Plant Archives Vol. 24, Special Issue (GABELS), 2024 pp.296-305 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.043

EFFICACY OF TRICHODERMA MEDIATED COPPER NANOPARTICLES AGAINST SEED BORNE MYCOFLORA OF CHICKPEA

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ABSTRACT

Chickpea (*Cicer arietinum*) is grown extensively in India as an important legume crop. Mainly, seed borne fungi affect chickpea crop leading to loss in quality and quantity of the seed. In the present study, *Trichoderma asperellum* mediated copper nanoparticles are used to manage the different seed borne mycoflora associated with the chickpea crop. The effect of *Trichoderma asperellum* mediated CuNPs on seed germination, root length, shoot length, seedling vigour, pre and post emergence mortality of chickpea was evaluated by rolled paper towel method at 10, 50, 100, 150, 200 and 250 ppm concentrations. The seed treatment with Tr. CuNPs @ 10 ppm and 50 ppm recorded maximum seed germination, highest root length, shoot length and seedling vigour index. It also found to decrease the adverse effect of seed mycoflora on pre and post emergence mortality of chickpea. Seed treatment of Carboxin + Thiram @ 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 chickpea. Also, seed treatment of Trichoderma based copper nanoparticles at higher concentration i.e. 100, 150, 200, 250 ppm showed adverse effect on seed as it hampered the germination, root length, shoot length and seedling vigour of chickpea. Thus, seedborne mycoflora associated with chickpea can be controlled using *T. asperellum* mediated copper nanoparticles as seed treatment at lower concentrations i.e. 10 and 50 ppm, respectively.

Keywords:- *T. asperellum*, copper nanoparticles, Carboxin + Thiram, chickpea.

Introduction

Pulses are vital to Indian agriculture because of their high nutritional content in addition to their economic importance. The most significant pulse among all is the chickpea (*Cicer arietinum* L.), which is high in protein (21%), carbs (61.57%), and vitamins (Amule *et al*., 2019). The chickpea, or *Cicer aretinum*, is a member of the Papilionaceous subfamily in the Leguminosae family. According to Vavilov (1921), the Mediterranean region and the Fertile Crescent which is now southeastern Turkey and Syria are the principal sites of origin for chickpeas, while South Asia and Ethiopia are the subsidiary centres.

The research on nanotechnology has been established since the end of the century. Since Nobel laureate Richard P. Feynman introduced the concept of "nanotechnology" in his well-known 1959 lecture "There's Plenty of Room at the Bottom", the field has seen several ground-breaking advancements. Materials of all kinds were manufactured at the nanoscale level by nanotechnology. According to Laurent *et al*. (2008), nanoparticles (NPs) are a broad class of materials that include particulate compounds with at least one dimension of less than 100 nm.

Nanotechnology offers fresh perspectives to improve the usability of these solutions in the treatment of plant diseases and has given new answers to issues in food science and agriculture (post-harvest goods). For this reason, nanoparticles made of silicon, gold, silver, copper, and other materials are discovered. The antibacterial, antifungal, and antiviral characteristics of metal nanoparticles, including copper, zinc oxide, titanium dioxide, and silver, have been the subject of extensive research recently. Metal and metal oxide nanoparticles (NPs) are among the nanomaterials that have shown promising results in treating plant diseases (Padmavathi *et al*., 2022). One of the most common elements, copper plays an essential role in normal functioning of all living things. When compared to modern antibiotics, coper nanoparticles exhibit stronger antibacterial qualities. In addition, they have been shown to have antifungal, antiviral, and anticancer effects in addition to their antibacterial one. Copper nanoparticles may prove to be a useful substitute in the fight against bacterial and fungal strains that are resistant to multiple antibiotics, even though the exact mechanism of action is unknown (Crisan *et al*., 2021).

Nowadays, the multidisciplinary field of nanotechnology works with various chemical compositions and sizes to synthesize green nanoparticles. Comparing metal and metal oxide nanoparticles to their bulk counterparts, they have been found to show more promising antifungal and antibacterial agents due to their small size (Singh *et al*., 2016). According to earlier research, biological synthesis increases the antimicrobial potential of nanoparticles by secreting antimicrobial metabolites. This leads to an overall 10–20-fold increase in efficacy of biologically synthesized nanoparticles over the chemically synthesized nanoparticles (Bawskar *et al*., 2015).

