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Reconnaissance for Natural Product Research in Nepal: Forest Biomaterials

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Preface

Madan Bhandari University of Science and Technology Development Board (MBUSTDB) is undertaking preparations for the establishment of a research-oriented world-class university. In this context, MBUSTB is engaging experts for identification of potential areas for research and teaching, which has the potential of directly contributing to economic development of the country.

This publication presents the outcome of a study related to the identification of research areas in the field of forest biomaterials, the outcomes of which have the potential of directly contributing to country's economic development. This study is a part of wider studies aimed at exploring the potential of natural products for biomedical, technological and agricultural applications.

MBUSTDB highly appreciates the remarkable hard work and dedication of the author – Dr. Sudip Pandey – for preparing this publication. MBUSTB appreciates and thanks all other individuals and institutions who contributed to bringing out this publication.

Prof. Rajendra Dhoj Joshi
Chairperson
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List of Abbreviations

GC-MS	Gas Chromatography-Mass Spectrometer
HPLC	High-Performance Liquid Chromatography
IADFs	Intra-Annual Density Fluctuation
NMR	Nuclear Magnetic Resonance
NTFPs	Non-Timber Forest Resources
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analysis
SM	Secondary Metabolites
WD	Wood Density
WHO	World Health Organization

Executive Summary

Timber and non-timber (Medicinal plants) are important resources for the Nepalese economy as Nepal harbour 45% of resources. These resources are not properly utilized and in most cases are exported to neighbouring countries for valuable product development. Also, these resources are having strong pressure due to changing climate change. Therefore, it is important to have knowledge of these resources for prosperity and development of the Country. To fulfil this gap in knowledge in resources and technology for product development, Madan Bhandari University of Science and Technology is established working for interlinking, socio, economic and the environment benefit. This report explores different research prospects and application of forest and agriculture for product development. The report is divided into different chapters with output based on specific Terms of Reference points. Chapter I provide a review on the effects of environmental parameters (temperature, Carbon dioxide, ozone, light and soil stress) on secondary metabolites production. Chapter II is related to climatic effects in wood anatomical structures of tress. In chapter III, IV and V, a review on the use of forest waste as biochar and biofertilizers are presented. Chapter VI particularly deal with different biofertilizers available in the Nepalese market and the technology used for their production is explained. Chapter VII focused on the applicability of nano fertilizers and their development to improve the nutrition dynamics of the soil-plant system for sustainable crop management. Lastly, Chapter VIII, IX and X is related to the identification of instruments that are working in a similar field of our interest. Moreover, it provides a list of different instruments and equipment for running a forest biomaterials science and engineering laboratory and organic agriculture.

Contents

CHAPTER 1: The influence of environmental conditions on secondary metabolites in medicinal plants: a literature review

Abstract

Secondary metabolites (SMs) of medicinal plants are the natural products used by plants for defence mechanism and interaction with insects and other organisms. Such compounds due to their ability to interact with living organisms have been used by humans as medicines, food, flavourings, biopesticides and fragrances. The synthesis and accumulation of such compounds are affected by multiple factors including intrinsic and extrinsic plant characteristics. This reviewed aimed to identify and analyse the present knowledge on the potential effects of environmental factors (temperature, CO_2, light, ozone, drought) on the synthesis and accumulation of SMs of some medicinal plants. We followed the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement guidelines and searched databases from PubMed, ScienceDirect, Google scholar published from January 2015 to May 2020. The included studies showed the influence of environmental parameters on SMs to plant is species-specific and on the chemical type. SMs induced by environmental factors are highly variable and are specific to different environmental stressors. For examples, phenolic metabolites seem to be induced by temperature and CO_2 but not by other factors, and an increase in alkaloids and flavonoids has been particularly detected under elevated Ozone. This review summarizes the reports of recent research articles involving environmental factors affecting SMs production, implications for healthcare, livelihoods, and conservation practice.

Keywords: Medicinal plants, Environmental factors, Secondary metabolites

1. Introduction

Climate change is a serious threat that is afflicting many parts of the world with influence on populations, animals and plants survival, especially in some geographical areas. For example; in the case of Latin America, which accounts for one of the earth's largest concentration of biodiversity, the risk of biodiversity loss is expected to increase as a result of climate change. Similarly, in terms of animals, there is a decline in the population of frogs and small mammals in Central America can be related to regional climate change (Kapos et al., 2008). In mountain regions, higher temperature leads to an upward shift of biotic zones and likely increase the frequency of forest fires (Hoffmann et al., 2019; Telwala et al., 2013). A specific class of plants that can be influenced by these changes are the medicinal plants and up to now millions of people still are depending for their cure and therapies on herbal medicine or traditional preparations. Since ancient times, more than half of the world population depends on medicinal plants to cure different human ailments (Mongalo et al., 2016). Traditional herbal medicine is getting significant attention in global health because each part (root, stem, leaves, flowers, fruits and seeds) of the medicinal plant are a rich source of natural metabolites and possess pharmaceutical properties. It is estimated that more than four billion people (80% of the world's population) living in the developing countries rely on medicinal products as a source of healthcare and traditional medical practice (Bandaranayake, 2006; Bodeker and Burford, 2006; Ekor, 2014). According to World Health Organization (WHO), approximately 45,000 different plants in more than 21,000 species of plants are being used for medicinal purposes that can be verified by the Ayurveda (Taur and Patil, 2011; World Health Organization, 2019). Medicinal plants are good sources of chemical substances like terpenoids, phenols, steroids, flavonoids, tannins and aromatic compounds which are widely used in pharmaceutical, cosmetics and food industry (Efferth and Greten, 2012). These chemical substances are commonly known as secondary plant metabolites which are not essential for growth and development of the plant but are considered as defence compound to interact with its environment for adaptation (Ramakrishna and Ravishankar, 2011). There are over 2,140,000 secondary metabolites (Thirumurugan et al., 2018) and are divided into different groups consisting of 29,000 terpenoids, 12,000 alkaloid derivatives, 8,000 phenolics (Kumar et al., 2019), steroids, flavonoids, tannins, and aromatic compounds and many more are in the process of identification. The demand for medicinal plants is profoundly increased in the recent decade due to enormous chemical diversity, few side effects and economic values (Anand et al., 2017; Verpoorte and

Memelink, 2002). Many studies have shown that secondary metabolites play role in treatment or prevention of many serious diseases or syndromes like diabetes, tuberculosis, ulcers, asthma, cancer, Alzheimer's disease, and cardiovascular diseases, Parkinson's disease (Basu and Imrhan, 2007; Crozier et al., 2009; Davies and Espley, 2013; Fang et al., 2011; Miller and Snyder, 2012).

Besides, the critical role of medicinal plants in different aspects of human lives, their growth and development are affected by environmental conditions like temperature variation, light intensity, elevated carbon dioxide, ultraviolet radiation, ozone, drought, salinity, and flood (Gupta et al., 2019; Imadi et al., 2015; Pessarakli et al., 2019). These environmental factors are important as each plant species needs special environmental conditions for growth. According to IPCC (2014), mean annual temperature is increasing faster to a rate of 0.06-0.1^{o}C/yr. with CO_2 increase of 407.4 parts per million. Change in climatic conditions not only influences the normal behaviours of plants but also their physiology, ultimately affecting the secondary metabolites. Studies showed that some environmental factors like temperature (Shohael et al., 2006; Singh and Sharma, 2020), elevated CO_2 (Ghasemzadeh et al., 2010; Ibrahim and Jaafar, 2012; Zhang et al., 2015; Ziska et al., 2008), Ozone (Bortolin et al., 2016; Pellegrini et al., 2015), UV light (Chen et al., 2018; Takshak and Agrawal, 2016), drought (Amirjani, 2013; Liu et al., 2011) adversely affects the metabolites, growth and productivity in plants. However, these studies are not enough and are sporadic in comparison with other commercial crops. On the other hand medicinal species has strong impact in life of many people and entire populations. Significant changes due to climate and ecological modifications can occurred in the synthesis of secondary metabolites thus is possible in the theory that a medicinal species that is source of important active compound can change the amount or the type of secondary metabolites production with problems for industries and medicinal products. In this regard more research is needed on medicinal plants at the molecular level to better understand the specific role of each environmental parameter as they are potential sources of bio-molecules and nutraceuticals.

In this study, we made a systematic review of the recent literature published in Google Scholar, PubMed and science direct to evaluate the influence of climatic parameters on secondary metabolites of medicinal plants. The main aim of this review is to enhance our understanding of the adaptability of plant SMs to key environmental factors. This helps in optimization of cultivation techniques at ambient environmental conditions with maximization quality and quantity of SM in plants efficiently and sustainably.

2. Materials and methods

We used a standard method called PRISMA for systematic literature review which includes resources eligibility and exclusion criteria, the systematic review process, and data abstraction and analysis (Liberati et al., 2009). The review was based on a systematic search of research articles from electronic databases: PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Google Scholar (https://scholar.google.com/#d=gs_asd) and ScienceDirect (https://www.sciencedirect.com/search/advanced) (Table 1). We choose these databases as they cover about 256 disciplines with +300 minor disciplines (e.g., health science, life science, social science physical science) such as science, social science, arts and humanities. The advanced search tool in databases was used for rigorous search on assigned topics (Appendix I). All the searched articles were imported into Mendeley reference management software and duplicates were deleted.

2.1 Inclusion and exclusion criteria

All original articles collected from search engine were compiled. We selected only peer-reviewed articles which were based on climatic variation and its effects on secondary metabolites of the plant with specific methods and results. The printed and online journal registered on journal website were included for study. Valuable sources of information from news articles, case studies, technical notes, educational materials were excluded as they are not peer-reviewed. To make a concise and systematic review we choose only articles published in English between January 1st 2015 to May 31st 2020. Studies which were related to study topics but not to climatic parameters were excluded (Table 2).

2.2 Data extraction

All the searched articles were imported into Mendeley reference management software (Mendeley Desktop version 1.19.4). A total of 258 articles were found through databases: 133 articles in Google scholars, 93 articles in PubMed and 32 articles in ScienceDirect. From this database, 37 duplicates articles were removed. The first screening of the articles consisted of reading the title and abstracts and 51 articles were identified eligible for full text, and 38 research studies were finally included in the data analysis. This review process is based on the PRISMA flowchart (Fig. 1). Moreover, the references of the analysed papers were accessed to identify other studies that had not been found in the consulted databases. Thus, 31 new papers added, totalling 289 articles were used in the preparation of this review articles.

Information was extracted from all articles, including study methodology, aim and importance of secondary metabolites where available using data extraction form. Title, abstract and full-text reviews of the publication were done independently by two researchers (SP and PP). The abstract, geographical location of the study, the methods used were manually screened based on the eligibility criteria and exclusions cross-checked by a member of the team. We also contacted the authors for additional information whenever necessary. Disagreement about any article's eligibility was resolved by consent.

2.3 Quality assessment

Review of the articles was done to identify if there is any potential systematic error in data extraction. The quality assessment is based on different categories: rationale for research studies, reproducibility, robustness in methodology, and significance of the study. With this criterion, a table was made and assessment was made with Yes, or No corresponding to each category. Agreement and disagreement of rating were discussed and final scores were given to the publication. Moreover, articles with same plant species with similar climatic parameters were grouped into a single set and data extraction were made from those groups. We reviewed reference lists of some review articles with similar heading to assess articles of our interest.

3. Results

The review was conducted in 38 articles with 7 articles published in 2015, 9 in 2016, 6 in 2017, 8 in 2018, 4 in 2019 and 4 in 2020. The articles were from 12 countries (China (n =13), Brazil (n =5), India (n=4), Iran (n=4), Canada (n=1), Saudi Arabia (n=1), Malaysia (n=1), Italy (n=4), Mexico (n=1), Australia (n =1), Germany (n =2) and Egypt (n=1)) analyzing the impacts of climate changes on secondary metabolites of medicinal plants found in the area (Table 3). The review was based on 30 different journals which are considered reputed in medicinal plant study.

Climatic parameters i.e. temperature (n=6), elevated carbon dioxide (n=9), elevated ozone (n=5), light intensity (n=11) and drought stress (n=7) were considered in the study (Fig 2). High-performance liquid chromatography (HPLC), GC-MS, NMR were the most prevalent instruments used for metabolites profiling. Leaves (48%), whole plants (15%), and roots (13%) were commonly used parts for assessing climatic effects. Though the percentage is quite low some researcher also used stem, shoot, flower and fruits.

Both *in vitro* and *in vivo* methods were utilized in most of the plants to have comparative effects of climatic parameters on quality and quantity of SMs. Articles considered in the review showed plants species was extensively used for curing different illness i.e. respiratory tract infection, stomach diseases, cancer, parkinsonism, depression etc. Most of the author considered in the study was a natural product expert with funding support from research institutes and government authorities. The biological analysis of plants used in the study showed the dominant of herbs and sub herbs. Most of the researcher raised concern on climatic issues in the context of medicinal plants and provide a necessary way for getting the better quality and quantity of SMs in medicinal plants which are useful in pharmaceutical industries, food beverages and cosmetic etc. This raised concern to many scientists to work further to have in-depth knowledge on it.

4. Discussion

The environmental factor is the major limiting factor for survival and growth of medicinal plants. Studies showed plant of the same species grown in the different environment have different concentration of a particular secondary metabolite (Radušienė et al., 2013; Ramakrishna and Ravishankar, 2011). This is because the plant has to produce specified quantity and quality of SMs to counter the environmental stress. Thus, the study on each environmental factor is important to know the adaptability and availability of plant in a particular region.

4.1 Temperature stress

Change in temperature affects plant growth and metabolic pathways involved in signalling, physiological regulation and defence responses (Al Jaouni et al., 2018; Pandey et al., 2018). Temperature as major weather variables can significantly influence the composition of SMs with disruption in photosynthesis activities to tolerate stressful condition. For instance, the composition of Alkaloid compounds in Duboisia plants demonstrated a minor increase with temperature (Ullrich et al., 2017). Zhang et al. (2019) indicated an increase in tanshinones accumulation in *Salvia miltiorrhiza* Bunge with an increase in temperature. Likewise, in *Tithonia diversifolia,* there is an increase in phenolic compounds with temperature (Sampaio et al., 2016). A study in global gene regulation of unsaturated fatty acid and jasmonic biosynthesis pathways were deduced in the low temperature in *Camellia japonica* (Li et al., 2016). In contrary, high temperature reduce silymarin content in *Silybum marianum* roots showing biomass and SMs accumulation is a temperature-dependent process (Rahimi and Hasanloo, 2016). Also, Yuan et al. (2020) observed the response of *Dendrobium officina*le in three different cultivation modes namely, wild, bionic and greenhouse. The result showed polysaccharide, total alkaloid and total flavonoid was higher in wild followed by bionic and greenhouse. They found metabolites content decrease with the increase in temperature indicating it as a shade lover and too high temperature is detrimental for growth. Mostly it has been seen from all previous studies that the accumulation of SMs is significantly increased under temperature stress condition (Table 3).

4.2 CO_2 stress

Carbon dioxide is considered as a major greenhouse gas hampering the physiology of medicinal plants. Since the industrial revolution, the concentration of it is increasing rapidly from 270 parts per million (ppm) to 407.4 ppm (World Meteorological Organization and Global Atmosphere

Watch, 2019). Plant adapt to change in environment through metabolic plasticity, however, this affects the SMs which are the basis for their medicinal activity (Yang et al., 2018). For example, *Hypericum perforatum* known for its use in moderate depression was treated with elevated CO_2 and found both growth and biomass to be increased after 140 days compared to ambient conditions. In the same experiment, Hypericin concentration significantly decreases by 22% in elevated CO_2 which further decrease to 19.30% under combined effects of elevated CO_2 and temperature (Sharma et al., 2020). Similarly, phenological stages (bud and flower formation) were advanced by 4 days under 140 days of CO_2 enrichment as compared to ambient condition. A study conducted by Paudel et al. (2016) in *Arabidopsis thaliana* (L.) Heynh found a distinct metabolite signature under elevated CO_2 with a lower concentration of defence compounds such as Glucosinolates. They suggest that changing atmospheric condition and nitrate fertilization may affect plants ability to identify and cope with oxidative stress (e.g. insect damages). Atmospheric CO_2 level and nitrate fertilization play an important role in shaping the constitutive and wound-induced metabolic profile in Arabidopsis leaves. *Paris polyphylla* var. *yunnanensis*, a traditional Chinese medicinal plant showed stronger photosynthetic activity and higher content of bioactive compounds in western Yunnan than in cultivar from central Yunnan under elevated CO_2. In western Yunnan the growth rate increases higher at first and decrease with further CO_2 increase. In contrary, in central Yunnan growth rate is lower at first and increase afterwards suggesting western Yunnan cultivars to be sensitive to atmospheric CO_2 concentration. Diosgenin increase and Pennogenin decrease in both locations showing opposite response with elevated CO_2 for 30 days (Qiang et al., 2020). A study on *Centella asiatica* used as medicinal herbs for its multiple therapeutic properties showed improved photosynthetic efficiency initially with a higher concentration of flavonoids under CO_2 levels at 400 and 800 $\mu mol\ mol^{-1}$. Furthermore, there was an increase in flavonoids concentration in the irradiated plants with rising CO_2 concentration from 400 to 800 $\mu mol\ mol^{-1}$ suggesting *C. asiatica* grown under CO_2 is more capable of overcoming the detrimental impacts of gamma radiation (Siavash Moghaddam et al., 2017). In *Stevia rebaudiana* Bertoni, elevated CO_2 increased Steviol glycosides content, low-calorie sweeteners (Hussin et al., 2017). It was observed that elevated CO_2 enhanced photosynthetic rate and water use efficiency thereby reducing the threat of oxidative stress.

A similar study conducted on *Mentha piperita* L. showed an increase in herbal biomass (48%) and flavonoids concentration with the application elevated CO_2 of 360 ppm and 620 ppm (Al Jaouni et al., 2018). In a typical study on *Hibiscus sabdariffa* var. UKMR-2 elevated CO_2 levels from 400 to 800 µmol mol^{-1} showed an increase in calyx yields and total phenol concentration (Ali et al., 2019). Moreover, it is also predicted that an increase in CO_2 may result in greater height and higher fresh yields than ambient CO_2. Kaundal et al. (2018) subjected *Valeriana jatamansi* Jones to elevated CO_2 levels (550 µmol mol^{-1}) and observed increased in essential oil content by 17.7%. Chemical constituents such as patchouli alcohol, bornyl acetate, β-patchoulene, germacrene D significantly increase at elevated CO_2 and decrease with increasing temperature compared to ambient condition. This finding indicates that elevated CO_2 in future could have positive effects while temperature has negative effects on oil composition in *V. jatamansi*. In vitro study performed with Brazilian ginseng (*Pfaffia glomerata*) showed increased phytoecdysteroid 20-hydroxyecdysone under CO_2 enrichment (1000 µL CO_2L^{-1}). This study was important in demonstrating the best culture conditions and to increase the development and production of 20-hydroxyecdysone in the species (Ferreira et al., 2019). The overall trend in such finding showed important of secondary metabolites of medicinal plants with respects to CO_2 besides seasonal variation, time duration and nutrient availability. This can provide insight into the role of elevated CO_2 in altering the metabolic plasticity of medicinal plants providing appropriate conservatory practices in the long run.

4.3 Ozone stress

Ozone is considered as a bio protector from ultraviolet radiations, however, at ground level, they affect both animals (Jaffe, 1967; J. J. Zhang et al., 2019) and plants (Felzer et al., 2007; Grulke and Heath, 2020). *Hypericum perforatum* (St. John's wort) showed an increment of total phenols and flavonoids with activation of peroxidase activity by ozone (110ppb, 5 hrs.) (Pellegrini et al., 2018). This suggests that ozone can be considered as a potential contributor for enhancing the concentration of several antioxidants which are beneficial properties of medicinal plants. Similarly, a study on ecophysiological and antioxidant traits of *Salvia officinalis* under ozone stress (120 ± 13 ppb for 90 consecutive days) showed an increase in phenolic content; notably Gallic acid (2-fold increase), Caffeic acid (8-fold increase) and Rosmarinic acid (122% increase on 60^{th}

day of treatment) (Pellegrini et al., 2015). *Capsicum baccatum* plants were studied by Brazilian scientist to check effects of chronic ozone exposure, showing a decrease in capsaicin (50%) and dihydrocapsaicin in ozone exposed pericarp (Bortolin et al., 2016). Furthermore, capsaicin content was reduced in the seeds of the plant while no change was observed in dihydrocapsaicin as compared to control plants. Also, total carotenoid and phenolic content in the pericarp increased by 52.8 and 17% respectively. A study on *Melissa officinalis*, a traditional medicinal plant with large number of uses including dementia and anxiety showed an increase in total anthocyanins to a substantial extent along with phenolic and Rosmarinic acid in plants subjected to ozone treatment (200 ppb, 3h) (D'Angiolilloa et al., 2015; Tonelli et al., 2015). Literature search on ozone influence on quality of plants revealed many studies on edible crops while limited specifically related to medicinal plants. Thus, more studies with wide perspectives and plan are needed for depicting the role of this treatment as enhancer of production of secondary metabolites as well as to understand the role of increased oxidative stress conditions on the conservation and management of cultivated as well as spontaneous medicinal plants.

