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REVIEW OF LITERATURE

This chapter comprises the general description about the medicinal plant fenugreek. A brief description of marine polysaccharides, which show plant growth promoting activity in their depolymerised form, is presented. Besides, the effect of irradiated polysaccharides, such as those of irradiated sodium alginate (ISA) and irradiated chitosan (IC) on various aspects of plant processes has been reviewed. In addition, a brief discussion on gibberellic acid (GA$_3$), kinetin (Kn) and phosphorus (P) is presented with regard to their effects on growth and production of active constituents in medicinal plants.

2.1. General description: A general account of *Trigonella foenum-graecum* L. is presented below.

2.1.1. *Trigonella foenum-graecum* L. (Fenugreek)

*Trigonella foenum-graecum* L. commonly known as Fenugreek, is an annual herb of family Fabaceae (Figure 2). It is cultivated in North Africa, Pakistan, China, India, Egypt and the Mediterranean countries (Duke 1986). The leafy shoots of fenugreek are used as a green vegetable throughout India. In India, it is comprehensively cultivated in the states of Madhya Pradesh, Uttar Pradesh, Rajasthan, Gujarat, Maharashtra and Punjab. Fenugreek is an annual dicotyledonous plant attaining a height of about 20-130 cm. The stem is smooth, green to purple and is often characterized by pinkish colour due to anthocyanin accumulation under field condition. It has a well developed taproot with extended fibrous roots. Leaves are light green, pinnate and arranged alternately with three ovate leaflets. Flowers are 1-2, axillary in position and may be white or yellow. The inflorescence is compound umbel, terminal. Pods are 3-15 cm long with persistent beak, with each pod having 10-20 seeds. Seeds are greenish brown in colour, 2.5-5×2-3.5 mm in size, oblong in shape and carry a deep groove, giving the seeds a hooked appearance. Fenugreek is propagated by seeds.
2.1.2. Systematic position:

**Kingdom**: Plantae

**Division**: Magnoliophyta

**Class**: Magnoliopsida

**Order**: Fabales

**Family**: Fabaceae

**Sub-family**: Papilionaceae

**Genus**: Trigonella

**Species**: foenum-graecum Linn.

2.1.3. Uses of fenugreek

Diabetes mellitus (DM) commonly referred to as diabetes is a metabolic disorder characterized by high level of blood sugar. Diabetes results either because of less insulin secretion (insulin dependent diabetes) or failure of body cells to respond to insulin (insulin independent diabetes). According to IDF (2013) there are 382 million people living with diabetes and by 2035 this will rise to 592 million. Although the drugs biguanides and sulphonylurea are valuable in the treatment of diabetes mellitus, their use is restricted by their limited action, pharmaco-kinetic properties, secondary
failure rates and accompanying side effects (Krentz and Bailey, 2005). Fenugreek is one such plant that has been extensively used in different systems of medicine as a source of antidiabetic compounds obtained from its seeds, leaves and extracts (Raju et al. 2001; Srinivasan 2006; Khalki et al. 2010).

Fenugreek has anti-diabetic, anti-parasitic, anti-fertility, anti-cancer, antimicrobial and hypocholesterolemic effects (Al-Habori and Raman, 2002), and particularly in India, it is used as a stimulant for the lactating mothers (Tiran 2003). The seed powder of fenugreek exhibits anti-diabetic properties (Broca et al. 2004; Preet et al. 2005; Modak et al. 2007; Yadav et al. 2010; Baquer et al. 2011; Ramadan et al. 2011), hypocholesterolemic effects (Venkatesan et al. 2003; Suboh et al. 2004), anti-cancer effect (Devasena and Menon, 2003; Shabbee et al. 2009). Fenugreek is known to possess antioxidant properties (Jung et al. 2006; Sinha et al. 2007; Nautiyal et al. 2008; Gupta and Prakash, 2009; Marathe et al. 2011; Xue et al. 2011). The ethanolic extract of fenugreek shows potent anti-bacterial activity (Premananath et al. 2011). The seed of fenugreek is a rich source of fibre which is mainly comprised of galactomannans (Madar and Stark, 2002), polyphenolic compounds (Saleh et al. 2010). The chemical constituents of fenugreek include flavonoids, saponins, polysaccharides, and alkaloids like trigonelline and choline (Yoshikawa 1997). Fenugreek is also used as a rotation crop; it increases nitrogen fixation in the soil and is used as a livestock feed (Sadeghzadeh-Ahari et al. 2009; Mehrfararin et al. 2011).

2.2. Marine polysaccharides


2.2.1. Sodium alginate

Alginate is a naturally occurring polysaccharide of guluronic acid (G) and mannuronic acid (M) residues, and is the major structural component of marine brown
algae (*Phaeophyceae*). It is also found as capsular polysaccharide in soil bacteria, such as some species of *Azotobacter* and *Pseudomonas*. The chemical compound, sodium alginate is the sodium salt of alginic acid with empirical formula NaC₆H₁₀O₆. The monomeric units of sodium alginate viz. β-D-mannuronic acid and α-L-guluronic acid are linked through α-1→4 bonds (Figure 3). It was confirmed by the process of the partial acid hydrolysis that the monomers of sodium alginate are arranged in three types of block structure and these may be homopolymeric block (M block, G block) or heteropolymeric block (MG block) (Haug et al. 1967). MG block is known for most flexible chain formation, while M block for its strong immuno-stimulating property. The gel forming property of the sodium alginate is because of the G block, which form stiff chains and are cross linked by divalent cations. The composition of the monomers and their sequence govern the functional properties of sodium alginate. Alginites have widespread application in the food and drink, pharmaceutical and bioengineering industries (Gacesa 1988). Alginate is used in many biomedical applications due to its biocompatibility, low toxicity, relatively low cost, and mild gelation by addition of divalent cations such as Ca²⁺ (Gombotz 1998). The main source of sodium alginate is marine brown algae (*Phaeophyceae*) such as *Laminaria hyperborea*, *Macrocystispyriforma*, *Laminariadigitata*, *Ascophyllumnodosum*, *Laminaria japonica*, *Edonia maxima*, *Lessonia nigrescens* and *Sargassum spp.* The colour of sodium alginate ranges from white to yellowish-brown, and is available in filamentous, granular and powdered forms. It has the property of absorbing water quickly and can absorb 200-300 times water than its weight. It is also produced by two bacterial genera *Pseudomonas* and *Azotobacter*, which played a major role in the unravelling of its biosynthesis pathway. Bacterial alginate is useful for the production of micro- or nano-structures suitable for medical applications.
Figure 3. Schematic diagram of β-D-mannuronic acid (M units) and α-L-guluronic acid (G units), and sodium alginate.

2.2.2. Chitosan

Chitosan is a copolymer of glucosamine and N-acetylglicosamine linked through α-(1→4) glycosidic linkages as shown in Figure 4 (Kumar 2000) and is the deacetylation product of chitin extracted from crustaceous shells, exoskeletons of insects and cell walls of fungi and shrimps (Shahidi et al. 1999). It is the second most abundant natural polymer on earth after cellulose. On an average, the molecular weight of commercially produced chitosan is between 3800 and 20,000 Daltons. A common method for the synthesis of chitosan is the deacetylation of chitin using sodium hydroxide (in excess) as a reagent and water as a solvent.

Chitosan and its derivatives have been widely used in medicine, biotechnological applications and in wastewater treatment (Kumar 2001). Chitosan has been used for agro product preservation, seed coating and as fertilizer (Tay et al. 1993). Chitosan induces the activity of enzyme chitinase and enhances seed germination and crop yield (Inui et al. 1991; Vasyokova et al. 2001; Tham et al. 2001) and reduces the transpiration of plants (Bittelli et al. 2001). It stimulates the plant growth and improves disease and insect resistance of plants (Doareset al. 1995). Both chitin and chitosan are known to induce defence responses in plants, which include lignification (Barber et al. 1989), ion flux variations, cytoplasmic acidification,
membrane depolarization, protein phosphorylation (Felix et al. 1998; Kikuyama et al. 1997; Kuchitsu et al. 1997) and activation of chitinase and glucanase enzymes (Roby et al. 1987; Kaku et al. 1997). Chitosan also induces phytoalexin biosynthesis (Ren and West, 1992; Yamada et al. 1993), generation of reactive oxygen species (Kuchitsu et al. 1995), biosynthesis of jasmonic acid (Nojiri et al. 1996), and the expression of unique early responsive and defence-related genes (Minami et al. 1996; Takai et al. 2001). Additionally, the Chitosan serves as a carbon source of soil microbes and accelerates the conversion of organic matter into inorganic matter thereby facilitates the root system of plants to absorb more nutrients from the soil. Roots are able to absorb the chitosan after being decomposed by the soil bacteria and chitin enzyme (chitinase) secreted by the roots (Somashkcar and Richard, 1996; Brian et al. 2004). Use of chitosan in agriculture reduces the dependence on chemical fertilizers and increases the crop production; in different kinds of plants by 15-20% (Hong et al. 1998). Ohta et al. (1999) is of the opinion that promotion of growth by chitosan might be the effect of presence of nitrogen in it because it contains about 8.7% N. However according to Suzuki and Shinshi (1998) the growth promotion by chitosan is because of its elicitor effect.

