Plant Archives Vol. 24, Special Issue (GABELS), 2024 pp.80-90 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2024.v24.SP-GABELS.013

BIOEFFICACY OF COPPER NANOPARTICLES AGAINST SEED BORNE MYCOFLORA OF PIGEON PEA

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ABSTRACT

The present study investigates management of seed borne mycoflora associated with pigeon pea using biosynthesized CuNPs prepared using *Trichoderma asperellum* metabolites. *Trichoderma asperellum* is environment friendly fungus and is well known for producing extracellular enzymes and metabolites in greater quantity than other fungi. Thus, was used to biosynthesize copper nanoparticles, The effect of Tr.CuNPs as seed treatment on germination, root length, shoot length and seedling vigour, pre and post emergence mortality of pigeon pea was evaluated using rolled paper towel method at 10, 50, 100, 150, 200, 250 ppm concentrations. Seed treatment with Tr.CuNPs @ 10ppm and 50 ppm recorded maximum germination, highest root length, shoot length and seedling vigour index. It also found to minimize the adverse effect of seed mycoflora on pre and post emergence mortality of pigeon pea. Seed treatment of Thiram + Carboxin @ 3 g/kg was equally effective in terms of improving seed germination, root length, shoot length, seedling vigour index and reducing pre and post emergence mortality of pigeon pea. Seed treatment of Trichoderma based copper nanoparticles at higher concentration i.e. 100, 150, 200, 250 ppm showed adverse effect on the seed as it hampered the germination, root length, shoot length and seedling vigour of pigeon pea. Thus, copper nanoparticles synthesized from *T. asperellum* can be used for seed treatment at lower concentrations as it shows high seedling vigour and seed germination as compared to chemical and biological seed dressers.

Keywords: *T. asperellum*, copper nanoparticles, Thiram + Carboxin, seed vigour.

Introduction

The pigeon pea (*Cajanus cajan*), also referred to by other colloquial and commercial names, including red gram, angola pea, congo pea, yellow dhal, and oil dhal, is a significant grain legume crop found in tropical and subtropical regions. India is the world's largest producer of pigeon peas, which are eaten as split peas that have been decorated, or "dal." It is common to consume it as a green vegetable and whole dry seed in other nations. Its leaves are utilized as animal fodder, and the leftovers from milling make a great meal for household pets. About 20–22% protein and significant amounts of important minerals and amino acids can be found in pigeon pea seeds. The major anti nutritional components of seeds, such as tannins and enzyme inhibitors, are eliminated during dehulling and boiling processes. This legume loses a significant amount of quality during seed storage. More than a hundred infections pathogens have been identified to impact the pigeon pea crop, according to studies conducted by several researchers on the mycoflora linked with pigeon pea seeds and their relevance. The most frequent fungi linked to stored seed are *Fusarium*, *Alternaria*, *Phytophthora*, *Rhizoctonia*, and *Cercospora*. These pathogens are mostly in charge of causing seed deterioration, a decrease in germination capacity, and the development of seedlings. Mallesh *et al*. (2008) and Pradhan *et al*. (2016).

As a cutting-edge antibacterial material, copper nanoparticles are useful in the fields of agriculture. We have reported the antifungal activity of copper nanoparticles against specific crop pathogenic fungi in this work. Significant antifungal efficacy was shown by copper nanoparticles against the plant pathogenic fungi *Fusarium oxysporum* (MTCC No. 1755), *Curvularia lunata* (MTCC no. 2030), *Alternaria alternata* (MTCC no. 6572), and *Phoma destructiva* (DBT-66). According to Usman *et al*. (2012), synthesized copper nanoparticles can be employed as a novel antifungal agent in agriculture to control the plant pathogenic fungus.

Copper was known to man since time immemorial as an essential element. Copper has drawn attention for its antimicrobial properties and various other advantages in several fields. Considering the myriad advantages of the copper compound, currently, they are of special interest to many for the synthesis of CuNPs. Plant diseases are a significant barrier to its production, and efforts to address this issue have lasted for many years. Many useful compounds (fungicides, antibiotics, etc.) that can effectively manage the variety of diseases caused by phytopathogens have been created and introduced with persistent, unrelenting effort. However, because of high toxicity and the possibility that the fungicides applied by farmers may be wasted owing to wind or surface runoff, these traditional ways of managing phytopathogens have had an adverse effect on the environment and the farmer's income. Unfortunately, because of their site of action, systemic fungicides have contributed to the development of resistance against pathogens, even though their introduction marked a significant turning point in the management of infections. Thus, there is an urgent need to develop substitute antifungal medicines, such as nanofungicides. Kasana *et al*. (2017).

