Indian Journal of Plant Sciences ISSN: 2319–3824(Online)
An Open Access, Online International Journal Available at http://www.cibtech.org/jps.htm
2017 Vol.6 (2) April-June, pp.1-11/Fazili et al.
Research Article

EFFECT OF DIFFERENT TREATMENTS OF GAMMA-IRRADIATED SODIUM ALGINATE (ISA) ON GROWTH AND BIOCHEMICAL COMPOSITION OF *MENTHA ARVENSIS* L.

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ABSTRACT
Large numbers of essential oil bearing plants are the important sources of therapeutic agents used for medicinal purposes. Natural polysaccharides like sodium alginate, chitosan, carrageenan after undergoing depolymerization brought about various plant growth accelerating effects. Sodium alginate after irradiating with Co-60 gamma rays has proven to be an important growth promoter for a varied agriculture crops and allied fields. Therefore, the present study was aimed as to study the effect of different fractions of irradiated sodium alginate (ISA) on plant growth, physiological and biochemical parameters and essential oil production of *Mentha arvensis* L. after 120 days of plantation (DAP). The fractions of sodium alginate were obtained by the application of column chromatography using Sephadex as a stationery phase. Out of seven spray treatments, T4 proved to be highly effective as it enhanced the growth attributes by over 53.53%, physiological and biochemical parameters by over 24.37% respectively over the control in *Mentha arvensis* L. However, with the foliar spray of T5 there was a gradual decrease in growth, physiological, biochemical values as compared to control.

Keywords: *Mint, Fractions, Sephadex, Column Chromatography*

INTRODUCTION
Out of large number of essential oil bearing plants, mint serves a good source of oil containing crop. Besides this, the essential oils extracted from some of the medicinal plants have been used in synthesis of organic compounds of high economic value (The Wealth of India, 1992; Sangwan *et al.*, 2001). *Mentha arvensis*is L. exported on large scale from India along with its processed derivative and because of its insecticidal properties, the natural menthol (Misra *et al.*, 2000). *Mentha arvensis* L. is cultivated in India in the foot hills of Himalaya and Punjab, Bihar, Uttar Pradesh and Himachal Pradesh. India is also the largest mint oil producer, with annual approximate production of 15000 – 20000 tons (Chand *et al.*, 2004).
Of the total world production of essential oils, 15 products constitute 90 per cent of the total, *Mentha arvensis* L. and *Mentha piperita* L. (peppermint) (Waterman, 1993). *Mentha arvensis* L. belongs to family Lamiaceae has achieved the commercial importance for its essential oil derivatives.
Degraded polysachharides (oligomers) when applied to the plants promote various developmental processes in plants including physiological, biochemical and growth parameters (Hien *et al.*, 2000; Abad *et al.*, 2009; Aftab *et al.*, 2011; Khan *et al.*, 2011; Sarfaraz *et al.*, 2011; Naeem *et al.*, 2012a, b; Relleve *et al.*, 2000).
Natural polysaccharides like chitosan, carrageenan and sodium alginate are known to be the one of the best plant potent enhancers and effective in defensive responses (John *et al.*, 1997; Mercier *et al.*, 2001). Enhanced effects of alginates derived oligosaccharides were found in maize seed germination (Hu *et al.*, 2004). The chemical compound sodium alginate is the sodium salt of alginic acid. A colorless or light yellow powdery or crystalline polysaccharide, sodium alginate is a gum, extracted from the cell walls of brown algae.

MATERIALS AND METHODS

Plant Materials, Location of Cultivation and Growth Conditions

The Pot experiments, were conducted under controlled conditions at Department of Botany, Aligarh Muslim University, Aligarh.

Each earthen pots (25 cm diameter x 25 cm height) was filled with 5 Kg homogenous mixture of soil and organic manure in the ratio of 4:1. The soil analysis was done at agricultural farms soil testing unit (Quarsi, Aligarh, U.P., India). The soil used in the study contains following physico-chemical properties (Table 1).

