Suitability of Nano-sulphur for Biorational Management of Powdery mildew of Okra (Abelmoschus esculentus Moench) caused by Erysiphe cichoracearum

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Abstract

New nano-sulphur synthesized at IARI and three other commercial products namely commercial sulphur (Merck), commercial nano-sulphur (M K Impex, Canada) and Sulphur 80 WP (Corel Insecticide) were evaluated in vitro for fungicidal efficacy at 1000 ppm against Erysiphe cichoracearum of okra. All the sulphur fungicides significantly reduced the germination of conidia of E. cichoracearum as compared to control. Least conidial germination was recorded in IARI nano-sulphur (4.56%) followed by Canadian nanosulphur (14.17%), Merck sulphur (15.53%), sulphur 80 WP (15.97%) and control (23.09%). Non-germinated conidia count was also high in case of IARI nano-sulphur followed by Canadian nano-sulphur, Merck sulphur and Sulphur 80WP. Apart from inhibition of conidial germination, cleistothecial appendages were also disrupted in contact with nano-sulphur and the cleistothecia became sterile. The study proved that IARI nano-sulphur is more effective than the commercial formulations and could be applied at lower amount for controlling powdery mildew disease for its better efficacy.

Keywords: Sulphur; Fungicides; Nano formulations; Powdery mildew; Okra

Introduction

Powdery mildew is a serious disease of okra, beans, southern peas, squash, cucumbers, muskmelons, and pumpkins in almost all the areas of the country. The disease is caused by the fungus Erysiphe cichoracearum DC ex Merat [Sphaerotheca fuliginea (Schlecht ex Fr.) Poll.]. Other species of Erysiphe like E. ploygoni causes powdery mildew in beans, southern peas, and English peas and Sphaerotheca macularis affects strawberries. Okra or Bhindi or Lady's finger (Abelmoschus esculentus Moench) is an important vegetable crop. Most of the okra cultivars are susceptible to powdery mildew disease, and depending upon the age of the plant at the time of infection, yield losses range between 17 and 86.6% [1]. The disease is favored by low temperature (11-28°C) and dry weather conditions and early infection has more effect on the plant growth and yield than late infection [2]. Effective control of the disease is possible with fungicial applications and the recommended fungicides are benomyl (0.1%), wettable sulfur (0.2, 0.3 and 0.5%) [2,3]. Applications of potassium silicate [K2SiO3 (200 mg/L)] and the alternation of systemic fungicide Bayleton WP 5 [Triadimenol (25 mg/L)] and Thiovit 80% WP (sulfur (2.4g/L)) with 12 day interval were also highly protective against powdery mildew [4]. Among these fungicides, elemental sulphur (S°) is universally known as the most effective one for managing powdery mildews and this fungicide has been used as foliar spray @ 2-4 g per litres (2000-3000 ppm).

As against achieving effective control of powdery mildew disease by using sulphur fungicides, phytotoxicity may be an adverse effect of this fungicide even at recommended doses or due to over use. Hence, it was advised for restricted use of sulphur fungicides in the fruit plants like apple [5] and apricot, vegetables like spinach, melon and squash. To avoid the risk of phytotoxicity, threat to non-targeted organisms and the environment, an idea was conceived to have better control of powdery mildews using lower or safe doses of fungicides. Recently, nanofomulations (particle size <100 nm) of pesticides have been able to draw much attention due to their higher efficacy even at very low doses, because nanoparticles could be more chemically reactive and bioactive than larger particles [6]. We have developed nano-sulphur having 50-90 nm particle size by liquid synthesis method [7] and also standardized a simple method for estimation of active ingredient of sulphur nanoformulation [8]. Subsequently, sulphur nanoparticles were characterized by using dynamic light scattering (DLS) study, transmission electron microscopy (TEM), scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDAX) [8]. The reasons of choosing sulphur for developing into nanoform are that firstly, micronized elemental sulphur (S°) has been in use as a fungicide since long time more particularly in controlling powdery mildew diseases of crops and secondly, this element is having multipropoged applications such as fertilizer, pharmaceutical, anti-microbial agent, insecticide, fungicide, high density charger in lithium ion battery as well as rubber and fibre industries [9-15].

