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Antimicrobial efficacy of Nano-formulation and Inducer chemical against *Phomopsis* Blight of Brinjal

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Abstract
Phomopsis vexans is one of the most important pathogen of brinjal which cause leaf blight and fruit rot of brinjal that lead to heavy yield loss in field condition. Hence, in present study, an efficient research studies carried out related to management of agricultural important pathogen of brinjal crop. Under *in-vitro* study seven chemical namely silver Nanoparticle (AgNPs), $AgNO_3$, Silvox(H_2O_2+ $AgNO_3$), Chitosan, Salicylic acid, H_2O_2, Isoprotiolane(check chemical) were evaluated in different concentration against fungal pathogen *Phomopsis vexans*, under *in-vitro* and *in-vivo* condition. In *in-vitro* condition out of seven chemical $AgNO_3$@ 4.89 ppm appeared to be most effective in respect of mycellial growth inhibition against *Phomopsis vexans*. The H_2O_2 and Salicylic acid exhibited maximum disease suppression due to prophylatic use at 50ppm concentration (H_2O_2-64.72% & Salicylic acid-68.45%) as compared to check chemical Isoprothiolane.

Keywords: *Phomopsis vexans*, silver nanoparticle (AgNPs), chitosan, brinjal, salicylic acid

1. Introduction
Brinjal (*Solanum melongena* L, Solanaceae) is an agronomically important non-tuberous crop native to southern India, and one of the most important vegetable crops in India (Zeven & Zhukovsky 1975, Sekara *et al*. 2007) [12, 10]. It affected by a large no of fungal disease among them Fruit rot and leaf blight disease caused by *Phomopsis* vexans (Perfect stage: *Diaporthe vexans*) is of major concern in brinjal producing areas of India as it reduces yield and marketable value of the crop by 20−30% (Das 1998, Khan 1999) [6]. In order to control this fungi, agrochemicals have been used for a long time. But wide spread use of agrochemicals has certainly decreased the outbreak of fungal diseases but at the same time has contributed to the development of resistant pathogens (Lamsal *et al*., 2011) [9]. The potential applications of nanomaterial, inducer chemicals and new systemic fungicides in crop protection helps in the development of efficient strategies for the eco-friendly management of plant pathogens under sustainable agriculture. In recent years, nanotechnology has been increasingly applied to the development of novel antimicrobials for the management of pathogen affecting agricultural crops, humans and animals. A number of patents and products integrating nanomaterial's into agricultural practices (e.g., nano pesticides, nano fertilizers, and nano sensors) have been developed. A number of different types of induced resistance have been defined based on differences in signalling pathways and spectra of effectiveness, including systemic resistance and induced systemic resistance. Such resistance can be induced in plants by application of a variety of biotic and abiotic agents. The resulting resistance tends to be broad-spectrum having long lasting, effect for disease by between 20 and 85%. Although research in this area has been increased over last few years. There have also been a number of studies required for understanding of how best to use induced resistance along with nano-formulations in practical crop proctection.

Materials and Methods
The present research was conducted in Bidhan Chandra Krishi Viswavidyalaya (Department of Plant Pathology). Potato-dextrose-agar (PDA) medium was used for isolation of fungus *Phomopsis vexans.*

Identification of the Fungal Culture: Morphological and cultural characters of isolated fungi was recorded and compared with standard text for establishing their identity (Booth and Sutton, 1984; Chowdhry, *et al*., 2000) [4, 5].

***In-vitro* evaluation of Chemicals Using Poisoned Food Technique:** Potato dextrose agar amended with different nano formulation, plant inducer, ROS molecule and the final concentration of different nano formulations, ROS molecules and plant inducer are 1 ppm, 5 ppm, 10 ppm, 15 ppm, 20 ppm and control. The control is amended with sterile distilled water without any formulation with three replication of each treatment. Observation on radial growth were measured in both nano-formulation and different plant inducer chemicals amended media and untreated control and extended of incubation of mycelia growth by each formulation was calculated by estimating the percent reduction in mean mycelia radial growth over that control (Vincent, 1947).

$$\text{Inhibition \%} = \frac{C-T}{C} \times 100$$

The poisoned food technique (Falck, 1907) [7] was followed to evaluate the efficacy of chemicals, in this technique different concentrations of the test chemicals are mixed with the nutrient medium (PDA) and then test organism is allowed to grow in such medium.