Numerous fungal species have been identified from chickpea, including *Alternaria alternata*, *Aspergillus amstelodami*, *A. flavus*, *A. fumigatus*, *A. nidulans*, *A. niger*, *A. sydowi*, *A. wentii*, *Botrytis cinerea*, *Cladosporium macrocarpum*, *Curvularia lunata*, *Fusarium equiseti*, *F. moniliforme*, *F. oxysporum*, *F. semitectum*, *Macrophomina phaseolina*, *Myrothecium roridum*, *Penicillium notatum,* Rhizoctonia sp., and *Rhizopus arrhizus*. Chickpea crops can be severely damaged by these fungi, and once they start, they can be challenging to eradicate. Fungicides are needed early in the growing season to prevent early infection and disease spread once the crop is established. The need for additional fungicide applications will depend on the variety's resistance and the frequency of rain during the growing season (Agarwal *et al*., 2011).

Over the past few years, the copper compound has been used as fungicides, pesticides, and fertilizers (Ahamed *et al*., 2014). Several studies examined the toxic effects of CuONPs on different crops, while their reports suggested that CuONPs were not toxic up to certain concentrations and efficiently suppressed the disease (Shaheen *et al*., 2021). Hence the present study has been undertaken to investigate the effect of biosynthesized copper nanoparticles on pre and post emergence seedling mortality of Chickpea by tackling the associated seed borne mycoflora at different concentrations.

Material and Methods

Collection of Chickpea seeds

The seed samples of Chickpea, JAKI-9218 were collected from Pulse Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra).

Procurement of copper nanoparticles

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

Seed treatment of Tr mediated CuNP's.

The copper nanoparticles prepared from *Trichoderma asperellum*were dissolved in DMSOsolvent in equal volume and a stock solution was prepared.Six different concentrations of Tr. CuNPs viz., 10ppm, 50ppm, 100ppm, 150ppm, 200 ppm and 250ppm were prepared by adding respective suspension of CuNPs in 100 ml of sterilized distilled water.For seed treatment, the required seeds of JAKI-9218 were soaked in respective concentrations of copper nanoparticles for 2-3 minute and then were used for seed treatment.

Evaluation of Tr. CuNPs

Rolled paper towel method were used to assess the effect of Trichoderma based copper nanoparticles on seed germination, root length, shoot length and seedling vigour of chickpea and pre & post emergence mortality of chickpea 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* dissolved in $DMSO_4$ and six different concentrations of Tr. 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 JAKI-9218 were soaked in respective concentrations of copper nanoparticles for 2-3 minute and then used.

Seed Germination

Seeds of chickpea were treated with different concentration of Tr. 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°C for 7 days. The count of germination was taken on 7^{th} 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).

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

Pre and post emergence mortality

Pot culture experiment was conducted with three replications for nine treatments. Pot was 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 ten days after sowing. Observation on post emergence mortality was recorded up to 30 days after sowing.

Pre emergence seed rotting (%)

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

Post emergence seedling mortality %

$$(\text{PESM}) = \frac{\text{Number of seedlings died}}{\text{Total number of seedlings}} \times 100$$

Results and Discussion

Collection of Chickpea seeds

The seed samples of Chickpea, JAKI-9218 were collected from Pulse Research Unit, Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (Maharashtra).

Procurement of copper nanoparticles

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

Detection and identification of seed borne mycoflora of chickpea seeds.

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

Table 1 : Comparative evaluation of different detection methods of seed mycoflora of Chickpea

Seed borne mycoflora	Frequency of occurrence of seed borne mycoflora (%) of Chickpea		
	Standard blotter paper method	Agar plate method	Rolled paper towel method
Alternaria alternata	2.25	1.6	-
Aspergillus flavus	20.5	22.2	9.75
Curvularia lunata	1.00	-	-
Aspergillus niger	30.22	26	10.25
Fusarium oxysporum	17.7	17.5	8
Rhizoctonia spp.	-	1.5	-
Rhizopus spp.	19.5	16.5	8.25
Penicillium spp.	-	3.22	2
Total frequency of mycoflora	91.17	88.52	38.25

Effect of Trichoderma based copper nanoparticles on germination of Chickpea.