![Figure 4. Structure of chitosan](image)

2.3. Plant Growth Regulators (PGRs)

Plant growth regulators are the organic compounds, which influence the growth and development of plants at low concentration (Davies 1995). The naturally occurring growth substances are called as plant hormones, which are produced within the plant. Plant hormones are synthesized at specific site/s and then they are transported to other tissues, where in very low concentrations they evoke the specific biochemical,
physiological and/or morphological responses. The present review of literature confines to gibberellic acid and cytokinin (kinetin) only, used in the study.

2.3.1 Gibberellic Acid

Gibberellins are naturally occurring tetracyclic diterpenoid acids consisting of an ent-gibberellane skeleton (containing 20 carbon atoms) or a 20-nor-ent-gibberellane skeleton (containing only 19 carbon atoms because C-20 is missing). Gibberellins that have the full diterpenoid complement of 20 carbon atoms are referred to as C20-GAs (e.g. GA12). Presently, there are 136 fully characterized gibberellic acids (GAs), designated as gibberellin A1 (GA1) through gibberellin A136 (GA136) that have been identified and isolated from 128 different species of vascular plants, and also from seven species each of bacteria and fungi (MacMillan 2002). Gibberellic acid (Figure 5) regulates plant growth, affecting various growth and developmental processes, such as stem elongation, seed germination and dormancy, flowering, sex expression, induction of enzymes, senescence of leaves and fruits, etc.

![Gibberellic Acid Structure](image)

*Figure 5. Structure of Gibberellic acid*

2.3.2 Cytokinins

Cytokinins are a class of plant hormones, which promote cell division in plant cells, chloroplast maturation, senescence, cell growth, differentiation and other physiological processes. They co-ordinate certain metabolic activities, sink capacities and the mobilization of materials in leguminous and non-leguminous plants during development. The first discovered cytokinin was an adenine (aminopurine) derivative named as kinetin (6-furfuryl-aminopurine); it was isolated as a DNA degradation product. Kinetin was isolated from autoclaved herring sperm DNA in 1955.
Cytokinins are of two types: (1) adenine type, which includes kinetin, zeatin and 6-benzylaminopurine (BAP) and (2) phenylurea-type, like diphenylurea or thidiazuron. Kinetin (Figure 6) is soluble in strong aqueous acids, alkalis, and glacial acetic acid; it is slightly soluble in ethanol, acetone, butanol and ether and is partially insoluble in distilled water. It is an amphoteric compound with pKa values of 4 and 10. The first naturally occurring cytokinin was identified and purified from immature maize kernels and was named as Zeatin [6-(4-hydroxy-3-methylbut-2-enamino) purine]. Cytokinins are present in all plant tissues; they are abundant in the root tip, the shoot apex and immature seeds. The endogenous concentration of cytokinins is very low (in nM range). Cytokinins can act over long distances as well as in the direct vicinity of the cytokinin producing cells (paracrine signaling). They may also act on the cells that produce them (autocrine signaling). Naturally occurring cytokinins are derived from adenine and carry an isoprenoid or aromatic side chain on their N6 terminal. Among the two adenine derivatives of natural cytokinins, isoprenoidecytokinins are most abundant. They are either isopentenyl (iP)-type cytokinins, having an isopentenyl N6-side chain or Zeatin type cytokinins, having a hydroxylated isopentenyl N6-side chain. The side chain of a Zeatin-type cytokinin occurs in either cis or trans configuration, depending on which of the two methyl groups is hydroxylated. The trans form is usually more active than cis configuration. Aromatic cytokinins (e.g., benzyladenine) have greater stability and are often used in tissue culture studies. In addition, there are the structurally unrelated phenylurea-type cytokinins (e.g., diphenylurea, thidiazuron), a class of synthetic cytokinins. There are different forms of cytokinins in different plant species. In rice, the isoprenoidecytokinin (cis-zeatin) is the major form, whereas in Arabidopsis trans-zeatin and iP forms predominate. Structural variation in the isoprenoid and aromatic side chains of cytokinins affect the interaction of cytokinins with their receptors.

![Figure 6. Structure of Kinetin](image-url)
2.4 Response of plants to depolymerised polysaccharides:

Polysaccharides such as sodium alginate and chitosan are degraded into lower molecular weight oligomers by high energy gamma radiations. These oligomers when applied to plants as foliar sprays affect different biological and physiological activities in plants like shoot elongation, root growth, flower production, alleviation of heavy metal stress and seed germination; they enhance the essential oil content, stimulate the production of active constituents of medicinal plants and reduce the harvesting period of crops and use of chemical fertilizers and insecticides.

2.4.1 Effect of irradiated sodium alginate (ISA) on different aspects of plants.

Tomoda et al. (1994) investigated the effect of depolymerised sodium alginate on barley roots. They found that depolymerised alginate promoted the elongation of barley roots, in particular that of the radicle. They observed that the effective concentration for elongation of roots was 100-300 µg/mL, with no inhibition even at the highest concentration. They noted the root elongation rate from 2.9 to 5.3 mm/h and 2-3 fold increase in the alcohol dehydrogenase activity in treated plants under hypoxic conditions.

Naotsugu et al. (1998) measured the molecular weight of irradiated alginate by carrying out the UV analyses and ESR spectra. They found that through irradiation treatment, alginate degradation occurs mainly at glycosidic linkage.

Akimoto et al. (1999) investigated the effect of different concentrations of alginate oligomers (AO), chitosan oligomers (CO) and oligo-galacturonic acid (OGA) on the physiological activities, membrane permeability and protoplast formation of Catharanthus. They concluded that alginate acted as an endogenous elicitor and that the effects of alginate were similar to that of galacturonic acid because both the chemicals had structural similarity.

Iwasaki and Matsumura (2000) degraded sodium alginate by alginate lyase obtained from Corynebacterium spp. They obtained a mixture of di- to octasaccharides of sodium alginate which promoted the elongation in lettuce root when applied at the concentration range of 200-3000 µg/mL. In a lettuce bioassay study, when these degraded products of sodium alginate (tri-, tetra-, penta-, and hexasaccharides) were applied, they exhibited the growth promoting activity.
Hien et al. (2000) investigated the effect of the irradiated alginate products (with molecular weight less than $10^4$ Daltons) on the growth-promotion of rice and peanut. They degraded alginate by gamma-rays irradiation using Co-60 as source in liquid state (aqueous solution) and in solid state (powder form). Low concentration of degraded alginate (4% solution irradiated at 100 kGy) was effective for the growth-promotion of plants and the suitable concentration was 50 ppm for rice and 100 ppm for peanut. The dry matter was increased by 60% in peanut by the application of degraded alginate. To check the effect of foliar spraying of degraded alginate, field experiments were carried out on tea, carrot and cabbage. The productivity of these crops was increased by 15-40% with the foliar application of 20-100 ppm of the degraded alginate.

Kim et al. (2002) studied the process of upgrading and utilization of carbohydrates such as chitosan, alginate, carrageenan, cellulose and pectin, in order to recycle them and thereby, reduce the environmental pollution. They degraded these carbohydrates by irradiation; they observed that the degradation products (oligomers) were able to induce various kinds of biological activities in plants such as anti-microbial activity, promotion of plant growth, suppression of heavy metal stress, and induction of phytoalexins, etc.

