that the RWC was more in all the treatments and at all the stages under shaded conditions as compared to open light. There was a progressive decline in RWC with DAS and the decline was more rapid in paraquat at all the concentration. At 4 DAS the RWC had reduced to almost 50 per cent of the control at higher concentrations (4000 ppm) of paraquat. Among the herbicides, the maximum RWC was recorded, in 2,4-D at all the concentrations under both open light and shaded conditions. However, at 8 DAS no significant differences were observed between 2000 ppm concentration of 2,4-D and glyphosate. Similarly, the treatments 2,4-D (3000 ppm), paraquat (2000 and 3000 ppm) and glyphosate (3000 ppm) did not differ significantly with each other under open light conditions. Whereas, under shaded conditions the treatments, 2000 ppm of 2,4-D, 2000 and 3000 ppm of glyphosate were insignificant.

4.3.3 Chlorophyll content (light grown)

The data on the chlorophyll content (mg dm^{-2} leaf area) indicated significant differences due to treatments at all the DAS (Table 27 and Fig. 6). Significant differences were also observed in chl. a, chl.b and total chlorophyll contents. However, no significant differences were observed in chl.a :

Table 27 : Effect of herbicides on chlorophyll content (mg dm-2) in Eupatorium grown in open light conditions

Treatments	Concentration (ppm)	Days After Spray											
		2				4				6			
		Chl.a	Chl.b	Total	Chl.a:b	Chl.a	Chl.b	Total	Chl.a:b	Chl.a	Chl.b	Total	Chl.a:b
Control	----	25.4	9.3	34.7	2.7	25.9	10.3	36.2	2.5	26.2	10.5	36.7	2.5
2,4-D	2000	17.2	7.2	24.4	2.4	15.2	6.3	21.5	2.4	11.4	4.3	16.2	2.4
2,4-D	3000	15.9	6.3	22.2	2.5	13.1	5.3	15.4	2.5	11.1	4.5	15.6	2.5
2,4-D	4000	14.7	5.6	20.3	2.6	11.5	4.6	16.1	2.5	9.9	3.9	13.8	2.5
Paraquat	2000	10.2	4.1	14.3	2.5	8.8	3.7	12.5	2.4	6.7	2.9	9.6	2.3
Paraquat	3000	8.3	3.2	11.5	2.6	7.4	3.2	10.6	2.3	5.6	2.4	8.0	2.4
Paraquat	4000	6.7	2.7	9.4	2.5	5.9	2.4	5.3	2.4	4.5	1.9	6.4	2.3
Glyphosate	2000	14.7	5.9	20.6	2.5	12.7	5.1	17.8	2.5	10.0	4.2	14.2	2.4
Glyphosate	3000	12.8	5.4	18.2	2.4	11.1	4.6	15.7	2.4	9.1	3.7	12.8	2.5
Glyphosate	4000	11.7	4.7	16.4	2.5	9.7	3.9	13.6	2.5	8.0	3.5	11.5	2.3
S Em±		1.9	1.2	1.9	0.3	1.5	1.1	2.2	0.4	1.3	0.7	1.5	0.2
C D at 5%		5.8	3.5	5.5	NS	4.5	3.3	6.7	NS	3.8	2.2	4.3	NS

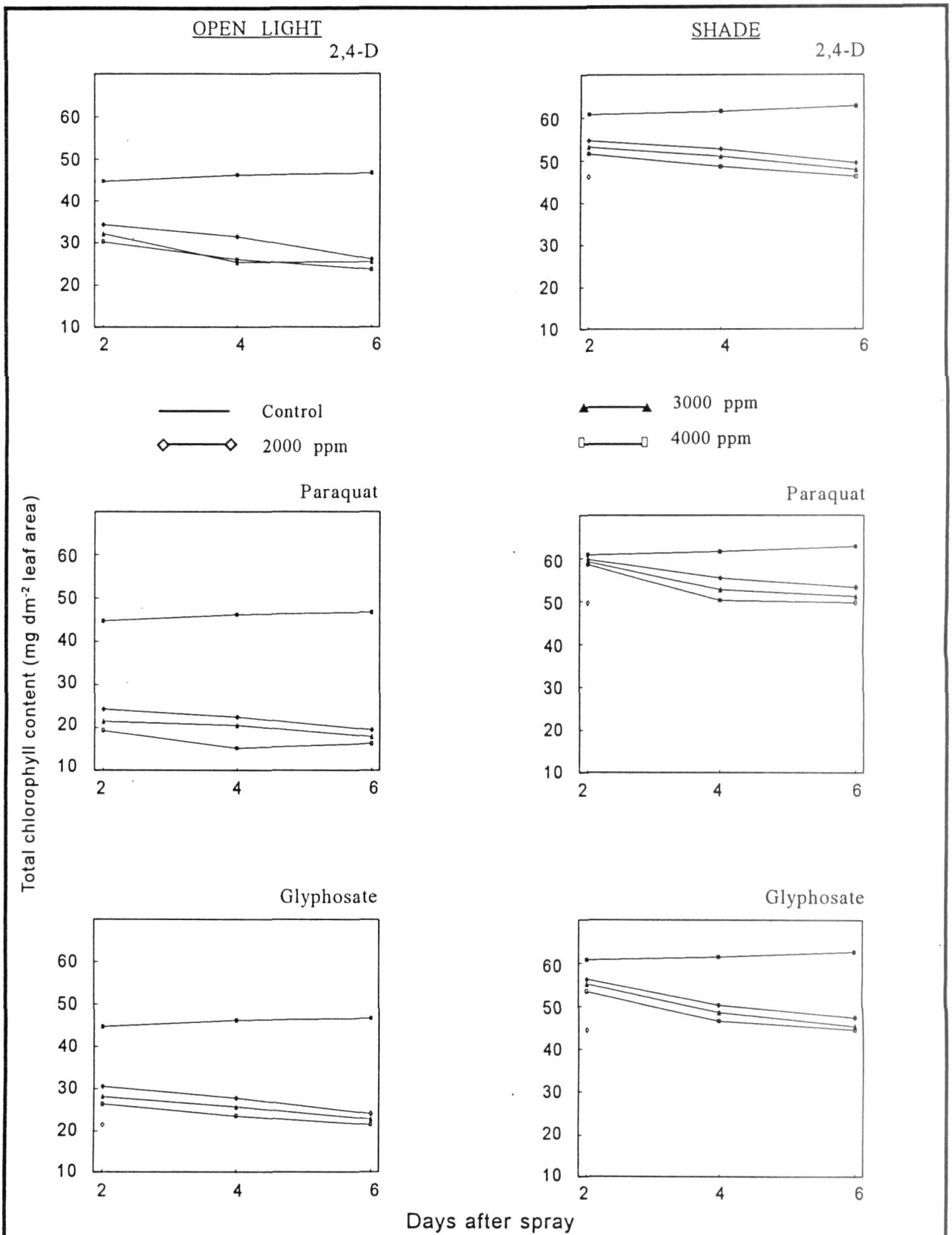


Figure 6. Effect of herbicides on total chlorophyll content in Eupatorium grown in open light and shade conditions

chl.b ratio at any stage. The chlorophyll content was maximum in the beginning (2 DAS) and decreased at 4 and 6 DAS in all the herbicide treatments except control, where it increased from 2 to 6 DAS. At 2 DAS, the maximum chl a and chl b contents were measured in 2000 ppm of 2,4-D followed by 3000 ppm among the herbicides. The chlorophyll content was maximum in control as compared to other treatments at all the stages. The lowest chl. a and b contents were observed in 4000 ppm of paraquat at all the stages. It was further observed that no significant differences were observed between 2000 ppm concentrations of any herbicide.

At 4 DAS no significant differences in chl. a, b and total between different concentrations of the same herbicide. Similarly, no significant differences were observed between the same concentrations of 2,4-D and glyphosate. Paraquat at all the concentrations recorded lower chlorophyll content over other herbicides. A similar trend was noticed even at 6 DAS. The total chlorophyll content ranged from 36.2 mg in control at 4 DAS to 1.9 mg in 4000 ppm paraquat at 6 DAS.

4.3.4 Chlorophyll content (shade grown)

It was observed that the chlorophyll content was more under shaded conditions as compared to light grown conditions the herbicide treatment (Table 28 and Fig. 6). While, the chl. a, b ratio declined under shaded condition over light grown

Table 28 : Effect of herbicides on chlorophyll content (mg dm-2) in Eupatorium grown in shade conditions

Treatments	Concentration (ppm)	Days After Spray											
		2				4				6			
		Chl.a	Chl.b	Total	Chl.a:b	Chl.a	Chl.b	Total	Chl.a:b	Chl.a	Chl.b	Total	Chl.a:b
Control	----	32.0	18.8	50.8	1.7	31.7	19.8	51.5	1.6	32.2	19.5	52.7	1.7
2,4-D	2000	27.5	17.2	44.7	1.6	26.3	16.5	42.8	1.6	24.3	15.2	39.5	1.6
2,4-D	3000	27.1	16.1	43.2	1.7	25.8	15.2	41.0	1.7	23.3	14.5	37.8	1.6
2,4-D	4000	26.7	14.9	41.6	1.8	24.8	13.8	38.6	1.8	23.2	13.0	36.2	1.8
Paraquat	2000	31.4	18.3	49.7	1.7	27.9	17.5	45.4	1.6	26.6	16.6	43.2	1.6
Paraquat	3000	31.2	18.0	49.2	1.7	26.9	15.8	42.7	1.7	25.2	15.9	41.1	1.6
Paraquat	4000	30.7	17.8	48.5	1.7	25.3	14.9	40.2	1.7	24.9	14.7	39.6	1.7
Glyphosate	2000	28.6	17.7	46.3	1.6	25.4	14.9	40.3	1.7	23.5	13.9	37.4	1.7
Glyphosate	3000	28.5	16.7	45.2	1.7	24.8	13.8	38.6	1.8	22.7	12.6	35.3	1.8
Glyphosate	4000	28.3	15.5	43.4	1.8	23.3	12.9	36.6	1.8	22.2	12.3	34.5	1.8
S Em±		1.8	1.3	2.2	0.1	1.2	1.1	1.9	0.2	1.8	1.2	2.1	0.3
C D at 5%		5.5	3.7	6.5	NS	3.5	3.2	5.7	NS	5.2	3.6	6.2	NS

condition. Similar to that of light grown condition the chl content decreased from 2 to 6 DAS in all the herbicide treatments except in control where there was increase in both chl-a, b and total chlorophyll contents. While, there was marginal decrease in chl.b content from 4 DAS (19.8 mg) to 6 DAS (19.5 mg). The maximum total chlorophyll content was to the tune of 52.7 mg in control and the minimum was 34.5 mg in 4000 ppm glyphosate. There was hardly any variation in chl.a, b and total chlorophyll contents between the concentrations of each herbicide at all the stages. Similarly no significant differences were observed between the same concentrations of different herbicides with respect to chl a, b and total chlorophyll contents. Chlorophyll a:b ratio was found to be non-significant throughout. At 2 DAS the maximum chla, b and total chlorophyll was observed in all the concentrations of paraquat as compared to other herbicide. While, the lowest chlorophyll contents were observed in 2,4-D in all the concentrations. At 4 DAS, again paraquat at all the concentrations, recorded higher chl.a, b and total chlorophyll contents while, glyphosate at 3000 ppm (38.6 mg) and 4000 ppm (36.6 mg) had the lowest total chlorophyll content. At 6 DAS paraquat continued to maintain higher chl.a, b and total chlorophyll contents and glyphosate had the lowest chlorophyll contents in all the concentrations.

4.3.5 Electrical conductivity (ds/m/dm^2 leaf area)

It was observed that the electrical conductivity (EC) increased with increase in the number of days of spray (DAS) in all the treatments and differed significantly between the treatments at all the stages except at 2 DAS under both open light and shaded conditions (Table 29). Among the treatments paraquat at all concentrations had higher EC at all the stages under both open light and shaded conditions. While, the lower EC was observed in 2,4-D at all the concentrations at all the stages. It was further observed that the EC increased continuously from 2 to 8 DAS at all the stages irrespective of the herbicide and their concentrations. The EC values ranged from 0.4 ds/m/dm^2 leaf area (2000 pm 2,4-D at 2 DAS) to 1.25 ds/m/dm^2 leaf area at 8 DAS in 4000 ppm paraquat) under open light conditions. It was generally seen that the EC was more under open light conditions in all treatments as compared to shaded conditions.

4.3.6 OD_{525} of leaf leachate

The data on changes in OD_{525} of leaf leachates with herbicide spray at different DAS are given in Table 30. It was observed that the significant differences were observed between herbicide treatments and control at all the stages except at 2 DAS both in open light and shaded conditions. Further, there was a continuous increase in OD of leaf leachates from 2 to 8 DAS in herbicide treatments in both the open light and

Table 29 : Effect of herbicides on electrical conductivity (ds/m /dm^2 leaf area) of leaf leachates in Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	0.51	0.49	0.54	0.59	0.42	0.43	0.42	0.50
2,4-D	2000	0.54	0.57	0.62	0.68	0.40	0.42	0.46	0.50
2,4-D	3000	0.58	0.64	0.69	0.75	0.44	0.47	0.53	0.58
2,4-D	4000	0.63	0.67	0.76	0.83	0.48	0.56	0.61	0.65
Paraquat	2000	0.77	0.86	0.95	1.15	0.56	0.61	0.67	0.71
Paraquat	3000	0.82	0.90	1.04	1.19	0.0.63	0.68	0.72	0.79
Paraquat	4000	0.88	1.97	1.13	1.25	0.74	0.79	0.84	0.88
Glyphosate	2000	0.56	0.59	0.65	0.71	0.46	0.49	0.53	0.57
Glyphosate	3000	0.61	0.67	0.73	0.79	0.50	0.52	0.56	0.61
Glyphosate	4000	0.65	0.69	0.76	0.88	0.52	0.55	0.60	0.68
S Em±		0.16	0.02	0.01	0.09	0.14	0.04	0.01	0.03
C D at 5%		NS	0.05	0.03	0.26	NS	0.11	0.03	0.09

Table 30 : Effect of herbicides on OD (at 525 nm) of leaf leachates in Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	0.123	0.134	0.142	0.146	0.080	0.092	0.109	0.154
2,4-D	2000	0.145	0.174	0.189	0.213	0.094	0.124	0.138	0.154
2,4-D	3000	0.167	0.182	0.205	0.228	0.123	0.147	0.159	0.135
2,4-D	4000	0.201	0.235	0.274	0.296	0.154	0.178	0.185	0.196
Paraquat	2000	0.295	0.322	0.353	0.389	0.098	0.115	0.127	0.139
Paraquat	3000	0.319	0.360	0.395	0.434	0.101	0.128	0.141	0.158
Paraquat	4000	0.374	0.396	0.442	0.478	0.115	0.136	0.152	0.167
Glyphosate	2000	0.163	0.189	0.209	0.234	0.099	0.120	0.137	0.154
Glyphosate	3000	0.218	0.245	0.283	0.310	0.138	0.149	0.163	0.180
Glyphosate	4000	0.301	0.337	0.376	0.407	0.156	0.168	0.182	0.196
S Em±		0.091	0.023	0.024	0.043	0.053	0.060	0.130	0.012
C D at 5%		NS	0.067	0.067	0.116	NS	0.18	0.36	0.033

shaded conditions and the rate of increase was more in open light compared to shaded conditions when compared for any given herbicide concentration. Among the herbicides the OD was maximum at all the concentrations of paraquat at all the stages under open light. Significant differences were observed between the lowest (2000 ppm) and the highest (4000 ppm) concentrations of all the herbicides at all the stages. No significant differences were observed between 2000 and 3000 or 3000 and 4000 ppm concentrations of different herbicides at all the stages. The same concentrations of different herbicides also differed significantly with each other.

