CHILLING AND SALINITY INDUCED METABOLIC RESPONSES IN DIFFERENT CULTIVARS OF *VIGNA UMBELLATA* T.

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ABSTRACT

The physiological and biochemical responses of four *Vigna umbellata* cultivars under two abiotic stress conditions, chilling and salinity were studied. These resulted in the metabolic dysfunction and reduction in growth of the plants under stress conditions. However, there was enhanced synthesis of protein, proline, soluble carbohydrates etc. during the short duration of the present study. The loss of membrane integrity resulted in increased membrane lipid peroxidation and membrane injury. Accumulation of free radicals had toxic effects on the protein nature of the scavenging enzymes. Of the *V. umbellata* studied the black cultivar was most cold tolerant and the grey cultivar of *Vigna umbellata* was the most salt tolerant.

KEY WORDS: Vigna umbellata, abiotic stress, oxidative stress, free radicals, metabolic dysfunction

INTRODUCTION

Legumes are one of the richest sources of vegetable protein and form an important component of stable diet all over the world. Rice bean (*Vigna umbellata* T.) is a less known legume crop that has been identified as a suitable addition to the existing list of pulses (Maggo et al., 1999). This plant is used in the especially in the Darjeeling Himalayas and generally, all over the temperate Himalayas as an important crop offering high quality protein and increasing the input of combined nitrogen in soil. In this part of the Himalayas this crop has traditionally been used for rotation with cereal crops in order to increase the soil fertility (Chhetri and Mukherjee, 2003).

Salinity affects various aspects of plant growth and metabolism such as osmotic adjustment, ion uptake, pigment content, protein and amino acid metabolism etc. Injury to plants from salt stress may be due stress induced membrane damage, lipid peroxidation caused by free radicals (Cakmak and Horst, 1991). The most commonly reported effect of salt stress on amino acid metabolism is an accumulation of proline. There is strong correlation between increased cellular proline levels and the capacity to survive both water deficit and the effect of high environmental salinity (Sairam and Tyagi, 2004). MDA which is one of the decomposition products of PUFA of biomembranes also showed greater accumulation under salinity treatment. (Choudhuri and Choudhuri, 1993). Salt stress and dehydration stress show a high degree of similarity with respect to physiological, biochemical, molecular and genetic effects. Sub-lethal salt stress is ultimately an osmotic effect, which is apparently similar to that brought in by water deficit and to some extent by cold as well as heat stresses (Almoguera *et al*., 1995).

Low temperature affects cellular metabolism and may even cause injury in plant tissue at different developmental stages. The effects depend on severity of exposure and the plant species (Levitt, 1980). Membrane systems of the cells are the primary site of freezing injury in plants (Steponkus, 1984). At low temperature, greater membrane lipid unsaturation appears to be crucial for optimum membrane function. The accumulation of sucrose and other simple sugars that typically occurs with cold acclimation protects the membranes against freeze induced membrane damage in-vitro (Strauss and Hauser, 1986). There is also evidence that certain proteins participate in the stabilization of membranes against

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freeze induced injury. However, protein denaturation is a regular phenomenon in plants exposed to low temperature which could result in cellular damage (Guy *et al*., 1998).

Understanding the responses of plants to their external environment is an attractive target for improving stress tolerance (Madhusudhan *et al.*, 2002). Studies on the physiological and biochemical responses of *Vigna umbellata* under abiotic stress conditions is lacking. An understanding of the physiological mechanisms and identification of specific characteristics conferring stress tolerance could play a major role in the development of new varieties suitable for such stress conditions (Chhetri and Mukherjee, 2003).

MATRIALS AND METHODS

Four cultivars of rice bean seeds, *Vigna umbellata T*. (viz., red=Vum-R, yellow=Vum-Y, Black=Vum-B and Grey=Vum-G) were collected and brought to the laboratory. The seeds could be visually identified into four different types due to their colour. Each of the cultivars were divided into three different groups of 40 seeds each and were washed thoroughly for 10 minutes in a liquid detergent followed by washing in 0.1% HgCl2 for 5 minutes; then the seed lots were washed 3 times in sterile distilled water. All the seeds lots were soaked for 12 hrs in distilled water and blotted dry. One set of the seed lots containing 40 seeds of each of the 4 cultivars of rice bean were taken in petridishes over filter papers moist with distilled water and placed for cold treatment under darkness at 4°C for 24 hrs for chilling pretreatment, similarly, another set was incubated in darkness at room temperature for 24 hrs while being irrigated with 100 µM NaCl solution, all the while. The third group of the seed lots that served as the control group were similarly incubated in the darkness for 24 hrs at room temperature, but they were irrigated with distilled water only. All the physiological and biochemical estimations were performed after 7 days of pretreatment except the dry weight and photosynthetic pigments which were estimated 14 days after treatment. The temperature during the analysis was 22 ^{o}C ± 2 ?C and the RH of lab during the analysis was75% ± 2%.

Soluble protein was extracted from the germinating seed tissue wtih 50 mM Tris-acetate buffer (pH 7.0) and estimated with Folin-Ciocalteau reagent (Lowry *et al*., 1951) as modified by Bollag et al. (1996). For the extraction of proline, the seed tissue was homogenized 3% sulfosalicylic acid and estimated with acid-Ninhydrin reagent using pure proline as standard as per the method Bates *et al*., (1973). Soluble carbohydrate was extracted and estimated from germinating endosperm tissue with anthrone reagent following the method of McCready *et al*. (1950).

For the extraction of catalase and peroxidase, the plant material was homogenized with 0.2 M chilled Na-phosphate buffer (pH-6.8) The enzyme assay was carried out according to the method of Snell and Snell (1971) and Kar and Mishra (1976) respectively. The enzyme activities in both the cases were calculated according to Fick and Qualset (1975).

Membrane injury index (MII) was estimated by measuring the electrical conductivity of seed leachate as per the method of Sullivan (1972). Membrane lipid peroxidation was determined in terms of MDA concentration according to Heath and Packer (1968).

Photosynthetic pigments (chlorophyll a, b and caroteinoids) were extracted and estimated from fresh leaf samples following the method of Lichtenthaler (1987).

Mean tolerance indices (MTI) and Relative growth index (RGI) were calculated from 10 seedlings (7 days old) according to Paliouris and Hutchinson (1991).

RESULTS AND DISCUSSION

Protein content is the indicator of general vigour and vitality of seedlings. During the course of analysis it was found that the soluble protein content showed a steady increase in the cold stress conditions in all of the four cultivars of V. umbellata tested. Of course, the soluble protein level decreased in all the cultivars of the plants on exposure to salinity (Table-1). During the chilling treatment the plant must have produced cold responsive proteins to tide over the cold stress conditions. But salinity treatment to a level of 100µg/ml may have

become too toxic and so the protein level was brought down (Chhetri et al., 2004). It may be due to breakdown of protein synthesis mechanism or due to reduced incorporation of free amino acids into protein (Hsiao, 1970).

The role of carbohydrates in the development of chilling tolerance has been demonstrated in tomato seedlings where chilling sensitivity changes according to the contents of the carbohydrates (King et al., 1988). The synthesis of various low molecular weight sugars, such as glucose, fructose and sorbitol is a common feature of low temperature acclimation (Sakai and Larcher, 1987). Soluble carbohydrate showed an increase in its content in both cold and salinity pretreatments. It may be assumed that even the short exposure to chilling or a slight saline condition during the treatment was sufficient to upset the integrity of cellular membrane that resulted in the leaching of soluble carbohydrates (Table-1).