Data presented in Table 2, Fig 1 and Plate 1 revealed the effect of different concentrations of Tr. CuNPs i.e. 10 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm on seeds germination of Chickpea. Tr. CuNPs enhance the seed germination at lower concentrations, whereas high concentration results adverse effect. Among all treatments T_2 (10 ppm) was found significantly superior and recorded highest seed germination (87.33%). Seed treated with Carboxin + Thiram @ 3 g/kg (T_8) recorded 86.00% seed germination which found at par with T_2.

Table 2 : Effect of Trichoderma based copper nanoparticles on seed germination of Chickpea.

Tr. No.	Seed Treatments	Germination (%)	Abnormal seedling (%)	Rotten seed (%)
T_1	*Trichoderma asperellum* @ 4g/kg	85.33 (67.50)*	8.33 (2.87)**	5.66 (2.36)**
T_2	Tr. CuNPs @ 10 ppm	87.33 (69.17) *	6.66 (2.58)**	5.66 (2.37)**
T_3	Tr. CuNPs @ 50 ppm	83.33 (65.92) *	9 (2.99)**	7.66 (2.76)**
T_4	Tr. CuNPs @ 100 ppm	82.33 (65.15) *	10.00 (3.16)**	7.66 (2.76)**
T_5	Tr. CuNPs @ 150 ppm	80.33 (63.67) *	12.33 (3.50)**	7.33 (2.70)**
T_6	Tr. CuNPs @ 200 ppm	74.66 (59.78) *	16 (3.99)**	9.33 (3.05)**
T_7	Tr. CuNPs @ 250 ppm	67.33 (55.14) *	17.66 (4.20)**	15.00 (3.87)**
T_8	Carboxin + Thiram @ 3 g/kg	86.00 (68.03)*	7.66 (2.76)**	07.009 (2.64)**
T_9	Control	84.66 (66.95) *	9.33 (3.05)**	09.00 (2.44)**
	SE (m) ±	**0.8089**	**0.7201**	**0.5774**
	CD (0.01%)	**2.443**	**0.427**	**0.449**

The figures in parenthesis* indicate arc sin transformed values and the figures in parenthesis** indicate square root transformed values.

Whereas seed treatment with Tr. CuNPs @ 200 and 250 ppm significantly declined seed germination i.e. 74.66% and 67.33% respectively than control (84.66%).

The abnormality of seedling was increased with increased in concentration of Tr. CuNPs. Least abnormal seedlings i.e. 6.66% was also observed in T_2 (10 ppm) followed by T_8 (Carboxin + Thiram 3 g/kg) i.e. 7.66% whereas, maximum abnormal seedling was observed in T_7 and T_6 at highest concentrations @ 250 ppm and 200 ppm i.e. 17.66% and 16% respectively.

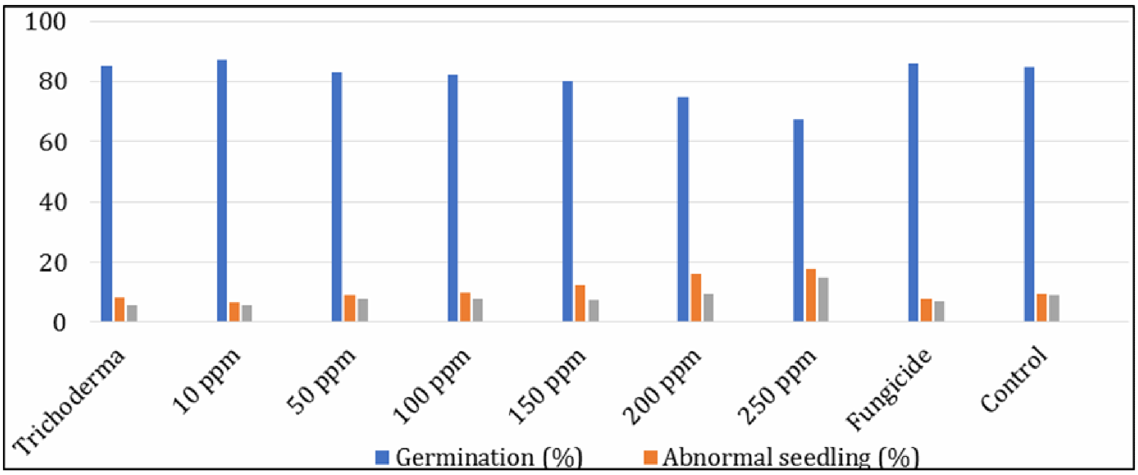

Fig. 1 : Effect of Trichoderma based CuNPs on germination of Chickpea.

