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# LS – An International Journal of Life Sciences

**Vol. 2, Issue 2, May-August 2013**

## Contents

**LS - An International Journal of Life Sciences**
DOI : 10.5958/j.2319-1198.2.2.012



# Comparative Anatomical Studies of Some Genera of Cucurbitaceae Juss.

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**ABSTRACT**

Comparative anatomical studies were carried out on four species of Cucurbitaceae Juss.: *Citrullus lanatus* (Thunb.) Matsum and Nakai (watermelon and brown-seeded melon), *Cucumis melo* L. (true melon), *Cucumeropsis mannii* Naud. [Syn. *C. edulis* (Hooker f.) Cogn. (white-seeded melon)) and *Cucurbita moschata* (Duch. Ex Lam.) Duch. Ex Poiret (squash melon)] found in Nigeria. This work presents the anatomical features of the melons in this family together for the first time. Parts of each species (petioles, pedicels and tendrils) were hand-sectioned and observed using a photomicroscope. Additional anatomical features of taxonomic value are variations in the number of vascular bundles, the presence of schlerenchymatous pericycle and endodermal cells and the presence of sclerenchymatous sheath in the medullary rays of the pedicels.

**Keywords**: Anatomy, *Citrullus*, *Cucumis, Cucurbita, Cucumeropsis*, Cucurbitaceae, petioles, pedicels, tendrils

## 1. INTRODUCTION

The *Citrullus* genus is represented by one species in Nigeria, *C. lanatus* (Dalziel, 1937). It is a plant genus of desert vines, and the only Cucurbitaceae genus having pinnatifid leaves. Members bear small sepals, solitary staminate flowers, a corolla, that is 5-parted to the base, and fleshy fruits, and included the watermelon (of three different varieties) and the brown-seeded melon, having bitter pulp, which are the sub-species of the species, *C. lanatus*, widely cultivated in Nigeria. *Cucumis* is a genus of twinning, tendril-bearing plants, which includes the cucumber, true melons, the honey melon and *Cucumis anguria*, the West Indian gherkin (Ghebretinsae *et al*., 2007). The leaves are not or rarely divided beyond the middle. The fruits are smooth or at most green-lined or hairy, a ground trailer. The genus, *Cucumeropsis,* is represented by one species in Nigeria, the white-seeded egusi melon, *Cucumeropsis mannii* (Okoli, 1984). The leaves are smooth or nearly so on the lower surface, with a broad sinus and distant lobes at the base. The male flowers are umbellate on a distinct long peduncle. The fruit is about 30 cm long and 10 cm in diameter. *Cucurbita* is a genus of about 20 species. Five *Cucurbita* species, *C. pepo, C. moschata, C. maxima, C. argyrosperma* and *C. ficifolia,* comprise the principal cultivated squash and/or pumpkin crops. Both mature and immature fruit are the most important edible plant parts, although for some species, seed, flowers, roots and even leaves are consumed. All the different plant species in this study have nutritional and medicinal uses. In Nigeria, they are used for different purposes in different parts of the country. They occupy a special place in the life and culture of many ethnic groups (Okoli, 1984). *Citrullus lanatus*, watermelon, is cultivated extensively for its fruits and seeds. The sweet, red juicy pulpy flesh of the watermelon is eaten as a dessert. In some parts of Nigeria, the skin and the mesocarp, including the seeds, are eaten as a delicacy. The fruit contains 93% water, with small amounts of protein, fat, minerals and vitamins

and the major nutritional components of the fruit are carbohydrates (6.4/100 g; Rubatzky and Yamaguchi-Mas, 1997). In some cases, the extracted juice is fermented to produce an alcoholic drink and the fruits have been served as a reserve water source in parts of Nigeria where there is drought or where the source of water is contaminated.

The bitter variety of *C. lanatus*, brown-seeded melon, after shelling and grinding the seeds, are used for thickening soup and the bitter pulpy flesh is sometimes cooked and fed to pigs. In Akwa Ibom State, Nigeria, the seeds of the brown-seeded melon are ground and mixed with pepper (*Capsicum annum)* and salt, baked and eaten. We particularly find it very delicious and irresistible. Sometimes, during traditional marriage ceremonies, they are ground and baked in form of a cake and presented as a traditional cake.

*Cucumis melo*, sweet melon, is a 'fruit' rather than a vegetable; the sweet, delicately flavoured, juicy flesh of the pepo is eaten raw, often as a dessert. In the eastern part of Nigeria, the small white seeds are roasted and eaten as a delicacy.

*Cucumeropsis mannii*, the white-seeded egusi melon, has edible and oily seeds, which are used like the seeds of *Citrullus lanatus* – brown-seeded melon (Okoli, 1984). The seeds are also fried and chewed as a delicacy in the South part of Nigeria. The squash melon, *Cucurbita moschata*, is edible. The young leaves are cooked and eaten as a vegetable. The leaves are sometimes used as a replacement for Okra (*Abelmoschus esculentus* (L.) Moench) in soups and stews. The flesh of the fruit is cooked and eaten with palm oil (*Elaeis guineensis* Jacq.). The dried shell of the fruit is sometimes used as a container (Hugues and Phillippe, 1989). This paper aims at providing additional anatomical features of the different species of the family Cucurbitaceae that may be of taxonomic value in their identification and classification.

## 2. MATERIALS AND METHODS

Fresh plant materials collected by the authors during field trips to various parts of Nigeria shown in Table 1 and their seeds germinated in plastic bags in the botanical garden of the University of Port Harcourt were used for the anatomical studies. The plants were transplanted directly into the soil in the botanical garden. Mature petioles, pedicels and tendrils were collected from the middle portion of the plant. Hand sections were made according to the method of Okoli (1987). A camel hair brush was used in collecting good and high-quality sections. Sections were stained by dipping the slides in 1% safranin for 5 min, and rinsed with distilled water to remove excess stain. They were then mounted temporarily in glycerine and viewed under the microscope. Photomicrographs of good sections were taken with a Leitz Diaplan photomicroscope fitted with Leica WILD MPS 52 camera at ´10 microscope objective lens.

**Table 1: Sources of plant materials used for the study**

| Taxa | Serial number of species studied | Collector and accession/herbarium number | Collection date | Locality |
|---|---|---|---|---|
| *Citrullus lanatus* Thunb. Matsum and Nakai (watermelon) | 001 | Ajuru, 023 UPH | 20/02/2010 | Rumuokoro, Port Harcourt |
| *C. lanatus* (brown-seeded melon) | 002 | Ajuru, 025 UPH | 24/02/2010 | Rumuokoro, Port Harcourt |
| *Cucurbita moschata* (Duch. Ex Lam.) Duch. Ex Poiret | 003 | Ajuru, 030 UPH | 01/04/2010 | Port Harcourt fruit garden, Kaduna Street, Port Harcourt. |
| *Cucumeropsis mannii* Naud. (syn. *C. edulis* (Hooker f.) Cogn.) | 004 | Ajuru, 031 UPH | 03/04/2010 | Ozuaha farmland, Ikwerre L.G.A., Port Harcourt. |
| *Cucumis melo* L. (sweet melon) | 005 | Ajuru, 035 UPH | 15/09/2011 | Port Harcourt fruit garden, Kaduna street, Port Harcourt |

## 3. RESULTS

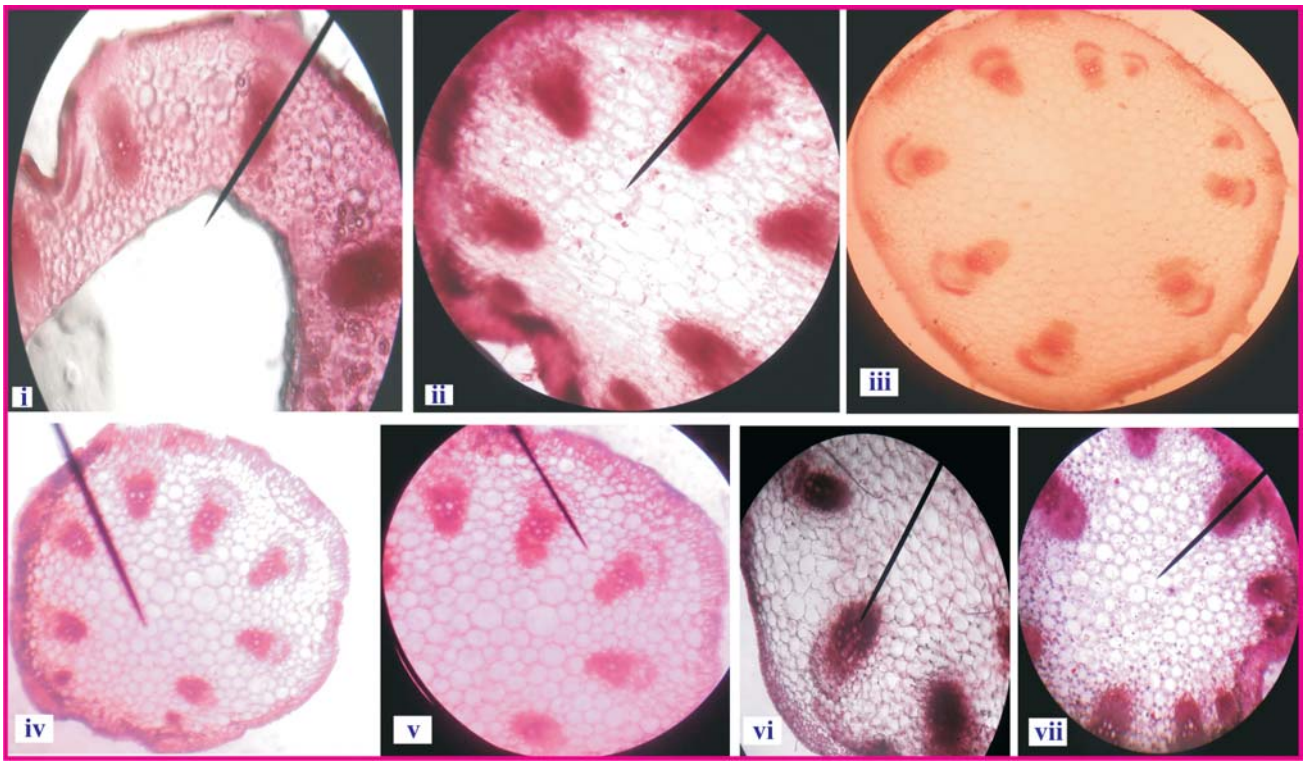
### 3.1. Petiole Anatomy

The arrangement of tissues in the petioles of the species studied is shown in Plates I–VII. They all possess a layer of epidermal cells, followed by four to six layers of collenchyma cells.

The amphiphloic bicollateral vascular bundles are arranged in a single ring in the cortical parenchyma tissue. In *Cucurbita moschata*, *Cucumeropsis mannii and C. lanatus,* the watermelon with light green exocarp, as well as the one with light green exocarp with deep green vertical stripes, the bundles are nine in number. They are eight in *Cucumis melo* and *Citrullus lanatus*, - watermelon with deep green exocarp colour; but six in *C. lanatus* – brown-seeded egusi melon. In all the species studied, there are crescentric caps of sclerenchyma over the peripheral phloem of each of the bicollateral bundles. The shape of the transverse sections of each of the species is different. The shape is reniform in *C. moschata*, notched ovate in *C. lanatus*, brown-seeded melon, and *C. mannii*, and spherical in all the watermelons and *C. melo.*

### 3.2. Tendril Anatomy

In all the species (Plates viii–xiv), the tendril has a single layer of epidermal cells. The epidermal layer is followed by four or five layers of collenchymatous tissue in all the species. There is a layer of endodermis, which is sclerenchymatous, and two to three patches of pericyclic sclerenchyma cells distributed around the vascular bundles. The bicollateral vascular bundles are arranged



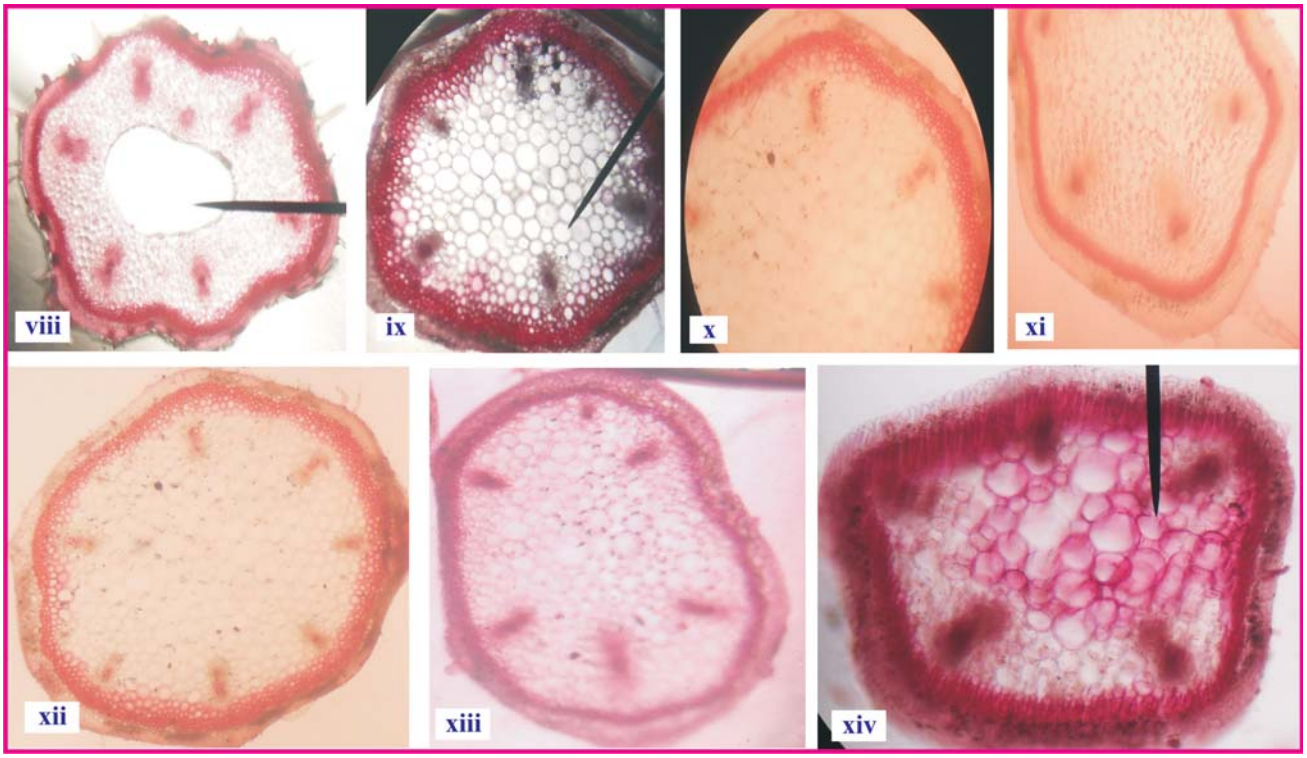

**Petiole anatomy**

**Plates** (i) *C. moschata* (note: crescentric caps of sclerenchyma over the peripheral phloem in the bicollateral vascular bundles in all the species); (ii) *C. lanatus* – -brown-seeded melon; (iii*) C. lanatus* – watermelon with light green exocarp colour; (iv) *C. lanatus* – watermelon with deep green exocarp colour; (v) *C. lanatus* – watermelon with light green exocarp and deep green vertical stripes; (vi) *C. melo*; (vii) *C. mannii*

in a single ring in the cortical parenchyma tissue, and are seven in number in *C. moschata* and *C. lanatus*, watermelon with light green exocarp and those with light green exocarp but with deep green vertical stripes,, six in *C. melo* and *C. lanatus*, brown-seeded melon and watermelon with deep green exocarp colour, and five in *C. manni*. The pith cavity in some species like *C. melo*, *C. lanatus*, brown-seeded melon and the watermelons, and *C. mannii* are completely filled up with oval-shaped parenchyma cells. But in *C. moschata*, there is a hollow at the centre of the pith cavity devoid of any cells. Also, the arrangement of the vascular bundle is peripheral in all the species, with the vascular tissues occurring in the ridges and not the furrows, except in *C. moschata*, where there is a vascular tissue at the furrow.

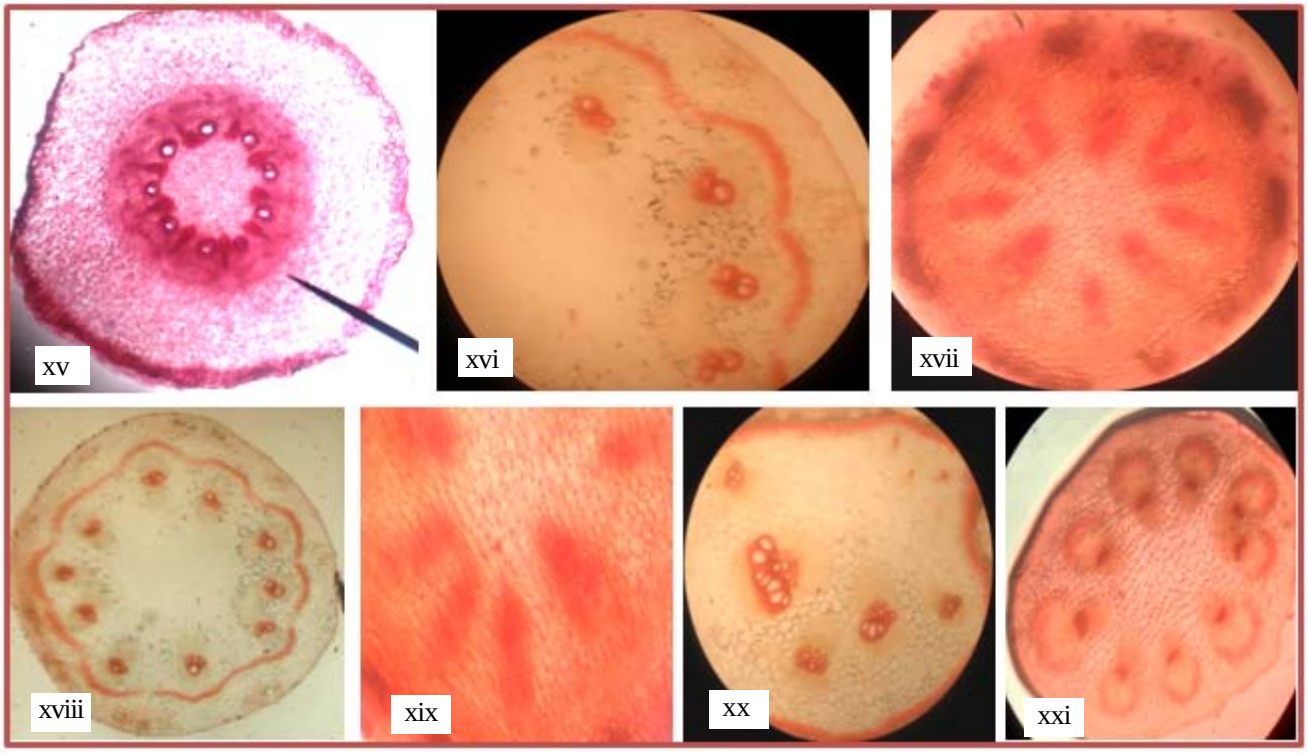
### 3.3. Pedicel Anatomy

The tissue distribution in the pedicels studied is shown in Plates xv–xxi. The epidermal layer in all the species is one-celled thick, closely followed by three or four layers of collenchyma cells in all the species, except in *C. melo*, where there are two or three layers of sclerenchyma cells beneath the epidermal layer. The collenchyma cells are closely followed by seven or eight layers of cortical parenchyma cells. There is a layer of endodermal cells, followed by two or three layers of pericyclic cells in all the species. The arrangement of the vascular bundles is peripheral in all the species except *C. moschata* and *C. melo*. In *C. moschata*, the vascular bundles occur at the centre, interspersed with bundle sclerenchyma sheath. In *C. melo*, they are arranged in an arc, on the abaxial part of the pedicel, with one bundle larger than the others. There are about 5 vascular bundles in *C. melo*, 9 in *C. moschata* and C. *mannii*, 10 in all the watermelon types and 11 in *C. lanatus* - brown-seeded melon. The pith cavity is completely filled up with the ground parenchyma cells.




**Tendril anatomy**

**Plate** (viii) *C. moschata*; (ix) *C. lanatus* – brown-seeded melon; (x) *C. lanatus* – watermelon with light green exocarp colour; (xi) *C. lanatus* – watermelon with deep green exocarp colour; (xii) *C. lanatus* – watermelon with light green exocarp and deep green vertical stripes; (xiii) *C. melo*; (xiv) *C. mannii.*




**Pedicel anatomy**

**Plate** (xv) *C. moschata*; (xvi) *C. lanatus* – brown-seeded melon; (xvii) *C. lanatus* – watermelon with light green exocarp colour; (xviii) *C. lanatus* – watermelon with deep green exocarp colour; (xix) *C. lanatus* – watermelon with light green exocarp and deep green vertical stripe; (xx) *C. melo*; (xxi) *C. mannii*

## 4. DISCUSSION

The variations that exist among the species of Cucurbit studied are of taxonomic value as reported by Stace (1980), Abdul Rahaman and Oladele (2010a and 2010b), Ogunkunle and Oladele (2008) and Ndukwu and Okoli (1992). The anatomical structures of all the species as observed in this study are in agreement with the findings of Esau (1965) on herbaceous plants. The primary plant body is made up of the dermal system, the epidermis, the fascicular tissue system composed of the xylem and phloem, and the fundamental or ground system represented by the cortex and pith. The pith lies to the inside of the stele and as in other herbaceous stems, it is wide in diameter and continues to occupy a high proportion of the stem throughout life. This is also seen in *Helianthus tuberosus* L. and *H. annuus* L. (Metcalfe and Chalk, 1979). All the species in this study are said to be drought- resistant (Oomen and Grubben, 1977). The presence of the cuticle, sclerenchymatous sheath in the petiole, tendril and pedicel supports this view. The medullary rays alternate with the bundles linking up with the cortex instead of forming a continuous cylinder. This then explains why the plants are creeping/trailing and also a climber in the case of *C. mannii*. The observed anatomical similarities among the Cucurbit species studied indicate phylogenetic relatedness of the taxa. These anatomical differences observed in each species must have been as a result of evolution, conferring heritable variation that could be exploited for taxonomic purposes (Ajuru and Okoli, 2012). The present study is an attempt to provide additional anatomical features of the different species of the family Cucurbitaceae that may be of taxonomic value in their identification and classification, as earlier described by Hutchinson and Dalziel (1954).

In conclusion, based on anatomical features, the species of melons studied can readily be distinguished from one

another as a result of variation in the number of layers of tissues and vascular bundles present in them. This information can be useful in herbal medicine and Forensic Science, where identification and authentication of plant specimens are essential.

## ACKNOWLEDGEMENT

We are grateful to the Curators of the Herbarium at the Obafemi Awolowo University, Ile-Ife, Ife, Osun State, Nigeria, for their assistance during the study.

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**LS - An International Journal of Life Sciences**
DOI : 10.5958/j.2319-1198.2.2.013



# Impact of Cumin Variety (GC-4) under Semi-Arid Conditions of Rajasthan

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**ABSTRACT**

Impact assessment of demonstrations was carried out on cumin (variety-GC-4) and net additional return over farmers practice during *Rabi* seasons of 2010-11 and 2011-12. The data revealed that the yield in demonstration plots increased from 16.22 to 18.91% over farmers practice during the study period. Similarly, the economic analysis of data indicated higher B:C ratio. The adoption of variety GC-4 ranged from 78 to 98% in operational as well as in nearby villages. The use of improved variety GC-4 of cumin under semi-arid conditions of Nagaur proved superior with respect to adoption by farmers and productivity levels.

**Keywords:** Cumin, front line demonstration, adoption, B:C ratio, impact

## 1. INTRODUCTION

Indian agriculture has the significant history. Today, India ranks second worldwide in farm output. India is the largest producer and exporter of seed spices in the world. Seed spices are mostly grown in western part of India having arid and semi-arid climatic region. Rajasthan and Gujarat are two major states of India and known as seed spices bowl. Among the seed spices, cumin is one of the high value but low volume seed spice crop of arid and semi-arid region of Gujarat and Rajasthan, which requires less inputs, i.e., irrigation, fertilizers, labour, etc., but it is high risky crop due to sensitiveness with biotic and abiotic factors. In India, the area under cumin is 5,27,132 ha with the total production of 2,83,000 tonnes with an average productivity of 537 kg/ha (Jangir and Rathore, 2007). In state like Rajasthan, area under cumin is spreading in semi-arid regions but the productivity of the crop is low as compared to Gujarat. The yield potential of cumin is low primarily due to less availability of better genotype for cultivation under the prevailing agro-climatic conditions (Anwer *et al*., 2011) and second, due to poor management of production sites (Sharma and Meena, 2012). Improved agronomical practices along with good quality seed are the key factors to increase the productivity levels. Keeping these facts in view demonstrations were laid out by Krishi Vigyan Kendra, Nagaur, to evaluate the impact of high yielding variety (GC-4) along with seed treatment during *Rabi* seasons of 2010-11 to 2011-12.

## 2. MATERIALS AND METHODS

The demonstrations were carried out at farmers field in village Nimbri and Gaju of Nagaur during *Rabi* seasons from 2010-11 to 2011-12. The soil of demonstration sites was mostly sandy loam in texture with low organic carbon (0.17-0.25%). The available Nitrogen Phosphorus and Potash were 138-147, 13-17 and 164-204 kg/ha, respectively. pH of irrigation water was 8.6 with high Electrical conductivity (±3.42/dsm). The sowing of cumin variety GC-4 was done in between second week of November to last week of November in both the years. Seed treatment was done with carbendazim @ 2 g per kg seed. The other crop management practices were performed as per standard recommendations of the

region. One block of farmer's practice (farmers own seed without any treatment) was also kept for comparison. Harvesting of crop was done during second fortnight of April and grain yield was recorded.

## 3. RESULTS AND DISCUSSION

### 3.1. Yield

The yield data presented in Table 1 indicated that the yield in demonstration plots increased from 16.22 to 18.91% over farmers practice during the study period. The variation in yield data during the years was aberrant due to weather condition like frost and sudden development of cold waves. However, owing to dry conditions, the arid region of north western parts of Rajasthan like Nagaur offers an excellent opportunity for high-quality seed spices. Thus, congenial weather condition together with quality seed can be an effective tool to achieve better yield.

**Table 1: Year-wise impact of cumin GC-4 on yield/ha**

| Year | No. of farmers | Area (ha) | Average yield (q/ha) | | % increase in yield |
|---|---|---|---|---|---|
| | | | Demon-stration | Farmer's practice | |
| 2010-11 | 10 | 5.0 | 7.31 | 6.29 | 16.22 |
| 2011-12 | 15 | 7.5 | 6.24 | 5.06 | 18.91 |

The higher yield in demonstration plots might be due to higher yield potential and wilt tolerance of the variety (GC-4) during crop seasons. Similarly, in arid region, where intense solar irradiations and high temperature are amply available during summer months, harnessing solar heat has been found effective in minimising cumin wilt incidence in the field (Singh *et al*., 2012; Sharma and Meena, 2012).

### 3.2. Economic Analysis

The adoption of any technology in modern agriculture can only be feasible and acceptable to farmers if it is economically viable. In seed spices, the potential yields are not achieved due to poor seed replacement rate and slow initial growth (Jangir and Rathore, 2007). The cultivation of traditional crops has become uneconomical due to increasing cost of cultivation under such circumstances, therefore farmers always look forward for more remunerative option. The economic analysis data of cumin (GC-4) presented in Table 2 indicated that variety GC-4 gave higher B:C ratio and net additional return over farmers practice during both the years of crop season. The study reveals that use of improved variety (GC-4) may provide a net additional return up to Rs.70,517 and Rs.36,960 per hectare during year 2010-11 and 2011-12, respectively.

### 3.3. Impact and Horizontal Spread of Cumin (GC-4)

The level of adoption and horizontal spread of cumin variety GC-4 was assessed in operational area and nearby villages during 2011-12. As a result of survey, it was 98% adoption of improved variety in operational village Nimbri. The beneficiary farmers were advised to sale their seed among farmers. The "farmer to farmer" approach for horizontal spread of seed resulted in significant spread of seed in near by villages. The survey data are summarised in Table 3. The adoption of variety

**Table 3: Horizontal spread of cumin variety (*N*=100)**

| Name of operational villages | Per cent adoption | No. of nearby villages |
|---|---|---|
| Nimbri | 98 | 10 |
| Gaju | 78 | |

**Table 2: Economic analysis of cumin GC-4 under semi-arid condition of Nagaur**

| Year | Demonstration | | Farmers practice | | B:C ratio | | Net additional return (Rs.) |
|---|---|---|---|---|---|---|---|
| | Cost (Rs. per ha) | Average gross return (Rs. per ha) | Cost (Rs. per ha) | Average gross return (Rs. per ha) | Demontration | Farmer's practice | |
| 2010-11 | 24,513 | 95,030 | 23,200 | 81,770 | 1:2.86 | 1:2.52 | 70,517 |
| 2011-12 | 25,440 | 62,400 | 24,000 | 50,600 | 1:2.45 | 1:2.10 | 36,960 |

GC-4 ranged from 68 to 90% in nearby villages of operational area.

In the light of above findings, it may be concluded that use of improved variety GC-4 of cumin under semi-arid conditions of Nagaur proved superior in respect to adoption by farmers and productivity levels.

