MATERIALS AND METHODS

The Test Crops – The test crops selected for the experiment work are tomato and brinjal. These occupy an important place among vegetables in India. Vegetables are essential for a balanced diet and maintenance of good health. Vegetables have a key role in neutralizing the acid produced during digestion of proteinous and fatty food. They provide valuable roughage which promote digestion and help to prevent constipation and are the major source of dietary fiber, minerals and vitamins. In addition, they contribute proteins, fats and carbohydrate to the human nutrition and are rich in calcium, phosphorus, iron and magnesium. These are essential requirements for the body. Though vitamins occur in small quantities in vegetables, but they produce profound and specific physiological effects (Umrao et al., 2008).

The current status of vegetable production in India is approx. 94.34 million tonnes (13.5% of world production) with an area of 6.25 million hectares, being second in the world after China. But this production is capable to supply only 175 g vegetables per capita per day after deduction the quantity exported (0.5 million tonnes) and post harvest losses of about 30%, which is much lower than what is recommended by the dieticians (300 g per capita per day). Vegetables constitute hardly 8-10% of total food intake, which is distressingly low as compared to other countries like Japan with 45%. Hence, it is necessary to increase the production and productivity of vegetable to meet the demand of growing population to ensure better nutrition by adopting improved technology (Singh et al., 1998)
Solanaceae is a family of flowering plants that contains a number of important agricultural crops as well as many toxic plants. The Solanaceae family is characteristically ethnobotanical, i.e. extensively utilized by human. It is an important source of food, spice and medicine. Plants are herbs, shrubs, trees or sometimes vines. Many species of Solanaceae are used as food, the two most species of the family for the global diet are the tomato, *Solanum lycopersicum* L. (syn), *(Lycopersicon esculentum*, *Mill)* widely grown for its savory fruit and the brinjal, *(Solanum melongena* L.) an important source of phytonutrients.

**TEST CROP- TOMATO**

*(Regional names: tomate, tomate extranjero, tomate de arbol, tomate granadilla, granadilla, pix, and caxlan pix (Guatemala); In 1970, the construed name "tamarillo" was adopted in New Zealand and has become the standard commercial designation for the fruit). (Solanum lycopersicum, syn. & *Lycopersicon esculentum*).*

**SCIENTIFIC CLASSIFICATION**

- **Kingdom**: Plantae
- **Subkingdom**: Tracheobionta
- **Superdivision**: Spermatophyta
- **Division**: Magnoliophyta
- **Class**: Magnoliopsida
- **Subclass**: Asteridae
- **Order**: Solanales
- **Family**: Solanaceae
- **Genus**: Solanum
- **Species**: *Solanum lycopersicum* L.
The tomato ‘‘Queen of vegetable’’ is native of Peru Eucader area. The word tomato not used until 1695, is said to be derived from ‘‘Aztec Xitomate’’. It was eaten for a longtime in Southern Europe before it was used by people of North Europe and the United States. The plant was first domesticated in India from the last century. It has now become a popular vegetable and is cultivated extensively, in the vicinity of large town and cities (Pandey, 2000).

Tomato is a warm season crop, not only sensitive to frost but does not thrive at low, non-freezing temperature, high temperature, by low humidity and dry winds, which damage its floral parts and there is no fruit set. The tomato withstands drought fairly well but fruits are subject to blossom end rot and to growth cracks if moistures supply falls drought. It cannot grow in regions of higher rainfall. The tomato grows on all soils from light sandy to heavy clay. The tomato crops responds very well to manurial and fertilizer application. A crop of tomato producing about 37 tones/ ha of ripe tomatoes removes 80 kg of nitrogen, 20 kg of phosphorus and 130 kg of potassium (Nath et al., 1987). Some popular varieties of tomato are as fallows- Pusa Ruby, Pusa Early Dwarf, Sioux, Marglobe, Roma and Punjab Chuhara (Pandey, 2000).

HISTORY AND DISTRIBUTION

The Tomato History has origins traced back to the early Aztects around 700 A.D; therefore it is believed that the tomato is native to the Americans. It was not until around the 16th century that Europeans were introduced to this fruit when the early explorers set sail to discover new lands. The tomato is native to South America. Genetic evidence shows the
progenitors of tomatoes were herbaceous green plants with small green fruit and a center of diversity in the highlands of Peru. One species, *S. lycopersicum* was transported to Mexico, where it was grown and consumed by the Mesoamerican civilizations. French botanist Tournefort provided the Latin botanical name *L. esculentum* to the tomato. It translates to ‘wolfpeach’-peach because it was round and luscious and wolf because it was considered poisonous. The English word tomato comes from the Spanish word *tomatl* first appearing in 1595, literally ‘the swelling fruit’. Most likely the first variety to reach Europe was yellow in color, since in Spain and Italy they were known as *pomi d’ oro* meaning yellow apple. Italy was the first to embrace and cultivate the tomato outside South America. Research on processing tomatoes is also conducted by the California Tomato Research Institute in Escalon California. In California, growers have used a method of cultivation called dry-farming, especially with Early Girl tomatoes. The high acidic content of the tomato makes it a prime candidate for canning. Soon after this discovery, it gradually became very popular all over the world. It is now grown in Malaysia, Indonesia, Philippines, Central, East and West Africa, tropical America, the Caribbean and throughout the tropics. It holds second place among the vegetable produced in the world, only exceeded by the potato. Today, the United States, Russia, Italy, Spain, China, and Turkey are among the top selling commercial producers of tomatoes.

**CULTIVATION**

The tomato is now grown worldwide for its edible fruits with thousands of cultivars having been selected with varying fruit types, and for optimum growth in differing growing conditions. Cultivated tomatoes vary in size, from tom berries, about 5 mm in diameter,
through cherry tomatoes, about the same 1-2 cm (0.4-0.8 in) size as the wild tomato, up to beefsteak tomatoes 10 cm (4 in) or more in diameter. The mostly widely grown

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commercial tomatoes tend to be in the 5-6 (2.0-2.4 in) diameter range. Most cultivars produce red fruit, but a number of cultivars with yellow, orange, pink, purple, green, black, or white fruit are also available. Tomatoes grown for canning and sauces are often elongated, 7-9 cm long and 4-5 cm diameter; they are known as plum tomatoes, and have a lower water content. About 130 million tons of tomatoes were produced in the world in 2008. China, the largest producer, accounted for about one quarter of the global output, followed by United States and Turkey. For one variety, plum or processing tomatoes, California accounts for 90% of U.S. production and 35% of world production.