Table-1.

Effect of seed treatment with salinity (100 µM NaCl) and chilling stress (48 hrs at 4°C) on soluble protein and soluble carbohydrate content (mg/g fresh wt) of four *Vigna umbellata* T. (Vum-G=grey, Vum-Y=yellow, Vum-R=red and Vum-B=black) cultivars

Seed types	Protein (mg/g fresh wt)			Soluble carbohydrate (mg/g fresh wt)		
	Control	Chilling	Salinity	Control	Chilling	Salinity
Vum-G	31.01 ± 1.40	32.6 ± 1.21	28.2 ± 0.58	14.0 ± 1.12	23.8 ± 1.78	21.0 ± 1.66
Vum-Y	30.8 ± 3.32	33.6 ± 0.90	28.0 ± 2.06	11.4 ± 0.41	24.4 ± 1.55	21.4 ± 0.87
Vum-R	28.6 ± 1.31	31.4 ± 1.84	26.0 ± 1.38	15.6 ± 0.60	25.2 ± 1.21	22.5 ± 1.00
Vum-B	34.0 ± 0.72	36.0 ± 2.37	25.2 ± 1.09	12.0 ± 1.35	23.0 ± 1.00	20.3 ± 1.32

(Values are mean ± SE of 3 replicates)

In all the four cultivars of rice bean proline content showed a increased accumulation following both chilling and salinity stress conditions (Table-2). The role of proline in protecting plants from osmotic stress is well known. Proline level has been reported to increase in response to low temperature (Songstad et al., 1990) Proline is one of the osmolites in water stressed plants and accumulated in increased amounts, both by activation of its biosynthesis and inactivation of its degradation (Yoshiba et al., 1997). Any sort of abiotic stress ultimately effects in denying water abundance in the plants. Thus accumulation of proline during the present study is justified. During the present study the accumulation of proline in cold stressed plant was almost double to that of control and the accumulation was of the same in salinity stress is double to that of cold stressed plants. Clearly, the salt stressed conditions predisposed the proline accumulation to offset the osmotic imbalance caused by salinity.

Assessment of one of the products of lipid peroxidation in terms of MDA accumulation revealed a gradual rise in MDA content from control to cold stressed to salt stressed conditions (Table-2). Hypothermia and salinity

Table-2.

Effect of seed treatment with salinity (100 µM NaCl) and chilling stress (48 hrs at 4°C) on proline content (mg/g fresh wt) and membrane lipid peroxidation expressed in terms of MDA accumulation (n mole/g fresh tissue) in four *Vigna umbellata* T. (Vum-G=grey, Vum-Y=yellow, Vum-R=red and Vum-B=black) cultivars

Seed types	Proline (mg/g fresh wt)			MDA accumulation (n mole/g fresh tissue)		
	Control	Chilling	Salinity	Control	Chilling	Salinity
Vum-G	1.8 ± 0.11	3.7 ± 0.20	7.3 ± 0.37	28.25 ± 2.94	33.87 ± 1.04	34.83 ± 1.11
Vum-Y	1.8 ± 0.15	3.0 ± 0.25	4.7 ± 0.35	31.25 ± 1.05	35.64 ± 0.47	39.19 ± 2.28
Vum-R	1.6 ± 0.25	2.8 ± 0.15	4.0 ± 0.45	17.25 ± 1.45	35.80 ± 0.70	43.38 ± 1.96
Vum-B	2.5 ± 0.36	4.1 ± 0.117	6.2 ± 0.43	21.75 ± 0.59	32.25 ± 1.54	36.61 ± 0.51

(Values are mean ± SE of 3 replicates)

may bring about hydrolysis of membrane components which cannot be repaired by other synthetic processes. Thus MDA, which is a decomposition product of polyunsaturated fatty acids of biomembranes, shows greater accumulation in response to abiotic stress conditions. Considering the parameters of proline content and MDA accumulation the black cultivar of *V. umbellata* (Vum-B) was found to be most robust followed by the gray cultivar (Vum-G) that can withstand both chilling and salinity stress.

Cold treatments affected the membrane permeability and the composition of membranes. Studies on freezing injury on Rye indicated that plasma membrane was primarily affected (Steponkus, 1984). The cell membrane in non-halophytes when placed under hyper-saline condition is severely damaged. NaCl stress also mimics the effects of oxidative stress as a secondary stress and results in enhanced peroxidative damage to the membrane system. Salinity was responsible for membrane injury and leakage as a function of NaCl stress. In the present study when membrane stability was tested in terms of membrane injury, it was found that both type of abiotic stress had considerable damaging effects on cell membrane. However, Vum-B was the most tolerant genotype showing resistance to both chilling and salinity stress factors (Table-3). However, when stress effects were considered upon other physiological parameters, Vum-G was found most suitable for chilling tolerance and Vum-Y for salt tolerance in terms of relative growth index. Similarly Vum-G and Vum-R were found to be most tolerant of chilling and salinity stress in terms of mean tolerance index (Table-3).

It is well known that peroxidases are often the first enzymes to alter their activities during stress, and in several cases enhanced activities have been observed under stress (Srivalli et al., 2003). Toxic H2O2 is produced in plants as a response to various stresses. One of the important pathways of generation is the dismutation of superoxide by SOD. Superoxide may again react with H2O2, generating a series of more reactive free oxygen radicals that damages membrane and cellular macromolecules. Peroxidase acts as scavenging enzyme destroying H2O2 (Bhattacharjee and Mukherjee, 1995). In all the cultivars of *V.umbellata*, peroxidase level declined drastically when subjected to stress conditions. The decline was most pronounced in Vum-R and the least decline was seen in Vum-B (Fig-1).

Table-3.