4.4. Light exposure and ultraviolet radiation stress

Light and UV visible radiations are essential for plant metabolism and life due to photosynthesis. Therefore, the survival of plants depends on their ability to sense different light spectra and ultraviolet (UV) light present in the solar radiation (Kazan and Manners, 2011). Also, the light at different intensity impacts on the levels of a broad range of secondary metabolites in the complex biochemical interaction (Kazan and Manners, 2011; Rozema et al., 1997).

A study on plants of the genus Mahonia (*Mahonia bodinieri* (Gagnep.) Laferr and *Mahonia breviracema*) well-known traditional Chinese medicine used for the treatment of tuberculosis, dysentery, periodontitis, pharyngolaryngitis, eczema and wounds showed higher foliar biomass under I_{50} (50% of sunlight) followed by I_{30} (30% of sunlight) giving a higher yield of alkaloids than under I_{10} (10% of sunlight) and I_{100} (Full sunlight) (Kong et al., 2016; Li et al., 2018). Therefore, I_{30} and I_{50} were not only beneficial to increase biomass but also suitable for synthesis and accumulation of SMs indicating noticeable effects of photoperiod and light intensity. Rarely, the opposite situation was also reported like in *Flourensia cernua*, a Mexican traditional medicine used to treat indigestion, respiratory tract infection, tuberculosis showed higher total phenolic compounds under partial shade than on fully irradiated conditions (Estell et al., 2016). In some plants, the higher irradiance is helpful for plant growth and SMs production (Zhang et al., 2015). For example, the amount of scutellarin (flavone glycoside) in *Erigeron breviscapus* and chlorogenic acid (phenols) in *Centella asiatica* (L.) Urb was higher in sun-developed leaves than in shade-developed leaves (Alqahtani et al., 2015; Zhou et al., 2016). Both this species (*E. breviscapus* and *C. asiatica*) are well known for their medicinal applications and understanding the role of UV irradiation can be crucial for selecting proper cultivation sites, as well as for the collection of spontaneous populations. *Hyptis marrubioides*, a medicinal plant used against gastrointestinal infection was cultured *in vitro* supporting the culture using different wavelengths (white, blue, green, red, and yellow) (Pedroso et al., 2017). They found white and blue lights promote the flavonoids rutin accumulation, whereas red light induces plant growth and increase leaf number and dry weights. This provides a theoretical basis for studies related to quality control of growth and production of secondary metabolism of *H. marrubioides*. Also, this aspect strongly shows the influence of different latitude and/or potential cultivation conditions on the medicinal plants. Furthermore, it opens a significant potential role in the cultivation of such species in indoor systems where light exposure can be controlled by specific lamps and systems.

Ultraviolet (UV) light is also an important abiotic factor which stimulates the production of secondary metabolites, and hence many studies were conducted considering the factor. For example, the concentrations of flavonoids and phenolic acids increased in response to increasing UV-B radiation of Chrysanthemum (Ma et al., 2016). Similarly, a study on *Coleus forskohlii* leaves by Takshak and Agrawal (2015) showed an increase in flavonoids and phenolic under UV-B stress. When the plant *Prunella vulgaris* L. Spica were irradiated with UV-B, the production of total flavonoids, rosmarinic acid, caffeic acid was enhanced (Chen et al., 2018). However, these contents differ in development stages and best harvest stage was between budding and full-flowering for the best medicinal values. Nascimento et al (2015) found that the level of phenolic profile and flavonoids content in *Kalanchoe pinnata* leaves increase in response to UV-B irradiation. The content was further increased in the combination of white light providing higher diversity of phenolic compound and a larger amount of quercitrin. These studies highlighted the importance of photoperiod and light intensity for photosynthesis, growth and accumulation of secondary metabolites in medicinal plants. Thus, medicinal properties yield might be achieved by proper adjustment of light quality and quantity in future.

4.5 Drought stress

Drought stress is an important environmental factor affecting the content of secondary metabolites in plants. A larger number of studies manifested that plants exposed to drought stress accumulate higher concentration of secondary metabolites than those cultivated under well-watered conditions (Kleinwächter and Selmar, 2015).

In *Scutellaria baicalensis* Georgi, a traditional medicinal herb mild drought stress increase baicalin but decrease under severe stress (Cheng et al., 2018). This result demonstrates that an appropriate degree of drought stress may promote baicalin accumulation by stimulating the expression and activities of the key enzymes involved in the biosynthesis of the compound. Therefore, for appropriate protective enzyme activity and increase baicalin content, *Scutellaria baicalensis* soil moisture should be properly controlled. A similar study was conducted on *Glycyrrhiza glabra* L. at slight, moderate and intense drought (Hosseini et al., 2018). It showed drought promoted the synthesis of glycyrrhizin with an increase in glycyrrhizin biosynthesis pathway. But, a large decrease in root biomass at extreme drought condition led to a general decrease in the amount of glycyrrhizin content compared to slight and moderate drought. Further, they found glycyrrhizin yields in response to drought differ between genotypes of plants. Rastogi et al., (2019) found *Ocimum tenuiflorum* was able to sustain the severe drought for 30 days. They provide insight into transcriptomic changes so we can identify the putative genes of medicinal plants. Alhaithloul et al. (2020) reported that in *Mentha piperita* and *Catharanthus roseus* drought stress decrease total phenols, flavonoids and saponin content, however, level of other SMs including tannins, terpenoids and alkaloids increased under stress in both plants. In contrast to this, Liu et al. (2017) demonstrated an increased accumulation of alkaloids and the expression of genes regulating secondary metabolite accumulation in drought-stressed *Catharanthus roseus.* There was a study by Ashrafi et al. (2018) on *Thymus vulgaris* (drought-sensitive) and *Thymus Kotschyanus* (drought-tolerant species) under different drought condition. In both the plants, sugars, amino acids, and energy metabolism were significantly affected by drought stress thus, new technologies such as transcriptomics and proteomics could be helpful to understand in-depth knowledge on enhanced drought tolerance. Zhang et al. (2017) showed the root biomass, total saponin content increased at first and decreased afterwards in *Stellaria dichotoma* L. var. lanceolata Bge. Thus, moderate water stress was suitable for roots biomass formation and active ingredient accumulation which was affected by endogenous hormones and water status. These all studies revealed the concentration of SMs in plants significantly differ under different drought stress condition. However, there are still few researches on medicinal plants and need more studies.

5. Conclusion

Medicinal plants are rich sources of chemically active constituents which are used as raw materials in nutraceutical, fragrance, dyes, cosmetic and pharmaceutical. The constituents commonly known as SMs are used for adaptation by the plant during stress condition such as temperature, carbon dioxide, ozone, light and drought. These abiotic stresses not only modify plant structurally and anatomically but also lead to fluctuation in their antioxidant quantities. Thus, knowledge of abiotic stress and SMs help to protect the plant sources which are under pressure due to excessive exploitation. In this review, we have provided evidence on how secondary metabolites show diversified and changeable responses to different environmental stresses. Interestingly, we have found individuals stress selectively alter the content of several SMs in plants. This clearly showed the synthesis of natural products can be altered by different abiotic stresses. However, further research is needed at a molecular level to understand a synergistic effect of multiple environmental factors using new techniques such as metabolomics, proteomics and transcriptomics for improvement of the growth and productivity of plants.

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Tables

Table 1: Search manual used

Search term used	PubMed	Google Scholar	ScienceDirect
Secondary metabolites and medicinal plants	59	85	17
Medicinal plants and climate change	6	39	3
Secondary metabolites and temperature	28	9	12
Total	93	133	32

Table 2: The inclusion and exclusion criteria

S. N	**Criterion**	**Eligibility**	**Exclusion**
1	Literature type	Research articles (journal)	Review journal articles, book, book chapter, book series, conference paper, report, proceeding
2	Language	English	Non-English
3	Timeline	Between 2015 and 2020	<2014
		Full-length paper peer-reviewed journal	Published abstract

Table 3: Publications identified in the main databases (PubMed, Google Scholar and ScienceDirect) through systematic review

Country	Plants	Parts Used	Secondary Metabolites	Environment Factor	Concentration Change	References
Germany	*Duboisia myoporoides*	Whole plant	Alkaloids	Temperature	Increase	(Ullrich et al., 2017)
China	*Salvia miltiorrhiza* Bunge	Roots	Tanshinone	Temperature	Increase	(C. Zhang et al., 2019)
Brazil	*Tithonia diversifolia*	Leaf, stems	Phenols	Temperature	Increase	(Sampaio et al., 2016)
China	*Camellia japonica*	Leaf	α-linolenic acid, Jasmonic acid (Fatty acid)	Low temperature	Increase	(Li et al., 2016)
Iran	*Silybum marianum* (L.) Gaertn	Roots	Silymarin	Temperature	Decrease	(Rahimi and Hasanloo, 2016)
China	*Dendrobium officinale*	Stems	Total alkaloids and Total flavonoid	Temperature	Decrease	(Yuan et al., 2020)
India	*Hypericum perforatum* L.	Flowers and Fruits	Hypericin	Elevated carbon dioxide [CO_2]	Decrease	(Sharma et al., 2020)
Canada	*Arabidopsis thaliana*	Leaf	Glucosinolates	Elevated carbon dioxide [CO_2]	Decrease	(Paudel et al., 2016)
China	*Paris polyphylla* var. yunn anaensis	Rhizome	Saponins (Diosgenin and Pennogenin)	Elevated carbon dioxide [CO_2]	Increase	(Qiang et al., 2020)
Iran	*Centella asiatica*	Leaf	Flavonoid	Elevated carbon dioxide [CO_2]	Increase	(Siavash Moghaddam et al., 2017)
Germany	*Stevia rebaudiana* Bertoni	Whole plant	Steviol Glycosides	Elevated carbon dioxide [CO_2]	Increase	(Hussin et al., 2017)
Saudi Arabia	*Mentha piperita* L	Shoots	Flavonoids	Elevated carbon dioxide [CO_2]	Increase	(Al Jaouni et al., 2018)

Malaysia	*Hibiscus sabdariffa*	Calyx	Total phenolic and Total Anthocyanins	Elevated carbon dioxide [CO_2]	Increase	(Ali et al., 2019)
India	*Valeriana jatamansi* Jones	Roots	Essential oil (patchouli alcohol, bornyl acetate, β-patchoulene, germacrene D)	Elevated carbon dioxide [CO_2]	Increase	(Kaundal et al., 2018)
Brazil	*Pfaffia glomerata* (Brazilian ginseng)	Shoots	Saponins and Phytosteroids	Elevated carbon dioxide [CO_2]	Increase	(Ferreira et al., 2019)
Italy	*Melissa officinalis*	Shoots	Total carotenoids	Elevated Ozone	Increase	(D'Angiolilloa et al., 2015)
Italy	*Hypericum perforatum*	Leaf	Total phenols and flavonoid	Elevated Ozone	Increase	(Pellegrini et al., 2018)
Italy	*Salvia officinalis*	Leaf	Phenols (Gallic acid, Caffeic acid, Rosmarinic acid)	Elevated Ozone	Increase	(Pellegrini et al., 2015)
Brazil	*Capsicum baccatum* L. var. pendulum	Fruit	Capsaicin and dihydrocapsaicin	Elevated Ozone	Decrease	(Bortolin et al., 2016)
Italy	*Melissa officinalis*	Shoots	Rosmarinic acid	Elevated Ozone	Increase	(Tonelli et al., 2015)
China	*Mahonia bodinieri* (Gagnep.) Laferr	Whole Plants	Alkaloids	Light intensity	Increase	(Kong et al., 2016)
China	*Mahonia breviracema*	Leaf, Stem and Root	Alkaloids	Light intensity	Increase	(Li et al., 2018)
Brazil	*Hyptis marrubioides* Epling	Leaves	Flavonoid: Rutin	Light intensity	Increase	(Pedroso et al., 2017)
Mexico	*Flourensia cernua* DC	Leaf	Total phenolic	Light intensity	No effects	(Estell et al., 2016)
China	*Glechoma longituba* (Nakai) Kupr	Leaves	Ursolic and Oleanolic acid	Light intensity	Decrease	(Zhang et al., 2015)

China	*Erigeron breviscapus*	Leaf	Phenols: scutellarin	Sunlight	Increase	(Zhou et al., 2016)
Australia	*Centella asiatica*	Whole plant	Phenols: Chlorogenic acid	Full sunlight	Increase	(Alqahtani et al., 2015)
China	*Chrysanthemum*	Flower	Phenols (Phenolic Acid)	Ultraviolet-B Radiation	Increase	(Ma et al., 2016)
India	*Coleus forskohlii*	Leaves	Flavonoids and Phenolics	Ultraviolet-B Radiation	Increase	(Takshak and Agrawal, 2015)
China	*Prunella vulgaris* L. Spica	Whole plant	Total flavonoids, Rosmarinic acid, caffeic acid	Ultraviolet-B Radiation	Increase	(Chen et al., 2018)
Brazil	*Kalanchoe pinnata+F17*	Leaves	Phenolic Profile and Flavonoid Content	Ultraviolet-B Radiation	Increase	(Nascimento et al., 2015)
China	*Scutellaria baicalensis*	Whole plant	Baicalin (Phenols)	Drought stress	Increase	(Cheng et al., 2018)
Egypt	*Mentha piperita*	Leaves	Phenol and Flavonoid	Drought stress	Decrease	(Alhaithloul et al., 2020)
Iran	*Thymus vulgaris* and *Thymus kotschyanus*	Seed, Leaf	Malic acid and succinic acid, acetic acid, citric acid, fumaric acid	Drought stress	No effects	(Ashrafi et al., 2018)
Iran	*Glycyrrhiza glabra* L. (licorice)	Roots	Glycyrrhizin	Drought stress	Increase	(Hosseini et al., 2018)
China	*Stellaria dichotoma* L. var. lanceolata Bge	Leaves and roots	Total saponins, Total flavonoid	Drought stress	Increase	(Zhang et al., 2017)
India	*Ocimum tenuiflorum*	Leaves	Phenol: Eugenol	Drought stress	Decrease	(Rastogi et al., 2019)
China	*Catharanthus roseus*	Leaves	Alkaloid	Drought stress	Increase	(Liu et al., 2017)

Figures

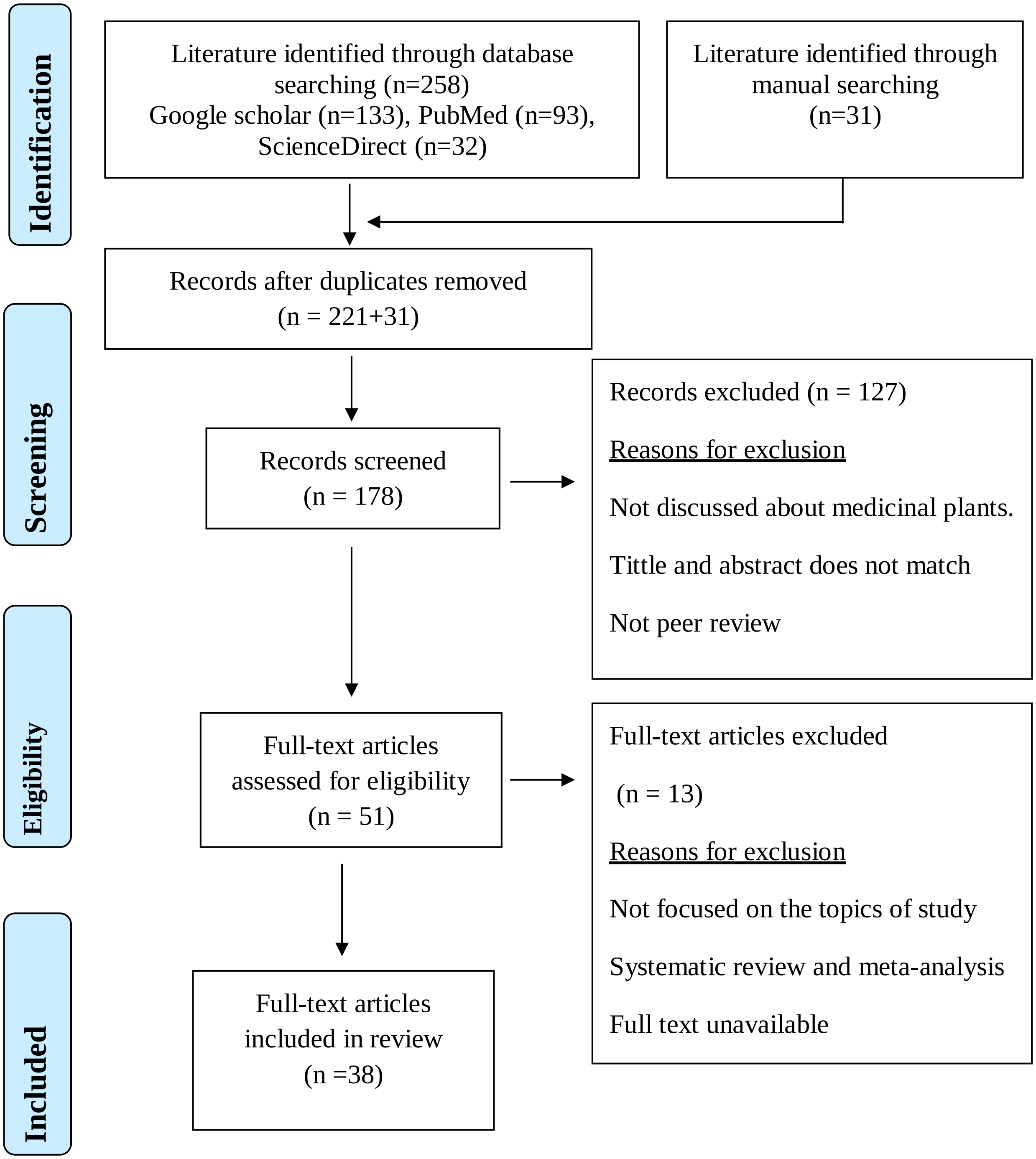

Fig 1: Preferred reporting items for systematic reviews and meta-analyses (PRISMA) flow diagram of the study

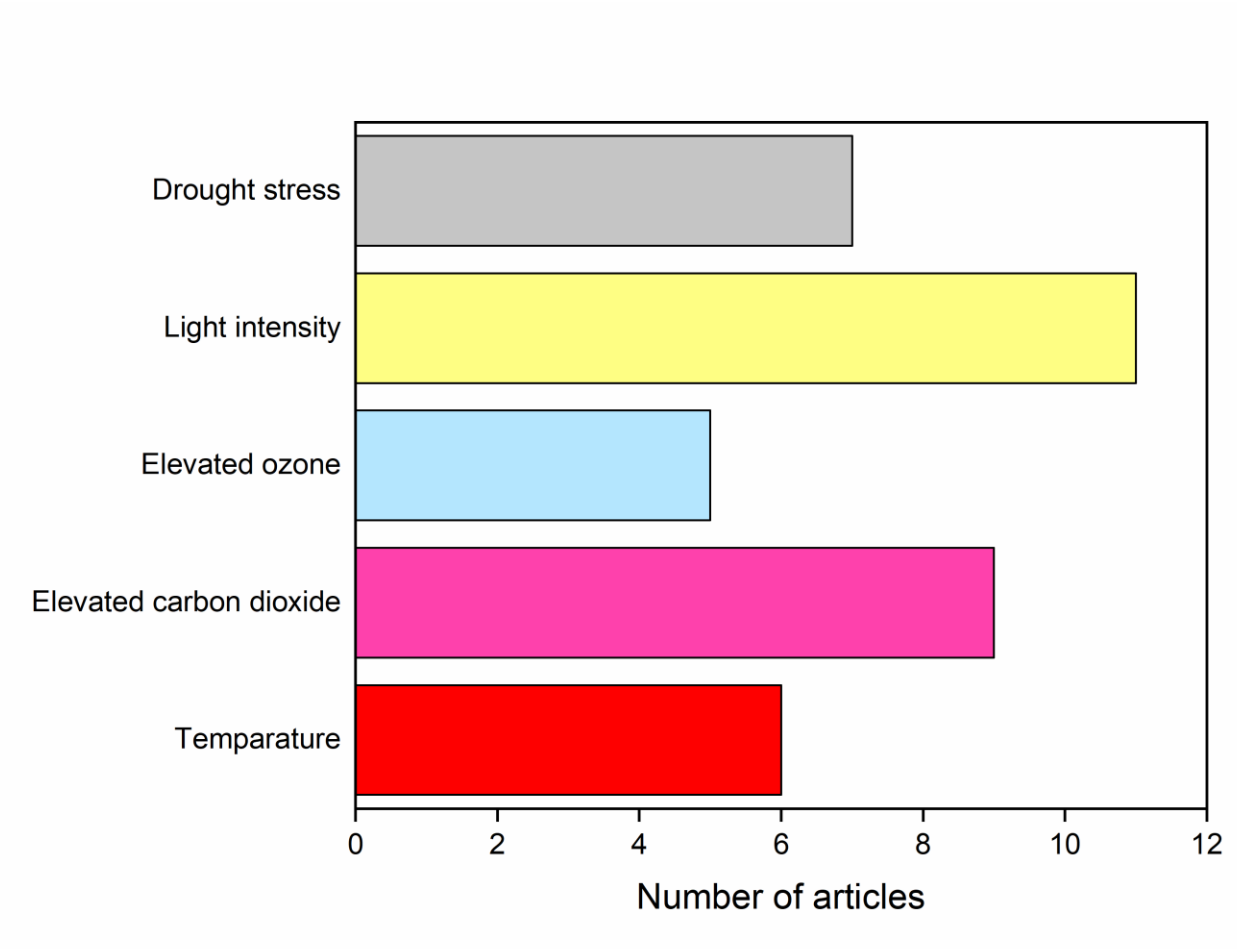

Fig. 2: Climatic parameters considered in the study with the number of articles on assigned topics

CHAPTER 2 - Climatic influence on tree wood anatomy – a review

Abstract

Effects of climate changes on physiology and wood anatomy are reported as a wide response to rising temperature, increase carbon dioxide and erratic precipitation on high altitude. Studies suggest that climate change is already having negative impacts on plant resources modifying their function such as nutrient cycling and carbon sequestration. Moreover, change in climatic parameters affects structure, function and diversity of plants specifying the importance of understanding these processes. Here, we review and synthesize literature on the influence of climatic parameters on wood anatomy at high altitude using recent (2015-2020) published research on the topic. The literature review showed an increasing trend of research on high altitude plant covering tree physiology and wood anatomical factors. The change was observed in high altitude plant in their growth, migration (to high and/or to low altitude), forest community structure. These changes can alter the functional composition of plant communities through disruption in the hydrological cycle, nutrients cycling and carbon storage. However, more research covering holistic approach on mountain tree communities is necessary to understand the key knowledge gaps that are of utmost importance for future research in the mountain ecosystem.

