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Original Research

Evaluation of the nutrient profile of *Trachyspermum ammi* L. seed under the influence of nanoparticles during germination

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Abstract: *Trachyspermum ammi* L. commonly known as Ajwain is an annual herb belonging to the family Apiaceae. It is enormously grown in Egypt, Iran, Pakistan, Afghanistan, and India as well as European region. Seeds of Ajwain were highly administered by traditional healers and usually employed for different ailments. Nanomaterials are known to have plant growth promoting effects, which could find applications in agriculture. In this study, the nanoparticles (NPs) showed the potential to enhance the primary metabolites when administered during germination. Therefore, nanoparticles elicitation can be used to increase the productivity, nutritional values and metabolite contents in *Trachyspermum ammi* L. This study aimed to provide new insight of the potential growth promoting effects of the nanoparticles () on plant system. Different concentrations of two nanoparticles, that is, iron pyrite (FeS_2) and molybdenum disulphide (MoS_2) at three different concentrations of 25ug/ml, 50ug/ml and 75ug/ml were tested on the seeds of *Trachyspermum ammi* L. The data indicated that nanoparticles enhanced the seedling growth as greener leafs and increased lengths of epicotyl and hypocotyls were seen. These nanoparticles also showed the potential to increase the contents of primary metabolites during germination and the total soluble protein content in seed was increased in nanoparticles-treated seeds as compared to control. The total protein profiling by SDS-PAGE indicated significant differences in number and molecular weights of protein bands upon exposure to nanoparticles.

Key words: Ajwain; Nanoparticles; Germination; Primary metabolites; Seedling.

Introduction

The projected worldwide population will be around nine billion by 2050. To provide healthy nutrition and the cure of various diseases, plant derived compounds will play a major role. Medicinal plants are the wealthy bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food additives, folk medicines, pharmaceutical intermediates and chemical aspects for synthetic drugs (1). In addition to major primary metabolites (carbohydrates, lipids and amino acids), higher plants are also able to generate a wide range of low molecular weight compounds – the secondary metabolites. In general we can say that the production of these beneficiary compounds is low (less than 1% dry weight) and greatly depend upon the physiological and developmental stage of the plant (2). Primary metabolites productivity directly depends on an optimal nutrient management system. The past few decades of accelerated metabolite production approach have resulted in extreme use of chemical fertilizers, sprays, which in the turn resulted in the human health nutrient degradation and pollution. This problem could be overcome by the development of sustainable strategy for enhancing the production of primary metabolites. One such approach which could be used effectively is the elicitation of the nutrients in plants by nanoparticles (NPs). This reduces the time, cost and the use of chemical fertilizers. Nanoparticles are anthropogenous in nature and get directly used in growth substrate (3). Carbon nano structures are also used to boost the plant growth (4,5). Most study is carried out in order to obtain the uptake and conversion in the systemic site such as fruits, leaves and roots of the plants (6,7). There is very little study till date, which attempted the exploitation of nanoparticles as a seed treatment agent (3). Some physical and chemical processes have been standardized in the previous literature but the alteration in the content of primary metabolites during germination through a nanoparticle is a novel approach.

In the present study, *Trachyspermum ammi* L. (Ajwain) seeds which is an aromatic herb having several medicinal uses were taken. It is very widely grown in black soil, mostly along the river banks in Egypt as well as many other countries like India, Iran and Afghanistan as declared by Boskabady and Shaikhi 2000 (8). It is highly esteemed as a curative agent for flatulence, flatulent colic, tonic dyspepsia, diarrhoea - in short, as a digestive support and also as an antiseptic (9). The oils produced by this plant seem to help in the adaptation to its environment and are appropriately produced in higher quantities when plants meet maximum conditions, biotic and a biotic stresses (10). Environmental aspect affecting the growth rates, have drastic effect on fructose production (11). As previously mentioned that stress change the plant respiration, photosynthesis, translocation, ion uptake, carbohydrates, nutrient metabolism and hormones (12). Stress also increased the secondary metabolite production in a variety of medicinal plants, like *Artemisia annua* and *Catharanthus roseus* roots

Table 1. Morphological changes during different phases of germination in *Trachyspermum ammi* L. in the presence of nanoparticles.