CLIMATIC AND SOIL REQUIREMENTS

The tomato is a warm season crop. The crop does well under an average monthly temperature of 21° C to 23° C. Temperature and light intensity affect the fruit set, pigmentation and nutritive value of the fruit. It does not sensitive to frost only, but also does not thrive at low, non-freezing temperature, high temperature, by low humidity, and dry winds damage floral parts, and there is no fruit set. Tomato pollen grains germinate best at 29.4° C as well as at 21° C, poorly at 10° C and very poorly at 37.3° C. The tomato withstands drought fairly well but fruits are subject to blossom end rot and to growth cracks if moistures supply follows drought. It cannot grow in regions of higher rainfall, it requires low to medium rainfall. Avoid water stress and long dry period as it causes cracking of fruits. Bright sunshine at the time of fruit set helps to develop dark red colored fruits (Nath et al., 1987).
**MATERIALS AND METHODS**

**TEMPERATURE REQUIREMENT**

<table>
<thead>
<tr>
<th>Sr. no</th>
<th>Stages</th>
<th>Temperature (°C)</th>
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<tr>
<td></td>
<td></td>
<td>min</td>
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<td>max</td>
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</tr>
<tr>
<td>1.</td>
<td>Seed germination</td>
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<td>16-29</td>
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<td>2.</td>
<td>Seedling growth</td>
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<td>21-24</td>
<td>32</td>
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<td>3.</td>
<td>Fruit-set(day)</td>
<td>10</td>
<td>15-17</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(night)</td>
<td>18</td>
<td>20-24</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>Red-colour</td>
<td>10</td>
<td>20-24</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td></td>
<td>development</td>
<td></td>
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</tr>
</tbody>
</table>

The tomato grows on all soils from light sandy to heavy clay. Light soils are good for an early crop, while clay loam and slit loam soils are suited for heavy yield. If the soil is acidic, liming soils is advocated, tomato do best in a soil that has a soil reaction from pH 6 to 7. Soil depth 15 to 20 cm proves to be good for healthy crop (Nath et al., 1987). Deep tillage can allow for adequate root penetration in heavy clay type soils, which allows for production in these soil types. The soils with proper water holding capacity, aeration, free from salts are selected for cultivation. The addition of organic matter to mineral soil will increase yield.

**PLANTING REQUIREMENTS**
In the northern plains, where frost occurs during winter, usually two crops taken, whereas in frost-free areas where winter is not severe, 3 crops in a year are possible. In regions where frost occurs, first transplanting done in February, and second crop transplanting done in July (rain has set). In the Southern plains, (no frost) first transplantation done in December-January while the second transplantation in June-July and third in September-October (depending upon irrigation facilities). Good tomato seeds remain viable for 4 years, germination is 85-90%, seed quantity of 430-550 g is sufficient to plant one hectare of land. The seeds are sown in nursery bag, when the plants are about 4-5 week old and 12-15 cm in height; they are transplanted to rows at 60-75 cm apart. Initial trial indicated that planting in double-rows systems (30cm×30cm×1cm) on raised beds provide higher yield of healthy fruits besides facilitating in intercultural operations. The transplanting is done in small flat beds in light soils where irrigation is available and on shoulders in shallow furrows where irrigation water is scanty. In heavy soils, it is usually transplanted on ridges (Nath et al., 1987).

MANURIALS AND FERTILIZER REQUIREMENTS

The tomato crop responds very well to manurai and fertilizer application. As the fruit production and quality depends upon nutrient availability and fertilizer application so balance fertilizer are applied as per requirements. The nitrogen in adequate quantity increases fruit quality, fruit size, colour and taste. It also helps in increasing desirable acidic flavor. A crop of tomato producing about 37 tones/ha of ripe tomatoes removes 80 kg of nitrogen, 20 kg of phosphorus and 230 kg of potassium. Mono Ammonium Phosphate may be used as a starter fertilizer to supply adequate phosphorus during
germination and seedling stages. The fertilizers are placed in bands 7.5-10 cm deep on both
sides rows before making furrows. The nitrogenous fertilizer is applied in 2 equal split
doses, first-given before transplanting; second dose is applied around each plant about 45
days after transplanting. Calcium availability is also very important to control soil pH and
nutrient

**MATERIALS AND METHODS**

 availability. Sandy soils will require a higher rate of fertilizer, and more frequent
applications of these fertilizers due to increased leaching of essential nutrients. Normally
tomato crop requires 120 kg Nitrogen (N), 50 kg Phosphorus (P\textsubscript{2}O\textsubscript{5}) and 50 kg Potash (K\textsubscript{2}O).

Nitrogen should be given in split doses. Six sprays of 1% urea at weekly intervals, starting
at the seedling stage, produced significantly increases yield of tomato. Soil and tissue
analyses should be taken throughout the growing and production season to insure essential
nutrients are in their proper amounts and ratios. In the present situation it has been realized
that the use of inorganic fertilizers should be integrated with renewable and environmental
friendly organic fertilizers, crop residues and green manures (Nath et al., 1987).

**INTERCULTURAL OPERATIONS**

The irrigation arranged such that soil remains moist. Tomatoes need very careful irrigation
that is sufficient water at the right time. It is necessary to maintain even moisture content.
During summer season, irrigation at every 3-4 days interval is necessary, in winter 10-15
days interval is sufficient. A period of drought followed by sudden heavy watering during
the fruiting period may cause cracking of fruits. Use a cultivars to create a hole 3-4 inches
in depth, space out holes about 18 inches apart to allow the plants to grow and expand.
Cover the tomato with soil up to the top of roots.
Inter-tillage and hoeing done at regular intervals so as to keep the field free of weeds and to facilitate soil aeration and proper root development, Treflon, at the rate of 0.25 kg/ha was effective in controlling the weeds of tomato. What you will find is the tomato plants do well even if you forget about them. Allow tomatoes to ripen on the vine for the best results. You should get multiple harvests from your plants throughout the summer months. Planting tomatoes is a great way to have a healthy vegetable on your table all summer long.

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Mulching with straw, black polythene and many other materials has been found beneficial in moisture conservation, in controlling weeds and some diseases. As grow, the intercultural operation should be shallow so that damage to roots, present 5 cm below the soil, avoided. Staking plants has proved to be beneficial. In rainy and winter season, diseases of fruit and foliage reduced (Nath et al., 1987).

**HARVESTING**

The harvesting of tomatoes depends on different stages-

**Mature green stage:** Fruits are bulk packed in ventilated containers for shipment. Those picked to be shipped are picked at the mature green stage and sprayed with ethylene 48 hours prior to shipping. Tomatoes harvested at the mature green stage will ripen into a product indiscernible from vine-ripened fruit.

**Pink color fruits:** For local sale of tomatoes may be vine ripened to a firm ripe or a full red color before harvesting.

**Ripened tomatoes:** should be harvested at the breaker stage to ensure the best quality.

**Fully ripe:** Processing tomatoes are picked fully ripe.

Harvesting every day may be desirable during the peak of the season. Remove discarded
tomatoes from the field to avoid the spread and buildup of diseases and insect pests (Nath et al., 1987).

**NUTRITIONAL VALUE**

Earlier tomato is considered as poisonous and may contain acids. It always blamed to be cancer culprit. It is now consider as to have unsurpassable nutritional and health giving qualities. Tomato is a rich source of minerals, vitamins and organic acid, essential amino acids and dietary fibers. It is a rich source of vitamin A, B, B$_2$ and C; it also contains

**MATERIALS AND METHODS**

minerals like iron, phosphorus, calcium and carbohydrates. Tomato contains lycopene and Beta-carotene pigments, one of the most powerful natural antioxidants. Tomato contains lycopene and Beta-carotene pigments, one of the most powerful natural antioxidants. It consists of 93 g moisture 23 kcal energy, 1.9 g Protein, 0.1 g Fat, 3.6 g Carbohydrates, 0.7 g Fibre, 0.7 mg Thiamine, 0.01 mg Riboflavin, 0.4 mg Niacin, 31 mg Ascorbic acid, 15 mg Magnesium, 2 mg Oxalic acid, 36 mg Phosphorus, 45.8 mg Sodium, 114 mg Potassium, 0.19 mg Copper, 24 mg Sulphur, 38 mg Chlorine, 20 mg Calcium and 1.8 mg Iron (Pandey, 2000).

