Synthesis, Characterization and Bioassay of Nanocarbendazim – An Ecofriendly Benzimidazole Fungicide

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The environmental effects of pesticides illustrates more number of upshots of using pesticides. The impact of modern agriculture on the environment is due to over use of pesticides and its negative impact. Over sixty percent of agricultural land is at its risk of pesticide pollution. The aim of this study is to synthesize Nanocarbendazim, a Benzimidazole fungicide used for control of soil borne diseases. Polymeric Nanoformulation of a Carbendazim by using polycapralactone as capping agent. The encapsulated Benzimidazole complex was characterized by using analytical techniques like UV-Visible spectroscopy, Dynamic light scattering and Transmission electron microscope . The particle size distribution was materialized at 60-75nm. The bioassay was conducted against Aspergillus niger. The bioassay exemplified improved results as compared to the commercial pesticide

Keywords: Aspergillus niger; Carbendazim; Encapsulation; Polycaprolactone.

Pesticides are substances or mixtures of substances that are mostly used in agriculture and public health protection programs to protect plants from diseases, pests, and weeds. Many of the pesticides have been associated with health and environmental issues that can significantly reduce crop yield and quality[1] . As fungal diseases are a major threat to crop production[2] (Fisher et al., 2012), The unfailing exploit of fungicides can potentially comprise a peril to the ecosystem mainly if residues overcome in the production as well as in the soil[3] . Carbendazim(CBM) is an odorless fungicide, white crystalline solid, exist as an aqueous suspension, aqueous dispersion, flowable water-dispersible granule and a wettable powder. It is a systemic fungicide belonging to the Benzimidazole family. The application of CBM fungicide in agriculture gave good results due to its high efficiency and stable chemical properties[4].

Consequently, CBM slowly degenerates into the environment and grounds the presence of pesticides in vegetables, fruits, juices, and affects consumer safety[5] . A range of toxicology studies on the dose of CBM may grounds uncharacteristic pathological occurrence in the body, especially in the immune system[6-7]. Screening CBM pesticide presence in agricultural products is an important issue at the moment and it is prohibited in the United States[8] and European Union (EFSA 2021).

Researchers have made substantial pains to ascertain different methods for successful CBM determination. The conventional methods

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applied sofar include high-performance liquid chromatography (HPLC), liquid chromatography-tandem mass spectrometry (LC-MS) and gas chromatography-tandem mass spectrometry (GC–MS)[9-12]. The disadvantages are like highly sensitive, most effective, so expensive equipment, the need for professional personnel and complicated pretreatment steps etc[13]. At present, there have been a lot of efforts to extend alternative methods one of such alternatives is the carrier systems for pesticides that can amend the discharge profiles and enhance the efficacy of the formulations for the efficient control of agricultural pests[14-15]. These encapsulation processes have wide applications in foods, paints, cosmetics, textiles, paper, etc. Few methods reported on polymer nanocomposites due to their properties[16-19]. The polycaprolactone can be used to synthesize the nanocapsules possessing an oily interior that is capable of efficiently encapsulating hydrophobic compounds of carbendazim[20-21]. Polymeric nanoparticles application in agriculture was advantageous due it low toxicity, slow release etc[22-28].

The aim of the present study was to prepare and characterize the polymeric nanocapsule, polycaprolactone that was used as a carrier for CBM. The CBM nanoparticle was confirmed based on particle size, morphology and surface topology by applying analytical techniques. The effectiveness of nano-CBM and release profiles of the fungicides were determined in vitro on *Aspergillus niger* and the results showed that encapsulated nanocarbendazim in contrast with bulk pesticide has an extraordinary function. Hence study offers the possibility of plummeting unfavorable effects in ecosystems and shrinking the risks to human health.

MATERIALS AND METHODS

Analytical grade reagents and chemicals were used in this study. Double distilled water was used throughout this research process. CBM was openhandedly gifted by Raghavendra Agro. Ltd, India. The polycaprolactone was procured from E. Merck, India.

