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Influence of chitosan and indole-3-butyric acid in improving chemical, biochemical and yield contributing parameters of soybean

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Abstract
In order to examine effects of different concentrations of growth regulators (chitosan and IBA) on biochemical and yield contributing parameters on soybean a field experiment was carried out in the field of botany section, college of agriculture, Nagpur during 2016-17. Foliar application of chitosan and IBA @ 25, 50, 75, 100 and 125 ppm each were given at vegetative stage (30 DAS) and the data were recorded at 30, 45, 60 and 75 DAS coinciding with vegetative stage, flowering stage and before harvest stage. Application of chitosan and IBA enhanced biochemical parameters *viz.,* chlorophyll content, NPK content in leaves, protein content in seeds, yield contributing characters *viz.,* number of pods plant^{-1}, 100 seed weight, seed yield plant^{-1} and plot^{-1}. Analysis of data revealed that 25 ppm chitosan considered as most effective concentration in enhancing all biochemical and yield contributing parameters.

Keywords: soybean, chitosan, IBA, foliar application, biochemical parameters, and yield

Introduction
Soybean is one of the important oilseed as well as leguminous crop. It is gaining importance in India and other developing countries to ward off malnutrition. It is the cheapest and richest source of high quality protein. It supplies most of the nutritional constituents essential for human health. Hence, soybean is called as “Wonder crop” or “Golden bean” or “Miracle bean”. Soybean protein contents all the essential amino acids vital for human diet.

Besides protein and oil, soybean contains 20.9 % carbohydrate, 60 % polyunsaturated fatty acids (52.3 % linolenic acid + 7.29 % linoeic acid), Vitamin A, Vitamin B, Vitamin C, D, E, K, 0.69 % phosphorus, 0.0115 % iron, 0.0024 % calcium and all the essential amino acids. Amongst oil seed crops it has highest content of lysine (5 %), a limiting factor in cereals. So it is called “Poor mans meal” it’s a really true.

Chitosan is polysaccharide and composed of 2-deoxy-2-(acetylamino) glucose unit (N-acetyl glucosamine). Chitosan is a linear polysaccharide composed of randomly distributed β-(1-4)-linked D-glucosamine (deacetylated unit) and N-acetyl-glucosamine (acetylated unit) combined by 1,4 glycosidic linkages, forming a long chain linear polymer and due to this NH_2 group chitosan is more versatile in properties.Chitosan produced as processing waste from shellfish krill, oyster squid and fungi.Primarily, chitosan is used for plant defence (Walkar, 2004) and yield increase. In agriculture, chitosan is typically used as a natural seed treatment and plant growth enhancer. It is one of the most abundant biodegradable (Gooday, 1990) [11] materials in the world.

IBA is a plant growth regulator, used to promote and accelerate root formation of plant clippings and to reduce transplant shock of non-food ornamental nursery stock. IBA is also used on fruit and vegetable crops, field crops and ornamental turf to promote growth and development of flowers and fruits and to increase crop yields. IBA has been classified as a biochemical pesticide because it is similar in structure and function to the naturally-occurring plant growth hormone indole-3-acetic acid.

Considering the above fact present work was undertaken to study the response of chitosan and IBA on chemical and biochemical parameters and yield of soybean.

Materials and Methods
Experiment was laid out in randomized block design with eleven treatments and three

replications. Plot size of individual treatment was gross 2.10 m x 2.20 m and net 1.50 m x 2.00 m. Seeds were sown at the rate of 75 kg ha^{-1} by dibbling method at spacing of 30 cm x 10 cm on 11th July, 2016. Treatments comprised of control (T_1), 25 ppm chitosan (T_2), 50 ppm chitosan (T_3), 75 ppm chitosan (T_4), 100 ppm chitosan (T_5), 125 ppm chitosan (T_6), 25 ppm IBA (T_7), 50 ppm IBA (T_8), 75 ppm IBA (T_9), 100 ppm IBA (T_{10}) and 125 ppm IBA (T_{11}). The foliar application of chitosan and IBA was given at 30 DAS on soybean.