10 ppm

250 ppm

Plate 1 : Effect of Trichoderma based CuNPs on germination of Chickpea

Also, the minimum rotten seeds (5.66%) were found in T_2 i.e. seed treatment of Tr. CuNPs @ 10 ppm whereas, seed treated with Tr. CuNPs at higher concentration showed rotting of seed.

The results obtained were agreed with the findings of Kasana *et al.* (2017), who evaluated that the copper nanoparticles enhance the germination and growth of some plants at lower concentrations, whereas high concentrations result in retarded growth. Parallel result was obtained by Kausar et.al. (2022), who found a condition dependent effect of CuNPs when NPs applied to wheat. Some varieties of wheat showed maximum germination and growth rate at 50 mg CuNPs/ L, while other variety showed that 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.Similar findings were recorded by Hafeez *et al.* (2015) and Shende *et al.* (2017), who observed the beneficial effects of CuNPs at certain concentrations.

Effect of Trichoderma based copper nanoparticles onroot/shoot length and seedling vigour index of Chickpea.

Different concentrations of Tr. CuNPs i.e., 10 ppm, 50 ppm, 100 ppm, 150 ppm, 200 ppm and 250 ppm were evaluated to know their effects on root length, shoot length and seedling vigour index of Chickpea using the rolled paper towel method and the data are presented in Table 3, Fig 2,3 and shown in Plate 2. Data analysis revealed that, as compared to the control, lower concentrations of CuNPs had a significant impact on root length, shoot length, and seedling vigour index of Chickpea. However, higher concentrations of CuNPs, had a negative impact on seedling vigour index, root length and shoot length.

Highest seedling vigour index recorded with seed treatment of Tr. CuNPs @ 10 ppm (T_2) i.e. 1424.18 fallowed by Carboxin + Thiram @ 3 g/kg (T_8) i.e. 1361.14 and Tr CuNPs @ 50 ppm i.e. 1339.53 which was found at par with each other. Also, in case of seed treatment with *T. asperellum* @ 4 g/kg it was found non-significant (1313.33). Whereas, at higher concentrations of Tr. CuNPs i.e., 200 ppm and 250 ppm recorded (877.58) and (604.82) seedling vigour index than control (1212.32).

Table 3: Effect of Trichoderma based CuNPs on root /shoot length and seedling vigour index of Chickpea.

Tr. No.	Seed Treatments	Germination (%)	Root length(cm)	Shoot length(cm)	Seedling vigour index
T_1	*Trichoderma asperellum* @ 4g/kg	84.66 (66.95)*	7.31 (2.70)**	8.19 (2.86)**	1313.33
T_2	Tr. CuNPs @ 10 ppm	87.33 (69.17)*	7.62 (2.76)**	8.68 (2.94)**	1424.18
T_3	Tr. CuNPs @ 50 ppm	85.33 (67.50)*	7.38 (2.71)**	8.30 (2.88)**	1339.53
T_4	Tr. CuNPs @ 100 ppm	83.33 (65.920)*	7.15 (2.67)**	7.47 (2.73)**	1219.30
T_5	Tr CuNPs @ 150 ppm	80.33 (63.67)*	6.15 (2.48)**	6.86 (2.61)**	1045.27
T_6	Tr. CuNPs @ 200 ppm	74.66 (59.78)*	5.45 (2.33)**	6.30 (2.51)**	877.58
T_7	Tr. CuNPs @ 250 ppm	67.33 (55.14)*	3.86 (1.92)**	5.13 (2.26)**	604.82
T_8	Carboxin + Thiram @ 3g/kg	86.00 (68.03)*	7.47 (2.73)**	8.36 (2.89)**	1361.14
T_9	Control	82.33 (65.15)*	7.15 (2.67)**	7.56 (2.75)**	1212.32
	SE (m) ±	**0.8089**	**0.0186**	**0.1506**	**21.46**
	CD (0.01%)	**2.443**	**0.161**	**0.122**	**87.36**

The figures in parenthesis* indicate arc sin transformed values and the figures in parenthesis** indicate square root transformed values.