**MEDICINAL VALUE**

As tomato is rich in lycopene, it has been shown to protect against oxidative damage in many epidemiological and experimental studies. Tomato consumption has been associated with decreased risk of breast cancer, (Zhang et al., 2009) head and neck cancers and might be strongly protective against neurodegenerative diseases (Suganuma et al., 2002). Tomato contains lycopene and Beta-carotene pigments, one of the most powerful natural antioxidants. Tomatoes and tomato sauces and puree are said to help lower urinary tract symptoms and may have anticancer properties (Polvika et al., 2010). It also lowers high
blood pressure. As tomato contains more amounts of vitamin-C and therefore it is mostly used for children for relieve from scurvy. Tomato helps in blood formation as well as remove toxic elements from the body by taking raw tomato juice and tomato salad. Because of its low carbohydrates contents, it is very good food for diabetic patients and for those who want to reduce their body weight. It is said to be very effective in controlling the percentage of sugar in urine of diabetic patients. Being a rich source of vitamin A, tomatoes are a dependable preventive against night blindness, short sightedness and other diseases of the eye caused by the deficiency of the vitamins.

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Tomato leaves are useful in optic nerve and eye weakness. Tomatoes are highly beneficial in the treatments of obesity. One or two ripe tomatoes taken early morning, without breakfast, for a couple of months are considered a safe method of reduction in weight and at the same time, it also supplies the essential food elements to preserve the health.

**USES**

Tomatoes are now eaten freely throughout the world, and their consumption is believed to benefit the heart among other organs. Fresh ripe fruits are refreshing and appetizing and are consumed raw in salads or after cooking. Unripe fruits are cooked and eaten. Tomatoes are also consumed in the form of juices, paste, ketchup, sauces, soups, and powder.

**Tomato juice:** Ripe tomatoes with bright red color and high acidity are used for the expression of juice. Tomato juice is highly esteemed as an appetizing and nourishing beverage. It is sometimes seasoned to produce cocktail known as Tomato juice Cocktail.

**Tomato ketchup:** It is prepared from ripe tomatoes of deep color by cooking the pulp in kettle with spices. Cooking is continued till the desired consistency is obtained. Vinegar, salt, sugar, and sometimes pectin are added.
**Tomato soup:** For preparing tomato soup, the pulp is partly neutralizing by adding sodium bicarbonate solution and then concentrated in a pan; spices, and butter or cream is added. When the desired consistency is obtained, salt, and sugar are added the mass boiled for another two minutes (Pandey, 2000).

**TEST CROP- BRINJAL**


**MATERIALS AND METHODS**

**SCIENTIFIC CLASSIFICATION**

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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<tr>
<td>Family</td>
<td>Solanaceae</td>
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<tr>
<td>Genus</td>
<td>Solanum</td>
</tr>
<tr>
<td>Species</td>
<td><em>Solanum melongena</em> L.</td>
</tr>
</tbody>
</table>

*S.melongena* is a plant of the family of *Solanaceae* and genus *Solanum*, native of Southern India and Sri Lanka. It is an herbaceous prickly or sometimes unarmed perennial 0.6-2.4 m tall, cultivated throughout India as an annual for its edible fruit. Leaves are ovate, sinuate or lobed with 10-20 cm long and 5-10 cm board, the leaves sometimes spiny. Semi wild types can grow much larger, to 225 cm with large leaves over 30 cm long and 15 cm broad.
The flowers are white to purple, with a five-lobed corolla and yellow stamens. The stem is often spiny. The fruit is fleshy, has a meaty texture, and is less than 3 cm in diameter on wild plants, but much larger in cultivated forms, with thick calyx; seeds many discoid. The raw fruit has a somewhat disagreeable taste, but when cooked, becomes tender and develops a rich, complex flavor and firm texture. It is difficult to fix the ancestry of cultivated eggplants, but hybrids-vigour and continuous selection must have played an important role in the evolution and development of the various cultivated types which bear large edible fruits and are adapted to a wide range of climatic conditions.

MATERIALS AND METHODS

HISTORY AND DISTRIBUTION

The genus *Solanum* is predominantly central and South American and most of the species originated there, but the brinjal plant is probably a native of South Asia. Some authors have suggested that it is a native of Africa and even Arabia. From the study of ancient records it appears that the plant is native to India and was first cultivated in this country; later its cultivation spread through Iran to Egypt and other North Africa countries and to Turkey and the Balkans. In China, its cultivation has been known for the last 1500 years. The name aubergine, is the French, a diminutive of auberge, variant of alberge ‘a kind of peach’ or from the Spanish alberchigo, alverchiga, ‘an apricocke’. In India, it is used in everyday cooking. It is used in a paste in India believed to heal wounds and cuts. People also use it as a meat substitute (*Nath et al., 1987*).

CULTIVATION AND SOIL REQUIREMENTS

Brinjal is a warm season crop and is very susceptible to frost, late round types being less susceptible to frost than the early long ones. It is grown almost throughout the year in the
plains but on the hills it is grown only during the summer, the crop extending up to September. Deep fine rich loam (pH ≠ 6) with proper drainage is most suitable. In clayey soils the plants remain stunted and bear small fruits. The growth is luxuriant in the soils rich in organic manure and the plants bear more fruits. The field for transplanting the seedlings should be well-manured with organic manure and ploughed 4-5 times before transplanting. A daily mean temperature of 200°C to 270°C is most favorable for its successful production. It is hardy plant and can be grown on different kinds of soil but does best on silt loams and clay loams. For an early crop sandy or sandy loam soil is preferred with pH ranging from 5.5 to 6.5.

MATERIALS AND METHODS

MANURIALS AND FERTILIZER REQUIREMENTS

A good crop of brinjals needs about 80-110 kg, each of nitrogen and phosphorus and 55 kg, of potash per hectare. At the time of field preparation about 40-50 tones of well-rotten farmyard manure or sludge may be added, together with 400 kg of superphosphate, 200 kg of ammonium sulphate and 100 kg of potassium sulphate or nitrate of potash. All fertilizers were applied as basal dressing except nitrogen, of which 50 per cent was applied as basal dose and the remaining after 45 days transplanting.

High yields of brinjal are under optimum moisture condition. Timely irrigation is necessary for good fruit set and its development. Irrigation may be done every 3-4 day during summer, and after 12-15 days during winter. Drip irrigation is beneficial to reduce water use and used controls 100-110 cm of irrigation are required for successful brinjal crop. Hoeing should be done to check the weeds, taking care not to damage the roots. The operation may be fairly deep in the young crop, but it should be shallow as the plants develop (Nath et al., 1987).
HARVESTING AND STORAGE

Fruits are harvested when they are still immature, possessing a bright glossy appearance, before the flesh becomes tough and the seeds begin to harden. They tend to shrink rather quickly; hence they are harvested in the afternoon and sprinkled with water till they are sent to marketing. The fruits are edible from the time they are quarter grown until they are near ripe. When full ripe they become greenish yellow.

Harvesting is done by cutting the fruit from the stem. They are served from the plant by shear or knives. The fleshy calyx and a short piece of the peduncle are left attached to the fruit. The yield generally varies from 9.5 to 13 tones/ha, depending on the type and sowing season. The size of the fruit may be increased to some extent by spraying the plants with growth regulators. The fruit may be stored for 1-2 days in summer and 3-4 days in winter, provided they are stored in shade and kept moist. Treating brinjals with wax emulsion containing suitable fungicides also extends the shelf life by 30-40 per cent. The treatments consist of dipping fruits for about a minute in freshly prepared 3 per cent aqueous fungicidal wax emulsion (Pandey, 2000).