However, under shaded condition same concentration of different herbicides were on par with each other but together were significantly higher over control at all the stages. Glyphosate at 3000 and 4000 ppm and 2,4-D at 4000 ppm had higher OD at all the stages.

4.3.7 Total soluble sugars (mg g dry wt^{-1}) in leaves

The data on changes in total soluble sugars in leaves after herbicide spray indicated that there was a decrease in total soluble sugars with an increase in the concentrations of herbicides at all the stages under open light (Table 31). Among the treatments, maximum total soluble sugars was observed in 2,4-D sprayed plants at all the

Table 31 : Effect of herbicides on total soluble sugars (mg g dry wt^{-1}) in leaves of Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	22.6	21.7	21.5	22.8	18.6	17.9	18.8	19.3
2,4-D	2000	22.1	21.6	20.9	18.8	18.1	18.5	17.9	17.4
2,4-D	3000	21.4	21.3	19.5	17.6	18.3	18.7	17.2	16.3
2,4-D	4000	20.5	20.2	18.3	16.4	17.4	18.1	16.5	16.1
Paraquat	2000	21.7	22.4	20.7	18.5	19.0	17.6	17.1	17.2
Paraquat	3000	20.8	21.5	19.4	17.2	18.3	17.2	16.8	16.5
Paraquat	4000	20.2	20.1	17.9	16.2	18.7	16.3	16.1	15.7
Glyphosate	2000	20.3	19.5	18.5	16.4	18.2	17.3	17.0	16.1
Glyphosate	3000	19.7	18.6	17.7	15.2	18.2	17.1	16.3	15.4
Glyphosate	4000	19.1	18.2	16.6	14.4	18.1	16.5	16.1	14.8
S Em±		1.0	0.3	0.6	0.3	0.8	0.3	0.3	0.5
C D at 5%		NS	0.9	1.6	0.9	NS	0.7	0.8	1.3

stages. However, no significant differences were observed at 2 DAS under both open and shaded condition. The maximum (22.05 mg) total soluble sugars among the herbicides were noticed at 2 DAS and the minimum (14.38 mg) in 4000 ppm of glyphosate under open light conditions.

At 4 DAS the treatments 2000 and 3000 ppm of 2,4-D and 3000 ppm of paraquat did not differ significantly. Similarly no significant differences were observed between the control and 3000 ppm of paraquat and 3000 ppm and 4000 ppm of glyphosate. At 6 DAS the maximum total soluble sugars were recorded in control (21.53 mg) followed by 2000 and 3000 ppm of paraquat (20.73 mg) and 2000 ppm of 2,4-D (20.88 mg). The treatments 4000 ppm of 2,4-D and paraquat, 2000 and 3000 ppm of paraquat did not differ significantly with each other. A similar trend was noticed at 8 DAS also with no significant differences between the same concentration of 2,4-D and paraquat.

Under shaded conditions, the maximum total sugars were noticed in control at 6 and 8 DAS , while it was maximum in 3000 ppm of 2,4-D at 4 DAS. No significant differences were observed between 2000 and 3000 ppm or 3000 ppm and 4000 ppm concentrations of any of the herbicides tested. The lowest total soluble sugars (14.82 mg) were recorded in 4000 ppm of glyphosate at 8 DAS.

4.3.8 Crude Protein Content (% of dry weight)

It was observed that the crude protein content decreased with an increase in the number of days after herbicide spray in all the treatments including control under both open light and shaded conditions (Table 32). Among the herbicides the maximum crude protein content was observed in 2000 ppm of glyphosate (26.45%) followed by 2000 ppm 2,4-D (26.52%) and paraquat (26.30%) and 3000 ppm of glyphosate (26.27%), which were on par with each other at 4 DAS. Whereas, at 6 DAS the treatments 2000 and 3000 ppm of paraquat and 2000 ppm of glyphosate did not differ significantly with each other. Similarly 3000 ppm of glyphosate, 4000 ppm of paraquat and 3000 ppm of 2,4-D were on par with each other.

No significant differences were observed between 2,4-D and paraquat at 2000 ppm concentrations and the lowest crude protein content of 19.56 per cent was recorded in 4000 ppm of glyphosate at 8 DAS.

Under shaded condition treatments did not differ significantly with each other at 2 DAS and differed significantly at all other stages. It was further observed that the differences between the treatments were not evident between 2000 and 3000 ppm concentrations of any of the herbicides irrespective of the stages. However, treatments differed significantly between 2000 and 4000 ppm concentrations

Table 32 : Effect of herbicides on crude protein content (% of dry wt) in leaves of Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	27.1	26.1	25.3	26.4	22.5	22.4	21.9	21.8
2,4-D	2000	26.5	25.4	26.1	24.8	22.3	21.5	19.6	18.7
2,4-D	3000	26.0	24.8	23.6	21.7	21.9	21.4	19.1	18.0
2,4-D	4000	25.4	23.5	22.4	20.5	21.7	20.1	19.0	17.4
Paraquat	2000	26.3	25.7	24.8	24.1	21.4	20.2	19.0	18.1
Paraquat	3000	25.4	25.1	24.2	23.7	21.1	19.8	18.6	17.6
Paraquat	4000	25.2	24.4	23.5	23.0	20.3	18.9	18.3	17.0
Glyphosate	2000	26.5	25.7	24.2	23.4	21.7	21.0	19.2	18.2
Glyphosate	3000	26.3	24.3	22.9	22.1	21.4	21.1	18.6	17.6
Glyphosate	4000	25.6	23.1	20.7	19.6	20.6	20.2	18.0	17.0
S Em±		1.4	0.3	0.4	0.4	0.9	0.3	0.3	0.2
C D at 5%		NS	0.9	1.0	1.2	NS	0.8	0.8	0.7

of all the herbicides. Similar to that of open light conditions, the minimum crude protein content (17.03%) was recorded in 4000 ppm of glyphosate.

4.3.9 Free amino acid content (mg g dry wt^{-1})

The free amino acids content differed significantly under open light at all the stages (Table 33). There was an increase in free amino acid content from 2 to 6 DAS in control and declined slightly at 8 DAS. With respect to glyphosate at all the concentrations there was increase in free amino acids from 2-8 DAS. Whereas, in 2,4-D and paraquat there was a continuous decline in free amino acid content irrespective of the concentration of the chemical. Among the herbicides maximum contents were observed in glyphosate at all the concentrations. In general, free amino acids content decreased with an increase in the concentration in 2,4-D and paraquat, while the trend was reverse in glyphosate at all the stages.

Under shaded conditions, a similar trend was observed as has been recorded under open light with glyphosate recording the maximum free amino acid contents (5.75 mg) and 4000 ppm of 2,4-D having the minimum content (1.43 mg). It was further seen that the free amino acids content was more under shaded conditions as compared to open light at all the stages irrespective of the herbicides and their concentrations.

Table 33 : Effect of herbicides on free amino acids (mg g dry wt^{-1}) in leaves of Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	1.76	1.83	2.08	1.92	3.98	3.16	2.88	3.25
2,4-D	2000	1.64	1.61	1.28	1.02	3.12	2.67	2.25	2.07
2,4-D	3000	1.35	1.27	1.03	0.83	2.75	2.46	2.08	1.65
2,4-D	4000	1.26	1.09	1.97	0.71	2.24	2.12	1.76	1.43
Paraquat	2000	1.81	1.93	1.42	1.13	2.73	2.44	2.12	1.92
Paraquat	3000	1.73	1.82	1.34	1.09	2.42	2.27	2.00	1.74
Paraquat	4000	1.68	1.57	1.18	0.92	2.15	2.06	1.89	1.45
Glyphosate	2000	1.97	2.15	2.61	3.23	3.16	3.57	4.95	4.33
Glyphosate	3000	2.03	2.73	3.53	0.85	3.48	3.93	4.38	4.71
Glyphosate	4000	2.64	2.94	3.78	4.36	3.75	4.22	4.57	5.47
S Em±		0.12	0.08	0.06	0.07	0.56	1.41	0.08	0.17
C D at 5%		0.32	0.23	0.16	0.20	NS	NS	0.21	0.49

4.3.10 Total free phenols (mg g dry wt-1)

The data on total free phenols in leaves indicated significant differences between the treatments at all the growth stages under both open light and shaded conditions (Table 34). It was observed that total free phenols decreased from 2 to 8 DAS in all the herbicide treated plants under open light. Whereas, under shaded conditions, total free phenols increased from 2 to 8 DAS in 2,4-D and paraquat at all the concentrations while in glyphosate there was a continuous decrease in total free phenols at all the stages. At 2 DAS the maximum free phenols were recorded in 4000 ppm of paraquat and the minimum in 2000 ppm glyphosate. The treatments 4000 ppm 2,4-D, 2000 ppm paraquat, 3000 and 4000 ppm were on par with each other. Further, the treatment 2000 ppm 2,4-D did not differ significantly with control. At 4 DAS the treatments 3000 ppm 2,4-D, 2000 ppm paraquat and 4000 ppm glyphosate did not differ significantly with each other. Similarly control, 2000 ppm 2,4-D and 3000 ppm glyphosate had no significant differences among them. At 6 and 8 DAS, lowest total phenols were observed at 4000 ppm glyphosate. It was interesting to note that control and 2000 ppm 2,4-D did not differ significantly at any of the growth stages under both open light and shaded conditions except at 6 and 8 DAS under shaded conditions. There was an increase in the total free phenols with an increase in the concentration of paraquat at all the growth stages under both the conditions.

Table 34 : Effect of herbicides on total free phenols (mg g dry wt^{-1}) in leaves of Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	7.53	8.05	8.97	8.32	5.25	5.36	4.89	5.41
2,4-D	2000	7.36	8.14	8.04	8.00	5.36	5.78	6.28	6.33
2,4-D	3000	8.01	8.36	7.62	7.06	5.85	6.23	6.63	6.27
2,4-D	4000	7.89	8.67	7.25	6.77	6.12	6.57	7.25	7.48
Paraquat	2000	6.97	6.38	6.45	6.45	5.38	5.83	6.24	6.85
Paraquat	3000	6.64	6.87	6.27	6.48	6.17	6.24	6.86	7.23
Paraquat	4000	6.82	7.06	5.35	6.07	7.32	7.76	8.09	8.24
Glyphosate	2000	7.47	7.83	7.89	8.46	5.14	4.85	4.58	4.06
Glyphosate	3000	7.72	8.01	9.27	9.47	4.78	4.13	3.75	3.12
Glyphosate	4000	7.81	8.27	10.34	10.51	4.22	4.00	3.42	4.05
S Em±		0.09	0.07	0.31	0.29	0.26	0.19	0.32	0.25
C D at 5%		0.29	0.20	0.83	0.83	0.76	0.54	0.95	0.69

4.3.11 Phenyl alanine ammonia layse (PAL, µg cinnamic acid hr^{-1} mg $protein^{-1}$)

The activity of PAL differed significantly between, the herbicides and their concentrations (Table 35 and Fig.7) at all the stages both under open light and shaded conditions except at 2 DAS under shaded condition. The PAL activity increased with an increase in the concentration of all the herbicides and the increase was more pronounced in glyphosate under open light. The maximum PAL activity was noticed in 4000 3000 ppm of glyphosate at 8 DAS (56.4 mg) followed by 4000 ppm of paraquat (55.2 mg) which were on par with each other. No significant differences were observed between the same concentrations of different herbicides but the differences were evident at higher concentrations (4000 ppm) of all the herbicides indicating the significant influence of herbicides at higher concentrations in inducing PAL activity.