Our sulphur nanoparticles exhibited significantly superior fungicial properties than the conventional sulphur against a food contaminant Aspergillus niger [7]. Therefore, further investigation was carried out on the efficacy of newly synthesized nano-sulphur against...
crop diseases like powdery mildew of okra caused by *E. cichoracearum*.

**Materials and Methods**

**Collection and maintenance of the pathogen**

Okra leaves affected by *E. cichoracearum* showing the typical symptoms of powdery mildew disease were collected from vegetable farm of IARI, New Delhi. Profusely growing conidia were collected for artificial inoculation by using a camel hair brush and suspended in sterile distilled water containing TWEEN 20. The conidial suspension was then centrifuged at 3000 rpm for 5 min twice, in order to separate conidia from conidiophores [16]. The concentration of the conidia was adjusted to 3×10^4 conidia ml^-1 using a haemocytometer. This suspension was sprayed using an atomizer on the healthy leaves of 60 days old potted okra plants maintained in the poly house. After inoculation, the plants were covered by polythene bags for 24 h to maintain high humidity for disease development.

**In vitro evaluation of fungicides and spore germination**

Four non-systemic fungicides viz. commercial sulphur (Merck), commercial nano-sulphur (M K Impex, Canada) and Sulphur 80 WP (Corel Insecticide) and IARI nano-sulphur were evaluated at 1000 ppm (or 0.1%) concentrations under in vitro conditions against *E. cichoracearum*. Conidial germination was tested by two methods. In the first method, two percent water agar medium was used as a basal medium that was poured over a clean grease free glass slide. Fungicidal suspensions of 1000 ppm were prepared in sterile distilled water under aseptic condition. Powdery mildew spores were added to each fungicidal suspension, adjusted the spore concentration as described earlier and smeared (three times per slide) on agar surface by using camel hairbrush. These slides were incubated at 25°C for 24 h. Three replications were maintained for each treatment. In control, conidial suspension was prepared in sterile distilled water, spread on the 2% water agar surface and conidial germination was observed under microscope (Magnus MLXi, Olympus).

In the second method, the efficacy of fungicides was assessed based on the conidial germination of *E. cichoracearum* employing detached leaf method [17]. Fresh okra leaves were washed in sterile distilled water and air dried. Solution of the fungicide prepared at 0.1% concentration was sprayed on the adaxial surface of the leaf using an atomizer and allowed to air dry. The treated leaves were inoculated with three drops of conidial suspension of *E. cichoracearum* (3×10^4 conidia ml^-1) and spread uniformly on the leaf surface using camel hairbrush. The leaves smeared with the conidial suspension without fungicide was served as a control. In each treatment, three leaves were placed in a petridish having moist tissue paper and incubated at 25°C. Each treatment was replicated thrice. After 72 h, powdery growth was observed on the inoculated leaves. Conidia from the superficial growth were dusted (five sweeps from each leaf for one slide) on water agar coated slide using a hair brush, incubated again for 24 h and observed under fluorescent microscope (at 10X). The germinated and non-germinated conidia and also their total number were counted from three microscopic fields. The percent germination was calculated by using the formula: 

\[ \text{PG} = \frac{(A/B) \times 100}{\text{where, PG=} \text{Per cent germination, } A=\text{Number of conidia germinated and } B=\text{Total number of conidia examined.}} \]