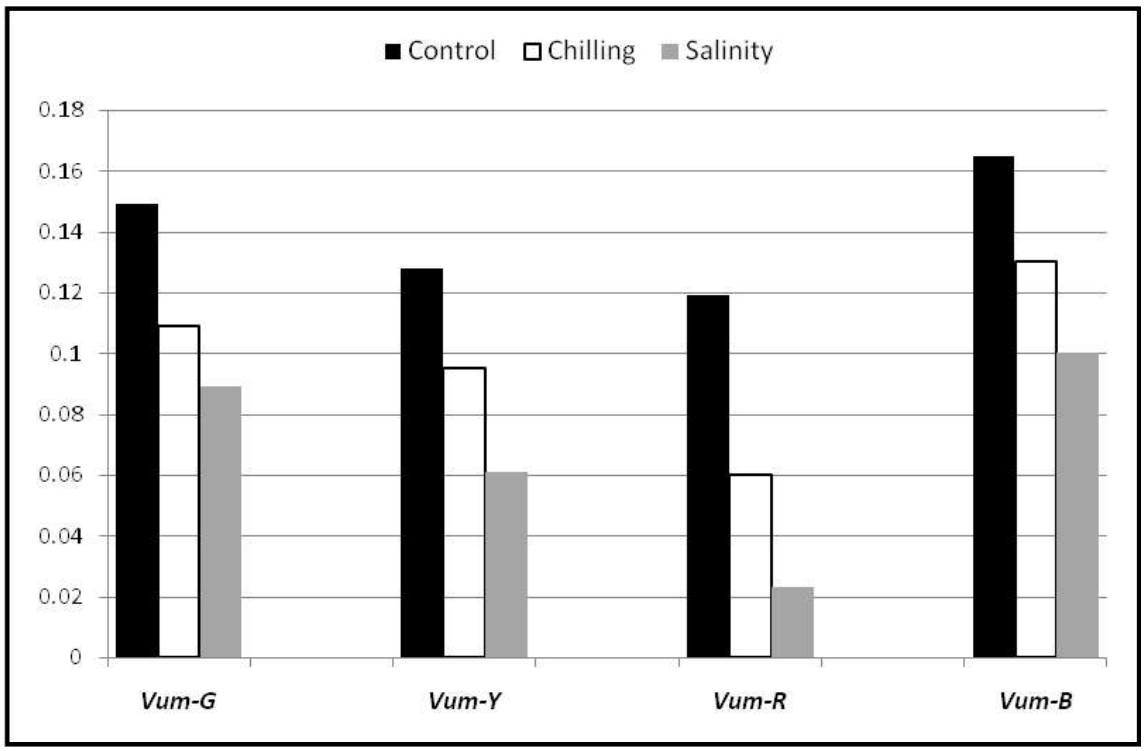
Effect of seed treatment with salinity stress (100 µM NaCl) and Chilling (48 hrs at 4°C) on Membrane injury index (MII), Relative growth index (RGI) and Mean tolerance index (MTI) in four cultivars of *Vigna umbellata* T. (Vum-G=grey, Vum-Y=yellow, Vum-R=red and Vum-B=black varieties).

Seed Types	Type of analysis					
	Membrane injury index (MII)		Relative growth index (RGI)		Mean tolerance index (MTI)	
	Chilling	*Salinity*	*Chilling*	*Salinity*	*Chilling*	*Salinity*
Vum-G	50.00 ± 2.13	62.00 ± 2.51	146.93 ± 2.06	106.61 ± 7.66	130.30 ± 6.78	63.04 ± 2.42
Vum-Y	57.00 ± 3.51	66.00 ± 4.58	108.82 ± 4.27	82.65 ± 3.88	128.26 ± 7.30	65.21 ± 1.84
Vum-R	53.00 ± 1.00	69.00 ± 0.89	132.30 ± 4.80	95.38 ± 2.58	115.21 ± 5.28	67.39 ± 2.15
Vum-B	64.00 ± 2.64	69.00 ± 2.31	124.46 ± 3.14	98.93 ± 1.21	171.73 ± 1.79	60.60 ± 2.25

(Values are mean ± SE of 3 replicates)

Fig-1.

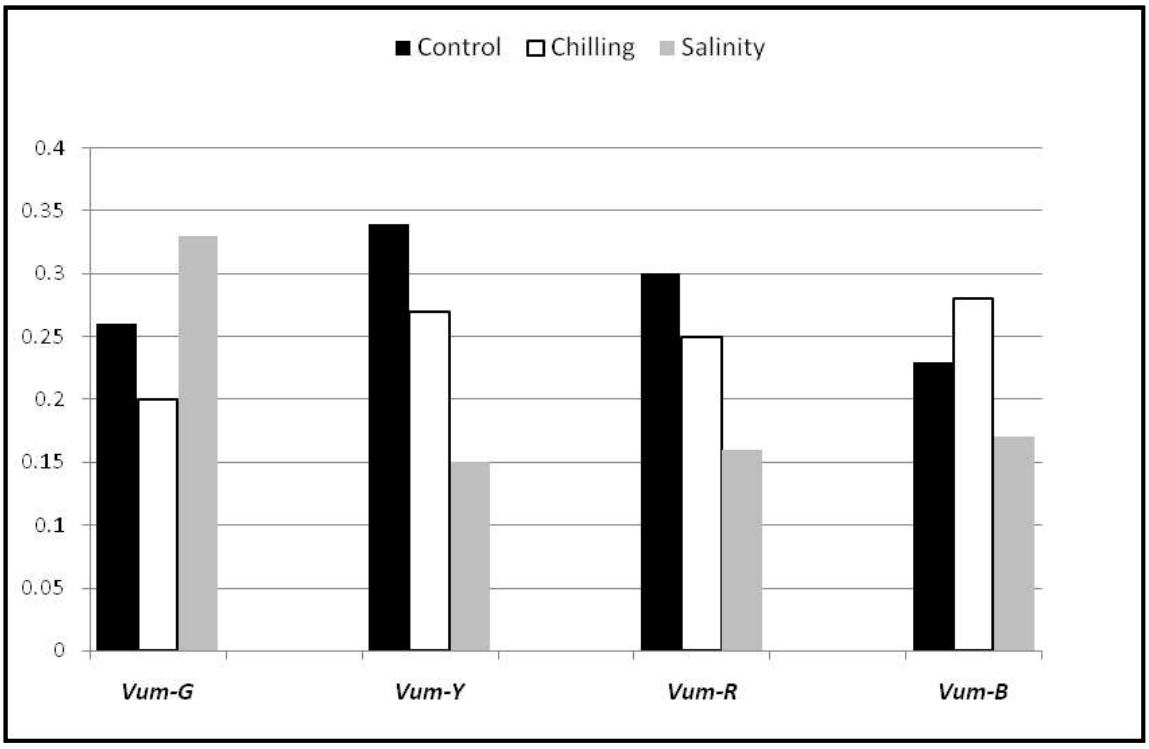
Effect of seed treatment with salinity (100 µM NaCl) and chilling stress (48 hrs at 4°C) on Peroxidase activity (units/minute/g fresh wt) of four *Vigna umbellata* T. (Vum-G=grey, Vum-Y=yellow, Vum-R=red and Vum-B=black) cultivars



Catalase is another radical scavenging enzyme which showed decreased activity under chilling stress conditions except in case of Vum-B where the activity was increased. Similarly, under NaCl imposed stress conditions, the activity of the enzyme declined except in case of Vum-G (Fig-2).

Fig-2.

Effect of seed treatment with salinity (100 µM NaCl) and chilling stress (48 hrs at 4°C) on Catalase activity (units/minute/g fresh wt) of four *Vigna umbellata T.* (Vum-G=grey, Vum-Y=yellow, Vum-R=red and Vum-B=black) cultivars

