

Nano-pesticides - A recent approach for pest control

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ABSTRACT

Indiscriminate use of synthetic chemical pesticides has led to several environmental problems such as resistance and environment pollution. Nanotechnology visualized as a rapidly evolving field has potential to revolutionize agriculture and food systems. We utilized nano-technological interventions for developing nanopesticides with potent fungicidal and miticidal properties. Nanosulphur and nanohexaconazole were prepared using encapsulation technique. The Dynamic Light Scattering (DLS) studies, Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) studies confirmed the nano-structure of nanopesticides. Nanoparticles of sulphur and hexaconazole thus characterized were estimated for the presence of active ingredient in the formulation adopting analytical techniques like Gas Chromatography / High Pressure Liquid Chromatography (HPLC). The bio-assay studies revealed many fold improved activity of nanopesticides against fungi and mites as compared to commercial sulphur.

Keywords: Nanotechnology, nanosulphur, nanohexaconazole, miticidal, fungicidal, biosafety, analysis

Introduction

The application of synthetic pesticides has caused threat to non-target organisms and the environment due to their overuse (Savary *et al.* 2006). Since the release of xenobiotic results in the increase of environmental risk, the goal should be to use such compounds carefully so that they cause least negative impact on the environment (Savary *et al.* 2012) into which they are released. To remove harmful effects on the non-target organisms, encapsulation of the active ingredient with other materials such as a polymer can allow sensitive ingredients to be physically enveloped into a protective matrix in order to protect core materials from adverse reactions due to factors like air or light. Traditional strategies like integrated pest management used in agriculture are insufficient, especially in changed climate scenario and application of persistent older

pesticides have adverse effects on animals and human beings apart from the decline in soil fertility. An outcry is exhibited against the use of pesticides due to their hazardous effects on human as well as environment (Gao *et al.* 2012; Sparks *et al.* 2012). There is a great concern regarding the nano materials which have potential to exert hazardous effects on human and the environment and when we have a nano-pesticide, it becomes a double edged weapon. Nanomaterials need to be evaluated, so that this novel technology does not meet the same apprehensions and bottleneck as faced by genetically modified crops.

Nanoparticles (Karunaratne *et al.* 2012; Khot *et al.* 2013) present possibilities for more efficient and effective control of pests, but our relative lack of information on how they act and how they can be contained are giving regulators pause before allowing their release

into the environment. Nanopesticides hold promise for reducing the environmental footprint left by conventional pesticides. As EPA has noted, “these novel products may allow for more effective targeting of pests, use of smaller quantities of a pesticide, and minimizing the frequency of spray-applied surface disinfection. These could contribute to improved human and environmental safety and could lower pest control costs”. Nanotechnology research (Anders & Glotzer 2012) opens up opportunities of agricultural productivity enhancement involving nanoporous zeolites for slow release and efficient dosage of water and fertilizer, nanocapsules for herbicide delivery and vector and pest management and nanosensors for pest detection. The atom by atom arrangement allows the manipulation of nanoparticles thus influencing their size, shape and orientation for reaction with the targeted tissues.

We prepared nanopesticides (Gopal *et al.* 2011a, 2011b; Kumar *et al.* 2011; Choudhary *et al.* 2010) of fungicides and insecticides and compared their efficacy with the conventional products. Nano-hexaconazole was characterized by SEM, TEM, and FT-IR etc. and it was found to be less than 100 nm in size. Patent application on Nano-hexaconazole has been filed. Nano-hexaconazole is five times more effective in controlling pathogens and nanosulfur is ten times more effective for control of mites as compared to its WDP formulations. We have to ensure the materials, we introduce in environment, are evaluated before launching.

