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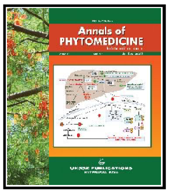

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Synthesis of green nanoparticles from leaves of *Cannabis sativa* L. and its effective role on growth performance of tomato

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Abstract

There is a rising demand for eco-friendly, non-toxic synthetic biological procedures for the synthesis of nanoparticles. The goal of the experiment was to synthesize silver nanoparticles using fresh leaves of *Cannabis sativa* L. and their characterization and antifungal activities examined under *in vitro* conditions. Several biotic factors present in seeds and adversely affect their viability and germination. Green synthesis of silver nanoparticles applying procedure at specific set of conditions. Tomato seed treated with green synthesized nanoparticles significantly increased the germination rate and reduced the mortality of seeds. The highest germination as 92.66% and minimum seedling mortality with the value of 5.2% were found in seeds treated with silica nanoparticles at 100 ppm (T12) compared to non-treated seeds and remaining all treated seeds also indicate better germination as compared to control. The length, fresh and dry weight of root and shoot were highest observed in treatment T12 (silica 100 ppm) treated plants. As might be expected, green synthesized silver nanoparticles could be used as antimicrobial and plant growth promoting agent.

1. Introduction

In current era, food security across the globe is threatened by climate change. Many countries are impacted by this problem. However, India is especially at risk due to insufficient coping systems and a tropical monsoon climate. Almost all crops are expanding, but a number of biotic and abiotic factors negatively affect their productivity. The tomato (*Lycopersicon esculentum* Mill.) is one of the most important vegetables grown worldwide due to its great medical and nutritional qualities, including essential amino acids, vitamins, minerals, lycopene, fibre, and a dietary supply of antioxidants (Yadav *et al.,* 2022). Many plant pathogens impose serious disease that inflicts economic yield losses. In India, up to 45% loss in yield of tomato fruits has been reported due to effect of biotic agents (Ramyabharathi *et al.,* 2012). Several pathogenic agents reside in soil, so their management by conventional methods, such as chemical, biological, cultural, and resistance varieties, is challenging. However, each of these approaches has significant drawbacks and cannot completely control the disease (Kumar *et al.*, 2023). Therefore, there is need to serve an alternative method which can manage the soil and seed born harmful microbes in an appropriate and sustainable manner in near future (Worrall *et al.,* 2018; Kumar *et al.,* 2018).

Cannabis sativa L. is a medicinal plant belong to Cannabaceae that has received praise from all over the world for its healing properties. Mainly two cannabinoids contained in the plant, tetrahydrocannabinol (THC) and cannabidiol (CBD), interact with the body's endocannabinoid system to affect how painful stimuli are interpreted. Indeed, because of their antibacterial, antifungal, and anti-inflammatory properties, several of these compounds are used as pharmaceutical medicines (Hanus and Hod, 2020; Bergman *et al*., 2019). *C. sativa* leaves extract have diverse antifungal activities, depending on their composition, which have attracted the interest of researchers and medical professionals.

One of the new emerging approaches is nanoparticles, which support to the search for alternative, eco-friendly and non-chemical tools for crop improvement. Nanoparticles are materials that varying between the range of 10 to 100 nm. One way to potentially enhance motivation for the development of such affordable, high-performing nanopesticides that are biodegradable and least harmful to people and the environment is by the use of innovative green synthesis nanomaterials (Schiavi *et al.,* 2021). Green synthesized nanoparticles have the potential to protect the plant against pathogens and reduce the disease development through direct application on seeds, foliage and roots. *In vitro* evaluation of silver nanoparticles inhibits the growth of several microbes including, *Alternaria brassicae, Alternaria alternate, Sclerotinia* sp., *Curvularia lunata* and *Rhizoctonia solani* (Desai *et al.,* 2021). Consequently, particular antimicrobial properties of silver nanoparticles that can prevent the expression of proteins involved in ATP synthesis (Yamanaka *et al.,* 2005). In order to achieve food security, sophisticated nanoengineering is a crucial technique today for boosting agricultural productivity and maintaining sustainability. The effectiveness of inputs, particularly those connected to plant protection approaches, is increased by nanotechnology, which also minimises important losses (Shang *et al.,* 2019). Nanomaterials could provide better coverage when applied to crops, enhancing the antibacterial effects of pesticides (Takehara

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et al., 2023: Suman *et al.,* 2022). Keeping all the points in view, the present study conducted to synthesis of green nanoparticles from leaves of *C. sativa* and its effect on seed germination, seedling mortality and morphological behaviours of tomato.

