ENVIRONMENTAL PHYSIOLOGY OF DOMESTICATION OF HERBAL PLANTS UNDER FRAGILE ECO-SYSTEM

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LIST OF ABBREVIATIONS

WS	Water stress
NS	Non stress
SS	Salinity stress
DAT	Days after transplanting
DAS	Days after sowing
PEG-6000	Polyethylene glycol-6000
PGRs	Plant growth regulators
GA	Gibberellic acid
BA	Benzyl adenine
IBA	Indole butyric acid
CCC	Cycocel
%	Percentage
Dil. sul. acid	Dilute sulphuric acid
Lab.	Laboratory
cm	Centimeter
mm	Millimeter
g	Gram
Min.	Minute
LAI	Leaf area index
N	Nitrogen
P	Phosphorus
K	Potash

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ENVIRONMENTAL PHYSIOLOGY OF DOMESTICATION OF HERBAL PLANTS UNDER FRAGILE ECO-SYSTEM

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ABSTRACT

A study was conducted on four medicinal plants namely *Achyranthus aspera, Andrographis paniculata, Plumbago zeylanica* and *Amaranthus cruentus* under laboratory, pot and field conditions. It was observed that under laboratory conditions the simulated osmotic stress progressively enhanced germination percentage, seedling growth and vigour parameters upto –5.0 bar osmotic potential, thereafter a declining trend was observed. This treatment caused an osmopriming effect. The salinity stress progressively reduced seed germination percentage and seedling growth and the effects were in increasing order at least upto EC_{12}. The adverse effects of osmotic stress and salinity stress could be mitigated by application of synthetic plant growth regulators (PGRs) including GA (150 ppm), IBA (40 ppm), BA (40 ppm) and CCC (150) out of these PGRs GA proved most effective and CCC least effective with respect to seeds germination and allied characteristics. Similarly the adverse effects of salinity stress was also reduced by these PGRs but in this study CCC proved most effective and GA counteracted the adverse effect of salinity up to least extent. Dormancy of seeds was broken and seed germination was improved by rubbing the seeds by sand as well as by treating them with dilute H_2SO_4 in all the four species of medicinal plants. In a separate study under pot conditions the water stress significantly reduced the seedling emergence. In this experiment various parameters of growth and productivity were also adversely affected by water stress. In a field experiment conducted for two consecutive years it was further observed the soil drought suppressed various growth and productivity parameters including plant height, number of branches, plant stand, leaf area and leaf area index (IAI). Total chlorophyll content, photosynthetic rate, transpiration rate, relative water content (RWC), phytomass productivity and economic (crude drug) yield in. Rough Chaff flower, King of bitter, Chita or Chitrack and Amaranth. Contrary to growth and productivity parameters, the therapeutic yield as indexed by total alkaloid contents in economically useful plant parts was enhanced under soil moisture drought conditions. Results were interpreted on the basis of old and modern published literature and it was concluded that these scientific information may be further utilized in domestication and cultivation of medicinal plants.

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1. INTRODUCTION

Ayurveda is the ancient and holistic system of medicine in the world. The objectives of Ayurveda are to cure, prevent and maintain human health. Ayurveda is committed to health and longevity through use of herbs for treatment, vitalizer, aphordisiac and other purposes. Mineral and animal products are also used as medicine in Ayurveda, but herbal use is most important. Herbal medicine are available from plants growing wild or cultivated (upto limited extent). Although some species are cultivated like *kateli, Lajwanti, aonla, ashwagandha* etc. and species which are also a main part of medicine. Rest of the medicines are forest produce. Due to deforestation not only trees are eradicated but in the shadow of trees shrubs, under shrubs, climbers, rhizome etc. are being eradicated which form the back bone of the Ayurvedic treatment.

Since the time immemorial, Indian subcontinent has been reputed as the treasure house of valuable medicinal plants of the world on account of vast diversity in climatic condition (Padulosi *et al*., 2002 and Jakhar *et al*. 2004). However, population explosion coupled with environmental degradation has brought about a substantial depletion in the forest and natural vegetation. This has culminated in 'threat' to the very existence of several plant species of therapeutic value. Hence, an alarm has been raised to adopt effective strategies for conserving and further development of biodiversity of medicinal plants. (Franz, 1993; Brucher, 1985; Schroder, 1998). Basic, application oriented basic and applied aspects of

environmental biology of medicinal plants, form the foundation of such strategies (Govil *et al*., 2002; Singh and Tyagi 2004). These scientific informations are considered of immense importance for domestication, cultivation and improvement of medicinal plants (Singh *et al*., 2003 and Jakhar *et al*., 2003).

The scientific interest in such studies has been recently developed due to the importance of such investigations in understanding the nature of biological processes involved in plant production. This discipline of the life sciences has also opened new vistas to understand the nature of inter relationship between organisms including plants and their physical, chemical and biotic environments (Larcher, 2002). The influence of environmental factors on the growth of plants has been observed for centuries. However, the interest in the quantification of such influences, at various stages of plant development, is of recent origin (Nilsen and Orcutt, 1996).

The international market of medicinal plants is over 60 billion US dollars per annum. This trade is increasing at the rate of 7.0 per cent. India at present exports herbal materials and drugs to the tune of Rs 446.30 crore only per annum which can be raised upto 3000 crore by 2005 and upto 10,000 crore upto 2010 (estimated targets Sarin, 2003, Jakhar *et al.* 2003 and Singh and Tyagi, 2004). However, this target can not be completed with concerted efforts of various scientific and administrative organizations. For such purpose we are bound to bring more and more plants of therapeutic value under cultivation web. For domestication studies environmental physiology or eco-physiological studies are of utmost importance.

Relevance of such observations in Agriculture and Horticulture has been emphasized repeatedly by several workers. Villareal (1980) realized that plant productivity in developing countries like India has been much lower then in the developed countries. This is mainly due to the fact that excessive priorities have been given to cereal, very little to fruits and vegetables and practically negligible to plants having medicinal values. Kramer (1980) emphasized that physiological processes of plants should be investigated in relation to environmental parameters so as to obtain production oriented results.

Atal and Kapur (1982) and Swaminathan (1982) have also advocated the need of bringing more species of therapeutically valuable plants under cultivation. For domestication and successful cultivation of medicinal plants, value of environmental biological investigation has been repeatedly emphasized (Kavalijian, 1980; Jain and Sastry, 1982), Krishnan (1980) has mentioned that after the identification of medicinal plants for pharmaceutical industry, the work of ecophysiologist immediately starts. He emphasized that the study of physiological constraints is the first task for medicinal plant domestication programme. According to him, out of several constraints, the study on causes and cure of low and erratic seed germination, prolonged crop duration, low productivity and difficult propagation are to be dealt on priority basis. Giving the examples of a few medicinal plants (including *Atropa, Rauwolfia, Cathearanthus* and *Papaver*), he emphasized that the study of basic physiological processes in relation to agro-climatic conditions of the locality in question and under various environmental

stresses, should be conducted. The information so obtained will be of immense importance for a plant improvement programme.

There is a global interest and expending market of plant based raw materials for manufacturing of drugs, pharmaceutical, perfumery products, cosmetics and aroma compounds used in food flavours and fragrances. The demand in organized sector of industry has led to their introduction in the agriculture which beside meeting the demand at reasonable price that has also enable the producer to maintain, potency and composition of the pharmaceuticals.

Now, it is right time to boost up cultivation programme for the medicinal plants because the demand of medicinal herbs is increasing day by day not even in pharmaceutical and cosmetic industries, but all over use of herbal remedies has been increased in the world. All four species of medicinal plants *viz*., *Achyranthus aspera*, *Amaranthus crucntus*, *Andrographis paniculata* and *Plumbago zeylanica* which have been taken in present investigation have vital importance in respect to their medicinal use “Rough chaff flower or chirchitta” (*Achyranthus asperra* L.) the plant is reported to possess antidiabetic and antircheumatic properties and used beneficially in abdominal tumors. The seed powder is used in renal dropsy and generalized anasarca. The leaf is used as a remedy for boil and abscess. Leaf juice is useful in stomachache, bowel complaints, piles and skin eruptions. The paste of leaves is used to treat bites of poisonous insect, wasps and bee. The decoction of the whole plant is given in painfull delivery. The juice of the plant is used to stop bleeding of wounds. The roots have been used as stomachic and digestant and is said to be useful for the treatment of

pheumonia. Ramdana or Amaranth" (*Amaranthus cruentus* L.) leaves have medicinal value. It is used to treat intestinal hemorrhage, ulcers, diarrhoea, dysentery, piles and excessive menstrual flow. It has a cleansing effect and helps to reduce tissue swelling. The plant is a blood purifier. The leaves are also eaten as vegetables, "King of bitters" (*Andrographis paniculata* Burm. f. Wall. ex Necs) leaves and stem constitute the drug. It is a bitter tonic and possess antiyphiod and antibiotic properties. It is used to treat liver and digestion complaints, general weakness, fever, dysentery and excessive gas formation in stomach. "chitrak or chitawar" (*Plumbargo zeylanica* L.) roots are used as a masticating for toothache. The plants is acrid, caustic, produces. Irritations and even rubefaction when applied to skin. The roots are used to promote appetite and improve digestion. It is also used to treat piles, diarrhoea and anascasa. The medicinal plants descried above have good potential for export to other developed and developing countries at the same time they are utilized in Indian pharmaceutical industries. There domestication and subsequent cultivation will certainly lower down the dependence on wild resources and in this way will be beneficial for biodiversity conservation of plant resources of arid and semi-arid regions of India including Rajasthan. This will also be advantageous for the environmental protection. Presently the feasibility is only from forests therefore, unscientific exploration of plant material is increasing and some important species have been declared threatened and endangered. So far as Rajasthan is concerned some plants like *Sterculia urens, Anoglosis latifolia, Chlorophytym borivilinium* and *Tecomella undulata* have been declared endangered. The domestication/cultivation of medicinal plants will not only

improve the economic status of farmers but also it will be right way for enhancing availability of medicinal plants. However, a little interest is being taken in their cultivation. Thus, the status of forest, medicinal plants in Rajasthan is decreasing. The factors responsible for imbalance in demand and supply of various forest produces are attributed mainly to unbridle growth of human and livestock population. Shrinkage of natural resources is predominantly based on the expansion of agriculture (mainly field crops) and increasing industrialization and urbanization. So, we should enhance primary productivity. The existing average growing stock of the state forest is 9.84 cm per hectare as against 74 cm per hectare of the country. There is a tremendous potential to enhance the growing stock by providing proper protection and using site specific technologies. It has already been established that the growing stock under irrigated conditions in the Indra Gandhi Canal Project areas could be enhanced to the level of 150 cm per hectare.

Screening of the published literature and scientific information collected from CIMAP, TDAI, CSIR, ICAR etc have clearly revealed that no systematic and scientific studies seem to have been conducted on ecophysiological aspects of experimental plant species (*viz*., *Achyranthus aspera*, *Andrographis paniculata*, *Amaranthus crucntus* and *Plumbago zeylanica*). These plants enjoy a considerable therapeutic repute in allopathic, homoeopathic and ayurvedic systems of medicine (Singh and Tyagi, 2004). Barring a few areas, these species are predominantly, collected from wild sources for Indian pharmaceutical industry and foreign export trade. These investigations therefore, were carried out in semi-arid ecosystem represented by Jobner (India) with following objectives :

1. To study drought and salt tolerance in medicinal plants.
2. To study the photosynthetic and transpirational aspect of medicinal plants.
3. To study the methods for enhancing phytomass productivity and economic yield in medicinal plants.

2. REVIEW OF LITERATURE

Environment is a complex of a factors and the interaction of factors is so complicated that it is impossible to isolate any single component of factors that does not influence the other. Complex environment, subdivided it in to units like physical environment and biotic environment (Daubenmire, 1976). Levitt (1980) classified the stress in to two main groups-biotic and abiotic. Infection of plants and competition with other organism were called as biotic stress while physical factors influencing the plants were included under abiotic stresses. Ecophysiological studies on plants may be regarded as comparatively new. Levitt (1980, 81) recently mentioned that there is a whole new field in stress physiology waiting for investigation, specially, taking the whole plant system, as experimental material. However, the experimental medicinal plant species wild and non cultivated therefore, the study of the growth, development and productivity responses of experimental plant species under simulated stresses was considered a subject of paramount importance for a plant domestication oriented programme.

Hence, efforts in this review have been made to avoid the repetition of what has already been published and scientific information relevant to present piece of research work have been collected and documented. But a brief review of the work done on various aspects of environmental physiology and biology of plants in recent past is being given in the following paragraphs.

2.1 Seed germination and seedling growth under drought and salinity stresses

Water stress is closely interrelated with plant growth and development. Water is required for all life processes and often functions as the limiting factor for several ecophysiological plant processes (Mayer *et al.*, 1974). Adaptability of plants is determined by the availability of water (Misra, 1980). All the physiological processes are influenced by water directly or indirectly (Leopold and Kriedemann, 1975).

The scientific information on seed germination are comprehensively available for some field crop plants (Hadas, 1976) but such information on medicinal plants are scanty. Atal and Kapur (1982) have emphasized the need of cultivation of medicinal and aromatic plants but they have mentioned that for such efforts a knowledge of germination behaviour is necessary. This type of study is still inadequate particularly with reference to Indian medicinal plants (Krishnan, 1980). Koller (1972) showed the importance of environmental factors controlling seed germination. Kozlowski (1972) also review the literature on germination and some other related aspects. Koch (1977) has reviewed the literature on such aspects and has given some information on seed germination of plants which are weeds but may be exploited as the source of important drugs.

Effects of moisture stress were investigated by several workers on germination. Hadas (1977) made an extensive study of the effects of moisture stress on seed germination in some leguminous plants and reported that the percentage of

germination of seeds was declined with increasing moisture stress under field condition as well as in petridishes. Singh and Afria (1985) and Singh *et al.* (1986) have also studied the effect of moisture stress on seed germination percentage in all the three major cereals, some legumes and medicinal plants.

Hadas (1977) and Atal and Kapur (1982) pointed out that the scientific information on seed germination and subsequently seedling growth of some crop plants are comprehensively available but such information about medicinal plants are scantly till now. Alvin and Kozlowski (1977) studied the germination and seedling growth of several plants under climatic stresses. Singh and Singh (1981a, b) also studied the nature of seedling growth of several plant under natural as well as stress conditions. The growth behaviour of seedlings in responses to environmental stresses such as water stress has been studied by Singh and Singh (1981a,b and 1982a,b). Similar studies on the seedling growth and other aspects of growth in relation to water stress were also carried by Hsiao *et al.* (1976) using different plant materials. Recently, the importance of the study of the level of water stress which critically affects seed germination and the growth of subsequently developed seedlings, has been realized to make a physiological evaluation of drought resistance characters in plants (Hall *et al.*, 1976 and Carlson, 1980). The quantification of effects of the adverse environmental conditions is of considerable importance for raising a agricultural successful agricultural crop as they determine the limit of yield and productivity of crops (Gelmond, 1978 and Agarwal, 1980).

Levitt (1980) reported that environmental conditions such as water, temperature and radiation stresses affect the seedling growth and germination of plants. Taylor *et al.* (1982) also studied the germination and seedling growth characteristics of three species of tomato as affected by water deficit and it was shown that shoot growth was affected to a greater extent than the root growth of seedlings by moisture stress.

Chippa *et al.* (1992), Mishra and Sharma (1994) observed that salinity levels exceeding electrical conductivity of 4 mmhos cm^{-1} was detrimental for germination of seeds and growth.

Agarwal and Yadav (1956) stated that barley grows normally in the EC range of 0-3.5 mmhos/cm and growth is stunted in the EC range of 3.2-7.8 mmhos/cm. crop do not grow above the EC of 7.8 mmhos/cm and pH 9.3.

The physiological basic of variability for salinity tolerance of cereal and legume crops have been extensively investigated (Mizara and Tariq, 1993 and Shannon and Nobble, 1995).

Asana and Kale (1965) observed the application of different qualities of irrigation water was found responsible for reducing germination percentage, tillering, plant height, leaf area on the main shoot, absolute growth, root growth in the wheat.

Verma (1994) observed that salinity delays as well as decreases germination of most of the crops. In pearl millet a number of studies indicate that

the depressive effects of salinity and sodicity on seed germination and early seedling growth.

Although most of the results indicate that the germination, fresh and dry weight decreased with increased levels of salinity yet varietal differences have also been reported in rice (Grieve and Fujiyama, 1987).

Rathore *et al.* (1977) determined the effect of 4 salinity levels of 0, 24, 32 and 40 mmhos/cm on seed germination of 22 varieties of barley and revealed that increased salinity levels delayed and decreased germination percentage.