Nanotechnology is a relatively new science that is both intriguing and mysterious, with a lot of potential applications in agriculture. Nanotechnology can decrease toxicity and increase the effectiveness and shelf life of an antifungal treatment, making disease management more environmentally friendly and sustainable. Regarding plant protection, according to Kanhed *et al*. (2014) and Yaswanth *et al*. (2021), nanoparticles alone can be utilized as direct antifungal agents. Potential applications of nanotechnology in crop production include the control of plant diseases. Because they are so small, nanoparticles may influence a pathogen that is comparable to that of chemical pesticides, or they may be utilized as a vehicle for the pesticide's active ingredient to reach the intended pathogen. With the application of nanotechnology, disease diagnosis, pathogen detection, and residual analysis may become considerably more accurate and expedient. However, according to Jamkhandi *et al*. (2019), certain copper compounds can be harmful if used excessively. Nanotechnology has suggested substitutes for copper compounds to lessen this adverse effect and enhance agriculture.

Copper nanoparticles that have been biosynthesized exhibit strong antimicrobial activity, preventing the growth of harmful bacteria and fungus. Certain plants respond better to lesser concentrations of copper nanoparticles in terms of germination and growth, while higher concentrations cause the plants to grow more slowly. To address the related seed-borne mycoflora at various concentrations, the current study has been designed to examine the impact of biosynthesized copper nanoparticles on seedling growth, vigour, pre- and post-emergence seedling mortality of pigeon pea.

Materials and Methods

Collection of pigeon pea seeds

The Pigeon pea seeds sample i.e. PKV-TARA was collected from Pulse Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra).

Procurement of copper nanoparticles

The biosynthesized copper nanoparticles from *Trichoderma asperellum* metabolites were procured from Department of Plant Pathology, Dr. PDKV, Akola, Maharashtra, India

Seed treatment of biosynthesized CuNP's

The copper nanoparticles prepared from *Trichoderma asperellum* were dissolved in DMSO solvent in equal volume and a stock solution of 1000 ppm was prepared. Six different concentrations of biosynthesized CuNPs viz., 10ppm, 50ppm, 100ppm, 150ppm, 200ppm and 250ppm were prepared by adding respective suspension of stock solution in 100 ml of sterilized distilled water. For seed treatment, the required seeds of PKV-TARA were soaked in respective concentrations of copper nanoparticles for 2-3 minute and then were used for seed treatment.

Evaluation of biosynthesized CuNPs

Rolled paper towel method were used to assess the effect of biosynthesized copper nanoparticles on seed germination, root length, shoot length and seedling vigour of pigeon pea and pre & post emergence mortality of pigeon pea was assessed by conducting pot culture experiment.

Treatment details:

Design : CRD (Completely Randomized Design)

Replications : Three

Treatments : Nine

Tr. No.	Treatment Details	Conc. used
T1	*Trichoderma asperellum* (Talc based formulation)	@ 4 g /kg
T2	Biosynthesized Trichoderma based CuNPs	@ 10ppm
T3	Biosynthesized Trichoderma based CuNPs	@ 50ppm
T4	Biosynthesized Trichoderma based CuNPs	@ 100ppm
T5	Biosynthesized Trichoderma based CuNPs	@ 150ppm
T6	Biosynthesized Trichoderma based CuNPs	@ 200ppm
T7	Biosynthesized Trichoderma based CuNPs	@ 250ppm
T8	Carboxin 37.5% + Thiram 37.5% WS	@ 3 g /kg
T9	Control	--------

Procedure for seed treatment

Copper nanoparticles prepared from *Trichoderma asperellum* metabolites were dissolved in DMSO solvent and six different concentrations of biosynthesized CuNPs viz., 10ppm, 50ppm, 100ppm, 150ppm, 200ppm and 250ppm were prepared by adding respective suspension of CuNPs in 100 ml of sterilized distilled water. For seed treatment, the required seeds of PKV-TARA were soaked in respective concentrations of copper nanoparticles for 2-3 minute and then used.

Seed Germination

Seeds of pigeon pea were treated with different concentration of biosynthesized CuNPs as per treatments. Total 400 seeds of selected variety taken of which randomly selected 100 seeds were placed on layers of moist germination papers with help of forceps, at ten seeds per row. The rolled towel papers will be kept in slanting position along the wall of laboratory tables and incubated at 26+ 2°Cfor 7 days. The count of germination was taken on 7th day. Morphologically normal seedlings were examined. The germinated seeds were counted in each treatment for the measurement of germination percentage (GP). The GP was determined as GP = GN/SN × 100, where GN and SN are the total germinated seeds and tested seeds, respectively as per the formula suggested by Kausar *et al*. (2022).