Preparation of Nano-CBM

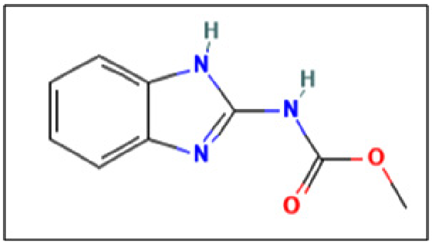
CBM was fully grounded in a mortar. 200 mL of Acetone solution of pesticide sample (one gram of grounded pesticide was dissolved in 200 mL of Acetone) and 200 mL of polycaprolactone water solution (6:4) were mixed in an ultrasonic bath for 40 minutes for the dispersion of CBM particles in polycaprolactone. Continuous stirring of solution was done for 6 hours at 1200 rpm, and then the excess solvent was removed by using Rota-evaporator.

Instrumentation:

Name of the Instrument	Make
Dynamic light scattering (DLS)	Horiba, nanopartica, Japan, s2100.
Transmission electron microscopy	Jeol JEM 2100, Japan
UV-Visible spectrophotometer	Shimatzu(UV 2700i)

RESULTS AND DISCUSSION

At present nano plant protection products symbolize the emerging agritech development in the world. Nanotechnology show a novel solution and solve pest problems. The development of Nanopesticides and their bioassay was increasingly



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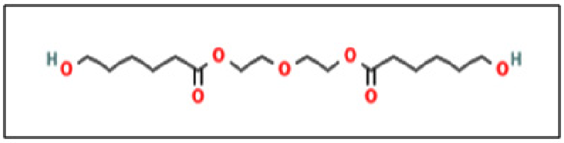

B

Fig. 1. Structures of A) Carbendazim B) Polycapralactone

popular and possess advantages like improved efficacy and enhanced adhesion to plant foliage. Nanoformulations are expressly premeditated to increase the solubility of insoluble or poorly soluble active ingredients and to release the biocide in a proscribed and embattled manner. The flow of formation of nano-CBM was shown in figure 2.

Table 1. Antifungal activity of CBM

Medium selected	PDA-Potato dextrose agar
Incubation temperature	37^0C
Period	10 days
Control selected	Sterile de-ionized water
fungus selected	*Aspergillus niger*

DLS Analysis

The particle size distribution was measured with Dynamic light scattering (DLS). DLS was based on the scattering intensity based on Rayleigh scattering. One milli litre of nano-encapsulated carbendazim was suspended in 5mL of water. The resultant hydro dispersed suspension was analyzed with DLS at 25- 40ºC. The particle size distribution was appeared around 60–75 nm (Shown in figure 3).

TEM Analysis

Field-emission gun was used to produce an electron beam which was made up of heating filament.TEM instrument obeys Gaussian law. Transmission Electron Microscopy(TEM) dealt with internal structural elucidation of agglomerated nano particles. High resolution and diffraction

