Harnessing the Power of K-Immune 10%, A Novel Bio-stimulant for Enhancing Crop Resilience and Yield: A Multi-crop Study

Abstract

This study investigates the efficacy of K-Immune 10%, a novel combination of chitosan oligosaccharides and kelp, in addressing agricultural challenges exacerbated by unsustainable farming practices including excessive chemical inputs. Experiments were conducted on greengram, mustard and okra to assess its impact on seed germination, plant growth, stress tolerance and crop yield. In greengram, seed treatment of K-Immune at 8 ml/L concentration significantly enhanced seedling vigour and speed of germination without affecting germination percentage. For mustard, K-Immune applied at 30 ml/15L water twice increased seed yield by 42.09% over the RFD, while also providing effective disease control. In okra, K-Immune at 40 ml/15L water applied twice boosted fruit yield by 22.91% and substantially reduced pest and disease damage. The study demonstrates the potential of K-Immune as a bio-stimulant and plant protectant, offering a sustainable alternative to conventional agrochemicals. By enhancing crop resilience and productivity while minimizing environmental impact, K-Immune can be strategically applied between regular pesticide sprays to serve as a crucial tool in combating the development of pesticide resistance.

Keywords: K-Immune 10%, chitosan oligosaccharides, kelp, sustainable agriculture, germination, crop yield, plant protection.

1. Introduction

Agriculture today is confronted with unprecedented challenges, intensified by unsustainable farming practices, climate change and the rising demand for food. Conventional agricultural practices, including the indiscriminate use of chemical fertilizers and pesticides, have led to environmental degradation and the development of pesticide resistance in pathogens. In response to these challenges, there is a growing interest in sustainable and eco-friendly alternatives that can enhance crop productivity while minimizing environmental impact. One such promising solution is K-Immune 10%, a novel combination of chitosan oligosaccharides and kelp.

K-Immune 10% harnesses the power of two natural components: chitosan oligosaccharides, derived from the deacetylation of chitin (the second most abundant naturally occurring polysaccharide after cellulose (Rinaudo, 2006), and kelp, a nutrient-rich seaweed. Chitosan oligosaccharides are primarily obtained from the exoskeletons of arthropods such as crabs and shrimp, as well as from certain fungi (Sanford, 2003). The chitosancombined with the mineral-rich profile of kelp, confers K-Immune 10% with remarkable properties that make it valuable in agriculture and beyond.

K-Immune 10% enhances plant growth, nutrient uptake and resistance to biotic and abiotic stresses, making it a versatile tool for seed treatment and foliar application. Its biodegradable, non-toxic nature provides an eco-friendly solution, supporting crop resilience and reducing reliance on chemical pesticides. This study evaluates its impact on seed germination, stress tolerance and yield improvement.

2. Materials and Methods

2.1 Experiment on Germination

The impact of K-Immune on greengram germination was investigated through a series of experiments. Initially, a pilot study was conducted using 50 greengram seeds soaked overnight in K-Immune solutions ranging from 2 ml/L to 10 ml/L.

Following the pilot study, the experiment was refined to explore the effects of shorter soaking durations. Two sets of 50 seeds each were soaked in various concentrations of K-Immune (2 ml/L to 10 ml/L) for 10 minutes and 30 minutes. After soaking, seeds were immediately placed on filter paper in petri dishes for germination.

Germination was monitored daily, with the number of germinated seeds recorded. Seedling vigour was assessed by measuring seedling length on day 6. Water-soaked seeds served as a control in all experiments.