Under shaded conditions also, PAL activity increased with an increase in the concentration of herbicides. The PAL activity decreased from 2 to 8 DAS in al the concentrations of 2,4-D and glyphosate, while in glyphosate, it increased between 4 and 8 DAS. At 4 DAS, the differences in PAL activity were significant between the 3000 and 4000 ppm concentrations of paraquat, while, it was insignificant between all the concentrations of glyphosate and 2,4-D. A similar trend was

Table 35 : Effect of herbicides on phenylalanine ammonia lyase (PAL, µg cinnamic acid hr^{-1} mg $protein^{-1}$) activity in leaves of Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	40.3	42.7	43.4	42.3	37.2	37.3	37.5	38.7
2,4-D	2000	43.4	41.2	42.3	43.4	37.6	39.4	37.6	36.3
2,4-D	3000	40.6	44.6	45.7	46.3	38.2	40.3	36.2	34.7
2,4-D	4000	42.3	46.3	46.3	47.2	38.4	40.8	34.8	32.3
Paraquat	2000	39.2	43.4	48.3	50.2	36.1	37.5	39.7	40.2
Paraquat	3000	42.3	45.7	50.4	51.6	37.2	39.9	43.4	45.3
Paraquat	4000	44.7	49.3	54.3	55.2	38.5	42.3	46.3	47.2
Glyphosate	2000	39.9	45.3	51.6	37.5	37.7	36.2	35.3	34.4
Glyphosate	3000	43.3	47.7	53.3	54.3	38.2	35.4	33.2	31.2
Glyphosate	4000	47.8	52.2	57.8	56.4	37.1	34.6	33.5	40.3
S Em±		1.2	0.9	0.8	0.9	0.9	1.2	0.8	0.7
C D at 5%		3.4	2.6	2.4	2.7	NS	3.3	2.3	2.5

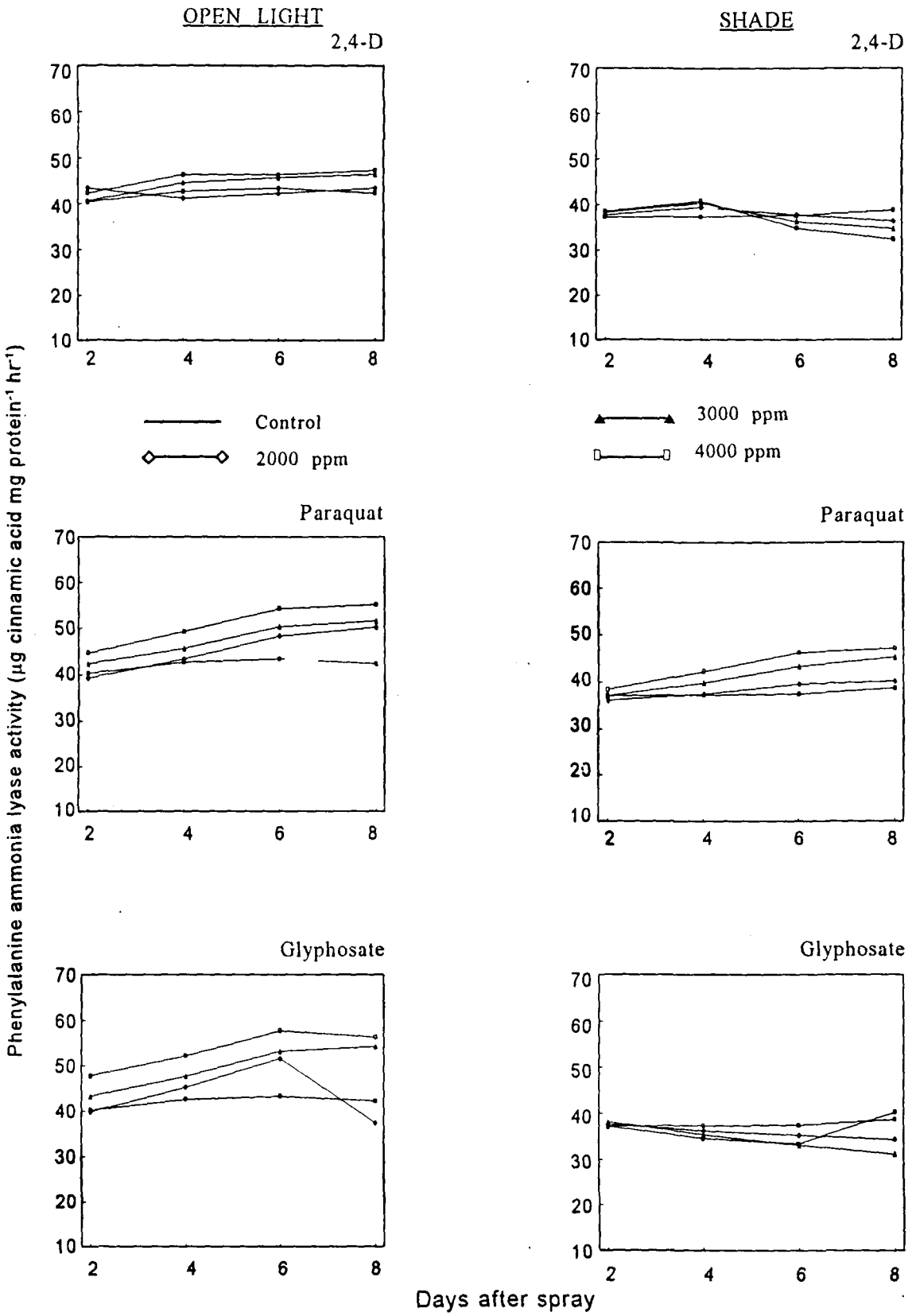


Figure 7. Effect of herbicides on phenylalanine ammonia lyase activity in leaves of Eupatorium grown in open light and shade conditions

observed even at 6 and 8 DAS except that PAL activity differed between the concentrations significantly in all the concentrations. The maximum PAL activity was recorded in 4000 ppm of paraquat (47.2 mg) and the lowest in 3000 ppm glyphosate (31.2 mg).

4.3.12 Tyrosine ammonia layse activity (TAL, µg p-coumaric acid hr^{-1} mg $protein^{-1}$)

The observations on Tyrosine ammonia layse activity as influenced by herbicide treatments are presented in Table 36 and depicted in Figure 8. There were significant difference due to herbicides and their concentration under both open light and shaded conditions at all the stages except at 2 and 4 DAS under shaded conditions. The data indicated that there was increase in TAL activity from 2 to 8 DAS in all the treatments with maximum activity in 4000 ppm of glyphosate (94.9 mg). It was further observed that there was an increase in the activity of TAL with an increase in the herbicide concentration at all the DAS. The lowest TAL activity was noticed in 2000 ppm glyphosate and it almost doubled with an increase in the concentration (4000 ppm) as compared to 2000 ppm and it tripled at 8 DAS between 2000 and 4000 ppm. Significant differences between 3000 and 4000 ppm were observed only in glyphosate at all the stages. Whereas, with respect to other herbicides significant differences were observed only between 2000 and 4000 ppm concentrations.

Table 36 : Effect of herbicides on tyrosine ammonia lyase (TAL, µg P-coumaric acid hr^{-1} mg $protein^{-1}$) activity in leaves of Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	62.2	67.4	73.2	72.7	46.3	48.4	49.3	48.4
2,4-D	2000	63.9	64.6	63.9	62.8	47.2	46.1	48.2	48.4
2,4-D	3000	60.4	65.8	65.2	65.1	45.5	49.8	52.1	53.3
2,4-D	4000	57.3	65.9	66.5	66.9	44.4	50.2	55.5	54.3
Paraquat	2000	60.8	68.9	67.5	68.8	47.3	47.8	52.4	53.6
Paraquat	3000	67.3	70.7	71.3	72.2	48.2	50.6	56.7	55.8
Paraquat	4000	68.5	73.3	72.2	72.7	48.9	51.8	51.5	62.3
Glyphosate	2000	56.4	48.2	45.6	38.4	47.3	46.7	44.6	41.2
Glyphosate	3000	61.6	73.5	87.3	90.3	50.7	53.6	57.3	60.3
Glyphosate	4000	65.2	78.4	91.4	94.7	55.6	56.3	63.2	67.3
S Em±		0.9	1.0	1.2	1.3	3.8	3.7	2.9	2.7
C D at 5%		2.5	2.7	3.2	3.6	NS	NS	7.7	7.5

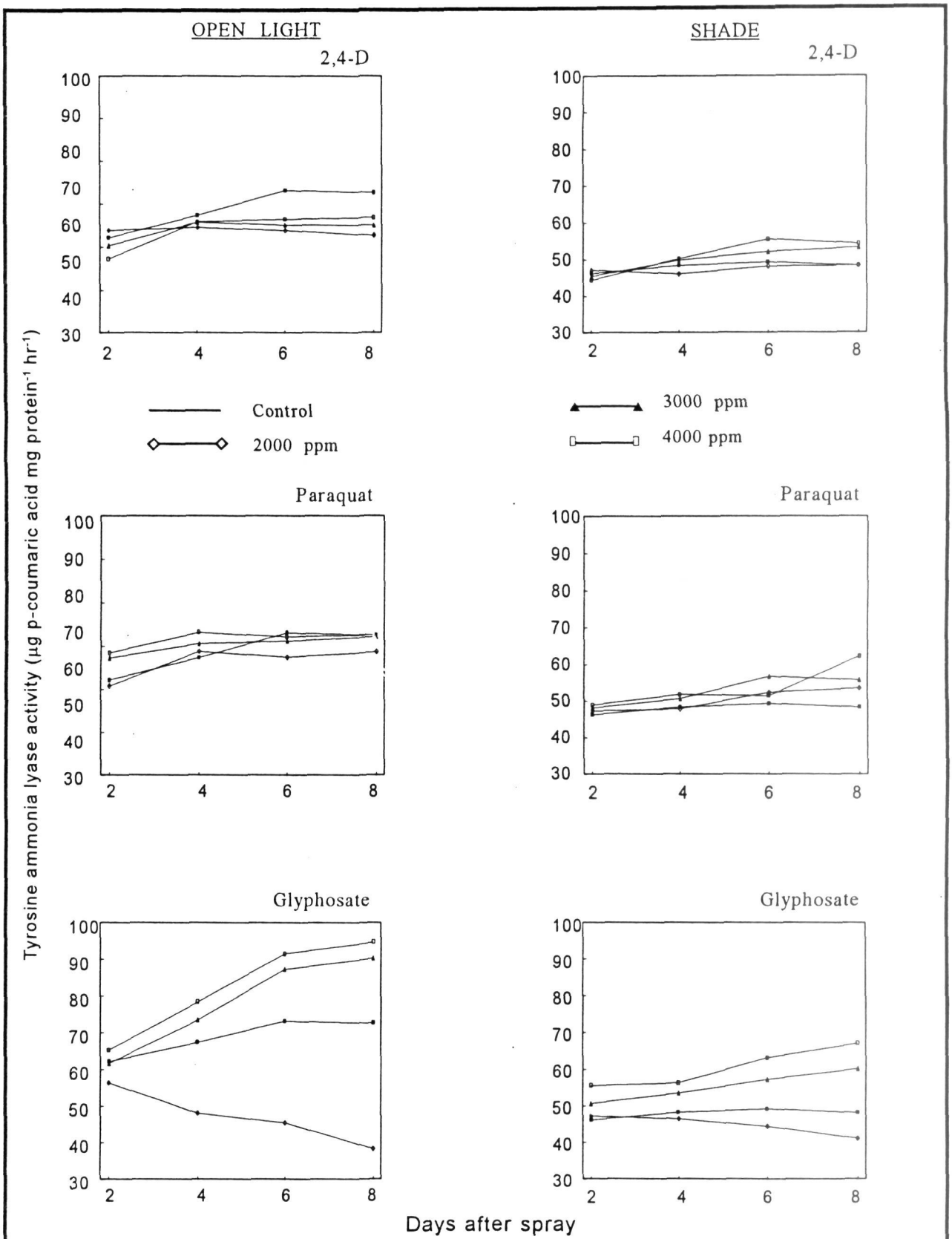


Figure 8. Effect of herbicides on tyrosine ammonia lyase activity in leaves of Eupatorim grown in open light and shade conditions

The activity of TAL decreased drastically in control and other herbicide treated plants under shaded conditions as compared to open light. Similar to that of open light there was increase in TAL activity with an increase in the concentrations of herbicides and with an increase in the DAS. Among the herbicides, TAL activity was low in glyphosate at 2000 ppm and high at 4000 ppm. No significant differences were observed between any of the concentrations in 2,4-D and paraquat whereas, in glyphosate differences between 2000 and 4000 ppm were quite evident and significant.

4.3.13 Peroxidase activity (OD_{420} min^{-1} $mg\ protein^{-1}$)

The data presented in Table 37 and in Figure 9, indicate the peroxidase activity with herbicide treatments both under open light and shaded conditions. The activity was more in open light condition than in shade. There was a continuous increase in peroxidase activity with increasse in concentration of herbicide and DAS. However, maximum increase was recorded in paraquat followed by glyphosate and 2,4-D in open light but under shade condition maximum increase was in glyphosate, followed by 2,4-D and paraquat.

In open light condition the proxidase activity was lowest in control at 2 DAS (0.432) and highest in 4000 ppm paraquat (1.763) at 8 DAS. All herbicide treatments significantly increased the peroxidase activity over control at all the stages.