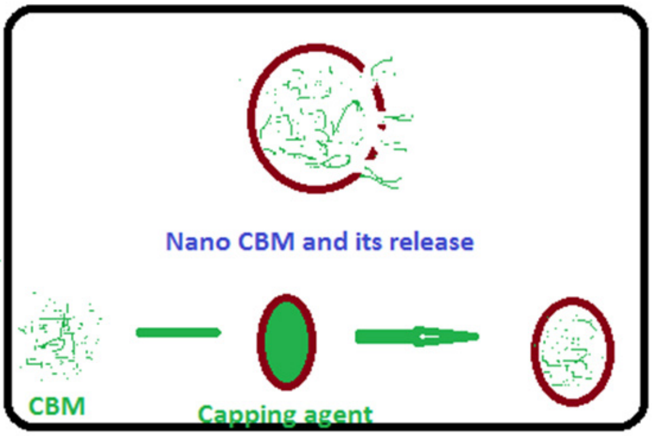

Fig. 2. Formation of nano-CBM

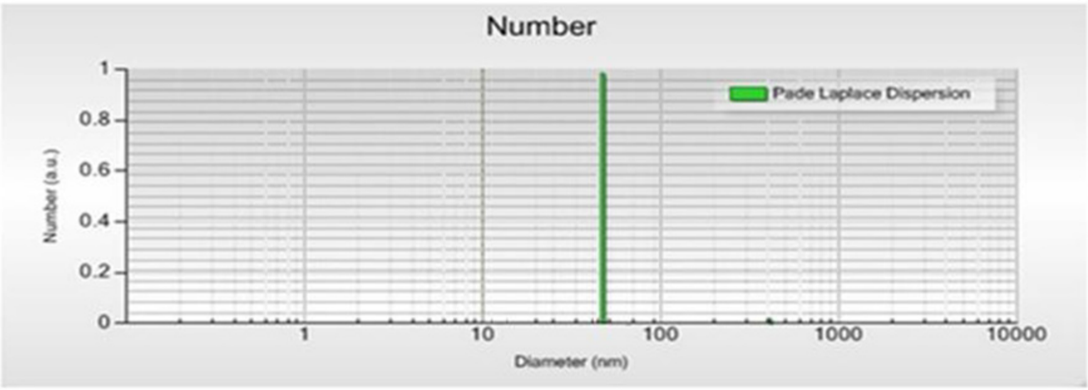

Fig. 3. Particle size distribution of nano-CBM

imaging are the advantages of TEM. The Nano-CBM pesticide morphology was observed by TEM. Deposition of nano encapsulated CBM on a carbon coated copper grid was done in the solution and leave the grid to evaporate the solvent for hours before analysis. Disperse the sample in a low boiling point non-solvent.The presence of spherical to hexagonal shape particles was observed.

The TEM image of Nano-CBM was depicted in figure 4. TEM images demonstrates clusters having number of Nano-CBM particles.

UV-visible Spectral Analysis

The polymer based nanoencapsulation was favourable for getting physical stability.The stability of encapsulated CBMwas ascertained by its maximum absorption. UV spectra value of encapsulated CBM was obtained at 278 nm where as the commercial CBM has maximum absorption at 266 nm.The UV results are shown in figure 5.

Assay for antifungal activity

The antifungal activity of Nano-CBM samples was examined by disc diffusion method[29]. Initially all Samples(5,10.15ppm) diluted with de-ionized water on to the PDA mediums. On to the PDA medium the filter paper discs dipped with different concentrations. The average number of colonies from sample-treated spore suspensions (fungi) was compared with the number on the water control (percent colony formation). The size of the inhibition zone diameter was measured and the effectiveness of CBM and Nano-CBM against Fungi revealed that Nano-CBM showed best results as compared to the commercial CBM(shown in figure 6). The conditions maintained for antifungal assay were given in table 1

