ISSN: 2456 – 9259
PLANTICA: 2(3), July, 2018

PLANTICA

PLANTICA

Journal of Plant Science

PLANTICA

The Official Publication of

Association of Plant Science Researchers

www.jpsr.in

PLANTICA

Journal of Plant Science

ISSN: 2456 – 9259

Vol. – 2, No. – 3 **July, 2018**

(A Quarterly Journal)

Official Publication of

Association of Plant Science Researchers
Dehradun, Uttarakhand, India

www.jpsr.in

PLANTICA - Journal of Plant Science, Volume 2 (3) July, 2018
The Official Publication of Association of Plant Science Researchers - APSR

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PLANTICA – Journal of Plant Science
(The Official Publication of Association of Plant Science Researchers)
ISSN: 2456 – 9259
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Research Article

PHYTOCHEMICAL SCREENING, TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITIES OF *CATHARANTHUS ROSEUS* LEAVES

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Received: March, 2018 – Accepted: June, 2018

Abstract

Medicinal plants are the most exclusive source of life saving drugs for majority of the world's population.Catharanthus roseus was investigated from the ancient time for their phytochemical components and their therapeutic effects. In the present study photochemical, antioxidant, total phenolic content and ash content of Catharanthus roseus was carried out by standardized methods. The results showed that almost all the photochemical components are present in the plant and it is notable to note that the DPPH Radical scavenging activity showed in a dose dependent manner and highest inhibition at 500 µg/ml. The significant antioxidant activity showed at 62.5% at a concentration of 100 µg/ml. The total phenolic content and ash content determination of the plant leaves revealed that the plant has a high medicinal property, particularly as anti inflammatory activity.

Key words: Phytochemical, antioxidant activity and phenolic content

Introduction

Since ancient times, people have been exploring the nature particularly medicinal plants in search of new drugs. Medicinal plants are used by 80% of the world population for their basic health needs (Hashim*et al*., 2010). Traditional systems of medicines are prepared from a single plant or combinations of more than one plant. Those efficacies depends upon the current knowledge about taxonomic features of plant species, plant parts and biological property of medicinal plants which in turn depends upon the occurrence of primary secondary metabolites (Vinoth*et al*., 2011).

Plants synthesize a wide range of chemical compounds which are classified based on their chemical class, biosynthetic origin and functional groups into primary and secondary metabolites. Phytochemicals are bioactive compounds found in plants that work with nutrients and dietary fiber to protect against diseases. It is crucial to know the type of phytochemical constituent, thus knowing the type of biological activity which might be exhibited by the plant (Agbafor and Nwachukwu, 2011). Antioxidant principles from medicinally important plants possess enormous potential in correcting imbalance mediated oxidative stress and various degenerative diseases (Londhe*et al*., 2009).

Catharanthus roseus which is an important medicinal plant of the family apocynaceae is used to treat many of the

fatal diseases. The synonyms of the plant name include Vincarosea, Ammocallisrosea and Lochnerarosea, other English names occationally used for the plant include Cape Periwinkle, Rose Periwinkle, Rosy Periwinkle and "Old Maid" (Monika Sain and Vandana Sharma, 2013).

It is cultivated mainly for its alkaloids, which are having anticancer activities (Jaleel *et al*., 2006) &used to produce modern chemotherapeutic agent for their pain relieving properties (KratikaKumari and Sharmita Gupta, 2013). The two classes of active compounds in Vinca are alkaloids and tannins.Leaves and twigs of *Catharanthus roseus* have been reported to have hypoglycaemic activity in streptozotocin induced diabetic rats (Singh *et al*., 2001). It has more than 400 alkaloids, some of which are approved as antineoplastic agents to treat leukemia, hodgkin'sdisesase, malignant lymphomas, neuroplastoma, wilm'stumour and other cancers (Taylor and Fransworth, 1975).

The present investigation was carried out to study the biochemical and phytochemical activities of the aqueous leaves extracts of *Catharanthus roseus*, a plant rich in secondary metabolites. Furthermore, the study aims to find out antioxidant properties of the aqueous extract, the total ash content and moisture content evaluation of the dry powder.

Objectives of the study:

1. To Prepare the aqueous extracts of the leaves of the plant *Catharanthus roseus*
2. To carry out Phytochemicalanalysis.
3. To evaluate antioxidant activity of the aqueous extract.
4. To estimate the total phenolic content (TPC).
5. To estimate the ash content and moisture content of the dry powder.

Materials and Methods

Collection of Plant Material:

Extraction Method:

The fresh leaves were washed under tap water and then dried under shades and in hot air oven at 50^0C. The dried samples were then crushed into powder. About 20 g of crude powder was mixed with 250 ml of distilled water. The homologous mixing of the extracts was done by keeping in a magnetic stirrer for overnight. After extraction, the solvent was removed to yield a viscous dark brown residue of aqueous extract. The extracts were filtered using Whatman No 1 filter paper in a Buchner funnel. The extracts were then concentrated at 40-45^0C to a constant weight.

Phytochemical Screening Methods:

Carbohydrates: Benedict's test
Alkaloids: Hagers test:
Proteins: To 0.5 ml of crude extract, added few drops of concentrated HNO_3 and mixed well.
Flavanoids: About 5 ml of dilute ammonium hydroxide solution was mixed with a portion of the aqueous extract followed by addition of concentrated H_2SO_4.
Tannins: About 0.5 gm of dry powdered samples was boiled in 20 ml of water and then filtered. Few drops of 0.1 % ferric chloride were added.
Phenols: Ferric chloride test: 0.5 ml of crude extract was mixed with a few drops of 5% neutral ferric chloride solution.
Diterpenoids: Copperacetatetest
Triterpenoids: Dry crude extract is mixed with 2 ml chloroform and 1 ml acetic anhydride. Mixed well and 1ml concentrated H_2SO_4 was added.
Saponins: Extract was diluted with 20ml distilled water and shaken by hand for 15

minutes. A foam layer was obtained on the top of the test tube.

Steroids: 1mg of crude extract were treated with 10 ml chloroform and filtered. Added 5 ml concentrated H_2SO_4 through the sides of the test tube. Upper layer turns red and H_2SO_4 showed yellow with green fluorescence.

Antioxidant Activity Assay Methods:

DPPH radical scavenging assay: The free radical scavenging activity of *Catharanthus roseus* was determined (Gadow*et al*., 1997). Freshly prepared methanolic solution of DPPH (634 µm) was incubated at ambient temperature with sequential extracts of *Catharanthus roseus* and A515 was measured using a colorimeter.

Total antioxidant capacity assay: Total antioxidant capacity was measured according to the method described by Preito*et al*., 1999. Various concentrations of crude extracts were dissolved in distilled water in eppendorf tubes. They were mixed with 1ml of reagent solution containing 0.6 M sulphuric acid, 2.8 mM sodium phosphate and 4 mM ammonium molybdate. The tubes were capped and incubated at 90^0C for 90 minutes. After cooling to room temperature, the absorbance at 695 nm against blank was measured.

Estimation of total phenol: 0.1ml of the aqueous extract was mixed with 1 ml of Folin's phenol reagent and 1 ml of 20% sodium carbonate. The mixture was allowed to incubate at 45° C for 45 min and the intensity of blue colour developed was read at 760 nm in a colorimeter.

Ash content determination: Two grams of the dry powder was weighed into a previously weighed crucible. The crucible and the content were ignited in a pre-heated furnace to 600ºC for 1 hr.

Moisture content determination: Two grams of the dry powder was weighed into a previously ignited and weighed in crucible and placed in hot air oven at 105ºC for 2 hrs. The crucible was removed, cooled in a desicator and weighed. The process was repeated.

Results and Discussion

Phytochemical analysis of *Catharanthus roseus*:

Table 1.1: Phytochemical screening of the aqueous leaves extracts of *Catharanthus roseus*

S.N.	Phytochemicals	Inference
1	Carbohydrates	+
2	Protein	+
3	Tannins	+
4	Flavanoids	+
5	Triterpenes	+
6	Diterpenes	+
7	Steroids	+
8	Alkaloids	+
9	Saponins	+
10	Phenolic compounds	+

***(+)** = *presence of particular Phytochemical*

From the table 1.1 it is cleared that all the photochemical components are present in the *Catharanthus roseus* in aqueous extract and the result clearly indicate the medicinal properties of the plant.

Antioxidant activity

DPPH Radical Scavenging Assay:

Table 1.2 showed the results of DPPH radical scavenging activity and its showed that the scavenging activity ic on the dose depending manner and the highest scavenging activity was at concentration 500µg/ml. IC50 value of aqueous extract of Catharanthus roseus DPPH radical scavenging activity was obtained as 450 µg/ml. (Fig: 1)

Table 1.2: DPPHradical scavenging activity of aqueous leaf extracts of *Catharanthus roseus*

Concentration of Drug (µg/ml)	% Inhibition
100	17.65
200	33.33
300	38.89
400	44.44
500	55.56

Total Antioxidant Capacity Assay:

The EC50 value of aqueous extracts by Total Antioxidant Capacity Assay of *C. roseus*was obtained as 148.50 µg/ml (Table 1.3)

Table 1.3: Total antioxidant capacity of aqueous leaf extracts *Catharanthus roseus*

Concentration of Drug (µg/ml)	Antioxidant activity (%) of aqueous extract
100	45.60
200	62.50
300	77.78
400	83.33
500	98.72

This indicates that the leaves extract could produce total antioxidant capacity in a concentration dependent manner.

Total phenolic content (TPC)

The TPC value obtained is found to be 33.34 mg%.

Ash content determination

The total ash content value of the aqueous leaf extracts of *Catharanthus roseus* obtained is 0.01552

Moisture content determination

The moisture content value of the aqueous leaf extracts of *Catharanthus roseus* obtained is 0.998.

In our Investigations, phytochemical constituents of leaf extracts of *C. roseus*aqueous extracts revealed that the presence of alkaloids, carbohydrates, flavonoids, phenols, phytosterols, terpenoids ,saponins and tannins. These compounds are described as potent biologically active compounds found in medicinal plant parts which are precursors for clinically useful drugs (Steiling*et.,al*1993). Aqueous extract of *C. roseus*leaf extracts was further studied for antioxidant properties (Rhee, 2009).

The DPPH radical scavenging assay demonstrated the free radical scavenging activity of *C. roseus*. The leaf extract of *C. roseus*showed excellent radical scavenging activity. There are many evidences that natural products and their derivatives have efficient anti-oxidative characteristics, consequently linked to anti-cancer, hypolipidemic, anti aging and anti-inflammatory activities (Kapoor *et al.*, 1969). Results obtained in our study confirmed the antioxidant activity of *C. roseus*.

High amount of phenolic content indicates the ability of plant to treat inflammatory diseases and can be implicated in wound healing. Pharmacists usually target the plant with high phenolic content to treat different diseases (Petti and Scully, 2009). The results of the current study showed that the *C. roseus* could produce a significant total phenolic value.

Ash and moisture content value was determined with a purpose to find out the total amount of inorganic solutes present in the medicinal plant. Higher amount of total ash suggests a high value mineral composition comprising potassium, calcium and iron as the main elements (Tambe*et al*., 2012).

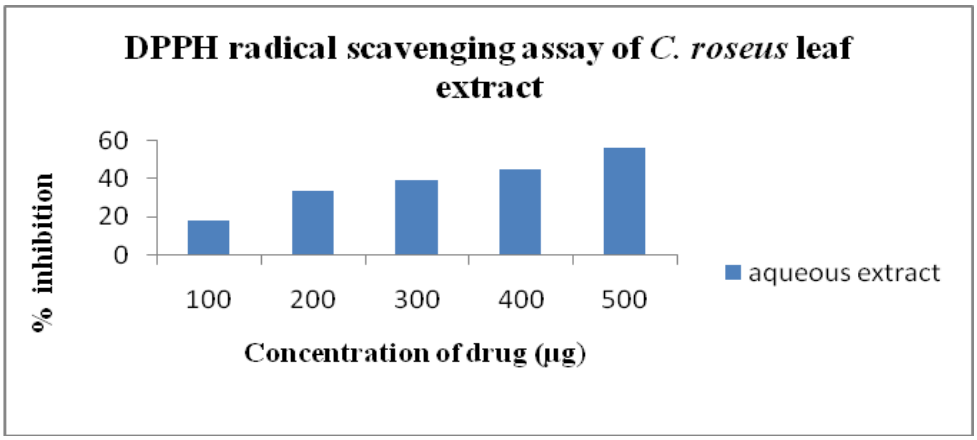

Fig. 1: DPPH radical scavenging assay of *C. roseus* leaf extract

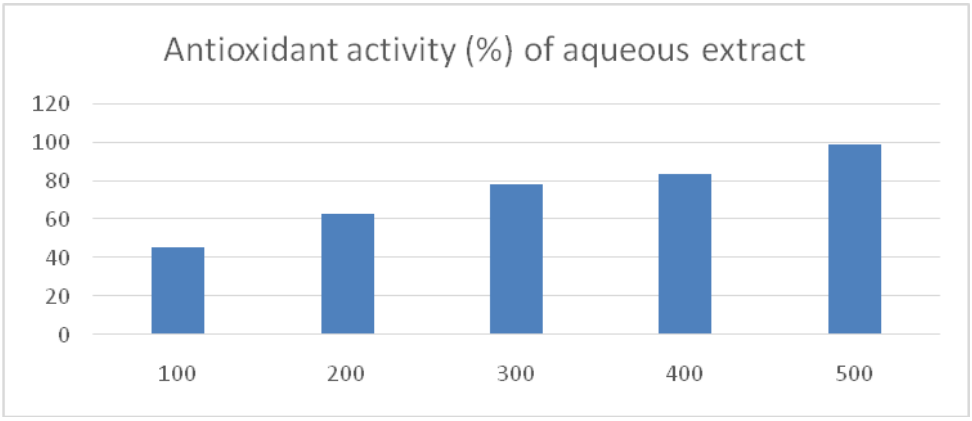

Fig. 2: Antioxident activity (%) of aqueous extract

The present study provides support to the plant's traditional and alternative use against various diseases and infections. Further, the biomolecules present in the extract which are active against these microbes needs to be characterized. Use of natural products has been encouraged due to less or no side effects, cost effectiveness and development of resistance to conventional synthetic antibiotics. Hence, this study holds importance in using medicinal plants as an alternative source for treating various diseases.

Conclusion

The result of our studies revealed *Catharanthus roseus* possesses various phytochemicalssuch ascarbohydrate, protein, tannins,flavanoids, triterpenoids, diterpenoids, steroids, alkaloids, saponins and phenolic compounds. The result of present study put forward the antioxidant property of *C.roseus*leaves which can defend against the oxygen free radicals.High amount of phenolic content reflects the ability of the plant to treat inflammatory diseases and can be implicated in wound healing.Total ash and moisture content analysis were also carried out.

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PLANTICA – Journal of Plant Science
(The Official Publication of Association of Plant Science Researchers)
ISSN: 2456 – 9259
Plantica, Vol. 2 (3), 2018: 170 – 179
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Research Article

PHYTOMONITORING OF DUST LOAD AND ITS EFFECT ON FOLIAR MICRO MORPHOLOGICAL CHARACTERISTICS OF URBAN TREES

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Received: March, 2018 – Accepted: June, 2018

Abstract

Dust load is considered as one of the most widespread air pollutants. In this study evaluated the dust load and dust capturing capacity of different plant species and their effect on leaf micro morphological characteristics of trees. Result showed significant variation at site and season of dust load and dust capturing capacity of different plants species. Variation of Micro morphological characteristic was also observed at site and season. Seasonally variation of dust load was higher in winter than summer and monsoon. In this study the highest dust deposition rates were observed in *Ficus virens* > *Ficus religiosa* > *Cassia fistula* and *Azadirachta indica* whereas dust capturing capacity was highest in *Azadirachta indica* > *Ficus virens* > *Ficus religiosa* > *Cassia fistula.* Micro morphological characteristic i.e. number of stomata, number of epidermal cells and Stomatal index was negatively affected by dust load. On the basis of maximum dust removal efficiency and less affected morphological and micro morphological characteristics of plants (*Azadirachta indica*) can be used in the reduction of dust pollution by acting as natural filters and promoted for plantation along the roadside having greater dust removal efficiency.

Key words: Dust load; Dust capturing capacity; Dust removal efficiency; Stomatal variability

Introduction

Air pollution is a burning problem of the urban environment and it is one of the leading providers to the disease burden in world develop and developing countries (Mukherjee and Agrawal, 2017). Increasing anthropogenic (industrialisation and urbanization) activities is one of the main agents of pollutant discharge into the environment and introduced several harmful substances into the atmosphere. Urban trees one of the solutions to this devastating problem identification of existing natural resources that can provide suitable mitigation in a sustainable manner. Urban trees are one of the key contenders to provide several health benefits beside their ecosystem services of environment (Ulmer et al., 2016, Vailshery et al., 2013). Once the pollutants are released to the atmosphere, only the plants are the optimism and wash up the pollutants by adsorbing and metabolizing them from the atmosphere. Consequently, the plants, role in the air pollution reduction have been increasingly recognized in recent years. Plants act as a sink and even as living filters to minimize air pollutant by developing characteristic

response and symptoms. Furthermore, roadside plant is in direct contact with air pollutant, and may act as natural filter for these pollutants (Pandey et al. 2005; Sharma et al. 2007).

