Mechanical synthesis of Nano carrier based bio-formulation of *Trichoderma harzianum*and their bio-efficacy against Fusarium wilt of chick pea

ABSTRACT

The terms "nano science" and "nanotechnology" have grown in popularity in comparison to micron-sized particles because they are more durable, efficient and have a high surface-to-volume ratio. In this regard, an experiment was conducted to assess the efficiency of a nano carrier-based formulation of *Trichodermaharzianum* against the chickpea wilt pathogen *Fusarium oxysporum* f. sp. *ciceris.* Carrier materials such as talc, lignite, and fly ash were reduced to nano size using a high-speed cryo ball mill and particle sizes were determined using a particle size analyzer, scanning electron microscope and x-ray diffraction. The results revealed all of the concentrations of nano carrier formulated (talc, lignite, and fly ash) *T. harzianumviz*.,100, 200, 300, 400 and 500 ppm concentrations were found to effective in inhibiting the growth of the *F. oxysporum* f. sp. *ciceris* with 100 per cent mean mycelial inhibition except nano talc formulated *Trichoderma* which shows mycelial inhibition of 2.56 per cent at 50 ppm concentration.

Keywords: Talc, lignite, fly ash, *T. harzianum*and *Fusarium oxysporum* f. sp. *ciceris.*

INTRODUCTION:

Nanotechnology encompasses the study and application of minuscule entities ranging from 1 to 100 nanometers in size, with widespread utility across diverse scientific disciplines including chemistry, biology, physics, engineering, medicine, agriculture and related fields. The synthesis of nanoparticles represents a vibrant area of exploration within the realm of nanotechnology. Various chemical, physicaland biological techniques have been employed for the production of nanoparticles. The physical methods of nanoparticle synthesis, such as ball milling, laser ablation and physical vapor depositioninvolve the generation of nanoscale particles through mechanical or energy-based processes. For instance, in ball milling materials are ground or mixed in a ball mill to achieve particle size reduction to the nanoscale, leveraging mechanical forces to transform larger particles into nanoparticles with distinct characteristics. The use of ball milling in nanotechnology allows for precise control over particle size, shapeand compositionleading to advancements in nanomaterial synthesis and characterization (Elmer and Jason, 2018).

Ball mill-synthesized carrier materials may provide a suitable matrix for the formulation and delivery of bio-control agents such as *Trichoderma*. The physical properties of the carrier materials, including particle size, surface area and porositycan impact the adhesion, survival and efficacy of *Trichoderma* in combating Fusarium wilt in chickpea.*Trichoderma* as a bio-control agent can inhibit the growth of *Fusarium* through mechanisms such as competition for nutrients, production of antifungal compounds and induction of plant defense responses (Yao *et al*., 2023 and Mukhopadhaya*et al*., 2020).

The formulation of *Trichoderma* with carrier materials like talc, lignite and fly ash synthesized *via* ball milling can enhance the stability and dispersibility of the bio-control agent, potentially improving its bio-effectiveness against Fusarium wilt in chickpea. However, the specific bio-effectiveness of these formulations would depend on factors such as the compatibility of *Trichoderma* with the carrier materials, the concentration and application method of the formulation, environmental conditionsand the susceptibility of the chickpea plants to *Fusarium* infection. Hence the present research is planned to evaluate the efficacy of ball mill-synthesized carrier materials formulated with *Trichoderma* against Fusarium wilt in chickpea under *in-vitro* conditions.

MATERIALS AND METHOD:

Synthesis and characterization of nano carrier materials

Nanoparticles were synthesized mechanically using a ball mill. A ball mill grinds and blends materials for use in mineral dressing processes, paints, pyrotechnics, ceramicsand selective laser sintering (Cao, 2004). It is a top-down method that involves a container packed with hardened steel or tungsten carbide balls. Ball milling was performed to reduce the micron size of the carrier materials *viz*., talc, lignite and ash with ball to powder (BPR) weight ratio of 2:1. The milling was carried out continuously with different time intervals for all the materials at the impact frequency of 30 Hz (1800 rpm).