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**LS - An International Journal of Life Sciences**
DOI : 10.5958/j.2319-1198.2.2.014



# Comparative Study of $S_1$ and Open Pollinated Progenies of Pearl Millet [*Pennisetum glaucum* (L.) R. Br. emends Stuntz] for Green Fodder Yield and its Components

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**ABSTRACT**

A comparative study of 36 $S_1$, $S_1$-OP-$S_1$ and $S_1$-OP-OP progenies of pearl millet [*Pennisetum glaucum* (L.) R.Br. emends Stuntz] for green fodder yield over one season revealed highly significant differences among progenies for days to heading, plant height, tillers per plant, leaves per plant, third leaf area, internode extrusion, leaf:stem ration, stem thickness and green fodder yield per plant. The genotypic and phenotypic coefficients of variation were more or less similar for all the characters. High (50–70%) to vary high (>70%) heritability was coupled with high (>50%) genetic advance as percentage of mean was observed for green fodder yield per plant in all three types of progenies, confirming that genotypic variance has contributed substantially to the total variance. Whereas, high heritability was coupled with medium to low was observed. Based upon character association and path analysis, it may be concluded that plant height, tillers per plant and leaves per plant were the major green fodder yield component, because it has high positive significant association and positive direct effect on green fodder yield in all the three types of progenies. Based on the results of the *per se* performance of three progenies considering for various traits, the genotypes IP-141, IP-196-1, IP-111-1, IP-181-1 and IP-204-1 were found to be superior for earliness, plant height, tillers per plant, leaves per plant, third leaf area, stem thickness, leaf:stem ratio and green fodder yield per plant. Therefore, these progenies should be utilized in further breeding programme for developing superior varieties.

**Key words:** Pearl millet, $S_1$ progeny, $S_2$ progeny, OP progeny

## 1. INTRODUCTION

India has about 0.05% of the global pastures against more than 15% of the animal population (Swaminathan, 1998). To sustain this animal wealth as well as its productivity a large quantity of fodder is required throughout the year. Therefore, forage crops are important for the economy of our country as these crops constitute major nutritional base of our livestock. The efficacy of both milch as well as drought animals largely depend upon the supply of adequate quantity of quality feed and fodder, in which green fodder plays a vital role.

In recent years, shortage of fodder has emerged as an important problem particularly in the Rajasthan, which calls for the attention of the researchers to initiate efforts that ensure regular supply for the development of dairy farming and improving our cattle health. Pearl millet can be grown under dry land conditions and indeed in some of the hottest and driest region where no other crop can be grown and play critical role in food security. Generally, a good quality forage should have high content of protein and digestible nutrients and low content of fiber and lignin. Pearl millet yields better forage as compared to sorghum and maize because of the absence of hydracyanic acid. Its ability to produce high biomass even with minimum soil moisture makes it a highly desirable crop in semi-arid and arid zones of Rajasthan. Therefore, the development of new high yielding varieties of green fodder

requires knowledge of the existing variability or diversity and also the extent of association among the yield contributing characters for yield and yield-related attributing characters is also necessary.

## 2. MATERIALS AND METHODS

During *kharif* 2008, 120 S, lines from various sources were obtained from Agricultural Research Station (ARS), Durgapura, and International Crop Research Institute for Semi-Arid Tropics (ICRISAT), Patancheru, India. While keeping half seed in safe storage, these 120 $S_1$ lines were evaluated during *kharif*, 2008. In each line, randomly selected plants were selfed as well as left to open pollinate. Thus, from each line two types of progenies were obtained $S_2$ and $S_1$ (open pollinated, OP). In the years 2009, these two types of progenies were raised for evaluation in paired rows of each line for evaluation. Randomly selected plants within each $S_2$ progeny were again selfed to obtain seeds for $S_3$ generation. At the same time, these were also left to open pollinate. Similarly, in the OP progeny, randomly selected plants were selfed as well as left to open pollinate. Because of adverse weather condition the seed set was poor in $S_2$ progeny rows. Hence, no seed was harvested but the seed set was appreciable in OP progenies, so the seeds of selfing ($S_1$) and OP of each of the OP progeny of the original $S_1$ progeny were harvested. These were designated as $S_1$-open pollinated-$S_1$ ($S_1$-OP-$S_1$) and $S_1$-open pollinated-open pollinated ($S_1$-OP-OP), respectively. In the experiment from the original 120 lines of 2009, 36 lines were selected at random and $S_1$ (2009), $S_1$-OP-$S_1$ and $S_1$-OP-OP progenies of these selected lines were evaluated in three separate experiments in Randomised Block Design with three replications at Research Farm, SKN College of Agriculture, Jobner, during *kharif*, 2010. In each replication, each $S_1$, $S_1$-OP-$S_1$ and $S_1$-OP-OP progenies were sown in a plot of 3.0 x 0.80 m size accommodating a two rows of 3 m row length spaced 40 cm apart. The plant-to-plant distance of 10 cm within the rows was adjusted by thinning at three leaf stage. The crop was raised with recommended package of practices. Ten competitive plants were randomly selected in each type of progenies in each replication avoiding border plants at the time of initiation of early heading in each plot. Ten random plants were selected to record data for nine quantitative morphological traits *viz*., days to heading, plant height, tillers per plant, leaves per plant, third leaf area, internode extrusion, leaf-to-stem ratio, stem thickness and green fodder yield per plant. Statistical analysis was carried out following procedures of Panse and Sukhatme (1978). The heritability estimates of >70% is considered very high; 50–70% high; 30–50% moderate and <30% low (Hallauer and Miranda, 1981).

## 3. RESULTS AND DISCUSSION

### 3.1. Variability Studies

Genetic variability play a vital role in the improvement

**Table 1: Analysis of variance for green fodder yield and yield contributing character in $S_1$, $S_1$-OP-$S_1$ and $S_1$-OP-OP progenies**

| Characters | Mean sum of squares | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Replication | | | Genotypes | | | Error | | |
| | $S_1$ progenies df.=2 | $S_1$-OP-$S_1$ progenies df.=2 | $S_1$-OP-OP progenies df.=2 | $S_1$ progenies df.=35 | $S_1$-OP-$S_1$ progenies df.=35 | $S_1$-OP-OP progenies df.=35 | $S_1$ progenies df.=70 | $S_1$-OP-$S_1$ progenies df.=70 | $S_1$-OP-OP progenies df.=70 |
| Days to heading | 52.02 | 49.84 | 26.03 | 40.19** | 59.65** | 21.61** | 20.28 | 16.3854 | 8.4085 |
| Plant height (cm) | 200.73 | 215.89 | 2.49 | 699.44** | 718.78** | 1122.72** | 72.81 | 172.3694 | 6.5937 |
| Tillers per plant | 0.05 | 0.043 | 0.06 | 0.09** | 0.14** | 0.24** | 0.02 | 0.0534 | 0.0359 |
| Leaves per plant | 1.23 | 1.40 | 1.58 | 4.06** | 5.61** | 10.25** | 1.40 | 1.2257 | 0.7171 |
| Third leaf area | 18.57 | 296.93* | 633.52 | 493.40** | 590.82** | 2932.73** | 71.06 | 85.5788 | 235.9745 |
| Internode extrusion | 1.88 | 1.83 | 1.25 | 1.98** | 3.45** | 0.91* | 0.92 | 0.845 | 0.5094 |
| Leaf:stem ratio | 0.0043 | 0.0035 | 0.0006 | 0.0059** | 0.0088** | 0.0055** | 0.0026 | 0.0014 | 0.0007 |
| Stem thickness | 0.75 | 2.10 | 1.89 | 2.10** | 2.68** | 2.89** | 0.76 | 0.7805 | 0.712 |
| Green fodder per plants | 371.66 | 232.19 | 90.65 | 2423.49** | 2626.79** | 6623.65** | 423.29 | 109.549 | 63.0718 |

Abbreviation: OP=open pollinated., df = Degree of freedom

*Significant at *P*=0.05;**significant at *P*=0.01 and $S_1$-OP-$S_1$ progenies=$S_2$ progenies, $S_1$-OP-OP progenies=open pollinated progenies

of crops since it offers scope for natural and artificial selection to tailor genotypes suitable for diverse agro-ecological conditions. Thus, more the genetic variability in the base material more is the chance of improvement, the variability parameters presented in Tables 1 and 2, showed highly significant differences among all the three types of progenies *viz*., $S_1$, $S_1$-OP-$S_1$ and $S_1$-OP-OP, for all characters, indicating sufficient variability existed within the progenies used to bred and suggested ample scope for selection of superior and diverse progenies of pearl millet. The phenotypic expression of the characters is the results of interaction between the genotypes and environment. Genotypic coefficient of variation (GCV) measures the range of variability available in a crop and also enables to compare the amount of variability present in different characters. The high phenotypic coefficient of variation and GCV for green fodder yield per plant was noted in $S_1$-OP-$S_1$ and $S_1$-OP-OP progenies, while it was moderate for green fodder yield per plant in $S_1$ progenies. It was moderate for internode extrusion and stem thickness in $S_1$-OP-$S_1$ progenies and tillers per plant and third leaf area in $S_1$-OP-OP progenies, indicating the possibilities of improving these characters through phenotypic selection for the development of superior variety or hybrids.

High heritability and high genetic advance indicates the predominance of additive gene action and greater response to phenotypic selection and improvement of such traits could be anticipated. In the present study, very high heritability coupled with high genetic advance as percentage of mean was observed for green fodder yield per plant in $S_1$-OP-$S_1$ and $S_1$-OP-OP progenies suggested that these traits are governed by additive gene action, whereas the characters *viz*., leaves per plant, internode extrusion and leaf:stem ratio had high heritability accompanied with moderate genetic advance as percentage of mean in $S_1$-OP-$S_1$ progenies and very high heritability coupled with moderate genetic advance as percentage of mean was observed for plant height, leaves per plant and third leaf area, whereas the characters *viz*., tillers per plant, leaf:stem ratio and stem thickness had high heritability with moderate genetic advance as percentage of mean was observed in $S_1$-OP-$S_1$ progenies. In $S_1$ progenies, high heritability coupled with moderate genetic advance as percentage of mean was observed for green fodder yield per plant, whereas the characters

**Table 2: Estimates of genetic parameters in fodder pearl millet in $S_1$, $S_1$-OP-$S_1$ and $S_1$-OP-OP progenies**

| Characters | $S_1$ progeny | | | | $S_1$-OP-$S_1$ progeny | | | | $S_1$-OP-OP progeny | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Coefficient of variance | | Herita-bility broad sense (%) | Expected advance expressed as percentage of mean | Coefficient of variance | | Herita-bility broad sense (%) | Expected advance expressed as percentage of mean | Coefficient of variance | | Herita-bility broad sense (%) | Expected advance expressed as percentage of mean |
| | Pheno-typic | Geno-typic | | | Pheno-typic | Geno-typic | | | Pheno-typic | Geno-typic | | |
| Days to heading | 8.82 | 4.38 | 24.65 | 4.48 | 9.26 | 6.33 | 46.81 | 8.93 | 5.89 | 3.45 | 34.36 | 4.17 |
| Plant height (cm) | 9.95 | 8.57 | 74.15 | 15.21 | 12.08 | 8.66 | 51.38 | 12.79 | 11.48 | 11.38 | 98.26 | 23.25 |
| Tillers per plant | 15.31 | 10.18 | 44.26 | 13.96 | 19.61 | 11.68 | 35.48 | 14.34 | 24.85 | 20.13 | 65.62 | 33.61 |
| Leaves per plant | 15.03 | 9.35 | 38.71 | 12.76 | 15.91 | 11.74 | 54.41 | 25.26 | 17.67 | 15.96 | 81.6 | 43.56 |
| Third leaf area ($cm^2$) | 9.51 | 7.75 | 66.45 | 14.93 | 11.06 | 9.01 | 66.31 | 16.38 | 24.20 | 21.54 | 79.21 | 38.24 |
| Internode extrusion (cm) | 17.07 | 9.02 | 27.92 | 9.63 | 31.69 | 22.58 | 50.77 | 21.13 | 12.41 | 5.69 | 21.02 | 5.62 |
| Leaf:stem ratio | 17.79 | 9.70 | 29.73 | 10.91 | 18.79 | 15.01 | 63.79 | 24.7 | 14.89 | 12.25 | 69.57 | 21.05 |
| Stem thickness (mm) | 18.17 | 11.04 | 36.93 | 13.83 | 21.41 | 14.33 | 44.81 | 19.77 | 19.77 | 14.05 | 50.53 | 20.58 |
| Green fodder yield per plant (g) | 27.86 | 21.79 | 61.17 | 35.12 | 32.58 | 30.64 | 88.45 | 59.38 | 40.98 | 40.40 | 97.2 | 82.06 |

Abbreviation: OP=open pollinated

*viz*., tillers per plant, leaves per plant and stem thickness had moderate heritability coupled with low genetic advance as percentage of mean. The characters like days to heading and internode extrusion depicted low heritability with low genetic advance as percentage of mean, indicating the influence of non-additive gene action in controlling the expression of these traits. Hence, simple phenotypic selection for these traits may not be fruitful as these traits are highly influenced by the environment.

### 3.2. Correlation Studies

The genotypic and phenotypic correlation coefficient worked out among different characters including green fodder yield per plant revealed that, in general, the phenotypic correlation coefficients were higher than their respective correlation coefficients at genotypic levels, which is a results of environmental effects (Tables 3, 4 and 5). Genotypic correlation coefficient provides a measure of genetic association between characters and thus helps in identifying the traits, which are important and need to be considered for improvement of yield. The green fodder yield per plant showed positive and significant association with plant height, tillers per plant, leaves per plant, third leaf area, internode extrusion and stem thickness, while non-significant and negative correlation were observed with days to head and leaf:stem ratio in $S_1$ progenies. In $S_1$-OP-$S_1$ progenies, the green fodder yield per plant showed the positive and significant association with plant height, tillers per plant, leaves per plant, third leaf area, leaf:stem ratio and stem thickness and association with remaining character days to heading showed negative and significant and with internode extrusion it was non-significant. In $S_1$-OP-OP progenies, the green fodder yield per plant showed the positive and significant association with plant height, tillers per plant, leaves per plant, internode extrusion, leaf:stem ratio and stem thickness, while negative and non-significant association was observed with days to heading. Similar to the present findings, significant positive association of third leaf area with green fodder yield per plant was reported earlier by Gupta and Athwal (1966), Narooka (1985) in full-sib, half-sib and $S_1$ progenies and Kumawat (1993) in $S_1$ progenies.

Inter-relationship among different traits revealed that significant and positive association of plant height with tillers per plant, leaves per plant, third leaf area, internode extrusion, stem thickness and leaf:stem ratio; of tillers per plant with leaves per plant, stem thickness and leaf:stem ratio; of leaves per plant with leaf:stem ratio; of third leaf area with internode extrusion and stem thickness in $S_1$ progenies. Whereas inter-relationship in $S_1$-OP-$S_1$ progenies showed positive and significant association of plant height with leaves per plant, third leaf area and leaf:stem ratio; of tillers per plant with leaves per plant and leaf:stem ratio; of leaves per plant with leaf:stem and third leaf area with stem thickness. In $S_1$-OP-OP progenies, inter-relationship showed positive and significant association of plant height with tillers per plant, leaves per plant, third leaf area, internode extrusion and stem thickness; of tillers per plant with leaves per plant, third leaf area, internode extrusion, leaf:stem ratio and stem thickness; and of leaves per plant with third leaf area, internode extrusion and stem thickness.

Perusal of direct and indirect effects of various characters on green fodder yield per plant (Tables 6, 7 and 8) indicate that, in general, there is an agreement between direction and magnitude of direct effect of various characters and correlation with green fodder yield per plant, i.e., the characters that had high correlations also showed high direct effect on green doffer yield. Indirect effects are opposing, thus a significant improvement in green doffer yield can be expected through selection in the component traits with high positive direct effects. Present studies revealed that plant height, leaves per plant and leaf:stem ratio had high direct effect on green fodder yield per plant.

The conclusion that could be reached from the variability, correlation and path coefficient is that plant height, tillers per plant, leaves per plant and leaf:stem ratio are the most important component characters for the green fodder yield. The other important characters are third leaf area, internode extrusion and stem thickness that should be considered as selection criteria in a selection programme. All these component characters had high variability, heritability, genetic advance, and hence, these may be successfully improving the green fodder yield.

Based on the results of the *per se* performance of the three progenies considering together for various fruits, the genotypes IP-141, IP-196-1, IP-111-1, IP-104-1 and IP-181-1 were observed superior in all the three types

**Table 3: G and P correlation coefficients among different characters of $S_1$ progenies**

| Characters | | Days to heading | Plant height (cm) | Tillers per plant | Leaves per plant | Third leaf area ($cm^2$) | Internode extrusion (cm) | Leaf : stem ratio | Stem thickness per plant (mm) | Green fodder yield (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to heading | G | | 0.0444 | -0.2063 | -0.4528 | 0.2698 | -0.2994 | -0.1011 | -0.5767 | -0.2718 |
| | P | | -0.0404 | 0.0405 | -0.0867 | 0.0078 | -0.1478 | -0.0739 | -0.2721** | -0.0874 |
| Plant height (cm) | G | | | 0.3146 | 0.4076 | 0.5629 | 0.5843 | 0.7459 | 0.5179 | 0.9229 |
| | P | | | 0.2066* | 0.3391** | 0.4689** | 0.3881** | 0.2443* | 0.3060** | 0.7206** |
| Tillers per plant | G | | | | 0.9008 | 0.1757 | 0.3389 | 0.2696 | 0.3815 | 0.1709 |
| | P | | | | 0.6685** | 0.0585 | 0.0757 | 0.196* | 0.1137 | 0.3692** |
| Leaves per plant | G | | | | | 0.1412 | 0.3202 | 0.6982 | 0.2233 | 0.3493 |
| | P | | | | | 0.0235 | 0.1205 | 0.3427** | 0.1456 | 0.4980** |
| Third leaf area ($cm^2$) | G | | | | | | 0.5992 | -0.5794 | -0.1656 | 0.5998 |
| | P | | | | | | 0.3315** | -0.1476 | 0.2957** | 0.3550** |
| Internode extrusion (cm) | G | | | | | | | -0.7879 | 0.3471 | 0.5481 |
| | P | | | | | | | -0.1702 | 0.1817 | 0.2728** |
| Leaf:stem ratio | G | | | | | | | | -0.3905 | -0.65702 |
| | P | | | | | | | | -0.0697 | -0.16809 |
| Stem thickness (mm) | G | | | | | | | | | 0.6984 |
| | P | | | | | | | | | 0.3540** |
| Green fodder yield per plant (g) | G | | | | | | | | | |
| | P | | | | | | | | | |

Abbreviations: G=genotypic; P=phenotypic. *Significant at *P*=0.05; **significant at *P*=0.01

**Table 4: G and P correlation coefficients among different characters of $S_1$-OP-$S_1$ progenies**

| Characters | | Days to heading | Plant height (cm) | Tillers per plant | Leaves per plant | Third leaf area ($cm^2$) | Internode extrusion (cm) | Leaf : stem ratio | Stem thickness per plant (mm) | Green fodder yield (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to heading | G | | -0.4642 | 0.0072 | -0.2126 | -0.0835 | -0.1782 | 0.2127 | -0.0448 | -0.3273 |
| | P | | -0.3276** | 0.0030 | -0.0665 | -0.0446 | 0.0126 | 0.0455 | -0.0999 | -0.2077* |
| Plant height (cm) | G | | | 0.0194 | 0.3193 | 0.5506 | 0.0423 | -0.4284 | 0.1934 | 0.5528 |
| | P | | | 0.0084 | 0.2542* | 0.3989** | -0.1090 | 0.2607** | 0.1348 | 0.3854** |
| Tillers per plant | G | | | | 0.8434 | -0.1978 | 0.2706 | 0.4587 | 0.4138 | 0.5615 |
| | P | | | | 0.6979** | -0.0298 | 0.0681 | 0.2612** | 0.0735 | 0.3514** |
| Leaves per plant | G | | | | | -0.2705 | -0.002 | 0.3210 | 0.1836 | 0.4671 |
| | P | | | | | -0.1381 | -0.043 | 0.2412* | 0.0344 | 0.2512* |
| 3rd leaf area ($cm^2$) | G | | | | | | 0.1783 | -0.2502 | 0.4772 | 0.4017 |
| | P | | | | | | 0.1772 | -0.1508 | 0.3902** | 0.3815** |
| Internode extrusion (cm) | G | | | | | | | -0.2724 | 0.1864 | 0.0518 |
| | P | | | | | | | -0.0703 | 0.1747 | 0.0568 |
| Leaf : stem ratio | G | | | | | | | | -0.1593 | 0.5753 |
| | P | | | | | | | | 0.0257 | 0.3565** |
| Stem thickness (mm) | G | | | | | | | | | 0.3036 |
| | P | | | | | | | | | 0.2515* |
| Green fodder yield per plant (g) | G | | | | | | | | | |
| | P | | | | | | | | | |

Abbreviations: G=genotypic; OP=open pollinated; P=phenotypic**;** *Significant at *P*=0.05; **significant at *P*=0.01

Table 5: G and P correlation coefficients among different characters of $S_1$-OP-OP progenies

| Characters | | Days to heading | Plant height (cm) | Tillers per plant | Leaves per plant | Third leaf area ($cm^2$) | Internode extrusion (cm) | Leaf : stem ratio | Stem thickness per plant (mm) | Green fodder yield (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to heading | G | | -0.1961 | -0.1205 | -0.1034 | -0.3124 | -0.3391 | -0.3852 | -0.0795 | -0.2325 |
| | P | | -0.1120 | -0.1169 | -0.1163 | -0.2342* | -0.0163 | -0.1703 | -0.0769 | -0.1116 |
| Plant height (cm) | G | | | 0.3124 | 0.3764 | 0.4152 | 0.5971 | 0.0334 | 0.6523 | 0.5812 |
| | P | | | 0.2361* | 0.3304** | 0.3451** | 0.2762** | 0.0307 | 0.4597** | 0.5727** |
| Tillers per plant | G | | | | 0.8885 | 0.5341 | 0.6480 | 0.2960 | 0.3334 | 0.8572 |
| | P | | | | 0.6843** | 0.4125** | 0.3169** | 0.2193* | 0.2110* | 0.6729** |
| Leaves per plant | G | | | | | 0.3412 | 0.6451 | 0.2084 | 0.3744 | 0.7398 |
| | P | | | | | 0.2451* | 0.2389* | 0.1454 | 0.2770** | 0.6561** |
| Third leaf area ($cm^2$) | G | | | | | | 0.1425 | 0.4152 | 0.2541 | 0.3412 |
| | P | | | | | | -0.0231 | 0.253* | 0.1425 | 0.1254 |
| Internode extrusion (cm) | G | | | | | | | -0.4074 | 0.3094 | 0.6421 |
| | P | | | | | | | -0.0935 | 0.2855** | 0.2982** |
| Leaf:stem ratio | G | | | | | | | | 0.0723 | 0.4766 |
| | P | | | | | | | | -0.1651 | 0.3947** |
| Stem thickness (mm) | G | | | | | | | | | 0.5775 |
| | P | | | | | | | | | 0.41068** |
| Green fodder yield per plant (g) | G | | | | | | | | | |
| | P | | | | | | | | | |

Abbreviations: G=genotypic; OP=open pollinated; P=phenotypic; *Significant at *P*=0.05; **significant at *P*=0.01

**Table 6: Direct (diagonal) and indirect (above and below diagonal) effects at genotypic and phenotypic level of different characters towards green fodder yield per plant in $S_1$ progenies of pearl millet**

| Characters | | Days to heading | Plant height (cm) | Tillers per plant | Leaves per plant | Third leaf area ($cm^2$) | Internode extrusion (cm) | Leaf : stem ratio | Stem thickness per plant (mm) | Corr. coeffi. with green fodder yield per plant (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to heading | G | **-1.4067** | 0.0246 | -0.3376 | 1.1019 | 0.3245 | 0.0870 | -0.0527 | -0.0128 | -0.2718 |
| | P | **-0.0822** | -0.0322 | 0.0032 | -0.0324 | -0.0005 | 0.0264 | 0.0406 | -0.0102 | -0.0874 |
| Plant height (cm) | G | -0.0625 | **0.5552** | 0.5148 | -0.9919 | 0.6770 | -0.1699 | 0.3886 | 0.0115 | 0.9229 |
| | P | 0.0033 | **0.7963** | 0.0162 | 0.1269 | -0.0301 | -0.0693 | -0.1343 | 0.0115 | 0.7206** |
| Tillers per plant | G | 0.2902 | S0.1747 | **1.6363** | -2.1920 | 0.2113 | -0.0985 | 0.1405 | 0.0085 | 0.1709 |
| | P | -0.0033 | 0.1645 | **0.0786** | 0.2502 | -0.0038 | -0.0135 | -0.1077 | 0.0043 | 0.3692** |
| Leaves per plant | G | 0.6370 | 0.2263 | 1.4740 | **-2.4334** | 0.1698 | -0.0931 | 0.3638 | 0.0050 | 0.3493 |
| | P | 0.0071 | 0.2700 | 0.0525 | **0.3742** | -0.0015 | -0.0215 | -0.1883 | 0.0055 | 0.4980** |
| Third leaf area | G | -0.3795 | 0.3125 | 0.2875 | -0.3436 | **1.2027** | -0.1742 | -0.3019 | -0.0037 | 0.5998 |
| | P | -0.0006 | 0.3734 | 0.0046 | 0.0088 | **-0.0642** | -0.0592 | 0.0811 | 0.0111 | 0.3550** |
| Internode extrusion | G | 0.4212 | 0.3244 | 0.5546 | -0.7792 | 0.7206 | **-0.2907** | -0.4105 | 0.0077 | 0.5481 |
| | P | 0.0122 | 0.3090 | 0.0059 | 0.0451 | -0.0213 | **-0.1785** | 0.0935 | 0.0068 | 0.2728** |
| Leaf:stem ratio | G | 0.1422 | 0.4141 | 0.4412 | -1.6990 | -0.6968 | 0.2290 | **0.5210** | -0.0087 | -0.6570 |
| | P | 0.0061 | 0.1945 | 0.0154 | 0.1282 | 0.0095 | 0.0304 | **-0.5496** | -0.0026 | -0.1681 |
| Stem thickness | G | 0.8113 | 0.2875 | 0.6243 | -0.5434 | -0.1992 | -0.1009 | -0.2035 | **0.0222** | 0.6984 |
| | P | 0.0224 | 0.2437 | 0.0089 | 0.0545 | -0.0190 | -0.0324 | 0.0383 | **0.0377** | 0.3540** |

Abbreviations: G=genotypic; P=phenotypic; **Significant at *P*=0.01

**Table 7: Direct (diagonal) and indirect (above and below diagonal) effects at genotypic and phenotypic level of different characters towards green fodder yield per plant in $S_1$-OP-$S_1$ progenies of pearl millet**

| Characters | | Days to heading | Plant height (cm) | Tillers per plant | Leaves per plant | Third leaf area ($cm^2$) | Internode extrusion (cm) | Leaf : stem ratio | Stem thickness per plant (mm) | Corr. coeffi. with green fodder yield per plant (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to heading | G | **0.6785** | -1.9164 | -0.0159 | 0.2873 | 0.2654 | -0.3040 | 0.7890 | -0.1113 | -0.3273 |
| | P | **-0.0862** | -0.1420 | 0.0011 | 0.0133 | -0.0101 | 0.0007 | 0.0211 | -0.0054 | -0.2077* |
| Plant height (cm) | G | -0.3150 | **4.1283** | -0.0428 | -0.4315 | -1.7499 | 0.0722 | -1.5892 | 0.4807 | 0.5528 |
| | P | 0.0282 | **0.4336** | 0.0031 | -0.0508 | 0.0905 | -0.0059 | -0.1206 | 0.0073 | 0.3854** |
| Tillers per plant | G | 0.0049 | 0.0801 | **-2.2040** | -1.1398 | 0.6286 | 0.4616 | 1.7016 | 1.0284 | 0.5615 |
| | P | -0.0003 | 0.0036 | **0.3655** | -0.1394 | -0.0068 | 0.0037 | 0.1209 | 0.0040 | 0.3514** |
| Leaves per plant | G | -0.1442 | 1.3182 | -1.8588 | **-1.3514** | 0.8597 | -0.0034 | 1.1908 | 0.4563 | 0.4671 |
| | P | 0.0057 | 0.1102 | 0.2551 | **-0.1997** | -0.0313 | -0.0023 | 0.1116 | 0.0019 | 0.2512* |
| Third leaf area | G | -0.0567 | 2.2731 | 0.4359 | 0.3655 | **-3.1782** | 0.3041 | -0.9282 | 1.1860 | 0.4017 |
| | P | 0.0038 | 0.1730 | -0.0109 | 0.0276 | **0.2269** | 0.0097 | -0.0698 | 0.0213 | 0.3815** |
| Internode extrusion | G | -0.1209 | 0.1746 | -0.5964 | 0.0027 | -0.5667 | **1.7057** | -1.0105 | 0.4633 | 0.0518 |
| | P | -0.0011 | -0.0473 | 0.0249 | 0.0086 | 0.0402 | **0.0545** | -0.0325 | 0.0095 | 0.0568 |
| Leaf:stem ratio | G | 0.1443 | -1.7686 | -1.0110 | -0.4338 | 0.7952 | -0.4646 | **3.7097** | -0.3959 | 0.5753 |
| | P | -0.0039 | -0.1130 | 0.0955 | -0.0482 | -0.0342 | -0.0038 | **0.4628** | 0.0014 | 0.3565** |
| Stem thickness | G | -0.0304 | 0.7984 | -0.9120 | -0.2481 | -1.5166 | 0.3179 | -0.5910 | **2.4853** | 0.3036 |
| | P | 0.0086 | 0.0584 | 0.0269 | -0.0069 | 0.0885 | 0.0095 | 0.0119 | **0.0545** | 0.2515* |

Abbreviations: G=genotypic; OP=open pollinated; P=phenotypic; *Significant at *P*=0.05; **significant at *P*=0.01

**Table 8: Direct (diagonal) and indirect (above and below diagonal) effects at genotypic and phenotypic level of different characters towards green fodder yield per plant in $S_1$-OP-OP progenies of pearl millet**

| Characters | | Days to heading | Plant height (cm) | Tillers per plant | Leaves per plant | Third leaf area ($cm^2$) | Internode extrusion (cm) | Leaf : stem ratio | Stem thickness per plant (mm) | Corr. coeffi. with green fodder yield per plant (g) |
|---|---|---|---|---|---|---|---|---|---|---|
| Days to heading | G | **-0.0543** | -0.1086 | -0.1822 | 0.0749 | 0.2088 | -0.0028 | -0.1591 | -0.0093 | -0.2325 |
| | P | **-0.0004** | -0.0516 | -0.0617 | -0.0178 | 0.0960 | 0.0010 | -0.0631 | -0.0140 | -0.1116 |
| Plant height (cm) | G | 0.0106 | **0.5537** | 0.4724 | -0.2728 | -0.2775 | 0.0049 | 0.0138 | 0.0761 | 0.5812 |
| | P | 0.0000 | **0.4603** | 0.1245 | 0.0507 | -0.1414 | -0.0164 | 0.0114 | 0.0836 | 0.5727** |
| Tillers per plant | G | 0.0065 | 0.1730 | **1.5121** | -0.6439 | -0.3569 | 0.0053 | 0.1222 | 0.0389 | 0.8572 |
| | P | 0.0000 | 0.1087 | **0.5274** | 0.1050 | -0.1690 | -0.0188 | 0.0813 | 0.0384 | 0.6729** |
| Leaves per plant | G | 0.0056 | 0.2084 | 1.3435 | **-0.7247** | -0.2280 | 0.0052 | 0.0861 | 0.0437 | 0.7398 |
| | P | 0.0000 | 0.1521 | 0.3609 | **0.1535** | -0.1004 | -0.0142 | 0.0539 | 0.0504 | 0.6561** |
| Third leaf area | G | 0.0170 | 0.2299 | 0.8076 | -0.2473 | **-0.6683** | 0.0012 | 0.1715 | 0.0296 | 0.3412 |
| | P | 0.0001 | 0.1589 | 0.2175 | 0.0376 | **-0.4098** | 0.0014 | 0.0938 | 0.0259 | 0.1254 |
| Internode extrusion | G | 0.0184 | 0.3306 | 0.9798 | -0.4675 | -0.0952 | **0.0081** | -0.1682 | 0.0361 | 0.6421 |
| | P | 0.0000 | 0.1271 | 0.1671 | 0.0367 | 0.0095 | **-0.0594** | -0.0347 | 0.0519 | 0.2982** |
| Leaf:stem ratio | G | 0.0209 | 0.0185 | 0.4476 | -0.1510 | -0.2775 | -0.0033 | **0.4130** | 0.0084 | 0.4766 |
| | P | 0.0001 | 0.0141 | 0.1157 | 0.0223 | -0.1037 | 0.0056 | **0.3707** | -0.0300 | 0.3947** |
| Stem thickness | G | 0.0043 | 0.3612 | 0.5041 | -0.2713 | -0.1698 | 0.0025 | 0.0299 | **0.1166** | 0.5775 |
| | P | 0.0000 | 0.2116 | 0.1113 | 0.0425 | -0.0584 | -0.0170 | -0.0612 | **0.1818** | 0.4107** |

Abbreviations: G=genotypic; OP=open pollinated; P=phenotypic; **Significant at *P*=0.01

of progenies and could be used for further breeding programme, due to high mean value of plant height, tillers per plant, leaves per plant, green fodder yield and earlier in flowering.