Deposited materials on the leaves have some effect on the overall biochemical, physiological and morphological aspects of plants and reduce the plants growth and development. Several investigation have been performed on the physiological and biochemical response of plants growing in an industrial region (Joshi et al. 2009; Gupta et al. 2009; Sharma and Tripathi 2009; Gupta et al. 2012). Plants remove air pollutants primarily by uptake via leaf stomata and once inside the leaf, gases diffuse into intercellular spaces and absorbed by water films. Plants, grown in such a ways to function as pollutants sinks are collectively referred to as greenbelts which have limits to their tolerance towards air pollutants (Cheng 2003). Greening by plantation, which makes use of vegetation to remove, detoxify or stabilize persistent pollutants, is a green and environment friendly tool for clean environment. The screening of effective plants for particulate sink is very essential for air pollution abatement in urban environment. The objective of the present investigation is firstly to estimate and analyse the dust deposition and its removal efficiency and changes in micro morphological characteristic of selected plant species growing at study areas Gandhinagar Gujarat.

Materials and Methods

Study area and plant sampling

Gandhinagar is a capital of Gujarat situated at Western India and it's located approximately 23 km north of Ahmedabad city. Population of Gandhinagar approximately 195, 985 (2001) and Area: 177 km² by Government body: Gandhinagar Municipal Corporation Sources include: UN data. In our investigation, study of four dominated plant species *Azadiractha indica, Cassia fistula, Ficus religiosa, and Ficus virens* were selected and sample randomly in triplicate of leaves which continuously exposed to more polluted area (industrial area & traffic area) and the another sample of leaves were collected from less polluted area (residential area) in Gandhinagar, Gujarat. Selection of plants species at sampling sites is depending on their availability. The Maximum number of plant species present at selected sites.

Dust deposition and capturing capacity

Initial weight of oven dried patriplates was taken (W_1). After weighing dry patriplates, a single leaf of each sampled tree species was washed in dry petri-plates and dried in the oven at 80^0 C for overnight to evaporate the water present in the petri-plates due to leaf washing. After drying again the weight of petri-plates was taken (W_2). Washed leaf was store in refrigerator for physiological analysis. The dust deposition on the leaves was measured using following formula:

$W = W_2 – W_1$

Where, W = Dust content (g), W_1 = Weight of patriplates without dust, W_2 = Weight of patri-plates with dust.

Dust capturing capacity of the selected plant species was measured applying dust load on the leaf surface and leaf area on the following formula:

$W = W_2 – W_1 / A$

Where, W = Dust content (mg/cm^2), W_1 = Weight of patriplates without dust, W_2 = Weight of patri-plates with dust, A = Leaf Area (cm^2)

Leaf morphology and biomass

Leaf area plays an important role in plant growth determination and photosynthesis. For this study graphical method was used for leaf area measurement**.** Dry weight of the same leave was recorded after drying it in hot air

electric oven 60 degree centigrade for 48 hours.

Leaf micro morphological characteristic

Leaf micro morphological character was measured with the help of microscope. The abaxial surfaces of leaf were separated with the help of dissecting needle & forceps and washed with clean water. Then each specimen was stained with safranin (1% aqueous) for 3 to 10 minutes. The excess stains were washed with deionized water and then the stained cuticle was mounted in glycerin jelly and observed with microscopic. The number of stomata, epidermal cells and stomatal index was calculated as per the (Salisbury, 1927) equation, i.e.: (S I= S × 100/S + E) with the help of compound light microscope.

Where: SI = stomatal index, S = No of stomata/unit leaf area, and E = number of epidermal cells/unit leaf area.

Statical analysis

For analyze to quantitative changes in different parameters of selected plant species due to dust deposition. The Statistical tool using Test of Duncan's Multiple Range and correlation coefficient with the help of SPSS (SPSS Inc., version 17.0).

Results and Discussion

Assessment of dust falls on different plants species at various sites and season.

Results of the study showed higher dust deposition on leaf of all the tree species at all the sites during winter season and minimum during monsoon (Fig. 1). As expected, highest dust deposition on leaves was observed at thermal power plant (industrial zone) than pathikashram (traffic zone) and Chiloda (residential zone). Among the tree species, maximum dust was deposited on *Ficus virens* at all the sites and all the season while *Azadirachta indica* showed least dust deposition during study period at all the sites. Analysis of the present study shows that the dust fall on the leaves of all the plants species was higher at most polluted sites during each sampling, which was due to more pollutants releasing through industries and traffic activities while in controlled area i.e. Residential area dust particles settled down on leaves generally come from the surrounding soils due to high wind speed. Same study was reported by (Selmi et al. 2016; Vailshery et al. 2013) high dust deposition on the leaf surface at urban and industrial site.

Dust capturing capacity of different plants species at various sites and season.

Dust capturing capacity was measured in the term of mg/cm^2 dust deposited on leaf area. It was highest in *Azadirachta indica* and lowest in *Cassia fistula* (Fig. 2) during all the sampling period. Maximum dust capturing efficiency was observed at industrial site than traffic and residential site. A study was denoted by Saha and Padhy, (2011) dust accumulation patterns on *Shorea robusta* and *Madhuca indica* foliage in Lal pahari forest.

Effect of dust load on leaf morphology and biomass of different plants species at the various sites and season

Leaf area

Effect of dust load on the leaf area during study period at various sites is presented in figure 3. Leaf area was increased from summer to winter in all the tree species at all the sites. Leaf area was reduced at high polluted sites in all experimental trees. Maximum reduction of leaf area was observed in *Cassia fistula* at polluted site and minimum in *Azadirachta indica* and plant *Ficus viren* and *Ficus*

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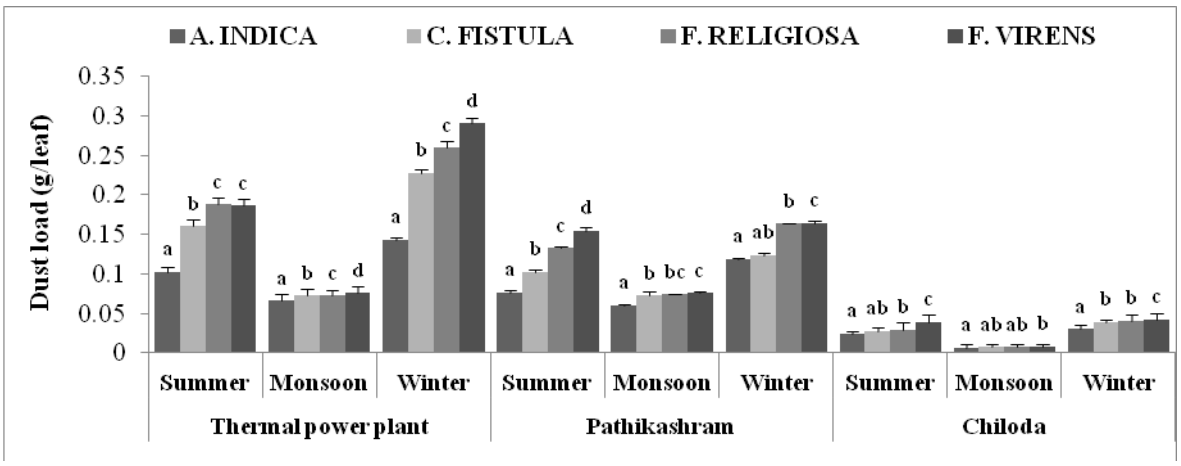

Figure 1. Dust load on foliage of selected trees at various sites (Thermal power plant = Industrial zone, Pathikashram = Traffic zone, Chiloda = Residential areas) during summer, monsoon and winter (Each value given in M ± SD^3, and Value within each column of same letter showed not significantly different ($p< 0.05$) using Duncan's Multiple Range Test).

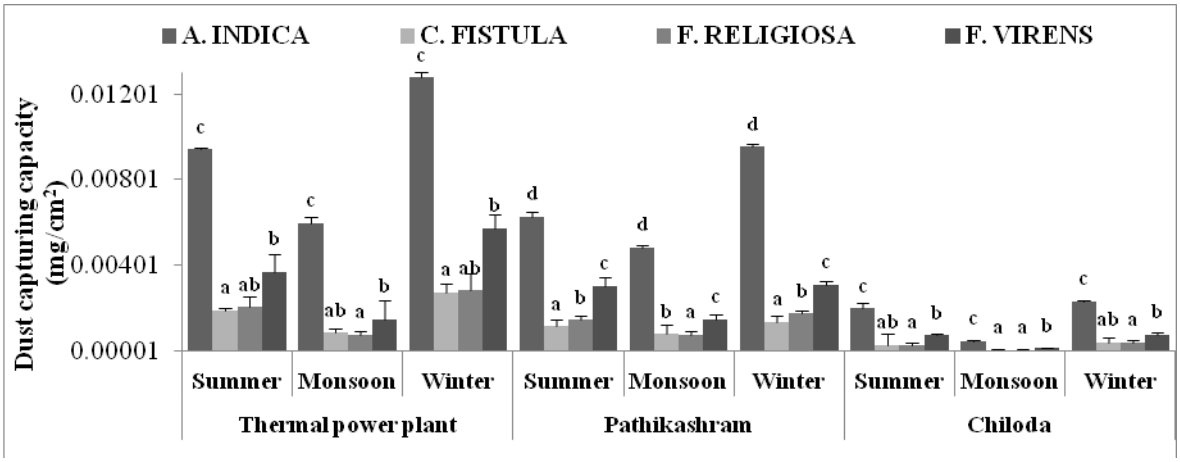

Figure 2. Dust Capturing capacity of selected trees at various sites (Thermal power plant = Industrial zone, Pathikashram = Traffic zone, Chiloda = Residential areas) during summer, monsoon and winter (Each value given in M ± SD^3, and Value within eachcolumn of same letter showed not significantly different ($p< 0.05$) using Duncan's Multiple Range Test).

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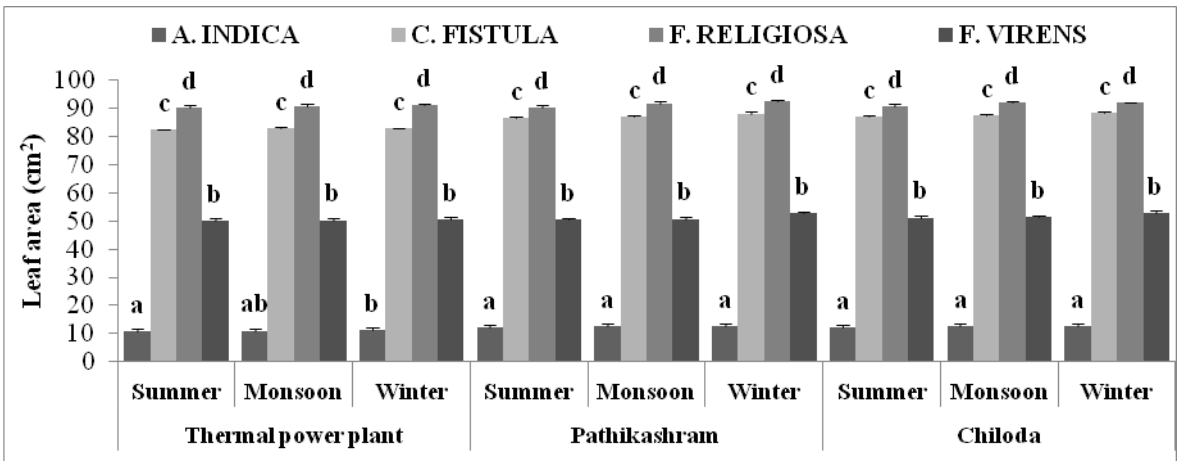

Figure 3. Effect of dust load on leaf area selected trees at various sites (Thermal power plant = Industrial zone, Pathikashram = Traffic zone, Chiloda = Residential areas) during summer, monsoon and winter (Each value given in M ± SD^3, and Value within each column of same letter showed not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

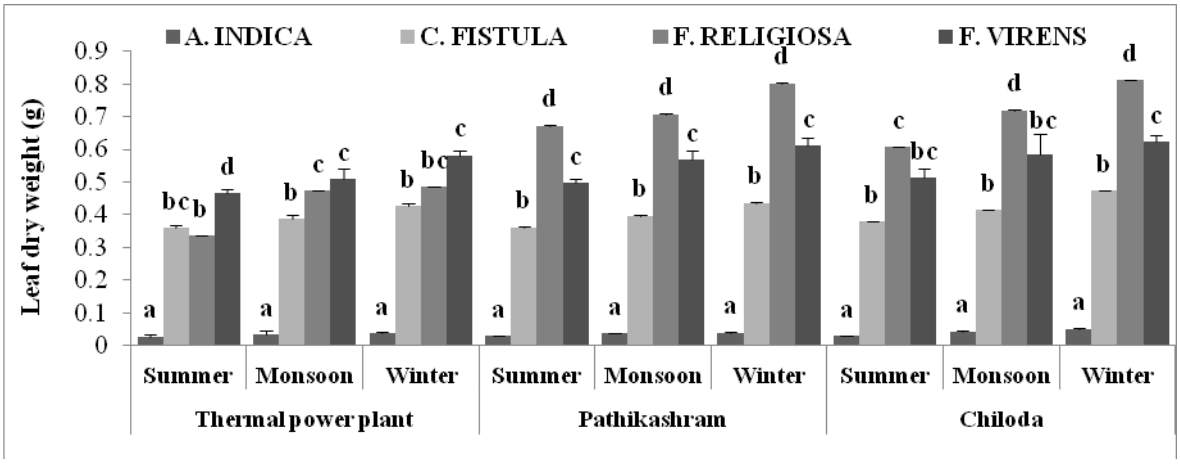

Figure 4. Effect of dust load on leaf dry weight of selected trees at various sites (Thermal power plant = Industrial zone, Pathikashram = Traffic zone, Chiloda = Residential areas) during summer, monsoon and winter (Each value given in M ± SD^3, and Value within each column of same letter showed not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

PLANTICA – Journal of Plant Science
(The Official Publication of Association of Plant Science Researchers)
ISSN: 2456 – 9259
Plantica, Vol. 2 (3), 2018: 170 – 179
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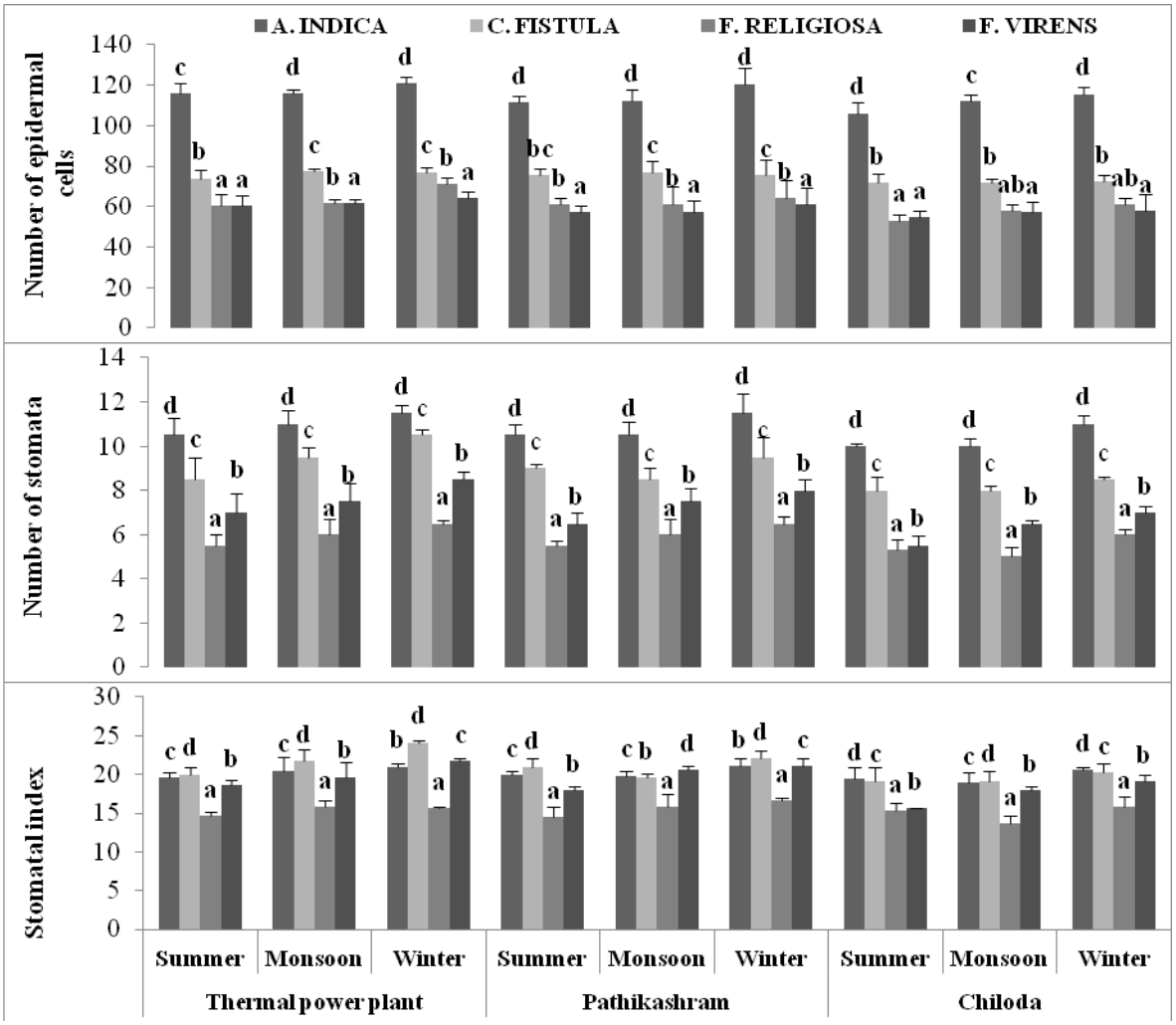

Figure 5. Effect of dust load on No. of epidermal cells, No. of stomata and stomatal index of selected trees at various season (Thermal power plant = Industrial zone, Pathikashram = Traffic zone, Chiloda = Residential areas) during summer, monsoon and winter (Each value given in M ± SD^3, and Value within each column of same letter showed not significantly different ($p < 0.05$) using Duncan's Multiple Range Test).