The chemical and biochemical parameters *viz*., leaf chlorophyll, nitrogen, phosphorus, potassium and seed protein content were estimated and recorded. Total chlorophyll content of dried leaves was estimated by colorimetric method as suggested by Bruinsma (1982) [4]. Nitrogen content in leaves was determined by micro-kjeldhal's method as given by Somichi *et al.* (1972) [23]. Phosphorus content in leaves was determined by vanadomolybdate yellow colour method as given by Jackson (1967) [13]. Potassium content in leaves was determined by flame photometer by di-acid extract method given by Jackson (1967) [13]. Nitrogen content in seed was determined by micro-kjeldhal's method as given by Somichi *et al*. (1972) [23] and same was converted into crude protein by multiplying 'N' per cent with factor 6.25. Number of pods $plant^{-1}$, 100 seed weight (g), seed yield $plant^{-1}$ (g) and $plot^{-1}$ (kg) were also recorded. The data were analysed as per the method suggested by Panse and Sukhatme (1954) [17].

Results and Discussion

Chemical and biochemical parameters

The chemical and biochemical studies with respect to chlorophyll, N, P and K content in leaves at different stages of observations as well as protein content in seed were estimated and data regarding these parameters have been presented here below.

Leaf chlorophyll content

The greenness of the leaf is generally considered to be a parameter contributing to yielding ability of the cultivar. Leaves constitute most important aerial organ of the plants, playing a major role in the anabolic activities by means of the so called 'green pigments' or 'chlorophyll' is the sole medium of photosynthetic progress which in turn is the major synthesis pathway operative in plants.

The treatment effects were found statistically significant at 45, 60 and 75 DAS stages of observations except 30 DAS.

Data indicated that, at 45 DAS chlorophyll content was significantly increased in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) when compared with treatment T_1 (control) and remaining treatments under study. Treatment T_4 (75 ppm chitosan) was found significantly superior over treatment T_1 (control) and rest of the treatments.

At 60 DAS the treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) noted significantly maximum chlorophyll content over treatment T_1 (control) and rest of the treatments under study. Treatments T_4 (75 ppm chitosan), T_{11} (125 ppm IBA), and T_5 (100 ppm chitosan) were found significantly superior over control. Remaining treatments were found at par with control (T_1). The range of chlorophyll content at 60 DAS in soybean was 2.03 - 2.34 mg g^{-1}. The per cent increase in chlorophyll content at 45 DAS in treatment T_2 (25 ppm chitosan) over T_1 (control) was 15.27.

At 75 DAS the treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) were most effective in increasing chlorophyll content in leaves when compared with treatment T_1 (control) and rest of the treatments under study. Also treatments T_4 (75 ppm chitosan), T_{11} (125 ppm IBA) and T_5 (100 ppm chitosan) were found significantly superior over treatment T_1 (control) and remaining treatments. The range of chlorophyll content at 75 DAS in soybean was 2.01 - 2.32 mg g^{-1}. The per cent increase in chlorophyll content at 75 DAS in treatment T_2 (25 ppm chitosan) over T_1 (control) was 15.42.

The chlorophyll contented respond positively to the application of chitosan. Cuibu and Shibayama (2001) [5] also reported the higher chlorophyll content in the plants treated with chitosan. Our results also indicate significant increases in both nitrogen and potassium content in plant shoots, which may be play an important role in increasing the number of chloroplasts per cell, cell size and number $unit^{-1}$ area as well as increased synthesis of chlorophyll (Possingham, 1980) [19]. The significant effect of chitosan on plant growth may be attributed to an increase in the key enzyme activities of nitrogen metabolism and increased photosynthesis which enhanced plant growth (Gornik *et al.,* 2008; Mondal *et al.,* 2012) [12, 15]. IBA treatment might retard chlorophyll destruction and increase their biosynthesis or stabilize the thylakoid membrane. These might be the reasons for increase in chlorophyll content in the present study.

Shraiy and Hegazi (2009) [21] conducted an experiment to study the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA at 25 and 35 DAS significantly increased total chlorophyll in leaves.

Rabbi *et al*. (2016) [20] carried out an experiment to investigate the impact of foliar application of chitosan on mungbean. The experiment comprised five levels of chitosan viz., 0 (control), 25, 50, 75 and 100 ppm sprayed at 30 and 40 DAS. Results showed that spraying of chitosan @ 50 ppm significantly increased chlorophyll content in leaves.