Aoyagi et al. (2002) investigated the distribution of oligomers of alginites (AO) in cultured plant cells by using AO conjugated with monopotassium 7-aminoo-1, 3-naphthalenedisulfonate (AO-ANDS). When AO-ANDS was added to cell culture of catharanthus at the concentration of 0.5 g L$^{-1}$, it was traced not only in the cell walls but also in the cell membrane and cytoplasm as indicated by fluorescence microscopy study. They cultivated catharanthus in a medium containing oligo-galacturonic acid, which is an endogenous elicitor; this was also traced in the cell wall, cell membrane and cytoplasm of catharanthus cells. Similar results were also obtained in the case of Wasabia japonica cells.

Luan et al. (2003) degraded the alginate by irradiation at 10-200 kGy using 4% (w/v) aqueous solution of alginate; the degraded product was used as a growth promoter for plants in the tissue culture study. They reported that alginate irradiated at 75 kGy, with molecular weight of approx. $1.43 \times 10^4$ Da, had the highest positive effect on the growth of flower plants, namely, Limonium, Lisianthus and Chrysanthemum. Shoot multiplication rate of the tested plants increased from 17.5 to
40.5\% compared with control when plants were treated with the irradiated alginate at concentrations of 30-200 mg/L. Irradiated alginate supplemented at 100 mg/L enhanced the shoot height (9.7-23.2\%), root length (9.7-39.4\%) and fresh biomass (8.1-19.4\%) of the treated plants compared with the untreated ones.

Hu et al. (2004) investigated the influence of different concentrations of alginate-derived oligosaccharide (ADO) on maize seed germination. They reported the effect of ADO concentration on $\alpha$- and $\beta$-amylase activities in different stages of seed germination. The activities of both $\alpha$- and $\beta$-amylase reached maximum at 0.75\% ADO. Compared with the control, root growth on day 3 and 7 showed increases of 34 and 18\% respectively and shoot growth of 46\% on day 7. It was found that 0.75 and 1.50\% ADO increased the protease activity significantly compared to the control.

Hu et al. (2005) depolymerised the alginate by using the enzyme lyase (alginate lyase) and studied the antibacterial activity of depolymerised alginate against 19 bacterial strains. They obtained a series of mannuronic acid (M-block) and guluronic acid (G-block) fractions as a result of lyase depolymerisation; according to them, M-block and G-block fractions showed antibacterial activity against certain tested bacteria, whereas M-block fractions exhibited broader spectra of action and showed more potent inhibition of bacterial growth than shown by G-block fractions. They also reported that among all the fractions, the fraction having molecular weight equal to 4.235 kDa exhibited the broadest spectrum of inhibition and high inhibitory activity against *Escherichia coli*, *Staphylococcus aureus* and *Bacillus subtilis* bacteria.

**Alginate Lyase Specificity**

![Alginate Lyase Specificity](image)

*Figure 7. Specificity of alginate lyase on alginic acid*
El-Rehim (2006) observed the effect of polyacrylamide/sodium alginate (PAAm/Na-alginate) treated soil on the growth responses of faba bean (Vicia faba). The growth of the bean plant cultivated in a soil treated with PAAm/Na-alginate was better than that cultivated in soil treated with only PAAm. The sodium alginate copolymer partially underwent the radiolytic and enzymatic degradation to produce oligo-alginate, which acted as a plant growth promoter. It was argued that PAAm as a soil conditioner and degraded sodium alginate as growth elicitor could improve the agricultural performance of faba bean in the field, providing the plant with improved water status and vigorous growth, respectively.

Aoyagi et al. (2006) observed that in combination with Alteromonas macleodii (exogenous bacterial elicitor), 0.1% (w/v) alginate oligomers (AO: endogenous elicitor as well as scavenger of active oxygen species) minimized the cell growth inhibition but enhanced the production of PDase (5'-phosphodiesterase) (0.474 U/mL) about 20 times higher than the control (no addition). They found that the mixed alginate elicitors significantly promoted PDase production (2.67 U/mL) in catharanthus: as a result, the productivity was increased by 120-fold compared to the control, without inhibiting the growth of cells.

Mollah et al. (2009) reported the effect of sodium alginate irradiated by gamma-radiation (Co-60) at the dose of 12.5-50.0 kGy on the growth performance of red amaranth (Amaranthus cruentus L.). 150 ppm concentration of sodium alginate, irradiated at 37.5 kGy dose, proved the best that increased the plant dry matter by 50% than the control. There was significant effect of irradiated sodium alginate on plant height (17.8%), root length (12.7%), number of leaves (5.4%) and maximum leaf area (2.0%) compared to the control.

Idrees et al. (2011) irradiated sodium alginate at 520 Kilo Gray using Co-60 gamma rays as source and the degraded alginate products were sprayed on foliage of catharanthus to study their effectiveness as a plant growth promoter. As per their study, irradiated sodium alginate showed promotive effect on the growth and alkaloids production of catharanthus. They reported that irradiated sodium alginate at 80 ppm concentrations significantly enhances the growth, photosynthesis and other physiological activities in plants, including alkaloids production.
Sarfaraz et al. (2011) conducted a pot experiment on fennel to study the effect of gamma-rays degraded sodium alginate on the growth, biochemical and yield attributes. They observed that foliar spray of radiation- degraded alginate improved the growth characteristics (shoot and root length, number of leaves and fresh and dry weight), biochemical attributes (total chlorophyll content, carotenoids content and proline content) and yield parameters (umbels per plant, umbellets per umbel, 100-seed weight and seed yield). According to the results, 80 ppm concentration of ISA proved to be the best spray-treatment.

Khan et al. (2011) studied the effect of foliar application of irradiated sodium alginate on the overall performance of opium poppy. They used different concentrations of irradiated sodium alginate (ISA) and observed that treated plants with ISA-120 ppm concentration showed the highest response and gave 35.5, 48.7, 35.5, and 43.6% higher value of shoot length, root length, fresh weight and dry weight of plant, respectively. Due to 80 ppm of irradiated sodium alginate (ISA-80 ppm), there was observed significant improvement in the physiological parameters, viz. chlorophyll a content (20.5%), chlorophyll b content (33.9%), carotenoids content (29.4%), leaf nitrogen content (23.0%), nitrate reductase activity (28.7%) and carbonic anhydrase activity (28.2%) over the control. They performed HPLC analysis of crude opium alkaloids and found that codeine content was doubled while thebaine content and noscapine contents increased significantly in plants treated with 120 ppm of irradiated sodium alginate.

Afia et al. (2011a) investigated the effect of foliar application of irradiated sodium alginate (ISA) on artemisia. They used different concentrations of ISA ranging from 20-120 mg L⁻¹. Among different concentrations, ISA 80 mg L⁻¹ proved to be the best, which enhanced the growth attributes, photosynthetic capability, enzyme activities and artemisinin content of the plant significantly. It was concluded that ISA-enhanced H₂O₂ content in the leaves that resulted in the enhancement of leaf-artemisinin content.

Nacem et al. (2011) studied the effect of irradiated sodium alginate (ISA) on growth, physiological parameters, herbage yield, essential oil content and essential oil yield of Mentha arvensis L. Different concentrations of ISA used by them included 25, 50, 75, 100 and 125 mg L⁻¹, as foliar sprays. Among all the concentrations, ISA applied at 100 mg L⁻¹ proved to be the best at 100 and 120 days after planting.
Idrees et al. (2012a) studied the effect of irradiated sodium alginate (ISA) on seed germination of fennel. They used ISA in the range of 20-100 mg L\(^{-1}\). Among all the concentrations, 80 mg L\(^{-1}\) proved to be the best. 25 seeds of fennel were soaked for 15 h in 20, 40, 60, 80 and 100 mg L\(^{-1}\) of ISA. It was concluded that seed soaking with aqueous solution of ISA at 80 mg L\(^{-1}\) significantly improved various seed germination parameters like nitrate reductase activity, protease activity, germination, viability, relative water content of seed and α and β amylase activities in the germinated seedlings of fennel.

Idrees et al. (2012b) investigated the effect of four levels of irradiated sodium alginate (ISA) viz. 20, 40, 60, and 80 mg L\(^{-1}\) on growth and yield of essential oil and citral content of lemongrass. At both the stages of sampling (120 and 150 days after planting), ISA application significantly enhanced the essential oil production and the yield of citral. Among all the levels, ISA 60 mg L\(^{-1}\) proved the best for most of the parameters studied.