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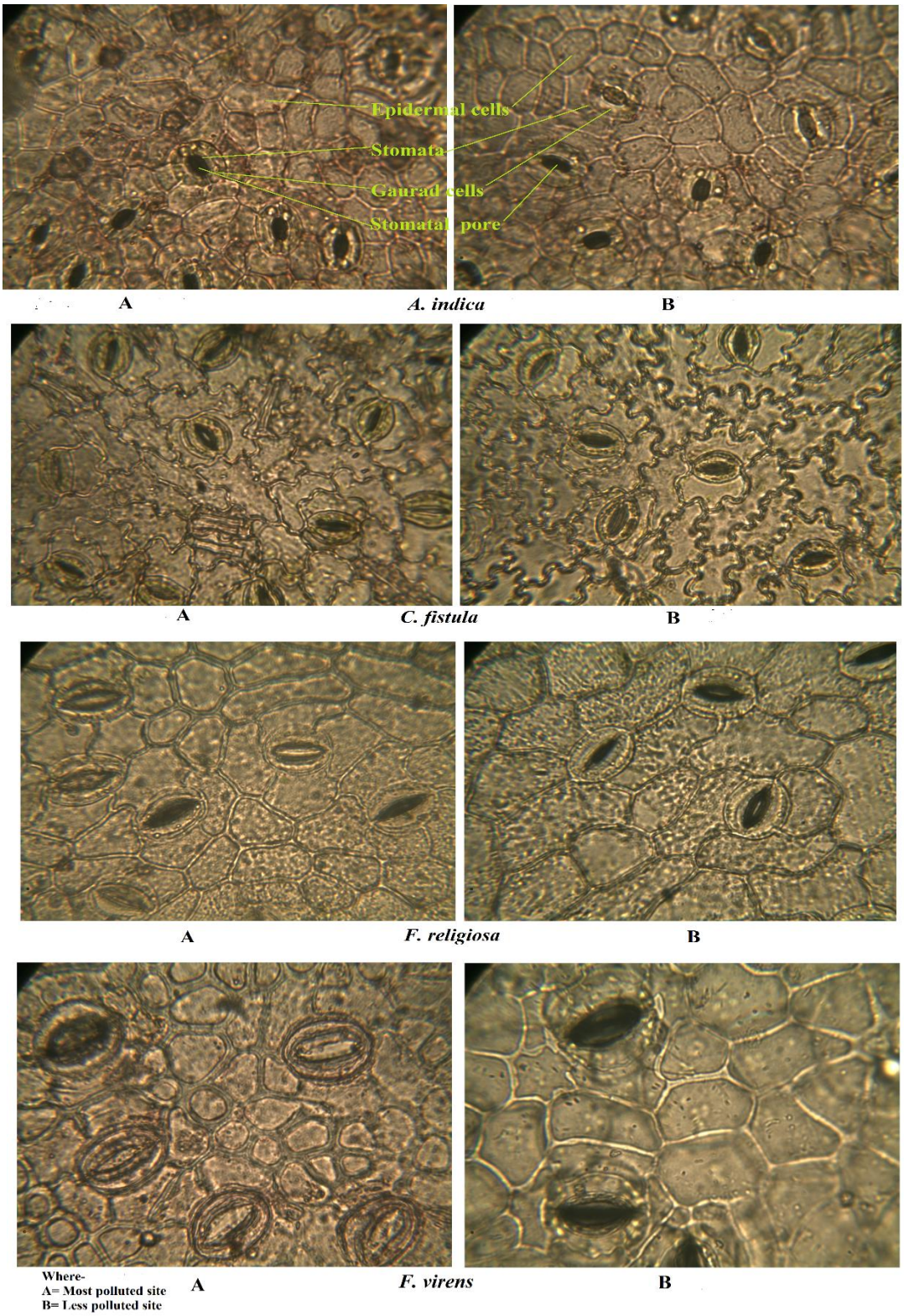

Figure 6. Effect of dust load on stomata of different plants species at more polluted and less polluted site (Thermal power plant = Industrial zone, Chiloda = Residential area).

religiosa showed moderate reduction due to dust deposition (Figure 3). In statistical analysis leaf area was negatively correlated with dust load in all plant species given in table 1.

Leaf dry weight

Leaf dry weight of all plant species was negatively correlated with dust load at various sites is presented in figure 4 and table 1. Leaf dry weight was increased from summer to winter in all the tree species at all the sites. Leaf dry weight of all the experimental trees was reduced at high polluted sites (thermal power plant) as compared to those growing in the less polluted sites during all the sampling periods. But, this reduction was not prominent in *Azadirachta indica* (Figure 4). Atmospheric dust affects the foliage properties of the trees and may lead to reduction of leaf area and resulting less photosynthesis, decrease in stomatal densities, width and stomatal pore (Pourkhabbaz et al. 2010).

Effect of dust load on leaf micro morphological characteristic of different plants species at various sites and season

Our result showed the changes in micro morphological characteristic seen in (figure 5 and 6) the most polluted site shows maximum number of stomata than less polluted sites. Number of epidermal cells was also higher at most polluted site (figure 5). Stomatal index was calculated higher at most polluted site (industrial area) than less polluted site (residential area). Trends of stomatal index of plant was higher to lower i. e. *Cassia fistula* > *Azadiractha indica*> *Ficus virens* > *Ficus religiosa*. Micro morphological character of plant leaf was illustrated in figure 6. Qadir et al. (2016) was also reported the increasing stomatal index due to higher dust load on leaf of *Azadirachta indica*.

Conclusion

This study concluded that the dust loads and their effect on micro morphological characteristic of plant species at Gandhinagar Gujarat. Result showed high dust load caused negative effect on leaf physiology, biomass and micro morphological characteristic. Maximum dust load was observed at industrial area (Thermal power plant) as compared to traffic (Pathikasharam) and residential area (Chiloda) of Gandhinagar. Seasonally variation of dust load was higher in winter season than summer and monsoon. Plant wise dust deposition was observed from higher to lower i.e. *Ficus virens* > *Ficus religiosa* > *Cassia fistula* and *Azadirachta indica*. Plant *Azadirachta indica* was showed maximum duat capturing capacity as compared to *Ficus virens* > *Ficus religiosa* and *Cassia fistula*. Micro morphological characteristic (No. of stomata, No. of epidermal cells and stomatal index) was highly affected by dust load. Higher stomatal index value showed high sensitivity of tree species.

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Table 1. Correlation between dust load and different parameters of selected tree species at various site

S. No.	Parameters	*Azadirachta indica*							*Cassia fistula*						
		Dust load	**Dust capturing capacity**	**Leaf area**	**Leaf dry weight**	**No. of epidermal cells**	**No. of stomata**	**Stomatal index**	**Dust load**	**Dust capturing capacity**	**Leaf area**	**Leaf dry weight**	**No. of epidermal cells**	**No. of stomata**	**Stomatal index**
1.	**Dust load**	1							1						
2.	**Dust capturing capacity**	0.994	1						0.999*	1					
3.	**Leaf area**	-0.709	-0.784	1					-0.857	-0.877	1				
4.	**Leaf dry weight**	-0.997*	-0.999*	0.763	1				-0.979	-0.971	0.735	1			
5.	**No. of epidermal cells**	0.971	0.992	-0.857	-0.987	1			0.922	0.906	-0.591	-0.981	1		
6.	**No. of stomata**	0.999*	0.997*	-0.733	-0.999*	0.979	1		0.999*	0.996	-0.830	-0.988	0.941	1	
7.	**Stomatal index**	0.995	0.977	-0.632	-0.983	0.941	0.990	1	0.999*	1.000**	-0.879	-0.969	0.904	0.995	1
		Ficus religiosa							***Ficus virens***						
1.	**Dust load**	1													
2.	**Dust capturing capacity**	1.000**	1						1.000*	1					
3.	**Leaf area**	-0.682	-0.688	1					-0.923	-0.930	1				
4.	**Leaf dry weight**	-0.731	-0.737	0.998*	1				-0.899	-0.907	0.998*	1			
5.	**No. of epidermal cells**	1.000**	1.000**	-0.677	-0.726	1			0.942	0.949	-0.999*	-0.994	1		
6.	**No. of stomata**	0.943	0.940	-0.399	-0.461	0.945	1		0.995	0.993	-0.882	-0.853	0.906	1	
7.	**Stomatal index**	0.695	0.689	0.052	-0.017	0.700	0.895	1	0.961	0.955	-0.780	-0.742	0.813	0.983	1

Where,

**** Correlation is significant at the 0.01 level (2-tailed).**

*** Correlation is significant at the 0.05 level (2-tailed).**

PLANTICA – Journal of Plant Science
(The Official Publication of Association of Plant Science Researchers)
ISSN: 2456 – 9259
Plantica, Vol. 2 (3), 2018: 180 – 199
www.jpsr.in

Review Article

CHEMICAL CONSTITUENTS, THERAPEUTIC USES, BENEFITS AND SIDE EFFECTS OF *BISTORTA VIVIPARA*: A REVIEW

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Received: January, 2018 – Accepted: May, 2018

Abstract

Bistorta vivipara is a perennial herb belongs to the family Polygonaceae. It is highly distributed in Iceland, high elevation mountains regions and also grown in Alpine meadows, fields and Tibetan plateaus. It is commonly known as Alpine bistort, Serpent grass, *Polygonum viviparam* and viviparous knotweed. It originates from short, thickened rhizomes which is the rich source of starch. The best time to study this plant lasts from late June to early September. This herb contains a lot of medicinal values to treat dysentery, gastric problems, urinary tract disorders as well as pharyngitis. The root parts are highly used to cure piles, wounds, ulcers, vomiting and biliousness. It is a rich source of chemical constituents like volatile oils, flavonoids, gallic acid, tannins and saponins. It also has many bioactive effects viz; antibacterial, antioxidant, antitumor and antiarthritic properties. In this review paper the medicinal properties, side effects, chemical constituents of *Bistorta vivipara* has been explored. The whole plant parts are used to cure diseases including roots too.

Key words: Antibacterial, biliousnous, dysentery, flavonoids, gallic acid, *Polygonum viviparam*

Introduction

Bistorata vivipara is generally considered as an Alpine species, are mainly found in high latitude regions. It is a perennial herb belongs to the family *Polygonaceae* (Sanchez et al. 2009). It is extensively distributed in mountain regions, Alps, Pyrenees and the Tibetan plateaus. It is commonly known as bistort alpine, viviparous knotweed and serpent. It originates from short and thickened rhizome. The stem is 10-30cm in length and terminates with narrow, dense, single flowering spike. It is simple, erect, smooth and bears single few leaves. The best survey period starts from late June to early September. This plant is highly known for its medicinal uses. The root or rhizome part is a rich source of starch and it is used to treat a number of disorders such as bronchitis, piles, ulcers, wounds, vomiting and biliousness. Along with these it is also used to several other diseases like dysentery, gastrointestinal disorders and pharyngitis. This plant having a large number of chemical constituent's volatile oils, flavonoids, gallic acid, saponins and tannins (Peng et al., 2013). In addition *Bistorta vivipara* is also used to perform bioactive effects viz; anticancerous, antibacterial, antiulcer, anti-inflammatory, antiarthritic and antioxidant. This plant is also known as blood flow rectifier. It is used to cure

internal as well as external bleeding. It is highly astringent in nature that helps to prevent hemorrhages from lungs and stomach. Bistort roots are styptic and used to cure fever, sore throats and ulcers (Lee et al. 2012).

Basic Information:

General Name:	Bistort
Botanical name:	*Bistorta vivipara*, *Polygonum bistorta*
Hindi name:	Amli, Kutrya
Chinese name:	Quan Shen
Other names:	Common bistort, snake weed, poor man's cabbage, snakeroot, oderwort, meadow bistort, dragonwort, easterman giants, easter mangiants, passion dock, sweet dock, patience dock, pudding grass.

Classification:

Kingdom:	Plantae
Order:	Caryophyllales
Family:	Polygonaceae
Genus:	Bistorta
Species:	Vivipara

Basic plant information:

Life cycle:	Perennial
Origin:	Native
Status:	State threatened
Habitat:	Part shade, coniferous swamp, wooded shorelines, alpine meadows
Bloom season:	June-August
Plant height:	3-12 inches

Habitat and Distribution:

Bistort is mainly found in wet areas, lakes, beaches and at a lesser extent in bedrock crevices along the rocky shores. Sometimes it is also grown near the other shrub species like Alnus species. These herbs prefer Alnus species because it provides unique microclimate for their growth. This plant grown in great number in meadows, fields, Iceland and along the river banks and roads. These plants are highly grown under higher altitudes and also prefer moist soil, rich in silicic acid. This plant has a circumpolar distribution. It is found from Peninsula and Britain in the west, through the Pyrenees, Alps, Turkey, Central Europe, Russia and east in Northern China, Japan and Canada.

Description:

Bistorta vivipara is a perennial herb, which can grow up to 1 meter. The roots are fleshy and cylindrical in shape. The leaves are green in colour on top while grey-green underneath. The shape of the leaves is oval and pointed at the end. The basal leaves have a cylindrical petiole and lanceolate blade which is pointed at its tip. These are 2-10 cm in length as well as long and stiff. The seeds of this plant are shiny and dark brown in colour and triangular in shape. Bistort blooms in the month of June and July. The taste of its leaves is sour while roots or rhizomes are bitter and are astringent. The roots are the rich source of starch which is highly used as a food. The flowering stem of this plant is short and approximately 15cm in length. The single spike of flower is of 2-10 cm with small pinkish or white flowers.

Floral characters:

The flowers are radially symmetrical. There are five sepals, petals or tepals in a single flower. The petals and sepals are fused and forming a tube or cup like structure. The number of stamen varies from 5-8 in different-different species with purple anthers and 3 fused carpels. The lower ones

are replaced by bulbils. The colour of flower is white in initial stages but it transforms into pinkish or dark red colour at maturity. Flowers of bistorta plant are with corollaceous perianth, in a cylindrical spike. Flowering time starts from June until autumn.

Bistort combines with:

Anemone chinensis	Ash Bark
Chrysenthemum L.	Coptis
Japanese honeysuckle	Silkworm
Uncaria rhynchophylla	Wild turmeric

Nutrients in Bistorta:
Flavonoids, Phlobaphene, Starch, Vitamin A, Gallic acid, Polyphenols , Tannic acid and Vitamin C.

Types: A- Stilbene type, **B-** Flavonoid type, **C-** Drimane sesquiterpenoid, **D-** Phenol type, **E-**Coumarin and isocoumarin types**, F-** Lignan type, **G-** Chromone type, **H-** Alkaloid type, **I-** Sterol type, **J-** Anthraquinone type, **K-** Xanthone type, **L-** Fatty alcohol acetate type**, M-** Naphthalene type, **N-**Ester type**.**

Species: a- P. multiflorum, **b-** P. cuspidatum, **c-** P. aviculare, **d-** P. amphibium, **e-** P. persicaria, **f-** P. hyrcanicum**, g-** P. amplexicaule, **h-** P. capitatum, **i-** P. jucundum, **j-** P. viscosum, **k-** P. hydropiper, **l-** P. hydropiper f. purpurascenes, **m-** P. minus, **n-** P. punctatum, **o-** P. bellardii.

Edible parts of Bistort:

Sr. No.	Part used	Effects
1.	Leaves	It is directly used as raw or cooked. These produces a pleasant tart taste after cooked.
2.	Seeds	These are also used as raw or cooked. These are small in size and shiny, used as pickle in Nepal. Seeds are highly rich in starch content.
3.	Roots	These are also used as raw or cooked form. Roots are the rich source of starch and pleasant in taste, small in size. They taste become best after roasting.
4.	Bulbils	It is used as raw only, and from lower part of flowering stem.