Leaf nitrogen content

Nitrogen is key component in mineral fertilizers and has more influence on plant growth, appearance and fruit production or quality than any other essential elements. Nitrogen is an important constituent of protein and protoplasm and essential for the growth of plants. Its storage leads to chlorosis and stoppage of growth and its presence in moderate doses is essential for plant growth and fruiting. An abundant supply of essential nitrogenous compound is required in each plant cell for normal cell division, growth and respiration. The N present mostly as protein is constantly moving and under concentration of N is found in young, tender plant tissues like tips of shoots, buds and new leaves (Jain, 2010) [14].

It is observed from the data that there was significant variation in leaf nitrogen content due to foliar sprays of different concentrations of chitosan and IBA at 45, 60, 75 DAS except 30 DAS.

At 45 DAS nitrogen content was significantly more in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) when compared with treatment T_1 (control) and other remaining treatments under study. While, treatment T_4 (75 ppm chitosan) was found significantly superior over treatment T_1 (control) and rest of the treatments. The range of N content at 45 DAS in soybean was 2.47-2.75 %.

At 60 DAS nitrogen content was significantly maximum in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10}

(100 ppm IBA) and T_3 (50 ppm chitosan) in a descending manner when compared with treatment T_1 (control) and other remaining treatments under study. While, treatments T_9 (75 ppm IBA) and T_4 (75 ppm chitosan) were also found significantly superior over treatment T_1 (control). Remaining treatments were found at par with control. The range of N content at 60 DAS in soybean was 2.40-2.65 %.

At 75 DAS nitrogen content was significantly maximum in treatment T_2 (50 ppm chitosan) followed by the treatment T_{10} (100 ppm IBA) when compared with treatment T_1 (control) and other remaining treatments under study. While, treatments T_3 (50 ppm chitosan), T_9 (75 ppm IBA) T_4 (75 ppm chitosan) and T_{11} (125 ppm IBA) were found significantly superior over treatment T_1 (control) and rest of the treatments. The range of N content at 75 DAS in soybean was 1.97-2.23 %.

From this data it is observed that leaf nitrogen content was increased up to 60 DAS and reduced thereafter at 75 DAS. The decrease in N content might be due to fact that younger leaves and developing organs, such as grains act as strong sink demand and may draw heavily N from leaves (Gardner *et al*., 1988) [19].

The above findings are consonance with the findings of Poonkodi (2003) [18]. He stated that decrease in N content in leaves might be due to translocation and utilization of nutrients for flower and pod formation in black gram.

Chitosan contain nitrogen in the basic unit of its formula ($c_{11}h_{17}o_7n_2$), which is considered one of the most important nourishing elements for plant (Sheikha and Malki, 2011) [25]. Chitosan content high percentage of nitrogen (6.89%) compared to synthetic substitute of cellulose. It was reported that chitosan improved the transportation of nitrogen in the functional leaves which enhanced plant growth and development (Chibu and Shibayama, 2003; Gornik *et al*., 2008) [5, 12]. It is an important constituent of protein, nucleic acid, prophyrins, chloroplast, etc. Similarly IBA increase the ability of cell division in meristematic zone of plant and hence ability of plant to absorb nutritive material (Ghodrat *et al*., 2012) [10]. These might be the reasons for increase in leaf nitrogen content in the present investigation.

Amin *et al*. (2013) [1] tester two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1} applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly enhanced nitrogen of chickpea (*Cicer arientinum* L.)

Sharifa (2013) [24] carried out a field experiment on common bean to study the effect of different concentrations of chitosan (100, 200 and 400 ppm) and found that foliar application of 200 ppm chitosan increased inorganic nitrogen content in leaves.

Leaf phosphorus content

Phosphorus is an important constituent of protoplasm and nucleic acid and protein also, it is essential for the formation of seed.

Data pertaining to phosphorus content in leaves were estimated at four stages of observations i.e. 30, 45, 60 and 75 DAS. Phosphorus has been recognized as an important environmental factor limiting crop growth and production. Significant results were recorded at all the stages of observations viz., 45, 60 and 75 DAS except 30 DAS.

At 45 DAS significantly more leaf phosphorus content was recorded in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) when compared with treatment T_1 (control) and other remaining treatments under study. Also, treatments T_4 (75 ppm chitosan) and T_{11} (125 ppm IBA) were found significantly superior over treatment T_1 (control) and rest of the treatments. The range of phosphorus content at 45 DAS in soybean was 0.38-0.55 %.