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**LS - An International Journal of Life Sciences**
DOI : 10.5958/j.2319-1198.2.2.015



# Inconsistent Impact of Nanoparticles on Food Chain

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**ABSTRACT**

Nanotechnology has proved to be beneficial for its medical, ethical, mental, legal and environmental applications. It encompasses several fields, e.g., engineering, biology, chemistry, computing, material science, military applications and communications. The incumbent technology identifies unique properties of materials with dimension in the range of 1-100 nm. Though these properties yield many far-reaching societal benefits in several fields, including improved manufacturing methods, water purification systems, energy systems, physical enhancement, nanomedicine, better food production methods and nutrition, and large-scale infrastructure auto-fabrication, but they may also pose threat and risks to environment and human health demanding great concern for safety issues. To lessen the deleterious impacts of nanoparticles (NPs) on environment, especially on the food chain, it is imperative to develop potential toxicity tests, which would aid in the rapid detection of the lethal effects of NPs (screening) and epidemiologic studies must be conducted on exposed populations. The uptake, bioaccumulation, biotransformation and risks of nanomaterials (NMs) for the food crops are still unclear. Very few reports exist that provide the in-depth information regarding interaction of NMs and plant species, largely at the very early growth stages of the plants.

**Keywords:** Biotransformation, bioaccumulation, epidemiology, uptake, nanomaterials, nanomedicine.

## 1. INTRODUCTION

Nanotechnology aims to deliver novel products and processes to enhance their performance and creating various interventions in a wide range of areas, nanoengineering has witnessed its contribution in nearly all industrial sectors, e.g., pharmaceuticals, electronics, automobiles, textile, chemical manufacturing, information technology and communications as well as biotechnology, with extensive integration of nanoparticles (NPs) in numerous commercial products. The increased use of engineered NPs in variable sectors owes to their unique properties and is likely to help the release of several toxic materials into the environment. Consequently, some concerns have been expressed regarding the risks to environmental as well as human health.

Based on their synthesis, NPs are categorised in to two broad groups: engineered NPs and incidental (or adventitious) NPs. The former NPs are those having highly specified properties and are further formed with four more preferred methods: gas-phase synthesis, vapour deposition, and colloidal and attrition methods. Contrarily, incidental NPs are formed in a relatively uncontrolled manner with heterogenous physical and chemical properties. The unifying factor for the two types is that both NPs have large surface volume ratio and this characteristic feature generally increases their potential for reactivity, which finally enhances their toxic behaviour. Hence, it is reported that the methods preferred for engineered synthesis of NPs may pose serious problems in inhalation, dermal absorption and ingestion (Aitken *et al*., 2004).

The synthesis of NPs, via engineered nanomaterials (NMs), is of prime importance. However, it calls for techniques to improve their bioavailability and toxicity. In this context, it may be stated that the interactions between engineered NMs and higher plants demand great attention because plants interact intimately with soil, water and the atmosphere. Thus, such an interaction constitutes the main route for their accumulation through the food

chain. The detailed process through which the NPs ingress the plants is not fully understood. Thus, we need to unravel the rapid practice of constantly producing the new materials and their accelerated applications (Miralles *et al*., 2012).

Detailed study pertaining to mobility, reactivity, exotoxicity and persistency were likely to provide details of their toxicity. Due to the lack of availability of quantitative analytical techniques for measuring NP in natural systems, there is a serious crave for information about their occurrence in the environment. Some ecotoxicological studies have shown that certain NPs affect organisms under environmental conditions, though most of them are found at elevated concentrations (Nowack and Buchell, 2007).

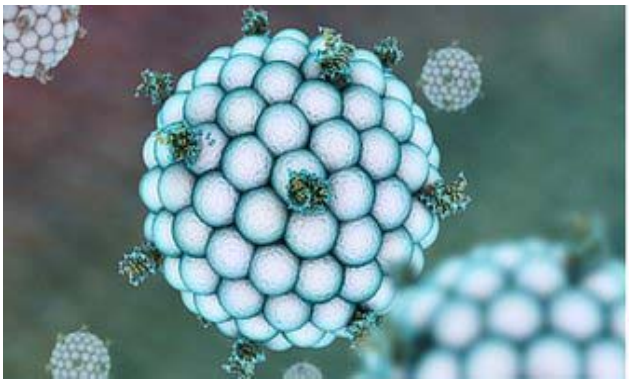

**Figure 1: Nanoparticles, illustrated in this computer-generated image, are just millionths of a millimetre in size**

Nanoscale pollutants can easily enter the crop roots, triggering cascade of reactions, which at the end effect the plant growth and health causing detrimental effects like stunted plant growth, boost the plants' absorption of pollutants, increasing the need for crop fertilizers, etc.

The mode of uptake, bioaccumulation, biotransformation and risks caused by the use NMs in the food crops are still not well understood. Although, scanty of literature is available to define the interaction of NM's with plant species very scanty information exists. One solitary report has explained the effect on *Arabidopsis thaliana* and the effect of ZnO NPs on ryegrass has also been demonstrated. Both the studies pertain to the effect of NMs on seed germination and seedlings growth.

There is general a lack of information on biotransformation of NMs in food crops and their possible communication to the next generation. Contrarily, evidence explaining the possible biomagnification of NPs in the food chain is also scarce.

Human beings are routinely exposed to ultrafine particles and fine particles due to air pollution and occupational cohorts relevant to the exposure to mineral dusts, fibres, welding fumes, combustion products and poorly soluble, low-toxicity particulates such as titanium dioxide and carbon black (Maynard and Kuempel, 2005; Nel *et al*., 2006). The hazards caused with these exposures (including engineered NPs) have also been studied and identified in animal models (Donaldson *et al*., 2004, 2006) providing a strong relationship between the physical properties like surface area, oxidative stress and pro-inflammatory effects of NPs in the lung. These reports state that greater the oxidative stress, the more likely the risk of inflammation and cytotoxicity (Nel *et al*., 2006; Oberdörster *et al*., 2005).

On the other hand, titanium dioxide particles, which are poorly soluble and having low-toxicity, cause an increased risk for lung cancer in animals on the basis of particle size and surface area (Oberdörster *et al*., 2005). Exposure to carbon nanotubes has adverse effects, e.g., the development of fibrosis and other pulmonary diseases (Lam *et al*., 2006; Oberdörster *et al*., 2005). NPs are Tran located to brain and activate platelets and enhance vascular thrombosis (Radomski *et al*., 2005). Admittedly, none of these findings are conclusive about the nature and extent of the hazards, but call for precautionary measures.

Unarguably, plants have evolved along with natural NMs, hence, the use of engineered NMs in several instruments and commodities have increased the likelihood of plant exposure to NMs (Pan and Xing, 2010). There is several form of transformation of these ENMs into plants, e.g., either through direct application, accidental release, contaminated soil/sediments or atmospheric fall outs. However, insufficient information exists about the impact of ENMs on food crops (Darlington *et al*., 2009; Pidgeon, 2009), and selective studies have been done to describe the toxic effects of ENMs on crop plants such as rape (*Brassica napus*), radish (*Raphanus sativus*), lettuce (*Lactuca sativa*), corn (*Zea mays*) and cucumber

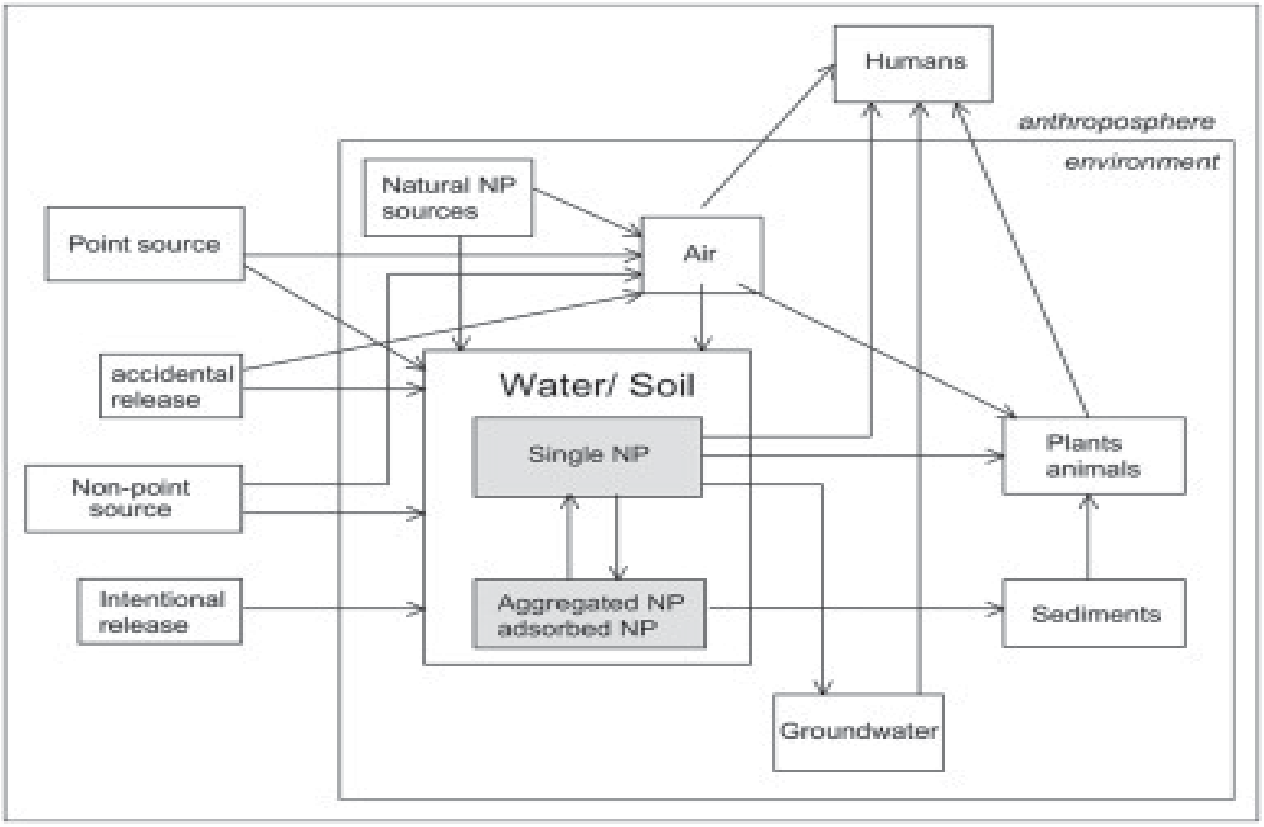


**Figure 2: Nanoparticle pathway from anthrophosphere into the environment, reaction in the environment and exposure to human.**

(C*ucumis sativus*), among others (Lin and Xing, 2007; Lee *et al*., 2008; Bareena *et al*., 2009).

### 1.1. Mode of Uptake of NMs by Plants

The information on the uptake of carbon-based and metal-based (MB) NMs by plants have attracted much significance in recent years and most studies correspond to the germination stage and cell cultures. Figure 3 depicts the steps during the uptake of nano-based pollutants by an organism.

### 1.2. Uptake of NPs

The selective uptake, translocation and accumulation of NPs into plants mainly depend on the plant species and the physical and chemical properties of the NPs. Figure 4 shows their mode of entry in the food chain.

There are several ways in which NPs can enter plant cells; with the help of carrier proteins, process of

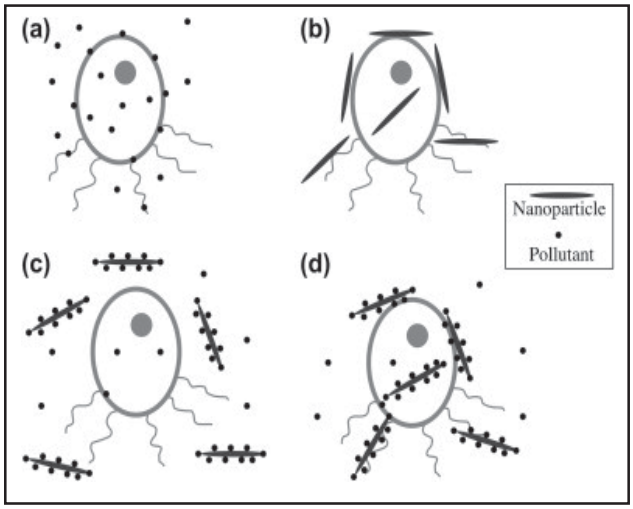


**Figure 3: (a) Adsorption and uptake of the pollutant, (b) adsorption and uptake of nanoparticles, (c) adsorption (or absorption) of pollutants onto nanoparticle and reduction in pollutant uptake by organism and (d) adsorption of nanoparticles with adsorbed pollutant and possible uptake of the pollutant nanoparticles.**

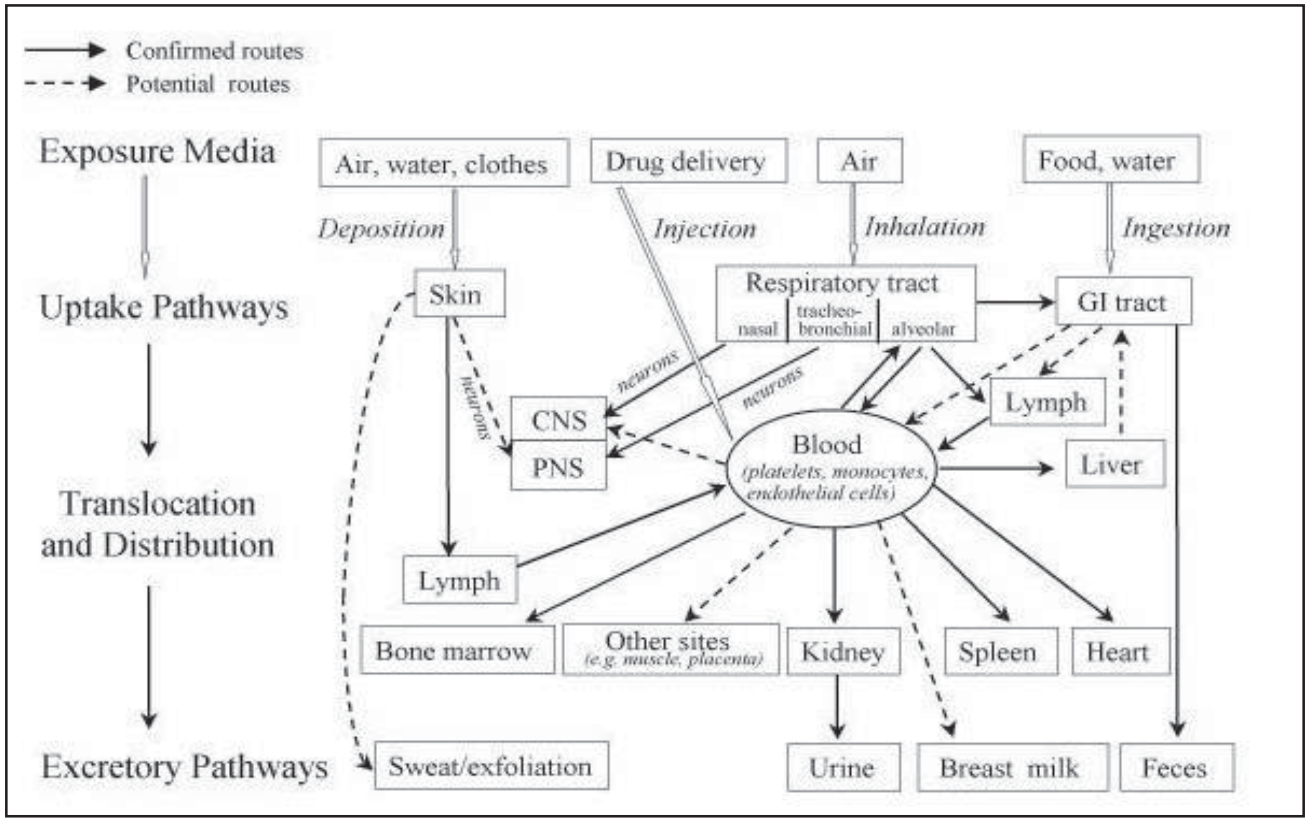


**Figure 4: Mode of entry of nanoparticles in the food chain.**

aquaporins, ion channels, endocytosis or even creating new pores. Sometimes NPs may form complexes with membrane transporters or root exudates and subsequently are transported into the plants (Watanabe *et al*., 2008).

The MB NPs are taken up by the plants easily through the ion transporters (Hall and Williams, 2003). After entering the plant cell, these NP's may be transported apoplastically or symplastically from one cell to the other and this movement was facilitated by plasmodesmata (Figure 5).

### 1.3. Uptake of Carbon-Based NMs

Nanotubes are the tube-like structure formed due to the spherical arrangement of carbon atoms made up of single layer of graphite. Nanotubes exist in a honeycomb manner and rolled up to form a cylindrical structure, which have the diameter of one nanometer. The property of the nanotubes depends on how cylindrically rolled in it (Kushwaha and Malik, 2012). Carbon nanotubes (CNTs) are used as smart delivery systems for various bio-molecules/genes/drugs into the cells and therefore studies have been undertaken to find out the mechanism of their uptake and transport into the intact plant cells. Shen *et al*. (2010) using *Arabidopsis thaliana* leaf cell have shown the impact of single-walled carbon nanotubes (SWCNTs) revealing that endocytosis-like structure was formed in the plasma membrane during the subsequent process. Studies on cell suspensions of *Nicotiana tobaccum* also showed the potential penetration of SWCNTs in the intact cell wall and cell membrane through fluidic phase endocytosis (Liu *et al*., 2009).

The role of single-walled CNTs in root elongation has been described where the effect of functional and non-functional nanotubes on root elongation in six different crop species, including cabbage, carrot, lettuce, cucumber, onion and tomato is reported. Here nanotubes are functionalised using poly 3-aminobenzene sulphonic acid. With the help of analytical technique, Scanning

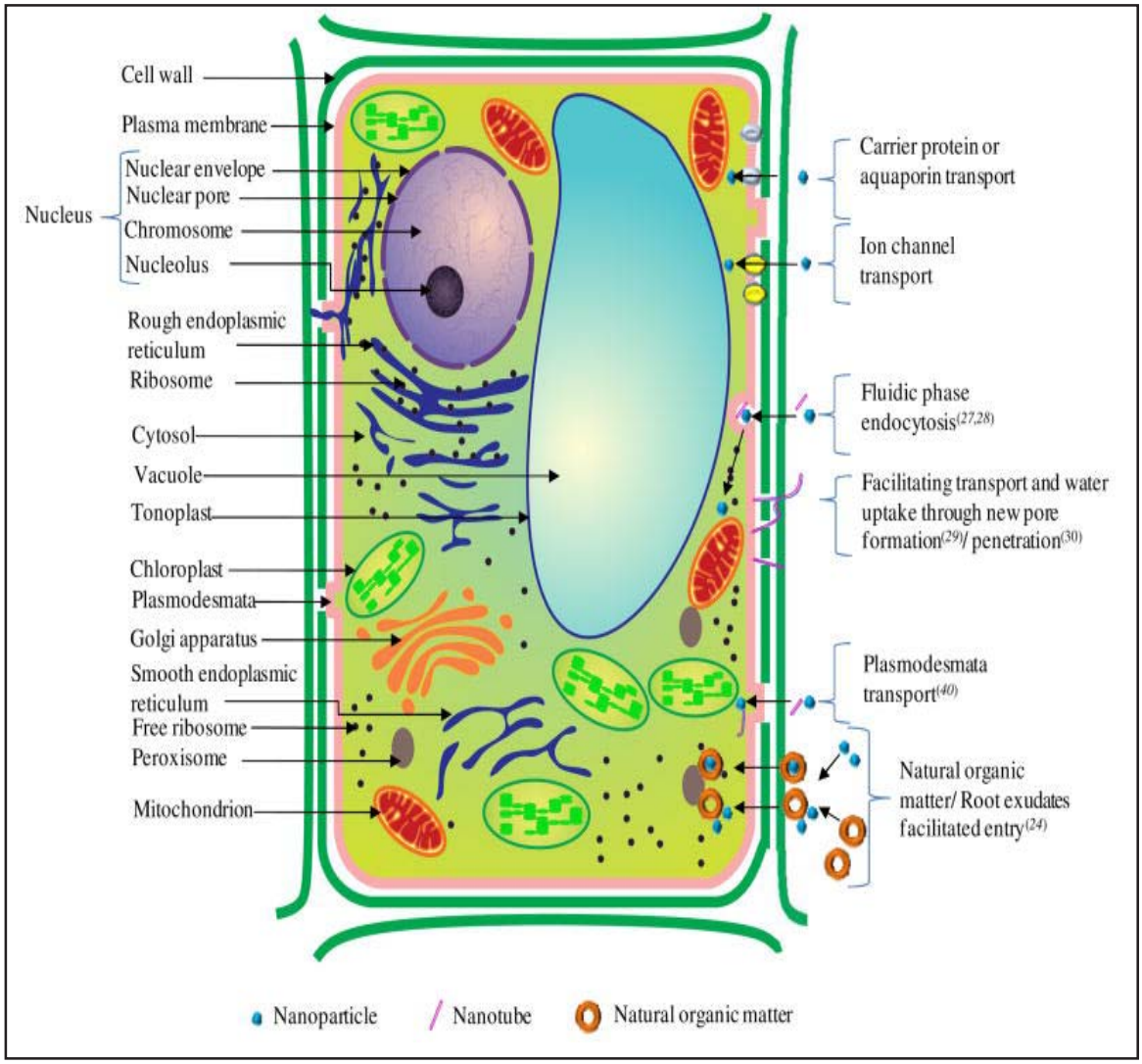


**Figure 5: Probable modes of cellular uptake of the nanoparticles by a plant cell (Cyren *et al.*, 2011).**

Electron Microscopy (SEM) root elongation was measured at 0, 24 and 48 h of exposure measuring the uptake of CNT as well as their interaction with the roots. The results show that CNTs does not show any effect on carrot and cabbage in either of the form but functionalised CNTs showed marked effect in inhibiting the root elongation in lettuce. Contrarily, enhancement in root elongation was observed in onion and cucumber (Canas *et al.*, 2008). During the whole analysis it was found that the SWCNTs adhere on the external surface of the primary and secondary roots in the form of nanotube sheets.

Some reports also support the use of multiwalled carbon nanotubes (MWCNT), where their penetration was observed in the seeds and root systems of the developed tomato seedlings (Khodakovskaya *et al.*, 2009). It was analysed that the MWCNTs penetrates the seed coat by creating pores in it enhancing the water uptake. Visual observations were also made revealing the penetration of MWCNTs through the root surface and ultimately pierce the epidermal and root hair cell walls and root cap in wheat seedlings (Wild and Jones, 2009).

From the above instances, it can be hypothesised that CNTs interact with the proteins and polysaccharides on

the cell wall and bring out hypersensitive responses mimicking plant pathogens due to their small size, eventually leading to cell mortality (Tan *et al*., 2009 and Liu *et al*., 2009). This hypersensitive response causes prevention of the entry of MWCNTs through the plant cell walls.

Chen *et al*. (2010) reported that the hydrophobic fullerenes $C_{70}$ blocked the cell wall pores in *Allium cepa* cell suspensions, which results in the negligible uptake of the NPs by the cells. Whereas when the small size and greater hydrophilicity of the fullerols $C_{60}(OH)_{20}$ was used it allows their permeability through *A. cepa* cell walls and eventually accumulates at the interface, between cell wall and plasma membrane.

Lin *et al*. (2009 a and b) reported the uptake, accumulation and the translocation of suspended fullerene $C_{70}$ and MWCNT in rice plants, and suggested that they followed the transmission route of water and the nutrients through the xylem.

### 1.4. Possible Effect of CNTs on Plants

Studies by Lin and Xing (2007) have shown that MWCNTs do not have any significant effect on seed germination of rape, radish, lettuce, corn and cucumber at 2000 mg/l after 5 days of treatment. Similar results are reported, with negligible phytotoxic symptoms or increased physiological response, by Wild and Jones (2009) on living wheat roots. Contrarily, MWCNTs at 10-40 mg/l have accelerated seed germination in tomato. Canas (2008) showed adverse affect on the root elongation in tomato. In brief, variable responses have been reported in different crop species.

### 1.5. Uptake of Metal Oxide-based MNs

(i) Uptake of $TiO_2$ NPs by *A. thaliana* and *Zea mays*

Kurepa *et al*. (2010) have reported the uptake and translocation of ultra small $TiO_2$ by *A. thaliana* seedlings. During the studies $TiO_2$ complexes with Alizarin red S nanoconjugate was taken up and was translocated by *A. thaliana* seedlings to cell-specific distribution. Reports suggested that the roots of *A. thaliana* release some mucilage-like substance that formed a pectin hydro-gel capsule surrounding the root, either inhibiting or facilitating the entry of the $TiO_2$ complexes with Alizarin red S or sucrose. Contrarily, Asli and Neumann (2009) investigated the uptake and translocation of $TiO_2$ NPs by excised roots of *Zea mays*. Here NPs were not taken up by the root cells, due to its large size compared to the small size of the pore diameter in the root cell wall, indicating that the high stability of $TiO_2$ NPs makes the digestion process difficult for $TiO_2$-treated plant samples.

(ii) Uptake of zinc oxide NPs by soybean and rye grass

Holden illustrates the use of two types of NPs to explain their effects on the growth of soybean. During the study, the effects of zinc oxide and cerium oxide NPs are demonstrated. Zinc oxide is a common component of cosmetics and ultimately ends up as a contaminant of solid waste generated by sewage treatment and is widely used as manure.