Effect of Different Chemicals against Different Foliar Diseases on Brinjal Crops under Net House Condition

Under net house condition, different chemicals were applied 1 day before spore inoculation and 1 day post inoculation on brinjal crop. Forty five days old plant of Brinjal, with different nano formulations and plant inducer chemicals molecule with different concentrations i.e., 25 ppm and 50 ppm. The control is modifying with sterile distilled water without any formulation with three replication of each treatment. Spore obtained from the actively growth expanding of 7- 9 days old colony of each isolated pathogens and inoculated to plants. Observations were taken after 10 days. Disease severity calculated by scoring the diseases following 0-9 scale of Mayee and Datar (1986).

$$\text{Disease Severity (\%)} = \frac{\text{sum of all numerical rating}}{\text{No.of leaves examined} \times \text{Maximum disease rating}} \times 100$$

The disease severity was recorded at 7, 14, 21 days and 28 days after onset of disease in all tested crops.

Results and Discussion

***In-vitro* evaluation of chemicals against isolated foliar fungal pathogens *Phomopsis vexans* on Brinjal**

From the Table: 1 and Plate: 1 reveals that out of seven chemicals considered for the *in-vitro* study. $AgNO_3$ @ 4.89 ppm appeared to be most effective in respect of mycellial growth inhibition against the pathogen *Phomopsis vexans* followed by H_2O_2 (7.24 ppm). It was also observed that more or less all the chemicals proved themselves to be better than check chemical Isoprothiolane (25.23 ppm). Hence, these above observations are in agreement with the findings of Young-ki Jo *et al.*, 2009 [11]. They noticed that role of $AgNO_3$ had some inhibitory effects against the microbial colony formation, disease progress of pathogen by direct contact of silver with spore and germ-tube and inhibit their viability.

Table 1: Regression and correlation of toxicity of chemicals against *Phomopsis vexans (*Mycelia growth inhibition)

Chemicals	Mycellial growth inhibition(MGI)		
	Regression equation (RE)	Co-efficient of determination (R^2)	ED-50 (ppm)
Silver nanoparticle (AgNPs)	y = 0.649x + 4.148	R^2 = 0.644	20.54
H_2O_2	y = 0.788x + 4.330	R^2 = 0.945	7.24
Silvox	y = 0.711x + 4.209	R^2 = 0.766	12.95
Chitosan	y = 1.100x + 3.576	R^2 = 0.854	19.70
Salicylic acid	y = 0.947x + 3.931	R^2 = 0.855	13.45
$AgNO_3$	y = 1.406x + 4.022	R^2 = 0.931	4.89
Isoprothiolane (check chemical)	y = 1.306x + 3.168	R^2 = 0.984	25.23

X= Probit value of percentage inhibition; Y (µg/ml) =Antilog of the value obtained

