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EFFECT OF CHITOSAN OLIGOSACCHARIDE, ALGINATE OLIGOSACCHARIDE AND OLIGOGALACTURONIDE ON MORPHO PHYSIOLOGICAL TRAITS IN SOYBEAN UNDER FIELD CONDITIONS

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ABSTRACT

Oligosaccharides have been proved effective in promoting plant growth in a number of crops by boosting their secondary metabolites. However, the research of using various oligosaccharide in qualitative traits improvements for soybean is limited. Therefore, our study was conducted to explore the effect of different oligosaccharides on growth, yield, protein, and oil content in soybean. Two field experiments were conducted in 2021 and 2022 at the Bangladesh Institute of Nuclear Agriculture (BINA) Mymensingh, Bangladesh. Three oligosaccharides namely Chitosan oligosaccharides (COS), Alginate oligosaccharides (AOS) and Oligogalacturonides (OGA) were applied with 50 ppm, 70 ppm and 100 ppm on Binasoybean-2, respectively. In both years, higher soybean yield was obtained from different concentrations of OGA, which increased nearly 7-12% in 2021 and 10-11% in 2022 over control. Moreover, 100 ppm OGA provided a seed yield was 2639 kg ha^{-1}, which was the most optimal concentration for yield than other treatments. Oligosaccharides are all able to enhance protein content, and 100 ppm AOS (40.77%) produces the highest protein content which is 16-17% higher than control at 2022. Furthermore, the oil content shows a similar trend to the protein content, and the highest oil content was still generated by 100 ppm AOS (19.78%). Among the fatty acids, maximum oleic acid was obtained from 50 ppm COS (3.43%) and linoleic acid from 100 ppm COS (28.67%). Taken together, the use of oligosaccharide might be a sustainable approach to maximize soybean yield and quality, OGA has been shown to significantly increase soybean yields, with a recommended application concentration of 100 ppm.

Key words: Oligosaccharides, soybean, protein, oil and fatty acid.

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INTRODUCTION

Soybean (Glycine max L.) is recognized as the best source of plant-based high-quality proteins and oils, which is extensively cultivated in many parts of the world (Abdelghany *et al.,* 2020), and the market value is expected to reach US$255.3 billion by this year (https://apps.fas.usda.gov/psdonline/circulars/oilseeds.pdf). The primary components of soybean seed oil include various fatty acids, such as saturated palmitic acid (PA), stearic acid (SA), unsaturated oleic acid (OA), linoleic acid (LA), and linolenic acid (LNA). Among them, LA and LNA oxidized easily, leading to cardiovascular diseases. On the other, monounsaturated fatty acid OA has good autoxidative stability and increases the oil quality. Therefore, from a technological and nutritional perspective, soybean oil with a higher OA and lower LA has become an important topic in scientific research (Liu *et al.,* 2022). Fatty acid synthesis is considered an important step in seed oil formation, and compared to the model plant *Arabidopsis*, soybean has 63% more genes involved in this process, posing a challenge for breeders to improve genetic materials enriched in fatty acids for soybean. However, along with genetic background, management practices can also regulate the metabolic pathway of fatty acid composition by controlling phytohormone activities (Scilewski da Costa Zanatta *et al.,* 2017). So, management practices like the use of plant growth regulators (PGR) or elicitors might be an alternative that also promotes various secondary metabolites in plants (Rao *et al.,* 2002; Zhao *et al.,* 2020). The efficiency of plant growth regulators (PGRs) or elicitors largely depends on the application methods, which include foliar application, drenching, seed priming, pasting, capillary string, and injection (Sajjad *et al.,* 2017). Among various management practices, foliar spraying ensures uniform application, rapidly boosts crop growth, and allows multiple applications to achieve specific objectives (Gutte *et al.,* 2018). Moreover, the overuse of synthetic type growth promoters led to serious environmental and human health problems (Boussemart *et al.,* 2013). This highlights the need for eco-friendly plant growth regulators to improve crop yield and quality (Yang *et al.*, 2021).

In recent years, the use of oligosaccharides as natural biostimulants in crop cultivation has gained significant attention (He *et al.,* 2021; Rajib *et al.,* 2024 and Jiang *et al.,* 2024). Among the various oligosaccharides, our focus is on COS, AOS and OGA. COS is obtained from hydrolysis or degradation of chitosan. Recent studies found that foliar spray of COS could increase vegetative growth by enhancing photosynthesis (Jia *et al.,* 2020), yield and biochemical contents in plants (Ramakrishna *et al.,* 2017). In addition, COS draws the researcher's attention due to its excellent physical properties, higher solubility, biocompatibility and nontoxicity. On the other hand, biologically active OGA are derived from homogalacturonan, a major element of cell wall pectin (Howlader *et al.,* 2020). The exogenous application of OGA enhances the biological nitrogen fixation capacity of beans (Lara-Acosta *et al.,* 2023), seedlings growth of tomato (Zhou *et al.,* 2023) and the yield of cauliflower (Rajib *et al.,* 2024). Meanwhile, AOS was also given much attention because of its uncommon properties, low molecular weight, higher solubility and coating function, and it was extracted through hydrolysis of alginate (Acevedo *et al.,* 2012; Liu *et al.,* 2019). Previous research supported that AOS can increase the yield of rice (Zhang *et al.,* 2014) and wheat (Yang *et al.,* 2021). Nevertheless, the research related to the foliar application of more than one oligosaccharide on a single crop was very limited. In addition, the role of oligosaccharides on the qualitative characteristics of soybean was also not studied earlier. Therefore, our research aimed to find out the effect of oligosaccharides (COS, OGA and AOS) on the yield and quality parameters of soybean.

