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Influence of Biosynthesized Nanosilver and Panchagavya on the efficiency of *Pisum sativum* L. Crops

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Abstract
Pisum sativum L. is one of the important and nutritious vegetable crops consumed in India. It has been postulated that the effect of silver nanoparticles (AgNPs) and panchagavya has an effect on the growth of a pea. Hence, the study was designed to test the ability of AgNPs and panchagavya on the morphological and biochemical status of the pea plant. The morphological parameters like shoot and root length, number of leaves per plant, leaf surface area, relative water content percentage and biochemical contents like protein, carbohydrate and total chlorophyll in leaf samples are observed. Firstly the project was focused on the synthesis of AgNPs and panchagavya. AgNPs were synthesized from *Azadirachta indica* and characterized by a UV-Vis spectrophotometer which shows the highest peak at 439 nm. In SEM analysis, the crystallized size of AgNPs has observed in the range of 51.54 nm to 82.5 nm, these values confirm that nanoparticles are successfully synthesized. Panchagavya was synthesized from the five products of desi cow. As the treatments of AgNPs and panchagavya provided there were changes observed in morphological and biochemical level. After 45 days of seedlings, observations were taken, there was an increase in the morphological and biochemical parameters in the pea plant. Hence, the present study concludes that the silver nanoparticles and panchagavya show more effective results as compared to the control on the morphological and biochemical content of pea.

Keywords: *Azadirachta indica*, FTIR, panchagavya, *Pisum sativum*, silver nanoparticles, SEM

1. Introduction
Nanotechnology is science related to nano-materials, which help overcome the size limitation or branch of technology that deals with dimensions and tolerances of less than 100 nm. Nanotechnology is recognized as an emerging technology for the 21st century, in addition to the already areas of information technology and biotechnology [13]. Nanotechnology open up the wide chances in different fields like medicine, pharmaceuticals, electronics, and agricultural field [11]. Nanoparticles defined as a small object or particle that behaves as the whole unit in items of its transport and properties. The most prevalent nanoparticle can be prepared either by nanoparticle synthesis or by processing nanomaterial into nanostructured particles. Synthesis of the nanoparticle can be carried out by either a chemical method or a physical method or biological method [18]. Silver is one of the most commercialized Nano-material with five hundred tons of silver nanoparticles production per year and is estimated to increase in next few years including its profound role in field of high sensitivity biomolecular detection, catalysis, biosensors and medicine; it is been acknowledged to have strong inhibitory and bactericidal effects along with the anti-fungal, anti-inflammatory and anti-angiogenesis activities [1]. Over the past several years, plants, algae, fungi, bacteria, yeast, and viruses have been used for the production of inexpensive, energy-efficient and eco-friendly metallic nanoparticles. The major advantage of nanoparticle synthesis using plant extracts is that they are easily available, safe and nontoxic in most cases. The added advantage of this resource in Nano synthesis is the ready availability of natural capping agents from the plant itself [4]. Silver nanoparticles (AgNPs) have proved to be most effective because of its good antimicrobial efficacy against bacteria, viruses and other eukaryotic micro-organisms [5]. The synthesis of silver nanoparticles using the plant parts is known as green synthesis. The formation of silver nanoparticles will be crystalline nature. The water-soluble organic materials present in the plants are responsible for the reduction of silver nitrate to silver nanoparticles. Greener syntheses of nanoparticles also provide advancement over other methods as they are simple, one step, cost-effective, environment-friendly and relatively reproducible and often results in more stable materials [8]. The tree *Azadirachta indica* is of family Meliaceae, growing in the tropical and subtropical region and native to India, Pakistan and Bangladesh. There are over 16.6 million neem trees in India. Presently in 72 countries, neem trees can be seen successfully growing stage, in Central America, South America, North America, Asia, Africa, and Australia [20]. The chemical constituents contain many biologically active compounds that can be extracted from neem, including alkaloids, flavonoids, triterpenoids, phenolic compounds, carotenoids, steroids and ketones, the biologically most active compound is azadirachtin, it is a mixture of seven isomeric compounds labeled as azadirachtin A-G and azadirachtin E is more effective. The four best limonoids compounds are Azadirachtin, Salannin, Meliantriol, and Nimbin. Limonoids contain insecticidal and pesticidal activity. All parts of the tree have medicinal importance. It has been used in Ayurvedic medicine for more than 4000 years due to its medicinal properties [6]. Pea is a widely cultivated crop species and the second most important food legume worldwide after a common bean. According to APEDA reports of 2017-18 the total of 20,699,736 tons production of the pea in the world. In India total of 5,345,000 tons production of pea and second rank of India