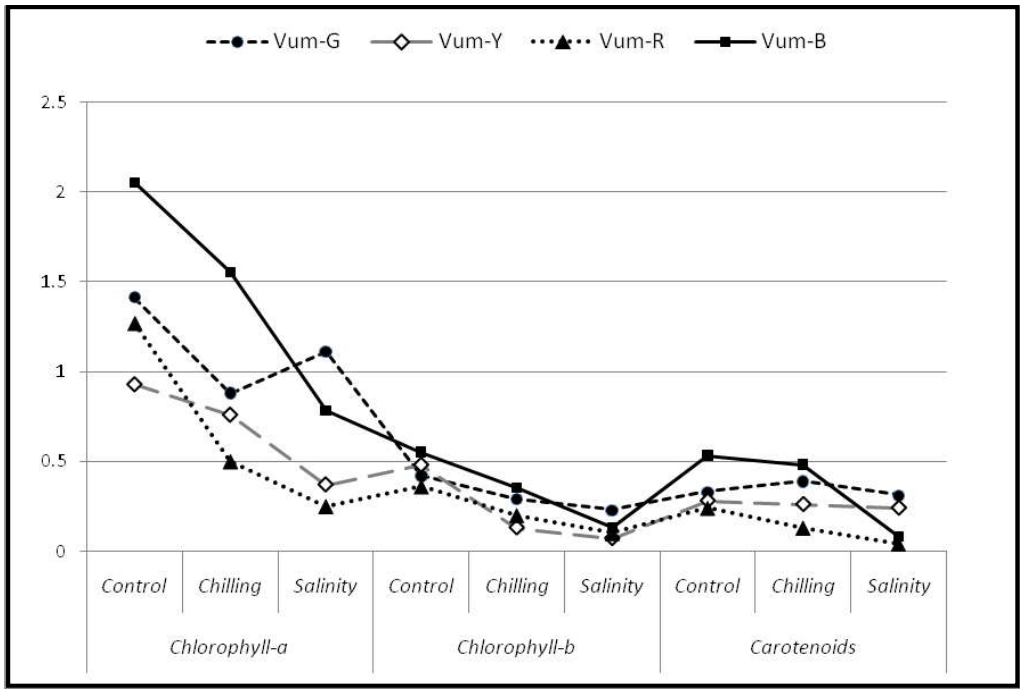


Photosynthetic pigments like chlorophyll-a, chlorophyll-b and carotenoids in the leaves of 14 day old *V. umbellata* cultivars showed decline under chilling and salinity treatments. Almost similar trend of decline was observed in all the four cultivars of the plant when subjected to stress conditions. Resistance to decline in chlorophyll-a under cold stress was more prominent in Vum-B and the same against salinity was most pronounced in Vum-G, otherwise, the change in photosynthetic pigments did not show any trend (Fig-3).

The study presented here showed that chilling and salinity stress conditions rapidly disrupted the physiological and biochemical activities of Vigna umbellata seedlings even in a short spell of one week's time. The accumulation of protein, proline and sugars was a cellular response against stress conditions. The loss of membrane integrity resulted in increased membrane lipid peroxidation and membrane injury. Accumulation of free radicals had toxic effects on the protein nature of the scavenging enzymes. All this resulted in the metabolic dysfunction and reduction in growth of the plants under stress conditions. The extent of stress injury and its physiological manifestations could easily be understood by the study of protein, proline, sugar and MDA accumulation along with the study of CAT, POX, MII, RGI, MTI etc. Of the V. umbellata studied it could be safely concluded that Vum-B was most cold tolerant and Vum-G was the most salt tolerant cultivars.

Fig-3.

Effect of seed treatment with salinity (100 µM NaCl) and chilling stress (48 hrs at 4°C) on photosynthetic pigments: chlorophyll-a, chlorophyll-b and carotenoids (mg/g fresh wt) of four cultivars of *Vigna umbellata T.* (Vum-G=grey, Vum-Y=yellow, Vum-R=red and Vum-B=black).



ACKNOWLEDGMENT

We are thankful to the Principal, Darjeeling Government college, Darjeeling for prroviding laboratory facilities.

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PREVALENCE OF FOOD ALLERGY IN CONSECUTIVE PATIENTS BASED ON SKIN PRICK TEST & IMMUNODIFFUSION TECHNIQUE

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ABSTRACT

Detection and diagnosis of the offending food allergens were undertaken in Jabalpur. Skin prick testing is usually the first test recommended and also immunodiffusion technique was tested. When an allergy is suspected and the estimation of protein was done from food samples by Lowry's method. During this study all 5 food allergens were tested. Analysis shows that out of 5 food allergen, all patients with allergic disorder are showing allerginisity to all food allergen and showed maximum positive SPT towards penut that is 42.10% and detected as positive immunodiffusion reaction in which 44.44% of allergic patient are sensitive towards peanut.

Key words- Allergy, Allergens, Immunity, Hypersensitivity.

INTRODUCTION

Allergy is a clinical expression of atopic disease, including asthma, rhinitis, eczema and food allergy (Stephen *et al*. 2008). Allergy can occur in large number of condition. Allergic state includes hay fever, bronchial asthma, rhinitis and many forms of dermatology in conditions such as urticaria pruritis and eczema.

Food allergies have increased significantly in the past decade. Food allergy is defined as an immune system-mediated adverse reaction to food. Although any food can cause an allergic reaction, which account approximately 90% of reactions in individuals. Food allergy determine by skin prick test and immunodiffusion technique. Food protein estimated also done during this study by Lowry's method.

MATERIAL AND METHODS

Skin Prick Test (Shivpuri, 1974)

Skin prick test (Shivpuri, 1974) is usually the first test recommended when an allergy is suspected. This test measures specific IgE antibody attached to cells in the skin important in allergies called "mast" cells.

Detection and diagnosis of the offending allergens were undertaken in collaboration with Dr. Sandeep Jain consultant Jabalpur Hospital and Prasann ENT and Allergy Clinic situated at Nagar Nigam road, Marhatal Jabalpur.

The skin prick test is usually carried out on the inner forearm, as 3 or 4 or up to about 20 allergens can be tested. The arm is coded with a marker pen for the allergens to be tested. A drop of the allergen (extract) solution is placed by each code. The skin is then pricked through the drop using the tip of a lancet. The size of the wheal varies with the average being 3-5 mm in diameter eg.

+ve histamine buffer sample =5mm.
-ve phosphate buffer sample =3mm.

Usually reactions of 2+to4+were obtained to be significant.

Protein estimation:-

Estimation of food protein was done by Lowery's method (Lowery et al. 1951). Protein concentration of all food samples was compared with the standard curve of bovine serum albumin (BSA).

Immunodiffusion reaction:-

The fungal antibodies test for the qualitative screening test for the detection of precipitating antibodies in patient sera. The type and number of band formed are helpful in interpreting result of other serological test. An indicative test is presumptive evidence of current or different infection.

RESULT AND DISCUSSION

Food allergy is defined as an adverse immune response to food-protein. The development of allergies to food-proteins also depends on the structure of the protein, does of the antigen, and the genetic susceptibility of the host. Typical diets contain tens of thousands of different proteins, and efforts to understand the unique physiochemical and molecular properties of food- allergens are ongoing. (Breiteneder, 2005; Hausen, 2008; Lehrer and Bannon, 2005).