Day	Control	FeS_2			MoS_2		
		25µg/ml	50µg/ml	75µg/ml	25µg/ml	50µg/ml	75µg/ml
0	No change observed	No change observed	No change observed	No change observed	No change observed	No change observed	No change observed
1	Imbibition of water	Imbibition of water	Imbibition of water	Imbibition of water	Imbibition of water	Imbibition of water	Imbibition of water
2	Initiation of sprouts	Initiation of sprouts	Initiation of sprouts	Imbibition of water and swelling	Initiation of sprouts	Initiation of sprouts	Initiation of sprouts
3	Increase in sprout size (2.0cm)	Increase in sprout size (2.5cm)	Increase in sprout size (1.2cm)	Initiation of sprouts	Increase in sprout size (3.0cm)	Increase in sprout size (2.5)	Increase in sprout size (1.0cm)
4	First leaves observed	First leaves observed	First leaves observed	Increase in sprout size (1.5cm)	First leaves observed	First leaves observed	First leaves observed

(13) (*Trachyspermum ammi* was cultivated and found in water-deficient areas (14). Engineered nanoparticles have unique characteristics including size, structure and composition. The applications of these nanoparticles could uplift the economy in the sectors of pharmaceutics, consumer products, cosmetics, transportation, energy and agriculture (15). Only little studies exist on the effects of nanoparticles on higher plants and bulk of the studies reported the positive impacts of nanoparticles on plant growth with a few isolated studies pertaining to negative effects. Several studies have demonstrated that TiO_2 nanoparticles activated the photosynthesis and nitrogen metabolism and thus highly improved the production of spinach at a concentration as low as 20 mg/l (16). Seed germination was not distressed except for the inhibition of nano scale zinc (nano-Zn) on rye grass and zinc oxide (nano-ZnO) on corn at concentration of 2000 mg/l. Inhibition on root growth varied greatly among the nanoparticles and plants. Chlorophyll contents were investigated in the sunflower seedlings treated with magnetic nanoparticles (17). Aim of the present study was to determine the optimum concentration of the two tested nanoparticles, that is, iron pyrite (FeS_2) and molybdenum disulphide (MoS_2) for germination, plantlet growth and sugar contents in *Trachyspermum ammi* L.

Materials and Methods

Collection of *Trachyspermum ammi* seeds and nanoparticles

The seeds were procured in January 2015 from the local grocery store in Lucknow, India and the nanoparticles were chemically synthesized (3) in Lab 12, Department of Biological Sciences and Bioengineering at IIT Kanpur, Kanpur.

Germination of seeds

Seed lots used for the different experiments showed germination capacities ranging from 80–98%. For germination studies, seeds were placed on four layers of damp filter paper at 25°C and incubated in dark till the initiation of sprouting after which they were placed at a light intensity of 100 µmol m^{-2} s^{-1} and at 14/10 h (day/night) photoperiod till the complete plantlet with two leaves was obtained. Germination, defined as 1 mm radicle emergence, was followed for four days; no contamination by microorganisms was observed during this time. The seeds were then divided into two equal groups, namely, control and test. Control seeds were kept overnight in de-ionized water, while the test seeds were kept in an aqueous suspension of synthesized nanoparticles in 90 mm petri dishes. The dose was optimized in our previous experiments.

Determination of wet and dry weights

Total fresh weight was determined daily after removing excess of water by placing the seeds in between blotting paper. Dry weight was examined by drying the seeds in various stages of germination in shade and grinding it. This was the starting material for all biochemical determinations and the amount of total water-soluble sugars, total fructans and total proteins during germination were estimated in the fresh biomass of the sample.

Biochemical analyses

Total soluble carbohydrate content was determined in seed and its different phases of germination. Soluble sugars were extracted following the method adopted by Homme *et al.,* 1992 (18) and determined with anthrone reagent (19). Total fructans were calculated in various phases by using resorcinol reagent (20) and the total protein contents were determined using Lowry's method of estimation (Lowry *21*).

Results

The seeds were taken on moist filter paper in the dark. The sprouts were seen on the second day of germination after which the there was an emergence of the radicle. An increase in the length of hypocotyl and epicotyl was analysed daily till 4d when a complete plantlet was formed. Total fresh and dry weights were determined and there was a continuous increase in the fresh weight due to imbibition from 1d to 4d but the dry weight showed uniformity (Table 1, Figure 1).