Bistorta vivipara collection at Keylong Valley

Various chemical constituents in Polygonaceae family:

Sr. No.	Compounds Name	Types	Species	Tissues	References
1.	2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucopyranoside	A	a	Tubers, roots	Cheung et al (2014)
2.	(E)-2,3,4',5-Tetrahydroxystilbene-2-β-D-(6"-galloyl)-glucopyranoside	A	a	Roots	Kim et al (2008)
3	(E)-2,3,4',5-Tetrahydroxystilbene-2-β-D-(2"-galloyl)-glucoside	A	a	Roots	Kim et al (2008)
4.	(E)-2,3,5,4'-Tetrahydroxystilbene-2-O-(4"-O-α-D-glucopyranosyl)-β-D-glucopyranoside	A	a	Roots	Li et al (2013a)
5.	(E)-2,3,5,4'-Tetrahydroxystilbene-2-O-(6"-O-β-D-glucopyranosyl)-β-D-glucopyranoside	A	a	Roots	Li et al (2013a)
6.	(E)-2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucopyranosyl-4'-O-α-D-glucopyranoside	A	a	Roots	Li et al (2013a)
7.	(E)-2,3,5,4'-Tetrahydroxystilbene-2-O-β-D-glucopyranosyl-5'-O-α-D-glucopyranoside	A	a	Roots	Li et al (2013a)
8.	(E)-2,3,5,4'-Tetrahydroxystilbene-2-O-(2"-O-β-D-fructofuranosyl)β-D-glucopyranoside	A	a	Roots	Li et al (2013a)
9.	Polygonumoside A	A	A	Roots	Yan et al (2014)
10.	Polygonumoside B	A	a	Roots	Yan et al (2014)
11.	Polygonumoside C	A	a	Roots	Yan et al (2014)
12.	Polygonumoside D	A	a	Roots	Yan et al (2014)
13.	Polyflavanostilbene A	A	b	Rhizomes	Li et al (2013b)
14.	(Z)-2,3,4',5-	A	a	Roots	Kim et al (2008)

	Tetrahydroxystilbene-2-β-D-glucoside				
15.	Myricetin 3-O-β-D-glucuronide	B	c	Herbs	Granica et al (2013)
16.	Mearsetin 3-O-β-D-glucuronide	B	c	Herbs	Granica et al (2013)
17.	Querctin-3-O-β-D-galactopyranoside	B	i	Herbs	Lin et al (2009)
18.	8-Methoxyquercetin	B	i	Herbs	Lin et al (2009)
19.	Apigenin	B	i,b	Herbs(i), roots (ii)	Lin et al (2009)
20.	Luteolin	B	i	Herbs	Lin et al (2009)
21.	Kaempferol	B	h	Herbs	Zhang et al (2012c)
22.	Quercetin-3-O-(6”-galloyl)-β-D-galactoside	B	j	Whole plant	Datta et al (2000)
23.	5,3’,4’,5’-Tetramethoxy-6,7-methylenedioxyflavone	B	e	Herbs	Lajter et al (2013a)
24.	3,5,3’,4’,5’-Pentamethoxy-6,7-methylenedioxyflavone	B	e	Herbs	Lajter et al (2013a)
25.	(+) Catechin	B	f,g,b	Aerial parts, roots	Moradi- Afrapoli et al (2012a)
26.	(-) Gallocatechin	B	f	Aerial parts	Moradi- Afrapoli et al (2012a)
27.	Amplexicine	B	g	Aerial parts	Tantry et al (2012)
28.	Quercetin-3-O-α-L-(3”,5”-diacetyl-arabinofuranoside)	B	f	Aerial parts	Moradi- Afrapoli et al (2012a)
29.	Quercetin-3-O-α-L-(3”-acetyl-arabinofuranoside)	B	f	Aerial parts	Moradi- Afrapoli et al (2012a)
30.	Quercetin-3-O-α-L-arabinofuranoside (avicularin)	B	f	Aerial parts	Moradi- Afrapoli et al (2012a)
31.	Myricetin-3-O-αL-(3”,5”-diacetyl-arabinofuranoside)	B	f	Aerial parts	Moradi- Afrapoli et al (2012a)
32.	Myricetin-3-O-αL-arabinofuranoside	B	f	Aerial parts	Moradi- Afrapoli et al (2012a)
33.	Quercetin-3-O-(2”-O-galloyl)-β-D-glucopyranoside	B	h	Herbs	Zhang et al (2012c)

34.	Quercetin-3-O-(3”-O-galloyl)-β-D-glucopyranoside	B	h	Herbs	Zhang et al (2012c)
35.	Quercetin-3-O-(2”-O-galloyl)-α-L-rhamnopyranoside	B	h	Herbs	Zhang et al (2012c)
36.	Quercetin-3-O-(3”-O-galloyl)-α-L-rhamnopyranoside	B	h	Herbs	Zhang et al (2012c)
37.	Quercitrin	B	h,b	Herbs, roots	Zhang et al (2012c)
38.	Kaempferol-3-O-α-L-rhamnopyranoside	B	h	Herbs	Zhang et al (2012c)
39.	Polygodial	C	k,1,m,n	Whole plant, leaves, stem	Asakawa et al (2012)
40.	Isopolygodial	C	k,1,m,n	Whole plant, leaves, stem	Asakawa et al (2012)
41.	Drimenol	C	k1,k3,l, m,n	Whole plant, leaves,	Asakawa et al (2012)
42.	Warburganal	C	k3	Leaves	Asakawa et al (2012)
43.	Drimenin	C	k1,k3,l	Whole plant, leaves	Asakawa et al (2012)
44.	Isodrimenin	C	k1,k3,l	Whole plant, leaves	Asakawa et al (2012)
45.	Cinnamolide	C	k3,m,n	Leaves, stem	Asakawa et al (2012)
46.	Confertifolin	C	k3,l	Whole plant, leaves	Asakawa et al (2012)
47.	Isodrimeninol	C	k3	Leaves	Asakawa et al (2012)
48.	Polygonumate	C	k2	Whole plant	Asakawa et al (2012)
49.	7-Ketocoisodrimenin	C	k2	Whole plant	Asakawa et al (2012)
50.	Futronolide	C	k2	Whole plant	Asakawa et al (2012)
51.	Winterin	C	k2	Whole plant	Asakawa et al (2012)

52.	Dendocarbin L	C	k2	Whole plant	Asakawa et al (2012)
53.	7β-Hydroxyiso-angeloyloxy-7-epi-futronolide	C	k2	Whole plant	Asakawa et al (2012)
54.	Changweikagnic acid A	C	k2	Whole plant	Asakawa et al (2012)
55.	Chlorogenic acid	D	g,b	Aerial parts, roots	Tantry et al (2012)
56.	Caffeic acid	D	g	Aerial parts	Tantry et al (2012)
57.	Gallic acid	D	g, b	Aerial parts, roots	Tantry et al (2012)
58.	p-Hydroxybenzaldehyde	D	i	Herbs	Lin et al (2009)
59.	p-Hydroxybenzoic acid	D	g	Rhizomes	Xiang et al (2012)
60.	p-Hydroxybenzoic ethanol	D	g	Rhizomes	Xiang et al (2012)
61.	Vanillin	D	g	Rhizomes	Xiang et al (2012)
62.	Isovanillic acid	D	g	Rhizomes	Xiang et al (2012)
63.	3,4,5-Trihydroxy-benzoic acid-butyl ester	D	g	Rhizomes	Xiang et al (2012)
64.	4-Hydroxy-3-methoxycinnamic acid	D	g	Rhizomes	Xiang et al (2012)
65.	1-O-β-D-(6'-O-galloyl)-glucopyranosyl-3-methoxy—hydroxybenzene	D	h	Herbs	Zhang et al (2012c)
66.	Ellagic acid	D	h	Herbs	Zhang et al (2012c)
67.	Polygonolide	E	k3	Leaves	Asakawa et al (2012)
68.	5,6-Dihydropyranobenzopyrone	E	g	Aerial parts	Tantry et al (2012)
69.	Scopletin	E	g	Aerial parts, rhizomes	Tantry et al (2012)
70.	(+)-Lyoniresinol-3a-O-[α-L-rhamnopyranosyl-(1"-6")]-β-	F	o	Aerial parts	Abd El-Kader et al (2013a,b)

	D-glucopyranoside				
71.	(+)-Isolariciresinol-3a-O-[α-L-rhamnopyranosyl-(1”-2”)]-α-L-rhamnopyranosyl-(1”-6”)]-β-D-glucopyranoside	F	o	Aerial parts	Abd El-Kader et al (2013a,b)
72.	3,5,7-Trihydroxychromone	G	i	Herbs	Lin et al (2009)
73.	5,7-Dihydroxy-4H-chromen-4-one	G	h	Herbs	Zhang et al (2012c)
74.	(S)-2-(2’-hydroxypropyl)-5-methyl-7-hydroxychromone-7-O-α-L-fucopyranosyl(1-2)-β-D-glucopyranoside	G	a	Roots	Zhao et al (2014)
75.	N-trans-ccaffeoyl-tyramine	H	f	Aerial parts	Moradi-Afrapoli et al (2012b)
76.	Hirsutine	H	h	Herbs	Zhang et al (2012c)
77.	β-Sitosterol-3-O-acetate	I	o	Aerial parts	Abd El-Kader et al (2013a,b)
78.	β-Sitosterol	I	i,a	Herbs, roots	Lin et al (2009)
79.	β-Sitosterol-3-O-β-D-glucoside	I	a	Roots	Xu et al (2006)
80.	Physcion	J	a	Roots	Kim et al (2008)
81.	Emodin	J	a	Roots	Kim et al (2008)
82.	Emodin-8-O-β-D-glucoside	J	a	Roots	Kim et al (2008)
83.	Physcion-8-O-β-D-glucoside	J	a	Roots	Kim et al (2008)
84.	1,8-Dihydroxy-3,6-dimethoxy-xanthone-5-O-[α-L-rhamnopyranosyl-(1”-2’)]-β-D-glucopyranoside	K	o	Aerial parts	Abd El-Kader et al (2013a,b)
85.	17-Hydroxypentacosanyl acetate	L	o	Aerial parts	Abd El-Kader et al (2013a,b)
86.	Torachrysone-8-O-β-D-glucoside	M	a	Roots	Kim et al (2008)
87.	Diisobutyl phthalate	N	g	Rhizomes	Xiang et al (2012)

Traditional uses of *Bistorta vivipara*:

For External Bleeding: The herb is usually applied to reduce or prevent internal as well as external bleeding from the human body. We can prevent bleeding by making a paste of its leaves and applied to the injured part. This is a herbal remedy to cure ulcers, gastritis, enteritis and also for irritable bowel syndrome.

For Gum Diseases: By taking 2 tablespoon rootstock powders in a cup of water and boil for 10-15 minutes to form liquid. Use it as a mouthwash against gum problems. It is also used to cure inflammations of mouth.

For Wounds: In this we can use a herb as both paste as well as liquid form. For preparing liquid solution, take 2-3 tablespoon of rootstock in powder form and boil it for 10 minutes. After forming a solution we can directly apply it on wounds. But in paste form the rootstocks are crushed with pestle mortar and adding few drops of water into it and applied at the wound regions to cure the infection or disease.

For Sore Throat: For treating this disease, take 1 tablespoon rootstock and half cup of water and boil it for 5-8 minutes. Take a mouthful at one time and half cup in a day. It is also used as a lukewarm to gargle.

For Small Pox: Leaves are used to cure this disease, by preparing decoction of leaves and applied on the whole body. This is applied 3 times in a day with the help of cotton. This herb shows high value of antibacterial and antiviral in leaves.

For Urinary Tract Disorders: This plant is best used to cure urinary tract diseases such as cystitis and incontinence. A decoction is prepared from the leaves to reduce leucorrhea and menstrual pain in women. This is also used to reduce bleeding during menstrual periods and also relieves pain. The young leaves can be used as salad or cooked as spinach because these are the rich source of Vitamin A and C. these two vitamins are highly responsible to cure urinary tract diseases.

For Irritable Bowel Syndrome: This syndrome mainly affecting the large intestine and causes a lot of other problems like abdominal pain, cramping, bloating, diarrhea or constipation. To cure these problems the bistorta herb act as a herbal remedy and its active ingredients viz; flavonoids, tannins, flobafen, starch and calcium play very important role. The high amount of these ingredients is present in rootstock part of the herb.

Anti-Inflammatory: This herb is also well known for treating inflammatory diseases of mouth and throat. The tannins and flavonoids are the main constituents for curing disease like inflammatory, bacterial and viral diseases.

For Diarrhea: To cure this disease, Bistorta herb may be combined with other herbs like Agrimony (*Agrimonia eupatoria*), Spotted Geranium (*Geranium maculatum*) or English Oak (*Quercus robur*). Although bistorta herb is known for curing many other diseases like bleeding, snake bites but it is highly well known as herbal medicine for curing diarrhea. This herb has a large amount of tannins which has a strong astringent and antibacterial property to cure diarrhea.

For Skin Diseases: Bistorta is a good herb to cure skin diseases like skin infection, skin eruptions, measles and burns. It also reduces blood flow from wounds and cuts. We can

prepare a paste or lotion for fast relief. This lotion is used to cure ulcers by applying directly on the skin. This herb is used to treat Diabetes.

For Hemorrhoids: *Bistorta vivipara* is known as natural blood flow rectifier. It is highly useful to cure bleeding disorders. It is mainly used to cure hemorrhages in lungs or stomach because it is highly astringent in nature. It also used to reduce nasal bleeds and nasal polyps. The dried rootstock, in the form of extract is used to cure bleeding from both internal as well as external body parts.

For Purulent Otorrhea: This disease is also known as ear drainage which causes inflammation in external or middle ear. This may be serious or purulent. It is also associated with symptoms like fever, pain, hearing loss and ear bleeding. *Bistorta vivipara* roots are used to cure this disease.

Bioactivities and Therapeutic Uses:

Sr. No	Therapeutic effects	Chemical drug	Plant species	Bioactivity	References
1.	Cardiovascular System	Resveratrol	*Polygonum cuspidatum*, grapes, peanuts and berries	It interacts with multiple targets of cardiovascular disease model to induce a reduction in cardiovascular risk parameters.	Tang et al 2014
				Resveratrol also induces endothelial hyperpermeability by hyperglycemia, which causes atherosclerosis in diabetes.	Tian et al 2013
		Polydatin	*P. cuspidatum*	It improves high-glucose-induced hyperpermeability through caveolae pathway.	Ding et al 2014
				Polydatin attenuates cardiac hypertrophy through modulation of cardiac calcium handling and calcineurin-NFAT signaling pathway.	Xu et al 2014a
		TSG (2,3,4,5-Tetrahydroxy-stilbene-2-o-β-D-glucoside)	*P. multiflorum*	TSG shows an inhibitory effect against angiotensin II, induced proliferation of vascular smooth muscle cell.	Yao et al 2013
				TSG treatment is used to	

				cure atherosclerosis by inducing key proteins such as calreticulin, vimentin, HSP 70, lipocortin 1, Apo A-1.	
		Emodin	*P. multiflorum*	Downregulated the activity of intracellular ROS and suppressing Src-MEK 1/2-ERK 1/2 signaling pathway.	Lim et al 2014
		Physcion			Liu et al 2016
		Catechin-3-O-gallate	*P. cuspidatum*	Inhibits toxic tension after suppressing PKSδ-mediated, inhibitor of myosin phosphatase.	Chen et al 2012
		Ellagic acid and Corilagin	*P. cuspidatum*	Increases the level of HTGL and lipolysis of triglyceride.	Xiao et al 2013
		Cinnamoyl-phenethyl amides	*P. chinese*	Shows inhibitory effect against neuraminidase (NA) activity with IC50 value of 129.8, 44.8 and 21.3 µmol/lt.	Moradi-Afrapoli et al 2012b
		Confertifolin	*P. hyrcanicum*	It shows anti-diarrheal activity.	Maheswaran and Ignacimuthu 2014
			P. hydropiper	It shows anti-trypanosomal activity.	
				Essential oil of this herb used to cure dengue fever	

