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# Ecological Study of Macroinvertebrate Communities in Three Limnocrene Freshwater Springs of Kashmir Himalaya

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**ABSTRACT**

Benthic macroinvertebrate communities during 2005-2006 were studied in three perennial limnocrene freshwater springs located in single ground water area in Kokergund Yaripora of District Kulgam ( Kashmir).Though most of the invertebrate orders, as well as individual taxa, showed differences in relative abundance between habitat types, yet Tumbernag spring differed from other two springs in sustaining a different assemblage of macroinvertebrates. The macroinveretebrates were most abundant in Khudanag and least abundant in the Tumbernag.However, the taxonomic richness in the investigated springs was highest in Nagrad and lowest in Tumbernag. During the entire study, a total of 19 taxa belonging to Diptera (04), Trichoptera (03), Coleoptera (01), Megaloptera (01), Ephemeroptera (01), Amphipoda (1), Ostracoda (02), Oligochaeta (03), Hirudinae (01) and Mollusca (02) were encountered. Analysis of variance to the data showed that the taxa like *Chironomus* sp., *Limnodrilus* sp., *Tubufex tubifex, Branchiura sowerbyii, Erpobdella octoculata* and *Gammarus pulex* were found in all the four seasons without any significant variation among seasons. Certain taxa like *Rhyacophila* obscura and *Tabanus* sp. were absent in spring season while *Glossosoma* sp. was not recorded in summer season. However, no single taxa was restricted to a particular season. On the other hand, the taxa like *Simulium, Tabanus, Rhyacophila, Limnephilus* and *Glossosoma* were restricted to only one particular biotope. Most studied taxa showed differences in relative abundances between the seasons as well as between the spring biotopes.

***Key words:*** Spring water, ecology, macroinvertebrates, Kashmir Himalaya

## INTRODUCTION

The location of the springs at the interface between several distinct ecosystems creates a heterogeneous mosaic of aquatic, semi-aquatic and semi-terrestrial microhabitats, which has led many ecologists to suggest that springs are hot spots for aquatic biodiversity (Cantonati *et al.*, 2006; Scarsbrook *et al.*, 2007; Staudacher and Füreder, 2007).Habitat heterogeneity is usually higher in helocrene springs where water emerges in a swampy area, medium in rheocrene springs, where water emerges directly into a

stream channel, and lower in limnocrene springs where water emerges into a pool(Lindegaard et al., 1998; Cantonati *et al.*, 2006).A number of reviews have been published in 1990s (Williams *et al.*, 1990; Ferrington, 1995; Botosaneanu, 1998), which has helped to understand the ecology of springs and the importance of some key factors controlling the composition and structure of their biological communities, especially benthic invertebrate assemblages. Classification of spring habitats into ecological typologies is central for spring management, conservation and monitoring (Barquín and Scarsbrook, 2007) and to improve our understanding of spring ecology (Glazier, 1991). Many ecologists have attempted to classify spring habitats using spring physical and chemical attributes (Roca 1990; Glazier, 1991; Hoffsten and Malmqvist, 2000), spring invertebrate communities(Glazier and Gooch, 1987; Roca and Castillo, 1993; Lindegaard *et al.*, 1998;Hahn, 2000; Meyer *et al.*, 2003; Ilmonen and Paasivirta, 2005) or both (Zollhöfer *et al.*, 2000). Our knowledge of spring ecosystems in Kashmir valley lags far behind and only few preliminary reports on spring ecology are available (Qadri and Yousuf, 1979; Rashid, 1982; Yousuf *et al.*, 1983; Qadri and Yousuf, 1988; Bhat and Yousuf, 2002; Latief *et al.*, 2003; Pandit *et al.*, 2001, 2002,2005 a &b,2007) and for this purpose baseline data on macroinvertebrates of three limnocrene springs in Kashmir Himalaya was carried out for formulating any conservation strategy.

## STUDY AREA

The investigated springs are located in Kokergund Yaripora of Kulgam district and fall within the single large groundwater area. These springs are perennial and are situated around 33°44´ N latitude, 075°.01´ E longitude and at an altitude of about 1670±2 m( a.m.s.l. ).These springs are surrounded by large trees comprising of *Populus, Ulmus, Acacia, Jugalanus, Platanus* etc. The springs are devoid of any macrophytic growth. All the three springs namely Nagrad,Tumbernag and Khudanag are alluvial type falling in sixth order classification based on discharge(Meinzer, 1923) with mean annual discharge of 0.401L/s, 0.172L/s and 0.299L/s respectively(Table 1). The area of spring was considered as the area of open water. The pristine naturalness of these springs has diminished because of anthropogenic modification such as concrete embankments for water storage which is used for various domestic purposes such as drinking water, irrigation, washing etc.

**Table1.General characteristics of three limnocrene freshwater springs in Kokergund Yaripora, Kulgam**

| S.No | Parameter | NAGRAD | TUMBERNAG | KHUDANAG |
|---|---|---|---|---|
| 1 | Altitude | 1665m | 1667m | 1667m |
| 2 | Lat. & Long. | 33°44´N;075°.01´E | 33°44´N;075°.01´E | 33°44´N;075°.01´E |
| 3 | Mean annual discharge L/S | 0.401 | 0.172 | 0.299 |
| 4 | Spring order | 6 | 6 | 6 |
| 5 | *Spring type I. (Meinzer, 1923)* | | | |
| | *(a) Hydraulic characteristics* | Gravity-Contact type | Gravity-Contact type | Gravity-Contact type |
| | (b)Topography | Pool type | Pool type | Pool type |
| | (c)Permenance | Perrenial | Perrenial | Perrenial |
| | (d)Character of opening | Filtration type | Filtration type | Filtration type |
| | *Spring type II. Thieneman (1924)* | Limnocrene | Limnocrene | Limnocrene |
| 6 | Spring area(1-5)* | 3 | 1 | 2 |
| 7 | Naturalness(0-3)** | 2 | 2 | 2 |
| 8 | Substrate composition | Pebble, gravel, sand, less organic matter | Gravel with less sand | Gravel, sand, mud, organic matter |

*Classes of spring area are: 1=<5$m^2$, 2= 5-10$m^2$, 3= 10-20$m^2$, 4=20-40$m^2$ and 5= 40-100$m^2$
**Spring naturalness are: 1= severe pressure/ damage from humans in spring or vicinity, 2= minor pressure/ damage in or near the spring, 3= almost or totally undisturbed spring in its surrounding.

## MATERIAL AND METHODS

The field study was carried out during 2005-06 and the benthic samples were taken in summer (June), autumn (September), winter (December) and spring (March) with the help of D-net having 0.2mm mesh size. The organisms were collected while disturbing the substratum by kicking or forcing ahead the net (Hoffsten and Malmqvist, 2000).

**Table 2. Seasonal variations in invertebrates (ind/m²) in three limnocrene freshwater springs**

| Class/Order/ Taxa | Summer (June) | | | Autumn (September) | | | Winter (December) | | | Spring (March_ | | | ANOVA Seasons P Value | ANOVA Siters P Value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Nagrad | Tumber-nag | Khuda-nag | Nagrad | Tumber-nag | Khuda-nag | Nagrad | Tumbernag | Khuda-nag | Nagrad | Tumber-nag | Khuda-nag | | |
| Mollusca | | | | | | | | | | | | | | |
| *Lymnea* sp. | 4 | 0 | 0 | 3 | 0 | 1 | 3 | 0 | 0 | 6 | 0 | 0 | 0.962 | 0.000 |
| *Corbicula* sp. | 3 | 0 | 1 | 4 | 0 | 0 | 4 | 0 | 0 | 8 | 0 | 1 | 0.852 | 0.001 |
| Annelida | | | | | | | | | | | | | | |
| *Branchiura sowerbyii* | 25 | 12 | 280 | 40 | 16 | 456 | 35 | 21 | 396 | 35 | 15 | 180 | 0.921 | 0.000 |
| *Limnodrillus* sp. | 15 | 8 | 300 | 10 | 6 | 280 | 10 | 8 | 130 | 18 | 5 | 300 | 0.000 | 0.952 |
| *Tubifex tubifex* | 150 | 6 | 200 | 140 | 8 | 240 | 70 | 5 | 130 | 180 | 8 | 280 | 0.792 | 0.000 |
| Arthropoda *Erpobdella octoculata* | 20 | 12 | 30 | 32 | 20 | 45 | 8 | 2 | 56 | 25 | 18 | 60 | 0.782 | 0.003 |
| *Gammarus pulex* | 40 | 32 | 200 | 45 | 30 | 180 | 60 | 40 | 250 | 32 | 8 | 120 | 0.860 | 0.000 |
| *Illyocypris* sp. | 10 | | 5 | 15 | 0 | 0 | 5 | 0 | 2 | 3 | 0 | 0 | 0.731 | 0.017 |
| *Cyclocypris* sp. | 15 | 0 | 2 | 10 | 0 | 0 | 6 | 0 | 1 | 1 | 0 | 4 | 0.810 | 0.027 |
| *Baetis rhodani* | 5 | 0 | 6 | 11 | 0 | 16 | 3 | 0 | 15 | 3 | 0 | 10 | 0.752 | 0.003 |
| *Rhyacophila obscura* | 2 | 0 | 0 | 6 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0.686 | 0.037 |
| *Limnephilus* sp. | 0 | 0 | 6 | 0 | 0 | 21 | 0 | 0 | 14 | 0 | 0 | 20 | 0.909 | 0.001 |
| *Glossosoma* sp. | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0.596 | 0.100 |
| *Elmidae* sp. | 0 | 0 | 3 | 2 | 0 | 5 | 2 | 0 | 6 | 0 | 0 | 12 | 0.843 | 0.007 |
| *Corydalus* sp. | 1 | 0 | 2 | 2 | 2 | 1 | 3 | 0 | 2 | 2 | 0 | 0 | 0.633 | 0.127 |
| *Tipula* sp. | 3 | 0 | 2 | 4 | 0 | 5 | 2 | 0 | 2 | 1 | 0 | 2 | 0.532 | 0.014 |
| *Chironomus* sp. | 100 | 30 | 200 | 80 | 25 | 130 | 100 | 125 | 80 | 40 | 15 | 62 | 0.407 | 0.192 |
| *Simulium* sp. | 3 | 0 | 0 | 1 | 0 | 1 | 2 | 0 | 0 | 1 | 0 | 0 | 0.908 | 0.007 |
| *Tabanus* sp. | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0.802 | 0.007 |
| Total | 397 | 100 | 1237 | 406 | 107 | 1384 | 317 | 201 | 187 | 355 | 69 | 1051 | | |

The samples were also taken 5by sweeping the top substrate of half a square meter once or twice ahead of D-net(Ilmonen and Paasivirta, 2005).The organisms were sorted out and the material was sieved through a sieve( mesh size 500μm) and preserved in 4% formalin and 70% alcohol depending upon the type of organisms to be preserved. The soft-bodied organisms were preserved in 70% alcohol while the shelled organisms like molluscs in 4% formalin (Borror *et al.,* 1976). The identification of specimens was done with the help of standard taxonomical works (Edmondson,1959; Borror *et al.,* 1976; Pennak, 1978;Ward,1992;Engblom and Lingdell, 1999; Yildiz and Balik, 2005; Rossetti *et al.,* 2006).

## RESULTS AND DISCUSSION

In all 19 taxa of invertebrates, insect as well as non-insect fauna, belonging to Diptera (04), Trichoptera (03), Coleoptera (01), Megaloptera (01), Ephemeroptera (01), Amphipoda (1), Ostracoda (02), Oligochaeta (03), Hirudinae (01) and Mollusca (02) were recorded from three springs. Nagrad and Khudanag registered more taxa (17 and 16) than Tumbernag (07). Diptera among the insects and Annelida among noninsect fauna were the best represented groups with four and three taxa respectively. *Chironomus* sp., *Limnodrilus* sp. , *Tubifex tubifex, Branchiura sowerbyii, Erpobdella octoculata* and *Gammarus pulex* were found in all the three springs throughout the sampling period without any significant variation among seasons (F-1.09, P-0.407,df-3; F-0.11, P-0.952,df-3; F-0.35.p-0.792,df-3; F-0.16,P-0.921, df-3; F-0.36,P-0.782,df-3; F-0.25, P-0.860,df-3) for the above species respectively. Seasonal distribution of taxa was not prominent as no single taxa was restricted to any particular season.However, *Rhyacophila obscura and Tabanus* sp. were absent in spring season while *Glossosoma* was absent in both the summer and spring seasons. The forms like *Simulium* sp. *Tabanus* sp. and *Rhyacophila* sp. having variance values (F-9.21,p-0.007,df-2; F-9.0,P-O.O7,df-2; F-4.84,P-0.037,df-2) were restricted to Nagrad spring while as *Limnephilus* sp. and Glossosoma sp.(F-19.55,P-0.001,df-2;F-3.0,P-0.100,df-2). were confined to Khudanag spring.

Among the spring invertebrates, *Chironomus* sp, *Tubifex tubifex,Limnodrilus, Erpobdella octoculata, Gammarus pulex* and *Branchiura sowerbyii* were the most dominant forms, being present in all the three springs .However, species like *Simulium* sp., Tabanus sp, *Lymnaea* sp. *Corbicula* sp., *Rhyacophila obscura* and *Glossosoma* sp. were least represented as only few individuals were encountered. Seasonal variation of benthic fauna reveals the maximum growth and abundance of spring invertebrates to be in the autumn at Nagrad(406 ind./$m^2$) and Khudanag (1384 ind/$m^2$) and winter at Tumbernag (201 ind/$m^2$). Among the sites the maximum mean seasonal population was obtained at Khudanag(1156 ind/$m^2$) followed by Nagrad (369 ind/$m^2$) and decreasing to a minimum of 119 ind./$m^2$ at Tumbernag.

All the three limnocrene freshwater springs are located in similar geologic strata and the differences between them in substrate composition are largely the result of local environmental factor, being an element of manipulation as well (Table 1.). These included the minor variations in current speed which affected the fine sediment transport with settlement besides being a source of allochthonous material especially leaf litter

in Nagrad and Khudanag springs. In case of Tumbernag the bed has been modified by the addition of sand which accumulated between and on stones, as a result of which the range of habitats available to benthos was reduced(Marrshall and Winterbourn, 1979). Average number of invertebrate taxa collected per spring was 10 oscillating between 7 and 19 taxa. This number(7-19) is slightly lesser than the average invertebrate taxa collected from springs in the Pyrenees(6-30; Roca and Castillo, 1993) or in karstic regions of Poland(6-25; Dumnicka *et al.* 2007) and Slovenia(8-26; Mori and Brancelj, 2006).Large spring size and high discharge has been shown to increase the invertebrate number of taxa elsewhewre (Cantonati et al. 2006) and we believe this may be the case in our study because Nagrad spring has larger size and higher mean annual discharge than Khudanag and double that of the Tumbernag spring(Table1).Lower invertebrate diversity in spring habitats when compared to similar nearby runoff-fed streams has been reported elsewhere (Ward and Dufford, 1979; Anderson and Anderson, 1995).However, very slightly lower invertebrate diversity(16) in nearby runoff-fed stream(Saeskoon) against 19 in the studied springs has been reported(Bhat and Pandit, 2006).The lower invertebrate diversity in spring habitats of Cantabria, Spain in comparison to nearby runoff-fed streams has been attributed to the result of the intereaction between factors such as glaciation,flow constancy and predation(Barquin and Death, 2004).Predation by amphipods like *Gammarus pulex* may be important factor reducing the invertebrate diversity in the studied springs as has been reported elsewhere(Glazier, 1991; Barquin and Death, 2004;Zollhöefer et al., 2000).

In the present study large populations of *Gammarus pulex* showed codominance with certain oligochaetes like *Limnodrillus* sp., *Tubifex tubifex*, *Erpobdella octoculata* and *Branchiura sowerbyii* and Dipterans like Chironomus sp.However,Oligochaeta and Diptera dominated the fauna more prominently in the Nagrad and Khudanag and less prominently in Tumbernag spring.The presence of fine sediments with their high organic content and abundance of microbial colonization sites might be expected to provide favourable conditions for burrowing oligochaetes as the fine sediments fill the interstices between larger bed materials, decrease turbulence and reduce the number of dead spaces near the surface, (Lopez and Levinton, 1978).The authors believe that the physical heterogeinity of the substrate is reduced as is being reflected by a reduction in species richness. In contrast, springs charecterised by pebble,gravel sand leaf litter(Nagrad) and gravel,sand,mud,organic detritus(Khudanag) relatively maintain fairly high level of physical diversity,support populations of richer fauna which include amphipods like *Gammarus pulex* and insect larvae. Marshall and Winterbourn (1979) have shown that growth and productivity of Tubufex tubifex is greater in fine than coarse sediments and at nutrient enriched as opposed to non-enriched sites. In general, oligochaete communitiues have been observed in soft depositing substrates rather than stony beds. However, exception is the study of Lafont(1977) who found that Naididae,Lumbriculidae and Tubificidae made up 48, 23 and 22% of oligochaete fauna in stony substrates of running waters where as Tubificidae accounted for 92% of worms in soft sediments and still waters. Amphipoda like Gammarus pulex demonstrated numerically after *Chironomus , Tubifex tubifex,Limnodrilus* and *Branchiura sowerbyii* in these studied springs were subdominants also been obtained by Barquin and Death(2004).The results of this study are well in concordance with the previous studies carried out in other biogeographical areas depicting the differences in the macroinvertebrate

assemblages between different spring types especially with respect to flow conditions and substrate characteristics. Our results confirm that substrate composition plays an important role for macroinvertebrate assemblages as it has been widely documented in literature (Bonettini and Cantonati, 1996;Hahn, 2000;Ilmonen and Paasivirta,2005).The present study reveals that the springs like Nagrad and Khudanag, with good percentage of leaf litter in substrate composition, harbour relatively fairly high diversity and density of *Gammarus pulex*, a fact also revealed by Cumminus *et al.* (1973) and Pandit *et al.*(1978).

The population density varied greatly and reached upto 1384 ind/$m^2$ in Khudanag spring in autumn followed by 406 ind/$m^2$ in Nagrad in autumn and 201 ind/$m^2$ in Tumbernag in winter.The higher invertebrate densities in spring habitats seems to be a globally recognized pattern (Barquin and Death, 2006).The mean annual density of invertebrates at Nagrad (369),Tumbernag(119) and Khudanag(1190) and their density ranges from317-406,69-201 and 957-1384 at three respective sites are lower than the invertebrate density reported elsewhere(Cantabrian springs- 6313-28615 ind/$m^2$; Barquin and Death, 2009; Karstic springs of Switzerland- (2250-14225 ind/$m^2$ ; Fumetti *et al.*, 2007) and Austrian alpine springs-(3880-9750 ind/$m^2$; Staudacher and Fureder, 2007), helocrene springs in Denmark(>70000 ind/$m^2$; Lindegaard and Thorup, 1975; Thorup and Lindegaard,1977), Alluvial springs in Switzerland(>200000 ind/$m^2$; Zollohöefer,2000).The invertebrate density pattern in the springs under study may be related to the proportion of leaf litter deposited, being higher in Khudanag than in Nagrad, Tumbernag spring being devoid of any significant amount of leaf litter. It may also be opined that spring area is also believed to have some effect on invertebrate density pattern as also suggested by Cantonati *et al.* (2006). Most invertebrate orders as well as individual taxa showed marked differences in their relative abundances between the habitat types, although the most common taxa were represented in all springs except in Tumbernag. This result is in concordance with a study of a single spring in Denmark with variable benthic substrate types and flow conditions (Thorup and Lindegaard, 1977).The study reveals that discharge is one governing factor determining both the substrate composition and the macrozoobenthic assemblages in springs. The springs are also linked because of the influence of the substrate on macroinvertebrate assemblages.

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# Macrophytic Diversity in Anchar Lake, Kashmir

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## ABSTRACT

The present floristic survey focuses on the diversity of macrophytes in Anchar lake, Kashmir. A total of 41 macrophyte species were recorded during the growing season in 2005. The emergent macrophytes had the highest diversity (24 species), followed by rooted floating leaf- type (07), submergeds (07) and free floating (03). The maximum number of macrophyte species were recorded in the months of July and August (41) while a minimum of 32 species were recorded in the month of April. A comparison of present data with earlier ones reveals that there has been a considerable decline in the macrophytic diversity of Anchar lake. The lake which has been submitted to an increasing eutrophication for the past several years is overgrown mainly by the aggressive interlope`rs like *Typha* spp., *Phragmites australis*, *Sparganium ramosum* and *Scirpus* sp.

***Key words :*** Macrophytes, diversity, eutrophication, Anchar lake.

## INTRODUCTION

The role of macrophytes in freshwater aquatic systems has received increased attention over the last 10-15 years, primarily due to their widespread decline in many lakes as a result of sustained cultural eutrophication (Egertson *et al.*, 2004). Macrophytes are excellent indicators of lake condition for many reasons including their relatively high levels of species richness, rapid growth rates, and direct response to environmental change. Individual species show differential tolerance to a wide array of stressors. Thus, as environmental conditions vary, community composition shifts in response. Aquatic plant communities have been shown to change in response to hydrologic alterations (Spence, 1982), nutrient enrichment (Craft and Richardson, 1998), sediment loading and turbidity (Sager *et al.*, 1998), and metal and other pollutants. These patterns have been interpreted and used to diagnose lake impacts because they represent a diverse assemblage of species with different adaptations, ecological tolerances, and life history strategies. The composition of the plant community can thus, reflect (often with great sensitivity) the biological integrity of the aquatic ecosystems. The appreciation of the positive ecological values of macrophytes has led to a better understanding of their diversity and role in natural ecosystem for their scientific management. In Kashmir a few studies pertaining to macrophytes have been reported (e.g. Kaul and Zutshi, 1967; Kaul *et al.*, 1978; Kak, 1987,89; Pandit, 1984,92,99,2001). Keeping in view the importance of macrophytes in aquatic ecosystems, the present study was undertaken to add some more information on their diversity in an eutrophic valley lake.

## STUDY AREA

Anchar lake is a shallow basined valley lake, having fluviatile origin. The lake is situated to the north-west of Srinagar at a distance of about 14 km. It is fed by the cold water river, Sindh which enters the lake on its northern end, while the southern end receives water from Khushalsar lake. The lake has a number of small outlet channels that drain the lake water into the nearby Shalabough wetland.

## MATERIAL AND METHODS

The lake was surveyed during the growing season of the macrophytes (April-October, 2005). The identification of macrophytes was done upto genus or/species level by adopting the standard works (Sculthorpe, 1967; Kak,1978; Cook, 1996 ).

## RESULTS AND DISCUSSION

During the present survey of the lake, a total of 41 macrophyte species(excluding Bryophyta) belonging to 23 families and 33 genera were recorded (Table1). The maximum number of species were recorded in the months of July and August (41) and a minimum of 32 species were recorded in April. All the four ecological groups viz. emergents, rooted floating type, free floating and submergeds were recorded from the lake. The diversity of emergent species was highest (24 species) while comparatively low diversity was recorded for other three groups of macrophytes : rooted floating type (07 species), submerged (07 species) and free floating (03 species).. A comparison of present data with the earlier ones, such as Zutshi *et al.,*(1967) who reported 99 macrophytic species (including Bryophyta) from Anchar lake, reveals that there has been a considerable decline in the macrophytic diversity of Anchar lake.

Due to intensified anthropogenic activities, the depth of the lake has been reduced considerably and as such much of the lake has been converted to marsh land. The lake is intensely overgrown and the typical zonal distribution of the macrophytes is lacking, resulting in complex physiognomy of macrophytes.