The seedling length of wheat is reduced because of salinity stress. Shah *et al*. (1973), Morogova (1979), Alekseeva (1981); Bliss *et al.* (1984), Muralia (1989) and Taneja *et al*. (1992) and oat Verma and Yadava (1986). Seedling emergence and its establishment of susceptible genotypes are appreciably affected by increasing salinity in wheat Chhipa and Lal (1985) and Kuipear and Schuit (1987) and Kuipear and Schuit (1987) and rice Dutta and Pradhan (1981).

Salinity also affects fresh and dry weight of seedling of wheat (Sharma, 1987; Hanna *et al*., 1978), barley (Gill and Dutt, 1982 and Hassan *et al*., 1970), maize (Ashraf and Mc Neillu, 1989) horsegram (Sudakar *et al*., 1990) and oat (Verma and Yadava, 1986).

Pakroo and Kashirad (1981) observed reduced shoot dry weight, shoot/root ratio, plant height, leaf area in sunflower with increased NaCl salinity.

However, Dutta and Dayal (1988) reported decreased fresh weight of shoot and root with higher level of salt stress.

2.2 Effect of osmotic stress on germination and seedling growth parameters

To study the effect of water stress on seed germination and seedling growth of polyethylene glycol (PEG-6000) is used for the experimental control of external water potential (simulated osmotic stress) has been proved to be very effective method (Hadas, 1976).

Singh and Singh (1982a) reported a reduction in germination percentage and seedling growth of maize composites with decrease in external water potentials. Decrease in germination percentage was noticed maximum (about 60%) in response to -10 bars external water potential. It was also observed that increasing moisture stress at all the tested external water potential levels stimulated a significant and progressive reduction in shoot and root lengths.

Similarly a reduction of seed germination percentage and germination relative index was observed in response to moisture stress of all the four tested (-3.0, -5.0, -7.5 and -10.0 bars) external water potential levels in lentils. The seedling growth and vigour were also suppressed by stimulated moisture stress (Singh and Singh, 1982 b). Same trends in above parameters have also been reported by Hadas and Stibble (1973) in grass; Hadas (1976) in Vetches; Srivastava and Ahluwalia (1978) in Soybean and Singh & Singh (1982) in Wheat hybrids. The retardation of germination process under increasing moisture stress has been attributed to a fall in

mobilization of carbohydrates which otherwise supply the raw material for a successful metabolism is an important part of seed germination and seedling growth (Singh and Singh, 1981 b).

Singh and Kakralya (1995) carried out an investigation to study the efficacy of osmo-conditioning treatments for ameliorating tropical fabacious crops (pigeon pea and chick pea) and concluded that osmo-conditioning with -7.5 bars osmotic potential for 48 hours in pigeon pea and for36 hours in chick pea improved the seed germination, seedling vigour and seedling emergence. Osmo-primed seeds depicted better crop stand establishment, protein and seed yield under field conditions. Reduction in germination of different crops under moisture stress has been reported (Winter *et al*., 1989 and Goswami and Baruah, 1994).

In experiments conducted with ten rice cultivars to study the effect of moisture stress on seed germination using external water potential treatments with PEG-6000 in laboratory and seedling growth at two regimes in net house, Deka and Baruah (1999) recorded decreased cumulative germination and seedling growth with increasing intensity of stress in the cultivars except Iharsal ahu and Maibee II, which showed higher radicle and plumule length with germination under stress conditions.

Singh and Singh (1983 a) conducted an experiment on rice to study water stress in terms of various external water potentials during seed germination and early seedling growth under osmoticum solution of polyethylene glycol. The cumulative germination percentage and water uptake by germinating seeds was

declined progressively in response to decreasing external water potentials. However, the retardation of shoot and root length was significant only in response to treatments with moisture stress of higher order (-10 bars). It has also been observed that seedling growth and vigour are reduced under moisture stress in gram (Singh and Afria, 1984).

2.3 Water stress and it's management through PGRs application

The hormonal control of water stress in plant growth and development is most fascinating and fast growing field of plant physiology. Although few plant hormones, classed as auxins, gibberellins, cytokinins, abscisic acid and ethylene, are well established during the last 30 to 40 years, a detailed study has recently been made on the action and chemistry of plant growth regulators (Krishnamoorthy, 1981).

Among the physiological constraints that affect the crop yield, poor germination and low seedling emergence are worth mentioning, especially under rainfed and saline conditions (Singh and Afria, 1990). The role of gibberellins acid in germination of seeds of various crops including bean, wheat, barley etc. was discussed by (Krishnamoorthy, 1981).

Sen Gupta and Chattopadhyay (1955) studied the effect of IAA, IBA and NAA on seed germination in *Corchorus capsularis*, *Hibiscus sabdarifolia* and *Crotolaria juncea*.

Abscisic acid has been shown to act as dormancy imposing agent which inhibits the seed germination in crop plants (Milborrow, 1974). Ethylene was

reported to act as the promoter of seed germination as well as a dormancy breaking agent in pineapple by Abeles (1973).

Auxin promoted root elongation in lower concentration and strongly inhibited the same in higher concentration (Pilet *et al.*, 1979). Chaudhary and Singh (1960) reported a promotary effect of gibberellic acid and auxin on tomato plants in relation to growth and development.

Singh and Kakralya (1992) reported differentially enhanced germination in pigeonpea cultivars with varying concentrations of GA, BA, IBA, B-9 (Alar) and ETH (Ethrel). Similar trends were also noticed for seedling growth, emergence and survival responses. Lower concentrations of IBA, B-9 and ETH promoted while higher doses retarded the aforementioned parameters. BA and GA (50 & 100 mg/l) have shown enhancing effects on seedling growth and establishment. It was suggested that 50 mg/l of ETH or GA or BA may be applied as pre-sowing seed soaking treatments so as to achieve better germination and crop stand of pigeonpea genotypes. Khan *et al.* (2000) found that gibberellic acid and kinetin both alleviated some of the inhibitory effects of salinity on shoot growth in *Suaeda fruticosa* while root growth was promoted by kinetin.

Misra and Srivastava (2000) studied that changes in plant growth and development, micronutrient accumulation and essential oil yield and comparison were studied in Japanese mint (*Mentha arvensis* cv. MS 77) growing under selected water stresses from deficiency to sufficiency. Water stress resulted in significant reduction in CO_2 exchange rate, total assimilatory area, fresh and dry matter,

chlorophyll carotenoids, Fe, Mn, Zn and essential oil yield. Some changes in essential oil composition were observed, but these changes generally were not correlated with water stress.

Singh *et al.* (2001) have shown that moisture stress reduced the activity of nitrate reductase (NR), the first enzyme of nitrogen assimilation in senna. Recovery was full when stress was given at seedling but increase at later stages. Similarly two days stress showed the lowest reduction in NR activity and in soluble protein. Benzyl adenine (BA) and ascorbic acid (AA) treatments enhanced the NR activity at all phonological stages and in all levels of moisture stress.

2.4 Salinity stress and it's management through PGRs application

Growth and developmental processes in plants are controlled by many factors (both external and internal) and their interaction (Leopold and Kriedemann, 1975 and Levitt, 1980). Wiltwer (1971) and Arnon (1975) have shown that the adverse effects of certain stresses may be reduced or eliminated by careful and timely use of certain plant growth substances. However, this seems to be a new challenge to plant physiologists who should provide field applicable and economic remedies after intensive experimentation (Mc Laren, 1982; Grierson *et al.*, 1983).

Salt stress increased proline content in wheat (Gupta and Shrivastva, 1989) and decreased transpiration rate in wheat (Sharma, 1989). Salinity decreased the net photosynthesis, stomatal conductance in pearlmillet (Garg *et al.*, 1999-2000). Salt stress also decreases significantly the transpiration rate, stomatal

conductance, RWC in wheat (Khatkar *et al.*, 1999-2000) and RWC, osmotic potential decreased but increased membrane permeability of coleoptile of wheat under salinity (Angrish *et al.*, 1999-2000).

Bragg *et al.* (1984) observed apparent effect of CCC in increasing root growth and restricting the shoot growth in wheat. Gabar and Mahmoud (1986) studied the effect of cycocel on seed germination and growth of *guar* in field trials. Soaking of seed in cycocel solution increased germination percentage in some of the treatments on saline and saline alkali soils. Aufhammer *et al.* (1993) observed that soaked seeds of winter barley cv. *Noveta* for 15 hours in cycocel solution at concentration of 40, 400 and 4000 mg L^{-1} but had negligible effect on barley germination and emergence.

Afria and Narnolia (1999) reported that in four genotypes of wheat namely, Kharchia-65, KRL-1-4, HD-2285 and C-306 germination percentage decreased by salinity which significantly increased by the application of cycocel upto 750 and 1500 mg L^{-1} as compared to control.

Afria and Bagdi (1999-2000) reported that saline irrigation significantly decreased the germination percentage and seedling growth in barley cv. BL-2 while pre-soaking of seed with cycocel solution at concentration 0, 500, 1000 and 1500 mg L^{-1} was found to increase germination percentage and seedling growth significantly as compared to control except shoot length which was found reduced.

Singh *et al.* (1985) observed significant response of kinetin application on germination percentage, coleptile length and root length at different hours of sowing at concentration 10 mg L^{-1} on wheat under different salinity levels 0, 4, 8, 12 and 16 MMhos /cm. Germination relative index was also increased significantly at 10 mg L^{-1} concentration of kinetin. Gupta *et al.* (1999-2000) observed that two foliar spray of cytokinin (1.0, 5.0 and 10.0 mg L^{-1}) on wheat cv. Kalyansona (drought sensitive) and C-306 (drought tolerant) at 25 and 45 DAS results showed that there was a concentration dependent increase in cell membrane and chlorophyll stability in both the cultivar at boot and anthesis stages, which were significantly correlated with the grain yield.

2.5 Physiology of seed dormancy

The mature seeds of some plants do not germinate even though, all the component of favourable environment is supplied to them. Such seeds are known as dormant. The period in which such seeds do not germinate, is called dormancy period (Misra, 1980). The successful cultivation of plants largely depends on the quality and germination behaviour of seeds (Nikolaeva, 1969).

The physiological aspects of various types of dormancy of seeds have been studied by some workers (Nikolaeva, 1969; Black *et al.*, 2000; Singh and Purohit, 2000; Singh *et al.*, 2001). Nikalaeva (1969) emphasized that the cause of dormancy must be worked out to study both fundamental and applied aspects of germination of wild as well as cultivated plants, Villers (1972) used the term

dormancy to describe the state of arrested development whereby the organ or organism by virtue of its structure and chemical composition may possess one or more mechanisms preventing its own germination.

Bishnoi (1997) and Mahala (2002) reported that among non-cultivated arid zone plants, hard seed coat, impermeable to water, is important cause of dormancy.

Nielson and Orcutt (1996) emphasized that breaking of the dormancy and including seeds to germinate may be accomplished in a number of plant species by low temperature treatment.

Gupta (2000-2001) reported that seed impermeability to water is a major constraint in germination in families like leguminosae, malvaceae and chenopodiaceae seed topography plays an important role in water uptake followed by germination. Mechanical scarification and acid treatments erase this coat-imposed dormancy. Not much is known about the changes that occur in seed coat structure when these treatments are applied. The results of the present study indicate that in the case of the medicinal plants tested, *Abelmoschus moschatus*, *Argyreia nervosa* and *Urena labata* these treatments affect the hilar/micropylar region and seed testa.

Durrani *et al.* (1997-2000) studied that germination studies indicated that *Pistacia khinjuk* had delayed germination which was promoted by mechanical and chemical scarification applied alone or in combination with hormones such as pro-vide and pro-gib. These treatments resulted improving 80 to 83 per cent

germination. It appeared that besides hard seed coat some hormonal dormancy was present. For the reforestation purpose scarified seeds treated with hormones may provide improved seedling stands.

Gupta (2000-2001) revealed that the seeds of *Abelmoschus moschatus, Argyreia nervosa* and *Urena labata* were subjected to various scarification treatments. It is observed that in *A. nerovsa* acid scarification directly acted on the hilum region where as in the wet heat treatment at 100 degree C, the seed coat ruptured near the hilum region. Presoaking the seeds of *A. maxicana* at 40 degree C, for 72 hours softened the seed coat uniformly thereby giving manifold increase in germination over control. In *A. moschatus*, sand paper scarification caused seed coat cracking, thereby allowing the inhibition of water.

Popay and Roberts (1970) reported that dormancy of seeds of *capsella* could be overcome by stratification treatment. Rao and Reedy (1978) found that the seeds of *Indigofera linifolia* could be induced to germination by hot and cold temperature treatments. Villiers (1972) made a detailed study of various aspects of seed dormancy in many wild and cultivated plant species. Ovacharrow (1977) pointed out that in several cases considerable differences are observed in seed germination behaviour under laboratory and field conditions.

Breaking the dormancy by chemical and mechanical scarification treatments was reported by Joshi and Nigam (1970). Sen (1977) observed the stimulation of seed germination by chemical scarification in *Trianthema* seeds. Anderson (1968) reported that a number of seeds of other plant species failed to

show any response to such treatments. He also reported that a considerable extent of variability exists in the response of seeds to treatment with inorganic chemicals.

2.6 Plant biomass under normal and stress condition

With pharmaceutical point of view, the yield generally comprises the dried plant parts (powered or intact). This is termed as crude drug yield (CDY). In modern pharmaceutical industry, therapeutic yield (TY) is considered as the amount of active principles (including alkaloids, glucosides, tannins, terpinoids and certain other so called 'secondary plant products') present in dried crude drug (Atal and Kapur, 1982). However, the screening of published literature, has clearly revealed that information on crude drug yield and therapeutic yield responses of medicinal plants to environmental stress are extremely scanty. Therefore, efforts were made to evaluate these yield parameters as influenced by certain stresses.

Many agronomists and physiologists have reported that biological as well as economic yield of the plant are highly affected by environmental stresses (Slayter, 1973, Levitt, 1980 and Cooper and Hammer, 1996). Yield is a complex quantitative character which is the result of the complex interplay of the genetically operative hereditary make up of the plant and the environmental factors. In the set of prevailing conditions any one or all the factors may act to the extent of stress in plants. However, radiation, temperature and moisture stresses have been studied more frequently under field, green house and phytotronic conditions (Evans, 1973, Palmer, 1973; Singh Gopal, 1973 etc.). The action of temperature stress in influencing the yield of plants was investigated by Bierhuizen (1973) in tomato and

cucumber. Palmer (1973) in maize and Begg and Barton (1973) in pearlmillet in also conducted similar studies. Slayter (1973) concentrated his attention on the study of water stress in wheat crop. Fischer (1973) and Sen and Bansal (1978) studied some arid and semi-arid zone plants as affected by moisture stress.

Bhargava (1979) studied the role of environmental complex in biomass productivity and turn over in desert ecosystem. Pandey and Sant (1980) in their investigation of monthly fluctuation in standing crop biomass and not primary productivity of *Dichanthium annulatum* grasslands on protected and grazed plots, found that production of above ground biomass was highest in the rainy season and that of below ground biomass was highest in the winter season on both protected and grazed plots.

Al-Niemi (1981) reported decreased dry matter per plant in soyabean with increasing NaCl concentrations. Elhaak *et al.* (1997) reported high dry matter content in *Euphorbia paralias* during the dry season (summer & autumn) compared with the wet seasons (winter & spring). The decrease in leaf and stem dry weights was more affected by soil salinity than that of seawater spray. The percentage of dead leaves increased with the stress treatments. This was also reported by Nobel *et al.* (1984) and Srivastava & Jefferies (1995) who found that leaf death may approach or exceed their initiation. The high percentage of dead leaves did not correspond to a decrease in dry weight in *E. paralias*, which may indicate efficient dry matter production.

2.7 Plant water relation and plant productivity

The water relations and metabolism of land plants depend to a large extent on the diffusion of water vapour and gases through the small pores, the stomata that occur on their aerial parts. Stomates are considered portals through which Co_2 enters the leaf and through which a large quantity of water also evaporates in the form of vapour from the leaf to the atmosphere. The exclusion of the stomatal pores is probably the most important structural innovation contributing to the adaptation of plants to the arid environment. Stomates show amazing versatility in their reaction to the environment and respond to all factors that are of physiological importance.