Table 37 : Effect of herbicides on peroxidase activity (OD_{420} hr^{-1} mg protein^{-1}) in leaves of Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	0.432	0.451	0.467	0.442	0.326	0.335	0.357	0.348
2,4-D	2000	0.537	0.586	0.632	0.674	0.387	0.395	0.443	0.486
2,4-D	3000	0.642	0.723	0.787	0.873	0.423	0.473	0.521	0.593
2,4-D	4000	0.753	0.835	0.928	1.245	0.487	0.552	0.531	0.635
Paraquat	2000	0.647	0.682	0.754	0.823	0.378	0.421	0.462	0.485
Paraquat	3000	0.756	0.823	0.962	1.241	0.431	0.478	0.512	0.563
Paraquat	4000	0.972	1.264	1.635	1.763	0.535	0.557	0.572	0.612
Glyphosate	2000	0.526	0.631	0.763	0.785	0.461	0.495	0.571	0.624
Glyphosate	3000	0.724	0.785	0.847	0.924	0.528	0.616	0.734	0.775
Glyphosate	4000	0.835	0.943	1.138	1.354	0.633	0.684	0.764	0.879
S Em±		0.061	0.056	0.059	0.063	0.036	0.039	0.047	0.043
C D at 5%		0.173	0.157	0.165	0.178	0.107	0.112	0.134	0.125

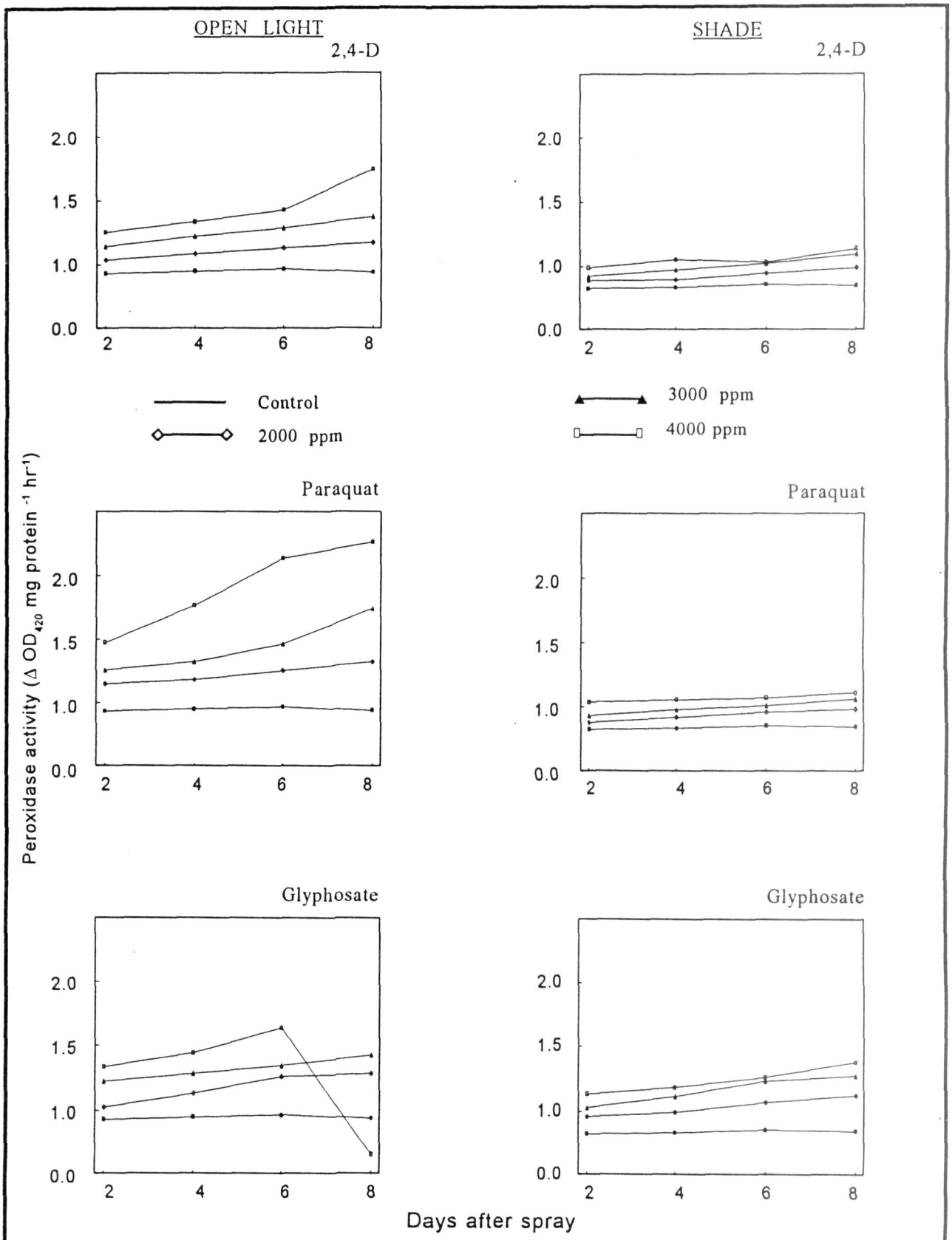


Figure 9. Effect of herbicides on peroxidase activity in leaves of Eupatorium grown in open light and shade conditions

Under shaded condition also there was significant difference between treatments at all the stages. The lowest activity (0.326) was in control at 2 DAS and the highest (0.879) was in 4000 ppm glyphosate at 8 DAS. At 8 DAS same concentations of 2,4-D and paraquat did not differ significantly but only lowest (2000 ppm) and highest (4000 ppm) of these herbicides showed significant difference.

4.3.14 Nitrate reductase activity (μmole NO_2 g fr wt^{-1} min^{-1})

Nitrate reductase (NRase) activity recorded at different DAS of herbicides treatments showed significant differences at all the stages except at 2 DAS under shade (Table 38 and Fig.10). The activity in control (unsprayed) plants under open light was nearly two times more than under shaded condition. Further, there was a continuous decrease in NRase activity with increase in both concentration of herbicides and the time after spray. However, the rate of decrease between two consecutive stages was more in open light than in shade. Among the different herbicides lowest activity (0.97) was recorded with 4000 ppm of paraquat in open light at 8 DAS while 4000 ppm of glyphosate recorded lowest (0.73) in shade.

At 2 DAS in open light treatments of 2000 and 3000 ppm of 2,4-D and paraquat were on par with control. Similarly, 3000 ppm of 2,4-D, 4000 ppm paraquat and 2000 ppm glyphsate

Table 38 : Effect of herbicides on nitrate reductase activity (μ mole NO_2 g fr. Wt.$^{-1}$ min^{-1}) in leaves of Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control	----	4.16	4.32	4.73	4.28	2.74	2.85	3.96	2.35
2,4-D	2000	4.27	3.71	3.25	2.78	2.56	2.72	2.27	2.13
2,4-D	3000	3.93	3.62	2.84	2.13	2.32	2.12	2.98	1.74
2,4-D	4000	3.24	2.75	2.16	2.76	2.24	2.05	1.87	1.67
Paraquat	2000	4.25	3.95	3.22	2.85	2.76	2.52	2.31	2.15
Paraquat	3000	4.87	3.74	2.81	2.72	2.45	2.10	1.84	1.53
Paraquat	4000	3.42	2.15	1.43	0.97	2.32	2.98	1.67	1.42
Glyphosate	2000	3.15	2.73	2.12	2.66	2.82	2.71	2.43	2.12
Glyphosate	3000	2.84	2.15	1.67	2.24	2.24	1.86	1.57	1.36
Glyphosate	4000	2.25	1.86	1.35	2.10	2.14	1.75	1.31	0.73
S Em$\pm$		0.25	0.26	0.31	0.19	0.23	0.22	0.19	0.15
C D at 5%		0.63	0.78	0.86	0.54	NS	0.63	0.57	0.41

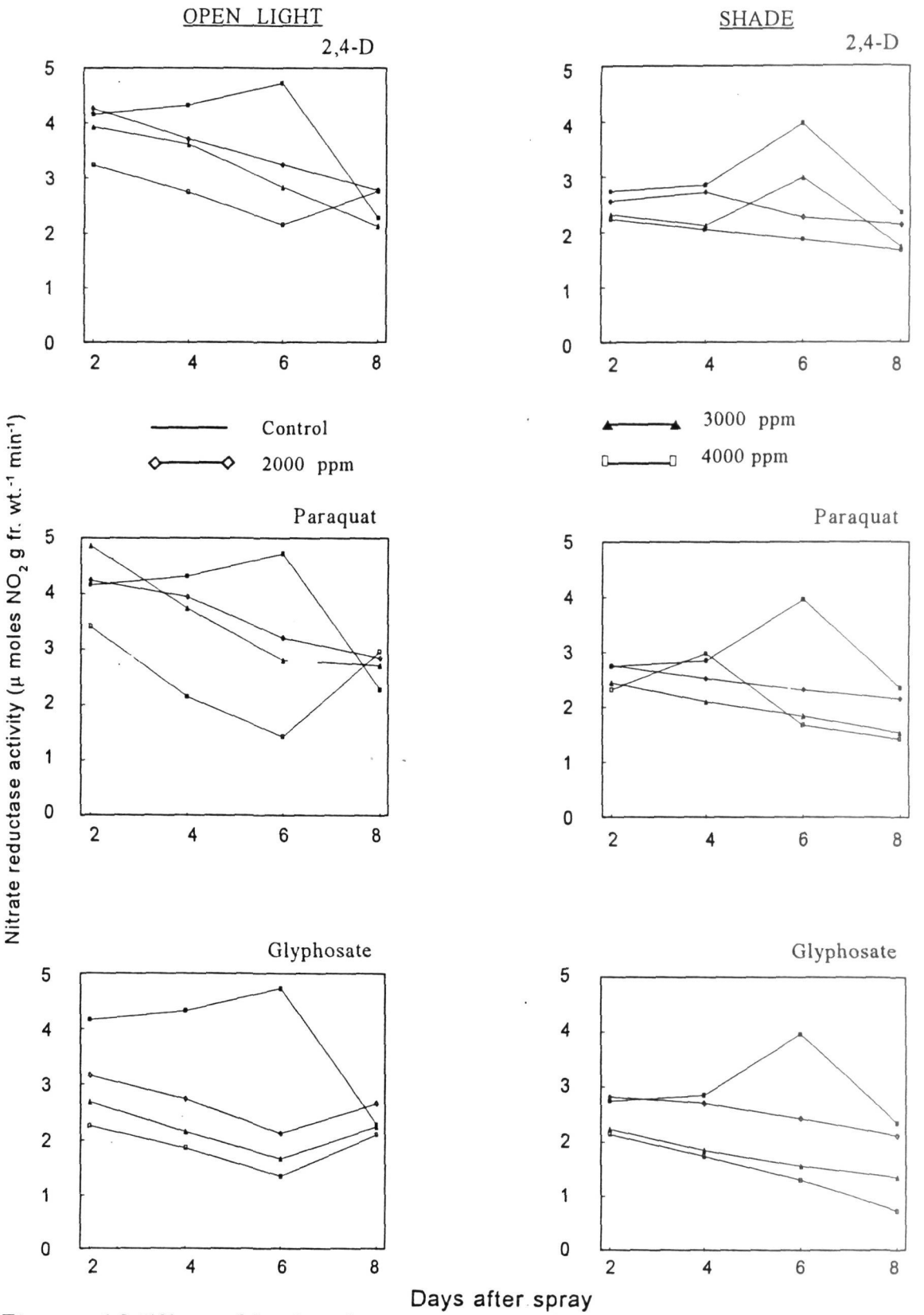


Figure 10.Effect of herbicides on nitrate reductase activity in leaves of Eupatorium grown in open light and shade conditions

were on par with each other but were significantly lower than control. The lowest activity (2.25) was in 4000 ppm glyphosate.

At 4 DAS in open light condition, 4000 ppm 2,4-D and paraquat, 2000 and 3000 ppm glyphosate treatments were on par with each other but significantly lower activity than the control. The lowest activity (1.86) was in 4000 ppm glyphosate. Similar trend was recorded at 6 DAS. At 8 DAS all herbicide treatments had significantly lower activity over control. Treatments 2000 ppm 2,4-D and paraquat were on par with each other. Similarly, 4000 ppm paraquat having lowest activity and was on par with 3000 and 4000 ppm glyphosate.

Under shaded condition, at 4 DAS 2000 ppm of all the herbicides were on par with control. Similarly, treatments 3000 and 4000 ppm of 2,4-D paraquat and glyphosate were on par with each other but differed significantly with control. Same trend was noticed at 6 DAS also. At 8 DAS, lower concentration (2000 ppm) of all the herbicides and between 3000 and 4000 ppm of 2,4-D and paraquat respectively, there was no significant difference. Whereas, glyphosate at all the concentrations differed significantly recording lowest activity at 4000 ppm.

4.3.15. Transpiration rate (μmol H_2O $m^{-2}s^{-1}$)

The transpiration rate was recorded at 5 and 10 DAS under the both open light and shaded conditions and presented in Table 39. It was observed that the transpiration rate

Table 39 : Effect of herbicides spray on transpiration rate (μmol H_2O $m^{-2}s^{-1}$) in Eupatorium grown in open light and shaded conditions

Treatments	Concentration (ppm)	Growth Conditions			
		Open light		Shade	
		5	10	5	10
		<---------- Days After Spray ---------->			
Control	----	31.4	57.5	26.3	43.6
2,4-D	2000	30.5	26.3	25.8	24.9
2,4-D	3000	20.8	16.7	15.0	14.3
2,4-D	4000	10.9	9.8	8.6	8.0
Paraquat	2000	9.9	5.3	18.7	10.7
Paraquat	3000	7.3	3.3	11.5	8.6
Paraquat	4000	4.5	1.5	9.3	6.5
Glyphosate	2000	19.8	16.1	11.5	9.8
Glyphosate	3000	15.2	10.6	9.9	8.3
Glyphosate	4000	13.4	5.7	6.8	6.2
S Em±		1.9	2.7	2.0	2.5
C D at 5%		5.3	7.8	5.9	7.2

increased from 5 to 10 DAS in control under both light and shaded conditions and were significantly superior over all other treatments except with 2000 ppm of 2,4-D at 5 DAS. There was a significant decrease in the transpiration rate due to herbicides over control. The lowest transpiration rate was recorded in all the concentrations of paraquat at both DAS under open light, whereas, under shaded condition the transpiration rate was minimum in glyphosate. It was further observed that there were no significant differences between the concentrations in paraquat at 5 and 10 DAS under both open light and shaded conditions. Similarly, glyphosate under shaded condition did not differ significantly between the concentrations. The lowest transpiration rate of 1.5 μ mol was observed at 4000 ppm paraquat under open light.

4.3.16 Leaf temperature (°C)

There was significant difference in herbicide treatments with respect to leaf temperature under both open light and shaded conditions (Table 41). However, differences were more in open condition than in shaded for corresponding herbicide concentrations. The leaf temperature increased from 5 to 10 DAS in all the treatments under both the conditions. It was also observed that with an increase in the concentration of herbicide there was increase in leaf temperature and such increase was prominent under open light. The maximum leaf

Table 40 : Effect of herbicides on stomatal conductance (cm s^{-1}) in Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions			
		Open light		Shade	
		5	10	5	10
		<---------- Days After Spray ---------->			
Control	----	2.44	3.03	1.38	2.38
2,4-D	2000	1.92	2.13	1.37	1.15
2,4-D	3000	1.89	1.56	0.99	0.80
2,4-D	4000	1.03	1.31	0.62	0.60
Paraquat	2000	0.87	0.42	1.12	0.87
Paraquat	3000	0.54	0.91	1.09	0.78
Paraquat	4000	0.43	0.17	0.76	0.45
Glyphosate	2000	0.76	0.63	1.01	0.86
Glyphosate	3000	0.64	0.61	0.97	0.64
Glyphosate	4000	0.42	0.58	0.52	0.41
S Em±		0.37	0.48	0.43	0.54
C D at 5%		1.16	1.17	1.34	1.61

temperature was recorded at all the concentrations of paraquat (33.4, 34.6 and 34.8 respectively, at 2000, 3000 and 4000 ppm) as compared to other herbicides under open light at 10 DAS. The lowest leaf temperature was recorded in 2000 ppm paraquat (28.3°C) at 5 DAS under shaded condition. It was further observed that the differences between the two consequentive concentrations were not significant.