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**Table 1. List of macrophyte species recorded from Anchar lake during 2005**

| S.No. | EMERGENTS |
|---|---|
| 1. | *Alism plantago-aquatica* Linn. |
| 2. | *Bidens cirnua* |
| 3. | *Carex* sp. |
| 4. | *Cyperus* sp. |
| 5. | *Eleocharis palustris* Linn. |
| 6. | *Gallium* sp. |
| 7. | *Hippuris vulgaris* Linn. |
| 8. | *Lycopus europus* Linn. |
| 9. | *Menyanthese trifoliate* Linn. |
| 10. | *Myriophyllum verticillatum* Linn. |
| 11. | *Nasturtium officinale* R.B.r. |
| 12. | *Paspalum paspaloides* (Michx) |
| 13. | *Phragmites australis* Trin. |
| 14. | *Polygonum amphibium* Linn. |
| 15. | *Polygonum hydropiper* Linn. |
| 16. | *Ranunculus lingua* Linn. |
| 17. | *Ranunculus scleratus* Linn. |
| 18. | *Sagittaria sagittifolia* Linn. |
| 19. | *Scirpus* sp. |
| 20. | *Sium latijugum* C.B.C.L. |
| 21. | *Sparganium ramosum* Huds. |
| 22. | *Typha angustata* Bory & Chaub. |
| 23. | *Typha latifolia* |
| 24. | *Veronica* sp. |
| **ROOTED FLOATING-LEAF TYPE** | |
| 25. | *Hydrocharis dubia* (Blume) Bacquer |
| 26. | *Marsilea quadrifolia* Linn. |
| 27. | *Nelumbo nucifera* |
| 28. | *Nymphaea alba* Linn. |
| 29. | *Nymphoides peltatum* (Gmel) Kuntze |
| 30. | *Potamogeton natans* Linn. |
| 31. | *Trapa natans* Linn. |

**SUBMERGEDS**

32. *Ceratophyllum demersum* Linn.
33. *Hydrilla verticillata* (L.f.)Royle.
34. *Myriophyllum spicatum* Linn.
35. *Potamogeton crispus* Linn.
36. *Potamogeton lucens* Linn.
37. *Potamogeton pusillus* Roxb.
38. *Potamogeton pectinatus*

**FREE –FLOATING TYPE**

39. *Azolla pinnata*
40. *Lemna* spp.
41. *Salvinia natans* Linn.

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At present, the overriding influence on this lake is eutrophication caused by the human activities in its catchment. The nutrient loading is high and derived mainly from sewage and agricultural land. The changes in vegetation caused by eutrophication have been well documented (Ozimek,1978). Excessive nutrient loadings can affect the plant communities in a variety of ways (Weisner,1990), shifting the species composition away from species that take up nutrients slowly, to those that are able to exploit nutrient pulses more rapidly or which have high nutrient requirements (Wetzel and Van der Valk, 1998). The present survey revealed that emergent species were the most common and overgrown growth forms in Anchar lake. Growth of emergent aquatics can become very dense with eutrophication (Moss, 1979). Emergents like *Phragmites australis, Sparganium ramosum, Scirpus* sp. and *Typha* spp. which are the known aggressive interlopers, form continuous or at some places patchy mixed or monospecific stands, and thus colonize large areas of Anchar lake. Nutrient enrichment often results in growth of species tolerant to high nutrient loadings e.g. *Typha, Phragmites* (Galatowitsch *et al.,* 1999). The emergent vegetation has expanded rapidly across the lake, presumably as a response to lake shallowing caused by enhanced infilling of the lake, mainly due to the eroded soil and silt carried by the River Sindh.

Among the three free floating macrophytes recorded from Anchar lake, *Lemna* spp. and *Azolla pinnata* were found to have an explosive growth in the open waters of the lake. *Lemna* spp. often increases in density and coverage epecially during late spring and early June, in response to increased nutrients (Pandit *et. al.,* 1978; Vaithiyanathan and Richardson, 1999).Later invasive plants, especially non-native invaders, such as *Azolla,* usually form a solid cover which creates compact, thick, floating mats, that shade the water column below them, restricting the submerged growth and thus altering the species composition of the lake . Their explosive expansion, thus, pose a great threat to the lake ecosystem.

The present floristic survey revealed that the number of floating leaved species was less as compared to the emergent species. This may be because in high alkalinity lakes, the floating leaved species are replaced by emergent macrophytes ( Makela *et al.,* 2004).

The low species diversity of submergeds encountered during the present study, may be attributed to the high water turbidity of the lake. This is in accordance with the results of other studies (Sand-Jensen *et al.,*2000). Turbidity (i.e. decreased water clarity) almost by definition means decreased availability of light to submerged aquatic vegetation, killing many species. Conversely, increased water clarity may result in increased cover of submerged aquatics ( Scheffer *et al.,* 1993). During this study, *Ceratophyllum demersum* was the most common submerged macrophyte encountered in the lake. *Ceratopyllum* is known to grow well in organically polluted waters (Kulshreshtha, 1982). Similarly, *Potamogeton pectinatus,* recorded during the present survey, is known to be tolerant to murky waters ( Nichols and Lathrop, 1994). The declining of submerged vegetation may be considered to be one of the symptoms of advanced eutrophication of Anchar lake as also reported by Phillips *et al.,* (1970) for other lakes. Similar observations have been made by Pandit (1992) while working on takes and wetlands of Kashmir.

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# Distribution and Abundance of Macrozoobenthos in Dal Lake of Kashmir Himalaya

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## ABSTRACT

The distribution and abundance of macrozoobenthic fauna in relation to several physico-chemical factors was investigated in Dal lake for the period between January 2006 to December 2006. Five sites were selected on the whole, one each from the four basins of the lake and fifth one from the floating gardens, on the basis of morphometric and biological features of the lake. The physico-chemical factors including temperature, pH, alkalinity, carbon dioxide, dissolved oxygen, chlorides and nutrients were studied. The organisms showed seasonal variation in relation to these physico-chemical parameters and all the parameters had a combined effect on the occurrence, periodicity and population of the various contributors of macrobenthic community.

***Key words*:** Macrozoobenthos, Kashmir Himalaya, morphometric, occurrence, periodicity, population

## INTRODUCTION

The valley of Kashmir also referred to be a "paradise on earth" has been known for its scenic beauty. The high–attitude valley of Kashmir is of tectonic origin, lying between 33° 25′ to 34° 50′ N & 74° to 75° E. It abounds in a great array of fresh water bodies like lakes, ponds, rivers, springs, streams etc. In addition to socio-economic, cultural and ecological values it sustains the economy of the state. It is well known for its high altitude lakes like Alipather, Sheshnag, Kounsarnag, Tarsar, Marsar etc. and low land lakes like Dal, Anchar, Manasbal, Wular etc.

Dal Lake a world renowned picturesque tourist spot and an inseparable part of Kashmir's glorious heritage and culture is aptly referred to as a cradle of Kashmir civilization, situated in an expanding urban environment and nested within the Srinagar city, in the back drop of Zabarwan range with lush green slopes descending to woodlands and vast orchards adding splendor and magnificent ambience of the lake. Dal Lake, the urban valley lake of fluviatile origin is situated at an altitude of 1886m (ASL) between 34°5′ - 34°6′ N latitude and 74°8′ - 74 °9′ E longitud'es, in the heart of Kashmir Valley on the northeast of the state summer capital Srinagar at the foot of Zabarwan mountains. The total water surface area of the lake is 11.45km$^2$, of which 4.1km$^2$ is floating under gardens, 1.51km$^2$ and 2.25km$^2$ are land and marsh respectively, whereas the total volume estimated is 9.05×10 m$^3$ and the ratio between the mean and maximum depth (m) ranges

between 0.20 and 0.25 indicating the gentle slope of the lake bed. This open drainage eutrophic lake is multibasined with the Hazratbal, Bod-dal, Gagribal and Nigeen as its four basins, which differ markedly in their area, volume, depth and shorline development indices etc.

The lake has been experiencing significant ecological changes in the form of deterioration of catchment, excessive nutrient loading and encroachment. Huge quantities of nutrients are added to the lake every year in the form of wash off from the adjoining areas of the city, the large population of the residents within the lake and above all from the mushroom growth of houseboats moored in the lake. The immediate visible effect of these on the biology of the lake is seen, in the luxuriant growth of macrovegetation including *Potamogeton natans, Ceratophyllum demersum, Polygonum amphibium, Lemna* sp.*, Salvinia natan,* and above all *Azolla* sp.

In aquatic ecosystem aquatic biota is closely dependent on the physical, chemical and biological characteristics of water, each of which acts as a controlling factor A number of hydrobiological studies have been carried out in this lake with respect to water chemistry, sediment chemistry, plankton, vegetation and others, but so far no attempt has been made to relate the benthic fauna found in its sediments with the water chemistry, which forms a very important component of the lake and plays a great role in the sediment-interface metabolism and thereby determine the condition of the substrate and the life-supporting condition thereupon. In aquatic ecosystem every component gets disturbed as a result of racing eutrophication and studying each of these can help us to evaluate the impact of pollution, thereby enabling us to formulate the management strategies to curb this menace**.** It was with this aim that the present investigation on the population abundance and seasonal behaviour of macrozoobenthos under the operative influence of physico-chemical factors was undertaken.

## MATERIAL AND METHODS

On the basis of morphometric and biological features, five sampling sites were selected one each from the four basins and the fifth one from amongst the floating gardens. Sediment and water sampling collection was done at all the sites on monthly basis for evaluating macrozoobenthos and physico-chemical characteristics respectively. The detailed analysis of water samples was carried out according to the standard methods developed by Welch (1963), Mackereth (1963), Golterman and Clymo (1969) and APHA (1989).

Sampling of benthos was done with the help of Ekman-Birge dredge of 225 $cm^2$ sampling area. At each site the sampling was done at three different places, differing in depth and vegetation and then pooled together. Thereafter, the sample was filtered through a sieve of No. 40 (250 meshes $cm^{-2}$). The organisms retained in the sieve were then preserved in 10% formaldehyde solution before being sorted out, identified, counted and expressed as individuals $m^{-2}$(Welch 1948).

The sites selected for the collection of data from the lake are as follows:

Site I : Boddal basin

Site II : Gagribal basin.

Site III: Hazratbal basin.

Site IV: Nigeen basin.

Site V : Near Floating Gardens

## RESULTS AND DISCUSSION

The macrozoobenthic animals collected belonged to three metazoan phyla viz.Annelida Mollusca and Artropoda. The greater density of the benthic community was recorded mainly amongst annelids and insects both in respect of their taxa and abundance. This was followed in a decreasing order by molluscs and crustaceans. Within these three major groups a total of 30 different species belonging to 5 classes, 9 orders and 13families were recorded.

Annelida were represented by 13 species of which 12 belonged to class Oligochaeta and 1 species to class Hirudinea. Arthropoda were represented by 12 species, 10 belonged to class Insecta and 1 to Crustacea. Mollusca was exclusively represented by gastropods. Of the 5 species recorded, 4 belonged to family Lymneidae and 1 to Planorbidae.The sequence of dominance of these groups at various sites was of the following order:

Annelida>Insecta>Mollusca>Crustacaea at Sites 1, 2 and 4

Mollusca>Annelida>Insecta>Crustacea at Site 3

Annelida>Mollusca>Insecta>Crustacea at Site 5

**Table 1. Distribution and abundance (Individuals/m²) of dominant macrozoobenthos in Dal lake**

| Speices | Sites | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Limnodrillus hoffmesteri* | I | -- | -- | 44 | 30 | 15 | -- | -- | -- | -- | -- | -- | 15 |
| | II | 59 | 207 | 74 | 119 | 15 | 15 | -- | -- | -- | 30 | 44 | 44 |
| | III | 59 | 30 | 193 | -- | -- | -- | -- | -- | -- | 30 | 45 | 74 |
| | IV | -- | -- | 44 | 74 | 44 | -- | -- | -- | 59 | 59 | 30 | 44 |
| | V | 104 | 44 | 89 | 59 | -- | -- | -- | -- | -- | 59 | 15 | 15 |
| *Tubifex* sp. | I | -- | -- | 74 | 44 | -- | -- | -- | -- | -- | 44 | -- | -- |
| | II | -- | 133 | 56 | 44 | 33 | 33 | -- | -- | -- | -- | 22 | -- |
| | III | 44 | 33 | 122 | 44 | 22 | -- | -- | -- | -- | -- | 44 | 56 |
| | IV | -- | 15 | 44 | 89 | -- | -- | -- | -- | 30 | 15 | 15 | 59 |
| | V | 59 | -- | 74 | 44 | -- | -- | -- | -- | -- | 44 | 30 | 30 |
| *Aulodrilus* sp. | I | -- | -- | -- | 59 | -- | -- | -- | -- | -- | -- | -- | -- |
| | II | -- | 44 | -- | 44 | -- | 33 | -- | -- | -- | 30 | 44 | 44 |
| | III | -- | -- | -- | -- | -- | -- | -- | 22 | -- | -- | 33 | -- |
| | IV | -- | -- | 59 | -- | -- | -- | -- | -- | -- | 30 | -- | 15 |
| | V | 30 | 45 | 30 | 15 | -- | -- | -- | -- | -- | -- | -- | -- |
| *Chaetogaster* sp. | I | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | II | -- | 45 | -- | 22 | -- | -- | 22 | -- | -- | -- | -- | 33 |
| | III | -- | 22 | 67 | 22 | -- | -- | -- | -- | -- | -- | 33 | -- |
| | IV | -- | -- | -- | -- | 30 | -- | -- | -- | -- | -- | -- | -- |
| | V | -- | 30 | 14 | 30 | -- | -- | -- | -- | -- | -- | -- | -- |
| *Aeolosoma* sp. | I | 15 | 30 | 30 | -- | -- | -- | -- | -- | -- | 30 | -- | -- |
| | II | 22 | 189 | 56 | 33 | -- | -- | -- | -- | -- | -- | 33 | -- |
| | III | 22 | -- | 122 | 44 | -- | -- | -- | -- | -- | 15 | -- | 44 |
| | IV | -- | -- | 59 | -- | -- | -- | -- | -- | -- | 15 | -- | 30 |
| | V | 59 | 30 | 59 | 30 | -- | -- | -- | -- | -- | -- | -- | -- |

| Speices | Sites | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *Branchiura sowerbyii* | I | -- | -- | -- | -- | 15 | 20 | 20 | -- | -- | -- | -- | 15 |
| | II | 40 | 30 | 45 | -- | 15 | 15 | -- | -- | -- | 30 | 44 | 44 |
| | III | 20 | 10 | 10 | -- | -- | -- | -- | -- | -- | 10 | 45 | 55 |
| | IV | -- | -- | 10 | -- | -- | -- | -- | -- | -- | -- | -- | 11 |
| | V | 15 | 18 | -- | -- | -- | -- | -- | -- | -- | -- | 59 | 15 |
| *Nais communis* | I | 15 | -- | 45 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | II | 18 | 89 | 43 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | III | 33 | 27 | 67 | 22 | -- | -- | -- | -- | -- | -- | -- | 44 |
| | IV | 15 | -- | 30 | -- | 15 | -- | -- | -- | -- | -- | -- | -- |
| | V | 30 | 30 | 30 | -- | 30 | -- | -- | -- | -- | -- | -- | -- |
| *Dero dorsalis* | I | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | II | 11 | 89 | 33 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | III | 44 | 33 | 22 | 67 | 22 | -- | -- | -- | -- | -- | -- | -- |
| | IV | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | V | -- | -- | -- | -- | 30 | -- | 45 | -- | -- | -- | -- | -- |
| *Dero digitat* | I | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | II | -- | -- | -- | -- | -- | -- | 22 | -- | -- | -- | -- | -- |
| | III | -- | -- | -- | -- | -- | -- | -- | 33 | -- | -- | -- | -- |
| | IV | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | V | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| *Aulophorus* sp. | I | -- | 15 | -- | 30 | 15 | -- | -- | -- | -- | -- | -- | 15 |
| | II | -- | 56 | 22 | 15 | -- | -- | -- | -- | -- | -- | -- | -- |
| | III | -- | -- | -- | -- | -- | -- | 11 | 22 | -- | -- | -- | -- |
| | IV | -- | -- | 45 | 45 | -- | -- | -- | -- | -- | -- | -- | -- |
| | V | 15 | 30 | 45 | 30 | 30 | -- | -- | -- | -- | -- | -- | 15 |
| *Pristina longiseta* | I | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | II | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | III | 22 | -- | -- | -- | -- | -- | -- | 11 | -- | -- | -- | -- |
| | IV | -- | -- | -- | 59 | 30 | -- | -- | -- | -- | -- | -- | -- |
| | V | 30 | 15 | -- | -- | -- | -- | -- | 15 | -- | -- | -- | 15 |

| Speices | Sites | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Sep | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | I | -- | -- | 30 | -- | -- | -- | -- | -- | -- | -- | -- | -- |
| | II | -- | -- | 44 | 45 | -- | -- | -- | -- | -- | -- | 33 | -- |
| *Stylaria* sp. | III | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | -- | 40 |
| | IV | -- | -- | -- | 45 | -- | -- | -- | -- | -- | 59 | -- | -- |
| | V | -- | -- | 30 | 30 | 30 | -- | -- | -- | -- | -- | -- | -- |
| | I | -- | -- | 30 | -- | -- | 15 | 30 | -- | -- | -- | -- | -- |
| | II | -- | -- | -- | -- | -- | 44 | 56 | 12 | -- | -- | -- | -- |
| *Nepa* sp. | III | -- | -- | -- | -- | -- | 08 | 12 | 03 | -- | -- | -- | -- |
| | IV | -- | -- | -- | -- | 15 | -- | 45 | -- | 06 | -- | -- | -- |
| | V | -- | -- | -- | -- | 30 | -- | 45 | -- | -- | -- | -- | -- |
| | I | -- | -- | -- | -- | -- | 30 | 15 | -- | -- | -- | -- | -- |
| | II | -- | -- | -- | -- | -- | 45 | 67 | 16 | 45 | -- | -- | 89 |
| *Hydrophilus* sp | III | -- | -- | -- | -- | -- | 06 | 12 | -- | 30 | 08 | 45 | 74 |
| | IV | -- | -- | -- | -- | -- | 38 | 15 | -- | 31 | -- | 30 | -- |
| | V | -- | -- | -- | -- | 30 | 43 | 30 | -- | 15 | -- | -- | 30 |
| | I | -- | -- | -- | -- | -- | -- | 05 | 10 | -- | -- | -- | -- |
| | II | -- | -- | -- | -- | -- | 24 | 30 | 05 | -- | -- | -- | -- |
| *Tendipes* | III | -- | -- | -- | -- | -- | 12 | 22 | 12 | -- | -- | -- | -- |
| *tentans* | IV | -- | -- | -- | -- | -- | 22 | 12 | -- | 31 | -- | -- | -- |
| | V | -- | -- | -- | **46** | 17 | 42 | 11 | -- | -- | -- | -- | -- |
| | I | -- | 15 | 178 | -- | -- | -- | 15 | 06 | -- | -- | -- | -- |
| | II | -- | -- | -- | 45 | 15 | 30 | -- | 30 | -- | -- | -- | -- |
| *Chironomus* | III | 104 | 15 | 30 | 15 | -- | -- | -- | 05 | -- | 12 | 30 | 54 |
| sp. | IV | -- | 15 | 15 | 155 | 44 | -- | -- | 15 | -- | 30 | 15 | -- |
| | V | 256 | 104 | 267 | 89 | -- | 12 | -- | 09 | -- | 118 | 77 | 324 |
| | I | -- | -- | -- | -- | -- | -- | 15 | 30 | -- | -- | -- | -- |
| | II | -- | -- | -- | -- | 15 | 15 | 20 | -- | -- | -- | -- | -- |
| *Chaborus* sp. | III | -- | -- | -- | -- | 33 | 21 | 34 | -- | -- | 30 | -- | -- |
| | IV | -- | -- | -- | -- | 44 | 25 | 11 | -- | -- | 11 | -- | -- |
| | V | -- | -- | -- | -- | 10 | 13 | 22 | 09 | -- | -- | -- | -- |
| | I | -- | -- | -- | -- | -- | 26 | 40 | -- | -- | -- | -- | -- |
| | II | -- | -- | -- | -- | 15 | 15 | 45 | -- | -- | -- | -- | -- |
| *Pseudochirono-* | III | -- | -- | -- | -- | -- | 33 | 21 | -- | -- | 30 | -- | -- |
| *mus* sp. | IV | -- | -- | -- | -- | -- | 21 | 11 | 10 | 42 | -- | 30 | 44 |
| | V | -- | -- | -- | -- | 31 | 11 | 231 | 76 | -- | -- | 27 | -- |

The benthic fauna collected from the five sites varied with respect to the type of vegetation and the impact from the human settlements as the site 2 was located in the thick growth of macrovegetation and the site 4 was very close to human habitation where the greatest concentration of sewage from the surrounding houseboats was flushed into the lake. It recorded the maximum abundance of organisms as compared to

other sites. These organisms were represented by annelids of which *Limnodrillus* sp., *Tubifex* sp., *Branchiura sowerbyii,Aelosoma* sp., *and Stephenosoniana* sp., were most conspicuous and among the insecta *Chironomus* sp., *Pseudochironomus* sp., *Tendipes tentans* and *Chaborus* sp. showed predominance. These may be regarded as direct indicators of organic pollution in fresh waters. Wilham and Dorros (1968) and Adholia *et al.* (1990) reported that oligochaetes particularly *Tubifex* sp. are the common large inhabitants of mud enriched with organic matter. In the present study the oligochaete species richness in Dal lake confirms that the lake is high recipient of organic pollution load. Many workers (Oliver,1971; Brinkhurst & Cook,1974; Saether,1979; Milbrink,1980 and Bazzanti,1983) have also designated *Limnodrillus* and *Tubifex* as indicators of pollution in other studies. The presence of C*hironomus* sp. in Dal lake again confirms its organic rich waters. Similar results have also been obtained by Bay *et al.* (1966), Hilsenshoff (1966), Pandit *et al.* (1985), Kaushik *et al.* (1991) and Pandit (1992). Temperature which is generally regarded as one of the most important factors in the aquatic ecology showed a gradual increase from January onwards and favoured the growth of vegetation in the lake bottom which in turn influenced the increase in benthic population. Though eurythermal, molluscs were more common during summer when the temperature of the water was higher and decomposition processes at the sediment interface did not appear to be negative factor. The annelids, however, appeared to be rather stenothermal and cold water species as they did built up their peak population in the winter months only. Such a mode of occurrence of molluscs and annelids has been reported in several other studies (Kajak, 1956; Hunter, 1956 and Michael, 1968).

The concentration of the dissolved oxygen remained low below saturation level. The maximum values were found towards the colder months of autumn and winter and minimum during the high temperature of summer. There are reports of the lake water being in a super saturation condition earlier (Zutshi and Vass, 1978) but the decreasing trend of saturation is indicative of a definite shift in the trophic status of the lake and greater decomposition at the bottom level in the sediments due to the organic nutrients entering the lake from various sources. Further it may also be due to longer duration of photosynthetic activity during summer, when large amounts of oxygen are released and the high temperature reduces the solubility of the gas in water. The low dissolved oxygen and high temperature of summer months appeared to have a direct influence on the population abundance and species composition of oligochaetes in the lake. The number of these organisms was very low as compared to molluscs and insects (Gizinski, 1974).

The pH, which is responsible for providing the appropriate living conditions for the organisms, remained usually on the alkaline side throughout probably due to high total alkalinity more so when the bicarbonate system prevailed. Such a phenomenon has been reported by Freisner and Fuller (1966). The more highly buffered waters supported more macroinvertebrates. Zischki *et al.* (1983) concluded that low pH values decrease community diversity while increasing the relative abundance of the tolerant species. pH showed irregular fluctuations and the range from 7.0 in summer to 9.43 in winter did not vary greatly with changing season (Table. 2). However, the minimum values were recorded in summer months. The decrease

in pH was probably the result of high temperature and thereby decomposition of organic matter increased and increased concentration of carbondioxide.

The chlorides showed a direct relationship with the temperature with maximum concentration being recorded in summer and the minimum towards the colder months. Nakao (1982) observed that the seasonal changes in chlorinity corresponded with the variation in population density of benthos. Such a relationship is also supported by the observations of Kroon *et al.,* (1985). Silicates in the lake were greatly affected by the amount of silt brought in the water from exterior as has been reported by Hall *et al.* (1977). This was the case with Hazratbal basin site which receives large quantities of silt through Telbal Nallah. The high values at Nigeen in summer and autumn were probably due to the presence of large number of molluscs. Michael (1968) is of the opinion that availability of lime supplied by decomposing of shells result in high concentration of silicates. However, the low values in winter could be due to low inflow of water and the retreating of snails during winter into deeper parts of the littoral region. It is known that in polluted waters the oligochaetes are present in high percentage of the total benthic fauna (Goodnight and Whitley, 1965; Howmiller and Beeton, 1971). In Dal lake the relative abundance of these worms was rather high and more so at Gagribal basin and floating garden basin and the take has crossed the initial stages of entrophication.

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# TERRESTRIAL ECOLOGY

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# Growth and Productivity of Wicker Willow (*Salix triandra* L.) Plantation In Kashmir

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## ABSTRACT

An experiment on growth and productivity of wicker willow plantation (*Salix triandra* L.) was carried out on Farmers Field at Batipora (Taibal) in Srinagar district of Kashmir in India. The experiment was laid out in RBD with four treatments fresh plantation ($T_1$), two year old coppice ($T_2$), three year old coppice ($T_3$), four year old coppice ($T_4$), which was replicated six times.