Key-words: climate change, high altitude, tree physiology, wood anatomy,

1. Introduction

Climate change is the biggest challenge of this century and is exerting pressure on both plants and animals (Root et al., 2003; Willis, 2017). Increase in global temperature (≈0.2ºC/decade) along with the rise in CO_2 (2.5+/-0.8 ppm/year) in the atmosphere with the change in precipitation regime may change the structure and function of trees (IPCC, 2014; Menezes-Silva et al., 2019; Vennetier et al., 2013). This climatic change interacts with trees during their life leaving a permanent imprint in the woody tissues. Therefore, trees are considered as most valuable natural archives of past environmental conditions (Bradley, 2011; Franke et al., 2013; Swetnam and Brown, 2011) showing a unique source for annual variability in forest biomass and carbon allocation (Babst et al., 2014; Wimmer, 2002). Thus, there is increasing interest of researcher in analyzing tree-ring characteristic to better understand tree growth responses to environmental variability and extreme events (Battipaglia et al., 2014; Carrer et al., 2017; Pandey et al., 2018; Puchi et al., 2020; Rossi et al., 2011). Recent advancement in dendroecology, namely dendroanatomy aim at deciphering the effects of climate on the whole xylogenetic process, i.e. complex process of differentiation and division of cambium with environmental input and formation of new woody tissues (Fukuda, 1996; Liang et al., 2016; Rossi et al., 2012). Dendroanatomy is defined as the analysis of xylem-cell features along dated tree-rings which provide a long term perspective on wood formation process (Fonti et al., 2010). With the advancement in sample processing and image, analysis allows studying the detailed measurement of multiple traits in wood with longer time (Prendin et al., 2017; von Arx and Carrer, 2014). This allows researcher in studying inter-annual variability of vessels/tracheids lumen area or cell wall thickness retrospectively analyzing the cambium phenology with changing environment (Carrer et al., 2017; Castagneri et al., 2017a; von Arx et al., 2016).

Afterwards, many studies have explored long term effects of climate variability on the corresponding year to year change in wood anatomical traits and its consequence for Gymnosperm (Lange et al., 2020; Pacheco et al., 2016; Puchi et al., 2020) and Angiosperm (Castagneri et al., 2017b; Pandey et al., 2018) physiology and growth. For instance, some authors reported a drought decrease lumen area leading to declining in stem hydraulic conductivity (Liang et al., 2013; Olano et al., 2014) whereas other found the opposite (Eilmann et al., 2009; Martin-Benito et al., 2017). Two dominant Mediterranean shrub species, *Erica multiflora* and *Globularia alypum* grown in same drought conditions showed contrasting phenology indicating its effects to be species-specific (Bernal et al., 2011; Llorens et al., 2004). Researchers also found some tree species in extreme drought conditions produce intra-annual density fluctuation (Pacheco et al., 2016; Zalloni et al., 2016) or distorted and collapsed cells (Arend and Fromm, 2007), early cessation of cell division (Eilmann et al., 2011) and light rings characterized by narrow latewood brand (Liang et al., 2006). Drought stress indicates missing rings in Himalayan species (*Betula utilis* D. Don) (Liang et al., 2014; Pandey et al., 2018) and Tibetan species (alpine Juniper shrub) (Liang et al., 2012) providing compelling evidence of moisture-triggered xylogenesis. Restricted water availability is not only limiting factors for growth in warm and dry areas but also low-temperature control growth at high latitudes and elevation (Rossi et al., 2013).

Elevated carbon dioxide in ring-porous species showed an increase conduit size in earlywood and wood density due to higher proportion of latewood (Domec et al., 2010) whereas in coniferous species there is an increase in radial growth but not a large change in wood density and tracheids dimensions (Mccarthy et al., 2007; Watanabe et al., 2016). This shows climatic parameters impacts in xylem anatomy of trees and ultimately determine its future scenario. Yet, more studies are needed focusing on different species from different geographical regions.

In this review, we briefly present an overview of inter and intra-annual variation in wood structure in response to climatic parameters. We particularly focus on dendroecology-based xylem anatomy to assess the state of the art in the emerging field and future research. The paper discusses how changing climatic parameters have interactive effects on tree growth, xylem anatomy and wood density of trees from different geographical regions. Lastly, it gives an idea about the research conducted till now and way forward specifying the research gaps

2. Materials and methods

The literature search was done using an electronic database, google scholar and web of science using the term wood density and climate; xylem anatomical traits and climate; radial growth and climate as keywords. The data were considered valid if any of the above terms appear in the title of a downloaded record. The database was used as they provide the core collection and also contain the most relevant and influential journal in its records (Olawumi and Chan, 2018). Publications obtained from databases were selected based on different criteria i) published from 2015 to 2020

ii) published in English iii) exclusion of book, review articles and report iv) consider only articles which include at least one tree-ring chronology and analyze the relationship with instrumental climate data. Duplication of articles obtained from different databases was removed. A manual search of the related articles was done to find if there are any articles missed. Using the combined search approach, 240 relevant articles were identified. However, 28 articles were selected based on geographical region and wood anatomical parameters. The selected articles based on the criteria were imported into Mendeley databases software for proper processing.

3. Results

The studies included 25 species (14 angiosperm and 12 gymnosperms) from 8 families (Table 1). There were six articles on wood density, 14 on xylem anatomy and 8 on radial growth making a total of 28 articles. Studies showed climatic parameters, temperature and precipitation as main driving factors for growth and development of plants. The response differs based on origin, climatic condition, the orientation of tree species. There was a more apparent response by gymnosperm species and angiosperm showed some tolerance to climatic changes.

4. Discussion

4.1 Climate change and wood anatomy

Wood is a porous and fibrous structural tissue found in the stems and roots of trees and woody plants. A tree is composed of a bundle of vessels (for transporting food and waste product within trees) and its wall composed of cellulose glued together with lignin. These woods are classified based on cellular structure as softwood (derived from conifer tree – Gymnosperm) and hardwood (derived from broad-leaved trees – Angiosperm). The name as hardwood and softwood does not depend on the softness of material but based on presence of pores in cellular structure in timber (hardwood – the presence of pores; softwood – absent of pores) (Fig. 1).

These wood structures are affected with synergic effects of endogenous (genetic) and exogenous (environment) factor (Downes et al., 2009; Downes and Drew, 2008a). Therefore, it is necessary to critically evaluate and discuss the effects of ongoing environmental changes to wood anatomical traits. Wood anatomical traits include radial growth, wood density, fibre or tracheid length and microfibril angle (Barnett and Jeronimidis, 2003). Radial growth (ring width, earlywood width, latewood width, latewood proportion) and wood density (ring average density, earlywood density, latewood density) are considered as an important trait in assessing wood quality.

4.2 Radial growth

Radial growth is defined as the ability to grow in girth by the formation of wood. The average growth increment in diameter, represented by the annual tree-ring, is defined as the rate of radial growth, representing the volume of wood. This growth in the plant is the result of both endogenous (physiological, genetic factors) and exogenous factors (climatic parameters) (Downes and Drew, 2008b; Lenz et al., 2010). For instance, the climate-growth responses of Himalayan treeline are spatiotemporally and species-specific (Schickhoff et al., 2015). Studies in western and central Himalaya have revealed a strong sensitivity of tree growth to pre-monsoon temperature and humidity conditions (Gaire et al., 2019; Pandey et al., 2018; Panthi et al., 2017; Schickhoff et al., 2015). This study signifies higher winter temperature caused earlier snowmelt and increased water supply for growth. Marques et al. (2016) study two Iberian pine species (*Pinus sylvestris* and *Pinus nigra*) subjected to Mediterranean conditions in Eastern Spain and found *P. sylvestris* growth was enhanced by warm spring temperature and *P. nigra* growth was improved by positive spring water balance. Similarly, silver fir (*Abies alba* Mill.) showed negative effects with summer drought and positive effects with spring temperature of current years (Latreille et al., 2017). Black spruce (*Picea mariana* [Mill.] B.S.P.) from Alaska suffer from drought stress during warm, dry summers due to moisture stress from topography and seasonality of drought (Wolken et al., 2016). South American species (*Pilgerodendron uviferum* and *Nothofagus pumilio*) radial growth is most strongly associated with warm spring-early summer and reduced spring precipitation (Álvarez et al., 2015; Holz et al., 2018). These studies indicated that best radial growth predictor is temperature and precipitation which seems to have dominant effects on growth responses. Similar, studies help to understand how global warming may influence tree growth.

4.3 Xylem anatomical traits' association with climate

Xylem is vascular tissues that can transport water and dissolved minerals from the soil to leaves and to support tree structure. The anatomy of xylem affects functional properties such as hydraulic safety and efficiency (Lachenbruch and Mcculloh, 2014; Schuldt et al., 2016). Therefore, changes in xylem anatomy strongly determine trees performance, survival and their capacity to fix carbon (Pandey et al., 2020; Sperry and Love, 2015). Xylem is a complex tissue which is composed of tracheids (narrow cells), vessels (wider cells), xylem parenchyma (live plant cells) and xylem fibres (dead plant cells) (von Arx et al., 2016; Wimmer, 2002).

Castagneri et al. (2017a) found tracheids of *Picea abies* from Italian Alps get influenced by climate conditions in the growing season. They found that early-summer temperature affects cell enlargement at higher elevation whereas at lower elevation water availability helped in enlargement of the cell. Xylem hydraulic traits of native *Quercus robur* L. was more sensitive to previous-summer drought than those of alien *Robinia pseudoacacia* L. This suggests that *R. pseudoacacia* L. might be more competitive under future drier conditions (Nola et al., 2020). Two deciduous species namely *Quercus ithaburensis* and *Quercus boissieri* from the southeastern Mediterranean showed abundant precipitation and low temperature from November to April benefit the xylem formation (Castagneri et al., 2017b). They dry years strongly limit vessels size and number in *Q. ithaburensis* compared to *Q. boissieri*, making one as high resilience and other as high resistance. *Betula nana* L vessels lumen area from western Greenland showed influence by spring and summer temperature whereas, vessels grouping was driven by winter temperature (Nielsen et al., 2017). Similarly, Hollesen et al (2015) documented that *Betula nana* growth is positively influenced by winter temperature as important as summer.

Lange et al (2020) studied *Picea glauca* (Moench) Voss lumen area and cell wall thickness and found higher sensitivity to drought indicating a plastic adaptation to shift in growth-limiting conditions mainly stabilizing latewood cells. A study on *Betula utilis* D. Don from high altitude forest of Nepal showed Vessels areas to be positively associated with March precipitation and fibres area negatively correlated with temperature during the previous and current season (Pandey et al., 2018). This showed that fibres get narrower when vessels are wider. A study on black spruce (*Picea mariana*) from the boreal forest of Canada showed cell number and cell wall thickness to be positively affected by spring and summer daily mean and maximum temperature at northern sites. Moreover, they found latent impacts of water availability on xylem traits (Puchi et al., 2020). A similar study was conducted by Rosner et al (2016) on Norway spruce (*Picea abies*) which showed trees from the coldest site was thinner with largest lumen area indicating the species to be probably less resistant under extreme climatic events. Also, Norway spruce from Italian Alps, Slovenia and the Czech Republic showed lumen dimension of earlywood tracheids to be positively affected by precipitation in the previous autumn and early summer of the current growing season (Castagneri et al., 2015; Gricar et al., 2015). Carrer et al. (Carrer et al., 2017) investigated wood anatomical traits of two high elevation conifers (*Larix decidua* Mill., *Picea abies* (L.) Karst.) and found xylogenesis in the species benefits from warm temperature depending on the timing of cell production. Studies on wood anatomy of *Embothrium coccineum* (Evergreen) and *Nothofagus antarctica* (Deciduous) from southern Chile showed an increase in vessels density with dryness without changes in estimated hydraulic conductivity (García-Cervigón et al., 2018). Another Chilean deciduous broadleaf tree *Nothofagus pumilio* (Poepp. et Endl.) Krasser showed an increase in vessels density and decrease in vessels composition with lower temperature, however, the xylem specific hydraulic conductivity remained constant across elevation and latitude (García-Cervigón et al., 2020). Himalayan treeline species *Betula utilis* D. Don showed a strong dependency on spring precipitation whereas *Abies spectabilis* D. Don with summer temperature showing contrasting response to secondary growth with the climatic regime (Pandey et al., 2020; Sigdel et al., 2018).

Certain environmental events will affect the normal activities of cambium. Intra-annual density fluctuation (IADFs) are layers of cells within tree rings identified by different shape, size and wall thickness with the characteristic of latewood like cells in earlywood or earlywood like cells in latewood. Formation of IADFs can be considered as a strategy of trees to adjust wood anatomical traits to short term variation in environmental condition maintaining the balance between hydraulic efficiency and safety against embolism. Several dendrochronological studies reported high IADFs frequency in species growing in Mediterranean area which is considered one of the vulnerable regions to climate change (Pacheco et al., 2016; Zalloni et al., 2016). Also, ray parenchyma of *Juniperus thurifera* showed its interlink to climatic conditions at critical stages during the xylogenetic process (Olano et al., 2013). In conclusion, we can observe environmental factors have an important effect on xylem anatomical traits indicating their great potential for dendroecological studies.

4.4 Wood density and climate

Wood density (WD) is the basic measurement of the content of dry biomass within the green (or fresh) volume of a tree. It is related to trees ability to withstand embolism and provides important ecological and physiological insights for foresters, ecologist and physiologist. Tree species allocate a different amount of carbon to produce xylem structure and the trade-off between the allocation of biomass and cost associate in its production determine the quality of wood. Wood density has been linked with different physiological and ecological parameters i.e. the difference in mechanical properties of wood, hydraulic properties of xylem, defence against attack by pathogens, canopy architecture and the ratio of leaf area to stem cross-sectional area (Chave et al., 2009). Wood density is also affected by recent climatic changes producing an effect on wood structure and quality. The analysis of wood density-climate relationship showed that the mean density of *Pinus halepensis* was negatively correlated with spring temperature and with precipitation during the previous winter and spring (Olivar et al., 2015a). A survey done in 175 species located in dry, mesic and humid forests of New Caledonia showed a positive relation between wood density and aridity; but some species in dry sites showed low wood density (Ibanez et al., 2017). A study conducted on Mexican *Pinus Cooperi* showed a higher density of wood and basal area with higher accumulation of carbon. Also, wood density showed a positive correlation with precipitation and negative with temperature during the spring season (Pompa-García and Venegas-González, 2016). *Facus sylvatica* radial growth and wood density was found to be negatively influenced by drought and positively by water availability (Vannoppen et al., 2018). A researcher from Portugal revealed both wood radial growth and density highly benefit from the strong decay of cold days and increase with minimum temperature (Kurz-Besson et al., 2016). Camarero and Gutierrez (2017) found mixed responses of intra-annual width and wood density to climate. They found minimum wood density negatively responded to spring precipitation particularly in dry sites and positively with mean maximum temperature and sunshine duration during late summer and early autumn. This shows that wood density which is an indicator of wood quality can be triggered by changing climatic parameters.

5. Conclusion

Climate warming is shown to be more apparent in high altitude impacting tree growth and xylem anatomical traits. Therefore, any change in xylem anatomy and growth would influence the quality of wood. However, many factors should be considered when studying the effects of climate warming. The review showed that the formation of xylem is more sensitive to environmental factors. This sensitivity depends on species like deciduous species did not show a significant difference in wood structure however, evergreen species showed higher variation indicating a higher sensitivity to varying climatic conditions. This study provides special issues representing the importance of a multidisciplinary approach in wood science to provide an answer to a question related to tree performance under changing environmental conditions. This provides knowledge on climate-growth relationship from field to laboratory experiment which can be used for conservation of forest health, wood biomass and wood quality.

