Abstract

Plants are the backbone of traditional systems of medicine and are also revered for their contribution to development of modern drugs. The therapeutic potential of plants depends on the quality and quantity of phytoconstituents present. Plant growth and the biosynthesis of plant metabolites are greatly influenced by biotic and abiotic factors. Scientific investigations have shown that various abiotic stresses including salt stress, flooding, drought, fertilization, shade, soil types etc. influence plant growth and formation of active constituents. Thus, optimization of abiotic stresses may help in increasing levels of plant metabolites and thus enhancing the bioactivity. The present review summarizes the importance of various abiotic stresses on plant growth and production of bioactive constituents. Literature shows that plants respond to abiotic stresses by modifying their morphology, physiology and phytochemical nature. Changes in plant growth and their bioactive metabolites are reported with alteration in abiotic stresses. This knowledge however is not translated to the fields during cultivation of valuable medicinal plants. From this review the authors conclude that, alteration of environmental factors during growth/cultivation of medicinal plants may ensure supply of plants with increased marker content which ensures better activity.

Keywords: Abiotic stress; plant growth; plant metabolites; secondary metabolites

1. Introduction

Medicinal plants are the mainstay for both traditional as well as modern system of medicine (Mehta et al., 2011) [73]. The World Health Organization states that 80% of the world population relies on traditional medicine that is derived mainly from plants. Moreover 30% of conventional drugs are of natural origin (WHO, 1999) [114]. The trend towards use of herbal medicines is increasing and thus the demand for medicinal plants is increasing in developing as well as developed countries. This has led to an increase in the number of herbal drug manufactures (Mehta et al., 2011) [73]. For the herbal drug industry regular supply of good quality raw material is necessary. Cultivation is one way of ensuring regular supply of uniform quality medicinal plants. The quality and medicinal properties of plants are attributed to the presence of different phytoconstituents present therein (Brinkhaus et al., 2000; Mukherjee et al., 2007) [17, 83]. The amount of bioactive constituents i.e. the secondary metabolites, present in plant is often low and they play a major role in adaptation of plants during stress conditions (Bannett and Wallsgrove, 1994; Bartwal et al., 2013; Murthy et al., 2014) [13, 12, 85]. The biosynthesis of plant metabolites is influenced by biotic and abiotic factors. The production of plant metabolites gets altered by variations in environmental factors or stresses (Akula and Ravishankar, 2011) [4].

Environmental factors that affect the growth as well as biochemical expression of plants are termed as stress (Lichtenrhalter, 1996) [63]. Any change in environmental factors produces change in the plant growth, morphology or biochemistry of plants. These factors or stresses may be biotic or abiotic. Biotic stresses include effect of living organisms e.g. fungi, insects, viruses, bacteria, herbivores, weeds etc. (Mittler, 2006) [78]. Abiotic stresses are environmental and non-biological in nature e.g. climate [rainfall (e.g. annual rainfall and distribution; floods/drought), temperature (heat, cold, chilling), light (day length, shade, high light intensity)] and soil conditions [i.e. physical properties (particle size, water holding capacity), chemical nature (pH, salinity, organic and inorganic nutrients (fertilizers) and microbiological properties (e.g. nitrogen fixing bacteria)]. Figure 1 summarizes major factors that affect plant growth and metabolism.

Literature shows that these affect the plant growth and production of plant metabolites (Chinnusamy et al., 2004; Cramer et al., 2011) [23]. Change in concentration,
(increase/decrease) of secondary metabolites in response to stress such as light, salinity, cold, drought, flooding shade, metal, fertilization etc. is well documented. The question arose in our minds that can we alter abiotic stresses during cultivation of medicinal plants with a view to increase the production of secondary metabolites. If we understand which environmental factor/stress enhances the production of secondary metabolites, that particular factor may be altered during cultivation thereby producing plants with increased amount of secondary metabolites. Hence we examined the available literature on effect of various environmental factors on medicinal plants. This review summarizes the effect of various abiotic factors on the plant growth and production of secondary metabolites and also suggests how these factors may be used during medicinal plant cultivation.

