RESEARCH ARTICLE Agricultural Reviews, Volume 46 Issue 3: 507-511 (June 2025)

In vitro Seed Germination Studies in Barley Exposed to Zinc Oxide Nanoparticles

A. Pathak[1], T. Mehta[1], N. Thakur[1]

10.18805/ag.R-2637

ABSTRACT

Background: Nanotechnology is an emerging field that could lead to novel applications in the fields of biotechnology and agriculture. The present study was conducted to test the efficacy of zinc oxide (ZnO) nanoparticles on the germination of barley seeds.

Methods: The current study used a chemical method to prepare ZnO nanoparticles. An absorption band at 322 nm using UV/ Visible spectroscopy was obtained due to the formation of ZnO nanoparticles. The seeds were given 100, 200 and 500 ppm of ZnO nanoparticles. Seeds in the control group were given distilled water. Zinc was also given as zinc nitrate (0.1 M) to the seeds of the zinc-treated group.

Result: ZnO nanoparticles at higher concentrations (*i.e.*, 200 ppm and 500 ppm) were found to be toxic to plants, whereas their lower concentrations (100 ppm) boosted the yield and growth of the plant as obtained form different germination indices parameters. The present study showed that ZnO nanoparticles have a significant impact on the seed germination potential and could provide an alternative source for fertiliser or growth enhancers that may improve sustainable agriculture.

Key words: Barley, Growth, Nanoparticles, Seed germination, Zinc.

INTRODUCTION

Barley (commonly called “Jau”) is the main cereal grain in India. It is a very healthy grain that is rich in minerals and vitamins. It has a number of industrial applications and is also used as an animal feed. Due to increasing population in India, there is huge demand of such grains. To meet this increasing demand due to a growing population, it has become important to increase the production of crops. To meet these needs, researchers are trying to develop innovative eco-friendly techniques and methods for increasing the efficiency of crop production. Nanotechnology is one of the options nowadays to enhance the nutritional value of crops. Some engineered nanoparticles could be used as fertiliser and are being visualised as having a potential to revolutionise agriculture and food systems and thus improve the condition of the poor. Nanoparticles have unique physicochemical properties and have the potential to boost plant metabolism (Thakur *et al*., 2022). Reports in literature suggest that nanoparticle may also be used as an essential plant nutrient (Thakur *et al*., 2021; Rizwan *et al*., 2017; Moghaddasi *et al*., 2017) as they can interact with plants at various levels, thereby causing many morphological and physiological changes (Pathak *et al*., 2021; Khodakovskaya *et al*., 2012).

Zinc is an important micronutrient that regulates various physiological responses and metabolic processes in plants (Upadhyaya *et al*., 2017). At a zinc concentration of 25 mg/L, improvement in growth and physiology of cluster beans has been observed, whereas at 50 mg/L zinc, which is considered to be a higher concentration, reduced growth with an adverse effect on plant physiology has been observed (Manivasagaperumal *et al*., 2011). Zinc is known to play an important role in protecting and maintaining the stability of cell membranes. It is also used for protein synthesis, membrane function, cell elongation and tolerance to environmental stresses (Cabot *et al*., 2019). Plants emerging from seeds with low zinc have poor seedling vigour and field establishment in zinc-deficient soils. Germination also involves the movement of metal ions like zinc, so that it may be utilised efficiently. Due to the adverse potential and also having no negative effect on the ecosystem, it has been reported that zinc oxide nanoparticles are used worldwide in a number of applications (Rizwan *et al*., 2017). However, the majority of studies on nanoparticles to date concern toxicity. Comparatively few studies have been conducted on nanoparticles that show that they are also beneficial to plants.

Therefore, the present study was taken up to investigate the growth promotory or inhibitory effects of various concentrations of ZnO nanoparticles on the growth of barley seeds. The observations from the present study will prove useful in understanding the possible role of ZnO nanoparticles in barley during seed germination and will give an insight into the role of ZnO nanoparticles in understanding the physiology of barley seed germination.

[1]Department of Biotechnology, Goswami Ganesh Dutta Sanatan Dharma College, Chandigarh-160 032, India.