2.2 Experiment on Yield and its Components

Two experiments, one each on mustard and okra, were conducted to determine the impact of K-Immune on yield and yield components along its ability to control biotic stresses. The experiments were conducted in Randomized Block Design (RBD) with seven treatments at AGROCEL Industries Private Limited, R&D Farm, Koday, Mandvi, Kachchh.The experiments site represented sandy loam soil with a light brown colour, well-drained and fairly retentive of moisture. The soil was low in available nitrogen, optimum in available phosphorus and medium in available potassium. In mustard, three doses of K-Immune (15 ml, 22.5 ml and 30 ml per 15 litres of water) were applied either once (45 DAS) or twice (35 and 55 DAS), in addition to the recommended fertilizer dose (RFD). The treatments were compared against a control having RFD only. The biometric observations were recorded on five randomly selected plants within each net plot and statistically analysed as per method described by Panseand Sukhatme (1985). Disease severity in mustard was assessed on five randomly selected plants using a 12-point rating scale (Kanzariaand Dhruj, 2018) at the final stage of pod development as follows:

List 1: A 12-point rating scale used for Disease severity in mustard

Rating Scale	Infection/phenotypicclass
0	Healthy(i.e.nodiseasesymptomsontheplant)
I	Few whitishspecks(1to5)onleaf
II	Upto25percentleafareacoveredwithwhitishspecks
III	>25to50percentleafareacoveredwithwhitish fungalgrowth
IV	Morethan50percentareaofleafcoveredwithwhitishfungalgrowthandfewwhitishspecksonstem
V	Morethan50percentareaofleafandupto25percentareaonstemcoveredwithwhitish growth
VI	Morethan50percentareaofleafandstemcoveredwithwhitish growth
VII	Morethan50percentareaofleafandstemcoveredbywhitishgrowthwithfewwhitishspecksonbranches
VIII	Morethan50percentareaofleaf,stemandupto25percentareaofbranchescoveredwithwhitishgrowth
IX	Morethan50percentareaofleaf,stemandbranches coveredwithwhitishfungalgrowth
X	Morethan50percentareaofleaf,stemandbranchescoveredwithwhitishfungalgrowthwithfewwhitishspecksonsiliquae
XI	Morethan50percentareaofleaf,stem,branchescoveredwithwhitishfungalgrowthandupto25percentareaofsiliquae coveredwithwhitish growth

XII Morethan50percentareaofleaf,stem,branchesandsiliquaecoveredwithwhitishgrowth(i.e.entireplantcoveredwithwhitishfungalgrowth)

Percent disease index (PDI) was calculated as [∑Total Rating/Total plants observed x Maximum disease rating] x 100. The PDI was used to calculate Disease Control Percent as [(PDI in Check – PDI in Treatment)/PDI in Check] x 100.

The experiment on okra also comprised three doses of K-Immune 10% (30, 40 & 50 ml/15L) in addition to RFD applied once (30 DAS) and twice (30 and 50 DAS); and RFD as check.

For measuring damage attributed to biotic stresses, 10 leaves were selected from five randomly selected plant and plotted on graph paper to measure total leaf area and damaged area due to biotic stresses, specifically Alternaria leaf spot and caterpillars. The percent damage was calculated as the proportion of damaged leaf area relative to the total area, expressed as percentage.

3. Results and Discussion

3.1 Experiment on Germination in Greengram

3.1.1 Overnight Soaking Experiment

The overnight soaking experiment revealed no significant differences in germination percentages across treatments, with rates ranging from 92% to 96% by day 6 (Table 1). However, seedling vigour, as measured by seedling length on day 6, showed marked differences. Seeds treated with 8 ml/L K-Immune exhibited the highest average seedling length of 7.53 cm, with a maximum length of 10.2 cm. In contrast, the control (water-soaked) seeds had an average length of 2.54 cm and a maximum of 4.2 cm. Seedling length increased progressively up to the 8 ml/L concentration, after which a sharp decline was observed, suggesting an antagonistic effect at higher concentrations (10 ml/L).

Table 1. Effect on germination of Green gram (*Vigna mungo* L.) after soaking for 30 minutes in different concentrations of K-immune and kept overnight

Treatment	Germination % as on					Seedling length on 6th Day (cm)	
	Day 2	Day 3	Day 4	Day 5	Day 6	Average	Maximum
Control	0	6	92	92	94	2.54	4.2
2 ml/L	16	46	92	92	94	4.76	6.2
4 ml/L	24	86	88	94	94	5.03	6.4
6 ml/L	16	62	86	90	92	5.04	6.5
8 ml/L	18	84	94	94	96	7.53	10.2
10 ml/L	16	36	94	92	96	4.50	7.4

3.1.2 10-Minute Soaking Experiment

When seeds were soaked for 10 minutes, seedling vigour increased progressively with K-Immune concentration. The highest concentration (10 ml/L) resulted in an average seedling length of 5.7 cm and a maximum of 8.5 cm, compared to 2.34 cm and 3.20 cm respectively for the control (Table 2).