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Table 1: Articles included in the study

Country	Name of plant	Family	Plant type	Measured parameter	Environmental parameter considers	References
Spain	*Pinus halepensis*	Pinaceae	Gymnosperm	Wood density and tree ring width	Temperature and Precipitation	(Olivar et al., 2015b)
Mexico	*Pinus cooperi*	Pinaceae	Gymnosperm	Wood density	Temperature and precipitation	(Pompa-García and Venegas-González, 2016)
Belgium	*Fagus sylvatica* and *Quercus petraea*	Fagaceae	Angiosperm	Wood density and radial growth	Drought and water availability	(Vannoppen et al., 2018)
Portugal	*Pinus pinaster* L. var. *maritima* Aiton	Pinaceae	Gymnosperm	Wood density	Temperature	(Kurz-Besson et al., 2016)
Spain	*Abies alba*	Pinaceae	Gymnosperm	Wood density	Temperature and precipitation	(Camarero and Gutiérrez, 2017)
New Caledonia	*Pisonia gigantocarpa*	Nyctaginaceae	Angiosperms	Wood density	Temperature and precipitation	(Ibanez et al., 2017)
Italy	*Picea abies*	Pinaceae	Gymnosperm	Xylem anatomical traits (Tracheids)	Temperature and precipitation	(Castagneri et al., 2017a)
Italy	*Robinia pseudoacacia L.* and *Quercus robur L.*	Fabaceae Fagaceae	Angiosperms	Xylem hydraulic traits (Vessels)	Temperature and Precipitation	(Nola et al., 2020)
Italy	*Quercus ithaburensis* and *Quercus boissieri*	Fagaceae	Angiosperms	Xylem hydraulic (Vessels)	Drought	(Castagneri et al., 2017b)
Greenland	*Betula nana L.*	Betulaceae	Angiosperms	Xylem anatomical traits (vessels)	Temperature and precipitation	(Nielsen et al., 2017)
Greenland	*Betula nana L.*	Betulaceae	Angiosperms	Xylem anatomical traits (vessels)	Temperature and precipitation	(Hollesen et al., 2015)
North America (Alaska and Canada)	*Picea glauca* (Moench) Voss	Pinaceae	Gymnosperm	Xylem anatomical traits (lumen area and cell wall thickness)	drought	(Lange et al., 2020)

Nepal	*Betula utilis* D. Don	Betulaceae	Angiosperms	Xylem anatomical (Vessels and fibres)	Temperature and Precipitation	(Pandey et al., 2018)
Canada	Black spruce (*Picea mariana*)	Pinaceae	Gymnosperm	Wood anatomical traits (CWT and tracehids size and umber)	Temperature and Precipitation	(Puchi et al., 2020)
Norway	Norway spruce (*Picea abies*)	Pinaceae	Gymnosperm	Xylem anatomical traits (Cell wall)	Climatic events extreme (drought)	(Rosner et al., 2016)
Slovenia	Norway spruce (*Picea abies*)	Pinaceae	Gymnosperm	Cell Lumen area	Temperature and Precipitation	(Gricar et al., 2015)
Italian Alps	Norway spruce (*Picea abies*)	Pinaceae	Gymnosperm	Cell Lumen area	Temperature and Precipitation	(Castagneri et al., 2015)
Italian Alps	*Larix decidua* Mill. and *Picea abies* (L.) Karst.	Pinaceae	Gymnosperm	Xylem anatomy	Temperature	(Carrer et al., 2017)
Chilean	Nothofagus pumilio (Poepp. et Endl.) Krasser	Nothofagaceae	Angiosperms	Xylem anatomy (Vessels)	Temperature and moisture	(García-Cervigón et al., 2020)
Chile	*Embothrium coccineum* (Evergreen) and *Nothofagus antarctica* (Deciduous)	Proteaceae Nothofagaceae	Angiosperms	Xylem anatomy (Vessels	Precipitation	(García-Cervigón et al., 2018)
Nepal	(Betula utilis D. Don) and Himalayan fir (Abies spectabilis (D. Don	Betulaceae Pinaceae	Angiosperms Gymnosperm	Tree growth	Temperature	(Sigdel et al., 2018)
Nepal	(Betula utilis D. Don) and Himalayan fir (Abies spectabilis (D. Don	Betulaceae Pinaceae	Angiosperms Gymnosperm	Carbon and Oxygen isotope	Temperature and Precipitation	(Pandey et al., 2020)
Spain	*Juniperus thurifera* L., Cupressaceae)	Cupressaceae	Gymnosperm	IADFs	Temperature and Precipitation	(Pacheco et al., 2016)

Spain	*Pinus sylvestris* and *Pinus nigra*	Pinaceae	Gymnosperm	Radial growth	Temperature and Precipitation	(Marqués et al., 2016)
Alaska, USA	Black spruce (*Picea mariana* [Mill.] B.S.P.)	Pinaceae	Gymnosperm	Radial growth	Drought	(Wolken et al., 2016)
France	silver fir (*Abies alba* Mill.)	Pinaceae	Gymnosperm	Radial growth	Drought and Temperature	(Latreille et al., 2017)
Chile	*Nothofagus pumilio*	Nothofagaceae	Angiosperms	Radial growth	Temperature and Precipitation	(Álvarez et al., 2015)
South America	*Pilgerodendron uviferum*	Cupressaceae	Angiosperms	Radial growth	Temperature and Precipitation	(Holz et al., 2018)

Figures

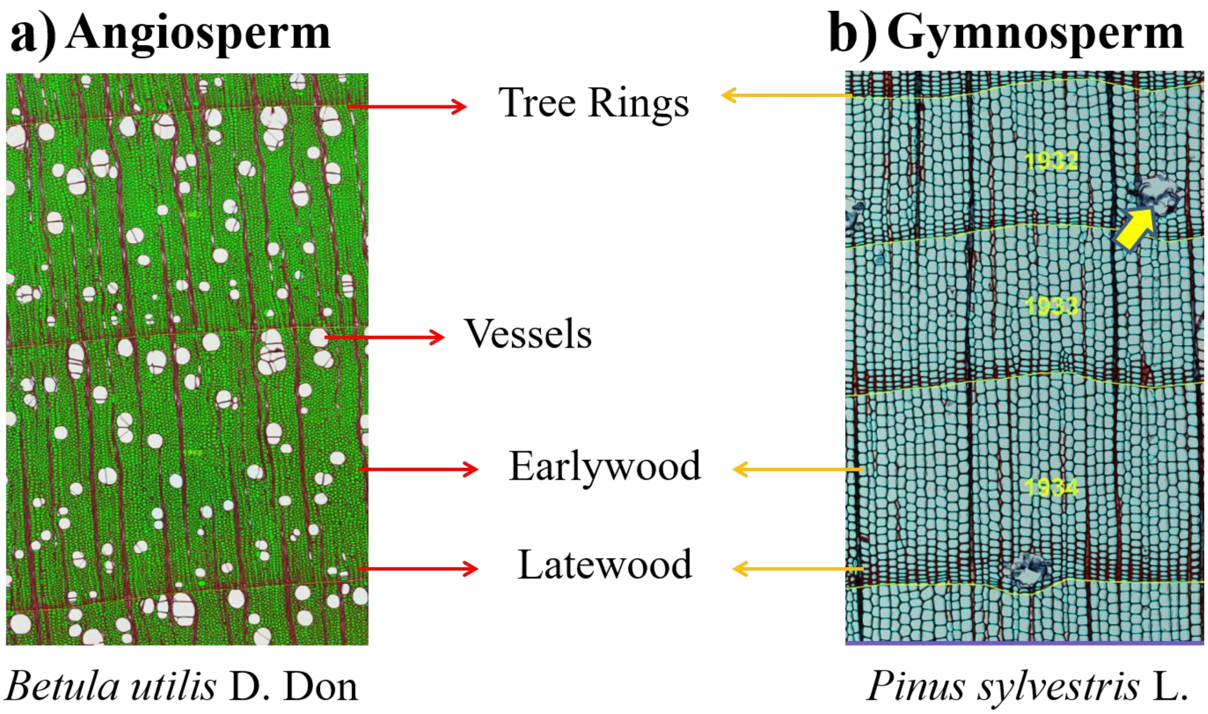

Fig 1: Cut-out image showing xylem anatomical features (vessels, latewood, earlywood, tree rings) (a) Image of *Betula utilis* D. Don, Angiosperm (b) Image of *Pinus sylvestris* L., Gymnosperm. The arrow in (b) depicts a parenchyma cell of a resin duct

CHAPTER 3 - Biofertilizers – An effective compost for soil quality improvement and plant growth

Abstract

Plant growth and development depend on the combination and concentration of essential nutrients in the soil. A deficiency of any of the nutrients results in decreased productivity and fertility. Therefore, people utilized chemical fertilizers to maintain productivity without taking care of its negative effects on ecology and the environment. Biofertilizers has been identified as an alternative for increasing soil fertility and crop production in a sustainable way. The fertilizers contain beneficial microbes which help in promoting the growth of plants by increasing the supply of essential nutrients. This review aims to understand the different types of microbes utilize as biofertilizers and their potentialities in another sector. The review showed microorganism-based fertilizers can enhance nutrient uptake, promote growth and protect plants from pests and diseases. Many studies showed microbial intervention can also be used to clean contaminated sites with heavy metals, industrial effluents, and to kill pathogens infecting plants. Lastly, it discussed the limitation and prospects of biofertilizer research for sustainable development and environmental management.

Key-words: Biofertilizers, Micro-organism. Soil fertility, Sustainability

1. Introduction

The human population is increasing raising a big threat to food security as the land of agriculture is limited and even getting reduced with time. Therefore, it is necessary to increase agriculture productivity to meet the demand of a growing population (Le Mouël and Forslund, 2017; United Nations: Department of Economic and Social Affairs, 2019). This increases the use of chemical fertilizers despite its harmful effects on the environment, human and animals (Ajmal et al., 2018). Also, continuous application of chemical fertilizers leads to the decay of soil quality and fertility and might lead to the collection of heavy metals in plant tissues, affecting the fruit nutritional value and edibility (Chaney, 1989; Savci, 2012; Sharma et al., 2014). Therefore, in recent years, many biofertilizers have been introduced that act as natural stimulators for plant growth with market value and health benefit (Mie et al., 2017).

Biofertilizers are substances that contain microorganisms, which when added to the soil increase fertility and promotes plant growth (Bargaz et al., 2018; Vessey, 2003) (Itelima, 2018). It not only enhances soil quality but also provide a conducive environment for a microorganism that is beneficial for plants (Ahmad et al., 2018). Biofertilizers are applied as soil inoculants which multiply and participate in nutrients cycling and benefit crop productivity (Singh et al., 2016). Recently, many studies and research have been focused on developing and commercializing agro-waste based biofertilizer. It was reported that the use of biofertilizers elevates crop yield around 10-40% by increasing content of proteins, essential amino acids, vitamins and nitrogen fixation (Bhardwaj et al., 2014). A study showed biofertilizers with pesticide degrading strains bacteria (*Azospirillum, Azotobacter, Bacillus, Enterobacter, Gordonia, Klebsiella, Paenibacillus, Pseudomonas, Serratia*, etc.) help to combat the deleterious effects of pesticides (Shaheen and Sundari, 2013). El-Hadad et al. (2011) reported that some bacterial biofertilizers including the nitrogen-fixing bacteria showed the highest reduction in nematode population in tomato plants infected with the root-knot nematode. Also, they found that these biofertilizers increased shoot length, many leaves, shoot/root dry weight compared to plant which is not inoculated with biofertilizers. Likewise, Khan et al. (2012) found the growth yield and quality of nematode infested chilli (*Capsicum annum* L.) increased significantly when they were inoculated with biological nitrogen fixer (*Azospirillum* and *Azotobacter*).

Nowadays, biofertilizers are considered as key agricultural components to improve crop productivity and sustainable agro-ecosystem. Biofertilizers can produce plant hormones like gibberellins and cytokinin reducing stress in plants and stabilizing their yields (Bhardwaj et al., 2014). Proline, an organic acid is accumulated in the plant during physiological response is degraded by bacteria and improves drought resistance (Verbruggen and Hermans, 2008). Similarly, Chickpea cultivars treated with plant growth-promoting rhizobacteria (PGPR) showed negative effects of drought stress with increasing biomass (Kumar et al., 2016). A meta-analysis with 112 experiments showed an increase in both yield and plant nutrient by inoculation of arbuscular mycorrhizal fungi (AMF) under open field condition (Berruti et al., 2016). Augusto et al. (2013) showed that a high level of phosphorous availability in soil drives plant growth and also biological nitrogen fixation. These studies showed various types of biofertilizers provide optimum nutrients to crop plant causing nominal damage to the environment and enhance the biodiversity of the soil. Studies showed global biofertilizers market size was valued at USD 1.0 billion in 2019 and it is anticipated to reach USD 1.66 billion by 2020 (Grand View Research, 2020; Timmusk et al., 2017).

This review discusses how microbes can be used as biofertilizers to reduce our dependence on agrochemical. Moreover, highlights potentialities of biofertilizers in different sectors including agriculture, ecology, and remediation that can craft biofertilizer as a promising tool for sustainable agriculture development. Lastly, it tries to find the gap and prospects of biofertilizers research.

2. Use of microbes in biofertilizers

A microorganism which adds or make available different nutrients to the plants are called as biofertilizers. They form important components of integrated nutrient management and applied to soil through seeds, roots, or directly to soil where microbes multiply and mobilize the inert nutrient. Generally, biofertilizers may be classified into four types 1. Nitrogen supplementing microorganisms 2) Phosphorous solubilizing microorganisms 3. Composting microorganism and 4. Plant Growth Promoting Rhizobacteria (PGPRs) (Pathak and Kumar, 2016).

2.1.1 Nitrogen supplementing microorganisms

These are the group of microorganisms (*Rhizobium, Azotobacter, Acetobacter*) which have the capability of fixing atmospheric nitrogen from the atmosphere. A plant can utilize nitrogen only in the form of nitrate, therefore, there should be some medium of nitrogen conversion for its utilization. Some microorganism utilizes nitrogen as food and converts it to ammonia through the activity of an enzyme called nitrogenase (Kim and Rees, 1994). This ammonia is then converted into nitrate by nitrification and assimilated into the plant system. Both symbiotic and nonsymbiotic microorganism has been reported to fix nitrogen. Symbiotic nitrogen fixation is a result of a mutualistic relationship between bacteria and leguminous plants. In this case, microbes first colonize the root and form nodules in which nitrogen is converted to ammonia or its product that is supplied to plants as nitrogen sources (Ahemad and Kibret, 2014). Microbes in return obtain carbon sources from the plants specifically in the form of dicarboxylates. Non-symbiotic nitrogen fixation is mostly performed by the free-living diazotrophic microbes (*Pseudomonas, Acetobacter, Azotobacter*). The study showed inoculation of nitrogen-fixing microorganism to crop plants serves as an integrated strategy for growth stimulation, disease suppression and maintenance of nitrogen level in the soil of agricultural fields (Tsukanova et al., 2017).

2.1.2 Phosphate solubilizing microorganisms (PSM)

Phosphorus (P) is the second most important plant nutrients other than nitrogen for plant growth. It plays an important role in various metabolic activities (energy transfer, respiration, photosynthesis, signal transduction, and macromolecular synthesis (Kalayu, 2019; Sharma et al., 2013). Phosphorus is available in soil in inorganic and organic form but its utilizable form is free low. This is because more than 95% of P exists in insoluble and immobilized forms which plant cannot absorb. The plant absorbs P in monobasic ($H_2PO_4^-$) and dibasic ($H_2PO_4^{-2}$) form (Bhattacharyya and Jha, 2012). To overcome this problem, there is a certain microorganism (*Aspergillus spp, Arthobacter, Bacillus, Erwinia, Pseudomonas, Microbacterium Rhizobium* etc.) which have the capability of resolubilizing that insoluble phosphate, making them available to the plants. These biofertilizers are considered important as they promote plant growth and productivity by supplying phosphorus in usable forms in environment-friendly and inexpensive modes.

2.1.3 Composting microorganism

Waste from forest and agriculture field has a sufficient amount of plant nutrients but they are not easily available to plants. There should be some decomposition process with the involvement of microorganism. Composting microorganism is usually available in the atmosphere and help in the decomposition of dead organic matter. *Trichoderma, Penicillium, and Aspergillus* are some of the microorganisms which is efficient in composting heap of agriculture waste faster and of good quality.

2.1.4 Plant Growth Promoting Rhizobacteria (PGPRs)

Plant growth-promoting rhizobacteria ((*Pseudomonas, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus,* and *Serratia)* are a heterogeneous group of microorganisms known to improve plant growth by their ability to colonize the rhizosphere besides their effects as biocontrol agent and producers of plant hormones (Joseph et al., 2007; Kloepper et al., 1980). These microbes provide a wide range of services and benefits to the plant and in return, the plants provide the microbial community with reduced carbon and other metabolites. The study showed inoculating plants with PGPR can be an effective strategy to stimulate crop growth (Backer et al., 2018). There are two main classes of PGPRs, i.e., extracellular plant growth-promoting rhizobacteria (ePGPR) and intracellular plant growth-promoting rhizobacteria (iPGPR) (Martínez-Viveros et al., 2010). ePGPR (*Serratia, Azospirillum, Azotobacter, Bacillus, Chromobacterium* etc.) typically colonize the rhizosphere or space on the surface of the root cortex and iPGPR ((*Rhizobium, Bradyrhizobium, Allorhizobium, Mesorhizobium*) mostly reside in the specific nodulated parts of roots cells (Backer et al., 2018; Martínez-Viveros et al., 2010). PGPRs are known to synthesize different types of antibiotics and possess novel attributes in heavy metal detoxification (bioremediation) (Egamberdieva and Lugtenberg, 2014; Xie et al., 2016).

3. Potentialities of biofertilizers

Biofertilizers not only help to boost crop productivity but also have potentialities in other sectors. They are divided into two broad headings described below;

3.1 Bioremediation

It is defined as a process of removing hazardous wastes biologically under controlled conditions to an innocuous state (Prince, 2003). In this process, microorganisms are utilized to reduce, eliminate, transform and detoxify the unwanted products present in soils, sediments, water and air (Saranya et al., 2017; Ward and Singh, 2004). In developing countries, there is a tradition of using wastewater and sewage sludge directly into the soil due to its high nutrient concentration (nitrogen, phosphorus and organic matter). However, the long-term application can alter the physical, chemical and biological properties of soil and lead to high concentrations of heavy metals (Gottschall et al., 2009; Kharche et al., 2011). There are several methods used to detoxify this effluent but most of them are expensive and not environment friendly (Zhang et al., 2004). For this, we can utilize microorganism, which makes a significant impact by removing contaminants from soils and reducing their toxic effects on the environment (Das and Adholeya, 2012). Pandey et al (2018) found ligninolytic mushroom *Lenzites elegans* can absorb synthetic dye by forming laccase enzyme. Similarly, *Bacillus cereus* and two strains of *Pseudomonas aeruginosa* have been found to decolourize bleached kraft paper-mill effluents (Tiku et al., 2010). *Rhodococcus* biosurfactants have been used for the bioremediation of oil-contaminated agricultural soils after an accidental oil spill (Christofi et al., 1998). Studies showed certain biofertilizers (*Phosphoren, Microbien, Cerealin* and *Azospirillum*) may act as a potential agent for soil inoculation to bioremediate pesticides contaminated soil (van Veen et al., 1997). Likewise, Azolla, a free-floating, fast-growing, and nitrogen-fixing pteridophyte seems to be an excellent candidate for removal, disposal, and recovery of heavy metals from the polluted aquatic ecosystems (Umali et al., 2006).

3.2 Biopesticides

Biopesticides are the compounds that are used to manage agricultural pests through specific biological effects rather broader chemical pesticides. Biopesticides contain natural organism or substances derived from natural materials such as animal, plants, bacteria, or certain minerals including their genes of metabolites. Microorganism (e.g. bacteria, fungi, viruses, algae) have been successfully used in controlling insect pest, plant pathogens and weeds (Harding and Raizada, 2015; van den Bosch et al., 1982). For instance, microbial pesticides suppress pests through the production of a toxin specific to the pest that causes the diseases (Sharma and Malik, 2012).

Application of biofertilizers such as *Trichoderma harzianum, P. fluoresecens,* and *Bacillus subtilis* helps to cure the plant diseases caused by the pathogens like *Pythium spp., Rhizoctonia spp*., and *Sclerotium spp* and enhance plant growth (Mahanty et al., 2017). Bhattacharya and Jha (2012) reported that the interaction between some *Rhizobacteria* and plant roots can prevent pathogenic fungi, bacteria, and viruses from affecting the host plant. Studies showed many individual bacterial components such as lipopolysaccharides (LPS); flagella; siderophores; cyclic lipopeptides; 2,4 diacetyl phloroglucinol; and homoserine lactones and volatile compounds such as 2,3-butanediol and acetonin can induce induced systematic resistance (ISR) in the host plant and eventually enable the host plant to combat against a variety of plant pathogens (Lugtenberg and Kamilova, 2009; Pieterse et al., 2014). There are some biocontrol bacteria which produce different enzymes such as cellulases, proteases and lipases that can lyse come a portion of the cell wall of pathogenic fungi thus prevents disease spreading by killing the fungus (Beneduzi et al., 2012).

4. Limitation of biofertilizers

Though biofertilizer is eco-friendly and possesses a lot of advantages, there is some limitation associated with this technology in its application. For examples, biofertilizers are plant-specific with lower nutrient density therefore need skilled manpower and may not benefit farmers because of inadequate awareness. Also, there may be constraints on application and implementation of biofertilizers which may affect technology at stages of production, marketing or uses. Other constraints limiting the use of biofertilizer technology may be unavailability of suitable strains, and unavailability of the suitable carrier, short shelf life, susceptibility to high temperature.

5. Conclusion

Biofertilizers are an important component of sustainable organic farming and alternative of chemical fertilizers that are associated with various environmental hazards. The fertilizers activate the microorganism found in soil in a cheaper, effective and environmentally friendly manner restoring the soil natural fertility. This help farmer to increase yield enhanced soil quality and induced drought tolerance in plants. However, for the success of biofertilizers technology, more research and development are needed to make agriculture practices more sustainable and economical. The technology should be economically viable and should be easily accepted by society.

6. Future perspectives of biofertilizers

Biofertilizers is the newly emerging field and use as an integral component of agriculture practice. They have been successfully used in a few developing countries and expected to grow with time. Hence, there are wide research opportunities for its overall development with integration in the agriculture sector.

- Effective and competitive multi-functional biofertilizers for a variety of crops
- Make people aware that bacteria are not always harmful but are beneficial to the environment and our soil
- Should focus research on a new approach of growth, storage, shipping, formulation and its application
- Research on genetically engineered strains for more efficacy in stimulating plant growth
- Research on quality control for their proper application in the field and to ensure their benefit
- Agronomic, soil and economic evaluation of biofertilizers for diverse agricultural productions should be done.

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CHAPTER 4 - Use and effectiveness of biomaterials for production of biochar and its technology enhancement – a review

Abstract

Biochar is a stable carbon-rich product synthesized from biological materials through different heating techniques. Its yield and quality vary significantly with the production technology and process parameters, which also affect its performance in agro and forestry systems. The main aim of this review is to understand the use and effectiveness of biochar along with technology development for production. The review showed a yield of biochar decrease with the faster heating rate or with the availability of oxygen. Also, the study showed biochar can be utilized for improvements in soil health, plant growth, carbon sequestration, and greenhouse gas mitigation, however, its beneficial aspects depend on the type of biochar used, the rate of application, soil type, and climate. Therefore, more research in understanding the relationship among biochar production technologies, their properties, and performance in agro and forestry system is needed.

