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Evaluation of Fabricated Slow Release Nano Encapsulated Herbicide for Bio-Safety Issues

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ABSTRACT

Background: Nanotechnology has the great potential to make ensure the valuable impact on season free weed control, eco environment impact analysis and management in these emerging areas especially pulses.

Methods: A laboratory experiment was carried out at the Department of Nano Science and Technology, TNAU, Coimbatore, Tamil Nadu with the objective of fabrication of slow release nanoencapsulated herbicide and its bio-safety issues.

Result: Our investigation on toxicological research should be aimed to define nanomaterial hazards and levels of exposure along the life cycle of the earthworms and microbes without affecting the environment.

Key words: Encapsulation, Environment impact analysis, Nano herbicide, Nanotechnology.

INTRODUCTION

Nanotechnology has the potential to revolutionize the agricultural with new tools for the weed free condition, molecular treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients *etc*. Smart delivery systems will help the agricultural industry combat all the aspects. In the near future nano structured catalysts will be available which will increase the efficiency of herbicides and pesticides, allowing lower doses to be used (Biswal *et al*., 2012). As shown in the past, science and technology have a significant and necessary role to play in these areas (Spielman and Pandya-Lorch, 2009). Whether by increasing productivity of fields, improving food and water quality, or helping to improve market access, new technologies can enable faster advancements in these directions and provide high returns to investments. Although there are debates on the tradeoff between investing in the development and diffusion of well-known past technologies versus that of new and potentially revolutionary technologies, several examples show that both of these strategies may be beneficial and are sometimes complementary. In some cases, technological advances driven by demand, *viz*., mobile phones can have an enormous impact and even replace previous technologies (such as land-based telephones) before their complete diffusion.

Platforms for bilayer membranes that can be used for protein analysis can be fabricated by layering of sodium silicate and poly (allyl amine hydrochloride) on gold followed by calcinations in a furnace. Lipid bilayers can fuse to the silicate layer and be used to detect specific proteins (Biswall *et al*., 2012).

The capsules were prepared by layer-by-layer (LbL) adsorption on decomposable $CaCO_3$ cores using model polyelectrolytes, namely poly-styrene sulfonic acid (PSS) and poly (allylamine hydrochloride) (PAH). Salt-mediated LbL made microcapsule fusion has been reported recently with a different poly-cation resulting in merging of the capsule's content and formation of anisotropic "Janus-like" capsules indicating no polymer exchange between the capsules (Pechenkin *et al*., 2012). The question for public authorities is how to approach the prioritization of research and development efforts with biosafety analysis without hampering the environment.

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MATERIALS AND METHODS

A laboratory experiment was carried out at the Department of Nano Science and Technology, TNAU, Coimbatore, Tamil Nadu with the objective of fabrication of slow release nanoencapsulated herbicide and its bio-safety issues.

Nanoencapsulation of pendimethalin herbicide

Synthesis of $MnCO_3$ core material

Equal volume of 0.33 M ammonium bi carbonate (NH_4HCO_3) was mixed with equal volume of 0.33 M manganese sulphate monohydrate solution ($MnSO_4$). Equal volume of 0.5 per cent

ethanol solution was added to the above mixture in the round bottom flask. The resulting solution was vigorously stirred for 10 min. It was then left undisturbed for 10 min and incubated for 1 hour in water bath at 75°C.

The solution was centrifuged to settle down the particles completely and the particles were washed thrice with distilled water followed by centrifuging after each wash. Then the particle was isolated by filtering the outcome of final water wash with filter paper Whatman no. 41. The filter paper along with particles settled on it was dried in compact vacuum desiccators. After drying the particles were scrapped and stored in a vial.

Loading pendimethalin in the $MnCO_3$ core template

Took 20 mg of $MnCO_3$ core particles and added 25 ml of 20 ppm pendimethalin and stirred it for 15 min in magnetic stirrer. Then the suspension was allowed to dry for overnight. This enables pendimethalin to adsorb on the $MnCO_3$ core particles surface. The dried particles were collected and stored in vial.

Encapsulation of pendimethalin adsorbed $MnCO_3$ core material

$MnCO_3$ microcapsules are prepared by altering layer by layer (LbL) adsorption of opposite charge polyelectrolyte onto the $MnCO_3$ microsphere templates. Prepared polyelectrolyte solution *viz*., Poly (allylamine hydrochloride), Sodium poly (styrene sulfonate) and Poly vinyl pyrolidone weighing each 20 mg was added to the 20 ml of 0.5 N NaCl solution in separate beakers and dissolved it completely. Then pH was adjusted for the solution to 6.5-7.0 by using hydrochloric acid and sodium hydroxide.

