Research Article

Improving growth and yield of mungbean (Vigna radiata L.) through foliar application of silver and zinc nanoparticles

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Citation
Allah Wasaya, Tauqeer Ahmad Yasir, Naeem Sarwar, Omer Farooq, Ghulam Rasool Sheikh and Abdul Wahid Baloch. Improving growth and yield of mungbean (Vigna radiata L.) through foliar application of silver and zinc nanoparticles. Pure and Applied Biology. Vol. 9, Issue 1, pp790-797.
http://dx.doi.org/10.19045/bspab.2020.90085

Abstract
Nano-particles are being used in every aspect of modern life and their application is rapidly expanding even in agriculture. Silver (Ag) and Zinc (Zn) nano-particles are hypothesized to enhance growth and productivity of crop plants. Therefore, current experiment was conducted to evaluate the impact of foliarly applied Ag and Zn nano-particles on growth characteristics and yield attributes of mungbean. This experimental study was comprised of sixteen treatments having 3 replications. Three concentrations of Ag nano-particles (10, 20 and 30 ppm) and Zinc nano-particles (2, 4 and 6 ppm) were used as sole treatment as well as in combinations and compared with the untreated control. Foliar application of nano-particles significantly affected growth, yield and yield related traits. Number of branches per plant, pods per plant, chlorophyll contents and grain yield was improved in plots where Ag and Zn nanoparticles were applied in combination @ 20 ppm + 6 ppm concentration, respectively. About 26% increase in seed yield was recorded under combined application of Ag and Zn nanoparticles (i.e. 20 ppm + 6 ppm). A significant reduction in branches per plant, number of pods per plant, chlorophyll contents and seed yield was observed in control plots and it was at par with higher concentration of applied silver and zinc nanoparticles i.e. 30 ppm + 6 ppm, respectively. The results of the current study suggest that Ag and Zn nanoparticles should be applied in combination @ 20 ppm + 6 ppm respectively to improve mungbean production under arid regions.

Keywords: Foliar spray; Nano-particles; Seed yield; Vigna radiata

Introduction
Mungbean (Vigna radiata L.) is an important legume crop and due to its good nutritional value it fulfills the need of increasing world population [1]. In Pakistan, mung bean was cultivated on an
area of 219700 ha with a total production of 157.4 thousand tones and average yield of 716 kg ha\(^{-1}\). Though mung bean is growing throughout the country, Punjab is the major pulse producing province in Pakistan [2]. For sustainable agriculture production, mung bean crop plays a significant role to sustain the soil fertility through biological nitrogen fixation. It stabilizes the atmospheric nitrogen and increases the soil fertility [3]. Its seed consists of proteins (24.2%), carbohydrates (60.4%) and fat (1.3%) which are important part of human diet [4].

Modern technology is the best to meet the feeding requirements of increasing world population by improving food quality including pulses [5]. Nanotechnology has a potential to solve the problems related to food security [6]. Silver and Zinc nanoparticles are most commonly used nanomaterials in various fields, including agricultural sector [7]. Due to their small size (between 1 and 100 nm) and unique chemical and physical characteristics, these are used in daily life including agriculture [7, 8]. Application of nano-particles in agriculture as well as the farming industry may change the field through pest identification and encourage crop ability to absorb further nutrients [9]. Application of nano-particles provides a new aspect for selecting and distributing such assets, which enhances product significance [10]. Silver nano particles (SNPs) are good materials and have an enormous effect on crop development including flowering, root-shoot proportion, seed growth, root formation and root stiffness [11]. It was observed that use of nano-materials such as ZnO, FeO and Ag nano-particles improved crop yield in various crop plants species viz., Brassica species [12] and Solanum lycopersicum [13]. Carbon based graphene nanoparticles have been shown to activate physiological mechanisms at reduced doses and act as growth regulating compounds in food plants including Coriandrum sativum and Allium sativum [14]. A lot of research on the impact of silver nanoparticles (SNPs) on microbial and animal cells have been recorded, but only a few plant experiments have been carried out [15, 16]. AgNP increased the growth profile of plants (root and shoot size, leaf length and width), biological and physiological attributes (chlorophyll, carbohydrates and proteins, antioxidant enzymes) of Brassica juncea, bean and wheat [17, 18]. Nanoparticles increase crop nutrient utilization efficiency and reduce environmental pollution. It also improves plant growth by increasing the resistance of plants as well as uptake of nutrients by deeper root system [19].

There are a number of factors, which can be explored for boosting mungbean yield but application of nanoparticles is of prime importance for yield increment in mungbean of arid area. With the changing climate for last few years, the use of nanotechnology plays a major role for improving growth and yield of agricultural crops. Therefore, this experimental study was conducted to evaluate the role of zinc and silver nanoparticles for enhancing growth and yield of mungbean under arid climatic conditions.