2. Materials and Methods

2.1 Green synthesis and collection of nanoparticles

2.1.1 Green synthesis of silver nanoparticles (NPs)

In general, the methods and procedures for the green synthesis of silver nanoparticles can be summarized as follows: obtain leaves extract of *C. sativa*, extract heat on hot plate at 80-90°C for 50-60 min, filtration using through Whatman No. 1 filter paper, 0.2 M of $AgNO_3$ (212.996 g/mol) solution used as precursor, addition of leaves extract in $AgNO_3$ solution, yellowish colour of leaves extract changes to black which indicates silver nanoparticles formation which can be the centrifuged at 5000 rpm for 20 min, as per Deka *et al.* (2021). Green synthesised nanoparticles characterized by Scanning Electron Microscopic (SEM) analysis, all work carryout in specific set of conditions.

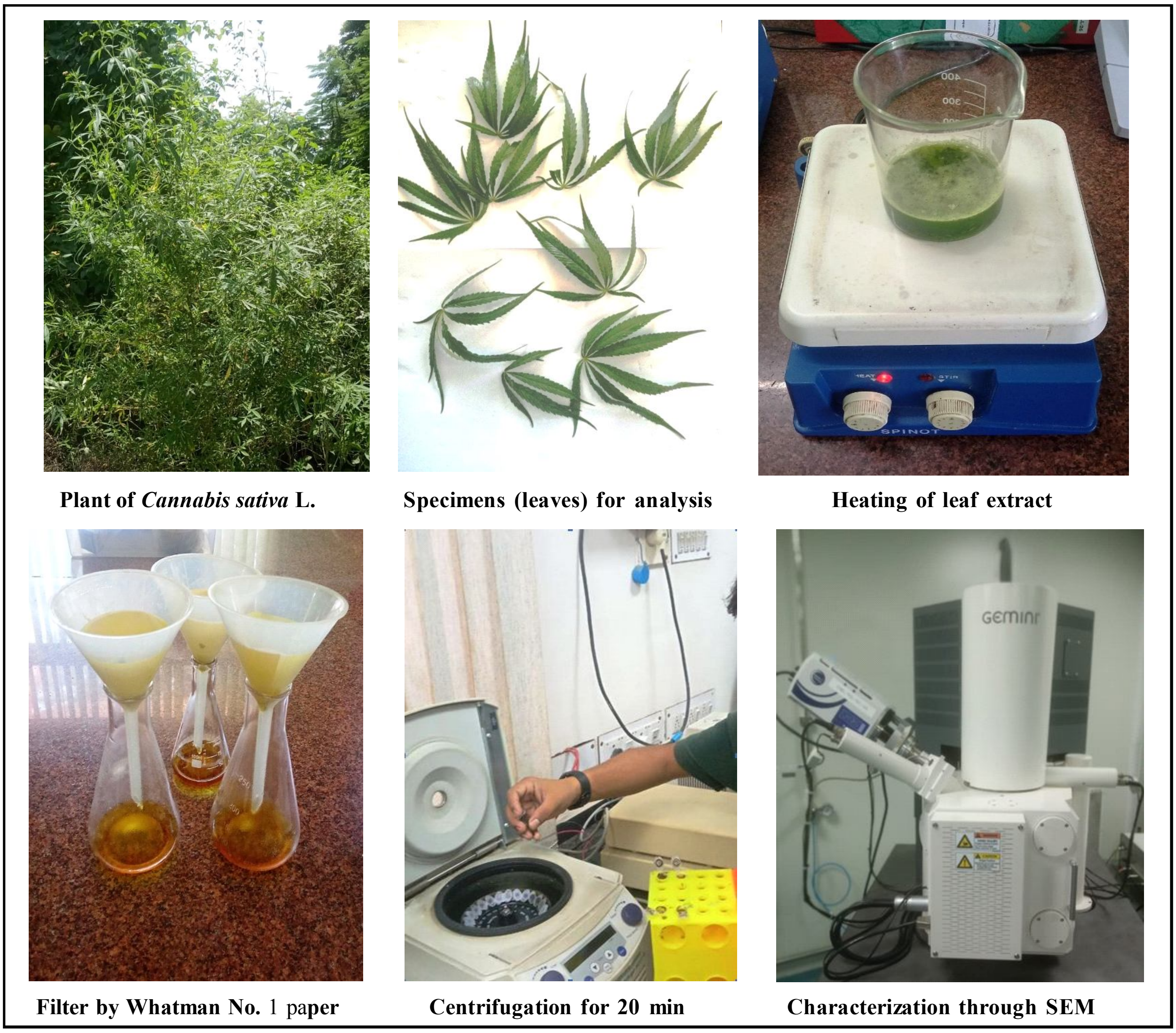


Figure 1: Collection of sample and laboratory procedure of green synthesis of silver nanoparticles.

2.1.1.1 Characterization

The characterization of the synthesized particles is an important part of proving whether they are nanoparticles or not. If, the particles size varies between the ranges of 1 to 100 nm, means it is nanoparticles. Nanoproperties of synthesized silver particles confirmed through Scanning Electron Microscope (SEM) image in Figure 2(a) from the Centre for Nanoscience, Indian Institute of Technology, Kanpur.

2.1.2 Collection of agri-tech nanofungicides (NFs)

Agri-tech nanofungicides a product (containing nanoparticles of silver + copper + zinc) were procured from the market prepared by Nano Research Lab Jamshedpur, Jharkhand, India. After fungicidal activities confirmation of nanoparticles further investigations as nanofungicides. Three different nanomaterials, *viz.,* silver nanoparticles, agri-tech nanofungicides and silica nanoparticles with each four different concentrations as 25, 50, 75 and 100 ppm were used.

Treatment details