Corresponding Author: A. Pathak, Department of Biotechnology, Goswami Ganesh Dutta Sanatan Dharma College, Chandigarh-160 032, India. Email: ashima.pathak@ggdsd.ac.in

How to cite this article: Pathak, A., Mehta, T. and Thakur, N. (2025). *In vitro* Seed Germination Studies in Barley Exposed to Zinc Oxide Nanoparticles. Agricultural Reviews. 46(3): 507-511. doi: 10.18805/ag.R-2637.

Submitted: 15-04-2023 **Accepted:** 24-08-2023 **Online:** 12-09-2023

MATERIALS AND METHODS

Synthesis of ZnO nanoparticles

In order to synthesise ZnO nanoparticles, a wet chemical method using zinc nitrate and sodium hydroxide precursors was used (Talam *et al*., 2012). In this experiment, an alcoholic zinc nitrate solution (0.5 M) was prepared and a 0.9 M aqueous ethanol solution of sodium hydroxide (NaOH) was added drop-wise into it with high speed stirring and then it was allowed to settle overnight. The next day the supernatant was removed and the remaining solution was centrifuged at 6000 rpm for 5 minutes and precipitates were removed. These precipitates were washed with distilled water and ethanol solution and then dried at 60°C in an oven. After drying, ZnO nanoparticles were obtained (Talam *et al*., 2012). The characterization was done by taking the UV-Vis absorption spectrum of the zinc nitrate solution after the addition of NaOH. Also, the absorption spectrum of ZnO dispersed in water was obtained.

Pot experiments

Barley seeds were obtained from Punjab Agricultural University (PAU), Ludhiana, India. The experiments were performed in Biotechnology Department of Goswami Ganesh Dutta Santa Dharma College in the month of February and March in 2021. All the seeds were surface sterilised before germination by treating them with a 5% bavistin solution for 10 minutes and then washed several times with distilled water to remove the traces of bavistin from the seeds. The seeds were divided into five groups. There were 20 seeds in each treatment group. In the zinc treated group, the seeds were treated with a 0.1M zinc nitrate solution (Pathak *et al*., 2018). The nanoparticles were suspended in distilled water and dispersed by providing ultrasonic vibration. Seeds of nanoparticle-treated groups were given 100, 200 and 500 ppm concentrations of ZnO nanoparticles. A similar experiment without nanoparticles was conducted as a control. The seeds belonging to all the groups were given respective treatments daily by watering them and all these treatments were continued for 3 weeks. During the period of three weeks, the number of seeds germinated each day was noted. After 3 weeks, seedlings were harvested and the shoots and roots of seedlings were separated. Seedling growth in terms of root length, shoot length, leaf length and leaf lamina was recorded and the results were compared with the controls to see the effect of nanoparticles on seed germination and early seedling growth.

Germination indices parameters like PI (Promptness index), GSI (Germination stress tolerance index), PHSI (Plant height stress tolerance index), RLSI (Root length stress tolerance index), seedling vigour index (SVI) and relative root elongation (RRE) were calculated by using different formulae (Dharanguttikar *et al*., 2015).

Statistical analysis

All the experimental values are expressed as means of 20 seeds. Comparisons between the control and treated groups were done by one-way ANOVA.

RESULTS AND DISCUSSION

Nanotechnology has emerged as a new discipline and nanoparticles have become the centre of attraction for researchers because of their unique physico-chemical properties compared to bulk particles (Gatoo *et al*., 2014). Nanoparticle characterization is considered an important parameter for confirming whether nanoparticle formation has occurred or not. UV-visible absorption spectroscopy is widely being used to examine the optical properties of nanosized particles. The absorption spectrum of ZnO nanopowder is shown in Fig 1. It exhibits a strong absorption band at about 322 nm. This confirmed the proper synthesis of ZnO nanoparticles (AL-Asady *et al*., 2020; Shamhari *et al*., 2018).