4.3.17 Stomatal conductance (cm s^{-1})

The data on stomatal conductance indicated significant differences due to treatments at 5 and 10 DAS under both open light and shaded conditions Table 40). The stomatal conductance decreased with an increase in the concentration of herbicides. It was further observed that the control had significantly higher stomatal conductance over paraquat and glyphosate at all the concentration and 2,4-D at higher concentrations under open light, where under shaded condition no significant differences were observed between control and any of the herbicide at 5 DAS. At 10 DAS, control had significantly higher stomatal conductance over all other herbicides and their concentrations except 2000 ppm of 2,4-D. It was lower under open light as compared to shaded condition.

Table 41 : Effect of herbicides on leaf temperature (°C) in Eupatorium grown in open light and shade conditions

Treatments	Concentration (ppm)	Growth Conditions			
		Open light		Shade	
		5	10	5	10
		<---------- Days After Spray ---------->			
Control	----	29.8	31.4	28.3	30.8
2,4-D	2000	29.8	31.1	28.9	30.3
2,4-D	3000	30.0	32.5	28.9	31.3
2,4-D	4000	30.7	32.6	28.4	29.1
Paraquat	2000	29.6	33.4	28.3	29.5
Paraquat	3000	30.4	34.6	28.9	30.4
Paraquat	4000	31.7	34.8	29.1	30.9
Glyphosate	2000	31.0	32.2	29.4	31.8
Glyphosate	3000	31.0	33.0	28.9	29.6
Glyphosate	4000	30.9	34.3	28.6	29.9
S Em±		0.2	0.3	0.2	0.2
C D at 5%		0.7	0.9	0.5	0.5

4.4 Experiment IV : Use of carriers to increase the efficacy of herbicides under different growth conditions

4.4.1 Relative water content (RWC %)

The data on relative water content (RWC) indicated, significant differences due to treatments at all the stages under both open light and shaded conditions (Table 42 and Fig.11). The RWC was maximum in control at all the DAS and there was a slight decline in RWC from 2 to 8 DAS under open light. Whereas, under shaded condition it increased from 2 to 4 DAS and declined thereafter. Among the herbicides, the maximum RWC was recorded in 2,4-D with any of the carriers used at 2 DAS. However, the differences between $(NH_4)_2$ SO_4 and KNO_3 were insignificant. A similar trend was observed at 4 DAS also. It was further observed that 2,4-D in combination with urea recorded that low RWC as compared to KNO_3 and $(NH_4)_2$ SO_4. At later stages, glyphosate + $(NH_4)_2$ SO_4 was more effective in reducing the RWC followed by paraquat + KNO_3. Among the herbicides treatment only with carriers maximum RWC was recorded in 2,4-D + $(NH_4)_2$ SO_4 (77.4%) and the minimum (40.6%) in paraquat + KNO_3.

It was observed that the RWC values were generally more under shaded conditions as compared to open light in all the treatments. The maximum RWC was recorded in 2,4-D + $(NH_4)_2$ SO_4 (81.25%) which was on par with all the treatments except

Table 42 : Effect of herbicides with carriers on leaf relative water content (RWC, %) of Eupatorium grown in open light and shade conditions

Treatments	Growth Conditions							
	Open light				Shade			
	2	4	6	8	2	4	6	8
	<---------- Days After Spray ---------->							
Control	85.6	84.7	84.3	83.8	83.3	86.5	86.3	95.4
2,4-D +$(NH_4)_2$ SO_4*	77.4	72.5	63.7	53.4	81.2	77.4	64.4	60.7
2,4-D + KNO_3	76.3	70.8	60.5	51.7	80.6	70.4	58.7	55.3
2,4-D + Urea	72.4	66.4	57.3	48.2	80.4	75.7	67.3	61.0
Paraquat +$(NH_4)_2$ SO_4	73.5	68.7	60.4	49.7	78.5	76.2	60.3	54.4
Paraquat +KNO_3	70.2	70.8	53.8	40.6	78.4	67.8	52.6	46.4
Paraquat +Urea	74.3	70.4	63.7	59.5	79.7	77.4	60.7	55.7
Glyphosate+$(NH_4)_2$ SO_4	73.6	64.8	52.5	43.2	77.9	67.5	48.4	42.3
Glyphosate+KNO_3	73.9	68.2	57.1	46.8	79.5	73.6	56.2	47.2
Glyphosate+Urea	74.8	67.6	58.2	47.3	80.2	74.8	64.5	58.3
S Em±	0.9	0.8	0.8	0.9	1.2	1.0	1.0	0.9
C D at 5%	2.5	2.4	2.3	2.6	3.6	2.6	2.8	2.6

* - The concentrations of herbicides and the carriers used were 2000 ppm and 1 per cent, respectively

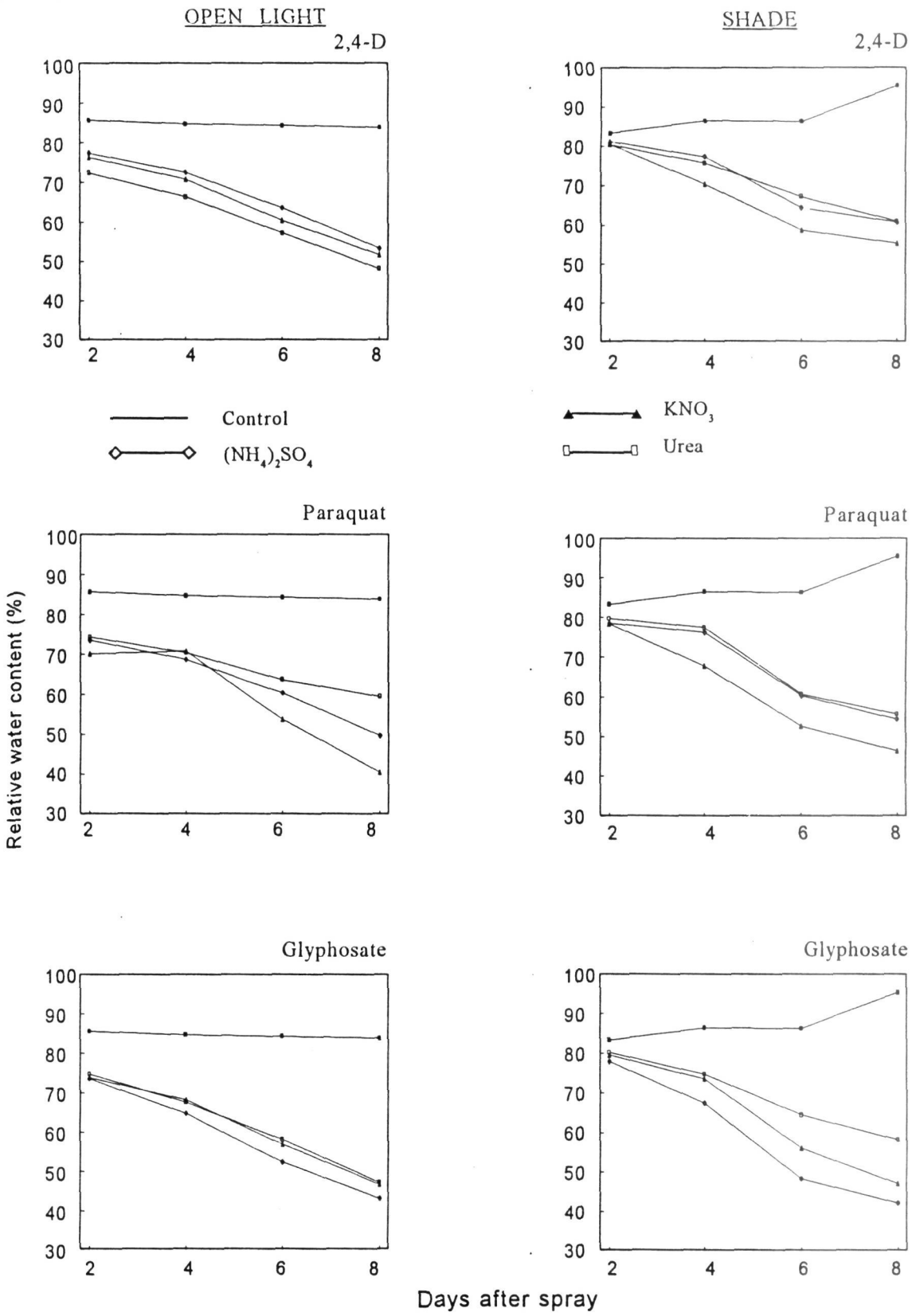


Figure 11. Effect of herbicides with carriers on leaf relative water content of Eupatorium grown in open light and shade conditions

glyphosate + $(NH_4)_2$ SO_4 (77.9%) at 2 DAS. Similarly glyphosate + $(NH_4)_2$ SO_4 had the minimum (42.3%) RWC at 8 DAS which was significantly lower over all other treatments. However, the treatments 2,4-D + KNO_3, paraquat + $(NH_4)_2$ SO_4 and paraquat + Urea did not different significantly with each other at any of the stages under shaded condition.

4.4.2 Electrical conductivity (EC) (ds/m/dm^2 leaf area)

The data on the effect of herbicides with carriers on EC presented in Table 43, indicated significant differences between the treatments at all the DAS under open light and shaded conditions. The EC was least in control as compared to all other treatments. Not much variation was observed in EC at different stages both under open light and shaded condition. It was further observed that the EC increased continuously between the stages in all the treatments under both the conditions. The maximum EC was recorded in glyphosate + $(NH_4)_2$ SO_4 at all stages under both the conditions. Similarly paraquat in combination with KNO_3 and 2,4-D in combination with urea gave higher EC than other combinations at all the stages under open light. The least EC was recorded in 2,4-D, paraquat, glyphosate in combination with $(NH_4)_2$ SO_4 urea and KNO_3 respectively at all the stages. However, the lowest EC among various combinations and herbicides was recorded in 2,4-D with $(NH_4)_2$ SO_4 (0.72 ds/m/dm^2 leaf area) and the maximum with the same carrier

Table 43 : Effect of herbicides with carriers on electrical conductivity (ds/m /dm^2 leaf area) in Eupatorium grown in open light and shade conditions

Treatments	Growth Conditions							
	Open light				Shade			
	2	4	6	8	2	4	6	8
	<---------- Days After Spray ---------->							
Control	0.53	0.52	0.54	0.53	0.43	0.44	0.43	0.45
2,4-D +$(NH_4)_2$ SO_4*	0.72	0.79	0.84	0.93	0.52	0.58	0.63	0.69
2,4-D + KNO_3	0.78	0.81	0.89	1.02	0.54	0.61	0.65	0.71
2,4-D + Urea	0.83	0.88	0.96	1.17	0.67	0.72	0.79	0.85
Paraquat +$(NH_4)_2$ SO_4	0.89	1.03	1.05	1.21	0.64	0.70	0.74	0.78
Paraquat +KNO_3	0.93	1.27	1.43	1.86	0.66	0.73	0.79	0.83
Paraquat +Urea	0.80	0.93	0.98	1.20	0.72	0.78	0.84	0.89
Glyphosate+$(NH_4)_2$ SO_4	1.08	1.31	1.56	2.13	0.74	0.79	0.85	0.97
Glyphosate+KNO_3	0.73	0.86	0.93	1.06	0.62	0.63	0.71	0.78
Glyphosate+Urea	0.79	0.89	1.02	1.11	0.64	0.67	0.75	0.81
S Em±	0.06	0.04	0.11	0.16	0.03	0.03	0.02	0.02
C D at 5%	0.17	0.12	0.32	0.43	0.09	0.09	0.05	0.06

* - The concentrations of herbicides and the carriers used were 2000 ppm and 1 per cent, respectively

i.e., Ammonium sulphate with glyphosate (2.13 ds/m²/dm leaf area). It was observed under shaded condition that similar to that of open light EC increased from 2 to 8 DAS and was low with 2,4-D + Ammonium sulphate and higher with glyphosate + Ammonium sulphate at 2 and 8 DAS respectively. In general 2,4-D + urea, paraquat + $(NH_4)_2SO_4$, paraquat + KNO_3 and paraquat + urea did not differ significantly with each other at any of the stages.

4.4.3 OD_{525} of leaf leachate

The data on the OD of leaf leachate indicated that there was an increase in OD from 2 to 8 DAS under both open light and shaded conditions. Herbicides with different carriers also had significant differences among themselves at all the stages under both the conditions (Table 44). It was further observed that OD values were low under shaded conditions as compared to open light in all the herbicides combination at all the stages. Further, the data revealed that the maximum OD values were obtained in different combination of herbicide and carriers. For example the maximum OD was recorded in 2,4-D + urea, paraquat + KNO_3 and glyphosate + $(NH_4)_2SO_4$ in comparison to other combinations tested with each herbicides. Whereas, the minimum OD values were registered in 2,4-D + $(NH_4)_2SO_4$, paraquat + $(NH_4)_2SO_4$ and glyphosate + KNO_3 indicating differential impact of carriers in increasing the efficacy of different herbicides under both open light and shaded conditions except the combinations of glyphosate + urea under

Table 44 : Effect of herbicides with carriers on OD_{525} of leaf leachates in Eupatorium grown in open light and shade conditions

Treatments	Growth Conditions							
	Open light				Shade			
	2	4	6	8	2	4	6	8
	<---------- Days After Spray ---------->							
Control	0.131	0.136	0.140	0.145	0.085	0.097	0.112	0.124
2,4-D +$(NH_4)_2$ SO_4*	0.150	0.168	0.248	0.367	0.110	0.125	0.143	0.174
2,4-D + KNO_3	0.160	0.285	0.345	0.393	0.124	0.138	0.165	0.192
2,4-D + Urea	0.181	0.327	0.383	0.455	0.115	0.142	0.189	0.216
Paraquat +$(NH_4)_2$ SO_4	0.145	0.258	0.285	0.325	0.112	0.136	0.162	0.185
Paraquat +KNO_3	0.183	0.294	0.354	0.427	0.125	0.149	0.186	0.207
Paraquat +Urea	0.156	0.272	0.296	0.354	0.116	0.138	0.167	0.188
Glyphosate+$(NH_4)_2$ SO_4	0.193	0.313	0.365	0.425	0.156	0.175	0.194	0.228
Glyphosate+KNO_3	0.129	0.197	0.243	0.270	0.134	0.146	0.158	0.172
Glyphosate+Urea	0.149	0.228	0.268	0.284	0.110	0.127	0.133	0.153
S Em±	0.012	0.024	0.018	0.027	0.012	0.004	0.019	0.006
C D at 5%	0.029	0.068	0.049	0.079	0.032	0.011	0.051	0.018

* - The concentrations of herbicides and the carriers used were 2000 ppm and 1 per cent, respectively

shaded condition. The highest OD under shaded condition was with glyphosate + $(NH_4)_2SO_4$ (0.228) and the lowest with 2,4-D + $(NH_4)_2$ SO_4 (0.110).