Since water is the dominant climatic factor, the ecophysiology of water relations and stomatal behavior become two of the most important parameters used to judge the capability of a particular species to adjust under prevailing climatic and edaphic conditions (Mohammed, 1988). Rain water is the only source of available moisture in the Rajasthan desert. Although the monsoon season starts here in mid June and extends to October, the rains are very erratic and scanty. The occurrence of a rather long intermission between successive rain showers, sometimes ranging from a few days to weeks is not uncommon here. The roots of annual and perennial species from the months of June and July to November and December exploit whatever rainwater is retained by the soil. After this, annual species start to disappear because of moisture scarcity in the upper soil layers and their inability to exploit moisture from the deeper soil layer through shallow root systems. By this time, perennial species also start showing various

symptoms of water scarcity, which are very much reflected by remarkable reduction in the transpiring surface (Sen and Mohammed, 1994).

Osmotic adjustment or osmoregulation enables plants to maintain growth as the plant water potential decreases. Adjustment occurs through decreases in osmotic potential by solute accumulation in the cell as the leaf water potential decreases, with the net result that the cell turgor potential is kept relatively high, thus maintaining turgor-dependent processes, such as leaf growth and stomatal opening (Sambo & Ashton, 1985).

Elhaak *et al.* (1997) conducted a green house experiment on *Euphorbia paralias* L. under saline conditions (0, 200 and 400 nm soil salinity level) and sea water spray and reported that salinity caused decrease in daily mean transpiration, shifting in the time of maximum transpiration to be at the time of maximum evaporative demand of the atmospheres, several peaks of transpiration were attained corresponding to favorable conditions and noticeable night transpiration was observed.

This indicated control of water loss in response to the salinity. However, the low rate was found to be affected through first, shifting the time of maximum rate might be from the time of maximum evaporative demand at noon to early morning or late afternoon second, by exhibiting more than one peak of transpiration during the day night cycle including a night one, and third through decrease in the overall day mean transpiration rate. These changes in transpiration patterns were known to decrease plant's water loss as reported by Elhaak, (1980).

2.8 Secondary metabolites in plants under normal and stress conditions

Arnon (1975) and Kaicker *et al.* (1978) expressed the view that economically valuable component of the crop is only a small fraction of the total dry matter produced and moderate water/ temperature/radiation etc. stresses may show favorable effects on the pharmaceutical yield. They assumed that under these conditions, the decomposition of starch and proteins may favour the formation of so called secondary plant products including alkaloids. Similar results were obtained by Svab *et al.* (1979) and Verzar *et al.* (1978) the temperature, water and radiation stress are generally reported to show an adverse effect on yield and productivity of plants (Levitt, 1981). Cooper and Hammer (1996) laid on emphasis on the study of yield or productivity of the plants in relation to drought. Landsberg and Cutting (1977) pointed out that various conditions of environmental stress influence the plant productivity in different ways.

Recently, Singh *et al.* (2000) studied the effect of environmental stresses on the dry matter accumulation of some plants of therapeutic importance in India.

The interaction of plant growth substances and environmental stress in affecting the plant productivity in medicinal plant materials and appropriate combination of water and temperature stress with growth substances suitable for increasing the therapeutic yield of medicinal plants, such studies recently suggested by Singh *et al.* (2000), Shankhla (2002) and Mahala (2002).

Baiza *et al.* (2000) found that relationship between alkaloid production and growth measured as biomass increase and cellular division frequency in *D. stramonium* in vitro root cultures has been discussed. Differences between transformed and non-transformed lines were observed. The differences in growth have been attributed neither to different cell division rates, nor to the presence of larger meristenms, but to the development and growth of lateral roots and the presence of active intercalary meristematic zones in each line. The maximum alkaloid production occured when the cultures were not growing, IARI, New Delhi.

A number of workers have experimented with crop plants and concluded that various parameters of plant growth, development, photosynthetic efficiency and economic yield are adversely affected by various categories of abiotic stresses especially the condition of water deficit. Fernandez and Miller (1985), Kurdali *et al.* (1997), Erskine *et al.* (1999), Borrell *et al.* (2000), Byrd and May (2000), Li *et al.* (2000), Polley *et al.* (2002), Yang *et al.* (2002) and Singh and Tyagi (2004).

Conclusion

After screening of the available literature revealed various lacunae in our knowledge about seed germination and seedling growth behaviour, type and causes of dormancy, vegetative growth behaviour and pharmaceutical and therapeutic yields. It may be indicated that a substantial gap of information exists in our understanding of environmental biology of medicinal plants. This was seen particularly true for the medicinal plants of India in general and *Achyranthus aspera*, *Andrographis paniculta*, *Plumbago zeylanica* and *Amaranthus cruentus* etc. in particular. Such gap certainly hampers the productivity and subsequently the export potential of medicinal plants (Sarin, 2003; Rawat, 2003; Singh *et al.*, 2003 and Jakhar *et al.* 2003).

3. MATERIAL AND METHODS

Investigation reported in this document was carried out in post graduate laboratory, cage house and NARP field, Department of Plant Physiology, S.K.N. College of Agriculture a prestigious Campus of RAU, Jobner, in the *kharif* seasons of 2003 and 2004 to study some parameter of environmental physiology of domestication of herbal plants under fragile eco-system.

The experiments were conducted under ambient laboratory conditions, under pot (cage house) conditions and also under normal field conditions. Observations were on seeds, seedlings and plants of the experimental species as briefly described below :

(1) *Achyranthus aspera* L. (Amaranthaceae)

Rough chaff flower (English), Chirchitta (Hindi). *Achyranthus aspera* plant is reported to posses antidiabetic and antircheumatic properties, beneficial in abdominal tumors. Seed powder is used in renal dropsy and generalized anasarca. The leaf is used as a remedy for boil and abscess. Leaf juice is useful in stomachache, bowel complaints, piles and skin eruptions. The paste of leaves is used to treat bites of poisonous insects, wasps and bee. The roots have been used as stomahic and digestant useful for the treatment of pneumonia.

(2) *Andrographis paniculata* Brum.f. wall.ex. Nees (Acanthaceae)

King of bitters (English), Kalmegh (Hindi). The leaves and stem constitute the drug. It is a buffer tonic and possess antityphiod and antibiotic properties. It is used to treat liver and digestion complaints, general weakness, fever, dysentery and excessive gas formation.

(3) *Plumbago zeylanica* L. (Plumbaginaceae)

Chita (English), Chitrak (Hindi) roots are used as a masticating for toothache. The plant is acrid, caustic, produces irritation and even rubefaction when applied to skin. The roots are used to promote appetite and improve digestion. It is also used to treat piles, diarrhoea and anascasa.

(4) *Amaranthus cruentus* L. (Amaranthaceae)

Amaranth (English), Ramdana (Hindi). Leaves have medicinal value. It is used to treat intestinal hemorrhage, ulcers, diarrhoea, dysentery, piles and excessive menstrual flow and also help to reduce tissue swelling.

Seeds of the experimental medicinal plants species were obtained from the medicinal plant project, Department of Plant Breeding and Genetics, S.K.N. College of Agriculture, Jobner- Jaipur (Raj.). These seeds were thoroughly cleaned and shade dried and experiments were conducted as per description as given below in brief.

3.1 Experiments under ambient laboratory conditions

These experiment were conducted to investigate the effect of simulated water stress and simulated salinity stress and their mitigating effects use of synthetic plant growth regulators (PGRs) on germination and seedling growth

responses in experimental medicinal plant species. All the observations were recorded on final count. Different test solutions were prepared as follows:

3.1.1 Simulated water stress

In this part of the experiment 0.0, -1.0, -3.0, -5.0 and –7.0 bars were taken as levels of water potential with PEG-6000 (polyethylene glycol - 6000) or carbowax. Solutions of different levels of water potential were prepared by dissolving 0, 89, 158, 195 and 240 g of carbowax in every 1000 ml of distilled water for different bar of water potentials (Michel and Kaufman, 1973). Solution with 0.0 bar was taken as control.

3.1.2 Simulated salinity stress

EC levels were maintained by dissolving the NaCl, $CaCl_2$, and $MgSO_4$ salts in distilled water keeping the ratio of 24 : 10: 6 for NaCl, $CaCl_2$, and $MgSO_4$, respectively. In this part of the experiment salinity levels were taken 0 (control) 4, 8, 12 and 16 per cent solution of different salinity levels.

3.1.3 Determination of the therapeutically valuable secondary metabolites

This determination was done as per method initially described by Harborne (1973) but some modifications were incorporated as per technique standardized by Singh and Tyagi (2004) who published such modifications in 2004 (Singh and Tyagi, 2004). The outline of the method is as follows :

The plant material (either the whole plant or economic part of plant were thoroughly washed with water and sun dried followed by oven drying at 80^0C for about 48 hours. This dried matter was grind in a mechanical grinder. After

grinding, 5 g of powder was taken and dissolved in or ethyl alcohol at room temperature in conical flask and was gently shaken for about one hour. The solution so prepared was dried. Then, the whole substance was placed in a big beaker and was allowed to evaporate. After three hours, the excess of solvent was evaporated and remaining substance was treated with dilute sulphuric acid. All secondary metabolites were dissolved in sulphuric acid, then filtered. The filtrate so obtain was treated with Marquis Reagent (formaldehyde + water 60 :40). Finally the filtrate Marquis reagent was obtained as a clear solution. This solution was colorimetrically studied for the quantitative determination for total content of secondary metabolites. The range of wave length is 300-700 nm finally. The O.D. was compared with colrimetric reading to know the amount present in 5 g powder and total content of secondary metabolites was calculated on per gram weight basis.

3.1.4 Details of parameters studied (under ambient laboratory conditions)

I Seed germination percentage

The seed germination in *Plumbago zeylanica* and *Amaranthus crucntus* species started on 3rd day under ambient condition with or without salinity/somotic stress treatment. However, the process of germination was started on 5th day in *Achyranthus aspera* and *Andrographis paniculata* species. The days of final count are those when there was no further increase in germination percentage for 48 hours. Such days were recorded to be 21 days in all the species.

Number of seeds germinated were counted at three stages for each treatment and germination percentage were calculated by the following formula :

$$\text{Germination percentage} = \frac{\text{No. of seeds germinated at the stage}}{\text{Total No. of seeds sown}} \times 100$$

II Seedling length

It was measured directly with the help of metre scale. Seedling length was considered from root tip of the main root to the shoot apex in petriplates.

III Seedling vigour

Seedling vigour was calculated by the formula given by Abdul-Baki and Anderson (1973) and modified by Singh and Singh (1983).

Seedling vigour = Germination percentage x Seedling length

IV Seedling dry weight

Seedling after weight for fresh weight for each treatment were put in the oven at 75 ^{0}C over night. The weight of oven dried seedling sample was measured with the help of single pan electrical balance and this was repeated till the constant weight obtained.

3.1.5 Study of seed dormancy and breaking methods

Seed dormancy is a condition in which viable seeds fails to germinate even under suitable conditions due to several internal and external factors. In present investigation methods of breaking the dormancy by chemical and mechanical scarification treatments were adopted as Joshi and Nigam (1970). These treatments were given to seeds which had been stored at ambient conditions for three months after harvest.

I Mechanical scarification method

In this method the seeds of experimental plant species were rubbed with sand for 10 and 30 minutes. These seeds were subsequently tested for germniability by the method described by Agarwal and Dadlani (1995). By rubbing process seed coat cover is loosened and dormancy is broken down.

II Chemical method

In this method dilute sulphuric acid (H_2SO_4) was used as chemical treatment. Seeds of experimental plant species were soaked in dilute H_2SO_4 for one and two minutes followed by quick washing of seeds in running water and drying in shade.

3.2 Experiment under pot conditions

A separate experiment was conducted in large cemental pots to study the effect of water stress on various vegetative and economic characters. Pots were maintained in cage house of Department of Plant Physiology, S.K.N. College of Agriculture, Jobner. Plants were raised from seeds in these pots filled with sandy loam soil. The soil was completely dried before seed sowing. Seeds were sown at uniform depth and distance to evaluate the seedling emergence character. Subsequently other parameters were also studied. The water stress in these pots was simulated by conventional method of withholding the irrigation as described by Singh and Kakralya (1995). The seedling emergence percentage was observed at 29^{th} day after seed sowing, at this stage the number of seedlings emerged become constant atleast for three consecutive days. The leaf area, plant height and economic yield were recorded by the method as described for field experiments

(mentioned in field experiment). Some plant water relation parameters and photosynthetic rate were also studied on these pot grown plants following the standard methods of observation also as mentioned for field experiments.

3.3 Experiment under normal field conditions

This experiment was conducted to study the growth, water relations, yield and its attributes and also to investigate the secondary plant metabolites of the experimental plant species.

3.3.1 Preparation of nursery

The polythene bags were maintained in condition which was free from water deficits and nutritional stress. Emerging seedlings were supplied with normal water at regular interval. Seedling preparation for transplanting in nursery under these polythene bags was practiced in summer upto the last week of June of each experimental year and these seedlings were carefully taken from the polythene bags and transplanted into the field in 1st week of July. Before transplantation the field was well prepared by adding FYM and adequate tillage. Before the actual operation of transplantation, the field was flood irrigated with normal water and then transplanting was done by maintaining row to row distance 30 cm and plant to plant distance 30 cm upto seedling establishment, transplanted seedlings were not allowed to experience water deficit, under field experiment. Plot size 2 m X 2 m. Each species was having three replicates of treated control. Where irrigation was given as normal intervals in another groups of replicates the irrigation was

discontinued for alternate missing of irrigation. This deficit treatment was based on the method described by Bruckner and Phroberg (1987) who adopted the technique for evaluation of drought tolerance and adaptability in wheat genotypes. This water deficit treatment continued upto mid flowering stage of each species. Plant sampling for various observations was also done as per method by the technique originally described by Bruckner and Phroberg (1987) as modified by Mahala (2002) for medicinal plants.

3.3.2 Details of the parameters studies

I Plant stand establishment

The total number of plants per plot were counted at different intervals and the average was worked out.

II Plant height at regular intervals

Plant height of the experimental medicinal plant species was measured with the help of meter scale. It was considered from the ground surface to top of the plant.

III Number of branches per plant

The number of branches per plant from randomly selected five plants in each plot were counted at four different stages.

IV Leaf area and leaf area index

One plant was selected from each plot for leaf area and leaf area index at maturity stage. All the green leaves of each plant were pressed carefully so

that there was no folding of any leaf area. Then leaf area was measured in square cm with the help of LI-3100, area metre. The LAI was calculated by the following relationship (Watson, 1947).

$$LAI = \frac{\text{Leaf area (cm)}^2}{\text{Land area (cm)}^2}$$

V Biological yield

The unthreshed produce from net plot area after through sun drying was weight for recording the biological yield and expressed in terms of yield in gm per plant.

VI Economic yield

The economically used parts of the plant produced from net plot area after through sun drying was weighed for recording the economic yield and expressed in terms of yield in gm per plant.

VII Photosynthetic rate (µ mol m^{-2} s^{-1})

Photosynthetic rate was measured directly by using infrared gas analyzer (LI 6200, LICOR, USA).

VIII Estimation of total chlorophyll content (mg g^{-1} fr.wt.)

Total chlorophyll content (the sum of chlorophyll ‘a’ and chlorophyll ‘b’) of all randomly selected plants of experimental plant species was estimated at pre-flowering stages according to the method of Witham *et al.* (1971).

Sample extract was prepared from 100 mg of leaf with 10 ml of 85 per cent acetone and the homogenate was centrifuged at 5000 rpm for 10 minutes. The clear supernatant was transferred to a 25 ml measuring cylinder. The residue was again re-extracted with 5 ml of acetone and centrifuged and then the supernatant was transferred to the measuring cylinder. The final volume of the supernatant was made to 20 ml with the acetone.

Finally, the optical density of chlorophyll ‘a’ and ‘b’ was measured at 663 and 445 nm, respectively using spectrophotometer, by taking 85 per cent acetone as control.

The total chlorophyll content in mg L^{-1} was calculated by the formulae :

$$\text{Total chlorophyll (mg } L^{-1}) = 20.2\ A_{645} + 8.02\ A_{663}$$

Where,

A_{645} and A_{663} were absorption (optical density) of chlorophyll ‘b’ and ‘a’, respectively. From this value, chlorophyll content in mg g^{-1} of fresh weight of the leaf sample was calculated using the expression :

$$\text{Total chlorophyll (mg } g^{-1}) = \frac{\text{Total chlorophyll (mg } L^{-1}) \times \text{Total volume of the extract}}{1000 \times \text{Sample weight (g)}}$$

IX Transpiration rate (m mol m^{-2} s^{-1})

Leaf transpiration rate was measured directly by using infra red gas analyzer (LI 6200, LICOR, USA). It represented the sum of adaxial and abaxial surfaces of the leaf.