Table 2. Effect on germination of Green gram (*Vigna mungo* L.) after soaking for 10 minutes in different concentrations of K-immune

Treatment	Germination % on					Seedling length on 6th Day (cm)	
	Day 2	Day 3	Day 4	Day 5	Day 6	Average	Maximum
Control	2	86	88	90	90	2.34	3.2
2 ml/L	2	82	90	92	92	3.22	4.2
4 ml/L	2	82	90	92	92	3.63	5.6
6 ml/L	6	84	86	86	86	5.54	7.1

8 ml/L	2	70	78	86	86	5.52	8.3
10 ml/L	0	82	92	92	94	5.70	8.5

3.1.3 30-Minute Soaking Experiment

Extended soaking for 30 minutes showed positive effects on seedling length up to 8 ml/L concentration, with average and maximum lengths of 6.03 cm and 8.1 cm, respectively, compared to 2.2 cm and 3.1 cm for the control (Table 3). However, phytotoxic symptoms were observed at 10 ml/L concentration, with reduced average (3.04 cm) and maximum (5.80 cm) seedling lengths.

Table 3. Effect on germination of Green gram (*Vigna mungo* L.) after soaking for 30 minutes in different concentrations of K-immune

Treatment	Germination % on					Seedling length on 6th Day (cm)	
	Day 2	Day 3	Day 4	Day 5	Day 6	Average	Maximum
Control	12	80	82	82	84	2.20	3.1
2 ml/L	6	92	96	96	96	4.41	5.2
4 ml/L	14	84	84	88	88	5.98	7.4
6 ml/L	14	90	92	92	92	5.74	7.2
8 ml/L	28	82	84	84	86	6.03	8.1
10 ml/L	6	88	90	90	92	3.04	5.8

In summary, while K-Immune treatment did not significantly affect germination percentage, it had a substantial impact on germination speed and seedling vigour. The optimal concentration was found to be 8 ml/L, promoting both rapid germination and robust seedling growth (Figure 1). Consistent with the present findings, Al-Tawahaand Al-Ghzawi (2013) have also reported that priming lentil seeds with chitosan, regardless of the concentration, improves both seed germination and salt tolerance. Rubina*et al.* (2018) noted that pre-soaking soybean seeds in chitosan increased the hypocotyl length and fresh weight of soybean sprouts and positively affected the content of bioactive compounds in the sprouts. Gürsoy (2020) demonstrated that soaking seeds in chitosan significantly increases germination rates, along with the length and weight of hypocotyls and radicles across various crops. Abobatta(2023) found that chitosan seed treatments enhance germination rates by forming a protective layer around the seeds, which helps retain moisture and improves water uptake.

Figure 1. Day wise germination evincing no difference in % germination after Day 6. However, early germination is evident