4.4.4 Total soluble sugar content (mg g dry wt^{-1})

The data on total soluble sugar content indicated that the herbicides with different carriers had differential response and were significant at all the stages under both open light and shaded conditions (Table 45 and Fig. 12). The total soluble sugars were maximum in control as compared to other treatments at all the stages under both the conditions. There was a significant reduction in total soluble sugars due to the combination of carriers with different herbicides. It was observed that the maximum total soluble sugars were recorded in 2000 ppm of 2,4-D, paraquat and glyphosate in combination with urea, KNO_3 and $(NH_4)_2$ SO_4 respectively, whereas minimum total soluble sugars were recorded in these herbicides with $(NH_4)_2$ SO_4 urea and KNO_3 respectively at all the stages under both open light and shaded conditions. Under open light, the minimum total soluble sugar was recorded in paraquat + KNO_3 (14.18 mg) at 8 DAS and under shaded condition it was with glyphosate + $(NH_4)_2$ SO_4 (14.21 mg) at 8 DAS.

4.4.5 Total free phenols (mg g dry wt^{-1})

Total free phenol content in Eupatorium leaves as influenced by herbicides with carriers are given in Table 46.

Table 45 : Effect of herbicides with carriers on total soluble sugar content (mg g dry wt^{-1}) in leaves of Eupatorium grown in open light and shade conditions

Treatments	Growth Conditions							
	Open light				Shade			
	2	4	6	8	2	4	6	8
	<---------- Days After Spray ---------->							
Control	21.78	20.97	18.98	22.47	18.25	19.16	19.87	20.16
2,4-D +$(NH_4)_2$ SO_4*	20.46	20.15	18.98	17.47	18.57	19.07	18.56	17.16
2,4-D + KNO_3	19.57	19.67	16.53	16.05	18.15	18.72	17.78	17.00
2,4-D + Urea	18.61	17.03	15.87	15.21	18.00	17.42	16.35	16.04
Paraquat +$(NH_4)_2$ SO_4	19.86	19.15	18.24	17.26	17.67	19.42	17.05	16.72
Paraquat +KNO_3	19.15	16.43	15.36	14.18	17.34	17.00	16.13	15.26
Paraquat +Urea	20.56	19.97	19.03	17.34	18.25	18.42	17.73	17.14
Glyphosate+$(NH_4)_2$ SO_4	19.32	17.41	15.74	14.67	18.75	17.17	15.36	14.21
Glyphosate+KNO_3	20.74	19.00	17.86	17.12	18.26	18.00	17.47	17.13
Glyphosate+Urea	20.17	18.68	18.03	17.56	18.42	18.21	17.73	17.41
S Em±	0.28	0.31	0.29	0.34	0.48	0.32	0.38	0.39
C D at 5%	0.82	0.90	0.85	1.02	NS	0.93	1.06	1.16

* - The concentrations of herbicides and the carriers used were 2000 ppm and 1 per cent, respectively

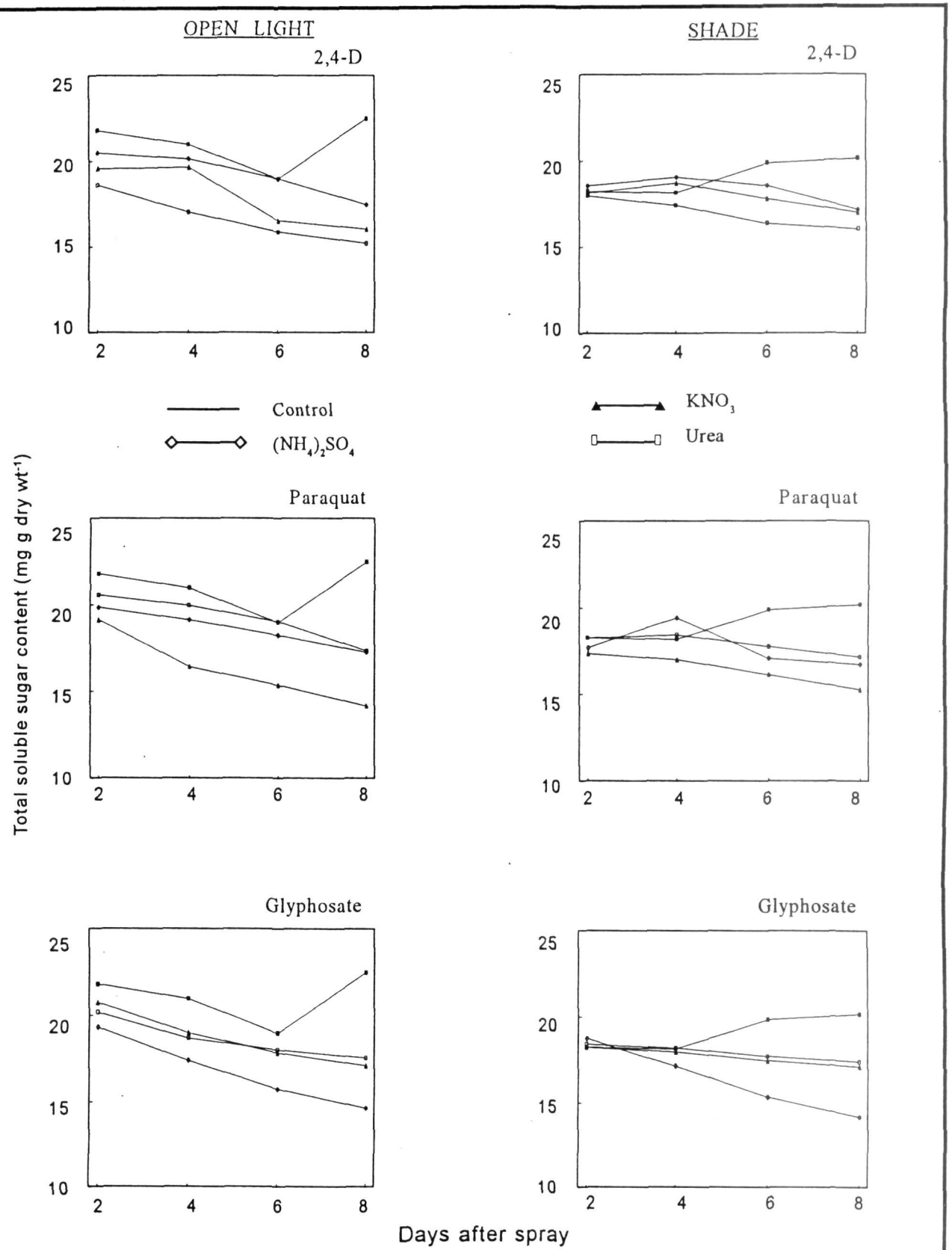


Figure 12. Effect of herbicides with carriers on total soluble sugar content in leaves of Eupatorium grown in open light and shade conditions

Table 46 : Effect of herbicides with carriers on total free phenols (mg g dry wt^{-1}) in leaves of Eupatorium grown in open light and shade conditions

Treatments		Growth Conditions							
		Open light				Shade			
		2	4	6	8	2	4	6	8
		<---------- Days After Spray ---------->							
Control		7.35	7.86	8.27	8.43	5.47	5.68	5.28	5.56
2,4-D	+$(NH_4)_2$ SO_4*	7.72	7.15	6.41	6.27	5.68	4.97	4.97	4.07
2,4-D	+ KNO_3	7.58	7.10	6.23	6.13	6.15	5.37	5.37	4.63
2,4-D	+ Urea	8.24	8.77	8.04	7.38	7.35	7.82	7.82	5.73
Paraquat	+$(NH_4)_2$ SO_4	8.05	7.54	7.35	7.21	7.56	7.23	7.23	6.23
Paraquat	+KNO_3	10.74	9.25	8.31	6.15	9.27	8.72	8.72	6.34
Paraquat	+Urea	7.67	8.21	7.44	7.10	8.35	9.45	9.45	7.46
Glyphosate	+$(NH_4)_2$ SO_4	8.26	10.43	8.26	6.12	7.52	8.14	8.14	5.54
Glyphosate	+KNO_3	7.87	7.16	6.35	5.78	6.25	5.72	5.72	4.85
Glyphosate	+Urea	8.57	9.35	8.13	7.46	6.93	7.76	7.76	6.22
S Em±		0.31	0.40	0.30	0.27	0.58	0.72	0.72	0.38
C D at 5%		0.89	1.12	0.85	0.76	1.68	2.01	2.01	1.11

* - The concentrations of herbicides and the carriers used were 2000 ppm and 1 per cent, respectively

The data indicated that phenol content was more in open light grown plants than that in shaded condition. Significant differences were noticed with the herbicide treatments at all the stages both in open light and shaded condition. In open light condition, there was a gradual decrease in phenol contents (from 2 to 8 DAS) among herbicide treatments except glyphosate + $(NH_4)_2$ SO_4 and 2,4-D, paraquat and glyphosate with urea treatments in which first a slight increase from 2 to 4 DAS and then a decline was observed.

At 2 DAS maximum phenol content (10.74) was in paraquat + KNO_3 treatment and it was in glyphosate + $(NH_4)_2$ SO_4 at 4 DAS. Treatments 2,4-D with $(NH_4)_2$ SO_4 and KNO_3 paraquat with $(NH_4)_2$ SO_4 and urea and glyphosate with KNO_3 were on par with control at 4 DAS. At 6 and 8 DAS, most of the treatments showed reduction in phenol content with lowest in 2,4-D + KNO_3 (6.23) followed by glyphosate + KNO_3 (6.35).

Under shaded condition, between 2 and 4 DAS, treatments 2,4-D + Urea, paraquat + urea, glyphosate + Urea and glyphosate + $(NH_4)_2$ SO_4 showed an increase in phenol content. Whereas, rest all treatments showed a continuous decrease with increase in DAS. At 8 DAS, the lowest phenol content (4.07) was recorded in 2,4-D + $(NH_4)_2$ SO_4 which was on par with 2,4-D + and KNO_3 and glyphosate + KNO_3 treatments.

V. DISCUSSION

V DISCUSSION

Eupatorium (*Chromolaena odorata* K & R) has been considered as the most troublesome and obnoxious weed next only to *Cyperus* and *Cynadon* species. Being a perennial weed, Eupatorium produces many suckers from the underground rhizome like structures apart from producing millions of air borne seeds which could travel to any distance in air and establish in uninfested areas. Several efforts have been made to combat the menance of its aggression in several areas but no conclusive evidences have been drawn with respect to its permanent eradication.

The activity of herbicides depends on their concentration and their effective translocation into the plant system and interference with several metabolic pathways. It has long been known that the efficacy of herbicides could be increased by mixing with the compatible carriers. The carriers selected should be such that they should be compatible, cheap and easily available. Lot of work has been done on these aspects in several perennial and annual weeds and absolutely no work has been done in Eupatorium.

Growth conditions have marked influence in modifying not only the plant growth and development but also are known to influence the translocation and efficiency of herbicides

leading to the impairment in metabolic activity of plants. The growth conditions are also known to alter the internal structure which inturn could lead to several physiological and biochemcial changes.

With this background in mind, the present experiments were conducted to find out various morphological, physiological, biochemical and anatomical changes induced by changing the growth conditions and foliar application of herbicides with or without carriers under naturally grown and shaded conditions. The present investigation was also aimed at finding out the influence of herbicides on seed production aspects and the results obtained are discussed in this chapter.

5.1 Use of different herbicides and time of application for control of Eupatorium

It was intended to find out the effect of different herbicides and their concentrations in contrast environments on various morphological parameters and partitioning of dry matter so as to assign specific reasons for their differential response or similarity.

5.1.1 Herbicidal effect on plant height and dry matter accumulation

5.1.1.1 Plant height

Considering the nature of propagation of Eupatorium, herbicides selected to control the spread of Eupatorium should be such that it should get translocated in plants, particularly in roots. Several workers have tried different herbicides to control the infestation of Eupatorium. But, the information available on the relative effectiveness of different chemicals, their concentrations, the time of application at different locations is very meager. The present investigation revealed that there was a substantial reduction in plant height and partitioning of dry matter in leaf and stem and such response varied among the herbicides and locations. It was observed that among various herbicides, glyphosate at 7500 ppm was more effective in reducing the plant height followed by glyphosate at 5000 ppm sparyed during August, September and October. The extent of reduction in plant height was maximum during August spray and declined thereafter upto October. The maximum reduction in plant height during the August spray was mainly because of no rainfall during the month of November. Though, there was maximum rainfall during August (655.2 mm), the difference between the maximum and minimum air temperature was less

(20.6°C minimum and 24.3°C maximum) which might have helped in the better translocation of herbicides resulting in the effective control. It was further observed that, the per cent reduction in plant height increased gradually from 0 to 90 DAS in all the months. such increase in the per cent reduction could be attributed to the exposure of sprayed plants to rain free environment and lower difference between the maximum and minimum temperatures. It could also be attributed to slow incorporation of the herbicides into the plant system.

It was also observed that the per cent reduction in plant height increased with an increase in the concentration of herbicides at both Yellapur and Prabhunagar locations, indicating the requirement of herbicides at higher concentrations for effective control. Similar observations on decrease in plant height due to increase in the concentration were reported by Nair (1973), Risidino (1975) Ca deno *et al.* (1986), Mumbareddy (1989), Doddamani (1992) and Singh *et al.* (1992).