Germination frequency was recorded after 8, 16 and 21 days. It was observed from Table 1 that the percent seed germination decreased in the zinc-treated group as compared to the control group. Also, the per cent germination was found to decrease with an increasing concentration of ZnO nanoparticles. Seed germination is an important phenomenon in modern agriculture because it is a thread of life for plants that guarantees their survival (Siddiqui *et al*., 2014). Results pertaining to seed germination and early seedling growth clearly indicate that ZnO nanoparticles at lower concentrations promoted seed germination, but at higher concentrations reduced seed germination and seedling growth. An increase in germination at low concentrations of nanoparticles may be due to the absorption and utilisation of ZnO nanoparticles by the seeds. Also, there could be the generation of superoxide and hydroxide anions by ZnO nanoparticles that might have encouraged the intake of water and oxygen needed for quick germination (Pathak and Bedi, 2015). ZnO nanoparticles have the potential to overcome seed germination issues in plant species that have low seed germination due to zinc alone.Therefore, a decrease in seed germination at a higher nanoparticle concentration might be due to the physical or chemical toxicity exerted by ZnO nanoparticles on plants, depending on their chemicacomposition, size, surface energy and plant species (Talam *et al*., 2012).

After harvesting, root length, shoot length, leaf length and lamina of control as well as different treated groups were taken. The root length was found to decrease in the zinc-treated group as compared to the control group. The 100 ppm of nanoparticle treatment also caused a significant decrease in root length as compared to the control plants. However, the root length was found to increase at 200 ppm treatment and it again decreased significantly at 500 ppm nanoparticle treatment (Table 1). On the contrary, the shoot length was found to decrease after zinc treatment, further significantly decreased at 100 and 200 ppm nanoparticle treatments but was found to be increased at 500 ppm nanoparticle treatment. Zinc treatment also caused a decrease in leaf length in barley plants. However, the leaf length was found to increase with an increase in the concentration of ZnO nanoparticles. Further, zinc treatment again caused a decrease in leaf lamina in barley plants.

However, the leaf length was found to increase with an increase in the concentration of nanoparticles. Plant growth and development starts with the germination of seeds, followed by root elongation and shoot emergence. These are regarded as the earliest signs of growth and development. Therefore, it is important to understand the course of plant growth and development in relation to nanoparticles. The results obtained in the present study are in accordance with the results obtained by Lawre and Rasker (2014), who revealed that the higher dose of ZnO nanoparticles suspension reduced root and shoot growth of gram and mung seedlings, which may be due to toxicity levels of nanoparticles. ZnO nanoparticles were also reported to be one of the most toxic nanoparticles that could terminate root growth of radish, rape, ryegrass, lettuce, corn and cucumber (Tymoszuk *et al*., 2017; Lawre and Raskar, 2014). This can also be accepted because more ions are released from particles over time and accumulate in plantlets, making them more toxic. However, Prasad *et al*. (2012) found that lower concentrations of ZnO nanoparticles were beneficial to seed germination in peanut, Sedghi *et al*. (2013) in soybean, Ramesh *et al*. (2014) in wheat and Raskar and Laware (2014) in onion. The higher plant growth with nanoparticles might also be due to the mobilisation of nutrients in the soil as well as an increase in microbial population, especially in the rhizosphere (Raliya and Tarafdar, 2013). In the case of root/shoot ratio, an increasing trend was seen from lower (100 ppm) to higher concentrations (200 ppm) of ZnO nanoparticles (Table 1). However, the root/shoot ratio decreased concomitantly from 200 ppm to 500 ppm of ZnO nanoparticles. This indicates that at this higher concentration, the root length promotion is comparatively less when compared to shoot length. This indicates that roots were affected more by higher concentrations of ZnO nanoparticles as compared to percent seed germination and shoot growth.

The results with respect to shoot and root length as well as root to shoot ratio are also consistent with previous studies that report nanoparticles had less of an effect on seed germination than on seedling growth (Lin *et al*., 2007; Ruffini *et al*., 2009). This may be explained by the protective effect of the seed coat (Adhikari *et al*., 2015). Since roots are the first target tissue to confront pollutants, toxic symptoms seem to appear more strongly in roots than in shoots (Garriga *et al*., 2014). The result clearly indicates that ZnO nanoparticles are effective in enhancing plant growth and yield. Studies on other types of nanoparticles have also shown that the engineered nanoparticles can boost seed germination, growth and development of plants (Siddiqui *et al*., 2014; Lahiani *et al*., 2013). As zinc is the structural component of phosphorous (P)-mobilizing phosphatase and phytase enzymes, it can be hypothesised that application of nanoZnO may help in more secretion of these enzymes, which are involved in the mobilisation of

