

Original Research Article

Screening for optimum concentration of chitosan through seed priming in mungbean genotypes

Comment [m1]: the

Abstract

Four different concentrations of chitosan and four mungbean genotypes were studied to find out the optimum concentration of chitosan seed priming. The experiment results revealed that germination percentage, shoot length, root length, fresh weight, dry weight, and seedling vigor index differed significantly among the treatments and genotypes. The greatest impact was observed at lower concentrations which was 0.15% in all four genotypes showing consistent improvements in all the studied parameters. The highest concentration of 0.35% appears to be at par with the control and shows no statistical significance. However, root length was affected in IPM 2-14 and IPM 2-3 at high concentrations. In terms of seedling vigor index, IPM 2-3 showed significant reductions at 0.35%, while other genotypes recorded improvement. Based on our results, we conclude that chitosan seed priming has genotype-specific responses, and lower chitosan concentrations have significant positive effects on all parameters and provide important results for deployment in sustainable agricultural practices.

Comment [m2]: Should have a one sentence stating the problem you are trying to solve.

Comment [m3]: to determine

Comment [m4]: of

Keywords: Chitosan, Germination parameters, Mungbean, Seed priming

1. Introduction

Mungbean (*Vignaradiata* L.) is widely used globally as a major component of many crop systems. The plant is primarily grown in India and other Asian nations as green gram or golden gram. Due to its high protein content, it is commonly consumed as sprouts or dried seeds[1]. The plant has an amazing ability to fix atmospheric nitrogen to soil up to 251 kg/ha by maintaining a symbiosis with *Rhizobium* bacteria. It also enhances soil fertility, supporting crop production[2]. Due to its short growing period (60-70 days), mungbean is also suitable for intercropping between two cereal crops[3]. Despite the high yield potential of mungbean, its average productivity remains astonishingly low at 0.5 t/ha. Poor crop management practices, abiotic and biotic constraints and the absence of quality seeds of improved varieties are contributing factors to low productivity[2,4].

Comment [m5]: (*Vigna radiata* L.)

Comment [m6]: Delete

Comment [m7]: contribute

In recent years, advancements in the field of seed technology have led to increased interest in the use of chitosan in agricultural applications. These applications should take into consideration the nature, origin, application form, and physical characteristics of chitosan as these affect the physiological characteristics of seed[5–7].

Comment [m8]: Delete

Comment [m9]: consider

Chitosan is a natural polysaccharide obtained from the deacetylation of chitin. Chitin is the most abundant polysaccharides found in planet next to cellulose which is present in the exoskeleton of arthropods, such as crab, shrimp and some fungi[8]. Due to the presence of amino groups in chitosan, it can be manipulated structurally with various bioactivities for use in various agricultural systems such as defense inducers of biotic and abiotic stresses as well as enhancing crop growth and yield[9].

Comment [m10]: on the planet

Comment [m11]: and

Using the priming technique, chitosan increased wheat seed germination by 18%, germination rate by 53%, and seedling vigor by 27%[10]. A significant increase in both germination rate and growth parameters was also reported for rice[11]. Additionally, chitosan seed primed seedlings in pearl millets were shown to improve germination rate by 13% and vigor by 18% [12]. Previous research findings such as in bean seeds, artichoke seed and soybean are reported to improve seedlings parameters [13–15].

Thus the use of chitosan in seed technology is a strategy that takes advantage of its characteristics to formulate coatings that are an integral part of the management of crops; its application is intended to enhance the physiological and functional responses during the early stages of crop growth. So, the present study aimed to identify the optimum concentration of chitosan seed priming in different mungbean genotypes through observations on germination and seedling parameters.

Comment [m12]: Do you have a problem of growing mungbean? Document your problems.

Comment [m13]: of

2. Materials and Methods

Comment [m14]: Where was the work done. Give latitude, longitude.