Two nanoparticles (FeS_2 and MoS_2) were used at three different concentration of 25µg/ml, 50µg/ml and 75µg/ml in the study. On the first day of germination, imbibitions of water and swelling of the seeds were observed in both the control and the treated seedlings. On the second day, initiation of sprouting was observed in the treated seedlings as well as the control seeds. On the third day, the length of the sprouts was found

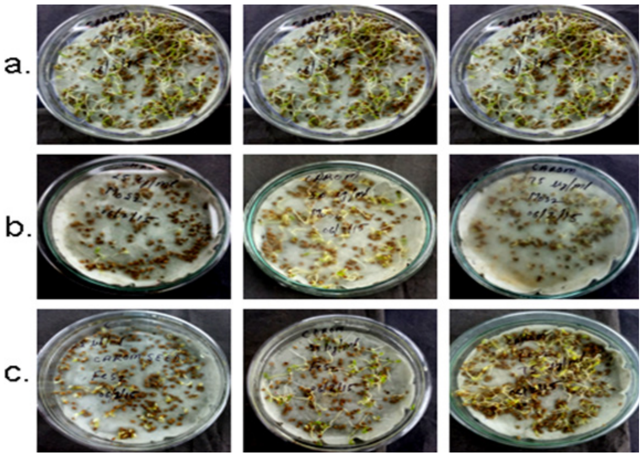

Figure 1. Growth of the *Trachyspermum ammi* L seeds under nanoparticle treatment and control: a. Control, b. FeS_2 (25 µg/ml 50µg/ml, 75µg/ml) and c. MoS_2 (25µg/ml,50µg/ml,75µg/ml).

to be greater in FeS_2 NPs-treated seeds (25µg/ml) and MoS_2 NPs-treated seeds (25 and 50 µg/ml) as compared to control. On the fourth day, the length of the sprouts increased in the NPs-treated seedlings as compared to the control seedlings following the same trend. The same pattern of growth was observed on the fifth day and the emergence of first leaves was seen. Results of the study revealed that among the tested concentrations i.e. 25µg/ml, showed the best response on the germination of *T. ammi* followed by 50µg/ml in case of MoS_2 as the length of sprout was more as compared to control. In case of FeS_2 best growth was observed at a concentration of 25µg/ml. At a concentration of 75µg/ml of both the nanoparticle showed reduced growth of sprout.

In present study, there was a continued synthesis of sterols throughout germination. The control seeds of *Trachyspermum ammi* L. demonstrated high phytosterol content as compared to the NP treated seeds. MoS_2 nanoparticles reported highest activity when compared to the FeS_2 nanoparticles-treated seeds as shown in Figure 2a.

Sucrose is the major non-reducing sugar in the seeds formed during germination due to hydrolysis of complex sugars. In present study, there was a continued synthesis of non-reducing sugars throughout germination under the nanoparticle treatment at different concentrations. MoS_2 nanoparticules reported higher activity when compared to FeS_2 nanoparticles treated seed as shown in Figure 2b. Although the amount of sucrose was considerably elevated in the MoS_2 nanoparticle-treated seed as compared to the control and non-treated *Trachyspermum ammi* L. seeds. The control seeds of *T. ammi* demonstrated high ketose content as compared to the NPs-treated seeds. In the case of MoS_2, 75µg/ml reported highest activity when compared to the other concentrations of MoS_2 whereas in the case of FeS_2,50µg/ml showed the highest activity among the rest of concentrations as shown in Figure 2c. The amount of ketoses in non-treated seed was found to be highest among all the treatments.

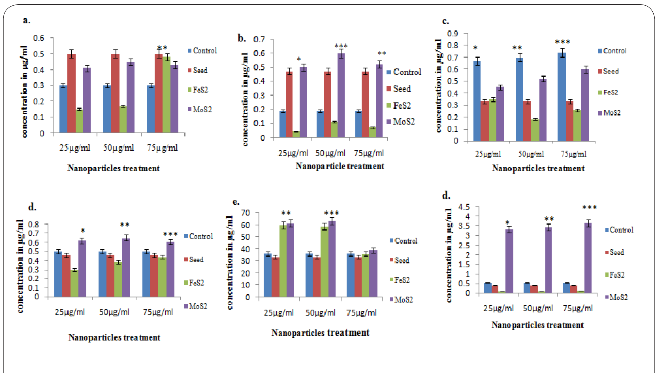

Figure 2. Content of metabolites in nanoparticle-treated seeds of *T. ammi.* a. Phytosterols, b. Non-reducing sugars, c. Ketoses, d. Starch, e. Total fats and f. Aldoses. * - significant, ** - more significant, *** - most significant