X Relative water content :

Leaf segments (1 cm^2) were initially weighed and floated over the distilled water for 24 hours and the turgid weight was recorded. Dry weight was obtained after drying the leaf segments at 80 ^{0}C for 48 hours. The relative water content was calculated by given formula (Salvik, 1974).

$$RWC = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Statistical analysis

All the observations were taken in triplicates and data were analyzed statistically using randomized block design (RBD) in field experiment and complete randomized design (CRD) in laboratory and pot experiment as described by Raghav Rao, 1983. Standard error of mean (SEm$\pm$) and critical different (CD) were calculated as follows :

$$SEm\pm = \frac{\sqrt{\text{Error mean square (EMS)}}}{\text{Replications (r)}}$$

$$CD = \sqrt{2} \times SEm \times t\ 0.05 \text{ at d.f.}$$

Where ‘t’ the table value of ‘t’ at 5 per cent level of significance with error degree of freedom.

4. Experimental Results

4.1 EXPERIMENT UNDER LABORATORY CONDITION

4.1.1 Effect of simulated water stress on seed germination and seedling growth parameters

I Seed germination percentage

Data presented in table 1 and fig. 1 showed that germination percentage was significantly increased by water stress treatment (PEG-6000) upto –5.0 bar level. The germination percentage was recorded 73.33, 68.67, 68.0 and 76.00 in the *Achyranthus aspera, Andrographis paniculata, Plumbago zeylanica* and *Amaranthus crucntus* species, respectively at control level, but at –5.0 bar level it was recorded 86.67, 89.33, 85.33 and 92.62 in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylanica* and *Amaranthus cruentus*, respectively. In this experiment it was also showed that further increase in PEG-6000 (osmotic potential) level for water stress decreased the germination percentage but it was still more as compared to control in all the experimental medicinal plant species.

The enhancement in germination percentage by increasing level (upto –5.0 bar) of PEG-6000 upto highest extent was found in *Andrographis* species and minimum extent was found in *Achyranthus* species among all the four medicinal plant species.

II Seedling length (cm)

It was observed that seedling length was non significantly increase by seed water stress treatments with PEG-6000 osmotica (Table 2 and fig. 2). It was found that maximum increase at -5.0 bar level of osmotica among all medicinal plant species (10.33, 10.67, 10.33 and 9.67 cm in *Achyranthus, Andrographis, Plumbago* and *Amaranthus* species, respectively). However, further increase in water stress levels (upto –7.0 bar) it was decrease in all the experimental plant species. It was also observed that at –5.0 bar level of osmotica the highest extent was found in *Andrographis paniculata* and was lowest extent in *Achyranthus aspera* species.

III Seedling vigour

Data presented in table 2 and fig. 2 revealed that seedling vigour increase with increasing levels of osmotica (PEG-6000), at –5.0 bar it was recorded 895.56, 952.89, 881.78 and 895.78 in *Achyranthus, Andrographis, Plumbago* and *Amaranthus* species, respectively which was significant increase as compared to control. The pattern of increase was found similar in all the experimental species. However, further increase in osmotica (PEG –6000) level *i.e.* –7.0 bar it leads to decrease and this decreasing pattern also similar in all the experimental species.

The increase in seedling vigour at –5.0 bar level was recorded highest in *Andrographis paniculata* and lowest in *Achyranthus aspera* species.

IV Seedling dry weight (g)

Water stress significantly enhanced the dry weight of seedlings in all the species upto –5.0 bar level table 2 and fig. 2. Seedling dry weight was recorded 0.260, 0.200, 0.300 and 0.250 g in *Achyranthus, Andrographis, Plumbago* and *Amaranthus* species, respectively at control. It was recorded 0.360, 0.320, 0.410 and 0.360 g at –5.0 bar level which was maximum increase in dry weight among all the species. The further increase in PEG-6000 level for water stress decreased the seedling dry weight but it was also still more as compared to control in all experimental plant species. At the –5.0 bar level of water stress the maximum increase was found in *Andrographis* species and lowest was in *Achyranthus* species among all experimental plant species.

4.1.2 Effect of simulated salinity stress on seed germination and seedling growth parameters

I Germination percentage

Data presented in table 3 and fig. 3 revealed that the germination percentage in the experimental plant species suppress significantly and progressively over control level of salinity stress. However, the magnitude of decrease was found highest in *Achyranthus* species at all the levels of salinity. The more intensive rate of decrease was recorded at EC_{12} level of salinity stress among all the species (52.67, 49.33, 48.00, 57.33 in *Achyranthus, Andrographis,*

Plumbago and *Amaranthus* species, respectively). The maximum decrease in germination percentage was recorded at EC_{16} level of salinity stress (49.33, 46.00, 44.00 and 52.62 in *Achyranthus, Andrographis, Plumbago* and *Amaranthus* species, respectively). Recorded data also revealed that germination percentage began to suppress intensively after EC_4 level of salinity stress in all the experimental medicinal plant species.

II Seedling length (cm)

Data presented in table 4 and fig. 4 showed that seedling length significantly decrease with increasing levels of EC (salinity stress), it was recorded 9.50, 9.17, 9.17 and 8.67 cm in *Achyranthus, Andrographis, Plumbago* and *Amaranthus* species, respectively at control. The highest decrease in seedling length in all species was recorded (7.17, 6.17, 6.50 and 5.17 cm in *Achyranthus, Andrographis, Plumbago* and *Amaranthus* species, respectively) at EC_{12} level of salinity. However, at EC_{12} level of salinity the highest decrease extent was found in *Amaranthus* and lowest was recorded in *Andrographis* species, thus the species *Andrographis* proved better salinity tolerable in comparison to others, because reduction of seedling length by salinity stress was found minimum in this species.

III Seedling vigour

Seedling vigour significantly decrease with the increasing levels of salinity stress (Table 4 & Fig. 4) in all the experimental plant species. It was recorded 658.63, 599.08, 586.88 and 624.00 in *Achyranthus, Andrographis, Plumbago* and *Amaranthus* species, respectively at control. The suppression in

seedling vigour was highest recorded 377.64, 304.37, 312.00 and 296.40 in *Achyranthus, Andrographis, Plumbago* and *Amaranthus* species, respectively at EC_{12} salinity level, however, at EC_{12} level the highest decrease extent was recorded *Amaranthus* and lowest was recorded in *Achyranthus* species.

IV Seedling dry weight (g)

Data given in table 4 and fig. 4 revealed that seedling dry weight was significantly reduced with the increasing levels of salinity stress. At control it was recorded 0.286, 0.220, 0.331 and 0.293 (g) *Achyranthus, Andrographis, Plumbago* and *Amaranthus* species, respectively.

Although the maximum decrease in seedling dry weight was found at EC16 at level of salinity but the drastic decrease in seedling dry weight was recorded at EC12 level of salinity in all the experimental species. At EC 12 level of salinity it was recorded 0.212, 0.146, 0.235 and 0.175 in *Achyranthus, Andrographis, Plumbago* and *Amaranthus* species, respectively. The magnitude of highest per cent decrease at EC12 level of salinity was observed in *Plumbago* species and lowest was recorded in the species of *Achyranthus aspera.*

4.1.3 Effect of PGRs treatment on germination and related parameters under water stress

The experiment was conducted to study the effect of water stress and their ameliorative effects by synthetic plant growth regulators on germination and related parameters under ambient conditions of laboratory.

I Seed germination percentage

It was observed that simulated water stress upto the level of -10.0 bar drastically reduced the germination upto maximum extent and therefore the efficacy of different PGRs in reducing the adverse effects of water stress was studied in the comparison of effects shown by –10.0 bar water potential (Table 5 & Fig. 5). It was found that gibberelic acid (GA 150 ppm) reduced the adverse effects of water stress upto maximum extent. In *Achyranthus aspera* where 64.67 per cent seeds germinated in the condition when GA (150 ppm) was applied in combination with water stress (-10.0 bar) in this species cycocel (CCC 150 ppm) was found least effective in mitigating the adverse effect of water stress.

In the species of *Andrographis paniculata* 59.00 per cent seeds were germinated in GA (150 ppm) treatment, where as under cycocel (CCC 150 ppm) treatment only 52.33 per cent seeds germinated, the quantitative trend of results was similar in other two species also with respect to effect of different treatments with plant growth regulator. However, in *Amaranthus cruentus* (GA 150 ppm) treatment could alleviate the effect upto the extent of 70.67 per cent which was statistically at par with untreated control.

These results presented further indicated that the adverse effects of water stress was ameliorated by synthetic plant growth regulators (PGRs) in physiological active concentration upto maximum concentration of gibberellic acid (GA 150 ppm) and upto lowest extent by cycocel (CCC 150 ppm) among all the species.

It was observed that in water stress (-10.0 bar) condition the GA (150 ppm) treatment ameliorated adverse effects of water stress upto maximum extent in *Plumbago zeylanica* species and upto lowest extent in *Amaranthus cruentus* species whereas the cycocel (CCC 150 ppm) ameliorated adverse effects upto maximum extent in *Plumbago zeylanica* and upto lowest extent in *Amaranthus cruentus* species.

II Seedling length (cm)

The experiment was conducted to study the ameliorative effects of water stress (-10.0 bar) level on seedling length parameter by application of physiologically active concentration of synthetic plant growth regulators. It was observed that water stress (-10.0 bar) drastically reduced the seedling length in all the experimental plant species. The species *Plumbago zeylanica* proved to be most sensitive to adverse effects of water stress on seedling length. In this species reduction in the seedling length was found up to the extent of 23.33 per cent over control. However, in *Achyranthus aspera* species there was reduction of 18.55 per cent only by water stress. Appraisal of data presented in table 6 and fig. 6 showed that application of physiologically active concentration of synthetic plant growth regulators mitigated the adverse effects of water stress (-10 bar level). It was also observed that gibberelic acid (GA 150 ppm) mitigate the adverse effects of water stress upto maximum extent in *Andrographis paniculata* followed by *Plumbago zeylanica, Amaranthus cruentus* and *Achyranthus aspera* species. Among all the synthetic plant growth regulators that gibberelic acid (GA 150 ppm) ameliorated the adverse effects of water stress (-10.0 bar) upto maximum extent followed by

indole butyric (IBA), benzyl adenine (BA) and cycocel (CCC) in all the experimental plant species.

III Seedling vigour

The experiment was conducted to study the ameliorative effects of water stress (-10.0 bar) level on seedling vigour by application of physiologically active concentration of synthetic plant growth regulators. It was observed that simulated water stress (-10.0 bar) reduced the seedling vigour in all the experimental plant species. The species *Plumbago zeylanica* proved to most sensitive to adverse effects of water stress on seedling vigour. In this species suppression in the seedling vigour was found upto the extent of 44.86 per cent over control. However, in the species of *Achyranthus aspera* there was reduction of 38.71 per cent only due to water stress (Table 7 & Fig. 7).

Appraisal of data revealed that application of synthetic plant growth regulators alleviated the adverse effects of water stress (-10 bar). It was observed that GA (150 ppm) mitigate the adverse effects of water stress upto maximum extent followed by IBA (40 ppm), BA (40 ppm) and CCC 150 (ppm) in all the experimental plant species. The effect of GA 150 ppm was found most effective in mitigating effects of water stress upto maximum extent in *Plumbago zeylanica* species and upto lowest extent in *Andrographis paniculata* species over treated control.

IV Seedling dry weight (g)

The experiment was conducted to study the ameliorative effects of water stress (-10.0 bar) level on seedling dry weight (g) by application of physiological active concentration of synthetic plant growth regulators. It was observed that water stress (-10.0 bar level) drastically suppressed the seedling dry weight in all the experimental medicinal plant species. The adverse effects of water stress (-10.0 bar) on seedling dry weight was maximum found (20.56 %) in *Amaranthus cruentus* species and lowest was recorded in *Plumbago* species which was 15.41 per cent over control.

Appraisal of data given in table 8 and fig. 8 revealed that application of physiologically active concentration of synthetic plant growth regulators ameliorated the adverse effects of water stress (-10 bar). It was observed that application of gibberelic acid (GA 150 ppm) alleviated the adverse effect of water stress upto maximum extent in *Andrographis paniculata* species. In case of *Amaranthus cruentus*, *Achyranthus aspera* and *Plumbago zeylanica* also the GA (150 ppm) was found most effective followed by IBA (40 ppm), BA (40 ppm) and CCC (150 ppm).

4.1.4 Effect of PGRs application on germination and related parameters under salinity stress

The experiment was conducted to study the effect of salinity stress and their ameliorative effects by synthetic plant growth regulators on germination and related parameter under ambient laboratory conditions. The salinity stress upto the level of EC_{16} reduced the germination and related parameters upto the maximum extent and therefore, it was considered as treated control, unlike water

stress CCC (150 ppm) proved more effective followed by BA (40 ppm), IBA (40 ppm) and GA (150 ppm) in reducing the adverse effects of salinity stress under laboratory conditions. The experimental medicinal plant species showed quantitative differences in their responses to treatments with synthetic plant growth regulators (PGRs).

I Germination percentage

The experiment was conducted to study the ameliorative effects of salinity stress (EC_{16}) on seed germination percentage by application of synthetic plant growth regulators. It was observed that salinity stress (EC_{16}) drastically reduced the germination percentage in all the experimental medicinal plant species. The species *Plumbago zeylanica* proved to most sensitive to adverse effect of salinity stress on germination percentage. In this species reduction in the germination percentage was found upto the extent of 34.05 per cent over untreated control, whereas, in *Achyranthus aspera* species there was reduction of 27.30 per cent only over untreated control only.

Appraisal of data given table 9 and fig. 9 revealed that application of physiologically active concentration of synthetic plant growth regulators alleviated the adverse effects of salinity stress (EC_{16}). It was observed that cycocel (CCC 150 ppm) mitigated the adverse effects of salinity stress upto maximum extent in *Plumbago zeylanica* species followed by *Amaranthus cruentus*, *Andrographis paniculata* and *Achyranthus aspera* species. Among application of all the synthetic plant growth regulators cycocel (CCC 150 ppm) ameliorated the adverse effects of

salinity (EC_{16}) upto maximum extent followed by BA (40 ppm), IBA (40 ppm) and GA (150 ppm) in all the experimental medicinal plant species.

II Seedling length (cm)

The experiment was conducted to study the mitigating effects of salinity stress (EC_{16}) on seedling length (cm) by application of physiologically active concentration of synthetic plant growth regulators. It was observed that salinity stress (EC_{16}) sharply suppressed the seedling length (cm) in all the experimental medicinal plant species. The adverse effects of salinity stress (Ec_{16}) on seedling length was found upto maximum extent (47.13 per cent) and lowest extent (43.82 per cent) over control in *Amaranthus cruentus* and *Plumbago zeylanica* species, respectively.

Appraisal of data presented in table 10 and fig. 10 showed that application of physiologically active concentration of synthetic plant growth regulators mitigate the adverse effects of salinity stress (EC_{16}). It was observed that cycocel (CCC 150 ppm) mitigated the adverse effects of salinity stress upto maximum extent in *Amaranthus cruentus* species. In case of *Plumbago zeylanica*, *Andrographis paniculata* and *Achyranthus aspera* species also the cycocel (CCC 150 ppm) treatment was found most effective followed by BA (40 ppm), IBA (40 ppm) and GA (150 ppm) in all the experimental medicinal plant species.

III Seedling vigour

The experiment was conducted to study the ameliorative effects of salinity stress (EC_{16}) on seedling vigour by the use of synthetic plant growth regulators. It was observed that salinity stress drastically reduced the seedling vigour in all the experimental plant species. The reduction in seedling vigour due to salinity stress was recorded upto highest extent (62.95%) in *Plumbago zeylanica* and upto lowest (59.60%) extent in *Andrographis paniculata* species over untreated control.

Appraisal of data presented in table 11 and fig. 11 revealed that application of physiologically active concentration of synthetic plant growth regulators alleviate the adverse effects of salinity stress. It was also noted that cycocel (CCC 150 ppm) mitigate the adverse effects salinity stress upto maximum extent in *Amaranthus cruentus* species followed by *Plumbago zeylanica*, *Achyranthus aspera* and *Andrographis paniculata* species over treated control. Among all the synthetic PGRs the CCC (150 ppm) ameliorated the adverse effects of salinity stress upto maximum extent followed by BA (40 ppm), IBA (40 ppm) and GA (150 ppm) in all the experimental plant species over treated control.

IV Seedling dry weight (g)

The experiment was conducted to study the mitigating effects of salinity stress (EC_{16}) on seedling dry weight by application of physiologically active concentration of synthetic plant growth regulators.