At 60 DAS significantly maximum leaf phosphorus content was noticed in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) when compared with treatment T_1 (control) and other remaining treatments under study. While, treatment T_4 (75 ppm chitosan) was found significantly superior over treatment T_1 (control) and other remaininig treatments. The range of phosphorus content at 60 DAS in soybean was 0.58-0.79%.

At 75 DAS leaf phosphorus content was significantly maximum in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) when compared with treatment T_1 (control) and other remaining treatments under study. Also, treatments T_4 (75 ppm chitosan) and T_{11} (125 ppm IBA) were found significantly superior over treatment T_1 (control) and other remaining treatments. The range of phosphorus content at 75 DAS in soybean was 0.43-0.63 %.

The inferences drawn from the data, it is clear that leaf phosphorus content was gradually increase up to 60 DAS and reduced thereafter at 75 DAS. The increase in phosphorus content might be because of translocation of leaf phosphorus and it's utilization for development of food storage organ.

Phosphorus is one of the most important nutrients in the growth and development of plants. It plays a key role in cellular energy transfer, respiration and photosynthesis. Phosphorus uptake decreases with decreasing soil moisture in various crops such as papper (Turner 1985) [26] and wheat (Ashraf *et al.,* 1998) [2]. The role of chitosan in increasing ionic content may be due to its effects on stabilizing cellular membranes through increasing antioxidants substances, saving cell membranes from oxidative stress and hence, improving plant cell permeability and ultimately enhances nutrient uptake. These might be the reason for increase in phosphorus content in the present study by the application of chitosan. Similarly IBA increased enzymatic activity and translocation processes from leaves to seeds, linking or converting to other plant metabolites (Amine *et al.,* 2013) [1]. These might also be the reasons for increase in leaf phosphorus content in the present investigation by the application of IBA.

Amin *et al*. (2013) [1] studied two plant growth regulators viz., putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1}, applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly increased phosphorus content of chickpea (*Cicer arientinum* L).

Deotale *et al.* (2016) [7] applied putrescine and IBA (50, 75, 100, 125 and 150 ppm each) with one control on soybean and observed that two foliar sprays of 100 ppm putrescine and 100 ppm IBA at two stages ie. before flowering and 10 days after flowering were found to be most effective in enhancing phosphorus content in leaves.

Leaf potassium content

Potassium is an essential macronutrient for plants involved in many physiological processes. It is important for crop yield as well as for the quality of edible parts of crops. Although K is not assimilated into organic matter, K deficiency has a strong impact on plant metabolism. Plant responses to low K involve changes in the concentrations of many metabolites as well as

alteration in the transcriptional levels of many genes and in the activity of many enzymes.

Data pertaining to potassium content in leaves were estimated at various stages of observations viz., 30, 45, 60 and 75 DAS. Significant results were recorded at all the stages of observations viz., 45, 60 and 75 DAS except 30 DAS.

At 45 DAS significantly maximum leaf potassium content was observed in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) when compared with treatment T_1 (control) and other remaining treatments under study. While, treatments T_4 (75 ppm chitosan) and T_{11} (125 ppm IBA) were found significantly superior over treatment T_1 (control) and rest of the treatments. The range of potassium content at 45 DAS in soybean was 1.22-1.54%.

At 60 DAS significantly maximum leaf potassium content was observed in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) when compared with treatment T_1 (control) and other remaining treatments under study. While, treatment T_4 (75 ppm chitosan) was found significantly superior over treatment T_1 (control). Remaining treatments were found at par with control. The range of potassium content at 60 DAS in soybean was 2.08-2.46%.

At 75 DAS significantly maximum leaf potassium content was recorded in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) when compared with treatment T_1 (control) and other remaining treatments under study. Also, treatments T_4 (75 ppm chitosan) and T_{11} (125 ppm IBA) were found significantly superior over treatment T_1 (control) and rest of the treatments. The range of potassium content at 75 DAS in soybean was 1.33-1.92%.

It is from the data, that leaf potassium content was increased gradually up to 60 DAS and decreased at 75 DAS. It might be because of diversion of potassium towards developing parts i.e. pods of the soybean crop at advanced stage.