While using a new technology, safety of the user and its effect on environment has to be considered. We tested nano-hexaconazole and initiated work for preparing a protocol to test the safety of nanomaterial to be applied in field. The present study also evaluated the effect of nano-hexaconazole on total microbial count, soil enzymes, nitrifying bacteria, blue-green algae and seed germination. Comparing the results of various enzyme activities, like soil dehydrogenase (DHA), fluorescein diacetate (FDA), alkaline phosphatase (Alk P), acidic phosphatase (Acid P) and microbial count after application of a nanomaterial was taken as a criterion for testing, whether such materials are causing adverse effect on soil health or not. The results are encouraging and our nanopesticide, unlike metal nanoparticles, is found to be safe. Therefore, nanotechnology has potential to provide green and efficient alternatives for the management of pests in agriculture without harming the nature.

This paper is focused on traditional strategies used for the management of insect pests, limitations of use of chemical pesticides and potential of nanomaterials in insect pest management as modern approaches of nanotechnology.

Materials and Methods

Nanosulphur and nano-hexaconazole were prepared by our patented method (Gopal *et al.* 2011a, 2011b) characterized using SEM, TEM and spectral techniques and bioefficacy studies were carried against fungi and mites (Gogoi *et al.* 2013; Kumar *et al.* 2011; Choudhary *et al.* 2010). The analysis of active ingredients in

nanoencapsulated pesticide was done using spectroscopy and chromatography (Kumar *et al.* 2011). In extraction procedure, saline solution was used to break the emulsion formed in the separating funnel. The extracted solution (lower transparent layer) was passed through anhydrous sodium sulphate to make it free from water. Effect of nanohexaconazole on soil health and beneficial organisms was studied using standard method (Gopal *et al.* 2012).

Results and Discussion

Nanosulphur

Nanosulphur was prepared and characterized using DLS and TEM studies which showed their size to be within 1-100 nm range. HPLC analysis confirmed the presence of sulphur in the prepared nanosulphur formulations. Nanosulphur fungicide displayed effective control of powdery mildew, *Erysiphe cichoracearum* as compared to control (Figure 1).

Significantly higher miticidal activity was observed with nanosulphur as compared to commercial sulphur against red spider mite, *Tetranychus urticae* (Table 1).

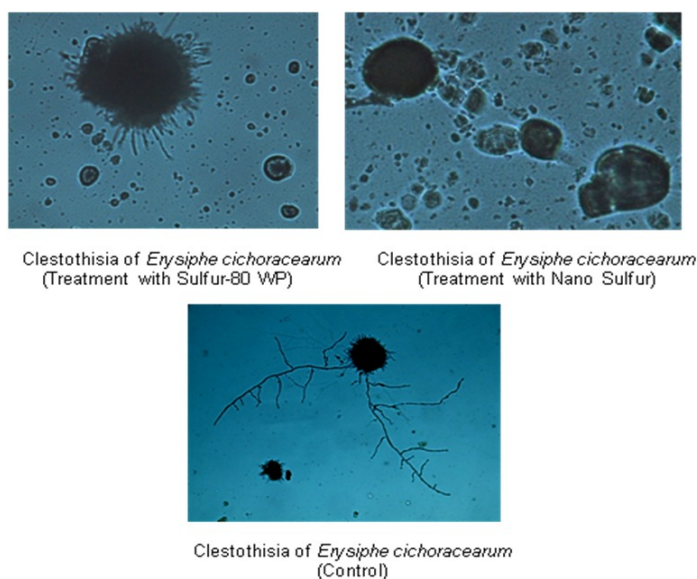


Fig 1. Efficacy of nano-sulphur against *Erysiphe cichoracearum*

Table 1.

Miticidal activity of nanosulphur and commercial sulphur against red spider mite

Compound	Heterogeneity		Regression equation	LC ₅₀	Fiducial limits		RT
	χ^2	df			Min	max	
Commercial sulfur	1.85	3	7.74+2.50x	0.080	0.071	0.090	-
Nanosulfur	1.59	4	6.40+0.63x	0.005	0.004	0.008	16

Nanohexaconazole

Hexaconazole was ball-milled and converted to nanohexaconazole formulation by encapsulating with poly ethylene glycol-400. Results of Dynamic light Scattering (DLS) and Scanning electron microscopy (SEM) study showed that average particle size of nanohexaconazole was about 100 nm (Figure 2). The particle size remained unchanged even after different dilutions as can be seen in DLS graphs.