It was observed that salinity stress (EC_{16}) reduced the seedling dry weight (g) in all the experimental plant species. The species *Amaranthus cruentus*

proved to be most sensitive to adverse effects of salinity stress because maximum reduction 49.83% over untreated control was recorded in this species followed by *Andrographis paniculata* (49.49%) *Achyranthus aspera* (42.25%) and *Plumbago zeylanica* (41.77%) species.

Appraisal of data presented in table 12 and fig. 12 indicated that use of synthetic plant growth regulators alleviate the adverse effects of salinity stress (EC_{16}). It was also noted that cycocel (CCC 150 ppm) alleviative the adverse effects of salinity stress upto maximum extent followed by BA (40 ppm), IBA (40 ppm) and GA (150 ppm) in all the experimental plant species. The effect of cycocel (CCC 150 ppm) was found most effective in mitigating effects of salinity stress upto maximum extent in *Amaranths cruentus* species and upto lowest extent in *Achyranthus aspera* species over treated control.

4.1.5 Effect of physical and chemical treatments on dormancy and seed germination

It was found that per cent germination in stored seeds in all species was less without physical and chemical treatment. Table 13 and fig. 13 revealed that both dormancy breaking methods (Sand rubbing and dilute H_2SO_4) enhanced the germination percentage in all the medicinal plant species. It was recorded that application of sand rubbing method is less effective in comparison to the soaking of seeds by dilute sulphuric acid (dilute H_2SO_4), however, the dilute H_2SO_4 treatment was increase significant germination percentage and among them 2 minute soaking also better then one minute.

4.2 EXPERIMENT UNDER POT CONDITION

4.2.1 Growth parameters

The plant growth parameters which were studied on experimental species under pot conditions, the water stress was simulated by conventional method of withholding the irrigation.

I Seedling emergence (%)

Data presented in table 14 and fig. 14 revealed that the seedling emergence percentage was significantly reduce by water stress in the *Achyranthus* and *Andrographis* spices, however, it was found significantly at par in the species of *Plumbago* and *Amaranthus.* The highest per cent reduction (12.11) in seedling emergence per cent by water stress was recorded in the species of *Andrographis paniculata* and lowest (6.24) reduction due to water stress in seedling emergence percentage was recorded in the species of *Amaranthus cruentus*, thus the species *Amaranthus* could tolerate the water stress upto maximum extent and species *Andrographis* tolerate water stress upto minimum extent among all the medicinal experimental plant species with respect to seedling emergence percentage.

II Plant height

Perusal of data presented in table 14 and fig. 14 showed that in pot conditions the water stress significantly suppress the plant height at all the plant growth stages in all the four experimental plant species, but only at preflowering stage it was found significantly at par in the species of *Plumbago zeylanica.*

At the stages of maturity plant height (cm) was recorded 87.20, 40.60, 54.00 and 79.40 (cm) in the condition of non stress and 69.40, 29.80, 37.80 and

62.70 (cm) in the condition of water stress in *Achyranthus aspera, Andrographis paniculuata, Plumbago zeylanica* and *Amaranthus cruentus* species, respectively.

At maturity stage the maximum (30.0%) and minimum (20.41%) per cent reduction in plant height by water stress was recorded in *Plumbago zeylanica* and *Achyranthus aspera* species, respectively.

Thus the species *Achyranthus aspera* could tolerate the water stress upto maximum extent because the reduction in plant height was minimum in water stress conditions among all the experimental plant species, but the height of species *Plumbago zeylanica* adversely affected by water stress in comparison to other species.

III Leaf area per plant (cm^2)

It was observed that water stress significantly reduced the leaf area per plant in all the experimental plant species (Table 15 & Fig. 15) under pot conditions. The highest (14.62%) reduction in leaf area was recorded in *Plumbago zeylanica* and it was found lowest (9.21%) in *Amaranthus cruentus*, thus the species *Amaranthus cruentus* provided better in comparison to other experimental species, because in this species reduction in leaf area per plant due to water stress was found minimum.

4.2.2 Yield parameters

I Economic yield

Data presented in table 15 and fig. 15 revealed that the economic yield was significantly suppress by water stress in all the experimental plant species. It was recorded 63.25, 31.00, 33.15 and 26.40 (g) in *Achyranthus aspera*, *Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus*, respectively in the condition of non stress. However, it was found 54.65, 26.50, 28.20 and 20.60 (g) in *Achyranthus aspera, Andrographis paniculata*, *Plumbago zeylania* and *Amaranthus cruentus*, respectively in the condition of water stress. The highest (21.97%) per cent reduction and lowest (13.60%) per cent reduction was recorded in the species of *Amaranthus cruentus* and *Achyranthus aspera*, respectively by simulated water stress, thus the species *Achyranthus aspera* could tolerate water stress upto maximum extent because minimum per cent reduction in economic yield was found in this species.

4.2.3 Physiological and water relation parameters

I Photosynthetic rate (µ mol m^{-2} s^{-1})

Perusal of data presented in table 16 and fig. 16 revealed that in all the experimental plant species photosynthetic rate (µ mol m^{-2} s^{-1}) significantly suppressed by water stress at all the plant growth stages.

At the maturity stages it was recorded 15.99, 25.72 and 19.50 (µ mol m^{-2} s^{-1}) in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and

Amaranthus cruentus, respectively in the non stress condition and in water stress condition it was recorded 6.10, 12.42, 8.42 and 8.55 (µ mol m^{-2} s^{-1}) in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively.

The per cent decrease in photosynthetic rate upto highest (61.18%) extent was found in *Achyranthus aspera* and upto lowest extent was recorded in *Andrographis paniculata* species, thus the species *Andrographis paniculata* could tolerate water stress in comparison to other experimental medicinal plant species.

II Transpiration rate (m $molm^{-2}$ S^{-1})

Data presented in table 17 and fig. 17 showed that transpiration rate was significantly reduced by water stress at all the plant growth stages in all the experimental medicinal plants species. At maturity stages the transpiration rate was recorded 1.01, 1.59, 1.62 and 1.72 (m $molm^{-2}$ S^{-1}) in *Achyranthus aspera*, *Andrographis paniculata*, *Plumbago zeylania* and *Amaranthus cruentus* species, respectively in the condition of non stress. However, it was found 0.60, 0.62, 0.75 and 0.73 (m $molm^{-2}$ S^{-1}) in *Achyranthus aspera, Andrographis paniculata*, *Plumbago zeylania* and *Amaranthus cruentus* species, respectively.

In the condition of water stress at maturity stage due to water stress the maximum (61.01%) reduction and minimum (40.59%) reduction in transpiration was observed in *Andrographis paniculata* and *Achyranthus aspera* species, respectively. Thus the species *Andrographis paniculata* was proved more sensitive to simulated water stress and species *Achyranthus aspera* could tolerate

simulated water stress upto maximum extent in pot conditions because per cent reduction in transpiration rate was lowest recorded in this species in comparison to other experimental plant species.

III Relative water content (RWC) %

In pot conditions the relative water content was significantly decrease in all species at all the time period/stages (Table 18 & Fig. 18). At final stage it was recorded 60.83, 70.18, 69.53 and 74.59 per cent in *Achyranthus aspera*, *Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively in non stress condition. However, at final stage in conditions of water stress it was recorded 51.40, 61.52, 61.80 and 66.47 per cent in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively.

The per cent decrease in relative water content (at final stage) upto highest (15.50%) extent and upto lowest (10.89%) extent was recorded in *Achyranthus aspera* and *Amaranthus cruentus* species, respectively thus the species *Amaranthus cruentus* could tolerate water stress condition upto better extent as compared to other experimental plant species.

4.3 EXPERIMENT UNDER FIELD CONDITIONS

4.3.1 Growth parameters

Proposed plant growth parameters were studied on plants of experimental species raised under normal field condition (non saline soil) having EC 1.05 and pH 7.8. These experiments were also conducted for two consecutive

years in the same experimental field. The water stress was simulated by conventional method of with holding the irrigation.

I Plant height (cm)

It was observed that water stress significantly reduced the plant height at all the stages of plant sampling for recording observations (Table 19 & Fig. 19). The plant height at harvest was 90.17, 43.13, 56.03 and 81.07 cm in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively. Under the condition of non stress, whereas the height was 72.73, 32.70, 40.83 and 64.62 cm in *Achyranthus aspera*, *Andrographis paniculata*, *Plumbago zeylania* and *Amaranthus cruentus* species, respectively under the condition of water stress.

The critical appraisal of these data also indicated that the water stress suppress the plant height upto the highest extent in *Plumbago* species and upto the lowest extent in *Achyranthus* species. Likewise in the second year the overall trend of reduction in plant height was quantitatively similar in all the four experimental plants species.

Considering the average of two years it was also observed that suppression was similar to that of individual years. Indicating that the medicinal plant species *Plumbago* was adversely affected by water stress upto maximum extent and the species *Achyranthus* could tolerate the water stress upto better extent because the reduction was minimum in this species by simulated water stress.

II Number of branches per plant

Data presented in table 20 and fig. 20 reveled that water stress significantly reduced the number of branches per plant at all the stages of experimental plant species except *Amaranthus cruentus* species because this species is single branch, thus *Amaranthus cruentus* shows non significant results at all the stages. The number of branches at harvest was 9.13, 25.60 and 11.93 in *Achyranthus, Andrographis,* and *Plumbago* species, respectively, under the condition of non-stress, whereas the number of branches was 6.40, 20.27 and 8.47 in *Achyranthus, Andrographis* and *Plumbago* species, respectively under the conditions of water stress. The critical appraisal of these data showed that the water stress suppress the number of branches upto the highest extent in *Achyranthus aspera* and upto the lowest extent in *Andrographis paniculata*. Likewise, in the second year the overall trend of reduction in number of branches was quantitatively similar in all the three species. Considering the average of two seasons it was also observed that suppression was similar to that of individual year. These data also revealed that the medicinal plant species *Achyranthus aspera* was adversely affected by water stress upto maximum extent and the *Andrographis paniculata* species could tolerate the water stress upto better extent in simulated water stress.

III Plant stand establishment

Data presented in table 21 and fig. 21 revealed that water stress significantly reduced the plant stand establishment at all the stages of plant sampling for recording observations. The plant stand establishment at harvest was 31.00, 29.33, 34.00 and 33.67 in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively, under the non-stress conditions, whereas the plant stand establishment was 27.00, 25.00, 30.00 and 29.00 in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania*

and *Amaranthus cruentus* species, respectively under the water stress condition. The critical appraisal of these data also indicated that the water stress suppress the plant stand establishment upto the highest extent in *Andrographis paniculata* and upto the lowest extent in *Plumbago zeylanica*. Likewise, in the second year the overall trend of reduction in plant stand establishment was quantitatively similar in all the four species. Considering the average of two years it was also observed that suppression was similar to that of individual year. These data also indicate that the medicinal plant species *Andrographis paniculata* was adversely affected by water stress upto maximum extent and the species *Plumbago zeylanica* could tolerate the water stress upto better extent because the reduction was minimum in this species by simulated water stress.

IV Leaf area per plant (cm^2)

Data presented in table 22 and fig. 22 showed that water stress significantly reduced the leaf area of plants. The leaf area was 189.89, 225.53, 296.60 and 270.10 in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively, under the non stress conditions, whereas, the leaf area was 158.42, 194.73, 252.52 and 239.53 in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively under the water stress condition. The critical appraisal of these data also indicated that the water stress reduce the leaf area upto the highest extent in *Achyranthus aspera* species and upto the lowest extent in *Amaranthus cruentus* species. Likewise, in the second year the overall trend of reduction in leaf area was quantitatively similar in all the four species. Considering the average of two years it was also observed that reduction was

similar to that of individual year. This was also showed that the species *Achyranthus aspera* was adversely affected and species *Amaranthus cruentus* could tolerate the water stress upto better extent among all the experimental medicinal plant species.

V Leaf area index (LAI)

Data presented in table 22 and fig. 22 revealed that water stress significantly suppressed the leaf area index (LAI) of plant sampling for recording observations. It was 1.18, 1.39, 1.85 and 1.67 in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively in the non stress condition, whereas, in the condition of water stress leaf area index was found 1.00, 1.22, 1.55 and 1.49 in species of *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively. The critical appraisal of these data also indicated that in condition of water stress the extent of suppression of leaf area index upto the highest extent in *Plumbago zeylanica* species and maximum tolerance of water stress was found in *Amaranthus cruentus* species because minimum reduction occur in this species.

4.3.2 Physiological parameters

I Photosynthetic rate (μ mol m^{-2} s^{-1})

Data presented in table 23 and fig. 23 showed that water stress significantly reduced the photosynthetic rate at all the stages of plant sampling for recording observations. The photosynthetic rate at maturity stages was 16.93,

25.80, 20.07 and 19.53 (µ mol m^{-2} s^{-1}) in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively, under the non-stress condition, whereas in condition of water stress photosynthetic rate was 5.77, 12.47, 8.50 and 8.53 (µ mol m^{-2} s^{-1}) in the *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively. The critical appraisal of these data also indicated that the water stress suppress the photosynthesis rate upto highest extent in *Achyranthus aspera* species and upto lowest extent in *Andrographis paniculata* species. Likewise, in the second year the overall trend of reduction in photosynthetic rate was quantitatively similar in all the experimental plant species. Considering the average of two years it was also observed that suppression was similar to that of individual year. These data also indicated that the medicinal plant species *Achyranthus aspera* was adversely affected by water stress upto maximum extent and the species *Andrographis paniculata* could tolerate the water stress upto better extent because the reduction was minimum in this species by simulated water stress.

II Total chlorophyll (mg g^{-1} fr. wt.)

Data given in table 24 and fig. 24 showed that water stress significantly reduced the chlorophyll of plant sampling for recording observations. The chlorophyll was 2.48, 2.63, 2.67 and 2.29 (mg g^{-1} fr.wt.) in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively, under the non stress condition, whereas as the chlorophyll was 2.30, 2.41, 2.50 and 2.14 (mg g^{-1} fr.wt.) in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively

under the water stress condition. The critical appraisal of these data also indicated that the water stress suppress the chlorophyll upto the highest extent in *Andrographis paniculata* and upto the lowest extent in *Plumbago zeylanica* species. Likewise in the second year the overall trend of reduction in chlorophyll was quantitatively similar in all the experimental species. Considering the average of two seasons the trend of reduction was similar to that of individual year. These data also indicate that the medicinal plant species *Andrographis paniculata* was adversely affected by water stress up to maximum extent and the species *Plumbago zeylanica* could tolerate upto the water stress better extent because the reduction was minimum in this species by simulated water stress.

4.3.3 Water relation parameters

II Transpiration rate (m mol m^{-2} s^{-1})

Water stress significantly reduced the transpiration rate at all the stages of plant sampling for recording observations. The transpiration rate at maturity stage 1.05, 1.61, 1.60 and 1.74 (m mol m^{-2} s^{-1}) in *Achyranthus aspera*, *Andrographis paniculata*, *Plumbago zeylania* and *Amaranthus cruentus* species, respectively, under the condition of non-stress, whereas, the transpiration rate was 0.51, 0.65, 0.74 and 0.76 (m mol m^{-2} s^{-1}) in *Achyranthus aspera*, *Andrographis paniculata*, *Plumbago zeylania* and *Amaranthus cruentus* species, respectively in the condition of water stress (Table 25 Fig. 25). The critical appraisal of these data also indicated that the water stress suppress the transpiration rate upto highest extent in *Andrographis paniculata* and upto the lowest extent in *Achyranthus aspera* species. Likewise, in the second year the overall trend of reduction in

transpiration rate was quantitatively similar in all the four species. Considering the average of two years it was also observed that suppression was similar to that of individual year. Indicating that the medicinal plant species *Andrographis* was adversely affected by water stress upto maximum extent and the species *Achyranthus* could tolerate the water stress upto better extent.

III Relative water content (%)

Relative water content (RWC) was significantly reduced by water stress at all the stages of experimental plant species (Table 26 & Fig. 26). The relative water content (RWC) was found 60.97, 70.30, 69.60 and 74.71 per cent in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively in the condition of non-stress. In the condition of water stress it was 51.44, 61.57, 61.90 and 66.55 per cent in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively. The critical appraisal of these data also indicated that the water stress reduced the relative water content (RWC) up to the maximum extent in *Achyranthus* species and lowest extent in *Amaranthus* species. Likewise in the second year the overall trend of reduction in relative water content was similar in all the four experimental species. Considering the average of two years it was also revealed that the reduction was also similar to that of individual year. These data showed that the medicinal plants species *Achyranthus aspera* was adversely affected by water stress upto maximum extent and the species *Amaranthus* could tolerate the water stress upto better extent because the per cent reduction in RWC due to water stress was minimum in this species.