The differential response of herbicides on plant height was attributed to their differential mode of action. The decrease in plant height due to herbicide treatment was because of inhibition of apical and intercalary meristematic cells which inturn reduce considerably the apical and internodal elongation, respectively. The development of mesoyphyll tissue, thus was equally affected. In the present

investigation, 2,4-D ester form was used mainly because of its low toxicity to human beings and animals. Chen *et.al.* (1971) reported that the phytotoxicity of 2,4-D is due to differential alteration of RNA species and interference in protein synthesis.

The trend of decrease in plant height due to the application of herbicides did not vary between Prabhunagar and Yellpaur which was mainly because of the similarity in the weather conditions, with which the crop was exposed after the spray. It can be seen from the meteorological data (Table 1) that during November when the final plant height was recorded did not receive any rain at both the locations. Although at Yellapur, the rainfall received during the earlier months of spray was more as compared to Prabhunagar, it did not have much influence on the final per cent reduction in plant height. But, it is clear that the higher plant height in control (unsprayed) and other treatments (Sprayed) at Yellapur over Prabhunagar was mainly because of high rainfall, low minimum and maximum temperatures which are conducive for the luxuriant vegetative growth. It can also be inferred from the investigation that it is not the initial plant height which is important but it is the herbicides and their concentrations which are more important for effective control of Eupatorium.

The maximum per cent reduction in glyphosate at all the concentrations as compared to the respective concentrations of 2,4-D and paraquat was mainly because of its mode of action which is systemic and non selective. It is found to inhibit the biosynthesis of aromatic amino acids, more particularly the phenyl alanine and the metabolism of chorismic acid (Jaworski, 1972).

5.1.1.2 Drymatter accumulation in leaf and stem

The dry matter production is the integral of all metabolic process and influenced by both intrinsic viz., photosynthesis, translocation of photosynthates, source-sink relationship and exterinsic factors, mainly light intensity, temperature and humidity. If any one of these factors is affected it will have a profound influence on plant growth, development and dry matter production.

Herbicides applied to the plants result in the reduction in the total dry matter through their interference in plant growth by way of blocking the electron transport chain, altering the membrane integrity, stomatal inhibition of photosynthesis, inhibition of protein synthesis and cell division (O'Brein *et al.*, 1968 and Halliwell, 1982).

In the present investigation, application of herbicides at higher concentration (7000 ppm) was more effective in reducing the dry matter accumulation both in the

stem and leaf. Of the three herbicides tested, glyphosate at 7500 ppm recorded maximum reduction in the dry weights indicating increased efficacy of this herbicide over the other two.

However, the per cent distribution of dry matter in the leaf was lowest in 7500 ppm of paraquat as compared to the respective concentrations of other herbicides indicating its rapid absorption through the foliage. Klood and Gosnill (1965) reported that the translocation of paraquat is apoplastic through the xylem. Its mode of action in green plants appears to be mediated by the generation of phytotoxic hydrogen peroxide and super oxide radicals. At lower concentrations, the reactive super oxide radicals are produced in insignificant quantities and are being quenched, neutralized and detoxified by the natural protective mechanisms, there by causing elastic strain on biomembranes, which by and large are reversible in nature and thus, damage occurred at lower concentrations is less as compared to higher concentrations of paraquat.

There was continuous increase in leaf dry matter upto 60 days after spray and then declined drastically. The distribution of dry matter in the stem was more affected by 7500 ppm of glyphosate and 2,4-D at all the stages as compared to paraquat, because it is a contact herbicide and

was more effective in decreasing in leaf dry weight.

Results of Mogali (1982), Mumbareddi (1989) and Doddamani (1992) also revealed the similar response with respect to dry matter accumulation and distribution in the stem and leaf due to the application of glyphosate, 2,4-D and paraquat. Ivens (1974) suggested that differential accumulation of dry matter in stem and leaf was due to selective nature of herbicides. Similarly, Maligeppagol (1990) and Patil (1990) reported a reduction in the foliage dry weight in Cynodon due to the spray of glyphosate and 2,4-D mainly due to strong phytotoxic effect of these chemicals which are responsible for affecting the phenol metabolism.

Campbell *et al.* (1976) reported that the application of glyphosate on quack grass (*Agropyron repens* L.) resulted in the modification of the ultrastructure of mesophyll chloroplasts, complete disruption of mitochondrial cristae, swelling of chloroplasts and polar disorientation of grana, which altogether resulted in the impaired photosynthesis and other metabolic process leading to decreased leaf and stem dry weights. Glyphosate induced an increase in PAL activity (James and Davidson, 1976) and reduction in protein synthesis through depletion in aromatic aminoacids pool as well as through the production of phytotoxic levels of amino and secondary phenolics (Jaworski, 1992) which have been associated with growth.

It was further observed that the per cent reduction in leaf and stem dry weights was more in Yellapur as compared to Prabhunagar. This could be attributed to the environmental conditions prevailing during both pre and post spraying periods. It can be seen from the meteorological data that Yellapur received more rainfall compared to Prabhunagar, leading to luxuriant growth of crop and more foliage, through which there could have been better absorption and translocation of herbicides in the plant system. It is also possible that lesser difference between maximum and minimum temperatures at Yellapur over Prabhunagar could have triggered the action of herbicides leading to maximum per cent reduction in leaf and stem dry weights.

5.2 Effect of herbicides under different growth conditions

The efficacy of herbicides depends on both intrinsic and external environmental conditions such as light intensity, rainfall, relative humidity and water availability etc., Results of the experiment conducted separately to find out the efficacy of herbicides under open light and shaded conditions on various physiological, biochemical, morphological and anatomical changes in Eupatorium are discussed hereunder.

5.2.1 Physiological parameters

It was observed that relative water content (RWC) in the leaf decreased with an increase in concentration of herbicides under both light and shaded conditions and the maximum decrease in RWC was noticed in paraquat as compared to other two herbicides in all the concentrations. The extent of decrease in RWC was more under open light as compared to shaded condition, mainly because of active transport of herbicides under open light. With an increase in light intensity, there was an increase in leaf temperature, which could lead to higher rate of metabolic activity within the plants. The RWC decreased from 2 to 8 DAS and the extent of decrease was more in light over shade. This could be attributed to increase in desiccation of plants, such effect was more in paraquat since, it is a contact herbicide causing plasmolysis of cells.

Chlorophyll a, b and total chlorophyll contents decreased with an increase in the concentration both under light and shade conditions and the extent of decrease were more in light. Paraquat was found to have more influence in decreasing the chloropyll content and it was almost 50 per cent of the content over other two herbicides at higher concentrations indicating the leathal effect of paraquat as compared to 2,4-D and glyphosate. The decrease in the chlorophyll content due to herbicides was attributed to a decrease in the RWC.

Halliwell (1982) reported that the application of paraquat results in the disintegration of thalakoid membranes, swelling of frets and blockage of the ETC. Such influence on the ultrastructure of chloroplast and grana thalakoids was attributed to the excess production of H_2O_2 free radicals and super oxide radicals and causing damage to the natural protective mechanism. The influence of shade could not cause greater reduction in the chlorophyll content at low concentrations of herbicides and however, decrease in the chlorophyll content at higher concentration even under shade was significant. Decrease in the extent of reduction under shade condition was attributed to the slow uptake and translocation of herbicides. Since paraquat is a contact herbicide, it caused greater damage at higher concentration even under shade. The insignificant difference in the ratio of chlorophyll a:b could be attributed to the differential activity of herbicides on both chlorophyll a and chlorophyll b contents.

The electrical conductivity (EC) of leaf leachates indicate the extent of membrane integrity. More the EC more will be the loss of membrane integrity (Vanstone and Stobbe, 1977). In the present investigation, it was seen that the EC was maximum at 4000 ppm of paraquat under both light and shade conditions, indicating the maximum effect of paraquat on membrane integrity as compared to 2,4-D and glyphosate. The

increase in the EC due to herbicide treatments could also be attributed to decrease in the turgidity of plants as has been evidenced by significant decrease in the RWC and the leakage of ions. It is evident that when the biomembranes are disintegrated, they loose their basic property of semipermeability which could lead to leakage of ions.

Anthocyanin content will have the maximum absorption peak at 525 nm and is soluble in water. The OD of leachate at 525 nm indicates the leakage of anthocyanins due to herbicidal effect. The OD was maximum at all the concentrations of paraquat in comparision to the respective concentrations of 2,4-D and glyphosate indicating the greater damage to the membrane integrity due to paraquat. However, under shade conditions, paraquat did not have greater impact on leakage of anthocyanin and was more with glyphosate. The extent of increase in OD was more in open light as compared to shade indicating the slow action of herbicides under low light intensity. It is inferred from the present finding that systemic herbicides like 2,4-D and glyphosate are more effective under shade conditions and contact herbicides under open light conditions.

Soluble sugar content was more in light grown plants than shade grown plants which is quite natural because of increased rate of phtosyntheis in light. The effect of

herbicides on changes in sugar content indicated greater reduction in the sugar content under light condition as compared to shade. Although, all the herbicides caused a significant reduction in sugar content, the degree of reduction was more in glyphosate followed by paraquat and 2,4-D treatments in both light and shade conditions. Lower levels of total soluble sugars in herbicide treatments could be attributed to the utilization of carbohydrate reserves during the period when the demand is more and the supply is less due to impairment in photosynthetic activity (Trlica and Singh, 1979). The decrease in total soluble sugars could also be attributed to reduced chlorophyll content, which is necessary for photosynthesis, hydrolytic decomposition of starch, inuline and proteins, increased respiration and disrupted water metabolism (Gurr *et al.*, 1979; Gruzdev *et al.*, 1980 and Sharma *et al.*, 1986).

Significant reduction of crude protein content due to spraying of herbicides has been noticed both under light and shade conditions and the extent of reduction was more under light. Among the herbicides, glyphosate was more effective in reducing crude protein content as it is a systemic herbicide and is known to inhibit protein synthesis through reduced supply of aromatic amino acid pool, decrease in RNA content, which is necessary for protein systnehsis (Coole *et al.*, 1978; Hoagland and Duke, 1983 and

Villers and Koacher, 1983). Similr reduction in the crude protein content in plants due to the foliar application of glyphosate, 2,4-D and paraquat have been reported by Patil and Bangal (1984), Maligeppagol (1990) Patil (1990) and Doddamani (1992).

It was also observed in the present investigation that there is a drastic reduction in free amino acid content due to the application of herbicides. Glyphosate treatment caused increased level of free amino acid content which may be because of non utilisation of amino acids (other than aromatic amino acids that are known to be reduced) for protein synthesis. Whereas, the reduction in amino acid pool by 2,4-D and paraquat could be attributed to the increased rate of respiration. The extent of decrease in amino acid content by herbicides was more in light than in shade which may be because of increased rate of respiration and photorespiration in light. Similarly, glyphosate has been postulated to act by inhibiting the aromatic amino acid synthesis in shikimic acid pathway in plants (Jaworksi, 1972). Its effect on duck weed *Lemna gibba* and on *Rhizobium japonicum* caused reduced levels of aromatic amino acids needed for normal protein synthesis and thus growth was reduced (Hoagland, 1980).

It was observed that the application of herbicides altered the metabolism of phenolic compound and the enzymes associated with phenol metabolism in the present

investigation. Among the herbicides, the total phenols increased with an increase in the concentration and such increase was marked in glyphosate. Under open light, there was a slight decrease in the phonelic content with an increase in the herbicide concentration and DAS. The increase in total free phenols could be attributed to a significant increase in PAL and TAL activities and this was clear in open light. Whereas, under shaded condition, no much difference was observed between the stages and at lower concentration. Similarly, Duke and Hoagland (1978), Duke *et al.* (1979 and 1980), and Hoagland *et al.* (1978 and 1979) reported increased hydroxyphenolic compound and increased ammonia levels due to glyphosate. On the contrary, it was reported that although, PAL activity increased dramatically in glyphosate treated maize roots and in light and dark grown soyabean axis, increase in ammonia levels and decreased levels of free phenyl alanine correlate well with increased PAL activity. The increase in PAL and TAL activities may be caused by enzyme activation, increase in synthesis or decrease in enzyme degradation (Engelsma, 1974; Huffakar and Peterson, 1974; Johanson and Smith, 1978 and Smith *et al.*, 1977).

Significant increase in peroxidase activity due to herbicide treatment was observed. The per cent increase over control was more under open light than in shade and was maximum with paraquat. There was a gradual increase in the

peroxidase activity between the stages. Such increase in the peroxidase activity could be attributed to reduced protein synthesis and RWC, loss in the membrane integrity and decreased amino acid pool. Similar results of increase in the peroxidase activity due to the application of 2,4-D and glyphosate were reported by Thangaraj and Sakharamarao (1973) and Halliwell (1982).

Nitrate reductase (NRase) is an important enzyme in the nitrogen metabolism required for the reduction of nitrate to nitrite. The activity of NRase decreased drastically due to herbicide treatment and the decrease was more with an increase in the concentration of herbicides. The low activities under shaded condition as compared to light, both in control and herbicide treated plants could be mainly because of low photosynthetic rates, which limit the supply of energy and reducing equivalents required for NRase activity. Similarly, herbicide treated plants also had low NRase. Among them, maximum reduction was seen in paraquat. This was mainly attributed to maximum reduction of RWC and chlorophyll content. Chlorophyll molecules are required for absorption of light which is the first act of photosynthesis. Klepper (1979) reported that the herbicides which inhibit photosytnthesis, act by causing nitrate accumulation within the cells. Similarly, Finke *et al.* (1977) studied the effect of various herbicides on *in vivo* nitrate and nitrite

reductase in barley and bean and found the differential inactivation of these enzymes by different herbicides.

The effect of different herbicides on biophysical characters indicated that there was a significant reduction in the transpiration rate (TR) and stomatal conductace (SC) while the leaf temperatures increased significantly. The decrease in the TR and SC was maximum in paraquat since paraquat is a contact herbicide which sets in fast desiccation of the leaves (Plate 6). Due to fast desiccation and loss of turgidity, there is a partial or complete closure of stomates leading to an enormous increase in the resistance which is indicated by a decrease in stomatal conductance. Under shaded condition, the TR and SC were more, mainly because of increase in RWC over open light and better maintenance of turgidity. The decrease in transpiration rate or the stomatal conductance in paraquat could be attributed to the fast action of the contact herbicides. Putnam (1976) has also reported that transpiration is rapidly affected by glyphosate.