2. Method
Review of literature was prepared by using online searching on Google scholar, PubMed, Science direct, www.manybooks.net, http://www.pharmatext.org, www.getfreebooks.com etc. from 2000 to 2019.

3. Effect of abiotic stress on plants
Plants produce secondary metabolites to cope or adapt with any change or stress. Secondary metabolite production is often enhanced under stress. The emphasis of this review is to understand how environmental factors influence plant growth as well the nature and quantity of phytoconstituents. Effect of some abiotic factors influencing plant growth and metabolism are discussed below:

3.1. Climatic factors
Climate is a primary feature influencing plant growth and metabolism. Climatic conditions cannot be controlled or changed completely, but it is possible to select suitable climate for cultivation of a particular plant. Though plants grow best in their native environment, they adapt to altered environment and can exist in a range of external conditions by modifying their morphology, anatomy or biochemical response. Moreover these days using greenhouses for growing plants provide controlled conditions since greenhouses provide cover for crops and the amount of water and sunlight the plants receive can be controlled. Water availability, temperature and day length/ light intensity are critical components of climate.

3.1.1. Water availability
Excess or lack of water can lead to change in plant growth and survival. Water deficit leads to decreased photosynthesis in plants and hence decreased productivity (Zlatev and Lidon, 2005) [121]. Water scarcity leads to slowing down of root and leaf growth, delays flowering and fruit set and fall in seed number, size and viability. Water scarcity also triggers production of secondary metabolites (Kumar and Sharma, 2018) [59]. Drought stress occurs when the available water in the soil is reduced to critical levels and an atmospheric condition adds to continuous loss of water i.e. water deficit, usually accompanied by high temperatures and intense solar radiation (Xu et al., 2011) [115]. Drought is considered a major abiotic factor affecting plants. It is well documented that exposure of plants to drought leads to enhanced secondary metabolite production (Isah, 2019) [50]. Drought causes oxidative stress and is reported to increase the amount of flavonoids and phenolic acids in willow leaves. Drought stress influenced changes in the ratio of chlorophyll a and b and carotenoids. Plant tissues containing anthocyanins are usually resistant to drought but anthocyanins are reported to accumulate under drought stress at cold temperatures (Chalker - Scott, 1999; Akula and Ravishankar, 2011) [19, 4]. Excess water or flooding is tolerated by some plants and not by others. Excessive moisture reduces oxygen levels and impedes respiration in the roots; leads to buildup of CO2, CH4 and N2 gases as well as toxic compounds (Armstrong et al., 1994) [8]. Plant become more prone to microbial attack (Heath,
2000) [44]. If leaves and stems are submerged it leads to inhibition of photosynthesis (Liao and Lin, 2001; Caudle and Maricle, 2012) [62, 18]. Extent of damage by flooding depends on the time of the year and age of plants. Very young and old plants are more sensitive. During winters plants are not actively growing hence they are more tolerant to flooding (Colmer and Voeselek, 2009; Rapacz et al., 2014; Kosová et al., 2018) [26, 96, 56].

3.1.2. Temperature variations
Temperature strongly influences metabolic activity and plant ontology i.e. anatomy, morphology, growth and development of plant. Temperature is a key factor in regulating physiological processes. High temperatures can disrupt functional integrity of the photosynthetic apparatus at the chloroplastic level (Allakhverdiev et al., 2008) [6]. It can induce premature leaf senescence. Very high temperatures lead to water stress heading to nutrient deficiency. Several studies have examined the effects of increased temperatures on secondary metabolite production of plants. Temperature variations have multiple effects on the metabolic regulation, permeability, and rate of intracellular reactions in plant cell cultures. Elevated temperatures increase leaf senescence and root secondary metabolite (ginsenosides) concentrations in the herb Panax quinquefolius. Temperatures elevated by 5°C reduced photosynthesis and biomass production of P. quinquefolius (Jochum et al., 2007) [13].