MATERIALS AND METHODS

NUTRITIONAL VALUE

Eggplants are rich sources of dietary fiber, vitamins and minerals and contain very less calories. This makes it an ideal component of the low fat diets and the diets of those working on weight loss. Other essential minerals contained in eggplant include potassium, manganese and copper. Eggplants are very important sources of phytoneutrients, which is obtained from their deep purple colour, the important phytoneutrient like flavonoids, caffeic and chlorogenic acid. The flavonoid Nasunin in eggplant has high levels of antioxidant properties and is known to be a scavenger of free radicals, thus protecting the cells of the
body. Nasunin is also known for its ability to protect the fat surrounding the cell membrane of the brain. The chlorogenic acid it contains is known to be the potent antioxidant that displays antimicrobial, antiviral and antitumor abilities and plays an important role in prevention of many diseases. Analysis of the edible portion of fruit (all except stalk and calyx) gave the following values: moisture-92.7; protein-1.4; fat-0.3; minerals-0.3; fibre-1.3; and other carbohydrates-4.0 g/100 g. the minerals constituents presents are (mg./100 g. edible matter): Ca-18; Mg-16; P-47 (phytin P-3); Fe-0.9; Na-3; K-200; Cu-0.17; S-44; and Cl-52; small quantities of manganese (2.4 mg./100g.) and iodine (7µg./kg.) are reported to be present. The vitamins presents are: vitamin A; thiamine-0.04 mg; riboflavin-0.11 mg; nicotinic acid-0.9 mg. vitamin C-12 mg; and choline-52 mg/100g of edible matter.

**MATERIALS AND METHODS**

Although not rich source of vitamin B₂, brinjals contain a higher per cent of the vitamin than many other vegetables. Toasting is reported to raise the nicotine acid content at the expense of trigonelline which is present in it. The vitamin C content of the fruit varies according to variety. Leaves contain more vitamin C than in the fruits (Nath et al., 1987).

**MEDICINAL VALUE**

The value of brinjal is enhanced as a vegetable during autumn when approaching ripeness and are a fairly good source of calcium, phosphorus, iron and vitamin B. Brinjals are rich in iodine contents, they are given in liver complaint, they stimulate interhepatic metabolism of cholesterol. The roots are antiasthmatic and stimulant, juice employed for otitis, pounded and applied to ulcers in the nose. The leaves are sialagogue, and used in bronchitis asthma and dysuria. Seeds yield a fatty oil and used as a stimulant (Pandey, 2000).
is reported to stimulate the intrahepatic metabolism of cholesterol. Extract of the plant inhibit the growth of several types of bacteria. Both leaf and fruit, fresh or dry, produced a marked in blood cholesterol level. Dried fruit is reported to contain a goitrogenic principle (Nath et al., 1987).

USES

The fruits are given as vegetable. They are usually cut into slices and fried or boiled (Pandey, 2000). The fruit can also be stuffed with meat, rice, or other fillings and then baked. As a native of plant, it is widely used in India cuisine, for example in Sāmbhar, dalma, chutney, curries, and achaar. Roasted in hot ashes, mashed and seasoned with salt, onion, chilies, and lime juice or curds and mustard oil, they are made into bharta. Sliced fruits are sometimes dried in the sun and stored.

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TEST CHEMICAL: 28-HOMOBRASSINOLIDE

Brassinosteroids, (BRs) the first steroidal hormones with growth promoting nature was obtained from Brassica napus L. that had been collected by bees (Grove et al., 1979). BRs are a group of naturally occurring polyhydroxy steroidals. BRs are probably ubiquitous in the plant kingdom. They are potent natural plant growth regulators with pleiotrophic effects
as they influence varied development processes like growth, germination, rhizogenesis, flowering, and senescence. BRs also confer resistance to plants against various abiotic stresses (Prusakova, 1999). Natural BRs so far identified have a common 5α-cholestan skeleton, and their structural variations come from the kind and orientation of functionalities on the skeleton (Fujioka and Sakurai, 1997). The compound can be classified as C27, C28 or C29 BRs depending on the alkyl substitution pattern of the side chain (Yokota, 1997). A sequential numerical suffix designates the BRs occurring in nature. BR1 denotes HBL and others follow the sequence BR2, BR3, BR4,........BRn. BRs are present in plants at extremely low concentrations. Young growing tissues contain higher levels of BRs than mature tissues (Yokota and Takahashi, 1986). The richest source are pollen and immature seeds where its concentration ranges between 1-100 ng per g fresh mass (Takatsuto, 1994). Till now 42 BRs and four BR conjugates have been characterized (Fujioka, 1999). However, all the BRs are not always biologically active (Sakurai and Fujioka, 1993). Only brassinolide (BL), 24-Epibrassinolide (EBL), 28-Homobrassinolide (HBL) are the three biologically active BRs, being widely used in physiological studies.

**MATERIALS AND METHODS**

g fresh mass, whereas shoots and leaves have about 0.01-0.1 ng per g fresh mass (Takatsuto, 1994). Till now 42 BRs and four BR conjugates have been characterized (Fujioka, 1999). However, all the BRs are not always biologically active (Sakurai and Fujioka, 1993). Only brassinolide (BL), 24-Epibrassinolide (EBL), 28-Homobrassinolide (HBL) are the three biologically active BRs, being widely used in physiological studies.

**HISTORY**

The recognition that pollen extracts causes growth promotion paved the way for the discovery of BRs in plants. In 1970, Mitchell and co-workers screened pollen from nearly sixty species, and half of them caused growth of bean seedlings. The growth promoting substances from various pollen sources were named as ‘brassins’’. The search for its active factors (s) was collectively approached in 1974 by USDA scientists working at Northern Regional Research Centre (NRRC), Peoria; Eastern Regional Research Centre (ERRC),
Philadelphia and Bettsville Agricultural Research Centre (BARRC), Maryland. Bee-collected pollens (500 lb) were processed through a pilot plant-size solvents (2-propanol) extraction procedure at ERRC and succeeded in partial purification at BAARC. However, it was crystallization at NRRC and was subjected to X-ray analysis to establish its structure. This biologically active plant growth promoter was found to be steroidal lactone (C_{28}H_{48}O_{6}), MW= 480 and was named as "brassinolide". As the first steroidal hormone with growth promoting nature was obtained from *B.napus*, the name 'brassinosteroids' was given to this new class of substance.

**CHEMICAL STRUCTURE**

Steroids play a role as essential hormones in plants as well as in animals. Plants produce numerous steroids and sterols, some of which are recognized as hormones in animals

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(Geuns, 1978; Jones and Roddick, 1988; Bishop and Koncz, 2002). Brassinolide (BL) is the most bioactive form of the growth-promoting plant steroids, termed brassinosteroids (BRs). Grove *et al.* (1979) purified 4 mg of BL from 40 kg of bee-collected rape pollen to determine its structure, which shows similarity to animal steroid hormones (Figure 1).
Figure-1: The structure of brassinolide, a commonly occurring BR with high biological activity, showing numbered positions mentioned in the text. In natural BRs, hydroxylation can occur in ring A at positions 3-, and/or 2-, and/or 1-; also found are epoxidation at 2,3-, or a 3-oxo-group. In ring B, alternatives are 6-oxo- and 6-deoxo- forms. In the side chain methyl-, ethyl-, methylene-, ethylidene-, or nor- alkyl groups can occur at 24-, and the 25-methyl- series is also represented.