**Materials and methods**

**Experimental site and weather details**

The current study was carried out at Research farm of BZU, Bahadur Sub-Campus Layyah, Pakistan (latitude 31.25\(^{\circ}\) N and longitude 73.09\(^{\circ}\) E) during summer 2017. The climate of this area is arid to semi-arid. Experimental soil was sandy loam having soil physiochemical properties as pH 8.2, EC 1.07 ds m\(^{-1}\), organic matter 0.49%, available phosphorus (P) 8 ppm and available potassium (K) 86 ppm. The weather data for the crop season under study is given in (Fig. 1).
Crop husbandry and experimental details
A pre-sowing irrigation of about 10 cm was applied to wet the field. After three days of pre-sowing irrigation, when field achieved favorable moisture condition seedbed was prepared. A tractor mounted cultivator was used for seedbed preparation. To prepare fine seedbed 2-cultivations with cultivator along with one planking was done to place the seed at proper depth. The mung bean variety AZRI-2006 was obtained from Arid Zone Research Institute (AZRI) Bhakkar and was dropped at 2 cm depth using hand drill on June 15, 2017. Different treatment combinations viz. Control (water spray), Silver nano-particles (SNPs) 10 ppm; SNPs 20 ppm; SNPs 30 ppm; Zinc oxide (ZnO) was used as 2; 4 and 6 ppm; SNPs 10 ppm + ZnO 2 ppm; SNPs 10 ppm + ZnO 4 ppm; SNPs 10 ppm + ZnO 6 ppm; SNPs 20 ppm + ZnO 2 ppm; SNPs 20 ppm + ZnO 4 ppm; SNPs 20 ppm + ZnO 6 ppm; SNPs 30 ppm + ZnO 2 ppm; SNPs 30 ppm + ZnO 4 ppm and SNPs 30 ppm + ZnO 6 ppm were applied through foliar spray. This experiment was randomized according to RCBD with factorial arrangement having experimental unit size 5 × 2.4 m. Mungbean was sown through hand drill using seed rate of 25 kg ha⁻¹. There were total 8-rows in each plot having R×R distance of 30 cm. Thinning was done manually 30-days after sowing (DAS) of crop by maintaining a P×P distance of 10 cm. NPK were applied at sowing with the rate of 25, 50 and 25 kg ha⁻¹ nitrogen (N), phosphorus (P), potassium (K), respectively using Urea, DAP and SOP as source. Manual hoeing was done to control weeds while all other cultural practices were kept normal and uniform. Overall three irrigations were applied to mature the crop.

Data collection
Data on chlorophyll contents, number of branches per plant, pods per plant, seeds per pod, pods weight (g), grain yield and biological yield was recorded following standard procedures. Ten plants from each treatment were randomly selected to measure the chlorophyll content using portable SPAD-502 Chlorophyll Meter (Minolta Co., Ltd.). The reading was taken from three different points (bottom, middle and tip of leaf) and then averaged to get final SPAD-Chlorophyll value. Ten (10) plants were randomly selected to count branches per plant and pods plant⁻¹. Total number of branches and pods were counted from these randomly selected plants and then averaged to get number of branches and pods per plant. At physiological maturity 10 plants were harvested from each plot and their pods were separated, counted and then weighed to find total weight of all pods and then averaged to get pod weight. After calculating pod weight, all the pods were threshed manually and total number of seeds were counted and then averaged to calculate seed per pod. Similarly from these threshed pods about 1000-seeds were counted with the help of automatic seed counter and then weighed to measure 1000-seed weight. At physiological maturity, harvesting was done during first week of September, 2017 using hand sickle and all plants of each plot were harvested, sun-dried for 6-days and then weighed to measure biomass per plot and then converted into kilogram per hectare. After this all the harvested and dried plants from whole plot were manually threshed to separate seed and straw and weighed to measure straw and seed yield per plot then converted into kilogram per hectare. Harvest index (H.I.) was calculated by using following formula [20].

\[
H.I. (\%) = \frac{Grain \ Yield}{Biological \ Yield} \times 100
\]