The FTIR studies (Fig. 3) showed that there was no change in the chemical structure of hexaconazole as evident from the presence of peaks due to same functional group which was more intense around 3420 cm^{-1} due to the presence of poly ethylene glycol in nanohexaconazole.

In order to maintain the quality of nanohexaconazole, an improved method for estimation of its active ingredient was developed.

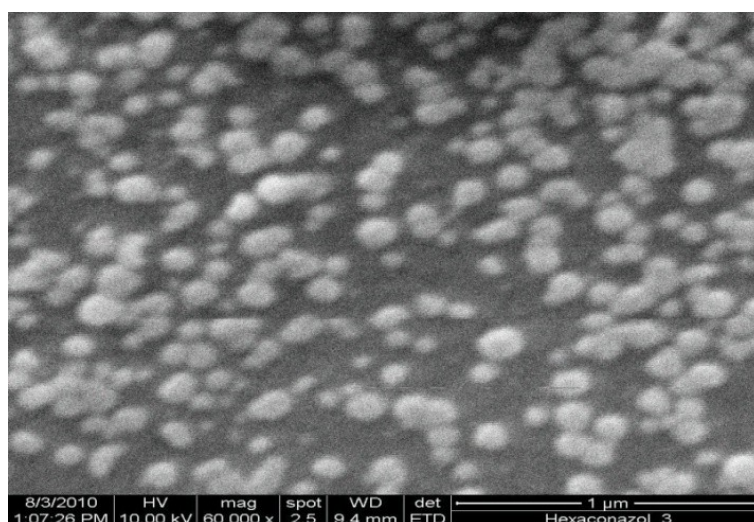


Fig 2. Scanning electron micrographs of nano-hexaconazole

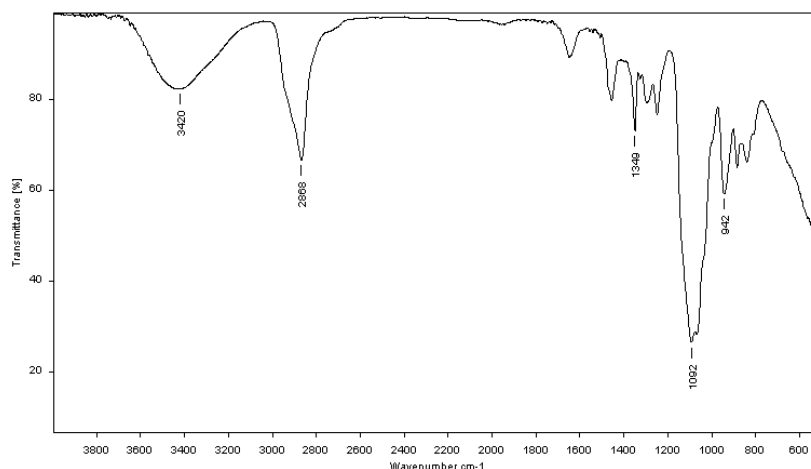


Fig 3. FTIR of nanohexaconazole

The limit of detection (LOD) was 2.5 ppm in case of technical hexaconazole and purified hexaconazole which was extracted from technical grade fungicide. The accuracy and precision of analytical method was ascertained by estimating prepared nano-hexaconazole of known concentrations. The study for linearity, repeatability and reproducibility shows that this is a rapid and efficient method for estimating hexaconazole in nanoformulation.

The fungicidal bioassay data (Table 2) against *Rhizoctonia solani* revealed that nano-hex-

aconazole was about two times more effective as compared to commercial hexaconazole. The activity of nanofungicide varied depending on various isolates of *R. solani* collected from different parts of India.