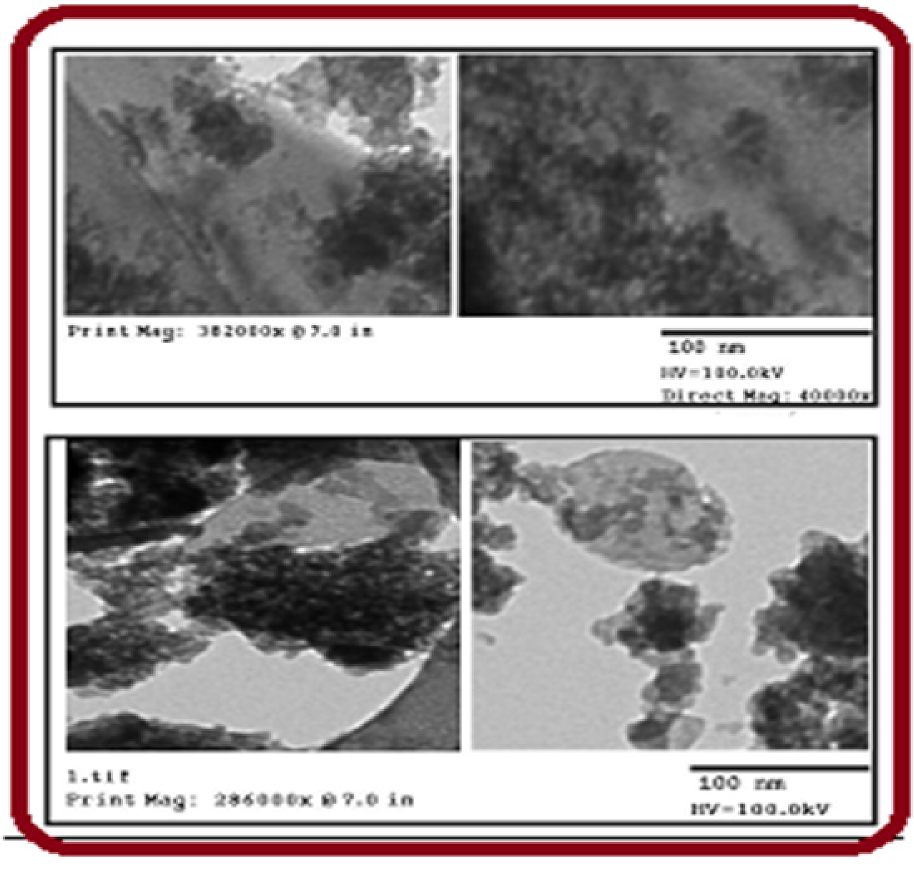

Fig. 4. TEM images of Nano- CBM pesticide

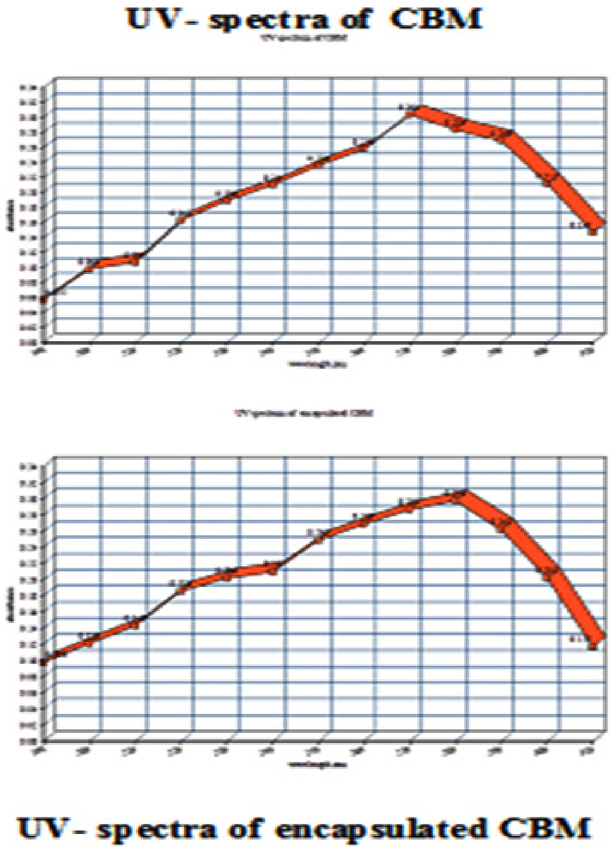

Fig. 5. UV-Spectra of CBM and Encapsulated CBM

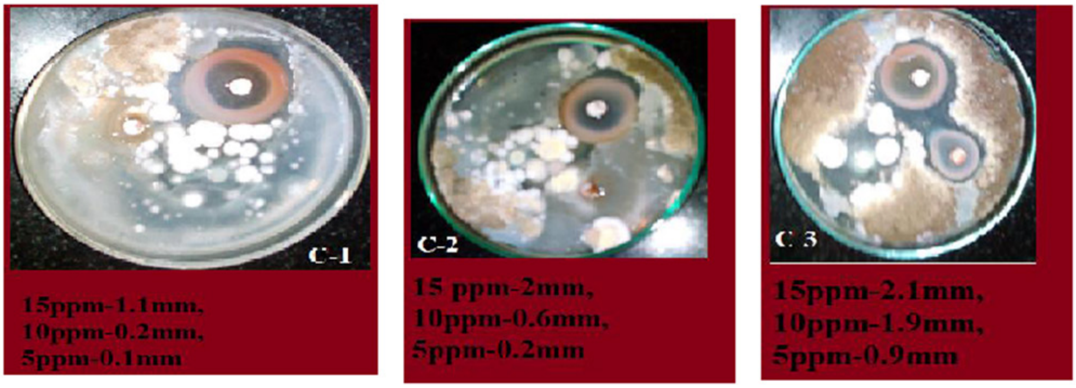

Fig. 6. Bioassay of Nano -CBM against *Aspergillus niger*
*(*C-1:Unformulated CBM, C-2 and 3: formulated CBM)

CONCLUSION

The impact of modern agriculture on the environment is due to over use of pesticides and its negative impact. Over sixty percent of agricultural land is at its risk of pesticide pollution. A better alternative to solve this environmental problem is to introduce nanopesticides.Nano-CBM was formulated using polycapra lactone as encapsulating agent. The DLS, TEM, and UV characterization studies supports the encapsulation and stability of Nano-CBM. The precious recommended procedures are lined way to auxiliary development and practical application of polymeric Nano formulation of pesticides with mammoth potential.

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Conflict of Interest

The authors declare that there is no conflict of interest involved in the manuscript.

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