Typically, 20 ml of polyelectrolyte solution was added to 40 mg of dry pendimethalin adsorbed $MnCO_3$ microparticles and the suspension was gently stirred in magnetic stirrer for 15 min. Then the suspension was centrifuged at 1000 rpm for 15 min. Later the centrifuged particles rinsed three times with 0.1 N NaCl to remove the unbounded particles. The same procedure was repeated with oppositely charged polyelectrolyte. Then alternate layer was formed using PAH, PSS and PVP. Then the suspension was centrifuged and allowed to dry for overnight. The particles were collected and stored in a vial. The different combination of encapsulation details are given below,

T_1- Single layer coating - Poly (Allylamine hydrochloride) (PAH).

T_2- Double layer coating - PAH + Sodium poly (Styrene sulfonate) (PSS).

T_3- Three layer coating I - PAH + PSS + Polyvinylpyrrolidone (PVP).

T_4- Three layer coating II - PVP + PSS + PAH.

Scanning electron microscope (SEM)

The SEM is a type of electron microscope that images the sample surface by scanning it with a high-energy beam of electrons in a raster scan pattern. All samples must be of an appropriate size to fit in the specimen chamber and are generally mounted rigidly on a specimen holder called a specimen stub. The SEM (Quanta 250, FEI, Netherlands) can examine any part of a 6-inch (15 cm) semiconductor wafer and some can tilt an object of that size to 45°. For taking images, about 0.5 to 1.0 g of encapsulated herbicide sample was dusted on the carbon conducting tape. Then the stub was mounted on sample stage and the images were taken in 2, 400 to 30, 000 magnification under 15.00 to 30.00 KV.

Energy dispersive X-ray spectroscopy (EDAX)

EDAX is an analytical technique used for the elemental analysis or chemical characterization of a sample. It is one of the variants of X-ray fluorescence spectroscopy, which relies on the investigation of a sample through interactions between electromagnetic radiation and matter, analyzing-rays emitted by the matter in response to being hit with charged particles**.** Its characterization capabilities are due in large part to the fundamental principle that each element has a unique atomic structure allowing X-rays that are characteristic of an element's atomic structure to be identified uniquely from one another. The quantitative analysis of encapsulated herbicide samples was done by FEI QUANTA 250 EDAX. About 1-2 g of encapsulated herbicide sample was dusted on the carbon conducting tape. Then the tap was mounted on sample stage and the images were taken using FEI QUANTA 250 EDAX.

Bio safety evaluation

Studies on the safety of encapsulated pendimethalin on earthworms

The effect of direct and solvent evaporation encapsulated pendimethalin nano herbicide on earthworm *E. eugeniae* was tested by following the artificial soil test method proposed by Edwards and Bohlen (1992). The culture of *E. eugeniae* was obtained from a vermicompost unit at Central Farm of TNAU, Coimbatore.

Garden soil and FYM [mixed in the ratio (2:1)] were taken in the tubular plastic tubs (12×4 cm) and treated with different treatments as given above. Twenty earthworms washed cleanly with water were placed on the top of the substrate. After (every 120 hrs.) 5 days, 50 g of FYM was mixed inside the container and water lost by evaporation was replaced daily. The numbers of live earthworms were counted and the weight of the worms was recorded before release and after experimental period of 30 days. Earthworms were considered dead if they did not respond to a gentle mechanical stimulus.

Studies on the safety of encapsulated pendimethalin on microbial analysis

Population dynamics of different types of microorganisms in soil samples collected from pot culture experiment were studied. Soil samples were serially diluted up to a desired level and unless otherwise mentioned, 100 µ ml suspensions were added to 15-20 ml of the desired medium separately.

The plates were incubated at room temperature generally for 3 to 7 days and the number of colonies was counted. Media used for the estimation of population dynamics of different microbial communities furnished in Table 1.

The data collected from the experiments were analyzed statistically adopting the techniques described by Pansey and Sukhatme (1999). The data were tested at five per cent level of significance.

RESULTS AND DISCUSSION

The nano encapsulated herbicide particles were analysed and tested scanning electron microscope (SEM) and different bio-safety analysis using standard procedure and the results are discussed here under.

SEM and energy dispersive X-ray spectroscopy (EDX) studies

SEM studies were carried out in every step of the sample preparation to study the surface morphology, shape and size of the fabricated particles. Energy dispersive X-ray spectroscopy (EDS or EDX) is an analytical technique used for the elemental analysis or chemical characterization and elemental composition of a sample.

Characterization of $MnCO_3$ core

$MnCO_3$ microparticles were prepared by mixing $MnSO_4$ and NH_4HCO_3 solutions. Ethanol was added to decrease the dielectric constant of the system and the solubility of the inorganic salts and results in the formation of modified porous surfaced $MnCO_3$.