Maximum root length (7.62 cm) was recorded in T_2 (10 ppm) which was followed by T_8 (Thiram + Carboxin) i.e. 7.47 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.

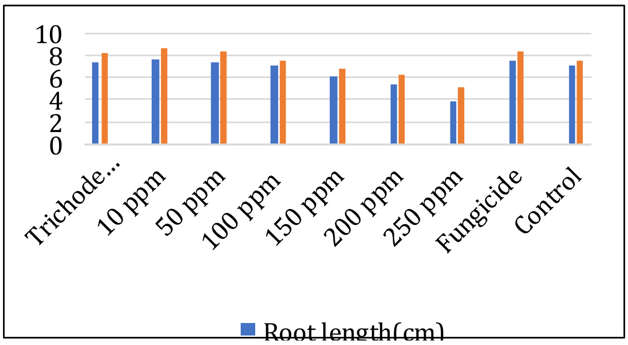

Fig. 2 : Effect of Trichoderma based CuNPs on root/shoot length of Chickpea.

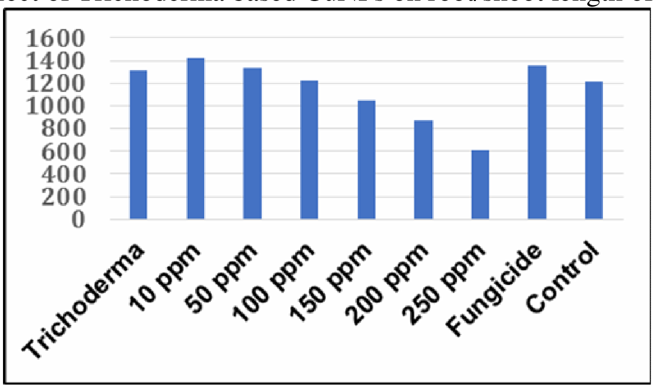

Fig. 3 : Effect of Trichoderma based CuNPs on seedling vigour of Chickpea.