Low temperature is one of the most harmful abiotic stresses affecting temperate plants. These species have adapted to variations in temperature by adjusting their metabolism during autumn, increasing their content of cryoprotective compounds to maximize their cold tolerance. Low temperatures or cold environment causes reduction in permeability of root surface membranes; delays stomatal opening thus lowering daily photosynthesis. Cold stress increases production of phenolics and subsequently they are incorporated into the cell wall as suberin/lignin, acting as a water barrier and increase resistance to the cold (Akula and Ravishankar, 2011) [4]. In apple tree adaptation to cold climate was found to be associated with a high level of chlorogenic acid (Zhao et al., 2011) [117]. The effect of cold stress on polyamine accumulation was reported. When leaves of Triticum aestivum L. are exposed to a cold temperature, accumulation of putrescine (6–9 times), increased (Kovacs et al., 2010). Lower soil temperatures cause an increase in levels of steroidal furostanol and spirostanol saponins (Szakiel et al., 2011) [108].

3.1.3. Light
It is well known that light is a physical factor which can affect plant physiological processes and metabolite production. A positive correlation between increasing light intensity and levels of phenolics has been reported in vitro as well as in field studies (Chalker - Scott, 1999) [109]. Autotrophs depend on light for survival. Photoperiod (duration of light), intensity and quality of light change their primary metabolic processes thereby changing production of primary and subsequently secondary metabolites. With increasing light intensity, uptake of oxygen, phosphorus and potassium is increased. Decreased light is a limiting factor in plant growth. Shading leads to reduced rate of photosynthesis (Gordon, 1969) [37]. Long day conditions and direct sunlight enhance the flavor and production of volatile oil in Mentha species (Clark and Menary, 1980) [24]. It is reported that phenols and flavonoids accumulate in the skin and leaves of fruits as their biosynthesis is stimulated by light (Treutter, 2006) [118]. UV-B is shown to induce the production of flavonols in Norway spruce, silver birch and grape leaves (Fischbach et al., 1999; Tegelberg et al., 2004) [32, 109]. UV (300–400 nm) increased flavonoids in the roots of pea plants (Akula and Ravishankar, 2011; Siipola et al., 2015; Shiozaki et al., 1999) [4, 102, 100]. Catharanthus roseus plants exposed to UV-B light show significant increases in the production of vinblastine and vincristine. In apples, UV light from 280–320 nm synergistically stimulates anthocyanin synthesis when it was combined with red light (Binder et al., 2009) [114].

3.2. Edaphic factors
These are abiotic factor relating to the physical or chemical composition of the soil found in a particular area. Different plants have different soil and nutritive requirements. Soil properties vary from one place to another in terms of type of soil particles, pore spaces, water holding capacity, pH, presence of lime, inorganic nutrients (e.g. N, P, K, Mg, Ca, S, Cu etc.), humus content, heavy metals and salinity. Fine soil, rich in inorganic nutrients and humus with a permeable substratum, pH range 6.5-7.5 and a good degree of moisture is favorable for growth of most plants. Specific pH, nutrient deficiency/ supplementation may elicit a response in plants in terms of altered secondary metabolite production.

3.2.1. Nutrients
Nitrogen, phosphorus and potassium are the primary nutrients necessary for plant growth. Nitrogen fertilizers improve plant vigor, water utilization, number of seeds, size of leaves and stems, number of roots. Nitrogen supplementation increases volatile oil production in Mentha piperita but there a decrease in menthol content (Marotti et al., 1994; Singh et al., 1989) [71]. Physical size as well as alkaloid content of Datura stramonium is proportional to nitrogen supplied. In opium poppy similar results were obtained. A deficiency of nitrogen and phosphorus results in increased production of phenolics which are a part of the chemical defense of the plants. Phenolic compound biosynthesis is also increased by shortage of potassium, sulfur and iron (Mogren et al., 2006; Alizadeh et al., 2010) [78, 5].