Aftab et al. (2013) studied the effect of irradiated sodium alginate (ISA) in combination with different grades of nitrogen on artemisia. As per results, ISA 80 (80 mg L\(^{-1}\)) together with (80 kg N ha\(^{-1}\)) enhanced the content and yield of artemisinin by 38.1 and 80.5%, respectively, over the control.

El-Sawy et al. (2013) studied the effect of oligo-chitosan (oligomers of chitosan) and oligo-alginate (oligomers of alginate) and their combination on the growth of maize plants. Foliar application of oligo-alginate and/or oligo-chitosan, obtained by irradiating the alginate and chitosan by gamma rays at 45 kGy, showed an increase in grain yield of 47 and 40%, respectively. The authors concluded that these oligosaccharides might act as plant growth promoters in the field of agriculture.

Aftab et al. (2014) investigated the effect of gamma-irradiated sodium alginate and various phosphorus doses on growth, physiological and biochemical characteristics and production of artemisinin in artemisia. ISA\(_{80}\) (80 mg L\(^{-1}\) of ISA) applied with P\(_{40}\) (40 kg P ha\(^{-1}\)) enhanced the growth and yield of artemisia and also the artemisinin yield of plants significantly.

Ali et al. (2014) investigated the effect of radiolytically depolymerized sodium alginate (ISA) on physiological activities, yield attributes and composition of essential oil of lemon scented gum. The treatments were applied as foliar spray of deionized
water only (control), seed soaked with ISA (90 mg L\(^{-1}\)) and foliar spray of ISA with 30, 60, 120 and 240 mg L\(^{-1}\). The application of ISA at 120 mg L\(^{-1}\) significantly enhanced the parameters studied. It also enhanced the essential oil content (33.3%), essential oil yield (86.7%), citronellal content (63.4%) and citronellal yield (205.5%) as compared to the control.

Naeem et al. (2014) conducted a pot experiment to investigate the effect of foliar doses of irradiated sodium alginate (ISA), triacontanol (TRIA) and 28-homobrassinolide (HBR) on growth, yield and quality of mint. Combined application of 100 ppm ISA + 10\(^{-6}\)M TRIA + 10\(^{-7}\)M HBR proved to be the best treatment that improved most of the plant growth attributes, physiological and biochemical parameters, herbage yield and the content and yield of active constituents of mint significantly.

Idrees et al. 2015 conducted two field experiments for two consecutive years on lemongrass to study the effect of five concentrations of ISA (0, 20, 40, 60, and 80 mg L\(^{-1}\)) in terms of plant growth and content and yield of essential oil and oil-citral. Among different ISA levels, ISA60 mg L\(^{-1}\) proved to be the best which enhanced the essential oil yield by 103.41 and 94.86% over the control at 120 and 150 days after planting (DAP), respectively. ISA-60 mg L\(^{-1}\) significantly improved the citral yield by 181.03% over the control at 120 DAP.

2.4.2 Effect of irradiated chitosan on different aspects of plants

Ohta et al. (1999) investigated the effect of chitosan treatment on the plant growth and flower quality of Eustoma grandiflorum (Raf.) Shinn. They found that application of 1% (w/w) chitosan, mixed with soil, remarkably enhanced the plant growth. The plants, grown in the soil treated with chitosan, flowered 15 days earlier than the control plants. Moreover, number as well as weight of cut flowers was greater than that of the control plants.

Tham et al. (2001) investigated whether the irradiated chitosan could reduce the adverse effects of vanadium toxicity in plants, viz. soybean, rice, wheat and barley. They observed that by the application of radiation-degraded chitosan damages were reduced. The recovery of growth and reduction of vanadium levels in seedlings were obtained by the treatment carrying 10-100 μg/mL of chitosan irradiated at 70-200 kGy of gamma rays on 1% solution.

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Luan et al. (2001) studied the effect of irradiated chitosan on growth performance of flower plants namely *Limonium latifolium*, *Eustoma grandiflorum* and *Chrysanthemum morifolium* in a tissue culture study. They observed that irradiated chitosan showed a strong growth-promotion effect and increased the shoot and root length as well as fresh biomass of flowers. 50 ppm concentration of irradiated chitosan obtained by irradiation at 75-100 kGy in 10% solution proved to be optimum for the growth. The optimum concentrations of chitosan, irradiated at 100 kGy were 100 ppm for *C. morifolium*, 30 ppm for *E. grandiflorum* and 40 ppm for *L. latifolium*.

Hałeez et al. (2003) studied the effect of irradiated chitosan on growth of Chinese Kale (*Brassica oleracea* var. *alboglabra*). They observed that chitosan irradiated at 200 kGy showed significant increase on the growth rate of Chinese Kale. They used 10, 50, and 100 ppm concentration of irradiated chitosan in hydroponic culture. As per results, the irradiated chitosan reduced the harvesting period of certain plants; it also reduced the dependency of plants on insecticide and chemical fertilizers.

Luan et al. (2005) studied the effect of irradiated chitosan on plants under in vitro conditions. Chitosan with an 80% degree of deacetylation and of average molecular mass (of approx. 48 kDa) was irradiated with γ-rays at doses up to 200 kGy in a 10% (w/v) solution; as a result, the $M_w$ of chitosan was reduced from 48 to 9.1 kDa by irradiation. It was found that supplementation with irradiated chitosan increased the fresh biomass of shoot clusters (7.2-17.0%) as well as the shoot multiplication rate (17.9 to 69.0%) for *Chrysanthemum morifolium* (florist's chrysanthemum), *Limonium latifolium* (limonium or sea-lavender), *Eustoma grandiflorum* (lisianthus) and *Fragaria ananassa* (modern garden strawberry). The optimum concentrations of irradiated chitosan were found to be approximately 70-100 mg L$^{-1}$ for *chrysanthemum*, 50-100 mgL$^{-1}$ for *Lisianthus* and 30-100 mg L$^{-1}$ for *Limonium*. The optimum concentrations of irradiated chitosan resulted in a significant increase in the fresh biomass (68.1% for *Chrysanthemum*, 48.5% for *Lisianthus*, 53.6% for *Limonium* and 26.4% for strawberry), shoot height (19.4% for *Chrysanthemum*, 16.5% for *Lisianthus*, 33.9% for *Limonium* and 25.9% for strawberry) and root length (40.6% for *Chrysanthemum*, 66.9% for *Lisianthus*, 23.4% for *Limonium* and 22.6% for strawberry). Irradiated chitosan also increased the activity of chitinase in the treated plants.
Quang et al. (2006) conducted an experiment to study the effect of irradiated chitosan on the growth performance of barley and soybean. They used chitosan irradiated at 25-200 kGy in 10% solution and observed positive effect on the growth of barley. They found that that among various degraded samples the fraction F2, with Mw in the range of 1-3 kDa, significantly increased the activity of phytoalexin enzymes, namely phenylalanine ammonia lyase (87%) and chitinase (186%) in barley and soybean; the treatment also increased by 15.8% seed yield of soybean.

Chmielewskia et al. (2007) depolymerized the chitosan by radiation, by chemical degradation and by the combination of the two methods. The efficiency of these methods was verified by viscometric analysis and it was concluded that chemical-radiation method was much more appropriate from economical point of view. In a seed-soaking experiment, they observed that the chitosan fraction, with molecular weight of 47,000 Da, at the concentration of 0.1g/kg of seed, showed maximum growth in plants. Due to the treatment, the average growth of above-ground plant parts was increased to about 16-22%, diameter of roots was increased to about 11-13%, and mass of root was increased to about 51-65% in comparison to the control.

Wisniewska-Wrona et al. (2007) used microcrystalline chitosan to obtain oligo-amino-sacharides by the enzymatic degradation. They studied the effect of degraded chitosan and of its degradation products on biological properties of radish plants in respect with seed germination ability and capability to retard the bacterial, mycotic and viral diseases. Their results showed that all of the tested chitosan forms stimulated the germination of radish seeds: the chitosan oligomers in concentration of 0.01% were characterised as the most advantageous treatment. It increased the length of the germs by about 55% and that of the mass by about 26% in relation to control. Chitosan oligomers stimulated the germination of the radish test seeds at a lower preparation dose in comparison with microcrystalline chitosan.