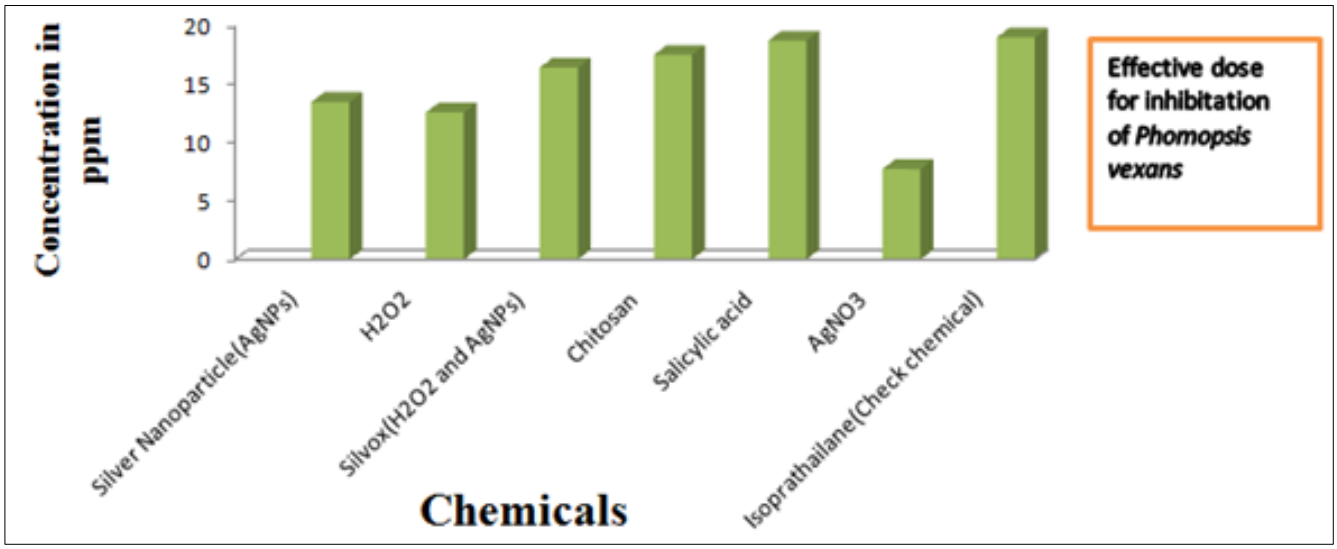
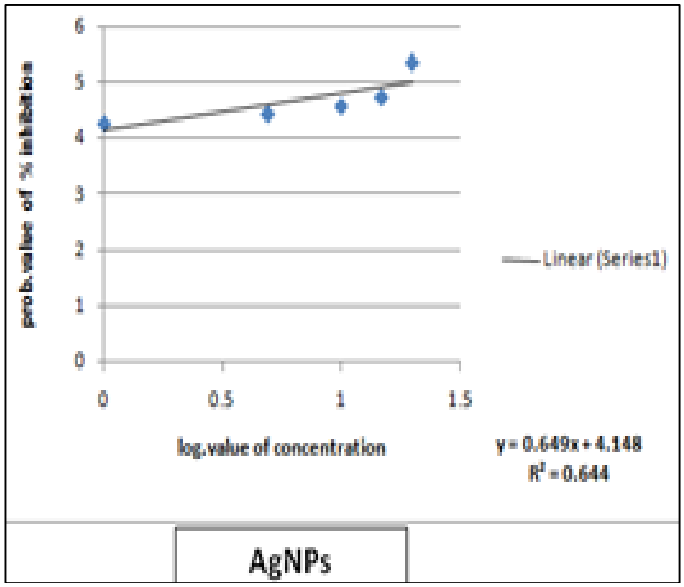
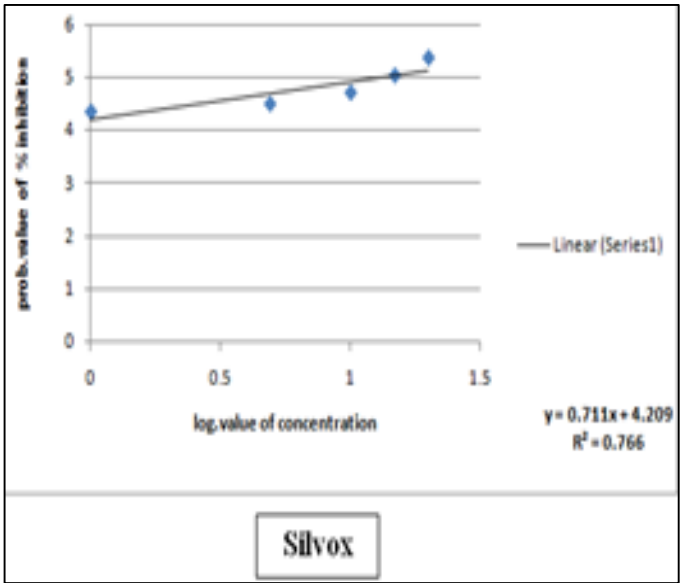