3.3. Metal stress
Heavy metal application may effect the composition of chemical constituents in plants. Quality and efficacy of the natural plant products is seriously altered in many medicinal plants (Nasim and Dhir, 2010; Lizhong and Cullen, 1995) [18, 66]. Plants exposed to heavy metal stress show differential responses in synthesis and accumulation of pharmacologically active molecules. Usage of heavy metals in optimum concentration acts as abiotic elicitors that improve the biosynthesis of specific bioactive compounds (Namdeo, 2007) [86]. It is reported that heavy metals treatment increases the biosynthesis of secondary metabolites in many plant species.

3.4. Salinity/Salt stress
Plant metabolomic studies show varied responses of plants to salinity. Salt stress is a condition in which osmotic forces are exerted on plants by applying excessive salt solutions in soils, due to which the plant growth rate may be decreased but content of secondary metabolites increases significantly. Salt environment leads to cellular dehydration, which causes
osmotic stress and removal of water from the cytoplasm resulting in a reduction of the cytosolic and vacuolar volumes, this leads to increased accumulation of osmolytes like proline, glycine betaine, soluble sugars etc which elicit production of secondary metabolites. Salt stress also triggers ionic stress and leads to enhanced production of reactive oxygen species (ROS); in response to these changes plants produce enhanced amounts of antioxidant phenolic compounds, alkaloids, tannins (Sytar et al., 2018) [106]. For example, increase in polyphenol content in different plant parts under salinity has been reported in a number of plants eg. *Cakile maritima* (Asteraceae), *Mentha pulegium* L. (Labiatae) (Dixon et al., 1995; Ksouri et al., 2007; Oueslati et al., 2010) [29, 58, 92]. Numerous studies have been carried out, in the last 2 decades, to study the effect of different abiotic stresses on plant growth and production of secondary metabolites, and their outcomes are summarized in Table 1.

### Table 1: Summary of studies demonstrating the effect of abiotic stresses on plant growth and secondary metabolites in situ.

