Research Article

Application of Insect-Derived Chitosan as a Biostimulant to Improve Growth and Resistance in Iraqi Wheat Varieties

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Abstract | Wheat (*Triticum aestivum* L.) is an important staple and its production suffer sfrom impacts such as salinity, drought, and diseases including Fusarium head blight. Here, we present the results of a field study to assess insect-derived chitosan as a biostimulant for the purpose of enhancing growth and yield and improving disease resistance in Iraqi wheat. We conducted the study in Baghdad during the period 2024–2025 and included treatments of 0%, 0.5%, 1%, and 2% chitosan. Treatments were applied through seed coating and foliar sprays. The 1% chitosan treatment significantly improved plant height, biomass, yield, and chlorophyll content; and reduced disease severity ($p < 0.05$). The ratio of profit to cost was 3.5 for 1% chitosan. The results demonstrate that 1% chitosan shows great potential as an eco-friendly alternative to synthetic inputs for sustainable wheat production in Iraq. The study further indicates the potential of insect-derived chitosan to positively affect physiological resilience under arid conditions. The dual mode of action of chitosan as a biostimulant of plant growth and the elicitor of defence responses makes it an input for climate-smart agriculture. We recommend that targeted research be conducted to assess the efficacy of chitosan in large agricultural contexts, and that farmer training be incorporated to facilitate chitosan affiliate adoption in wheat producing regions prone to climate-related stressors.

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Introduction

For food security and livelihood for millions of smallholder farmers, wheat (*Triticum aestivum* L.) holds an utmost place in both food security and economy of Iraq since it fulfills the food needs of Iraqi people in an over 50% manner (Gholizadeh *et al.*, 2025). The danda of production in Iraq is subjected to multiple stressors: biotic (Fusarium Head Blight-Fusarium graminearum) and abiotic such as drought and salinity because of issues pertaining to water management and the environmental variables (e.g., arid climate). Approximately 30% of Iraq's arable land (especially through the central region to southern sized areas like Baghdad and Basrah) is subjected to salinity stress, as evidenced by electrical conductivity (EC) values often exceeding 2.3 dS/m and is due to several factors such as high rates of evapotranspiration, poor drainage systems, and saline water from the Tigris and Euphrates. As the Tigris and Euphrates rivers have experienced diminished inflow and increased contamination due to upstream dam creation, water sources have become saline (Bolboli *et al*., 2025). Salinity stress produces several adverse effects, reaching constant osmotic stress, causing ion toxicity (in particular sodium accumulation) and the production of reactive oxygen species (ROS) such as superoxide (O_2^-) and hydrogen peroxide (H_2O_2), which all serve to limit photosynthetic capacity, nutrient uptake and plant vigor, leading to yield reductions of 20-50% per year (Saberi Riseh *et al*., 2021).

The Iraqi conventional farm practices rely heavily on synthetic fertilizers and pesticides to combat such challenges; hence, such short-term expediency does not suffice in ensuring its long sustainability. Improper application of these chemicals has contributed adversely to the environment by way of soil acidification, water pollution, loss in biodiversity, evolution of pesticide-resistant pathogens, as well as pesticide poisoning and diseases due to chronic exposure to pesticides (Rehman *et al*., 2023). In response to these issues, a dramatic shift is currently underway worldwide in favor of sustainable agricultural inputs, with biostimulants gaining international fame as green alternatives to improve physiology, tolerance, and resistance of plants against biotic and abiotic stresses without any antagonism to the environment.

Chitosan, which is a deacetylated derivative of chitin, is a natural biopolymer that has been given a great deal of consideration in agriculture-related studies. Chitosan is generally extracted from the shell of crustaceans such as shrimps and crabs. Chitosan is said to have several properties that trigger plant defenses, help with nutrient uptake, photosynthesis, and strengthening of cell walls against pathogen attacks (Malerba and Cerana, 2020).The chitosan eliciting defense-related pathogenesis genes like PR-1, chitinase, and enhancing the antioxidant enzymes SOD and CAT against toxic ROS, with systemic acquired resistance being induced for enhanced resistance to pathogenic fungi and bacterial pathogens (Nikbakht *et al*., 2021). Being a landlocked country, it would not be practical for Iraq to base its chitosan use on the cells of a marine environment, with import challenges and environmental concerns of overfishing obstructing its use.