Synthesized nanoparticles were characterized to know the shape, size and other parameters using UV-Visible spectrophotometer (UV-Vis), Zetasizer, scanning electron microscope (SEM) and X-ray diffractometer (XRD).

Formulation of nano carrier material with *T. harzianum* culture filtrates

Mechanically synthesizednano talc, lignite and fly ash powders were employed to produce bio-formulations with *T. harzianum* culture filtrates. Further, formulations were carried out in accordance with the procedure followed in the Biocontrol Laboratory, Department of Plant Pathology, College of Agriculture Raichur, namely, 2.5 kg of carrier material is compounded with 1 litre of liquid suspension of *T. harzianumi.e.* 1mL of *T. harzianum* liquid suspension was mixed with 2.5 g of each nano particle separately (Plate 1) under aseptic conditions. The wettable powder (WP)-based formulations were appropriately dried under shade at room temperature (25-30°C) and formed into a fine powder. The finished formulations were kept in an airtight glass.

Bio-efficacy of formulated product against *Fusarium oxysporum* f. sp. *ciceris.*

The poisoned food technique was used to assess the efficacy of *T. harzianum*bio-formulations. The desired concentrations of formulations ranging from 50 to 500 ppm were made and combined with appropriate media where, 15 mL of poisoned mediumwas poured into each sterilized Petri plate. A 5 mm mycelia disc was removed from the periphery of a seven-day-old pathogen culture, placed in the centre and incubated at 27±10°C until the fungus reached the control plate's periphery. The control plate did not contain formulated concentration and each treatment was replicated three times. The colony's diameter was measured in two directions and an average was calculated. The percent inhibition of growth was calculated by using the formula given by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

Where,

I = Per cent inhibition (mm)

C = Radial growth of fungus in control (mm)

T = Radial growth of fungus in treatment (mm)

RESULT AND DISCUSSION:

Talc, lignite and fly ash nanoparticles were synthesized through high speed cryo ball mill and characterized by various analytical techniques. Dynamic light scattering (Zetasizer) analysis data showed that the average particle diameter of talc, lignite and fly ash nanoparticles was found to be 73.46 nm, 41.55 nm and 53.92 nm, respectively, which were used for the further characterization and applications (Fig 1, 2and 3). The UV-Visible spectrum of talc, lignite and fly ash nanoparticles recorded the maximum absorption sharp band edge at 311.00 nm, 359.00 nm and 353.50 nm, respectively. XRD pattern confirmed the amorphous nature of synthesized talc, lignite and fly ash nanoparticles. SEM analysis data showed that talc nanoparticles were spherical with uniform shape distribution whereas the lignite and fly ash nanoparticles were oblong and irregular in shape with the rough surface morphology (Plate 2, 3 and 4).

The data pertaining to the inhibition of pathogen on different nano carrier material and concentration of formulated *T. harzianum* are presented in the Table 1. The observations were recorded 7 DAI. The mycelial inhibition of *F. oxysporum*f. sp. *ciceris*with respect to nano carrier material was observed in the study. The maximum inhibition was recorded in all the three carrier materials with complete inhibition of 100 per cent.Among the different concentrations tested, maximum mycelial inhibition was noticed in all the concentrations from 50 ppm to 500 ppm which were significantly superior over the other treatments except with the treatment of talc @ 50 ppm concentration which recorded the inhibition of 2.56 per cent. Complete mycelial growth with no inhibition was observed in control.

The interaction between the concentrations and the carrier material varied with the inhibition percentage. Complete inhibition was noticed among all the carrier materials with the concentrations ranging from 100 ppm to 500 ppm. Whereas, at 50 ppm concentration both lignite and fly ash showed 100 per cent inhibition except the talc with the inhibition percentage of 2.56 (Table 1, Plate 5 and Fig. 4).