- Treatment 1-Silver nanoparticles 25 (ppm); Treatment 2-Silver nanoparticles 50 (ppm);
- Treatment 3-Silver nanoparticles 75 (ppm); Treatment 4-Silver nanoparticles 100 (ppm);
- Treatment 7-Agri-tech NFs 75 (ppm); Treatment 8-Agri-tech NFs 100 (ppm);
- Treatment 9-Silica nanoparticles 25 (ppm); Treatment 10-Silica nanoparticles 50 (ppm);
- Treatment 11-Silica nanoparticles 75 (ppm); Treatment 12-Silica nanoparticles 100 (ppm);
- Control-Non-treated
- Treatment 5-Agri-tech NFs 25 (ppm); Treatment 6-Agri-tech NFs 50 (ppm);

2.2 Seed treatment and sowing

Seeds of tomato (Navodaya variety) were collected from the Centre of Excellence, Department of Vegetable Science, CSA University of Ag. and Tech., Kanpur. Seeds are soaked in different concentrations (25, 50, 75 and 100 ppm) of three nanomaterials (silver, agri-tech and silica based) for 30 min. After that, remove excess moisture and shade dried. Nanoparticles treated seed sown on plastic tray filled with soil and spray water time to time according to need.

2.3 Effect of nanoparticles on growth performance of tomato

Nanoparticles were used as seedling treatment and single foliar application (40 days after transplanting), to find out the most effective nanoparticles against the growth behavior of tomato, *viz.*, seed germination, seedling mortality, root and shoot length, fresh and dry weight of root and shoot, *etc*.

2.4 Statistical analysis

The Completely Randomized Design (CRD) used during conducting our experiment with the mean of 3 replications. All the examined was carried out at 5% level of significance.

3. Results

3.1 Characterization of green synthesized nanoparticles through Scanning Electron Microscope (SEM) analysis

The nano size of green synthesized materials confirmed through the view of Scanning Electron Microscope, the particles size of silver examined about 100 nm representing in Figure 2(a) and the particles size of silica seen about 90 nm in Figure 2(b) nano properties confirmed.

