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The Use of Nano-Silver Particles to Increase Yield of Broad Bean

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Present work was carried out to determine the role of silver nanoparticles to improving yield of *broad bean* SNPs (10-100 nm) were synthesized by green method using extract of *Alfalfa*. Broad bean seeds were soaked and seedling was foliage sprayed by 0, 40, 80 and 120 ppm SNPs. The experiment was arranged as split-split plot randomized complete block design with three replicates. This study shows that silver nanoparticles have definite ability to improve growth and yield of crops. Nevertheless, a comprehensive experimentation is needed to establish the most appropriate concentration, size and mode of application of silver nanoparticles for higher growth and maximum yield of broad bean.

Keywords: Nano-silver particles, green pod yield, *broad bean.*

Generally, metal nanoparticles can be prepared and stabilized by physical and chemical methods; the chemical approach, such as chemical reduction, electrochemical techniques, and photochemical reduction is most widely used[1,2]. Studies have shown that the size, morphology, stability and properties (chemical and physical) of the metal nanoparticles are strongly influenced by the experimental conditions, the kinetics of interaction of metal ions with reducing agents, and adsorption processes of stabilizing agent with metal nanoparticles[3,4]. Hence, the design of a synthesis method in which the size, morphology, stability and properties are controlled has become a major field of interest[5].

Engineered nanoparticles have three different unique characteristics, size, structure and properties. These nanoparticles received a particular attention for their positive impact in improving many sectors of economy, including consumer products, pharmaceutics, cosmetics, transportation, energy and agriculture etc., and are being increasingly produced for a wide range of applications within industry. Higher plants strongly interact with their atmospheric and terrestrial environments and are expected to be affected as a result of their exposure of NSPs. Only a few studies are available on the effects of nanoparticles on higher plants. The majority of the reported studies point to the positive impacts of nanoparticles on plant growth with a few isolated studies pertaining to negative effect[7,8]. Sastry et al. have reported that fungus *Verticillium* sp. and *Fusarium oxysporum*, when exposed to gold and silver ions, reduced the metal ion fairly rapidly and formed respective metallic nanoparticles. Klaus et al. have observed that the *Pseudomonas stutzeri* AG259, isolated from a silver mine, when placed in a concentrated solution of silver nitrate produced

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silver nanoparticles of well-defined size and distinct morphology within the periplasmic space of the bacteria[9]. Another study by Lin and Xing investigated phytotoxicology of nanoparticles (multi-walled carbon nanotube, aluminum, alumina, zinc and zinc oxide) on seed germination and root growth of six higher plant species. Seed germination was not affected except for the inhibition of nanoscale zinc (nano-Zn) on rye grass and zinc oxide (nano-ZnO) on corn at 2000 mg/l. Inhibition on root growth varied greatly among nanoparticles and plants[10].

Silver nanoparticles, like its bulk counterpart, is an effective antimicrobial agent against various pathogenic microorganisms. Though various chemical and biochemical methods are being explored for silver nanoparticles production[11], microbes arevery much effective in this process. known to reduce the metals, most of them are found to be spherical particles as reported earlier[12–13]. The resistance conferred by bacteria to silver is determined by the 'sil' gene in plasmids[14] while a nitrate-dependent reductase and a shuttle quinone extracellular process were reported for the reduction of silver ions by several Fusarium oxysporum strains[15]. In fact, nanotechnology is a promptly emerging discipline considerably influencing every field of science and biology. Discovering comprehensive application profile of nanoparticles may transform research in crop science and warrant food security by enhancing crop productivity. Sifting the possible aids of SNPs present work was conducted to document the role that SNPs can play in yield of Broad bean.

EXPERIMENTAL

Synthesis of Silver Nanoparticles

Silver nanoparticles were synthesized by treating 100 ml aqueous extract of *Alfalfa* with 100 ml of 4 mM silver nitrate ($AgNO_3$) solution. The aqueous extract of Alfalfa was prepared by dissolving 10 g dried powder of Alfalfa in 200 ml distilled water and kept for 20 hrs with continueous shaking. The conversion of silver ions to silver nanoparticles was monitored by colour change which truned from yellowish to dark brown, an indication of formation of silver nanoparticles (Fig. 1). Then this treated solution was subjected to UV-Visible spectroscopy in the range of 350 to 700 nm after 0 and 8 hrs. It showed an absorption broad beank at 426 nm which is a characteristic broad beank of silver nanoparticles in the solution due to surface plasmon resonance (Fig. 2). The size and shape of the silver nanoparticles was determined by Transmission electron microscopy. The solution of silver nanoparticles was centrifuged at 14000 rpm for 4 min and centrifuged silver nanoparticles were then suspended in distilled water. A drop of suspension was placed on a carbon coated copper grid and allowed to complete dry under lamp. TEM analysis was performed to determine the size of nanoparticles. TEM micrographs indicated that size of the silver nanoparticles is ranging from 10-100 nm with spherical shapes (Fig 3).

