OLYMERIC NANOFORMULATION OF A NON-SYSTEMIC DITHIOCARBAMATE PESTICIDE: PREPARATION, CHARACTERIZATION, AND BIOASSAY

N.V. S. Venugopal1, B. Padmavathi1 and D.Swetha2
1Department of Chemistry, GITAM School of Science, GITAM (Deemed to be University)
Andhra Pradesh, India.
2Assistant Professor, VNRVJIET, Bachupally, Hyderabad, Telangana state, India.
 Corresponding Author: vnutulap@gitam.edu

ABSTRACT
The current agricultural practice is to develop novel Nano agrochemicals in the form of Nano pesticides. Many researchers in the world have foretold an upheaval in present agricultural practice. This paper aims at the application of nanotechnology for plant protection in the form of Nano pesticides. Our study endowed with a novel polymeric Nano formulation of a dithiocarbamate nonsystemic agricultural fungicide called Mannose (MCB) by using polycaprolactone as a capping agent. The encapsulated dithiocarbamate complex was characterized by using analytical techniques like UV-Visible spectroscopy, Dynamic light scattering, Scanning electron microscope, and Transmission electron microscope. The particle size distribution emerged at 17-44nm. The bioassay was conducted against Aspergillus flavus, Aspergillus niger, Sclerotium rolfsii, and Rhizoctonia solani. The bioassay illustrated better results as compared to the commercial pesticide.

Keywords: Dithio-Carbamatepesticide, Polycapralactone, Bioassay, Aspergillus flavus, Aspergillus niger, Sclerotium rolfsii, Rhizoctonia solani.

INTRODUCTION
The usage of high concentrations of different types of pesticides at present leads to several environmental problems. Pesticides are obligatory for an increase in receiving a high yield of agricultural products. The excessive usage of agrochemicals contaminates water and soil resources. Over the past decade, nanotechnology application research in the field of agriculture has become very popular, especially the development of nano pesticides. Mancozeb (MCB) is a thiocarbamate nonsystemic agricultural fungicide with a protective action on contact. It is a trusted protectant fungicide and controls different diseases like rust petal blight, and mildew on apples, flowers, vegetables, and turf. MCB was successful towards plant pathogens. MCB application to water tends to sediment and remain as a solid material and high adsorption was observed towards the soil. Nanotechnology certainly leads to a revolution in having the best agricultural practices and reducing human and environmental problems.1 Nanopesticides received less and delayed attention as compared to other areas of the food chain and processing.2 The development of nano pesticides has been remarkable and showed better results for crop production.3-4 MCB damages the DNA and is capable of crossing the placental barrier.5 In rats it was evident that LD 50 of more than 5000 ppm to 11200 ppm and MCB is nontoxic through the oral route.6-7

Fig-1: Structure of MCB

The encapsulation process has broad purposes, especially in paints, foods, cosmetics, etc. and it protects the core material from adverse conditions. Many researchers developed and characterized nano pesticides and studied their performance against bacteria and fungi.8-17 In the present study we have reported a rapid
and eco-friendly nano pesticide suitable for non-systemic agricultural crops. Nano formulation of a dithiocarbamate nonsystemic agricultural fungicide called Mancozeb (MCB) was prepared by using polycaprolactone as a capping agent. Bioassay was conducted against *Aspergillus flavus*, *Aspergillus niger*, *Sclerotium rolfsii*, and *Rhizoctonia solani*.

**EXPERIMENTAL**

Polycaprolactone was procured from E. Merck, India. MCB was munificently gifted by Raghavendra Agro Ltd, India. Throughout the experimental work double distilled water was used. All other chemicals used in the investigation were of Analar grade.

**Instrumentation**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Name</th>
<th>Make</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Dynamic light scattering</td>
<td>Vasco, Cordouan</td>
</tr>
<tr>
<td>2.</td>
<td>Scanning Electron microscopy</td>
<td>Zeiss EVO 18</td>
</tr>
<tr>
<td>3.</td>
<td>UV-Vis spectrophotometer</td>
<td>Shimadzu UV 1650</td>
</tr>
</tbody>
</table>

**Grounding of Nano-MCB**

MCB was fully grounded in a mortar and pestle. 100ml of pesticide sample containing one gram of grounded pesticide and 200mL of polycaprolactone were clearly mixed in an Ultra sonicate bath for one hour. Complete dispersion of MCB in polycaprolactone was done with constant stirring for six hours at 1200 rpm. After continuous stirring the formed Nano pesticide solution was kept overnight for complete formation of Nano encapsulation.