Boonlertnirun et al. (2008) used chitosan for increasing the rice yield. They used four treatments, which comprised: no chitosan (control), seed soaking with chitosan solution, seed soaking and soil application with chitosan solution and seed soaking and foliar application with chitosan solution. They observed that seed soaking and soil application of chitosan (four times through the cropping season) significantly increased the rice yield compared to other treatments; whereas, seed soaking and
foliar spray with chitosan (four times through the cropping season) tended to show the ability on disease control.

Hewajulige et al. (2009) investigated the use of gamma-irradiated chitosan regarding the extension of storage life of papaya (varieties ‘Ratna’ and ‘Red Lady’), the control of the Colletotrichum gloeosporioides and the cause anthracnose disease in papaya. The powdered chitosan was exposed to different radiation doses (viz. 5, 10, 25, 50, 75, 100 and 150 kGy), using Co-60 gamma rays. They observed that 1% chitosan solution, irrespective of radiation dose, inhibited the growth of fungal strains in comparison to control (distilled water). The irradiated chitosan treatment also increased the shelf life of papaya fruits. Due to irradiated-chitosan treatment, there was 80% marketable quality of ‘Ratna’ papaya fruits, while there was 70% marketable quality of ‘Red Lady’ papaya fruits.

Asghari-Zakaria et al. (2009) treated the plantlets of ‘Agria’ cultivar of potato (Solanum tuberosum) in vitro using soluble chitosan at different concentration (0, 5, 15, 50, 150, 500, 750 and 1000 mg L⁻¹). They added the chitosan solution to the MS tissue culture medium. Application of 500 mg L⁻¹ of soluble chitosan increased the shoot fresh weight and resulted in improved acclimatization of the plantlets in the greenhouse as expressed by significant increase in mini-tuber number and yield, compared to the control. The 5 and 15 mgL⁻¹ of soluble chitosan led to a significant increase in root fresh and dry weight of in vitro plantlets; whereas, higher concentrations, especially 500 mg L⁻¹, significantly decreased the root fresh weight of in vitro plantlets. The results proved that soluble chitosan could be successfully incorporated for potato seed production by in vitro plantlets.

Ya-jing Guan et al. (2009) investigated the effect of seed priming with 0.25, 0.50 and 0.75% (w/v) chitosan solutions at 15 °C on the growth and physiological processes of two maize inbred lines, HuangC (chilling-tolerant) and Mo17 (chilling-sensitive). Seed priming with chitosan under low temperature stress though did not affect the germination percentage, but it enhanced the germination index, reduced the mean germination time (MGT), and increased the shoot height, root length, and shoot and root dry weight in both maize lines. They observed a decrease in malondialdehyde (MDA) content and relative permeability of the plasma membrane, but concomitantly there was noticed an increase in the concentrations of soluble sugars, proline, peroxidase (POD) activity, and catalase (CAT) activity in chilling-
sensitive as well as chilling-tolerant maize seedlings after priming with all the concentrations of chitosan. It was found that priming with 0.50% chitosan solution for about 60-64 hours showed the best response. Hence, it was concluded that seed priming with chitosan might improve the seed germination in maize and could improve theseedling growth under low temperature stress.

Abdel-Mawgoud et al. (2010) investigated the effect of foliar application of chitosan on the growth, yield and fruit quality of strawberry plants. They used different concentrations of chitosan, viz. 0 (control), 1, 2, 3 and 4 cm³/L. They applied the foliar spray treatments three times at four weeks interval, starting at ten weeks after transplanting. Chitosan application improved the plant height, number of leaves, fresh and dry weight of leaves and yield components, with 2 cm³/L giving the best results.

Sheikha et al. (2011) investigated the effect of chitosan on plants of bean (Phaseolus vulgaris). They watered plants with chitosan solution at concentrations of 0 (control), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0%. Application of chitosan enhanced the shoot and root length, fresh and dry weight of shoots and roots, leaf area and leaf chlorophyll content.

Boonlertmirun et al. (2012) studied the effect of chitosan on rice (Oryza sativa). The experiment was conducted with complete randomized block design and in total they used four treatments, viz. T-1: chitosan at the concentration of 80 mg L⁻¹ in combination with mixed chemical fertilizer between urea-1 (46-0-0) and urea-2 (16-20-0) at the rate of 312.5 kg ha⁻¹, T-2: mixed chemical fertilizer between urea-1 (46-0-0) and urea-2 (16-20-0) at the rate of 312.5 kg ha⁻¹, T-3: foliar application of chitosan at the concentration of 80 mg L⁻¹ and T-4; no application of chitosan and mixed chemical fertilizer. Five replications were performed. It was observed that chitosan application in combination with mixed chemical fertilizer showed positive effects on leaf greenness, dry weight and yield of rice plants.

Mondal et al. (2012) conducted an experiment to investigate the effect of foliar application of chitosan on growth, biochemical parameters, yield attributes and fruit yield of okra (Abelmoschus esculentus). Five different concentrations of chitosan were used, viz. 0 (control), 50, 75, 100 and 125 mg L⁻¹. They concluded that chitosan enhanced the growth, biochemical parameters and yield attributes of okra. Among all
the concentrations, 100 and 125 mg L\(^{-1}\) of chitosan had superiority over other treatments for plant growth, yield components and fruit yield.

Shehata et al. (2012) carried out an experiment to study the effect of foliar application of chitosan (1, 2, 3 and 4 mL L\(^{-1}\)) and yeast (1, 2, 3 and 4 g L\(^{-1}\)) on growth, yield, quality and chemical constituents of cucumber in two successive seasons of 2010 and 2011. Foliar application of chitosan with yeast significantly enhanced the vegetative growth, yield and quality of cucumber. Foliar application of chitosan at the rate of 4 ml L\(^{-1}\) gave the highest contents of leaf P and K (%) in the two seasons of study. Finally, it was concluded that chitosan application at 4 ml L\(^{-1}\) recorded the highest vegetative growth, yield and quality of cucumber plants.

Hossain et al. (2013) conducted an experiment to study the effect of different concentrations of irradiated chitosan (300, 500 and 1000 mg L\(^{-1}\)) on plants of tea (*Camellia sinensis*). They applied chitosan as foliar sprays at 7 days interval. They concluded that application of irradiated chitosan increased the productivity of tea plants by 38.0% and reduced the total fungal count significantly.

Yacob et al. (2013) studied the effect of degraded chitosan on riceplants. Chitosan was degraded by gamma rays within the dose range of 25-75 kGy. Ubbelohde capillary viscometer was used to determine the effects of irradiation on the molecular weight and viscosity of the chitosan. At 50 kGy dose, the molecular weight dropped from 67,352 Da to 14,946 Da. They used chitosan in the range of 20-100 mg L\(^{-1}\). It was concluded that irradiated chitosan enhanced the plant growth by 15-20% as compared to the chemical growth promoters.

2.5 Effect of plant hormones and mineral nutrients

2.5.1 Response of plants to Gibberellic acid (GA\(_3\)) application

Wu et al. (1993) investigated the effect of GA\(_3\) on the activity of invertase enzyme in the cell wall of pea (*Pisum sativum*) plants. Northern blot analysis showed that the amount of invertase mRNA (1.86 kb) was rapidly induced to the maximal level 4 h after GA\(_3\) treatment. The mRNA level at 4 h in GA\(_3\)-treated peas was fivefold higher than that of the control group. Maximal increase in the activity of pea cell wall invertase, elicited by GA\(_3\), occurred 8 h after GA\(_3\) treatment. The study indicated that the expression of the pea shoot cell-wall invertase gene could be regulated by GA\(_3\) at transcriptional and/or translational levels.
Khan and Ansari (1997) investigated the effect of GA$_3$ spray on the yield parameters and fatty acid composition of rapeseed mustard (*Brassica juncea* cv. Varuna). They conducted two field experiments during 1994-95 to study the effect of $10^{-5}$M GA$_3$, at 40 days after sowing, with basal application of 0, 40, 80 and 120 kg N ha$^{-1}$ (Exp 1) and of 0.15, 30 and 45 Kg P ha$^{-1}$ (Exp 2). When GA$_3$ was used in combination with 80 kg N ha$^{-1}$, it resulted in significant enhancement in yield characteristics. In the 2$^{nd}$ experiment, GA$_3$+30 kg P ha$^{-1}$ enhanced the yield individually; the interaction was not significant.