The stability for nano-hexaconazole was done at three different temperatures namely 4, 25, and 54°C. The weight and concentration of active ingredients were checked and there were no significant variation in weight and concentration of active ingredients. It was stable up to the four months at ambient temperature (Fig 4).

Table 2.

Fungicidal activity of nano-hexaconazole and conventional hexaconazole against isolates of *R. solani* (host: rice)

Isolates of <i>R. solani</i> (Location)	Nano-hexaconazole					Commercial hexaconazole				
	Heterogeneity		ID ₅₀	Fiducial limits		Heterogeneity		ID ₅₀	Fiducial limits	
	df (n-2)	χ^2		Low	High	df (n-2)	χ^2		Low	High
R-TN (Tamil Nadu)	3	4.159	0.154	0.097	0.245	3	4.745	0.364	0.235	0.564
R-4500 (Punjab)	3	1.732	0.145	0.097	0.217	3	6.859	0.422	0.277	0.642
R-A ₂ (Faizabad)	3	1.045	0.158	0.104	0.241	3	4.633	0.968	0.535	1.752
R-D ₁₄ (Dehradun)	3	5.037	0.232	0.153	0.354	3	7.552	0.671	0.407	1.104
R-KAPR (Kapurthala)	3	2.068	0.107	0.067	0.171	3	6.580	0.388	0.251	0.599

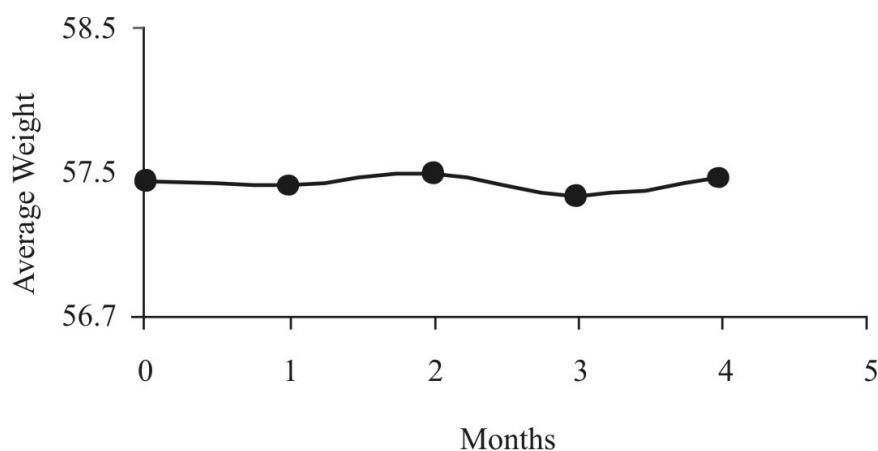


Fig 4. Average weight loss of nano-hexaconazole at room temperature in hard water

Various studies regarding effect of nanohexaconazole on soil health and beneficial organisms were carried (Gopal *et al.* 2012). It performed equivalent or better than commercial hexaconazole and was safe to soil health and other beneficial organisms. There was no adverse effect on (a) various soil enzyme activities (b) nitrogen fixing bacteria and blue green algae (c) total soil microbial count (d) nitrifying bacteria, the two key microorganisms namely *Nitrosomonas* and *Nitrobacter* species (e) germination of mustard (f) trichoderma species in the presence of nanoparticles of nanohexaconazole.

The study revealed the processes for the preparation of nanopesticide. The bioactivity of nanopesticide was many folds higher as compared to conventional formulations against powdery mildew fungi (*Erysiphe cichoracearum*) and adult red spider mite (*T. urticae*). The biosafety issues of these nanopesticides are also addressed. The process of registration of pesticides will need to be modified and also a protocol for handling them has to be developed specially for nanomaterial. Newer technologies can be adapted with surveillance and social control for sustainable agriculture.

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