Table 1: Physio-Chemical Properties of Soil

Parameters	Values
Texture	Sandy Loam
pH	7.5
Conductivity dsm^{-1}	0.58
Available Nitrogen (mg/Kg Soil)	97.40
Available Phosphorous(mg/Kg Soil)	142.00
Available Potassium (mg/Kg Soil)	7.06

A uniform recommended basal dose of N, P and K (25:11:21 mg Kg^{-1} soil, respectively) was applied to the soil in the form of urea, potassium di-hydrogen orthophosphate and muriate of potash respectively, at the time of planting. The experiment was performed in simple randomized block design with each treatment has a minimum of 5 replicates including the control. The pots were watered as and when required.

Column Chromatography

Column chromatography in chemistry is a method used to purify individual chemical compounds from mixtures of compounds. The column was loaded with Sephadex G-25 as a stationery phase and double distil water (DDW) was used as a mobile phase. The solution of irradiated sodium alginate was poured in the column for isolation of the oligosaccharides (in form of fractions) with difference in molecular weights.

A 80 ppm concentration was prepared by dissolving 8mg of the ISA in 100 ml Double Distil Water (DDW). Optical Density of oligosaccharides isolated was noted at 280 nm and the fractions resembling in Optical Density were pooled together with the help of graphical representation.

Infra-Red Spectroscopy Analysis

The Infra-Red Spectroscopy analysis of the seven treatments of irradiated sodium alginate was done in the instrumentation centre (Department of Chemistry), AMU, Aligarh, UP.

Estimation of Growth Attributes

Standardized plant suckers of *Mentha arvensis* L. (var. Kushal) was brought from Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. The experiments were conducted in the randominised block design in earthen pots (25 cm diameter x 25 cm height). The pots were placed in the net house of the Department of Botany, Aligarh Muslim University, Aligarh.

Five replicates of each treatment were planted in the fields. The first foliar spray was given 40 days after sowing (DAS). At least, five foliar sprays were given after the halt of two weeks. The plants were uprooted carefully before they were dried in hot air oven at 80° C for 24 h before recording of the plant dry weight. The response of crops was analyzed in terms of the growth parameters, physiological and biochemical parameters, essential oil content and yield of oil. The plant growth parameters plant height, plant fresh weight, plant dry weight, leaf area per plant, leaf yield per plant were determined after 120 days.

The leaf area was calculated using graph paper sheet (Watson, 1958). The mean area per leaf, thus determined, was multiplied with the total number of leaves to measure the total leaf area per plant. Yield attributes were recorded at the time of harvest.

Estimation of Physiological and Biochemical Attributes

Estimation of Total Chlorophyll and Carotenoid Contents

Using the method of **Mac Kinney (1941) and Machlachlan and Zalik (1963)** total content of leaf chlorophyll and carotenoids was estimated. Using 80 % acetone, the fresh tissues from their interveinal area of leaves were grinded using mortar- pestle. Following the grinding, the optical density were recorded at 663 nm, 645 nm and 470 nm to determine chlorophyll a, chlorophyll b and total carotenoid content, respectively using the spectrophotometer (Shimadzu UV-1700, Tokoyo, Japan). The total chlorophyll content was also calculated. These photosynthetic pigments were measured in mg g^{-1} leaf FW.

Estimation of Carbonic Anhydrase (CA) Activity

The method described by Dwivedi and Randhawa (1974) was used to estimate the activity of carbonic anhydrase in fresh leaves of the plants. 200mg of fresh leaves were transferred to Petri plates. In10 ml of 0.2 M cysteine hydrochloride solution, the leaf pieces were dipped and kept for 20 minutes at 4°C. The leaf pieces were dipped in 1mL of 0.2 M cystein hydrochloride solution for 20 min at 4°C. The solution appearing at the cut surfaces of the leaf pieces was then removed with the help of a blotting paper followed by their transfer immediately to a test tube containing 4 mL of phosphate buffer of pH 6.8. To it, 4 mL of 0.2 M sodium bicarbonate solution and 0.2 mL of 0.022 bromothymol blue were added. The reaction mixture was titrated against 0.05 N HCl using methyl red as indicator. The enzyme activity was expressed as M CO2 kg^{-1} leaf FW s^{-1}.