in the world to the production of pea and Maharashtra total of 51.00 tons production of pea [20]. It is also one of the first domesticated crops in the Old World and one of the first genetic research materials. Garden pea (*Pisum sativum* L.) is a valuable nutritive product for human consumption [3]. Panchagavya is an organic product, used in Indian medicine since time immemorial. It is a Vedic formulation for increased productivity, disease resistance in plants and the potential of utilizing Panchagavya as biofertilizer was tested on various pulses [15]. Panchagavya has played an important role in providing resistance to pests and diseases, resulting in increased overall yields. Panchagavya possesses the properties of fertilizers and biopesticides. Panchagavya has resulted in a positive effect on the growth and productivity of the crop [16].

2. Materials and Methods

2.1 Collection of Sample

Neem *(Azadirachta indica)* leaves were collected from the campus of Modern College of Agricultural Biotechnology, Kule-Dakhane, Paud, Pune and GS-10 variety of pea was collected from Gurudatta Krishi Bhandar, Paud- 412108. Cow dung, cow urine, cow milk, cow ghee, and cow curd were collected from desi cow farm Paud and jaggery, sugarcane juice and coconut water were collected from the Paud market.

2.2 Preparation of neem leaf extract

10 gm of neem leaves were collected and washed with distilled water (3 times) for 5 min and dried. Dried leaves were cut and crushed in mortar and pestle containing 100 ml of sterile distilled water for 15 min. The extract was filtered with whatmann filter paper no.1 to the removal of dust. Filtered extract was kept at 4º C temperature for further use [14].

2.3 Synthesis of silver nitrate solution

10 ml filtered extract was added to the 90 ml aqueous solution of 1mM silver nitrate and incubated in dark for 37˚C for 24 hrs. The incubated solution was centrifuge for 10 to 20 minutes at 13000 rpm. The supernatant was transferred into the Petri plates. The sample was measured for 300-800 nm using a UV-visible spectrophotometer [14].

2.4 Drying of neem AgNPs

The *Azadirachta indica* AgNPs solution was air-dried in a hot air oven at 80º C for 8 hrs to the obtained dry powder of nanoparticles. This powder was collected in clean vials and stored for further use [14].

2.5 Characterization of neem AgNPs

2.5.1 Detection and characterization of AgNPs

The *Azadirachta indica* leaves extract treated with $AgNO_3$ was observed for the color change as compared to control.

2.5.2 UV- vis spectroscopy of neem AgNPs

The reduction of pure Ag+ ions was monitored by measuring the UV- visible spectrum of the silver nanoparticles. UV-visible spectral analysis was done by using UV-visible spectrophotometer UV-2450. The broad absorbance peaks between 300-900 nm confirmed the formation of AgNPs respectively. 1mM $AgNO_3$ solution was used as the blank solution for these measurements. $AgNO_3$ aqueous solution was loaded in the first compartment and auto-zero was done in the spectrophotometer. After that 100 µl sample adds for the 1 ml of deionized water containing cuvettes was loaded in the second compartment. Spectral analysis was carried out between 300-900 nm for silver nanoparticles [13].

2.5.3 SEM analysis of neem AgNPs

The dried *Azadirachta indica* AgNPs were allowed to dry completely and grinded to powder. NPs were loaded in the sample holder (vacuum chamber). SEM analysis was done at 20 kV in a vacuum and a secondary electron image was obtained. The morphology of the *Azadirachta indica* AgNPs was analyzed [13].

2.5.4 FTIR analysis of neem AgNPs

The solution of AgNPs was centrifuged and washed with distilled water to remove any unreacted materials. AgNPs centrifuged at 8000 rpm for 20 min. Obtained pellet, was dried in a desiccator containing calcium chloride. Liquid AgNPs were loaded in glass plates. The light absorbed by the sample was detected by the detector. The percentage of transmittance of NPs was obtained between 600 to 4000 cm. According to an absorption band pattern, the functional groups were detected [13].

2.6 Preparation of panchagavya

Fresh cow dung (1.5kg) was mixed with cow ghee (250gm). It was incubated for 2 days. The cow urine (700ml) was added to 2.5 liters of water. Stirring was done (morning and evening, daily for 1 week). Sugarcane juice (700ml) or jaggery was mixed in water at 1:6 ratio. Cow milk (700ml) and Cow curd (700ml) was added. Coconut water (700ml) and (50 g) yeast was 12 ripe bananas. Stirring was done (morning and evening, daily for 3 weeks). Panchagavya was ready to use [10].