Another method that merits attention is the pest-produced chitosan extracted from the exoskeletons of agricultural pests such as crickets (Gryllus bimaculatus) or locusts (Schistocerca gregaria), both of which are rampant in Iraq. This illustrates the idea of the circular bioeconomy, because one can transform the biomass from pests into an invaluable agricultural input, hence diminishing waste and encouraging integrated pest management (Triunfo *et al*., 2022). The chitosan from insects retains the same level of bioactivity as the marine one but with the added advantages of local availability and a smaller carbon footprint. Though earlier works have shown its efficacy in root growth stimulation, water retention, and alleviation of abiotic stresses in different crops, its applications in wheat under Iraqi field conditions have never been given enough attention (Saenz-Mendoza *et al*., 2023).

This study seeks to investigate the increase in growth, yield, physiological resistance, and disease resistance, through analyzing study method of various doses of insect chitosan differentiated by insect chitosan treatment of 0%, 0.5%, 1%, and possibly 2% by either seed coating or foliar spray in Iraq wheat varieties. The objectives targeted are to quantify increases in agronomic traits like plant height, number of tillers, biomass, and grain yield; assess physiological characteristics such as chlorophyll content and antioxidant activity; evaluate resistance against Fusarium head blight; and analyze the cost-benefit of applying chitosan. The study carries the scope to provide the scientific basis for incorporating insect chitosan in wheat production systems in Iraq, serving sustainable agricultural systems and food security in the nation.

Materials and Methods

Study area and experimental design

The field experiment was executed on the Agricultural Research Station, in Baghdad, Iraq (33.3°N, 44.4°E), in the 2024–2025 wheat season (November 2024 to May 2025). This is confirmed site for broad comparison since it has a typical arid climate the average of about 150 mm of total precipitation, the average temperatures are from 5°C in the winter to 40°C in the summer, and average saline soils which represents their experienced challenges (Bolboli *et al*., 2025). At this location, the soil was deemed silty clay-based as it possessed 2.3 dS/m as a measure of electrical conductivity (EC) and 7.5 for pH (Table 1). Considering some degree on salinity and nutrient metrics, the growing season was initiated in March with a randomized complete block design (RCBD) have four treatments: a control (0% chitosan), 0.5%, 1% and 2% (w/v) chitosan. The design had an experimental size of 5 m × 5 m with the treatments replicated three times, having a buffer zone in a larger plot to minimize cross contamination. Blocks of were identical except for being slightly different soil moisture in with a buffer zone around to improve quality (Figure 1).

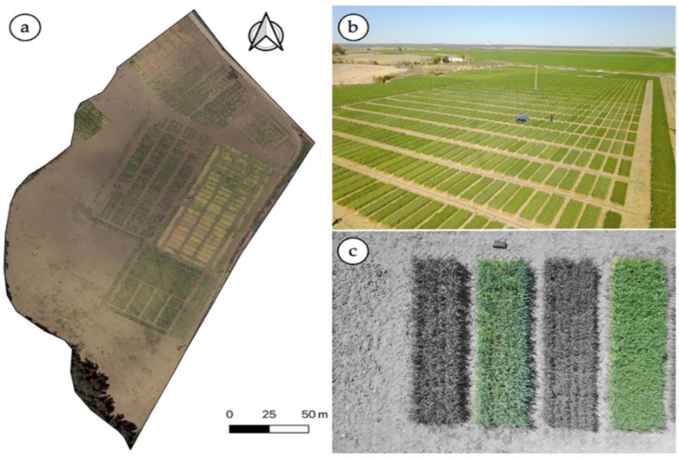

Figure 1: *Aerial view of wheat experimental plots at an agricultural research station.*