In a greenhouse experiment, soybean plants were grown in the presence of the NPs, and the plant growth was traced. Further, the accumulation of the NPs in different parts of the plant was also evaluated. With zinc oxide NPs, plant growth was better over control (plants grown in the absence of NPs). However, zinc also accumulated in the edible parts of the plants, the leaves and the beans. Zinc oxide NPs are shown to be toxic to cultured mammalian cells. Subsequently, low levels of zinc oxide were used in further experiments.

Lopez-Moreno (2010) investigated the uptake and accumulation of ZnO NPs (8 nm) by soybean (*Glycine max*) seedlings by treating with ZnO NPs in the range of 500-4000 mg/l. It was matter-of-fact that the Zn uptake by the seedlings was significantly higher at 500 mg/l. The possible reason behind such mechanism may be that at this concentration the NPs had lesser aggregation than at high concentrations (1000-4000 mg/l). This formation of aggregates makes passage through the cell pore walls difficult and thereby reducing the uptake and accumulation of NPs.

Using X-ray absorption spectroscopy (XAS) analysis of the ZnO NP-treated samples revealed the presence of $Zn^{2+}$ inside the plant tissues, but the spectrum resembled more the one of Zn acetate and nitrate than ZnO NPs. ZnO NPs are expected to be a source for $Zn^{2+}$ found within tissues but the study fails to highlight whether the $Zn^{2+}$ was contributed by the biotransformation of the ZnO NPs on/in roots. However, it may be surmised

that root exudates ionised the ZnO NPs on the root surface, as no traces of ZnO NPs were shown by the XAS spectra.

SEM studies in ryegrass confirmed that the ZnO NPs are adsorbed and aggregated on the root surface (Lin and Xing, 2008) revealing their presence in the apoplast, cytoplasm and nuclei of the endodermal cells and the vascular cylinder using TEM analysis.

(iii) Uptake of ceramic oxide NPs by *soybean and Alfa alfa*

Soybean plants grown hydroponically with cerium oxide NPs were stunted in growth because the cerium entered the plant roots and inhibited the process of nitrogen fixation. Soybean roots host bacteria that fix atmospheric nitrogen into a form that the plant can use for growth. The cerium NPs appear to completely inhibit the ability of nitrogen fixing bacteria for this function.

Some workers believe that: NMs are not "equally hazardous" or conversely "equally safe" stating that the effects of the NPs, seems to be dependent upon their physical and chemical characteristics, to help predict toxicity or safety based on these characteristics (Ball, 2012). Studies using ceramic oxide NPs are performed where their effect on seedlings of alfalfa (*Medicago sativa*), corn and tomato are evaluated (*Lycopersicon esculentum*). The observation supports the accumulation of Ce in tissues with the increasing concentration of $CeO_2$ NPs (Lopez and Moreno, 2010). Contrarily, some workers have demonstrated that when $CeO_2$ NPs was sprayed as aerosol or suspension on corn leaves, NPs are absorbed by the leaves but fail to translocate to new leaves.

(iv) Uptake of nickle hydroxide NPs by *Prosopis* sp.

Parsons *et al*. (2010) investigated the uptake, translocation and accumulation (as oxidised form) of Ni $(OH)_2$ NPs in *Prosopis* sp. through XAS. Plants treated with NPs coated with citrate before or after synthesis showed Ni NPs only in roots with no effect on plant size or chlorophyll production.

(v) Uptake of iron oxide NPs

Detailed description of the uptake of magnetite ($Fe_3O_4$ NP, 20 nm diameter) by pumpkin seedlings in hydroponic conditions using magnetometer is given by Zhu *et al*. (2008), where the signal for magnetic NPs were detected in roots, stems and leaves of pumpkin plants. The uptake of the NPs was found to be effected by growth medium, as no uptake was observed when grown in soils and reduced uptake was observed when grown on sands. This change occurs may be due to the adherence of the $Fe_3O_4$ NPs to the soil and sand grains revealing that the uptake of NPs was seen to be species dependent. On the other hand, Wang *et al*. (2010) did not notice any uptake of $Fe_3O_4$ NPs (25 nm) by the pumpkin plants.

Concluding the above studies, it can be hypothesised that it is difficult for the large size NPs to penetrate through the cell walls and transport across the plasma membranes. The cell wall pore sizes vary from 2-20 nm, while the size of ions and water molecules are about 0.28 nm. Thus, ions and water find their ways through ion channels and aquaporins, respectively (Woehlecke *et al*., 1995; Rondeau-Mouro *et al*., 2008).

### 1.6. Possible effect of Metal Oxide NPs on plants

Till now, scanty of knowledge is available regarding estimation of the toxic effect of metal oxide NPs. So far, only $Fe_3O_4$, $CeO_2$, $SiO_2$, $TiO_2$ and ZnO, have been studied in a few plant species.

$TiO_2$ NPs are used in maize cell leading to the reduction in the hydraulic conductivity in the primary roots; thereby, leading to reduced transpiration and leaf growth (Asli and Neumann, 2009). This phenomenon is attributed by the physical interaction between the colloidal particles and a physical inhibition of the apoplastic flow through the cell walls, rather than toxic effects.

On the other hand, when soybean plant was supplied with the mixture of $SiO_2$ and $TiO_2$ NPs, an increase in the nitrate reductase activity was observed enhancing the uptake of water and fertilizer, and thus stimulating the antioxidant system (Lu *et al*., 2002). In spinach, $TiO_2$ NPs are reported to increase chlorophyll formation, photosynthesis and plant dry weight, among others (Zheng *et al*., 2005; Hong *et al*., 2005)

ZnO NPs does not show any potential deleterious effect on the seed germination at the concentration of in soybean plant, rather it is found to be an effective promoter in root elongation at lower concentration but

at higher concentration showed inhibitory effect (Lopez-Moreno *et al*., 2010). Besides having variable effect on seedlings growth, ZnO NPs have been associated to cortical cells, highly vacuolated and collapsed along with the shrinking and partial death of the vascular cells (Lin and Xing, 2008).

Conflictingly, nanoceria are found to be accountable for reducing seed germination in alfalfa, soybean, tomato and cucumber at high concentration (Lopez-Moreno *et al*., 2010), but corn germination was significantly inhibited even at the low concentrations stating that corn was seen to be more sensitive to ZnO and $CeO_2$ NPs, demonstrating toxicity symptoms even at concentration that were not found to significantly affect other food and forage crops (Lin and Xing, 2007; Lopez-Moreno *et al*., 2010). Same results are obtained when $CeO_2$ NPs are interacted with tomato and alfalfa seedlings at higher concentrations.

The iron oxide NPs increase soybean pods and leaf dry matter (Sheykhbaglou *et al*., 2010), also act as facilitators for iron and photosynthates transfer to the leaves of peanut (Liu *et al*., 2010) and are also responsible for root elongation in pumpkin, with positive/no significant negative effect. These NPs are supposed to induce oxidative stress and higher anti-oxidative enzyme activity in pumpkin (Wang *et al*., 2010). The uptake of $Fe_3O_4$ NPs is facilitated by root surface or absorbed by the roots disturbing the metabolic activities in roots, leading to local instability of the cell wall and/or membrane and eventually producing oxidative stress.

### 1.7. Uptake of MB NPs

Over the years, new techniques have been developed for the synthesis of NPs, especially silver NPs synthesis and their applications has gained enormous impetus. Stampoulis (2009) reported the use of Ag NPs and their uptake by *Cucurbita pepo*. Plants exposed to different concentrations of Ag NPs, had shoots 4.7 times higher than controls possibly due to the ion release from Ag NPs in the shoots. In *Brassica juncea* Ag NPs were used but no accumulation of Ag was observed (Haverkamp and Marshall, 2009).

Contrarily, when carbon-coated Fe NPs were applied to the leaf petioles of pumpkin plants, Ag were found only in the epidermal cells close to the application site (Corredor *et al*., 2009).

Lee *et al*. (2008) investigated the uptake and translocation of Cu NPs in mung bean and wheat using agar growth medium. Their data revealed that the Cu NPs were able to cross the cell membrane and agglomerated in the cells confirming a responsive relationship between the bioaccumulated NPs in plant tissues and growth media.

There are umpteen reports available regarding the reduction of metal ions into NPs and their subsequent accumulation in edible plants. Based on some studies it is strongly suggested that Au, Ag and Pt NPs are accumulated in the seedlings of some plants.

### 1.8. Possible Effect of Metallic NPs on Plants

Some workers have reported that Si NPs, with sodium dodecyl sulfate, cause complete inhibition of the germination of *Cucurbita pepo* seeds; whereas, Si NPs in the absence of SDS, resulted in 80% germination.

### 1.9. Storage of NPs in Plants

Very few studies are available regarding the accumulation of the NPs in the plants. It is enigmatic how and where within the plants are the NPs absorbed and stored though reported from plant cells and tissues.

There is also no vivid report whether NPs in the plants are transmitted to the second generation or not. Regarding the latter concern, should NPs be found in the second generation plants, there is the possibility that these plants would become adapted and more responsive, and hence, accumulating more of the respective NPs. The bioavailability of the accumulated NPs to the next trophic level, e.g., in humans and ruminants is also unknowable.

### 1.10. Biotransformation of NPs in Plants

Biotransformation of ZnO and $CeO_2$ NPs is reported by Lopez-Moreno *et al*. (2010) in soybean plants where with $CeO_2$ NPs up take occurs by soybean plant but no indications of biotransformation is observed ,while using ZnO, their presence is confirmed in the plant in an ionic ($Zn^{2+}$) state.

Interestingly, XAS studies have demonstrated the biotransformation of the $Ni(OH)_2$ NPs.

### 1.11. Genotoxic and Cytotoxic Effects of Engineered NPs in Plants

Properties of NPs being small, highly reactive, catalytic in nature and high surface-volume ratio have many positive implications but have tendency to cross the cell barriers and interact with intracellular structure, contributing to potential cytotoxic and genotoxic effects through inducing oxidative stress (Kovacic and Somanathan, 2010). This aspect has not been well studied.

Lower concentrations of MWCNTs in rice cell suspensions are shown to cause cell death due to apoptosis; though higher concentration affected cell mortality due to necrosis and leakage of cytoplasmic content and membrane disruption (Tan *et al*., 2007, 2009). However, some positivity was also reported when the rice cells in the suspensions were exposed to MWCNTs they exhibit self-defence response, just by sacrificing a small population of cells that aggregates with the NPs and finally precipitates providing protection to the residual cells in the culture (Tan *et al*., 2007).

SWCNTs possess potential cytotoxic effects in rice and *Arabidopsis* protoplast cells. Higher concentration of such NPs caused abundant endonucleolytic cleavage of DNA in *Arabidopsis* cells indicating their genotoxic behaviour towards plant systems (Shen *et al*., 2010).

The effect of these applied NPs is also observed in the second generation of the rice plants. The most recent report on cytotoxicity of metal oxide NPs was presented by Lopez-Moreno *et al*. (2010), experimenting with $CeO_2$ and ZnO NPs in soybean seedlings. Some new DNA bands were observed in soybean roots treated with ZnO NPs at 4000 mg/l. Whole assay was performed using random amplified polymorphic DNA analysis. The resultant genotoxicity may rise either due to the interaction of the DNA with the Zn ions leached out from the ZnO NPs or with its direct interaction with the ZnO NPs. Contrarily, when the same analysis was made with the soybean roots treated with $CeO_2$ at 2000 and 4000 mg/l four and three new bands are observed, respectively. The result analysis exhibited deleterious effect on genetic stability to some extent.

Genotoxic effect of $TiO_2$ is confirmed using comet assays and DNA laddering technique. The presence of chromosomal aberrations and interphase micronuclei in *A. cepa* plants validated the occurrence of cellular fragmentation in the previous cell cycle.

From the whole analysis, it may be concluded that the genotoxic and the cytotoxic effects are due to the generation of superoxide radicals resulting in lipid peroxidation in the *A. cepa* cells (Ghosh *et al*., 2010). In *A. cepa* root meristematic cells, Ag NPs have been reported to possess mitodepressive, mitoclassic and clastogenic properties. Dose-dependant decrease has been noticed in the frequency of mitotic index in the Ag NP-treated *A. cepa* cells. At varying concentration of Ag NPs, different kinds of chromosomal aberrations, e.g., stickiness, chromosomal breaks, gaps, disturbed metaphase and cell wall disintegration at different doses (Kumari *et al*., 2009; Babu *et al*., 2008). The interaction of Ag NPs with tubulin-SH group may probably be responsible for the ineffective mitotic spindle function. As explained by Kumari *et al*. (2009), the stickiness in the metaphase and anaphase stages may be attributed to degradation or depolymerisation of chromosomal DNA or by intermingling of inter chromosomal chromatin fibers, which leads to sub-chromatid connections between chromosomes (Sudhakar *et al*., 2001). The induction of chromosomal breaks and micronuclei by Ag NPs indicates the clastogenic potential of the xenobiotic, which may lead to a loss of genetic material (Raun and Lilum, 1992). Thus, these may be regarded as an end point of irreversible genotoxicity on the chromosomes. Although the genotoxicty of the Ag NP is well established, the causative mechanism has not been reflected in this study. Moreover, no studies have been made to clarify if the toxicity is caused by the Ag NPs themselves or the Ag(II) ions released from the NPs in the external/biological media.

The water-based magnetic NPs (50-300 µl/l) coated with perchloric acid has been reported to decrease the nucleic acid level in the cells of corn revealed an inhibitory effect on biosynthesis.

## 2. CONCLUSION

In all, the analysis of all the demonstrated studies it is concluded that the NPs possess the ability to penetrate the cells of living plants, and are able to translocate to the remote positions through the vascular system. Still more, concerted efforts are needed to clearly understand

and make known, whether they exhibit any genotoxic effect in plants by themselves or through their biotransformation within the plants. More focused studies are required for evaluating the differences in toxicity of MB NPs with their respective bulk counterparts, the increased concentration of the element, if it is an essential micronutrient, and the effect of the ions produced inside or outside the organism exposed to the NPs. These facts constitute an important step in elucidating the mechanisms of interaction of NPs with the plant cells and thus, in designing strategies for using NPs for targeted delivery of substances. On the other hand, safer strategies must also be prepared to identify and evaluate the toxic effect and toxicity level of these NPs before being exposed to the outer environment. In this sense, methodological improvements are required to make the system suitable for agronomical purposes.

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**LS - An International Journal of Life Sciences**
DOI : 10.5958/j.2319-1198.2.2.016



# Depth-wise Distribution of Microbial Populations, Macro and Micronutrients under Different Land Use Systems in Submontaneous Tract of Punjab

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**ABSTRACT**

The research conducted at Punjab Agricultural University (PAU), Zonal Research Station for Kandi area, Ballowal Saunkhari, to investigate the surface and profile distribution of macronutrients, micronutrients and microbial parameters in four land use systems. The results of our study revealed that forest land use system had significantly higher levels of macronutrients, micronutrients and microbial parameters compared with cultivated land use system (CLUS), pasture land use system (PLUS) and undisturbed land use system (ULUS). Further, among the four land use systems, FLUS and CLUS were found fertile and more productive. The higher levels of macronutrients, micronutrients and microbial parameters in CLUS were due to addition of fertilizers and farm yard manure, whereas higher levels of microbial parameters in FLUS were due to the regular addition of organic matter in the form of leaf litter. The results further reported that PLUS and ULUS exhibited low magnitude of soil fertility parameters and thus were less productive. Also the soil samples drawn from profiles of CLUS and FLUS in the Ballowal Saunkhari watershed (BSW) have higher levels of soil fertility parameters compared with the remaining two land use systems. Higher levels of soil fertility parameters in profiles of FLUS and CLUS were associated with higher content of clay and organic matter. The magnitude of soil fertility parameters generally decreased with depth in profile.

**Keywords:** Micronutrient, macronutrient**,** land use system, surface and profile distribution of nutrients, microbial parameters

## 1. INTRODUCTION

The submontaneous tract of Punjab and the adjoining undulating piedmont plains in the South of *Siwalik* hills popularly known as the *Kandi* zone, covers part of Roop Nagar, Nawanshahar, Hoshiarpur, Gurdaspur and Patiala districts of the state. The total area of this belt is 0.5 million hectares constituting about 10 % of the geological area of the state. Although this belt has a distinct advantage of having higher rainfall than other parts of the state, but because of topographical and lithological constraints, the irrigation facilities in this zone are very limited and most of the area is rainfed. As a consequence, this belt is characterized by existence of very distinct land use systems. Cultivated, forest, pasture and undisturbed lands are four major land use systems constituted in Ballowal Saunkhari watershed (BSW).

Different chemical, physical and microbial parameters play an important role in determination of soil fertility of different land use systems in this watershed. However, the major improvement in the soil chemical conditions arises from the fact that organic matter is the basic resource of several nutrient elements. The micro and macronutrients are readily available within the pH range of 6.0–7.5. Karlen *et al.* (1997) compared pH and electrical conductivity (EC) values under three different land use systems in surface soils (0–10 cm) and suggested that cultivated system had higher pH (6.3) compared with forest system (5.9) followed by organic system (5.8). Reganold and Palmer (1995) studied three land use systems based on cation exchange capacity (CEC) of

soil in the surface layer and reported that pasture system (24.1 Cmol(+)/kg) and biovegetation (24.4 Cmol(+)/kg) system recorded almost same CEC compared to conventional vegetation system, which recorded 16.9 Cmol (+)/kg CEC value. Karlen *et al.* (1997) and Reganold and Palmer (1995) observed higher levels of organic matter in pasture system (5.13%) followed by conventional vegetation system (3.06%). Karlen *et al.* (1994) studied higher levels of available N, P, K and total N under undisturbed system as compared to in cultivated land use system (CLUS) followed by deep tillage system. Dhaliwal *et al. (*2012) reported that the available N, P and K were higher in pasture system compared with organically managed system followed by cultivated system. However, total N and total P were higher in pasture and organically managed systems, respectively. Rattan *et al.* (1999) reported higher amount of available Zn (6.00 mg/kg), Cu (2.33 mg/kg), Fe (30.70 mg/kg) and Mn (29.10 mg/kg) in organic system as compared with amount of Zn (3.90 mg/kg), Cu (1.42 mg/kg), Fe (2.30 mg/kg) and Mn (25.0 mg/kg) in conventional system. Dhaliwal *et al.* (2009a) reported that available Zn and Cu were higher in FLUS compared with ULUS and PLUS. Gilley and Doran (1997) reported higher levels of microbial biomass nitrogen (MBN) under conserve reserve programme system compared to conventional tillage system. In soil profiles, MBN level decreases with depth (0–30.5 cm).

Very little attention has so far been paid to monitor the distribution of macro, micronutrients and microbial parameters under different land use systems existing in Ballowal Saunkhari watershed (BSW) existing in Kandi region. The productivity of the soil in this watershed area is directly related to the maintenance of soil fertility parameters over time. A detailed investigation of the processes leading to delineation of various soil fertility parameters of soil can help in finding ways and means to achieve sustainable high production levels in different land use systems. Keeping in view that several distinct land use systems exist in the region with different fertility status, the present investigation was carried out with the following objectives to study the surface distribution of macro micronutrients and microbial parameters and to get information related to depth-wise distribution of micro and macronutrients in profiles of different land use system existing in the BSW.

## 2. MATERIALS AND METHODS

The research was carried out in BSW in Kandi region of Punjab adjoining the Zonal Research Station for Kandi area (PAU) in Shaheed Bhagat Singh Nagar district. Four land use systems identified in BSW selected for study were: cultivated land use system (CLUS); undisturbed land use system (ULUS); pasture land use system (PLUS) and forest land use system (FLUS). CLUS is characterized by the addition of chemical fertilizers and farm yard manure, whereas FLUS is characterized by regular addition of organic matter in the form of leaves of Subabul (*Leucaena leucocephala*), Kikker (*Acacia catechu*), Khair (*Acacia catechu willd*) and Tahli (*Dalbergia sissoo*) existing in the watershed. On the other hand, PLUS and ULUS are characterized by poor grass stands. Also, these land use systems are located on highly degraded site of the watershed where erosion is a severe problem.

### 2.1. Soil Sampling and Analysis

Fifteen spots were randomly selected from each land use system in the watershed. Soil samples from 0 to 15 cm depth were collected from each spot. One profile was exposed in each of the four land use systems. Profile samples were also collected from each land use system to know the depth-wise movement of various soil fertility parameters. These surface and profile soil samples were analyzed in the laboratory using standard procedures. Soil pH and CEC were determined by the procedures given by Jackson (1973), whereas, EC was determined with method of Richard (1954). Rapid titration method (wet digestion method) was used for organic carbon determination (Walkley and Black, 1934). Available nitrogen, phosphorus and potassium were determined by the methods described by Subbiah and Asija (1956), Olsen *et al.* (1954) and Merwin and Peech (1950), respectively. The total N and total phosphorus contents of the soil were determined by the methods of Dalal *et al.* (1984) and Jackson (1967) respectively. The available Zn, Cu, Fe and Mn were assessed by the method of Lindsay and Norvel (1978). Total K, Zn, Cu, Fe and Mn were determined by the method described by Page *et al.* (1982). Soil texture was determined by standard international pipette method of Day (1965). Aggregate stability was determined by the method of Yoder (1936).

The potentially mineralizable nitrogen (PMN), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN) in the soil were estimated following the method described by Keeney (1982), Anderson and Domsoh (1978) and Keeney and Nelson (1982), respectively.

### 2.2. Statistical Analysis

The statistical analysis was carried out for probability associated with Student's t-test for comparing different macro, micronutrients and microbial parameters of soil fertility within different land use systems (Elhance *et al.,* 1999).

## 3. RESULTS AND DISCUSSION

### 3.1. Surface Distribution of Physical, Chemical and Microbial Parameters

The data presented in Table 1 show that soils in FLUS possessed significantly higher water-holding capacity (WHC; 57.4 %), porosity (62 %) and aggregate stability (0.84) followed by PLUS and ULUS. Soil in FLUS also contained more clay content compared to those in the other two systems. However, the differences were not significant if CLUS was compared with FLUS and PLUS was compared with ULUS. A significant coefficient of correlation between organic carbon and clay content (0.71**) was observed in FLUS. These results obtained for WHC, porosity and aggregate stability showed that the soils in FLUS and CLUS were more productive as compared to PLUS and ULUS. Our results reported that soils in CLUS and FLUS possessed significantly higher WHC, porosity, aggregate stability (mean weighted diameter- MWD) and infiltration rate as compared to in PLUS and ULUS. Soils in CLUS and FLUS also reported more clay and silt as compared to those in the other two systems. However, the differences were not significant if CLUS was compared with FLUS or PLUS was compared with ULUS. The results obtained were insignificant if same land use system was compared for two consecutive years. A significant coefficient of correlation between organic carbon and clay content (0.71**) was observed in FLUS. These results obtained for WHC, porosity, aggregate stability and infiltration rate showed that the soils in FLUS and CLUS were of better quality as compared to PLUS and ULUS for both the years. These observations are similar to those obtained by Dhaliwal *et al.* (2008a) for texture, WHC, porosity and aggregate stability (MWD). Bulk density (Db) and penetration resistance and were significantly less (Table 1) in CLUS and FLUS compared to in PLUS and ULUS. The later systems had relatively higher values in both the years. Similar trends of results were observed from the same site after 1 year. The results pertaining to bulk density (Db) and penetration resistance revealed that CLUS and FLUS had better quality soils as compared to PLUS and ULUS for both the years. These systems had poor soil quality. The results are in accordance with those reported by Dhaliwal *et al.* (2008b) and Rawat *et al.* (1998) for bulk density.

The data presented in Table 1 show that soils in CLUS and FLUS possessed significantly higher levels of EC, cation exchange capacity and organic carbon as compared to in PLUS and ULUS. FLUS exhibited the highest organic carbon (0.56%) level and the lowest pH (7.64) compared to the other three land use systems. The results pertaining to EC, CEC and organic carbon (OC) were not significant when CLUS was compared with FLUS and PLUS was compared with ULUS. The increase in organic carbon (OC) and decrease in soil pH in FLUS were due to regular addition of organic matter through tree leaves, whereas, the increase in pH, EC and CEC in CLUS was due to addition of chemical fertilizers. Organic carbon and pH were significantly correlated (0.37*) in FLUS. These land use systems thus possessed better soil quality compared to PLUS and ULUS, which are located on highly eroded and degraded region of the watershed. Similar observations were made by Dhaliwal *et al.* (2008b) for pH, CEC, EC and organic carbon. They reported higher levels of EC, CEC and pH in CLUS and higher OC and low pH values in FLUS. Available nitrogen (264 kg/ha) and potassium (384 kg/ha) were significantly higher in FLUS, whereas, available phosphorus (18.4 kg/ha) was significantly higher in CLUS compared with PLUS and ULUS (Table 1). The results pertaining to available N, P and K were not significant if FLUS was compared with CLUS. Higher levels of available N, P and K in CLUS were possibly due to addition of fertilizers. In FLUS, recycling of nutrients due to falling leaves of leguminous tree species (*Subabul, Kikker, Khair and Tahli*) were the possible reasons for high available N, P and K value. A significant coefficient of correlation (0.44**) between available N

**Table 1: Surface distribution of macronutrients, micronutrients and microbial parameters in four land use systems of Ballowal Saunkhari watershed**

| Parameters | | CLUS* | ULUS* | PLUS* | FLUS* |
|---|---|---|---|---|---|
| | | *Physical parameters* | | | |
| Texture | Sand (%) | 63.7 | 80.5 | 74.4 | 64.1 |
| | Silt (%) | 16.1 | 10.8 | 14.7 | 15.4 |
| | Clay (%) | 20.2 | 8.7 | 10.9 | 20.5 |
| pH | | 7.75 | 7.80 | 7.72 | 7.64 |
| EC (ds/m) | | 0.27 | 0.21 | 0.20 | 0.25 |
| Cation exchange capacity (me/100 gm) | | 14.2 | 8.6 | 8.5 | 13.8 |
| OC (%) | | 0.48 | 0.34 | 0.42 | 0.56 |
| WHC (%) | | 56.4 | 44.6 | 47.6 | 57.4 |
| Db ($g/cm^3$) | | 1.57 | 1.58 | 1.59 | 1.53 |
| Porosity (%) | | 58 | 49 | 52 | 62 |
| Aggregate stability (MWD) | | 0.82 | 0.61 | 0.64 | 0.84 |
| | | *Macronutrient parameters* | | | |
| Available nitrogen (kg/ha) | | 261 | 220 | 210 | 264 |
| Available phosphorus (kg/ha) | | 18.4 | 10.1 | 11.3 | 17.9 |
| Available potassium (kg/ha) | | 374 | 309 | 341 | 384 |
| | | *Micronutrient parameters* | | | |
| Available zinc (mg/kg) | | 0.84 | 0.58 | 0.64 | 0.96 |
| Available copper (mg/kg) | | 0.58 | 0.36 | 0.40 | 0.56 |
| Available iron (mg/kg) | | 9.36 | 6.56 | 7.42 | 9.40 |
| Available manganese (mg/kg) | | 12.96 | 7.80 | 8.36 | 11.80 |
| | | *Microbial parameters* | | | |
| PMN (mg/kg/7d) | | 9.34 | 7.14 | 7.35 | 9.64 |
| MBC (mg/kg) | | 100.3 | 90.6 | 93.7 | 124.7 |
| MBN (mg/kg) | | 32.6 | 23.1 | 26.3 | 36.5 |

*Abbreviations; Bd, bulk density; CLUS, cultivated land use system; EC, electrical conductivity; FLUS, forest land use system; MBC, microbial biomass carbon; MBN, microbial biomass nitrogen; OC, organic carbon; PLUS, pasture land use system; PMN, potentially mineralisable nitrogen; ULUS, undisturbed land use system; WHC, water-holding capacity.

and organic carbon was observed in FLUS. These results are in accordance with those of Dhaliwal *et al.* (2009a) and Gilley *et al.* (1997).

Significantly higher levels of available zinc (Zn), copper (Cu), iron (Fe) and manganese (Mn) were observed in surface soils of FLUS and CLUS followed by PLUS and ULUS (Table 1). In the FLUS, organic carbon was significantly correlated with available Zn (0.47**), Fe (0.66**) and Mn (0.67**). On the basis of micronutrient content of soils, it is observed that soils in FLUS and CLUS were having higher levels of Zn, Cu, Fe and Mn compared with PLUS and ULUS. Dhaliwal *et al.* (2009b) and Rattan *et al.* (1999) have also recorded similar observations in FLUS and CLUS.

PMN (9.64 mg/kg/7d), MBC (124.7 mg/kg) and MBN (36.5 mg/kg) levels were significantly higher in FLUS when compared with CLUS followed by PLUS and ULUS (Table 1). The differences were not significant when FLUS was compared with CLUS and ULUS was compared with PLUS. Significant coefficients of correlation of organic carbon with PMN (0.37*) and MBC (0.51**) were observed in CLUS and FLUS, respectively. The data showed that soils of FLUS and CLUS are of better productivity compared with PLUS and ULUS. Similar results have been reported by Dhaliwal *et al.* (2009c) for PMN, Gilley and Doran (1997) and Dhaliwal *et al.* (2012) for MBC and MBN.