The initial nutrient status of the soil showed pH neutral to acidic, EC medium, organic carbon high with available NPK, low, high, medium, respectively. Planting distance 15 x 30 cm was maintained in all the treatments. Sprouting vigour was found maximum in $T_3$ (three year old coppice) and $T_4$ (four year old coppice) which started and completed earlier than $T_2$ (two year old coppice) and $T_1$ (Fresh plantation). Maximum growth and productivity was observed with different age of coppice (i.e., two year and four year old coppice, respectively). The number of shoots per coppice was the deciding factor in contributing yield, number of shoots (7.66) and dry yield (25.10 t/ha). In post harvest processing rods were boiled in water for 10-12 hours and cooled for another 10-12 hours and peeling was done, the bark contributed nearly 25 per cent of weight. The dimensions of processed rods falls into medium category in length and thick category in diameter as per the standards.

***Key words:*** Growth, productivity, processing, grading, chemical properties, *Salix triandra* L.

## INTRODUCTION

Willows are the fast growing pioneer species, their allocation of the assimilate transport is concentrated to the growing points of the shoots in the early stages of life (juvenile phase) and later willows invest more and more in the root system. This phenomenon is one of the reason for early harvesting of willows to maintain the juvenile stages. As a result there is high woody biomass production in the stems of willows (Christersson, 2005) Willow have been used by people for many centuries to build shelters, make fences, boats, agricultural implements, tooth picks, preparation of artificial limbs and baskets (Wadoo, 2005.)

Willows are easily propagated by stem cuttings. The cuttings are prepared out of vigorous 2-3 years old shoots free from disease and defects. These are obtained when the trees enter in the dormancy stage and are cut to the size of 20 cm length and mid point diameter 10-20 mm. Cuttings obtained in February to March and prepared from centre half of the shoot.

Forest officers of Kashmir imported famous English bat willow (*Salix alba* cv. Calva = *Salix coerulea*, Smith) from England in 1917 and raised it in Kitreteng wetland nursery (Bijbehara Forest Division of Kashmir). They also imported some varieties of wicker willows from England such as *Salix triandra, Salix purpurea, Salix amygdalina, Salix hypopofolia, Salix viminalis, Salix flabellaries* etc. These are not planted by the departmental agencies as these plantations can be found in Harn, Shalbugh and other areas of Tehsil Ganderbal in the private lands of farmers raised for wicker works where water is readily available (Wadoo, 2005).

The *Salix triandra* L. (Almond willow), most commonly planted wicker willow species in Kashmir is a deciduous shrub upto 5 m height rarely a small tree, bark reddish flaking off in autumn like that of Chinar, leaves 5-10 cm, narrow, finely toothed, dark green and shining above, bluish or green beneath. The leaves have a distinct smell and taste of swe*et al*monds (hence known as almond willow). Winter twigs are brownish green shining above, buds narrow pointed, flowers March-May and some times July and August. Catkins 2.5 to 5 cm long, male catkin with three stamens (Krussnan,1978). The long branches of the almond willow are cut out after one year growth and processed in boiling water, then their bark is peeled off to make the rods water proof and more durable and the wickers are provided as a raw material to small scale industries of Kashmir for the manufacturing of chairs, tables, sofa sets, baskets of various shapes and designs, flower vases and a variety of other fancy items (Fig. 1)

Due to its fast growth, easy rooting, recurrent harvest and having commercial value for wicker works in Kashmir valley. The wicker willow *Salix triandra* L. is preferred over the main food crop of the valley i.e., paddy, due to lack of care and high returns. Till date no study has been done on wicker willow cultivation in Kashmir. Therefore, the present study has been undertaken on the growth and productivity of wicker willow plantation in Srinagar district of Kashmir, where wicker willow is cultivated on wet sites. The survey of the area revealed that wicker willow has been found in all the districts of Kashmir except Kupwara. Also Srinagar district was found in the fore front of wicker willow cultivation (maximum families, area, yield and income).

## MATERIAL AND METHODS

The experiment was carried out on the farmer's field during the year 2005 at Batipora (Hazratbal), which is located at an altitude of 1587 m asl. with a latitude of 34. 08 °N and 74.83 °E. During the growing period the maximum mean temperature was recorded in June (30.72 °C) and minimum in November –0.80 °C whileas, rainfall was highest in the month of July (28.3 mm) and minimum in November (3.05 mm) and relative humidity was maximum in October (78.02 %) and minimum in June (58.56 %).

The data on different parameters for growth and productivity of *Salix triandra* L. commonly known as wicker willow were collected from four treatments $T_1$ (fresh plantation) which was planted in the first week of March and remaining treatments two year old coppice ($T_2$), three year old coppice ($T_3$) and four year old coppice ($T_4$) were earlier harvested, once, twice and thrice. The common planting distance was maintained at 30 x 15 cm. However, each treatment was replicated six times in RBD (Randomized Block Design).

The number of coppice counted from the marked plots (1 $m^2$) and were termed as plant density/$m^2$. The coppice in marked sample plot was tagged and numbered one, two, three, etc. the sample plots were visited after five days interval to record the sprouting percentage. The shoots were counted from the randomly tagged coppice and were expressed as shoots/coppice. The shoot height and collar diameter were recorded at an interval of one month from the date of shoot emergence in cm from the randomly tagged coppice stands of 1 $m^2$ with the help of metallic scale verneir callipers. Also the number of leaves/shoots were recorded at an interval of one month in 1 $m^2$ sampling plot from the randomly tagged coppice and were expressed as leaves/shoot.

The maturity indices as perceived by the farmers was taken as harvesting stage and period of harvesting as harvesting time. From each sampling plot shoots were taken from an area of $0.25^2$ m to determine the fresh and dry weight of shoots prior to leaf fall and after leaf fall and expressed as kg/0.25 $m^2$ which was converted to fresh weight (tonnes/ha). The shoots taken were oven dried at a temperature 105 ± 2 °C for 72 hours and weighed (2). The weight obtained was reported as dry weight kg per 0.25 $m^2$ and then converted to dry weight t/ha. The moisture percentage in shoots prior and after leaf fall was determined by the formula:

$$\text{Percent moisture} = \frac{\text{Fresh weight} - \text{dry weight}}{\text{Fresh weight}} \times 100$$

After harvest samples were boiled in standard sized boiling tanks (15' x 5' x 5') made of cast iron and heated directly by fuel wood for 10-12 hours and then left for cooling for another 10 to 12 hours. After cooling samples were peeled by fastening the bark of rods between two wooden sticks held at cross with each other and partly embedded in the soil. Then the rods were air dried under sunlight for a weeks time, which was then graded depending upon the diameter and height in (cm) as per the standards into thin, medium and thick with metallic scale and vernier calliper. (Romero-Ablos, 1988).

**Fig. 1. Willow raw material for small scale industries.**

The soil samples were taken randomly from all the coppice stands of different ages at 0-30 cm soil depth. The samples were air dried under shade, crushed and passed through 2 mm sieve. The sieved samples

were analysed for different chemical properties. The methods used for analysis of various chemical properties are as follows:

| Chemical properties | Methods employed |
|---|---|
| pH (1 : 2..5) | Jackson (1973) |
| EC ($dsm^{-1}$) | Jackson (1973) |
| Organic carbon (%) | Walkey and Black (1934) |
| Available nitrogen (kg/ha) | Subbiah and Asija (1956) |
| Available phosphorus (kg/ha) | Olsen *et al.* (1954) |
| Available potassium (kg/ha) | Jackson (1973) |

## RESULTS AND DISCUSSION

The studies conducted on the soil analysis depicted in Table 1 which revealed that the soil pH decreased and organic carbon increased with the increase in age of the coppice. The pH recorded was suitable for the growth of wicker willow (*Salix triandra* L.) The increase in organic carbon and decrease in pH was due to the reason that leaves of the deciduous trees decomposes readily, which increased the organic carbon and during decomposition of leaves the acids were released which consequently decreased the soil pH. These results were in agreement with the works of Sennerby - Forsse (1990), Cannell (2004) and Savill (2004). The electrical conductivity increased with the increase in age of the coppice and was found in the range of medium to high as depicted in Table1. EC was minimum (0.16 $dsm^{-1}$) in $T_1$ (Fresh plantation) and maximum in $T_4$ (four year old coppice) (0.23 $dsm^{-1}$) which was best for the growth and development of the willows which are in conformation with Crough and Honeyman (1986).

The available nitrogen, phosphorus and potassium was found in the range of low, high and medium as depicted in Table 1. The concentration of available nitrogen was low due to the reasons that the short rotation forest species grow rapidly and bind great amount of nutrients in their biomass and also losses due to leaching, denitrification and amonification of nitrogen is high. While as phosphorus concentration increases due to the addition of litter fall which results into formation of polyhumic substances and coating of aluminium and iron ions thus the higher content of phosphorus in soil may be attributed to the higher content of organic matter in the soil which prevents the fixation of phosphorus ions. While as potassium falls in the medium range due to the reason that potassium is always in the exchangeable form in the soil. So it clearly indicates that nutrient status of the soil is very important in short rotation forestry which is in confirmation with the findings of Hyfonen (1996).

The studies conducted on the growth of *Salix triandra* L. on various parameters revealed that sprouting vigour in $T_1$ (fresh plantation) was slow than the other treatments as deficited in Table. 2. While as

on 30th March, 100 per cent sprouting was recorded in $T_3$ (three year old coppice) and $T_4$ (four year old coppice) stand. Whereas, in fresh and one year old coppice it was observed upto 4th April. Statistically the significant difference were found in $T_1$ (Fresh plantation) and $T_2$ (two year old coppice) on 15th, 20th and 25th March while as, $T_3$ (three year old coppice) and $T_4$ (four year old coppice) differed significantly on 15th March. The results obtained indicate that more sprouting vigour in $T_2$ (two year old coppice), $T_3$ (three year old coppice) and $T_4$ (four year old coppice) than $T_1$ (fresh plantation) was obviously due to the higher carbohydrate levels and well established root system in these coppice stands than the fresh cuttings $T_1$ (fresh plantation), which diverted its reserve in root establishment. Also coppicing effect increases the hormonal changes in the remaining stumps, as willow sprouts from the auxillary buds of recent sprouts and enhances the sprouting due to the hormonal changes. These results are in agreement with the rescarches of Taylor *et al.* (1982), Blake (1983) and Kauppi *et al.* (1987).

**Table 1. Initial nutrient status of the soil**

| Nutrients | Depth 0-30 cm | | | | Range | Method employed |
|---|---|---|---|---|---|---|
| | $T_1$ (Fresh plantation) | $T_2$ (2 yr. old coppice) | $T_3$ (3 yr. old coppice) | $T_4$ (4 yr. old coppice) | | |
| pH (1 : 2.5) | 6.56 | 6.25 | 6.10 | 6.03 | Neutral to mild acidic | Jackson (1973) |
| EC $dsm^{-1}$ | 0.16 | 0.18 | 0.22 | 0.23 | Medium | Jackson (1973) |
| OC (%) | 1.14 | 1.40 | 1.65 | 1.78 | Medium to high | Walkley and Black (1934) |
| Available N (kg/ha) | 236.50 | 213.50 | 217.50 | 231.00 | Low | Subbiah and Asija (1956) |
| Available P (kg/ha $P_2O_5$) | 28.66 | 33.00 | 36.16 | 36.16 | High | Olsen *et al.* (1954) |
| Available K (kg/ha) $K_2O$} | 224.00 | 223.66 | 224.50 | 225.33 | Medium | Jackson (1973) |

**Table 2. Sprouting percentage of wicker willow (*Salix triandra* L.)**

| Treatment/month | Per cent sprouting | | | | |
|---|---|---|---|---|---|
| | 15th March | 20th March | 25th March | 30th March | 4th April |
| $T_1$ (Fresh plantation) | 1 | 25 | 75 | 98 | 100 |
| $T_2$ (2 yr. old coppice) | 2 | 40 | 84 | 99 | 100 |
| $T_3$ ( 3 yr. old coppice) | 2 | 42 | 88 | 100 | 100 |
| $T_4$ (4 yr. old coppice) | 3 | 44 | 90 | 100 | 100 |
| **Mean** | **2** | **37.75** | **84.25** | **99.25** | **100** |
| **CD (0.05)** | **0.12** | **4.06** | **6.35** | **NS** | **NS** |

In the present study number of shoots per coppice is presented in table 3 which revealed that maximum shoots per coppice were recorded in $T_4$ (7.66) on 15th April and minimum on 15th November (6.97) followed by $T_3$, $T_2$ and $T_1$. Table 3 also reiterates that mortality percentage was highest in $T_4$ with 9.00 per cent followed by $T_3$ (5.68%), $T_2$ (4.66%) and lowest in $T_1$ (4.41%), in one growing season. So from the results it can be concluded that both number of shoots as well as mortality percentage increased with increase in age of the coppice. It is obviously due to the reason that the fast growing species needs complete over head light so with the increase in shoots the maximum shoots does not capture complete light, thus resulting lower photosynthetic efficiency which in turn accounted for reduction in net assimilation rate, so mortality percentage was increased. These results are in conformity with the findings of Tolonen (1988), Bullard *et al.* (2002), Smallukas and Noreika (2005) and Wadoo (2005).

**Table 3. Average number of shoots/coppice of wicker willow (*Salix triandra* L.) and mortality(%)**

| | Average number of shoots | | |
|---|---|---|---|
| **Treatment/month** | **15th April** | **15th November** | **Mortality (%)** |
| $T_1$ (Fresh plantation) | 1.36 | 1.30 | 4.41 |
| $T_2$ (2 yr. old coppice) | 3.00 | 2.86 | 4.66 |
| $T_3$ (3 yr. old coppice) | 4.57 | 4.31 | 5.68 |
| $T_4$ (4 yr. old coppice) | 7.66 | 6.97 | 9.00 |
| **CD (0.05)** | **0.33** | **0.30** | **0.95** |

The perusal of data in Tables 4, 5 and 6 clearly indicated that *Salix triandra* L. showed three main period of growth namely 15th April to 15th June, 15th July to 15th August and 15th August to 15th November. The average shoot height, average collar diameter and number of leaves increased significantly rapid during

the first two stages and then in 3rd stage it increased with decreasing rate and finally becomes very steep. The highest average mean increment in shoot height (55.7 cm), collar diameter (0.61 cm) and number of leaves (22.45) was recorded from 15th June to 15th July. The average maximum shoot height (142 cm), collar diameter (1.12 cm) and number of leaves (6.79) was recorded in $T_2$ (two year old coppice). Statistically, the increase in average height, collar diameter, number of leaves differed significantly in $T_1$ (fresh plantation), $T_3$ (three year old coppice) and $T_4$ (four year old coppice) upto August while as in $T_1$ (fresh plantation) it increased significantly upto 15th September. Maximum mean average height (187.64 cm), collar diameter (1.51 cm) and number of leaves (75.05) was recorded on 15th November and minimum value of average height, collar diameter and number of leaves on 15th April (20.15 cm), (0.10 cm) and (8.07), respectively. These values were attained in a period of one growing season which indicated the fast growth of this species. The shoot height, collar diameter and number of leaves were found minimum in $T_3$ (three year old coppice) and $T_4$ (four year old coppice) due to the reasons that the number of shoots were higher than that of other $T_1$ (fresh plantation) and $T_2$ (two year old coppice) and the nutrients gets distributed in the more number of shoots. In $T_1$ (fresh plantation) the root system was not fully developed so maximum nutrients were used by the cutting for the development of root system. These results are in conformity with the findings by various scientists (Fricsson, 1981; Cannell *et al.,* 1988 and Smallukas and Noreika, 2005).

The results indicated that maximum growth occurred during the warmer seasons i.e, June and July with rich precipitation (28.3 mm rainfall), and the species under study was found to invest more photosynthetic products to above ground parts during the early growth which ultimately facilitated plants to capitalize more light and allow fast above ground growth (Perttu *et al.*, 1984; Cannell *et. al.*, 1988).

Harvesting indices is reported to be one of the important factor for the management of coppice stand. The harvesting was observed best when the colour of the lower leaves started to change from green to brown and finally turned to yellowish. While as harvesting time started when complete or greater than 75 per cent of leaf fall occur/shedding took place i.e., during dormant period. It is obviously due to the reason that when days becomes shorter and nights longer indicates the harvesting stage. So with the decrease in photoperiod, temperature and number of leaves, photosynthesis decreased and content of abscisic acid increases, which indicated dormant period was best time of harvesting of *Salix triandra* L. These results are in agreement with those recorded by (16,271. The relevant data on green and dry weight of shoots before and after leaf fall is presented in Tables 7 and 8 which reveales that with the increase in age of the coppice the green and dry weight increased significantly. The maximum green weight of shoots was obtained in $T_4$ (four year old coppice) (54.3 t/ha.) and lowest in $T_1$ (fresh plantation) (16.7 t/ha), while as dry weight figures obtained after leaf fall for the treatments were subsequently maximum in $T_4$ (four year old coppice) (23.21 t/ha) and lowest in $T_1$ (fresh plantation) (6.78 t/ha). The maximum shoot weight in $T_4$ (four year old coppice) was obviously due to the maximum number of shoots than other treatments. The variation in yield before leaf fall and after leaf fall was due to the reason of continued photosynthesis. The green and dry yield reached maximum when complete leaf fall took place i.e., when photosynthesis ceases. The moisture per cent calculated did not differ significantly among the treatments because in all the treatments growth was of one

year and moisture per cent ranged between 56.32 to 55.14. Due to the obvious reasons that one year shoots grows fast to capture light and LAI reaches maximum which increases the rate of photosynthesis. These results were in agreement with the findings of Ericsson (1981) and Szezukawski *et al.,* (2005).

**Table 4. Average shoot height of wicker willow (*Salix triandra* L.)**

| Treatment/month | Average shoot height (cm) | | | | | |
|---|---|---|---|---|---|---|
| | **$T_1$ (Fresh plantation)** | **$T_2$ (2 yr. old coppice)** | **$T_3$ (3 yr. old coppice)** | **$T_4$ (4 yr. old coppice)** | **Mean** | **Monthly increment (cm)** |
| 15th April | 19.28 | 20.96 | 20.52 | 19.83 | **20.15** | - |
| 15th May | 47.04 | 60.80 | 54.01 | 53.79 | **53.91** | 33.76 |
| 15th June | 84.12 | 107.05 | 95.49 | 88.53 | **93.80** | 39.89 |
| 15th July | 113.13 | 167.84 | 155.51 | 141.52 | **149.50** | 55.7 |
| 15th August | 174.42 | 188.30 | 178.92 | 160.76 | **175.60** | 26.1 |
| 15th September | 189.21 | 195.12 | 183.96 | 165.80 | **183.52** | 7.92 |
| 15th October | 194.29 | 197.87 | 186.58 | 170.62 | **187.34** | 3.82 |
| 15th November | 194.58 | 198.06 | 186.64 | 171.27 | **187.64** | 0.30 |
| **Mean** | **129.51** | **142.00** | **132.71** | **121.52** | - | - |

$CD_{(0.05)}$

Month = 5.75 Treatment = 4.07 Month x treatment =11.51

**Table 5. Average collar diameter of wicker willow (*Salix triandra* L.)**

| Treatment/Month | Average collar diameter (cm) | | | | | |
|---|---|---|---|---|---|---|
| | $T_1$ (Fresh plantation) | $T_2$ (2 yr. old coppice) | $T_3$ (3 yr. old coppice) | $T_4$ (4 yr. old coppice) | *Mean* | Monthly increment (cm) |
| 15th April | 0.09 | 0.11 | 0.10 | 0.10 | **0.10** | - |
| 15th May | 0.23 | 0.30 | 0.26 | 0.26 | **0.26** | 0.16 |
| 15th June | 0.50 | 0.78 | 0.64 | 0.60 | **0.63** | 0.37 |
| 15th July | 1.17 | 1.33 | 1.27 | 1.20 | **1.24** | 0.61 |
| 15th August | 1.44 | 1.52 | 1.39 | 1.30 | **1.41** | 0.17 |
| 15th September | 1.55 | 1.64 | 1.42 | 1.33 | **1.48** | 0.07 |
| 15th October | 1.62 | 1.65 | 1.43 | 1.35 | **1.51** | 0.03 |
| 15th November | 1.63 | 1.65 | 1.43 | 1.35 | **1.51** | 0.00 |
| **Mean** | **1.03** | **1.12** | **0.99** | **0.94** | - | - |

$CD_{(0.05)}$

Month = 0.10 Treatment = 0.07 Month x treatment = NS

**Table 6. Average number of leaves/shoot of wicker willow (*Salix triandra* L.)**

| Treatment/ month | Average number of leaves | | | | | Monthly increment (No) |
|---|---|---|---|---|---|---|
| | $T_1$ (Fresh plantation) | $T_2$ (2 yr. old coppice) | $T_3$ (3 yr. old coppice) | $T_4$ (4 yr. old coppice) | *Mean* | |
| 15th April | 7.71 | 8.38 | 8.23 | 7.95 | **8.07** | - |
| 15th May | 1.81 | 24.31 | 21.60 | 21.51 | **21.56** | 13.49 |
| 15th June | 33.64 | 42.81 | 38.19 | 35.40 | **37.51** | 15.95 |
| 15th July | 53.91 | 67.13 | 62.20 | 56.60 | **59.96** | 22.45 |
| 15th August | 69.76 | 75.31 | 71.56 | 64.30 | **70.23** | 10.27 |
| 15th September | 75.67 | 78.04 | 73.58 | 66.31 | **73.40** | 3.17 |
| 15th October | 77.71 | 79.14 | 74.63 | 68.24 | **74.93** | 1.53 |
| 15th November | 77.82 | 79.22 | 74.65 | 68.50 | **75.05** | 0.12 |
| **Mean** | **51.88** | **56.79** | **53.08** | **48.60** | - | - |

$CD_{(0.05)}$

Month = 2.34 Treatment = 1.65 Month x treatment = 4.68

Processing is important for storage of raw material. After boiling for 10-12 hours withies or rods debarked by fastening the bark in the two wooden sticks embedded 1/3 in the soil. Then dried for a week time under sun light. Boiling softens the bark and bark tannins was absorbed into stem wood which gives buff tint to rod and make it water proof and increases its durability. As well as prevents the raw material from deterioration, increases strength and quality. The sun drying is found to be most cost effective and the rods dried 10 times slower with bark than debarked because maximum moisture per cent is present in bark (Gigler *et al.,* 200).

**Table 7. Average green and dry weight of shoots (t/ha) and moisture percentage of wicker willow (*Salix triandra* L.) before leaf fall on 15th September**

| Treatment | Green weight (t/ha) | Dry weight (t/ha) |
|---|---|---|
| $T_1$ (Fresh plantation) | 16.7 | 6.78 |
| $T_2$ (2 yr. old coppice) | 34.6 | 14.20 |
| $T_3$ (3 yr. old coppice) | 42.4 | 18.24 |
| $T_4$ (4 yr. old coppice) | 54.3 | 23.21 |
| **CD (0.05)** | **0.73** | **0.17** |

**Table 8. Average green/dry yield in (t/ha) of wicker willow (*Salix triandra* L.) after leaf fall on 15th November**

| Treatment | Green yield (t/ha) | Dry yield (t/ha) |
|---|---|---|
| $T_1$ (Fresh plantation) | 17.5 | 7.85 |
| $T_2$ (2 yr. old coppice) | 35.2 | 15.76 |
| $T_3$ (3 yr. old coppice.) | 43.5 | 19.00 |
| $T_4$ (4 yr. old coppice) | 56.3 | 25.10 |
| **CD (0.05)** | **1.41** | **1.62** |

Raw material after processing were graded on the basis of rod length and diameter. The average rod length varied from 162.9 to 187.3 cm and average diameter 1.26-1.54 cm as depicted in Table 9. It indicates that average length falls in the medium and diameter in the thick grade – these dimensions are in agreement with the dimensions of standards reported by (RomeroAblos (1988).

Average rod length and diameter (Table 10) after processing were measured which revealed that the maximum length and diameter was found in $T_2$ (two year old coppice) (187.30 and 1.54 cm) followed by $T_1$ (fresh plantation) (186.50 and 1.52 cm), $T_3$ (three year old coppice) (175.74 and 1.34 cm) and $T_4$ (four year old coppice) 162.90 and 1.26 cm). Statistical analysis revealed that length of the rods differed significant among the treatments, while as collar diameter was not found significant among the treatments. The rod

length and collar diameter was found less than that recorded before processing due to obvious reasons that during processing the bark and succulent tips were removed. The bark and tips removed contributed nearly 25 per cent of its total weight. These results were in confirmation with the findings of Decei (1975).