| Plant Name | Abiotic stress | Observation | Reference |
|------------|----------------|-------------|-----------|
| *Achillea fragrantissima* L. (Asteraceae) | Salinity | Increase in tannin and alkaloid content | Abd et al., 2009 |
| *Artemisia annua* L. (Asteraceae) | Drought | Water deficits of 38 and 62 hours increased leaf artemisinin content | Marchese et al., 2010 |
| *Atriplex hortensis* (Amaranthaceae) | Heavy metals i.e. Cu, Ni, Pb, Zn | Decrease in growth rate of the roots and shoots of the plant | Kachout et al., 2009 |
| *Beta vulgaris* L. (Amaranthaceae) | Salinity (150 and 500 mM NaCl for 12 days) | Activity of antioxidant enzymes i.e. Glutathione reductase, Ascorbate peroxidase was stimulated with NaCl treatment | Bor et al., 2003 |
| *Brassica juncea* (Brassicaceae) | Metal (Copper chloride) | Level of Indole phytoalexins increased under the effect of CuCl₂ | Mithofer et al., 2004 |
| *Brassica napus* L. (Brassicaceae) | Flooding | Crop yield, the growth of seedlings unaffected by freshwater, but negatively affected by seawater | Hanley et al., 2019 |
| *Buplerum chinense* DC. (Asteraceae) | Fertilizers (0.3 g N + 0.4 g P₂O₅·kg⁻¹) | Increase in saiko-saponin a and d | Zhu et al., 2009 |
| *Camelia sinesis* L. (Theaceae) | Salinity | Polyphenols content of the halophytes increases | Ksouri et al., 2007 |
| *Calendula officinalis* L. (Asteraceae) | Fertilizer (180 kg N ha⁻¹) | Yield of marigold was increased with nitrogen treatment | Moosavi et al., 2014 |
| *Canola sinensis* L. (Asteraceae) | Drought | Increase in caffein level | Mello et al., 2006 |
| *Cassiopic tetragona* (Eriaceae) | Shade | Increase level of epicatechin and epigallocatechin in tea leaves | Hagiwara and Wright, 2015 |
| *Catharanthus roseus* L. (Apocynaceae) | Metal treatment with Cadmium (Cd) concentration ranging from 0.05 to 0.4mM | Increase in amount of Ajmalicine | Zheng and Wu, 2004 |
| *Chrysanthemum boreale* M. (Asteraceae) | Fertilizer(CaCO₃) | At 1.5 ton CaCO₃/haec terpene content was increased sesquiterpenes (30.4%) and monoterpenoids (9.5%), compared to the control. | Lee et al., 2005 |
| *Coriandrum sativum* L. (Apiaceae) | Drought | Water deficit increased percentage of petroselinic, oleic and palmitic acids | Yeganehpoor et al., 2017 |
| *Cucumis sativus* L. (Apiaceae) | Salinity | NaCl + Silicon treatment significantly increased the dry weight of shoots and roots | Zhu et al., 2004 |
| *Datura stramonium* (Solanaeaceae) | Metal | Concentration of Lubimin, 3-OH-I-Lubimin is increased with CuCl₂ and CdCl₂ treatment | Mithofer et al., 2004 |
| *Echinacea angustifolia* DC (Asteraceae) | Fertilizers | Nitrate and ammonium in the molar ratio of 1:1 supplied to the plant, the concentrations of chlorogenic acid, echinacoside and caffeic acid were significantly higher in roots of the plant | Montanari et al., 2008 |
| *Echinacea purpurea* (Asteraceae) | Drought | Increase in total phenol content | Gray et al., 2003 |
| *Elymus (Egeria) densa* Planch (Hydrocharitaceae) | Metal | Increase the level of chlorophyll and carotenoids in leaves with cadmium treatment | Melava et al., 2012 |
| *Eurotulipus globulus* (Myrtaceae) | Metal | Inoculation of *Pseudomonas orientalis* and *Chaetomium curvum* on the plant significantly increased plant growth and mitigated oxidative stress. | Ortiz et al., 2019 |
| Plant Name                  | Stressed Condition | Environmental or Nutritional Stress | Effects | Scientific Reference |
|----------------------------|--------------------|-------------------------------------|---------|-----------------------|
| *Fragaria ananassa* (Rosaceae) | Fertilizers        | Increased biomass and yield         |         | Anttonen et al., 2006 |
| *Glycine max* L. (Fabaceae)  | Shade              | Increase in lignin content          |         | Liu et al., 2018      |
| *Gossypium hirsutum* (Malvaceae) | Salinity           | Total carbohydrate percentage and oil percentage increases significantly with N-P-Fertilizers | Ahmed et al., 2010 |
| *Helianthus annuus* L. (Asteraceae) | Fertilizers       | Increase in the content of anthocyanins and flavones | Aziz et al., 2007 |
| *Hordeum vulgare* (Poaceae)  | Salinity           | Increase in the content of flavonoids |         | Gulzar et al., 2003   |
| *Hypericum brasiliense* (Clusiaceae) | Flooding          | The increase in the content of betulinic acid and phenolic compounds i.e. quercetin, rutin is increased | DeAbreu and Poulo, 2005 |
| *Ilex paraguariensis* (Aquifoliaceae) | Shade            | Decrease in Methylxanthine like caffeine, theobromine content with increasing shading | Coelho et al., 2007 |