Key-words: biochar, soil health, agro and forestry system, heating techniques, soil remediation

1. Introduction

Biochar is a stable carbon-rich, fine-grained and porous material. It is usually produced by thermal decomposition of biomass under oxygen-limited conditions at temperature <900ºC (Lehmann et al., 2006). It has received increasing attention due to its ability to store a large amount of carbon, increase crop yield, reduce soil emission of greenhouse gases, improve soil quality, decrease nutrient leaching and reduce irrigation and fertilizer requirements (Lehmann, 2007; Nguyen et al., 2009). Biochar is produced from various feedstocks (forest and agricultural biomass) at different pyrolysis conditions. Masek et al. (2018) reported that different feedstocks resulted in different magnitudes of surface area, pores and functional groups in biochar, and all these variables affect sorption characteristics of biochar.

Recently, there has been growing interest in the scientific community on biochar research because of its multidisciplinary areas for scientific research and engineering applications. Also, biochar is a low-cost effective compared to activated carbon. Many studies have highlighted the benefit of using biochar in terms of mitigating global warming, soil amendment, enhancing crop yield, and carbon storage (Clough et al., 2013; Hua et al., 2009; Qian et al., 2015). Biochar contributes to economic sustainability through sequestering carbon in soil and by reducing the net emission of greenhouse gases (Laird, 2008; Lehmann et al., 2006). Moreover, there has also been considerable interest in using biochar to remove pollutants from aqueous solutions (Tan et al., 2015). This opens up the opportunity of converting invasive plants into biochar and protect the environment. Therefore, the conversion of biomass into biochar as a sorbent is a win-win solution for both improving waste management and protecting the environment (Matuštík et al., 2020; Odling et al., 2020).

However, biochar impacts on soil, environmental, and agronomic characteristics have not been systematically studied. While biochar has the potential to generate revenue and enhance the sustainability of agriculture and the environment, the agricultural and bioenergy industries will be reluctant to pay for biochar until its precise effects on soil properties and crop production are shown. Complete development of biochar as a commercial product must establish concrete benefits of the product to soil properties and crop production and link these benefits to biochar properties and their appropriate use and economic value.
Thus, the objectives of this review were to compare biochar production technologies and link the processes to biochar yield and properties with their benefits to agro and forestry systems. Technologies for biochar production and application of biochar in improving soil health, plant growth, carbon sequestration, and greenhouse gas mitigation were summarized in this review.

2. Biochar application in agro and forestry systems

2.1 Biochar for soil improvement

2.1.1 Improvement in soil physicochemical properties

Biochar application on soil can increase the net soil surface area, increase soil aeration and improve soil bulk density, porosity, and packing (Chan and Xu, 2012; Palansooriya et al., 2019). Also, biochar directly changes the relationship of soil-water by increasing soil aggregate stability, soil preparation workability, water infiltration, and water holding capacity (Purakayastha et al., 2019; Qambrani et al., 2017). This decrease in bulk density and increase in soil porosity contributes to the movement of water, heat, gases in soils and helps in the improvement of soil quality (Lian and Xing, 2017). Biochar also alter soil pH value, particularly helpful in eliminating soil acidity by increasing cation exchange capacities (K, Ca, Mg, and Na from biochar), through functional group effects (–COO– and -O- contribute greatly to biochar alkalinity) and increase the availability of primary and secondary nutrients like K, P, Ca, Mg (Kookana et al., 2011). An increase in cation exchange capacity such as K, Ca, Mg, and Na from biochar increases nutrient availability to plant roots (Laird et al., 2010) facilitating microbial activity and accelerating chemical reaction in the rhizosphere.

2.1.2. Improvement in soil nutrition and fertility

Biochar directly serves as a source or sink for available nutrients as it contains nutrients derived from feedstock (Palansooriya et al., 2019). Incorporation of biochar into soil proves to be an effective method for enhancing nutrient cycling, affecting root growth and overall plant performance (Palansooriya et al., 2019). Zhou et al (2015) showed biochar to indirectly alter the soil nutrient content and availability with a slow release of fertilizers reducing leaching and runoff. A study by Haider et al (2017) showed biochar can have an indirect influence on soil N cycling resulting in N leaching and an increase in recovery of N fertilizer. This adsorption of the inorganic form of N onto biochar decrease ammonia and nitrate losses from the soil and can potentially allow the retention of nutrients. Since biochar is a C-rich substrate with a high C/N ratio, biochar application into the soil can trigger microorganisms to decompose the native soil organic matter. Additionally, biochar also influences soil phosphorus transformation increasing soil microbial activity and help in reducing soil acidity (Xu et al., 2018). Biochar is a huge source of potassium and helps in the retention of potassium in the soil due to its high cation exchange capacity (Purakayastha et al., 2019). Therefore, the application of biochar has many additional benefits for plant nutrient cycling, such as increasing retention and use efficiency, reducing leaching, thereby improving soil fertility.

2.1.3. Improvement of plant growth

Biochar affects the physical properties of soil that may subsequently have a direct effect on plant growth. The effectiveness of biochar application on improving crop productivity in fertile or healthy soils is commonly much lower than in nutrient-poor and degraded soils (Hussain et al., 2017; Laghari et al., 2016). Generally, a key obstacle for plants growing especially in poor soils is root establishment and growth. The improved soil properties ultimately influence the root area and encourage great root development; the expanding volume of plant roots in soil is beneficial for the capture of more nutrients and improved plant growth (Uzoma et al., 2011). Studies showed amending soils with biochar addition improved the antioxidant response of quinoa in addressing the complex conditions of drought and salt accumulation by increasing plant promoting hormones (Ramzani et al., 2017; Thomas et al., 2013). In addition to the biotic stresses, biochar application can trigger microbial activities to mitigate plant pathogenicity that threatens plant health; the release of microbial inhibitors like volatile organic compounds can deter soil pathogens thereby, enhancing plant growth (Zhu et al., 2017).

2.2 Biochar for environmental remediation

In addition to soil fertility improvement, biochar can be used as a remediation agent to alleviate soil pollution. Biochar in soil displays various interactions with inorganic and organic pollutants, these interactions affecting the mobility and bioavailability of the pollutants could be beneficial for remediating contaminated soils (Younis et al., 2016).

2.2.1. Removal of heavy metals

Heavy metals in the water environment mostly come from anthropogenic activities such as smelting, mining, and electronic manufacturing effluents. Biochar has been suggested to be used for heavy metals removal from contaminated water. Removal mechanisms vary depending on the valence state of the target metal at different solution pH (Li et al., 2017). Numerous studies have shown that the capability of biochar to mitigate pollution attributes not only to the surface sorption but also to various functional groups and inorganic ions present in the biochar that may make a great contribution to stabilize metals in soils metals (Wang et al., 2018; Xu et al., 2013). A study by Zhang et al (2015) showed that there was almost an equal amount of sorption of Cd and total released cations (Na, K, Mg, Ca) from the biochar, indicating the cation exchange as a leading role in Cd sorption. Zhou et al (2013) showed that the biochar modified by chitosan had favourable removal efficiency for three heavy metals (Cd^{2+}, Pb^{2+}, and Cu^{2+}) from solutions. Two dyes used in wood carpet dyeing (Lanasyn Orange and Lanasyn Gray) could be highly sorbed on nanoporous biochar derived from bamboo cane (Pradhananga et al., 2017).

2.2.2. Removal of pesticides

Biochar can be utilized as distinctive remediation of pesticide contamination treatment, thereby making ecological balance and improve human health (Dai et al., 2019). Klasson et al. (2013) found almond shell biochar with a sorption capacity of 102 mg g^{-1} for dibromochloropropane, a nematode insecticide. Biochar produced from maize straw was found to absorb thiacloprid through pore-filling and hydrophobic interaction (Zhang et al., 2018). Char produced from sawdust help to remove above 89% of Tetracycline (Zhou et al., 2017).

2.2.3. Removal of antibiotics

Pharmaceutical wastewater is difficult to decompose in the natural environment and regarded as emerging environmental contaminants (Carvalho and Santos, 2016). The research was conducted on reducing the toxicity of antibiotics by biochar. Humic acid-coated magnetic biochar derived from potato stems and leaves sorb three typical fluoroquinolones (FQs) i.e. enrofloxacin (ENR), norfloxacin (NOR), and ciprofloxacin (CIP) by hydrophobic, electrostatic, and formation of hydrogen bonds (Zhao et al., 2019). Mohanty et al. (2014) improved sand biofilters with 5 wt% biochar amended to increase the *Escherichia coli* removal capacity and prevent their mobility during continuous, intermittent flows.

2.3 Biochar for carbon sequestration

Carbon sequestration is a procedure that carbon is captured, and soil organic carbon content is increased, leading to an increase in soil carbon sink and a change in land management (Powlson et al., 2011). Biochar has been widely accepted as a promising C sequestration tool for enhancing soil carbon sink, because biochar possesses high levels of resistance to soil chemical and biological degradation, as biochar production through the thermochemical conversion of biomass increases the recalcitrance and stability of the carbon (Herath et al., 2015). It has been estimated that the mean residence time of biochar labile fraction (pool size = 97%) in 556 days (Wang et al., 2016). Therefore, biochar application to soil potentially sequesters soil carbon for hundreds or thousands of years. The addition of biochar in the soil can also increase microbial biomass carbon and reduce the metabolic quotient due to its influence on C and N availability.

2.4. Biochar for the mitigation of greenhouse gas emissions

The agricultural sector is a primary contributor to atmospheric greenhouse gas (GHGs) emissions (Kavitha et al., 2018). Biochar application in soil has been useful not only in carbon sequestration but also in decreasing gaseous emissions. Biochar positively aids in the reduction of GHGs emissions such as CH_4, N_2O, NH_3, and CO_2 in ecologically and economically sustainable systems (Vithanage et al., 2015). A study showed CO_2 emission from soil respiration is almost ten times higher than that of fossil fuel burning (Spokas et al., 2009). Therefore, it is crucial to reduce CO_2 emissions from agricultural soil for the mitigation of climate change. Turning biomass into more stable carbon as biochar help to store the captured carbon in the soil for long periods (Gwenzi et al., 2017; Yuan et al., 2019). Moreover, the application of biochar in soil improves nutrient and water retention, which in turn results in energy saving, reduces irrigation frequency and fertilizer use, and indirectly reduced greenhouse gas emission (Sohi et al., 2010).

3. Technology enhancement

3.1 Technology improvement in biochar

Biochar quality and quantity affect both biochar physical (e.g., increase surface area and improve pore structure) and chemical properties (e.g., introduce the certain functional group and induce activated oxygen species on biochar surface) promoting the adsorption or degradation of different contaminants depending on the specific approach employed. Pyrolysis technique is important in this process as this helps to improve the characteristics of resultant biochar. Therefore, fast and efficient pyrolysis techniques are necessary instead of conventional techniques. There is different pyrolysis technique developed, listed below;

3.1.1 Microwave-assisted pyrolysis

This is the process of transforming biomass with electromagnetic energy without direct physical contact between a heat source and heated material (Liu et al., 2016). High water content feedstock favors the absorption of microwaves since water can rotate and align in dipoles, resulting in collision and friction (Kong et al., 2019; Liu et al., 2016). This microwave radiation can affect both biochar morphology and chemical characteristics. Mašek et al. (2013) observed that microwave-assisted pyrolysis could achieve higher degrees of carbonization of biochar at lower pyrolysis temperatures compared with conventional slow pyrolysis, affecting the physical and chemical properties. Nair and Vinu (2016) found that narrow and deep pores (pore diameter 3.5 nm and pore volume 0.13 cm^3 g^{-1}) were generated with the assistance of microwaves. A study by Paunovic et al. (2019) showed the number of oxygen-containing functional groups decreased through microwave irradiation resulting in higher absorption capacity due to electrostatic interaction. Biochar produced by microwave-assisted pyrolysis has been used for the adsorption of heavy metals, organic contaminants. However, several studies showed biochar produced from microwave pyrolysis not satisfactory for metal adsorption as surface complexation was suppressed (Elaigwu et al., 2014). Biochar produced from this pyrolysis method can be utilized as bio-fertilizers for increasing fertility and remediation of soil contamination with organic pollutants (Mohamed et al., 2016).

3.1.2 Stem assisted pyrolysis

Stem-assisted pyrolysis increases specific surface area and pore volume due to the removal of tar and other by-products on biochar surface (Krerkkaiwan and Fukuda, 2019; Lam et al., 2019). It is easy to apply and is relatively energy-efficient compared with other methods aimed at increasing surface area. A study showed biochar produced from steam-assisted pyrolysis can be used for sulfur dioxide removal from flue gas (Braghiroli et al., 2019). Similarly, application of pine sawdust biochar produced with steam activation showed a significant reduction in cumulative CO_2 and N_2O emission from forest soil (Pokharel et al., 2018). Sewu et al (2019) utilized the spend mushroom substrate waste for producing biochar through steam-activated pyrolysis and found it effective in colour removal of waster waste. This showed that steam-assisted pyrolysis effectively immobilizes organic aromatic contaminants.

3.1.3 Wet pyrolysis

This method introduces oxygen-containing functional groups such as hydroxyl and carboxyl onto the surface of biochar. In this method, biomass is mixed with phosphoric acid and stirred for 2 hours. Afterwards, the mixture is poured into the autoclave and heated at 200^oC for 5 hours. The biochar produced is mixed with sodium carbonate (to neutralize the system and promote the precipitation of heavy metals) and rinsed several times forming a large number of phosphoric and oxygenic groups (Zhou et al., 2019). They showed biochar has a high adsorption capacity for lead and cadmium compared to conventional pyrolysis and is energy-efficient and cost-effective.

3.1.4 NH_3 ambience pyrolysis

In this method, NH_3 reacts with oxygen-containing species (e.g., ketones, aldehydes, esters, furans) of biomass to form nitrogen (N) containing heterocyclic compounds (i.e., pyrrole, pyridine, piperidine, indole) (Chen et al., 2018). Biochar produced from such a process could significantly reduce the bandgap energy of TiO_2/Fe_3O_4/biochar composite, making it possible for visible light with relatively low energy to excite electrons from the valence band to the conduction band (Mian et al., 2019). However, the mechanism is not fully understood and needs more research on the topics.

3.1.5 Co-pyrolysis

Co-pyrolysis is an effective means to modify biochar properties and to synthesize composite products. Also, this procedure can be an effective way to reduce the environmental risks that may be associated with biochar produced from metal-rich feedstocks because of improved immobilization of inherent metals within the biochar matrix. A study by Buss et al (2019) showed a significant potential of co-pyrolysis biochar in reducing heavy metals while maintaining nutrients, such as potassium in their available form. The study also found that co-pyrolysis could improve biochar pore structure and increase surface area enhancing biochar functionality in environmental applications (Fan et al., 2018). Co-pyrolysis biochar was used to synthesize layered double hydroxides (LDH) composite by preloading AgAl-LDHs on the surface of risk husk powder through precipitation (Lee et al., 2019).

Besides, there are novel modification strategies of biochar from conventional (magnetic and acid/alkaline modification) to new methods (contamination adsorption in waste-water to heavy metal stabilization in soil). Magnetic biochar has been widely used as a sorbent for wastewater treatment and soil remediation because of a generation of reactive oxygen species. Biochar can be activated with acid on the specific surface area, pore volumes, and functional groups. A study showed that apart from adsorption, acid-activated biochar can also promote plant growth through elevating soil nutrient bioavailability (Sarkar et al., 2019; Wang et al., 2019). This acid activation, however, may not be an appropriate modification method if the biomass itself contains heavy metals. Oxidant modification of biochar can increase the content of oxygen-containing functional groups, promoting the complexation of heavy metals. Huff and Lee (2016) activated pinewood biochar with hydrogen peroxide which decreases methylene blue. This was because oxygen-containing groups weakened the forces of delocalized π interactions, which was the major mechanism of methylene blue adsorption. The effectiveness of this method depends greatly on the target contaminant type and contaminant removal mechanism. Lastly, the electrochemical modification method is a simple and rapid method for introducing specific functional groups and impregnating chemicals onto the surface of raw biochar compared with other modifications. This method can be used for MgO impregnation and is effective for soil metal stabilization processes (Shen et al., 2019). Therefore, this could be a simple method to fabricate these immobilizing agents Many studies showed the use of biochar in different environmental and health sectors through technological advancement. Qian et al. (2015) reviewed that biochar can be used as a catalyst for syngas cleaning and conversion of syngas into liquid hydrocarbons, and as a solid acid catalyst for biodiesel production. Recent research suggests a novel strategy to use oxygen plasma to upgrade biochar at a temperature lower than 150°C (Gupta et al., 2015), demonstrating a strong potential to expand the horizon of biochar application to various smart materials. Thus, biochar is a treasure from an old chest to revolutionize its application in global environmental and human health management.

4. Future outlook for utilization of biochar

Biochar is not widely applied and still in the test stage of research. At present, the biochar application in developing countries is not well established due to the lack of industrial chain and awareness among the people. Therefore, arduous research work should be carried out to solve the potential environmental problems and expand biochar applications. Feedstock/biomass are extensive and easily available however need proper grinding, cleaning and pyrolyzed techniques. Modification in any of these steps enhances sorption effects. Therefore, future researches should attempt to find a compromise between optimizing the production process and maximizing the applicability of biochar to minimize the cost. Moreover, research on the careful selection of feedstocks, production conditions, and modification methods should be done to acquire biochar with better performance. More efforts would be needed to link biochar properties to soil and crop responses in both climate-controlled environments and in the field.

5. Conclusion

This review gives a systematic overview of the broad application and effectiveness of biochar. The yield and quality of biochar from the thermochemical conversion process of biomass are vastly different due to differences in the amount of oxygen available, heating rate, and reaction temperature. Biochar from agro-forest waste showed a beneficial impact in improving soil health, better plant growth, carbon sequestration, and reduced greenhouse gas emissions. However, more studies are needed relating to biochar in the establishment of protocols, production standards, and standardized characterization methods for new biochar-based materials. Also, the relationship between the chemical and physical properties of biochar is still poorly understood therefore, more research should be conducted to provide solutions to those problems.

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CHAPTER 5 - Biochar as an important ingredient for organic fertilizers in the improvement of soil fertility, plant growth and crop production – a review

Abstract

Biochar is a valuable product that can be produced in combination with bio-energy in a cascading approach to make the best use of available resources. It is persistent and beneficial to the soil. This review systematically analyzed and summarize the beneficial aspects of biochar for soil quality improvement and plant growth. Also, this review provides an overview of the research conducted on biochar till data. This study showed that the effects of biochar depend on the quality of materials and the type of soil where it is going to be utilized. Also, it provides the overview and prospects of biochar research in Nepal. The application of biochar in carbon sequestration should be further investigated at similar experimental conditions to obtain consistent results. A study suggested that the effect of biochar on soil microbes should be further investigated to elucidate the dominant reason for the improvement of soil fertility based on different soil and feedstock. In summary, biochar has a wide application prospect in environmental remediation and should be further investigated.

Key-words: biochar, carbon sequestration, plant growth, soil fertility

1. Introduction

Soil fertility is the ability of soil to sustain plant growth and optimize crop yield. This can be enhanced through organic and inorganic fertilizers to the soil. It combines several soil properties (biological, chemical and physical), all of which affect directly or indirectly nutrient dynamics and availability. Inorganic fertilizers have played a significant role in increasing crop production since the "green revolution" (Liu et al., 2010); however, they are not a sustainable solution for maintenance of crop yields (Vanlauwe et al., 2010). Long-term overuse of mineral fertilizers may accelerate soil acidification, affecting both the soil biota and biogeochemical processes, thus posing an environmental risk and decreasing crop production (Aciego Pietri and Brookes, 2008). Organic amendments, such as compost and biochar, could therefore be useful tools to sustainably maintain or increase soil organic matter, preserving and improving soil fertility and crop yield.

Biochar is a black, stable, carbon-rich material obtained from thermochemical conversion (slow, intermediate, and fast pyrolysis or gasification) of biomass in an oxygen-limited environment. It can be produced from a range of feedstock, including forest and agriculture residues, such as straw, nutshells, rice hulls, wood chips/pellets, tree bark, and switchgrass (Sohi et al., 2009). Biochar has been described as a possible tool for soil fertility improvement, potential toxic element adsorption, and climate change mitigation (Ennis et al., 2012; Stewart et al., 2013). Several studies have shown that biochar application to soil can (i) improve soil physical and chemical properties (Mukherjee and Lal, 2013; Sohi et al., 2010) (ii) enhance plant nutrient availability and correlated growth and yield (Biederman and Stanley Harpole, 2013; Jeffery et al., 2011) (iii) increase microbial population and activities (Jaafar et al., 2014; Lehmann et al., 2011), and (iv) reduce greenhouse gas emissions through C sequestration (Crombie et al., 2015).