Chitosan of low molecular weight and deacetylation $\geq 75\%$ were obtained from Sigma-Aldrich Co (St Louis, MO, USA). Four genotypes of mungbean viz. Samrat, IPM 2-14, IPM 2-3 and HUM 2 were procured from Indian Institute of Pulse Research, Kanpur, India and genotype HUM 2 from Department of Genetics and Plant Breeding, Banaras Hindu University, Varanasi, India. All the chemicals used were analytic grade and used further used without purification. The chitosan was made to dissolved in 0.1% acetic acid solution under constant stirring overnight and was adjusted to pH 6 using 1N sodium hypochloride. Seeds were primed in different concentrations of chitosan viz. 0.05%, 0.15%, 0.30% and 0.45% and

Comment [m15]: Delete

control with distilled water for 6 h. The soaked seeds were washed and air dried to regain the original weight. Then 20 seeds of each genotype were placed in two layers of whatman no. 1 filtered paper placed in petri dishes moistened with distilled water and finally placed in controlled germination incubator at 28°C±1°C. All the treatments were replicated three times. After 7 days, germination and seedlings parameters were measured. Treatments details are as follow-

Comment [m16]: of Whatman

T0- Control

T1- CS (0.05%)

T2- CS (0.15%)

T3- CS (0.25%)

T4- CS (0.35%)

Comment [m17]: Did the cultures incubate under the light conditions? Type of light, intensity..

2.1 Germination (%)

Germination percentage was observed in both the mungbean genotypes by the given formula:

$$\text{Germination \%} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

2.2 Seedling vigor index (SVI)

Seedling vigor index measured in 7 day old seedling calculated by the formula given as under:

Comment [m18]: 7-day

$$\text{SVI} = \text{germination \%} \times \text{Seedling lengths (Root length + shoot length)}$$

2.3 Measurement of root length and shoot length

Root length and seedling length in wheat seedling were measured by the help of centimeter scale.

2.4 Fresh weight of roots and shoot (g plant⁻¹)

The fresh weight and dry weight of seedlings were measured and expressed in g plant⁻¹. Roots and shoots were separated before fresh weight was assessed via a Sartorius BT-224S electronic balance. Freshly weighed seedlings were placed in the envelopes after which they were placed in a hot air oven for one hour at 100°C. The temperature was then decreased to 65°C until the constant weight of the samples could be achieved.

Comment [m19]: Delete

Comment [m20]: weighed seedlings

Comment [m21]: Add comma

2.5 Statistical analysis

All data presented are subjected to one-way ANOVA and treatment means were compared with Duncan multiple range test (DMRT) at significant level of 5% ($p < 0.05$) through SPSS version 26 (IBM).

3. Results

3.1 Germination %

Germination % of all the four genotypes is presented in Table 1(a). Germination % was found to increase with the increasing chitosan concentration. But there was variation in the magnitude of response between the genotypes. In Samrat and IPM 2-14, all chitosan treatments (T1-T4) showed significant increase in germination % ($p < 0.05$). While, in IPM 2-3, T1, T2 and T3 recorded maximum germination %. In HUM 2, T1, T2 and T3 showed maximum increase while control (T0) and T4 produced non-significant value ($p < 0.05$).

Comment [m22]: significant

3.2 Shoot length

Data revealed that significant differences were found among the treatments as presented in Table 1 (b) and Figure 1. In Samrat, the shoot length significantly increased ($p < 0.05$) in T1, T2 and T3 with maximum value obtained in T2. However, T4 resulted in significant reduction ($p < 0.05$). Similarly, in IPM 2-14, shoot length significant increased in all the chitosan treatments. Maximum was found in T2. In IPM 2-3, there was no significant effect ($p < 0.05$) between T1 and T2. In HUM-2, T2 had significant effect as compared to other treatments. Overall, T2 recorded optimum effects in all the genotypes.

Comment [m23]: Data revealed significant differences among the treatments, as presented in Table 1 (b) and Figure 1.

Comment [m24]: a

Comment [m25]: results

3.3 Root length (cm)

Root length was measured in four genotypes of mungbean (table 1(c) and Figure 1). Chitosan treatment induced significant increase in root length of all the genotypes. Significant variation was observed in Samrat where highest increase was found in T1, T2 and T3, however, they exhibited non-significant effects between them at $p < 0.05$. In IPM 2-14, T0, T1 and T3 showed no significant differences between them at $p < 0.05$. However, there was a significant reduction in T3 and T4. Also, in IPM 2-3, significant increase ($p < 0.05$) was observed in T2 and T3. Lowest value was observed in T4. In HUM-2, T1 and T2 showed significant increase ($p < 0.05$). However, T3, T4 and T0 showed non-significant effect between them.