Estimation of Nitrate Reductase Activity

Nitrate reductase activity was estimated by the intact tissue assay method developed by **Jaworski (1971).** The reaction is based on the reduction of nitrate to nitrite according to the following biochemical reaction:

$$NO^-_3 + NADH + H^+ \rightarrow NO^-_2 + NAD^+ + H2O$$

The total amount of nitrite (NO_2^-) formed was determined spectrophotometrically. Fresh pieces of chopped leaves (200mg) were transferred and kept in plastic vial. The reaction mixture that was contained in every vial, carried 2.5 mL of phosphate buffer (pH 7.5), 0.5 mL of 0.2 M potassium nitrate solution were also added followed by the addition of 2.5 mL of 5% isopropanol. The increase in leaf- N and leaf- P content mediate increase in the uptake of variety of nutrients and thus result in the increment in NR activity when mantained in controlled conditions. The vials were thus kept for incubation for almost 2 h in dark at 30°C for the maximum enzyme activity. After incubation, 0.4 mL of the total content were transferred from the vial to the test tube. In addition to it, 0.3 mL solution each of 1% sulfanilamide and 0.02% N-(1-naphthyl) ethylenediamine dihydrochloride (NED–HCl) was added and were thus kept for almost 20 minutes at room temperature for color development. The O.D was recorded at 540 nm using the spectrophotometer. The NR activity was expressed as nano moles of nitrite produced per gram of fresh weight of leaf tissue per hour (nM NO_2^- g^{-1} FWh^{-1}).

Statistical Analysis

Five replicates of each treatment were present in the pots. SPSS-17 statistical software (SPSS Inc., Chicago, IL, USA) was used for data analyses. Means were compared using Duncan's Multiple Range Test (DMRT) at $p \leq 0.05\%$. Standard error (± S.E.) was incorporated to isolate the means in the tables.

RESULTS AND DISCUSSION

Plant Growth Parameters

It was revealed from the results (Table 2) that foliar application of various treatments of irradiated sodium alginate enhanced the growth attributes such as plant height, fresh weight, dry weight, leaf area per plant,

leaf-yield per plant in *Mentha arvensis* L. Application of T4 of irradiated sodium alginate (ISA) proved to be the best followed by treatments T5>T3>T2 and T1 respectively as compared to control (T7) which showed poor response in comparison to the above treatments. The overall increase in plant height over the control was by 47.82%, leaf area of the plant over the control by 43.96%, leaf yield of the plant over the control by 56.00%, fresh weight of the plant over the control by 51.45%, dry weight of the plant over the control by 68.42%, respectively. In a similar study the effect of natural polysaccharides such as sodium alginate, carrageenan, and chitosan has been positive for plant growth parameters (Abad *et al.*, 2009; Aftab *et al.*, 2011; Hegazy *et al.*, 2009; Hien *et al.*, 2000; Hu *et al.*, 2004; Khan *et al.*, 2011; Kume *et al.*, 2002; Luan *et al.*, 2003; Mollah *et al.*, 2009; Natsume *et al.*, 1994; Relleve *et al.*, 2000; Sarfaraz *et al.*, 2011; Tomoda *et al.*, 1994). The application of treatments (T4) spray showed the optimal results for growth parameters which drastically decreased with T5. The least response was observed in the control plants which showed the minimum plant growth and development. Exogenous factors along with various plant growth promoters are directly or indirectly involved in growth and development of the plant. Oligosaccharide such as carrageenan in association with the endogenous growth elicitors may function as a signal to initiate the biosynthesis of different enzymes and activate many responses along with exploiting of gene expression (Ma, Li, Bu, & Li, 2010) . There has been enough evidence in regard to the growth-promoting effect of irradiated sodium alginate (Sharma *et al.*, 2006; Khan *et al.*, 2007, 2009; Naeem *et al.*, 2009, 2011, 2012c; Swamy and Rao, 2006, 2008, 2009, 2011; Talaat and Abdallah, 2011; Naeem *et al.*, 2012d), indicating that oligosaccharides like irradiated sodium alginate increase the plant height and dry matter accumulation in various plants.