Some recent studies have indicated that combined applications of biochar with organic or inorganic fertilizers could lead to enhanced soil physical, chemical, and biological properties, as well as plant growth. In particular, several composted materials represent a sustainable source of available nutrients that could enhance plant growth, ameliorating soil physicochemical characteristics and microbiological properties (Liu et al., 2012; Schulz and Glaser, 2012). Liu et al. (2012) showed that the combined application of compost and biochar had a positive synergistic effect on soil nutrient contents and water-holding capacity under field conditions. Also, the combination of biochar with compost has proved to be suitable, allowing the reduction of fertilizer inputs, stabilizing the soil structure, and improving its nutrient content and water retention capacity (Agegnehu et al., 2015; Schmidt et al., 2014). Again, these studies underline that compost and biochar combination could enhance compost properties, leading to a higher added value and a much better carbon sequestration potential due to the long-term stability of biochar (Schulz and Glaser, 2012).

However, the literature shows that compost effects, as also reported above for biochar, can differ on soil biophysical-chemical properties and plant growth and yield based on feedstock types, methods of producing, and application (Bernal et al., 2009). The objectives of this review are to understand the importance of biochar as an important ingredient for organic fertilizers for the improvement of soil fertility and crop production.

2. Biochar as organic fertilizers from natural resources

Biochar is carbon-rich in organic matter and inorganic salt (humic and fluvic substance) and N, P and K which can serve as fertilizer and assimilate by plants and microorganism. Lin et al (2012) showed that biochar produced from *Acacia saligna* at 380 ^{o}C and sawdust at 450 ^{o}C contained humic of 17.7 and 16.2%, respectively. Biochar made from *Lantana camara* at 300 ^{o}C contained available P (0.64 mg kg^{-1}), available K (711 mg kg^{-1}), available Na (1145 mg kg^{-1}), available Ca (5880 mg kg^{-1}) and available Mg (1010 mg kg^{-1}) (Masto et al., 2013). Similarly, fresh biochar had the potential of nutrient availability and could release large amounts of N (23 – 635 mg kg^{-1}) (Mukherjee and Zimmerman, 2013). Biochar not only increase nutrient content but also increase available water capacity, soil porosity (Nelissen et al., 2015) and increase crop production and prevent soil degradation (Amézketa, 1999). Lu et al (2014) showed an increase in soil aggregation (8 to 36%) and soil pore structure (20%) after the application of rice husk biochar. These studies show that biochar has great potential to be utilized as nutrient-rich organic fertilizers.

2.1 Biochar for soil fertility and plant growth

Biochar has shown not only to improve soil physicochemical properties but also to change soil biological properties through the cycling of soil organic matter (Liang et al., 2010). It can improve soil structure, enhance nutrient cycles and increase pore space with nutrient retention and immobilization ultimately promoting plant growth (Warnock et al., 2007). Microorganisms, such as rhizosphere bacteria and fungi may facilitate plant growth directly through the cycling of soil organic matter. Domene et al. (2014) indicated that microbial abundance could increase from 366.1 to 730.5 µg C g^{-1} after addition of 30 t ha^{-1} biochar. Likewise, microbial abundance increased by 5 – 56% with the increase of corn stover biochar rates (from 0 to 14%) for the different preincubation times (2 – 61 days) (Domene et al., 2015). The possible reason for the increase of microbial abundance due to higher availability of nutrients, less competition, the increased habitat suitability and refuge with the addition of biochar.

The effects of biochar on soil microbial community depend on biochar and soil type. Some organic pyrolytic product, such as phenolic and polyphenolics may be present in biochar and are harmful to soil microorganism. A study by Warnock et al (2007) showed a decrease in mycorrhizae and total microbial biomass after application of biochar. Microbial abundance and activities are reduced due to the retention of heavy metals and pesticides release from biochar (Gell et al., 2011). Moreover, some biochar might pose a direct risk to soil biota and their functions and a decrease in crop yields (Liesch et al., 2010). Biochar application without proper washing procedure to remove organic and inorganic matters was reported to cause discoloration of leaves of clover plants (Turner, 1955). Therefore, we cannot conclude that biochar application always has positive effects on soil biota.

More research is needed with fundamental mechanisms and the utilization of biochar are poorly understood. Therefore, research should focus on the following aspects.

- Research on the interaction between biochar and soil microbial communities especially focusing on greenhouse gas release (CH_4 and N_2O) from the soil.
- Many factors restrict the use of biochar and soil and its application rates; therefore, field trials should be conducted selecting a suitable variety to understand the interaction between biochar, soil, microbes and plants roots.
- The life cycles of biochar in the soil are poorly understood, so we can pay more attention to the decomposition rate of biochar is soil.

2.2 Biochar research initiatives in Nepal

There are few research and limited knowledge of biochar application in Nepal. There are hardly any peer-reviewed journal articles that are based on comprehensive experimentations of biochar in Nepali soils (Table 1). Nepali farmers are traditionally practicing open-burning of agricultural wastes before the cropping season with a belief that ashes of biomass help enriching soils. Research is conducted on temperate climates, in countries of Asia (especially in China, Indonesia, Japan), Europe, Australia, USA and South America. There is research done by Nepalis researchers but they are not based on Nepalis soils. Also, field experiments conducted in one part of the world could not be the same on others due to diverse soils and climate. According to Mukherjee and Lal (2014), biochar advocacy is growing worldwide without adequate scientific knowledge on basic soil processes and cost-benefit analysis of biochar application to soil. Therefore, more research on biochar is needed despite its beneficial effects on the soil environment particularly to assess its effectiveness in different soil profiles, climatic conditions and crop varieties. In Nepal, there is a volume of renewable feedstock from agriculture and forest which is critical sources for promoting biochar. We can choose the feedstock which is underutilized biomass available from farm fields, households, markets of agro-processing mills. As our focus is on hill economy, we can choose available forest litters and invasive species of grass and shrubs and also ricks husks wooden dust from mills. The feedstock can be chosen depending on seasons and ecological conditions.

Table 1: Agencies undertaking biochar programs in Nepal

Agency	**Types of activity**	**The funding source, program areas, years and key interventions**
Asian Development Bank in collaboration with Nepal Agriculture Research Council and Nepal Academy of Sciences and Technology	Action research, piloting	Nordic Development Fund, 3 eco-regions – high mountains, mid-hills and Terai, 2014 -16, production tests, trials on soil amendment, carbon sequestration, and energy-saving stoves.
Department of Environment Science and Engineering, Kathmandu University	Action research	Helvetas Intercooperation, Sindhupalchok, Kavre and Lalitpur of Nepal, 2013-15,

Multi-stakeholders Forestry Programme	Community support	Jointly funded by Swiss, UK and Finland, 2013-14, support to communities of smallholding farmers for livelihood improvement
Nepal Agroforestry Foundation	Scientific research	Norwegian Geotechnical Institute and the Norwegian University of Life Sciences, 2012 onwards. Nepal is one of the 4 countries where the project has been launched.
Local Initiative for Biodiversity Research and Development	Action research, piloting	DanChurchAid, 2014-2016, support for livelihood improvement among low-income households

3. Conclusion

Biochar application into soil has great potential for improving soil fertility and promoting plant growth. However, the choice of biochar should be flexible to understand its actual role in biomass yield and soil fertility. This review showed biochar has a huge surface area, well-developed pore structure, amounts of exchangeable cations and nutrient elements. Therefore, biochar should be considered as catalyzers, not as a fertilizer. Thus, biochar offers an alternative technique for enhancing not only productivity of agriculture lands but also for carbon sequestration through added organic matter in the soil which is locked for centuries. Lastly, biochar research could provide very positive effects in improving soil quality and crop productivity in Nepal as agriculture and forest sector offers a huge number of resources.

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CHAPTER 6 - Biofertilizers available and techniques used for its development

Abstract

Biofertilizer is a substance which contains living micro-organisms which when applied to seeds, plant surface, or soil, colonize the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plants. This review provides an overview of biofertilizers available in Nepalese's market and their development. The review showed Nepalese's company mainly produced solid bio-fertilizers with few producing the liquid one. The living microorganism is used in the preparation of Biofertilizers which have a specific function to enhance plant growth and reproduction. Biofertilizers are considered a viable option for farmers to increase productivity maintaining a clean environment.

Key-words: biofertilizer, micro-organisms, soil fertility, plant growth

1. Introduction

The green revolution was introduced as modern technology to provide a steady supply of food to a growing population. This called for use of chemical fertilizers in large amount harming soil structure, microflora, quality of water, food and fodder (Eliazer Nelson et al., 2019; Pingali, 2012). This called for searching an alternative such as biofertilizer. Biofertilizers are natural and organic fertilizers that keep in the soil with all the nutrients and live microorganisms required for the benefits of the plants (Patel et al., 2014). Many research showed that biofertilizers as important components of integrated nutrient management, as they are a cost-effective and renewable source of plant nutrients to supplement the chemical fertilizers for sustainable agriculture (Bhardwaj et al., 2014; Bulgarelli et al., 2013). This drive the growth of the biofertilizers market and projected to reach US$ 3.3 billion by 2025 ("Biofertilizers Market Size, Share & Analysis Report [2020-2027]," n.d.). There are different types of biofertilizers available in the market, however, a meta-analysis showed P solubilizers and N fixer are the popular ones as their combination increase yield (Schütz et al., 2018). Research shows that yield of various crops can be increased by about 25% and use of inorganic N and P fertilizers be reduced by about 25-50% and 25% respectively through the application of biofertilizers (Masso et al., 2015). Although the concept of biofertilizers is widely studied, the utilization of this technology has not reached its full potential for several reasons. Continuous application of biofertilizers enables the microbial population to remain and build in the soil and helps in maintaining the soil fertility contributing to sustainable agriculture

(Masso et al., 2015). The biofertilizers can be manufacture in solid or liquid form depending on the application in the plants. Based on form, liquid fertilizers are estimated to account for a major value due to its higher shelf life as compared to carrier-based biofertilizers ("Biofertilizers Market Industry Trends, Opportunities, & Statistics | COVID-19 Impact on Biofertilizers Market," 2020). Liquid biofertilizers have a life expectancy of 2 to 3 years, making it more convenient and affordable to farmers in developing countries. Moreover, liquid biofertilizers have better tolerance limits for adverse conditions (Ji et al., 2017).

This review aims to understand the different types of biofertilizers available in the market and techniques used for the production of those fertilizers. Therefore, this provides concise information on biofertilizers type and technology used.

2. Biofertilizers available in the markets

The global market for biofertilizers has witnessed significant demand during the last few years and projected to reach USD 2,653.48 million at compound annual growth rate (CAGR) of 14.42% during the forecast period ("Biofertilizers Market Industry Trends, Opportunities, & Statistics | COVID-19 Impact on Biofertilizers Market," 2020). The market is driven primarily by the increasing organic farmland as well as the rising acceptance of biofertilizers among farmers. Based on types biofertilizers are dived into different types based on the product (nitrogen-fixing, phosphate solubilizing and Potash mobilizing); microorganisms (Azotobacter, Azospirillum, Rhizobium Cyanobacteria); application, crop type (Cereals, Grains, Pulses and Oilseeds and fruits and Vegetables) and region (Table 1). Based on regions, North America is stated to account for a leading share of 28.7% by 2024 in the biofertilizers market (Research Nester, 2017).

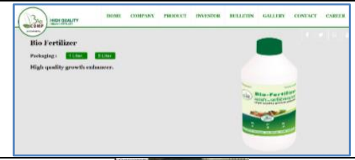
Table 1: Key market segments for biofertilizers

ATTRIBUTE	DETAILS
By Product	• Nitrogen Fixing Biofertilizers • Phosphorus Biofertilizers • Compost Biofertilizers • Liquid Biofertilizers
By Microorganism	• Rhizobium • Azotobacter

	• Azospirillum • Blue-green algae • Mycorrhiza • Phosphate solubilizing bacteria • Other microorganisms
By Applications	• Seed Treatment • Soil Treatment • Others
By Crop Type	• Fruits & Vegetables • Pulses & Oilseed • Cereals & Grains
By Region	• North America (U.S., Canada and Mexico) • Europe (UK, Germany, France, Spain, Italy and Rest of Europe) • Asia-pacific (China, Japan, India, Australia, South Korea and Rest of Asia-Pacific) • LAMEA (Brazil, Saudi Arabia, South Africa and Rest of LAMEA).

In Nepal, farmers apply almost all possible types of fertilizers required for crop production. Fertilizers such as compost, farmyard manure, green manure and town waste are traditionally utilized. In urban areas of Nepal, vermicomposting is gaining popularity as it converts organic matter both physically and chemically increasing soil porosity (Amgai et al., 2018). There are different private firms producing biofertilizers in Nepal, some of them are listed below, (Table 2).

Table 2: Different fertilizers available in Nepal

S. N	Company	Product	Location/Contact	Figure
1	Biocomp Nepal (https://www.biocompnepal.com/biof.html)	Liquid fertilizers from plant extract	Khokana, Lalitpur; +977-9802054440	
2	Organic-Biofertilizer Pvt. Ltd - OBiFert	Solid fertilizers	Bharatpur, Chitwan; +977-1-5542654	
3	National Biotech Pvt. Ltd	Solid fertilizers	Godavari Metropolitan, Lele; +977-9851229232	
4	Annapurna Organic Fertilizer (https://gandakiurja.com/services)	Solid fertilizers	New Road Pokhara; 061-620800	
5	Dhanvarsha Industries and Biotechnology Pvt. ltd	Solid fertilizers	Nepalgunj 20, Banke	
6	Bansun Agro-Organics Pvt. Ltd. https://organicnepalcoop.com/bansun-agro/	Solid fertilizers	Jugedi, Bharatpur Metropolitan City-29, Chitwan District, Nepal.	
7	Praramva Biotech Pvt. Ltd (http://praramvabiotech.com/)	Dust Ramkot - 6, Kalanki Kathmandu,	Ramkot - 6, Kalanki Kathmandu, Nepal	

3. Different techniques used for the development of biofertilizers

Biofertilizers are the product of the fermentation process, constituting efficient living soil microorganisms. They are considered eco-friendly and cost-effective to enhance agricultural production sustainably. For biofertilizer development, there are two processes i.e., solid-state and submerged fermentation. Each type of biofertilizers is prepared by a selection of efficient microbial strain, its cultivation using a specific nutrient medium, scale-up and formulation using solid or liquid base. The efficiency of biofertilizers is defined by its host specificity of the microbial strain and properties of soil and environmental conditions. Microbial taxonomy, genetic engineering, metabolic engineering, computer science and nanotechnology play a significant role in the fermentation of biofertilizer. The common raw materials used for making bio-organic fertilizers are:

1. Agricultural waste: straw, rice bran, etc.
2. Animal waste: chicken manure, pig, cattle and sheep manure, etc.
3. Industrial waste: vinasse, a sugar residue, etc.
4. Household waste: kitchen waste, vegetable market and slaughterhouse waste, etc.
5. Municipal sludge: river silt, sewage sludge, etc.

The basic steps used for the production of biofertilizers are

1) **Organic materials fermentation process**: It is preliminary steps which are conducted through self-propelled compost turner and hydraulic compost turner to accelerate the fermentation process.
2) **Crushing process**: Compost prepared should be grinded before the refining process. The general process involved in crushing is vertical chain crusher and double-shaft horizontal crusher.
3) **Mixing process**: Afterwards the compost is thoroughly mixed using horizontal/vertical mixer.
4) **Granulator process**: This process is conducted according to customers detailed requirements. Disc granulator machine can be chosen to granulate the materials evenly.
5) **Drying process**: Generally, the moisture of fertilizers should be less than 25%. Therefore, it should be dried with a certain degree of humidity and particle size.
6) **Rotary drum cooling machine:** This is used for cooling fertilizer to make its particles stronger.
7) **Rotary drum screening machine**: In this process, the large particles were separated to return them for secondary crushing and granulating. Rotary drum coating machine is a commonly used machine to coat fertilizer and prevent the fertilizer from sticking together.

8) **Packaging:** Fertilizer packaging machine can package bags quantitatively and automatically. We also need some auxiliary equipment for connection such as belt conveyor, bucket elevator, etc.

4. Conclusion

Biofertilizers being an essential component of organic farming which play a vital role in maintaining long term fertility and sustainability. They are considered a viable option for farmers to increase productivity per unit area in organic farming for an era of prosperity and a clean environment. Literature showed biofertilizer have better efficiency, multiple functionalities and longer shelf life. In the future, pretest of the soil community may predict the competitive chance of biofertilizer in specific soil and help to efficiently produce adapted biofertilizers for each specific application.

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CHAPTER 7 - Literature review on the use of nano fertilizers and its prospects for sustainable agriculture

Abstract

This review focused on the applicability of nano fertilizers and its development to improve the nutrition dynamics of the soil-plant system for sustainable crop management. Rapid use of chemical fertilizer has caused serious degradation of soil fertility, environmental pollution, loss of biodiversity with economic loss. Therefore, research was conducted on finding a productive method of fertilization for agriculture practice. Literature showed nano biofertilizer in plant and soil systems are efficient for the enhancement of agriculture productivity. Nano biofertilizers act synergistically providing higher retention of soil moisture and essential plant nutrients due to nanomaterial coating as well as the revitalization of the bioorganic component.

Key-words: nano biofertilizer, soil fertility, agriculture, fertilization

1. Introduction

Nano fertilizers are synthesized or modified form of traditional fertilizers which can be produced from different biological materials using nanomaterials for controlled and slow release of nutrients for the development of soil fertility, productivity, and quality of agricultural products (Pirzadah et al., 2020; Zulfiqar et al., 2019). The high reactivity of nanomaterials helps plants to improve and effective absorption of nutrients. Studies showed utilization of nano fertilizers in a correct way increase nutrient efficiency, prevents leaching and diminishes overall environmental risks (Chen and Wei, 2018; Solanki et al., 2015). Effectiveness of nano fertilizers depends on three main factors i.e., intrinsic (nano formulation, surface coating), extrinsic (soil depth, soil pH, soil texture, temperature and microbial activity) and route of administration (through plant roots of leaves) (Solanki et al., 2015; Zulfiqar et al., 2019). Figure 1 outline the comparative application of different fertilizers from conventional to nano fertilizer.

There are three different ways for encapsulation of nutrients with nanomaterial (Aamir Iqbal, 2020).

- Entrapped/encapsulated within the nanomaterials
- Coated with a layer of nanomaterials
- Delivered in the form of nano emulsions

Nano fertilizers have been classified into three groups 1. Nano formulation of micronutrients 2. Nano formulation of macronutrients and 3. Nutrients-loaded nanomaterials (Kah et al., 2018). Out of these three categories, nutrients loaded nanomaterials are more popular as compared to others. This is because nutrients-loaded nanomaterials are safer to workers and environmentally friendly and can release fertilizers in a precise manner according to the requirement. Nanocarriers technology have been employed in other applications, including pesticides, food, and drug delivery (Bernela et al., 2018; Sotelo-Boyás et al., 2017).