Root and shoot length (cm) and vigour index

To estimate growth, the primary root and shoot length of eight days old seedlings were measured. The normal seedlings were selected at random from each replication and the shoot and root length from the collar at the tip of the primary root was measured and the respective mean values were recorded. The formula suggested by Abdul Bakri and Anderson (1973).

Vigour Index = (Root length + Shoot length) × (Germination Percentage)

Pre and post emergence mortality

Pot culture experiment was conducted with three replicates, pots were filled with a mixture of soil and sand. Treated seeds were then sown @10 seeds/ pot. Proper care was taken and observations on pre and post emergence mortality was recorded. Pre emergence mortality was recorded on 10 days after sowing. Observation on post emergence mortality was recorded up to 30 days after sowing.

$$\text{Pre emergence seed mortality}(\%)\,(\text{PESR}) = \frac{\text{Number of seed not germinated}}{\text{Total number of seed sown}} \times 100$$

$$\text{Post emergence seedling mortality}\,\%(\text{PESM}) = \frac{\text{Number of seedlings died}}{\text{Total number of seedlings}} \times 100$$

Results and Discussion

Collection of pigeonpea seeds

The Pigeon pea seeds sample i.e. PKV-TARA was collected from Pulse Research Unit, Dr.Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra).

Procurement of copper nanoparticles

The biosynthesised copper nanoparticles from *Trichoderma asperellum* metabolites were procured from Department of Plant Pathology, Dr. PDKV, Akola.

Detection and identification of seed borne mycoflora of pigeon pea seeds.

The seed borne mycoflora of pigeon pea seeds were detected mainly by three methods i.e. Standard Blotter Method, Agar Plate Method and Rolled paper towel method. The seed borne mycoflora associated with pigeonpea seeds are mentioned below in Table no. 1.

Table 1 : Comparative evaluation of different detection methods of seed mycoflora of pigeon pea

Seed borne mycoflora	Seed borne mycoflora (%)		
	Standard blotter paper method	Agar plate method	Rolled paper towel method
Alternaria alternata	4.05	15	12
Aspergillus flavus	19.4	16.6	15.5
Curvularia lunata	-	3.5	-
Aspergillus niger	28.22	24.2	25.45
Fusarium spp.	9.5	20.2	20.2
Rhizoctonia spp	2.2	00	-
Rhizopus spp.	22.5	00	15
Penicillium spp.	-	16.5	-
Cladosporium spp.	3.22	4.5	-
Colletotrichum spp.	-	2.25	-
Total frequency	89.09	102.65	88.12

Effect of biosynthesized copper nanoparticles on seed germination of pigeon pea

The experiment was conducted to investigate the effect of different concentration of biosynthesized CuNPs on seed germination of pigeon pea. Total nine treatments including six different concentrations of biosynthesized CuNPs i.e. 10 ppm, 50 ppm, 100 ppm,150 ppm, 200 ppm and 250 ppm were tested and compared it with chemical fungicide i.e. Thiram + Carboxin 37.5 DS @ 3g/kg and bio fungicide *T. asperellum* @ 4g/kg as seed treatment.

Data presented in Table 2, Fig 1 and Plate 1. revealed the effect of different concentrations of biosynthesized CuNPs i.e. 10 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm on seed germination of pigeon pea. Tr.CuNPs enhances seed germination at lower concentrations. Among all treatments T_2 i.e. 10 ppm was found significantly superior and recorded highest seed germination (87%). Seed treated with Carboxin 37.5g+Thiram 37.5g @3g/kg ($T_{8)}$ recorded 86.00% seed germination which found at par with T_2. Whereas seed treatment with Tr.CuNPs @ 200 ppm and 250 ppm significantly declined seed germination i.e. 77.00% and 71.00% respectively. In control seed germination was only 84.33% which is at par with T_3 and T_1 (50 ppm) it recorded 84.66 % and 85.33 % seed germination respectively. This result indicates that there is significant decrease in seed germination as the concentration of copper nanoparticles increases.

The number of abnormal seedlings increased with increase in concentration of Tr.CuNPs. Minimum abnormal seedling i.e. 9.00% was also observed in T_2 (10 ppm) and T_1 (*T. asperellum* @4 g/kg) i.e.(8.33%) followed by T_8 (Thiram + Carboxin @ 3g/kg) i.e. 9.66% whereas, maximum abnormal seedling was observed when concentrationincreased @ 250 ppm and 200 ppm i.e. 18.00% and 15.66% respectively. Minimum rotten seeds i.e. (4.66%) were found in T_2 i.e. seed treatment with Tr.CuNPs @ 10ppm whereas, seed treated with Tr.CuNPs at 100,150,200,250 ppm concentration showed maximum rotting of seed.