The present study based on the relationship between food protein and clinical incidence of food-allergy. This study showed protein concentration of five food product which are currently known as a severe food allergen. These are peanut, kabulichana, cashew nut, milk and lemon during this study at was found that peanut cause maximum allergenicity as well as it contains maximum protein that is 424µg / µl. after that kabulichana 412 µg / µl, cashew nut 392µg / µl, milk 215µg / µl and lemon 150 µg / µl. (Verma et al. 2010), reported that protein concentration and peanut as 424µg / µl. **(Table-2)**

Savage *et al*. (2007) reported that allergies to peanut in older children or adults are thought to continue through out life. Total five food allergen was tested in present work and all were obtain from all cure pharma pvt. Ltd. Bahadurgarh, haryana. Out of five food allergen peanut showed maximum allergenicity that is 42.10% with 2+ to 4+ reaction. Jennifer *et al*. (2009) reported that among 918 children, 29.7% had positive SPT at least one food. Among 1200 adults, 21.9% were sensitized to at least one food. Overall the most common sensitizing food allergens were shellfish (16.7) and peanut (12.3%). After peanut food allergen lemon and cashew nut showed the maximum allergenicity that is respectively 26.32% and 13.6% in 2+ to 4+ reactions. Apart from this Davoren and Peake (2005) also reported that anaphylaxis to cashew nut was more common than to peanut (74.1%, 30.5%) and children with cashew allergy are at risk of anaphylaxis. **(Table-1)**

The prevalence of food allergy is around 5-8% in children (Van et al. 2006) and 1.2% in adult (Buttriss, 2002 and Sicherer *et al*. 2001). During the present study it was reported that the adult age people which are from 21-40 year age group are showing more allergenicity (50%) to food- allergen. Patriarca *et al*. in (2006) reported the case of a 38-year-old woman with a 10-year history of abdominal symptoms after eating peanuts and mild-to-moderate sensitization. About 30% patients are sensitive to food-allergen there belong to extreme age group. The adolescent group that is 0-28 is showing less allergenicity (20%) towards food-allergen. Clark *et al*. (2009) described 4 children (aged 9-13 years) with varying degrees of peanut allergy.

Bock et al. (2001) reported on 63 such fatalities. They documented that these happen to male (56%) more than females (44%) and 75% and 86% of the victim had known asthma or history of prior reaction, respectively. Similarly in present study out of 20 allergic patients 55% allergic patients are male and 45% allergic patients are female.**(Table-3,4)**

Allergic-rhinitis is one of the most frequent systemic inflammatory diseases observed in children. Its prevalence is closely related with age, gender and life style. (Kilpelainen et al. 2006 and Gallagher *et a*l. 1997) In this present investigation patients are mainly suffering from 3- allergic disorder that are allergic- rhinitis, urticaria problem and asthma. In total allergic patients 50% patients are suffering from allergic rhinitis, 45% patients have urticaria problem and 5% allergic patients are asthmatic. **(Table-5)**

Immunodiffusion technique is a serological method which is based on the principle of precipitation. The result of present study showed that the serum of almost all patients with allergic disorder are showing precipitation to food allergen and detected as positive reaction in which 44.44% of allergic patient are sensitive towards peanut and 22.22% of allergic patient are detected to be are allergic to lemon. Tariq et al. (1996) estimated the prevalence of sensitization to peanuts is quoted at 1.3% and found that this sensitization can occur at very young age and usually life long. Similarly Baars and Savelkoul (2008) also studied that Citrus/Cydonia comp. can potentially restore the disturbed immune state of rhinitis patients, which essentially could be sufficient to make allergic symptoms disappear permanently. Third maximum allergenicity showed by cashewnut that is 14.82% in allergenic patient.**(Table-6)**

TABLE -1

SKIN PRICK TEST AMONG ALLERGIC PAITENTS TO 5 DOMINATING FOOD ANTIGENS

FOOD ANTIGENS	NO. OF PAITENTS SHOWING POSITIVE REACTIONS				TOTAL NO OF 1+ TO 4+ REACTIONS		TOTAL NO OF 2+ TO 4+ REACTIONS		TOTAL NO OF 3+ TO 4+ REACTIONS	
	+1	+2	+3	+4	NO.	%	NO.	%	NO.	%
CASHEWNUT	-	1	4	-	5	12.83	5	13.16	4	11.43
KABULICHANA	-	-	3	-	3	7.69	3	7.89	3	8.57
LEMON	1	-	10	-	11	28.2	10	26.32	10	28.58
MILK	-	1	3	-	4	10.25	4	10.53	3	8.57
PEANUT	-	1	15	-	16	41.03	16	42.10	15	42.85

+Ve Histamine buffer Sample -5mm
- Ve Phosphate buffer Sample -3mm
Then 5mm-3mm =2mm

Reaction	Symbol	Prick test
-	-	No. of W.F. or less then 1mm diameter.
+1	+	W.F.,2mm or More then 2mm
+2	+ +	W.F. 4mm or More than 4mm.
+3	+ + +	W ,F, 4mm-6mm.
+4	+ + + +	W.F. More than 6mm.

TABLE-2

SKIN PRICK TEST AMONG ALLERGIC PAITENTS TO 5 DOMINATING FOOD ANTIGENS

S. No.	Food Sample	Protein concentration µg/ml
1	Cashew nut	392
2	Kabulichana	412
3	Lemon	150
4	Milk	215
5	Peanut	424
6	Standard BSA	0.345

TABLE- 3
AGE, SEX AND ALLERGIC INCIDENCE AMONG PATIENTS

S. No.	Age groups of patients	Allergic male		Allergic female		Total no. of both sex	Percentage
		No.	%	No.	%		
1	0-20	2	18.19	2	22.22	4	20
2	21-40	5	45.45	5	55.56	10	50
3	41-60	4	36.36	2	22.22	6	30
	Total	11	100	9	100	20	100

TABLE- 4
AGE AND ALLERGIC INCIDENCE AMONG PATIENTS

S. No.	Age groups of patients	No. of patients suffering from allergy	Percentage %
1	0-20	4	20
2	21-40	10	50
3	41-60	6	30

TABLE NO - 5
QUANTITATIVE EVOLUTION AND PERCENTAGE DISTRIBUTION OF ALLERGIC DISORDER

S No.	Symptoms	No. of Cases	Percentage
1.	Allergic rhinitis	10	50
2.	Urticaria	9	45
3.	Asthma	1	5
	Total	20	100

TABLE NO. - 6
ALLERGIC PATIENT SHOIWNG POSITIVE IMMUNODIFFUSION REACTION AGAINST FOOD ANTIGEN

S.NO.	FOOD ALLERGEN	0-20	21-40	41-60	TOTAL	PERCENT
1	CASHEWNUT	1	2	1	4	14.82
2	KABULICHANA	0	1	2	3	11.12
3	LEMON	3	2	1	6	22.22
4	MILK	0	2	0	2	7.40
5	PEANUT	2	6	4	12	44.44
					27	100

The food protein triggering the allergic response is termed as food allergen that causes food allergy. Almost 12 million people have food allergies annually and the prevalence is rising in the contemporary world. Food contains various nutrients. Proteins are one of the most important nutrients. It is required for many functions in the body. In view of these finding, it is concluded that in Jabalpur area allergic patients are more susceptible for peanut than other food allergen.

ACKNOWLEDGMENT

We are thankful to, the Head, Department of Biological Sciences, R.D. University, Jabalpur, for giving moral support and lab. facilities.

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PHYTOPHARMACOLOGICAL SCREENING AND EVALUATION OF POTENTIAL ANTIMICROBIAL ACTIVITY OF *ACACIA CATECHU* WILLD.