Statistical analysis
Fisher’s analysis was used to analyze the data and after this analysis least significant difference test at 5% probability was used to compare the means [21]. All the data were analysed using Statistix software version 8.1.
Results
Effect of foliar application of Zn and Ag nano-particles on SPAD-chlorophyll contents, number of branches per plant, biological yield, pods per plant, pods weight, seed per plant, seed yield and 1000-seed weight of mung bean is presented in (Table 1). This study indicated that foliar application of zinc and silver nano-particles significantly affected the number of branches per plant, chlorophyll contents, pods per plant, pods weight, seeds per pod, seed yield, thousand seed weight and biological yield while harvest index was not significantly affected by zinc and silver nano-particles application in mugbean. Highest number of branches, pods per plant and seeds per pod were recorded in plants where silver and zinc nano particles were applied in combination @ 10ppm and 6ppm, respectively. Similarly, an increase of about 31% chlorophyll contents, 26% seed yield, 21% thousand seed weight and 16% biological yield was recorded from plots treated with silver and zinc nano particles @ 20 ppm and 6 ppm concentration, respectively compared with control. Number of branches, seed per pod, seed yield and biological yield were highest in T_{13} i.e. silver and zinc nano particles were applied @ 20 ppm and 6 ppm concentration, respectively that was statistically at par to T_{12} where silver and zinc Nano particles were applied at 20 ppm and 4 ppm, respectively (Table 1; Fig. 2). While lowest number of branches, pods per plant, chlorophyll contents, seed yield and 1000-seed weight was recorded from plots where simple water was sprayed. Minimum number of branches per plant, seeds per pod and biological yield was recorded in T_{1} that was statistically at par to treatment T_{2} where SNPs were applied at 10 ppm concentration (Table 1). All the growth and yield parameter were reduced in the treatment T_{16} where silver and zinc Nano particles were applied at higher concentration i.e. 30 ppm and 6 ppm respectively.

Discussion
Plant physiological and yield parameters such as SPAD-chlorophyll, 1000-seed weight, seed yield and biological yield was improved by applying Zinc and Silver nano-particles in combination (Table 1; Fig. 2). Improvement in chlorophyll (SPAD), relative water contents (RWC) and reduction of membrane leakage in plants treated with ZnO nano-particles were also observed in previous studies [22]. The application of Zn during vegetative and reproductive stages may supply Zn to the developing seeds, thus increasing the growth of the plant and also Zn content [22]. Plant root growth was increased by 21.4% under ZnO nano-particles application which enhanced the plant’s ability to extract soil water, while Zn deficiency decreased the ability of plants to use soil moisture reserves [23]. Improved chlorophyll, under Zn application might be due its role of inducing stress tolerance in crop plants (Table 1). Similarly, in field-grown chickpea addition of Zn had increased water potential and stomatal conductance [24].

Our results showed increasing trend in grains number, 1000-seed weight as well as seed yield (Table 1; Fig. 2). This increase in seed yield as well as yield parameters might be due to enhanced chlorophyll contents (Table 1) which indirectly improved photosynthetic process and enhanced production of photo-assimilates. The higher grain weight increases the strength of individual sinks, which can be shown as the output of sink activity and sink size [25].

Our results showed that ZnO NPs increased plant growth and photosynthesis more than control. It has been reported that application of Zn nano-fertilized leaf improves chlorophyll content of leaf better than control and ordinary Zn application [26].
Increased chlorophyll contents of plants were observed under SNPs application [27]. Foliar application of silver nanoparticles enhanced the yield attributes which ultimately enhanced crop yield. This increase in yield might be due role of silver as growth regulator [28]. Foliarly applied SNPs improved the yield parameters of plants because it plays an imperative role in photosynthetic activity of plants and also increases production of growth promotor Indole acetic acid (IAA) [29, 30]. Results of this study revealed that foliarly applied ZnO nanoparticles enhanced the yield and yield related parameters, the reason of this increase is that Zn is involved in activation of several enzymes responsible for enhancement in photosynthetic activity of plants that increases cell size and ultimately improves the plant height, cob length, seeds per pod, grain and biological yield of crop [31-33].

Figure 1. Climatic data for whole growing season during 2017
Table 1. Effect of foliar application of silver and zinc nanoparticles on SPAD-chlorophyll and yield components of mungbean