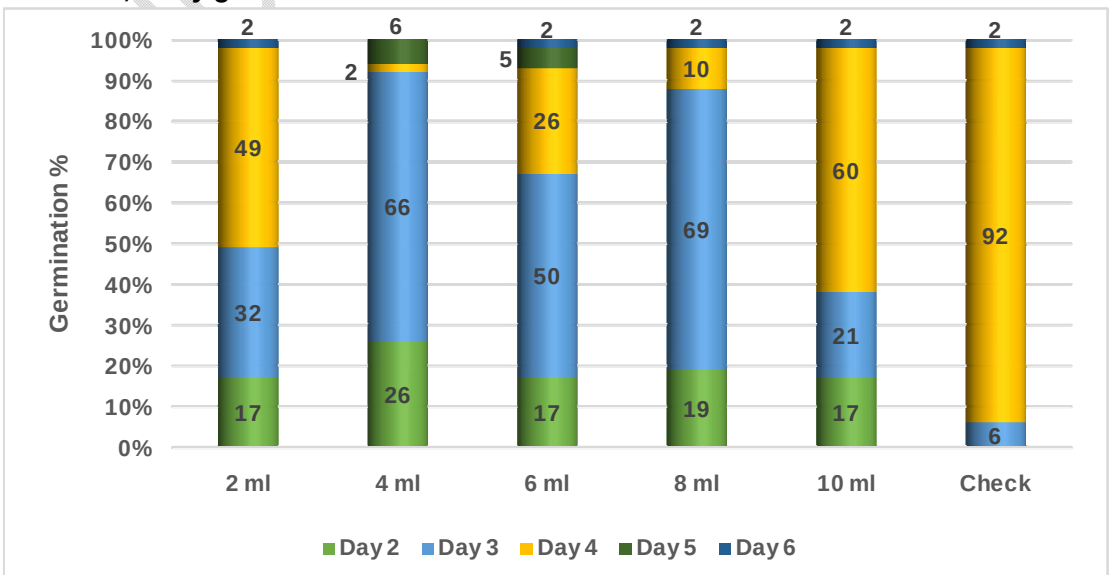


Figure 2. Average Length of Seedling (cm) measured on 6 DAS after 30 minutes soaking and kept overnight before sowing

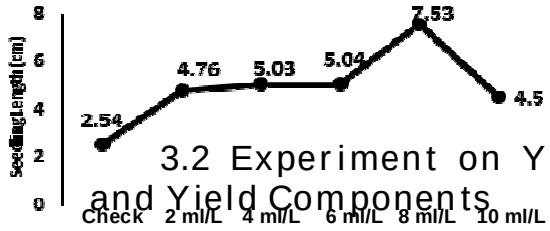
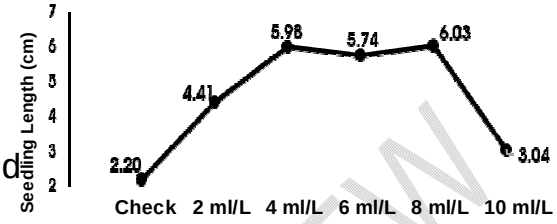


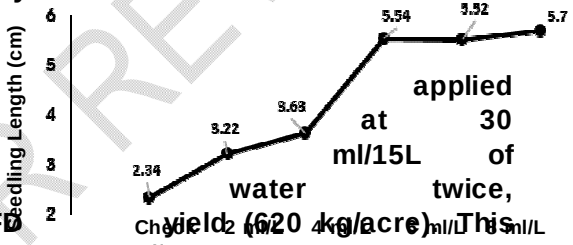
Figure 3. Average Length of Seedling (cm) measured on 6 DAS after 30 minutes soaking before sowing



3.2 Experiment on Yield and Yield Components

Figure 4. Average Length of Seedling (cm) measured on 6 DAS after 30 minutes soaking and kept overnight

Figure 5. Average Length of Seedling (cm) measured on 6 DAS after 10 minutes soaking before sowing



K-Immune was effective in significantly increasing both yield and yield components compared to the recommended fertilizer dose (RFD) (Table 4). The highest seed yield was achieved with K-Immune applied at 30 ml/15L of water twice, resulting in a 42.09% increase over the RFD yield (620 kg/acre). This was followed by increases of 28.06% with 30 ml applied once and 27.74% with 22.5 ml applied twice. Thus, seed yield in mustard increased progressively from 27.74% with two sprays of 22.5 ml/15L to 28.06% and 42.09% with one and two sprays of 30 ml/15L, respectively. The results suggested that two sprays of K-Immune are more effective than one for increasing seed yield. The increase in seed yield was primarily attributed to a significant rise in the number of primary and secondary branches per plant, as well as the number of siliquae per plant compared to RFD. Similar trends were observed for these yield components, with the maximum increase seen in the higher dose of 30 ml/15L sprayed twice, followed by 30 ml/15L sprayed once and 22.5 ml/15L sprayed twice.