2.	**Anti-cancerous activity**	Trans-piceid (TPc)	*P. cuspidatum* and grapes	TPc shows antiproliferative effects in Caco-2 cells of intestinal epithelial.	Storniolo et al 2014
		Methoxyst y-pandrone	*P. cuspidatum*	It inhibits signal transducer and activator of transcription 3 and nuclear factor-κB signaling.	Kuang et al 2014
		Resveratrol	*P. cuspidatum*	It inhibits invasion and metastasis of colorectal cancer cells via MALAT1-mediated Wnt/β-catenin signal pathway.	Ji et al 2013 Lajter et al 2013b
		Aqueous and organic compound	*Fallopia, Polygonum, Rumex, Persicaria* and *Oxyria sps.*	27 species shows anti-proliferative effect against HeLA, A431 and MCF7 cells.	
		n-hexane, chloroform and 50% methanol	*P.hydropiper, R.alpines, R.aquaticus, R.scutatus, R. thyrsiflorus*	Shows inhibitory effect against substantial cell growth and cell proliferation.	Hwangbo et al 2012
		3,5dihydro xy- benzyl	*Reynoutria japonica (Polygonum cuspidatum)*	Inhibitory effect against topoisomerase I than camptotherin.	
		Flavonoid glucuronid es		It also shows inhibition against DNA topoisomerase II than the positive control of etoposide.	Smolarz et al 2008 Wang et al 2014b
		Davidiin and Ellagitanin	*P. amphibium*	It shows antileukemic activity.	Mohd Ghazali et al 2014
		Myricetin-3-O-5”-	*P. cuspidatum*	It possesses antitumor activity and reducing hepatocellular tumor growth	

		acetyl-α-arabino-furanoside Resveratrol-4-O-D-(2' galloyl)-glucopyrano side Polydatin 8-Methyl-tryptanthrin	*P. minus* *P. cuspidatum* *P. cuspidatum* *P. tinctorium*	of EZH2. It exhibit marked cytotoxicity in HeLa and HepG-2 cells. It exhibit anti-hepatocellular carcinoma viability by inducing apoptosis via JNK and ERK pathway. It inhibits growth of lung cancer cells by inducing apoptosis and arresting cell cycle. It induces differentiation of P19CL6 embryonal carcinoma cells.	Xie et al 2014 Zhang et al 2014 Seya et al 2014
3.	**Antioxidant activity**	Polydatin Tetrahydroxy-stilbene EtoAc Xanthone and lignin glycosides 5,6-dihydro-pyranobenz	*P. cuspidatum* *P. multiflorum* *P. aviculare* *P. bellardii* *Polygonum*	It shows protection against carbon-tetrachloride, induced injury in mice. It helps to scavenging ROS and hydroxyl peroxide scavenging. It shows maximum scavenging activity for peroxynitrite by releasing flavonoids. It proved significant antioxidant potential by DPPH scavenging activity test.	Zhang et al 2012a Zhang et al 2012b Nugraho et al 2014 Abd El-Kader et al 2013a Tantry et al 2012

		o-pyrone, amplexicine Polysaccharide Flavonoids	*amplexicaule* *P.multiflorum* *P. aviculare*	It also exhibit antioxidant activity in a DPPH radical scavenging assay. It shows strongest antioxidant activity against free radicals, lipid oxidation and protein glycation. It exhibit antioxidant activity after cobining with other drugs like myricetin, myricitrin and desmanthin1.	Lv et al 2014
4.	**On Nervous system**	Resveratrol Myricetin-3-O-5”-acetyl-α-arabino-furanoside Hexane Tetrahydroxy-stilbene glucoside 8-hydroxy-calamenene Resveratrol and hexane	*P. cuspidatum* *P. minus* *P.multiflorum* *P. multiflorum* *P.ellipticum* *P.cuspidatum* and *P.multiflorum*	Reverses the effect of chronic mild stress, serum corticosterone level, BDNF expression in rats. It attenuates oxidative damage and ameliorates cognitive impairment in brain. It possesses antioxidant, anticholinesterase activity and also shows neuroprotective properties. It helps to improves tissue functioning. It attenuates neuro-inflammation through inhibition of microglia activation. It improves the cell death of RGC-5 cells. It activate Nrf2, GSH and Cys-Gly booster. GSH cleave to cysteine-glycine in order to maintain intracellular GSH level.	Liu et al 2014 Liu et al 2012a George et al 2014 Lee et al 2014 Zhang et al 2013 Jo et al 2013 Steele et al 2013
5.	**Other activities**	2,3,5,4-tetrahydrox		γ-glutamyl cysteine ligase enzyme is used to control	Cheung et al 2014

		y-stilbene-2-O-β-D-gluco-pyranoside	*P. multiflorum*	the synthesis of GSH. It inhibits tyrosinase, downregulated the expression of melanogenic proteins and reduction of TRP1 complex formation.	Sun et al 2013 Udani et al 2014
		Physta	*P. minus* and *Eurycoma longifolia*	It also promotes hair growth. It is more effective than placebo and enhancing sexual performance.	Kim et al 2013
		n-hexane, ethyl acetate, n-butanol and water	*P. cuspidatum*	It inhibits pancreatic lipase activity and adipogenesis.	Bothon et al 2013
		Semi-alcoholic components		It also shows antiobesity effect.	Wang et al 2013b
		Proanthory-anidines	*P. senegalensis*	It exhibit α-glucosidase inhibitors effect and antioxidant in nature.	Moradi-Afrapoli et al 2012a
		Phenols	*P.multiflorum*	It inhibits α-amylase and α-glucosidae activity and also reduces postprandial hyperglycemia.	
			P.hyrcanicum	It also inhibits the activity of α-glucosidase.	

Side Effects and Toxicity:

Although all varieties of polygonaceae family are harmless but sometimes it causes some reactions like irritation of the digestive tract, constipation, nausea and vomiting. This plant is highly beneficial when taken in small or proper proportion but it cause problems if we take this in high quantity. Polygonum multiflorum is the species which induces some toxic effects in liver and responsible for liver failure (Ma et al 2014). The mechanism of its toxicity is unknown yet, but some researchers observed that an idiosyncratic reaction occurs, which is responsible for forming genetic polymorphism of CYP1A2. The inhibitor emodin stimulates its activity by stilbene glucoside and inhibits the mRNA expression of UGT1A8 (Ma et al 2013). The other variety P. cuspidatum increases the brain

concentration of carbamazepine (CBZ) and CBZE after inhibiting the activities of CYP3A and MRP2 (Chi et al 2012).

Conclusion

It has been shown that medicinal plants are the good alternatives for many diseases and harmful conditions. They are more effective, less expensive and cause less or no side effects. Moreover they can be used by anyone without any prescription. Bistorta vivipara plant is highly useful to cure various harmful diseases like inflammation, infection, cancer, diarrhea etc. Approximate 70 chemical compounds including flavonoids, stilbenes, emodin, counmarins and ligans have been isolated from this plant. There is no enough data about its chemical constituents and its uses, so there is a need to investigate the various pharmacological uses and expand its constituents at a high level. So, everyone can aware about its pharmaceutical benefits. The genus Bistorta also contains biologically active substances and this is the reserve of medical remedies of a various directions of action. The each part of this plant is used as medicine either it is of above ground or below ground. The testing of all the organs may become the goal of their further study.

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PLANTICA – Journal of Plant Science
(The Official Publication of Association of Plant Science Researchers)
ISSN: 2456 – 9259
Plantica, Vol. 2 (3), 2018: 200 – 212
www.jpsr.in

Review Article

PLANT AND TREE SPECIES AS TOOLS FOR PHYTOREMEDIATION IN POLLUTED ENVIRONMENT: A REVIEW

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Received: January, 2018 – Accepted: May, 2018

Abstract

Plants act as a tool or green blanket to protect the environment from the effect of heavy metals, increasing day by day. Toxic metal pollution in water and soil is a major problem. Plants possess some important features which enable them to absorb heavy metals from soil and water. Plants also accumulate some toxic metals such as Silver (Ag), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Mercury (Hg), Lead (Pb) and Selenium (Se) etc. There are approximately 45 families have been identified which are responsible for absorbing heavy metals from the soil. Alongwith these plant species some agroforestry tree species also shows best results for accumulating heavy metals. Eucalyptus (Eucalyptus hybrid) and Poplar (Populus deltoids) trees are very suitable for phytoremediation due to its fast growth and having a large tissue. Poplar trees can breakdown atrazine from soil by using enzyme dehalogenase and laccase. Phytoremediation is the best method than the traditional methods. By using this technique we can protect soil, water as well as air resources in a single attempt. In this review article the ability of different plants to absorb the heavy metals from contaminated resources has been highlighted.

Keywords: Agroforestry, Brassica juncea, cadmium, hyperaccumulators, phytoremediation selenium.

Introduction

Heavy metal contamination in the environment is toxic to plant if its concentration is much higher. Natural ores in addition to modernization in industry are both the major cause of heavy metals in the environment. Their entry in the food chain is the most serious concern because of its direct effect on the human health. The reason behind their effect is their biomagnification in the biotic component of ecosystem. Beside it their accumulation also reduce the microbial activity in the soil. Heavy metals in plants are of two types (i) Essential (significant requirement in physiological functioning) such as Zn, Cu, Ni, Fe and Mn and (ii) Nonessential (not significant requirement in physiological functioning) such as Cr, Hg, Cr, As, Cd and Pb. They become toxic as their concentration exceeds the normal concentration requirements for proper functioning of physiological processes. Effective techniques for the removal of this heavy metal successfully remove them from their contaminated sites. But one serious limitation is that for when applied to large scale then they become much

costly. To overcome such serious problem phytoremediation is the one of the reliable technique to remove them in an ecofriendly way. Application of plant to removal of heavy metal is effective both in contaminated soil as well as water (Crawford RL 2006, Susarla S et al.2002, Ghosh M, Singh SP 2005). Selection of plant species on the basis of absorbing potential and their storage in its respective organ.

Plant species can be Excluders (favorably accumulate in root system as compare to their shoot system) or Hyperaccumulators (favorably accumulate in shoot system as compare to root system).The various techniques have been used in phytoremediation are used such as Phytostablization, phytoextraction, rhizofilteration, phytovolatalization etc. (Salt et al. 1995). Rhizosphere a better tool used by the plant to reduce their absorption opportunity, dissolution and free mobile movements of heavy metals. This phytostablization in combination with soil amendments beneficial in immobilization of these hyper accumulated heavy metals (Bolan NS et al. 2011). Phytodegradation another important metabolic tool used by the plants to neutralize the toxic effect of heavy metals. Heavy metal such as mercury and selenium can be diluted into the atmosphere through phytovalatalization. Through phytostablization the heavy metal can be made available for their extraction as extractable organ for commercial purposes (Salt et al. 1995, Kumar et al. 1995, Raskin et al. 1997, Padmavathiamma & Li 2007). This proves to it as a very novel approach because it also provide biomass in producing energy in addition to metal recovery (Zacchini et al. 2009).

Reason behind accumulation of heavy metals in contaminated sites

Human and nature both are the contributor of heavy metal in environment and we know well who is the main contributor? Obviously, human! There are several reason behind it some of them such as comparable to nature there is fast release of heavy metal from the man generated cycles ,mines are one of the potential sources, the discarded remain are also potential sources etc (J. J. D'Amore et al. 2005). Breakdown of parent material such as weathering are the natural sources (A. Kabata-Pendias and H. Pendias 2001, G. M. Pierzynski et al. 2000) but add heavy metals in very low concentration here human also accelerate their weathering. Also the human activities generated heavy metals are more prone to their availability to biotic system because of its mobile nature (M. Yli-Halla (2003). With the advancement in the human techniques the potential anthropogenic sources of heavy metals are also changes and even increases in concern to their time can be explain as below:

As we consider the basic demand of crop plant are fertilizers which are extensively use to reduce the deficiency of soil in comparison to plant requirement hence can be regarded as first anthropogenic source A. Scragg (2006) . Essential heavy metals such as Mn, Co, Zn, Ni, Fe, Mo, & Cu required for plant related to its proper growth (M.M. Lasat, 2000) provide by this source. Thus vast utilization of fertilizers for crop plant in order to get better yield we constantly increase the concentration of heavy metals and thus create potential toxic sites. With the use of single fertilizer other non required elements whose concentration may become toxic are also automatically added because initially they are manufacture in order to provide maximum benefit to plant. For example when we use

phosphatic fertilizer then it automatically supplement the non required elements such as Cd and some other fatal elements like F, Hg and Pb.

Advancement in the use of pesticides from the use of initiation in dual applied field's agriculture and horticulture are the great contributors of heavy metals in various sites. A data analysis from recent past reveals that in UK allow for fungus and insects around 10% of chemicals utilized which contains Cu, Hg, Mn, Pb or Zn. For instance Bordeaux mixtures and lead arsenate extensively used to controls fungus and insects respectively. Hence supplementing used for cattle ticks and pest of banana containing in Arsenate. Use of pesticides comparable to fertilizers generate more concentrated site in their respective applicable area (M. J. Mc Laughlin 2000). Livestock generated manure used from a very long interval of time becoming a potential source .With the modernization in the live stock to increase its benefit for commercial purpose. For example Cu and Zn growth promoting added to their feeds supplemented to the pig. When such waste product applied to field it automatically concentrates the heavy metal if such ma-nure used. Not only manures but, some other bio-solids such as compost, municipal sludge when come across to practices also supplement the environment with heavy metals (N T Basta 2005). Thus usage of these heavy metal containing materials in order to increase fertility of soil increase be- come unavailable because of their health hazards. Another component related to bio-solids are sewage sludge generated as a result of recycling process during treatment of wastewater. Data analysis revealed that around 5.6 million tons from United States and similarly Australia around 175000 tons of such type of sludge produced yearly. Such sludge is directly implicated to agricultural area in a purpose to reuse that material (M. J. McLaughlin 2000). Zn, Cu, Cd, Ni like heavy metals frequently occurs in such type of sludge and if this process of implication of sludge in the agricultural area increase then it definitely generate contamination sites. In certain condition by the process of leaching these heavy metal also able to reach the ground water and contaminate them (R G 2005). It is evidenced by analytical study shows the presence of Zn, Cd and Ni in ground water of New Zealand (C Keller et al. 2002, R G et al. (2004).

Industrialism and its modernization along with the mining and milling operations acts as potential source of heavy metals to generate contamination sites (P. S. DeVolder et al. 2003). Major heavy metals such as Zinc and Lead concentrated in these sites .Industries for different purposes discharges waste product directly into soil or water which increase their concentration. Other sources of heavy metals such airborne emission and long term irrigation. Air borne heavy metal contribution to air is due to the modernization in industrialism. Stack emission and fugitive emission both are the active contributor of air borne emission of metals into the atmosphere more or less respectively. The released particulates into the atmosphere get converted to another form such as oxides. From the atmosphere they comes to the soil through raining and can travel to long distances with the wind. Most common producing metals are Cd, Pb and Zn. Farmers continuously practices irrigation from rivers without aware of its contamination with heavy metals .The rivers contaminated by the cities and industries waste from a long time back. Hence such continuously irrigation practices may also generated contamination sites no doubt it may take time but the possible results surely occurred if it is

continuously increases in this way.

Salix spp. (willow) as ideal candidate for phytoremediation

The plant species Salix belong to family Salicaceae involving 400 members (Newsholme, 1992) commonly grown in wet lowland areas. These growing form showing variability such as some have creeping habit, some variable in their stem number either single or many. The single stemmed species such as Salix alba may reach up to the height of 20 m. The branches of most species when come in contact with the soil they fix themselves and grows very robously (Sommerville, 1992). Most of the Salix species and its hybrid have the potential to grow in the unfavorable soils and contaminated polluted sites (Dickinson et al., 1994).The reason behind the active role of Salix in phytoremediation is their practice of coppicing made it to produce more biomass (Riddell-Black, 1993). To perform such practices in the most commonly used species is Salix viminalis. High transpiration, nutrients absorption and specific absorption of heavy metals are the more important additional features of this plant. Heavy metal from the plant can be obtained fuel wood burning products such as its ash and smoke (Perttu and Kowalik, 1997; Dahl, 2000). In addition to recovery Salix also provide important material such as fodder for cattle, charcoal, alcohol like ethanol, paper production etc. Willow used in phytoremediation in either of the way

1. Willow is most important where its aerial part is an important component of food chain the reason is that in the survival area of the contamination site its aerial part have low uptake of metal .This decrease the entry of heavy metals into the food chains.
2. Regular wood harvesting allow the plant to remove the high amount of heavy metal from the contamination sites.

Because of its tree habit and large amount biomass production large amount of heavy metal removed even few times greater the hyperaccumulator species such as Thalspi caerulescens and Alyssum in case of Cd transport as it is 5 times higher, thus for this reason it can be regarded as ideal candidate for phytoremediation. And the accumulation capacity of heavy metals among the Salix members also varied from very low to higher .Among them Salix viminalis regarded as the most heavy metal accumulating species (Felix (1997). It had been observed that when Salix species were practiced for 3 years in a soil which is contaminated over 50 years with sludge containing heavy metals. From this it was concluded that Salix simply reduce the availability in the soil for biotic system. Relevant studies reveals that in 90 days practices of Salix showed that it remove up to 30% Cd (Greger, 1999) found in the available soil and also lowering in the soil concentration of Cd in 8 different soil sites. Some studies also indicated that Salix remove Cd concentration up to 65cms deep in the soil. According to some observers as comparable to other woody component of plant have compartmentalized metal Cd up to much higher concentration. In addition of Cd removal ,Salix used in treatment of Cu and Zn from its contaminated sites (Nissen and Lepp 1997).

Populus sp. (poplars)

The plant species belong to family Salicaceae used in order to remove heavy metals in addition to hybrids as well as cultivars. Experimental practices with this plant help to conclude that it show high efficiency in phytoremediation. Experiment done with the involvement of metal either

single or many but in combination respective to their different concentration. Most of these experiment perform in the cultured condition under the particular specific regulated media having desired conditions like availability and concentration of heavy metals to living tissue. From their responses in the cultured condition such as soilless cultures, in cell cultured condition as well as also in field practices revealing their ability for the valuable process of phytoremediation. The implication of in vitro techniques involving the treatment of higher concentration of heavy metal reveals their possible outcomes for better survival as according to a lot of authors (Castiglione S et al. 2007, Di Lonardo S et al. 2011, Katanić M et al.2015). It is however required to get the better results from the practices of plant in natural environment because of its tangled interaction with other biotic components (Kovačević B et al.2013).

The various experimental practices explaining the role of Poplars species in phytoremediation. Commercial as well as clonal study (Populous alba) of Di Lonardo at al. (2011) by treatment with these metals Zn, Cu, Cd and As showing the following capabilities of plant:

1. No change in biomass production even this plant can acquired a huge amount of heavy metals
2. Exclusion property of metal from the root is designated from the application of high concentration of metal to the root and shoots. Practices with clones of four species of white poplar reveals that Ni have some hindrance on the growth of plant by interfering in the photosynthetic apparatus (Katani et al. 2015). However it also observed that few genotypes have some tolerance in mild Ni concentrated soil supported by their phytoextraction and phytostablization mechanism of phytoremediation. White poplar also show significant accumulation of Pb concluded from the practices of their species in Pb contaminated sites (Kovačević B et al. 2013). Comparative analysis from results revealed that two of them have high lead concentration in shoot because of presence of translocation factor Pajević et al. (2014). Experimental study of Castiglione et al.(2007) indicate that commercial clone of Populus alba show tolerance to Zn but having lowered chlorophyll content and adventitious root formation .Plants resulted from the P. tremula L. × P. alba L. in the lab conditions showing greater tolerance to Al and Cu as comparable with the controlled cultured conditions Bojarczuk (2004).