### 3.2. Profile Distribution of Physical, Chemical and Microbial Parameters

The data pertaining to different soil fertility parameters in the profiles of CLUS (Table 2), ULUS (Table 3), PLUS (Table 4) and FLUS (Table 5) depicted that soil fertility (micro and macronutrients) and microbial parameters showed a lot variation in the profiles of BSW. Various

**Table 2: Profile distribution of macronutrients, micronutrients and microbial parameters in cultivated land use system of Ballowal Saunkhari watershed**

| Parameters | Depth (cm) | | | | |
|---|---|---|---|---|---|
| | 0–23 | 23–40 | 40–68 | 68–100 | 100–123 |
| | | *Macronutrients parameters* | | | |
| Available nitrogen (kg/ha) | 256.8 | 141.6 | 98.7 | 64.3 | 53.2 |
| Available phosphorus (kg/ha) | 15.7 | 7.8 | 3.8 | 3.2 | 2.3 |
| Available potassium (kg/ha) | 348 | 201 | 187 | 162 | 144 |
| Total nitrogen (%) | 0.09 | 0.10 | 0.08 | 0.12 | 0.10 |
| Total phosphorus (kg/ha) | 82.4 | 74.8 | 46.7 | 31.9 | 22.3 |
| Total Potassium (%) | 2.18 | 2.25 | 1.40 | 1.30 | 1.17 |
| | | *Micronutrients parameters* | | | |
| Available zinc (mg/kg) | 0.68 | 0.40 | 0.34 | 0.36 | 0.32 |
| Available copper (mg/kg) | 0.34 | 0.28 | 0.18 | 0.24 | 0.16 |
| Available iron (mg/kg) | 8.86 | 9.72 | 8.36 | 7.80 | 7.72 |
| Available manganese (mg/kg) | 9.96 | 9.18 | 7.36 | 9.34 | 9.10 |
| Total zinc (mg/kg) | 70.8 | 72.6 | 54.0 | 96.6 | 74.2 |
| Total copper (mg/kg) | 16.8 | 20.6 | 18.6 | 26.8 | 24.2 |
| Total iron (%) | 2.46 | 2.98 | 2.14 | 2.95 | 2.62 |
| Total manganese (mg/kg) | 696 | 741 | 652 | 818 | 754 |
| | | *Microbial parameters* | | | |
| PMN (mg/kg/7d) | 8.54 | 4.92 | 4.31 | 4.48 | 4.42 |
| MBC (mg/kg) | 90.8 | 60.4 | 50.7 | 56.1 | 54.3 |
| MBN (mg/kg) | 34.3 | 20.8 | 17.5 | 18.9 | 18.7 |

**Table 3: Profile distribution of macronutrients, micronutrients and microbial parameters in undisturbed land use system of Ballowal Saunkhari watershed**

| Parameters | Depth (cm) | | | | |
|---|---|---|---|---|---|
| | 0–18 | 18–40 | 40–75 | 75–110 | 110–134 |
| | | *Macronutrients parameters* | | | |
| Available nitrogen (kg/ha) | 192 | 76.3 | 36.4 | 24.2 | 16.9 |
| Available phosphorus (kg/ha) | 4.2 | 3.6 | 2.8 | 2.2 | 1.8 |
| Available potassium (kg/ha) | 265 | 138 | 160 | 144 | 133 |
| Total nitrogen (%) | 0.09 | 0.06 | 0.06 | 0.08 | 0.06 |
| Total phosphorus (kg/ha) | 70.8 | 58.4 | 44.3 | 48.4 | 33.5 |
| Total potassium (%) | 2.18 | 1.72 | 1.84 | 1.98 | 1.75 |
| | | *Micronutrients parameters* | | | |
| Available zinc (mg/kg) | 0.54 | 0.28 | 0.26 | 0.40 | 0.28 |
| Available copper (mg/kg) | 0.26 | 0.20 | 0.14 | 0.18 | 0.12 |
| Available iron (mg/kg) | 6.80 | 6.60 | 7.62 | 6.80 | 5.66 |
| Available manganese (mg/kg) | 8.52 | 7.90 | 8.68 | 7.92 | 4.76 |
| Total zinc (mg/kg) | 64.5 | 42.6 | 52.7 | 60.6 | 52.3 |
| Total copper (mg/kg) | 22.4 | 52.4 | 14.6 | 18.2 | 10.2 |
| Total iron (%) | 1.62 | 1.42 | 1.46 | 1.78 | 1.67 |
| Total manganese (mg/kg) | 576 | 584 | 662 | 784 | 746 |
| | | *Microbial parameters* | | | |
| PMN (mg/kg/7d) | 6.74 | 2.47 | 2.23 | 3.98 | 1.54 |
| MBC (mg/kg) | 67.3 | 36.5 | 27.4 | 32.8 | 18.6 |
| MBN (mg/kg) | 23.1 | 14.7 | 9.46 | 12.6 | 6.4 |

**Table 4: Profile distribution of macronutrients, micronutrients and microbial parameters in pasture land use system of Ballowal Saunkhari watershed**

| Parameters | Depth (cm) | | | | | |
|---|---|---|---|---|---|---|
| | 0–14 | 14–38 | 38–51 | 51–73 | 73–90 | 90–112 |
| | | | *Macronutrients parameters* | | | |
| Available nitrogen (kg/ha) | 198 | 136 | 86 | 70 | 42.3 | 64.6 |
| Available phosphorus (kg/ha) | 11.8 | 6.4 | 4.3 | 3.6 | 2.8 | 2.9 |
| Available potassium (kg/ha) | 328 | 242 | 144 | 134 | 176 | 194 |
| Total nitrogen (%) | 0.10 | 0.09 | 0.08 | 0.07 | 0.09 | 0.09 |
| Total phosphorus (kg/ha) | 75.8 | 40.6 | 56.4 | 32.5 | 41.7 | 40.8 |
| Total potassium (%) | 1.74 | 1.10 | 0.94 | 1.12 | 1.36 | 1.42 |
| | | | *Micronutrients parameters* | | | |
| Available zinc (mg/kg) | 0.62 | 0.38 | 0.34 | 0.23 | 030 | 0.34 |
| Available copper (mg/kg) | 0.28 | 0.20 | 0.18 | 0.12 | 0.13 | 0.14 |
| Available iron (mg/kg) | 7.36 | 6.92 | 7.10 | 6.62 | 6.42 | 6.86 |
| Available manganese (mg/kg) | 8.24 | 7.30 | 7.86 | 6.25 | 6.90 | 7.68 |
| Total zinc (mg/kg) | 76.4 | 50.0 | 47.6 | 37.8 | 48.0 | 54.2 |
| Total copper (mg/kg) | 20.6 | 14.8 | 10.8 | 12.1 | 8.7 | 10.4 |
| Total iron (%) | 1.58 | 1.46 | 1.34 | 1.62 | 1.47 | 1.96 |
| Total manganese (mg/kg) | 595 | 656 | 586 | 463 | 567 | 610 |
| | | | *Microbial parameters* | | | |
| PMN (mg/kg/7d) | 5.11 | 3.23 | 1.33 | 1.25 | 1.06 | 1.17 |
| MBC (mg/kg) | 62.8 | 37.4 | 16.3 | 15.4 | 13.3 | 14.2 |
| MBN (mg/kg) | 20.7 | 9.46 | 5.60 | 5.34 | 4.65 | 4.83 |

**Table 5: Profile distribution of macronutrients, micronutrients and microbial parameters in forest land use system of Ballowal Saunkhari watershed**

| Indicators | Depth (cm) | | | | | |
|---|---|---|---|---|---|---|
| | 0–20 | 20–50 | 50–70 | 70–88 | 88–108 | 108–114 |
| | | | *Macronutrients* | | | |
| Available nitrogen (kg/ha) | 245 | 196 | 118 | 163 | 124 | 135 |
| Available phosphorus (kg/ha) | 17.4 | 12.6 | 4.9 | 7.2 | 3.7 | 4.2 |
| Available potassium (kg/ha) | 372 | 261 | 241 | 271 | 243 | 261 |
| Total nitrogen (%) | 0.19 | 0.17 | 0.13 | 0.16 | 1.10 | 1.11 |
| Total phosphorus (kg/ha) | 92.3 | 86.2 | 66.3 | 71.4 | 38.5 | 44.2 |
| Total potassium (%) | 2.37 | 1.31 | 1.19 | 1.25 | 1.05 | 1.36 |
| | | | *Micronutrients* | | | |
| Available zinc (mg/kg) | 1.10 | 0.66 | 0.59 | 0.64 | 0.36 | 0.45 |
| Available copper (mg/kg) | 0.38 | 0.20 | 0.20 | 028 | 0.13 | 0.18 |
| Available iron (mg/kg) | 9.53 | 6.46 | 4.88 | 5.98 | 4.15 | 5.81 |
| Available manganese (mg/kg) | 11.96 | 8.90 | 7.84 | 7.98 | 6.12 | 6.33 |
| Total zinc (mg/kg) | 86.0 | 75.4 | 92.4 | 94.3 | 58.5 | 66.3 |
| Total copper (mg/kg) | 28.4 | 22.3 | 32.6 | 40.3 | 20.6 | 22.7 |
| Total iron (%) | 3.52 | 22.6 | 2.62 | 2.96 | 2.10 | 2.72 |
| Total manganese (mg/kg) | 691 | 700 | 828 | 854 | 750 | 838 |
| | | | *Microbial parameters* | | | |
| PMN (mg/kg/7d) | 10.90 | 7.63 | 4.61 | 5.82 | 4.07 | 4.42 |
| MBC (mg/kg) | 120.7 | 93.8 | 56.7 | 71.5 | 57.4 | 62.5 |
| MBN (mg/kg) | 34.6 | 32.4 | 19.6 | 24.5 | 18.9 | 21.6 |

macronutrients, micronutrients and microbial parameters of soil productivity were more prominently expressed in the surface horizon of each land use system and these parameters showed either increase or decrease within the land use system (Table 1). However, the magnitude of each soil parameters generally decreased with depth in all the four land use systems. In general, higher magnitude of soil fertility parameters were reported in profiles of FLUS as compared to in profile of CLUS followed by profiles of PLUS and CLUS. Increased levels of soil fertility parameters (macronutrient, micronutrient and microbial) were also reported at different depths in profiles in which higher magnitude of soil fertility parameters were recorded in different land use systems. The depths in profiles recorded were 68-100 cm (CLUS), 75-110 cm (ULUS), 90–112 cm (PLUS) and 70–88 cm (FLUS). Incidentally, these layers in profiles of different land use systems were associated with higher clay and organic matter content, EC, cation exchange capacity and low pH values. These observations were similar to those reported by Dhaliwal *et al.* (2009c) and Stockfisch *et al.* (1999).

The results pertaining to profiles of different land use systems revealed that profile in FLUS had higher levels of macronutrient (Av. N, P and K), micronutrient (Av. Zn, Cu, Fe and Mn) and microbial parameters (PMN, MBC and MBN) in surface layer as compared to profile in CLUS followed by profiles PLUS and ULUS. However, these parameters showed declining behaviour with depth. This showed that soils lying under FLUS and CLUS were of better fertility and more productive compared with those under PLUS and ULUS. The later land use systems have low levels of soil fertility parameters due to their existence on undulating topography with poor soil texture (loamy sand), which caused severe erosion. These observations were in accordance with Dhaliwal *et al.* (2008a), which reported declining behaviour of macro, micronutrients and microbial parameters with depth. The better soil fertility parameters in profiles of FLUS and CLUS were attributed to addition of fertilizers and farm yard manure (cultivated system) and organic matter via leaf litter (forest) which caused higher microbial activity. Higher levels of PMN (10.9 mg/kg/7d), MBC (120.7 mg/kg) and MBN (34.6 mg/kg) were reported in FLUS. These observations were similar to those reported by Dhaliwal (2008) and Reganold and Palmer (1995), which reported higher levels of PMN, MBC and MBN in FLUS and CLUS and lower values in ULUS.

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**LS - An International Journal of Life Sciences**
DOI : 10.5958/j.2319-1198.2.2.017



# Botany, Cultivation, Chemical Constituents and Genetic Diversity in Fennel (*Foeniculum vulgare* Mill*)*: A Review

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**ABSTRACT**

Fennel (*Foeniculum vulgare* Mill), wild or cultivated, is widely distributed throughout the world and belongs to the Apiaceae family. It is an old medicinal plant and has been commonly used as a traditional food and medicine. Fennel seeds are used for their anti-inflammatory, antispasmodic, antimicrobial properties and oestrogen-promoting action. Recent research has identified fennel as a valuable medicinal plant with potential for multipurpose uses and also as a source for preparing raw materials of pharmaceutical industry, especially steroidal hormones. A significant increase in quantity and quality yields through the suitable management of cultivation, chemical constituents and biotechnology practice could make an immediate and important contribution to farm and pharmaceutical industry income. To achieve these goals with regard to sustainable production, we reviewed a summary of biology, cultivation and biotechnology of fennel in this paper.

**Keywords**: *Foeniculum vulgare* Mill, Biotechnology, Cultivation, steroidal hormones

## 1. INTRODUCTION

*Foeniculum vulgare* Mill. (Apiaceae family) commonly known as fennel, is one of the widespread annual or perennial plants with aromatic odour massive popularity in traditional Chinese medicine.

*Foeniculum* is a genus of fewer than half a dozen species, and is best known as saunf in Hindi, treated by some botanists as the sole species of the genus. It is a well-known aromatic and medicinal herb, which is native to southern Europe and the Mediterranean region, where it has been used for centuries as a condiment and culinary spice as well as for medicinal purposes.

As an important economic crop, fennel has been used and traded internationally for centuries due to its therapeutic and culinary utilisation.

In Greece, it was a symbol of success. In Rome, the young fennel shoots were used as food. Though details about its introduction are obscure, it has occurred in California for the past 120 years and is presumed to have escaped from cultivation repeatedly.

### 1.1. Distinctive Characteristics

- Fennel is a large, long-lived, forming large clump up to 2 m tall herbaceous plant.
- It produces copious short-lived upright stems from a long-lived crown.
- Its alternately precise leaves are delicately alienated into many thread-like fragments and have a ferny manifestation.
- Its small yellow flowers are borne in large flat clusters at the tips of the twigs.
- When handled or crushed their leaf gives off a burly aniseed-like smell.

### 1.2. Varieties

*F. vulgare* has been intensively cultivated in China and can be divided into different subspecies or varieties on the basis of chemicals presents and its utilisation. The two most important subspecies of *F. vulgare* were *Vulgare* and *Piperitum,* where *Piperitum* have with bitter seeds,

and is characterised by the presence of rotundifolone, while *vulgare* with sweet seeds, varied with estragole, *trans*-anethole, limonene and fenchone, by which different chemotypes can be divided (Muckensturm *et al*., 1997). *Vulgare* variety is widely used for flavourings in baked goods, meat and fish dishes, ice cream, and alcoholic beverages, due to its characteristic anise odour.

Contrarily, according to some others botanists, *F. vulgare* constitutes two varieties. One is sweet fennel (*F. vulgare* var. *dulce*), which is annuals or biennials with small sweet-tasting fruits and the other one is bitter fennel (*F. vulgare* var. *vulgare*), which is a perennial with fruits having a bitter taste (Cosge *et al*., 2008; Miraldi, 1999).

Different populations of *F. vulgare* contain 10-nonacosanone as a specific chemical marker and *trans*-anethole is the major volatile constituent. This review focuses on the bioactive components of the fennel and their pharmacology, and also provides a platform for further study and utilisation of fennel.

### 1.3. Origin and Naturalised Distribution

Species of fennel have been found in southern Europe (i.e., France, Portugal, Spain, Albania, Bulgaria, Greece, Italy and Yugoslavia), the Azores, the Madeira Islands, the Canary Islands, northern Africa (i.e., Algeria, Egypt, Libya, Morocco and Tunisia) and western Asia (i.e., Afghanistan, Iran, Israel, Jordan, Lebanon, Syria, Turkey and Pakistan).

Fennel is an extensive species that is naturalised principally in the coastal and sub-coastal districts all through the southern and south-eastern parts of Australia. It is universal in Victoria, eastern New South Wales, the ACT, Tasmania, south-eastern South Australia and south-western Western Australia. Rarely, in some inland parts of eastern South Australia and northern New South Wales, in the cooler districts of south-eastern Queensland and on Norfolk Island.

Broadly naturalised in the UK, southern Africa, New Zealand the Pacific (i.e., Hawaii, Fiji, New Caledonia, Niue and French Polynesia), the USA, Mexico, Central America and South America. In India, it is mainly cultivated in states of Gujarat, Rajasthan and to some extend in UP, Punjab, Haryana, Bihar, Maharashtra and Karnataka. Gujarat is the leading state in Fennel production, which contributes about 85-90%. India exports a substantial quantity of fennel to the United States, Singapore, the United Kingdom, the United Arab Emirates, Sri Lanka, Malaysia, Saudi Arabia and Japan in a variety of forms including seeds, powder and volatile oil.

### 1.4. Botanical Perspective

It is an annual, biennial or perennial aromatic herb, depending on the variety. It is erect, glaucous green and grows to heights ranging from 3.3 to 12 feet tall (Klinger, 2000), with hollow stems. The leaves grow up to 40 cm long; they are finely dissected, with the ultimate segments filiform (threadlike), about 0.5 mm wide. The flowers are produced in terminal compound umbels 5-15 cm wide, each umbel section having 20-50 tiny yellow flowers on short pedicels. The fruit or seed of the fennel plant is brownish or greenish grey, ovate, ribbed and approximately 6-10 mm long and 1.5-2 mm broad. The stylopodium persists on the fruit. The constituent mericarps have ridges and furrows running along their length on the outer wall. The pericarp has oil canals that contain an essential oil. The endosperm is rich in fatty oil. It has a spicy licorice fragrance, which can be found in all parts of the plant.

Since all the fruits do not mature together, harvesting of the umbels has to be done four or five times at 10-15 days interval. Umbels should be plucked before the fruits fully ripen when they are just changing from deep green to slightly yellow. Picking umbels at half length fruit size of green colour is economical. The umbels should be dried under shade. Heaping of the umbels may be avoided as it may deteriorate the quality. The dried umbels are threshed and the seeds are separated and cleaned by winnowing.

### 1.5. Cultivation

It is cultivated throughout the temperate and sub-tropical regions of the country up to an altitude of 1825 m. It requires fairly mild climate and is cultivated as a winter crop. Dry and cold weather favours higher seed production. Fennel thrives well in the sunny, slimy, well-drained loams of Western India (Gujarat, Rajasthan and Uttar Pradesh), which are rich in minerals and lime.

However, heavy soils are more desirable than light soils for higher yields. Generally, fennel can grow in any type of soils, but water logged and salt-affected lands are unsuitable for its cultivation.

Seeds are sown from first week of October to mid November for seed production in North India. In certain areas, transplanting is practiced. Sowing in nursery beds is done in July-August followed by transplanting in September to the middle of October. Early sowing (by 15 October or 30 October) results in significantly higher number of umbels per plant and grain yield as compared to late sowing on 14 November. Germination occurs within about 2 weeks at a temperature of 18 °C. It can tolerate a range of annual precipitation from 0.3 to 2.6 m and soil pH from 4.8 to 8.3.

### 1.6. Chemical Constituents

Fennel seeds are used as spices and condiments. The nutritive analysis of fennel is given in Table 1 (Pruthi, 1979). The chemical constituents from the fennel include essential oil, fatty acid, phenylpropanoids, monoterpenids, sesquiterpenes and coumarins. It also contains triterpenoids, tannins, flavonoids, cardiac glycosides, saponins and other types of compounds.

### 1.7. Essential Oil

In western countries, essential oil of fennel fruits was used for flavouring purpose, cosmetic and pharmaceutical products (Bilia *et al*., 2000). The volatile compounds of fennel fruits were well studied by hydrodistillation, extraction with classical solvents, supercritical fluid extraction, headspace solvent microextraction and solid-phase microextraction (Damjanovic *et al*., 2005; Tschiggerl *et al*., 2010). The relative content of essential oil in fennel fruits was about 3.00% by weight. The prominent component of oil was *trans*-anethole (70.1%) and the most intense odour compounds of fennel fruits were *trans*-anisole, estragole, fenchone and 1-octen-3-ol (Diaz-Maroto *et al*., 2005; Mimica-Dukie *et al*., 2003). Radulovic and Blagojevic (2010) carried out (GC and GC/MS) analysis of *F. vulgare* Mill. (Fennel) root and schizocarp essential oils and diethyl ether extracts and identified 89 different components. The most abundant classes of constituents were the phenylpropanoids (69.5-85.5%) and

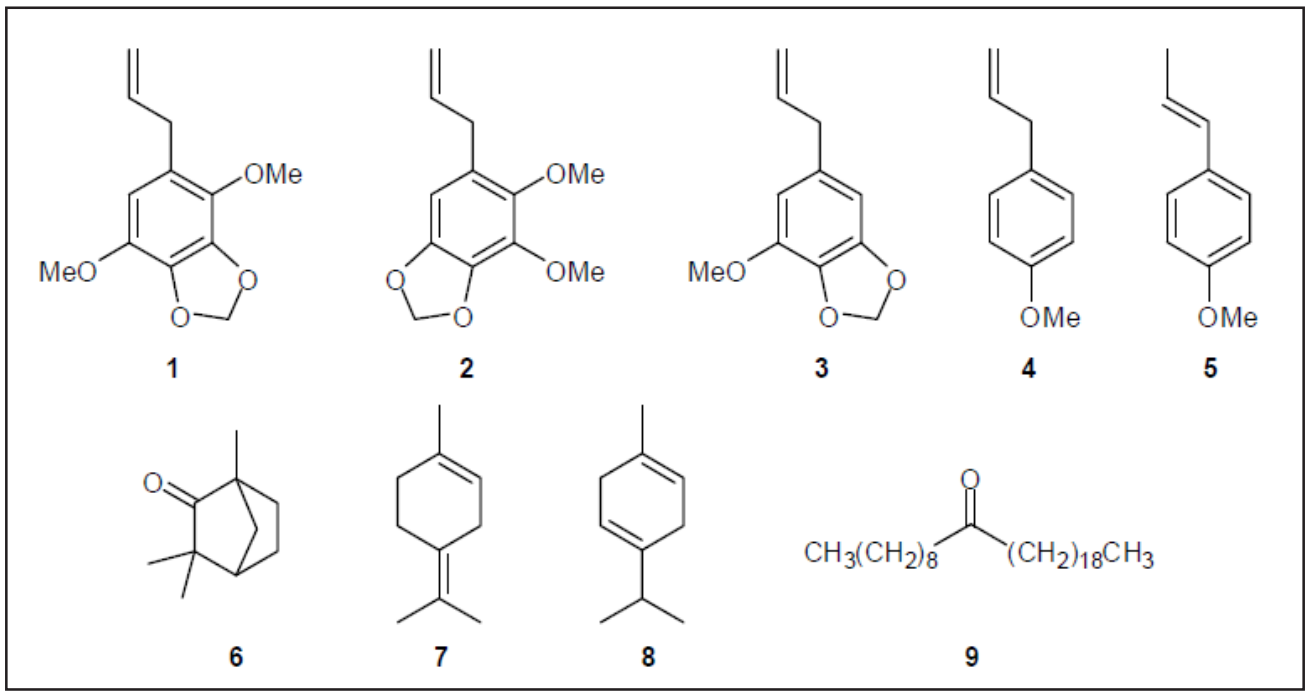


**Figure 1: The major compounds identified in the analysed oils and extracts: 1-apiole, 2-dillapiole, 3-myristicin, 4-methyl chavicole, 5-(*E*)-anethole, 6-fenchone, 7-terpinolene, 8-γ-terpinene and 9-10-nonacosanone.**

monoterpenoids (11.7-26.9%). The dominant volatile metabolites of the schizocarps were fenchone (13.3-18.8%) and (E)-anethole (66.1-69.0%). Contrary to that terpinolene (6.2-6.5%) and dillapiole (71.4-77.5%) were the major volatiles of fennel roots.

The composition of the essential oil depends upon various factors, including internal, environmental and agricultural practices as well as factors affecting the plant such as genetics and ecological conditions (Telci *et al*., 2006 and Fuente *et al*., 2003). According to Msaada *et al.* (2007), maturation stages play an important factor influencing essential oil composition, while suitable environmental and agricultural practices would also help in improving yield and quality.

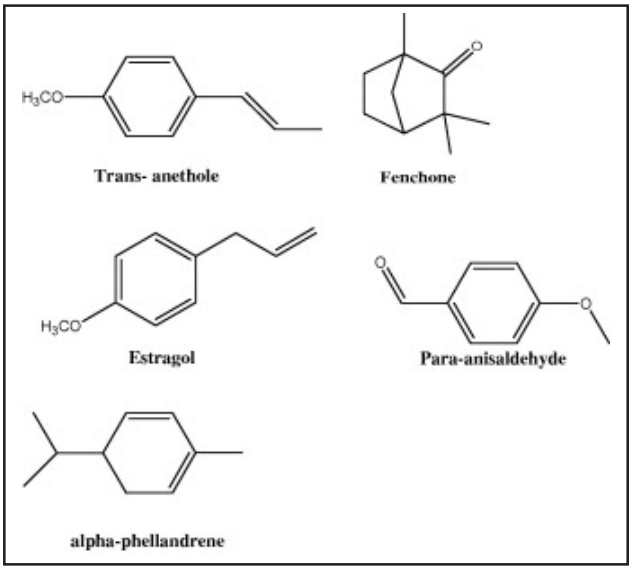


**Figure 2: The molecular structures of the major bioactive essential oil components of *F. vulgare*.**

Remarkable, chemodiversity occurs in the composition of the essential oil of *F. vulgare* depending upon the method of extraction and geographical origin. The accumulation of these volatile compounds inside the plant also varied, appearing practically in any of its parts viz., roots, stem, shoots, flowers and fruits (Gross *et al.*, 2009).

In one study, it was reported that the essential oil content and composition varies during the different maturation stages of *F. vulgare* and declines with fruit maturity. The content of trans-anethole, the main component, varied between 81.63% and 87.85% (Telci *et al*., 2009).

In an another study, it was stated that the phenylpropenes estragol and *trans*-anethole, which are the major constituents of the oleoresin of the aerial parts of *F. vulgare*, varied during plant development, being maximal in flowers and developing mericarps. These essential oils contribute to the pharmacological effects of the *F. vulgare* fruits.

Numerous studies have shown the effect of essential oil and its individual constituents in exhibiting novel pharmacological activities. (+) Fenchone and *P*-anisaldehyde were identified as the major acaricidal agents against *Dermatophagoides farinae* and *Dermatoghagoides pteronyssinus* and can be therefore used as potential house dust mite control agents or as lead compounds.

In another study, anethole has been reported to be active oestrogenic agent, while in some others it has been shown that polymers of anethole, i.e., dianethole and photoanethole are the actual oestrogenic agents. Anethole has been also reported to be a safe antithrombotic agent due to its antiplatelet activity, clot destabilising effect and vaso-relaxant action (Tognolini *et al*., 2007). However, estragole, as a main component of *F. vulgare*, has become a cause of concern, as structurally similar

**Table 1: Representing the data of the chemical composition of essential oils in different cultivars of *F. vulgare* (Shahat *et al*., 2011)**

| S.No. | Name of compound | *F. vulgare* | | |
|---|---|---|---|---|
| | | *Azoricum* | *Dulce* | *Vulgare* |
| 1. | α-Pinene | 1.65 | 3.26 | 3.61 |
| 2. | Camphene | 0.08 | 0.3 | 0.19 |
| 3. | Sabinene | 0.39 | 0.27 | 0.56 |
| 4. | β-Pinene | 0.12 | 0.14 | 0.21 |
| 5. | Myrcene | 0.18 | 0.66 | 0.32 |
| 6. | a-Phellandrene | 0.11 | 0.18 | 0.11 |
| 7. | o-Cymene | 0.46 | 0.38 | 0.71 |
| 8. | Limonene | 12.53 | 27.78 | 20.64 |
| 9. | Eucalyptol | 2.05 | 0.9 | 1.93 |
| 10. | g-Terpinene | 0.24 | 0.06 | 0.38 |
| 11. | Fenchone | 7.99 | 12.77 | 7.22 |
| 12. | Linalool | 0.29 | 0.09 | 0.11 |
| 13. | Camphor | 0.13 | 0.18 | 0.29 |
| 14. | Estragole | 11.99 | 6.24 | 57.94 |
| 15. | Fenchyl acetate | 0.13 | 6.34 | 0.21 |
| 16. | Cumic aldehyde | 0.18 | 0.06 | 0 |
| 17. | p-Anisaldehyde | 0.4 | 0.11 | 0.26 |
| 18. | trans-Anethole | 61.11 | 46.26 | 4.99 |

methyleugenol has been recently reported to be a potential carcinogen. This has led to the European Union to allow a new legal limit for estragole of 10 mg/kg in non-alcoholic beverages.

### 1.8. Fatty Acids

The fruits of fennel contain about 20% fatty acids and petroselinic acid is a characteristic fatty acid of fennel oil. The level of petroselinic acid could be as high as 70-80%. Barros *et al*. (2010) quantified 21 fatty acids. Polyunsaturated fatty acids were found to be the main group in all the fennel parts; linoleic acid predominated in shoots, stems and inflorescences, while a-linolenic acid predominated in leaves.