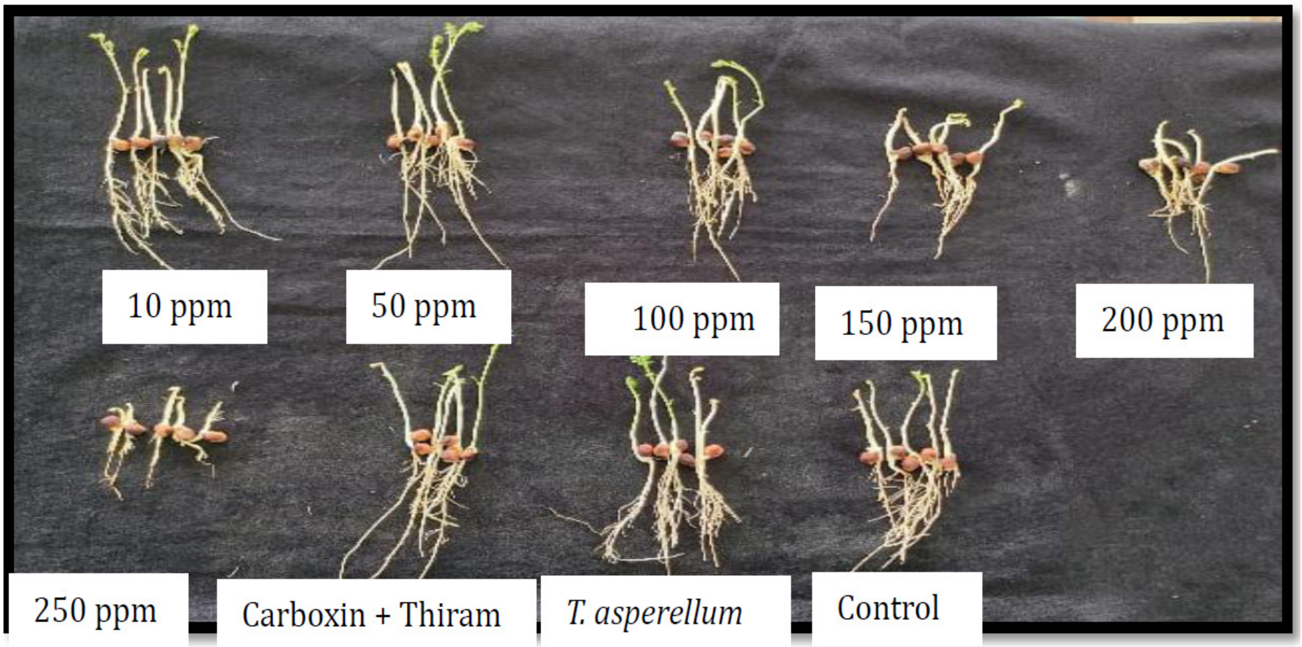

Plate 2 : Effect of Trichoderma based CuNPs on root/shoot length of Chickpea.

Similarly, higher concentration of Tr. CuNPs recorded reduced shoot length of Chickpea seedlings. Seed treatment @ 10 ppm was found superior. Also, significantly higher shoot length was observed in T_8 (8.36 cm) and T_3 (8.30 cm) which found at par with each other followed by T_1 (*T. asperellum* @ 4 g/kg).

Similar findings were given by Dorjee *et al.* (2022), who evaluated biosynthesized CuNPs on maize by seed treatment. A positive effect was observed on maize seedling characters *viz.*, shoot length, root length, number of roots per seedlings, fresh and dry weight. Also, Zakharova *et al.* (2019), analysed the influence of copper oxide nanoparticles (CuO NPs) dispersions (0.01...1 g/L) on seed germination capacity of wheat seedlings. Germination capacity improved by 14.5 per cent and a twofold increase in the root and stem length compared to the control have been recorded. At higher concentrations of NPs was found with toxic effects (decrease of root length). Similarly, Shende *et al.* (2017) and Kaur *et al.* (2018), recorded beneficial effects of nanoparticles in enhancing the seed germination and vigour index of crops.

Effect of Trichoderma based CuNPs on pre and post emergence mortality of Chickpea.

Data pertaining to the effect of different concentrations of copper nanoparticles on pre and post emergence mortality of Chickpea variety JAKI-9218 is presented in Table 4, Fig 4 and shown in Plate 3. Analysis of data indicated that higher concentrations of Tr. CuNPs recorded maximum pre and post emergence mortality of Chickpea in comparison with control. However, lower concentrations of Tr. CuNPs recorded minimum pre and post emergence mortality.

Least pre and post emergence mortality of Chickpea seedling i.e., 13.66% and 0.77% were observed in (T_2) Tr. CuNPs @ 10 ppm which found at par with chemical seed treatment Carboxin +Thiram @ 3 g/kg i.e., 17.33 % and 2.81 % pre and post emergence mortality were recorded respectively. However, treatment (T_7) with Tr. CuNPs @ 250 ppm recorded maximum pre and post emergence mortality (37.66% and 4.81%) fallowed by (T_6) @ 200 ppm (31.33% and 3.89%)) fallowed by (T_5) @150 ppm (22.66% and 3.02%) whereas, in control 17.33% and 2.81% pre and post emergence mortality is recorded.

Table 4 : Effect of Trichoderma based copper nano particles on pre and post emergence mortality of Chickpea.

Tr. No.	Seed treatments	Pre emergence mortality (%)	Post emergence mortality (%)
T1	*Trichoderma asperellum* @ 4g/kg	16.66 (24.08)*	1.99 (1.56)**
T2	Tr. CuNPs @ 10 ppm	13.66 (21.67)*	0.77 (1.09)**
T3	Tr. CuNPs @ 50 ppm	16.66 (26.48)*	1.58 (1.36)**
T4	Tr. CuNPs @ 100 ppm	17.00 (24.30)*	2.01 (1.54)**
T5	Tr. CuNPs @ 150 ppm	22.66 (28.42)*	3.02 (1.86)**
T6	Tr. CuNPs @ 200 ppm	31.33 (34.03)*	3.89 (2.09)**
T7	Tr. CuNPs @ 250 ppm	37.66 (37.85)*	4.81 (2.30)**
T8	Carboxin + Thiram @ 3g/kg	14.66 (22.50)*	1.16 (1.29)**
T9	Control	17.33 (24.59)*	2.81 (1.81)**
	SE (m) ±	**0.975**	**0.494**
	CD (0.01%)	**3.699**	**0.696**

The figures in parenthesis* indicate arc sin transformed values and the figures in parenthesis** indicate square root transformed values.