The synthesis of talc nanoparticles were in accordance with the findings of Kim *et al*. (2018) who synthesized nano-sized talc powder using high energy ball mill and also indicated that changes in the particle size with increasing milling time up to 720 min Similarly, the size of talc was reduced to sub micrometer by sonication method by Perez *et al*. (2005). The results obtained from*in-vitro* study were in partially agreement with reports made by Magar *et al*. (2020) that AgNPs synthesized from culture filtrates of different *Trichoderma* spp. at 100 and 150 ppm were found to be effective in inhibiting the mycelial growth of *F.oxysporum* f. sp.*ciceris* with high mycelial inhibition of 31.48 per cent.

CONCLUSION:

Physically synthesized carrier nanoparticles play a crucial role in enhancing the delivery and effectiveness of *Trichoderma*-based bioformulations in agriculture. These nanoparticles improve the stability, solubility and bioavailability of *Trichoderma*, while their controlled size and shape enable targeted delivery to specific plant tissues, enhancing bio-control activity Overall, the integration of physically synthesized nanoparticles in bioformulations with *Trichoderma* shows great potential for enhancing agricultural practices.

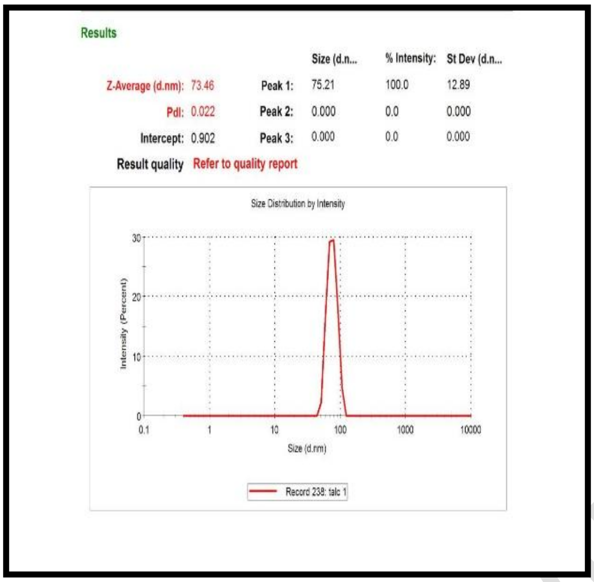


Fig.1. Average particle diameter of talc nanoparticles

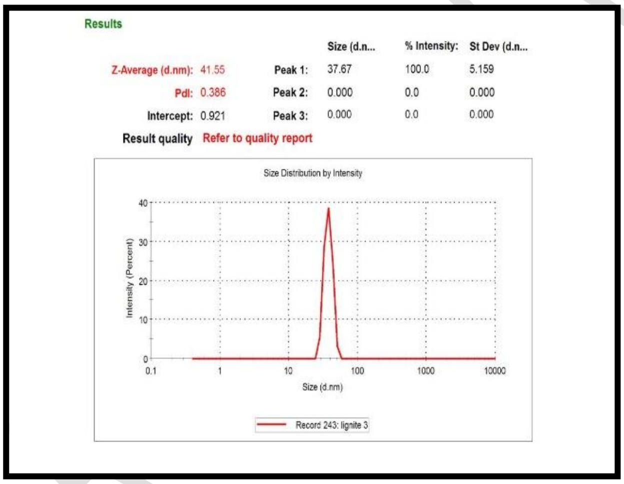


Fig.2. Average particle diameter lignite nanoparticles

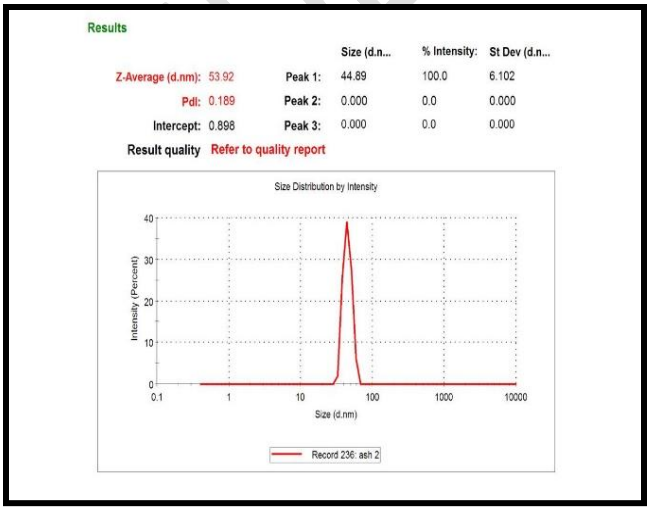