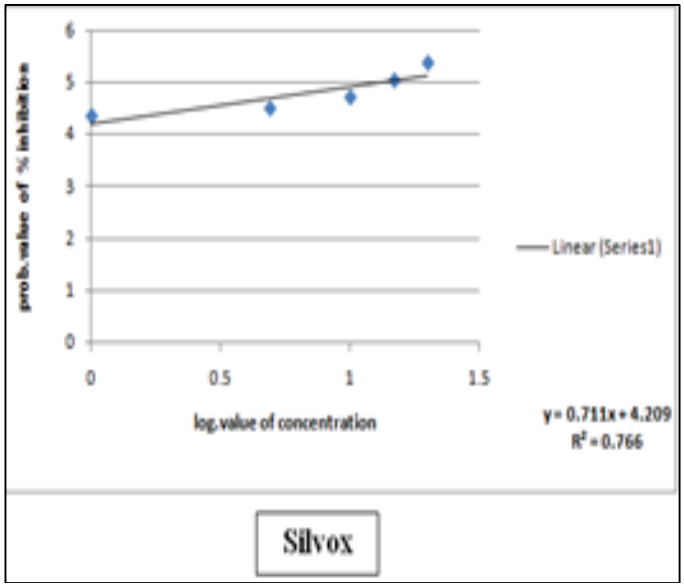
Fig 1: *In-Vitro* Testing of Chemicals against *Phomopsis vexans* (Mycelia Growth Inhibition)