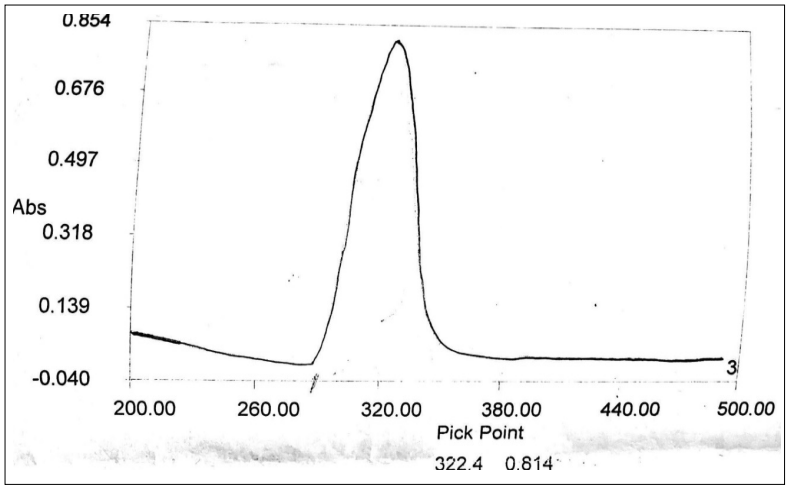


Fig 1: UV-Vis absorption spectrum ZnO nanoparticles.

Table 1: Effects of zinc and zinc oxide nanoparticles (NP) on seedling growth of the Barley.

Treatment	Germination (%)	Shoot length (cm)	Root length (cm)	Leaf length (cm)	Root/Shoot ratio	Lamina
Control	85	6.56	7.72	10.81	1.18	0.55
Zinc	75	3.2	5.98	7.11	1.86	0.38
100 ppm NP	92.85	4.23	2.92*	10.05	0.69	0.31
200 ppm NP	71.42	4.83	5.99	12.22	1.24	0.44
500 ppm NP	57.14	7.5	2.72*	12.34	0.36	0.53

Values are expressed as means of the readings.

*p.0.01 when compared with the control values.

Table 2: Effect of zinc and ZnO nanoparticles on PI, GSI, PHSI, RLSI, SVI and RRE of Barley.

Treatment	PI	GSI	PHSI	RLSI	SVI	RRE
Control	17.25	100	100	100	1213.8	
Zinc	11.75	68.12	64.88	77.46	688.5	77.46
100 ppm NP	6.0	34.78	68.55	37.82	663.8	37.82
200 ppm NP	6.5	37.68	91.83	77.59	772.7	77.59
500 ppm NP	6.0	34.78	89.92	35.23	583.9	35.23

Values are expressed as means of the readings.

phosphorus for plant nutrition from unavailable organic sources. Excess zinc in the soil can often compete with the plant's uptake of phosphorus, iron, or copper, resulting in deficiency in plants and, as a result, toxic ZnO nanoparticles at higher concentrations.The higher percentage of seed germination and significantly longer seedling length observed in barley seedlings obtained from ZnO nanoparticles treated plants can also be attributed to zinc movement from leaf tissues to seed during the seed development and maturation process.

Data with respect to the promptness index (PI) clearly showed that PI decreased with zinc treatment and also at all concentrations of nanoparticle treatment as compared to control, with a slight increase at 200 ppm (Table 2). A higher Germination stress tolerance index (GSI) (37.68) was observed in the 200 ppm ZnO nanoparticle treatment, while a lower GSI (34.78) was observed in the 100 and 500 ppm ZnO nanoparticle treatments. Zinc treatment alone also showed a decrease in GSI value. Plant height stress tolerance index (PHSI) values also showed a decrease at all concentrations of nanoparticle treatment compared to control.

A significant decrease in PHSI value, *i.e*., 68.55 at 100 ppm. However, a significant increase was noticed at 200 ppm and again at 500 ppm, a slight decrease was noted. A significant decrease in root length stress tolerance index (RLSI) values was observed at 100 ppm of ZnO nanoparticles treatment over control and a significant increase in RLSI values (77.59) was observed at 200 ppm, while again a decrease (35.23) was observed at 500 ppm concentrations of ZnO nanoparticles. Seedling vigour index (SVI) and relative root elongation (RRE) also showed a similar trend with decrease noticed at all concentrations of ZnO nanoparticles except at 200 ppm concentration, where a significant increase in SVI was noticed (Table 2).