It was reported that the sugar content of seeds gradually increased during germination. This indicates a higher mobilization of starch content in seedlings during germination (22). Starch showed high proportion in the 50ug/ml MoS_2 NP-treated seeds as compared to non-treated seeds as shown in Figure 2d. Crude fat content was calculated on dry-matter basis and it was seen that it increased during germination in MoS_2 NPs- treated seeds as compared to FeS_2 NPs-treated seeds Figure 2e.

Total aldoses estimation under the nanoparticles treatment at different concentration was determined including the control. The control seeds of *Trachyspermum ammi* L. demonstrated low aldose content as compared to the NPs-treated seeds. In case of MoS_2 , 50µg/ml reported highest content when compared to the other concentrations of MoS_2 nanoparticles whereas in case of FeS_2 nanoparticules, 50µg/ml showed the highest activity among the rest of concentrations in Figure 2f.

Protein estimation of the seeds under the nanoparticle treatment at different concentration was determined including the control. The control seeds of *Trachyspermum ammi* L. demonstrated high protein content as compared to the NP-treated seeds. In case of MoS_2, 50µg/ml reported highest content when compared to the other concentrations of MoS_2 whereas in case of FeS_2 75µg/ml showed the highest content as shown in Figure 3.

Protein Profiling of *Trachyspermum ammi* L seeds under the influence of nanoparticles during germination phases

When analyzed by SDS-PAGE, the samples showed an intense band of 28 kDa (Fig. 4, lane 3) in 50 µg/ml MoS_2 NP- treated sample. In contrast, there was no observable band in FeS_2 treatment at any concentration. Previous studies have shown that the stability of nanoparticles was dependent on molecular weight of protein (23).

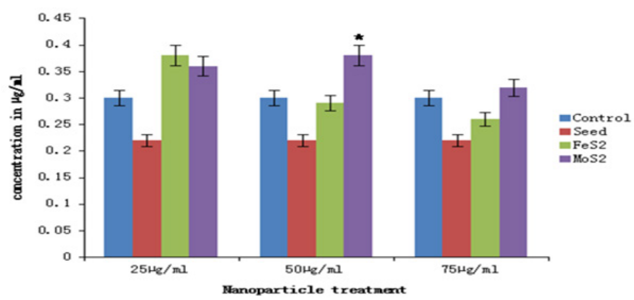

Figure 3. Protein content in the *Trachyspermum ammi* L seeds under the influence of nanoparticles during germination phases.

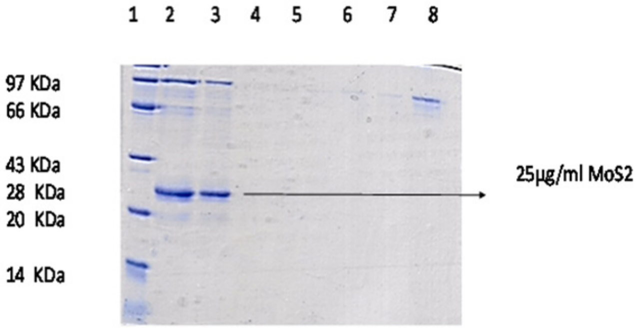

Figure 4. Protein profile of *Trachyspermum ammi* L. seeds treated with nanoparticles. Lane 1-marker, Lane 2-seed, Lane 3-25 µg/ml MoS_2, Lane 4- 50 µg/ml MoS_2, Lane 5-75 µg/ml MoS_2, Lane 6-25 µg/ml FeS_2, Lane 7-50 µg/ml FeS_2, Lane 8. 75 µg/ml FeS_2.

Discussion

The variation in the size of the *Trachyspermum ammi* L. sprouts may be because of the fact that nanoparticle treatment have expedite the motion of water and nutrients through a seed coat for enhancing the seed germination and growth of the seedling. At higher concentration, the nanoparticles are becoming toxic to the seeds. Molybdenum is an essential micronutrient required by many growth promoting enzymes as coenzyme. Therefore, nanoparticle elicitation can be used as a tool to increase the productivity and metabolite content (3).