**Table 9. Dimensions of basket willow of (*Salix triandra* L.) (wicker willow)**

| Grade | Required length (cm) | Observed length (cm) | Required diameter (cm) | Observed diameter (cm) |
|---|---|---|---|---|
| Thin | 80-160 | - | 0.2-0.4 | - |
| Medium | 180-280 | 162.90-187.30 | 0.5-1.1 | - |
| Thick | 300-400 | - | 1.2-1.5 | 1.26-1.54 |

**Table 10. Average rod (withies) diameter and length after processing of wicker willow (*Salix triandra* L.) (cm)**

| Treatment | *Diameter after debarking (cm) | Length after debarking (cm) |
|---|---|---|
| $T_1$ (Fresh plantation) | 1.52 | 186.5 |
| $T_2$ ( 2 yr. old coppice) | 1.54 | 187.3 |
| $T_3$ (3 yr. old coppice) | 1.34 | 175.74 |
| $T_4$ (4 yr. old coppice) | 1.26 | 162.90 |
| **CD $_{(0.05)}$** | **NS** | **6.49** |

* Diameter recorded from thick end

## CONCLUSIONS

Results from the study had showed that the soil pH was neutral to acidic, EC medium, organic carbon high with available NPK, low, high and medium respectively. Sprouting vigour was maximum in $T_3$ (three year old coppice) and $T_4$ (four year old coppice) which commenced and completed earlier than $T_2$ (two year old coppice) and $T_1$ (fresh plantation). The number of shoots per coppice was the deciding factor in contributing yield. However, the bark contributed nearly 25 per cent of the total weight. Dimension of processed rods falls in medium category in length and thick category in diameter.

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# An Assessment of Epigeal Invertebrate Community in Cement Polluted and Non-polluted Areas

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**ABSTRACT**

Comparative assessment of the epigeal macroinvertebrate community of cement polluted and non-polluted areas, with almost identical physiographic and geophysical features was carried out during 2002. The study revealed significant differences in species composition, relative density, importance value index and biomass. 17 species composed the community in non-polluted area as against only 7 species in the polluted area. The community of non-polluted area included six species of araneids; five orthopterans; two coleopterans and one species each of Diptera, Hymenoptera and Pulmonata. The polluted site community comprised of four araneid species; two orthopterans and a coleopteran species. Most of the epigeals appeared to be herbivores and higher species number and biomass in non-polluted area was most probably attributable to the availability of fresh vegetation without much particulate matter deposition on the herb layer. In terms of fresh biomass the fauna exhibited a fairly higher accumulation in non-polluted area.

***Key words*:** Macroinvertebrates, epigeal, cement polluted.

## INTRODUCTION

India, one of the leading developing countries, has undergone rapid industrialization in the few decades of near past. Besides steel and power the cement production of India is recognized as one of the most important industries. The consumption pattern of cement often denotes economic development of any nation. The rapid and unsafe growth of various industries in the last 50 years has however, resulted in remarkable deterioration of the biosphere. Decline in natural resources and increase in hazardous materials/chemicals in environment is estimated to cause about 7 to 10% loss of GDP in India. Macroinvertebrates constitute an important group of soil fauna playing vital multiple role in food chain. The changes in soil environmental complex have adversely affected the structure and function of soil macroinvertebrate populations. Air emissions can cause reductions in soil organism and shifts in trophic structure as studies have indicated an inverse relationship between ground beetle population numbers and sulfur dioxide emissions (Freitage and Hastings, 1973) and zinc in soil causing decline in earthworm densities (Bengtsson *et al.,* 1983).

In the agroclimatic region of Kashmir of Jammu and Kashmir state Khunmoh – Khrew Karewa belt has lately been identified for exploitation of cement because of the limestone lithological characteristics of the area. The present investigation was taken up as a case study to assess the impact the cement dust has on the epigeal invertebrate community structure in the area.

## STUDY AREA

The cement polluted and non-polluted sites were found to have the following salient features.

1. **Non-polluted site**
   This site was located (34° 04′ to 34° 11′N longitude and 74° 54′ to 75° 09′ E) longitude, just outside the official boundary of Dachigam National park.

2. **Polluted site**
   This site (34° 1′ N latitude to 75° 1′ E) longitude was located about 25km. South of Srinagar city at Khrew and was in the very close vicinity of cement factory.

   Both the cement polluted as well as non-polluted sites possessed the identical aspects i. e. were south-facing and were located at the same altitude i. e. 1680m from msl. The floristic community of the two areas was similar. An area of $100m^2$ was demarcated at each site for sampling of the epigeal invertebrates.

## MATERIAL AND METHODS

Monthly sampling was performed in both the areas for purpose of recording of the epigeal fauna after using random quadrat method. Collapsible quadrat of $1m^2$ area covered over by mosquito net was used. The parameters evaluated were relative density and importance value index following Dwivedi and Chattoraj (1984) and Misra (1989). Biomass (fresh weight $gm/m^2$) of epigeal invertebrates was evaluated by weighing organisms within 12 hours of their collection. The relative density was computed by the following formula:

## RESULTS AND DISCUSSION

### Community Composition and Occurrence of Species

The epigeal invertebrate community of the two study areas was comprised of 18 species. 94.44% i. e. seventeen species were found to comprise the community in the non-polluted area while in the polluted area the species recorded represented 33. 33% (7species). The group wise distribution of the 17 species of non-polluted area included: Six species of araneids; five species of orthopterans; two species of coleopterans, one species each of Diptera, Hymenoptera and Pulmonata. The distribution of 7 species ( 38.88% ) inhabiting the cement polluted area were four species of araneids, two species of Orthoptera and one species of coleopteran.

One of the reasons for the higher percent species occurrence of epigeal macroinvertebrates in the non – polluted area as against the cement dust polluted area of Khrew was probably attributable to availability of wider vegetation spectrum to the herbaceous / herbivorous fauna.

Most of the epigeal invertebrates were herbivores and the high species number in non-polluted area, most probably seemed to be to the availability of fresh, lush and without much particulate matter deposited herb layer, the products of which form their food. In terms of fresh biomass also the epigeal fauna showed significantly higher accumulation in non-polluted area. The population of *G. bimaculatus, G. africanus* and *H. goansis* was seen to exist in its highest relative density at non-polluted site, presumably because of availability of uncontaminated, sufficient and varied food in such a structurally heterogenous habitat.

A total of 5species of epigeal orthopteran species were recorded from the non-polluted site of Dachigam which were not similar to those of the earlier enumerations of Bhat and Qadri (1999) who have reported 16 species from the same area. The decline in species number might be due to the change in the environmental complex including the continuous drought of about five consecutive years. Only 2 orthopteran species were recorded from the polluted area lying just across the cement factory at Khrew. In the non-polluted area the species might also have locally migrated to suitable patches of habitats.

The orthopteran group was represented by the 5 specie which included *Acrida exaltata, Dicranophyma babaulti, Gastrimargus africanus, Gryllus bimaculatus* and *Leva* sp. The orthopteran group not only depicted remarkable difference in species composition between the two sites, but also the biomass ($gm/m^2$) of the species showing a difference of significant magnitude with lower biomass values recorded at the cement polluted site as compared to the non-polluted site.

**Relative density**

The highest relative density of 85. 71 (Table 1) in the non-polluted area was recorded for the orthopteran *Gryllus bimaculatus* and lowest of 66.66% for the araneid *Herphyllus goansis*. On the contrary *Herphyllus goansis* alone appeared to exhibit the highest relative density of 60 while lowest of 20% each was recorded for *Arctosa* sp; *Salticus* sp. (both araneid species); and the orthopteran *Gryllus bimaculatus* in polluted area. Higher relative density of most of these species is most probably related to their diurnally cryptozoic and nocturnal habit of emergence for their feeding etc.

**Importance Value Index**

In non-polluted area the highest value of 227.37 was exhibited by *Gryllus bimaculatus* followed by *Gastimargus africanus* (222.50), while the lowest importance value index of 25. 48 was recorded for *Scolopendra morsitans* followed by *Asillius* sp. exhibiting the value of 28.42. The highest values for species in polluted area were recorded for the cryptozoic araneid *H. goansis* (300.00), followed by the orthopteran *Leva* sp. (89.93) and lowest of 60.00 each for the non – cryptozoic araneid populations of *Pholcus* sp. and *Salticus* sp. (Table 2).

The importance value index for sixteen species is depicted in Fig. 1.

**Biomass**

The details of biomass (fresh weight gm / $m^2$) in respect of epigeal invertebrates are depicted in Table 3. There was a remarkable contrast with regard to the parameter between the polluted and non-polluted area. The dipteran *Asillius* sp. and the hymenopteran *Cimbex sp*. were detected as exclusives to the non-polluted area exhibited a biomass of 0.02 gm/$m^2$ and 0.24 gm/$m^2$ respectively. The land snail *Zebrina dextrosinister* with a biomass of 0. 80 g/$m^2$ was also found to exist only in the non-polluted area probably indicative of the sensitiveness of this land snail to cement pollution.

**Table1. Relative density of epigeal populations in areas with cement dust pollution and with no pollution**

| S. No. | | April | | May | | June | | July | | August | | September | | October | | November | | December | | January | | February | | March | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N |
| | **Aranieda** | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | *Arctosa* sp. | - | - | - | - | - | - | - | 50 | - | - | - | - | 20 | 15.4 | - | - | 20 | - | - | - | - | - | - | 14.3 |
| 2 | *Herphyllus goansis* | - | - | - | 13 | - | - | 43 | - | - | - | 12.5 | - | - | - | - | - | 60 | - | - | 66.7 | - | - | - | - |
| 3 | *Lycosa* sp. | - | - | - | - | - | - | - | - | - | - | - | 40 | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | *Mantis* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 20 | - | - | - | - | - | - | - | - |
| 5 | *Pholcus* sp. | - | - | - | - | - | - | - | - | - | - | - | - | 20 | - | - | 20 | - | - | - | - | - | - | - | - |
| 6 | *Salticus* sp. | - | - | - | 13 | - | - | - | - | - | - | - | - | 20 | - | - | - | - | - | - | - | - | - | - | - |
| | **Chilopoda** | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | *Scolopendra morsitans* | - | - | - | 6 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **Coleoptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | *Carabus* sp. | - | - | - | 31 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | *Copris* sp. | - | - | - | - | - | - | 29 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 10 | *Bolbocerus* sp. | - | - | - | - | - | - | - | 50 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **Diptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 11 | *Asillius* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 6.66 | - | - | - | - | - | - | - | - |
| | **Hymenoptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 12 | *Cimbex* sp | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 6.66 | - | - | - | - | - | - | - | - |
| | **Mollusca (Pulmonate)** | | | | | | | | | | | | | | | | | | | | | | | | |
| 13 | *Zebrina dextrosinister* | - | - | - | 13 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **Orthroptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 14 | *Acrida exaltata* | - | - | - | - | - | - | - | - | - | 13 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | *Dicranophyma babaulti* | - | - | - | 13 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 16 | *Gastrimargus africanus* | - | - | - | - | - | - | - | - | - | 60 | - | 13.3 | - | 84.6 | - | 26.7 | - | - | - | - | - | - | - | - |
| 17 | *Gryllus bimaculatus* | - | - | - | 13 | - | - | - | - | - | 13 | - | 13.3 | 20 | - | - | - | 20 | - | - | - | - | - | - | 85.7 |
| 18 | *Leva* sp. | - | - | - | - | - | - | 29 | - | - | 13 | - | 13.3 | 20 | - | - | 20 | - | - | - | 33.3 | - | - | - | - |

*P*= cement polluted area N* = Non – polluted area*

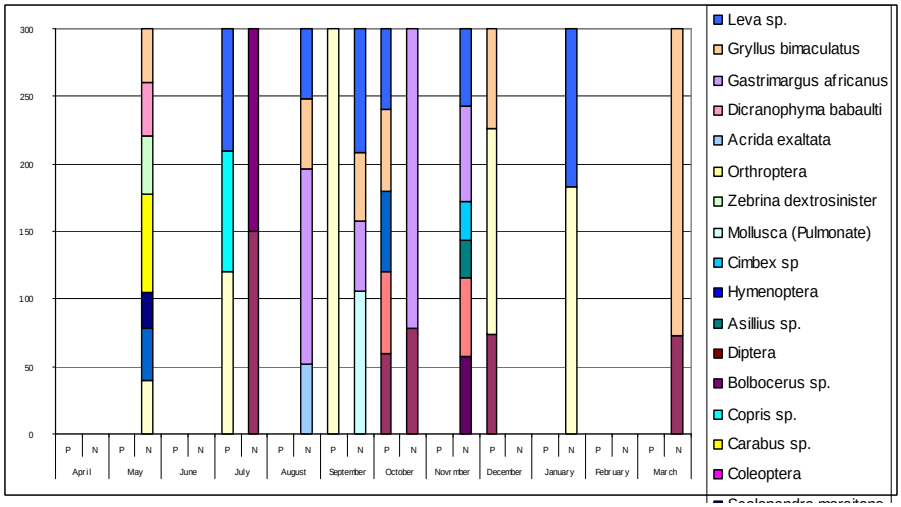
**Table 2. Monthly variations in IVI of 16 epigeal macroinvertebrate species in polluted and non-polluted areas**

| S. No. | | April | | May | | June | | July | | August | | September | | October | | November | | December | | January | | February | | March | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N |
| | **Aranieda** | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | *Arctosa* sp. | - | - | - | - | - | - | | 150 | - | - | - | - | 60 | 78 | - | - | 74 | - | - | - | - | - | - | 73 |
| 2 | *Herphyllus goansis* | - | - | - | 39 | - | - | 120 | - | - | - | 300 | - | - | - | - | - | 153 | - | - | 183 | - | - | - | - |
| 3 | *Lycosa* sp. | - | - | - | - | - | - | - | - | - | - | - | 106.4 | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | *Mantis* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 57.6 | - | - | - | - | - | - | - | - |
| 5 | *Pholcus* sp. | - | - | - | - | - | - | - | - | - | - | - | - | 60 | - | - | 57.6 | - | - | - | - | - | - | - | - |
| 6 | *Salticus* sp. | - | - | - | 39 | - | - | - | - | - | - | - | - | 60 | - | - | - | - | - | - | - | - | - | - | - |
| | **Chilopoda** | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | *Scolopendra morsitans* | - | - | - | 25 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **Coleoptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | *Carabus* sp. | - | - | - | 73 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | *Copris* sp. | - | - | - | - | - | - | 90 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 10 | *Bolbocerus* sp. | - | - | - | - | - | - | - | 150 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **Diptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 11 | *Asillius* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 28.4 | - | - | - | - | - | - | - | - |
| | **Hymenoptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 12 | *Cimbex* sp | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 28.4 | - | - | - | - | - | - | - | - |
| | **Mollusca (Pulmonate)** | | | | | | | | | | | | | | | | | | | | | | | | |
| 13 | *Zebrina dextrosinister* | - | - | - | 43 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **Orthroptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 14 | *Acrida exaltata* | - | - | - | - | - | - | - | - | - | 52 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | *Dicranophyma babaulti* | - | - | - | 39 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 16 | *Gastrimargus africanus* | - | - | - | - | - | - | - | - | - | 144 | - | 51.13 | | 223 | - | 70.2 | - | - | - | - | - | - | - | - |
| 17 | *Gryllus bimaculatus* | - | - | - | 39 | - | - | - | - | - | 52 | - | 51.13 | 60 | - | - | - | 74 | - | - | - | - | - | - | 227 |
| 18 | *Leva* sp. | - | - | - | - | - | - | 90 | - | - | 52 | - | 91.31 | 60 | - | - | 57.6 | - | - | - | 117 | - | - | - | - |

**Table 3. Fresh average biomass (gm/m$^2$) of epigeal invertebrate species from polluted and non-polluted areas.**

| S.No | Order/Species | April | | May | | June | | July | | August | | September | | October | | November | | December | | January | | February | | March | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N | P | N |
| | **Aranieda** | | | | | | | | | | | | | | | | | | | | | | | | |
| 1 | *Arctosa* sp. | - | - | - | - | - | - | | 0.1 | - | - | - | - | 0.1 | 0.1 | - | - | 0.1 | - | - | - | - | - | - | 0.1 |
| 2 | *Herphyllus goansis* | - | - | - | 0 | - | - | 0 | - | - | - | 0 | - | - | - | - | - | 0.1 | - | - | 0.08 | - | - | - | - |
| 3 | *Lycosa* sp. | - | - | - | - | - | - | - | - | - | - | - | 0.4 | - | - | - | - | - | - | - | - | - | - | - | - |
| 4 | *Mantis* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.09 | - | - | - | - | - | - | - | - |
| 5 | *Pholcus* sp. | - | - | - | - | - | - | - | - | - | - | - | - | 0 | - | - | 0.05 | - | - | - | - | - | - | - | - |
| 6 | *Salticus* sp. | - | - | - | 0 | - | - | - | - | - | - | - | - | 0 | - | - | - | - | - | - | - | - | - | - | - |
| | **Chilopoda** | | | | | | | | | | | | | | | | | | | | | | | | |
| 7 | *Scolopendra morsitans* | - | - | - | 0.4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **Coleoptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 8 | *Carabus* sp. | - | - | - | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 9 | *Copris* sp. | - | - | - | - | - | - | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 10 | *Bolbocerus* sp. | - | - | - | - | - | - | - | 0.3 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **Diptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 11 | *Asillius* sp. | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.02 | - | - | - | - | - | - | - | - |
| | **Hymenoptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 12 | *Cimbex* sp | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | 0.24 | - | - | - | - | - | | - | - |
| | **Mollusca (Pulmonate)** | | | | | | | | | | | | | | | | | | | | | | | | |
| 13 | *Zebrina dextrosinister* | - | - | - | 0.8 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| | **Orthroptera** | | | | | | | | | | | | | | | | | | | | | | | | |
| 14 | *Acrida exaltata* | - | - | - | - | - | - | - | - | - | 0.1 | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 15 | *Dicranophyma babaulti* | - | - | - | 0.4 | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - |
| 16 | *Gastrimargus africanus* | - | - | - | - | - | - | - | - | - | 5.5 | - | 0.24 | | 5.5 | - | 2.22 | - | - | - | - | - | - | - | - |
| 17 | *Gryllus bimaculatus* | - | - | - | 0.3 | - | - | - | - | - | 0.2 | - | 0.2 | 0.1 | - | - | - | 0.1 | - | - | - | - | - | - | 0.7 |
| 18 | *Leva* sp. | - | - | - | - | - | - | 0 | - | - | 0 | - | 0.29 | 0.1 | - | - | 0.14 | - | - | - | 0.12 | - | - | - | - |

P*= cement polluted area N* = Non – polluted area




**P*= cement polluted area N* = Non – polluted area**

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# MICROBIOLOGY

# Evaluation of Antimicrobial Activity of Aqueous Extract of *Marrubium vulgare* L.

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## ABSTRACT

The antimicrobial activity of aqueous extract of *Marrubium vulgare* L. whole plant was tested by disc diffusion method. Zones of Inhibition produced by aqueous extract in a concentration of 200, 400 and 600 mg/ml against selected bacterial and fungal strains was measured and compared with those of standard discs of antibiotic ciprofloxacin (10 µg/ml).

***Key words:*** Antimicrobial activity, Ciprofloxacin, *Marrubium vulgare.*

## INTRODUCTION

From centuries natural products have been used to prevent or cure infectious diseases. Many of these plants have been investigated scientifically for antimicrobial activity and a large number of plant products have been shown to inhibit the growth of pathogenic microorganisms. *Marrubium vulgare* L. (Lamiaceae) commonly known as "horehound" is naturalized in North and South America, the Mediterranean region and Western Asia. In India it is found in Kashmir at an altitude of 5,000-8,000 ft. It is a tall robust herbaceous perennial herb, 40-120 cm high, densely covered, especially when young, with a thick white cottony felt (Robert and Henry, 1880). It possesses expectorant, diaphoretic and diuretic properties. It is helpful for bronchial asthma and non-productive cough. It was formerly much esteemed in various uterine, visceral and hepatic ailments and in phthisis (Chopra *et al.*, 1956). The plant is reported to possess hypoglycemic (Roman *et al.*, 1992), antihypertensive (El-Bardai *et al.*, 2004), analgesic (DeSouza *et al.*, 1998), vasorelaxant (El-Bardai *et al.*, 2003b), anti-inflammatory (Sahpaz *et al.*, 2002a), antioxidant (Weel *et al.*, 1999), antioedematogenic (Stulzer *et al.*, 2006) and many other reported biological activities. The plant is reported to contain phenylethanoid glycoside, marruboside (Sahpaz *et al.*, 2002b), caryophyllene oxide, trans-caryophyllene (Asadipour *et al.*, 2005), caffeoyl-l-malic acid, acteoside (Sahpaz *et al.*, 2002a), vulgarol, β-sitosterol, lupeol and marrubiin (Amer, 1993) respectively.

The present study was undertaken to demonstrate the antimicrobial activity of aqueous extract of *Marrubium vulgare* whole plant against some bacterial and fungal strains.

## MATERIAL AND METHODS

### Collection of Plant Material

The whole plant of *Marrubium vulgare* was collected in the month of August from Nawhatta, Srinagar, Jammu and Kashmir.

Whole plant of *Marrubium vulgare* was dried in shade and crushed to fine powder. The plant material (100 g) was dried and crushed to coarse powder and then extracted with water by using cold extraction method till completely exhausted. The aqueous extract thus obtained was dried on the water bath to yield 18 gm of extract. Crude extract thus obtained was tested for the anti-microbial activity against various bacterial and fungal strains. These strains were obtained from MTCC Chandigarh, India.

### Screening for Antibacterial Activity

Sterile nutrient agar plates were prepared and incubated at 37°C for 24 hours to check for any sort of contamination. Sterile filter paper discs (Whatman No.1) of 6 mm diameter were soaked in three different dilutions of the aqueous extract and placed in appropriate position on the surface of the plate marked as quadrants at the back of the petri dishes. The *in-vitro* antimicrobial activity of *M. vulgare* in concentration of 200, 400 and 600 mg/ml was studied by disc diffusion method (Pelczar *et al.*, 1993) against *Escherichia coli, Bacillus subtilis, Staphylococcus aureus, Staphylococcus epidermidis, Proteus vulgaris* and *Candida albicans*. The petri dishes were incubated at 37°C for 18 hours and the diameter of the zone of inhibition measured in mm. The activity of the aqueous extract was compared with ciprofloxacin (10 µg/ml). The zone of inhibition was calculated by measuring the dimensions of the zone of no microbial growth around the disc. An average of three independent determinations was recorded (Table 1).

## RESULTS AND DISCUSSION

The aqueous extract of *M. vulgare* exhibited moderate to significant and concentration dependent antibacterial activity against tested bacterial strains using ciprofloxacin 10 (µg/ml) as standard. The study revealed that aqueous extract of the crude drug was very much effective against *S. aureus, S. epidermidis, P. vulgaris* (Gram - positive bacteria) and weakly effective against *E. coli* (Gram - negative bacteria). However, no activity was observed against *C. albicans*. Further phytochemical studies are needed to identify active constituents responsible for the observed activity.

**Table 1. Evaluation of antimicrobial activity of aqueous extract of *Marrubiumvulgare* L**

| Microorganisms | Zones of Inhibition* (mm) (mg/ml) | | | Ciprofloxacin (10 µg/ml) |
|---|---|---|---|---|
| | 200 | 400 | 600 | |
| *Proteus vulgaris* MTCC 426 | 0 | 09 | 15 | 22 |
| *Bacillus subtilis* MTCC 619 | 11 | 15 | 22 | 30 |
| *Staphylococcus epidermidis* MTCC 435 | 09 | 14 | 19 | 25 |
| *Staph. aureus* MTCC 740 | 09 | 14 | 19 | 22 |
| *Escherichia coli* MTCC 443 | 0 | 10 | 15 | 25 |

* Zone of inhibition (mm) are average of triplicate experiments. Disc diameter = 6 mm.

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# A Preliminary Study of Soil Bacteria of Kashmir University Campus

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**ABSTRACT**

A preliminary study on soil bacteria of Kashmir University Campus was carried out to assess their number and diversity. The campus comprises of two distinct areas which differ from each other markedly in terms of biotic and abiotic factors. The total viable count of Naseem Bagh Campus soil ($8\times10^4$ cfu/g) was found to be more than Main University Campus soil ($4.2\times10^4$ cfu/g). A total of 20 isolates of bacteria were recovered out of which 80% were Gram positive cocci. Almost all isolates showed fair utilization of carbohydrates like glucose, fructose. The isolates recovered from Main Campus exhibited higher tolerance to salt (0.5 – 3.5 % NaCl) concentration than those from Naseem Bagh Campus. Antibiotic sensitivity test done against four antibiotics Streptomycin (S), Amoxicillin (Am), Erythromycin (E), and Cloxacillin (Cx) revealed high degree of resistance, with only 8 isolates showing susceptibility.