Comment [m26]: a

Comment [m27]: Delete

Comment [m28]: The lowest

3.4 Fresh weight of the seedlings

Fresh weight was measured in four genotypes and found that chitosan treatment in all the concentrations significantly influenced the measured parameters (Table 1d). In Samrat, maximum fresh weight was found in T2 and T1 with non-significant value among them ($p < .05$). In IPM 2-14, there was significant ($p < .05$) increase in T2 while other treatments were found to be non-significant ($p < .05$). In IPM 2-3, T1 and T2 showed maximum increase in fresh weight. While, T0, T3 and T4 observed non-significant value ($p < .05$) among them. Lastly, in HUM 2, all chitosan treatments increased the fresh weight except T4 which was found to be non-significant with T0 ($p < .05$).

Comment [m29]: measured

Comment [m30]: a

Comment [m31]: Space to be

Comment [m32]: Delete

3.5 Dry weight of the seedlings

Dry weight of the seedlings were measured in all the genotypes showing significant variation among the genotypes and treatments (Table 1e). In Samrat, T2 was found to have maximum value while other treatments were found to be non-significant ($p < .05$) among them. In IPM 2-14, dry weight was found to be non-significant in all the treatments. In IPM 2-3, maximum was found in T2. However, lowest value was found in T4 as compared to T0. In HUM 2, significant increase was found in T2 and T3 ($p < .05$).

Comment [m33]: The dry

Comment [m34]: Delete

3.6 Seedling vigor Index

SVI increased in all the genotypes as chitosan concentrations increased (Table 1 (f)). Statistically significant differences were found in all the treatments in all the genotypes. In Samrat, maximum vigor index was found in T2 with significant value as compared to other treatments. Similarly, in IPM 2-3, T2 was found to have maximum value while lowest was observed in T4 and T0. In IPM 2-3, T2 observed maximum increase as compared to other treatments. While, maximum reduction was observed in T4. In HUM 2, T2 observed significant increase in vigor index. Overall, the optimum concentration was found to be T2 which was 0.15%.

Comment [m35]: Space

Comment [m36]: the

Comment [m37]: Delete comma

Comment [m38]: Delete

Table 1. Effect of different concentrations of chitosan on morphological seedling parameters a) germination percentage b) shoot length c) root length d) fresh weight e) dry weight f) Seedling vigor index. Mean values of three replicates are presented. Within a column, means followed by different letters are significantly different ($P > .05$) according to the DMRT post-hoc analysis

a)

| Treatment | Germination% | | | |
|-------------|--------------|-------------|-------------|-------------|
| | Samrat | IPM 2-14 | IPM 2-3 | HUM-2 |
| T0 | 90.00b | 91.67b | 86.67b | 86.67c |
| T1 | 96.67a | 96.67a | 96.67a | 96.67ab |
| T2 | 100.00a | 100.00a | 98.33a | 98.33a |
| T3 | 98.33a | 98.33a | 96.67a | 95.00ab |
| T4 | 96.67a | 96.67a | 86.67b | 90.00bc |
| SEm± | 1.29 | 1.49 | 1.66 | 2.23 |

b)

| Treatment | Shoot length (cm) | | | |
|-------------|-------------------|-------------|-------------|-------------|
| | Samrat | IPM 2-14 | IPM 2-3 | HUM-2 |
| T0 | 11.83c | 12.33c | 12.20cd | 11.67c |
| T1 | 12.90b | 13.17b | 13.50ab | 12.27c |
| T2 | 13.93a | 14.20a | 14.33a | 13.83a |
| T3 | 12.88b | 13.40b | 12.93bc | 12.93b |
| T4 | 11.17d | 11.87c | 11.41d | 12.17c |
| SEm± | 0.14 | 0.20 | 0.29 | 0.18 |

| Treatment | Root length (cm) | | | |
|-----------|------------------|----------|---------|--------|
| | Samrat | IPM 2-14 | IPM 2-3 | HUM-2 |
| T0 | 7.37c | 8.47a | 7.83b | 7.33c |
| T1 | 8.13ab | 8.80a | 8.00b | 8.17ab |
| T2 | 8.46a | 9.17a | 8.57a | 8.37a |
| T3 | 8.00abc | 7.10b | 8.33ab | 7.70bc |
| T4 | 7.67bc | 7.00b | 7.17c | 7.27c |