The result regarding PI shows that ZnO at lower concentration increased seed germination, promptness index and seedling growth. This indicates the lower concentration is not harmful to the cell division and early seedling growth. GSI indicates the speed of seed germination in the seedlings. Thus, the results of PI, GSI, PHSI, RLSI, SVI and RRE demonstrated that nano particles have both positive and negative effects on crops, depending on solution concentration, particle size and chemical and physical properties of nano particles.

CONCLUSION

It can be concluded from the present study that the effect of Zn nanoparticles in the germination of growing seedlings of barley was significant at 200 ppm. As a consequence, the ZnO nanoparticles treated seeds unto 200 ppm showed better potential for germination. Overall, our results can help the fertilizer industries to decide the production of nanofertilizers especially ZnO nanoparticles for plant nutrition which will help to reduce the zinc deficiency in plants and finally humans. However, field studies under different nanostructure sizes, shapes, conditions and plants may further enhance the mechanistic understanding of the applicability of nanoparticles in this field. Also, more studies are needed to explore the mode of action of nanoparticles, their interaction with biomolecules and their impact on the regulation of gene expressions in plants.

ACKNOWLEDGEMENT

We are thankful to the Principal, GGDSD College, Sector 32, Chandigarh, for providing the laboratory facilities in Department of Biotechnology.

Conflict of interest

All authors declared that there is no conflict of interest.

REFERENCES

Adhikari, T., Kundu, S., Rao, A.S. (2015). Zinc delivery to plants through seed coating withnano zinc oxide particles. Journal of Plant Nutrition. 39(1): 136-146.

AL-Asady, Z.M., AL-Hamdan, A.H., Hussein, M.A. (2020). Study the Optical and Morphology Properties of Zinc Oxide Nanoparticles. AIP Conference Proceedings. 2213: 020061. https://doi.org/10.1063/5.0000259.

Cabot, C., Martos, S., Llugany, M., Gallego, B., Tolrà, R., Poschenrieder, C. (2019). A role for zinc in plant defense against patho gens and herbivores. Frontiers in Plant Science. 10(1171): 1-15.

Dharanguttikar, V.M., Bharud, R.W., Borkar, B.H. (2015). Physiological responses of chickpea genotypes for drought tolerance under induced moisture stress. International Journal of Scientific and Research Publications. 5: 9.

Garriga, A.G., Sardans, J., Trujillo, M.P., Ubach, A.R., Oravec, M., Vecerova, K. *et al.* (2014). Opposite metabolic responses of shoots and roots to drought. Scientific Reports. 4: 6829. doi: 10.1038/srep06829.

Gatoo, M.A., Naseem, S., Arfat, M.Y., Dar, A.M., Qasim, K., Zubair, S. (2014). Physicochemical properties of nanomaterials: Implication in associated toxic manifestations. Bio Medical Research International. pp: 498420. https://doi.org/10.1155/2014/498420.

Khodakovskaya, M.V., de Silva, K., Biris, A.S., Dervishi, E., Villagarcia, H. (2012). Carbon nanotubes induce growth enhancement tobacco cells. ACS Nano. 6(3): 2128-2135.

Lahiani, M.H., Dervishi, E., Chen, J., Nima, Z., Gaume, A., Biris, A.S. *et al.* (2013). Impact of carbon nanotube exposure to seeds of valuable crops. ACS Applied Materials and Interfaces. 5: 7965-7973.

Lawre, S., Raskar, S. (2014). Influence of zinc oxide nanoparticles on growth, flowering and seed productivity in onion. International Journal of Current Microbiology and Applied Sciences. 3(7): 874-881.

Lin, D., Xing, B. (2007). Phytotoxicity of nanoparticles: Inhibition of seed germination a rootgrowth. Environmental Pollution. 150(2): 243-250.

Manivasagaperumal, R., Vijayarengan, P., Balamurugan, S., Thiyagarajan, G. (2011). Effect of copper on growth, dry matter yield and nutrient content of *Vigna radiata* (L.) Wilczek. Journal of Phytology. 3(3): 53-62.

Moghaddasi, S., Fotovat, A., Khoshgoftarmanesh, A.H., Karimzadeh, F., Khazaei, H.R., Khorassani, R. (2017). Bioavailability of coated and uncoated ZnO nanoparticles to cucumber in soil with or without organic matter. Ecotoxicology and Environmental Safety. 144: 543-551.