MATERIALS AND METHODS

Study Area: The experiment was conducted at the research field of the Bangladesh Institute of Nuclear Agriculture (BINA) Mymensingh, Bangladesh during 2021 and 2022. The soil of the study area was silty loam and belongs to Agro-Ecological Zone 9 under the general soil of non-calcareous dark gray at sonatola series. The soil of the experimental area was dominated by comparatively lower organic matter (1.44%) with 0.07% total nitrogen, 23.52 µg-1g soil available phosphorus, 0.40 meq-1100g soil potassium and 20.02 $\mu g^{-1}g$ soil sulphur (Kumar *et al.,* 2018).

Treatments with experimental procedure: Three different oligosaccharides (AOS, COS and OGA) obtained from the Dalian Institute of Chemical Physics (DICP), Chinese Academy of Sciences, Dalian 116023, China, were used with three different concentrations (50 ppm, 75 ppm and 100 ppm). All three oligosaccharides are water soluble. To prepare a 1000 ppm stock solution, 1 g of each oligosaccharide was dissolved in 1 L sterile deionized water and then a working solution was prepared from this stock solution. Binasoybean-2, one of the leading soybean varieties of Bangladesh was used to observe the effect of various oligosaccharides. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. The first experiment was conducted from 15th July 2021 to 20th November 2021. During this period, temperatures ranged varied from 32^0C to 17^0C, with an average of 26.6^0C (Figure 1).

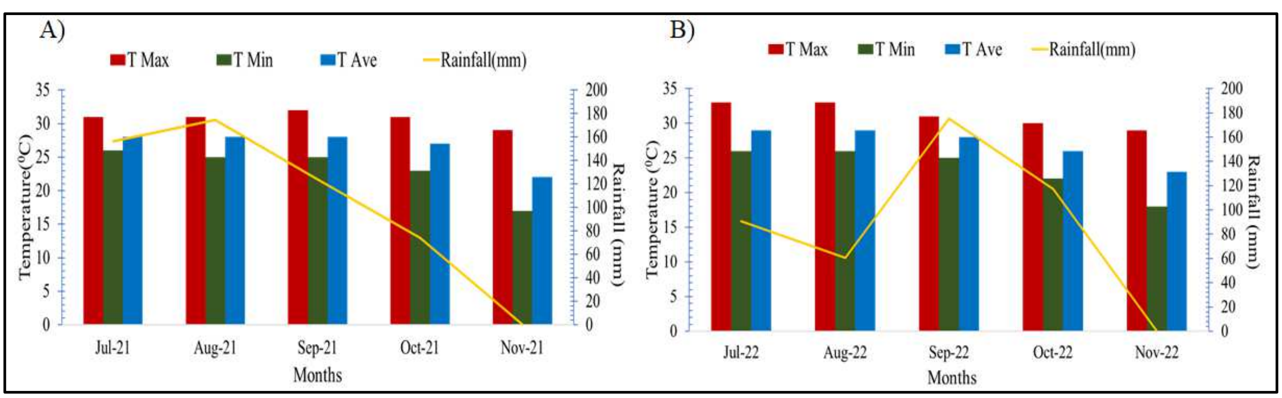

Figure 1. Meteorological condition of the experiment periods. Maximum temperature (T Max), minimum temperature (T Min), average temperature (T Ave) and rainfall scenario of the experimental area during study period 2021(a) & 2022(b).

The highest rainfall (in mm) was recorded during the first two months, peaking at the early vegetative stage. For the second trial, conducted from 15th July 2022 to 24th November 2022, the temperature variation was similar to the previous year. However, the lowest rainfall for both years was recorded in November (source: www.worldweatheronline.com/mymensingh).

In each year, freshly prepared oligosaccharide was applied at the R2 (full bloom) and R5 (seed formation) stages at a rate of 200 L ha^{-1}. The control was set by spraying an equal amount of distilled water (without any oligosaccharides). The unit plot size was 12 m^2 (4 m × 3 m), with plant spacing of 8-10 cm within rows and 25 cm between rows. Additionally, a distance of 0.5 m was maintained between plots and 1 m between blocks. Recommended management practices were followed to ensure proper growth and development, while fertilization was carried out according to the fertilizer recommendation guide (Ahmmed *et al.,* 2018).

Assessments of quantitative data: Various yield controlling morpho-physiological traits such as plant height (cm), number of branches $plant^{-1}$ (no), pods $plant^{-1}$ (no), pod length (cm), hundred seed weight (g) and seeds pod^{-1} (no) were considered here. In both years, the maturity was considered when the plants and pods in each plot turned yellowish or straw-colored, and nearly all the leaves had fallen. To collect data, ten plants were chosen from each plot following random sampling techniques without replacement (Gomez and Gomez, 1984), and the remaining whole plants were harvested together to determine seed yield (converted to kg ha^{-1}). Moreover, 500 g of seeds from each treatment were sampled for future qualitative analysis.