The results obtained were agreed with the findings of Ananda *et al*. (2019) who reported highest germination percentage i.e. 95% of tomato seedling when treated with 20 ppm Cu_2NPs and as the concentration of Cu_2NPs treatment increases the germination percentage of tomato seedling goes on decreasing. Copper nanoparticles enhance the germination and growth of some plants at lower concentrations, whereas high concentrations result in retarded growth as stated byKasana *et al*. (2017). Kausar et.al. (2022) found condition dependent effect of CuNPs when NPs applied to wheat as some varieties of wheat showed maximum germination and growth rate at 50 mg CuNPs/ L, while other variety showed increase at 25 mg CuNPs/ L, beyond these concentrations, the seed germination and growth of wheat declined. They conclude the application of CuNPs showed a beneficial effect in improving the growth of wheat at a certain concentration. Also Afrayeem *et al*. (2017), Shende *et al*. (2017) observed the beneficial effects of CuNPs on seed germination at certain concentrations.

Table 2 : Effect of Trichodermabased copper nanoparticles on seed germination of pigeon pea.

Tr. No.	Seed Treatment	Germination (%)	Abnormal seedling (%)	Rotten seed (%)
T_1	*T. asperellum* @ 4gm/kg	85.33 (67.48)*	8.33 (2.88)**	6.33 (2.50)**
T_2	Tr.CuNPs @ 10ppm	87.00 (68.87)*	9.00 (3.00)**	4.66 (2.15)**
T_3	Tr.CuNPs @ 50ppm	84.66 (66.95)*	10.00 (3.16)**	6.33 (2.51)**
T_4	Tr.CuNPs @ 100ppm	83.66 (66.16)*	12.00 (3.46)**	5.33 (2.29)**
T_5	Tr.CuNPs @ 150ppm	80.33 (63.67)*	13.00 (3.06)**	7.33 (2.70)**
T_6	Tr.CuNPs @ 200 ppm	77.00 (61.34)*	15.66 (3.95)**	8.33 (2.82)**
T_7	Tr.CuNPs @ 250ppm	71.00 (57.41)*	18.00 (4.23)**	11.33 (3.51)**
T_8	Thiram 37.5 + Carboxin 37.5 @ 3 g/kg	86.00 (68.02)*	9.66 (3.10)**	4.66 (2.15)**
T_9	Control	84.33 (66.02)	9.00 (3.00)**	6.66 2.58)**
	SE(m) ±	0.41	0.27	0.56
	CD (0.01%)	1.129	0.15	0.45

The figures in parenthesis*indicate arc sin transform values and the figures in parenthesis **indicated square root tronsform values