Structural features of these analogues look complicated, but they classified into two categories. The first category is grouped by the oxidation stages of the steroidal skeleton: 7-oxalactone, 6-ketone and 6-deoxo derivatives; pentahydroxyls at C₁, C₂, C₃, C₂₂ and C₂₃; tetra hydroxyl at C₂, C₃, C₂₂ and C₂₃ trihydroxyl at C₃, C₂₂ and C₂₃. The second category is grouped by the side chain skeleton: C₂₄ s-methyl, C₂₄ R-methyl, C₂₄-normethyl, C₂₄ methylene, C₂₄ s-ethyl, C₂₄-ethylidene and C₂₄ methylene, C₂₅ methyl. From the

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distribution of BRs, only brassinolide and castasterone are the most widely distributed biologically active BRs in the plant kingdom. Generally, 7-oxalactone and 6-ketone forms are the most active, while 6-deoxo derivatives are almost inactive. The BL differ from the variations at C-2 and C-3 in A-ring, the presence of a lactone, ketone or de-oxo functional group at C-6 in the B-ring, the stereochemistry of the hydroxyl groups in the side chain, and the presence or absence of a methyl (methylene) or ethyl (ethylene) group at C-24.

NATURAL OCCURRENCE AND DISTRIBUTION

The first BR, brassinolide (22R, 23R, 24S)-2a, 3a 22, 23-tetrahydroxy-24-methyl-B-homo-7-oxa-5α-cholestan-6-one, was isolated from pollen of B.napus (Grove et.al., 1979). Extensive investigations on the distribution of BRs-like active substances in plants led to them being found not only in pollen but also in insect galls, immature seeds, shoots and
leaves of a wide variety of plants. BRs have been found in all cases in which a rigorous examination of a plant species by GC-MS or LC has been undertaken; including at least 37 angiosperms, 1 alga, and pteridophyta (Fujioka, 1999). All are hydroxylated derivatives of cholestan, and given the possibilities of combination of sub structure in rings A and B and the side chain, the family probably has many more members. Structural features of these analogues look complicated but they may be classified into two categories. The first category is grouped by the oxidation stages of the steroidal skeleton: 7-oxalactone, 6-ketone and 6-dexo-derivatives; penta-hydroxys at C1, C2, C3, C22 and C23, tetrahydroxys at C2, C3, C22 and C23, tri-hydroxys at C3, C22 and C23. The second category is grouped by the side chain skeleton: C24S-methyl, CC24R-methyl C24-normethyl, C24-methylene, C24S-ethyl, C24-ethylidene and C25-methyl (Sakurai and Fujioka, 1993). Park et al. (1989) identified BL and castasterone (Abe et al., 1983) from the crown gall cells of Catharanthus roseus, are the most widely distributed biologically active BRs. The results of isolation and identification studies from plants conducted to date have shown the presence of BRs in almost every tissues or organ, such as pollen, seeds, flowers, fruits, shoots and leaves, in variable levels, though their presence in roots has not yet been confirmed. Among them reproductive organs (pollen and immature seeds) showed higher levels than vegetative tissues (Sakurai and Fujioka, 1993). So BRs are probably ubiquitous in the plant kingdom, and they certainly occur in shoots and seeds of the important experimental plant Arabidopsis thaliana (Fujioka et al., 1996; Schmidt et al., 1997).

ANALYSIS OF BRASSINOSTEROIDS
Micro analytical techniques by GC-MC or GC-SIM have been established for BRs (Takatsuto et al., 1982). The usefulness of the HPLC technique is described for the identification of BRs in buckwheat pollen (Takatsuto et al., 1990). Detection, identification and quantification of BRs in plant tissues or organs are quite important for investigating their occurrence, biosynthesis and metabolism with the help of GS-MS, GC-SIM, HPLC and Radio immuno-assay (Takatsuto, 1991). The extraction and purification of BRs depend on solvent partitioning and subsequent chromatographic separations. The choice and sequence of these may differ, with later fractionation often guided by bioassay. The most frequently used is the rice lamina inclination test (Wada et al., 1981, 1984). Analysis of purified fractions is mostly done by GC-MS and GC-MS-SIM (Takatsuto, 1994; Adam et al., 1996). FAB-MS is successful with pure BRs (Caballero et al., 1996), but not with partially purified extracts. LC-MS methods are also being explored (Gamoh and Takatsuto, 1994; Gamoh et al., 1996 a, b) and were found useful for the detection of epimerization (Nishikawa et al., 1995) and identification of teasterone esters (Asakawa et al., 1996). Assay by liquid chromatography, with UV, fluorescence, or electrochemical detection of derivatives, is also a sensitive method (Gamoh and Takatsuto, 1994). Fluorescence detection at long wavelengths is particularly useful for checking fractions from small samples of plant tissue. However, derivatization can be difficult in very dilute solutions, and conditions must be adjusted when very small-scale extractions and fractionations are attempted. For accurate quantification and calculation of losses, internal standards include appropriate d-labeled BRs in MS and selected “spikes” in LC analysis.

Identification of the conformations of BRs may be assisted by crystallographic (Kutschabsky et al., 1990) and NMR studies (Adam et al., 1996) and such knowledge will
be useful in receptor studies, and in understanding structure/activity relationships. These relationships have been examined by several groups, and while some differences were noted between bioassays, the 7-oxalactone and 6-keto forms were generally the most active, with distinct effects of the hydroxylation patterns in ring A and the side chain and of the alkylation pattern of the side chain (Mandava, 1988; Wang and Zhao, 1989; Yokota and Mori, 1992; Roddick, 1994; Wang et al., 1994; Zurek et al., 1994). A computer analysis of interatomic distances in energy-minimized structures of various BR structures showed that the distances between C16 on the ring and the C22, C23, C24, and C28 carbons as well as the O22 and O23 oxygen, were critical for optimal activity, suggesting that the overall dimensions of the side chain may be as important as the configuration at the individual chiral carbons (Zurek et al., 1994).

A quantitative structure/activity study has begun (Brosa, 1997), and the finding of high activity with inversion of the 2- and 3-hydroxyl groups together with a cis A/B ring

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junction is interesting, this is the arrangement in ecdysones. However, 20-hydroxyecdysone, alone or together with brassinolide, does not affect the rice lamina-bending assay (Sasse, 1999) confirming early work with ecdysones in other assays (West, 1980; Clouse et al., 1992) and suggesting the lamina inclination assay can discriminate between patterns of substitution in the side chain, even if conformations in the ring A region permit activity.

**ENDOGENOUS LEVELS**

BRs occur endogenously at quite low levels. Pollen, the original source of HBL, Young growing tissues contain higher levels of BRs than mature tissues (Yokota and Takahashi, 1986) and immature seeds are the richest source with ranges of 1-100 ng g⁻¹fw, while
shoots and leaves usually have lower amounts of 0.01-0.1 ng g⁻¹ fw (Takatsuto, 1994). Cultured crown gall cells of Catharanthus roseus have levels of BL and castasterone (30 ng·g⁻¹·fw) equivalent to that of pollen. However, all the BRs are not always biologically active, brassinolide, 24-epibrassinolide and 28-homobrassinolide are the three biologically active brassinosteroids, being widely used. Park et al. (1989) identified brassinolide and castasterone from the crown gall cells of C. roseus, their contents being at level comparable to that in pollen or immature seeds. BRs were found physiologically active in very low concentrations. Brassinolide is active at 0.01 µg in bean second-internode test (Grove et al., 1979) and at 0.0005µg-concentration in rice lamina inclination assay (Wada et al., 1981).

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BIOSYNTHESIS AND METABOLISM

Early and Late C6 Oxidation Pathways

A detailed understanding of how endogenous BR levels are regulated via synthesis, breakdown and conjugation is an essential component of a molecular model of BR action. A coordinated effort by several Japanese groups has led to rapid progress in the elucidation of the biosynthetic pathways leading to BRs in plant cell cultures and seedlings (Fujioka and Sakurai, 1997 a, b; Yokota, 1997). Campesterol was predicted to be the plant sterol progenitor of brassinolide based on side chain structure and the relative biological activities, co-occurrence, and molecular structure of teasterone, typhasterol, and
castasterone suggested that brassinolide was synthesized from campesterol through these intermediates (Yokota et al., 1991).