| | | | | |
|-------------|-------------|-------------|-------------|-------------|
| SEm± | 0.19 | 0.25 | 0.15 | 0.17 |
|-------------|-------------|-------------|-------------|-------------|

c)

d)

| Treatment | Fresh weight (g) | | | |
|-------------|------------------|-------------|-------------|-------------|
| | Samrat | IPM 2-14 | IPM 2-3 | HUM-2 |
| T0 | 0.297c | 0.361b | 0.317b | 0.290d |
| T1 | 0.388ab | 0.373b | 0.393a | 0.373b |
| T2 | 0.420a | 0.463a | 0.407a | 0.39a |
| T3 | 0.350b | 0.353b | 0.317b | 0.340c |
| T4 | 0.263c | 0.327b | 0.317b | 0.304d |
| SEm± | 0.16 | 0.15 | 0.01 | 0.01 |

e)

| Treatment | Dry weight (g) | | | |
|-------------|----------------|--------------|--------------|--------------|
| | Samrat | IPM 2-14 | IPM 2-3 | HUM-2 |
| T0 | 0.051b | 0.056a | 0.053b | 0.041b |
| T1 | 0.054b | 0.058a | 0.055b | 0.046b |
| T2 | 0.062a | 0.063a | 0.064a | 0.058a |
| T3 | 0.054b | 0.057a | 0.058b | 0.058a |
| T4 | 0.050b | 0.057a | 0.051c | 0.040b |
| SEm± | 0.002 | 0.003 | 0.002 | 0.002 |

| Treatment | Seedling vigor Index | | | |
|-----------|----------------------|-----------|----------|----------|
| | Samrat | IPM 2-14 | IPM 2-3 | HUM-2 |
| T0 | 1728.00c | 1905.87cd | 1735.33c | 1646.67c |
| T1 | 2032.68b | 2121.67b | 2077.33b | 1975.33b |

| | | | | |
|-------------|--------------|--------------|--------------|--------------|
| T2 | 2239.00a | 2336.67a | 2251.00a | 2183.17a |
| T3 | 2053.60b | 2016.33bc | 2056.17b | 1960.50b |
| T4 | 1821.67c | 1824.00d | 1609.02d | 1747.83c |
| SEm± | 42.49 | 40.88 | 26.96 | 46.95 |

f)

Figure.1 Photograph of seedlings primed with different concentrations of chitosan



4. Discussion

As demonstrated in various crops including wheat, maize, pepper, soybean and spinach, seed priming enhanced seedling establishment with uniform seed germination and improved resistance to environmental stresses [16,17]. The present experiment aimed to screen the

optimum chitosan concentration by observing germination and seedling parameters. According to our results, germination % and seedling vigor index were both increased across all chitosan treatments. Nonetheless, genotype-specific responses varied. In agreement with the previous report by Jogaiah et al. (2020)[18] seed priming with chitosan exhibited increased germination % and vigor index in cucumber seeds. Seed priming induces the expression of aquaporins, which increase the transportation of water across the membrane, facilitating tissue expansion and germination potential [17]. All chitosan treatments resulted in longer shoots and roots. However, genotypes respond differently to chitosan treatment. Higher concentrations of chitosan reduced shoot length in Samrat genotype and decreased root length in IPM 2-14 and IPM 2-3 genotypes. Based on these results, it is likely that a higher concentration of chitosan caused oxidative stress in embryo cells, which adversely affected seed germination potential and reduced antioxidative enzyme activities [19]. While low concentration of chitosan stimulates the germination potential and increases seedling establishment. Researchers have observed similar results in seed priming with chitosan nanoparticles at 0.5% in broad bean and observed significant reductions in germination parameters, and suggested that a lower concentration of 0.05% increased the antioxidant enzymes and total phenol content [20]. Rasheed et al. (2020) [21] found that seed priming with low molecular weight of chitosan increased shoot fresh weight, root fresh weight, and shoot/root dry weight, which was consistent with our own results. As reported in rice [22] and maize [23], seed priming with chitosan also promotes early seedling establishment and synchronized growth. Furthermore, chitosan increased shoot and root lengths, fresh and dry weights of the shoot and root, and leaf area of bean plants soaked in chitosan solution [24]. Hameed et al. (2014) [10] also found that seed priming with 0.50% chitosan increased the shoot and root lengths of wheat seedlings. Additionally, chitosan at 0.50% and 0.25% reduced mean germination time, increased shoot length, shoot dry weight, root length and root dry weight in maize genotypes as compared to controls [23]. Therefore, the present results are strongly supported by previous reports on germination and growth attributes.