Yield of Broad bean

The seeds of the broad bean varieties were planted in split-split plot randomized complete block design with three replicates. The main plots were assigned to broad bean varieties, subplots to modes of application of AgNPs and sub-sub-plots to silver nanoparticles concentrations (0, 40, 80 and 120 ppm). All the agronomic practices were carried out as and when needed. The silver nanoparticles were applied by three modes of application i.e. seed treatment, foliar spray and seed treatment plus foliar spray. Seed treatment was carried by soaking seeds in the solution of silver nanoparticles of 0, 40, 80 and 120 ppm for 8 hrs. The plants were sprayed by silver nanoparticles at phenological stage BBCH 11 and BBCH 60 in the early morning. Manual pump was used for spray in all cases. When plants were mature and ready for harvesting data number of seeds pod-1, number of pods plant-1, hundred seed weight, biological yield and green pod yield was recorded. Statistical analysis of data was done using the computer based statistical package MSTATC and treatment means were compared using DMRT Test at 5 % level of probability.

RESULTS AND DISCUSSION

The application of silver nanoparticles on yield paramters of broad bean revealed a significant difference among treatments. We first time report highly favorable effects of SNPs at 30 to 60 ppm on number of seeds pod-1, number of pods plant-1, hundred seed weight, biological yield and green pod yield in broad bean. Our study also shows that effect of SNPs is concentration dependent. When SNPs concentration increased from 30 to 60 ppm, it promoted the yield of broad bean. However yield dropped again when 90 ppm SNPs applied. Salama et al. also reported similar results[20]. They observed that increasing concentration of SNPs from 20 to 60 ppm led to an increase in shoot and root lengths, leaf surface area, chlorophyll, carbohydrate and protein contents of common bean and corn. Additional increase in level of SNPs resulted in reduction of these parameters. Nanoparticles stimulate growth at low dose but retard growth at high dosage. treated seedlings with ethylene and no flower bud formation was observed in treated seedling, however silver prevent the action of ethylene. In this research, silver nanoparticles treated plants produced increase number of pods per plant which was due to inhibition of undesired ethylene action by silver on flowering. It is also suggested that abscission may cause retardation in seed yield. The main cause of abscission is the imbalance in phytohormones e.g. ethylene and it has been proven that silver ions prevent ethylene to connect to its receptors in plant cell which inhibits its action. Seeds abscission is reduced which results in more seed yield. Another suggestion is that, increase in yield may be due to improvement of cellular electron exchange efficiency by silver nanoparticles which reduces the formation of reactive oxygen species by arresting electron leakage. As silver prevent the action of ethylene, so plants under the application of silver nanoparticles showed better growth and yield. A significant role of nanotechnology in agriculture was found but it may cause some negative impacts on our environment and ecosystem. The potential risks related with the discharge of nanomaterials in the environment are still indistinct to scientists. They emphasized their study on main exposure routes and determinants of nano toxicities involving particle size, surface, structure, chemical composition, and dosage. Control application of silver nanoparticles can minimize these negative impacts on environment. The latent application of nanomaterials in diverse agricultural applications needs further research investigation with respect to synthesis, toxicology and its effective application at field level. Present research and

Fig. 1. Change in colour of alfafa extract after adding $AgNO_3$ solution (right)

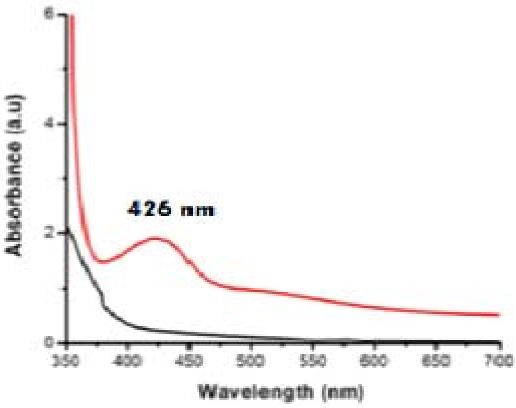

Fig. 2. UV-visible spectrographs of silver nanoparticles

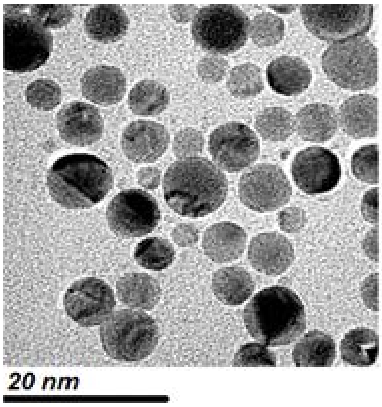

Fig. 3. TEM micrographs of silver nanoparticles

development is still at bench-top scale. Further struggles are mandatory in commercialization of nanomaterials for agricultural applications, which needs accurate protection necessities, testing concerns, risk assessment and regulatory management at global level. From economic point of view, biosynthesized silver nanoparticles can be a suitable choice because of easily synthesized and small amount can give large scale production. These are the best alternative to chemical fertilizer. So the application of these nanoparticles on other crops may revolutionize the agriculture and economy of country.

CONCLUSION

In order to understand the possible benefits of using nanoparticles in agriculture, it is importance to grow the crops under the effect of nanoparticles. Considering this, Positive effect of silver nanoparticles have been found, when they apply on *P. sativum*. High yield of broad bean varieties was recorded for the plants treated with 80 ppm AgNPs, which indicated that this concentration of silver nanoparticles is optimum for obtaining maximum yield. Sensible usage of SNPs can promote yield of crops.

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