Assessments of qualitative data: Protein and oil percentage were considered as the quality parameters of this study. The oil content was estimated using a modified version of the method described by King *et al.,* (1997). Ten grams of dry seeds were ground using a mortar and pestle. The powdered sample was transferred to an extraction thimble by adding 100 mL of petroleum benzene. The total weight of the thimble with the sample was measured and recorded. After the solvent evaporated, the thimble was weighed again. The oil content was calculated based on the difference between the two weights and expressed as a percentage. Additionally, the protein content was determined using the formula of Beljkaš *et al.,* (2010), based on the Kjeldahl method with minor adjustments. Initially, 2 grams of milled soybean seeds powder were hydrolyzed with 20 mL of concentrated sulfuric acid (H_2SO_4) in a digester. After digestion, 50 mL of distilled water was added to cool the solution, followed by 40% sodium hydroxide (NaOH). The titration was carried out using 0.1 M HCl, and the volume of acid used was recorded. The total nitrogen content in the raw material was multiplied by 6.25 to determine the total protein content. This process was repeated three times for each sample.

Sample preparation for GC: Various fatty acid contents (palmitic acid, oleic acid, linoleic acid, arachidate and eicosenoate) were measured from 2nd year experiment with the help of Chromatography system (3800 GC, USA). Sample preparation was performed following the guidelines set by Kamer *et al.* (1949), with minor adjustments. To do this the seeds of soybean were crushed into powder form. Then 375 mg powder was weighed on an electric balance and transferred into a test tube for extraction. 8 mL ethyl reagent (Petroleum Ether) was added into the tube and shaken, then the tube was left overnight. Afterwards, 5 mL of salt solution (NaCl 80 g and 3 g $NaHSO_4$ in 1L water) was added. A clear benzene phase was observed in the tube at the upper level. This benzene phase was carefully separated from the test tube, stored in a 2 mL airtight tube, and subjected for future GC reading through Flame Ionization Detector (FID). A mixed Fatty acid standard was used for the quantification of specific fatty acid content.

Statistical Analysis: Computing platform of 'R', version 3.3.3 was used for data analysis regarding the mean separation (Least Significant Difference test at $p \leq 0.05$) and correlation study of collected data. On the others, TBtools was used to be clustering the performance of various oligosaccharides under Euclidean distance methods.

RESULTS

Oligosaccharides positively promotes the growth of soybean: This study shows that applying oligosaccharides at different concentrations significantly improves soybean growth, including plant height, number of pods, and pod length (Table 1). Over two years of research, the tallest plants were recorded with 50 ppm OGA (62.76 cm) and 50 ppm COS (62.63 cm) in 2021, both similar to 75 ppm COS (61.56 cm), 75 ppm OGA (61.55 cm), and 100 ppm AOS (62.43 cm). A similar trend was observed in 2022, where 50 ppm OGA (63.22 cm) again resulted in the tallest plants, while the untreated control had the shortest. Significant differences were also noted in the number of pods $plant^{-1}$ and pod length, compared to the control. OGA produced the highest number of pods, with 100 ppm OGA yielding 67.39 pods in 2021. The lowest pod counts occurred with 50 ppm COS and 50 ppm AOS. For pod length, the control group had the shortest length (2.63 cm in 2021), while the longest (3.38 cm) was observed with 75 ppm COS. Similar results were noted in 2022. These findings indicate that oligosaccharides enhance key traits related to soybean yield.

The effect of oligosaccharide on soybean seed weight,

yield, oil and protein content: The present results demonstrated that foliar application of OGA over two years had a positive effect on soybean yield (Table 2). The highest hundred seed weight was recorded with 100 ppm OGA in 2021. Among the OGA treated plots in 2021, the maximum yield was obtained from the 100 ppm OGA treatment (2670.7 kg ha^{-1}). In 2022, the same treatment also produced the highest yield (2638.8 kg ha^{-1}). Overall, OGA application consistently increased yields in both 2021 and 2022.

Furthermore, different COS, AOS, and OGA concentrations led to higher protein content than the control in both years. In 2021, the lowest protein content was observed with 50 ppm COS (34.50%), while the highest was measured with 100 ppm AOS (40.03%). In 2022, the lowest protein content was recorded with 50 ppm OGA (33.66%), and the highest again with 75 ppm AOS (40.77%). However, the trend for oil content percentage showed different patterns across the treatments. In 2021, the highest oil content was consistently obtained with the 75 ppm AOS treatment, yielding 20.22% and 19.78%, respectively. Conversely, the lowest oil content in 2021 was recorded with 50 ppm OGA (16.67%), while in 2022, it was with 50 ppm COS (16.21%). However, it was still higher than the control (15.73%).

The heatmap effectively highlights the highest levels of similarity among variables or traits, clearly visualising the overall performance of different oligosaccharides and their concentrations. Separate heatmaps were constructed based on the two years of field performance. From the clustered results (Figure 2), we observed that the control group showed unique performance in 2021 (cluster i), while treatments with 50 ppm OGA, 50 ppm AOS, 75 ppm AOS, 50 ppm COS, and 75 ppm COS exhibited similar behavior (cluster iv).