***Key words:*** Soil bacteria, Kashmir University Campus, diversity

## INTRODUCTION

Fertile soil is inhabited by the root system of higher plants, many animal forms (e.g., rodents, insects & worms), and by tremendous numbers of micro-organisms. Soil microbial population is the key element in the biogeochemical cycling of nutrients in nature (Pelczar, *et. al.*1993). They make possible the cycles of carbon, oxygen, sulphur and nitrogen that take place in terrestrial and aquatic ecosystems. Micro-organisms are thus a source of nutrients at the base of all ecological food chains and webs.

The number and kind of bacteria found in different types of ecosystems vary and are influenced by the ecosystem processes maintaining plant primary productivity (Griffiths *et al.*, 2003). Most of the soil bacteria are decomposers that consume simple organic compounds, such as root exudates and fresh plant litter. The growth of bacteria in soil, like other microbes, is influenced by factors like the amount and type of nutrients available, moisture, degree of aeration, temperature, pH, etc. The existence of roots and extensiveness of the root system in soil also influence the numbers and kinds of bacteria in it.

The Campus of University of Kashmir, situated on the banks of famous Dal lake, comprises of two distinct areas namely Naseem Bagh Campus and Main Campus. The two campuses differ from each other in terms of vegetation cover, temperature, shade, amount of sunlight, etc., and hence differentiated into two

microclimatic habitats. A comparative study carried out on the physico-chemical characteristics of the soils of two campuses (Reyaz and Bhat, 2004; Kangroo and Bhat, 2005) have shown appreciable differences in the same. Since the physico-chemical and structural characteristics of soil provide many microenvironments in which bacterial populations can evolve (Ranjard and Richaume, 2001), a preliminary study on soil bacteria of the campus was considered valuable.

## MATERIAL AND METHODS

The present study was conducted between May 2007 to September 2007 in the two comparable microhabitats of Kashmir University i.e., Naseem Bagh (Site A) which is dominated by Chinar trees and can be regarded as a woodland habitat and the Main University Campus (Site B) which is almost a type of open grassland.

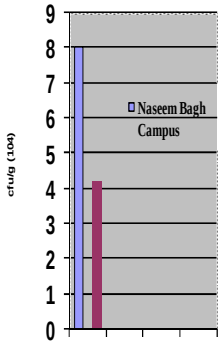
Composite soil samples were collected from the two sites by digging up to a depth of 5 inches with the help of spade. Samples were collected in sterile polythene bags and carried to the laboratory for bacteriological analysis. Analysis was done within 2 to 4 hours until which the samples were stored at room temperature.

Quantitative determination of bacteria was done by dilution plate method using spread plate technique (Cappucinno and sherman., 1992; Taylor *et al.,* 1983). Appropriate dilutions were spread on petri plates, two replicates for each dilution, containing nutrient agar as growth medium. The plates were incubated at $28\pm2^{o}C$ temperature in an incubator. A complete record of all the bacterial colonies appearing in each plate was maintained, and the bacterial count per gram of soil was calculated (Waksman, 1952).

Bacterial cultures isolated were pure cultured by four-way streaking and maintained on nutrient agar slants. Each isolate was given an isolate number on the basis of colony morphology, pigmentation, growth characteristics, etc. Growth behaviour of isolates was evaluated by carbon utilization test and salt tolerance test. Antibiotic sensitivity test of the isolates was carried out using Kirby-Bauer Method using antibiotic discs from Hi Media.

## RESULTS AND DISCUSSION

In the present investigation, heterotrophic bacteria were isolated from the soil of Kashmir University Campus. Total bacterial counts of Naseem Bagh Campus ($8\times10^4$ cfu/g) were found to be twice as much as found in Main Campus ($4.2\times10^4$ cfu/g) (Fig 1). This may be attributed to the differences in various biotic and abiotic factors (Reyaz and Bhat, 2004; Kangroo and Bhat, 2005) that have been found to influence the composition and diversity of soil bacterial communities (Piao *et al.,* 2000; Fierer and Jackson, 2006).




**Values are the mean of 5 samples**
**(cfu = colony forming units)**
**Fig. 1. Bacterial Population of Two Campuses (cfu/gm of soil)**

A total of 20 strains were isolated, purified and designated (as $A_1$, $A_2$, … …., $A_{10}$ for Site A and $B_1$, $B_2$, … … …, $B_{10}$ for Site B) on the basis of colony morphology, pigmentation and microscopic examination as shown in Table 1(a,b). Most of the colonies were small in size and either circular or irregular in shape. About 70% of strains isolated were observed as Gram positive cocci. Some spiral forms were also isolated from Site B. The isolates were further evaluated and characterized for their carbohydrate utilization pattern as shown in Table 2(a, b). All the isolates utilized glucose and fructose fairly well whereas lactose and mannitol were utilized only by 45% and 35% of the strains respectively. Similar results have been reported by Sheikh *et al.* (2006) on *Azotobacter spp*.

The growth behavior of the isolates against different concentrations of salt (NaCl -0.5-3.5%) was also evaluated (Table 3 a, b). It was observed that with the increase in salt concentration from 0.5% to 3.5%, the number of isolates showing growth decreased. For the ten isolates taken from Naseem Bagh campus, no inhibition in growth was observed at 0.5% salt concentration. At 1% concentration, 90% of strains showed maximum growth and at 2% and 3.5% salt concentration only 40% and 30% of the strains were tolerant respectively. Similar trend was observed in isolates from Main University Campus. Such decreasing trends to tolerance towards increasing salt concentrations have also been reported by Raj Kumar (1993) and Sheikh *et, al.,* (2006). However, in case of isolates from Main University campus higher tolerance to the increasing salt concentration was observed than those from Naseem Bagh Campus. This finding can be explained by the fact that this campus is manipulated and maintained for aesthetic purposes for which various types of manures and fertilizers were being continually added. The use of fertilizers leads to the formation of high salt

concentrations which can alter the structure and normal functioning of soil microbial complexes (Kravchenko, 1999; Lapygina *et al*, 2002).

**Table 1(a). Colony morphology and microscopic examination of the isolates from Site A**

| Isolate No | Size | Margin | Elevation | Color | Shape | Gram's Reaction | Cell Shape |
|---|---|---|---|---|---|---|---|
| $A_1$ | Moderate | Filamentous | Flat | White | Circular | +ve | B |
| $A_2$ | Very small | Entire | Raised | Yellow | Circular | +ve | C |
| $A_3$ | Very small | Undulate | Convex | White | Circular | +ve | C |
| $A_4$ | Small | Lobate | Flat | Red | Biconvex | +ve | C |
| $A_5$ | Small | Undulate | Raised | Yellow | Biconvex | +ve | C |
| $A_6$ | Small | Entire | Convex | White | Circular | -ve | C |
| $A_7$ | Large | Filamentous | Flat | White | Irregular | -ve | C |
| $A_8$ | Small | Undulate | Flat | Creamish | Irregular | +ve | C |
| $A_9$ | Small | Entire | Raised | White | Circular | +ve | B |
| $A_{10}$ | Small | Undulate | Umbonate | Red | Irregular | +ve | C |

B Bacilli C Cocci %age of cocci forms = 80%; %age of Bacilli forms = 20% %age of Gram +ve forms = 80%; %age of Gram -ve forms = 20%

**Table 1(b). Colony morphology and microscopic examination of isolates from Site B**

| Isolate No | Size | Margin | Elevation | Color | Shape | Gram's Reaction | Cell Shape |
|---|---|---|---|---|---|---|---|
| $B_1$ | Small | Entire | Convex | Red | Circular | -ve | C |
| $B_2$ | Small | Undulate | Flat | White | Irregular | -ve | C |
| $B_3$ | large | Filamentous | Flat | White | Irregular | +ve | C |
| $B_4$ | Small | Entire | Convex | Creamish | Circular | -ve | S |
| $B_5$ | Small | Entire | Convex | White | Circular | +ve | C |
| $B_6$ | Moderate | Undulate | Flat | Creamish | Irregular | +ve | C |
| $B_7$ | Small | Lobate | Umbonate | White | Irregular | +ve | B |
| $B_8$ | Small | Entire | Raised | Creamish | Circular | +ve | B |
| $B_9$ | Small | Entire | Raised | White | Circular | –ve | S |
| $B_{10}$ | large | Undulate | Raised | White | Irregular | +ve | C |

B Bacilli C Cocci S Spiral

%age of Cocci forms = 60%; %age of Bacilli forms = 20%; %age of Spiral = 20%

%age of Gram +ve forms = 60%; %age of Gram -ve forms = 40%

**Table 2(a). Utilization of different carbon sources by isolated strains from Site A**

| Isolate No. | Carbon source | | | |
|---|---|---|---|---|
| | Glucose | Fructose | Lactose | Mannitol |
| $A_1$ | ++ | ++ | + | - |
| $A_2$ | ++ | + | + | - |
| $A_3$ | + | + | - | - |
| $A_4$ | ++ | ++ | + | ± |
| $A_5$ | ++ | + | ± | - |
| $A_6$ | ++ | + | - | - |
| $A_7$ | ++ | ++ | + | ± |
| $A_8$ | + | + | + | ± |
| $A_9$ | ++ | ± | - | - |
| $A_{10}$ | ++ | ++ | + | - |

++ full growth; + moderate growth; ± suppressed growth; - no growth

**Table 2(b). Utilization of different carbon sources by isolated strains from Site B**

| Isolate No. | Carbon source | | | |
|---|---|---|---|---|
| | Glucose | Fructose | Lactose | Mannitol |
| $B_1$ | ++ | ++ | + | ± |
| $B_2$ | ++ | + | ± | - |
| $B_3$ | ++ | ++ | + | ± |
| $B_4$ | + | + | ± | - |
| $B_5$ | ++ | ++ | - | - |
| $B_6$ | ++ | + | ± | ± |
| $B_7$ | ++ | ++ | ++ | - |
| $B_8$ | ++ | + | - | - |
| $B_9$ | ++ | + | - | - |
| $B_{10}$ | ++ | ++ | ++ | ± |

++ full growth; + moderate growth; ± suppressed growth; - no growth

**Table 3(a). Growth behavior of isolates at different salt concentrations (Site A)**

| Strain Codes | Salt concentration (%) | | | |
|---|---|---|---|---|
| | *0.5* | *1* | *2* | *3.5* |
| $A_1$ | ++ | ++ | + | + |
| $A_2$ | ++ | ++ | + | ± |
| $A_3$ | ++ | + | ± | - |
| $A_4$ | + | ± | - | - |
| $A_5$ | ++ | + | ± | - |
| $A_6$ | ++ | ++ | + | + |
| $A_7$ | + | - | - | - |
| $A_8$ | ++ | + | ± | - |
| $A_9$ | ++ | ++ | + | + |
| $A_{10}$ | ++ | + | - | - |

++ full growth; + moderate growth; ± suppressed growth; - no growth

**Table 3 (b). Growth behaviors of isolates at different salt concentrations (Site B)**

| Strain codes | Salt concentration (%) | | | |
|---|---|---|---|---|
| | *0.5* | *1* | *2* | *3.5* |
| $B_1$ | ++ | ++ | + | + |
| $B_2$ | ++ | ++ | ± | - |
| $B_3$ | ++ | + | ± | - |
| $B_4$ | ++ | + | ± | - |
| $B_5$ | ++ | ++ | + | + |
| $B_6$ | ++ | + | + | - |
| $B_7$ | ++ | + | ± | - |
| $B_8$ | ++ | + | + | - |
| $B_9$ | ++ | + | + | ± |
| $B_{10}$ | ++ | ++ | + | ± |

++ full growth; + moderate growth; ± suppressed growth; - no growth

The isolated strains were tested for sensitivity against four antibiotics namely, Streptomycin (S), Amoxicillin (Am), Erythromycin (E) and Cloxacillin (Cx) (Table 4 a, b). The results revealed high degree of resistance against all four antibiotics tested for isolates from both the sites. Amongst the antibiotics tested susceptibility was exhibited only against Streptomycin, 60% in case of Site A and only 30% in case of Site B. In case of other antibiotics almost all the isolates showed resistance which is of the order Am>E>Cx for Site A and Cx> E>Am for Site B. In general, 70% of strains from Site A and 77.5% of strains from Site B were resistant against all four antibiotics tested. About 15% of strains from each site also exhibited intermediate response which implies that the application of a stronger dose would make them susceptible to the drug tested for. Riesenfeld *et al.* (2004) while working on uncultured soil bacteria concluded that soil bacteria are a reservoir of antibiotic resistance genes. Resistance of a single bacterial isolates to more than one antimicrobial drug has also been variously reported (Norelli *et al.,* 1991; Sayah et *al.,* 2005).

**Table 4(a). Antibiotic sensitivity behavior of isolates (Site A)**

| Bacterial strains | Antibiotic Agent | | | |
|---|---|---|---|---|
| | S | $A_m$ | E | $C_x$ |
| $A_1$ | S | R | R | R |
| $A_2$ | S | R | R | I |
| $A_3$ | R | R | I | I |
| $A_4$ | I | R | R | R |
| $A_5$ | R | R | R | I |
| $A_6$ | S | R | R | R |
| $A_7$ | R | R | R | R |
| $A_8$ | S | I | I | R |
| $A_9$ | S | R | R | R |
| $A_{10}$ | R | R | R | R |

S - Streptomycin $A_m$- Amoxicillin E – Erythromycin $C_x$ – Cloxacillin
R – Resistant I – Intermediate S – Susceptible

**Table 4(b). Antibiotic sensitivity behavior of isolates (Site B)**

| Bacterial strains | Antibiotic Agent | | | |
|---|---|---|---|---|
| | **S** | **$A_m$** | **E** | **$C_x$** |
| $B_1$ | S | R | R | R |
| $B_2$ | R | R | I | R |
| $B_3$ | I | R | R | R |
| $B_4$ | R | R | R | R |
| $B_5$ | S | R | R | R |
| $B_6$ | R | R | R | R |
| $0B_7$ | I | I | R | R |
| $B_8$ | R | R | R | R |
| $B_9$ | R | I | R | R |
| $B_{10}$ | S | I | R | R |

S - Streptomycin $A_m$- Amoxicillin E – Erythromycin $C_x$ – Cloxacillin
R – Resistant I – Intermediate S – Susceptible

From the present study it may thus be concluded that the bacteria isolated from the soils of the University Campus are mostly gram positive cocci and the counts obtained from Naseem Bagh Campus are twice as much as those obtained from Main University Campus. From carbohydrate utilization test it may be concluded that glucose and fructose are better utilized as sources of carbon than lactose and mannitol. The strains have revealed high resistance patterns against all the drugs tested with the exception of Streptomycin. Almost all isolates from Main Campus showed resistance to Cloxacillin. Since the problem of drug resistance is gaining importance, the present investigation may be of value in this direction. The salt tolerance test done shows some isolates to be more tolerant towards increased concentration of salt (NaCl).This test needs a detailed investigation to reveal the changing salt (NaCl) pattern in the soils and its effect on soil micro-organisms.

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# Interaction and Biodiversity of Vesicular Arbuscular Mycorrhizal Fungi Associated with Some Medicinal Plants of Mid-Mountain Range of Himachal Pradesh

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## ABSTRACT

The present investigation was focused on the study of endomycorrhizal status of some medicinally important plants growing in mid mountain range of Himachal Pradesh. Twenty one plants, belonging to fifteen families were examined for mycorrhizal status and diversity in natural habitat. A total of twenty four different vesicular arbuscular mycorrhizal (VAM) species, belonging to all the six genera of VAM fungi e.g.*Glomus, Acaulospora, Gigaspora, Sclerocystis, Scutellospora* and *Entrophospora* were isolated. It was found that mycorrhizal root colonization ranged from 33.93±4.2% to 89.62±10.0% and highest spore number was observed in *Rosmarinus officinalis* (841±25.9). VAM root colonization was observed in terms of presence of mycelium, vesicles and arbuscules in the cortical region of root.

***Key words:*** VAM, mycelium, vesicles, arbuscules, Himachal Pradesh, medicinal plants.

## INTRODUCTION

On our planet earth, we feel blessed to be surrounded by natural resources which benevolent God has provided us in abundance. Among all natural resources, one, that is rendering its valuable support to sustain human race, is plantation. Plants are one of the most important sources of medicine. Medicinal plants are important for the socio- economic upliftment of human being. Almost every civilization has a history of medicinal plant use. They have been subject of man's curiosity since time immemorial.

One of the richest reservoirs of biological diversity in the world is, the Indian Himalayan Region (IHR). Himachal Pradesh is a store house of biological wealth having potential for cultivation of herbs particularly medicinal and aromatic plants. Medicinal plants play an important role in the life of people living in rural and urban areas of Himachal Pradesh. Not only their primary health care need and dietary traditions are based on medicinal plants, but these also contribute towards the rural economy. With the increasing national and global trade in medicinal plants, this invaluable resource has come under pressure. This led to cultivation of these medicinal plants to support the increasing demand. About 85% of the world's flowering plants and trees, on which human life depend, form close root association with micro-organisms like fungi, mycorrhiza and bacteria. Mycorrhizal fungi are a key member of soil micro-biota and conduct activities which are crucial to plant establishment, development, nutrition and health.

VAM are recognized as most common type of mycorrhiza with diverse host range (Gerdemann, 1968). VAM fungi are known to be helpful in phosphorus uptake (Schweiger *et. al.,* 2007), bioremediation (Li *et al.,* 2006), enhancing plant growth (Javot *et al.,* 2007), Protection against toxicity (Aggarwal *et al.,* 1999), drought tolerance (Auge and Moore, 2005), increase in photosynthetic activity (Bethlenfolvay *et al.,* 1988) and fertility of soil (Charles *et al.*, 2006). The study of endomycorrhizal biodiversity on some medicinal plants is, therefore, necessary from efficient utilization and conservation point of view. Considering the importance of medicinal plants in Himachal Pradesh, an investigation was carried out to study the endomycorrhizal status of these medicinal plants and to select the predominant VAM fungi for future inoculation studies for production of quality seedling of important plants in nurseries and their better survival in adverse conditions.

## MATERIAL AND METHODS

**Study Site:**

Root and soil samples of medicinally important plants were collected from mid-mountain or inner Himalayan region of Himachal Pradesh during 2007-2008.

**Collection of soil sample:**

It was done by digging out a small amount of soil close to plant's roots, up to the depth of 15-30 cm and these soil samples were kept in sterilized polythene bags at 10°C for further processing.

**Isolation and quantification of VAM fungi:**

Isolation of VAM spores was done by using 'Wet Sieving and Decanting Technique' of Gerdemann and Nicolson (1963). 50 gram of soil was mixed in water in a small plastic container having a capacity of about 1000 ml. The soil was thoroughly mixed with water and allowed to settle down overnight. The water was decanted on a series of sieves in the following order 150µm, 120µm, 90µm, 63µm, 45µm. from top to bottom on which spore were trapped. The trapped spores were then transfered to the Whatmann filter paper No.1 by repeated washing with water and were counted. The quantification of VAM spores were done by 'Grid Line Intersect Method' (Adholeya and Gaur, 1994). The spores were picked by hypodermic needle under stereo binocular microscope. The spores were mounted on polyvinyl lactic acid alcohol (PVLA) for further studies.

**Identification of VAM fungi:**

For identification of VAM spores the following criteria was used like conventional morphological characters i.e. colour, size, shape, wall structure, surface ornamentations of spores and size of subtending hyphae, bulbous suspensor, the number and arrangement of the spores in the sporocarps. These VAM spores were identified by using the keys of Walker (1983); Schenk and Perez (1990); Morton and Benny (1990); Mukerji (1996); Morton and Redecker (2002).

**Colonization of VA Mycorrhiza:**

It was studied by 'Rapid Clearing and Staining Method' by Phillips and Haymann (1970). The root segment was washed with water to remove soil particles. It was then cut into 1cm small pieces. Root segment was washed with water and placed in 10% KOH solution at 90°C for half an hour or for 24 hours at room temperature. The KOH was decanted and root was washed with water till the brown colour is cleared. Then these segments were acidified with 1% HCl for 3-5 minutes. After this, the root segment was submerged in 0.5 % Trypan blue for 24 hours. After 24 hours the segment was destained with Lacto phenol. The root was examined in lactic acid or lactic acid: glycerol (1:1) solution. The percentage mycorrhizal root colonization was calculated by following formula:

$$\text{Percentage root colonization} = \frac{\text{Number of roots with infection}}{\text{Total number of root segments studied}} \times 100$$

## RESULTS AND DISCUSSION

Status of endomycorrhizal fungi associated with medicinally important plants of mid mountain region of Himachal Pradesh was determined. A total of twenty one plants, belonging to fifteen families (13 dicot and 2 monocot) were studied (Table 1).

**Table 1. Distribution of some studied medicinal plants of Himachal Pradesh**

| | **Group** | |
|---|---|---|
| | **Dicot** | **Monocot** |
| **Families** | **13** | **2** |
| **Genera** | **18** | **2** |
| **Species** | **19** | **2** |

In last couple of years, a number of reports have been focused on the study of biodiversity of VAM fungi. Mehrotra (2007) studied the diversity of VAM fungi in India and proved that *Glomus* species to be most common species. Singh and Jamaluddin (2007) reported three VAM fungi genera with eight species on *Vitex negundo*. Castillo *et al.* (2006) also studied the diversity of VAM fungi in evergreen forest, deciduous forest and grassland ecosystem of Southern Chill.

In the present investigation mycorrhizal root colonization was observed in the form of presence of mycelium, vesicles and arbuscules. Mycelia of different kinds like H-shaped, Y-shaped, coiled and parallel mycelia were obserbed in the root segments of various plants. Various types of vesicles i.e. round, oval, beaked and elongated were present in the cortical cells. Among the all twenty one plant studied, two plants were having only mycelium, three with mycelium and vesicles, six with mycelium and arbuscules and the ten plants with mycelium, vesicles and arbuscules.

It is envisaged from the observation that the mycorrhizal root colonization ranged from the minimum 33.93±4.2 % to maximum 89.62±10.0 % .The minimum root colonization was observed in *Swertia paniculata* and the maximum in *Hedychium spicatum* (Table 1). Variation in the degree of infection among different plants with in a family was observed by Thapar *et al.* (1992). Similarly, the range of variation in the percentage of VAM root colonization may be due to the effect of host chemicals on growth of VAM fungi (Rahman *et al.* 2003).

The range of sporulation also varied from lowest 50 ± 2.6 to highest 841 ± 25.9. The minimum spore count was observed in *Prunus amygladus* and the maximum spore number in *Rosmarinus officinalis* (Table 2).

The variation in the spore number resulted that the multiplication of spores depends upon species to species level. Muthukumar and Udaiyan (2001) reported that the plant senescence trigger the sporulation of VAM fungi. Similar were the observation of Allen (1991).

It is evident from Table 1 that rate of colonization could not be correlated with the spore number. Similar were the observation made by Scheltema *et al.* (1987). In the present study for e.g. *Hedychium spicatum* showed maximum (89.62 ± 10.0) VAM root colonization, but had fewer numbers of spores (88.3 ± 1.5), while *Citrus medica* possessed more spores number (143 ± 1.4), but had lower rate of root colonization (43.74 ± 1.2). As VAM fungal sporulation is dependent on a wide range of host fungal and environment factors, spore number in natural soil are not always correlated with root colonization level.