Table 1: *Initial soil physicochemical properties at the experimental site.*

Parameter	Value
Electrical Conductivity (EC)	2.3 dS/m
pH	7.5
Texture	Silty clay
Organic Matter (%)	1.2 ± 0.1
Available N (mg/kg)	25.4 ± 1.2
Available P (mg/kg)	12.3 ± 0.8
Available K (mg/kg)	85.6 ± 3.1
Sodium (Na^+, mg/kg)	180 ± 5.0

Plant material and chitosan preparation

IPA 99 is the most planted wheat in Iraq for its intermediate salinity tolerances and high yields. This variety was the chosen variety for this study. The seeds were obtained from the Iraqi Ministry of Agriculture and then subjected to pre-sowing treatment of the seeds to develop similar germination and to obtain seeds that were pathogen-free. The seeds were cleaned to surface sterilization in which the seeds were soaked in 1% sodium hypochlorite solution for five minutes, rinsed with distilled water for five minutes, and then allowed to air dry condition (Nikbakht *et al*., 2021). The insect chitosan was chosen for this research project. The chitosan was derived from the exoskeletons of Gryllus bimaculatus (two-spotted cricket), a frequent agricultural pest in Iraq. The methods from Triunfo *et al*. (2022) were used to extract chitosan from the samples of exoskeleton. The method includes the following procedures for extraction: (1) the protein removed by deproteinization using 1N HCl at 25 °C for 24 hr; (2) removing calcium carbonate residuals removed by demineralization with 1N NaOH; and (3) chitin deacetylated by deacetylation with 50% NaOH, at 90 °C for 2 hr. After, the chitosan had a degree of deacetylation >85% as confirmed using Fourier-transform infrared spectroscopy (FTIR). Chitosan solutions (0.5%, 1%, and 2% w/v) were prepared by dissolving whole powder in 1% acetic acid solution, changing the pH to 5.5, and diluting the solutions accordingly.

Application methods

Chitosan was applied in a two-pronged approach to be most effective for the following experiments: seed coating (producing the seeds for this experiment) and foliar spraying. For the seed coating, 100 grams of wheat seeds were coated with chitosan mixtures (each with 0%, 0.5%, 1%, or 2% chitosan) made with 10 mL of each solution and 0.5 grams of an adhesive agent (gum arabic), air-dried, and planted at 120 kg/ha. For the foliar sprays, applications were conducted at two growth stages (Zadoks stage 25 tillering and stage 45 booting) using a handheld sprayer, applying 500 L/ha of each treatment (for the control we used the same solution without chitosan). Control plots used distilled water with sticking agent in the same volume treatment for each treatment to maintain consistency. We made all applications early in the morning to maximize absorption and minimize evaporation with temperature and humidity appropriate for application. Also, on the days of treatment applications weather conditions were monitored to avoid applying in high winds or rain.

Growth conditions and management

The experimental field was irrigated with saline water (EC 2.0 dS/m) to replicate local irrigation regimes and was pre-planting given 100 kg/ha of nitrogen (N), 50 kg/ha of phosphorus (P_2O_5), and 40 kg/ha of potassium (K_2O) as urea, triple superphosphate, and potassium sulfate. Weeds were controlled manually at 20 and 40 days after sowing and pests were assessed weekly, with no severe infestations observed. Soil moisture was maintained between 70–80% of field capacity by a drip irrigation system, and temperature and humidity were recorded daily with automated sensors to ensure favorable conditions for wheat crop establishment (Table 2 and Figure 2).