2.7 Treatments of AgNPs and panchyagavya on pea plant

AgNPs and panchyagavya separately treatments were provided to the pea plant. Simply soil application and foliar sprayed was provided. Experiments done in triplicates with control.

2.8 Physiological analysis of pea plant

After ten days seed germination count was taken and seed germination percentage was calculated by using following formula; [2]

$$\text{Seed germination percentage (\%)} = \frac{\text{No. of seedlings emerged}}{\text{No. of seeds sown}} \times 100$$

After 15 days interval, seedlings shoot and root length measured by using scale. The number of leaves present in each plant were counted [17]. Pea leaf was placed on grid paper and outline was drawn. The squares covered more than half by the leaf was coloured. The number of coloured squares is equal to square centimetre (cm^{-2}) of the leaf area. From coloured square calculated the surface are of leaf [9]. Relative water content (RWC) percentage was calculated by using following formula, [19]

$$\text{RWC (\%)} = \frac{(\text{F.W}) - (\text{D.W})}{(\text{T.W}) - (\text{D.W})} \times 100$$

2.9 Biochemical analysis of pea plant

Protein was estimated by folin Lowry method [12]. Carbohydrate was estimated by anthrone method and

calculated by using following formula; [12]

$$\text{Amount of carbohydrate present in 100 mg of the sample} = \frac{\text{mg of glucose}}{\text{volume of test sample}} \times 100$$

Total chlorophyll content was estimated by arnon method and calculated by using following formula [7]

$$\text{Total chlorophyll (mg/g)} = 20.2(A645) + 8.02\ (A663) \times \frac{V}{1000 \times w}$$

3. Results and discussion

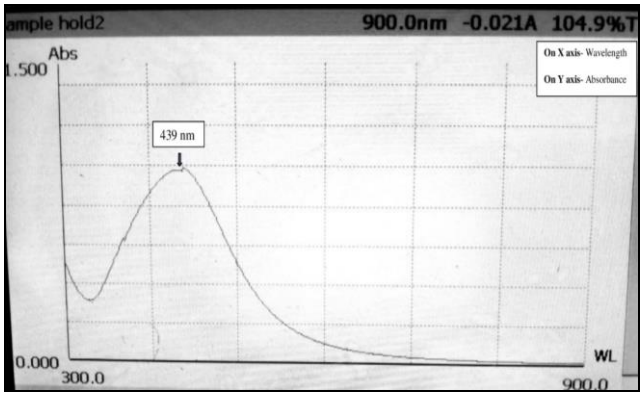
3.1 Synthesized and characterized AgNPs

3.1.1 Visual detection of neem AgNPs

In the visual detection of *Azadirachta indica* AgNPs change in color from yellowish to reddish-brown which indicated the synthesis of silver nanoparticles from *Azadirachta indica* leaf extract similar result was observed by Ahmed *et al*., 2016. After the synthesis of AgNPs, when the extraction color was changed. The extract was dried into a hot air oven, because of drying extract was converted into a dark and fine powder.

3.1.2 UV- vis spectral analysis of neem AgNPs

The *Azadirachta indica* sample was found to show the peak at 439 nm, which confirms the reduction of silver nitrate to silver nanoparticles. The wavelength which had obtained varies slightly to the peak value mentioned in the work carried out by Ahmed *et al*., 2016 in which the wavelength was found to be in the range of 436-446 nm. The graph obtained is shown in graph 1.



Graph 1: UV- Vis Spectroscopy for *A. Indica* AgNPs

3.1.3 SEM of neem AgNPs

SEM conferred the shape and size of AgNPs. It revealed that the particles were spherical and the crystallite size of *Azadirachta indica* AgNPs was observed in the range of 51.54 nm to 82.5 nm. Similarly Joany *et al.*, 2015 observed in the range of 43.23 nm to 80.5 nm. The SEM images obtained are shown in figure 1.