### 1.9. Phenolic Compounds and Flavonoids

Another important class of phytochemicals present in *F. vulgare* is phenols and phenolic glycosides. It contains

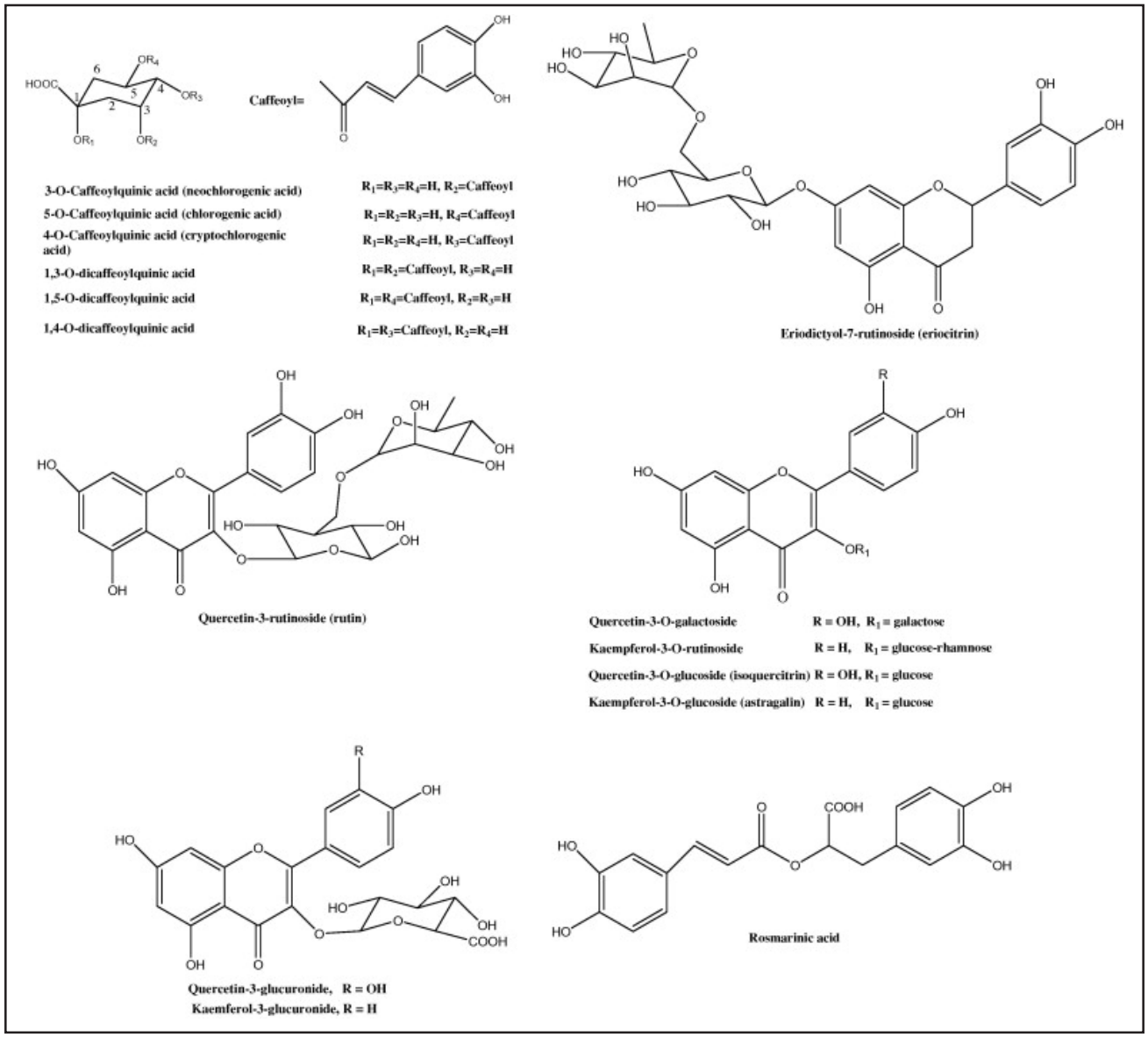


**Figure 3: Molecular structures of some phenols and phenolic glycosides isolated from *F. vulgare*.**

phenolic acids like 3-O-caffeoylquinic acid, 4-O-caffeoylquinic acid, 5-O-caffeoylquinic acid, 1,3-O-di-caffeoylquinic acid, 1,4-O-di-caffeoylquinic acid and 1,5-O-di-caffeoylquinic acid. The phenolic compounds present in *F. vulgare* are considered to be associated with the prevention of diseases thought to be induced by oxidative stress such as cardiovascular diseases, cancer and inflammation. These phenolic compounds have received tremendous attention among nutritionists, food scientists and consumers due to their roles in human health. Diglucoside stilbene trimers and benzoisofuranone derivatives have also been isolated from *F. vulgare* fruit together with *cis*-miyabenol C, *trans*-miyabenol C, *trans*-resveratrol-3-O-β-D-glucopyranoside, sinapyl glucoside, syringin-4-O-β-glucoside, oleanolic acid, 7α-hydroxycampesterol, (3β,5α,8α,22E)5,8-epidioxy-ergosta-6,22-dien-3-ol and 2,3-dihydropropylheptadec-5-onoate (Marino *et al*., 2007). An acylated kaemferol glycoside from flowers of *F. vulgare* has also been isolated (Soliman *et al*., 2002).

Flavonoids on the other hand are richly found in plants of Apiaceae family and aqueous extract of fennel fruits. The furocoumarins imperatorin, psoralen, bergapten, xanthotoxin and isopimpinellin were isolated from the methylene chloride extract (Nassra *et al*., 2010). The flavonoids isorhamnetin 3-*O*-α-rhamnoside, quercetin and kaempferol were isolated from the ethyl acetate extract, whereas quercetin 3-*O*-rutinoside, kaempferol 3-*O*-rutinoside and quercetin 3-*O*-β-glucoside were isolated from the methanol extract.

The flavonoids like eriodictyol-7-rutinoside, quercetin-3-rutinoside and rosmarinic acid have also been isolated from *F. vulgare* (Faudale *et al*., 2008; Park, 1996).

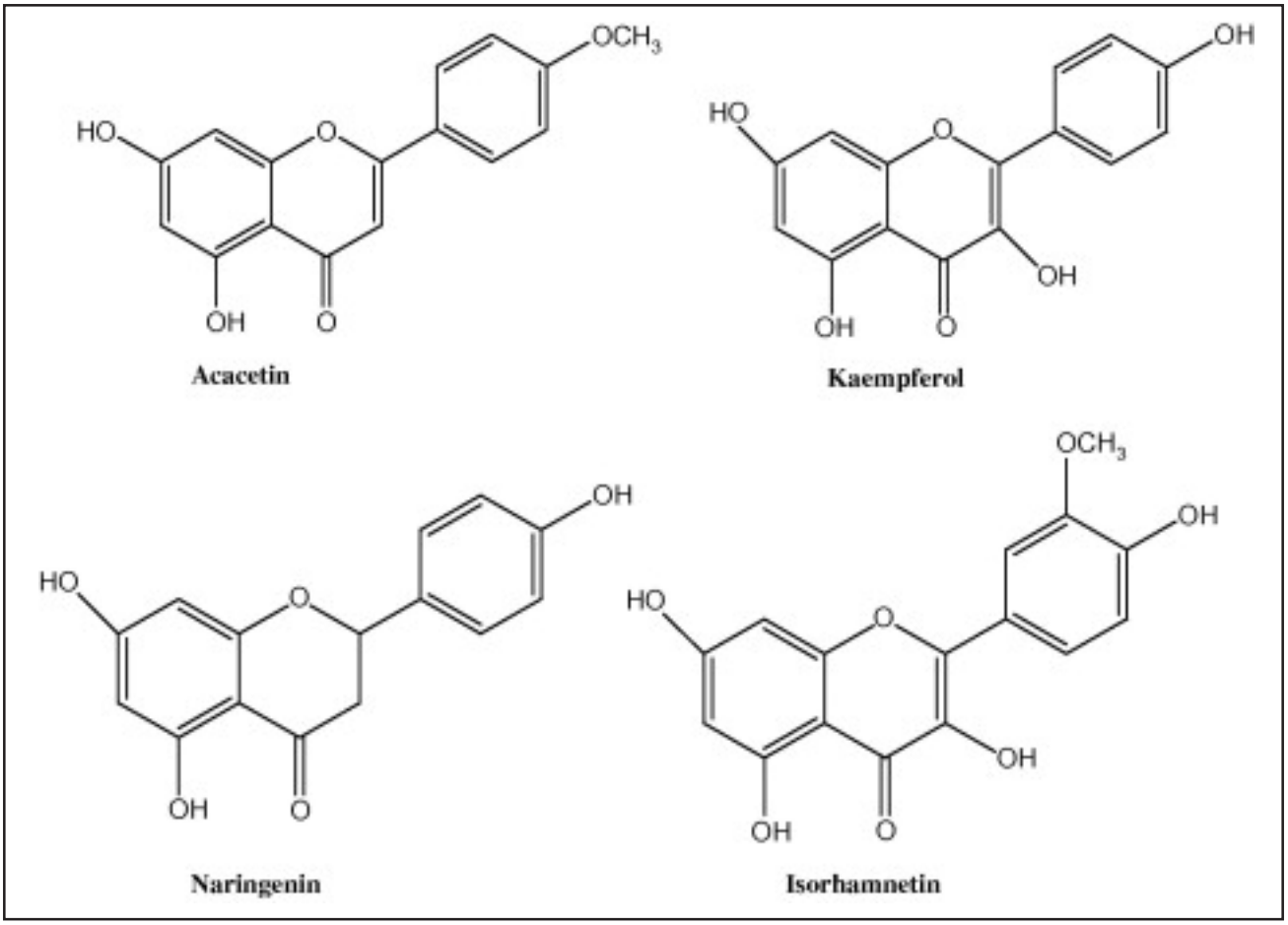


**Figure 4: Molecular structures of some flavonoid aglycons reported in *F. vulgare*.**

Quercetin-3-O-galactoside, kaempferol-3-O-rutinoside and kaempferol-3-O-glucoside have also been reported to occur in the aqueous extract of *F. vulgare*. Quercitin-3-O-glucuronide, kampferol-3-O-glucuronide, isoquercitin and isorhamnetin-3-O-glucoside have also been isolated from *F. vulgare*.

### 1.10. Therapeutic Uses

Fennel is a well-recognised medicinal plant because of its volatile oil, which is a stimulant, an aromatic and a carminative, which includes analgesic, purgatives, diuretic, emmenagogue, galactogogue, expectorant, hallucinogenic, stimulant, anti-inflammatory, etc. Its seeds are used for their anti-inflammatory, antispasmodic, antimicrobial properties and oestrogen-promoting action. They are widely used in the treatment of anaemia, menorrhagia, dysmenorrhoea, fibroids, stomach aches, sore throat, coughs, bad-breath, skin diseases, eye infections, intestinal worms and flatulence, gum infections, excessive weight and poor milk secretion in breast feeding women, to treat hiccup, nausea and is a

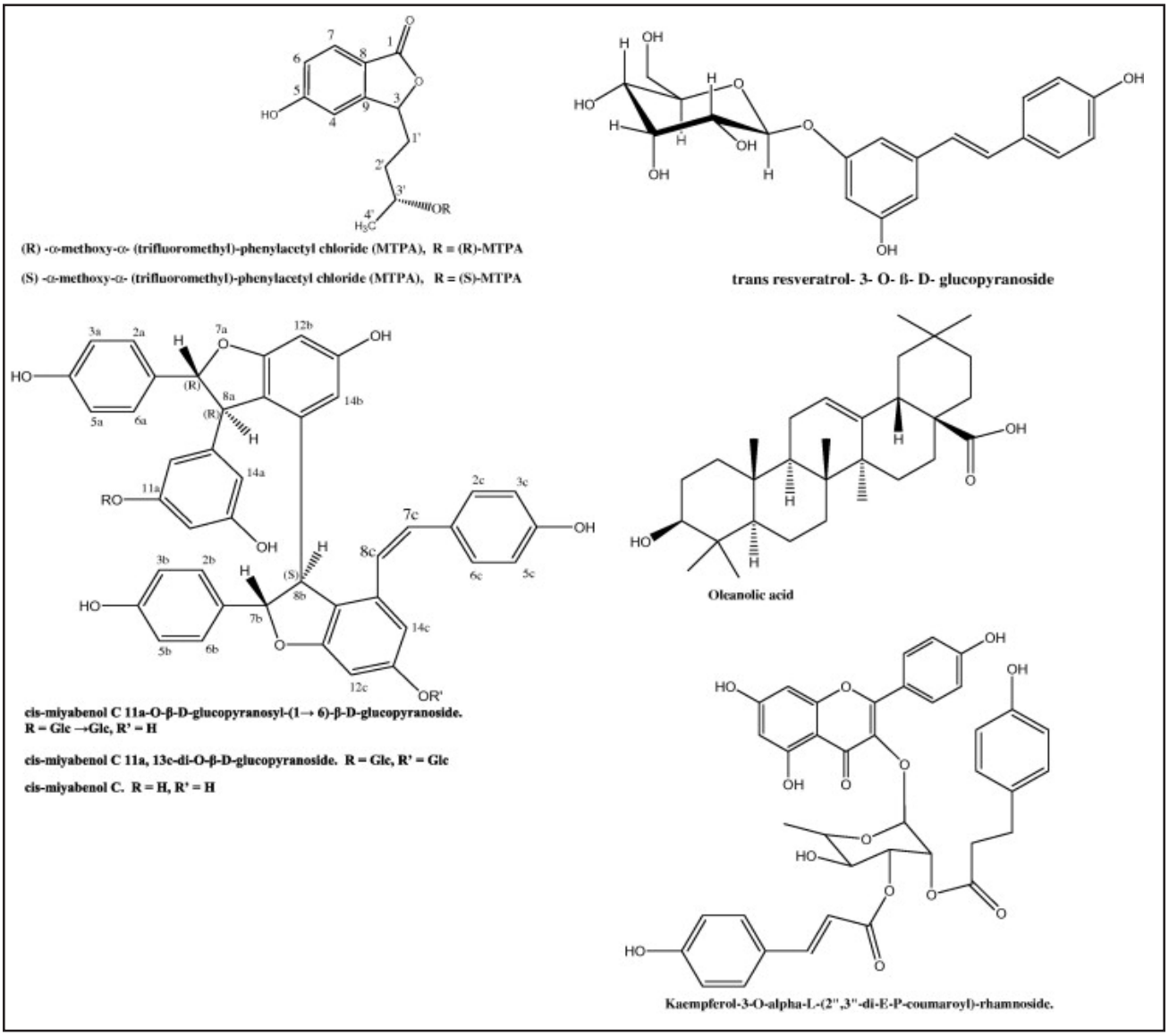


**Figure 5: Molecular structures of some flavonoid aglycons reported in *F. vulgare*.**

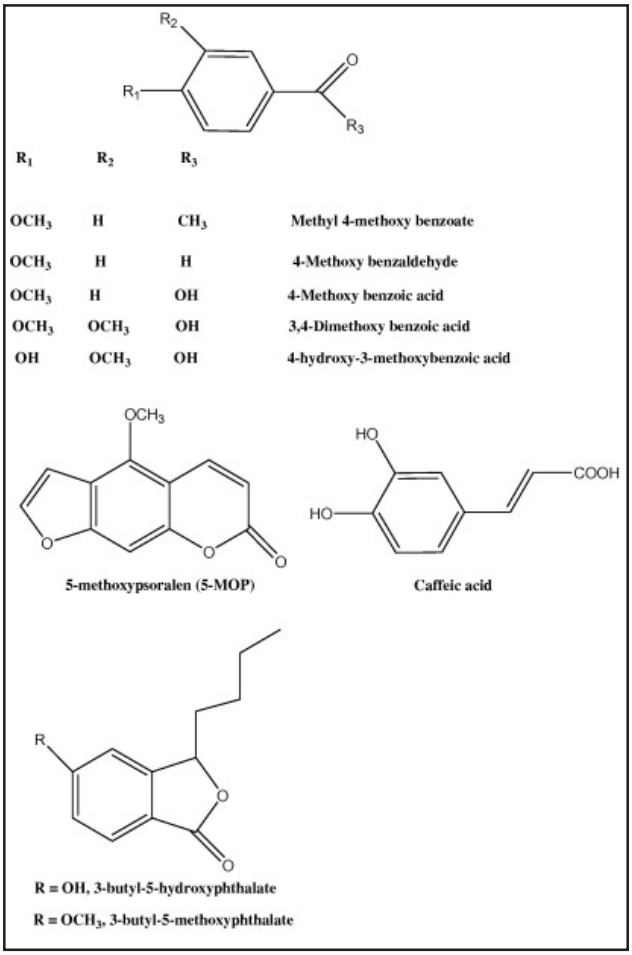


**Figure 6: Molecular structures of bioactive compounds from the methanol extract of *F. vulgare* seeds. These compounds have been reported to possess human liver cytochrome P 450 3A4 inhibitory activity with 5-methoxypsoralen showing the strongest inhibition.**

lung health support. Fennel is also supposed to mitigate gastritis, acidity, gout, cramps, colic and spasms, and as a diuretic agent, it increases the flow of urine.

Highly valuable for digestive system. Consuming fennel dried seeds immediately after eating food is a routine practice by most of the Indians Seeds, leaves and roots of fennel all are valuable medicinally, but fruits are the mainly imperative part of fennel as they defer essential oil, which is used in the treatment of various skin diseases, and as an antibacterial agent, while their powder is used in numerous medicinal preparations for domestic uses. Traditionally, in cases of eye infectivity fennel tea was used as chollyrium.

**Table 2: Nutritive value of fennel seeds**

| S.No. | Characteristic | Qty per 100 g seed |
|---|---|---|
| 1. | Moisture | 6.3 g |
| 2. | Protein | 9.5 g |
| 3. | Fat | 10.0 g |
| 4. | Carbohydrate | 42.3 g |
| 5. | Sodium | 0.09 g |
| 6. | Potassium | 1.7 g |
| 7. | Vitamin A | 1040 IU |
| 8. | Vitamin $B_1$ | 0.41 mg |
| 9. | Crude fibre | 18.5 g |
| 10. | Mineral matter | 13.4 g |
| 11. | Calcium | 1.3 g |
| 12. | Phosphorus | 0.48 g |
| 13. | Iron | 0.01 g |
| 14. | Vitamin $B_2$ | 0.36 mg |
| 15. | Niacin | 6.00 mg |
| 16. | Vitamin C | 12.00 mg |
| 17. | Food energy | 370 calories |

### 1.11. Genetic Diversity in *F. vulgare*

Studies on the performance of different collections of fennel were conducted by Singh *et al*. (2003) for identifying the suitable genotypes for direct introduction with higher seed yield per plant, seeds per umbel, umbel diameter, number of umbels per plant, number of primary branches per plant, number of secondary branches per plant, plant height and 100-seed weight. Significant differences and wide range of variation was observed among the genotypes for all the characters studied. The genotype IC-279039, JF-252, EC-279042 and EC-386375 were found promising in respect of grower's preference.

Thirty-seven diverse landraces of fennel assembled from 17 different states of India, UP (11) and 2 each from MP, Uttaranchal, Punjab, Orissa, J&K, Haryana, MS, Rajasthan, W Bengal, Gujarat; one each from AP, Chattisgarh, Himachal, Assam, Kerala and Bihar (Lal *et al*., 2006). Observations were recorded on the eight economic traits: days to flowering (50 %), plant height (cm), umbels/plant, diameter of main stem, umbels on main stalk, seed yield/plot (g), oil content (%) and t-anethole content (%) in the oil. Highly significant differences ($P$=0.01) for all of the eight traits indicated the presence of considerable divergence among the 37

landraces of fennel crop. Genetic diversity among the 37 landraces was relatively large, although 27.03% of the landraces could be grouped within one cluster I followed by clusters II and III (24.324%), cluster IV (13.514%) and cluster V (5.405%). Only 2.703% of the landraces were highly divergent forming two different clusters Cluster VI-VII.

Analysis of variability carried out for 15 characters in 36 diverse genotypes of fennel at Jagudan (Gujarat) revealed highly significant differences among genotypes for all the characters studied (Patel *et al*., 2008). High genotypic and phenotypic variances were observed for days to 50% flowering, days to 50% maturity, plant height, plant height up to main umbel, total branches per plant, number of seeds per main umbel and seed per plant. The highest genotypic coefficient of variation was observed for volatile oil content in seed followed by total branches per plant and number of seeds per main umbel. Heritability estimates were high for seed yield per plant, days to 50% flowering, number of primary branches per plant, total branches per plant, test weight and volatile oil content. High genetic advance as percentage of mean was recorded for seed yield per plant, days to 50% flowering, primary branches per plant, total branches per plant, effective umbels per plant, number of umbellates per umbel, number of seeds per main umbel, test weight and volatile oil content, suggesting that phenotypic selection for these traits would be effective. Overall, it was suggested that for improving yield in fennel, more emphasis should be given to plant height, primary branches per plant, total branches per plant.

Zahid *et al*. (2009) assessed genetic diversity of indigenous fennel (*F. vulgare* Mill.) germplasm from Pakistan by using RAPD markers. Fifty accessions of fennel were collected from different parts of Pakistan and evaluated for important characteristics like seed germination percentage, days for initiation of flowering, plant height, stem girth, nodal distance, umbel diameter, days to 50% maturity, days to harvesting, seed yield per row, weight of 100 seeds and harvest index (%). Genomic DNA of the accessions was extracted and subjected to RAPD analysis in order to ascertain their genetic diversity. Twenty-four out of thirty decamer primers generated 145 clear bands and 70 (48%) were polymorphic. Sixteen primers OPA-01, OPA-03, OPA-04, OPA-05, OPA-07, OPA-10, OPA-11, OPA-14, OPA-15, OPA-18, AC-11, AC-14, AC-15, AC-16, AC-18 and AC-20 gave polymorphism for different characters. About 66.6% of polymorphic primers generated the highest index to resolve genetic diversity even in small number of accessions. Seven accessions from Punjab, three from NWFP, one from Balochistan and one from Northern areas of Pakistan had appeared with promising characters.

An experiment was conducted to study the genetic divergence of 13 fennel varieties using Mahalanobsis D2 statistics (Meena *et al.*, 2010). Clustering pattern indicated that varieties from the same source were distributed over different clusters. This indicated that the geographic and genetic diversity are not necessarily related, therefore, the selection of varieties for hybridisation should be based on genetic diversity. Estimates of intra-cluster distance ranged from 1.00 to 6.09. It was highest in cluster VI and lowest in cluster II, IV and VI. Tocher's method of hierarchical cluster analysis was applied to group the varieties. The maximum inter-cluster distance between cluster III and I was 4.15 and 14.42, respectively. The varieties falling in cluster I were Hisar Swarup,GF-2, RF-101, RF-178 and RF-143, in cluster II was Rajendra Saurabha, in cluster III were Azad Saunf-1, GF-1, GF-11 and CO-1, in cluster IV was AF-1, in cluster V was RF-125 and in cluster VI was Pant Madurika. The study suggested that clusters I and II were quite divergent from rest of the clusters and also from one another. Among the 14 characters studied for genetic divergence, 80% flowering contributed the maximum accounting for 33.33% of total divergence, followed by angle of primary branch 32.05%.

Recently, in 2011 Grover *et al*. have evaluated the genetic diversity of seven varieties of *F. vulgare* using seven RAPD primers. A total of 70 clear bands were generated, out of which 35 (50%) were polymorphic. The total number of markers varied from 4 (GCC-181) to 13 (GCC-90 and GCC-132) with a mean of 10 markers per primer (Plate 1). The number of polymorphic markers for each primer varied from two (GCC-181) to seven (GCC-90, GCC-135 and GCC-176) with a mean of five polymorphic markers per primer. This study highlights that the high genetic diversity among varieties could be attributed to artificial selection, and not natural genetic differentiation.

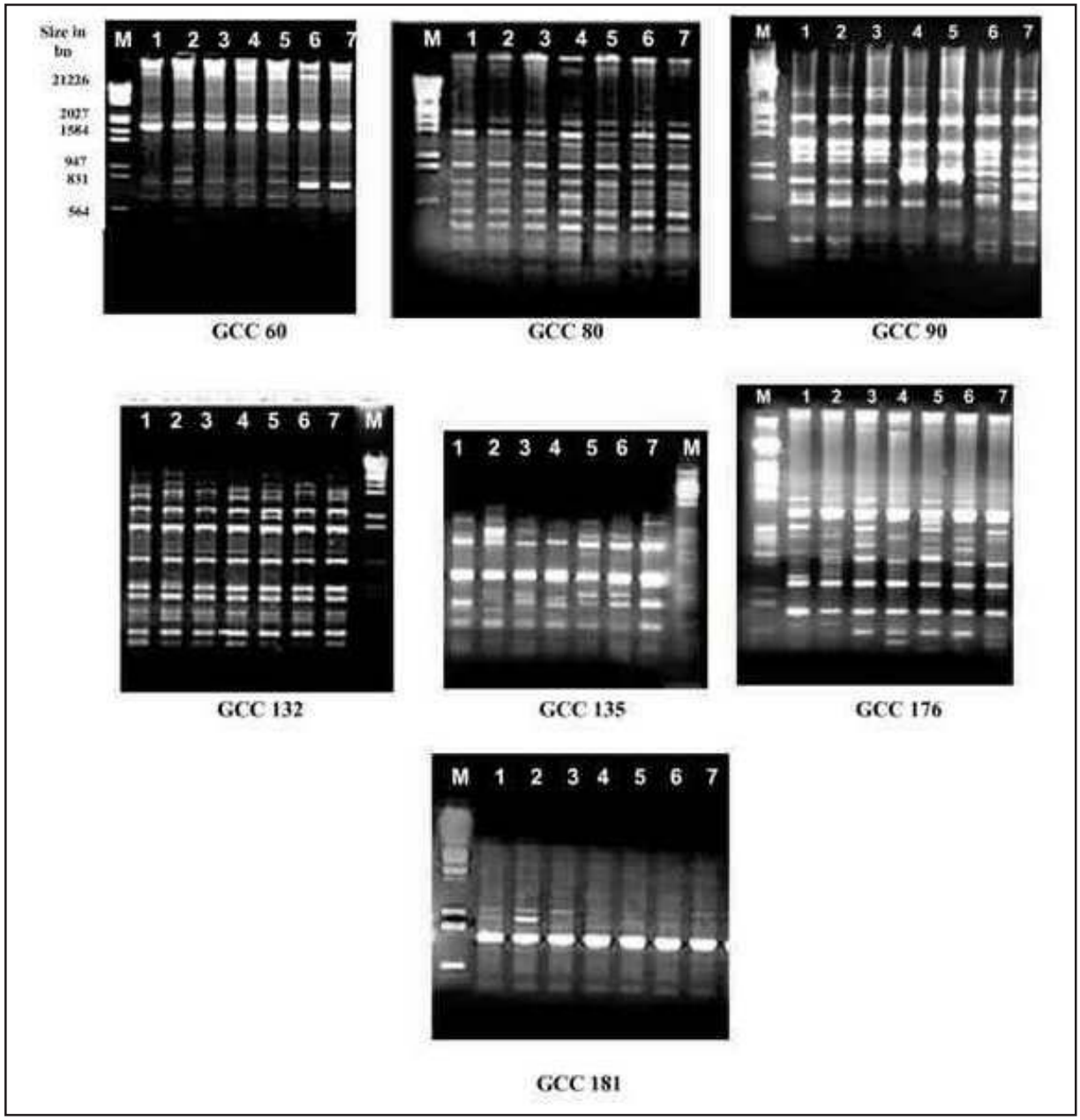


**Plate 1. RAPD profile of *F. vulgare* varieties obtained with GCC- 60, 80, 90, 132, 135, 176 and 181 primers**

## 2. CONCLUSION

Fennel has been supplemented as food and medicine with long history in central Europe and some of the Mediterranean region as well as in China. It is also a flavour food with health value. Numerous significant bioactivities compounds including *trans*-anethole, estragole, fenchone, sesquiterpenoids, coumarins and polyphenolics were isolated from this plant. The plant has potential beneficial therapeutic as well as antimicrobial actions, which makes their use more frequent against bacterial and fungal infections and colic pain. The essential oils of two of the fennel cultivars, i.e., *azoricum* and *dulce*, showed dramatically higher antioxidant activities than the essential oil of the *vulgare* cultivar.

Both the fruit and whole plant act as a potent source of useful chemical and biological materials in future. For further utilisation of this plant, systematic phytochemical and biological mechanic studies are needed.

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**LS - An International Journal of Life Sciences**
DOI : 10.5958/j.2319-1198.2.2.018



# Enrichment of Rice Cultivars with Fe at Different Plant Growth Stages through Ferti-Fortification

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**ABSTRACT**

An experiment was conducted at the farm research area, Department of Soil Science, Punjab Agricultural University, Ludhiana, to investigate the Fe content of five rice cultivars (PR113, PR116, PR118, PR120 and PAU 201) at maximum tillering, pre-anthesis and post anthesis stages through ferti-fortification. Plant samples collected at different stages of growth were digested in diacid mixture of $HNO_3$ and $HClO_4$ (3:1) for the analysis of total Fe. The concentration of Fe in these samples was determined using atomic absorption spectrophotometer (Varian AAS-FS 240 model). The results of our experimental study showed that concentration of Fe in different plant parts at different growth stages of rice crop increased with three foliar spray of $FeSO_4.7H_2O$ with 0.5% and 1% levels. The results indicated that at maximum tillering stage, the maximum Fe concentration was observed in PR113 (234.4 mg $kg^{-1}$) and PR116 cultivars (289.2 mg $kg^{-1}$) with 0.5% and 1% levels of $FeSO_4.7H_2O$ spray, respectively. At pre-anthesis stage, the maximum Fe concentration was reported in flag leaves of PR120 cultivar with 0.5% (155.5 mg $kg^{-1}$) and with 1% (175.2 mg $kg^{-1}$) levels of $FeSO_4.7H_2O$ spray, whereas in case of plant samples (without panicle), the maximum Fe concentration was observed in PR120 cultivar with 0.5% (279.9 mg $kg^{-1}$) and 1% (313.9 mg $kg^{-1}$) levels of $FeSO_4.7H_2O$ spray. The results further indicated that the maximum Fe accumulation was observed in panicle of PR120 (72.8 mg $kg^{-1}$) and PAU 201 cultivars (83.0 mg $kg^{-1}$) with 0.5% and 1% levels of $FeSO_4.7H_2O$ spray, respectively. On the other hand, at post anthesis stage, the maximum Fe concentration was reported in flag leaves of PR116 (375.4 mg $kg^{-1}$) with 0.5% level and PR120 (424.0 mg$kg^{-1}$) with 1% level of $FeSO_4.7H_2O$ spray. The results further revealed that with 0.5% and 1% levels of $FeSO_4.7H_2O$ spray, the maximum Fe concentration was reported in panicle samples of PAU 201 cultivar (69.0 mg $kg^{-1}$ and 122.0 mg $kg^{-1}$, respectively).