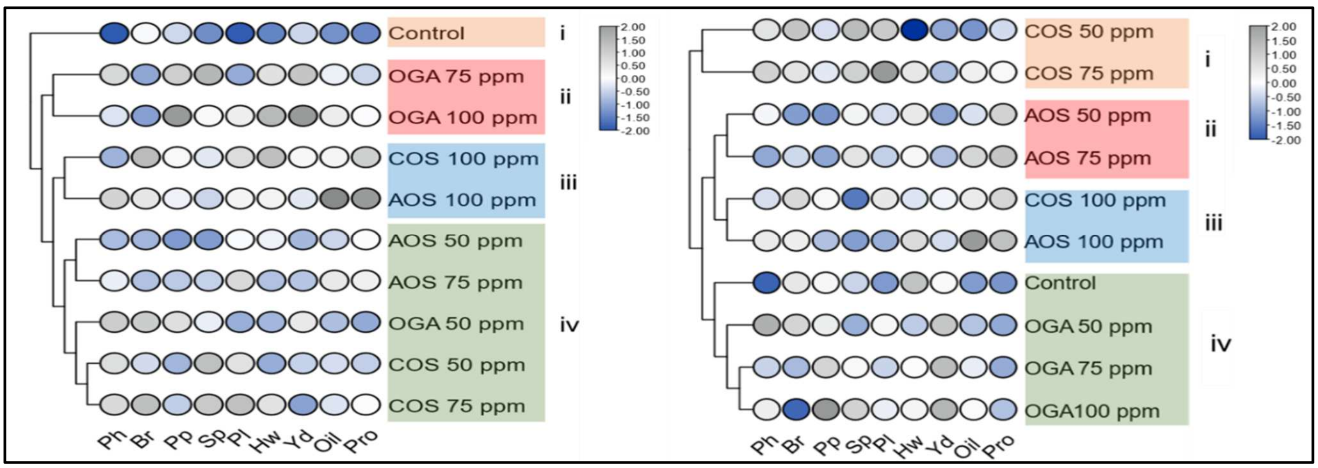

Figure 2. Cluster heat map for the performance evaluation of various oligosaccharide at different concentrations for 2021 (left) and 2022 (right). Clustering analysis expresses the visual representation of accessions indifferent groups based on similarity with different traits (i, ii, iii and iv). Ph = Plant Height, Br = Branches, Pp = Pods $plant^{-1}$, Sp = Seeds $plant^{-1}$, Pl = Pod length, Hw = Hundred Seed weight, Oil = Oil content (%) and Pro = Portent content (%).

The remaining treatments with higher concentrations of AOS and COS also showed comparable performance (cluster iii). In 2022, four distinct groups were identified, though they differed slightly from those in 2021. The control and all concentrations of OGA formed unique clusters in 2022 (cluster iv). Similar to 2021, higher concentrations of COS and AOS were grouped. These findings suggest that soybean yield, oil, and protein content are regulated by different mechanisms influenced by the three types of oligosaccharides. Additionally, varying concentrations of OGA may function through a similar pathway over the two years. Compared with the control, three oligosaccharides with different concentrations contained the same fatty acids, but the content was different (Figure 3). Among fatty acids, palmitic acid, oleic acid and linoleic acid have special importance. Comparatively lower palmitic acid was reported from 100 ppm COS which was 4.4% lower than control (Figure 4). Maximum palmitic acid was obtained from 75 ppm AOS (12.91%). The lowering of saturated fats like palmitic acid amount is also the target of soybean breeding which is obtained from the application of oligosaccharide. For oleic acid, it ranged from 2.79 % to 3.43%, and for linoleic acid, the value differs from 24.00% to 25.40%. Among all the oligosaccharides, the maximum content of oleic acid and linoleic acid was obtained from COS treatment and it was the highest with 50 ppm COS (3.43%) for oleic acid where 100 ppm COS ensure maximum linoleic acid (28.67%). This indicates that COS has influence on fatty acid content than other oligosaccharides.

Table 1: Effects of various oligosaccharides and their concentration on yield contributing agronomic traits of soybe (2021 and 2022