According to Nikolic et al. (2011), to determine the tolerance as: Tolerance index = fresh weight of shoot of plant grown in contaminated sites × 100/fresh weight of shoot of plant grown in controlled conditions.A comparative account deducted from the treatment of these metals such as Ni, Cd and Pb other Cd and Pb. Highest tolerances against heavy metals observed in the clonal practices of P.nigra & P. maximowiczii × P. nigra (Migeon and coworkers) Migeon A et al. (2012). Plant biomass production is not only the measurable factor from which tolerances to heavy metals is depend but it can be other factors such as water and photosynthetic system Luković J et al. (2012). Variation in the measurable factors such as concentration of pigment, enzymes like nitrate reductase observed in the tolerable heavy metal concentration in case of clone of P. deltoides and Populus× euramericana (Pilipović A et al. 2011). In the study made by Pietrini et al.(2010) it also observed that with the peak in the phytochelatins whereas there is reduction in transpiration and photosynthesis. Nikolić et al. (2009) reveals

that poplars have phytoextraction capability by the use of Ni and Cd in soilless cultures and also observed tissue specific accumulation in the plants .for example there is observation of accumulated form of Cd in stem tissue where as accumulated form of Ni in leaves. Experimental practices on treatment Cd, Zn, Cr and Cu rich waste biotic material concluded that there is phytostablization and phytoexraction are the two major strategies used by the plant in order to tolerate these metals. They translocate and accumulate in their respective organs such as Cu in roots, Zn in leaves whereas Cr unspecific translocation occurrence observed Sebastiani L et al. (2004).

Eucalyptus

Eucalyptus belongs to Myrtaceae family including 700 species having Australian natives. Because of its multiple potential capabilities involving high capability of growth, toleration to extreme condition like acidic nature of soil and least fertile soil plant species can be used better as an exotic one (Rockwood DL et al. 2008). Phytoremediation ability supported by soilless culturing pot as well as contaminated sites practices which are subjected to waste and heavy metals. Hybrid result from the Eucalyptus camaldulensis × Eucalyptus globulus having reduced root biomass under treatment of Cd. Their clonal analysis indicates that they have even reduced root growth around 50% under Cd treatment. Treated plant show a pattern of concentration decrease as move from root to leaves, as compared to root shoot have less concentration and leaves have even much less concentration comparatively Pietrini et al. (2015). Analysis of the treatment of Cd to the yonger plant of Eucalyptus camaldulensis providing observation that not much change in the biomass production in the soilless cultures comparatively to the mature plants which show decreased growth Fine et al. (2013). In the soilless culture there is addition of chelating agents such as EDTA & EDDS, out of these EDTA proven to be provide better results just because of its larger stability constant. These ligating agents complexed to stablizes the Cd metal. It has been suggested for the phytoremediation in the contaminated sites. It has also been observed that with the increased concentration of Cd plant appeared to generate notable symptoms such as chloretic and blackening in leaf, reduced root growth as well as wilting Gomes M et al.(2012).With the implication of novel approach, these negative effects of metal can be decreased by inoculating the plants with the fungus to allow the mycorrhizal association Arriagada C et al.(2005). Fungal associated plant have very little effect of Cd metal toxicity during the plant development Pajević et al. (2014) and it also facilitate the relocation of Cd in stem. In addition to role of plant in Cd phytoremediation it also show a considerable range of tolerance to Aluminum (Al) metal. Silva et al. (2004) work on six species in addition to its clonal analysis disclose that Al metal most concentrated in the root with reduced concentration in stem. This reduced concentration probably maintained by the reduced translocation into shoot in order to protect from the adverse effect of metal.

Table 1: Effect of different metals on Detoxification mechanism in plants

S. No.	Metal	Major source	Plant type	Family	Detoxification/chelation mechanism in plant	Sequestration	References
1.	As	Pesticide application in wood treatment	*Pteris- vittata* *Holcus lanatus*	Pteridaceae Poaceae	As can be reduced enzymatically to arsenite by arsenate reductase and non-enzymatically by glutathione. The ascorbic acid or GSH in yeast followed by the formation of an arsenite-thiol (AsO2--SH) complex. Phytochelatins (PCs) have also been proposed as chelators in *H. lanatus*	Sub-cellular frond (epidermal cells)	(Delnomdedieu et al. 1994) (Mukhopadhyay et al. 2000) (Raab et al. 2004)
2.	Cd	industrial and mining practices	*Thlaspi caerulescens, Arabidopsis hallerii* and *B. juncea*	Brassicaceae	In B. juncea the glutathione synthetase and γ-glutamylcysteine synthetase	Accumulates in the leaves *T. caerulescens*	(Boominathan and Doran 2003) Cosio et al. (2005)
3.	Cr	Industrial wastes	*Betula* *Salix* *Salsola kali*	Betulaceae Salicaceae Amaranthaceae	-	-	(Farmer et al. 1999). (Gardea-Torresdey et al. 2005).

4.	Cu	Mining and smelting	*Eichhornia crassipes*, *Salix nigra* *Elsholtzia splendens*, *Elsholtzia argyi* *Silene vulgaris* and *Mimiulus guttatus*	Pontederiaceae Salicaceae Lamiaceae Caryophyllaceae Phrymaceae	Metallothioniens, Phytocelatin 1, Phytochelatin 2 Phytochelatin3	-	(Murphy et al. 1999; Rauser 1999). (Jiang et al. 2004b), (Song et al. 2004) and (Harper et al. 1998).
5.	Hg	Mining practices of Hg-Au amalgamation	*Salix* spp. *Amanita muscaria*	Salicaceae Amanitaceae	-	cell wall of Roots (major) and Shoots (minor)	(Wang and Greger 2004) (Falandysz et al. 2003)
6.	Ni	Ni mining Serpentine soil,smelting wastes	*Alyssum lesbiacum* and *Thlaspi goesingense*	Brassicaceae	Vacuolar Nicotianamine and citrate-Ni association	cell wall and vacuole	(Vacchina et al. 2003) (Kramer et al. 2000)
7.	Pb	Lead paint, spills,mines, smelters	*Sesbania drummondii* *Piptathertan miliacetall*, *Brassica juncea*	Fabaceae Poaceae Brassicaceae		Soil amendments by using chelating agent enhance Pb uptake	(Blaylock et al. 1997) (Huang et al. 1997) (Wu et al. 1999).
8.	Se	petroleum	*Astragulus*	Fabaceae	Se non-accumulating species accumulate		(Pickering et al. 2003)

		refining	*bisulcatus*, *B. juncea*	Brassicaceae	seleno-Methionine (SeMet) and Semethylseleno-Met (Se-MeSeMet) while MeSeCys accumulates *A. bisulcatus*. If Se-MeSeMet or SeMet combined with the proteins and causes it non-functional Methylation of SeCys into MeSeCys by SMT reported in *A. bisulcatus*	-	(Banuelos et al. 1997) (McCluskey et al. 1986) (Virupaksha and Shrift 1965)
9.	Zn	Industrial applications	*T. caerulescens* *Arabidopsis halleri*	Brassicaceae	Zn-histidine Zn-citrate	Zn sequestration epidermal leaf cells vacuoles (*T. caerulescens)* and mesophyll cells (*A. halleri)*	(Frey et al. 2000) (Vazquez et al. 1992)

Conclusion

Phytoremediation is a promising green technology that can be used to improve heavy metal contaminated soils. In developing countries like Morocco, this technology can provide beneficial and low-cost solution to clean contaminated area, especially industrial sites (mines and landfills). The complexity of factors that control the efficiency of this technique, such as soils properties, plant species and climatic conditions, fact that more researches need to be conducted. More species that have remediative abilities need to be identified, especially the plants that can contribute to social and economic development of local population, such as industrial species. Also, in the future, research should focus on developing agricultural techniques to enhance phytoremediation efficiency and reduce time and cost of heavy metal removal from soils. The valorization of some industrial residue in order to increase the heavy metal phytoavailability can be investigated.

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PLANTICA – Journal of Plant Science
(The Official Publication of Association of Plant Science Researchers)
ISSN: 2456 – 9259
Plantica, Vol. 2 (3), 2018: 213 – 223
www.jpsr.in

Short Communication

SMALLEST LIFE STUDIES AT THE VARIOUS SOCIETIES IN UTTRAKHAND NORTH-WEST HIMALAYA

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Received: February, 2018 – Accepted: March, 2018

Abstract

The present investigation was conducted in Dandachali forest of Tehri Forest Division, North-Western part of Himalaya. Surveys and sampling of the vegetation were done using standard ecological assessment methods with an aim to study plant species like shrubs status at community level. 21 shrubs species were recorded in 6 forest communities, *viz*. *Pinus roxburghii- Quercus leucotrichophora* mixed, *Pinus roxburghii*, *Pinus roxburghii- Rhododendron arboreum* mixed, *Cedrus deodara- Pinus wallichiana* mixed, *Cedrus deodara- Rhododendron arboreum* mixed and *Rhododendron arboreum- Quercus leucotrichophora* mixed, between the altitudinal range of 1482 and 2200 m asl. The maximum shrubs density (4250.00 individuals per ha) was observed at *Pinus roxburghii- Rhododendron arboreum* mixed forest.

Keyword: Community, Shrubs, Diversity, Density

Introduction

The Compositional study is the characteristics, classification, relationships, and distribution of plant communities (The American Heritage Dictionary, 3rd ed.). A mixture of species which live in a habitat and are held together by common ecological tolerances, form a community. In other words, the study of plant community structure is called plant sociology or Phytosociology (Lal, P. 2015). The term Phytosociology was coined by Paczoski in 1896. The study of plant community implies knowledge of structure and composition of the component species. Therefore, compositional studies are essential for protecting the biodiversity and natural plant communities as well as understanding the changes experienced in the past and which takes place in the future (Lal *et al*. 2017). Himalayas are one of the largest and youngest mountain chains in the world and cover roughly 10% of India total land surface. The diverse climate and the varied environmental conditions are prevailing in Himalayas support diverse habitat and ecosystems with equally diverse life forms. Variations in terms of its size, climate and altitudinal ranges have created unique environment and characteristic to this region (Verma and Kapoor, 2013). The various changes in the Himalayan forests are appearing in their structure, density and

composition due to global warming (Gaur, 1982), uncontrolled lopping and felling of trees for fuel wood, fodder and grazing (Bargali *et al*., 1998; Kumar *et al*., 2009). These biotic pressures play an important role in forest community dynamics (Whitemore, 1984; Pickett and White, 1985). The present study is focused on the composition of shrubs in different plant communities.

Study Area

The study was conducted at Dandachali forest, Tehri Range of Tehri Forest Division, Tehri Garhwal district, Uttrakhand (State), a part of North West Himalaya. Tehri Forest Division covers 143268.90 ha total forest areas. Tehri Range lies in between 30^0-22'077" North latitude and 30^0-25'599" East longitude, which covers 16144.70 ha area. The Lohital beat under Tehri range covers 1117.30 ha area, comprises of 14 compartments. The study was carried out in Four Compartments of Lohital beat, such as Lohital-3 B (97.10 hectares area), Lohital-4 (46.50 hectares area), Lohital-11 (55.40 hectares areas), and Lohital-12 A (56.70 hectares areas), with a total of 255.7 ha area. The climate of the study area is humid temperate. The mean monthly minimum and maximum temperature varies between 2.2 ^{0}C -16.9 ^{0}C and 12.0 ^{0}C - 24.6 ^{0}C, respectively. The average annual rainfall of the study area is 1278.4 mm. Monsoon arrives in the last week of June and ends in September last.

Materials and Methods

Selection of Sites and Habitats for Vegetation Sampling

The study sites were chosen on the basis of altitudinal range, habitat(s), life forms, etc. The habitat was defined on the basis of vegetation dominance as well as physical characters. A site having closed canopy with high moisture percentage is considered moist habitat and with comparatively low moisture percentage is considered dry habitats, if a site experiencing high anthropogenic pressure is considered degraded habitat.

Assessment of the Forest Vegetation

Sampling of trees was done by laying out 10 quadrats of 10x10m size randomly. The shrubs species was sampled by placing 20 quadrats of 5x5m. The collection of data from the quadrates was made according to standard ecological methods (Curtis and McIntosh, 1950; Grieg-Smith, 1957; Kersaw, 1973; Muller-Dombois and Ellenberge, 1974; Dhar *et al*., 1997; and Samant and Joshi, 2004). The girth or circumference at breast height (cbh at 1.37m from ground) for each tree individual was recorded, the plant individuals were considered as trees on the basis of girth (cbh ≥ 31.5 cm) size (Saxena and Singh, 1982). The samples of unidentified plant species were collected, bought to the laboratory and identified with the help of local flora (Gaur, 1999) and subject experts.

Data analysis and Formulae used

Data analysis was done following standard ecological methods (Curtis & McIntosh, 1950; Grieg-Smith, 1957; Kersaw, 1973; Muller-Dombois & Ellenberge, 1974; Dhar *et al*., 1997; and Samant and Joshi 2004).

Density (D)

It represents the numerical strength of species in a community calculated as:

$$\text{Density (D)} = \frac{\text{Total number of individuals}}{\text{Total number of quadrates studied}}$$

Frequency (%F)

It is the indicator of number of samples in which the given species occurs, thus expresses the distribution of various species in the community.

$$\text{Frequency (\%)} = \frac{\text{Number of quadrates in which the species occurs}}{\text{Number of sampling unit quadrates studied}} \times 100$$

Abundance

$$\text{Abundance} = \frac{\text{Total number of species}}{\text{Number of quadrates in which species occurred}}$$

Relative density, relative frequency and relative abundance

These parameters were obtained from the per cent frequency, density and abundance according to the procedure given by Phillips (1959).

$$\text{Relative density (RD)} = \frac{\text{No. of individuals of the species}}{\text{No. of individuals of all species}} \times 100$$

$$\text{Relative frequency (RF)} = \frac{\text{No. of occurrence of the species}}{\text{No. of occurrence of all species}} \times 100$$

$$\text{Relative abundance (RA)} = \frac{\text{Abundance of individual species}}{\text{Total abundance}} \times 100$$

Importance value index (IVI)

The IVI, which is an integrated measure of the relative frequency, relative density and relative basal area, was calculated for all species of trees and shrubs separately for different sites in study areas.

IVI = Relative Density (RD) + Relative Frequency (RF) + Relative abundance (RA)

The abundance data of different sites were pooled to get community averages in terms of density and IVI. Communities were identified based on the IVI.

Species diversity

Species diversity ($\bar{H}$) was determined by Shannon Wiener's information statistics (Shannon and Weiner, 1963). Diversity is usually considered as a function of relative distribution of individuals among the species.

$$\bar{H} = - \Sigma (Ni/N) \log 2 (Ni/N)$$

Where, Ni is the total number of individuals of a species and N is the total number of individuals of all species in that stand.

Result and Discussion

Site and habitat characteristics

The physical characteristics of the study sites are presented in Table 1. These sites fall between $30^0$18.808'N and 30^0 17.995'N latitudes; 078^0 25.154'E and 078^0 25.009'E longitudes and cover an altitudinal range of 1482 to 2200 m amsl. The main habitats were identified as Dry degraded, Shade moist, Dry and Moist with a slope varying from 30 to 70^0. Seven sites represented Dry degraded habitats and Shady moist habitat. Only two sites were identified as Dry habitat. Seven sites were represented by North-West, Six sites by North east and Three sites by North aspect.

Community diversity

The community types, their altitudinal distribution, representation in sites, habitats, coordinates and major associates are presented in Table 2. A total of 6 forest communities were delineated on the basis of highest IVI value of tree species in the study area (Table 3). *Pinus roxburghii* community was represented in maximum 6-sites, followed by *Cedrus deodara* - *Rhododendron arboreum* mixed and *Pinus roxburghii -Rhododendron arboreum* mixed (#3, each), *Cedrus deodara- Pinus wallichiana* mixed (#2) and *Pinus roxburghii- Quercus leucotrichophora* mixed and *Rhododendron arboreum* - *Quercus leucotrichophora-* mixed (#1, each). These communities were represented in the North, North West and North East aspects. Similar to present study, Joshi and Samant (2004) were reported the different numbers of communities in different habitats in the Nanda Devi Biosphere Reserve, Western Himalaya.

1. *Pinus roxburghii- Quercus leucotrichophora* mixed

A total of 04 shrubs were recorded in this community. Total shrubs density was 590.00 Ind ha^{-1} respectively (Table 5). *Berberis aristata* (50.85 %), *Rhus parviflora* (30.51 %) and *Rhus cotinus* (16.10 %, each) were the main contributors in this site (Table 4). Similar study was conducted by Kumar and Batt (2006) in Garhwal Himalaya.