| Treatment | Branches Plant\(^{-1}\) | SAPD-Chlorophyll Value | Pods plant\(^{-1}\) | Seeds pod\(^{-1}\) | Thousand seed weight (g) | Harvest index (%) |
|-----------|--------------------------|------------------------|---------------------|-------------------|-------------------------|------------------|
| \(T_1\)   | 10.14 \(l\)              | 33.00 \(o\)            | 14.30 \(p\)         | 8.60 \(o\)        | 20.1                    | 33.0 \(l\)        |
| \(T_2\)   | 10.51 \(kl\)             | 34.10 \(n\)            | 15.00 \(o\)         | 9.02 \(no\)       | 25.5 \(k\)             | 35.06 \(k\)       |
| \(T_3\)   | 10.88 \(k\)              | 35.00 \(m\)            | 15.80 \(n\)         | 9.32 \(mn\)       | 29.4 \(jk\)            | 35.52 \(jk\)      |
| \(T_4\)   | 11.63 \(j\)              | 36.20 \(l\)            | 16.50 \(m\)         | 9.74 \(lm\)       | 30.2 \(j\)             | 36.32 \(ij\)      |
| \(T_5\)   | 11.53 \(j\)              | 37.00 \(k\)            | 17.00 \(l\)         | 10.11 \(kl\)      | 33.4 \(ij\)            | 36.77 \(hi\)      |
| \(T_6\)   | 11.9 \(ij\)              | 38.10 \(j\)            | 17.80 \(k\)         | 10.51 \(jk\)      | 37.6 \(hi\)            | 37.19 \(gh\)      |
| \(T_7\)   | 12.24 \(hi\)             | 39.20 \(i\)            | 18.50 \(j\)         | 10.82 \(ij\)      | 40 \(gh\)              | 37.69 \(fg\)      |
| \(T_8\)   | 12.61 \(gh\)             | 40.40 \(h\)            | 19.00 \(i\)         | 11.17 \(hi\)      | 43.8 \(fg\)            | 38.14 \(ef\)      |
| \(T_9\)   | 12.93 \(fg\)             | 41.30 \(g\)            | 19.80 \(h\)         | 11.50 \(gh\)      | 47.4 \(ef\)            | 38.74 \(e\)       |
| \(T_{10}\) | 14.33 \(bc\)             | 46.40 \(c\)            | 22.80 \(c\)         | 13.23 \(bc\)      | 65.8 \(bc\)            | 41.00 \(b\)       |
| \(T_{11}\) | 13 \(fg\)                | 42.00 \(g\)            | 20.30 \(g\)         | 11.87 \(fg\)      | 52 \(e\)               | 38.19 \(ef\)      |
| \(T_{12}\) | 14.63 \(bc\)             | 47.40 \(b\)            | 23.50 \(b\)         | 13.63 \(ab\)      | 70.4 \(b\)             | 41.18 \(b\)       |
| \(T_{13}\) | 15 \(a\)                 | 48.6 \(a\)             | 24.00 \(a\)         | 14.00 \(a\)       | 78.0 \(a\)             | 42.00 \(a\)       |
| \(T_{14}\) | 13.3 \(ef\)              | 43.10 \(f\)            | 21.00 \(f\)         | 12.19 \(ef\)      | 50.5 \(e\)             | 39.63 \(d\)       |
| \(T_{15}\) | 13.64 \(de\)             | 44.00 \(e\)            | 21.80 \(e\)         | 12.52 \(de\)      | 57.6 \(d\)             | 40.09 \(cd\)      |
| \(T_{16}\) | 14 \(cd\)                | 45.20 \(d\)            | 22.30 \(d\)         | 12.89 \(cd\)      | 61.2 \(cd\)            | 40.54 \(bc\)      |

\(\text{LSD (5\%)}\) 0.57 0.74 0.41 0.53 4.60 0.80 2.52

\(T_1\) = Control (water spray); \(T_2\) = Silver nano-particles (SNPs) 10 ppm; \(T_3\) = SNPs 20 ppm; \(T_4\) = SNPs 30 ppm; \(T_5\) = Zinc oxide (ZnO) 2 ppm; \(T_6\) = ZnO 4 ppm; \(T_7\) = ZnO 6 ppm; \(T_8\) = SNPs 10 ppm + ZnO 2 ppm; \(T_9\) = SNPs 10 ppm + ZnO 4 ppm; \(T_{10}\) = SNPs 10 ppm + ZnO 6 ppm; \(T_{11}\) = SNPs 20 ppm + ZnO 2 ppm; \(T_{12}\) = SNPs 20 ppm + ZnO 4 ppm; \(T_{13}\) = SNPs 20 ppm + ZnO 6 ppm; \(T_{14}\) = SNPs 30 ppm + ZnO 2 ppm; \(T_{15}\) = SNPs 30 ppm + ZnO 4 ppm; \(T_{16}\) = SNPs 30 ppm + ZnO 6 ppm

Figure 2. Effect of foliar application of silver and zinc nanoparticles on seed yield and biological yield of mungbean
Conclusion
Silver and Zinc nano-particles plays an important role in improving growth and productivity of crop plants to fulfill the increasing demand of pulses due to increasing population. The results of the current study suggest that Ag and Zn nanoparticles should be applied in combination @ 20 ppm + 6 ppm respectively to improve mungbean production under arid regions.

Author’s contributions
Conceived and designed the experiments: A Wasaya & TA Yasir, Performed the experiments: N Sarwar & O Farooq, Analyzed the data: AW Baloch & A Wasaya, Contributed reagents/ materials/ analysis tools: GR Sheikh & TA Yasir, Wrote the paper: A Wasaya.

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