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ABSTRACT

Acacia catechu Willd. member of family Fabaceae was screened for their phytochemical contents. Aqueous, ether, acetone and methanol extract of fresh leaves and bark were used for phytochemical screening. Prilimnary phytochemical screening showed that extracts contains alkaloids, tannins, flavonoids, proteins, saponins, carbohydrate and lipids. Antimicrobial activity of aqueous and methanol extract of bark were investigated by Disc Diffusion Method against three bacterial spp. *Stapylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* and three fungal spp. *Aspergillus niger*, *Alternaria alternata* and *Fusarium oxysporum*.This study depicts that methanol extract of bark was more effective. Metahnolic extract of bark show greater antibacterial activity against S*taphylococcus aureus* and greater antifungal activity against *Fusarium oxysporum*.

Key Words: *Acacia catechu,* Willd.., Phytochemical analysis, antimicrobial activity

INTRODUCTION

Plant compounds are of interest as a source of safer or more effective substitutes than synthetically produced antimicrobial agents. Phytochemical progress has been aided enormously by the development of rapid and accurate methods of screening plants for particular chemicals. The drugs contained in medicinal plants are known as active principles. The active principles are divided chemically into a number of groups among which are alkaloids, volatile essential oils, phenols and phenolic glycosides, resins, oleosins, steroids, tannins and terpenes (Mitcher *et al*., 1988; Habtermariam, 1993).

Acacia catechu Willd. (family: Fabaceae, sub family: Mimosiasae) commonly known as Katha or Karangali is widely used in India for its various pharmacological effects (Ismail and Asad, 2009). The chief phytochemical constituents are catechin and epicatchin (Goodwin and Mercer, 1972). The catechins have significant anti-toxicant and antimicrobial properties (Machado. *et al*., 2003). This plant material (bark) is used as anodyne, astringent, bactericide, refrigerant, stimulant, styptic, masticatory expectorant and antiphlogistic (Pingale, 2010).The bark extract of *Acacia catechu* is used in melancholia, conjunctivits, haemaptysis, catarrah, cough, pruritus, leprosy, leucoderma, skin diseases, helminthiasis, norexia, diarrhea, dysentery, foul ulcers, wounds, haemoptysis, haematemesis, haemorrages, fever, anaemia, diabetes and pharyngodynia. The objective of this study is to screen the phytochemical and antimicrobial activity of leaves and bark of *Acacia catechu* Willd.

MATERIAL AND METHODS

Preparation of Plant Extract

Fresh leaves and bark of *Acacia catechu* Willd.were collected from Dumna nature reserve, Jabalpur,collected material was washed thorouughly in running tap water, rinsed in distilled water and shade dried in open air. 8 gm of leaves and bark were taken in pestle & mortor with 15 ml distilled water and solvents i.e. ether, acetone and methanol. The material was crushed with distill water and solvents. The crude extract was filtered. The filtrate and crude extract is used for phytochemical screening and antibacterial activity studies.

Phytochemical Screening

Phytochemical screening procedures carried out were adapted from standard procedures to indentify the amino

acids and Phytochemical constituents as described by Edeoga *et al*. (2005); Sofowara (1993); Trease and Evans (1996) and Harborne (1973).

Test organism

Pure cultures of bacterial isolates *Stapylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* and pure culture of fungal isolates *Aspergillus niger*, *Alternaria alternata* and *Fusarium oxysporum* were collected from the Aeroallergens, Immunology and Angiosperm's Diversity Lab., Department of Biological Science, Rani Durgawati University, Jabalpur.

Antimicrobial Activity

Antimicrobial activity was carried according the standard procedures as described by Bauer *et al*. 1966.

RESULT

Table no.1 and 2 showed phytochemical screening of leaves and bark of *Acacia catechu* Willd. Phytochemical screening revealed that leaves and bark extract contains alkaloids, tannins, saponins, carbohydrates, lipids, proteins etc. **(Table 1,2)**

Table-No.1: Phytochemical analysis of *Acacia catechu* Willd. leaves

S.No.		TEST	AQUEOUS	ACETONE	METHANOL	ETHER
1		ALKALOIDS				
	A	Mayer's test	_	_	_	_
	B	Dragendroff's test	+	+	+	+
	C	Wagner's test	+	+	+	+
2		CARBOHYDRATE				
	A	Benedict test	_	_	+	_
	B	Fehling test	+	+	+	_
3		SAPONINS				
	A	Foam test	+	+	+	+
4		PROTEINS				
	A	Xanthoprotein test	_	_	_	_
	B	Biuret test	_	_	+	_
5		FLAVANOIDS				
	A	Flavanoids test	_	+	+	_
6		TANNINS				
	A	Ferric chloride test	_	_	+	+
	B	Lead acetate test	_		+	_
7		RESIN				
	A	Resin test	+	_	_	_
8		STEROL				
	A	Salkowaski test	+	_	+	+
9		LIPID				
	A	Glycerol test	_	_	+	_

Table-No.2: Phytochemical analysis of *Acacia catechu* Willd. Barks

S.No.		TEST	AQUEOUS	ACETONE	METHANOL	ETHER
1		ALKALOIDS				
	a	Mayer's test	_	_	_	_
	b	Dragendroff's test	+	+	+	+
	c	Wagner's test	+	+	+	+
2		CARBOHYDRATE				
	a	Benedict test	_	_	_	_
	b	Fehling test	_	_	_	_
					_	_
3		SAPONINS				
	a	Foam test	+	_	+	+
4		PROTEINS				
	a	Xanthoprotein test	_	_	_	_
	b	Biuret test	_	_	+	_
5		FLAVANOIDS				
	a	Flavanoids test	_	+	+	+
6		TANNINS				
	a	Ferric chloride test	_	_	+	+
	b	Lead acetate test	_		+	_
7		RESIN				
	a	Resin test	+	_	_	_
8		STEROL				
	a	Salkowaski test	+	_	+	+
9		LIPID				
	a	Glycerol test	_	_	+	+

Table no. 3 shows the results of the antibacterial activity showed by bark of *Acacia catechu* Willd.The result revealed that methanol extract of bark gave positive against these three bacteria. Staphylococcus aureus gave inhibition zone 1.5 cm in diameter for methanolic extract.

Table-No.3: Antibacterial activity of *Acacia catechu*, Willd.

S.No.	Plant Species	Bacterial Species	Inhibition Zone Diameter(cm)	
			Methanol	Water
1	*Acacia catechu*	*Bacillus subtillus*	0.8 cm	0.15 cm
		Pseudomonas aeruginosa	1.3 cm	0.25 cm
		Staphylococcus aureus	1.5 cm	0.5 cm

R= resistance

Table no.4 shows the results of the antifungal activity showed by bark of *Acacia catechu* Willd. The methanolic extract of *Acacia catechu* showed higher inhibition zone as compared to aqueous extract. Methanolic extract of bark showed greater antifungal activity against *Fusarium oxysporum*.

Table-No.4: Antifungal activity of *Acacia catechu*, Willd.

Species	Zone of inhibition(diameter-cm)	
	Leaf	Bark
METHANOL EXTRACT		
Aspergillus niger	0.3 cm	0.8 cm
Fusarium osysporium	0.4 cm	1.2 cm
Alternaria alternate	0.3 cm	0.7 cm
AQUEOUS EXTRACT		
Aspergillus niger	0.2 cm	R
Fusarium osysporium	0.5 cm	0.8 cm
Alternaria alternata	R	R

R= resistance

DISCUSSION

Alkaloids are found in aqueous, methanolic, acetone and ether extracts with positive Dragendroff's and Wagner's reagent test. Presence of saponins is found in bark extract. Flavonoids present in acetone and methanol extract. Tannins present in acetone solvents. Lipids present in aqueous, methanolic and acetone extracts.