4.3.4 Yield and yield attributes

I Biological yield (g)

Data presented in table 27 and fig. 27 revealed that water stress significantly suppressed the biological yield of plant sampling for recording observations. In the condition of non-stress it was found 65.00, 48.87, 74.73 and 55.67 g in the *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively, whereas, in the conditions of water stress it was found 57.07, 40.73, 61.93 and 46.80 g in the species of *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively. The critical appraisal of these data also showed that the conditions of water stress reduced the biological yield upto maximum extent in *Plumbago* species and upto lowest extent in *Achyranthus* species. Likewise, in the second year the overall trend of suppression in biological yield was similar in all the experimental medicinal plants species. Considering the average of two years it was also indicated that reduction was similar to that of individual year. This was clearly showed that the medicinal plants species *Plumbago* was adversely affected upto maximum extent and species *Achyranthus* could tolerate the water stress upto better extent among all experimental plant species.

II Economic yield (g)

Data presented in table 27 and fig. 27 showed that water stress significantly reduced the economic yield of plant sampling for recording observations. It was 65.00, 34.25, 36.60 and 29.20 g in *Achyranthus aspera*,

Andrographis paniculata, Plumbago zeylania and *Amaranthus cruentus* species, respectively, under the condition of non-stress. However, in the condition of water stress it was 57.07, 29.20, 30.00 and 22.75 g in the *Achyranthus aspera*, *Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively. The critical appraisal of these data also revealed that the water stress suppress the economic yield upto the highest extent in *Amaranthus cruentus* species and upto the lowest extent in *Achyranthus* species. Likewise, in the second year the overall trend of suppression in economic yield was similar in all the four experimental plant species. It was also observed in considering the average of two year the suppression was also similar to that of individual year. These data clearly showed that the medicinal plant species *Amaranthus* was adversely affected by water stress upto maximum extent and the species *Achyranthus* could tolerate the water stress upto better extent because the per cent suppression in economic yield was minimum in this species by simulated water stress.

III Therapeutic yield (mg/g dry weight)

Data presented in table 28 and fig. 28 revealed that water stress significantly enhance the therapeutic yield of plant sampling for recording observations. In the condition of water stress it was recorded 29.00, 26.40, 30.60 and 28.20 (mg/g dry weight) in *Achyranthus aspera, Andrographis paniculata*, *Plumbago zeylania* and *Amaranthus cruentus* species, respectively, whereas in the condition of non stress it was found 21.40, 20.80, 26.20 and 22.00 (mg/g dry

weight) in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylania* and *Amaranthus cruentus* species, respectively. The critical appraisal of these data also indicated that the enhancement of therapeutic yield by water stress upto the highest extent in *Achyranthus* species and upto the lowest extent in *Plumbago* species. Likewise the pattern overall of enhancement in therapeutic yield during second year was found quantitatively similar in all the four experimental plant species. Considering the average of two years it was also observed that increasing was similar to that of individual year, observed data also indicated that plant species *Achyranthus* was adversely affected by non-stress upto maximum extent and the species *Plumbago* could tolerate the non-stress upto better extent due to minimum suppression by non-stress.

5. DISCUSSION

There are substantial scientific evidences to mentioned that progress in various aspects of bio-chemistry, molecular biology and biotechnology has been satisfactory as far as cultivated plants are concern. However, little experimental work seems to have been conducted to bring more wild plants under cultivation web (Janick, 2001). It was therefore empathetically recommended that we must concentrate our efforts to bring more wild plants under cultivation. Janick (2001) advocated to conduct more studies on environmental physiology for domestication of wild plant species. This fact is much more applicable for medicinal plants which are still predominately extracted from wild resources and these biotech resources are depleting at alarming rate hence investigations conducted in this piece of research work are of immense importance with economic point of view under such situation whatever experimental investigation are conducted, the interpretation of so obtained results becomes difficult. This difficulty is widened when we want to discuss various parameters of plant growth and development of wild medicinal plants as studied under laboratory, pot and field conditions Singh and Tyagi (2004). Even then efforts are being made to interpret the results of present investigation in the light of published literature and also the scientific information obtained from various organization in India and abroad these organizations include Central Institute of Aromatical and Medicinal Plants (CIMAP), Lucknow, National Botanical Research Institute (NBRI), Lucknow, Regional Research Laboratory (RRL), Jammu, various ICAR institutes having their concerns with medicinal

plants, International with Plant Genetic Resources Institute, Rome (Italy), Centre For New Crops and Plant Products, Horticulture Landscape Architecture, Purdue University, West Lafayette, USA and Institute of Plant Sciences, Zurich, Switzerland.

Salient features of interpretations of various results is given below :

It was interesting to note that osmotic stress as simulated by various osmotica of polyethylene glycol –6000, progressively increased the germination percentage in seeds of *Achyranthus aspera, Andrographis paniculata, Plumbago zeylanica* and *Amaranthus cruentus* species but this enhancement was noted upto -5.0 bar only and there after a decaling trend was observed at least upto -10.0 bar (reported elsewhere). The promotory effect of simulated osmotic stress was also similarly recorded in case of other parameters including seedling length, seedling dry weight and seedling vigour. These result are inconformity with the findings of some earlier workers who experimented with seeds of genotypes of cultivated plants. Bailly *et al.* (1998) conducted systematic experiments on sunflower seeds and reported the improvement in seed germenability and seedling growth. Such beneficial impacts have been described as osmopriming effects. However, the satisfactory scientific explanation of such beneficial responses is yet to be explored. Bailly (1998) interpreted that such responses are mediated through the restoration of anti-oxidant mechanism, specially the activities of catalase (CAT) and glutathione reductase (GR). These enzymes are known to control at least partly the rate of lipid, peroxidiation by scavenging H_2O_2 and by producing the potent anti oxidant glutathione. Similar, reports have also been furnished by Singh and Afria

(1985) with chickpea, Singh and Singh (1995) with microsperma and macrosperma gram, Singh and Kakralya (2001) with groundnut, Kakralya *et al.* (2000) with greengram. Kakralya *et al.* (2000) selecting four genotypes of green gram reported that the suitable level of osmotic stress for getting beneficial responses differs from variety to variety and also influenced by other environmental conditions. Singh and Kakralya (2001) mentioned that such levels of osmotic stress should be experimentally determined for each genotypes and species. Singh and Tyagi (2004) selecting *Withania somenifera* and *Tephrosia purpurea* as experimental material showed that such beneficial responses may be economically viable for medicinal plants but the level of osmotic stress and the time of treatment should be critically evaluated. It was further emphasized that the beneficial responses observed in primed seeds are not merely confined to germination and related parameters but they are also reflected in yield and yield attributes of the crop raised from these osmoprimed seeds.

Seedling vigour is usually estimated on the basis of germination percentage and seedling length, therefore beneficial responses of plants to specific osmotic stress level are interpretable on the basis of results discussed earlier. Kakralya *et al.* (2000) and Mitharwal *et al.* (2002) selecting arid legumes as experimental materials further reported that the seedling vigour gave better index of the performance of primed seeds particularly when the seeds are subjected to adverse environmental conditions during subsequent storage and plantings.

A progressive decrease in seed germination percentage by increasing levels of simulated salinity stress in *Achyranthus aspera*, *Andrographis paniculata*,

Plumbago zeylanica and *Amaranthus cruentus* under laboratory conditions was found in agreement with findings of Waisel (1972) and Ungar (1978) and several other reports. The adverse effects of salinity on germination was also reported by Sharma (1980, 83) in experimental species mostly cultivated plants. The suppressive effects of salinity on germination process may be associated firstly with osmotic stress induced by high concentrations of salts in external medium and secondly to with ionic toxicity (cation accumulation) as also reported by Somani (1988), Patil *et al.* (1988) and Rajput and Sen (1990). Chhipa *et al.* (1992) with pearlmillet, Lal *et al.* (1993) with barley and wheat and Chhipa *et al.* (2002) with wheat also obtained similar results. Such suppressive effect of salinity stress on seed germination and seedling growth parameters were found to be medicated through ionic and osmotic stress both. The relative tolerance of different varieties of wheat is indexed through various morpho-physiological indices of salt tolerance crop plant. such indices may be utilized for screening the gremplasm for salt tolerance in crop plants. But their applicability in study relative salt tolerance in medicinal plants is yet to be intensively experimented under laboratory and field conditions.

Various levels of salinity stress also significantly reduced the seedling vigour and seedling length. The general trend of results in present investigation coincided with the observations recorded by Khan *et al.* (2000) and Patil *et al.* (1988). Khan *et al.* (2000) with *Cressa cretic*, Khan and Rizvi (1994) with *Atriplex*, Khan and Ungar (1996) with *Haloxylon recurrvum*, Gehlot and Sen (1999) with *Suaeda*, Roy and Srivastava (1999) with *Triticum* and Harsh and Godara (2001)

with some desert plant and Mahala (2002) with some phytoremadiative halophytes also obtained similar results.

The deteriorate effects of salinity stress on seedling length and seedling dry weight as observed in present investigation on medicinal plant species are in conformity with the findings of some earlier workers. Such adverse effects might be due to low water uptake by the cells of growing seedlings coupled with reduction in enzyme activities. Mahalaxmi *et al.* (1988), Naidoo and Raghunathan, (1990), Ungar (1998) and Khan *et al.* (2000) also obtained similar results.

Rehman *et al.* (2000) selective *Acacia* seeds and Mahala (2002) selecting seeds of *Atriplex* and *Suaeda* as experimental material further reported that the suppressive effects of salinity stress may be ascribed to the reduced biosynthesis, of certain growth promoting plant hormones which are otherwise necessary for normal process of seed germination and seed development.

Environmental stresses specially water stress (drought stress) and salinity stress are most important constraints in plant and crop productivity in developing as well as developed countries Nosberger *et al.* (2000).

The adverse effects caused by water and salinity stress are known to be operated through alteration of physiological and bio-chemical processes relating to growth and productivity. The potential of plant growth regulators in mitigating the adverse effect of abiotic stresses has been recently experimented in some crop plant species.

In present studies the interaction of PGRs with water stress (and also with salinity stress) was studied with respect to seed germination and seedling growth parameters and the test PGRs, were GA, IBA, BA and cycocel. It was observed that seed germination and seedling growth were tremendously reduced by water stress as simulated by –10 bar osmoticum of polyethylene glycol-6000. In the comparison of stress control GA mitigated the adverse effect upto maximum extent in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylanica* and *Amaranthus cruentus* followed by IBA, BA and CCC indicating thereby that cycocel (CCC) proved least effective through the effect was still significant. Such studies are however very uncommon in medicinal plant species. Buhan (2000) using several plant species and their seeds as experimental material also reported that synthetic GA, cytokinin and auxin reversed the damages caused by water stress and further increased the plant tolerance to abiotic stress Nilsen and Orcutt (1996) with bean cotton and tomato seeds and seedlings, Mahala (2002) with *Atriplex* and *Suaeda* seedlings also reported similar results. Singh and Tyagi (2004) with *Withania somnifera* and *Tephrosia. perpuria* also obtained similar results and further advocated that more work is required on commercial formulations of PGRs under field conditions prevailing in Indian agro-climatic zones.

The salinity stress upto the level of EC16 subsequently reduced germination percentage and seedling growth parameters in all the four species of medicinal plants taken as experimental material in present investigation.

The adverse effect of salinity stress was found more marked then that caused by water stress. Simultaneous application of cycocel proved most effective

in mitigating the adverse effect of salinity in the plant species namely *Achyranthus aspera, Andrographis paniculata, Plumbago zeylanica* and *Amaranthus cruentus* as inferred by a critical appraisal of data pertaining to seed germination and seedling growth. The ameliorative effects of PGRs in modulating the phenomena of plant growth and development under salinity stress has also been a subject of research findings reported by Afria *et al.* (1998) with clusterbean genotypes, Afria and Narnolia (1999) with wheat genotypes and Afria *et al.* (2001) with barley genotypes. Nathawat *et al.* (2001) selecting contrasting genotypes of clusterbean (both grain and vegetable genotypes) further reported that cycocel and other PGRs may be successfully used to mitigate the effect of salinity both under laboratory and filed conditions. However a clear understanding of physiological and biochemical mechanisms of such responses are yet to be achieved Larcher (2002), Singh and Tyagi (2004) and Singh and Kakralya (2004).

The condition of seed dormancy in seeds of *A. aspera, A. paniculata, P. zeylanica* and *A. cruentus* was adjusted on the basis of the visual observation which indicated that seeds did not germinate by usual seed testing method upto three months of storage (Dry storage at ambient laboratory conditions). The germination was found 70.00 %, 67.33, 62.00% and 75.00 % in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylanica* and *Amaranthus cruentus* species, respectively. The exact cause of seed dormancy in these species could not be known and this description of 'dormancy' in untreated seeds was simply based on the scientific information available in published literature on these and some taxonomically related wild species of plants Andersen (1968). Various methods of

breaking the seed dormancy were adopted from the technique originally given by Joshi and Nigam (1970) and later on modified by Agarwal and Dadlani (1995). It was observed that rubbing the seeds with sand or emersing the seeds in dilute sulphuric acid followed by a quick wash of the same in running water, significantly improved the seed germination percentage in these medicinal plants species. These observations are supported by some findings and earlier workers Andersen (1968) with some species of *Chenopodes, Abutilon* and *Ameranthus* also obtained similar results. Such studies have also been conducted by plant ecologist who selected wild plants as experimental material.

Joshi and Nigam (1970) with *Trianthema portulacastrum*, Babu and Joshi (1970) with *Borreria articularis,* Bishnoi (1997) with *Atriplex numularia* and Mahala (2002) with *Suaeda nudiflora* also obtained similar results. Singh and Tyagi (2004) selecting *Withania somnifera* and *Tephrosia perpuria* also reported seed germination promoting effects of rubbing the dry seeds with sand and treating the stored seeds with dilute sulphuric acid. Singh and Tyagi (2004) further interpreted that these treatments are operative through loosening of seed coat and improving the water uptake by germinating seeds. However, the biochemical and physiological explanations of such beneficial responses is to be intensively experimented in more species of otherwise wild medicinal plants.

In all the experimental plants species a marked distinctive adverse effect of water stress was observed on seedling emergence and leaf area per plant. Likewise the plant height at various stages of observations and economic yield on maturity stage were also reduced by water stress in the comparison of control

where, plants were not allowed to experience any water stress during their growth period. The adverse effect of water stress on seedling emergence and leaf area is a common observation and has been reported in several earlier studies conducted on crop plants. Basu and Chakarvarti (1984) with Solanum species, Singh *et al.* (2000) with several medicinal plants, Singh and Tyagi (2004) with *Withania somnifera* and *Tephrosia*. *purpuria* and Jakhar *et al.* (2004) with several medicinal plants of arid region also obtained similar results. Jakhar *et al.* (2004) and Singh and Tyagi (2004) interpreted that limited water supply or water defect during germination and vegetative growth period hampered the usual physiological processes leading to poor seedling emergence and retarded vegetative and reproductive growth. Such observations are common in crop plants experimented under pot and field conditions and such observations have been reviewed by earlier workers including Larcher (2002), Jakhar *et al.* (2004) and Singh and Tyagi (2004). The adverse effect of moisture stress on photosynthetic efficiency and transpiration rate are mediated through deleterious effects of moisture stress on photosynthetic machinery (chloroplast and chlorophyll biology and transpiration apparatus) Larcher (2002).

For studying various plant responses relating to growth and development, plants were grown in field no. 2 of Department of Plant Physiology, S.K.N. College of Agriculture, Jobner). Plants were cultured from the seedling which had been raised under nursery conditions in polythene bags from seeds. The field soil was characterized by EC 1.05 (dS/m), pH 7.8 and N 132.11, P 6.46, K 128.84 (kg/ha) before transplanting. The soil fertility was maintained by adding

sufficient FYM in the banging of the experiment in both the years. No other control environmental condition was practiced except moisture stress in soil which was simulated by the conventional technique of with holding the irrigation in treatment blocks. The interpretation of various observations is given below in brief.

The suppressive effect of plant height was clearly evident on the data obtained for plant height at all the stages of plant observations. This trend of suppression of plant height was quantitatively similar in both the years of experimentation in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylanica* and *Amaranthus cruentus* the reduction in plant height was found highest in *Plumbago zeylanica* and lowest in *Achyranthus aspera*. The suppressive action of soil moisture stress was also reported by Whitehead *et al.* (2000) in lentils, Condon *et al.* (2002) in wheat genotypes. Polley (2002) in forage legumes and Angadi and Entz (2002) in sunflower. Angadi and Entz (2002) selecting contrasting genotypes of sunflower interpreted that the reduced water supply restricted the growth and metabolism of photosynthetic apparatus which finally lead to reduced shoot growth.