Table 2: Effect of Foliar Spray of Irradiated Sodium Alginate (Fractions) on Growth Parameters of Mint (*Mentha arvensis* L.) at 120 Days after Plantation

Growth Attributes	Treatment Concentrations (80 ppm)						
	T1	**T2**	**T3**	**T4**	**T5**	**T6 (Unfractionated SA)**	**T7 (DDW) Control**
Plant Height (cm)	90.61 ± 1.59^{d}	97.81 ± 1.48^{c}	100.01 ± 1.16^{bc}	114.00 ± 1.28^{a}	104.00 ± 1.73^{b}	84.44 ± 1.91^{e}	77.12± 1.86^{f}
Leaf area	3447.04 ±2.48^{c}	3183.15 ± 33.55^{d}	3456.06 ± 13.76^{c}	4004.85 ±21.92^{a}	3730.99 ±10.68^{b}	2929.31 ±19.58^{e}	2781.90 ± 3.80^{f}
Leaf- yield (g)	20.63 ±0.24^{d}	19.08 ± 0.09^{e}	21.77 ±0.18^{c}	24.43 ±0.27^{a}	22.71 ±0.25^{b}	17.60 ± 0.26^{f}	15.66 ±0.21^{g}
Fresh Weight (g)	61.31 ± 1.26^{cd}	64.46 ± 1.88^{c}	70.20 ± 1.52^{b}	75.12 ± 4.03^{a}	68.34 ± 1.99^{b}	56.44 ± 1.45^{e}	49.52 ±2.67^{f}
Dry-Weight(g)	11.20 ±0.50^{cd}	12.60 ± 0.58^{bc}	13.10 ± 0.36^{b}	16.00 ± 0.64^{a}	11.02± 0.74^{c}	10.10 ± 0.46^{d}	9.50 ± 0.73^{d}

Physiological and Biochemical Parameters
Chlorophyll and Carotenoid Conent

The earlier studies observed the effect plant growth regulators and degraded polysaccharides on various physiological, biochemical and growth parameters in plants. It was observed that the sodium alginate which belong to the same class of Polysaccharides along with Carrageenan, Chitosan have tremendous growth, physiological and biochemical effects on the plants when given a foliar spray to them (Hien *et al.*, 2000; Aftab *et al.*, 2011; Khan *et al.*, 2011; Sarfaraz *et al.*, 2011; Naeem *et al.*, 2012a, b). It was revealed from the results that the foliar application of T4 significantly increased the Chlorophyll content (Chlorophyll a and Chlorophyll b) of *Mentha arvensis* L. followed by T5>T3>T2>T1>T6>T7. The

profound increase was seen in the total Chlorophyll content in plants by over 34.65% and Carotenoid content by 27.14% as compared to the control after 120 days of plantation followed by T5 which showed the total increase in Chlorophyll content (Chlorophyll a and Chlorophyll b) by 29.70% over the control. Increase in the Chlorophyll content due to the foliar spray of irradiated sodium alginate may be concorded to the irradiated sodium alginate effect on photosynthesis and overall growth of the plant. The Carotenoid content showed the maximum increase of 27.14% at T4 as compared to control followed by T5 that showed the total increase in Carotenoid content by 22.25% over the control in the experimental plants. As earlier studies suggest the positive effect of irradiated sodium alginate regarding photosynthetic pigments and rate of net photosynthesis (Aftab *et al.,* 2011; Khan *et al.,* 2011; Sarfaraz *et al.,* 2011). It has also been reported that irradiated sodium alginate mediate various physiological processes in plants, induce cell signaling, improving the irradiated sodium alginate mediated content of photosynthetic pigments and net photosynthetic rate (Farmer *et al.*, 1991).