**Table 2. Mycorrhizal quantification and root colonization of medicinal plants**

| S.No. | Botanical Name | Local Name | Family | Presence of VAM | % VAM root colonization | VAM spore no./50 gm.soil |
|---|---|---|---|---|---|---|
| **1.** | *Swertia paniculata* Wall. | Chirayata | Gentianaceae | + - + | 33.93 ± 4.2 | *81 ± 2.0 |
| **2.** | *Achillea millefolium* Linn. | Gandana | Asteraceae | + + - | 44.12 ± 7.4 | 74 ± 5.2 |
| **3.** | *Glaucium flavum* Crantz. | Yellow horned poppy | Papaveraceae | + - + | 71.38 ± 14.3 | 184 ± 7.2 |
| **4.** | *Gentiana kurooa* Royle. | Karu | Gentianaceae | + - - | 61.21 ± 1.0 | 112.6 ± 4.1 |
| **5.** | *Macuna prurita* (Linn.) Hook. | Kaunch | Papilionaceae | + + + | 86.10 ± 13.9 | 141.3 ± 3.0 |
| **6.** | *Centratherum anthelminticum* (Willd.) Kunze. | Kalijiri | Asteraceae | + + + | 59.01 ± 8.7 | 178 ± 6.2 |
| **7.** | *Digitalis lanata* Linn. | Tilpushpi | Scrophulariaceae | + - + | 72.57 ± 2.5 | 302 ± 5.0 |
| **8.** | *Thallictrum rugosum* Ait. | Pillijari | Ranunculaceae | + + + | 66.66 ± 11.1 | 100.6 ± 3.0 |
| **9.** | *Mentha spicata* Linn. | Sansfi | Liliaceae | + - + | 66.92 ± 11.7 | 112.6 ± 5.0 |
| **10.** | *Asparagus officinalis* Linn. | Geranium | Geraniaceae | + + + | 72.96 ± 14.6 | 253.6 ± 4.9 |
| **11.** | *Geranium wallichianum* Wall. | Lavender | Labiatae | + + + | 84.15 ± 8.9 | 137.5 ± 4.9 |
| **12.** | *Lavendula angustifolia* Mill. | Mushak-Bala | Valarianaceae | + + + | 76.51 ± 4.7 | 246.5 ± 6.3 |
| **13.** | *Valeriana jatamansi* Jones. | Rosmary | Labiatae | + + + | 88.07 ± 4.1 | 841 ± 25.9 |
| | *Rosmarinus officinalis* Linn. | | | | | |

| | | | | | | |
|---|---|---|---|---|---|---|
| **14.** | *Hedychium spicatum*Buch- Ham. | Kapur- | Zingeberaceae | + + + | 89.62 ± 10.0 | 88.3 ± 1.5 |
| **15.** | *Princepia utilis* Royle. | Bhekhra | Rosaceae | + - - | 57.90 ± 4.9 | 153.6 ± 6.0 |
| **16.** | *Urtica dioica* Linn. | Bichubuti | Urticaceae | + + - | 56.24 ± 5.5 | 142.6 ± 8.3 |
| **17.** | *Solanum surretence* Burm.f. | Laghu-kantkari | Solanaceae | + + - | 73.05 ± 8.1 | 166.6 ± 4.7 |
| **18.** | *Citrus medica* Linn. | Nimbu | Rutaceae | + - + | 43.74 ± 1.2 | 143 ± 1.4 |
| **19.** | *Prunus armeniaca* Linn. | Khumani | Rosaceae | + - + | 46.00 ± 0.9 | 109.6 ± 4.9 |
| **20.** | *Prunus amygladus* (Linn.) Batsch. | Badam | Rosaceae | + + + | 73.33 ± 6.6 | 50.0 ± 2.6 |
| **21.** | | | | | | |

* Mean of three replicates; M = Mycelium; V = Vesicle; A =Arbuscule; + = Present;- =Absent.

Table 3 indicates that a total of twenty four VAM species, belonging to six genera of VAM fungi i.e. *Glomus, Acaulospora, Gigaspora, Scutellospora, Entrophospora* and *Sclerocystis* were isolated from mid mountain medicinal plants of Himachal Pradesh. A variety of spores were screened out from the rhizospheric soil of these plants. The abundance of *Glomus* spore was more predominant than any other VAM fungi. Twelve species of *Glomus,* four species of *Acaulospora*, three species each of *Gigaspora* and *Sclerocystis* and one species each of *Entrophospora* and *Scutellospora* were isolated.

The occurrence of VAM spore depends upon different environmental conditions, plant species and type of soil (Trimurtulu and Johri, 1998). Chaturvedi *et al.* (2007) reported that *Glomus* species favour neutral and alkaline soil, whereas *Acaulospora* species are associated with acidic soils. Similarly, Muthukumar and Udaiyan (2007) recorded that *Gigaspora* species predominate in soil with high sand content.

It is confirmed from the result that VAM fungi colonized the most medicinal plants. Agricultural sustainability could be viewed as 'maximum plant production with minimum soil loss'. This type of study could be the beginning of further research pursuits that will utilize such symbiotic fungi to manipulate the host in different ways. The management of their population in the soil is an essential tool for overall plant health in the present scenario of sustainable crop productivity.

**Table 3. Natural occurrence of VAM spores with medicinal plants of Himachal Pradesh (Tentative identification of VAM spores)**

| | | Identification Code (I.C) | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| S No | Medicianl Plants | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 |
| 1. | *Swertia paniculata* | + | - | - | - | + | + | + | + | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + | + |
| 2. | *Achillea millefolium* | - | - | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - | + | + | - | - | - | - | - |
| 3. | *Glaucium flavum* | - | - | + | + | + | - | - | **-zs** | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - | - |
| 4. | *Gentiana kurooa* | + | + | - | - | - | - | - | - | + | - | - | + | - | - | - | - | - | - | - | + | - | - | - | + |
| 5. | *Macuna prurita* | - | - | - | + | - | - | - | - | + | - | + | - | + | + | + | + | - | - | + | - | - | - | - | - |
| 6. | *Centratherum anthelminticum* | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | + | + | - | + | - |
| 7. | *Digitalis lanata* | + | - | - | - | + | - | - | - | - | + | - | - | + | - | - | - | - | - | - | - | - | + | + | + |
| 8. | *Thallictrum rugosum* | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - | + | - | - | + | - | - |
| 9. | *Mentha spicata* | - | - | - | + | - | - | + | - | - | - | - | - | + | - | - | - | - | - | - | - | - | - | + | - |
| 10. | *Asparagus officinalis* | - | - | - | - | - | - | + | - | - | + | - | - | - | + | - | + | - | - | - | - | - | + | - | - |
| 11. | *Geranium wallichianum* | - | - | - | - | + | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | - |
| 12. | *Lavundula angustifolia* | - | - | + | - | - | - | - | - | - | - | - | - | + | - | - | - | + | - | - | - | + | - | - | - |
| 13. | *Valeriana jatamansi* | - | - | - | - | - | - | - | + | - | + | + | + | - | - | - | - | - | - | - | - | - | - | - | - |
| 14. | *Rosmarinus officinalis* | - | + | - | + | - | - | - | - | - | - | - | + | - | - | - | + | + | - | - | - | - | - | - | - |
| 15. | *Hedychium spicatum* | + | - | + | - | - | - | - | + | - | - | + | - | + | - | - | - | - | - | - | - | + | - | - | |
| 16. | *Princepia utilis* | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | - | + | - | - | - | - | - | + |
| 17. | *Urtica dioica* | - | - | + | - | + | - | + | - | - | - | - | + | - | + | - | - | - | + | - | - | - | - | - | |
| 18. | *Solanum surretense* | - | - | - | - | - | - | - | + | - | - | + | - | - | - | + | - | - | - | - | - | - | + | - | - |
| 19. | *Citrus medica* | + | - | - | - | - | - | - | - | - | + | - | + | - | - | - | - | - | - | - | - | - | - | + | - |
| 20. | *Prunus armeniaca* | - | - | - | + | + | - | - | - | - | - | - | - | + | - | - | - | - | - | - | - | + | - | - | - |
| 21. | *Prunus amygladus* | + | - | - | - | - | - | - | + | + | - | - | - | - | - | - | - | - | - | + | + | - | - | - | |

**I.C.**

| | | |
|---|---|---|
| 1. | *Acaulospora laevis* | Gerdemann &Trappe |
| 2. | *A.margarita* | Becker & Hall |
| 3. | *A.mellea* | Spain & Schenck |
| 4. | *A.rehnii* | Sieverding & Toro |
| 5. | *Glomus macrocarpum* | Tulasne & Tulasne |
| 6. | *G.scintilans* | Gerdemann & Trappe |
| 7. | *G.etunicatum* | Becker & Gerdemann |
| 8. | *G.constrictum* | Trappe |
| 9. | *G.intraradices* | Schenck & Smith |
| 10. | *G.mosseae* | Nicolson & Gerdemann (Gerdemann & Trappe |
| 11. | *G.pulvinatum* | Henning (Trappe & Gerdemann) |
| 12. | *G.caledonium* | Nicolson & Gerdemann Trappe & Gerdemann |
| 13. | *G.deserticola* | Trappe,Bloss & Menge |
| 14. | *G.geosporum* | Nicolson & Gerdemann (Walker) |
| 15. | *G.reticulatum* | Bhattacharjee & Mukerji |
| 16. | *G.nigra* | Redhead |
| 17. | *Gigaspora gregaria* | Schenck & Nicolson |
| 18. | *G.margarita* | Becker & Hall |
| 19. | *G.gigantea* | Nicolson & Gerdemann (Gerdemann & Trappe) |
| 20. | *Sclerocystis sinuosa* | Gerdemann & Bakshii |
| 21. | *S.duscii* | (Patouillard) Van Hohn) |
| 22. | *S.rubiformis* | Gerdemann & Trappe |
| 23. | *Scutellospora* sp. | |
| 24. | *Entrophospor*a sp. | |

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# PARASITOLOGY

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# Protective Immune Response of Kashmiri Native Sheep to *Haemonchus contortus* Induced by Parasite Membrane Associated Antigens

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## ABSTRACT

The membrane associated antigens of *Haemonchus contortus* were analysed for protective immunity in sheep. Sheep were challenged with infective 3rd stage larvae of *Haemonchus contortus* (500/kg bodyweight). Sheep vaccinated with membrane associated antigens showed significant reduction (64.085%) in mean faecal egg counts corresponding to infected control animals. There was general reduction in packed cell volume (PCV) and total serum proteins which was much faster in infected control group compared to vaccinated groups. On the basis of decreased faecal egg counts and haematobiochemical parameters, membrane associated proteins are considered to be the best protective antigens.

***Key words:*** Egg count, immune response, packed cell volume

## INTRODUCTION

Sheep are very susceptible to worms due to their close grazing behavior. Almost all sheep are parasitized by helminth parasites. The parasites that cause damage to sheep and goats are stomach worms like *Haemonchus contortus* and *Ostertagia circumcincta. Ostertagia circumcincta* feeds on abomasal contents while as *Haemonchus contortus* feeds on blood. Thus *Haemonchus contortus* (barber pole worm) is of primary concern. It is small about one cm long, blood sucking parasite that bores the lining of abomasum causing blood and protein loss. The barber pole worm is difficult to control because it has short direct life cycle, prolific egg producer and can go to hypobiontic (hibernating) state until environmental conditions are more favorable for its life cycle. Since primary mode of transmission for stomach worms is grazing therefore pasture management and use of anthelmintics are important aspects of controlling the internal parasites. Pasture control
strategies including the use of clean and safe pastures are not readily available. By using anthelmintics the parasite showed resistances to these drugs. Thus there arises a need to develop alternative control strategies like vaccines. Purified gut antigens of *Haemonchus contortus* used as immunogenic afforded a degree of protection against this parasite (Jasmer and Mecuire, 1991; Tavernor *et al.,* 1992; Jasmer *et al.,* 1993. Schallig *et al.* (1997) demonstrated that protective immunity in sheep is associated with low molecular weight antigens. The aim the present study was to analyze the protective immune response in Kashmir Native sheep induced by parasite membrane antigens.

## MATERIAL AND METHODS

**Preparation of Triton X-100 Extracts of** *Haemonchus contortus*: Parasites collected from infected abomasum of sheep were washed with 0.01 M phosphate buffer pH 7.2. Frozen parasites were thawed, ground up as a 10% w/v suspension in ice-cold homogenizing buffer (PBS containing 1nM EDTA and 1n M phenylmethyl sulphonyl fluoride), centrifuged (10000g for 20 minutes) and the pellets were homogenized again with buffer containing 0-1 % Teween 20. The last step was repeated and the pellet was resuspended and extracted in ice cold homogenizing buffer containing 2% (w/ver saline for 5 h at) reduced Triton X-100. (Aldrich chemical Co., Inc, St Louis, Mo, USA) without EDTA. The extract was centrifuged (1 h at 10000g) and the supernatant was clarified by passing through a 0-22μm filter and then stored at -20$^{o}$C. The concentration of crude somatic antigens was determined by Lowry *et al.* (1951)

**Experimental design:** Eight lambs aged between 5-6 months collected from local breeders of District Anantnag Kashmir , India, were used for the experiment. Lambs were rared and housed under controlled conditions. They were fed on the solid diet of maize and wheat husk. Before inoculation, animals were treated with broad spectrum anthelmintics, Albendazole (Valbazene, 10mg/kg) and Acaricide. At approximately 3 months of age, the animals were randomly allocated to two groups. Five lambs were allocated to the immunized groups and three lambs were treated as unimmunized control group. All the vaccinated animals received 50μg of antigenic solution in PBS in 1 ml Freund's complete adjuvant (Sigma). Each immunization included doses of inoculation (three intramuscularly, three subcutaneously). Both the groups were subsequently challenged with infective 3$^{rd}$ stage larvae (500/kg body weight) of *Haemonchus contortus* obtained from donor animal faeces cultured in an incubator at 27$^{O}$C for seven days according to the method of Roberts and Oscillvan (1950). Faecal samples were collected from the weak 3$^{rd}$ up to the end of experiment. Faecal egg counts were calculated by employing McMaster's egg counting technique. Throughout the experiment, blood samples were collected after every week from all the animals. Packed cell volume (PCV) was determined by microhaematocrit method. Total serum proteins concentration was estimated by Buiret method (Clinical diagnostic Kit method). The animals were weighed at the time of infection to determine the larval dose, and then every 14 days until the end of infection for the determination of weight gain.

## RESULTS AND DISCUSSION

All the animals developed infection which was confirmed by faecal examination. *Haemonchus contortus* eggs were detected in faeces on 28th and 24th day post infection in immunized and unimmunized groups respectively. The peaks of egg counts were found in faeces on day 32nd and 29th day of infection in group immunized and unimmunized groups respectively. The faecal egg counts observed in the present study are presented in Table1. Immunized animals showed 64.08% reduction in faecal egg counts corresponding to unimmunized control. Mean PCV values of both the groups of animals decreased from 4th week of infection (Fig. 1). However, this decrease was more evident in the unvaccinated group corresponding to control group. Mean total serum protein concentration was significantly higher (5.4± 0.3) in immunized group compared to 4.8 ± 0.2 of unimmunized group

**Table 1. Weekly faecal egg counts of immunized and unimmunized animal groups**

| S. No | Week | Immunized | Unimmunized |
|---|---|---|---|
| 1 | 1 | 0 | 0 |
| 2 | 2 | 0 | 0 |
| 3 | 3 | 0 | 0 |
| 4 | 4 | 200 | 2550 |
| 5 | 5 | 850 | 3800 |
| 6 | 6 | 2000 | 3600 |
| 7 | 7 | 1600 | 2700 |
| 8 | 8 | 1200 | 2400 |
| 9 | 9 | 800 | 1900 |
| 10 | 10 | 400 | 1700 |
| 11 | 11 | 200 | 1400 |
| 12 | 12 | 100 | 400 |
| Mean | | 612.5 | 1704.16 |
| ± | | 281.85 | 675.43 |
| Reduction | | 64.08% | |

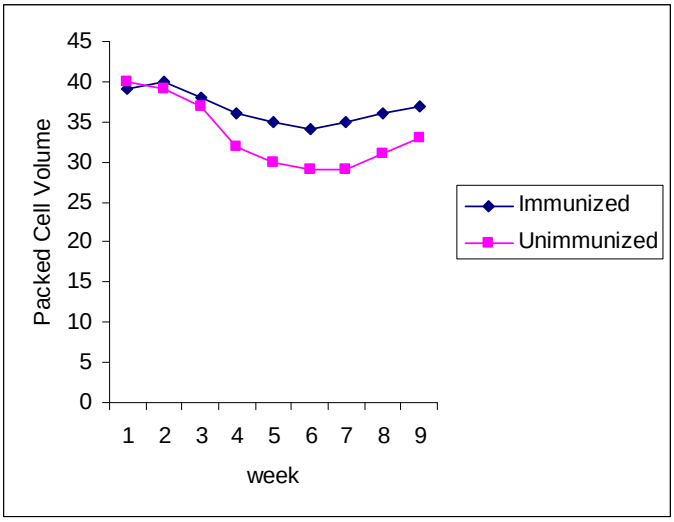


**Fig. 1. Variation in packed cell volume (PCV) in immunized and unimmunized groups**

The present study demonstrated that *Haemonchus contortus* membrane associated proteins can induce better protective response in sheep. In the present study the degree of protection achieved by vaccination as estimated by the reduction in faecal egg counts was similar to that observed by Munn *et al.* (1997). Values of the PCV observed in the present study were similar to Smith and Smith (1993) and Andrews *et al.* (1995) who observed decreased PCV values in infected animals. Various attempts have been made to induce a protective immune response to blood feeding parasite *Haemonchus contortus* with irradiated larvae, somatic extracts and excretory secretory products of its different stages of life cycle. Our results are in accordance with the findings of Jacobs *et al.* (1999) observed promising protection in lambs against *Haemonchus contotus* when vaccinated with surface antigen of L3 larvae. Considerable efforts have been applied to develop vaccines based on hidden antigens especially proteins molecules associated with the membrane of the gut. The immune response is not induced against these molecules during an infection as they are hidden. Vaccination with hidden antigen preparations has resulted in significant protection against *Haemonchus contortus* in young lambs, sheep and pregnant ewes (reviewed by Newton and Munn, 1999). The protection induced by this type of vaccination is based on the induction of antibodies directed against these hidden antigens (Smith, 1993).

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# BIOCHEMISTRY

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# Evaluation of Protease Inhibitory Activity in Pea (*Pisum sativum*)

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**ABSTRACT**

Protease inhibitory activity was detected from aqueous extract of *Pisum sativum*. Fresh pea seeds were taken, flash frozen in liquid nitrogen, ground with pestle and mortar and then thawed on ice. The crushed sample was mixed with protein extraction buffer and centrifuged .The protein concentration of sample was determined using Bradford method .The sample was analyzed for protease inhibitory activity by standard procedures. Suitable assay was performed and an opaque zone was formed when drop of extract was put on X-ray film with respect to control (trypsin and Buffer).Salt fractionation of pea extract was performed and inhibitory activities of the salt fractionated samples were checked and results were positive. SDS-PAGE of extract was performed with respect to crude extract of pea as standard. All the samples i.e. 30%, 60% and 90% get the bands at same location.

***Key words:*** Protease inhibitors, Pisum sativum, proteases

**Abbreviations:** PI: Protease inhibitors, SDS: Sodium dodecyl sulfate

## INTRODUCTION

Nature has provided us with certain regulatory mechanisms which prevent over secretion and hyperactivity of proteases. The proteins that inhibit the proteases and limit their activity by competitive inhibition are called protease inhibitors. Some protease inhibitors occur in plants naturally while some are synthetic as being found in processes of blood coagulation, fibrinolysis and complement cascade of animals. Protease inhibitors help in regulation of proteolytic processes and maintain intracellular metabolism of proteins. They help in self-defense mechanisms in plants against predators, pathogens and pests (Ryan, 1990). They are important tools of crop improvement targeting plant protection and human nutrition. They are also used as antiviral agents hence can be used in therapeutics against fatal viruses e.g. Picorna, Herpes, HIV.PIs have been considered to counter a act tumor progression and metastasis (Clemente *et al.,* 2005). PI genes are currently being used to develop anti fungal, antiviral and pathogen resistant transgenic crops (Valueva *et al.,* 1999; Krattiger and Anatole, 1997). Protease inhibitor genes are also involved in regulation of Programmed Cell death in plants (Mazal *et al.,* 1999). Several non – homolgous families of protease inhibitors are recognized among animal, plant and microorganism kingdoms. Protease inhibitors are abundant in storage organs and seeds of plants (Ryan, 1977). Majority of protease inhibitors studied in plant kingdom are

from Solanaceae, Leguminaceae and Graminaceae. Their synthesis is induced to high levels in response to stress, infection and wounding (Jongsma *et al.,* 1994).These inhibitor families that have been found are specific for each of the four mechanistic classes of proteolytic enzymes and are based on the active amino acid in their "reaction centre" (Kiowa *et al.,* 1997). These are Serine PI, Cysteine PI, Aspartic PI and Metallo PI.

Protease inhibitors have been worked out and isolated from many plants e.g. potato, tomato (Rancor,1968; Keilova and Tomasek,1976 ), black eyed pea (Louis Slade , et al., 1976), cow pea (Paulraj *et al.,* 2000), Mung beans (Maarten and Brumgartner,1978) , Medikus tubers (Zhang *et al.,* 2008), grass pea seeds, horse gram seeds, soya bean, and black gram (Maitra *et al.,* 2007). In this study an attempt is made to evaluate the protease inhibitory activity in pea (*Pisum sativum*), a frequently grown, edible, nutritious and tasty legume in Kashmir valley.

## MATERIAL AND METHODS

Pea plants were collected from local seller. Seeds were washed and cleaned thoroughly.15 mg of pea fresh weight, flash frozen in liquid nitrogen were ground with a pestle and mortar and thawed on ice. 15 ml protein extraction buffer (0.1 mM Tris chloride pH 7.6 and 10mM calcium chloride) was added to powdered sample and vortexed thoroughly. Centrifugation was carried out at 10,000 rpm for 20 min. Supernatant was preserved at 4 degree Celsius.

**Protein Estimation:** Protein content of sample was determined by Bradford method and samples were read at 595nm (Bradford, 1976).

**Dot Blot Analysis:** To expedite the recognition of PIs, a method utilizing surface of an X-ray film as proteolytic substrate is employed. Positive reaction is indicated by clear zone on the film after rinsing with water (Cheung *et al.,* 1991).

**Salt Fractionation:** Aqueous extract of pea was treated with different concentrations of ammonium sulfate (Mw: 132.14) to precipitate different proteins. Amount of ammonium sulfate required for achieving 0-30%, 30-60%, and 60-90% saturation has been taken from nomogram or calculated. Centrifugation was carried at 10,000 rpm for 20 min. Precipitate was re suspended in protein extraction buffer and kept for dialysis for 12 hours. Supernatant obtained from each salt saturations were preserved. PI inhibitory activity of each sample was detected using Dot blot assay.

**SDS -Poly Acrylamide Gel Electrophoresis:** SDS - PAGE of aqueous extract of pea was carried out with mini gel apparatus in Tris glycine buffer, pH 8.8 . SDS –PAGE was performed using method of Laemmli (1970). Gel was stained overnight and destained to view the bands.

## RESULTS AND DISCUSSION

Aqueous extract of pea was prepared to evaluate the protease inhibitory activity (Fig.1). The concentration of protein in aqueous pea extract was 338.75 mg%. Dot blot assays were performed in which drop of pea extract was put on X ray film with respect to buffer and trypsin as control. A clear zone was formed at zone of trypsin ,no effect was shown by buffer and an opaque zone was formed by extract .The opaque zone is formed as a consequence of the presence of inhibitors, which does not allow the proteases to digest the gelatin coated on X-ray film. Gelatin is a protein which can be degraded by proteases like trypsin. Salt fractionation of pea extract was performed and inhibitory activities of each of the salt fraction samples were checked. A drop of each of the sample was put on the X-ray surface with respect to control (Fig. 2-4). Opaque zone was formed which indicate presence of PIs in samples. 30% of salt fractionation sample used against trypsin showed little inhibitory activity because of lesser salt saturation and hence lesser purification (Fig.2). But 60% and 90% samples showed the presence of PIs which does not allow the digestion of gelatin on film (Fig. 3, 4). SDS-PAGE of salt fractionation pea extract samples was performed with respect to crude pea extract and we get four different bands over (Fig.5).It indicates presence of multi subunit PI protein. All the samples (30%, 60% and 90%) get the band at same location.

Results will be discussed in light of other family members of Leguminacea pea seeds contain a number of inhibitor proteins which have negative effects on digestibility. The researchers had shown that pea protease inhibitors can reduce the proliferation of adeno carcinoma cells in vitro and may provide benefit as dietary anti carcinogens. (Clemente *et al.,* 2005).The results also show presence of PIs in *Pisum sativum.* .The protease inhibition studies were performed on grass pea seeds, horse gram seeds, soya bean, and black gram using proteases from rohu fingerling.In case of grass pea seed, more than 50% inhibition of alkaline protease activity was recorded when the ratio of inhibitor to enzyme was 9.41$\mu gU^{-1}$. These results also reveal that grass pea seeds also contain protease inhibitor. (Maitra *et al.,* 2007). Another study done on pea indicates presence of carboxy protease belonging to metallo or metal-activated arid serine proteases family. It strongly means that there will be definite regulatory PIs against these proteases confirming our finding. (Craigh *et al.,* 2004). Similarly pigeon pea (*Cajanus cajan* L.) extracts have been analyzed for the protease inhibitors using Gel X ray film technique for detection of electrophoretically separated protease inhibitors. (Veerapa et al., 2006). Sangeeta *et al.* (2006) confirmed marked changes in protein content of pigeon pea (*Cajanus cajan* L.) during process of germination and seed development confirming that PIs are found in these seeds for the regulatory mechanisms. So legumes are considered to be rich source of PIs. Hence it confirms our findings and thus pea (*Pisum sativum*) can be used as a source of PIs but their isolation and characterization need further studies.