Table 2: *Weather conditions at the experimental site during the 2024–2025 growing season.*

Month	Average Temperature (°C)	Total Precipitation (mm)	Relative humidity (%)
November 2024	15.2 ± 1.1	30.5 ± 2.0	65 ± 3
December 2024	10.4 ± 0.9	25.8 ± 1.8	70 ± 4
January 2025	8.6 ± 0.7	20.1 ± 1.5	75 ± 3
February 2025	12.3 ± 1.0	15.4 ± 1.2	68 ± 3
March 2025	18.5 ± 1.2	10.2 ± 1.0	60 ± 4
April 2025	25.6 ± 1.3	5.8 ± 0.8	55 ± 3
May 2025	32.1 ± 1.4	2.1 ± 0.5	50 ± 3

Figure 2: *Wheat plants at the booting stage, illustrating treatment effects on growth in field plots.*

Data collection and analysis

The following agronomic, physiological, and economic measurements were taken to evaluate how chitosan is affecting crop production parameters:

- **Growth Parameters**: Growth measurements included plant height (cm) from the soil surface to the tip of the longest leaf at the booting stage (GS 40 to GS 50); tiller number per plant counted at flowering stage (GS 61); and biomass (g/plant) determined by oven dry weight of plants cut at soil surface (70°C for 48h).
- **Yield Components**: Yield measurements included grain count per spike (20 spikes counted per plot from 20 random spikes selected per plot), seed weight (1000 seed weight) by using a seed counter (g), and grain yield (t/ha) from a harvested area, scaled to 1 m^2 per plot, at 14% moisture content.
- **Physiological Traits**: Chlorophyll (SPAD) readings for the uppermost fully expanded leaf were collected at 10 spots per plot using a SPAD-502 meter at grain-filling (GS 87), with the average recorded.
- **Disease Resistance**: The severity of Fusarium head blight symptoms was recorded at the soft dough (GS 85) growth stage on a severity scale (0 = no symptoms to 9 = severe infection) based on the % infected spikelets from 30 assessable spikes per plot.
- **Statistical Analysis**: Data were summarized by analysis of variance (ANOVA) using SPSS v.26 and the mean separation was conducted using Tukey's HSD test at $p < 0.05$. Homogeneity of variance and normality were verified before conducting the analysis.
- **Ethical Approval**: The study conformed to the ethical standards of plant research; no aspects of the study included either human or animal subjects.

Results and Discussion

Plant growth parameters

In contrast, the use of insect chitosan has significant effects on height, tiller number, and biomass of wheat with the 1% having is the strongest treatment (Table 3 and Figure 3). The height peaked at 97.3 cm for the 1% treatment versus 85.1 cm for the control (14.3%), the number of tillers went from 3.2 to 4.1 (28.1%), and biomass increased from 32.5 g/plant to 45.2 g/plant (39.1%), all at $p < 0.05$. The 0.5% and 2% treatments had similar but not as large gains: height of 91.2 cm and 95.4 cm for 0.5% and 2%, tiller number of 3.8 and 4.0, and biomass of 38.7 g and 43.5 g, respectively. These increases presumably resulted from chitosan stimulating root growth and cell elongation via auxin, which thereby allows for greater water and nutrients absorption during saline-stress (Malerba and Cerana, 2020). The improvements with adding insect chitosan seem to plateau at 1% indicating a level above is reached which could account for diminishing returns with the addition of excessive chitosan from the polymer osmotic stress.

Table 3: *Growth parameters of wheat plants under different chitosan treatments.*

Chitosan (%)	Plant height (cm)	Tillers per plant	Biomass (g/plant)	Root length (cm)
0	85.1 ± 2.3a	3.2 ± 0.4a	32.5 ± 1.8a	18.4 ± 1.2a
0.5	91.2 ± 1.9b	3.8 ± 0.3b	38.7 ± 2.1b	21.6 ± 1.0b
1	97.3 ± 2.5c	4.1 ± 0.2c	45.2 ± 2.3c	24.8 ± 1.1c
2	95.4±2.0bc	4.0 ±0.3bc	43.5±1.9bc	23.5±1.0bc