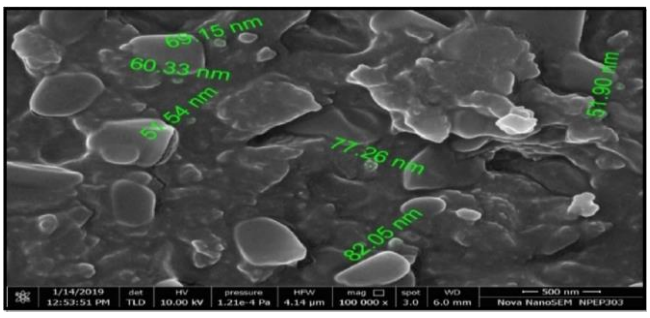
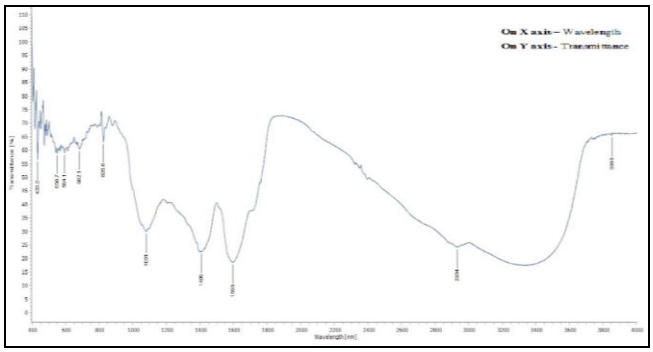


Fig 1: SEM of A. indica AgNPs at 500 nm

3.1.4 FTIR of neem AgNPs

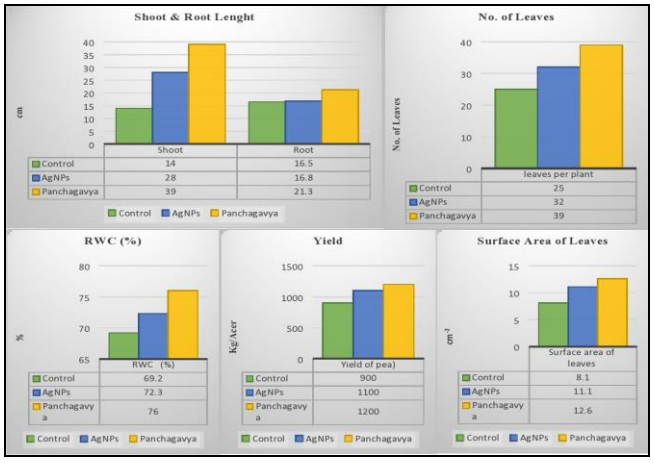
From graph 2, FTIR result showed absorption bands at 3855 cm^{-1}, 2934 cm^{-1}, 1596 cm^{-1}, 1409 cm^{-1}, 682.1 cm^{-1},which showed the first peak had shown 3855 cm^{-1} alcohol and phenol, second peak had shown 2934 cm^{-1} aldehydes, third peak had shown 1596 cm^{-1} primary amine, fourth peak had shown 1409 cm^{-1} alkyl halide and fifth peak had shown 682.1 cm^{-1} alkene functional groups were present in *Azadirachta indic*a leaf extract which played a major role in reduction and stabilization of AgNPs.



Graph 2: FTIR spectra for silver nanoparticle

3.2 Morphological analysis

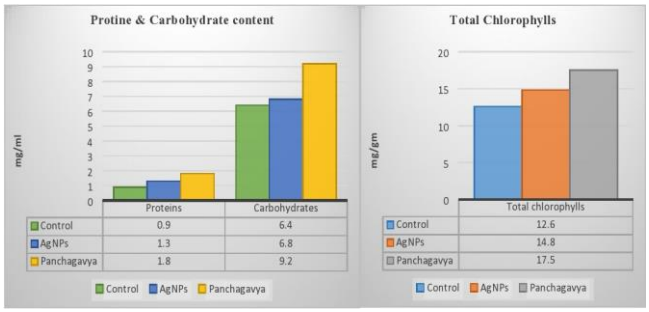
Before sowing seeds were treated with AgNPs, panchagavya separately and sown in different beds with control. After some days seed germination was recorded, it was found highest in panchagavya treated pea (90%) followed by AgNPs treated seeds (86%) as compared to control seeds (80%). Morphological analysis of pea was carried out in 15 days interval of foliar spray of panchagavya and AgNPs and it was observed that panchagavya and AgNPs showed positive results. The shoot length of the pea plant showed tremendous change in panchagavya and AgNPs treated pea as compared to control. After 45 days, panchagavya showed the highest shoot length, root length, surface area, relative water content percentage, yield as compared to AgNPs and control. The obtained statistics are mention in graph 3.



Graph 3: Morphological analysis of treated pea

3.3 Biochemical parameters

After 45 days of sowing biochemical analysis was done and observed that there was a great increment in carbohydrate, total chlorophylls content, and little bit protein content of panchagavya treated pea as compared to AgNPs and control. The obtained statistics are mention in graph 4.



Graph 4: Biochemical analysis of treated pea

4. Conclusions

Hence, the present study concludes that the panchagavya shows more effective results as compared to silver nanoparticles and control on the morphological and biochemical parameters of a pea.

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