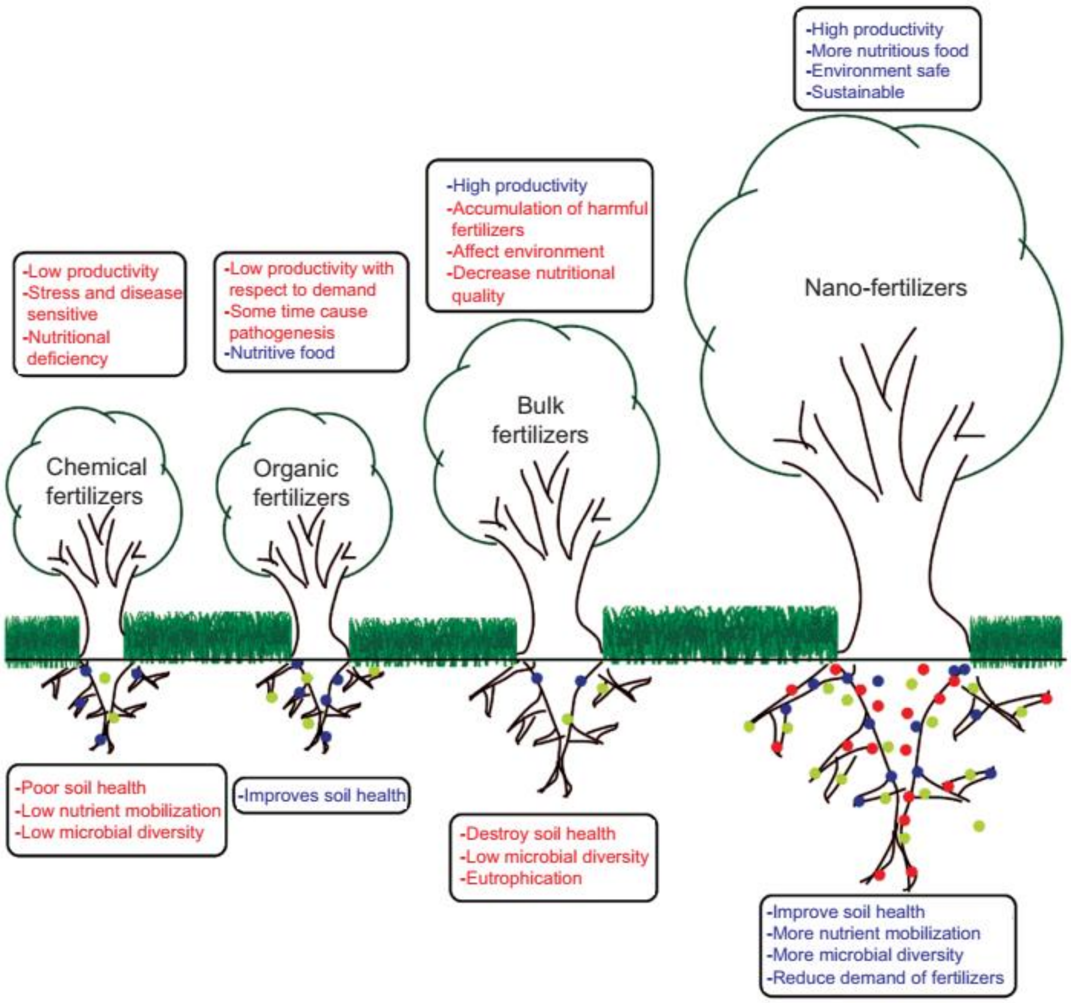

Figure 1. Comparative evaluation of possible advantages, gains, and losses of chemical fertilizers, organic fertilizers, bulk fertilizers, and nano fertilizers on plant growth and soil rhizosphere. [Red and blue text respectively denotes adverse and good impacts]

2. Manufacturing of Nano fertilizers

Nano fertilizers can be prepared by different approaches: physical (top-down), chemical (bottom-up), and biological (biosynthetic) approaches (Fig. 2). The physical approach is based on the size reduction of relatively large particles into smaller particles of the nanoscale by mechanical attrition (Prasad Yadav et al., 2012). The major limitation of this approach is the low control on the size of the nanoparticles and a greater quality of impurities. In the chemical approach, molecules in a solution are transformed into nanoparticles through some chemical reactions (e.g., precipitation methods, hydrosol methods) (Kumar et al., 2012). This process is chemically controlled; therefore, particle size can be controlled and impurities reduced (Pradhan and Mailapalli, 2017; Zahedi et al., 2020).

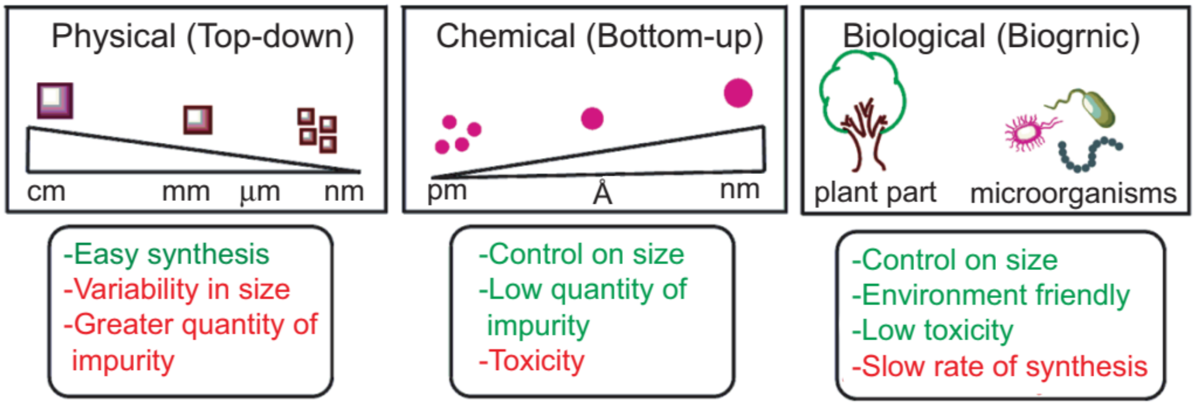

Figure 2. Representation of methods for the production of nano fertilizers: physical (top-down), chemical (bottom-up), and biological approaches and advantages and limitations represented in the lower text box with green and red colour, respectively.

Likewise, in biological process, nano fertilizers can be synthesized using plants, fungi, yeast and bacteria. In all the process of preparation of nano fertilizers proper care should be taken, therefore, it can be efficient as well as cost-effective.

3. Types of Nano fertilizers

Nanoparticulate carriers modify the role of fertilizers and help to improve crop yield. Nanoparticles can act as fertilizer or as delivery vehicles. Depending on the type of nutrient, nano fertilizers can be broadly divided into three types: macronutrient-based, micro nutrient-based and biofertilizer based.

- Macronutrients include primary (Nitrogen, Phosphorous and Potassium nano fertilizer) and secondary (Calcium, Magnesium and Sulfur nano fertilizer) fertilizer. Usually, primary macronutrients are consumed in higher quantities, secondary macronutrients are also very vital for plant growth.

- Micronutrient based nano fertilizers include Iron (Fe), Manganese (Mn), Zinc (Zn), Copper (Cu), Molybdenum (Mo) and Nickel (Ni). In comparison with the macronutrients, only trace levels of micronutrients are required for the healthy growth of crops another plant.
- Biofertilizer based Nano fertilizers encompass an intentional coexistence of a biocompatible nanomaterial and a biological source driven fertilizer encompassing high efficacy of both the ingredients. In this process, there is a slow and gradual release of nutrient contributing to improving nutrient usage as well as promoting crop yield and productivity (Duhan et al., 2017; Thirugnanasambandan, 2019). Biofertilizers are composed of biologically useful microorganism such as rhizobium, blue-green algae, mycorrhizae, bacterium azotobacter, Azospirillum, phosphate-dissolving bacteria. These microorganism act as catalysts not only by modulating the characteristics of nitrogen-fixing ability but also by improving the solubility of insoluble complex organic matter (Itelima, 2018).

The nanoscale formulation of a biofertilizer resolves the issues of poor on-field stability and shortage of beneficial bacterial strains through conferring structural protection to biofertilizer nutrients and plant growth-promoting bacteria, via nanoencapsulation mediated coating of nanoscale polymers (Golbashy et al., 2017). This nanoencapsulation approach could be used as a dynamic mechanism to elongate the structural protection of being delivered biofertilizer, enhance its chemical shelf life and dispersion in fertilizer formulation, allowing a controlled release (Vejan et al., 2016). Besides, improving nutrient release characteristics, the nano fertilizer technology also better the field performance and conclusively reduces the economic expenditure (through cost reduction as well as reduced application extents). Figure 3 depicts the typical role of nano fertilizers in enhancing plant growth and nutritional upkeep. Lastly, the diverse impacts of nano biofertilizers on soil texture and plant system enzymes manifest significant benefits in enabling improved growth and nutritional quality. Nanomaterials such as chitosan, zeolites and polymers facilitate considerable enhancement in the absorption of organic nutrients through the nanoencapsulation phenomenon, forming a sustainable rich source of nutrients for the plants (Qureshi et al., 2018).

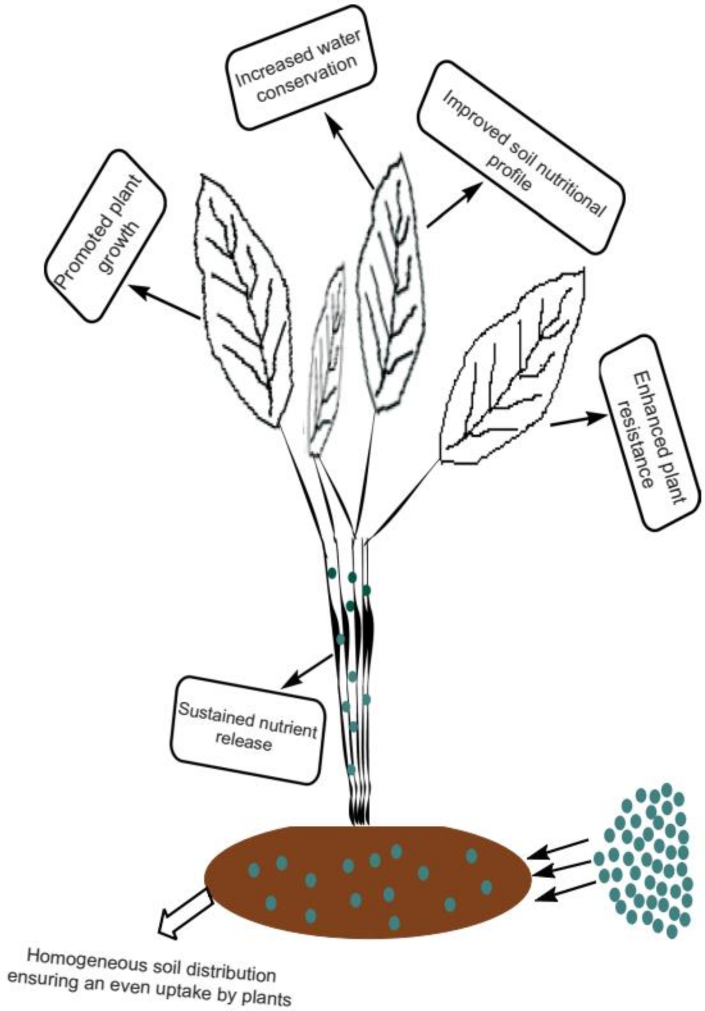

Figure 3. Nano fertilizer impacts on the different plant growth determining factors

4. Nano fertilizer for sustainable agriculture

Studies showed nano fertilizers significantly improve the crop growth through the optimization of photosynthesis, nutrient absorption efficacy, higher photosynthate accumulation and nutrient translocation, enabling enhanced productivity as well as quality. Pirzadah et al (2019) have specifically reported the characteristics effects of nano biofertilizers through the entrapment of biofertilizer (growth-promoting bacteria) within gold (Au) and silver (Ag) nanoparticles. The study showed higher crop growth upon administration of nanoparticles with bacteria compared to those with nanoparticles alone (Rahman and Zhang, 2018). Nanostructured fertilizer consisting of neem cake with PGPR provides efficacy toward promoting crop-harvest yields in several leguminous crops through earlier and greater seed germination as well as effective delivery of doped nutrients (Rahman and Zhang, 2018). Gatahi et al (2016) studied the effects of nano biofertilizers in bacterial wilt (caused by *Ralstoniasola nacearum*) infected tomato crop and reported it as pesticide resistance. A nano clay-based biological compound comprising *Trichoderma* and *Pseudomonas* sp. was investigated for controlling the fungal nematode disease in rabi crops and was noticed to confer crop resistance against abiotic stress (Mukhopadhyay, 2014). This shows that nano biofertilizer offers a sustainable, cost-effective, and competent integrated management of nutrients. However, there are some concern about nano biofertilizers in terms of transformation and accumulation of nanoparticles and associated safety concern for farmworkers.

5. Prospects

Nano fertilizers research is still a long way to go despite its advantageous uses. Developing countries like Nepal have extensive agricultural practices in a rural background. Therefore, getting back up in such circumstance is challenging to incorporate most of the scientific distinctions. So, there is a need to make grassroots efforts in awakening the farming community and farmers about the positives side of nano carrier-mediated fertilizer delivery. Therefore, scientists and media personnel must initiate harmonious and committed joint efforts along with reliable governmental support so that the exact scientific rationale for nano fertilizers usage is understood.

6. Conclusion

The scientific essence of nano fertilizers is to boost agricultural outputs, characterized by correct selection and uniform dispersal of seeds, thorough irrigation and adequate as well as regulated use of fertilizers. Many factors determine fertilizer distribution efficacy including soil type, chemical combination with other nutrients, leaching effect and uptake efficiency of plants. Excessive use of chemical fertilizer resulted in serious deterioration of soil fertility, enhanced environmental pollution, pesticides resistance. This called to find an amicable alternative to chemical fertilizer usage, enabling an environment-friendly approach in agriculture. Nanomaterials prepared from microbes to plant bears enormous potential for developing better, safer, and easily biodegradable fertilizer. For this, we need nanoparticles (capping agent) that do not cross-react with the soil environment. Therefore, optimum combinations of nanomaterials and biofertilizer help in the development of low-cost eco-friendly nano biofertilizers and pose a great potential to boost the agricultural output.

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CHAPTER 8 - National and International R & D Institutions on Forestry and Agriculture for Future Communication and Networking

Abstract
The report provides a list of different national and international organization working for the development of the forest and agriculture sectors. Five national government offices directly look after the national forestry and conduct research and training on forest enhancement. Similarly, a national organization like Nepal Agriculture Research Council (NARC) work actively for the development of organic agriculture in Nepal. Besides, different NGOs are working in the field of forestry and organic agriculture providing awareness and sustainable livelihoods to rural people. This report also includes different international organizations which are fully focused on that area. This provides a concise list of those organization and can be utilized by MBUSTB for future research and collaboration.

Key-words: national, international, forestry, agriculture

1. Introduction

There are different organization involved in the research and development of forestry and organic agriculture sectors. These organization at a national and international level are associated with conservation, development, communication and networking. Communication and networking are a matter of creating useful linkages both within and among communities, organizations and societies to mobilize resources and achieve various goals. Therefore, recognition of a national and international organization working in that field is important. In this report, I listed national and international organization with their purpose and address in the tabular form. This help for networking and future collaboration with those organization at a different level of studies.

2. National organization working in forestry and agricultural sector

There are many governments organizations as well as NGOs involved in the research and development of the forestry and agricultural sectors. These organizations are involved at community level research for the promotion of forest products and agriculture. National organizations involved in forestry and agriculture sectors are listed below,

Table 1: List of national organizations working in forestry and agriculture sector

S.N	Organization Name	Purpose of establishment	Address	Website	Remarks
1	Department of National Parks and Wildlife Conservation (DNPWC)	To conserve the country's major representative ecosystems, unique natural and cultural heritage	Babar Mahal, Kathmandu, Nepal	http://www.dnpwc.gov.np/en/	Government (Working in forestry sector)
2	Department of Forests and Soil Conservation	Work for sustainable utilization of forest resources	Babar Mahal, Kathmandu, Nepal	http://www.dofsc.gov.np/	Government (Working in forestry sector)
3	Forest Resource and Training Centre (FRTC)	Conduct scientific research and survey in the forestry sector	Babar Mahal, Kathmandu, Nepal	https://frtc.gov.np/home	Government (Working in forestry sector)
4	National Trust for Nature Conservation (NTNC)	To conserve nature and natural resources in Nepal while meeting the needs of the people in a sustainable way	Khumaltar, Lalitpur, Nepal	https://ntnc.org.np/	Government (Working in forestry sector)
5	Nepal Agricultural Research Council (NARC)	To conduct agricultural research in the country to	Singh Durbar Plaza,	https://narc.gov.np/	Government (Working in

		uplift the economic level of the people	Kathmandu, Nepal		agricultural sector)
6	Nepal Herbs	Popularize and promote the use of Nepali Herbal Products	Teku, Kathmandu, Nepal	http://nepalherbs.org.np/	Private (Working in forestry sector)
7	Federation of Forest-based Industry and Trade, Nepal (FenFIT-Nepal)	Advocacy program for promoting the forest-based industry and trade-friendly environment	Koteshwor, Kathmandu, Nepal	https://www.fenfitnepal.org.np	Private (Working in forestry sector)
8	Nepal Ban Nigam Ltd	To regulate the wood from the forest and its utilization	Babar Mahal, Kathmandu, Nepal	http://tcn.gov.np/	Government (Working in forestry sector)
9	Federation for Community Forestry Users (FECOFUN)	To promote cooperation and mutual good-will among forest user groups through sharing of experience	Duwakot, Bhaktapur, Nepal	http://www.fecofun.org.np/	Private (Working in forestry sector)
10	WWF Nepal	To tackle conservation and environmental problem	Baluwatar, Kathmandu, Nepal	https://www.wwfnepal.org/	INGO (Working in forestry sector)
11	Forest Action Nepal	To influence the public policy process and	Lalitpur, Nepal	https://www.forestaction.org/	Private

		empower forest and natural resource-dependent communities particularly the poor, marginalized and vulnerable, groups			(Working in both forestry and agricultural sector)
12	Asian Network for Sustainable Agriculture and Bioresources (ANSAB)	Biodiversity conservation and livelihood improvement in South Asia	Baneshwor, Kathmandu, Nepal	https://ansab.org.np/	Private (Working in both forestry and agricultural sector)
13	Rastrapati Chure Samrachayan Bikas Samati	Conservation and preservation of Chure forest	Khumaltar, Lalitpur, Nepal	https://chureboard.gov.np/	Government (Working in forestry sector)
14	Department of plant resources (DPR)	To conduct research and identification of herbal metabolites from the medicinal plant	Babar Mahal, Kathmandu, Nepal	https://dpr.gov.np/	Government (Working in forestry sector)

This are some of the government and private organization working in forestry and agriculture sector. Out of this, MBUSTB can work in collaboration Forest resources and training centre, national trust for nature conservation, Nepal Agricultural Research Council, Nepal Ban Nigam, WWF Nepal, Forest Action and Rastrapati Chure Samrachayan Bikas Samati.

3. International organizations working in forestry and agricultural sector

Similarly, there are different international organizations working in the field of forestry and agriculture. These organizations help by providing funding to national and local organization as well as conducting research and development. The list of the organizations working in the sector are listed below,

Table 2: List of international organizations working in forestry and agricultural sector

S.N	Organization Name	Purpose of establishment	Address	Website	Remarks
1	Food and Agriculture Organization (FAO)	To achieve food security for all and make sure that people have regular access to enough high-quality food to lead active, healthy live	Roma, Italy	http://www.fao.org/home/en/	Working in Nepal in both agriculture and forestry sectors
2	Regional Community Forestry Training Center for Asia and the Pacific (RECOFT)	To enhance capacities for stronger rights, improved governance and fairer benefits for local people in sustainable forest	Bangkok, Thailand	https://www.recoftc.org/	Working in Nepal in forestry sector

		landscapes in the Asia- Pacific region			
5	European Forest Institute (EFI)	Enhancing international forest research and providing decision-makers with unbiased forest-related information at a European level	Joensuu, Finland	https://efi.int/	Working in forestry sector mostly in Europe
6	The Center for International Forestry Research (CIFOR)	Conduct innovative research, develop partners' capacity, and actively engage in dialogue with all stakeholders to inform policies and practices that affect forests and people.	Bogor, Indonesia	https://www.cifor.org/our-work/about-cifor/	Working in Nepal in forestry sector
7	World Forestry Center	To supports the economic, ecological and social benefits of forests	Portland, USA	https://www.worldforestry.org/	Working in forestry sector
8	International Tropical Timber Organization (ITTO)	Promote the sustainable management and conservation of tropical forests and the expansion and diversification of international trade in tropical timber forests	Yokohama, Japan	https://www.itto.int/	Working in international arena in timber resources

10	FPInnovations	Support and provide a solution for the Canadian forest sector	Vancouver, Canada	https://web.fpinnovations.ca/	Private organization in Canada working in forest product development
11	SCION (Forest, product, Innovations)	Development of technology and conduct research for forestry and wood-derived materials	Rotorua, New Zealand	https://www.scionresearch.com/	Working in forestry sector in New Zealand
12	International Bamboo and Rattan Organization	To promote the well-being of producers and users of bamboo and rattan within the context of a sustainable bamboo	Beijing, China	https://www.inbar.int/	Working in China
13	Chinese Academy of Forestry	Multi-discipline and public research institution that is directly subordinate to the National Forestry and Grassland Administration (NFGA)	Beijing, China	http://en.caf.ac.cn/	Academic organization working in forestry sector in

					China and around world
15	International Maize and Wheat Improvement Center (CIMMYT)	Research and Development in Maize and Wheat for sustainable intensification	Houston, USA	https://www.cimmyt.org/	Working in agriculture sector in Nepal
17	Winrock International	Providing solutions to some of the world's most complex social, agricultural and environmental challenges	Virginia, USA	https://winrock.org/	International organization working in agriculture and forestry sector in Nepal

Nepal has already started a collaborative research and development in forestry and agriculture with different organization. MBSUTB can also start working with some international organization like FAO, World forestry center, The center for international forestry research, SCION, Chinese Academy of Forestry and FP Innovations Canada.

4. Conclusion

There are different national and international organization for the promotion and development of the forestry and agricultural sector. This report provides a summary of some of well know organizations working in this sector. The listing of the organization helps for future networking and collaboration for research within the University.

CHAPTER 9 - Formulation of Master and PhD level courses in Madan Bhandari University of Science and Technology

Abstract

This report provides an overview of the work performed for the formulation of master and PhD degree courses at Madan Bhandari University of Science and Technology. I was actively involved in arranging the required materials and formulation of the consultation meeting for forest biomaterials and organic agriculture. Moreover, I collected the secondary materials and help our honorary consultant Prof. Ning Yan from the University of Toronto and Prof. Andre van Amstel from Wageningen University. The consultation meeting was organized separately for forest and organic agriculture in different groups. After a series of consultation between policy level, business, academic and mixed stakeholder we came with the idea of building the two courses. The first course was defined as Forest Biomaterials Science and Engineering and another as Organic Agriculture. The course structure and probable research topics were defined for the two courses for master and PhD student.