Foliar application of chitosan especially CH_2 significantly increased nutrient elements and carbohydrates in plant tissue. The high cation exchange capacity of chitosan prevents nutrients from leaching. Chitosan absorbs the nutrients from chemical fertilizers and these exchanged nutrients slowly released to the plant. Similarly IBA increased enzymatic activity and translocation processes from leaves to seeds, linking or converting to other plant metabolites (Amine *et al.,* 2013) [1]. These might be reasons for increase in leaf potassium content in the present investigation by the application of chitosan and IBA.

Farouk and Amany (2012) [8] observed that foliar application of chitosan @ 250 ppm under water stress conditions significantly increased inorganic potassium content of cowpea plant.

Deotale *et al.* (2016) [7] tried two plant growth regulators putrescine and IBA (50, 75, 100, 125 and 150 ppm each) with one control on soybean and reported that two foliar sprays of 100 ppm putrescine and 100 ppm IBA at two stages ie. before flowering and 10 days after flowering were found to be most effective in increasing potassium content.

Protein content in seeds

Protein content of the seed is one of the considerable factor for seed quality determination.

Data indicated that protein content was significantly increased in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA) and T_3 (50 ppm chitosan) in a descending manner when compared with treatment T_1 (control) and other remaining treatments under study. While, treatments T_9 (75 ppm IBA) and T_4 (75 ppm chitosan) were found significantly superior over treatment T_1 (control). Treatments T_{11} (125 ppm IBA), T_5 (100 ppm chitosan), T_8 (50 ppm IBA), T_6 (125 ppm chitosan) and T_7 (25 ppm IBA) were found at par with treatment T_1 (control).

Chitosan contain NH_2 group in the basic unit of its formula which is an important constituent of protein, nucleic acid, prophyrins, chloroplast, etc. The stimulating effect of nitrogen on growth characters may be due to the major role of nitrogen on protein and nucleic acids synthesis and protoplast formation. Similarly IBA enhances enzymatic activity and translocation processes from leaves to seed (Amine *et al*., 2013) [1]. These might be the reasons for increased seed protein content in the present investigation.

Shraiy and Hegazi (2009) [21] carried out an experiment to study the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA at 25 and 35 DAS significantly increased protein content.

Sharifa (2013) [24] formulated a field experiment on common bean to study the effect of different concentrations of chitosan (100, 200 and 400 ppm) and observed that foliar application of 200 ppm chitosan increased protein content.

Yield and yield contributing parameters

Seed yield and its related parameters in soybean were influenced by the application of growth regulator which have different influence on the allocation of assimilates between vegetative and reproductive organs. In general crop yield depends on the accumulation of photo-assimilates during the growing period and the way, they are partitioned between desired storage organ of plant. In present study, it was revealed that the application of plant growth regulator significantly increased the number of pods, 100 seed weight and finally seed yield determining components in soybean.

Number of pods plant^{-1}

Data indicates that all the treatments were statistically significant over control. Number of pods plant^{-1} was significantly increased in treatment T_2 (25 ppm chitosan) followed by treatments T_{10} (100 ppm IBA) and T_3 (50 ppm chitosan) in a descending manner when compared with treatment T_1 (control) and other remaining treatments under study. Treatments T_9 (75 ppm IBA) and T_4 (125 ppm IBA) also showed their significance over treatment T_1 (control). While, treatments T_{11} (125 ppm IBA), T_5 (100 ppm chitosan), T_8 (50 ppm IBA), T_6 (125 ppm chitosan) and T_7 (25 ppm IBA) were found at par with T_1 (control).

Shraiy and Hegazi (2009) [21] studied the effect of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA at 25 and 35 DAS significantly increased number of pods plant^{-1}.

Mondal *et al.* (2013) [15] tested different concentrations of chitosan viz., 0 (control), 25, 50, 75 and 100 ppm at 25 and 35 DAS. They observed that foliar application of chitosan @ 50 ppm on mungbean significantly increased number of pods plant^{-1} over control.

100 seed weight

Data regarding 100 seed weight showed significant variation.

It was observed that foliar application of 25 ppm chitosan gave significantly higher 100 seed weight when compared with treatment T_1 (control) and remaining treatments under study. The range of increase in seed weight was 10.40 g in control (T_1) to 12.23 g in treatment receiving 25 ppm chitosan (T_2).