The SEM image shows (Fig 1) the surface topography of the manganese carbonate ($MnCO_3$) particles. The porous rough surface characteristic of $MnCO_3$ core particles aided in physical adsorption targeted herbicides. The results of the XRD (Fig 2) confirm the $MnCO_3$ core. Further the presence of the $MnCO_3$ core was confirmed with characteristic peak obtained in SEM-EDX.

Characterization of nanoencapsulated herbicide

In the direct encapsulation method the target per-emergence herbicide was implanted directly on the $MnCO_3$ core template synthesized already. Thus formed herbicide embedded core were encapsulated with different layers of polymers *viz.*, poly styrene sulfonate (PSS), Poly allyl amine hydrochloride (PAH) and Poly vinyl pyrolidone (PVP). The surface morphology of bilayers (PAH + PSS) nanoencapsulated herbicide formed by Direct method was observed in SEM (Fig 3). The porous nature of the core materials played an important role for adsorbing the herbicide molecules. Single bilayer of the polyelectrolytes (PAH and PSS) was coated onto the core $MnCO_3$ by Layer-by-Layer (LbL) assembly method. The LbL assembly is a kind of method wherein we can obtain a uniform sized spherical particle. The X-ray diffraction and Raman studies results conforms the unique

Table 1: Methods employed for biological analysis.

Particulars	Method	Reference
Biological analysis		
Total bacteria	Serial dilution method using nutrient glucose agar medium	Allen (1953)
Total fungi	Serial dilution method using rose bengal agar medium	Martin (1950)
Total actinomycetes	Serial dilution method using Kenknights agar medium	Allen (1953)

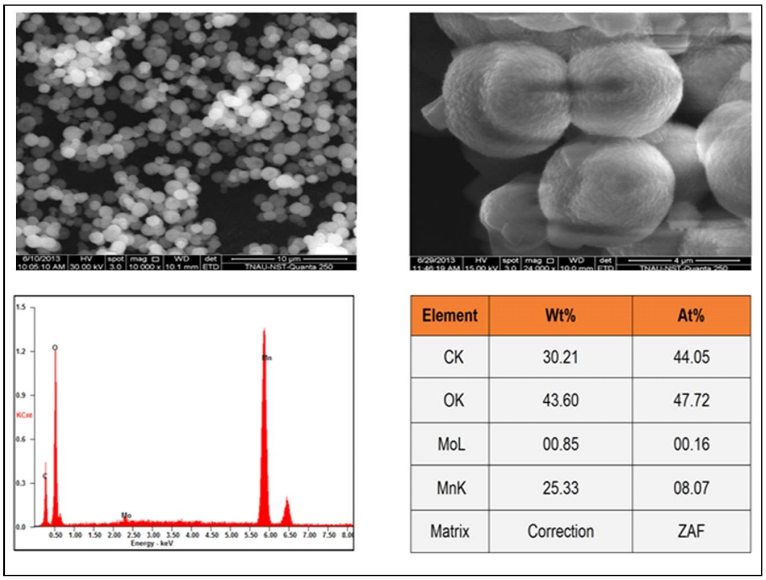


Fig 1: SEM image of $MnCO_3$ core, EDX graph and composition.

porous spherical superstructure and also covalent bonding (Kanimozhi and Chinnamuthu, 2012). The maximum yield and drug loading amount of hollow microspheres were 88.45% and 80±4.0%, respectively (Vandana Singh and Chaudhary, 2011).

Toxicity analysis of earthworms

The toxicity analysis of encapsulated pendimethalin by direct method did not influence the activity of earthworms (Table 2) significantly during the observation period of 30 days. Bilayer of direct encapsulated pendimethalin treatment showed that 92.50 per cent of the earthworms were healthy. The average weight of 20 earthworms in each treatment was increased two times compared to the initial weight (Table 2).

Toxicity analysis of beneficial soil microorganisms

The effect of direct encapsulation of pendimethalin by different layers of polymers on the population of bacteria,