Nitrate Reductase Activity

A promising increase in leaf-Nitrate Reductase activity by irradiated sodium alginate was observed in the plants in the (Table 2). This could be related to the increase in leaf –N and leaf – P content that might have possibly increased the concentration of nitrate in leaves. As reported by Hewitt & Afridi, (1959) the substrate nitrate essentially induces functional NR activity and thus producing nitrate sensing protein of unknown nature (Campbell, 2002). The application of T4 proved to be the best which showed maximum Nitrate Reductase acivity of 25.86% as compared to the control followed by T5 which showed the increase in Nitrate Reductase activity by22.43% over the control. The results obtained are in resemblance with finding of Naeem *et al.,* (2011), Aftab *et al.,* (2011) relating the irradiated sodium alginate mediated increase in overall plant growth and yield and quality parameters in *Artimisia annua* L. Thus, it can be maintained that the irradiated sodium alginate enhanced the NR activity because it improved the leaf- N, and – P contents.

Carbonic Anhydrase Activity

Carbonic anhydrase activity is one of the most abundant zinc containing proteins in plants. Its active role is found in photosynthesis, that is evident from its presence in photosynthesizing tissue. It catalyses the reversible hydration of CO_2 to RuBisCO in photosynthesis (Badger & Price, 1994). As obvious from the results (Table 3), T4 proved to be the optimal for plant growth which showed maximum CA activity of 34.24% over the control and it gradually decreased due to treatment (T5) with minimum Carbonic anhydrase activity recorded at (Control). Such a plant response to irradiated sodium alginate application is expected because the depolymerized natural polysaccharides have been reported to increase the stomatal conductance significantly (Naeem *et al.,* 2011) which might facilitate the diffusion of additional amount of CO2 through the stomata to be acted upon by Carbonic anhydrase, resulting in the enhanced Carbonic anhydrase activity. ISA- mediated de novo synthesis of Carbonic anhydrase, involve transcription/ translation of the genes associated as has been reported for the other natural polysaccharides (Knowles & Ries, 1981). Hopefully, the enhancement of Carbonic anhydrase activity in the plants treated with ISA might be responsible for the increase rate of CO_2 fixation which could have resulted in the increase in fresh weights and dry weights of the plants. Therefore, regarding ISA- mediated Carbonic anhydrase activity our findings are similar to those that claimed to the synthesis of certain enzymes in tissue culture as a result of application of natural polysachharides (Patier *et al.*, 1995; Akimoto *et al.*, 1999). Thus, the observed effect of ISA on the CA activity might be concorded with the previous findings of combined effect of a number of underlying facts associated with irradiated sodium alginate - mediated stimulation of physiological attributes.

Table 3: Effect of Foliar Spray of ISA Fractions on Physiological and Biochemical Parameters of Mint (*Mentha arvensis* L.) at 120 DAP

Physiological and Biochemical Parameters	Treatment /Concentration (80ppm)						
	T1	**T2**	**T3**	**T4**	**T5**	**T6 (Unfractinated Sodium Alginate)**	**T7 (DDW) Control**
Total Chlorophyll Content	1.19 ±0.01^{de}	1.23± 0.01^{cd}	1.27± 0.02^{bc}	1.36±0.02^{a}	1.31± 0.02^{b}	1.15 ±0.01^{e}	1.01±0.02^{f}
Total Carotenoid Content	0.44 ±0.03^{cd}	0.46± 0.04^{bc}	0.49±0.02^{b}	0.52±0.02^{a}	0.50± 0.02^{bc}	0.43 ±0.01^{de}	0.41±0.01^{e}
Carbonic Anhydrase Activity	245.00 ±1.74^{d}	262.00± 1.50^{c}	275.11± 2.04^{b}	285.00 ± 1.56^{a}	267.00 ±0.80^{bc}	234.21 ±2.46^{e}	212.30 ±1.07^{f}
Nitrate Reductase Activity	341.05 ±2.93^{e}	358.23 ±2.29^{d}	384.31±1.90^{c}	404.40 ± 1.91^{a}	393.80 ±2.92^{b}	332.40 ±1.33^{f}	321.10 ±2.93^{g}