The reduction of campesterol to campestanol and the oxidation of campestanol to 6-oxocampestanol (Figure-2) has been demonstrated by feeding experiments (Fujioka and Sakurai, 1997 a) and the hydroxylation of 6-oxocampestanol to cathasterone is presumed, but direct demonstration of this step by feeding experiments has not been accomplished, possibly because the endogenous pool of cathasterone is 500-fold less than that of 6-oxocampestanol (Fujioka et al., 1995). The large difference in pool sizes suggests that this conversion may be the rate-limiting step in brassinolide biosynthesis. Conversion of cathasterone to brassinolide via the intermediates (shown in Figure-3) has been demonstrated by feeding experiments (Fujioka and Sakurai, 1997 a).

In the final step, some differences were seen in seedlings; castasterone was converted to brassinolide in C. roseus, but not in tobacco and rice. However, since brassinolide and castasterone co-occur as endogenous BRs in rice seedlings, it is likely the full pathway is operational in this plant as well and that the exogenous labeled castasterone used in the feeding experiments may not have reached the site of brassinolide synthesis in the rice seedlings (Suzuki et al., 1995).

The conversion of teasterone to brassinolide did occur in lily cells (Asakawa et al., 1996), and the co-occurrence of teasterone, typhasterol, castasterone, and brassinolide in at least four other species (Phaseolus vulgaris, Lilium elegans, Citrus unshiu, and Thea sinensis) suggests that the complete pathway is widespread in plants (Suzuki et al., 1995).
Figure-2: Biosynthesis of early members of the BR biosynthetic pathway via mevalonate and the isoprenoid pathway. Campesterol is a bulk sterol, also found in membranes, while campestanol and later derivatives are considered committed to BR biosynthesis. Proposed blocks in BR biosynthesis in Arabidopsis (\textit{d}wf\textsubscript{1}, \textit{dim}1, \textit{cbb}1, \textit{lkb}; \textit{det}2, \textit{d}wf\textsubscript{6}) and pea (\textit{lkb}, \textit{lk}) mutants are indicated. The structure in brackets is a probable intermediate based on molecular genetic and biochemical studies (Redrawn from: \textit{Li et al.}, 1997).

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C6-oxidation is a very early step in this pathway (Figure-3). However, appreciable quantities of 6-deoxocastasterone have frequently been identified in extracts of BRs, and when other 6-deoxo-congeners were discovered, these compounds were also proposed as precursors to BRs (\textit{Griffiths et al.}, 1995; \textit{Sakurai and Fujioka}, 1996), and this has now been confirmed (\textit{Choi et al.}, 1996, 1997).
Figure-3: Biosynthesis of brassinolide via the early C6 oxidation pathway (left) and the late C6 oxidation pathway (right). Both pathways can occur together in the same plant. Steps marked by * have not been confirmed by feeding experiments. Putative assignments of biosynthetic and insensitive mutants in Arabidopsis (dWF4; cpd, cBB3, dWF3; bRI1, cBB2, dWF2), pea (lka), and tomato (dpy, cu-3) are indicated (Redrawn from: Fujioka and Sakurai, 1997 a).

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Thus, “early C6-oxidation” and “late C6-oxidation” pathways for the biosynthesis of BRs coexist in cultured cells of *C. roseus*, and in seedlings of tobacco and rice. Representatives from both pathways co-occur in many plants, e.g. *Arabidopsis* ([Fujioka et al., 1996](#)), so both could be widespread in the plant kingdom. The multiplicity of substitution patterns in the 2- and 3-positions of BRs, and in the alkyl substituent of the side chain, implies even more pathways and interconnections, and these remain to be elucidated, as does the detailed enzymology. Determining the specificity of the enzymes involved will assist our understanding, and the contribution of BR biosynthetic mutants has already been significant.

**TRANSPORT**

The possible transport of endogenous BRs has been studied by monitoring the movement of radiolabelled BRs after application to various parts of the plant ([Arteca, 1995; Adam and Schneider, 1999; Sasse, 1999, 2003; Bishop and Yokota, 2001; Ross et al., 2006](#)). However, it is interesting to note that there is some evidence for root-to-shoot transfer of exogenous BRs as in case of rice ([Yokota et al., 1992](#)). Similarly, in both cucumber and wheat seedlings, there appeared to be transferred to the shoot after applied to the roots ([Nishikawa et al., 1994](#)). Again, there is an evidence shows that there is an exogenous transfer of BRs from root-to-shoot in *Arabidopsis mutants* which can be restored to normal growth ([Choe et al., 1998](#)).

Although the available evidence rules out long-distance BRs transport within the plant, it is possible, indeed likely that endogenous BRs do move over short distances both within and between cells ([Symons et al., 2008](#)).
Additional evidence for short-distance cell-to-cell BRs transport comes from the BR-deficient d⁵ mutant of tomato (Bishop et al., 1996). BRs must be transported, either actively or passively, from their intracellular sites (s) of synthesis to the extracellular sites of perception (figure-4). Interestingly, Yamamoto et al. (2001) detected BRs in the medium in which Zinnia elegans callus tissue was cultured, consistent with BR movement out of these cells.

As a consequence, it is likely that short distance BR transport involves carrier mechanism. For instance BRs transport through the cystol could well be facilitated by binding of BRs conjugates, such as those formed by the binding of BRs to fatty acids or glucose (Fujioka and Yokota, 2003), may be candidates for transport (Sasse, 2003; Choe, 2006). Alternatively, BR transport could also be facilitated by the binding of these hormones to specific proteinaceous transporters (Sasse, 2003).
Figure-4: A proposed model of short-distance BR transport within and between neighbouring cells. The model outlines the movement of BRs from their intracellular sites of synthesis, across the plasma membrane, to the extracellular space where they can bind to BR receptors that are located on the exterior surface of the same, or neighbouring cells (Redrawn from: Symons et al., 2008).

SOURCES OF MATERIALS AND SCREENING

Certified tomato and brinjal seeds variety were purchased from the local market of Ballia (U.P) and test chemical 28-homobrassinolide (double) was collected from Godrej Agrovet Ltd., Vikroli, Mumbai. Healthy seeds of uniform size were selected by a primary method of floating them in water and viable seeds, which settled down in water, were taken for study. The selected doses of BRs (0.1, 1, 3, 5, 7 and 10 ppm) were applied as
foliar spray on the basis of preliminary screening experiment. These selected concentrations were prepared by adding glass-distilled water (GDW).

**Preparation of Stock Solution:** The Stock Solution was prepared by measuring 0.3 ml of given chemical with the help of pipette and then dissolved it in 30 ml of glass-distilled water (GDW) and stored in the dark for use.

**EXPERIMENT I: Screening test**

**Materials Required:** Plastic bags, Pipette, 28-homobrassinolide (double), \( \text{HgCl}_2 \), Distilled water.

**Procedure**

The screening experiment had been carried out to check the seed germination under the effect of the different concentrations of given chemical. Screening test was done in the laboratory in the month of October, 2008 in triplicate. Healthy seeds of uniform size were selected by a primary method of floating them in water and viable seeds, which settled down in water, were taken for study. Before sowing, the seeds were surface sterilized with 0.01% \( \text{HgCl}_2 \), then seeds were taken in the plastic glass containing sand and observed for 15 days (at every alternate days *i.e.*, 3 day, 5 day, 7 day, 9 day, 11 day and 13 day).