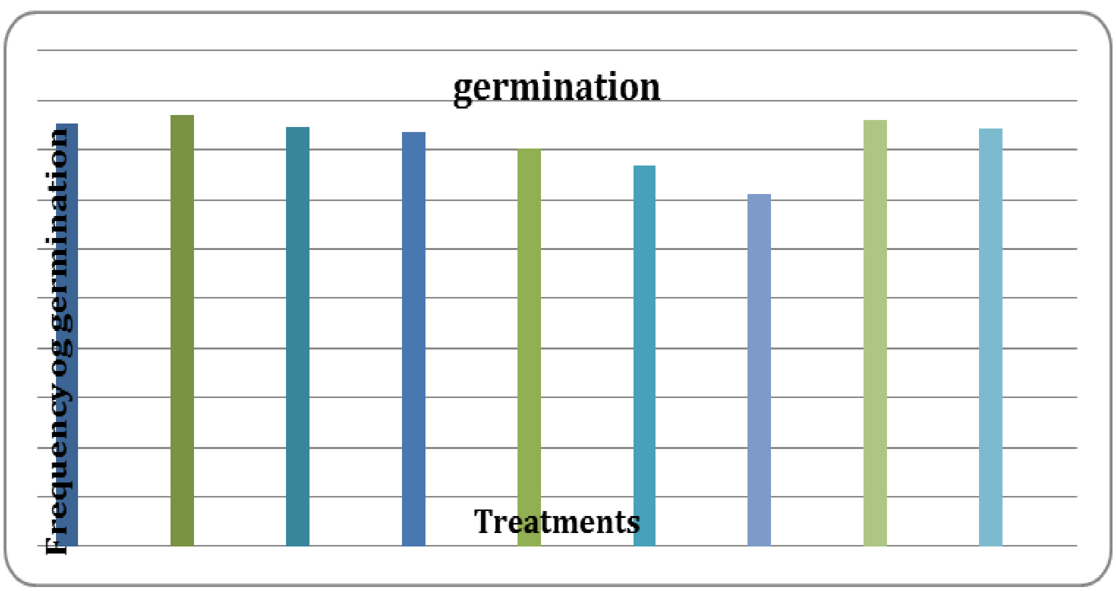

Fig. 1. Effect of biosynthesized CuNPs on seed germination of pigeon pea

Seed treatment @ 10 ppm biosynthesized CuNPs

Seed treatment @ 250 ppm biosynthesized CuNPs

Seed treatment control

Plate 1 : Effect of biosynthesized CuNPs on seed germination of pigeon pea.

Effect of biosynthesized copper nanoparticles on root /shoot length and seedling vigor indexof pigeon pea.

Different concentration of biosynthesized CuNPs @ 10 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm were evaluated to know their effect on root length, shoot length and seedling vigor index of pigeon pea. Data presented in Table 3, Fig 2, 3 and Plate 2 revealed that, as compared to the control, lower concentrations of CuNPs had a significant impact on root length, shoot length and seedling vigour index. However, higher concentration of biosynthesized CuNPs had a negative impact on shoot length and root length and seedling vigour index.

The highest seedling vigor index was recorded when seed was treated with Tr.CuNPs (T_2) @10 ppm i.e. 2018.54 followed by Thiram 37.5 + Carboxin @ 3g/kg (T_8) i.e. 1914.08 and Tr.CuNPs @ 50 ppm (T_3) i.e. 1835.92 which was found at par with each other. While seed treated with *T. asperellum* @ 4g/kg (2009.79) seedling vigour index was found non-significant. Whereas, at higher concentrations of Tr. CuNPs i.e., 200 ppm and 250 ppm recorded less (1472.86) and (1227.72) seedling vigour index than control (1815.56).

At higher concentrations i.e. Tr.CuNPs @ 150 ppm, 200 ppm, 250 ppm showed adverse effect on root length. Maximum root length (9.68cm) was recorded in T_2 (10 ppm) and in T_1 i.e. *T. asperellum* (9.34%) which was followed by T_8 (Thiram + Carboxin @ 3g/kg) i.e. 9.48 cm. Seed treatment with Tr. CuNPs@ 50 ppm and *T. asperellum*@ 4 g/kg found at par with each other and which was significantly higher than control. At higher concentration of Tr. CuNPs i.e.150 ppm, 200 ppm, 250 ppm showed adverse effect on root length. Seed treatment with Tr.CuNPs @ 50ppm and *T. asperellum* @ 4g/kg were found at par with each other which recorded root length (8.83 cm) and (9.34 cm) respectively and which was significantly higher than control (8.70 cm).

Similarly, higher concentration of Tr.CuNPs recorded reduced shoot length of pigeon pea seedling. Seed treatment @ 10 ppm was found superior. Also, significantly higher shoot length was observed in T_8 (12.68 cm) and T_3 (12.84 cm) which was found at par with each other and followed by T_1 (*T. asperellum* @ 4 g/kg) i.e. (13.76). But at higher concentrations, treatment had a toxic effect on seed germination and seedling growth Thus, 10 ppm concentration of Tr.CuNPs was best as seed treatment to pigeon pea for obtaining better seed vigour and seedling growth than others.

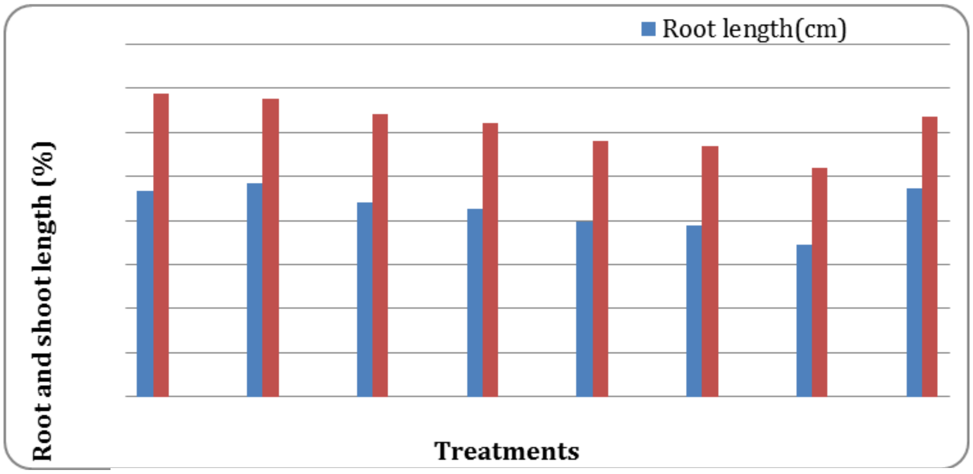

Fig.-2: Effect of biosynthesized CuNPs on root and shoot length of pigeon pea.