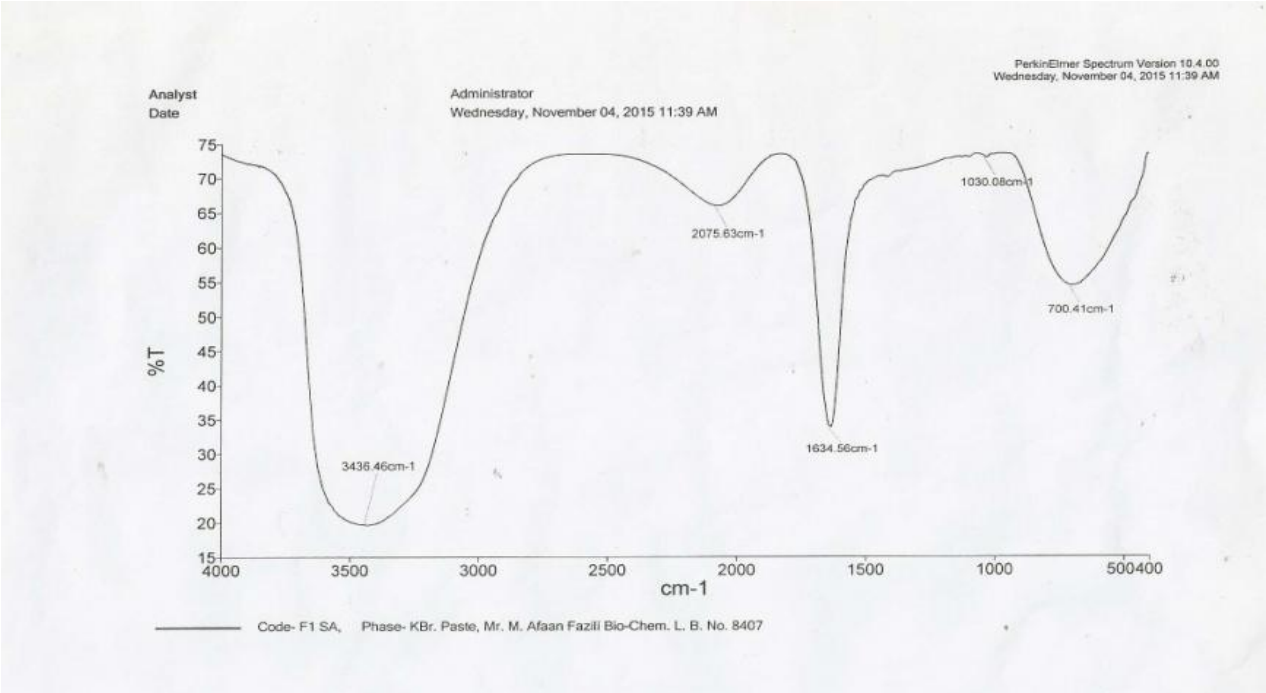

Figure I: IR Report of Treatment 1

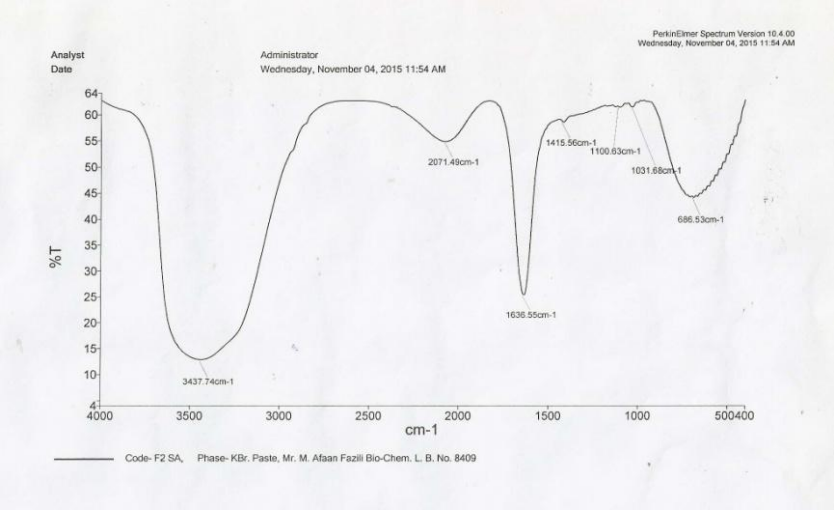

Figure II: IR Report of Treatment 2

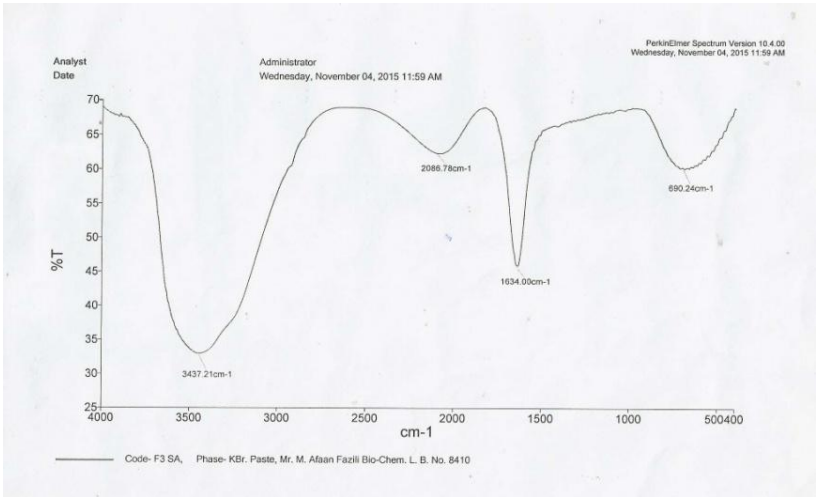

Figure III: IR Report of Treatment 3

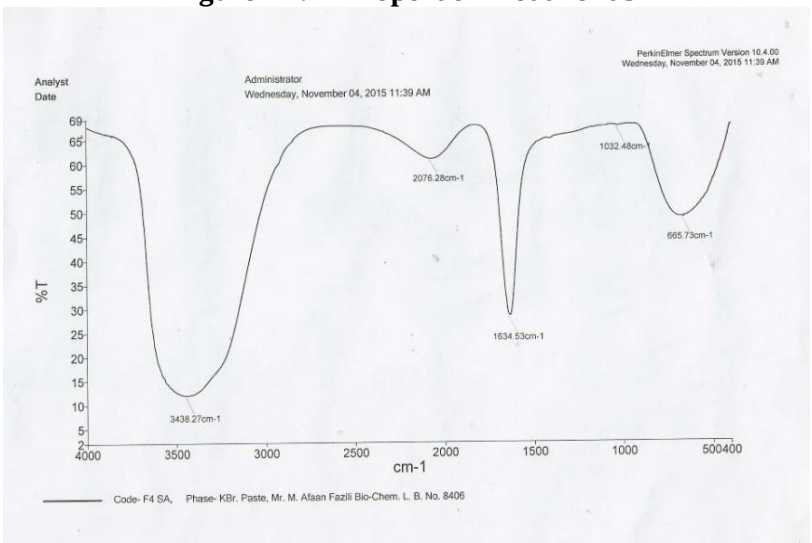

Figure IV: IR Report of Treatment 4

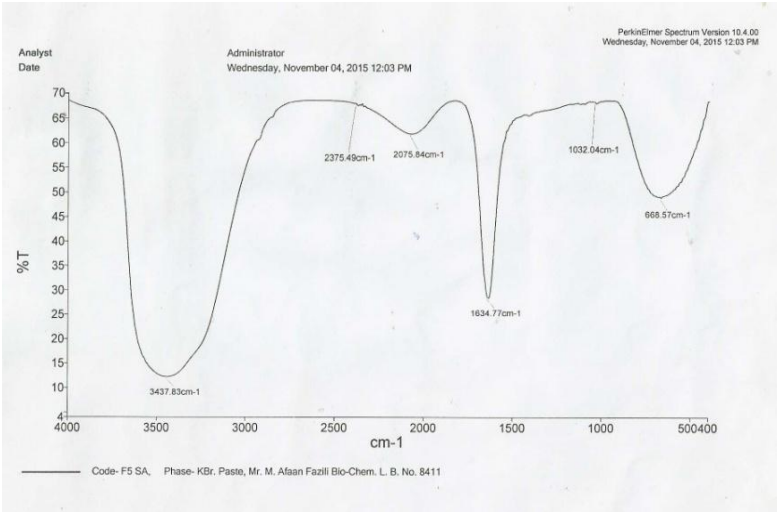

Figure V: IR Report of Treatment 5

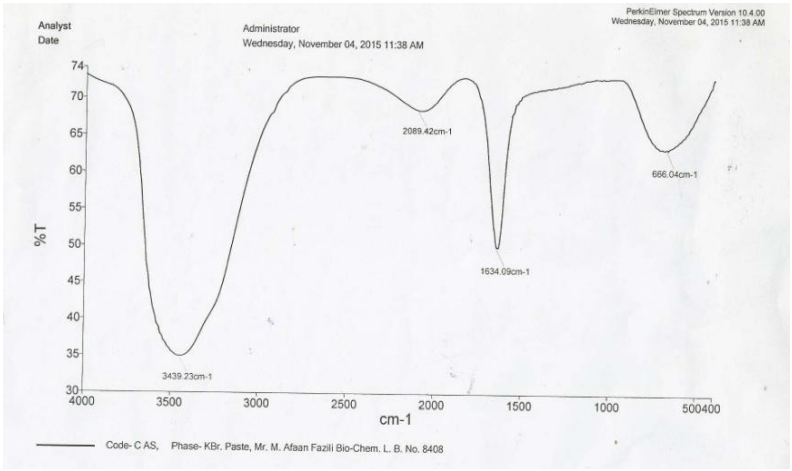

Figure VI: IR Report of Treatment 6 (Unfractionated Sodium A lginate)

Characterization of the treatments by Infra- Red Spectroscopy

Infra-Red Spectroscopy (IR) quantification and characterization of the treatments of 229 irradiated sodium alginate was done. The variation in peaks was noticed in the range of 400 - 4000 cm^{-1} for each treatment which are shown in the figures (I – VI) below. However, no IR analysis was performed for the Control (Treatment 7, Double Distilled Water) which shows disordered peaks in the range of 400- 4000 cm^{-1}.

Conclusion

The application of foliar sprays of irradiated sodium alginate resulted in enhancing the overall growth characteristics, physiological and biochemical parameters along with the improvement of essential oil content, essential oil yield and herbage yield. Among the various treatments T4 proved to be the optimal for overall growth, improvement in physiological and biochemical attributes over the Control. The trend in growth, physiological and biochemical parameters decreased with the increase in treatments like T5, T6. The fractionation of irradiated sodium alginate via column chromatography was done for the first time in order to isolate the exact polysaccharides which enhance the overall crop productivity, growth, physiological and biochemical attributes in the experimental plants and recommend it for further experimentation for the researchers. This research will help scientists to identify and isolate the actual

structure and compounds from the best fractions of irradiated sodium alginate. Besides, this technique is highly safe and non-hazardous as we used gamma radiations only to depolymerize the sodium alginate rather exposing the experimental plants.

ACKNOWLEDGEMENTS
The author is highly thankful to UGC, New Delhi, India for providing fellowship for the first author. The author is also thankful to CIMAP, Lucknow for providing the plant materials.

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