Treatments	Plant height (cm)		Branches^{-1} plant		Pods^{-1} plant (no.)		Seeds^{-1} pod (no.)		Pod length (cm)	
	2021	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
Control	48.00±2.22d	48.83±2.84c	3.20±0.5^{NS}	4.09±0.50^{NS}	53.88±4.24cd	55.90±3.41cde	2.24±0.33^{NS}	2.36±0.05^{NS}	2.63±0.10e	2.76±0.12e
COS50 ppm	62.63±2.50a	60.03±4.56ab	3.23±0.78	4.20±0.4	51.14±2.99d	57.66±4.38bcde	2.43±0.25	2.70±0.26	3.22±0.08abc	3.53±0.27ab
COS75 ppm	61.56±2.63ab	61.00±4.35ab	3.83±0.28	3.93±0.46	54.30±3.73cd	58.10±.03bcde	2.47±0.07	2.63±0.05	3.38±0.11a	3.83±0.15a
COS100 ppm	53.40±3.73c	57.13±2.99ab	3.59±0.63	4.04±0.44	57.64±3.85bc	59.68±4.15bcd	2.27±0.25	2.23±0.15	3.27±0.09ab	3.35±0.29bc
AOS50 ppm	54.06±1.55c	57.96±4.12ab	3.13±0.47	3.21±0.74	51.06±3.79d	54.30±1.96e	2.14±0.08	2.51±0.27	3.08±0.05cd	3.10±0.28cde
AOS75 ppm	58.10±3.02abc	55.16±1.78bc	3.10±0.10	3.51±0.36	53.95±2.68cd	55.11±3.15de	2.29±0.18	2.56±0.06	3.25±0.13abc	3.05±0.20cbc
AOS100 ppm	62.43±2.70a	59.50±4.46ab	3.60±0.29	3.84±0.11	55.73±4.17bcd	56.26±6.35cde	2.20±0.20	2.30±0.10	3.12±0.08bc	2.93±0.13de
OGA50 ppm	62.76±3.75a	63.22±6.26a	3.70±0.30	4.07±0.28	59.67±2.89bc	60.68±3.77bc	2.32±0.19	2.33±0.15	2.91±0.09ab	3.26±0.38bcd
OGA75 ppm	61.55±3.67ab	56.74±5.34ab	3.13±0.33	3.36±0.53	61.46±5.16ab	62.56±2.31ab	2.46±0.21	2.50±0.18	2.92±0.13ab	3.06±0.12cde
OGA100 ppm	56.80±1.45bc	59.26±5.54ab	3.06±0.47	4.08±0.60	65.52±2.15a	67.39±4.01a	2.39±0.25	2.62±0.25	3.12±0.16ab	3.15±0.11bcde

Same letter(s) for means over each column do not differ significantly at p≤ 0.05. NS is not significant.

Table 2. Effects of various oligosaccharides and their concentration on Soybean yield, oil and protein content (2021 & 2022.

Treatments	Hundred seed wt. (g)		Seed yield (kg ha^{-1})		Protein (%)		Oil (%)	
	2021	**2022**	**2021**	**2022**	**2021**	**2022**	**2021**	**2022**
Control	11.86±1.16^{NS}	12.21±1.21^{NS}	2347.4±126.08c	2384.66±105.9c	32.89±0.10g	34.18±0.43f	16.14±0.25f	15.73±0.28f
COS50 ppm	11.38±1.03	11.45±1.28	2349.2±116.89c	2397.6±116.5c	34.50±0.36de	36.70±0.33d	17.10±0.26d	16.21±0.24f
COS75 ppm	12.22±1.12	12.79±1.77	2350.0±105.36c	2426.0±107.86c	35.97±0.40cd	38.00±0.21c	17.24±0.27d	17.88±0.37c
COS100 ppm	12.01±1.58	12.42±1.09	2492.3±105.29abc	2495.1±129.57abc	38.00±0.67b	39.73±0.16b	17.76±0.21bc	17.98±0.30bc
AOS50 ppm	11.83±1.37	12.76±1.09	2356.0±107.38bc	2395.0±137.78c	35.81±0.26cd	39.88±0.36b	17.04±0.18de	17.19±0.19de
AOS75 ppm	11.43±1.34	12.63±1.07	2361.7±127.89bc	2429.2±117.7bc	36.33±0.13c	40.51±0.30ab	18.11±0.30b	18.47±0.44b
AOS100 ppm	11.93±1.28	12.90±1.31	2423.23±123.4bc	2465.6±107.94ab	40.03±0.18a	40.77±0.31a	20.22±0.28a	19.78±0.13a
OGA50 ppm	12.06±1.20	12.29±1.31	2513.9±114.97abc	2635.3±107.94ab	33.66±0.20f	35.30±0.43e	16.67±0.23e	16.80±0.34e
OGA75 ppm	11.96±1.23	12.59±1.16	2554.8±117.83ab	2653.0±93.15a	34.67±0.31e	35.37±0.35e	17.41±0.15cd	17.33±0.25d
OGA100 ppm	12.30±1.10	12.66±1.08	2638.8±127.02a	2670.7±102.036a	35.72±0.39d	36.00±0.10de	17.92±0.16b	17.66±0.26cd

Same letter(s) for means over each column do not differ significantly at p≤ 0.05. NS is not significant.

The effect of oligosaccharide on oil quality in soybean: Soybean seed oil has a tremendous nutritive value, and treated with different oligosaccharides have a significant effect on its saturated and unsaturated fatty acid content.