| *Labisia pumila* (Myrsinaceae) | Fertilizers       | Decrease total flavonoid and phenolics by nitrogen fertilization | Ibrahim et al., 2011 |
| *Lupinus albus* (Fabaceae)  | Metal              | Level of genistein and 2-OH genistein increased with CuCl₂ treatment | Mithofer et al., 2004 |
| *Lycopersicon esculentum* (Solanaceae) | Salinity        | Increase in the content of Eugenol |         | Rai et al., 2004      |
| *Matricaria chamomilla* (Asteraceae) | Metal           | Plant yield was enhanced when treated with heavy metals i.e. Zn, Co, Pb & Ni | Nasim and Dhir, 2009 |
| *Mentha arvensis* (Lamiaceae)  | Metal              | Growth rate and yield of plant is affected when plant is treated with Cadmium and N | Aina et al., 2007 |
| *Ononis arvensis* (Fabaceae)  | Metal              | Increase the level of flavonoids |         | Tumova et al., 2011   |
| *Oryzium vulgare* L. (Labiatae) | Flooding          | Increase in total polyphenol content in leaves | Oueslati et al., 2010 |
| *Ocimum basilicum* (Lamiaceae)  | Shade              | Decrease total volatile oil with increasing shading, increasing shading decreases linalool and eugenol while increases methyl eugenol content in oil. | Chang et al., 2008 |
| *Ocimum tenuiflorum* (Lamiaceae) | Metal             | Increase in the content of Eugenol |         | Rai et al., 2004      |
| *Populus nigra* or Black poplar (Salicaceae) | Drought          | Total biomass, leaf area, root mass increased and total phenolic glycosides increased in leaves by water deficit | Hale et al., 2005 |
| *Prunella vulgaris* L. (Labiatae) | Drought          | Increase in the content of audi and Hypophyllanthlas | Street, 2012 |
| *Rheum palmatum* (Polygonaceae) | Metal             | Amount of Anthracene derivatives increased with application of Cadmium chloride and Aluminium chloride | Kasparova and Siatka, 2004 |
Production of secondary metabolites depends principally on the change in ecosystem in which plant grows. Several factors like climate change, soil type, water availability, salinity, temperature, light and various other adverse conditions have direct effect on the plant biomass and biomarker content. For an instance, it was noticed that under the shade stress the plant yield increased in plants i.e. Glycine max L., Zea mays L., Vicia faba L., Phaseolus vulgaris etc. while in Ocimum basilicum, total volatile oil content decreased with shade stress. Under the effect of salinity stress tannin and alkaloid content, polyphenol content, flavonoid content increased in Achillea fragrantissima L., Cakile maritima, Hordeum vulgare respectively. In Labiatae pumila plant total flavonoid content decreased with nitrogen fertilization but on other hand, in Oryza sativa L., Origanum vulgare etc. yield and phytoconstituents content increased under fertilization stress. It was found that, eugenol and flavonoid content increased in Ocimum basilicum, only volatile oil content increased in leaves and roots of plant. This is important to lay emphasis not only on plant yield but also on the content of phytoconstituents. It is necessary to develop uniformity of content. Cultivation practices for many vital medicinal plants are documented. The authors recommend that while developing agrotechnology for medicinal plants it is important to lay emphasis not only on plant yield but also on the content of phytoconstituents. It is necessary to develop the cultivation conditions for optimum biomass yield and incorporate minor, inexpensive changes in the growth environment of the plants (e.g. giving water stress; adding saline once a month; shade stress) that can result in enhanced production of desired secondary metabolites. Once the factors and the changes necessary to elicit the production of the bioactive constituents are elaborated and demonstrated by agricultural/research institutes, knowledge transfer to the cultivators should ensue. Fig. 2 summarizes the application of modification of abiotic factors during cultivation of medicinal plants.
4. Concluding remarks
The objective of this review was to highlight the influence of different abiotic stresses on the production of secondary metabolites. Based on the study it may be concluded that minor alteration of environment factors that lead to increased phytoconstituents biosynthesis can be easily incorporated during the cultivation of medicinal plants.

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6. Conflict of interest
There is no conflict of interest to declare.

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