The percent disease index for powdery mildew ranged from 24.44% with 15 ml or 22.5 ml/15 L water sprayed twice to 27.22% with 22.5 ml sprayed once or 30 ml sprayed twice as compared to 33.89% for the RFD. The maximum disease control (27.88%) was achieved with 15 ml or 22.5 ml/15 L water sprayed twice. Therefore, 15 ml/15 L water sprayed twice was the most economical dose for controlling powdery mildew in mustard. However, from both seed yield and disease control perspectives, 22.5 ml/15 L water sprayed twice was the best treatment, resulting in significantly 27.74% higher yield and 27.88% disease control over RFD. Increasing the dose to 30 ml/15 L water sprayed twice resulted in the highest yield, 42.09% above RFD, with a satisfactory disease control of 19.67%. Ojaghian*et al*. (2019) demonstrated that chitosan reduces the mycelial growth and sclerotia formation of *Sclerotinia sclerotiorum*, the pathogen responsible for white rot in mustard. Shahrajabian*et al*. (2021) observed that chitosan acts as a plant growth promoter by increasing stomatal conductance, reducing transpiration, and promoting beneficial soil microorganisms. They also noted that it can be applied as a seed coating to improve nutrient delivery to plants.

3.2.2 Okra

The data presented in Table 5 demonstrated a significant impact of K-Immune 10% application on plant height, number of fruits per plant and fruit yield. All K-Immune treatments exhibited significantly higher plant height and number of fruits per plant compared to RFD, except for the single application of 30 ml/15L water at 30 DAS.

K-Immune treatment was effective in increasing fruit yield in okra (Table 5). When applied twice in addition to the RFD, it increased fruit yield varying from 8.37% in 30 ml/15 L water to massive 22.91% in 40 ml/15 L water over 10.22 tons/acre fruit yield obtained in RFD as check.The enhanced fruit yield in the K-Immune treatments was primarily attributed to an increase in the number of fruits per plant and fruit weight per plant.Furthermore, leaf damage due to caterpillars and Alternaria leaf spot was significantly reduced to 2.20% and 1.61%, respectively, when K-Immune was sprayed twice at 40 ml/15L water as compared to the RFD showing 23.93% caterpillar damage and 5.91% Alternaria leaf spot damage. Thus, it can be concluded that K-Immune supplementation in okra at 40 ml/15L water applied twice at 30 and 50 DAS, in addition to RFD, can significantly boost okra productivity by 22.91% while effectively controlling diseases and pests.

The effect of chitosan in enhancing seed germination rates, improving seedling growth and increasing overall plant vigourhas been demonstrated across multiple studies(Rahman*et al.*, 2018; Shibanaand Deepa, 2024).Chitosan oligosaccharides are effective against insect pests, achieving up to 80% mortality rates in lepidopterous and homopterous insects (Zhang*et al.*, 2003). Foliar application of chitosan in okra plants increased morphological parameters, growth rates, biochemical factors and yield attributes, resulting in up to a 27.9% higher fruit yield. Additionally, combining chitosan with other compounds has shown superior effects on vegetative characteristics and biochemical properties (Mondal*et al.*, 2012; Azimi*et al.*, 2023). Further, chitosan has been reported to induce defence responses in plants, helping them resist various pathogens and abiotic stresses while enhancing physiological properties and extending the shelf life of produce (Sharif*et al.*, 2018).More recently, Shibanaand Deepa (2024) observed that chitosan application, whether used as a foliar spray or soil drench, increases the populations of beneficial soil microflora, such as nitrogen-fixing bacteria and phosphate solubilizers, which enhance nutrient uptake and, consequently, improve plant growth and yield.