Similar results were also found during phytochemical investigation of the extracts which contained some phytoconstituents. Saponins, Tannins, Alkaloids, Flavonoids, Resins, Proteins, Lipids, Carbohydrates and Sterols (Verma *et al*. 2010).

The aqueous and methanol extract of bark and leaves of *Acacia catechu* shows antibacterial activities towards *Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa*. The methanol extract of bark of *Acacia catechu* showed greater *anti-bacterial* activitie against *Staphylococcus aureus*.

The composition of *Acacia catechu* extract had shown major components of terpene i.e. camphor (76.40%) and phytol (27.56%) along with other terpenes in minor amounts which are related with their high antibacterial and antifungal properties (Negi and daue 2011).

The methanolic extract of bark extract of *Acacia catechu* shows antifungal activity towards fungi - *Aspergillus niger, Fusarium oxysporum, Alternaria alternata*. Metahnolic extract of bark show greater antifungal activity against *Fusarium oxysporum*.

Similar results showed by Nagaraja *et al.* (2008) about the evaluation of anti-mycotic activity of *Acacia catechu* Willd.

Previously it was reported that bark and whole plant behaves as antimicrobial agents.(Brahmachari*et al*. 2006)

Catechin, rutin and isorhamnetin are reported as free radical scavengers and these compounds largely contribute to the biopotency of *Acacia catechu* Willd. Hence it is highly effective antioxidant.(Devi, 2011).

CONCLUSION

We conclude that the extract of the plant tested for phytochemical screening and Antimicrobial activity. The extract contains the active principles - terpenoids, tannins, alkaloids, saponins and glycosides. Methanolic bark extract of Acacia catechu Willd. produced antimicrobial activity against bacterial spp. *Stapylococcus aureus, Bacillus subtilis* and *Pseudomonas aeruginosa* and fungal spp. *Aspergillus niger*, *Alternaria alternata* and *Fusarium oxysporum*. This study observes that *Acacia catechu* has useful antimicrobial properties. It provide an ample opportunity to plant based drugs due to their considerable role in the ethnomedicine, most effectiveness against various microbial pathogens and their significant phytoconstiuents.

ACKNOWLEDGEMENT

We express our deep thanks to Dr. P. K. Singhal, Professor & Head, Department of Biological Science, Rani Durgawati University, Jabalpur (M.P). for his support and guidence.

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HERBACEOUS TAXA AND SOME EDAPHIC ATTRIBUTES OF DHARA CATCHMENT.

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ABSTRACT

Biological diversity is an indicator of the health of a particular habitat/ biogeographic area and its potential to sustain life. The study area forms an important phytogeographical region in the North Western Himalaya.

The present study was conducted to investigate the comparative assessment of edaphic factors and herbaceous vegetation on April - June. Species were studied by randomly laying 50 quadrates of 1×1 m2 size. The vegetation data recorded was quantitatively analyzed for frequency, density and abundance. The results indicated edaphic factors like Soil temperature, moisture content, pH, and organic carbon were observed highest at lower zone. The study reveals that study area is in much degraded stage and it needs management and conservation to protect Dhara catchment forest.

KEY WORDS: Edaphic attributes, Frequency, Density and Abundance.

INTRODUCTION:

The earth is home to a rich and diverse array of living organism, whose genetic diversity and relationships with each other and with their physical environment constitute our plant's biodiversity. The biodiversity is the natural biological capital of the earth and presents important opportunities for all nations (Kumar and Bhatt, 2006).

Forests are one of the most valuable ecosystems in the world containing over 60% of the world's biodiversity. This biodiversity has multiple social and economic values, apart from its intrinsic value, varying from the important ecological functions of forests in terms of soil and watershed protection to the economic value of the numerous products, which can be extracted from the forests. Forests play a crucial role in climate regulation and constitute one of the major carbon sinks on earth (Misri and Sindhu, 2004), preventing increase of green house effect.

Besides playing a vital role in the general economy of the country, they also act as valuable resources for providing subsistence to rural people (Swindel *et al.*, 1984). Further, protective influence of forests on habitat not only immediately under their canopy but also far considerable distances around has been generally accepted (Dhar *et al.*, 1988).

The Dhara catchment of world's famous Dal Lake falls in the north of Kashmir valley in the Jammu and Kashmir state. The area covering Faqir Gojar, Tulpatnar, Cheki-Dhara and Naganar micro watersheds extends from 3402'50" to 34014'7" N latitude, 74050'0" and 7508'35" E longitude. The area extending over an area of 21467 hectares is one of the badly deforested areas of the valley.

MATERIAL AND METHOD

Vegetation analysis: The study was conducted during summer (April-June, 2011). Dhara catchment was divided into three zones i.e. upper, middle and lower at 2640-2760, 2520-2640 and 2400-2520 msl respectively. Fifty quadrates of 1m2 size were laid randomly in these

three zones and herbaceous vegetation was studied in terms of frequency, density and abundance were calculated using by following formula-

$$\text{Frequency} = \frac{\text{Number of quadrate in which species occurred}}{\text{Total number of quadrate studied}} \times 100$$

$$\text{Density} = \frac{\text{Total number of individual species}}{\text{Total number of quadrate studied}}$$

$$\text{Abundance} = \frac{\text{Total number of individual species}}{\text{Total number of quadrate in which species occurred}}$$

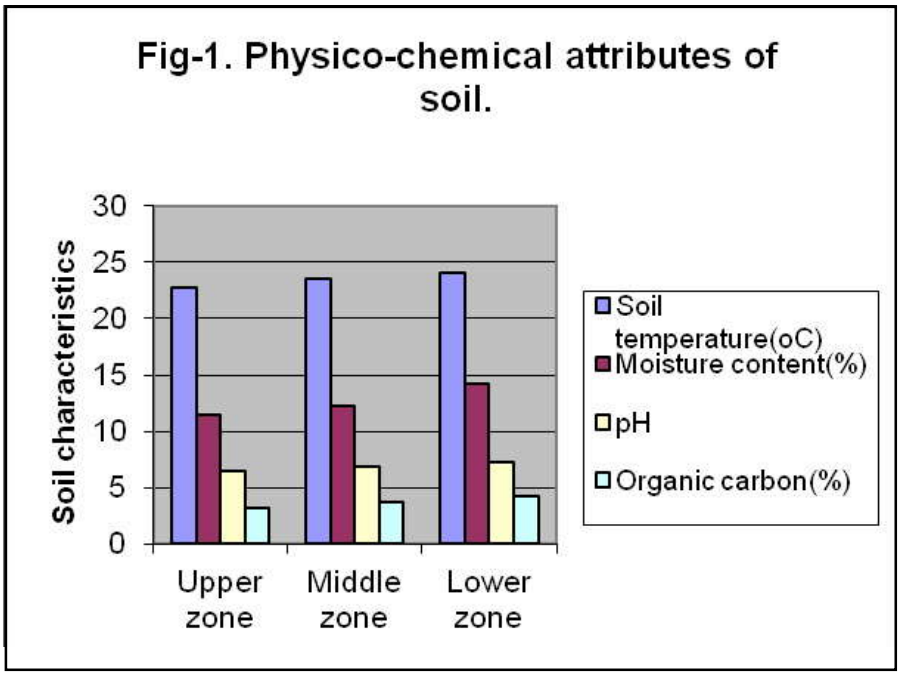
RESULTS AND DISCUSSION

Table-1. Frequency, density and abundance of herbaceous plants species at different zones.