Pathak, A., Bedi, G. (2015). Role of zinc on pesticides-induced oxidative damage and seed germination on Brassica species *in vitro*. Indian Journal of Applied Research. 5(11): 26-28.

Pathak, A., Kaur, R., Thakur, N. (2021). Germination studies and biochemical profile in seeds of wheat exposed to magnesium nanoparticles. International Journal of Pharmaceutical Sciences and Research. 12(12): 6638-6641.

Pathak, A., Virk, A.K., Sharma, S., Garg, P., Suman, Thakur, N. (2018). Studies on seed germination in capsicum seeds (Solan Bharpur) under abiotic stress. International Journal for Research in Applied Science and Engineering Technology. 6(3): 27-34.

Prasad, T.N.V.V., Sudhakar, P., Sreenivasulu, Y., Latha, P., Munaswamy, V., Reddy, K.R. *et al.* (2012). Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. Journal of Plant Nutrition. 35: 905-927.

Raliya, R., Tarafdar, J.C. (2013). ZnO nanoparticle biosynthesis and its effect on phosphorous- mobilizing enzyme secretion and gum contents in cluster bean (*Cyamopsis tetragonoloba* L.). Agricultural Research. 2(1): 48-57. DOI: 10.1007/s40003-012-0049-z.

Ramesh, M., Palanisamy, K., Babu, K. and Sharma, N.K. (2014). Effects of bulk and nano-titanium dioxide and zinc oxide on physio-morphological changes in *Triticum aestivum* Linn. Journal of Global Biosciences. 3: 415-422.

Raskar, S.V., Laware, S.L. (2014). Effect of zinc oxide nanoparticles on cytology and seed germination in onion. International Journal of Current Microbiology and Applied Sciences. 3: 467-473.

Rizwan, M., Ali, S., Hussain, A., Ali, Q., Shakoor, M.B., Rehman, M.Z., Farid, M., Asma, M. (2017). Effect of zinc-lysine on growth, yield and cadmium uptake in wheat (*Triticum aestivum* L.) and health risk assessment. Chemosphere. 187: 35-42.

Ruffini, C.M., Cremonini, R. (2009). Nanoparticles and higher plants. Caryologia. 62: 161-165.

Sedghi, M., Hadi, M., Toluie, S.G. (2013). Effect of nano zinc oxide on the germination of soybean seeds under drought stress. Annals of West University of Timisoara, ser. Biology, XVI. 2: 73-78.

Shamhari, N.M., Wee, B.S., Chin, S.F., Kok, K.Y. (2018). Synthesis and characterization of zinc oxide nanoparticles with small particle size distribution. Acta Chimica Slovenica. 65: 578-585.

Siddiqui, M.H., Al-Whaibi, M.H. (2014). Role of nano-SiO_2 in germination of tomato (*Lycopersicum esculentum* seeds Mill.). Saudi Journal of Biological Sciences. 21: 13-17.

Talam, S., Karumuri, S.R., Gunnam, N. (2012). Synthesis, characterization and spectroscopic properties of ZnO nanoparticles. International Scholarly Research Notices ISRN Nanotechnology. 1-6.

Thakur, N., Rana, S., Pathak, A. (2022). Impact of chemically synthesised copper nanoparticles on growth and biochemical profiling of wheat (*Tritium aestivum* L.). International Journal of Biology, Pharmacy and Allied Sciences. 11(6): 2638-2651.

Thakur, N., Chrungoo, S., Rana, S., Kaur, S., Kaur, S., Pathak, A. (2021). Effect of copper nanoparticles on *in vitro* seed germination of wheat (*Tritium aestivum* L.) vareties. International Journal of Pharmaceutical Sciences and Research. 12(8): 4307-4313.

Tymoszuk, A., Wojnarowicz, J. (2017). Zinc oxide and zinc oxide nanoparticles impact on in vitro germination and seedling growth in *Allium cepa* L. Materials (Basel).13(12): 2784. doi: 10.3390/ma13122784.

Upadhyaya, H., Roy, H., Shome, S., Tewari, S., Bhattacharya, M.K., Panda, S.K. (2017). Physiological impact of zinc nanoparticle on germination of rice (*Oryza sativa* L.) seed. Journal of Plant Science and Phytopathology 1: 62-70.