2. *Pinus roxburghii*

A total of 14 shrubs species were recorded in this community. Total shrubs density was 488.33 Ind ha^{-1} (Table 5), which is lowest among all studied communities. less no of shrubs density may be due to allelopathy effect of *Pinus roxburghii* to other species. Highest relative density was recorded for *Berberis aristata* 31.57 %, followed by *Myrsine africana* (31.06 %) and *Asparagus adscendens* (21.84 % Table 4). Hussain *et al*. (2008), had reported highest density (985.00 Ind ha-1) of *Berberis aristata* in Kumaon Himalaya.

3. *Pinus roxburghii- Rhododendron arboreum* mixed

13 shrub species were recorded in this community. Total shrubs density was 4250.00 Ind ha^{-1} (Table 5). *Myrsine africana* (38.00 %), *Rubus ellipticus* (12.31 %) and *Indigofera atropurpurea* (9.53 %) were the main contributors in terms of high relative density (Table 4). Similar resulrs was reported by Gairola *et al*. (2008) at Tunganat site.

4. *Cedrus deodara- Pinus wallichiana* mixed

Total shrubs density was 2112.50 Ind ha^{-1} (Table 5). *Myrsine africana* (29.69 %), *Rubus ellipticus* (17.52 %) and *Rubus niveus* (10.71 %) were the main contributors in terms of relative density (Table 5).

PLANTICA – Journal of Plant Science ISSN: 2456 – 9259 (The Official Publication of Association of Plant Science Researchers) Plantica, Vol. 2 (3), 2018: 213 – 223
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Table 1. Physical characteristics of study sites

Sr. No.	Altitude (m)	Habitat	Aspect	Latitude	Longitude	Dominated species
1.	1482	D, Deg	NE	30^0 18.808'N	078^0 25.154'E	*Pinus roxburghii - Quercus leucotrichophora*
2.	1525	D, Deg	NW	30^0 18.080'N	078^0 25.137'E	*Pinus roxburghii*
3.	1586	D, Deg	N	30^0 18.727'N	078^0 25.135'E	*Pinus roxburghii*
4.	1684	D, Deg	NE	30^0 18.570'N	078^0 25.090'E	*Pinus roxburghii*
5.	1784	D, Deg	NW	30^0 18.368'N	078^0 24.957'E	*Pinus roxburghii*
6.	1787	D, Deg	NW	30^0 18.472'N	078^0 25.066'E	*Pinus roxburghii*
7.	1791	D, Deg	NE	30^0 18.470'N	078^0 25.073'E	*Pinus roxburghii*
8.	1863	D	N	30^0 18.242'N	078^0 25.995'E	*Pinus roxburghii - Rhododendron arboreum*
9.	1873	SM	N	30^0 18.189'N	078^0 25.936'E	*Cedrus deodara - Pinus wallichiana*
10.	1928	SM	NW	30^0 18.101'N	078^0 25.145'E	*Rhododendron arboreum- Cedrus deodara*
11.	1968	D	NE	30^0 18.213'N	078^0 25.104'E	*Pinus roxburghii - Rhododendron arboreum*
12.	1987	SM	NE	30^0 18.197'N	078^0 25.061'E	*Rhododendron arboreum- Cedrus deodara*
13.	2015	SM	NE	30^0 18.204'N	078^0 25.059'E	*Rhododendron arboreum- Pinus roxburghii*
14.	2015	SM	NW	30^0 18.204'N	078^0 25.059'E	*Cedrus deodara - Pinus wallichiana*
15.	2116	SM	NW	30^0 17.893'N	078^0 25.004'E	*Cedrus deodar and R. arboreum*
16.	2200	SM	NE	30^0 17.995'N	078^0 25.009'E	*Quercus leucotrichophora – R. arboreum*

Abbreviations used: SM=Shady Moist; D=Dry; Deg=Degraded; N=North; NW=North West and NE=North East

Table 2. Community types, their distribution, habitats and major associated species in study sites

Community types	SR	AR (m)	Habitat	Aspect	Latitude	Longitude	Major associated spp.
Pinus roxburghii – Quercus leucotrichophora mixed	1	1482-1495	D, Deg	NE	30^0 18.808'N 30^0 18.900'N	078^0 25.154'E 078^0 25.204'E	*Pinus roxburghii, Quercus leucotrichophora, Berberis aristata, Rhus parviflora, Rhus cotinus*
Pinus roxburghii	6	1525-1791	D, Deg	NW, NE	30^0 18.080'N 30^0 18.470'N	078^0 25.137'E 078^0 25.073'E	*Lyonia ovalifolia, Myrica esculenta, Rhododendron arboreum, Berberis aristata, Myrsine Africana, Asparagus adscendens*
Pinus roxburghii-Rhododendron arboreum mixed	3	1863-2015	D, SM	N, NE	30^0 18.204'N 30^0 18.242'N	078^0 25.059'E 078^0 25.995'E	*Lyonia ovalifolia, Cornus capitata, Myrsine africana, Rubus ellipticus, Indigofra atropurpurea, Pogostemon plectranthoides*
Cedrus deodara - Pinus wallichiana mixed	2	1873-2015	M, SM	N, NW	30^0 18.204'N 30^0 18.189'N	078^0 25.059'E 078^0 25.936'E	*Rhododendron arboreum, Pinus roxburghii, Rubus ellipticus, Pogostemon plectranthoides, Rubus paniculatus*
Cedrus deodara-Rhododendron arboreum mixed	3	1928-2116	SM	NW, NW	30^0 18.101'N 30^0 17.893'N	078^0 25.145'E 078^0 25.004'E	*Lyonia ovalifolia, Pinus roxburghii, Myrsine africana, Rubus ellipticus*
Rhododendron arboreum-Quercus leucotrichophora mixed	1	2116-2200	SM	NE	30^0 17.893'N 30^0 17.995'N	078^0 25.004'E 078^0 25.009'E	*Cedrus deodara, Lyonia ovalifolia, Myrsine africana, Berberis aristata*

Abbreviations used: SR=Site representation; AR= Altitudinal range; SM=Shady Moist; D=Dry and Dgr=Degraded; N=North; NW=North West and NE=North East

PLANTICA – Journal of Plant Science ISSN: 2456 – 9259 (The Official Publication of Association of Plant Science Researchers) Plantica, Vol. 2 (3), 2018: 213 – 223
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Table 3. Community wise highest Importance Value Index (IVI) of species in different forest localities in study area

Species Name	Community types					
	1	**2**	**3**	**4**	**5**	**6**
Pinus roxburghii	166.69	271.57	140.87	18.74	27.76	-
Quercus leucotrichophora	133.31	1.79	-	12.10	7.51	91.37
Rhododendron arboreum	-	3.50	99.24	33.45	96.83	92.87
Cedrus deodara	-	-	13.42	127.25	101.85	56.71
Pinus wallichiana	-	-	-	60.12	8.94	20.60

Abbreviations used: 1= Pinus roxburghii- Quercus leucotrichophora mixed; 2= Pinus roxburghii; 3= Pinus roxburghii- Rhododendron arboreum mixed; 4= Cedrus deodara- Pinus wallichiana mixed; 5= Cedrus deodara- Rhododendron arboreum mixed; 6= Rhododendron arboreum- Quercus leucotrichophora mixed

Table 4. Community wise relative density (%) of shrubs in different forest localities in study area

Species Name	Community type					
	1	**2**	**3**	**4**	**5**	**6**
Berberis aristata	50.85	31.57	4.86	4.02	3.35	24.41
Coriaria nepalensis	-	5.12	0.59	0.56	0.42	-
Berberis lycium	-	3.07	-	-	-	-
Asparagus adscendens	2.54	21.84	0.82	4.35	4.02	-
Myrsine Africana		31.06	38.00	29.69	43.05	60.00
Desmodium gangeticum	-	0.85	-	2.23	1.68	-
Rubus ellipticus	-	3.75	12.31	17.52	14.24	3.53
Debregesia longifolia	-	0.17	-	-	-	-
Desmodium renifolium	-	0.17	-	0.56	0.42	-
Vibernum cotinfolium	-	0.17	-	-	-	-
Rhus cotinus	16.10	0.85	5.88	2.23	0.34	-

Indigofra atropupurea	-	0.34	9.53	6.70	5.78	-
Rubus niveus	-	0.68	4.82	10.71	3.52	4.71
Rosa brunanii	-	0.34	1.69	2.79	10.55	1.18
Rubus paniculatus	-		4.24	5.92	1.93	-
Pogostemon plectranthoides	-	-	8.86	7.03	6.53	0.29
Premna interrupta	-	-	-	-	0.08	-
Indigofra heterantha	-	-	8.16	5.69	4.27	-
Daphyne papyracea	-	-	-	-	-	5.88
Desmodium podocarpum	-	-	0.24	-	-	-
Rhus parviflora	30.51	-	-	-	-	-
Total	**100**	**100**	**100**	**100**	**100**	**100**

Abbreviations used: 1= Pinus roxburghii- Quercus leucotrichophora mixed; 2= Pinus roxburghii; 3= Pinus roxburghii- Rhododendron arboreum mixed; 4= Cedrus deodara- Pinus wallichiana mixed; 5= Cedrus deodara- Rhododendron arboreum mixed; 6= Rhododendron arboreum- Quercus leucotrichophora mixed

Table 5. Community wise density and species diversity (H^1) of shrubs in study area

Community type	**Shrubs**	
	Density (per ha)	**Species diversity (H^1)**
Pinus roxburghii - Quercus leucotrichophora mixed	590.00	1.09
Pinus roxburghii	488.33	1.63
Pinus roxburghii- Rhododendron arboreum mixed	4250.00	2.02
Cedrus deodara - Pinus wallichiana mixed	2112.50	2.20
Cedrus deodara- Rhododendron arboreum mixed	1993.30	1.93
Rhododendron arboreum- Quercus leucotrichophora mixed	1700.00	1.15
Maximum	**4250.00**	**2.20**
Minimum	**488.33**	**1.09**

5. *Cedrus deodara - Rhododendron arboreum* mixed

A total of 15 species of shrubs were recorded in the community (Table 7). Total shrubs density was 1993.30 Ind ha^{-1} (Table 5). *Myrsine africana* was the main contributors with maximum relative density (43.05 %), followed by *Rubus ellipticus* (14.24 %) and *Rosa brunonii* (10.55 % Table 5). Similar study was conducted by Kumar and Thankur (2008) at Solan forest division Himachal Pradesh.

6. *Rhododendron arboreum- Quercus leucotrichophora* mixed

A total of 07 shrubs species were recorded in this community. Total shrubs density was 1700.00 Ind ha^{-1} (Table 5). *Myrsine africana* (60.0 %), *Berberis aristata* (24.41 %) and *Rosa brunonii* (5.88 %) were the main contributors in terms of relative density (Table 7). Similar results were observed by Kumar (2012) in mixed forest in Garhwal Himalayas.

Species diversity ($\bar{H}$)

Community wise diversity of shrubs are shown in Table 5. Diversity of shrubs was ranged from 1.09-2.20. The highest diversity of shrubs was recorded in *C. deodara- P. wallichiana* mixed community (2.20), followed by *P. roxburghii- R. arboreum* (2.02) and *C. deodara –R. arboreum* mixed (1.93) communities. Numerous studies have been done on species diversity in temperate Himalaya (Saxena and Singh, 1982 and Giri *et al*., 2008).

Conclusion

Study of forest shrubs at community level and exposed its relationship with various community shows resource rich and resource poor condition to other species by the community. Plant community diversity reveals positive and negative collision. The results of this study would be useful to develop proper strategy for conservation and management of highly valuable species at different points of view like medicinal, fodder, edible fruit, and ecological.

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PLANTICA – Journal of Plant Science
(The Official Publication of Association of Plant Science Researchers)
ISSN: 2456 – 9259
Plantica, Vol. 2 (3), 2018: 224 – 233
www.jpsr.in

Research Article

EFFECT OF ORGANIC SUBSTANCES ON GROWTH OF DIFFERENT *PLEUROTUS* SPECIES

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Received: May, 2018 – Accepted: June, 2018

Abstract

The trend of cultivation of *Pleurotus species* on lignin, cellulose, hemicellulose and lignocellulose containing substrates with chemical supplements for the efficient nutritional attributes and yield is common. *Pleurotus* species has immense capacity to metabolize these wastes as substrate to nutritionally rich fruiting bodies compared to other species of mushrooms and contribute in enhancement of nutritional value of mushrooms in food industry. In current studies, the triple combination of wheat straw substrate, oil extracted lemon grass leaves as an additional basal substrate with biological supplement containing maize flour powder were analyzed on different *Pleurotus* species. Here, we found *P. sajor-caju* consumed less number of days for spawn running, pin head formation and showed better yield than *P. flabellatus and P. florida* with the triple combination. The mycelium running for *P. sajor-caju* occurred only in 10 days comparative to *P. flabellatus and P. florida*. The development of fruiting body for *P.sajor-caju* was observed in 16 days, earlier than *P. flabellatus and P. florida* showing fruiting body in 20 and 23 days respectively. The biological efficiency B.E of *P. sajor-caju* was found to be 25% higher than *P. flabellatus* and 19% higher than *P. florida*. The results were noticeably improved, when the combination of wheat straw, additional oil extracted lemongrass leaves waste and maize flour powder supplement were used rather than as an individual substrate or supplement in the order of *P. jsajor-caju* followed by *P. florida* and *P. flabellatus.*

Keywords: Cultivation of *Pleurotus* species, Agrowaste, Forestry waste, Spawn running, Primordia formation, Biological efficiency**.**

Introduction

Agriculture and forestry sectors are the major units for the production of agro and forestry wastage. These wastes can be used as substrate for the growth of variety of *Pleurotus* mushrooms species. These Oyster mushrooms (*Pleurotus*)species acts upon these waste through their hemicellulotic, lignocellulotic, lignolytic and cellulotic enzymes and convert the wastage into healthy staple food (Philippoussis et al. 2001; Olivieri et al. 2006; Li and Shah. 2016). The *Pleurotus* species (Dhingri) in Indian market is an edible mushroom that belongs to the spore bearing subdivision basidiomycotina and contributes as the third largest popular

food in several countries. *Pleurotus* spp. are the basidiomycetes white rot fungi which initiates their life cycle with basidiomycetes spore's germination in the substrate. (Tsujiyama et al. 2013; MD. Mijan Hossain. 2017). Oyster mushrooms has accompalished a satisfactory and competent market stability because of it's delectable flavour, longivity in shelf life, high protein and fibre content with it's nutritional and medicinal properties. *Pleurotus* species nutritional value comprises high protein of 25-50 %, nine essential amminoacids and very low 2-5 % fat content which makes it suitable as diet food for health concious individuals. The sugar content is moderate and ranges 17-47% including minerals such as calcium, pottaisum, sodium with vitamins such as niacin, riboflavin, vitamin B1, B5, B6, C and D (Caglarirmak, N. 2007; A. A. Syed et al. 2009).

Pleurotus spp. can be cultivated in short duration which simultaneously reduces the possibility of the infections by pathogens and attacks of insects and the pests making them as the best choice for growers, farmers and researchers. *Pleurotus* species such as *P. florida, P. flabellatus and P. sajor-caju* are the saprophytes that utilises conventional substrates as wheat straw, paddy straw, soybean straw and non-conventional substrates such as waste of fruits, vegetables, saw dust, leaves of bamboos, lemon grass, tea leaves for their development and production of fruiting bodies (Dehariya, Poonam et al. 2013). Agro and forestry waste based substrates have major contribution in the chemical, physiological, sensorial and functional based attributes of *Pleurotus* species (Oyetayo and Ariyo. 2013). Wheat straw and thatch grass which is a low cost agrowaste has been used from the long time as one of the most common substrate as the traditional recipe (Hussain et al., 2002; Pant et al., 2006; Fanadzo et al., 2010). The yield and biological efficiency (BE) could be enhanced by additional substrates as lemon grass with the wheat straw. These substrates supports and compensate the nutritional requirements for the *Pleurotus* species (Owaid et al., 2015). These additional substrates are metabolized by different enzymes including manganese peroxidase, ligninase, laccase and mannase which are produced during 2nd and 3rd day as the intitial stage of mycelial running takes place in the substrate (Rossi et al., 2001; Donini et al., 2009; Luz et al. 2012). The respective qualitative and quantitative factors like spawn running and B.E of the *Pleurotus* spp. were found to be enhanced upon the usage of wheat straw substrate as its increases the essential nutrients absorption surface area (Philippoussis et al., 2001; Pant et al., 2006; Fanadzo et al., 2010). Alam et al. 2010, used maize powder as supplement with rice bran as the substrate for the growth of milky mushrooms. The powdered pulses were also used to maintain Nitrogen content and C:N ratio for giving better yield (Ram Naraian et al. 2016). Vijay and Upadhyaya., 1985 suggested to use chicken manure, yeats mud, wheat and rice bran as supplements to increase yield of *P. flabellatus and P. sajor-caju* . In previous studies, wild grasses were used as suitable substrate for the cultivation of *Pleurotus* species mushroom (Das *et al.,* 2000). The usage of lemon grass has been carried out by (Martinez-Carrera, D. 1989) for the mushrooms. In previous work, nitrogen deficient lignocellulosic substrates were supplemented with powdered pulses to maintain nitrogen content. In this studies, maize powder is utilized as supplement to provide optimum nitrogen content, alongwith oil extracted waste lemon grass leaves as an additional substrate with wheat straw, for the evaulation of oil extracted lemon grass leaves with maize powder for the cultivation of as *P. florida, P. flabellatus and P. sajor-caju.*

The objectives of this study are**:**

1. Quantitative analysis of spawn running, pin

head formation and time for fruiting body of *Pleurotus* spp. for the combination of oil extracted lemon grass leaves with maize powder supplement and wheat straw

2. Biological yield and efficiency estimation on these substrates which contains oil extracted lemon grass leaves, wheat straw and maize powder supplement on different species of Pleurotus such has *P. florida, P. flabellatus and P. sajor-caju.*

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Materials and Methods

Spawn source of different *Pleurotus spp. (Pleurotus florida, Pleurotus flabellatus and Pleurotus sajor-caju)* were obtained from DMR (Directorate of Mushroom Research Solan H.P. The Substrates, additional basal substrates and biological supplements such as wheat straw, oil extracted lemon grass leaves waste and Maize powder were used respectively. For the process of sterilization, chemical process was used. Eventually, the prepared substrates were packed using PP bags.