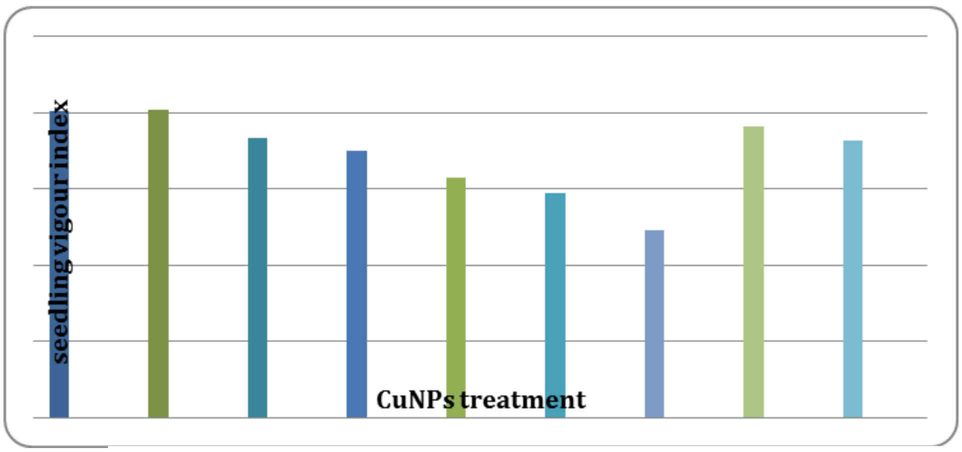

Fig. 3: Effect of biosynthesized CuNPs on seedling vigor index of pigeon pea.