**OBSERVATIONS**

Following Germination and Seedling Growth parameters were determined according to following formulae at final days of observation:

(A) **Germinability (\( %G \))** = \( \frac{\text{Total No. of Seeds Germinated} \times 100}{\text{Total No. of Seeds Sown}} \)

(B) **Standard Germination Test:** It was calculated as Germination Rate Index and Speed of Germination (*Maguire, 1962*)-

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(i) **Germination Rate Index (GRI)** = No. of normal seedlings of days x/ Days X

\[ X = \text{No. of days from seed soaking} \]

(ii) **Speed of Germination (SpG)** = n/t

Where, n = No. of seeds emerged on the day

\[ t = \text{time or days from soaking.} \]

(C) **Coefficient of Velocity of Germination (CVG)-Kotowski (1926)**

\[ \text{CVG} = \frac{\text{Sum of } n \times 100}{\text{Sum of } (nt)} \]

Where, \( n \) = No. of seeds emerged on the day

\[ t = \text{time or days of soaking} \]

(D) **Emergence Index (EI)** was calculated by the formula of by Baskin (1969).

\[ \text{EI} = \frac{(n_1/d_{n1})+(n_2/d_{n2})+(n_3/d_{n3}) \ldots \ldots \ldots (n_x/d_{nx})}{n} \]

Where, \( n \) = No. of seeds emerged on the day 1\(^{st}\), 2\(^{nd}\), 3\(^{rd}\), ….. \( n^{th} \) day.

\[ d_{n} = \text{No. of days from the day of sowing.} \]

\[ d_{nx} = \text{No. of days to the final count.} \]

(E) **Relative Seed Germination (RSG)**

\[ \text{RSG} = \frac{\text{No. of seeds germinated in the extract } \times 100}{\text{No. of seeds germinated in the control}} \]

(F) **Relative Root Elongation (RRE)**

\[ \text{RRE} = \frac{\text{Mean root elongation in the extract } \times 100}{\text{Mean root elongation in the control}} \]

(G) **Growth Index (GI)** = \% seed germination \times \% root elongation/100 (Tam and Tiquia, 1994)

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(H) **Seedling growth (SG)** = Total seedling Growth length in cm

\[ \text{No. of germinated seeds} \]

(I) **Vigour Index (VI)** = Germination % x Seedling length (Abdul-Baki and Anderson, 1973).

**CULTURAL PRACTICES**

The first set of experiment of brinjal was conducted in 2009; subsequent second and third set of experiment in 2010 and 2011 (mid of March-April) respectively, and the first set of experiment of tomato had been done in 2008, second and third in 2009 and 2010 (mid of September-October) were performed to repeat the experiment work. The data produced is the average of 9 replicate and three seasons. The set of experiment were conducted at Botanical Garden of the College, Ballia (25” N 45 latitude and 84” E 10 longitude, sea level 69.9 meter), India. The different concentrations of BRs used were 0.1, 1, 3, 5, 7, 10 ppm against control. The Stock Solution of BRs was prepared by measuring 5 ml of given chemical with the help of pipette and then dissolved it in 500 ml of glass distilled water. The soil testing has done by Soil Testing Department under Agricultural Department, Ballia, (U.P). For doing experiment, firstly normal size of fire clay pots were used, then pots were filled with equal weight (3 kg) of sandy loam soil taken from agriculture field of college campus S.M.M Town PG College, Ballia, (U.P). The soil has pH 6.8 and soil testing suggested that soil contain 0.56% organic carbon, 18 kg/hectare phosphate and 210 kg/hectare potash. For each treatment, triplicate pots were maintained and three plantlets were planted in each pot. The soil of each pot was thoroughly watered. The different amount of treatments was given as foliar spray at three stages of developments *i.e.* Pre flowering (20 DAP-50 ml), flowering (40 DAP-100 ml) and Post flowering (60 DAP-150 ml). The growth of test crops were recorded at 25 DAP, 45 DAP and 65 DAP.

All the parameters studied on growth, productivity were root length; shoot length; fresh and
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dry weights of root, shoot and leaves; number of leaves; leaf area; number of fruits; weight of fruits; shelf life period of fruits; length and girth of fruit; stomatal studies and biochemical parameters were Chlorophyll content in leaves; Carotenoids content in leaves; Protein content in fruits; Ascorbic acid content in fruit; and Total soluble sugar content in fruit.

Parameters studied

A-Growth

To see the effect of HBL on growth, the following parameters were recorded at 25, 45 and 65 DAP-

(a) Shoot length- The shoot length (cm) was measured by meter scale.
(b) Root length- The root length (cm) was measured by meter scale. For this, the plants of each concentration were uprooted at three stages of growth, and root length was measured.
(c) Number of Laterals per plant- The Number of Laterals was recorded by direct counting method.
(d) Leaf Growth- The growth of leaves was recorded in terms of number of leaves per plant by direct counting and Leaf area per plant in cm² by graph paper method. For measurement of leaf area, the 3rd, 5th and 7th leaves, from the base were taken and then placed on a graph paper, of which abaxial surface is coloured with ink, so as to make full impression of the leaf on the graph sheet. By counting the number of squares within the coloured-outline and multiplied with 1 cm², the area of single leaf was obtained, which was multiplied with the total number of leaves and leaf area per plant was obtained.
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(e) Fresh and Dry weights of plant- The fresh and dry weights (g) were recorded by an electronic weighing machine. For dry weight, the plant samples were wrapped in blotting paper and then kept in electric oven, first at 110°C for 10 minutes to stop metabolic functions and then at 85°C for 48 hrs.

(f) Biomass- Total biomass (mg/m²) was calculated, by using the following formula:

\[
\text{Total biomass} = \frac{\text{Dry weight of plant}}{\text{Area of sampling}} \times 100
\]

For this, plants of each concentration were uprooted from the soil and washed with DW to remove the soil particles. The fresh weight of the samples was taken with the help of electronic balance. After this, the plant samples were wrapped in blotting paper and then kept in electric oven at 85°C for 48 hours. For dry matter yield after 48 hours of incubation, the dry weight of the samples was measured.

B. Stomatal Studies

The stomatal studies performed by the Fevicol method (Nayeem and Dalvi, 1989). In this method, Fevicol (Pidilite industries Pvt. Ltd, Mumbai) is commonly used synthetic adhesive. It is a simple quick method, by which a sample can be studied in only 2-3 minutes. The method is described as follows:

a. Fevicol mixtures are gently applied on the leaf surface over an area of about 1.5-2.0 cm² and is allowed to dry for 1-2 min.

b. The dry fevicol is removed carefully with the pin or needle, till a fine film is separated from the leaf.

c. The fine film is removed and placed on a slide immediately.
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d. The Fevicol film, which has an impression on leaf surface, is cut into pieces, properly arranged and pressed on the slide using another plane slide.

e. The slide is ready for immediate microscopic observation and can also be stored for longer time.

f. The observations for stomatal frequency were made using Ocular and Stage micrometers and Camera (Sony Cyber Shot, 12.1 Mega Pixels) was used for taking photographs to study the anatomical variations in stomata.

To observe the variations in stomata the following parameters were recorded on both the surface of leaves.