Santos et al. (2000) conducted an experiment to study the effect of gibberellic acid on carrot (*Daucus carota*) growth and severity of Alternaria leaf blight. GA$_3$ applied at 2.5 to 250 mg L$^{-1}$, enhanced the dry weight of foliage. Further, GA$_3$ reduced the percentage of leaf area affected by Alternaria compared with untreated plants. Application of GA$_3$ significantly enhanced the growth of carrot plants in terms of plant height, width of petioles and length of leaves.

Shah et al. (2007b) studied the effect of gibberellic acid (GA$_3$) on the growth, physiology and yield of salt-stressed plants of mustard. Fifty days after emergence, there was recorded a reduction in leaf area, dry mass, leaf chlorophyll content, stomatal conductance and net photosynthetic rate due to the stress imposed by 25 or 50 mM of NaCl. It was concluded that $10^{-5}$ M of GA$_3$ mitigated the adverse effects of salinity stress on the overall performance and productivity of mustard plants.

Emongor (2007) carried out two field experiments to evaluate the effects of GA$_3$ on growth and development of two cowpea cultivar, 'Blackeye' and 'Tswarna'. It was found that exogenous application of GA$_3$, applied at 30, 60 or 90 mg L$^{-1}$, significantly increased the plant height, first node height, leaf area and leaf number/plant; it also increased the nodulation, plant dry matter accumulation, pod length, pod number/plant, 1000 seed weight, harvest index and seed yield.

Jaleel et al. (2007) studied the changes in antioxidant potentials and the production of indole alkaloid, ajmalicine in catharanthus employing GA$_1$ application. GA$_3$ treatments were given in two ways, namely, foliar spray and soil drenching methods on 30, 45, 60 and 75 days after planting (DAP). The antioxidant potential was studied in terms of antioxidant molecules like ascorbic acid (AA), α-tocopherol (α-toc) and reduced glutathione (GSH) and by the activities of antioxidant enzyme, viz.
superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT). It was concluded that GA$_3$ had a marked effect on the antioxidant potential and caused a significant enhancement in the production of ajmalicine alkaloid when compared to the control. These results suggested that GA$_3$ might be a useful tool to increase the antioxidant potential and alkaloid production in medicinal plants like catharanthus.

El-Naggar et al. (2009) conducted an experiment during successive seasons of 2007 and 2008 at Alexandria, Egypt, under green house conditions to investigate the effect of different concentrations of GA$_3$ (0, 25, 50 and 100 ppm) and orthophosphoric acid (H$_3$PO$_4$) (0, 50, 100 and 200 ppm of P$_2$O$_5$) on the growth and flower quality of *Dianthus caryophillus* cv. “Red Sim”. The results showed that the treated plants with GA$_3$ solely or with the combination of GA$_3$ and P-fertilizer stimulated the vegetative growth, flower production and flower quality in comparison to the control. It was concluded that 50 ppm of GA$_3$ in combination with 100 ppm of P$_2$O$_5$ could give the highest values regarding vegetative growth and flowering parameters. There was also observed a significant increase in total contents of chlorophyll, carotenoids, carbohydrates and phosphorus as a result of the combined spray of GA$_3$ and P-fertilizer in comparison to control (untreated plants).

Monsour et al. (2009) investigated the effect of GA$_3$ on the amount of terpenoids produced and the activity of the key enzymes, 1-deoxy-D-xylulose 5-phosphate synthase (DXS) and 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGR), involved in cytosolic mevalonic acid (MVA) pathway and plastidial methylerthritol phosphate (MEP) pathway. Gibberellic acid caused a decrease in DXS activity in both sexes and was accompanied by a decrease in chlorophylls, carotenoids and 9-tetrahydrocannabinol (THC) contents and an increase in α-tocopherol content. The treated plants with GA$_3$ showed an increase in HMGR activity. The increase in HMGR activity was followed by accumulation of stigmasterol and β-sitosterol in male and female plants and of campesterol in male plants.

Soni et al. (2010) investigated the effect of gibberellic acid on the seed germination of fenugreek. Seeds were soaked in different concentrations of GA$_3$, viz. 100, 200 and 400 ppm, for 12 hours; while, one set of the experiment was the control (seeds soaked in distilled water). They concluded that gibberellic acid stimulated the seed germination and other parameters, with 300 ppm giving the maximum values.
Shah et al. (2007) investigated the effect of foliar application of three concentrations of gibberellic acid (GA$_3$) or of kinetin (Kn), viz. 1, 10 and 100 μM, on net photosynthetic rate, nitrogen metabolism and the seed yield of black cumin. Control plants were sprayed with distilled water. According to the results, 10 μM solutions of both the hormones, especially of GA$_3$, appreciably increased the activities of nitrate reductase and carbonic anhydrase; it also enhanced the chlorophyll and total protein contents and net photosynthetic rate in the leaves in addition to significant increase in capsule number and seed yield plant$^{-1}$ recorded at harvest.

Alsokari (2009) studied the effect of kinetin on cadmium-treated sorghum plants. There was noticed a decrease in yield and yield attributes of sorghum plants in response to cadmium treatments; but when grains were pre-soaked in kinetin there was recorded a significant improvement in the parameters studied. Grain priming with kinetin increased the grain biomass and the content of carbohydrates, protein and ions in the seed of cadmium-treated sorghum plants.

Schmiderer et al. (2010) conducted an experiment on common sage (Salvia officinalis), investigating the effect of exogenously applied plant growth regulators, namely GA$_3$ and daminozide, on leaf morphology and essential oil formation in two-leaf stage plants during the period of leaf expansion. They observed that essential oil content increased with the increase in GA$_3$ concentrations, while it was decreased when gibberellin biosynthesis was blocked with daminozide. GA$_3$ applied at the highest level also led to a significant decrease of α- and β-thujone. The foliar application of GA$_3$ increased, while daminozide significantly decreased the gene expression of the monoterpene synthases.

Aftab et al. (2011b) investigated the effect of foliar application of GA$_3$ alone and in combination with different levels of soil applied nitrogen on artemisia. Application of GA$_3$ was effective in alleviating the growth, photosynthesis and enzyme activities of artemisia. N levels combined with GA$_3$ showed better responses and further improvement in these parameters. As compared to the control, there was 21.8% increase in content and 55.8% increase in the yield of artemisinin as a result of combined application of GA$_3$ (75 mg L$^{-1}$) and N (80 mg kg$^{-1}$ soil).

Cardoso et al. (2012) evaluated the influence of GA$_3$ on the vegetative and reproductive development of young plants of Phalaenopsis orchid hybrid genus.
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(Phalaenopsis FSNT ‘Dai-Itigo’ hybrid pink colour). GA₃ was applied as foliar spray at concentrations of 0, 125, 250, 500 and 1,000 mg L⁻¹. The application of GA₃ at 125 mg L⁻¹ showed the best results for the promotion of flowering and flower quality of this orchid hybrid.

Khadija et al. (2013) conducted a pot experiment at the glasshouse of the Universiti Putra (Malaysia) during 2010 - 2011 to determine the salinity tolerance of two rice varieties treated with GA₃. The results revealed that morphological traits such as plant height, tillers plant⁻¹, leaves plant⁻¹, leaf length and plant dry weight and the physiological attributes, namely, content of chlorophyll a, b and of total chlorophyll, photosynthetic rate, stomatal conductance and transpiration rate were reduced significantly with increasing saline condition in case of both the varieties. However, saline effects were ameliorated when rice plants were sprayed with GA₃. They concluded that GA₃, a safe plant growth regulator, could be effectively sprayed on rice variety MR219 in saline belts so as to improve the salinity stress in plants.

Lolaect al. (2013) conducted an experiment to study the effect of GA₃ on the yield and fruit quality of strawberry (Fragaria ananassa Duch., cv. Selva and Qeenelisa). They used 50, 100 and 150 ppm concentrations of GA₃ and found that 150 ppm of GA₃ had the greatest effect on the biomass and number of leaves and fruits. GA₃ spray increased the vegetative growth and number of stolons as a result of GA₃ spray at 100 and 150 ppm concentrations compared to the control. Furthermore, application of GA₃ resulted in delayed fruit ripening as reflected by lower TSS content and elevated amount of acidity, and contents of phenols and tannins in the fruits.