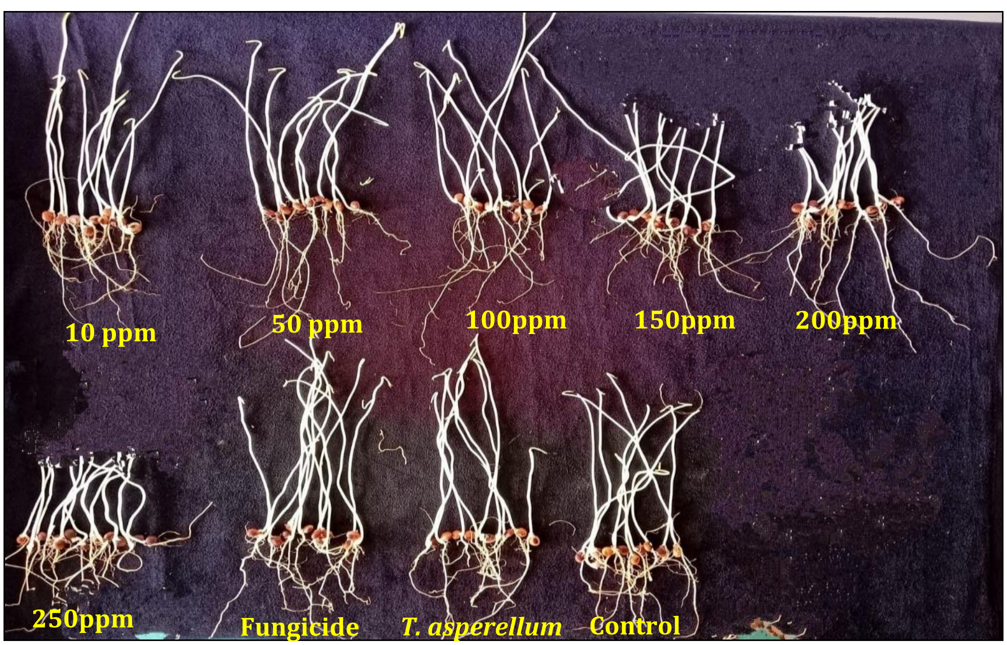

Plate 2: Effect of biosynthesized CuNPs on root and shoot length of pigeon pea

Similar observation was shown by Chandrashekhara *et al*. (2020) who revealed that CuNPs when treated @ 50 ppm i.e. lowest concentration has significantly shown higher percent of seed germination (75.33%), seedling vigor (1327), seedling dry weight (0.32 g) shoot (5.96 cm) and root length (11.66 cm) over all other treatment. Thus, as the CuNPs concentration increases the seed germination root/shoot length and seedling vigor index decreases. The reason for good germination could be due to the penetration of nanoparticles into the seed coat. Facilitating the influence of water inside the seed and activating the enzyme in early phase, thereby enhancing seed germination. Also, the results of Maithreyee *et al*. (2018) showed highest seedling vigor, shoot/root length, when treated with CuNPs at lower concentration as compared to higher concentration. Rajput *et al*. (2017) clearly denoted the toxic effects of CuONPs on cultivated crop plants by inhibiting seed germination, decreases in the shoot and root lengths, reduction in photosynthesis and respiration rate, and morphological as well enzymatic changes.

Prakash *et al*. (2017) also synthesized copper oxide nanoparticles (CuO NPs) of *Brassica napus* L. and observed that CuO NPs treatment significantly reduced shoot and root growth at higher concentrations. Thakur *et al*. (2021) synthesized copper nanoparticles (CuNPs) and revealed that lower concentration of CuNPs is potential enhancer of seed vigor whereas higher concentration of the nanoparticles proved to be detrimental seed vigor when treated to wheat crop. Shende *et al*. (2017) and Banik *et al*. (2017) recorded beneficial effects of nanoparticles in enhancing the seed germination and vigor index of crops.

Table 3 : Effect of biosynthesized copper nanoparticles of root and shoot length and seedling vigor index of pigeon pea

Tr. No.	Seed Treatment	Germination (%)	Root length (cm)	Shoot length (cm)	Seedling vigour index
T_1	*Trichoderma asperellum* @ 4 g/kg	85.33 (67.48) *	9.34 (3.05)**	13.76 (3.70)**	2009.79
T_2	Tr.CuNPs @ 10 ppm	87.00 (68.87)*	9.68 (3.11)**	13.52 (3.67)**	2018.54
T_3	Tr.CuNPs @ 50 ppm	84.66 (66.95)*	8.83 (2.97)**	12.84 (3.58)**	1835.92
T_4	Tr.CuNPs @ 100 ppm	83.66 (66.16)*	8.54 (2.92)**	12.40 (3.52)**	1752.16
T_5	Tr.CuNPs @ 150 ppm	80.33 (63.67)*	7.97 (2.82)**	11.63 (3.41)**	1574.57
T_6	Tr.CuNPs @ 200 ppm	77.00 (61.34)*	7.75 (2.78)**	11.37 (3.37)**	1472.86
T_7	Tr.CuNPs @ 250 ppm	71.00 (57.4)*	6.88 (2.62)**	10.41 (3.22)**	1227.72
T_8	(Thiram+ Carboxin @ 3g/kg	86.00 (68.02)*	9.48 (3.07)**	12.68 (3.55)**	1914.08
T_9	Control	84.33 (66.02)*	8.70 (2.94)**	12.82 (3.58)**	1815.56
	SE(m)±	0.41	0.24	0.31	37.35
	CD (0.01%)	1.129	0.16	0.18	152.04

The figures in parenthesis*indicate arc sin transform values and the figures in parenthesis ** indicated square root tronsform values.

Effect of biosynthesized copper nanoparticles on pre and post emergence mortality of pigeon pea

Data pertaining to the effect of different concentrations of copper nanoparticles on pre and post emergence mortality of pigeon pea variety PKV-TARA is presented in Table 4, Fig 3 and shown in Plate 3. Analysis of data indicated that higher concentrations of Tr. CuNPs recorded maximum pre and post emergence mortality of pigeon pea in comparison with control. However, lower concentrations of Tr.CuNPs recorded minimum pre and post emergence mortality.

Least pre and post emergence mortality of pigeon pea i.e. 11.66%, 2.28% were observed in Tr. CuNPs @ 10 ppm (T_2) which was at par with chemical seed treatment Thiram + Carboxin @ 3 g/kg i.e. 11.33%, 2.66% pre and post emergence mortality were recorded respectively. However, treatment (T_7) with Tr.CuNPs @ 250 ppm recorded maximum pre and post emergence mortality (30% and 5.58%), followed by (T_6) @ 200 ppm (28.33% and 5.26%) followed by (T_5) @ 150 ppm (24.66% and 4.08%) whereas, in control (19.00% and 3.42%) pre and post emergence mortality is recorded.