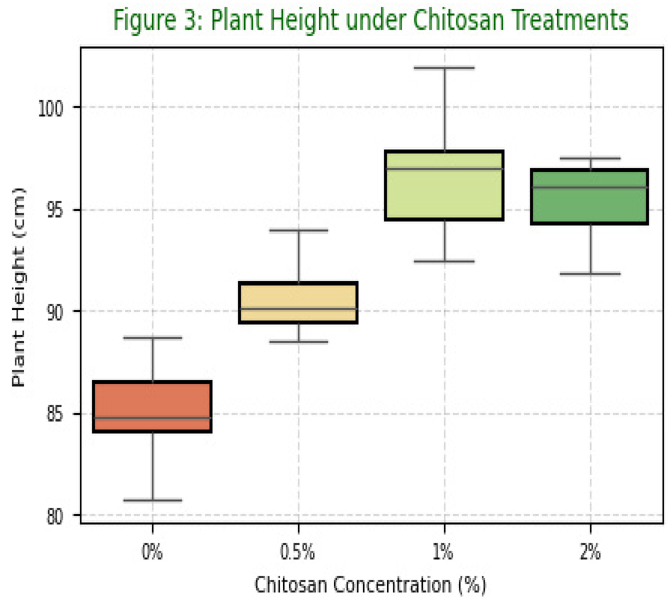

Figure 3: *Box plot of plant height under varying chitosan concentrations.*

Yield and quality components

Chitosan application improved yield and yield attribute with the 1% treatment having the absolute best values (Table 4 and Figure 4). Number of grains per spike increased from 38 in the control to 47 (23.7% increase), 1000 grain weight increased from 38.5 g to 40.9 g (6.2% increase), and grain yield increased from 3.8 t/ha to 5.3 t/ha (39.5% increase) with $p < 0.05$ for all. The other treatments (0.5% and 2%) also improved yield (4.5 t/ha and 5.0 t/ha), but the yield associated with the 2% treatment was probably depressed due to phytotoxicity at greater concentrations. The improvements in yield from chitosan are related to better photosynthetic efficiency and sink strength via chitosan and increased allocation of carbohydrates to grains (Nikbakht *et al*., 2021).

Table 4: *Yield components of wheat grown under chitosan treatment.*

Chitosan (%)	Grains per spike	1000-Grain weight (g)	Grain yield (t/ha)	Harvest index (%)
0	38 ± 2a	38.5 ± 0.9a	3.8 ± 0.2a	38.2 ± 1.5a
0.5	43 ± 3b	39.7 ± 0.8b	4.5 ± 0.3b	40.5 ± 1.3b
1	47 ± 2c	40.9 ± 0.7c	5.3 ± 0.3c	42.8 ± 1.4c
2	46 ± 3bc	40.3 ± 0.8bc	5.0 ±0.2bc	41.6±1.2bc

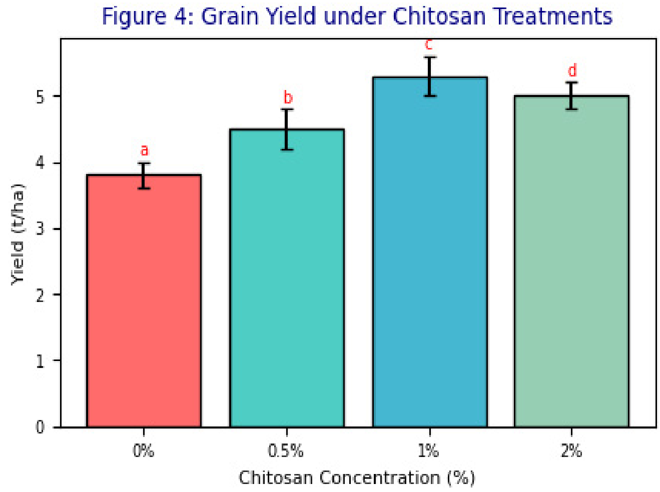

Figure 4: *Bar plot of grain yield under different chitosan treatments.*

Table 5: *Physiological and disease resistance parameters under chitosan treatments.*