Renu Dhupper (2013) examined the effect of three concentrations of GA₃ on germination and seedling growth behaviour of three tree species Albizia lebbeck, Acacia nilotica and Prosopis cineraria of desert region. Seeds were treated with different concentrations of GA₃, which included G1: untreated seeds (control), G2: 250 ppm of GA₃, G3: 500 ppm of GA₃ and G4: 750 ppm of GA₃. They concluded that seeds treated with 750 ppm of GA₃ resulted in maximum germination and seedling dry weight especially in Acacia nilotica and Prosopis cineraria followed by that in Albizia lebbeck, where as the germination was minimal in untreated plants (control).
Zalewska and Antkowiak (2013) studied the effect of GA₃ on growth and flowering performance of *Ajania pacifica* /Nakai/ Bremer et Humphries 'Bea'. They observed that two times spray with GA₃ accelerated the buds development of *Ajania pacifica*, thus, shortened the cultivation time by about two days. It was concluded that treatment with GA₃ at concentration 500 mgdm⁻³ stimulated the elongation of shoots more than that stimulated by 250 mgdm⁻³ of GA₃. Plants sprayed twice with GA₃ were taller than the control plants or those sprayed once. The study indicated the specificity of the response of *Ajania pacifica* to GA₃ treatment which appeared to be different from other ornamental species.

Yakubu et al. (2013) studied the effect of GA₃ concentrations on yield and yield traits of groundnut (*Arachis hypogaea* L.) under wet and dry conditions. The experimenters investigated the response of three varieties, viz. SAMNUT 21, SAMNUT 22 and SAMNUT 23 and five levels of GA₃ viz. 0, 100, 200, 300 and 400 mg L⁻¹, which were applied as foliar sprays at 3 and 6 weeks after sowing, on yield and yield traits of the crop. The results exhibited that 200 mg L⁻¹ of GA₃ significantly (*p<0.01*) reduced the number of days of first flowering and that of 50% flowering. The highest values of pod, kernel and haulm yields was obtained at 100 mgL⁻¹ of GA₃ during wet as well as dry season.

**2.5.2 Response of plants to Kinetin application**

Zahir et al. (2001) conducted a field experiment to investigate the effect of synthetic cytokinin (kinetin) and its physiological precursors (adenine+isopentyl alcohol) on growth and yield of rice. Five levels (ranging from 10⁻² to 10⁻⁶ M) each of kinetin and adenine (ADE) plus isopentyl alcohol (IA) were used in addition to an untreated control. The roots of rice seedlings were dipped in solutions of synthetic cytokinin or its precursors for an hour, just before transplanting. The precursors were more effective in comparison with pure cytokinin. As per results, plant height was increased by 6.5%, over the control, when treated with 10⁻⁵ M ADE+IA. Compared to the control, there was 34.7%, 38.5% and 21.6% increase in number of tillers, number of panicles and paddy yield respectively, due to application of ADE+IA at the rate of 10⁻⁴ M over the control. It was concluded that the supply of cytokinin or its precursors in the root zone could also improve the growth and yield of treated plants.
Werner et al. (2001) genetically engineered the cytokinin oxidase expression in transgenic tobacco (*Nicotiana tabacum*) plants to reduce their endogenous cytokinin content. Cytokinin-deficient plants developed stunted shoots with smaller apical meristems. The plastochrone was prolonged, and leaf cell production was only 3-4% that of the wild type tobacco, indicating an absolute requirement of cytokinin for leaf growth. In contrast, the effect on roots was opposite and the root meristem of transgenic plants was enlarged and gave rise to faster-growing and profusely branched roots. The results confirmed the important role of cytokinins in plant morphogenesis and their reverse effect on root and shoot.

Barnali and Srivastava (2006) studied the effect of different spray-concentrations of kinetin on in vivo nitrate reductase (NR) activity and level of soluble protein in the leaves and nitrogenase activity, leghaemoglobin content and soluble protein content in the nodules of *Vigna radiata*. The kinetin treatments were comprised of 1, 10 and 50mg/mL. The plants sprayed with 10mg/mL of kinetin resulted in maximum in vivo NR activity in the leaves on day-7 after spray; whereas, the soluble protein was maximum on day-14 after spray. They concluded that in the case of nodules the soluble protein and nitrogenase activity were maximum with 10 mg/mL of kinetin on day-14 after spray; whereas, the leghaemoglobin content was maximum on day-21 after spray.

Shah (2007a) conducted a field experiment to study the effect of foliar spray of kinetin on growth and productivity of black cumin. Plants were sprayed with the deionised water (control) or with $10^{-5}$M of kinetin. Further, kinetin application significantly enhanced the shoot length, leaf number, leaf area, branch number and dry weight per plant; it also enhanced the net photosynthetic rate, stomatal conductance and leaf chlorophyll content. As compared to the control, the kinetin treatment also resulted in significant enhancement in capsule number per plant, seed and biomass yield per plot; but, number of seeds per capsule, 1000-seed weight and harvest index remained unaffected.

Fatima et al. (2008) conducted an experiment to compare the effect of biofertilizers *Rhizobium leguminosarum* and three plant growth regulators (PGRs), viz., kinetin, indole-3-acetic acid (IAA) and abscisic acid (ABA) on the growth characteristics, yield attributes and N$_2$ fixation parameters of chickpea under natural conditions. The PGRs were applied at concentration of $10^{-5}$M as seed soaking...
and $10^{-6}$ M as foliar spray alone and in combinations with *Rhizobium* inoculum (strain TAL 1148 and TAL 620). Kinetin was most effective in increasing the growth characteristics (root biomass, shoot biomass, and grain yield) and nitrogen fixation parameters (specific nitrogenase activity of nodules, and total $N$-fixed per plant). It was concluded that both the efficiency and the longevity of the nodules seemed to be favourably affected by kinetin application.

Burke (2011) investigated the effect of foliar application of a commercial formulation of cytokinin (6-benzyladenine) on cotton (*Gossypium hirsutum*). Seedlings treated with 25 $\mu$mol/mol of 6-benzyladenine at approximately two weeks after planting exhibited increased hypocotyl diameters, enhanced lateral root proliferation, and breakage of apical meristem dormancy within one week of treatment. This study showed that application of 6-benzyladenine to cotton, early in the plant development, has the potential to increase yield and reduce water stress in cotton.

Kaya et al. (2010) conducted a field experiment to study the effect of foliar spray of kinetin (Kn) and indoleacetic acid (IAA) on salt stressed maize. 100 mM of NaCl was added to irrigation water to create the salt stress. Stress reduced the total dry matter, grain yield, chlorophyll content, and relative water content (RWC). but it increased the electrolyte leakage and proline accumulation in the maize plants. Foliar applications of both KIN and IAA overcame the adverse effects of NaCl; it reduced the concentration of Na$^+$ ions and increased that of Ca$^{2+}$ and K$^-$ in the tissue. However, the combination of two hormones did not significantly improve salinity tolerance in maize plants.

Lou et al. (2012) conducted an experiment on loquat (*Eriobotrya japonica*) to investigate the effect of kinetin (Kn) treatment on fruit color, size, weight and level of chlorophyll (Chl), total phenolics (TP), ascorbic acid (AA) and antioxidant activity in ‘Jiujiao’ loquat fruit during its development. The Kn-treated fruit exhibited significantly greener colour and higher levels of Chl, TP and AA than in the control fruit. There was also noted an increase in the size and weight of fruits. The Kn treatment provided a new fresh-keeping technology in loquat fruit on the tree and it also could postpone the harvest date and increase the fruit quality and yield of loquat fruit.
Nisha et al. (2012) investigated the effect of benzylaminopurine (BAP) on inflorescence production of a Dendrobium hybrid (Dendrobium Angel White). White plantlets were subjected to spray containing different BAP concentrations. The application of BAP increased the percentage of inflorescence production, induced earlier flowering and increased the inflorescence length, number of leaves and flowers produced. This study showed that BAP might be a potential plant growth regulator that could speed up the flowering process of the Dendrobium hybrid.

Vijayakumar et al. (2014) conducted a field experiment to investigate the effect of foliar sprays of brassinosteroids and kinetin on biochemical parameters and yield of chickpea under drought stress at the Agricultural College Farm, Bapatla, Andhra Pradesh, during rabi (winter) season of 2008-09 and 2009-10. The experiment was laid out with split plot design using three main plots, viz. control plot irrigated at 15 days intervals throughout crop period, water stress induced at vegetative stage, and water stress induced at flowering stage. Each main plot consisted of three subplots, viz. no spray, kinetin spray at 5 ppm and homobrassinolide spray at 1 ppm; the treatments were replicated four times. Water stress decreased SPAD chlorophyll values. Proline content, activities of antioxidant enzymes and SCMR values increased with homobrassinolide (1 ppm) and kinetin (5 ppm).