S. No.	Species name	Zone	F (%)	D(m^{-2})	Abundance
1.	*Artemisia scoparia*	Upper	20	0.4	1.4
2.	*Artemisia absinthium*	Upper	20	0.2	1.2
3.	*Thalictrum foliosum*	Upper	20	0.3	1.2
4.	*Rumex acetosa*	Upper	20	0.2	0.9
5.	*Nepeta clarkei*	Upper	20	1.3	1.2
6.	*Astragalus candolleanus*	Upper	10	0.1	0.2
7.	*Chenopodium album*	Upper	10	0.3	0.8
8.	*Lepchinnella microcarpa*	Upper	10	0.2	0.8
9.	*Nepeta eriostachya*	Upper	10	0.1	0.8
10.	*Stachys floccose*	Upper	30	0.6	2.6
11.	*Chenopodium vulgare*	Upper	30	1.5	2.0
12.	*Taraxacum officinale*	Upper	10	0.2	0.8
13.	*Marrubium vulgare*	Upper and middle	50	2.2	3.0
14.	*Morina longifolia*	Upper and middle	30	1.8	1.6
15.	*Bistorta amplexlcautis*	Middle	10	0.2	0.7
16.	*Plantago lanceolata*	Middle	10	0.2	0.6
17.	*Geranium pamiricum*	Middle	10	0.2	0.6
18.	*Astragalus candolleanus*	Middle	10	0.2	0.7
19.	*Chenopodium album*	Middle	10	0.2	0.6
20.	*Nepeta eriostachya*	Middle	10	0.2	0.5
21.	*Lychnisapetala*	Middle	10	0.2	0.7
22.	*Morina longifolia*	Middle	10	0.1	0.6
23.	*Stachys floccose*	Middle	10	0.4	0.9
24.	*Taraxacum officinale*	Lower	30	0.9	1.7
25.	*Nepeta eriostachya*	Lower	10	0.3	0.7
26.	*Stachys floccose*	Lower	40	1.2	1.9
27.	*Fragaria nubicola*	Lower	40	1.3	2.0
28.	*Astragalus candolleanus*	Lower	10	0.2	0.6

Table-2. Physico-chemical attributes of soil at different zones.

S. No.	Parameters	Upper zone	Middle zone	Lower zone
1.	Soil temperature (°C)	22.7	23.5	24.1
2.	Moisture content (%)	11.4	12.3	14.2
3.	pH	6.5	6.9	7.2
4.	Organic carbon (%)	3.2	3.7	4,2



The present study on herbaceous taxa and some edaphic attributes of Dhara catchment" was undertaken with the main objective to assess vegetation quality and status of soil. The findings of study are summarized as under:

Frequency : In Dhara catchment high frequency 30% was recorded in S*tachys floccose, Chenopodium vulgare* and 20% was obtained for *Artemisia scoparia, Artemisia absinthium, Thalictrum foliosum, Rumex acetosa, Nepeta clarkei* and low frequency was observed 10% for *Astragalus candolleanus, Chenopodium album, Lepchinnella microcarpa, Nepeta eriostachya, Taraxacum officinale* in upper zone. At upper and middle zone highest frequency was observed 50% for *Marrubium vulgare* and 30% was recorded in *Morina longifolia.* At middle zone frequency was observed 10% for *Bistorta amplexlcautis, Plantago lanceolata, Geranium pamiricum, Astragalus candolleanus, Chenopodium album, Nepeta eriostachya, Lychnisapetala, Morina longifolia, Stachys floccose.* Thus at lower zone high frequency was recorded 40% for *Stachys floccose, Fragaria nubicola* and 30% was recorded for *Taraxacum officinale* and low frequency was recorded 10% for *Nepeta eriostachya, Astragalus candolleanus.* **(Table-1)**

Density: In Dhara catchment high density 1.5 m-2 was recorded for *Chenopodium vulgare*, 1.3m-2 for *Nepeta clarkei*, 0.4 m-2 for *Artemisia scoparia* and 0.3 m-2 for *Stachys floccose*, 0.2 m-2 was recorded for *Artemisia absinthium, Rumex acetosa, Lepchinnella microcarpa, Taraxacum officinale* in upper zone. At upper and middle zone density was observed 2.2 m-2 for Marrubium vulgare and 1.8 m-2 for *Morina longifolia*. At middle zone high density was observed 0.4 m-2 for *Stachys floccose*, 0.2 m-2 for

Bistorta amplexlcautis, Plantago lanceolata, Geranium pamiricum, Astragalus candolleanus, Chenopodium album, Nepeta eriostachya, Lychnisapetala and low density was recorded 0.1 m-2 for *Morina longifolia*,. Thus at lower zone high density was recorded 1.3 m-2 for *Fragaria nubicola,* 1.2 m-2 for *Stachys floccose,* 0.9 m-2 for *Taraxacum officinale*, 0.3 m-2 for *Fragaria nubicola* and lowest density 0.2 m-2 was recorded for *Astragalus candolleanus*. **(Table-1)**

Abundance: In Dhara catchment high abundance 2.6 was recorded for *Stachys floccose*, 2.0 for *Chenopodium vulgare*, 1.4 for *Artemisia scoparia,* 1.2 for *Artemisia absinthium, Thalictrum foliosum, Nepeta clarkei*, 0.9 for *Rumex acetosa*, 0.8 for *Chenopodium album, Lepchinnella microcarpa, Nepeta eriostachya, Taraxacum officinale* and low abundance 0.2 for *Taraxacum officinale* in upper zone. At upper and middle zone abundance was observed 3.0 for *Marrubium vulgare* and 1.6 for *Morina longifolia.* At middle zone high density was observed 0.9 for *Stachys floccose*, 0.7 for *Bistorta amplexlcautis, Astragalus candolleanus, Lychnisapetala*, 0.6 for *Plantago lanceolata, Geranium pamiricum, Chenopodium album, Morina longifolia*, 0.5 for *Nepeta eriostachya.* Thus at lower zone high abundance was recorded 2.0 for *Fragaria nubicola*, 1.9 for Stachys floccosa, 1.7 for *Taraxacum officinale*, 0.7 for *Nepeta eriostachya* and lowest density 0.6 was recorded for *Astragalus candolleanus*. **(Table-1)**

Chemical attributes of soil: Soil temperature, moisture content, pH and organic carbon were observed minimum in upper zone (22.7, 11.4, 6.5 and 3.2 respectively) while in the lower zone all these parameters were recorded highest i.e. 24.1, 14.2, 7.2 and 4.2 respectively.**(Table-2, Fig.-1)**

CONCLUSION

Twenty eight species were found in studied area. The highest frequency, density and abundance were observed for *Marrubium vulgare* i.e. 50%, 2.2 and 3.0 respectively in upper and middle zone. The study reveals that study area is in much degraded stage and it needs management and conservation to protect Dhara catchment forest.

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