The pre and post emergence mortality recorded in (T_2) Tr.CuNPs @ 10 ppm was (11.66%) and (2.28%) respectively which was statistically at par with (T_8) *Trichoderma asperellum* @ 4g/kg(11.33%) and (2.66%) respectively. The pre and post emergence mortality was highest in T_7 (Tr.CuNPs250 ppm) i.e. 30.00% and 5.58% respectively which was at par with T_6 *(*200 ppm).

The results of this study clearly demonstrate that biosynthesized copper nanoparticles @ 10 ppm were best and effective for reducing the pre and post emergence mortality and, as the concentration increases, both pre-emergence and post-emergence mortality of seeds also increase.

Table 4: Effect of biosynthesized copper nanoparticles on pre and post emergence mortality of pigeon pea.

Tr. No.	Seed Treatments	Pre emergence mortality (%)	Post emergence mortality (%)
T_1	***Trichoderma. asperellum* @ 4g/kg**	12.33 (3.511)**	4.03 (1.74)**
T_2	**Tr.CuNPs @ 10 ppm**	11.66 (3.41)**	2.28 (1.47)**
T_3	**Tr.CuNPs @ 50 ppm**	16.66 (4.08)**	4.61 (2.14)**
T_4	**Tr.CuNPs @ 100ppm**	18.66 (4.03)**	3.64 (1.85)**
T_5	**Tr.CuNPs @ 150 ppm**	24.66 (4.96)**	4.08 (2.00)**
T_6	**Tr.CuNPs @ 200ppm**	28.33 (5.32)**	5.26 (2.42)**
T_7	**Tr.CuNPs @ 250ppm**	30.00 (5.47)**	5.58 (2.57)* *
T_8	**Thiram + Carboxin @ 3g/kg**	11.33 (3.36)**	2.66 (1.85)**
T_9	**Control**	19.00 (4.35)**	3.42 (1.85)**
	SE(m)±	**0.44**	**0.73**
	CD (0.01%)	**0.22**	**0.66**

The figures in parenthesis **indicated square root transform value

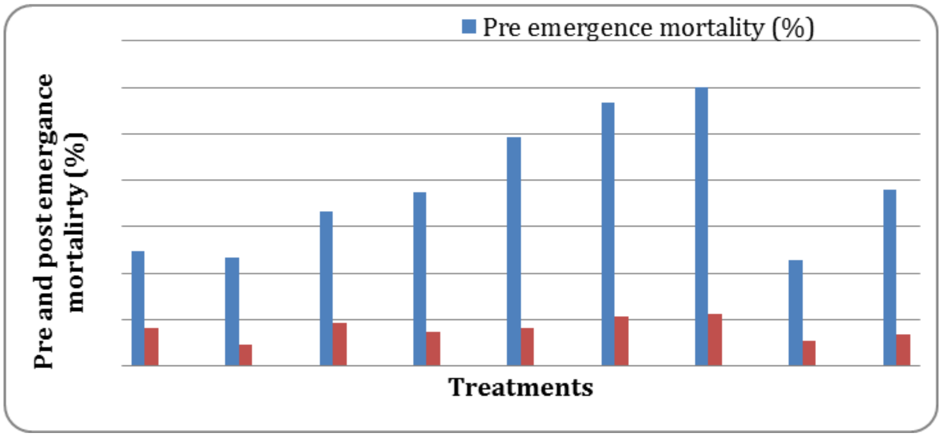

Fig. 4 : Effect of biosynthesized CuNPs on pre and post emergence mortality of pigeon pea

T1 T2 T3 T4 T5 T6 T7 T8 T9

Pot culture experiment

Pre emergence mortality Post emergence mortality

Plate 3: Effect of biosynthesized CuNPs on pre and post emergence mortality of pigeon pea

Conclusion

The above study concluded that seed treatment of biosynthesized copper nanoparticles from *Trichoderma asperellum* metabolites @10 ppm was found effective for nullifying the adverse effect of seed borne mycoflora on seed germination, root length, shoot length and seedling vigour index of pigeon pea seeds. Higher concentration of biosynthesized copper nanoparticles for seed treatment showed adverse effect on seed germination, root length, shoot length and seedling vigour index. Also, above study concluded that, as the concentration of copper nanoparticles goes on increasing the pre and post emergence mortality of pigeon pea seeds increased. Thus, based on above study, we can say that copper nanoparticles have an adverse effect on seed germination and seedling growth of pigeon pea when the concentration of copper nanoparticles is increased.

Acknowledgement

We are thankful to all staff of Department of Plant Pathology, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola for their valuable support to do my research work completely. Also special thanks to my research guide and all PhD seniors who guided me in writing this manuscript.

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