Comment [m39]: At the same time, a low

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is

Comment [m40]: The products used for this research are commonly and predominantly used in our research area and country

absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

5. Conclusion

It appears that the overall treatment which received 0.15% and 0.05% chitosan had significant effects on all genotypes. The higher concentration of chitosan negatively affected all genotypes' germination potential. The greatest impact was found in T2, which was 0.15% in all four genotypes, showing consistent improvements in all the parameters studied. We conclude that seed priming with chitosan at low concentrations could be beneficial to seedling establishment of mungbean plants and be used as sustainable crop management practice. Nonetheless, the origin and characteristics of chitosan play a key role in how plants respond to growth. Hence, more research related to different origins and properties of chitosan would be needed to provide insights into the beneficial effects of chitosan on the plants, and further studies should be focused on physiological and molecular aspects related to chitosan responses.

Comment [m41]: The overall treatment that received 0.15% and 0.05% chitosan had significant effects on all genotypes.

Comment [m42]: Delete

Comment [m43]: studied

Comment [m44]: a

Comment [m45]:

UNDER PEER REVIEW

References

Comment [m46]: You need to format references according to the PCTOC format.

1. Dash SR, Rautaray BK. Growth Parameters and Yield of Green Gram Varieties (*Vigna Radiata* L.) in East and South East Coastal Plain of Odisha, India. *Int J Curr Microbiol Appl Sci.* **2017**, 6;1517–1523, doi:10.20546/ijcmas.2017.610.181.
2. Pratap A, Gupta S, Basu PS, Tomar R, Dubey S, Rathore M, Prajapati US, Singh P, Kumari G. Towards Development of Climate Smart Mungbean: Challenges and Opportunities. In *Genomic Designing of Climate-Smart Pulse Crops*; Springer International Publishing: Cham, 2019; pp. 235–264.
3. Imtiaz AA, Shahriar SA, Baque MA, Eaty MNK, Falguni MR. Screening of Mungbean Genotypes under Polyethylene Glycol (PEG) Induced Drought Stress Condition. *Annu Res Rev Biol.* **2020**; 1–12, doi:10.9734/arrb/2020/v35i230184.
4. Chauhan Y, Williams R. Physiological and Agronomic Strategies to Increase Mungbean Yield in Climatically Variable Environments of Northern Australia. *Agronomy.* **2018**; 8, 83, doi:10.3390/agronomy8060083.
5. Lan W, Wang W, Yu Z, Qin Y, Luan J, Li X. Enhanced Germination of Barley (*Hordeum Vulgare* L.) Using Chitooligosaccharide as an Elicitor in Seed Priming to Improve Malt Quality. *Biotechnol Lett.* **2016** (38):1935–1940, doi:10.1007/s10529-016-2181-5.
6. Saharan V, Pal A. Chitosan Based Nanomaterials in Plant Growth and Protection; SpringerBriefs in Plant Science; Springer India: New Delhi, 2016; ISBN 978-81-322-3599-6.
7. Iglesias MJ, Colman SL, Terrile MC, París R, Martín-Saldaña S, Chevalier AA, Álvarez VA, Casalongué CA. Enhanced Properties of Chitosan Microparticles over Bulk Chitosan on the Modulation of the Auxin Signaling Pathway with Beneficial Impacts on Root Architecture in Plants. *J Agric Food Chem.* **2019** (67); 6911–6920, doi:10.1021/acs.jafc.9b00907.
8. Rinaudo M. Chitin and Chitosan: Properties and Applications. *Prog Polym Sci.* **2006**, 31; 603–632, doi:10.1016/j.progpolymsci.2006.06.001.
9. Hidangmayum A, Dwivedi P, Katiyar D, Hemantaranjan A. Application of Chitosan

on Plant Responses with Special Reference to Abiotic Stress. *Physiol Mol Biol Plants* **2019** (25); 313–326, doi:10.1007/s12298-018-0633-1.