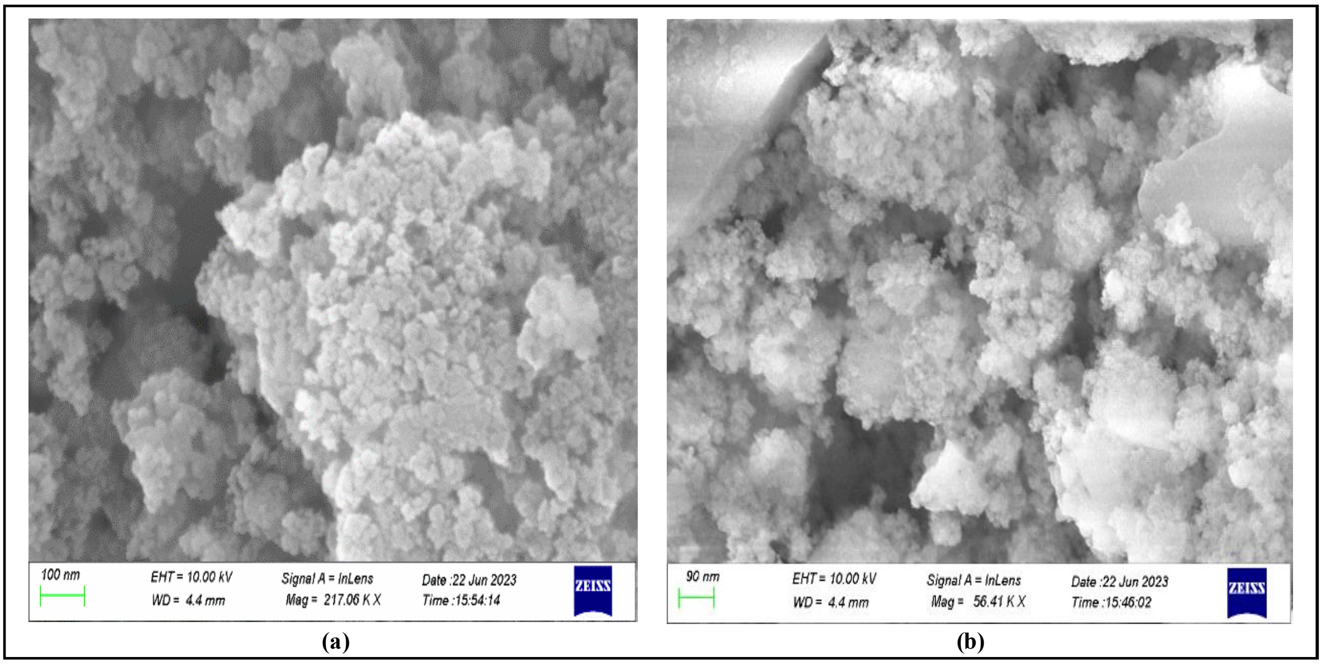


Figure 2: Scanning electron microscopic image of (a) silver and (b) silica nanoparticles.

3.2 Effect of green nanoparticles on growth performance of tomato plant

3.2.1 Effect of nanoparticles on seed germination (%) and seedling mortality (%) of tomato

In agriculture, germination is one of the most crucial stages for plant establishment and is essential for crop quality and production. The nanoparticle treated tomato seed improved germination while drastically lowering seedling mortality. The finding of the experiment showed, that the highest germination was found in T_{12} (silica 100 ppm) treated seeds about 92.66%, followed by T_8 (agri-tech 100 ppm), T_{11} (silica 75 ppm) and T_4 (silver 100 ppm) with the value of 89.32, 83.72 and 80.66%, respectively, against control about 54% which was least than all treated seeds. On the other hand, the minimum seedling mortality with 5.2% observed from seed treated by T_{12} (silica 100 ppm), followed by T_8 (agri-tech 100 ppm) representing as 10%, over the control. The mortality of seedling decreased as 96.20% against control recorded from seed treated with silica 100 ppm (T_{12}), followed by $T_8$with the value of 93.21%. Among the treatments, the least decreasing percentage of seedling mortality recorded from seed treated with silver nanoparticles 25 ppm (T_1) indicating as 43.2%. It is evident that all treated seeds with nanoparticles or nanofungicides have ability to increase germination against the control.