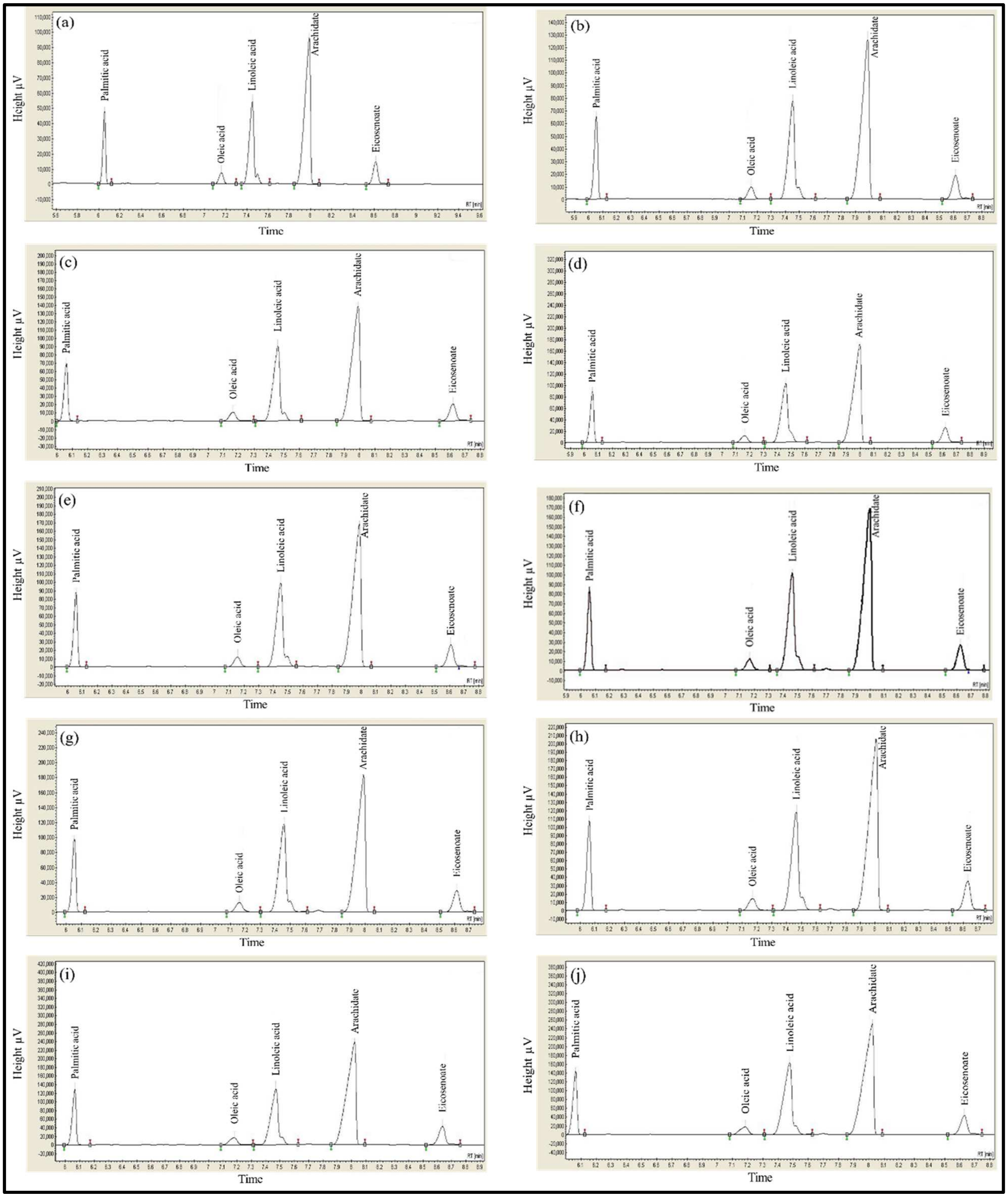

Figure 3. Chromatographic data view of different oligosaccharides, COS 50 ppm (a), COS 75 ppm (b), COS 100 ppm (c), AOS 50 ppm (d), AOS 75 ppm (e), AOS 100 ppm (f), OGA 50 ppm(g), OGA 75 ppm(h), OGA 100 ppm(i) and control (j).