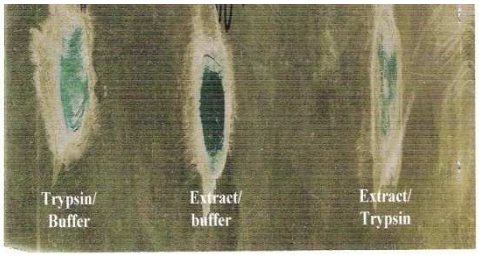


**Fig.1 : Pea extract showing inhibitory activity for protease trypsin**

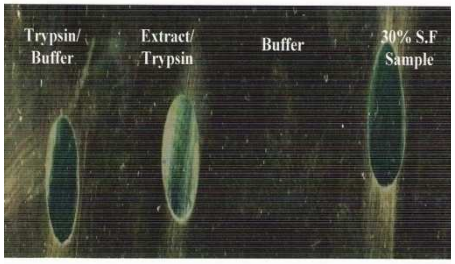


**Fig.2: 30% Salt fractionation sample showing little inhibitory activity.**

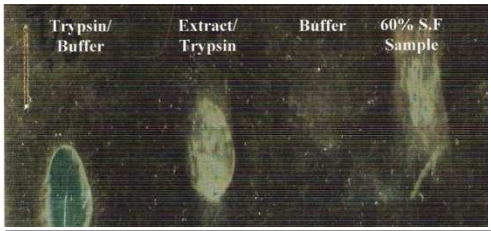


**Fig.3 : 60% Salt fractionation sample showing inhibitory activity.**


**Fig. 4 : 90% Salt fractionation sample showing inhibitory activity.**

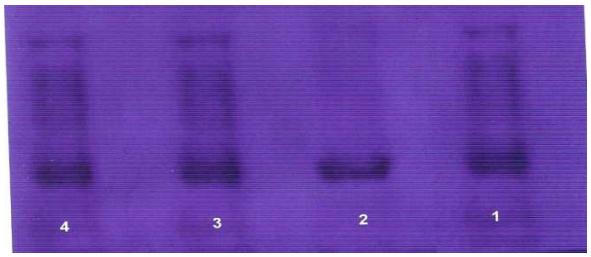


**Fig. 5: SDS-PAGE gel: Lane 1 to 4 represent from right to left represent crude extract, 90, 60, 30 % S.F samples**

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# GENETICS

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# Changes in Inversion Frequency by Inbreeding in *Drosophila melanogaster*

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**ABSTRACT**

*Drosophila melanogaster* flies were collected in different regions of Jhansi (U.P) and the inbreeding was allowed to occur under controlled conditions in BOD.The effects of inbreeding were seen and also its correlation with different inversions was worked out. The main finding was on egg viability which seems to decrease with inbreeding. Further the inbreeding results in depression of vigor because it increases homozygosity of a locus and thus brings together the deleterious recessive alleles.

***Key words**:* Correlations, egg viability, inbreeding depression, homozygosity, recessive alleles

## INTRODUCTION

Inversions are the structural changes in the chromosomes which involve the orientation of a segment of chromosomes in the reverse direction and hence the order of genes in this region is exactly the reverse of that found in the homologue (non-inverted). The inversion result from the breaking of a chromosome at two sites so that three segments are formed and that the middle one is rotated through $180^0$ i.e. joined in reverse direction. The inversions were first observed by Strutevant (1926) by the genetic map preparation of *Drosophila melanogaster*. The detection of inversions is relatively easy in Drosophila due to polytene chromosomes.

In *Drosophila* the traits that have been associated with inversion polymorphism include viability, development time, longevity, mating success, fecundity, bristle number, resistance to thermal stress and body size. These traits can be a good proxy in the study of inbreeding.(Chapko, 1979). Singh (1989) study showed a good variation of frequency of different gene arrangements in 24 Indian populations of *D. Ananassae*. The inbreeding has a great effect on crossing over and recombination is greatly suppressed in the regions of inversions (Ramel, 1962).

According to the dominance hypothesis, inbreeding depression is caused by expression of deleterious recessive alleles in homozygous individuals. In the present work the frequency and egg to larva viability were taken into consideration to study the effect of inbreeding in *Drosophila melanogaster*.

## MATERIAL AND METHODS

For the study of inversion frequency and inbreeding depression in *Drosophila melanogaster,* the flies were collected in different regions of Jhansi (U.P) like Shivaji Nagar and the local Sabzi Mandi of Jhansi.The flies were trapped by means of aspirators, nets and plastic funnels into empty bottles. The flies were then transferred into food bottles in the laboratory. The food had following composition:

| CONTENT | QUANTITY |
|---|---|
| Agar Agar | 13 gm |
| Maize powder | 160 gm |
| Crude sugar | 170 gm |
| Active yeast powder | 60 gm |
| Nipazine | 8 gm |
| Acid mix | 20 ml |
| Water | 2400 ml |

All the cultures were incubated at 25±1$^0$C and maintained under continuous lighting in BOD. The tests considered were for egg viability in *D. melanogaster* and its correlation with inversion frequency. The various steps in the experiment were:

(i) Random selection of 15 female and 10 male flies was done. These were called parents (P1). They were allowed to cross and lay eggs for 19 hours. Then after they were transferred into food vial fitted at the bottom of a plastic bottle. They were obtained by etherizing and were starved for 2 hours in empty bottle.

(ii) In the second step 10 male flies of P1 were crossed with 15 females of F1 (after allowing the virgin females to mature in food bottle). Egg counting was done after 19 hours.

(iii) In the third step, 10 male flies and 15 females of F1 were crossed and egg counting was done as usual after 19 hrs.

(iv) Subsequent squashing of the larvae using Lacto-0resien method was done. The inversions were observed in both parental and F1 larvae.

## RESULTS

All the four kinds of common cosmopolitian inversions were observed in the laboratory stock of *D. melanogaster*. None of them was pericentric and all of them were autosomal.Among the inversion 2LT inversions were more frequent i.e. maximum number of inversions were in the 2 L arm. In case of F1 generation 2L arm had 4 inversions out of total 6 inversions seen. The frequency of different inversions is given in Table 1.

**Table 1. Frequency of different gene arrangements in *D. melanogaster***

| | | 2 LT | 2 RNS | 3LP | 3RP |
|---|---|---|---|---|---|
| Parents | p | 0.86 | 0.95 | 0.95 | 0.98 |
| | q | 0.14 | 0.05 | 0.05 | 0.02 |
| F1 | p | 0.75 | 0.95 | 1 | 0.95 |
| | n | 0.25 | 0.05 | 0 | 0.05 |

Calculation of observed and expected number (i.e. Hardy and Weinberg proportions) of different karyotypes of the various inversions is given in the Tables 2 and 3 for parents and F1 respectively.

**Table 2. Observed and expected values of different gene arrangement in *Drosophila melanogaster* (parents)**

| S. No. | Inversion | +/+ | +/- | -/- |
|---|---|---|---|---|
| 1 | 2 LT | | | |
| 1.a | Observed expected | 37 | 12 | 01 |
| 1.b | $\chi^2$ | 36.98 | 12.04 | 0.98 |
| 1.c | | 0.0000108 | 0.0001329 | 0.0004082 |
| 2 | 2RNS | | | |
| 2.a | Observed expected | 45 | 05 | 0 |
| 2.b | $\chi^2$ | 45.12 | 4.75 | 0125 |
| 2.c | | 0.0003191 | 0.0131579 | 0.125 |
| 3 | 3LP | | | |
| 3.a | Observed expected | 45 | 05 | 0 |
| 3.b | $\chi^2$ | 45.12 | 4.75 | 0125 |
| 3.c | | 0.0003191 | 0.0131579 | 0.125 |
| 4 | 3RP | | | |
| 4.a | Observed expected | 48 | 02 | 0 |
| 4.b | $\chi^2$ | 48.02 | 1.96 | 0.02 |
| 4.c | | 0.0000083 | 0.0008163 | 0.02 |

df*=1;p>0.05

**Table 3. Observed and expected values of different gene arrangements in *Dmelanogaster* (F1)**

| S. No. | Inversion | +/+ | +/- | -/- |
|---|---|---|---|---|
| 1 | 2 LT | | | |
| 1.a | Observed expected | 6 | 3 | 1 |
| 1.b | $\chi^2$ | 2.85 | 3.7500 | 0.625 |
| 1.c | | 3.5683628 | 0.15 | |
| 2 | 2RNS | | | |
| 2.a | Observed expected | 9 | 1 | 0 |
| 2.b | $\chi^2$ | 9.025 | 0.95 | 0.025 |
| 2.c | | 0.0000693 | 0.0026316 | 0.025 |
| 3 | 3LP | | | |
| 3.a | Observed expected | 10 | 0 | 0 |
| 3.b | $\chi^2$ | 10 | 0 | 0 |
| 3.c | | 0.0 | 0.0 | 0.0 |
| 4 | 3RP | | | |
| 4.a | Observed expected | 9 | 1 | 0 |
| 4.b | $\chi^2$ | 9.025 | 0.95 | 0 |
| 4.c | | 0.0027701 | 0.0026316 | 0.0 |

df=1;p>0.05

It is evident from the data that there is no significant deviation in both the cases. However, because of selection of lesser number of flies the observed $\chi 2$ value at one place (3.5683628) in 2LT came near to the tabular value (3.84), i.e. near to the significant level. This shows that the egg laying and 2LT inversions have some correlation. Table 4 shows the correlation among the eggs laid and the different inversion frequencies.

**Table 4. Correlation among eggs laid and the different inversion frequencies**

| | Eggs laid | 2LT | 2RNS | 3RP | 3LP |
|---|---|---|---|---|---|
| Eggs laid | +1 | | | | |
| 2LT | -1 | +1 | | | |
| 2RNS | | | +1 | | |
| 3RP | +1 | -1 | | +1 | |
| 3LP | -1 | +1 | | -1 | +1 |

*df=1

It is seen that there is negative correlation between 2LT inversions and eggs laid. This indicates that lesser the 2LT inversion frequency, the more the egg production and vice-versa. The following results were obtained during the crosses:

(i) The average number of eggs produced by two replicates of cross between P1 males and P1 females was 150. Among these 112 hatched out and 41 did not.

(ii) The average number of eggs produced by two replicates of cross between P1 males and F1 females was 206. Among these 99.5 hatched and 56.5 did not.

(iii) The average number of eggs produced by two replicates of cross between F1 males and F1 females was 103.5 among which 19.5 hatched and 84 did not.

**Table 5. Number of eggs laid and their viability in different crosses of *D.melanogaster***

| | parents | | | P1 males crossed with F1 females | | | F1 males crossed With F1 females | | |
|---|---|---|---|---|---|---|---|---|---|
| | Total No. of eggs | No. of Unhatched eggs | No. of Hatched eggs | Total no. of Eggs | No. of unhatched eggs | No. Of hatched eggs | Total no. of eggs | No. of Unhatched Eggs | No. of Hatc-hed Eggs |
| A | 140 | 46 | 94 | 217 | 61 | 156 | 97 | 74 | 23 |
| B | 166 | 36 | 130 | 195 | 52 | 143 | 110 | 94 | 16 |
| Mean Of A & B | 150 | 41 | 112 | 206 | 56.5 | 99.5 | 103.5 | 84 | 19.5 |

## DISCUSSION

More than 326 inversions are known in *D. melanogaster* and most of them are paracentric (Lemunier *et al.,* 1986). On the basis of distribution and abundance, chromosome inversions in *D. melanogaster* have been divided into four types: (i) common cosmopolitian, (ii) rare cosmopolitian, (iii) recurrent endemic and (iv) unique endemic (Ashburner and Lemunier, 1976). All the four common cosmopolitian inversions occur in Indian natural populations of *D.melanogaster*. Most of the inversions detected during the present study occur at a low frequency as depicted in Table 1. This may be due to selection of lesser number of flies and environmental effect in Jhansi. It is interesting to note that very low frequency or complete absence of the common cosmopolitan inversions have been found in certain populations of *D. melanogaster* in Korea, Japan, Australia and USA (Mettler *et al.,* 1977).

No overlapping inversions were seen in the present study in *D.melanogaster,* a result which is in consonance with the previous works of Stralker (1976). Most inversions in *D. melanogaster* are paracentric.

In the present work inbreeding was allowed when the F1 males and females were allowed to cross and there was a marked difference in the eggs laid (i.e. egg count) and their viability in contrast to the P1 flies crossed (Table 1). Similar inbreeding depression results were recorded in *D. melanogaster* by other workers for example, Egg to adult viability showed I.D of 0.57 (Garcia *et al.,* 1994), wing length showed an I.D of 0.03

(Reeve, 1953). The association of body size and inversions in D.ananassae has been studied by Yadav and Singh (2003).

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# PLANT TISSUE CULTURE

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# Impact of BA and TDZ on *In Vitro* Shoot Proliferation of Two Indigenous Cultivars (Maharaji and Chambura) of Apple (*Malus Pumila* Mill.) in Kashmir

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**ABSTRACT**

Shoot apices of Maharaji and Chambura cultivars of apple (*Malus pumila),* obtained from young twigs of 40-50 year mature trees, were cultured on MS (1/2 strength) (1962) nutrient medium to assess the influence of different concentrations (0.5-5.0μM) of BA and TDZ separately and along with phloroglucinol(10μM) for multiple shoot formation. The explants of both the cultivars showed best response to BA (4.5μM) + PG (10μM) for shoot proliferation which was observed by direct multiple adventitious shoot regeneration at the base of the explant. The shoot lumps were subcultured 6-10 times, at an interval of 4 – 6 weeks, to increase the number of regenerated shoots. Each isolated shoot was then subcultured under the influence of a number of root inducing hormones. The best rooting response was scored on MS (½) +IBA (2.5μM) in both the cultivars. The plantlets thus obtained were transferred to pots containing peat vermiculite mixture (1:1) for hardening under laboratory conditions where survival rate was found to be 82% in Maharaji and 7% in Chambura.

***Key words:*** *In vitro,* Maharaji, Chambura, mature trees, shoot apices, multiple adventitious shoots, plantlets.

***Abbreviations:*** MS – Murashige and Skoog; BA – Benzyl adenine, PG – Phloroglucinol, IBA- Indole Butyric acid, IAA – Indole -3 Acetic Acid, NAA- Nephthalene Acetic Acid, 2,4-D – 2,4- Dichlorophenoxy Acetic Acid, Kn – Kinetin, TDZ- Thidiazuron.

## INTRODUCTION

Apple is a rosaceous fruit tree belonging to genus *Malus*. It is propagated in temperate regions of both northern and southern hemispheres of the world for its high economic value. The genus has five sections including 122 species and subspecies (Chadha and Awasthi, 2005). Over 700 accessions introduced from different parts of the world have been tried and tested in India from 1950 (Gosh, 2006). Natural varieties of cultivated apple belong to *Malus pumila* Mill. while its hybrid varieties belong to *Malus domestica* Bork. (Janick, 1996).

Nearly half of the production of apple is consumed as fresh fruit and most of the remainder is processed into apple juice, canned apple sauce, apple jam and apple butter. Dehydrated apples, apple flour, apple pie, apple dumpling, charoset (apple relish), apple haystacks are its other important commercial products. The fruit contains appreciable quantity sorbitol, and sugars (sucrose, glucose and fructose), organic acids (mainly malic and caproic acids) and vitamins. From medicinal view point, apple murraba, popular in India is regarded as a stimulant for heart. Fresh apple acts as purgative, prevents constipation, reduces incidence of dental caries, helps to control obesity and supplies extra energy for heavy exercise (Mitra, 1991). The pulp of apple fruit has been found to be the second richest source of phytochemicals like quercetin, catechin, phloridzin and chlorogenic acid, all of which are very strong antioxidants and reduce the risk of some cancers, cardiovascular diseases, asthma, and diabetes, inhibit cancer cell proliferation, decrease lipid oxidation, and lower cholesterol. Thus these prevent oxidative stress and delay ageing (Boyer and Liu, 2004).

Apple is propagated in all temperate zones of the world. In India the major apple producing regions include Kashmir, Himachal Predesh, Utter Predesh, Kumaon, Assam and Nilgiri Hills. Kashmir is the leading apple producing state in India with annual production of 10.38 metric tonnes (Anonymous, 2008). About 330 cultivars of apple were under cultivation in Kashmir Valley around 1978, but only few cultivars are seen at present in proliferating orchards. Because of poor returns, growers have stopped propagation of low yielding and less resistant varieties. This has led to drastic decline in the production of Maharaji, Chambura and other indigenous cultivars of apple and thus leaving their existence under threat.

Traditional method of propagation for apple is highly laborious, skilful and involves a lot of cost and wastage of time. Therefore, tissue culture technique seems to be the more reliable method for the production of self rooted clonal trees as it has the potential to provide large number of plants in less time. Although some *in vitro* work has been reported on other cultivars of apple from J & K state (Rizvi, 1999, Sharma *et al.,* 2004, Dalal *et al.,* 2006, Rizvi *et al.,* 2007) but no work has been done till date on Maharaji and Chambura cultivars.

Present work thus represents the first report on *in vitro* culture of Maharaji and Chambura cultivars which are receiving less attention in the valley. The work will form a plateform for the conservation of these cultivars.

## MATERIAL AND METHODS

Shoot apices of Maharaji and Chambura cultivars obtained from young twigs of mature fruit bearing trees (40-50 year old), growing in different orchards of Kashmir valley, were thoroughly washed under tap water using labolene (5%) and a wetting agent Tween-20. This was followed by their sterilization with sodium hypochlorite (10%) for 20 minutes and three times rinsing by double distilled water. The explants were then were placed in Kn (15µM) solution in flasks for 24 hours at 4°C (in refrigerator) with their mouths sealed for avoiding phenolic exudation, after which they were re-sterilized using $HgCl_2$ (0.1%) for 90 seconds, followed by rinsing with autoclaved water several times to remove the traces of $HgCl_2$. The explants were then trimmed to 0.5-0.8cm long tips for inoculation on MS nutrient medium supplemented with different concentrations (0.5-5.0µM) of BA and TDZ either separately or with PG(10µM). The cultures were placed in incubation room where temperature was maintained between 22-28°C with light intensity of 3000 lux maintained for 18 hours daily.

Initially, the explants were transferred onto fresh nutrient media regularly after every 24 hours at least 5-7 times, for controlling browning of the medium but later on the culture products obtained were subcultured after every 4- 6 weeks. The complete plantlets obtained were finally transferred from culture vials to small thumb pots for hardening.

## RESULTS

### Soot proliferation

Shoot apices (0.5- 0.8cm long) cultured under the influence of various BA and TDZ concentrations (0.5-5.0µM) separately or in combinations with PG(10µM) showed different degrees of response (Table-01). Best response in terms of shoot multiplication and proliferation was scored when medium was supplemented with BA (4-5µM) + PG (10µM) in both the cultivars tried. Phenolic exudations leading to browning of the medium and death of explants was avoided by chilling sterilized shoot tips in kinetin solution (15µM) over night, regular transfer of explants on fresh medium of same composition for 5-7 days and using the reduced (half) strength of MS salts. Culture establishment and shoot induction started after second week in Maharaji and third week in Chambura cultivar (Fig-1a & b). This was followed by direct multiple adventitious shoot

regeneration at the base of the explants (Fig-2a & b). The adventitious shoots thus produced were subcultured in lumps (6-10 times) after regular intervals of 4 – 6 weeks on same medium which continued to prolifer and increased shoot number by hundred folds (Table-01) (Fig-3a & b). During this period microshoots showed elongation as well. Both the selected cultivars responded best to BA (4.5µM) + PG (10µM) in terms of multiple shoot production. The average number of shoots produced per explant per subculture was 52±0.81 in Maharaji and 46±0.72 in Chambura.

**Rooting of micro-shoots**

Elongated micro-shoots were separated carefully from the lumps and subcultured on different rooting media where they showed varied response (Table-02). Best response was observed on MS (1/2) fortified with IBA (2.5µM) and PG (10µM) where 100 percent direct rhizogenesis was recorded in both the selected cultivars and average number of roots produced per shoot was 17±0.68 and 10±0.81 in Maharaji and Chambura respectively. When the concentration of IBA was increased to 5µM, percentage of response got drastically reduced to 51 and 36 in Maharaji and Chambura respectively. With IBA (2.5µM) root initials were seen in 2$^{nd}$ or 3$^{rd}$ week of subculture and profuse rooting was observed after six weeks of culture period (Fig-4a & b). The average number of roots produced per shoot was found to be 35±0.66 in Maharaji and 30±0.82 in Chambura with percent response of 80 and 70 respectively. Adventitious roots were also favoured by IBA (2.0µM) but percent response and mean root number were much lower than what was recorded with IBA (2.5µM). IBA (3.0-5.0µM) with PG (10 µM) initiated indirect roots. Rest of the rooting trials did not favour direct rooting but resulted in callusing at cut end (Table 02)

Complete plantlets worth transplantation were recovered in 8-10 weeks of rooting period. Roots started callusing soon after their initiation, when shoots were subcultured under the influence of triple auxin combination (IAA + IBA + NAA 1-5µM each) with or without PG (10µM). Rooted shoots (4-8cm long) were later on properly deflasked as per its established protocol and transferred to pots containing sand- soil mixture of 1:1 for acclimatization under high humidity under lab conditions (Fig-5a & d). The percentage of survival was observed to be 82% in Maharaji and 78% in Chambura cultivar.

**Table 1.** Impact of different concentrations of BA and TDZ used either separately or with PG(10µM) on the shoot apices from mature trees of different cultivars of apple cultured *in vitro* on MS (half-strength) nutrient medium.

| Phytohormones (µM) | Nature of Response | | Percentage of response | | Shoot Number Mean±SD | |
|---|---|---|---|---|---|---|
| | MJ | CH | MJ | CH | MJ | CH |
| CONTROL | NR | NR | 0 | 0 | NA | NA |
| BA (0.5) | NR | NR | 0 | 0 | NA | NA |
| BA (1.0) | NR | NR | 0 | 0 | NA | NA |
| BA (1.5) | NR | NR | 0 | 0 | NA | NA |
| BA (2.0) | CCE | CCE | 5 | 2 | NA | NA |
| BA (2.5) | CCE | CCE | 6 | 2 | NA | NA |
| BA (3.0) | CCE | CCE | 4 | 3.6 | NA | NA |
| BA (3.5) | CCE | CCE | 15 | 4 | NA | NA |
| BA (4.0) | ASP | ASP | 18 | 14 | 14±0.88 | 22±0.82 |
| BA (4.5) | ASP | ASP | 18 | 15 | 23±0.88 | 14±0.82 |
| BA (5.0) | ASP | ASP | 20 | 16 | 26±0.82 | 14±0.72 |
| BA (0.5) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| BA (1.0) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| BA (1.5) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| BA (2.0) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| BA (2.5) + PG (10) | CCE | CCE | 27 | 18 | NA | NA |
| BA (3.0) + PG (10) | CCE | CCE | 28 | 20 | NA | NA |
| BA (3.5) + PG (10) | CCE | CCE | 28 | 21 | NA | NA |

*Table 1 Contd..*

*Table 1 Contd..*

| | | | | | | |
|---|---|---|---|---|---|---|
| BA (4.0) + PG (10) | ASP | ASP | 92 | 80 | 48±0.82 | 42±0.71 |
| BA (4.5) + PG (10) | ASP | ASP | 90 | 75 | 52±0.81 | 46±0.72 |
| BA (5.0) + PG (10) | ASP | ASP | 90 | 72 | 52±0.76 | 46.±0.82 |
| TDZ (0.5) | NR | NR | 0 | 0 | NA | NA |
| TDZ (1.0) | NR | NR | 0 | 0 | NA | NA |
| TDZ (1.5) | NR | NR | 0 | 0 | NA | NA |
| TDZ (2.0) | CCE | CCE | 17 | 12.5 | NA | NA |
| TDZ (2.5) | CCE | CCE | 15 | 12.6 | NA | NA |
| TDZ (3.0) | CCE | CCE | 16 | 12.9 | NA | NA |
| TDZ (3.5) | ASP | ASP | 22.8 | 15.0 | 12±0.76 | 10±0.71 |
| TDZ (4.0) | ASP | ASP | 29 | 27 | 18±0.82 | 15±0.71 |
| TDZ (4.5) | ASP | ASP | 30 | 27 | 28±0.72 | 16±0.72 |
| TDZ (5.0) | ASP | ASP | 29 | 25 | 27±0.76 | 16.±0.82 |
| TDZ (0.5) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| TDZ (1.0) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| TDZ (1.5) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| TDZ (2.0) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| TDZ (2.5) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| TDZ (3.0) + PG (10) | CCE | CCE | 25 | 12 | NA | NA |
| TDZ (3.5) + PG (10) | CCE | CCE | 28 | 10 | NA | NA |
| TDZ (4.0) + PG (10) | CCE | CCE | 32 | 12 | NA | NA |
| TDZ (4.5) + PG (10) | CCE | CCE | 32 | 27 | NA | NA |
| TDZ (5.0) + PG (10) | CCE | CCE | 35 | 27 | NA | NA |

MJ- Maharaji, CH – Chambura. CCE - Callus at Cut End, ASP – Adventitious Shoot Proliferation
NR - No Response, NA- Not Applicable; Ten replicates/ treatment

Data scored after every six weeks and representing mean of ten subcultures.