The number of branch in all the experimental plant species was drastically reduced by water stress except in *Amaranthus cruentus* which is an unbranched species. The reduction in number of branches was highest in *Achyranthus aspera* and lowest in *Andrographis paniculata*. These observation are similar to those obtained by Fernandez and Millar (1985) with arid legumes Kurdali *et al.* (1997) with lentils, Erskine *et al.* (1999) in food legume species and Whitehead *et al.* (2000) in lentils. Erskine *et al.* (1999) selected contrasting

genotypes of arid legumes as experimental material and reported that the suppressive effect o limited water supply to plants during various stages of growth and development was operative through impaired nutritional absorption and also through arrested growth and physiological activity of root nodules and N fixing bacteria.

The seedling survival and the plant population under fragile agro-ecosystem are indicted by plant stand establishment as studied at various stages of plant growth and development. In present investigation the plant stand establishment was decreased by water stress at all the stages of observation. The reduction was found to be quantitatively similar in both the years of experimentation. The reduction in plant stand establishment was found highest in *Andrographis paniculata* and lowest in *Plumbago zeylanica*. These observations are inconformity with the results obtained by in several agronomical studies. Borrell *et al.* (2000 a & b) with sorghum, Byrd and May (2000) with switch grass, Biles and Cothern (2001) with cotton and Yang *et al.* (2002) with rice cultivars also obtained similar results reporting the adverse effect of water stress on plat population and plant stand establishment. Yang *et al.* (2002) using rice cultivars as experimental material reported that the deleterious effects of water stress on plant population are medicated through degradative changes and senescence of vegetative and reproductive parts.

Both these parameters are considered good markers of adverse effects of abiotic stresses on plants growth and development. In present studies also the suppressive effects of water stress were abdient in both the years of

experimentation with respect to leaf area per plant and also leaf index it was further noted that reduction in leaf area per plant and leaf area index parameters was more in *Achyranthus aspera* and *Plumbago zeylanica*, respectively than other species. The suppressive effects of soil moisture stress experienced by plants at vegetative and reproductive phages is a common observation in pot and field studies. Borrel *et al.* (2000 a & b) with sorghum, Byrd and May (2000) with switch grass and Yang *et al.* (2002) with hybrid rice have also reported the adverse effects of water stress on foliage characteristics and all the thirty experimental cultivars of hybrid rice showed similar trend of responses. It was further interpreted that accelerated senescence changes in leaves resulted in to reduction in total leaf area and leaf index under water stress.

In present studies the transpiration rate was recorded with portable photosynthesis measurement system (Model LI 6200, LICOR, USA). Whereas, the relative water content (RWC) per cent was determined by conventional method. The suppressive effects of water stress on transpiration rate was commonly evident in *Achyranthus aspera, Andrographis paniculata, Plumbago zeylanica* and *Amaranthus cruentus.* This adverse effect was observed at vegetative stage, preflowering stage, flowering stage and maturity stage. With respect to transpiration rate *Andrographis paniculata* was found most sensitive to moisture stress treatment. The relative water content was measured at three hours interval during the day period of at 6 P.M. in the evening the reduction in relative water content was highest in *Achyranthus aspera* and lowest *Amaranthus cruentus*. These

results are inconformity with the findings reported Borrell *et al.* (2000 a & b) with sorghum and Byrd and May (2000) with switch grass and Richards *et al.* (2002) with temperate cereals. Byrd and May (2000) using switch grass as experimental material reported that suppressive effects of water stress on water relation parameters was correlated with specific leaf weight and stomatal conductance. Wright *et al.* (1994) with groundnut, Brown and Byrd (1997) with peanut and Byrd and May (2000) with pearlmillet also obtained similar results.

The adverse effects of abiotic stress were also observed with reference to total chlorophyll content (measured at vegetative stage only) and photosynthetic rate in experimental medicinal plants species. The decrease in total chlorophyll content was highest in *Andrographis paniculata* and lowest in leaves of *Plumbago zeylanica*. However, the rate of photosynthesis measured at various stages of plant growth and development was lowered to the maximum extent in *Achyranthus aspera* and to the lowest in *Andrographis paniculata*. These observations are scientifically supported by the results obtained by Mahala (2002) with some desert halophytes, Condon *et al.* (2002) with several genotypes of wheat and Singh and Tyagi (2004) with two important medicinal plants (*W. sominifera* and *T. perpurea*). However, the molecular biology of these effects is yet to be understood. Polley (2002) selected many species of C_3 and C_4 plants and found that the adverse effects of soil moisture effects was common on both these photosynthetic groups. Yang *et al.* (2002) experimented with more than 30 genotypes of hybrid rice and interpreted that the adverse effects of water stress on

photosynthetic machinery (and photosynthetic process as such) was mediated through adverse effects on photosynthetic enzymes, chlorophyll biosynthesis, chloroplast structure, chloroplast turgidity and unfavourable stomatal physiology. It was further noted that the accelerated senescence changes were also involved in these adverse effects.

The biological and economic yields are important indices of cumulative effects of various physiological processes operative in plants under various environmental conditions interactivity coupled with genetic constitution of macrophytes. In present studies the biological yield includes total dry plant biomass (gm/plant) in different species including their under ground parts. However, the economic yield constitutes the crude drug yield as marked in domestic market. This yield varied from plant to plant e.g. in *Achyranthus aspera* total plant biomass of all part of the plant including roots. In *Androgrphis paniculata* the crude drug of the commerce constitutes dried leaves and stem in case of *Plumbago zeylanica* dried roots constitutes the economic yield and in case of *Amaranthus cruentus* the dried leaves constitutes the crude drug of the commerce. The biological yield as well as economic yield were substantially reduced by moisture stress in both the years of experimentation. This reductive trend was prevalent in all the experimental species but *Plumbago zeylanica* was found to be most responsive to the deteriorative effects of water stress with respect to biological yield. However, the economic yield was reduced upto maximum extent in *Amaranthus cruentus* the reduction was observed upto the lowest extent in case of *Achyranthus aspera* with respect to

biological as well as economic yield. The suppressive effects of water deficit or simulated drought stress has been a common observation by many crop biologists who selected diverse economic plant groups as experiment material. Kramer (1969) reported the results of a comprehensive study and showed the adverse effect of water stress on crop yields in plants like corn, wheat, rice, barley, soybean, pea and mustard etc. In later star studies more systematic work was preformed to quantify the yield losses under water stress. Nanda and Saini (1992) with chickpea, Geigenberger *et al.* (1997) with potato, Li *et al.* (2000) with spring wheat, Whitehead *et al.* (2000) with lentil, Richards *et al.* (2002) with temperate cereals also obtained similar results. Richards *et al.* (2002) interpreted that the deleterious effects of water stress on biological and economic yield are mediated through suppressed vegetative and reproductive growth, low plant height, reduced leaf area and leaf area index and low photosynthetic rate. These adverse effects are further enhanced with other biotic and abiotic stresses prevailing simultaneously. Richards *et al.* (2002) and Larcher (2002) specifically mentioned that a concrete scientific explanation of such expressions is very difficult. This is primarily because of complex interaction of genetics and environment in forming the biological and economical yields.

The therapeutic yield in present investigations was determined on the basis on the basis of criteria given by Padulosi *et al.* (2002) and subsequently discussed by Singh and Tyagi (2004) in similar group of economic plants. This constituted total alkaloid contents in dried sample plant samples as determined by

the conventional method (described in details in chapter on Material Method). Contrary to other parameters contributing to biological and economic yield, the therapeutic yield was significantly enhanced by simulated water deficit in all the four experimental plant species. This enhancement was quantitatively similar in both the years of experimentation. The enhancement was found highest in *Achyranthus aspera* where, the enhancement was upto 36.45 % over respective control on an average. The enhancement was found lowest in *Plumbago zeylanica* where total alkaloid content was increased upto 17.19 % over its respective control on an average. The improvement of contents of secondary plant metabolites in economic parts of plants in responses to water stress (and also by other abiotic stress not experimented in present investigation) has also been reported, through scantly, by some earlier workers. Pathak (1967) with members of Zygophylaceae and Ratra (1972) with members of Amaranthaceae also reported beneficial responses to abiotic stresses with references to secondary metabolites. Such studies are however, uncommon in edible crop plants. Balandrin (1985) with several medicinal plants. Graham and Graham (1991) with Dioscorea, Chadwick and Whelan (1992) with some temperate medicinal plants also obtained similar results.

Singh and Tyagi (2004) conducted a comprehensive study on the effects of abiotic stresses on therapeutic yield in economic parts of *W. somnofera* (dried roots) and *T. perpurea* (dried seeds) and reported that water stress given to pot as well as field growing plants stimulated the bio-synthesis and accumulation of more secondary plant metabolites. It was interpreted that the anabolic processes

which involved starch and protein degradation in plants may be responsible for favorable biosynthetic pathways of secondary plant metabolites. This explanation however, requires experimentation with more species of medicinal plants and also with modern methods of plant cultivation and bio-chemical analysis. The usefulness of such observations however can not be questioned this is because of the increasing demand of medicinal plants for domestic and international markets Singh (2004). Cracker and Simon (2002) and Hartmann *et al.* (2002) also advocated to conduct such studies on herbs, species and medicinal plants so as to ensure the quality of exportable crude drug products for pharmaceutical industries. Malik (2004), Jakhar *et al.* (2004), Bhandari (2004) and Mukherjee (2004) also recommended to conduct a comprehensive investigation on therapeutically valuable plants available in tropical, subtropical, temperate, humid, arid and semi-arid regions. Such investigations are essentially required to generate basic and applied scientific information for plant domestication and cultivation.

6. summary

The scientific information reported in this piece of work were conducted under the title "Environmental physiology of domestication of herbal plants under fragile eco-system". For species of therapeutically valuable plants were selected these were *Achyranthus aspera* L. (Amaranthaceae) English name-Rough chaff flower, Hindi name-Chirchitta, part use-whole plant, *Andrographis paniculata* Brum.f. wall.ex. Nees (Acanthaceae) English name-King of bitters Hindi name Kalmegh, part use-leaves and stem, *Plumbago zeylanica* L. (Plumbaginaceae) English name-Chita, Hindi name Chitrak, part use-mainly roots and *Amaranthus cruentus* L. (Amaranthaceae) English name-Amaranth, Hindi name- Ramdana, part use-mainly leaves.

Experiments were conducted to generate basic, application oriented basic and applied scientific information aiming at the domestication of these wild plants under fragile agro-ecosystem. For studying seed germination and seedling growth responses to water and salinity stresses experiments were conducted under laboratory conditions along with mitigating potential of certain plant growth regulators and some dormancy breaking measures. Under pot conditions some parameters of growth and development and plant water relations along with economic yield were investigated along with productivity under the condition of water stress. Under field conditions also various parameters of plant growth, development and productivity were studied on plants which were subjected to

water stress. Following features of special interest emerged on the basis of data evaluation.

(1) The osmotic stress as simulated by different orders of polyethylene glycol – 6000 (PEG –6000) improved the germination percentage and seedling growth and vigour parameters up to –5.0 bar osmoticum. These medicinal plant species indicated some quantitative differences in their responses to water stress under laboratory conditions. It also observed that osmotic stress (at least upto –5.0 bar) proved osmoconditioning treatment with respect to seed germination and seedling growth parameters.

(2) All the levels of simulated salinity stress progressively reduced the seed germination percentage and seedling growth/vigour parameters. The relative efficacy of gibberellic acid, indole beutyric acid, benzyle adenine and cycocel in counteracting the adverse effects of water stress were studied in a separate set of experiment. It was observed that GA (150 ppm), IBA (40 ppm), BA (40 ppm) and CCC (150 ppm) successfully counteracted the adverse effects of water stress in the context of seed germination and seedling growth parameters. GA proved most effective and CCC the least effective in ameliorating the responses.

(3) The adverse effect of salinity stress was also mitigated by plant growth regulators with reference to germinability and seedling growth. Cycocel was found most effective in reducing the adverse effects of salinity stress and GA proved least effective with this point of view.

(4) Treatment of seeds with dilute sulphuric acid (dilute H_2SO_4) and rubbing the seeds with sand were found effective in breaking the seed dormancy and improving the seed germination in all the four experimental plant species.

(5) Under pot conditions water stress as simulated by conventional method of withholding the water supply, adversely affected various parameters of plant growth and productivity.

(6) The relative performance of *Achyranthus aspera, Andrographis paniculata*, *Plumbago zeylanica* and *Amaranthus cruentus* was studied on the plants maintained under field conditions and subjected to soil moisture stress. It was clearly evident that various attributes of plant growth and productivity wee adversely affected by simulated drought. This conclusion was based on the critical appraised of statistical analysis of two years data. These data were related to plant height, number of branches per plant, plant stand establishment, total plant leaf area, leaf area index, transpiration rate, relative water content, chlorophyll content, photosynthetic rate, phytomass productivity (biological yield) and economic yield. The quantitative trend of results was similar in both the years of experimentation, hence, the pooled analysis also generated the similar inference.

(7) Though, the economic yield crude drug yield (CDY) was constituted by dry plant biomass of different plant parts in various species, the observation on the reduction of economic yield in responses to water stress was a common feature in experimental material. But quantitative differences at species level were quite obvious.

(8) The therapeutic yield was however enhanced by soil moisture drought in the economic parts of *Achyranthus aspera*, *Andrographis paniculata*, *Plumbago zeylanica* and *Amaranthus crucntus*.

The results summarized in above paragraphs have been interpreted on the basis of published literature. This was based on the literate cited on crop plants mostly. The effects of water and salinity stress, plant growth regulators and dormancy breaking treatments were discussed on the basics of laboratory experiments. The responses of plant to water stress under pot and field conditions have been discussed with reference to various parameters of plant growth, development and yield together with some parameters of plant water relations and photosynthesis. It was concluded that information obtained from present studies should be further tested on other plant species having therapeutic and export potential and requiring domesticational and cultivational approaches.