Table 1: Effect of seed treatment with nanoparticles or nanofungicides on germination (%) and seedling mortality (%) of tomato

Treatment details		Observations	
Name of nanomaterials	**Concentrations (ppm)**	**Germination (%)**	**Seedling mortality (%)**
Silvernanoparticles	$T_1 = 25$	62.66	43.2
	$T_2 = 50$	67.32	40.0
	$T_3 = 75$	70.00	37.2
	$T_4 = 100$	80.66	15.2
Agri-tech nanofungicides	$T_5 = 25$	74.66	27.2
	$T_6 = 50$	75.32	22.6
	$T_7 = 75$	77.32	16.6
	$T_8 = 100$	89.32	10.0
Silicananoparticles	$T_9 = 25$	72.66	32.0
	$T_{10} = 50$	77.33	19.2
	$T_{11} = 75$	83.72	12.6
	$T_{12} = 100$	92.66	5.2
Control	non-treated)	54.00	58.6
C.D.		4.782	2.469
SE(m)		1.311	0.906
C.V.		5.493	3.408

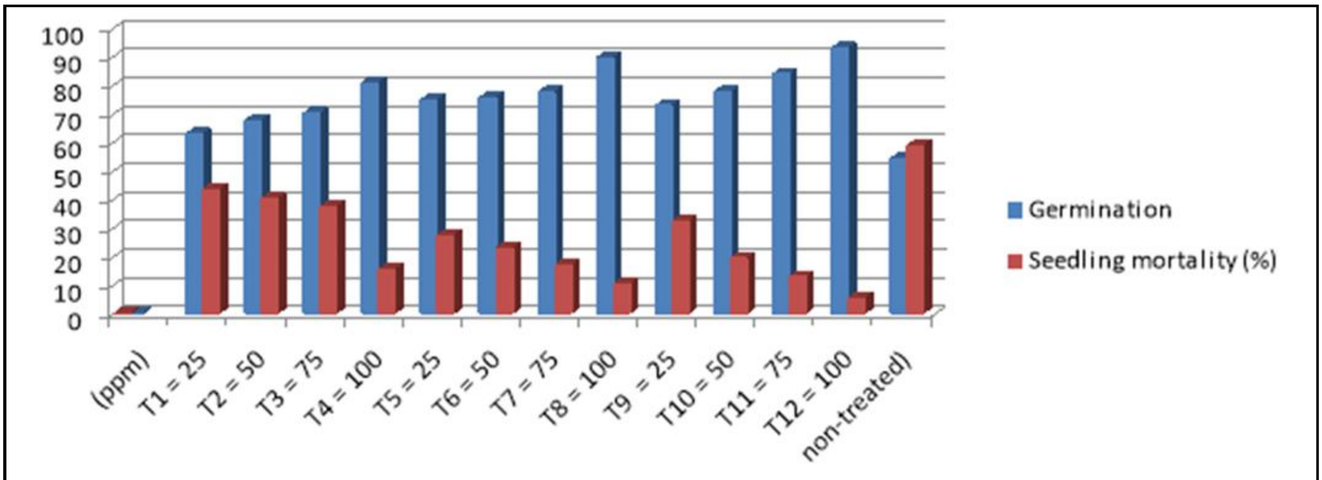


Figure 3: Effect of seed treated with nanoparticles/nanofungicides on germination and seedling mortality (%).

3.2.2 Effect of nanoparticles on morphological behaviours of tomato plant

3.2.2.1 Root and shoot length

The highest length of root and shoot were found in plant treated with T_{12} (silica 100 ppm) treatment indicating 32.12 and 36.02 cm, respectively. Plants treated by agri-tech 100 ppm (T_8), representing the length of root as 31.60 cm and shoot about 34.90 cm, which was the second highest among all the treatments. While, minimum length of root and shoot recorded in control as 17.00 and 20.50 cm, respectively. From the Table 2, it is cleared that treated plants significantly increased 88.94% root and 76.58% shoot length over control was observed from T_{12} (silica 100 ppm), followed by T_8 (agri-tech 100 ppm) as 85.88 and 70.24% and T_{11} (silica 75 ppm) indicating 78.41 and 74.87%, respectively.