Conclusion

K-Immune has demonstrated significant efficacy in enhancing both yield and disease management in mustard and okra crops compared to recommended fertilization doses. In mustard, 30 ml/15L applied twice boosted seed yield by 42.09%, while 22.5 ml/15L provided a balanced outcome, with a 27.74% yield increase and 27.88% disease reduction. In okra, 40 ml/15L applied twice at 30 and 50 DAS led to a 22.91% % increase in fruit yield and improved pest and disease control, notably against caterpillars and Alternaria leaf spot. K-Immune boosted plant height, fruit number and weight. Additionally, K-Immune at 8 ml/L significantly enhanced germination speed and seedling vigour, which is crucial for establishing resilient crops under challenging conditions. Overall, K-Immune offers a promising solution to the dual challenges of improving crop yields and managing diseases and pest pressures.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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References

Abobatta, W. F. (2023). Chitosan: A promising plant stimulant. *International Journal of Agricultural Science and Food Technology*. 9(4): 098-103.

Al-Tawaha, A. R. M. and Al-Ghzawi, A. L. A. (2013). Effect of chitosan coating on seed germination and salt tolerance of lentil (*Lens culinaris* L.). *Research on Crops*. 14(2): 489-491.

Azimi, N.; Gandomkar, A. and Sharif, M. (2023). Relationship between production condition, microstructure and final properties of chitosan/graphene oxide–zinc oxide bionanocomposite. *Polymer Bulletin.* 80(6): 6455-6469.

Gürsoy (2020). Effect of chitosan pretreatment on seedling growth and antioxidant enzyme activity of safflower (*Carthamus tinctorius* L.) cultivars under saline conditions. *Applied Ecology and Environmental Research*. 18(5):6589-6603.

Kanzaria, K. K. and Dhruj, I. U. (2018). Field Evaluation of Fungicides for the Management of Powdery Mildew (*Erysiphe cruciferarum*Opiz ex. Junell) Disease of Mustard (*Brassica juncea* (L.) Czern. &Coss.). *International Journal of Current Microbiology and Applied Sciences*. 7(8): 348-360.

Mondal, M. M.; Malek, M. A.; Puteh, A. B.; Ismail, M. R.; Ashrafuzzaman, M. and Naher, L. (2012). Effect of foliar application of chitosan on growth and yield in okra. *Australian Journal of Crop Science*. 6(5): 918-921.

Ojaghian, S.; Wang, L. and Xie, G. L. (2019). Resistance induction against white rot of tuber mustard using chitosans and acetyl salicylic acid. *Journal of Applied Biotechnology Reports*. 6(1): 26-33.

Panse, V.G. and Sukhatme, P. V. (1985). Statistical method for agricultural research workers. ICAR Publication, New Delhi.

Rahman, M.; Mukta, J. A.; Sabir, A. A.; Gupta, D. R.; Mohi-Ud-Din, M.; Hasanuzzaman, M. and Islam, M. T. (2018). Chitosan biopolymer promotes yield and stimulates accumulation of antioxidants in strawberry fruit. *PloS one*. 13(9): e0203769 pp. 1-14.

Rinaudo, M. (2006) Chitin and chitosan: Properties and applications. *Progress in Polymer Science.* 31: 603–632.

Rubina, M. S.; Vasil'kov, A. Y.; Naumkin, A. V.; Shtykova, E. V.; Abramchuk, S. S.; Alghuthaymi, M. A. and Abd-Elsalam, K. A. (2017). Sysis and characterization of chitosan-copper nanocomposites and their fungicidal activity against two sclerotia-forming plant pathogenic fungi. *Journal of Nanostructure in Chemistry*. 7: 249–258.

Sanford, P. A. (2003). Commercial sources of chitin and chitosan and their utilization. In: Varum KM, Domard A, SmidsrØd O (eds). *Advances in Chitin Science*. vol 6. NTNU, Trondheim, pp 35–42

Shahrajabian, M. H.; Chaski, C.; Polyzos, N.; Tzortzakis, N.; Petropoulos, S. A. (2021). Sustainable Agriculture Systems in Vegetable Production Using Chitin and Chitosan as Plant Biostimulants. *Biomolecules*. 11(6): 819-823.

Sharif, R.; Mujtaba, M.; Ur Rahman, M.; Shalmani, A.; Ahmad, H.; Anwar, T. and Wang, X. (2018). The multifunctional role of chitosan in horticultural crops; a review. *Molecules.* 23(4): 872-881.