Appendix- I

Monthly mean metrological observation of the experimental year

Month	**Temperature (^{0}C)**		**Relative humidity (%)**	**Rainfall (mm)**
	Minimum	**Maximum**		

January 03	04.80	20.93	60.70	0.40
February 03	09.88	25.43	54.13	6.83
March 03	13.45	32.25	45.88	0.00
April 03	20.20	39.62	36.10	1.62
May 03	24.10	41.60	37.25	0.00
June 03	28.55	40.00	39.18	2.25
July 03	24.86	33.66	25.09	35.00
August 03	23.25	32.90	22.43	34.65
September 03	24.15	36.03	16.74	8.05
October 03	19.58	34.86	14.46	0.00
November 03	11.52	29.55	14.89	0.00
December 03	5.95	25.47	11.07	0.00

Appendix- II

Monthly mean metrological observation of the experiment year

Month	Temperature (^{0}C)		Relative humidity (%)	Rainfall (mm)
	Minimum	Maximum		
January 04	4.9	22.04	68.10	0.00
February 04	6.97	28.27	53.50	0.00
March 04	13.35	36.28	41.50	0.00
April 04	21.58	38.60	34.90	0.6
May 04	27.20	41.15	32.38	1.15
June 04	27.10	37.88	42.75	2.05
July 04	26.00	36.76	54.30	14.32
August 04	24.12	31.50	81.10	46.30
September 04	22.67	35.7	55.75	1.25
October 04	15.9	31.74	60.20	4.36
November 04	9.18	30.20	48.00	0.00
December 04	6.48	24.85	51.04	0.00

Table 15 Effect of water stress on leaf area per plant (cm^2) and economic yield (g) under pot condition of experimental plant species

Species	Treatment	Leaf area per plant	Economic yield
Achyranthus aspera	NS	180.50	63.25
	WS	155.70	54.65
Andrographis paniculata	NS	219.50	31.00
	WS	190.60	26.50
Plumbago zeylanica	NS	293.40	33.15
	WS	250.50	28.20
Amaranthus cruentus	NS	262.70	26.40
	WS	238.50	20.60
SEm±		5.62	0.86
C.D. (P = 0.05)		16.80	2.58

Table 14: Effect of water stress on seedling emergence (%) and plant height (cm) of experimental plant species under pot condition

Species	Treatment	Seedling emergence (%)	Plant height (cm)		
			Pre-flowering	Flowering	Maturity
Achyranthus aspera	NS	50.33	58.60	82.60	87.20
	WS	46.67	54.40	67.40	69.40
Andrographis paniculata	NS	44.00	14.20	21.80	40.60
	WS	38.67	10.40	17.20	29.80
Plumbago zeylanica	NS	52.67	26.20	33.00	54.00
	WS	49.33	24.10	27.90	37.80
Amaranthus cruentus	NS	53.33	40.00	77.80	79.40
	WS	50.00	33.90	60.80	62.70
SEm±		1.18	0.92	1.20	1.52
C.D. (P = 0.05)		3.53	2.74	3.59	4.35

Table 16 : Effect of water stress on photosynthetic rate (µ molm^{-2} s^{-1}) under pot condition at four stages of experimental plant species

Species	Treat-ment	Vegetative	Pre-flowering	Flowering	Maturity
Achyranthus aspera	NS	38.13	25.93	20.91	15.99
	WS	16.03	8.01	7.44	6.10
Andrographis paniculata	NS	59.77	46.60	34.82	25.72
	WS	27.47	24.61	20.01	12.42
Plumbago zeylanica	NS	58.77	39.33	28.51	19.92
	WS	23.97	21.32	14.11	8.42
Amaranthus cruentus	NS	53.97	37.87	26.16	19.50
	WS	26.21	21.01	13.75	8.55
SEm±		0.97	0.74	0.50	0.30
C.D. (P = 0.05)		2.89	2.20	1.49	0.89

Table 17: Effect of water stress on transpiration rate (m molm^{-2} s^{-1}) under pot condition at four stages of experimental plant species

Species	Treat-ment	Vegetative	Pre-flowering	Flowering	Maturity
Achyranthus aspera	NS	2.40	2.17	2.01	1.01
	WS	1.74	1.71	1.40	0.60
Andrographis paniculata	NS	3.44	3.22	2.82	1.59
	WS	2.51	2.28	1.75	0.62
Plumbago zeylanica	NS	3.44	3.17	3.02	1.62
	WS	2.50	2.13	1.61	0.75
Amaranthus cruentus	NS	3.60	3.37	3.09	1.72
	WS	2.51	2.18	1.79	0.73
SEm±		0.07	0.07	0.06	0.03
C.D. (P = 0.05)		0.21	0.20	0.17	0.09

Table 18: Effect of water stress on relative water content (RWC %) under pot condition at different intervals of experimental plant species

Species	Treat-ment	09 : 0	12 : 0	15 : 0	18 : 0
Achyranthus aspera	NS	69.41	62.78	70.11	60.83
	WS	59.29	51.80	59.89	51.40
Andrographis paniculata	NS	78.33	71.74	79.12	70.18
	WS	69.99	63.17	70.48	61.52
Plumbago zeylanica	NS	69.94	71.20	75.60	69.53
	WS	58.31	64.89	69.14	61.80
Amaranthus cruentus	NS	79.38	79.09	81.33	74.59
	WS	72.79	70.42	70.74	66.47
SEm±		1.71	1.63	1.73	1.84
C.D. (P = 0.05)		5.10	4.87	5.18	5.50

Table 1 : Effect of simulated water stress on seed germination percentage of medicinal plant species under laboratory condition

Species	Water potential levels (bar)	Germination (%)		
		* 3 DAS/ 5 DAS	*5 DAS / 10 DAS	Final count
Achyranthus aspera	0 bar	0.00	26.67	73.33
	-1 bar	0.00	34.00	78.00
	-3 bar	16.67	42.00	82.67
	-5 bar	28.00	51.33	86.67
	-7 bar	22.67	42.00	78.67
Andrographis *paniculata*	0 bar	0.00	25.33	68.67
	-1 bar	0.00	35.33	73.33
	-3 bar	18.00	46.67	80.00
	-5 bar	23.33	62.00	89.33
	-7 bar	18.67	53.33	76.67
****Plumbago*** *zeylanica*	0 bar	0.00	56.67	68.00
	-1 bar	18.67	61.33	72.67
	-3 bar	33.33	70.67	76.67
	-5 bar	43.33	75.33	85.33
	-7 bar	35.33	65.33	76.67
**Amaranthus* *cruentus*	0 bar	23.33	66.67	76.00
	-1 bar	33.33	75.33	79.33
	-3 bar	38.67	82.67	88.67

	-5 bar	42.00	88.67	92.67
	-7 bar	38.00	80.00	85.33
SEm±		2.00	2.96	3.35
C.D. (P = 0.05)		5.71	8.42	9.56

Table 2 : Effect of simulated water stress on seedling length , seedling vigour and seedling dry weight of medicinal plant species under laboratory condition

Species	Water potential levels (bar)	Seedling length (cm)	Seedling vigour	Seedling dry weight (g)
Achyranthus	0 bar	9.50	696.67	0.260
aspera	-1 bar	10.00	780.00	0.270
	-3 bar	10.33	854.22	0.310
	-5 bar	10.33	895.56	0.360
	-7 bar	10.00	786.67	0.320
Andrographis	0 bar	9.33	640.89	0.200
paniculata	-1 bar	9.33	684.44	0.220
	-3 bar	10.00	800.00	0.260
	-5 bar	10.67	952.89	0.320
	-7 bar	10.33	792.22	0.280
Plumbago	0 bar	9.33	634.67	0.300
zeylanica	-1 bar	10.00	726.67	0.310
	-3 bar	10.33	792.22	0.350
	-5 bar	10.33	881.78	0.410

	-7 bar	10.00	766.67	0.350
Amaranthus	0 bar	8.67	658.67	0.250
cruentus	-1 bar	8.67	687.56	0.260
	-3 bar	9.00	798.00	0.300
	-5 bar	9.67	895.78	0.360
	-7 bar	9.33	796.44	0.310
SEm±		0.68	59.80	0.014
C.D. (P = 0.05)		1.94	170.41	0.039

Table 3: Effect of simulated salinity stress on seed germination percentage of medicinal plant species under laboratory condition

Species	**Treatments**	**Germination (%)**	
		***5 DAS / 10 DAS**	**Final count**
Achyranthus	EC0	24.67	69.33
aspera	EC4	22.67	66.67
	EC8	18.00	61.33
	EC12	12.00	52.67
	EC16	9.33	49.33
Andrographis	EC0	23.33	65.33
paniculata	EC4	20.67	62.00
	EC8	15.33	56.67
	EC12	9.33	49.33
	EC16	7.33	46.00

***Plumbago** *zeylanica*	EC0	53.33	64.00
	EC4	50.67	60.67
	EC8	45.33	55.33
	EC12	38.33	48.00
	EC16	35.33	44.00
**Amaranthus cruentus*	EC0	62.00	72.00
	EC4	59.33	68.67
	EC8	54.67	64.00
	EC12	48.00	57.33
	EC16	42.67	52.67
SEm±		0.83	1.54
C.D. (P = 0.05)		2.38	4.39

Table 4 : Effect of simulated salinity stress on seedling length, seedling vigour and seedling dry weight of medicinal plant species under laboratory condition

Species	**Treatments**	**Seedling length (cm)**	**Seedling vigour**	**Seedling dry weight (g)**
Achyranthus *aspera*	EC0	9.50	658.63	0.286
	EC4	8.83	588.70	0.261
	EC8	8.17	501.07	0.240
	EC12	7.17	377.64	0.212
	EC16	5.50	271.31	0.165

Andrographis	EC0	9.17	599.08	0.220
paniculata	EC4	8.50	527.00	0.205
	EC8	7.67	434.66	0.185
	EC12	6.17	304.37	0.146
	EC16	5.00	230.00	0.120
Plumbago	EC0	9.17	586.88	0.331
zeylanica	EC4	8.83	535.72	0.319
	EC8	8.33	460.90	0.300
	EC12	6.50	312.00	0.235
	EC16	5.50	242.00	0.195
Amaranthus	EC0	8.67	624.00	0.293
cruentus	EC4	7.67	526.70	0.262
	EC8	6.50	416.00	0.219
	EC12	5.17	296.40	0.175
	EC16	4.67	245.97	0.150
SEm±		0.17	10.26	0.006
C.D. (P = 0.05)		0.50	29.23	0.017

Table 13 : Effect of physical and chemical treatments on seed germination and dormancy braking under laboratory condition

Species	**Time**	**Germination %**
Achyranthus aspera		
Sand rubbing	0 minute	70.00
	10 minute	72.67

	30 minute	74.33
Dilute H_2SO_4	1 minute	74.67
	2 minute	80.00
Andrographis paniculata		
	0 minute	67.33
Sand rubbing	10 minute	70.33
	30 minute	72.67
Dilute H_2SO_4	1 minute	74.00
	2 minute	75.67
Plumbago zeylanica		
	0 minute	62.00
Sand rubbing	10 minute	62.67
	30 minute	64.33
Dilute H_2SO_4	1 minute	66.00
	2 minute	70.33
Amaranthus cruentus		
	0 minute	75.00
Sand rubbing	10 minute	76.67
	30 minute	80.00
Dilute H_2SO_4	1 minute	80.67
	2 minute	85.33
SEm+		1.72
C.D. (P = 0.05)		4.91

Table 5 : Effect of PGRs treatment on seed germination percentage under water stress condition in laboratory conditions

Treatments		Germination percentage			
		Experimental plant species			
		Achyranthus aspera	***Andrographis paniculata***	*Plumbago zeylanica*	**Amaranthus cruentus**
Control	0.0 bar	66.00	**60.33**	59.33	71.00
	-10 bar (water stress)	49.67	44.33	42.67	54.33
Water stress (-10 bar) + PGR GA (150 ppm)		64.67	*59.00*	58.00	70.67
Water stress (-10 bar) + PGR IBA (40 ppm)		62.00	56.33	55.67	68.00
Water stress (-10 bar) + PGR BA (40 ppm)		60.33	54.00	53.00	65.33
Water stress (-10 bar) + PGR CCC (150 ppm)		58.00	52.33	50.67	62.67
SEm±		1.39	1.13	1.14	1.40
C.D. (P = 0.05)		4.28	3.46	3.51	4.29

Table 6 : Effect of PGRs treatment on seedling length under water stress condition in laboratory conditions

Treatments		Seedling length (cm)			
		Experimental plant species			
		Achyranthus aspera	***Andrographis paniculata***	*Plumbago zeylanica*	*Amaranthus cruentus*
Control	0.0 bar	9.00	**8.67**	8.70	8.33
	-10 bar (water stress)	7.33	6.67	6.67	6.40
Water stress (-10 bar) + PGR GA (150 ppm)		8.70	*8.50*	8.50	8.00
Water stress (-10 bar) + PGR IBA (40 ppm)		8.55	8.20	8.00	7.80
Water stress (-10 bar) + PGR BA (40 ppm)		8.40	7.90	7.80	7.67
Water stress (-10 bar) + PGR CCC (150 ppm)		8.33	7.67	7.60	7.33
SEm±		0.19	0.18	0.17	0.16

C.D. (P = 0.05)	0.57	0.56	0.52	0.48

Table 8 : Effect of PGRs treatment on seedling dry weight under water stress condition in laboratory conditions

Treatments		Seedling dry weight (g)			
		Experimental plant species			
		Achyranthus aspera	***Andrographis paniculata***	*Plumbago zeylanica*	*Amaranthus cruentus*
Control	0.0 bar	0.290	**0.222**	0.331	0.287
	-10 bar (water stress)	0.243	0.180	0.280	0.228
Water stress (-10 bar) + PGR GA (150 ppm)		0.284	*0.217*	0.321	0.273
Water stress (-10 bar) + PGR IBA (40 ppm)		0.270	0.203	0.314	0.264
Water stress (-10 bar) + PGR BA (40 ppm)		0.256	0.192	0.301	0.247
Water stress (-10 bar) + PGR		0.251	0.186	0.289	0.238

CCC (150 ppm)				
SEm±	0.006	0.005	0.008	0.05
C.D. (P = 0.05)	0.018	0.015	0.024	0.017

Table 7 : Effect of PGRs treatment on seedling vigour under water stress condition in laboratory conditions

Treatments		**Seedling vigour**			
		Experimental plant species			
		Achyranthus aspera	***Andrographis paniculata***	*Plumbago zeylanica*	*Amaranthus cruentus*
Control	0.0 bar	594.00	**523.06**	516.17	591.43
	-10 bar (water stress)	364.08	295.68	284.61	347.71
Water stress (-10 bar) + PGR GA (150 ppm)		562.63	*416.50*	493.00	565.36
Water stress (-10 bar) + PGR IBA (40 ppm)		530.10	461.91	445.36	530.40
Water stress (-10 bar) + PGR		506.77	426.60	413.40	501.08

BA (40 ppm) Water stress (-10 bar) + PGR CCC (150 ppm)	483.14	401.37	385.09	459.37
SEm±	11.78	10.36	10.57	11.28
C.D. (P = 0.05)	36.18	31.83	32.48	34.68

Table 9 : Effect of PGRs treatment on seed germination percentage under salinity stress in laboratory conditions

Treatments		**Germination percentage (%)**			
		Experimental plant species			
		Achyranthus aspera	*Andrographis paniculata*	***Plumbago zeylanica***	***Amaranthus cruentus***
Control	EC0	68.33	**64.33**	62.67	70.00
	EC16 (salinity stress)	49.67	46.67	41.33	50.33
Salinity stress (EC16) + PGR GA (150 ppm)		59.00	*55.00*	50.00	61.00
Salinity stress (EC16) + PGR IBA (40 ppm)		60.33	57.33	53.33	63.33
Salinity stress (EC16) + PGR BA (40 ppm)		62.67	59.67	56.67	65.00

Salinity stress (EC16) + PGR CCC (150 ppm)	65.00	62.00	60.33	68.67
SEm±	1.42	1.21	1.18	1.37
C.D. (P = 0.05)	4.36	3.72	3.63	4.20

Table 10 : Effect of PGRs treatment on seedling length under salinity stress in laboratory conditions

Treatments		**Seedling length (cm)**			
		Experimental plant species			
		Achyranthus aspera	*Andrographis paniculata*	***Plumbago zeylanica***	***Amaranthus cruentus***
Control	EC0	9.00	**8.80**	8.90	8.70
	EC16 (salinity stress)	5.00	4.90	5.00	4.60
Salinity stress (EC16) + PGR GA (150 ppm)		7.10	*6.50*	7.10	7.00
Salinity stress (EC16) + PGR IBA (40 ppm)		7.67	7.00	7.60	7.50
Salinity stress (EC16) + PGR BA (40 ppm)		8.10	7.50	8.10	8.00

Salinity stress (EC16) + PGR CCC (150 ppm)	8.60	8.20	8.60	8.50
SEm±	0.17	0.17	0.15	0.15
C.D. (P = 0.05)	0.53	0.51	0.46	0.45

Table 12 : Effect of PGRs treatment on seedling dry weight under salinity stress in laboratory conditions

Treatments		**Seedling dry weight (g)**			
		Experimental plant species			
		Achyranthus aspera	*Andrographis paniculata*	***Plumbago zeylanica***	***Amaranthus cruentus***
Control	EC0	0.284	**0.222**	0.328	0.289
	EC16 (salinity stress)	0.164	0.121	0.191	0.145
Salinity stress (EC16) + PGR GA (150 ppm)		0.173	*0.129*	0.204	0.160
Salinity stress (EC16) + PGR IBA (40 ppm)		0.223	0.160	0.258	0.190
Salinity stress (EC16) + PGR		0.254	0.185	0.290	0.230

BA (40 ppm) Salinity stress (EC16) + PGR CCC (150 ppm)	0.276	0.216	0.325	0.281
SEm±	0.005	0.004	0.007	0.005
C.D. (P = 0.05)	0.016	0.013	0.021	0.015

Table 11 : Effect of PGRs treatment on seedling vigour under salinity stress in laboratory conditions

Treatments		**Seedling vigour**			
		Experimental plant species			
		Achyranthus aspera	*Andrographis paniculata*	***Plumbago zeylanica***	***Amaranthus cruentus***
Control	EC0	614.97	**566.10**	557.76	609.00
	EC16 (salinity stress)	248.33	228.68	206.65	231.52
Salinity stress (EC16) + PGR GA (150 ppm)		418.90	*357.50*	355.00	427.00
Salinity stress (EC16) + PGR IBA (40 ppm)		462.73	401.31	405.31	490.81
Salinity stress (EC16) + PGR		507.63	447.52	459.03	520.00

BA (40 ppm) Salinity stress (EC16) + PGR CCC (150 ppm)	559.00	508.40	518.84	583.70
SEm±	10.96	10.31	10.06	8.75
C.D. (P = 0.05)	33.68	31.69	30.91	26.87

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