**Keywords:** Maximum tillering, Pre-anthesis and Post anthesis stages, Fe content, Rice cultivars

## 1. INTRODUCTION

Rice (*Oryza sativa* L.) is the dominant staple food for more than 50% of the world's population (Wang *et al.*, 2005). Rice is the main source of nutrition for the poor people in many Asian countries (Beard and Finch 1985). In developing countries, rice accounts for 715 kcal/capita/day, which fulfills 27% of dietary energy supply, 20% of dietary protein and 3% of dietary fat (Huguet, 2007). Rice provides 23% more calories of energy than that provided by wheat and maize crops (Wang *et al.*, 2005). International Rice Research Institute (IRRI, 2006) reported that polished rice contains on an average only 2–5 mg $kg^{-1}$ Fe, whereas the recommended daily dietary intake of Fe for people is 10–15 mg $kg^{-1}$. In many Asian countries, rice provides 50–80% of the energy intake of the poor but it does not provide enough essential micronutrients to eliminate iron deficiency, anaemia.

Iron (Fe) is essential for plant growth and development. Vasconcelos *et al.* (2003) reported that the ferritin protein takes up Fe, stores it in a non-toxic form, and releases it when needed for metabolic functions as Fe stored in ferritin rice is bioavailable. Bioavailability is the fraction of the ingested nutrient that is utilised for normal physiological functions or storage (King, 2002). Lucca *et al.* (2001) observed that amount of bioavailable Fe is dependent both on Fe intake and its absorption. Silveira

*et al.* (2007) observed that ferrihydrite is the inorganic compound usually associated with Fe accumulation in ferritin. Ferritins are large proteins with a central activity that can store up to 4500 Fe atoms, which can be released when necessary. Therefore, proteins are believed to play a critical role in the cellular regulation of Fe storage and homeostasis. They also reported that the concentration of nutrients in the rice plant vary with the plant part and leaf age during the panicle differentiation stage. Fageria (2009) reported that Fe concentration in plant tissues varied with plant age, crop species and plant part analyzed. Fernandez and Ebert (2005) found that foliar applied compound penetration would occur via the cuticle through cuticular cracks and imperfections, through stomata, leaf hairs and other specialized epidermal cells. They reported that both the upper and the lower leaf surface are involved in the process of penetration of an applied solution. The structure and composition of cuticle as well as the morphology, distribution and size of the stomata differ among plant species and will play a role regarding the penetration of foliar sprays. Relative humidity and leaf water status are key factors governing foliar uptake of nutrients. Farrandon and Chamel (2008) observed that the ferrous sulphate applied on rice foliage showed also in IR68144, the Fe concentration in each part of shoot increased with nitrogen fertilizer application. Prasad (2009) observed that there are three ways to overcome micronutrient malnutrition. These were dietary supplementation with micronutrient, improving diet composition and fortification of cereal grains with micronutrient.

Bio-fortification leads to the development of micronutrient-dense staple crops using the best traditional breeding practices and modern biotechnology (Masuda *et al.,* 2008; Shivay *et al.,* 2008; Dhaliwal *et al.,* 2010). Ferti-fortification is a technique to increase Fe content in rice grains and brown rice. Bio-fortification and commercial ferti-fortification though are slow processes and show low efficiency of nutrient enrichment, but still these are highly complementary. Finally, breeding of cereal crops for higher trace mineral density in seeds will not incur a yield penalty (Hossain *et al.,* 2008). Keeping these points in view, the present investigation has been planned with the following objectives to study enrichment of rice cultivars with Fe at different plant growth stages, viz., maximum tillering, pre-anthesis, post anthesis and maturity stages.

## 2. MATERIALS AND METHODS

### 2.1. Location and Weather Parameters of Experimental Site

Field experiment was conducted at the research farm area, Department of Soil Science, Punjab Agricultural University, Ludhiana, during *Kharif* 2011 (June–October). Ludhiana is situated at the latitude of 30°54´ North and longitude of 75°48´ East at an altitude of 307.42 m above sea level. It represents agro-ecological zone No 4 and sub-zone No 1. Monthly average details of the important weather parameters of Ludhiana for the period May 2011 to October 2011, recorded from Agricultural Meteorological Observatory, Punjab Agricultural University, Ludhiana, were reported in Table 1.

### 2.2. Selection of rice cultivars

Five rice cultivars, namely PR113, PR116, PR118, PR120 and PAU 201, were selected for this experiment. The PR113 is short-statured, stiff-strawed cultivar having bold and heavy grains and its average grain yield is about 70 quintal of paddy per hectare, whereas the PR116 is semi-dwarf, lodging-tolerant cultivar, which possesses long slender translucent grains with very good cooking

**Table 1: Weather parameters of the experimental site during the period of study**

| Month | Temperature (°C) | | Relative humidity (%) | Sunshine (h) | Total rainfall (mm) |
|---|---|---|---|---|---|
| | Max. | Min. | | | |
| May 2011 | 39.4 | 25.0 | 45 | 9.0 | 34.4 |
| June 2011 | 35.4 | 25.3 | 68 | 7.3 | 352.9 |
| July 2011 | 33.7 | 26.8 | 78 | 5.1 | 114.2 |
| August 2011 | 32.4 | 26.1 | 82 | 5.1 | 513.4 |
| September 2011 | 31.8 | 24.1 | 84 | 6.3 | 177.1 |
| October 2011 | 32.1 | 17.5 | 67 | 8.5 | 0 |

quality and its average yield is 70 quintal of paddy per hectare. The PR120 is semi-dwarf cultivar with dark green erect leaves and it has long slender translucent grains with very good cooking quality and its average yield is 71.2 quintal of paddy per hectare. On the other hand, PR118 is lodging-tolerant cultivar with medium slender grains having good cooking quality and its average grain yield is 72.5 quintal of paddy per hectare. The average grain yield PAU 201 cultivar is 75 quintal of paddy per hectare and it has long, slender grains with good cooking quality. Three sprays of 0.5% and 1.0% ferrous sulphate ($FeSO_4.7H_2O$) were applied at maximum tillering stage, pre-anthesis stage and post anthesis stage (at 50% flowering). The experiment was laid out in randomised block design (RBD). Nitrogen (N) and phosphorus (P) fertilizers were applied to rice crop on soil test basis as per recommendations given by Punjab Agricultural University, Ludhiana. The N was supplied to the rice crop using urea (46% N), whereas P was applied through diammonium phosphate (DAP). Nitrogen was applied in three splits, first at the time of puddling and others two splits 3–6 weeks after transplanting, whereas whole of the DAP was applied at the time of puddling. No potassium (K) fertilizer was applied as the experimental soil was sufficient in its K status.

### 2.3. Diethylenetriaminepentaacetic acid (DTPA)-extractable Zn, Cu, Fe and Mn

Availability of Zn, Cu, Fe and Mn was assessed by extracting 10 g portion of soil sample with 20 ml of diethylenetriaminepentaacetic acid (DTPA) extractant (0.005 M DTPA+0.01 M $CaCl_2.2H_2O$+0.1 M triethanolamine (TEA) buffer adjusted to pH (7.3) as described by Lindsay and Norvell (1978). The determination of these four micronutrient cations was performed on the same extract with an atomic absorption spectrophotometer (Varian AAS-FS 240 model).

### 2.4. Plant Analysis

The plant samples were collected at maximum tillering, pre-anthesis and post anthesis stages of rice growth. These samples were collected three times, one week just after each spray of $FeSO_4.7H_2O$ at different plant growth stages. Samples collected were whole plant at maximum tillering stage, flag leaf, plant without panicle and panicle at pre-anthesis and post anthesis stages. These samples were washed with 0.2% liquid detergent followed by 0.1 N HCl solution and then with double distilled water. These washed plant samples were air dried by keeping them in paper bags and then at oven at 65°C for 3 days and dried samples were ground in Thomas wiley mill (Model ED-5). Whole plant, plant without panicle and panicle straw samples were digested in diacid mixture of $HNO_3$ and $HClO_4$ (3:1) for the analysis of total Fe (Page *et al.*, 1982). The concentration of Fe was determined using atomic absorption spectrophotometer given by Isaac and Kerber (1971). The concentration of Fe in these samples was measured on atomic absorption spectrophotometer (Varian AAS-FS 240 model).

### 2.5. Statistical Analysis

The experiment was laid out in RBD with three replications. The Fe concentration data were subjected to RBD for analysis of variance. The statistical analysis was done with the help of method described by Panse and Sukhatme (1985). Critical difference (CD) was used to compare the treatment effects at $P<0.05$.

## 3. RESULTS AND DISCUSSION

### 3.1. Concentration of Fe in Different Rice Cultivars at Maximum Tillering Stage

The concentration of Fe (mg $kg^{-1}$) in plant samples of different rice cultivars was reported at maximum tillering stage (40–45 days after transplanting) and data are presented in Table 2. It has been observed that foliar application of $FeSO_4.7H_2O$ with 0.5% and 1% levels significantly increased the Fe concentration in plant samples of different rice cultivars as compared to control. The data presented in Table 2 show significant interactions between different rice cultivars and treatments. The results further revealed that maximum and minimum concentration of Fe was reported in plant samples of PR113 (234.4 mg $kg^{-1}$) and PAU 201 (201.6 mg $kg^{-1}$) cultivars respectively, with 0.5% level of $FeSO_4.7H_2O$ spray. The concentration of Fe in plant samples of other cultivars (PR116, PR118 and PR120) ranged from 221.3 to 231.7 mg $kg^{-1}$ with 0.5% level of $FeSO_4.7H_2O$ spray. With the foliar spray of $FeSO_4.7H_2O$ with 0.5% level at maximum tillering stage, Fe

**Table 2: Concentration of Fe (mg kg$^{-1}$) in different rice cultivars at maximum tillering stage**

| Treatments | Rice cultivars | | | | |
|---|---|---|---|---|---|
| | PR113 | PR116 | PR118 | PR120 | PAU 201 |
| Control | 197.5 | 199.8 | 201.1 | 203.0 | 174.0 |
| 0.5% $FeSO_4$ | 234.4 | 221.3 | 227.0 | 231.7 | 201.6 |
| % Increase over control | 18.6 | 10.7 | 12.8 | 14.1 | 15.8 |
| 1% $FeSO_4$ | 264.5 | 289.2 | 247.3 | 266.4 | 248.6 |
| % Increase over control | 33.9 | 44.7 | 22.9 | 31.2 | 42.8 |
| CD (*P*=0.05) | 15.3 | 13.6 | 8.2 | 16.6 | 9.8 |
| Interaction (Cultivars x Treatments) | | | | | 9.0 |

concentration increased by 18.6%, 10.7%, 12.8%, 14.1% and 15.8% in plant samples of PR113, PR116, PR118, PR120 and PAU 201 cultivars, respectively, over control. Similarly, with the foliar application of $FeSO_4.7H_2O$ with 1% level at maximum tillering stage, the Fe concentration increased by 33.9%, 44.7%, 22.9%, 31.2% and 42.8% in plant samples of PR113, PR116, PR118, PR120 and PAU 201 cultivars, respectively, over control (Table 2). The data also showed that the highest and lowest concentration of Fe was observed in plant samples of PR116 (289.2 mg kg$^{-1}$) and PR118 (247.3 mg kg$^{-1}$) cultivars, respectively, with 1% level of $FeSO_4.7H_2O$ spray. The Fe concentration in plant samples of all the remaining cultivars (PR113, PR120 and PAU 201) with 1% level of $FeSO_4.7H_2O$ ranged from 248.6 to 266.4 mg kg$^{-1}$.

The results of our experimental study showed that different rice cultivars accumulate Fe in their leaves to different extent, which may be due to different Fe absorption capacities through the leaves of different rice cultivars. Similar results were obtained by Erdal *et al.*, (2004) in different varieties of strawberry with the foliar application of $FeSO_4.7H_2O$. The results showed that there was increase in Fe concentration in leaves of strawberry varieties by 75% as compared to control before blooming stage. This may also be due to fact that absorption rate of mineral nutrients by above-ground plant parts considerably differ not only among plant species but also between varieties within the same species (Wojcik, 2004). Zhang *et al.*, (2008, 2009) also reported that Fe status in plant leaves was significantly improved and more Fe was easily transported with foliar application of Fe. Habib (2009) observed that foliar application of Fe on wheat crop, at tillering and heading stages increased Fe concentration in plant as compared to control.

### 3.2. Concentration of Fe in Different Rice Cultivars at Pre-anthesis Stage

At pre-anthesis stage, flag leaf, plant without panicle and panicle samples were collected and Fe concentration (mg kg$^{-1}$) in these plant parts of different rice cultivars was observed. It was found that the foliar application of $FeSO_4.7H_2O$ with 0.5 per cent and 1 per cent levels significantly increased the Fe concentration in flag leaf, plant without panicle and panicle samples of different rice cultivars as compared to control, but results shown by panicle samples of PR113 and PR116 cultivars were non-significant. The data presented in Table 3 show significant interactions between different rice cultivars and treatments. The Fe concentration in flag leaves of PR113, PR116, PR118 and PAU 201 cultivars with 0.5% level of $FeSO_4.7H_2O$ spray ranged from 121.4 to 147.5 mg kg$^{-1}$ (Table 3). According to data, the maximum concentration of Fe was reported in flag leaves of PR120 cultivar (155.5 mg kg$^{-1}$) followed by PR113 cultivar (147.5 mg kg$^{-1}$) with 0.5% level of $FeSO_4.7H_2O$ spray. These cultivars showed significant results with a CD of 20.3 and 11.6 over control at *P*=0.05. With foliar spray of $FeSO_4.7H_2O$ with 0.5% at pre-anthesis stage, Fe concentration in flag leaves of PR113, PR116, PR118, PR120 and PAU 201 cultivars increased by 93.5%, 86.2%, 81.7%, 142.2% and 113.8%, respectively, over control. It has been further observed that maximum Fe concentration was reported in flag leaves of PR120 cultivar (175.2 mg kg$^{-1}$) followed by PR113 cultivar (161.2 mg kg$^{-1}$) with 1% level of $FeSO_4.7H_2O$ spray. At pre-anthesis stage, the foliar application of $FeSO_4.7H_2O$ with 1% level resulted in the increase of Fe concentration in flag leaves of PR113, PR116, PR118, PR120 and PAU

**Table 3: Concentration of Fe (mg kg$^{-1}$) in different rice cultivars at pre-anthesis stage**

| Treatments | Rice cultivars | | | | |
|---|---|---|---|---|---|
| | PR113 | PR116 | PR118 | PR120 | PAU 201 |
| | | | ***Fe concentration in flag leaves*** | | |
| Control | 76.2 | 68.6 | 66.8 | 64.2 | 62.3 |
| 0.5% $FeSO_4$ | 147.5 | 127.8 | 121.4 | 155.5 | 133.2 |
| % Increase over control | 93.5 | 86.2 | 81.7 | 142.2 | 113.8 |
| 1% $FeSO_4$ | 161.2 | 158.2 | 138.2 | 175.2 | 153.7 |
| % Increase over control | 111.5 | 130.6 | 106.8 | 172.8 | 146.7 |
| CD (*P*=0.05) | 11.6 | 7.1 | 9.2 | 20.3 | 7.4 |
| | | Interaction (Cultivars x Treatments) | | | 9.1 |
| | | | ***Fe concentration in plant without panicles*** | | |
| Control | 196.1 | 123.4 | 159.2 | 228.8 | 208.3 |
| 0.5% $FeSO_4$ | 241.0 | 140.3 | 275.8 | 279.9 | 234.7 |
| % Increase over control | 22.8 | 13.6 | 73.2 | 22.3 | 12.6 |
| 1% $FeSO_4$ | 290.7 | 197.5 | 293.2 | 313.9 | 286.8 |
| % Increase over control | 48.2 | 60.0 | 84.1 | 37.1 | 37.6 |
| CD (*P*=0.05) | 13.7 | 4.8 | 15.1 | 10.9 | 9.4 |
| | | Interaction (Cultivars x Treatments) | | | 8.4 |
| | | | ***Fe concentration in panicles*** | | |
| Control | 41.7 | 45.8 | 44.4 | 28.8 | 40.0 |
| 0.5% $FeSO_4$ | 45.1 | 50.8 | 60.0 | 72.8 | 61.6 |
| % Increase over control | 8.2 | 10.9 | 35.1 | 152.7 | 54.0 |
| 1% $FeSO_4$ | 52.4 | 53.8 | 64.9 | 78.2 | 83.0 |
| % Increase over control | 25.6 | 17.4 | 46.1 | 171.5 | 107.5 |
| CD (*P*=0.05) | NS | NS | 13.3 | 15.4 | 6.5 |
| | | Interaction (Cultivars x Treatments) | | | 7.6 |

201 cultivars by 111.5%, 130.6%, 106.8%, 172.8% and 146.7%, respectively, over control. The Fe concentration in flag leaves of other cultivars (PR116, PR118 and PAU 201) with 1% level of $FeSO_4.7H_2O$ ranged from 138.2 to 158.2 mg kg$^{-1}$. Similar results were obtained by Erdal *et al.* (2004) in different varieties of strawberry with the foliar application of $FeSO_4.7H_2O$. They reported that there was increase in Fe concentration in leaves by 62.5% as compared to control at first blooming stage of its different cultivars. Similarly, Hu-lin *et al.* (2007) observed the effect of different nitrogen fertilizer levels on Fe, Mn, Cu and Zn concentrations in shoot and grain quality of two rice varieties IR68144 (Fe-dense) and IR64 (non-Fe-dense). They observed that in IR64 rice variety, Fe concentration in leaves and peduncle increased with nitrogen application by 85.5 and 30.9% compared with control. They also reported that in IR68144, Fe concentration in each part of shoot increased with nitrogen fertilizer application.

With the foliar spray of 0.5% $FeSO_4.7H_2O$ at pre-anthesis stage, Fe concentration in plant samples (without panicle) of PR113, PR116, PR118, PR120 and PAU 201 cultivars increased by 22.8%, 13.6%, 73.2%, 22.3% and 12.6%, respectively, over control (Table 3). The Fe concentration in plant samples (without panicle) of different cultivars (PR113, PR118, and PAU 201) with 0.5% level of $FeSO_4.7H_2O$ spray ranged from 234.7 to 275.8 mg kg$^{-1}$. The data further showed that maximum and minimum plant concentration of Fe was reported in plant samples (without panicle) of PR120 (279.9 mg kg$^{-1}$) and PR116 (140.3 mg kg$^{-1}$) cultivars respectively, with 0.5% level of $FeSO_4.7H_2O$ spray. On the other hand, the foliar application of $FeSO_4.7H_2O$ with 1% level at pre-anthesis stage, resulted in increase of Fe concentration in plant samples (without panicle) of PR113, PR116, PR118, PR120 and PAU 201 cultivars by 48.2%, 60.0%, 84.1%, 37.1% and 37.6%, respectively, over control. The maximum concentration of Fe was reported in plant samples (without panicle) of PR120 cultivar (313.9 mg kg$^{-1}$) with 1% level of $FeSO_4.7H_2O$ spray, whereas

minimum concentration of Fe was reported in plant samples (without panicle) of PR116 cultivar (197.5 mg $kg^{-1}$) at this level of $FeSO_4.7H_2O$ spray. The Fe concentration in plant samples (without panicle) of other cultivars (PR113, PR118 and PAU 201) with 1% level of $FeSO_4.7H_2O$ ranged from 286.8 to 293.2 mg $kg^{-1}$. Therefore, greater variation in Fe concentration of plant samples (without panicle) of different rice cultivars has been observed with foliar application of Fe. These results were supported by Fageria (2009), who reported that Fe concentration in plant tissues varied with plant age, crop species and plant part analysed. Similarly, Nowack *et al.* (2008) conducted an experiment to study the uptake of Fe by wheat and their transfer to the grains in the presence of chelating agent. They found that the EDTA and EDDS chelating agents increased the Fe accumulation in the shoots of wheat crop.

Foliar spray of $FeSO_4.7H_2O$ with 0.5% and 1% levels increased the Fe concentration in panicles of different rice cultivars. The maximum concentration of Fe was reported in panicles of PR120 cultivar (72.8 mg kg) followed by PAU 201 cultivar (61.6 mg $kg^{-1}$) with 0.5% level of $FeSO_4.7H_2O$ spray. The concentration of Fe in panicles of PR113, PR116 and PR118 cultivars with 0.5% level of $FeSO_4.7H_2O$ spray ranged from 45.1 to 60.0 mg/kg. With foliar spray of 0.5% level of $FeSO_4.7H_2O$ at pre-anthesis stage, Fe concentration in panicles of PR113, PR116, PR118, PR120 and PAU 201 cultivars increased by 8.2%, 10.9%, 35.1%, 152.7% and 54.0% respectively, over control. The maximum concentration of Fe was observed in panicles of PAU 201cultivar (83.0 mg $kg^{-1}$) followed by PR120 cultivar (78.2 mg $kg^{-1}$) with 1% level of $FeSO_4.7H_2O$ spray. The concentration of Fe in panicles of other cultivars (PR113, PR116 and PR118) with 1% level of $FeSO_4.7H_2O$ spray ranged from 52.4 to 64.9 mg $kg^{-1}$. The foliar application of $FeSO_4.7H_2O$ with 1% level at pre-anthesis stage resulted in the increase of Fe concentration in panicles of PR113, PR116, PR118, PR120 and PAU 201 cultivars by 25.6%, 17.4%, 46.1%, 171.5% and 107.5%, respectively, over control. So, lot of variation has been observed in different plant parts. Similarly, Silveira *et al.* (2007) reported that the concentration of nutrients in the rice plant vary with the plant part and leaf age during the panicle differentiation stage.

### 3.3. Concentration of Fe in Different Rice Cultivars at Post-Anthesis Stage

At this stage, flag leaf, plant without panicle and panicle samples were analysed and data were presented in Table 4, which shows the significant interaction between different rice cultivars and treatments. It has been further observed that the foliar application of $FeSO_4.7H_2O$ with 0.5% and with 1% levels significantly increased the Fe concentration in flag leaf, plant without panicle and panicle samples of different rice cultivars as compared to control (Table 4). At post anthesis stage, the foliar spray of $FeSO_4.7H_2O$ with 0.5% increased Fe concentration in flag leaves of PR113, PR116, PR118, PR120 and PAU 201 cultivars by 110.7%, 138.6%, 130.6%, 113.0% and 109.4%, respectively, over control (Table 4). The concentration of Fe in flag leaves of cultivars PR113, PR118 and PR120 with 0.5% level of $FeSO_4.7H_2O$ spray ranged from 307.5 to 352.8 mg $kg^{-1}$. The data showed that maximum and minimum concentration of Fe was observed in flag leaves of PR116 (375.4 mg $kg^{-1}$) and PAU 201 (293.9 mg $kg^{-1}$) cultivars, respectively, with 0.5% level of $FeSO_4.7H_2O$ spray. These cultivars showed significant results with CD of 6 and 11.5 over control at $P$=0.05. However, with 1% level of $FeSO_4.7H_2O$ spray, the maximum Fe concentration was observed in flag leaves of PR120 cultivar (424.0 mg $kg^{-1}$), whereas minimum Fe concentration was reported in flag leaves of PAU 201 cultivar (383.9 mg $kg^{-1}$). The foliar application of $FeSO_4.7H_2O$ with 1% level at post-anthesis stage increased Fe concentration in flag leaves of PR113, PR116, PR118, PR120 and PAU 201 cultivars by 129.5%, 154.9%, 208.3%, 167.6% and 173.6%, respectively, over control. The Fe concentration in flag leaves of other cultivars (PR113, PR116 and PR118) with 1% level of $FeSO_4.7H_2O$ ranged from 384.2 to 411.0 mg $kg^{-1}$. Erdal *et al.*, (2004) obtained similar results in an experiment on different varieties of strawberry with the foliar application of $FeSO_4.7H_2O$. These results showed that there was increase in Fe concentration in leaves of strawberry varieties by 173.5% as compared to control at full blooming stage. Grusak *et al.* (1999) observed that during grain fill in rice, the flag leaf and upper leaves were the principle contributors of photo-assimilates. With 0.5% level of $FeSO_4.7H_2O$ spray, the Fe concentration in plant samples (without panicle) of different cultivars (PR113, PR116 and PR118) ranged

**Table 4: Effect of foliar sprays on Fe concentration (mg $kg^{-1}$) in different rice cultivars at post anthesis stage**

| Treatments | Rice cultivars | | | | |
|---|---|---|---|---|---|
| | PR113 | PR116 | PR118 | PR120 | PAU 201 |
| | | | ***Fe concentration in flag leaves*** | | |
| Control | 167.4 | 157.3 | 133.3 | 158.4 | 140.3 |
| 0.5% $FeSO_4$ | 352.8 | 375.4 | 307.5 | 337.5 | 293.9 |
| % Increase over control | 110.7 | 138.6 | 130.6 | 113.0 | 109.4 |
| 1% $FeSO_4$ | 384.2 | 401.1 | 411.0 | 424.0 | 383.9 |
| % Increase over control | 129.5 | 154.9 | 208.3 | 167.6 | 173.6 |
| CD (*P*=0.05) | 14.0 | 6.0 | 8.0 | 7.7 | 11.5 |
| | | Interaction (Cultivars x Treatments) | | | 7.4 |
| | | | ***Fe concentration in plant without panicles*** | | |
| Control | 183.3 | 143.3 | 146.7 | 126.3 | 165.8 |
| 0.5% $FeSO_4$ | 255.7 | 234.4 | 223.2 | 270.6 | 257.4 |
| % Increase over control | 39.4 | 63.5 | 52.1 | 114.2 | 55.2 |
| 1% $FeSO_4$ | 314.3 | 305.8 | 280.3 | 283.9 | 278.2 |
| % Increase over control | 71.4 | 113.3 | 91.0 | 124.7 | 67.7 |
| CD (*P*=0.05) | 6.9 | 7.4 | 13.8 | 5.5 | 8.7 |
| | | Interaction (Cultivars x Treatments) | | | 6.4 |
| | | | ***Fe concentration in panicles*** | | |
| Control | 38.7 | 35.0 | 33.6 | 34.6 | 32.2 |
| 0.5% $FeSO_4$ | 49.7 | 62.0 | 40.3 | 67.7 | 69.0 |
| % Increase over control | 28.4 | 77.1 | 19.9 | 95.6 | 114.2 |
| 1% $FeSO_4$ | 68.9 | 84.7 | 58.6 | 102.0 | 122.0 |
| % Increase over control | 78.0 | 142.0 | 74.4 | 194.7 | 278.8 |
| CD (*P*=0.05) | 9.5 | 9.9 | 3.7 | 14.4 | 10.5 |
| | | Interaction (Cultivars x Treatments) | | | 7.4 |

from 223.2 to 255.7 mg $kg^{-1}$. The maximum concentration of Fe was reported in plant samples (without panicle) of PR120 cultivar (270.6 mg $kg^{-1}$) followed by PAU 201 cultivar (257.4 mg $kg^{-1}$) with 0.5% level of $FeSO_4.7H_2O$ spray (Table 4) .With the foliar spray of 0.5% $FeSO_4.7H_2O$ at post-anthesis stage, Fe concentration in plant samples (without panicle) of PR113, PR116, PR118, PR120 and PAU 201 cultivars increased by 39.4%, 63.5%, 52.1%, 114.2% and 55.2%, respectively, over control. Whereas with the foliar application of $FeSO_4.7H_2O$ with 1% level, the Fe concentration in plant samples (without panicle) of PR113, PR116, PR118, PR120 and PAU 201 cultivars increased by 71.4%, 113.3%, 91.0%, 124.7% and 67.7%, respectively, over control. The Fe concentration in plant samples (without panicle) of cultivars PR118, PR120 and PAU 201 with 1% level of $FeSO_4.7H_2O$ ranged from 278.2 to 283.9 mg $kg^{-1}$. It has been observed that maximum Fe concentration was reported in plant samples (without panicle) of PR113 cultivar (314.3 mg $kg^{-1}$) followed by PR116 cultivar (305.8 mg $kg^{-1}$) with 1 per cent level of $FeSO_4.7H_2O$ spray. So, foliar application of Fe increases its concentration in plant samples (without panicle) of different rice cultivars. These results were supported by Farrandon and Chamel (2008), who observed that the ferrous sulphate applied on rice foliage showed an extensive fixation by the foliage at the point of application. With the foliar spray of $FeSO_4.7H_2O$ with 0.5 % level, Fe concentration in panicles of PR113, PR116, PR118, PR120 and PAU 201 cultivars increased by 28.4%, 77.1%, 19.9%, 95.6% and 114.2%, respectively, over control (Table 4). The maximum concentration of Fe was observed in panicles of PAU 201 cultivar (69.0 mg $kg^{-1}$) with 0.5% level of $FeSO_4.7H_2O$ spray, whereas minimum concentration of Fe was reported in panicles of PR118 cultivar (40.3 mg $kg^{-1}$) at this level of $FeSO_4.7H_2O$ spray. The concentration of Fe in panicles of other cultivars (PR113, PR116 and PR120) with 0.5% level of $FeSO_4.7H_2O$ spray ranged from 49.7 to 67.7 mg $kg^{-1}$. On the other hand, with 1%

level of $FeSO_4.7H_2O$ spray, Fe concentration in panicles of different cultivars (PR113, PR116 and PR120) ranged from 68.9 to 102.0 mg $kg^{-1}$. The foliar application of $FeSO_4.7H_2O$ with 1% level at post anthesis stage, the Fe concentration in panicles of PR113, PR116, PR118, PR120 and PAU 201 cultivars increased by 78.0%, 142.0%, 74.4%, 194.7% and 278.8%, respectively, over control. The maximum and minimum Fe concentration was observed in panicles of PAU 201 (122.0 mg $kg^{-1}$) and PR118 (58.6 mg $kg^{-1}$) cultivars, respectively, with 1% level of $FeSO_4.7H_2O$ spray. The concentration of Fe at post anthesis stage varied in different plant parts of different rice cultivars with foliar application of Fe. These results were in accordance with those obtained by Grusak (2002), who observed that plant mineral concentrations varied both among plant source (i.e., species, cultivars) and among plant tissues (e.g. leafy structures versus seeds), thereby demonstrating that genetic difference exists, which can contribute to the plant's ability to acquire and sequester minerals.