3.2.2.2 Fresh weight of root and shoot

The observations recorded 40 days after transplanting on fresh weight of root and shoot presenting in Table 3. Among the treatments, the highest fresh weigh of root and shoot was showed in plants treated with silica 100 ppm (T_{12}) with the value 16.0 and 98.50 g. The plants treated with agri-tech 100 ppm (T_8) were showing 14.53

and 92.47 g which was the second highest among the treatments. The other remaining treated plants were also increases the fresh weight of root and shoot than non-treated plants. The per cent increased over control of root and shoot observed highest in plants treated with T_{12} (silica 100 ppm) about 99.50 and 93.89%, followed by T_8 (agri-tech 100 ppm), values representing 81.17 and 82.02%, respectively. All nanoparticles treated plants statically significantly increased the total biomass.

Table 2: Effect of nanoparticles/nanofungicides on the root and shoot length (cm) of tomato

Treatment details		Observations			
Name of nanomaterials	Concentration (ppm)	Root length (cm)	Per cent increased over control	Shoot length (cm)	Per cent increased over control
Silver nanoparticles	T_1 = 25	24.00	41.17	28.70	40.00
	T_2 = 50	27.00	58.82	30.60	49.02
	T_3 = 75	27.20	60.00	31.70	54.63
	T_4 = 100	30.33	78.41	33.80	64.87
Agri-tech nanofungicides	T_5 = 25	28.86	69.74	32.70	59.51
	T_6 = 50	29.10	71.17	33.10	61.46
	T_7 = 75	29.50	73.52	33.50	63.41
	T_8 = 100	31.60	85.88	34.90	70.24
Silica nanoparticles	T_9 = 25	28.30	66.47	32.40	58.04
	T_{10} = 50	29.50	73.52	33.50	63.41
	T_{11} = 75	31.27	83.94	34.70	69.26
	T_{12} = 100	32.12	88.94	36.20	76.58
Control	non-treated	17.00		20.50	
C.D.		1.068		1.238	
SE(m)		0.364		0.501	
SE(d)		0.514		0.624	
C.V.		2.083		3.613	

Table 3: Effect of nanoparticles or nanofungicides on the fresh and dry weight of the root and shoot of tomato