The analysis of sterols could provide an effective tool for the quality control of vegetable oils and for the detection of oil and mixtures not predicated by the fatty acids profile (24). In present study, there was a continued synthesis of sterols throughout germination. The control seeds of *Trachyspermum ammi* L. demonstrated high phytosterol content as compared to the NPs-treated seeds. MoS_2 nanoparticles reported highest activity when compared to the FeS_2 NPs-treated seeds. The decline in the phytosterol content of the seeds might be due to the toxicity produced in the seed which retarded the growth of the biomolecules.

MoS_2 nanoparticules reported highest activity of the sugar as compared to FeS_2 NPs-treated seeds. Although the amount of sucrose was considerably elevated in the MoS_2 NPs-treated seeds as compared to the control and non-treated *Trachyspermum ammi* L. seeds. This increase might be due to the fact that during germination the hydrolysis of galacto-oligosaccharides into monosaccharide's or disaccharides took place. Monosaccharides or disaccharides are used as an energy source or hydrocarbon skeleton for the biosynthesis of macromolecules necessary for embryonic development during the early stages of germination (25). MoS_2 nanoparticles might act as a catalyst for the conversion of sugar alcohol to hydrocarbons.

The amount of ketoses in non-treated control seeds was found to be highest. The decline in ketose content of the NPs-treated seeds might be due to the toxicity produced in the seed which retarded the synthesis.

Starch showed high proportion in the 50ug/ml MoS_2 NPs-treated seeds as compared to non-treated seeds. These results are in accordance with some of the previous cytochemical observations of an increase of this reserve during germination and early plantlet growth (26). It was reported that the sugar content of seeds gradually increased during germination. This indicates a higher mobilization of starch content in seedlings during germination (22). Crude fat content was showed that it increased during the germination in MoS_2 NPs-treated seeds as compared to FeS_2 NPs-treated seeds. These results can be correlated with earlier reports (27).

The control seeds of *Trachyspermum ammi* L. demonstrated low aldose content as compared to the NPs-treated seeds. As aldoses are monosaccharides with small molecular weights, they pass through the cut off filters with ease and interact with nanoparticles. In addition, Panigrahi *et al*., 2004 (28) have shown that several simple aldoses can be used as reducing agents to synthesize common metal nanoparticles.

In the present study, the change in sugar contents can be correlated to the amylase bioactivity. At the early stages of germination, plants need high energy. The energy can be supplied from sugars which are produced by enzymatic hydrolysis of starch. The glucose and other free sugars formed apparently translocated to the growing plant during the scutellum, where the sugars are converted to sucrose (29). Sugars are indicators of cellular conditions under the nanoparticle treatment (30).

The results of this study demonstrated that the effect of MoS_2 is more severe than FeS_2. It was seen that protein decreases from MoS_2 exposure with respect to their control. Plants synthesize metal or metal based nanomaterials binding replicas when exposed to nanoparticle stress. The reduction in protein content of MoS_2 treated samples might have resulted from the reduced rate of photosynthesis.

When proteins were analyzed by SDS-PAGE, the sample showed an intense band of 28 kDa (Fig. 4, lane 3) in 50 µg/ml MoS_2 NPs-treated sample. In contrast, there was no observable band in FeS_2 nanoparticle treatment at any concentration. Previous studies have shown that stability of NP was dependent on molecular weight of protein (23). SDS-PAGE pattern showed drastic changes in both low and high molecular weight protein content due to germination. Protein extracted from seeds was more in relation to germinating seeds. Thus, the cytochemical changes in protein bodies may be due to changes in solubilization and/or interactions rather than metabolism (26). This finding may indicate that nanoparticle treatment with seeds is more protective than normal treated seed, probably due to the presence of other active components in the seeds.

It can be concluded that FeS_2 and MoS_2 nanoparticles could function as elicitors. Simple seed treatment with iron pyrite and molybdenum disulphide increases the production of primary metabolites by two way treatment. Firstly in the presence of FeS_2 nanoparticles and secondly in the presence of MoS_2 nanoparticles. MoS_2 increased the production of primary metabolites more rapidly at a particular concentration thus acting as an artificial catalyst to mimic the biochemical activity of the medicinal seed. Such innovative seed treatment strategy promises huge prospects in overcoming the pollution. Such conventional and judicious use of nanoparticles as seed treatment mediator could emerge as a novel strategy in the production of primary metabolites and improving the nutritional value of seeds.

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