## 4. CONCLUSION

The results of our experimental study showed that concentration of Fe in different plant parts at different growth stages of rice crop increased with three foliar spray of $FeSO_4.7H_2O$ with 0.5% and 1% levels. At maximum tillering stage, the maximum Fe concentration was observed in plant samples of PR113 and PR116 cultivars with 0.5% and 1% levels of $FeSO_4.7H_2O$ spray, respectively. Similarly, at pre-anthesis stage, the maximum Fe concentration was reported in flag leaves of PR120 cultivar both with 0.5% and 1% levels of $FeSO_4.7H_2O$. On the other hand, at post anthesis stage, the maximum Fe concentration was reported in flag leaves of PR116 cultivar with 0.5% level and PR120 cultivar with 1% level of $FeSO_4.7H_2O$ spray.

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**LS - An International Journal of Life Sciences**
DOI : 10.5958/j.2319-1198.2.2.011



# Arbuscular Mycorrhiza: A Biological Budding for Sustainable Agriculture

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**ABSTRACT**

Arbuscular mycorrhiza (AM) is possibly the most prevalent constituent of global symbiosis and is created by 70-90% of land plant species with fungi that belong to a monophyletic phylum, the Glomeromycota. The initiation of the AM fungal root colonisation begins with hyphae growing towards the plant root and extensively around it, where they subsequently attach to the root surface. This relationship results in an improved acquisition of nutrients (e.g., phosphate and nitrates) from the soil by the plant partners and, in exchange, allows the Arbuscular Mycorrhizal Fungi (AMF) to obtain the photosynthetically fixed carbon sources (e.g., sugars) necessary for their survival and propagation. AM symbiosis is very common as the fungi involved can colonise a vast taxonomic range of both herbaceous and woody plants, which are geographically cosmopolitan and ubiquitous, occurring over a broad ecological range in plants growing in the arctic, temperate and tropical regions. In this review, the development, host range, functioning of AM and its benefits for sustainable farming will be discussed.

**Keywords:** Arbuscular mycorrhiza, Global symbiosis, Host range, Sustainable farming, Glomeromycota

## 1. INTRODUCTION

Arbuscular mycorrhiza (AM) perhaps the most widespread component of terrestrial symbiosis (Fitter, 2005) and is formed by 70-90% of land plant species (Smith and Read, 2008) with fungi that belong to a monophyletic phylum, the Glomeromycota (Schübler *et al*., 2001). These fungi are obligate symbionts that infect the roots of most plants (Fitter and Moyersoen, 1996; Harley and Smith, 1983; Trappe, 1987). In this mycorrhizal association, the fungus colonises the roots of a host plant either intra- or intercellularly. The hyphae of the fungus are restricted to the cortical region of the roots and penetrate the host cell wall and grow within the cell but never penetrate the stele.

Morton (1988) classified the fungi that form Arbuscular mycorrhizal association in the order Glomales. The order is further divided into suborders based on the presence of: (i) vesicles in the root and formation of chlamydospores (thick wall, asexual spores) borne from subtending hyphae for the suborder *Glomineae* or (ii) absence of vesicles in the root and formation of auxiliary cells and azygospores (spore resembling a zygospore but developing asexually from a subtending hypha resulting in a distinct bulbous attachment) in the soil for the suborder Gigasporineae. The term vesicular-AM was originally applied to symbiotic associations formed by all fungi in the Glomales, but because a major suborder lacks the ability to form vesicles in roots, the term AMs are now preferred to these associations. The suborders are further divided into families and genera according to the method of spore formation.

Walker *et al*. (2007) have classified these fungi in to four orders comprising of 10 families and 13 genera belonging to the class Glomeromycetes of the Phylum Glomeromycota. These orders are Archaeosporales, Diversisporales, Glomerales and Paraglomerales. The

order Archaeosporales contains three families, Archaeosporaceae with the genus *Archaeospora* and *Intraspora*, Ambisporaceae with the genus *Ambispora,* and *Geosiphonaceae* with the genus *Geosiphon*. The order Diversisporales is represented by five families, Diversisporaceae with the genera Acaulospora and Kuklospora, Entrophosporaceae with the genus *Entrophospora*, Gigasporaceae with the genera Gigaspora and Scutellospora, and Pacisporaceae with the genus *Pacispora*. The order Glomerales includes only one genus, *Glomus*, similarly the order Paraglomerales is represented by one genus, *Paraglomus*.

### 1.1. AM Development

The initiation of the AM fungal root colonisation begins with hyphae growing towards the plant root and extensively around it, where they subsequently attach to the root surface. The hyphal tips start swelling, forming specific structures called appressoria between the epidermal root cells, followed by the development of infective penetration pegs emerging as hyphal tips from the appressoria (Mandelbaum and Piche, 2000). No appressoria are formed on dead roots or on various artificial fibres (Giovannetti *et al*., 1993), which indicates that the formation of this structure occurs only because of fungal recognition of a potential host plant. AM fungal hyphae, bearing the penetration pegs in their ends, penetrate the adjacent epidermal root cell walls followed by the cortical cell walls, to enter the root. The hyphae cross the hypodermis and start branching in the outer cortex.

At the penetration stage, the plant seems to recognise the fungal attachment, and it has been showed that the epidermal cells adjacent to the penetrating hyphae get slightly thicker walls (Garriock *et al*., 1989) at this stage, probably as a defence response to the fungal penetration. However, the thickenings do not contain callose or lignin (substances commonly produced in plant cells as a response to wounding or pathogen infection, providing the plant with a defensive structure) and do consequently not prevent the penetration of fungal hyphae through the walls (Harrison and Dixon, 1994). As the fungus grows, the host cell membrane invaginates and envelops the fungus, creating a new compartment called the apoplastic space, which prevents direct contact between the plant and fungal cytoplasm but allows for efficient transfer of nutrients between the symbionts (Sylvia, 2002).

After appresorium formation, roots can be colonised into two different ways (Smith and Smith, 1997). The arum type of AM colonisation, which is the most studied form, is characterised by intercellular spread of the hyphae until they reach the inner cortex, where the plant cell wall is penetrated and the fungus extensively ramifies to form an arbuscule. In paris-type of colonisation, fungal development is only intracellular and arbuscules are formed from hyphal coils. Numerous modifications have been observed in host cells during the development of arum-type arbuscules (Timonen and Peterson, 2002). The fungal cell wall becomes progressively thinner as the arbuscule develops and consequently in these cells, there is an extensive intracellular interface in which the two symbionts are in extremely close contact, separated only by their membranes and a narrow plant-derived apoplast (Bonfante-Fasolo and Perotto, 1990, 1992; Smith and Gianinazzi, 1988). This interface is thought to be the site at which phosphate and possibly carbon are transferred between symbionts, although some consider that the intercellular hyphae might be responsible for carbon uptake (Smith and Smith, 1989, 1990; Smith, 1993).

Following colonisation of the root cortex, fungal hyphae develop extensively within the soil. Early studies of the external mycelium had indicated that it is comprised of different types of hyphae, including large runner hyphae and finer absorptive hyphae (Friese and Allen, 1991). Once colonisation has occurred, short-lived runner hyphae grow from the plant root into the soil. These are the hyphae that take up phosphorus and micronutrients, which are conferred to the host plant. AM fungal hyphae have a high surface-to-volume ratio making their absorptive ability greater than that of plant roots (Tuomi *et al*., 2001). AMF hyphae are also finer than roots and can enter into pores of the soil that are inaccessible to roots (Bolan, 1991). The third type of AMF hyphae grows from the roots and colonises other host plant roots. These three types of hyphae are morphologically distinct (Wright, 2005). Recently, these findings have been confirmed, and an ultra structural examination of the fine absorptive hyphae has revealed features consistent

with a role in nutrient absorption (Bago *et al*., 1998). In addition to its role in the symbiosis, the extraradical mycelia contributes to soil stability by the aggregation of soil particles, probably mediated in part by glycoprotein called glomalin produced by the hyphae (Wright *et al*., 1996; Wright and Upadhyaya, 1996). These external AM hyphae extend from the root surface to the soil beyond the P depletion zone and so assess a greater volume of undepleted soil than the root alone (Hayman, 1983; Jackobsen, 1986; Plenchette and Fardeau, 1988). The small diameter of hyphae (20-50µm) allows access to soil pores that cannot be explored by roots. Therefore, a root system that has formed a mycorrhizal network has a greater effective surface area to absorb nutrients and explore a greater volume of soil than non-mycorrhizal roots.

In this association, all the fungal species form arbuscules-small tree-like, hyphal-filled, invaginations of the cortical cells-provide intimate contact between the plasmalemmae of the two symbiotic partners and are the point of material exchange between host and fungus. The arbuscules are relatively short lived, less than 15 days, and are often difficult to see in field-collected samples. Other structures produced by some AM fungi include vesicles, auxiliary cells and asexual spores. All the species, with the exception of *Gigaspora* and *Scutellospora*, form vesicles within the roots. These are thin-walled, lipid-filled, terminal swellings of hyphae with a storage/perennating function that usually form in intercellular spaces. However, vesicles can also serve as reproductive propagules for the fungus (Biermann and Linderman, 1983). Auxiliary cells are formed in the soil and can be coiled or knobby. The function of these structures is still unknown. Reproductive spores (Chlamydospores and Azygospores) can be formed either in the root or more commonly in the soil. These spores are asexual, formed by the differentiation of vegetative hyphae. For some fungi (e.g., *Glomus intraradices*), vesicles in the root undergo secondary thickening, and a septum (cross wall) is laid down across the hyphal attachment leading to spore formation, but more often spores develop in the soil from hyphal swellings. Spores may be formed singly, e.g., *Glomus mosseae,* in loose aggregation, e.g., *Glomus viscosum,* or sometimes in sporocarps, e.g., *Glomus taiwanensis*. According to Schenck and Perez (1990), sporocarp is a structure with spores borne closely together that may be surrounded by peridial hypha.

### 1.2. Range of Host Species

AM symbiosis is very common as the fungi involved can colonise a vast taxonomic range of both herbaceous and woody plants, which are geographically cosmopolitan and ubiquitous, occurring over a broad ecological range in plants growing in the arctic, temperate and tropical regions (Mosse *et al*., 1981). It can be found in a large majority of terrestrial plants (Newman and Reddell, 1987) and in almost a quarter of a million plant species (Gadkar *et al*., 2001). They have the widest host range and distribution of al1 the mycorrhizal associations (Harley, 1969). These arbuscular mycorrhizal associations are found in more than 90% of plants and 85% of plant families in all terrestrial environments. (Harley and Harley, 1987). There are at least 300,000 receptive hosts in the world flora (Kendrick and Berch, 1985). Some individual AM fungi may have access to thousands of hosts (Kendrick and Berch, 1985).

According to Gerdemann (1975), it is easier to list most plant families that do not form AM than to list those do. Families not forming AM include Pinaceae, Betulaceae, Orchidaceae, Proteacea, Funariaceae, Commelinaceae, Cruciferae, Zygophyllaceae, Dipterocaceae, Urticaceae, Myrtaceae, Fagacaeae and Ericaceae. Although Cactaceae, Bracicaceae, Chenopodiaceae, Cyperaceae, Polygonaceae, Amarantaceae and Juncaceae were earlier thought to be mycorrhiza free, most of the species were found to be infected under natural stressed rangeland conditions (Neeraj *et al*., 1991). The reason why some plants do not form mycorrhizas is not fully known, but it may be related to the presence of fungal toxic compounds in root cortical tissue or in root exudates. It may also be due to interactions between the fungus and the plant at the cell wall and/or middle lamella level (Tester *et al*., 1987). High concentrations of salicylic acid have been found to reduce mycorrhization (Medina *et al*., 2003), meaning that plants with a genetic basis for high salicylic acid content have evolved to be mycorrhiza free. Sometimes some immune species may occur within a normally susceptible family, e.g., *Lupinus consentinii* (Trinick, 1977), which has thick fringe of long root hairs

resembling the proteoid roots of members of the Proteaceae those are also immune. Some families that form both ectomycorrhiza and AM include Juglandiaceae, Tiliaceae, Myrtaceae, Salicaceae, Fagaceae and Caesalpiniaceae (Gerdemann, 1975). Janos (1980) has observed that most of the tropical rain forest trees are arbuscular mycorrhizal. Again, Harley (1969) has listed the Gymospermic plants having the AM association. Similarly, other researchers also observed arbuscular mycorrhization in Pteridophytes (Cooper, 1976) as well as in Bryophytes (Parke and Linderman, 1980). This AM colonisation has also been reported in floating (Bagyaraj *et al*., 1979) and submerged acquatic plants (Clayton and Bagyaraj, 1984). In general, AM are confined to the roots of plant, but they have been reported in diverse structures also, e.g., modified leaves of water fern *Salvinia cuculatta* (Bagyaraj *et al*., 1979), fruiting peg of peanut (Graw and Rehm, 1977) and modified scale-like leaves and rhizomes of ginger and canna (Selvaraj *et al*., 1986).

Many workers have clearly established that although AM fungi are not host specific and show extremely wide host range, but their affinity to the host is always preferential (Molina *et al*., 1992; Rogers *et al*., 1994). Because of the possible differences in the affinity of different symbionts with a particular host, the magnitude of mycorrhization is expected to vary with the symbionts. Moreover, the extent of benefit offered by the symbiont to the host varies according to stages of growth, and therefore, Smith and Walker (1981) have suggested that periodic sampling is of great importance in experiments related to selection of AM inoculants.

## 2. AM FUNCTIONING

### 2.1. Contribution of AMF in the Uptake of Nutrients

From the plant fungus association, AMF gain carbon (Smith and Read, 1997) and the host plant obtains a wide range of benefits, including enhanced uptake and transport of poorly mobile nutrients especially phosphorus (P) (Fitter, 1991). Some workers (Cress *et al*., 1979; Bolan *et al*., 1987; Tinker, 1978) have also reported that AMF hyphae are responsible for solubilisation of relatively immobile sources of P by exploring a large soil volume. AM fungal hyphae contribute to absorption and translocation of P from sites, in soil that are not accessible to plant roots (Sanders and Tinker, 1971), and depletion zone around non-mycorrhizal plants of a few millimetres (George *et al*., 1992).

Research has shown that when root exploration is restricted, up to 80% of the plant phosphorous can be delivered by the external arbuscular mycorrhizal hyphae to the host plant from a distance of more than 10 cm from the root surface. Hattingh *et al*. (1973) also observed that arbuscular mycorrhizal hyphae, could intercept labeled phosphorus, placed 27 mm from a mycorrhizal root, whereas it remained unavailable to non-mycorrhizal roots. These mycorrhizal roots have been known to absorb phosphorus faster per grams of root than non-mycorrhizal plants (Jackobsen *et al*., 1992).

There are some evidences that nitrogen is taken up by arbuscular mycorrhizal hyphae from inorganic sources of ammonium. It was also thought that the uptake of macronutrient nitrogen takes place in both inorganic and organic forms (George *et al*., 1995; Hawkins *et al*., 2000; Hodge *et al*., 2001). Both nitrate (Bago *et al*., 1996; Tobar *et al*., 1994) and ammonium (Ames *et al*., 1983; Frey and Schuepp, 1993; Johansen *et al*., 1992, 1996) can be taken up and used by AM fungi. There is no direct evidence for mobilisation and uptake of organic N (Hodge *et al*., 2000; Smith and Read, 1997). In various studies, the direct transport of N via the extraradical hyphae of AM fungi has been shown in systems, where the mass flow and diffusion of N from a compartment containing root-distant hyphae to another compartment containing roots and root near hyphae were reduced or prevented (Hawkins and George 1999; Johansen *et al*., 1992, 1994). In these cases, between 1 and 7% of the $^{15}NH_4$ $^{15}NO_3$ supplied to *Glomus mosseae* hyphae was recovered in the wheat host plant after 48 h (Hawkins and George, 1999), While 27-49% obtained over 27 days with the inoculation of *G. intraradices* in cucumber plant (Johansen *et al*., 1994).

AM fungus develop intensively inside roots and within the soil by forming an extensive extraradical network and this helps plants considerably in exploiting mineral nutrients and water from the soil. Researchers have demonstrated that AM fungi not only increases phosphorous uptake but also plays an important role in the uptake of other plant nutrients and water (Ellis *et*

*al*., 1985; Huang *et al*., 1985). These fungi contribute to the uptake of micronutrients, such as zinc (Thompson, 1990). Swaminathan and Verma (1979) also observed the uptake of Zinc by the AM fungi in some extent. Gildon and Tinker (1983) reported the uptake of Cu by AMF hyphae.

A number of workers have investigated on the role of AM on improvement of the absorption of several nutrients as indicated in Table 1.

**Table 1: Nutrient absorption by Arbuscular mycorrhiza**

| Nutrient | References |
|---|---|
| Phosphorus | Al-Karaki and Al-Radad (1997), Chandrashekara *et al*. (1995), Harley and Smith (1983) |
| Nitrogen | Liu *et al*. (2002) |
| Potassium | Liu *et al*. (2002) |
| Magnesium | Liu *et al*. (2002) |
| Copper | Gildon and Tinker (1983) |
| Zinc | Chen *et al*. (2003), Faber *et al*. (1991), Gildon and Tinker (1983), Jamal *et al*. (2002) |
| Calcium | Liu *et al*. (2002) |
| Iron | Caris *et al*. (1998) |
| Cadmium | Gonzalez *et al*. (2002), Guo *et al*. (1996) |
| Nickel | Guo *et al*. (1996), Jamal *et al*. (2002) |
| Uranium | Rufyikiri *et al*. (2002) |
| Arsenic | Orlowska *et al*.( 2012) |

### 2.2. Non-nutritional Benefits of AMF

Currently, AM fungi are acknowledged as biofertilizer (Lovato *et al.,* 1996), biological control of root pathogens, bioremediation, enhancing plant growth, salt tolerance and they also improve the productivity of medicinal compounds. A literature search reveals a few reports on the AM fungi association with micropropagated medicinal plants (Gaur and Adholeya, 1999; Joshee *et al.,* 2007) and 100% survival rate and better growth than non-mycorrhizal plant with the inoculation of *G. mosseae* (Yadav *et al.,* 2012).

Arbuscular mycorrhizae provide many non-nutritional benefits to crops, such as alleviation of water stress (Allen and Boosalis, 1983; Safir *et al*., 1971), environmental stresses on plant, improves plant tolerance to drought and polluted environments (Auge´, 2001), and accelerates plant establishment. At present, AMF are considered as an important component in the restoration and reestablishment of the vegetation in fragile or degraded ecosystems, and in the maintenance of plant biodiversity and ecosystem functioning (van der Heijden *et al*., 1998). Berta *et al*. (1990) and Ruiz-Lozano and Azcon (1995) reported that AMF enhances the water uptake of plants. According to Fitter (1988) and Davies *et al*. (2002), the influence of AMF on water uptake and transport may be a secondary consequence of enhanced host phosphorus nutrition, although these effects are not consistent. Some other workers observed that AM colonisation could enhance relative water content in zucchini leaves (Colla *et al*., 2008) and the water potential of maize plants (Feng *et al*., 2000).

The degree of AMF response increases with increasing intensity of drought stress (Sylvia *et al*., 1993). Thus, improvement of drought resistance under suboptimal plant growth conditions (Morgan *et al*., 1994) and under drought conditions, mycorrhizal colonisation promotes water relations of the host plants through stimulated plant nutrition (an indirect effect) and possibly through enhanced direct water uptake (Allen, 1982; Faber *et al*., 1991). Nelsen (1987) reported that drought tolerance of mycorrhizal onion plants was mainly due to improved P nutrition, which contributed to the healthy state of the host plant. Hardie and Leyton (1981) stressed that drought may be relieved by an increased rate of root growth and more efficient extraction of water from the soil because of increased P uptake. Greater P uptake promotes root growth, which in turn enhances the hydraulic conductivity and transpiration rate in AM soybean plants (Bethlenfalvay *et al*., 1988). Allen (1982) suggested that AM hyphae absorb and translocate water directly to their hosts, thus acting as a bridge between the dry zone around the roots and adjacent moist regions. Kothari *et al*. (1990) showed that rates of water uptake per unit root length and per unit time by AM inoculated maize plants were about twice that of non-AM plants and attributed this to hyphal transport. However, Graham *et al*. (1987) showed that improvement of water relations of AM citrus plants under drought conditions was due to the greater C cost and reduced hydraulic conductivity of mycorrhizal plants.

AM symbioses may also improve plant health through

increasing the protection against biotic and abiotic stresses (Barea and Jeffries, 1995; Bethlenfalvay and Linderman, 1992). Many researchers such as Roncadori and Hussey (1977) suggested that AM fungi could give protection against some soil-borne pathogens. It has been suggested that AM fungi increase host tolerance of pathogen attack by compensating for the loss of root biomass or function caused by pathogens, including nematodes (Pinochet *et al*., 1996) and fungi (Cordier *et al*., 1997). The impact of AMF on the reduction of soil-borne diseases has mainly been evaluated in studies on soil fungal pathogens, such as *Phytophthora*, *Aphanomyces*, *Fusarium* and *Verticillium* (Azcon-Aguilar and Barea, 1996), and nematodes causing, respectively, root rots and lesions and galls (Guillemin *et al*., 1993; Pinochet *et al*., 1996). Some studies have shown that mycorrhizal protection could also occur against *Erwinia carotovora* and *Pseudomonas syringae* (Garcia-Garrido and Ocampo, 1989). The life cycle of AMF depends on plant roots, and in return, they decrease disease in the latter and reduce population levels of pathogenic microorganisms in the soil, especially where the supply of P is limiting.

It is evident that an increased capacity for nutrient acquisition resulting from mycorrhiza association could help the resulting stronger plants to resist stress. Ruiz-lozano *et al*. (1996) in their study observed the influence of AMF on salt stress also. AM colonisation also improves plant growth by alleviating some stress conditions, including soil acidity (Habte, 1999). The external mycorrhizal mycelium, in association with other soil organisms, forms water-stable aggregates necessary for good soil quality (Bethlenfalvay *et al*., 1998). In this way, AMF helps in the improvement of soil structure also (Hamel *et al*., 1997; Schreiner and Bethlenfalvay, 1995).

### 2.3. AMF in Plant Growth Enhancement

AM fungi are known to affect growth of most plant species through various ways. According to Chang (1994) and Nemec (1987), AMF benefits to plant growth appear to be highest when colonisation occurs during early stages of plant growth. Moreover, AM colonisation may induce formation of lateral roots or increase root branching (Citernesi *et al*., 1998) further increasing the volume of soil explored. AM having a great influence on overall plant physiology contributes to improved plant health and growth, particularly under suboptimal conditions (Peuss, 1958; Hirell and Gerdeman 1980; Sharma *et al*., 1992). Clapperton and Reid (1992) while researching on the relationship between plant growth and increasing arbuscular mycorrhizal inoculum density reported that as the colonisation by arbuscular mycorrhizal fungi increased, so did root to shoot ratios. They concluded that this was due to the arbuscular mycorrhizal plants being able to translocate more carbon to the roots than non-mycorrhizal plants. The same has been reported by Kucey and Paul (1982); Douds *et al*. (1988) and Wang *et al*. (1989). Gerns *et al*. (2001) in their study observed the increased C assimilation and export from leaves when plants were inoculated with arbuscular mycorrhize. It has previously been established that *P. longum* growth is greatly enhanced by different AMF species such as *Glomus fasciculatum*, *G. versiforme*, *G. clarum*, *Glomus* sp. 2, *G. mosseae* and *G. etunicatum* (Gogoi and Singh, 2011). Higher height increment registered with inoculated plants could be because of enhanced inorganic nutrient absorption and greater rate of photosynthesis (Allen *et al*., 1981; Cooper, 1984). Chulan and Martin (1992) also reported a significant shoot dry weight increment when *Theobroma cocao* was inoculated with AM fungi. Aggangan and Dela cruz (1991) reported a dry matter yield increment of up to 631% when *L. leucocephala* was inoculated with AM. Similarly, Zajicek *et al*. (1987) reported a significant increment in dry matter yield when two forbs were inoculated with arbuscular mycorrhizal fungi. Further, Smith *et al*. (1979) observed that the extent to which typical arbuscular mycorrhizal fungi colonise root systems varies with the plant species. The association of AM fungi with plant roots alters plant soil interactions and enhances plant growth and nutrition under stressful edaphic conditions (Smith and Read, 1997).

AMF are also known to enhance plant growth through growth hormone production. According to Davies (1995), plant hormones are signal molecules, which regulate many developmental processes in plants and are therefore suitable candidates to function in the colonisation process (Barker and Tagu, 2000; Ludwig-Muller, 2000). The production of growth hormones such

as auxins, gibberellin-like substances and cytokinins by AMF has been well demonstrated by several workers (Allen *et al.,* 1980; Barea and Azcon-Aguilar, 1982), and due to production of these growth hormones, the rooting of plant increases. Kaldorf and Ludwig-Muller (2000) in their study on AM-inoculated maize roots showed an early increase in free indole-3-butyric acid (IBA) as well as an increase in IBA synthesis. This coincided with a significant increase in the percentage of lateral fine roots 10 days after inoculation.

### 2.4. AMF Interaction with Other Microorganisms

AM fungi also show positive synergistic interactions with other soi1 microorganisms. Where P is scarce, it has been found that plants inoculated with AM fungi either alone or in combination with phosphate-solubilising microorganisms (PSMs) have increased the P uptake (Raja *et al*., 2002). Once the arbuscular symbiosis has developed, AM hyphae influence the surrounding soil, which has been termed the mycorrhizosphe, resulting in the development of distinct microbial communities relative to the rhizosphere and bulk soil (Andrade *et al*., 1997). Within the mycorrhizosphere, AMF interact with beneficial rhizosphere microorganisms including free-living $N_2$-fixing bacteria and general plant growth-promoting rhizobacteria (PGPR; Biro *et al*., 2000). In a study, conducted on green gram (*Vigna radiate)*, Zaidi *et al*. (2004) observed that the dual inoculation of a symbiotic $N_2$ fixer *Azotobacter chroococcum* and AM fungus *Glomus fasciculatum* resulted in enhanced root infection, which stimulated plant growth, and increased N and P uptake. During interaction, the PSMs increase the availability of P and efficiency of $N_2$ fixation by phosphate-solubilising activity and releasing the plant growth-promoting substances (Kucey *et al*., 1989). The interaction of AMF with PGPR may be antagonistic as well as synergistic (Biro *et al*., 2000), but there seems to be a high degree of specificity between the plant, AMF and PGPR species involved in these interactions (Requena *et al*., 1997). Galleguillos *et al*. (2000) have observed the large increases in yield over uninoculated controls when AMF were inoculated with some PGPR. The legume-rhizobium symbiosis is strongly influenced by AMF and there are some evidences to suggest that legume nodules contain AMF communities quite distinct from those found in the roots of legumes (Scheublin *et al*., 2004). This Rhizobium symbiosis is dependant on high concentrations of P and so the enhanced P nutrition arising from the AM colonisation can result in an increase in nodulation and $N_2$ fixation (Vazquez *et al*., 2002). It was also observed that bacterial communities and specific bacterial strains promote germination of AM fungal spores and can increase the rate and extent of root colonisation by AM (Johansson *et al*., 2004). Recently, a study was conducted on AMF alteration of microbial mediation of litter decomposition. Plant nitrogen uptake was significantly increased if AMF accessed the litter, and (15) N analysis of the plant material indicated that 2-3% of plant nitrogen originated from litter (Herman *et al*., 2012). Hence, AMF is considered as an important component that can play a role in promoting sustainable agriculture (Hamel, 1996; Miller and Jastrow, 1992) and eco-sustainability (Allen, 1991).

## 3. ACKNOWLEDGEMENT

The authors are thankful to the Department of Biotechnology, New Delhi for providing fund for this study and also the Department of Botany, Rajiv Gandhi University for providing the essential library facilities to carry out the literature survey.

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