100 seed weight was also significantly increased in treatment T_2 (25 ppm chitosan) followed by treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan) and T_9 (75 ppm IBA) when compared with treatment T_1 (control) and remaining treatments under study. Treatments T_4 (75 ppm chitosan) and T_{11} (125 ppm IBA) also showed their significance over treatment T_1 (control). While, treatments T_5 (100 ppm chitosan), T_8 (50 ppm IBA), T_6 (125 ppm chitosan) and T_7 (25 ppm IBA) were found at par with T_1 (control).

Shraiy and Hegazi (2009) [21] tested the impact of acetylsalicylic acid (ASA) @ 10 and 20 ppm, indole-3-bytric acid (IBA) @ 50 and 100 ppm and gibberellic acid (GA) @ 50 and 100 ppm on pea (*Pisum sativum* L.). Application of ASA and IBA at 25 and 35 DAS significantly enhanced 1000 seeds weight over control.

Wagh (2015) tested different concentrations of putrescine and IBA (0, 50, 75, 100, 125 and 150 ppm) on soybean sprayed at 30 and 45 DAS. He observed that two foliar sprays of putrescine and IBA @ 100 ppm significantly increased 100 seed weight.

Seed yield plant^{-1} and plot^{-1}

Significantly maximum seed yield plant^{-1}and plot^{-1}was recorded in treatment T_2 (25 ppm chitosan) followed by the treatments T_{10} (100 ppm IBA), T_3 (50 ppm chitosan), T_9 (75 ppm IBA) and T_4 (75 ppm chitosan) when compared with control and remaining treatments under study. While, treatments T_{11} (125 ppm IBA), T_5 (100 ppm chitosan), T_8 (50 ppm IBA), T_6 (125 ppm chitosan) and T_7 (25 ppm IBA) were found at par with treatment T_1 (control).

Plant growth regulators are known to enhance the source-sink relationship and stimulate the translocation of photo-assimilates thereby helping in effective flower formation, fruit and seed development and ultimately enhance productivity of the crops. Growth regulators can improve the physiological efficiency including photosynthetic ability and enhance the effective partitioning of accumulates from source and sink in the field crops (Solamani *et al*., 2001) [22].

Bittelli *et al*. (2001) [3] reported that foliar application of chitosan decreased transpiration in pepper plants and reduce water use while maintaining biomass production and yield. The significant effect of chitosan on plant growth and yield may be attributed to an increase in the key enzyme activities of nitrogen metabolism and increased photosynthesis which enhanced plant growth (Gornik *et al.,* 2008; Mondal *et al*., 2012) [12, 15]. The increase in cowpea yield due to chitosan application may be due to its effects in stimulating physiological processes, improving vegetative growth, followed by active translocation of photoassimilates from source to sink tissue (Sharifa, 2013) [24]. Chitosan when externally supplied was observed to increase crop growth and ultimately the yield. It improves the nutritional status of plant system. Chitosan increases the absorption and translocation of nutrients in plant and ultimately influences yield.

Growth regulator IBA is proved to improve effective partitioning and translocation of accumulates from source to sink in the field crops. The plant growth regulators also increase mobilization of reserve food materials to the developing sink through increases in hydrolyzing and oxidizing enzyme activities and lead to yield increases. IBA increases the ability of cell division in meristematic zones of plant and hence, the ability of plant to absorb nutritive material which finally lead to the increase of grain yield (Ghodrat *et al*., 2012) [10].

Amin *et al*. (2013) [1] studied the effect of two plant growth regulators putrescine and Indole-3-butyric acid (IBA) @ 25, 50 and 100 mg l^{-1} applied either alone or in combinations. Spraying of putrescine and IBA @ 100 mg l^{-1} significantly increased seed yield of chickpea (*Cicer arientinum* L.).

Rabbi *et al*. (2016) [20] formulated an experiment to study the effect of chitosan (0, 25, 50, 75 and 100 ppm) on mungbean sprayed at 30 and 40 DAS. Results showed that application of chitosan @ 50 ppm significantly enhanced seed yield.