Shibana, S. N. and Deepa, S. N. (2024). Effect of Chitosan on Increasing Beneficial Soil Microflora and Disease Suppression in Turmeric Plants in Humid Tropics of Kerala, India. *International Journal of Plant & Soil Science*. 36(1): 170-76.

Zhang, M.I.; Tan, T.; Yuan, H. and Rui, C. (2003). Insecticidal and fungicidal activities of chitosan and oligo-chitosan. Journal of Bioactive and Compatible Polymers. 18: 391–400.

Table 4. Effect of K-Immune on growth, yield attribute, yield and powdery mildew control in mustard

Tr.	Specification	Primary branch/ plant	Secondary branch/ plant	No. of seeds/ siliqua	No. of siliqua/ plant	Seed yield (kg/acre)	Test weight (g)	PDI (%)	% control than RFD
T1	RFD + K-Immune 15 ml/15 L water 1X (45 DAS)	8.50	13.30	10.14	306.57	643	4.07	26.67	21.31
T2	RFD + K-Immune 15 ml/15 L water 2X (35//55 DAS)	8.97	14.60	10.89	363.07	708	4.26	24.44	27.87
T3	RFD + K-Immune 22.5 ml/15 L water 1X (45 DAS)	8.70	15.97	11.64	338.03	740	4.48	27.22	19.67
T4	RFD + K-Immune 22.5 ml/15 L water 2X (35//55 DAS)	9.67	17.07	11.66	378.47	792	4.39	24.44	27.87
T5	RFD + K-Immune 30 ml/15 L water 1X (45 DAS)	10.27	17.50	11.94	406.53	794	3.94	25.56	24.59
T6	RFD + K-Immune 30 ml/15 L water 2X (35//55 DAS)	11.80	19.03	12.13	426.97	881	3.94	27.22	19.67
T7	RFD (20:20:00:16 kg NPKS/acre)	7.87	12.87	10.55	298.67	620	4.03	33.89	--
	S.Em. ±	0.45	1.17	0.38	17.31	39.19	0.28	3.16	
	CD (5%)	1.38	3.60	1.16	53.32	120.75	NS	NS	
	CV%	8.28	12.83	5.80	8.33	9.18	11.65	10.22	

Table 5. Effect of K-Immune on growth, yield attribute, yield and biotic stress control in okra

Tr.	Specification	Plant height (cm)	Fruit diameter (mm)	Fruit length (cm)	No. of fruits/ plant	Fruit weight/ plant (g)	Fruit yield (t/acre)	% leaf damage due to Alternaria leaf spot	% leaf damage due to caterpillar
T1	RFD + Chito 30 ml/15 L water 1X (30 DAS)	217.00	18.17	18.13	13.33	200.93	8.95	4.27	5.79
T2	RFD + Chito 30 ml/15 L water 2X (30//50 DAS)	236.20	18.49	17.70	14.47	232.40	9.32	00	5.60
T3	RFD + Chito 40 ml/15 L water 1X (30 DAS)	227.33	18.34	18.05	14.60	190.13	9.34	2.77	6.64
T4	RFD + Chito 40 ml/15 L water 2X (30//50 DAS)	234.67	17.91	18.24	17.00	226.93	10.57	1.61	2.20
T5	RFD + Chito 50 ml/15 L water 1X (30 DAS)	249.13	18.76	18.83	16.00	226.53	9.34	00	9.97
T6	RFD + Chito 50 ml/15 L water 2X (30//50 DAS)	234.07	19.24	19.24	17.67	237.47	9.68	1.26	8.59
T7	RFD (60:20:20:00 kg NPKS/acre)	221.67	18.95	18.32	13.53	202.87	8.60	5.91	23.93
	S.Em. ±	5.06	0.65	0.83	0.59	29.92	0.25		
	CD (5%)	15.35	NS	NS	1.80	NS	0.77		
	CV%	3.84	6.11	7.89	6.92	24.37	4.63		