**Results and Discussion**

In the present study, newly synthesized IARI nano-sulphur and other forms of sulphur were tested in *in vitro* against powdery mildew of vegetables (okra) caused by *E. cichoracearum* and the results drawn out by executing two methods viz. poisoned food technique and detached leaf method (Table 1). All the sulphur fungicides highly inhibited conidial germination *E. cichoracearum* as compared to control in both the methods. In poisoned food technique, minimum (2.95%) germination of conidia was observed in IARI nano-S followed by Merck sulphur, Sulphur 80WP and Canadian nano-S. As far as percent inhibition of conidial germination and non-germinated conidia are concerned, IARI nano-S exhibited significantly higher inhibition followed by Merck sulphur, Sulphur 80WP and Canadian nano-S. The performance of the latter three forms of sulphur was at par with control in terms of all the studied parameters of *E. cichoracearum*. In the second method, i.e., detached leaf method, IARI nano-S out performed which was followed by Canadian nano-S, Merck sulphur and Sulphur 80WP. However, percent inhibition of conidial germination in IARI nano-S was non-significant with Canadian Nano-S. Although conidial germination as recorded in Sulphur 80WP was less (4.63%) than control (12.82%), there was no significant difference between their fungicidal properties. Therefore, in order to draw a common fungicial behavior among the latter three tested sulphur formulations other than IARI nano-S, data of both the methods were averaged and analyzed (Figure 1). It also revealed IARI nano-S as the best among all the sulphur forms to restrict conidial germination and thereby increasing the percentage of non-germinated conidia which was followed by Canadian nano-S, Merck sulphur and Sulphur 80WP. On the basis of

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Fungicides</th>
<th>Food poison technique</th>
<th>Detached leaf method</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Germination of conidia</td>
<td>% Non-germinated conidia</td>
</tr>
<tr>
<td>1</td>
<td>IARI Nano Sulphur</td>
<td>2.95 (9.09)</td>
<td>84.03 (67.86)</td>
</tr>
<tr>
<td>2</td>
<td>Merck Sulphur</td>
<td>11.94 (19.15)</td>
<td>47.49 (42.88)</td>
</tr>
<tr>
<td>3</td>
<td>Sulphur 80 WP</td>
<td>12.13 (19.71)</td>
<td>42.30 (40.41)</td>
</tr>
<tr>
<td>4</td>
<td>Canadian Sulphur</td>
<td>13.09 (19.76)</td>
<td>41.11 (39.61)</td>
</tr>
<tr>
<td>5</td>
<td>Control</td>
<td>16.25 (25.56)</td>
<td>33.41 (34.46)</td>
</tr>
<tr>
<td></td>
<td>CD (5%)</td>
<td>9.45 (19.17)</td>
<td>9.44 (9.44)</td>
</tr>
<tr>
<td>CV</td>
<td></td>
<td>22.26</td>
<td>6.91</td>
</tr>
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</table>

**Table 1:** Effect of various forms of sulphur (1000 ppm at 24 h) on the conidial germination of *Erysiphe cichoracearum*. 

The fungicidal property which needs a further study.

The mould fungi (Aspergillus flavus and Staphylococcus areus) and yeast (S. aureus) are amongst the most common food pathogens in the world. However, there are new food pathogens that have been identified recently and which are yet to be controlled. For example, E. cichoracearum, a fungal pathogen, is a new food pathogen that has been reported recently in the world. This pathogen is known to cause powdery mildew of okra (Abelmoschus esculentus Moench) in potted plants, especially in organic apple production. To control this disease, we have synthesized sulphur nanoparticles from hazardous H2S gas and used it as an antimicrobial agent and thus newly synthesized IARI nano-S is found four times better than the commercial product Sulphur 80WP and Merck sulphur and three times superior over Canadian nano-S. The efficacy of IARI nano-S as it was found only four times superior than the commercial formulation Sulphur 80WP.

In conclusion, nanoparticles of sulphur are undoubtedly better in having fungicidal property as compared to its counterparts of conventional micronized formulations available in the markets. However, refinement of the protocol for synthesis of the sulphur nanoparticles will be required for further improvement in the efficacy of IARI nano-S as it was found only four times better than the commercial formulation Sulphur 80WP.

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References