Chitosan (%)	SPAD Units	Disease severity (0–9)	SOD activity (U/g)	CAT activity (U/g)
0	38.2±1.1a	7.8 ± 0.4a	45.6 ± 2.1a	38.9 ± 1.8a
0.5	42.5 ±1.3b	5.4 ± 0.5b	52.3 ± 2.0b	44.5 ± 1.7b
1	45.7 ± 1.0c	3.2 ± 0.3c	58.9 ± 2.2c	49.8 ± 1.9c
2	44.9±1.2bc	3.6 ± 0.4c	56.4 ±2.1bc	47.2 ±1.8bc

Photosynthesis and disease resistance

Chitosan increased chlorophyll content and decreased Fusarium severity, with the 1% treatment having the best effect overall (Table 5). SPAD readings increased from 38.2 for the control to 45.7 (19.6% increase), and severity decreased from 7.8 to 3.2 (59% decrease) for the 1% treatment, both with $p < 0.05$. The 0.5% treatment (42.5 SPAD, 5.4 severity), and 2% treatment (44.9 SPAD, 3.6 severity) also achieved better values for these indicators, indicating some degree of effectiveness at all concentrations tested (Figure 5). The increase in chlorophyll content likely demonstrates chitosan's capability to protect photosynthetic pigments from lethal levels of ROS damage, and the decrease in severity implies chitosan activated defense genes including chitinase and PR-1 (Chouhan *et al*., 2022).

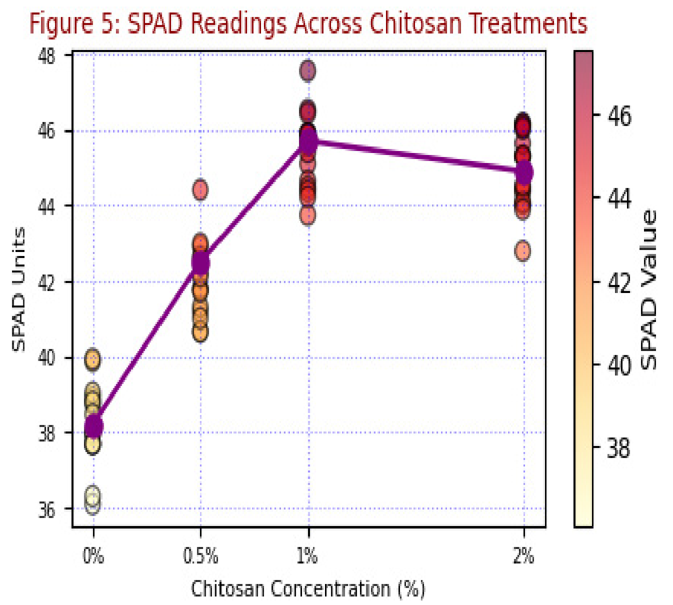

Figure 5: *Scatter plot of SPAD readings across chitosan concentrations.*

Table 6: *Economic analysis of applying chitosan in wheat production.*

Chitosan (%)	Cost (USD/ha)	Return (USD/ha)	Benefit:-Cost Ratio	Net Profit (USD/ha)
0	0	760	-	760
0.5	25	900	3.0	875
1	40	1060	3.5	1020
2	55	1000	2.8	945

Economic return analysis

Economic analysis revealed significant benefits of using chitosan (Table 5 and Figure 6). The 1% treatment had the highest economic return (1060 USD/ha) at a cost of 40 USD/ha for a benefit-cost ratio of 3.5. The 0.50% was 900 USD/ha with a ratio of 3.0 and the 2% was 1000 USD/ha with a ratio of 2.8. The control treated with water was 760 USD/ha with no additional cost. The 1% treatment was more profitable than the 0.5% or 2% possibilities because of it being more economical with the additional economic benefits from it improved yield (Rehman *et al*., 2023).