The stomatal frequency and index were calculated by using formula of Salisbury (1927)

1. Stomatal frequency=(Number of Stomata/Unit Area) x 100

2. Stomatal Index=(No. of Stomata/No. of epidermal cells + No. of Stomata) x 100

C. Productivity/Yield

For the study of productivity responses under HBL, the following parameters were recorded at two stages of growth, flowering (45 DAP) and post-flowering (65 DAP) stages:

1- Number of flowers/ plant

2- Days of Initiation of flowers

3- Number of Fruits/ plant

4- Weight of 100 Fruits (g)
5- Yield / plant (g)

6- Total fruits yield (q/ha)

All the above parameters were recorded in twelve replicates in three seasons of the experimental plants in each concentration, as well as in untreated control.

D. Biochemical Aspects

I. Chlorophyll content

The amount of chlorophyll estimated by the method of Arnon (1949), as follows-

(1) Preparation of Extract: For this 250 mg of fresh leaves were cut into small pieces and grounded with neutral sand and 10 ml of 80% acetone in a clean mortar. The content was centrifuged at 3000 rpm for 10 minutes. The procedure repeated with the residue for complete extraction. The volume of the pooled supernatant was raised to 25 ml with 80% acetone.

(2) Determination: The optical density of the supernatant of chlorophyll extract was taken with Spectrophotometer (Systronics-106) at 645 and 663 nm using 80% acetone as blank. The amount of total chlorophyll present in the extract was calculated in terms of mg of chlorophyll/g of leaf tissue, through following equation-

\[
\text{Total chlorophyll} = 20.2 \times \frac{\text{OD} 645}{} + 8.02 \times \frac{\text{OD} 663}{\text{V}} \times \frac{\text{a} \times 1000 \times w}{\text{V}}
\]

\[
\text{Chlorophyll ‘a’} = 12.7 \times \frac{\text{OD} 663}{\text{V}} - 2.69 \times \frac{\text{OD} 645}{\text{a} \times 1000 \times w}
\]

\[
\text{Chlorophyll ‘b’} = 22.9 \times \frac{\text{OD} 645}{\text{V}} - 4.68 \times \frac{\text{OD} 663}{\text{a} \times 1000 \times w}
\]
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Where, OD: is the optical density of supernatant of the extract at the wavelength specified,

\[ V: \text{the final volume of extract}, \quad w: \text{the fresh weight of leaves in mg.} \]

‘a’: the length of path light in the cell (1 cm.).

II. Carotenoids Content

For carotenoids estimation 1 g, fresh weight of leaves tissue was taken and crushed in a pestle and mortar with 20 ml of 80% Acetone (v/v) and a pinch of Na$_2$CO$_3$. The extract was centrifuged at 2500 rpm, for 10 minutes and the clear supernatant were made up to 10 ml with 80% Acetone. The absorbance of the extract was read at 440 nm. Total carotenoids content was estimated following the method given by Jensen and Jensen (1972).

Total carotenoids (mg/g fresh tissue) = DVF \times \frac{10}{2400}

\[ C = \text{Total Carotenoids}; \quad D = \text{OD of the extract at 440 nm} \]

\[ V = \text{Total volume in ml}; \quad F = \text{Dilution factor, if any} \]

III. Protein Contents in Fruits

Quantitative estimation of Protein was done following the method of Lowry et al. (1951). In which the 100 mg of fresh fruits was homogenized and weighted (1 g) material was extracted in 10 ml of 80% acetone and centrifuged at 4000 rpm for 10 min. Supernatant was discarded and precipitate was dissolved in 5 ml of 0.1N NaOH solution. It was again centrifuged at 1000 rpm for 15 min and supernatant was separated. To this 5 ml alkaline CuSO$_4$ solution and 0.5 ml of Folin-phenol reagent (freshly prepared) was added. After mixing all these, it was shaken immediately and stands for 30 min at room temperature, blue colour developed, then OD was read at 530 nm against blank. The protein content in
different samples were calculated by referring the reading to standard curve and expressed in mg/100 mg fresh or dry weight of materials.

**IV. Total Soluble Sugar Content in Fruits**

Sugar was extracted by the quantitative analysis of samples containing mixture of carbohydrate, particularly the sugar was done by Anthrone test, following the method of Morris (1948) and modified by Highkin and Frankel (1962) in fruits, which in a general test for carbohydrate. In this, the produced react with Anthrone reagent to give bluish green coloured complex. 100 mg of fresh and weighted material (fruits) was extracted in 10 ml of 80% ethanol. The material was then centrifuged at 6000 rpm for 10 min. Supernatant was separated and the procedure repeated twice with residue for complete extraction. All supernatant was pooled and the alcohol was removed by the evaporation in water bath at 80°C. The residue was made to a known volume (2 ml) with Glass Distilled Water (GDW). Suitable aliquots of extract (0.1 ml) was taken in triplicate and again made to known volume 1 ml GDW. To this extract, 4 ml of Anthrone reagent was added in ice bath, and then the solution was heated for 10 min., in water bath. The solution was cooled at room temperature and then the OD was read at 620 nm. The amount of TSS was calculated by referring the readings to standard curve and expressed in mg/100 mg fresh weight of fruits. Standard curve is prepared by using glucose.

**V. ASCORBIC ACID**

The ascorbic acid content in the fruit was measured by the method of (Dhopte and Manuel, 1989).

**Preparation of Extracting solution:** Metaphosphoric acid-acetic acid (HPO3-HOAC) was prepared by weighing 15 g of HPO3 sticks and dissolving in 40 ml of HOAC and 200 ml of
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distilled water. The resulting solution was diluted to 500 ml with DW, filtered and stored in the dark for use.

Preparation of Indophenols solution: 50 mg of 2,6-dichlorophenol powder was dissolved in 50 ml of DW containing 42 mg of NaHCO3. The resulting solutions were then diluted to 200 ml with DW, filtered and stored in the dark for use.

Preparation of standard ascorbic acid solution: 1 mg/ml of saturated ascorbic acid solution was prepared by dissolving 50 mg of ascorbic acid in 40 ml of HPO3-HOAC solution and made up to 50 ml in a volumetric flask.

Standradization of indophenols solution with standard ascorbic acid solution: Three 2 ml aliquots ascorbic acid solution was titrated against indophenols from the burette until a distant rose color persisted for about 5 seconds. Blank titrations were also carried out using 7 ml of HPO3-HOAC solution against indophenols.

Extraction of ascorbic acid from the samples/fruits: 100 mg of fruit sample was weight and transferred into conical flasks. 50 ml of the extracting solutions was added to each sample and trititated to form a suspensions and this allowed to stands for 30 minutes. The volume obtained was noted and designated as V ml. Warm extracting solutions (500° C) was used for samples A as it froths in cold extracting solutions. Sample aliquots (7 ml each) were obtained by the filtering the suspension. The filtrates obtained from sample were decolorized with Active Charcoal.

Titration of extracted solutions against indophenols: 7 ml of sample aliquots were measured into conical flasks and titrated against indophenols from the burette until a distinct pink to rose colour persisted for 5 seconds. The titration was repeated three times for each sample aliquot and average titre values obtained.
**MATERIALS AND METHODS**

**Calculations:** The concentration of ascorbic acid in each sample (Z mg/g of sample) was calculated using following formula:

\[ Z \text{ mg/g} = (X - B) \times \frac{V}{Y} \] (AOAC, 1980)

where, \(X\) = Average titre value obtained from sample titration.

\(B\) = Average titre value obtained from blank titration.

\(F\) = mg of ascorbic acid equivalent to 1 ml of indophenols solution.

\(E\) = Number of grams of fruit sample assayed

\(V\) = volume of initial assay solution.

\(Y\) = volume of sample aliquot titrated.

All the data collected on growth, productivity and biochemical analysis were subjected to statistical of variance using completely randomized design as described by (Panse and Sukhatme, 1985).