Culture cultivation

Pure culture of *P. florida, P. flabellatus and P. sajorcaju, were* obtained from DMR, Solan, H.P. The cultures were grown at 25°C and maintained on potato dextrose agar (PDA) plates and stored at 4°C in the laboratory. Further, subculturing was performed in every 15 days and used as inoculum for spawn Preparation.

Spawn preparation

Healthy wheat grains were collected and washed thoroughly in tap water and soaked overnight in water till they become soft. Then grains were boiled, drained off excess water and mixed with calcium carbonate at the rate of 2 % on dry weight basis of the grains. The grains were filled into glucose bottle, plugged with non-absorbent cotton and sterilized in autoclave at 121 °C for 30 min. Grains were then eventually inoculated independently in different bottles with actively growing mycelium of *P. florida, P. flabellatus and P. sajor-caju*, maintained on PDA and incubated at 25°C for mycelial growth until the grains were fully and completely get covered with mycelium of *P. florida, P. flabellatus and P. sajor-caju*. The modified method of Michael *et al.*, 2011 was used.

Preparation of substrates

Easily available, cheap and used agro or forest biowaste such as wheat straw containing high lignolytic, cellulotic, hemicellulotic, lignocellulotic content were used as a substrate for the cultivation of *Pleurotus* species. The oil extracted lemon grass leaves were used as an additional substrate to support the wheat straw substrate as additional nutrient source in ration of 3:1 as used previously by Hussain *et al.*, 2000. Maize powder was used as supplement in concentration of 3% with 3:1 ratio of lemon grass biowaste to wheat straw.

Soaking

Collected waste substrate like oil extracted lemon grass leaves, wheat straw were chopped in to 2-3 cm. pieces. The substrate materials were soaked in 100 liter of fresh water in a drum for 12 hours and fungicide used as a chemical sterilization process. The amount of 12 gm. of bevistin/ carbendazim and 125 ml. of formalin were mixed with 100 liter of water. After soaking, excess amount of water was drained, the substrates were taken out and further the spreading of the substrate in form of thin bed layer of 5-6 inch at concrete floor is performed to retain 60-65% moisture.

Spawning

After that drained straw was mixed with spawn and spawning was performed by

using a PP polypropylene bags of 16x24cm and 14 x 28cm. In this stage, substrate moisture contain was 65 to 70%. Two kg of substrate was used to fill up in each bag. Three layerings were performed per bag with spawning layering rate of 2.5% of wet substrate. One kg spawn was used for 10-12 kg wet straw. Further, spawned bags were kept in the dark incubation chamber for the next step of spawn running. Proper aeration at the optimum temperature of 20-30 °C for the process of spawn running was maintained.

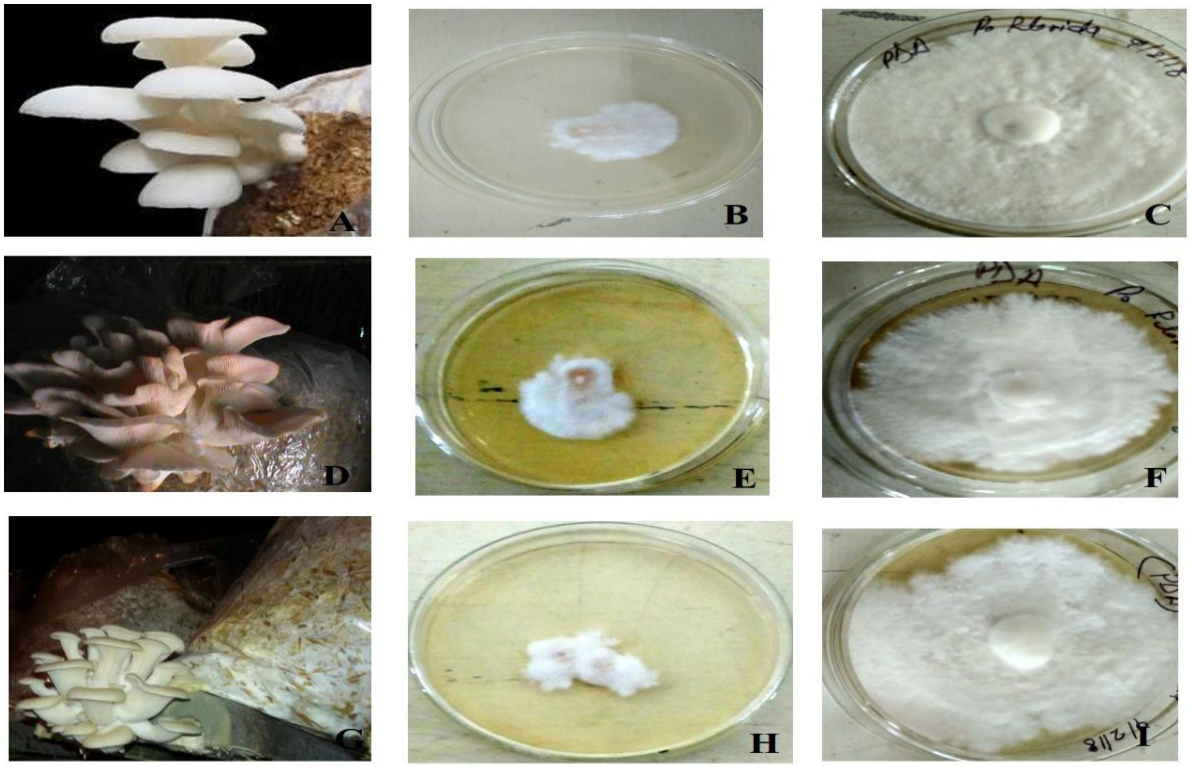

Figure 1. Mother culture of *Pleurotus* species on Potato Dextrose agar medium (PDA)- The different species of oyster *Pleurotus florida* (A-C) , *Pleurotus flabellatus* (D-F) and *Pleurotus sajor-caju* (G-H) mycelium showing different progressive stages on the PDA medium.

Cropping and Harvesting

After 10-20 days, when substrate was fully/completely colonized with mycelium that appears as white cottony growth, it was further transferred to the cropping room, which was fully equipped with oxygen supply and proper aeration. Furthermore, polythene covers were removed after 4-5 days, for the initiation of pinheads. The temperature of 20-24 and relative humidity of 85% was maintained. The process of watering in morning and evening atleast twice a day using sprayer system was performed. The total yield of the fruiting bodies was recorded per day from each bag followed by the recording of B.E.

Results and Discussion

The common substrate containing the combination of Wheat substrate, oil extracted lemon grass leaves and Maize powder results were examined and

analysed for the three different species of Pleurotus species such as *P. florida, P. flabellatus and P. sajor-caju.*

Development of mycelium from fruiting body in mother culture on PDA

The pure mother cultures were obtained from the three different species of *P. florida, P. flabellatus and P. sajor-caju* as shown in Figure 1. The complete mycelial growth from the intial stage to the final establishment of mycelia on PDA plates took 12 days, 10 days and 10 days for *P., P. flabellatus and P. sajor-caju* respectively were explained in Table 1.

Spawn running, pin head and fruiting body formation-

The common substrate containing Wheat straw (W.S), Oil extracted lemon grass leaves (OELGL) and Maize powder (MP) were used to evaluate the the growth and yield of *P. florida, P. flabellatus and P. sajor-caju* The different stages such as the preparation of bags, spawn running , pinhead formation and fruiting body development were shown in Figure 2, 3 and 4 for *P. florida, P. flabellatus and P. sajor-caju* species respectively.

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Table 1. Days for completion of mycelium of *Pleurotus florida, Pleurotus flabellatus* and *Pleurotus sajor-caju*, the varieties of *Oyster mushroom* on PDA (Potato dextrose agar) medium.

Mother culture (Species)	Medium for growth	Initial mycelium growth (days)	Final mycelium growth (days)
Pleurotus florida	**PDA**	**5th day**	**12th day**
Pleurotus flabellatus	**PDA**	**2nd day**	**10th day**
Pleurotus sajor-caju	**PDA**	**3rd day**	**10th day**

Figure 2. White Mushroom (*Pleurotus florida*) showing different stages on the substrate**:** Substrate containing wheat Straw (Calcium carbonate), oil extracted lemon grass leaves and Maize powder as supplement

PLANTICA – Journal of Plant Science
(The Official Publication of Association of Plant Science Researchers)
ISSN: 2456 – 9259
Plantica, Vol. 2 (3), 2018: 164 – 169
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Figure 3. Pink Mushroom (*Pleurotus flabellatus*) showing different stages on the Substrate-Substrate containing wheat Straw, lemon grass oil extracted leaves and maize powder as supplement.

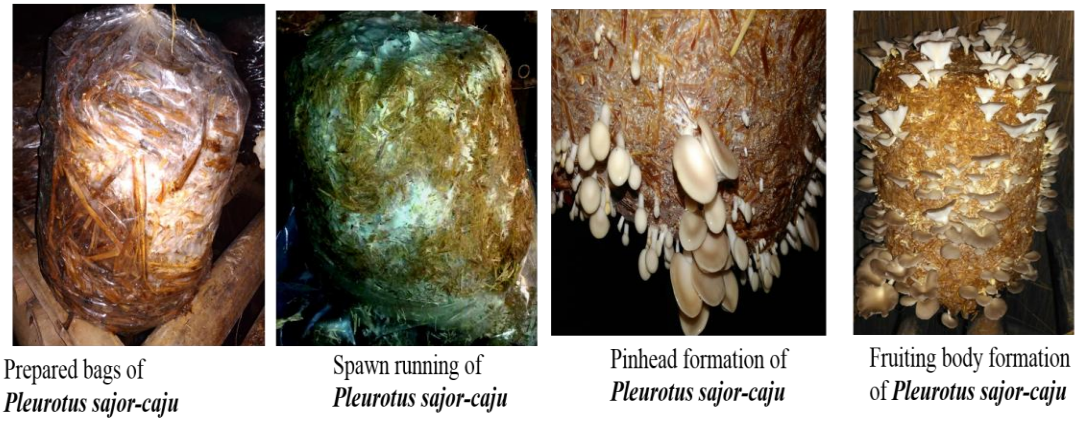

Figure 4. Grey Mushroom (*Pleurotus sajor-caju*) showing different stages on the Substrate- Substrate containing wheat Straw, oil extracted lemon grass leaves and maize powder as supplement.

The complete cycle for the growth of *P. florida* from Preparation of bags to the final fruiting stage took 23 days. The biological yield and efficiency were 560 gms per kg of dry substrate and the biological efficiency (BE) was found to be 56% on common substrate of wheat straw, oil extracted lemon grass leaves and maize powder supplement as mentioned in Table 2.

The cycle for the growth of *P. flabellatus* from bag's Preparation to the final fruiting bodies was completed comparatively earlier than *P. florida* in 20 days. The biological yield and efficiency were found to be 500 gms per kg of dry substrate and the 50% respectively on the common substrate of wheat straw, oil extracted lemon grass leaves and maize powder supplement as shown in Table 2. *P. sajor-caju* completed it's cycle from the Preparation of bags to the final fruiting spawn running to the formation of fruiting bodies occurred very early comparative to both *P. florida* and *P. flabellatus* with in only 16 days. The biological yield and efficiency were also found to be 750 gms per kg of dry substrate

and the 75% on the given substrate which is very higher than other two *Pleurotus* species tested for substrate containing wheat straw, additional substrate as oil extracted lemon grass leaves and maize powder supplement as shown in Table 2.

The time required for completion of spawn running, pin head and fruiting body formation for *Pleurotus* species such as *P. florida, P. flabellatus and P. sajor-caju* were compared in Table 2

.

Table 2. No. of days required for the completion of Spawn run, pinhead formation, fruiting body, yield and biological efficiency of *Pleurotus florida*, *Pleurotus flabellatus* and *Pleurotus sajor-caju* on substrates- The substrates containng Wheat Straw (W.S), oil extracted lemon grass leaves (OELGL) and maize powder (M.P) as supplement

Species	Substrate+ Basal substrate+ supplement	Spawn running (days)	Pinhead /Primordia formation (days)	Fruiting body formation/Harvest (days)	Total Harvest Yield (g/kg dry substance)	Biological efficiency (%)
Pleurotus florida	W.S+ OELGL+ MP	15th day	20th day	23th day	560 gms	56
Pleurotus flabellatus	W.S+ OELGL+ MP	12th day	16th day	20th day	500 gms	50
Pleurotus sajor-caju	W.S+ OELGL+ MP	10th day	12th day	16th day	750 gms	75

For the studies of *P. Sajor caju*, Mijan Hossain, MD., 2017, reported that either wheat straw or maize stalks and leaves were used as sole substrates that showed fruiting body formation in 28 days with biological yield and B.E as 601 g/Kg and 60.10% for wheat straw whereas fruiting body formation in 35 days with yield and B.E as 260 g/Kg and 26 % B.E. respectively for maize straw and leaves. The previous work was also performed on the combination of wheat straw and lemon grass which yielded 322 g/Kg and time duration required for fruiting completion was 19 days (Muhammad s. Mumtaz. et al., 2016). In our recent studies, the time duration for the production of fruiting body and biological yield of *P. sajor-caju* on our proposed combination of wheat substrate with oil extracted lemon grass leaves and maize powder supplement were found to be 16 days which is earlier than 19 days and 750 g/Kg of dry substrate respectively which is notabely higher when compared to previous studies. However, our results are similar to studies conducted by (Dehariya, Poonam., Vyas Deepak., 2013), that reported the combination of wheat straw with soybean straw showing yield of 873 g/Kg and maize stalks with soybean as 621.7 g/Kg. Therefore, our combination of wheat substrate with oil extracted lemon grass leaves and maize powder, shows comparable and effective yield to (Dehariya, Poonam., Vyas Deepak., 2013), supporting the significance of additional waste

substrates and supplements in enhacing the yield and biological efficiency in less time duration with solely used wheat straw as substrate,

Recently, Shalinee Prasad et al., 2018 reported that the use of perennial grass as the sole source, showed low yield and B.E of 197 g/Kg (B.E 26%) to 240 g/Kg (B.E 32%) but in the combination of wheat straw and perennial grass where grass is used as an additional substrate for the cultivation of *P. florida.* the biological yield was found to be 513.43 g/Kg and B.E as 68.45%. In the current studies, we report that our combination substrates shows higher biological yield as 560 g/Kg in 23 days with comparable biological efficiency (B.E) of 56%.

P. flabellatus on different substrates containing sawdust of Mango, Kadom,Mahogony, Jackfruit, Coconut, Jam Shiris sawdust with wheat bran showed 31 to 38 days for harvesting the crop and the yield was reported 83 to 150 g/Kg as reported by M. Z. Islam et al., 2009 . In the current work, we report that combination of wheat straw and lemon grass with maize powder supplement showed Biological yield and B.E of 500 g/Kg and 50 % in 20 days.

The completely organic combinational substrate consisting of wheat substrate with oil extracted lemon grass leaves as an additional supporting substrate and maize powder supplement showed minimum harvesting period for *Pleurotus sajor-caju*<*Pleurotus flabellatus*< *Pleurotus florida* as 16 days, 20 days and 23 days respectively. *The lowest time required for completion of spawn run was found for P. sajor-caju* i.e 16 days whereas *P. florida* took 23 days, which is higher running time than *P. flabellatus* that occurs in 20 days on this combination of substate, supporting substrate and supplement. The biological yield and biological efficiency were also found to be in order *Pleurotus sajor-caju*>*Pleurotus florida* > *Pleurotus flabellatus* with 750 g/Kg (75%), 560 g/Kg (56%) and 500 g/Kg (50%).

The previously used combination of wheat straw with lemon grass substrate for the cultivation of *P. sajor-caju, and P. flabellatus* showed higher production compared to solely used wheat straw where *it* showed 18.5 % increase in yield over control in ration of 3:1 for lemon grass to wheat straw combination as reported by (Hussain *et al.,* 2000). In this studies, we added the maize powder as a supplement and propose it to use as organic combination of substrate for the different variety of Pleurotus species such ***as*** *P. florida, P. flabellatus* and *P. sajor-caju.* The time duration was found to be low for harvesting of fruiting bodies, with satisfactory biological yield and notably high biological efficiency, making the oil extracted lemon grass leaves with organic maize powder supplement with wheat straw as suitable and efficient combinatorial medium for the cultivtion of *Pleurotus sajor-caju, Pleurotus flabellatus and Pleurotus florida.*

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