Key-words: forest biomaterials, organic agriculture, stakeholders,

1. Introduction

Madan Bhandari University of Science and Technology (MBUST) is established to become a world-class university and plan to accelerate the economic growth of the country. Therefore, the University board consult and collaborate with a national and international expert in designing and formulation of different master and PhD courses. The objectives of the courses are to educate people in the field of forest and organic agriculture incorporating cutting edge technology. This helps in fostering economic, environmental and socio-cultural benefits to the Nepalese people. This report includes outputs from different stakeholders meeting that was conducted for the formulation of forestry and organic agriculture courses.

2. Forest product program

Forestry in the broad sense is the science and art of managing forest resources to provide continuingly the goods and services which society demands from them. Because wood is a regenerative resource and because its lifecycle plays an important role in the quality of life (e.g. the cleaning and filtering of air and water), the multi-functional properties of this natural resource provide enormous potential in its application and use.

Nepal has almost 45% of the area covered by forest and vegetation. Government initiatives are focused more on the conservation of forest vegetation and biodiversity and its sustainable utilization as exemplified by community forestry. Mountain forests are well conserved with community forestry, and due to decreasing agricultural practices in farmlands, forest/woodland areas are increasing. Non-timber forest product utilization is building up gradually, utilization of timber and other forest products in a sustainable way is still not practiced in Nepal. A huge amount of timber and other forest products is simply decomposed and wasted while Nepal is importing a very high amount of timber and timber products as well as products of other forest materials.

To deal with this problem there is a need to research how forest raw materials can be converted into a profitable product is needed. This required a highly specialized academic program like the one practice in Europe, USA. Several different institutions are offering forestry-related qualifications for undergraduate and graduate level. This increases the level of qualifications of forestry professionals and civil society. However, these programs are largely having a traditional focus on forest land management and conservation and there is very limited inclusion of sound utilization of timber/wood and other forest products.

Therefore, MBUST plans to initiate academic and research programs on Forest Product Utilization focusing on forest products/wood science and technology/engineering so that Nepal could develop technical understanding and expertise in this field and manage its forest resources more efficiently along with its sustainable utilization. University will make strong ties with CFUGs, local communities and government agencies for proper utilization and economic upliftment of those communities. Recognizing a great prospect of research and product development from Nepali forests, and also based on the experiences and learning from international best practice in forestry, MBUST has conceptualized the idea of developing an academic and research program in forest product.

Four stakeholders meeting with the business community, policymakers, forest group and academic community were conducted to know the research area and its prospects in the forest. Conclusion drawn from the meeting is listed below:

2.1 Consultation with the business community

The consultation was conducted on September 27, 2020, with the forest business community. The comments and suggestions from the stakeholders are listed below;

- The forest business community is not strong in Nepal therefore there is a need to develop technology to strengthen their need.
- The University should focus research on technology and product development.
- Research on promotion and up-gradation of traditional technology with a focus on post-harvest, packaging and marketing is necessary.
- Proper government policy to prioritized and attract forest-based industry in need.
- There should be research on improving the quality of softwood through proper seasoning and treatment.

2.2 Consultation with policymakers

The consultation was conducted on September 29, 2020, with forest policymakers. The comments and suggestions from the stakeholders are listed below:

- The government policy should be focused on forest-based entrepreneurship
- The university should focus on developing proper and improvement of scientific forest management.
- The government should develop a policy that attracts the young generation in the forest sector.
- Forest policy should interlink socio-economic aspect.
- Some research on carbon policy from the forest such as REDD-plus should be considered in university courses.
- MBUST should bring innovative ideas and focus on NTFPs research.

2.3 Consultation with forest group

The consultation was conducted on October 01, 2020, with forest managers. The comments and suggestions from the stakeholders are listed below:

- The university should produce manpower who can increase the efficiency of workers in the forest sector.
- There should be a localization of research in forest resource based on small industry.
- There should be a focus on reclaimed or reconstructed wood, pulp and paper.
- There are policy gaps in forest sectors so people are not getting actual benefit from this sector.
- The university course should include a balance between environmental, social and economic sector.
- Research on the utilization of softwood as hardwood is necessary for its utilization.

2.4 Consultation with the academic community

The consultation was conducted on October 10, 2020, with the academic community. The comments and suggestions from the stakeholders are listed below;

- University courses should include new and innovative master and PhD courses in their curriculum.
- University should initiate a wood testing laboratory along with forest certification.
- The University should produce trained manpower who can develop employment opportunity.
- A world-class university should focus on innovation uniquely specific to Nepal.
- In the course curriculum, there is a need to have a course on physiology, entomology, pathology and wood science.
- Nature-based courses can also be included in the forestry courses.
- The courses should be based on internship and fieldwork rather than only theoretical.

3. Organic agriculture

Nepal hills and mountain occupy over 80% of the territory. Also, those areas have rich climatic and biodiversity which can be suitable for organic hill and mountain agriculture. Hills are suitable for agroforestry, horticulture and a variety of other cash crops like nuts, fruits, tea, ginger, turmeric etc. Mostly, Nepalese agriculture is based on traditional technology so there is a need for proper research and innovation in farming based on local people need. Therefore, MBUSTB aims to conduct research on farming and optimization of traditional varieties to support country aspirations for rapid economic growth. Stakeholders' consultation with farmers and business, academic and policy were conducted to know problem and prospects of research on organic agriculture. Conclusion drawn from the meeting listed below:

3.1 Consultation with farmers and business

The consultation was conducted on October 18 2020 with farmers and business group working in agriculture. The comments and suggestions from the stakeholders are listed below;

- The meeting focus on agricultural activities maintaining the quality of the soil.

- Most people focus on mixed agriculture system with organic fertilizers and without the use of artificial fertilizers, pesticides, antibiotics, and genetically modified organisms.
- Nepal government focused on the improvement of organic hill agriculture however more could not be done as we could not deal with the marketing of our products.
- Chain management needs to be improved which help to increase the value of the products.
- Some stakeholders indicate the importance of building the traditional seed varieties bank.
- Business group focus on proper certification systems for organic products in a cost-effective system where MBUSTB can take a lead.
- MBUSTB should focus on post-harvest technology and supply chain management
- More research should be done on organic fertilizers and pesticides for the success of organic farming.
- Research on ways to reduce dependency on pesticides through ecological interventions through a multi-cultural plantation
- Research and development in biofertilizers, nano fertilizers suitable for the crops.
- Should focus on Research, Development and Innovation that helps to promote organic agriculture

3.2 Consultation with academia

Based on consultation with academia the following research areas are considered for MBUSTB for developing Organic Agriculture master and PhD program.

- The graduate program should focus on developing student with skills and leadership qualities so they can start their own business and provide employment.
- The syllabus of the program should focus on topics such as water and soil management, product improvement, organic farm extension services and supply chain management.
- The program should be based on practical experience to improve the skills and competencies of the farmers in the hills of Nepal.
- The program should provide wide exposure to frontier science and technology through invited lectures from the national and international arena in the field of organic agriculture.
- Multi-disciplinary collaboration with the engagement of students with others background like food science, materials science, chemistry, hydrology and engineering is needed.

- ❖ The curriculum should include mandatory training in entrepreneurship, data analytics, modelling communication and writing are important.

3.3 Consultation with policy

- ❖ The course should focus on United Nations Sustainable Development Goals to promote biodiversity and organic agriculture.
- ❖ MBUSTB could link with a government agency at the national and provincial level to understand the problem and solving it at a local level.
- ❖ Linkage with international donor agencies like UN World Food Program, Bill and Melinda Gates Foundation, CIMMYT, International Fund for Agricultural Development etc. helps to develop a proposal and gets funding for research in sustainable farming.

4. Conclusion

After series of consultation with different stakeholders' group in forestry and agriculture, MBUSTB come up with two master and PhD program namely; Forest Biomaterials Science and Engineering and Organic Agriculture. The programs will be fully funded with 2 years master and 3 years PhD program. The program will help in the development of skilled manpower with entrepreneurship focusing on economic, environmental and social benefits. The program provides information on resources availability and upliftment of the hill and mountain economy. Students after completing the degree should come up with some type of product or nature-based solution in the field to promote the country economy. Lastly, more consultation in developing a concise course with a practical approach helps the program to become long-lasting and sustainable.

CHAPTER 10 - Cost estimation of the laboratory instruments needed for Forest Biomaterials Science and Engineering and Organic Agriculture

Abstract
This report provides an overview of different instruments purposed for research in Forest Biomaterials Science and Engineering (FBSE) and Organic Agriculture. FBSE laboratory instruments are divided into three subdivisions as wood processing laboratory, structural characterization laboratory and wet chemistry and chemical analysis laboratory. Similarly, organic agriculture laboratory instruments are categorized into organic agricultural laboratory, molecular laboratory, soil nutrients and biotechnology laboratory. The list of instruments was compiled based on the master and PhD degree courses assigned. All the instruments were compiled with detailed specification into their standard format. Afterwards, priority instruments were selected for buying in the first phase. The final list of the instruments was submitted separately in both the excel and word file for complete packaging for the tender process.

Key-words: Forest Biomaterials Science and Engineering, Organic Agriculture, Laboratory Instruments

1. Introduction

Laboratory instruments and their management is essential for having an accurate and reliable laboratory. Good equipment helps to maintain high laboratory performance, reduce variation in test results and improves the technologist confidence with lower repair costs. Therefore, a great deal of thought and planning is necessary for the selection and purchasing, installation, calibration and maintenance of instruments.

Usually, a director is appointed for the laboratory who can oversee all the equipment management systems along with necessary routine maintenance procedures. Also, we should be careful in selecting and acquiring equipment taking into consideration why and how will the equipment be used. Repair cost, equipment warranty, safety issues and operating language should also be taken into consideration while buying the instruments. Before equipment is installed, we should verify that all physical requirements (electrical, space, doors, ventilation and water supply) have been met.

2. List of instruments for Forest Biomaterials Science and Engineering (FBSE)

The laboratory instruments list was compiled based on the proposed research activities that were suggested in stakeholder consultation. FBSE laboratory is divided into three broad groups based on academic and research possibilities i.e., Wood processing laboratory, Structural characterization laboratory and Wet chemistry and chemical analysis laboratory. The list of instruments was presented in a table below with their tentative cost.

Forest Biomaterials Science and Engineering

Wood Processing Laboratory

S.N	Equipment's/Instruments	Specification	Quantity	Tentative cost
1	Viscometer	Digital Viscometer (Model: DV1MLV)	1	28,25,000.00 25,00,000.00 plus VAT @13% 3,00,000.00)
2	Fume Hood	INVENTA-EX-ST1200-LCV	1	NPR 650,000.00 + VAT
3	High temp. tube furnace "tempo"	temp. 1350°C/1400°C fitted with Energy Regulator for temp. Control etc. complete with transformer and Silicon Carbide Rods	1	Rs. 95,000.00 + VAT
4	Ceramic Kiln / Electric Muffle Furnace	1200°C, Stainless Steel, Heating Equipment Vertical Type	1	Rs. 550,000.00 + VAT
5	Bomb Colorimeter with Digital Backman Thermometer	Product Code: bhmce-0053 Brand Name: drs	1	Rs. 315,000.00+ VAT
6	Hot Press Machine for Veneering	BY214X8/16(4)H - 4'X8'	1	Rs. 18,50,000.00 + VAT
7	Mechanical Universal Tensile Tester	WAW-1000Y	1	28,50,000.00 + VAT
8	Charpy/lzod Impact Tester		1	350,000.00 + VAT
9	Dual Energy X-ray Absorptiometry Bone Densitometer		1	Rs. 950,000.00 +VAT
10	Muffle Furnace	Inner made of ceramic pot. Outer case made from thick gauge Mild Steel (CRCA) sheet with mild steel outer shell.	1	1,16,670.00
11	Grinders and Screening	Single drum grinders	1	Rs. 225,000.00 + VAT per piece

Forest Biomaterials Science and Engineering
Structural Characterization Laboratory

S. N	Equipment's/Instruments	Specification	Quantity	Tentative cost
1	Scanning Electron Microscope	Magnification Max 150,000 X; Signal Detection: SE Detector+BSE Detector; Accelerating Voltage:1kV to 30kV, High image resolution	1	Rs.1,10,00,000.00 + VAT
2	MAGNUS, Theia-i Binocular LED with battery backup Research Microscope	45500 Viewing Head: Siedentopf Binocular	1	Rs. 85,000.00 + VAT
3	USB DIGITAL CAMERA-MIPS-5MP (for Microscope)	Magnus Analytics	1	Rs. 45,000.00 + VAT
4	Leica Rotary Microtomes	Leica RM2125 RTS (100-240 V)	1	Rs 40,00,000.00
5	Teflon coated Haglöf Increment Borer (2-thread or 3-thread)	4 (2 thread) and 3 (3 thread)	4 (2 thread) and 3 (3 thread)	Rs 5,00,000.00
6	LINTAB™ 6 / TSAP-Win™ Software		1	Rs 20,00,000.00

Forest Biomaterials Science and Engineering
Wet Chemistry and Chemical Analysis Laboratory

S. N	Equipment's/Instruments	Specification	Quantity	Tentative cost
1	Differential Scanning Calorimeter (DSC)	DSC 204 F1Phoenix®	1	1,30,000,00.00
2	FT-IR spectrometer	Nicolet iS5 FT-IR, KBr Windows	1	73,45,000.00 (65,00,000.00 plus VAT @13% 8,45,000.00)
3	Gas Chromatography – Mass Spectrometry (GC-MS)	Triple Quadrupole GC-MS/MS	1	3,10,75,000.00 (2,75,000,00.00 plus VAT @13% 35,75,000.00)
4	High Performance Liquid Chromatography with Photodiode – Array Detection (PDA) detector	HPLC with Quaternary Pump/PDA Detector, Peltier Autosampler, Peltier Column Oven, Basic Software Model: Ultimate 3000 Manufacturer: Thermo Fisher, US	1	72,32,000.00 64,00,000.00 plus VAT@13% 832,000.00
5	Thermogravimetric Analysis (TGA)	TG 209 F1Libra®	1	169,50,000.00 1,50,000,00.00 plus VAT @13% 19,50,000.00
6	Vacuum Rotary Evaporator	Deluxe model	1	Rs. 405,000.00 + VAT
7	Soxhlet Apparatus	Mantle type 550 m l with glass parts 500 ml	1	Rs. 8,600.00 + VAT
8	UV/Visible Spectrophotometer	Model: Evolution 201	1	30,46,532.00 26,96,000.00 plus VAT @13% 350,532.00
9	Stainless Steel Heating Limpet Jacket Resin Polymer Chemical Reactor		1	Rs. 12,00,000.00 + VAT

3. List of instruments for Organic Agriculture (OA)

The laboratory instruments list was compiled based on the proposed research activities that were suggested in stakeholder consultation. Organic Agriculture laboratory is divided into three broad groups based on academic and research possibilities i.e. Soil and Plant Nutrient Analyzer (SPNA), Microbiology Laboratory (MBL), Molecular Biology (MoBL). The list of instruments according to the different laboratory is.

Organic Agriculture
Soil and Plant Nutrient Analyzer (SPNA)

S. N	Equipment's/Instruments	Specification	Quantity	Tentative cost
1	Flame photometer Model FP 8700	Kruss a. Kruss optronic Germany	1	1,200,000.00
2	Soxhlet assembly with heating mental	Borosil	1	75,000.00
3	Seal Auto analyzer 3 HR	Seal Analytical	1	1,800,000.00
4	Fully Automated Kjeldahl Nitrogen Analyzer, Kjeldahl Distillation and Titration System	Sonnen (K18)	1	1200,000.00
5	pH meter (PICCOLO® plus pH/Temperature Tester with 6.3" Probe - HI98113)	Hanna	2	50,000*2 = 1,00,000.00
6	Conductivity meter (Ultra-Pure Water (UPW) Tester (0.000-1.999 µS/cm) - HI98309)	Hanna	2	60,000*2 = 1,20,000.00
7	Atomic adsorption Spectrophotometer (AAS)	AA-6200	1	11,00,000.00
8	Muffle furnace (MF-200D)	Sonar	1	3,00,000.00
9	Hot air sterilizer oven (SDO -6096)	Sonar	1	90,000.00
10	Thermometer (Dual Input K-Type Thermocouple Thermometer - HI93532R)	Hanna	2	50,000*2 = 1,00,000.00

11	Seed germinator (PGC-30) – (825mm x 1200mm x 825mm, with 840 liters capacity)	Sonar	1	2,50,000.00

Organic Agriculture

Microbiology Laboratory (MBL)

S. N	Equipment's/Instruments	Specification	Quantity	Tentative cost
1	Vertical laminar Flow hood	Thermolab	1	300,000.00
2	Biosafety cabinet	Thermolab	1	400,000.00
3	Microscope	Olympus	2	1,00,000*2 = 2,00,000.00
4	Digital Colony Counter	Electronics India	1	80,000.00
5	Fluorescent Microscope	Olympus	1	6,00,000.00
6	Incubator (LB – 6096 P)	Sonar	1	3,00,000.00
7	Double distillation unit	Borosil	1	3,00,000.00
8	Autoclave	Thermolab	1	3,50,000.00
9	Antibiotic zone reader	Biotechniques India	1	30,000.00
10	B.O.D. Incubator (TB 40 S/G)	ThermoLab	1	4,50,000.00
11	Refrigerator (LFXC24726S)	LG	1	1,50,000.00

Organic Agriculture

Molecular Biology (MoBL)

S. N	Equipment's/Instruments	Specification	Quantity	Tentative cost
1	Flow cytometer	Biokrom	1	15,00,000.00
2	Nano drop 2000/C spectrophotometers	Thermo fisher scientific	1	10,00,000.00
3	Thermocycler (C1000 Touch^{TM})	Bio-Rad	1	7,00,000.00
4	Gel documentation system (Gel Doc 2000)	Bio-Rad	1	10,00,000.00
5	Centrifuge machine with temperature control	Bio-Rad	1	6,00,000.00
6	Water bath (WBI-4030D)	Sonar	2	75,000.00*2 = 1,50,000.00
7	UV-Spectrophotometer (uv1800)	Shimadzu	1	6,00,000.00
8	Refrigerator (LFXC24726S)	LG	1	1,50,000.00
9	Ultralow deep freezer (-20^{0}C)	REMI	1	2,50,000.00
10	Ultralow deep freezer (-80^{0}C)	REMI	1	6,00,000.00
11	Electromagnetic stirrer (10 MLH plus)	REMI	1	20,000.00
12	Microtube shaker	Tarson	1	40,000.00
13	Orbital Shaker with temperature control (CIS -18 Plus)	REMI	1	2,50,000.00
14	Rotary shaker (RS – 24 PLUS)	REMI	1	1,50,000.00

Madan Bhandari University of Science and Technology
Cost Estimate of Trial Organic Farm

S. N	Item	Cost (NRs)	Subtotal	Remarks
1	**Building and construction**: 1. Stable (200m^2 for 30 head cattle Manure Silo Manure and urine storage 2. Hen House for 1000 Chicken 3. Goat Stable (300 heads) 4. Shed (general) 5. Shed (Dry storage) 6. Fencing	 50,00,000.00 30,00,000.00 30,00,000.00 15,00,000.00 25,00,000.00 2,00,000.00 2,00,000.00 15,00,000.00	1,69,00,000.00	The cost is a tentative estimate and differs based on construction materials and design
2	**Greeneries with water and nutrient recovery system**	70,00,000.00	70,00,000.00	
3	**Organic theme park with lecture room**	65,00,000.00	65,00,000.00	
4	**Animals** Cattle cost (30 heads) Goat cost (300 heads) Chicken cost (1000)	 15,00,000.00 10,00,000.00 5,00,000.00	30,00,000.00	
5	**Equipment** 1. Electric tractor 2. Cart 3. Lawn Mower 4. Plough and Mulching 5. Chain Saw 6. Van 7. Soil and Tree work equipment	 15,00,000.00 50,000.00 1,20,000.00 1,50,000.00 35,000.00 18,00,000.00 10,00,000.00	46,55,000.00	
6	**TOTAL**		**3,80,55,000.00**	**264,835 Euro**
				NRs 3,80,55,000.00 @ 143.69 exchange rate on December 08, 2020
	Veterinary services	5,00,000.00/yr		
	Maintenance	40,00,000.00/yr		

4. Conclusion

Laboratories instruments for FSEB and OA have been well organized in a separate package according to the priority of utilization. The instruments were selected considering broad scope via, preventive maintenance, a procedure for troubleshooting and repair. This will help in maintenance in a high level of performance and greater confidence in the reliability of results. Lastly, proper laboratory safety and guideline has to be maintained within the laboratory with a proper management system.