**RESULTS AND DISCUSSION**

Nanoparticles have many advantages like better and more effective penetration, very less quantity of active components, quick dissolution, etc. Nanotechnology has a crucial role in agriculture. Nano-MCB reduces the toxicity even at very low concentrations in different agricultural fields. The encapsulating agent polycaprolactone certainly enhances the stability and releases slowly to hit the target.

**DLS Analysis**

Pade lap-lace dispersion is the key factor for the determination of the diameter of the nano-MCB. A laser beam passed through a dispersed nano-MCB sample and its angular variation intensity was measured. The scattering (angular) intensity data were analyzed for the size of the Nano-MCB particles.

**Method**

One gram of MCB sample is loaded into the sample holder and analyzed using a laser diffraction particle size analyzer using a dry accessory. Nano-encapsulated MCB (1ml) solution was suspended in 5ml of water and analyzed at 25°C - 40°C. The Nano-MCB particle size distribution was obtained at around 20.42 nm. The size distribution of untreated and nano MCB particles were shown in Fig.-3and 4.

**UV-Visible Study**

This study is needed to establish the stability of formed nano-encapsulated MCB. The maximum absorption of nano-MCB at 265nm and the commercial MCB at 225 nm were shown. The absorption maximum was depicted in Fig.-5.
Fig-3: Untreated MCB size distribution

Fig-4: Nano-MCB Size Distribution

Fig-5: Absorption maxima of MCB and Nano-MCB

SEM Analysis
To achieve a high-resolution image of the upper surface of Nano-MCB and their agglomerates SEM detectors are required. The nano-encapsulated MCB sample was anchored on a sample holder and inserted in SEM. Detection limits are on the order of 1-2% by weight due to spectral overlaps. A working voltage of 15KeV was used to analyze. Approximately 80% of the stub area was scanned for particles avoiding edges. Nano-MCB was placed on the stub and air-dried. The nano-MCB sample was subjected to sputtering using a sputter coater. The surface appeared as rough and the homogeneity was shown in shape among different scan regions. SEM image was illustrated in Fig.-6.

Application of nano-MCB on Aspergillus bacillus
The nano-MCB bioassay was conducted in Petri dish by disc diffusion method.\textsuperscript{18}
Plant pathogenic fungi selected: Aspergillus flavus, Aspergillus niger, Sclerotium rolfsii, and Rhizoctonia solani

Medium selected: potato dextrose agar (PDA)

Incubation temperature: 37°C for 10 days

Control selected: Sterile de-ionized water

Procedure
Initially all Samples (5, 10, 15ppm) were diluted with de-ionized water. On the PDA mediums, the filter paper discs dipped in different concentrations were inserted into the PDA medium. The size of the inhibition zone diameter was measured in mm (shown in Table-1). Nano-MCB shows the best results (shown in Fig.-7) when compared with commercial MCB.

Table-1: The Effectiveness of MCB and Nano-MCB against Fungi

<table>
<thead>
<tr>
<th>Test organism</th>
<th>Inhibition zone for MCB and Nano-MCB (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Plate -M(MCB)</td>
</tr>
<tr>
<td>Aspergillus flavus</td>
<td>1.2</td>
</tr>
<tr>
<td>Aspergillus niger</td>
<td>1.4</td>
</tr>
<tr>
<td>Sclerotium rolfsii</td>
<td>1.4</td>
</tr>
<tr>
<td>Rhizoctonia solani</td>
<td>1.4</td>
</tr>
</tbody>
</table>

Aspergillus flavus
CONCLUSION

Nano agro materials have significantly added to key triumphs in various fields. The biodegradable additive polymeric compounds were selected to reduce the size of pesticide molecules. Nano-MCB was prepared by using polycaprolactone as a capping agent. The biological evaluation study reveals that the nano-MCB was shown more antifungal activity. The result suggests that the synthesized nano-MCB pesticide undergoes strong interaction. The results obtained were useful for further development and practical application of polymeric nano pesticides with huge potential. Hence Nano pesticides are better substitute materials for commercial pesticides.

ACKNOWLEDGEMENT

We are deliberately thankful to the department of chemistry, GITAM school of science, and GITAM (Deemed to be the University) for their immense support and huge encouragement.

REFERENCES

5. Y. Shukla, A. Arora, *Journal of Environmental Pathology, Toxicology and Oncology*, 20, 127(2001), https://doi.org/10.1615/JEnvironPatholToxicolOncol.v20.i2.70


[RJC-6949/2021]