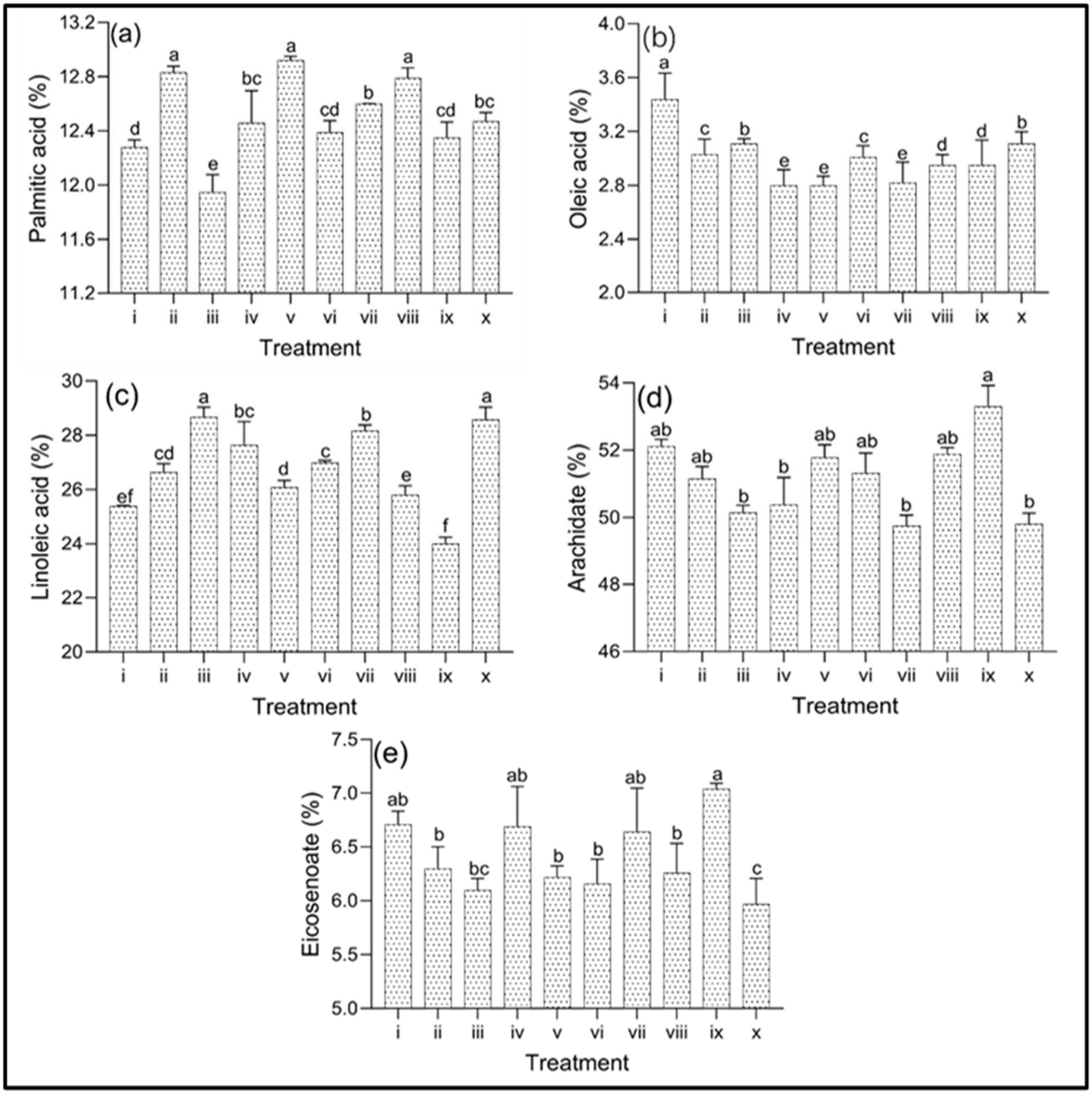

Figure 4. Fatty acid content (a) Palmitic acid, (b) Oleic acid, (c) Linoleic acid, (d), Arachidate and (e) Eicosenoate in response of various oligosaccharide (i = 50 ppm COS, ii =75 ppm COS, iii = 100 ppm COS, iv = 50 ppm AOS, v = 75 ppm AOS, vi = 100 ppm AOS, vii = 50 ppm OGA, viii = OGA, ix = 100 ppm OGA & x = Control). Same letter(s) do not differ significantly at 5% level of significance.

However, compared with control, the oleic and linoleic acid content was also comparatively lower in other oligosaccharides.

Among all of the fatty acid content, oleic acid was the most important one which has a negative correlation with palmitic acid (-0.38). The second important fatty acid was linoleic, and it also has a negative correlation with the presence of arachidate (-0.95) and eicosenoate (-0.54). These results indicated that the application of oligosaccharides has a positive relation with oil and portent content, with oleic acid that ensures the quality of soybean oil (Table 3).

Table 3 Correlation study of yield with qualitative parameter of soybean

	Oil	Protein	Palmitic acid	Oleic acid	Linoleic acid	Arachidate	Eicosenoate
Yield	0.04	-0.30	-0.01	-0.32	-0.25	0.22	0.26
oil		0.74***	0.08	-0.26	-0.15	0.24	-0.17
Protein			-0.08	-0.20	0.09	0.01	-0.15
Palmitic acid				-0.38*	-0.23	0.13	-0.13
Oleic acid					-0.08	0.08	-0.07
Linoleic acid						-0.95***	-0.54**
Arachidate							0.36*

***P≤0.001, **P≤0.01 and *P≤0.05.

DISCUSSION

Our study demonstrates that the foliar application of oligosaccharides significantly enhances several key agronomic traits in soybeans, including plant height, pod number, seed weight, and overall yield. The consistent increase in plant height with 50 ppm OGA, which showed a 30% improvement over the control, suggests that oligosaccharides can positively influence vegetative growth. Similar findings have been reported in other crops where chitosan improved plant height and biomass by enhancing cell division and elongation (Islam *et al.,* 2018; Mondal *et al.,* 2016). Furthermore, the application of oligosaccharides has been linked to enhanced seed yield in crops like cauliflower, where it improved curd development and biomass accumulation (Rajib *et al.,* 2024). This supports our observations of increased pod number and seed weight with 100 ppm OGA and AOS treatments, indicating that oligosaccharides may promote reproductive growth and biomass accumulation. This aligns with previous studies showing that higher oligosaccharide concentrations can activate distinct mechanisms for promoting biomass accumulation in crops like wheat and chili (Parvin *et al.,* 2019; Wang *et al.,* 2015). Additionally, the significant yield increases observed with 100 ppm OGA over two years imply that oligosaccharides could support nutrient partitioning towards reproductive organs. While our study did not directly measure physiological processes such as photosynthesis or nutrient uptake, previous research indicates that oligosaccharides enhance root growth and nutrient absorption, increasing the availability of essential nutrients critical for pod and seed development (Hossain *et al.,* 2024). The improved soybean yield observed in our study is consistent with findings showing that oligosaccharides can modulate phytohormonal balance, enhancing carbon allocation toward reproductive sinks (Sanyal *et al.,* 2023).