Fig.3. Average particle diameter fly ash nanoparticles

Table1. Efficacy of nano carrier (talc, lignite and fly ash) formulated *Trichodermaharzianum* on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*s

Sl. No.	Treatment	Mean mycelial inhibition (%)			
		Carrier material			
		Talc	Lignite	Fly ash	Mean
1	T_1- Control	0.00 (0.00)[a]	0.01 (0.39)[a]	0.51 (2.37)[b]	0.00* (0.00)**
2	T_2- Nanoparticles	1.34 (6.61)[c]	4.20 (11.78)[e]	1.11 (6.05)[c]	6.65 (8.15)
3	T3 - Nano formulated *T. harzianum*@ 50 ppm	2.56 (9.21)[d]	100.00 (90.00)[f]	100.00 (90.00)[f]	67.52 (63.07)
4	T_4 - Nano formulated *T. harzianum*@ 100 ppm	100.00 (90.00)[f]	100.00 (90.00)[f]	100.00 (90.00)[f]	100.00 (90.00)
5	T_5 - Nano formulated *T. harzianum*@ 200 ppm	100.00 (90.00)[f]	100.00 (90.00)[f]	100.00 (90.00)[f]	100.00 (90.00)
6	T_6 - Nano formulated *T. harzianum*@ 300 ppm	100.00 (90.00)[f]	100.00 (90.00)[f]	100.00 (90.00)[f]	100.00 (90.00)
7	T_7 - Nano formulated *T. harzianum*@ 400 ppm	100.00 (90.00)[f]	100.00 (90.00)[f]	100.00 (90.00)[f]	100.00 (90.00)
8	T_8 - Nano formulated *T. harzianum*@ 500 ppm	100.00 (90.00)[f]	100.00 (90.00)[f]	100.00 (90.00)[f]	100.00 (90.00)
TREATMENT CONCENTRATION (A)		**S. Em(±)**		**C. D. (1%)**	
		0.088		**0.250**	
CARRIER (B)		**0.054**		**0.153**	
CONCENTRATION × CARRIER (A × B)		**0.152**		**0.432**	

*Mean of three replications

**Values in the parentheses are arc sine transformed

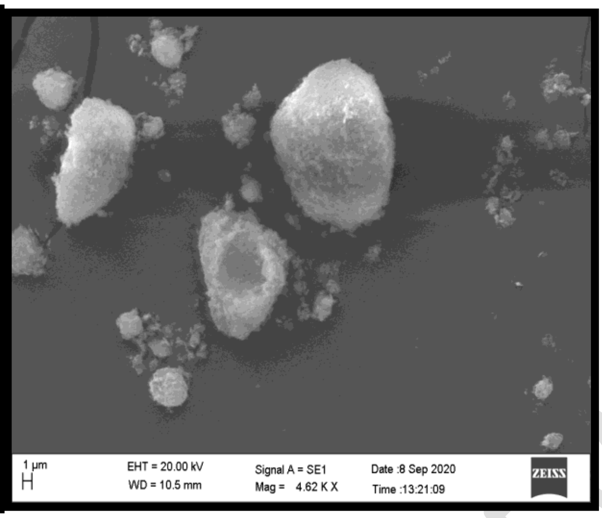
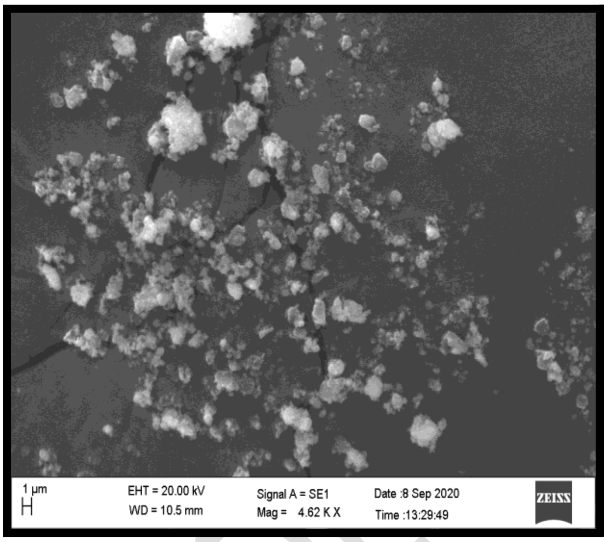