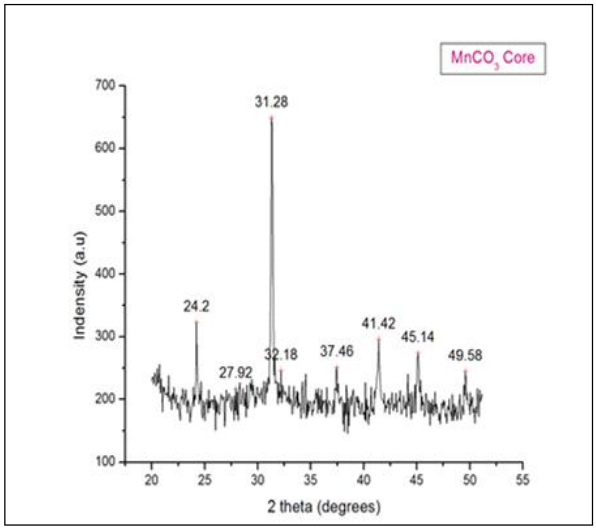
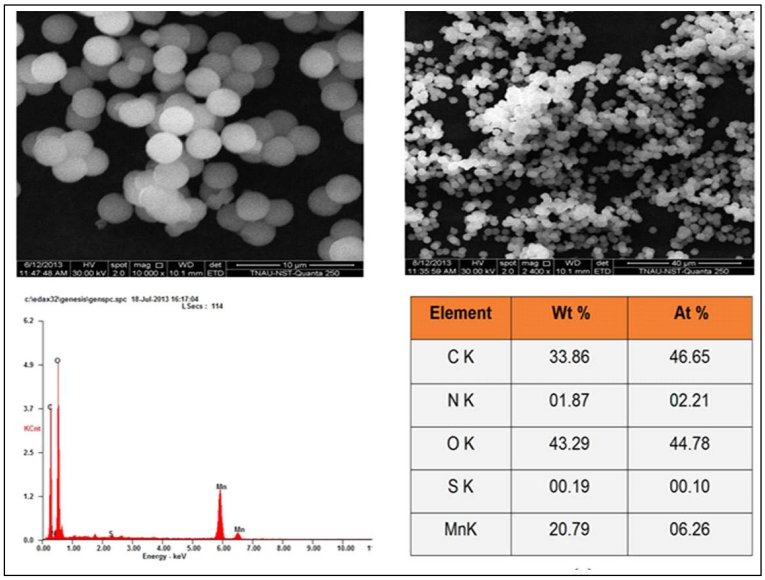


Fig 2: XRD-diffractrogram of $MnCO_3$ core.



Element	Wt %	At %
C K	33.86	46.65
N K	01.87	02.21
O K	43.29	44.78
S K	00.19	00.10
MnK	20.79	06.26

Fig 3: SEM image of nano encapsulated herbicide, EDX graph and composition.

Table 2: Effect of direct encapsulated pendimethalin on soil microorganisms.

Treatments	Bacteria ($\times 10^6$ CFU g^{-1})	Fungi ($\times 10^4$ CFU g^{-1})	Actinomycetes ($\times 10^2$ CFU g^{-1})
T_1- Single layer coating - Poly (allylamine hydrochloride) (PAH)	42.89	39.21	17.83
T_2- Double layer coating - PAH + Sodium poly (styrene sulfonate) (PSS)	42.66	39.16	17.69
T_3- Three layer coating I - PAH + PSS + Poly vinyl pyrrolidone (PVP)	42.78	38.46	17.64
T_4- Three layer coating II - PVP + PSS + PAH	40.57	38.72	17.12
T_5- Commercial formulation	42.87	37.79	16.86
T_6- Control	43.13	40.95	18.55
SEd	3.58	2.86	1.08
CD (P=0.05)	NS	NS	NS

Table 3: Effect of direct encapsulated pendimethalin on weight of earth worm and survival rate.

Treatments	Average weight of 20 earthworms (g)			Survival rate* (%)
	Initial weight	15th day	30th day	
T_1- Single layer coating - Poly (Allylamine hydrochloride) (PAH)	1.15	1.77	2.27	100.00 (91.25)
T_2- Double layer coating - PAH + Sodium poly (Styrene sulfonate) (PSS)	1.17	1.79	2.28	100.00 (92.50)
T_3- Three layer coating I - PAH + PSS + Poly vinyl pyrrolidone (PVP)	1.15	1.77	2.29	100.00 (91.25)
T_4- Three layer coating II - PVP + PSS + PAH	1.15	1.79	2.28	100.00 (92.50)
T_5- Commercial formulation	1.13	1.77	2.29	100.00 (90.00)
T_6- Control	1.17	1.80	2.30	100.00 (92.50)
SEd 0.06	0.02	0.02	5.67	
CD (P=0.05)	NS	NS	NS	NS

*Mean of three replications; Figures in parentheses are arc sine transformed values.

fungi and actinomycetes was studied in post-harvest soils of pot culture experiment. All the treatment noticeably recorded more population of fungi. Different treatments did not influence the soil beneficial microorganisms namely bacteria, fungi and actinomycetes (Table 3) significantly.

Microorganisms and earthworms in the environment are the clear indicators of environmental pollution. The change or fluctuation in population after the introduction of any foreign chemical is an indication of toxicity of that compound. Hence, tests were carried out to study the toxicity of nanoencapsulated pendimethalin against natural organisms.

Conflict of interest: None.

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