**Table 2. *In vitro* response of sub-cultured shoots of different cultivars of apple to rooting hormones used in MS (half-strength) nutrient medium.**

| Phytohormones (µM) | Nature of Response | | Percentage of response | | Root Number Mean±SD | |
|---|---|---|---|---|---|---|
| | MJ | CH | MJ | CH | MJ | CH |
| CONTROL | NR | NR | NR | NR | NR | NR |
| IBA (0.5) | NR | NR | 0 | 0 | NA | NA |
| IBA (1.0) | CCE | CCE | 0 | 0 | NA | NA |
| IBA (1.5) | CCE | CCE | 0 | 0 | NA | NA |
| IBA (2.0) | ARF | ARF | 32 | 21 | 12±0.85 | 13±0.88 |
| IBA (2.5) | ARF | ARF | 80 | 74 | 35±0.66 | 30±0.82 |
| IBA (3.0) | CR | CR | 52 | 38 | 15±0.80 | 13±0.82 |
| IBA (3.5) | CR | CR | 15 | 4 | 14±0.74 | 12±0.71 |
| IBA (4.0) | CR | CR | 15 | 4 | 15±0.81 | 11±0.82 |
| IBA (4.5) | CR | CR | 15 | 4 | 18±0.88 | 23±0.81 |
| IBA (5.0) | CR | CR | 15 | 4 | 26±0.82 | 24±0.72 |
| IBA (0.5) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| IBA (1.0) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| IBA (1.5) + PG (10) | NR | NR | 0 | 0 | NA | NA |
| IBA (2.0) + PG (10) | CR | CR | 52 | 37 | 25±0.82 | 13±0.81 |
| IBA (2.5) + PG (10) | ARF | ARF | 100 | 100 | 17±0.68 | 10±0.81 |
| IBA (3.0) + PG (10) | CR | CR | 72 | 68 | 18±0.65 | 19±0.82 |
| IBA (3.5) + PG (10) | CR | CR | 70 | 62 | 23±0.65 | 18±0.81 |
| IBA (4.0) + PG (10) | CR | CR | 72 | 62 | 33±0.65 | 28±0.81 |
| IBA (4.5) + PG (10) | CR | CR | 72 | 65 | 32±0.68 | 28±0.73 |
| IBA (5.0) + PG (10) | CR | CR | 51 | 36 | 28±0.67 | 24±0.77 |
| IAA (0.5) | NR | NR | 0 | 0 | NA | NA |
| IAA (1.0) | NR | NR | 0 | 0 | NA | NA |

*Table 2 Contd…*

*Table 2 Contd…*

| | | | | | | |
|---|---|---|---|---|---|---|
| IAA (1.5) | NR | NR | 0 | 0 | NA | NA |
| IAA (2.0) | NR | NR | 0 | 0 | NA | NA |
| IAA (2.5) | NR | NR | 0 | 0 | NA | NA |
| IAA (3.0) | NR | NR | 0 | 0 | NA | NA |
| IAA (3.5) | NR | NR | 0 | 0 | NA | NA |
| IAA (4.0) | CCE | CCE | 32 | 31 | NA | NA |
| IAA (4.5) | CCE | CCE | 32 | 32 | NA | NA |
| IAA (5.0) | CCE | CCE | 28 | 27 | NA | NA |
| NAA (0.5) | NR | NR | 0 | 0 | NA | NA |
| NAA (1.0) | NR | NR | 0 | 0 | NA | NA |
| NAA (1.5) | NR | NR | 0 | 0 | NA | NA |
| NAA (2.0) | NR | NR | 0 | 0 | NA | NA |
| NAA (2.5) | NR | NR | 0 | 0 | NA | NA |
| NAA (3.0) | CCE | CCE | 10 | 10 | NA | NA |
| NAA (3.5) | CCE | CCE | 20 | 22 | NA | NA |
| NAA (4.0) | CCE | CCE | 32 | 35 | NA | NA |
| NAA (4.5) | CCE | CCE | 28 | 25 | NA | NA |
| NAA (5.0) | CCE | CCE | 27 | 28 | NA | NA |

MJ- Maharaji, CH - Chambura,

CCE - Callus at Cut End, ARF– Adventitious Root Formation, CR – Callose Roots

NR - No Response, NA- Not Applicable, Ten replicates/treatment

Data scored after six weeks.

## DISCUSSION

Present investigation was carried out chiefly to explore organogenetic potential of shoot apices taken from mature trees of Ambri, Chambura, Maharaji, Golden Delicious apple cultivars to obtain clonal plantlets. The key factors governing cloning of apple through *in vitro* means were observed to be proper sterilization, pre-inoculation chilling, control over oxidative browning, strength of nutrient medium, appropriate hormonal concentration and hardening.

Fig-1a

Fig-1b

Fig-2a

Fig-2b

Fig-3a

Fig-3b

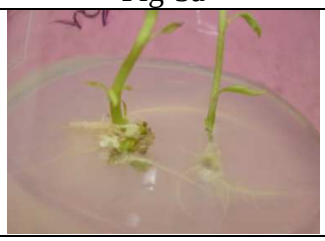

Fig-4a

Fig-4b

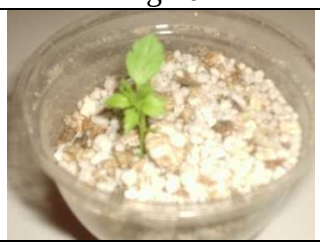

Fig-5a

Fig-5b

Figs 1-5: Morphogenetic response of shoot apices from mature trees of Maharaji and Chambura cultivars of apple (Malus pumila) to various phytohormones

**Legend:**

Fig-1a: Shoot induction in Maharaji cultivar from mature shoot apex on MS (1/2) + BA (5μM) + PG (10μM) (after 2nd week)

Fig-1b: Shoot induction in Chambura cultivar from mature shoot apex on MS (1/2) + BA (5μM) + PG (10μM) (after 3rd week)

Fig-2a: Direct multiple adventitious shoot formation in Maharaji cultivar on MS (1/2) + BA (5μM) + PG (10μM) (after 5th week)

Fig-2b: Direct multiple adventitious shoot formation in Chambura cultivar on MS (1/2) + BA (5μM) + PG (10μM) (after 5th week)

Fig-3a: Subcultured multiple shoots of Maharaji cultivar on MS (1/2) + BA (5μM) + PG (10μM) (after eight weeks)

Fig-3b Subcultured multiple shoots of Chambura cultivar on MS (1/2) + BA (5μM) + PG (10μM) (after eight weeks)

Fig-4a: Rooting of subcultured shoots of Maharaji cultivar on MS (1/2) + IBA (2.5μM) + PG (10μM) (after six weeks of subculture)

Fig-4b: Rooting of subcultured shoots of Chambura cultivar on MS (1/2) + IBA (2.5μM) + PG (10μM) (after six weeks of subculture)

Fig-5a: Deflasked plantlet of Maharaji cultivar in Sand-soil mixture 1:1 for acclimatization.

Fig-5b: Deflasked plantlet of Chambura cultivar in Sand-soil mixture 1:1 for acclimatization.

In present work, initial treatment of explants with sodium hypochlorite (10%) for 20 minutes followed by treatment with $HgCl_2$ (0.1%) for 90 seconds (after 24 hour chilling) was found to be highly effective for successful establishment of explants in all the cultivars under study. In contrast to this Zimmerman (1984) suggested use of calcium hypochlorite (20%) for controlling microbial contamination in apple cultivars.

In apple, browning of explants due to phenolic exudations has been found to be a main problem during culture establishment (Jones, 1967). This problem has been overcome by the use of PVP (Walkey, 1972) or PG (Jones and Hatfield, 1976) in the medium. In present investigation, overnight chilling of explants at 4°C in Kn (15µM) after initial sterilization, regular transfer (5-7 times) onto fresh nutrient medium of same composition and reduction of MS salt strength to half have been found to be effective steps for controlling browning.

Different nutrient media have been tried from 1958 onwards for establishment and proliferation of adventitious multiple shoots from shoot apices different cultivars of apple, like Knop's salt solution by Jones (1976), W-63 by Saad (1965), MiS by Messer and Lavee (1969), DP by Powel (1970), EL by Elliott (1972), FN by Fuji and Nito (1972), K(Knudson) by Jones and Hatfield (1976), QM by Snir and Erez (1980), LS by James and Thurbon (1981), 8P by Niizeki *et al.,* (1983), Nitsch's medium by Koudir *et al.* (1984), Lapovior medium by Le (1985), Gamborg's medium by Barberi and Moorini (1987), KSMP by Doughty and Power (1988), WPM by Orlikowska (1988), DKW by Wilson and James (2003). Most other persons like Zimmerman (1984), Anderson (1990), Dong *et al.* (1995), Caboni *et al.* (2000), Hoffmann *et al.,* (2001), Lambert & Tepfer (2001), Hofmann *et al.* (2001), Martins *et al* (2001), Sicurani *et al* (2001). Dobránszki *et al.,* (2002), Kadota *et al.* ( 2002), Cheng *et al.* (2003), Damiano and Monticelli (2003), Hao and Deng (2003), Hofer (2004), Sharma *et al.* (2004), Modgil *et al.* (2005), Allan *et al.* (2006), Dalal *et al.* (2006), Dandekar *et al.* (2006), Dantas *et al.* (2006), Goani *et al.* (2006), Bisogenin *et al.* (2008) have used MS (1962). In present experiment MS medium was tried which gave favourable results.

Strength of medium salts has been found to play vital role in culture establishment and shoot proliferation in M.7 apple root stock and half strength MS medium seems to be most effective (Werner and Roe, 1980; Bartish and Korkhovoi, 1997). Cheema and Sharma (1983) have, however, observed that half strength MS medium favoured the development of highly hydrated shoots, which were sensitive to injury and initiated much basal callusing. Such an observation has not been recorded in present studies using MS medium with ½ salt strength but instead our observations are in line with Werner and Roe (1980) and Bartiah and Korkhovoi (1997) in finding such a medium effective for culture establishment and shoot proliferation of apple. In contrast to this Cheema and Sharma (1983) have shown that MS medium with full

strength of salts fortified with BA (1.0mg/l) + IBA (0.2 mg/l) supported growth of shoot apices in apple which is supported by Sharma *et al.* (2004) who have used TDZ in addition to BA.

Shoot proliferation leading to multiple shoot proliferation was very much recorded in present work by using PG (10µM) and BA (4-5µM) in MS (1/2) medium. Earlier proliferation of shoots of M.7 and M.26 apple cultivars on Quoirin's medium (1974) using vitamins of Wetmore and Sorokin (1955) enriched with floridzin and PG ($10^{-3}$M), BA (0.5 mg/l), IBA (1mg/l) and IAA (1mg/l) was recorded by Jones (1976) which supports our results. Sharma *et al.* (2004) have found TDZ more effective than BA on Ambri cultivar which contradicts our findings on Maharaji and Chambura cultivars. Recently Dalal *et al.* (2006) have reported BA (2.22 µ*M*) effective for shoot proliferation which is in corroboration with our results.

In present investigation, best root initiation and elongation to obtain complete plantlets, was observed on MS (1/2) supplemented with IBA (2.5µM) + PG (10µM) which corroborates with the results of Zimmerman (1984); James and Thurbon (1979, 1981); Hicks, (1987); Correa *et al.* (1990); Nui *et al.* (1995) and Puntae and Martin (1997) but contradicts the findings by Dalal *et al* (2006) who have used very low concentration of IBA (0.49µ*M*) instead of 2.5µ*M* .

There has been a long controversy over the impact of PG on rooting. Zimmerman and Broome (1981) observed that phloroglucinol (a phenolic compound) favours rooting and reduces callus formation in apple cultivar Spartin, with different concentrations of IBA (0-4.9 µM). A number of other workers have also reported favourable effect of PG on rooting of different apple cultivars (James and Thurbon, 1979, 1981; Mehra and Saroj, 1979; Singha, 1982 and James, 1983). James and Thurbon (1979) reported auxin synergistic effect of PG in the process. Present findings also revealed that direct rhizogenesis took place in presence of IBA (2.5, 5µM) + PG (10µM). In contrast to this, Snir and Erez (1980) and Welender (1983) reported that PG (1mM) inhibited rooting in apple root stock $A_2$.

Use of shoot apices as explants for clonal micropropagation of apple has been found to be effective by Jones (1967), Pieniazek (1968), Elliot (1972), Powel (1970) Abbott and Whitely (1976), Zimmerman (1984) and Kumar and Kumar (1998). Present work also showed shoot tip explants to be highly effective in raising clonal (true to type) plants by direct regeneration. The presumption that more than 80,000 plants could be produced from single shoot tip in six months (Jones, 1967), can perhaps hold true after the refinement in presently established protocol, as very high number of direct multiple shoots were observed at basal end of subcultured shoots and the number continued to increase further after each subculture. Individual plantlets recovered in the present trials were looking healthy.

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# SHORT COMMUNICATIONS

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# Comparative Limnology of Telbal Nallah and Hazratbal Basin of Dal Lake in Kashmir

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***Key words:*** Limnology, Telbal Nallah, Dal Lake, Kashmir

The Dal lake is situated in the heart of the Srinagar at an elevation of 1586 m a.s.l and has surface area of 11.5km$^2$. It is multibasined lake with Nagin, Gagribal, Hazratbal and Boddal as its four basins. It is believed to be fed by a number of underground springs, but the main source is the Telbal Nallah that enters into the Hazratbal basin of the lake on the northern side at a place called Hanzheeul. (Fig. 1). Telbal catchment area lies at 34°4' to 34° 14′ N latitude and between 74° 48' to 75° 8'E longitude, covering an altitude range between 1600-4250m (a.m.s.l).

**Fig 1. Location map showing Telbal Nallah and Hazratbal basin of Dal lake**

In this communication an attempt is made to compare the physico-chemical features of Telbal Nallah with the Dal Lake (Hazratbal basin). The sampling was carried out during summer (June-August 2004) from the Telbal Nallah and Hazratbal basin of the Dal lake. For this purpose, dipping one liter polyethylene bottle just below the surface of water collected the water samples. Temperature, depth, transparency were recorded on the spot, while other parameters were analyzed in the laboratory within 24 hours in accordance with APHA, 1998; CSIR, 1974; and Mackereth (1963).

Data regarding various physico-chemical parameters of water is given in Table 1.

**Table I. Physico-chemical characteristics of water in Telbal Nallah and Hazratbal basin of Dal lake during June - August 2004**

| | Telbal Nallah | | | Dal Lake (Hazratbal Basin) | | |
|---|---|---|---|---|---|---|
| **Parameter** | **June** | **July** | **August** | **June** | **July** | **August** |
| **Depth (m)** | 1.7 | 1.5 | 1.2 | 2.40 | 2.41 | 2.42 |
| **Transparency (m)** | 0.42 | 0.72 | 0.41 | 1.54 | 1.57 | 1.56 |
| **Air temperature (°C)** | 27.0 | 27.0 | 26.0 | 28.0 | 26.0 | 27.0 |
| **Water temperature (°C)** | 23.0 | 24.0 | 24.0 | 26.0 | 24.0 | 25.0 |
| **pH** | 6.84 | 7.95 | 7.20 | 8.1 | 8.0 | 7.9 |
| **Conductivity (µS/cm)** | 217 | 255 | 212 | 316 | 320 | 319 |
| **Dissolved oxygen (mg/L)** | 5.4 | 5.7 | 5.6 | 6.0 | 5.9 | 6.4 |
| **Free/$CO_2$ (mg/L)** | 11.0 | 16.0 | 16.0 | 2.5 | 2.7 | 2.4 |
| **Chlorine (mg/L)** | 9.5 | 9.0 | 8.0 | 29.0 | 31.0 | 28.0 |
| **Total alkalinity** | 90.0 | 92.0 | 90.0 | 129 | 130 | 132 |
| **Total phosphate phosphorus (mg/L)** | 85 | 50.0 | 640 | 382 | 385 | 384 |
| **Nitrate-N (mg/L)** | 996 | 492 | 523 | 328 | 329 | 323 |
| **Ammonia-N (mg/L)** | 45.0 | 83.0 | 52.0 | 187 | 192 | 189 |

Water level of water body plays an important role in governing its water quality. The average lowest depth was found in Telbal Nallah against the highest in Dal lake. Further, the low transparency was found for Dal lake which may be attributed to: (i) entry of silt-laden water, (ii) rich concentration of nutrients, (iii) development of plankton blooms and (iv) macrophytc growth besides many exogenous and endogenous materials. The temperature of air affects the surface temperature and the two go hand in hand. Conductivity values were high in case of Dal lake. The specific conductivity is an indicator of the total nutrient level of a waterbody and is, therefore, used to indicate the trophic status. Using specific conductivity as an index of values more than 200µS/cm show higher enrichment level enrichment (Rawson, 1960). The conductivity values in both the water bodies reflect high ionic concentration. D.O values were comparatively high for Dal lake than Telbal Nallah. This is possibly due to the fact that in this season the longer hours of sunshine result in the prolonged photosynthetic activity of phytoplankton and macrophytes librating daygen (Qadri and Yousuf, 1978). The pH values were higher in lake water than Telbal Nallah. The increased alkalinity of lake water during summer is obviously related to the metabolic

activities of the autotrophs which by utilizing carbon dioxide and liberation of oxygen during photosynthesis reduce the H - ion concentration greatly (Kaul and Handoo, 1980). The alkalinity was high in case of lake water. As per the classification of Moyle (1945), both the types of waterbodies are under hard water type. Sites rich in $CO_2$ were comparatively less alkaline and decrease in its concentration result in an increase in pH. The high chloride concentratin in lake water may be attributed to the presence of large amounts of organic matter of both allocthanous and autocthanous organism (Pandit, 1999). The relatively low content of nitrate nitrogen in the Hazratbal basin may be attributed to profuse and luxuriant growth of macrophytes which utilize it during photosynthesis while the comparatively high content of ammonical nitrogen may be due to excessive use of nitrogen fertilizers in floating vegetable gardens and heavy anthropogenic pressure in the catchment area resulting in organic pollution. The high total phosphorus in lake water may be related to agricultural practice.

In conclusion, it was noted that lake water contains higher doses of nutrients as compared to Tebal Nallah which may be related to heavy anthropogenic pressures in catchment area resulting in discharge of human and agricultural wastes, stagnation of water and greater biological activities as a result of increased water temperature and availability of plant nutrients.

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# Suspended Particulate Matter in Industrial and Outskirt Residential Areas of Jammu

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***Key words:*** Air pulution, particulate matter, Jammu

Particulate matter is a discrete mass of any material, except pure water that exists as liquid or solid in the atmosphere and of microscopic or sub-microscopic dimensions. A large number of studies have been made on SPM from time to time by CPCB. This study was carried out during 2001 to investigate the level of SPM (suspended particulate matter) in outskirt residential and industrial areas of Jammu. (Fig.1)

For monitoring of SPM levels, these areas were divided into different sites:

A. Outskirt Residential area was divided into five sites:
   Site I: Nowabad Narwal Sunjwan Road, Site II: Karan Nagar,
   Site III: Krishna Nagar, Site IV Trikuta Nagar, Site V: Digiana.
B. Industrial area was divided into two sites:
   Site VI: Gangayal Induatrial Area, Site VII: Bari-Brahmana Industrial Area
   The area of study is shown in Figure 1.

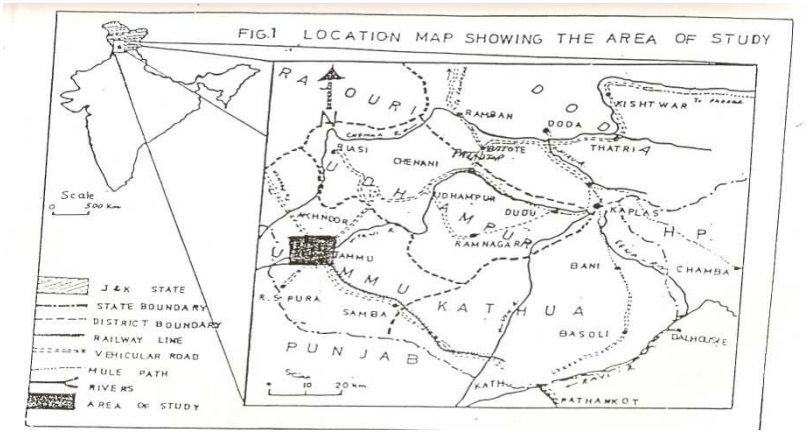


**Figure 1. Location map showing the area of study**

Sampling was done for 24 hours at each site with the help of balance, filter paper and High Volume air sampler and level of SPM was calculated by using following formula:

SPM ($\mu g/m^3$) = $(Fw_2-Fw_1)* 10^6/$ A.V.

Where:
Fw1 = Initial weight of filter paper
Fw2 = Final weight of filter paper
AV (Air Volume Sampled) = Sampling rate * Net sampling time
Sampling rate = (initial flow rate of air + Final flow rate of air)/2
Net sampling time = (Final time totaliser reading – Initial time totaliser reading) * 60.
Tables 1 and 2 show the SPM level in the Outskirt Residential Area and Industrial Area of Jammu respectively

**Table 1. SPM level in outskirt residential areas of Jammu**

| SITES | SPM (μg/m$^3$) |
|---|---|
| Site I. (Nowabad Narwal Sunjawan Road) | 175.163 |
| Site II. (Karan Nagar) | 63.924 |
| Site III. (Krishna Nagar) | 125.155 |
| Site IV. (Trikuta Nagar) | 96.160 |
| Site V. (Digiana) | 169.545 |

**Table 2. SPM level in industrial areas of Jammu**

| SITE | SPM(μg/m$^3$) |
|---|---|
| Site VI. (Gangyal Industrial Complex) | 239.154 |
| Site VII. (Bari-Brahmana Industrial Complex) | 447.059 |

In Jammu City, the increase in number of vehicles and traffic flow rate on the roads had added SPM in air to a considerable extent. The analysis of the data of SPM level collected in present study revealed that the industrial areas of Jammu have more SPM level than that of Outskirts Residential Areas of Jammu.

The level of SPM was observed to range from 63.924 μg/m$^3$ to 175.163 μg/m$^3$ in the Outskirt Residential areas of Jammu whereas the SPM level in industrial areas of Jammu ranged from 239.154 μg/m$^3$ to 447.059 μg/m$^3$. The critical analysis of the data at Outskirt Residential Areas of Jammu revealed that at Site-II (Karan Nagar) and Site-IV (Trikuta Nagar), the level of SPM was below 100 μg/m$^3$. This was due to raining a day before and on the day of sampling. From this it can be concluded that the humid atmosphere

reduces the level of SPM. Prasad *et at* (1998) while studying SPM level of Indian cities observed that air pollutant level in southern cities exhibited declining trend as compared with that of northern cities due to presence of humid atmosphere in coastal areas.

This clearly indicates that traffic flow rate is one of the major contributing factors of SPM in air. The presence of high SPM (239.154-447.059 μg/$m^3$) in industrial area was due to emissions from the chimneys of factories and other industrial units. The value of SPM level in the Outskirt Residential Area of Jammu was observed to be less than 200 μg/$m^3$ prescribed limits of the SPM in residential area and similarly the SPM level in the industrial area was also observed to be less than 500 μg/$m^3$ the prescribed limits of SPM in Industrial Area as per Ambient Air Quality-status and statistics, 1995 (CPCB, 1995)