Plate 1: Formulation of *T. harzianum*Plate 2. SEM Image of lignite nanoparticles



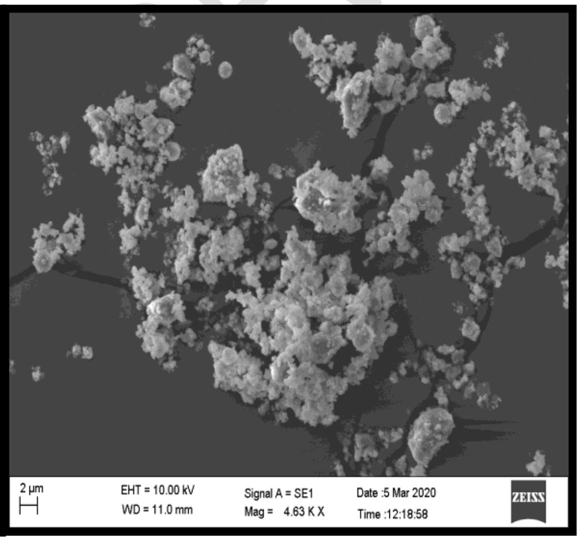


Plate 3. SEM Image of lignitenanoparticles Plate 4. SEM Image of fly ash nanoparticles

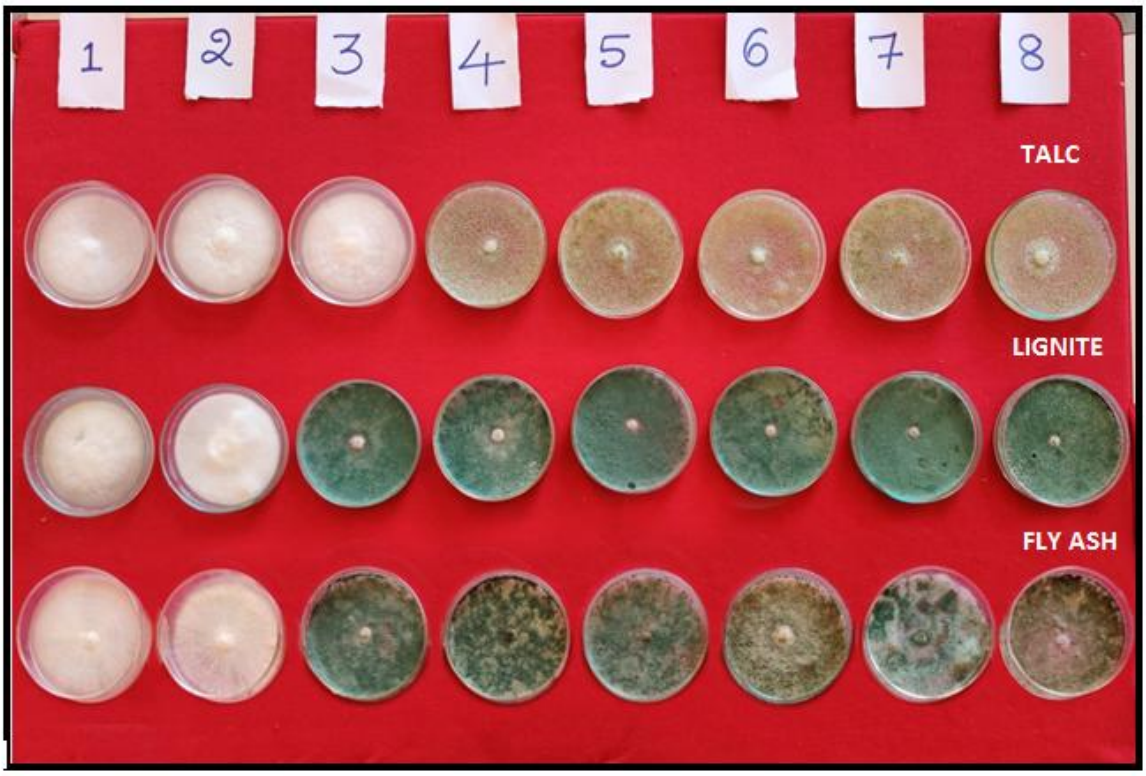


Plate 5. Efficacy of nano carrier (talc, lignite and ash) formulated *Trichodermaharzianum* on mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*s

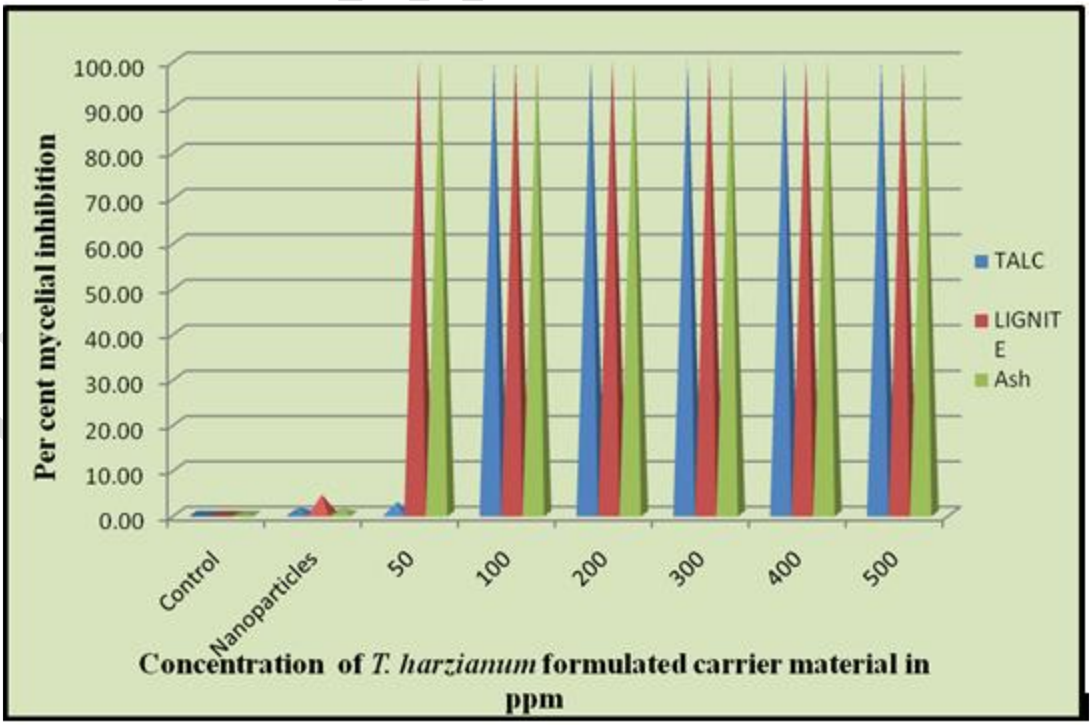


Fig.4.Efficacy of nano carrier (talc, lignite and fly ash) formulated *Trichodermaharzianum* on mycelial growth of *Fusariumoxysporum* f. sp. *ciceri*s

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