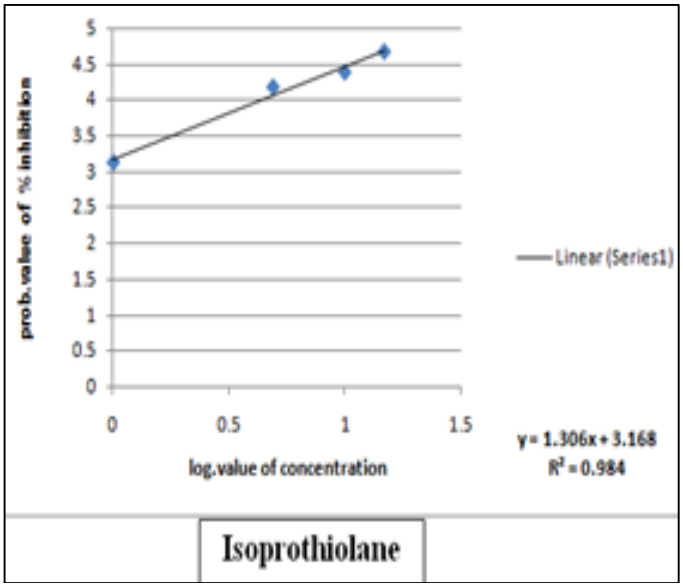
AgNPs



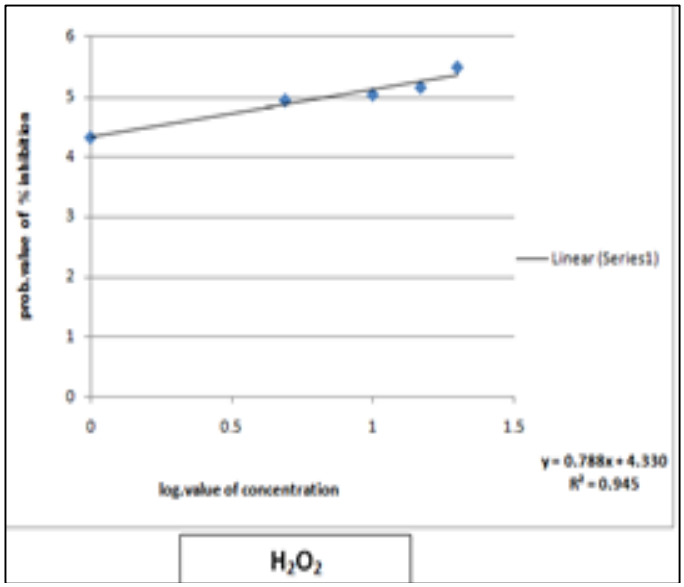
Silvox



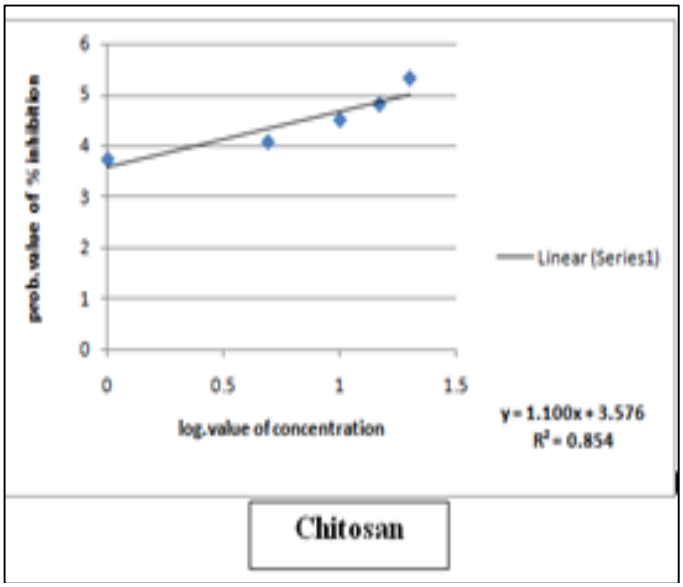
Silvox



Isoprothiolane



H_2O_2



Chitosan

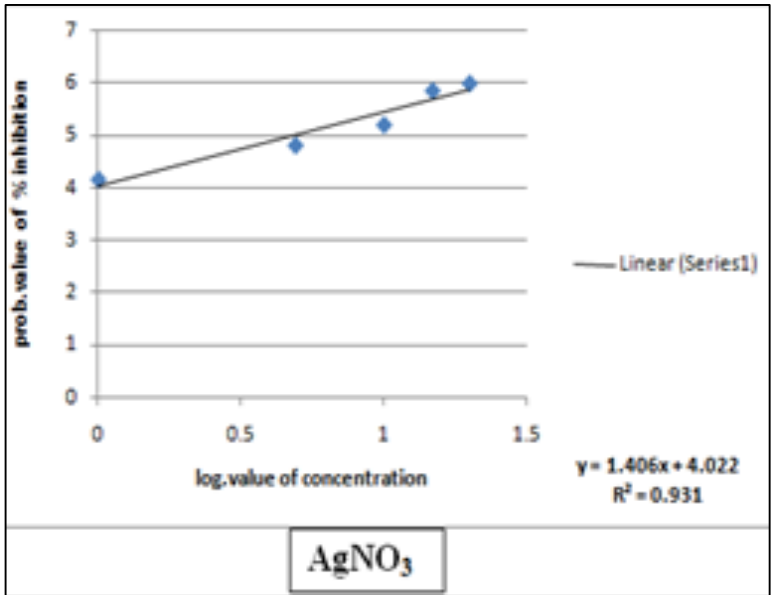


Fig 2: Prob. Value of Percentage Inhibition with Log Value of Concentration of Chemicals Used in *In-Vitro* Testing against *Phomopsis vexans*