2.5.3 Effect of phosphorus application on plants

Khiriya and Singh (2002) conducted a field experiment during the winter (rabi) season of 1996–97 and 1997–98 at CCS Haryana Agricultural University, Hisar (India), to study the effect of farmyard manure (0, 5, 10 and 15 tonnes ha\(^{-1}\)) and phosphorus (0, 20, 40 and 60 kg ha\(^{-1}\)) on two fenugreek genotypes (‘HM 65 and ‘NLM’). They found that ‘NLM’ was significantly superior to ‘HM 65’ in terms of more number of branches/plant, pods/plant and seeds/pod. It was concluded that increasing phosphorus levels (up to 40 kg P\(_2\)O\(_5\) ha\(^{-1}\)), significantly increased the yield-attributing characters, seed yield and quality parameters of fenugreek.

Naeem and Khan (2005) conducted a pot experiment at Aligarh (Uttar Pradesh), to find out whether phosphorus application could augment the growth, physiology and seed yield of Cassia tora. Out of five different concentrations of phosphorus, viz. 0, 0.1, 0.2, 0.3 and 0.4 g P per kg soil (P0, P1, P2, P3 and P4, respectively). P3 significantly enhanced the growth characteristics (fresh and dry
weight of plant, number of leaves and leaf-area per plant), physiological parameters (total chlorophyll content, leaf-N, -P and -K content, and leaf-nitrate reductase activity) and yield and quality parameters (pod length, number of pods, pod weight per plant, seed weight per pod, seed-yield, seed-yield-merit and seed-protein content).

Khan et al. (2005) conducted a field experiment on fenugreek to investigate the effect of different phosphorus levels and that of spatial arrangement with regard to plot-size and row-spacing. The treatments comprised of four phosphorus levels (0, 30, 45 and 60 kg P$_2$O$_5$ ha$^{-1}$). The net plot sizes were 1.8 × 6 m, 2.4 × 6 m and 3 × 6 m with row-spacing of 30, 40, and 50 cm, respectively. Phosphorus application improved the performance of fenugreek cropregarding number of seeds plant$^{-1}$. 1000 seed weight, biological yield, seed yield and harvest index, but pods per plant and number of branches remained unaffected. It was concluded that optimum dose of phosphorus for fenugreek crop was 45 kg P$_2$O$_5$ ha$^{-1}$, while the interaction effect of phosphorus and spatial arrangement was non-significant on growth and yield of fenugreek.

Jagdale and Dalve (2010) carried out an experiment on fenugreek. The treatments comprised of five levels each of nitrogen (0, 30, 60, 90 and 120 kg N ha$^{-1}$) and phosphorus (0, 15, 30, 45 and 60 kg P ha$^{-1}$). The results indicated that plant height, number of leaves and number of branches per plant were increased due to the application of 120 kg nitrogen and 60 kg phosphorus ha$^{-1}$.

Deshbhrrat et al. (2010) conducted a field experiment on pigeon pea during 2008-2009 to study the effect of sulphur (S) and phosphorus (P) on the yield, soil nutrient status and the tissue N and P content. The treatments comprised of three levels of sulphur (0, 20 and 40 kg S ha$^{-1}$) and four levels of phosphorus (0, 25, 50 and 75 kg P ha$^{-1}$). They observed a significant increase in grain yield (14.81 q ha$^{-1}$) and straw yield (41.26 q ha$^{-1}$) as a result of 20 kg S ha$^{-1}$ and 50 kg P$_2$O$_5$ ha$^{-1}$ applied with a common dose of nitrogen (30 kg N ha$^{-1}$). The increase in grain and straw yield was 102.77 and 52.87%, respectively, as compared to the control. The treatment also improved the number of pods per plant, maximum number of grains pod$^{-1}$ and test weight as compared to control.

Masood et al. (2011) conducted an experiment at the Agricultural Research Institute, Tarnab (Pakistan) during 2007 to investigate the effect of different
phosphorus levels [0 (control), 50, 100, 150 and 200 kg P ha$^{-1}$] on the yield and yield components of maize. Plant height, number of cobs plant$^{-1}$, number of grains cob$^{-1}$ and grain yield was positively affected by phosphorus levels. However, the effect was non-significant on number of plants m$^{-2}$, 1000 grain weight and biological yield of maize. 100 kg P ha$^{-1}$ proved to be the best among all the phosphorus levels.

Shaheen et al. (2012) conducted two field experiments at the National Research Centre Experimental Farm, Nobaria, Behira Governorate, Egypt, to study the effect of chemical and/or natural phosphorus fertilizer applied alone or with biofertilizers on vegetative growth, yield, quality and nutrient contents of onion bulbs cv. Giza 20. The onion plants which received the P fertilizer (90 P$_2$O$_5$ units/fed) along with biofertilizers (phosphatein) at the rate of 10 kg/fed were tallest, with largest leaves and largest bulbshaving largest neck diameter, maximum fresh and dry weight and the heaviest total bulbs yield.

Jat et al. (2012) investigated the effect of phosphorus and sulphur on growth and yield of fenugreek. Phosphorus applied at 60 kg P$_2$O$_5$ ha$^{-1}$ improved the growth of the crop in terms of plant height, dry matter accumulation plant$^{-1}$ and number of branches per plant. The crop fertilized with 60 kg P$_2$O$_5$ ha$^{-1}$ increased the seed and straw yield, biological yield and harvest index by 51.8, 19.8, 26.2 and 21.7% respectively over the control. The interaction of 40 kg P$_2$O$_5$ ha$^{-1}$ with 45 kg S ha$^{-1}$ gave the maximum seed yield (19.26 q ha$^{-1}$), net returns (Rs. 46,352 ha$^{-1}$) and B/C ratio (3.36).

Turuko and Mohammed (2014) conducted a field experiment at the Arba Minch farm field during the season of 2011 to investigate the responses of common bean to different levels of phosphorus fertilizer in terms of growth, dry matter yield and yield component of the crop. Different levels of phosphorus were 0, 10, 20, 30 and 40 kg ha$^{-1}$. They concluded that among different levels of phosphorus 20 kg P ha$^{-1}$ proved to be the best significantly enhanced the dry matter yield, yield components and growth parameters such as leaf area and number of branches per plant, whereas the effect on plant height was not significant.

Sarker et al. (2014) carried out an experiment to study the effect of lime (CaCO$_3$) and phosphorus on the growth and nutrient uptake by Indian spinach (Basella alba L.) in an acidic soil. They used four levels each of lime (0, 500, 1000
and 2000 kg CaCO₃ ha⁻¹) and phosphorus (0, 50, 100, and 150 kg P ha⁻¹) and their combination. They observed that the application of phosphorus and lime or the combination of the two had significant effect on the growth parameters, viz. fresh and dry weight of shoot and root, number of leaves and height and the uptake of N, K, P and Ca. The authors revealed that the application of lime and phosphorus could be used in combination to improve growth performance and nutrient uptake when spinach plants are grown in an acidic soil.

Uddin et al. (2014) conducted a 2-factor factorial experiment in a net-house to investigate the effect of graded levels of P fertilizer (0, 30 and 60 kg P ha⁻¹) together with Rhizobium [biological nitrogen fertilizer (BNF)] and/or phosphat-solubilising bacteria [biological phosphorus fertilizer (BPF)] on nutrient uptake, yield, and quality of chickpea. According to the results, P₆₀ (60 kg P ha⁻¹) proved superior or equivalent to P₃₀, while among the biofertilizer treatments, BNF+BPF gave the greatest values for nutrient uptake as well as for yield and quality parameters. Among the different combinations, 30 kg P ha⁻¹ applied with N and P biofertilizers (P₃₀ × BNF+BPF) was the most profitable interaction for N uptake as well as for yield and quality characteristics. P₃₀ × BNF+BPF resulted in significantly greater N uptake (27.3%), seed yield (21.1%), and the content of seed protein (2.9%) and carbohydrate (5.6%) as compared to the control.