10. Hameed A, Sheikh MA, Hameed A, Farooq T, Basra SM, Jamil A. Chitosan seed priming improves seed germination and seedling growth in wheat (*Triticum aestivum* L.) under osmotic stress induced by polyethylene glycol. *Philipp Agric Sci.* 2014;97(3):294-9.
11. Stanley-Raja V, Senthil-Nathan S, Chanthini KMP, Sivanesh H, Ramasubramanian, R, Karthi S, Shyam-Sundar N, Vasantha-Srinivasan P, Kalaivani K. Biological Activity of Chitosan Inducing Resistance Efficiency of Rice (*Oryza Sativa* L.) after Treatment with Fungal Based Chitosan. *Sci Rep* **2021**(11); 20488. doi:10.1038/s41598-021-99391-w.
12. Manjunatha G, Roopa K, Prashanth GN, Shekar Shetty H. Chitosan Enhances Disease Resistance in Pearl Millet against Downy Mildew Caused by *Sclerospora Graminicola* and Defence-Related Enzyme Activation. *Pest Manag Sci.* **2008** (64); 1250–1257, doi:10.1002/ps.1626.
13. Godínez-Garrido NA, Ramírez-Pimentel JG, Covarrubias-Prieto J, Cervantes-Ortiz F, Pérez-López A, Aguirre-Mancilla CL. Chitosan Coating on Bean and Maize Seeds: Release of Agrochemical Fungicide and Post-Storage Condition. *J Seed Sci.* **2021**,43. doi:10.1590/2317-1545v43254286.
14. Ziani K, Ursúa B, Maté JI. Application of Bioactive Coatings Based on Chitosan for Artichoke Seed Protection. *Crop Prot.* **2010** (29); 853–859 doi:10.1016/j.cropro.2010.03.002.
15. Zeng D, Luo X, Tu R. Application of Bioactive Coatings Based on Chitosan for Soybean Seed Protection. *Int J Carbohydr Chem.* **2012**;1–5, doi:10.1155/2012/104565.
16. Bose B, Kumar M, Singhal R.K, Mondal S. Impact of Seed Priming on the Modulation of Physico-Chemical and Molecular Processes During Germination, Growth, and Development of Crops. In *Advances in Seed Priming*; Springer Singapore: Singapore, 2018; pp. 23–40.
17. Chakraborty P, Dwivedi P. Seed Priming and Its Role in Mitigating Heat Stress Responses in Crop Plants. *J Soil Sci Plant Nutr.* **2021** (21);1718–1734,

doi:10.1007/s42729-021-00474-4.

18. Jogaiah S, Satapute P, De Britto S, Konappa N, Udayashankar AC. Exogenous Priming of Chitosan Induces Upregulation of Phytohormones and Resistance against Cucumber Powdery Mildew Disease Is Correlated with Localized Biosynthesis of Defense Enzymes. *Int J Biol Macromol.* **2020**(162):1825–1838, doi:10.1016/J.IJBIOMAC.2020.08.124.
19. Divya K, Jisha MS. Chitosan Nanoparticles Preparation and Applications. *Environ. Chem Lett* **2018** (16);101–112. doi:10.1007/s10311-017-0670-y.
20. Abdel-Aziz H. Effect of Priming with Chitosan Nanoparticles on Germination, Seedling Growth and Antioxidant Enzymes of Broad Beans. *Catrina Int J Environ. Sci.* **2019** (18); 81–86. doi:10.21608/cat.2019.28609.
21. Rasheed R, Ashraf MA, Arshad A, Iqbal M, Hussain I. Interactive Effects of Chitosan and Cadmium on Growth, Secondary Metabolism, Oxidative Defense, and Element Uptake in Pea (*Pisum Sativum* L.). *Arab J Geosci.* **2020**;13. doi:10.1007/s12517-020-05871-0.
22. Boonlertnirun S, Sarobol E, Meechoui S, Sooksathan I. Drought Recovery and Grain Yield Potential of Rice after Chitosan Application. *Kasetsart J Nat Sci.* **2007** (41); 1–6.
23. Guan Y, Hu J, Wang, X, Shao C. Seed Priming with Chitosan Improves Maize Germination and Seedling Growth in Relation to Physiological Changes under Low Temperature Stress. *J Zhejiang Univ Sci B.* **2009** (10); 427–433, doi:10.1631/jzus.B0820373.
24. Al S, Sheikha AK, Al-Malki FM. Growth and Chlorophyll Responses of Bean Plants to the Chitosan Applications; 2011; Vol. 50;