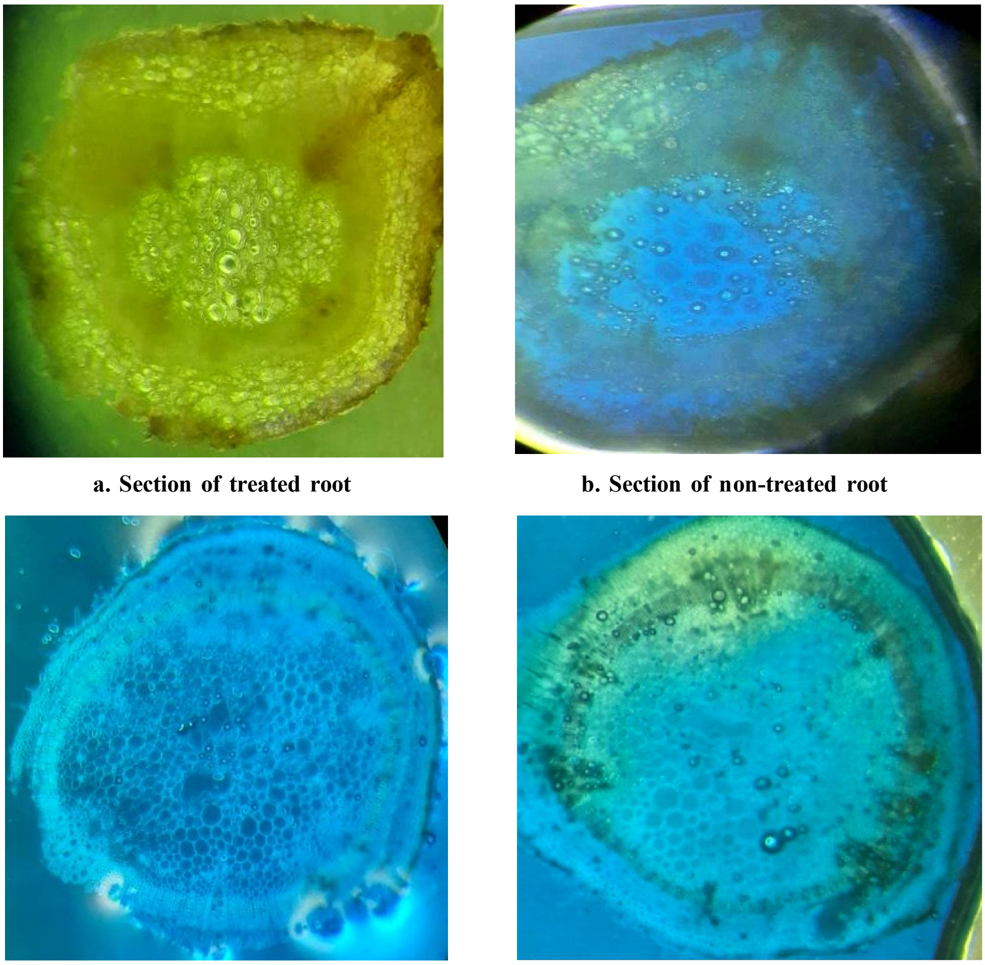
Treatment details		Root weight (g)			Shoot weight (g)		
Name of nanomaterials	Concentration (ppm)	Fresh weight (g)	Dry weight (g)	Per cent increased overcontrol	Fresh weight (g)	Dry weight (g)	Per cent increased over control
Silver nanoparticles	T_1 = 25	09.66	2.72	20.44	50.86	07.95	0.11
	T_2 = 50	09.91	2.83	23.56	51.47	08.20	01.31
	T_3 = 75	10.61	3.09	32.29	52.50	08.72	03.34
	T_4 = 100	12.07	4.34	50.49	84.12	13.90	65.59
Agri-tech nanofungicides	T_5 = 25	11.07	3.83	38.02	57.26	09.20	12.71
	T_6 = 50	11.15	3.96	39.02	71.05	09.80	39.86
	T_7 = 75	11.34	4.32	41.39	79.50	12.80	56.49
	T_8 = 100	14.53	4.70	81.17	92.47	16.24	82.02
Silica nanoparticles	T_9 = 25	10.90	3.17	35.91	54.40	08.70	07.08
	T_{10} = 50	11.33	4.21	41.27	77.02	11.92	51.61
	T_{11} = 75	13.10	4.50	63.34	87.30	14.00	71.85
	T_{12} = 100	16.00	5.23	99.50	98.50	17.23	93.89
Control	non-treated	08.02	2.21	-	50.80	06.61	-
C.D.		1.725			4.561	1.256	
SE(m)		0.669			1.553	0.496	
SE(d)		0.861			2.196	0.601	
C.V.		3.739			3.854	3.437	

3.2.2.3 Dry weight of root and shoot

On the other hand, the dry weight of root and shoot was presented in the Table 3, showed that the plant treated with silica 100 ppm (T_{12}) represented the highest dry weight of root and shoot as 5.23 and 17.23 g, the second highest was found in T_8 treatment (agri-tech 100 ppm) showing 4.70 and 16.24 g, followed by T_{11} (silica 75 ppm) and T_4 (silver 100 ppm) representing value about 4.50 and 14.0 g and 4.34 and 13.90 g, respectively. Among the treatments, T_1 (silver 25 ppm) was showing lowest dry weight of root and shoot as 2.72 and 7.95 g, respectively. While, non-treated plants (control) was observed least dry weight of root as 2.21 g and shoot about 6.61 g.

3.3 Transverse sections of root and shoot of treated (healthy) and non-treated (infected) tomato plants

The transverse section of root and shoot of non-treated and treated plant are representing in Figure 4 (a- transverse section of treated root: b- non-treated root: c- transverse section of treated stem and d- section of diseased stem, seen under Stereo Zoom Microscope at 10x magnification). Histochemical staining of the root section of tomato at 5 days after pathogen inoculation showed significant variation of lignin deposition between nanoparticles treated and non-treated plants. But, in case of wilt infected stem transverse section Figure 4(d) clearly indicated that the discoloration of tissues.



a. Section of treated root
b. Section of non-treated root
c. Section of treated stem
d. Section of non-treated stem

Figure 4: Transverse section of treated root (a) and non-treated root (b):treated shoot (c) and non-treated shoot (d).