Regarding seed composition, the results indicate that AOS enhances both oil and protein content, with 100 ppm AOS yielding the highest values in both years. This suggests that oligosaccharides regulate the expression of enzymes involved in lipid and protein biosynthesis, enhancing metabolic pathways related to these compounds. The positive correlation between oil and protein content is also supported by earlier studies indicating that biostimulants can enhance both traits simultaneously (Hong *et al.,* 2022; Pathan *et al.,* 2013).

The effects of oligosaccharides on the fatty acid profile of soybean oil were also noteworthy. The reduction in palmitic acid and the corresponding increase in oleic and linoleic acids, particularly in the 100 ppm COS treatment, suggest that COS modulates fatty acid metabolism. Previous studies have indicated that oligosaccharides can influence enzymes like fatty acid desaturase, which regulates the synthesis of unsaturated fatty acids (Rezaeizadeh *et al.,* 2019). Furthermore, the observed negative correlations among fatty acids indicate that oligosaccharides may enhance the synthesis of health-promoting unsaturated fatty acids while reducing saturated fats, which is consistent with findings from similar biostimulant applications (Khosro M., 2015; Sima *et al.,* 2020).

Although the precise biochemical pathways through which oligosaccharides exert these effects are not fully elucidated in our study, we propose a hypothetical pathway that needs to be validated in the future study (Figure 5). The results strongly suggest that these compounds influence key physiological and metabolic processes in soybean. Previous research has shown that oligosaccharides can act as signaling molecules, enhancing photosynthesis by increasing chlorophyll content and carbon fixation, ultimately contributing to higher yields and improved seed quality (Heldt and Piechulla, 2011; Li *et al.,* 2022).

Overall, this study underscores the significant role of oligosaccharides, particularly OGA and AOS, in improving soybean growth, yield, and quality. OGA consistently promoted plant height, pod number, and yield, while AOS notably enhanced oil and protein content, highlighting the potential of these biostimulants to optimize both productivity and nutritional value in soybean cultivation.

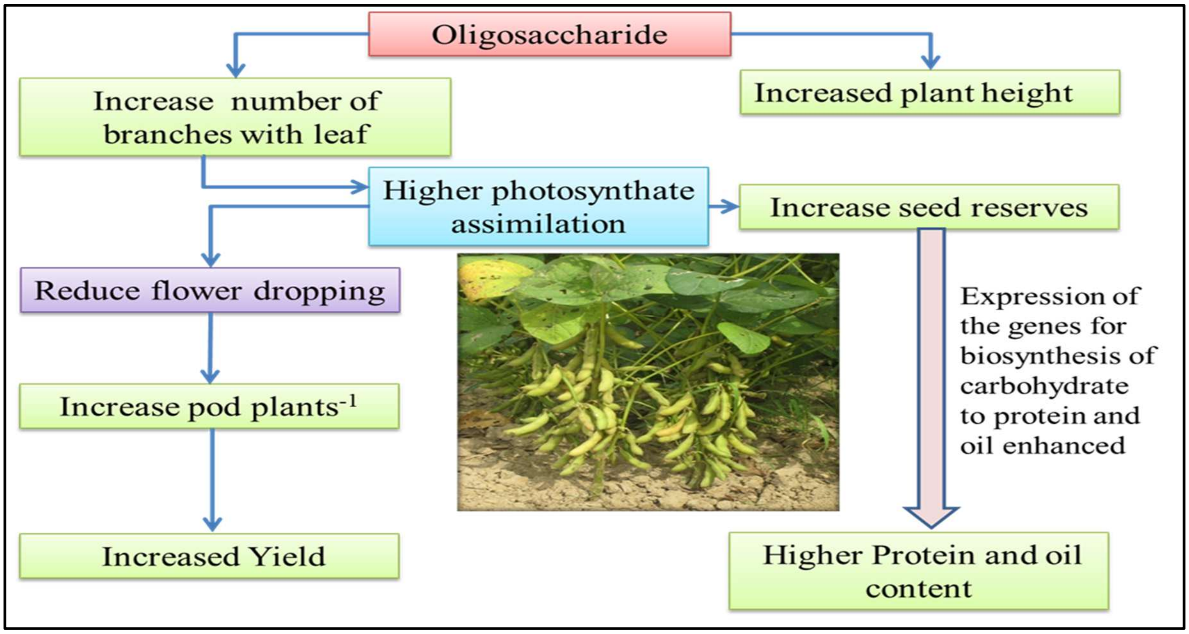

Figure 5. Hypothetical pathway of stimulation of yield and quality of soybean festinated by the foliar application of oligosaccharides.

Conclusion: The principal focus on sustainable agriculture is the utilization of natural, safe and environmentally friendly products. AOS, COS and OGA are all extracted from natural products and soluble in water at normal temperatures. The foliar application of oligosaccharides during flowering and pod formatting stages significantly increased growth and yield, along with promoted qualitative properties like protein of soybean. OGA significantly increased plant height, and number of pods plant^{-1} which leads to maximum production, followed by COS which is derived from the degradation of the widely distributed natural product chitosan. AOS has a crucial role on quality parameters, and 100 ppm AOS ensure maximum protein content, while 100 ppm of OGA provided maximum yield under field conditions. Even if we are unaware of the gene expression-regulated mechanism of oligosaccharide treatment against soybean, it should be the main focus for future research to improve the utilization of this kind of natural product.

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