The increase in leaf temperature due to herbicides could be attributed to a low stomatal conductance leading to low transpiration. Transpiration has been considered as a necessary evil, since it keeps the leaf surface cool apart from its role in photosynthesis. It has also been reported by



a. Control with different levels of . 2,4-D ·



b. Control with different levels of paraquat



c. Control with different levels of glyphosate

1. Control (unsprayed) 2. 2000 ppm 3. 3000 ppm 4. 4000 ppm

PLATE 6. COMPARISON OF LEAF APPEARANCE DUE TO THE APPLICATION OF HERBICIDES UNDER OPEN LIGHT AND SHADED CONDITIONS

Doddamani (1992) that the nutrient uptake is greatly reduced by the herbicides due to decrease in the transpiration pull.

5.3 Effect of herbicides on seed production in Eupatorium

Successful perpetuation of annual weeds takes place mainly through sexual reproduction. Eupatorium being a perennial weed, flowers once a year during November/December months. Although, Eupatorium regenerates from the underground root with the receipt of monsoon, seeds dispersed from mature plants form the major source of weed infestation in new lands. Eupatorium plant produces millions of small seeds that travel a long distance through wind by virtue of their pappus nature.

In order to find out the most effective chemical, its appropriate concentration and the time of spray, to check the seed production, different herbicides were sprayed. All the herbicides tried significantly reduced the seed parameters like number of seeds per head, seed weight and per cent seed germination. Spraying of herbicides at flower bud stage was found to be most effective than at later stage. This may be because of rapid absorption of herbicides by succulent flower buds at which stage herbicide may interfere with floral development. Among the herbicides tested, paraquat at 3000 ppm sprayed during flower bud stage did not

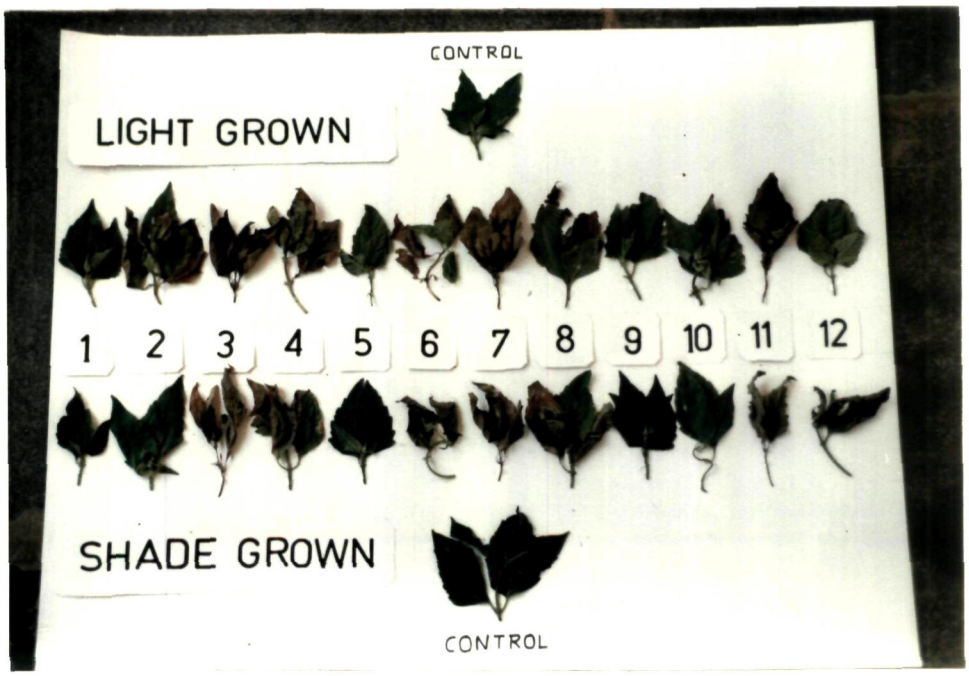
produce any seed that can germinate. This might have caused the inhibition of floral development, embryo development and seed filling. This has been further indicated by significant reduction in number of seeds formed and their test weight. Herbicides, 2,4-D and glyphosate could also be able to reduce seed production significantly over the control. Several workers have reported such results in some other weeds. Maun and Cavers (1969) reported complete prevention of the formation of viable seeds in curly dock (*Rumex crispus*) by spraying 2,4-D at 12 days before anthesis. He reasoned out that 2,4-D inhibited embryo and endosperm development in the seeds. However, spraying at later stages did produce normal seeds. Rehm (1952) reported that the application of 2,4-D in water melon (*Cucurbita pepo*) induced male sterility by preventing the development of male and female gametophytes. Fawcett and Slife (1978) obtained similar results with 2,4-D in Datura seeds.

5.4 Use of carriers for increasing the efficiency of herbicides

Herbicides that are applied to the foliage must penetrate the wax and cuticle layers, migrate to the phloem tissue and then be transported with the photosynthates to the areas where they can exert toxic action on plants. The absorption and translocation of herbicides depends on the

environmental and chemical factors (Richardson, 1977). Absorption of herbicides by leaves is affected by many factors such as the nature of chemical itself, its chemical form, and the effects of other chemicals, additives and surfactants. It has been demonstrated by several workers (Richardson, 1977; Sexsmith, 1953; Greenham, 1962; Brady, 1970 and Bird *et al.*, 1971) that the toxic effect of herbicides can be increased by using carriers. The most commonly used carriers are urea, KNO_3 and $(NH_4)_2SO_4$ and have been found to be effective in increasing the translocation efficiency of herbicides. Moreover, these chemicals are cheap, readily mix with herbicides and easily available and hence they were used in the present investigation with different herbicides.

The results revealed that there was a significant decrease in the relative water content due to the use of carriers in comparison to the control. The decrease in the relative water content was more under open light as compared to shade condition indicating that the translocation of herbicides was more effective under high light intensity. The comparison of leaves under different treatements given in Plate 7 gives an indication of the extent of desiccation and dryness set in by different herbicides in combination with the carriers. The carriers are specific to herbicides and it was



1. $(NH_4)_2\ SO_4$
2. 2,4-D + $(NH_4)_2\ SO_4$
3. Paraquat + $(NH_4)_2\ SO_4$
4. Glyphosate + $(NH_4)_2\ SO_4$
5. KNO_3
6. 2,4-D + KNO_3
7. Paraquat + KNO_3
8. Glyphosate + KNO_3
9. Urea
10. 2,4-D + Urea
11. Paraquat + Urea
12. Glyphosate + Urea

PLATE 7. COMPARISON OF LEAF APPEARANCE DUE TO THE APPLICATION OF HERBICIDES ALONG WITH CARRIERS UNDER OPEN LIGHT AND SHADE CONDITIONS

noticed that 2,4-D is more compatible with urea, paraquat with KNO_3 and glyphosate with $(NH_4)_2SO_4$ and were effective in decreasing relative water content. The decrease in relative water content due to application of herbicides with carriers was mainly because of increased desiccation of leaves.

Early work on the effect of carriers on herbicide uptake has been extensively reviewed by Hull (1970). Similarly, Turner and Loader(1978) reported that orthophosphoric acid and $(NH_4)_2SO_4$ increased the herbicide penetration through the leaf surface and help in the better absorption, translocation leading to increased glyphosate activity. Richardson (1977) also reported that the foliar absorption and translocation of 2,4-D and 2,4,5-T was affected by both environmental and chemical factors. Nitrogenous compounds, particularly urea and KNO_3 were found to be efficient carriers of 2,4-D and paraquat, respectively (Minshall, 1968 & 1969).

It was observed that the EC and OD_{525} were almost one and half times in herbicides mixed with carriers (Table 43) as compared to herbicides alone (Table 29) at 2000 ppm concentrations. Even the EC and OD_{525} at highest concentration of any herbicide was less than the herbicides at 2000 ppm concentration mixed with carriers. This clearly

indicates the role of carriers in improving the efficiency of absorption and translocation of herbicides leading to increased membrane permeability and more leakage of ions resulting in increased EC and OD_{525}. Among the treatments, glyphosate with $(NH_4)_2SO_4$ was more effective followed by paraquat with KNO_3 and 2,4-D with urea.

It is also clear that the carriers were not effective under shade as compared to open light confirming the role of environmental factors in absorption and translocation of herbicides in addition to chemical factors as has been reported by Several workers (Tangaraj and Sakharamrao, 1973; Richardson, 1977; McWhorter *et al.*, 1980 and Devendra *et al.*, 1986).

Herbicides in combination with carriers were effective in decreasing total soluble sugar contents significantly over the control. The total soluble sugars under shade among herbicide treatments were more as compared to open light, indicating the least influence of the carriers under shaded conditions. It is also clear that total soluble sugar content was less under shaded conditions mainly because of light limitation of photosynthesis. Such decrease in total soluble sugar content could also be due to decreased stomatal conductance (Table 40) leading to a decrease in the

influx of CO_2 necessary for the synthesis of photoassimilates. Significant reduction in total soluble sugars due to the combination of carriers with herbicides (Table 45) over herbicides alone (Table 31) could also be attributed to a decrease in stomatal conductance, increase in leaf temperature, decrease in chlorophyll content and relative water content. The application of herbicides along with carriers sets in the osmotic stress which may lead to the desiccation of leaf typical to that of plants undergoing drought stress.

Free phenolic content increased upto 4 DAS under both light and shade, then started declining under both the conditions due to the application of herbicides and carriers. Such response was mainly because when the herbicides are introduced to the plants, plants start synthesizing phenols as protective mechanism against any invasion. But such response does not persist for long due to the utilization of phenols in the respiratory metabolism. Among the herbicides and carriers, it was found that 2,4-D with urea, paraquat with KNO_3 and glyphosate with $(NH_4)_2SO_4$ were more effective in increasing free phenols. Such increase in total free phenols due to the application of herbicides and carriers in the beginning and their subsequent utilization in respiration have been reported (Cardeno *et al.*, 1976; McWhorter and Jordan, 1976, and Shaner, 1978).

5.5 Future line of work

1. Since most of the herbicides used in controlling weeds not only cause environmental hazards viz., atmospheric pollution, depletion of ozone layer, but also carsenogenic leading to health hazards and hence there is an urgent need to identify bioherbicides which could effectively kill this obnoxions weed.

2. In the present investigation, only limited carriers have been used that to at a fixed concentration. It is worth trying several other inexpensive and easily available carriers for increasing the herbicidal efficiency.

3. It is also necessary to find out the effect of increasing concentrations of carriers for effective control of Eupatorium so as to reduce the concentration of herbicides.

4. The cost:benefit ratio of using the herbicides and carriers at different concentrations and to find out its relationship with the per cent control of Eupatorium has to be worked out.

5. No work has been done to quantify photosynthetic rate, photorespiration, chlorophyll fluorescence and electron transport chain due to the application of herbicides in

Eupatorium. Hence, it is necessary to relate the changes in photosynthetic parameters to the suppression of weed growth and its control.

6. Considering the nature of seed production and its propagation, it is necessary to go for mass approach and integrated control for eradication of Eupatorium.

VI. SUMMARY

VI SUMMARY

The present investigation was aimed in finding out the effective herbicide and its concentration to control the naturally grown Eupatorium (*Chromolaena adorata* K&R) in different agro-climatic conditions. It was also intended to find out the critical time of application, use of different carriers to increase the efficacy of herbicide, to find out suitable herbicides for controlling seed production and also to find out the morphological, physiological, biochemical and anatomical changes induced by different herbicides and their concentrations both under open light and shaded conditions. To achieve the objectives set in, both field and pot experiments were conducted at different locations under differing growth conditions and the results obtained are summarised in this chapter.

1. Early onset of monsoon and more total rainfall at Yellapur resulted in early seed germination and fast growth of Eupatorium as compared to Prabhunagar. Due to this, herbicide sprayed during the month of August at Yellapur was found most effective in reducing the plant height and decrease in leaf and stem dry weight. Whereas, herbicides, sprayed during the month of september at Prabhunagar was found most effective in controlling the weed growth.

2. Among various herbicides, 2,4-D at 7500 ppm and glyphosate at 5000 ppm are the best concentrations of herbicides leading to effective control of Eupatorium in both the locations namely (Prabhunagar and Yellapur).

3. The foliar spray of herbicides at flower bud stage was most effective in controlling the seed production in Eupotorium as compared to full bloom stage due to greater reduction in number of capitula per shoot, 1000 capitula weight, number of cypsella per capitula, 1000 cypsella weight and seed germination.

4. Among various herbicides used, paraquat at 4000 ppm was found to be most effective followed by 2,4-D and paraquat both at 4000 ppm concentrations, in controlling seed production and spread in Eupatorium.

5. Decrease in various biochemical parameters viz., chlorophyll a, chlorophyll b, total chlorophyll, total soluble sugars, crude protein content, free amino acids content was noticed with increase in the concentration of herbicides. Among them, the extent of decrease was more in paraquat followed by glyphosate and 2,4-D.

6. Increase in the activity of phenyl alanine ammonia lyase, tyrosine ammonia lyase with an increase in the concentration of herbicide was observed and the activity of these enzymes was more under open light as compared to shaded conditions.

7. The transpiration rate and stomatal conductance were found to be more in open light as compared to shaded condition and was found to decrease with an increase in the concentration of herbicides and the extent of decrease was more under open light as compared to shaded conditions.

8. Among the herbicides, paraquat was found to be more effective in decreasing the transpiration rate and stomatal conductance.

9. The leaf temperature was found to increase with the increase in the concentration of herbicides and was found to decrease under shaded conditions as compared to open light in all the treatments.

10. Increase in electrical conductivity (EC) and optical density (OD) of leachates at 525 nm was noticed with the increase in the concentration of all the herbicides.

11. The relative water content was found to decrease more in glyphosate in combination with ammonium sulfate under both open light and shaded conditions.

12. Among various combination of herbicides and carriers, it was found that 2,4-D with urea, paraquat with KNO_3 and glyphosate with ammonium sulfate was found to be the best combinations under open light.

13. Herbicides significantly reduced the leaf and petiole thickness and the extent of reduction was more in glyphosate over other herbicides. The extent of reduction was also more under open light as compared to shaded conditions.

14. Growth conditions significantly altered the characters of leaf anatomy by increasing stomatal number, leaf thickness petiole diameter, epidermal thickness, pubescence, mesophyll thickness and number of mesophyll cells under open light as compared to shaded conditions.

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