4. Discussion

Germination is one of the most important phases in the development of plants and is essential for crop production. In the current study, all treated seeds with nanoparticles/nanofungicides exhibited better germination. The highest germination % was found in T_{12} (silica 100 ppm) treated seeds about 92.66% with least seedling mortality as 5.2% against the control. All nanoparticles treated seeds were statistically increased germination and reducing the seedling mortality. Deka *et al.* (2021) reported the silver nanoparticles synthesized using through leaves of *Azadirachta indica* and proved antioxidant and antimicrobial activities under *in vitro* studied against plant pathogenic fungi. Lahiani *et al.* (2015) also reported that the hastening of seed germination in different crops, *viz.*, rice, maize, soybean and tomato has been observed when treated with carbon containing nanomaterials. Another similar result found by Sheukh *et al.* (2022), who reported that the root extract of *Glycyrrhiza glabra* reduces the pathogenic activities in plants. The current results agreed with Rhaman *et al.* (2022) who reported that the seed treatment with nonomaterials is an economical and most efficient physiochemical technique which enhanced the seed germination.

The current study demonstrated that, the highest length of root and shoot recorded in plant treated with silica 100 ppm (T_{12}) indicated as 32.12 and 36.02 cm, respectively. While, minimum length of root and shoot was recorded in control plant as 17.00 and 20.50 cm, respectively. The highest as 88.94 and 76.58% increase over control was investigated in T_{12} (silica 100 ppm) treated plants. Tripathi *et al.* (2017) was found that positive effect of metal oxide nanoparticles on plant growth parameters (stem length) and productivity. The stimulatory effect of *C. globosum* on root and shoot length of wheat has also been documented by Biswas *et al.* (2008). The current results also supported by the finding of Seleiman *et al.* (2020) who concluded that the selenium nanoparticles primed tomato seedling showed a significant biomass variation between the treated and non-treated plants.

Highest fresh weigh of root and shoot was observed in plants treated with silica 100 ppm (T_{12}) with the value 16.0 and 98.50 g against 8.02 and 50.80 g noted in case of control (non-treated), respectively. The per cent increase over control the weight of root and shoot was maximum showed in all nanoparticles/nanofungicides treated plants. Ali *et al.* (2019) have been found that application of nanozinc oxide at low doses positively effect on physiological responses, such as shoot elongation, the fresh and dry weight in treated plants. The plant treated with ZnO NPs, positive responses on plant growth promotion have been reported by Venkatachalam *et al.* (2017). Ahmad *et al.* (2022) also agreed to current work, using nanomaterials against the *Alternaria mali, Botryosphaeria dothidea* and *Diplodia seriata* reduces pathogenic activities and enhanced the total biomass in apple orchards.

On the other hand, the dry weight of root and shoot showed that the plant treated with silica 100 ppm (T_{12}), had highest value of 5.23 and 17.23 g dry root and shoot weight, respectively. The second highest was found in T_8 (agri-tech 100 ppm) treatment as 4.70 and 16.24 g, respectively. The dry weight of root and shoot of all remaing treated plants were also increased than control. Josko and Oleszczuk (2013) also found that the increased fresh and dry weight of root and shoot in nanoparticles treated plants. Singh *et al.* (2015) also noted that tomato plants treated with bioagents grow more quickly and produce more true leaves, fresh and dry root, and shoot weights.

5. Conclusion

Strong evidence exists to support cannabinoids broad therapeutic range and qualities, such as its anti-inflammatory, antioxidant, anti-microbial, antifungal, and anticancer effects. The findings in this study provide more evidence that a wide range of *Cannabis* derivatives may have an impact on the reduction of plant pathogenic activities. In the near future, green synthesis nanoparticles may prove to be the best substitute against chemical fertilizers and synthetic pesticides for improving the quantity and quality of crops due to their eco-friendly, biodegradable, and non-chemical nature and the growing trends of their application in the field. The seed treated with green synthesized nanoparticles positively raised germination and reduced the mortality of tomato seedlings. All plants treated with nanoparticles or nanofungicides significantly increased the root, shoot length and total biomass (fresh and dry weight) of the plants. Thus, it is evident from the current results that the use of green synthesis nanoparticles can be used to manage tomato seedling mortality in a sustainable manner.

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Conflict of interest

The authors declare no conflicts of interest relevant to this article.

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