Similar results were obtained by Costa *et al.* (2016), who studied the physiological and biochemical behaviour of rice (*Oryza sativa*, var. Jyoti) treated with copper oxide nanoparticles (CuONPs) as they observed that the germination rate, root and shoot length and biomass decreased, while uptake of Cu in the roots and shoots increased at high concentrations of CuONPs. Similarly, 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. Also, similar findings were published by Hafeez *et al.* (2015) and Kausar *et al.* (2022) who evaluated the effect of CuNPs on seed germination.

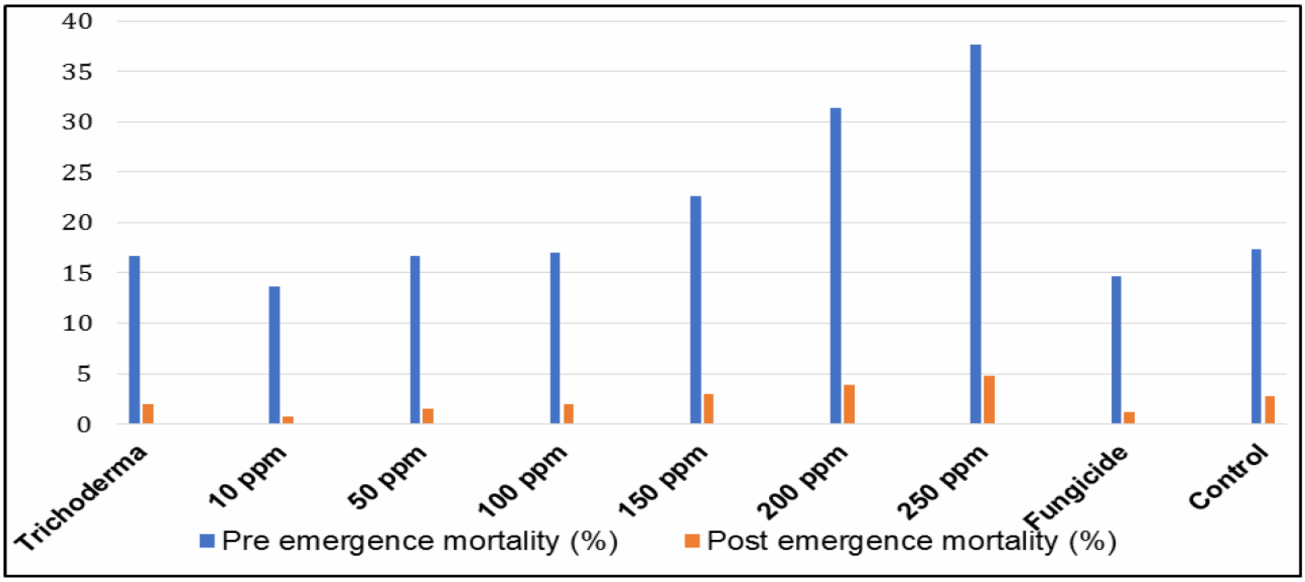

Fig. 4 : Effect of Trichoderma based CuNPs on pre and post emergence mortality of Chickpea

Pots as per treatments

a) Pre emergence mortality

b) Post emergence mortality

Plate 3 : Effect of Tr. CuNPs on pre and post emergence mortality of Chickpea

Conclusion

The above study concluded that seed treatment of Trichoderma based copper nanoparticles @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. Higher concentration of Trichoderma based copper nanoparticles for seed treatment showed harmful 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 chickpea seeds increased. Thus, based on above study results, we can say that copper nanoparticles have an adverse effect on seed germination and seedling growth of chickpea as the concentration of copper nanoparticles increases.

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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