Table 1: Effect of chitosan and IBA on chlorophyll, nitrogen and phosphorus content in leaves

Treatments	chlorophyll content in leaves (mg g^{-1})				Nitrogen content in leaves (%)				Phosphorus content in leaves (%)			
	30 DAS	45 DAS	60 DAS	75 DAS	30 DAS	45 DAS	60 DAS	75 DAS	30 DAS	45 DAS	60 DAS	75 DAS
T_1 (control)	1.33	1.70	2.03	2.01	1.55	2.47	2.40	1.97	0.33	0.38	0.58	0.43
T_2 (25 ppm chitosan)	1.36	2.04	2.34	2.32	1.57	2.75	2.65	2.23	0.35	0.55	0.79	0.63
T_3 (50 ppm chitosan)	1.32	1.98	2.27	2.24	1.53	2.68	2.58	2.13	0.34	0.51	0.71	0.59
T_4 (75 ppm chitosan)	1.44	1.86	2.21	2.18	1.56	2.61	2.51	2.08	0.37	0.47	0.68	0.54
T_5 (100 ppm chitosan)	1.28	1.78	2.15	2.13	1.59	2.55	2.45	2.05	0.32	0.44	0.65	0.51
T_6 (125 ppm chitosan)	1.35	1.75	2.12	2.11	1.54	2.52	2.42	2.01	0.34	0.42	0.61	0.48
T_7 (25 ppm IBA)	1.43	1.72	2.09	2.08	1.53	2.49	2.41	1.99	0.31	0.41	0.60	0.46
T_8 (50 ppm IBA)	1.38	1.76	2.13	2.12	1.55	2.54	2.44	2.03	0.34	0.42	0.63	0.50
T_9 (75 ppm IBA)	1.39	1.92	2.23	2.20	1.50	2.63	2.54	2.10	0.32	0.48	0.70	0.55
T_{10} (100 ppm IBA)	1.30	1.99	2.28	2.26	1.58	2.71	2.62	2.17	0.31	0.52	0.74	0.60
T_{11} (125 ppm IBA)	1.45	1.83	2.20	2.16	1.57	2.59	2.48	2.06	0.36	0.45	0.67	0.52
SE (m) ±	0.066	0.043	0.035	0.038	0.057	0.039	0.025	0.027	0.016	0.022	0.028	0.027
CD at 5%	-	0.126	0.103	0.112	-	0.114	0.074	0.080	-	0.064	0.082	0.080

Table 2: Effect chitosan and IBA on leaf potassium content, seed protein, number of pods plant^{-1}, 100 seed weight, seed yield plant^{-1} and plot^{-1}

Treatments	Leaf potassium content (%)				Protein content (%)	No. of pods plant^{-1}	100 seed weight (g)	Seed yield plant^{-1} (g)	Seed yield plot^{-1} (kg)
	30 DAS	45 DAS	60 DAS	75 DAS					
T_1 (control)	0.98	1.22	2.08	1.33	36.09	76.46	10.40	7.07	0.71
T_2 (25 ppm chitosan)	1.09	1.54	2.46	1.92	42.12	118.73	12.23	9.03	0.90
T_3 (50 ppm chitosan)	1.18	1.46	2.39	1.77	40.04	103.13	11.70	8.78	0.87
T_4 (75 ppm chitosan)	1.12	1.39	2.28	1.66	38.47	97.53	11.43	8.51	0.85
T_5(100ppm chitosan)	0.99	1.33	2.19	1.56	37.98	90.03	11.10	8.00	0.79
T_6 (125 ppm chitosan)	1.11	1.28	2.14	1.48	37.32	84.46	10.83	7.75	0.77
T_7 (25 ppm IBA)	1.07	1.24	2.11	1.39	37.05	81.07	10.57	7.58	0.76
T_8 (50 ppm IBA)	1.02	1.31	2.15	1.52	37.56	87.90	11.03	7.84	0.78
T_9 (75 ppm IBA)	0.97	1.43	2.33	1.69	39.12	99.20	11.57	8.65	0.86
T_{10} (100 ppm IBA)	1.15	1.49	2.41	1.88	40.78	109.47	11.80	8.87	0.88
T_{11} (125 ppm IBA)	1.08	1.37	2.26	1.60	38.17	92.87	11.27	8.28	0.82
SE (m) ±	0.064	0.039	0.046	0.081	0.703	5.445	0.235	0.246	0.022
CD at 5%	-	0.114	0.136	0.238	2.074	16.064	0.694	0.726	0.066

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