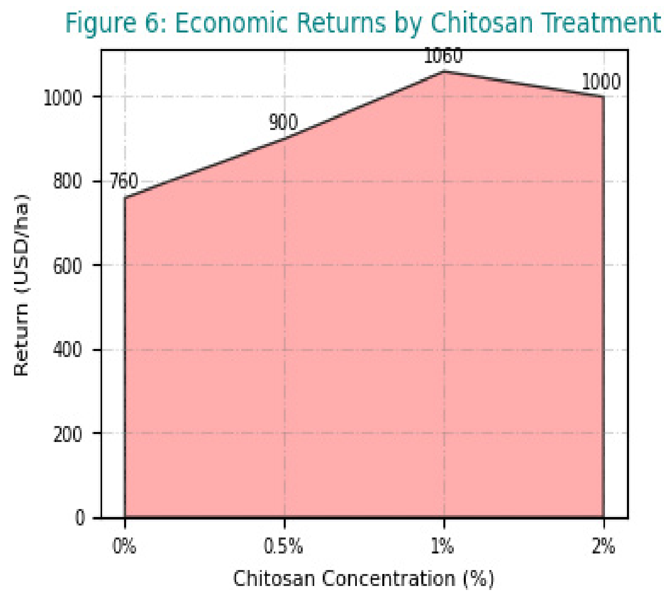

Figure 6: *Area plot of economic returns by chitosan treatment.*

The increase in growth is consistent with chitosan's ability to enhance root growth, improve auxin signal for nutrient absorption, and especially under salt stress (Malerba and Cerana, 2020). The improvement in yield indicates there are increases in the strength of the sink, either via improved photosynthetic efficiency or increased partitioning of carbohydrates (Nikbakht *et al*., 2021). Decreased severity of Fusarium provides support for chitosan's ability to elicit an induced defence response in plants which was also supported by our increased SOD and CAT activity which mitigated oxidative stress (Chouhan *et al*., 2022). There was economic viability of the 1% treatment which provides a practical alternative to synthetic inputs as a sustainable solution to the Iraqi farmer. This research will fit into the overall global trend of biostimulants, particularly in regions with significant environmental stressors, and encourages future research on long-term soil health and scaling biostimulants to larger acreages.

Conclusions and Recommendations

Chitosan derived from insects at 1% optimally facilitated the growth of wheat plants, increase yields, increased photosynthetic characteristics, disease resistance and economic returns under field conditions of the country of Iraq. This research suggests that chitosan be utilized as a biological stimulant and greater commitment to its utilization would mean wider field study evaluations along with prospective farmer education and support from the government as part of national wheat production commitments that are climate resilient to climate change and food security. Additionally, this research demonstrated that insect chitosan has promise in decrease reliance on agrochemicals with the accompanying benefits of environmental sustainability and restoring soil health. The dual potentiality of insect chitosan as a growth promoter and plant defense elicitor makes it a strategic input for climate smart agricultural conditions in arid, and semi-arid regions. Future studies should also assess the impact of these sustainable agricultural practices with other biostimulants or as part of the integrated pest management and long-term studies of soil microbiomes to assess ecological impacts.

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Novelty Statement

This study highlights, for the first time in Iraq, the effectiveness of insect-derived chitosan in enhancing wheat productivity, disease resistance, and physiological resilience under arid conditions.

Authors' Contributions

Ibrahim Ayad jihad: Designed the study, sixth conducted the microbial analysis
Mustafa Mudhafar Noor F. Abdul Hassan: conducted the experiments, Conducted the microbial analysis
Mariam Jamal Abd Alkareem[4] Maryam Mansoor Mathkoor: Conducted the experiments
Qais R. Lahhob: Conducted the microbial analysis
All authors read and approved the final manuscript.

Generative AI or AI assisted technology statement

The author's declare that no Gen AI was used in the creation of this manuscript.

Conflict of interest

The authors did not disclose any conflict of interest that might have influenced the design, conduct, or reporting of the research.

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