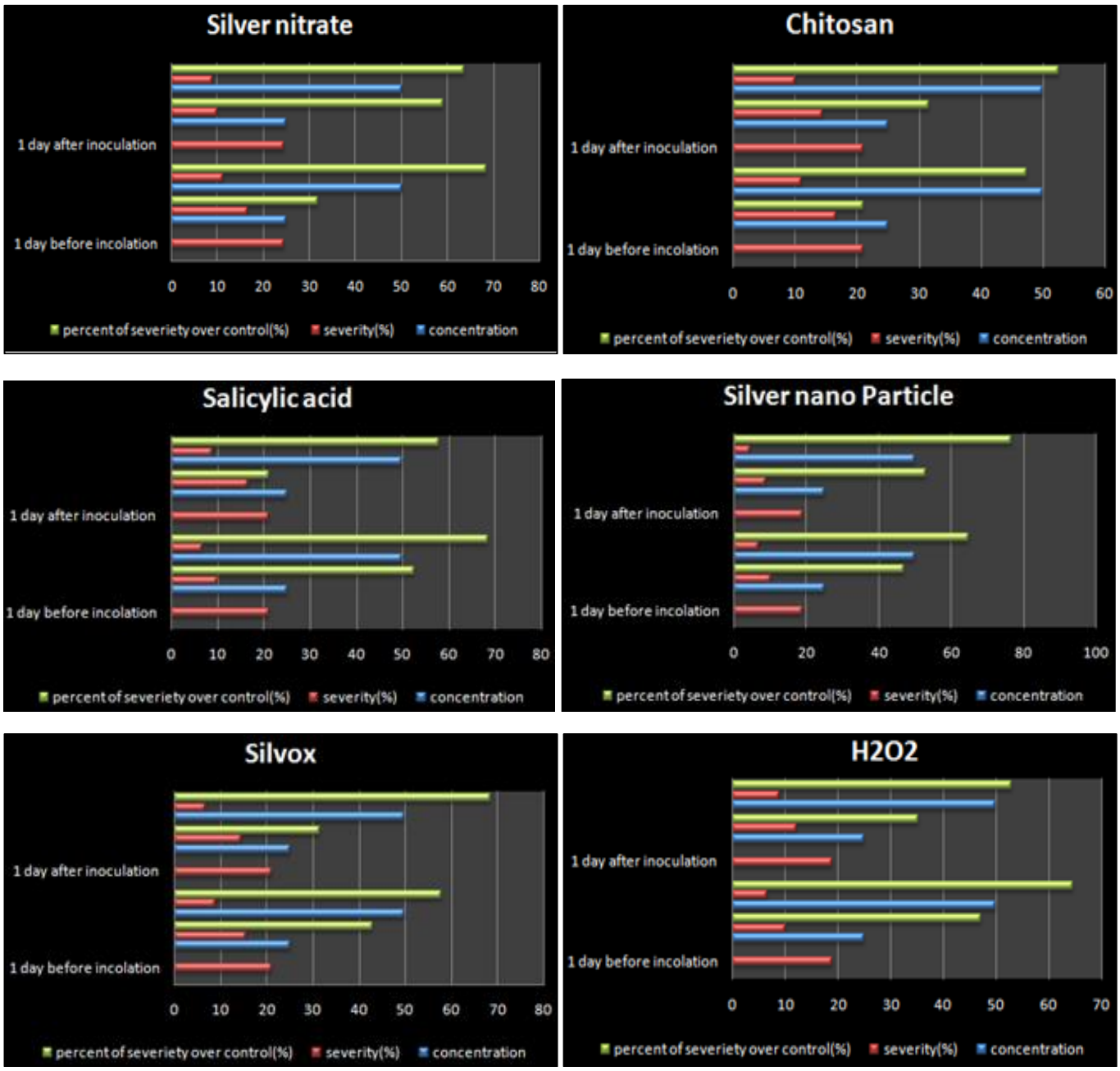
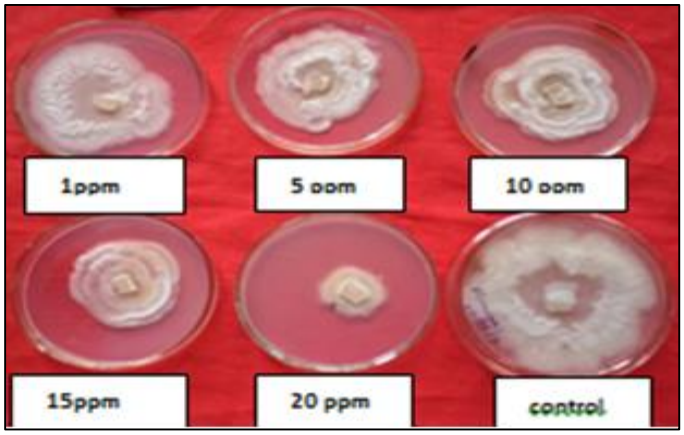
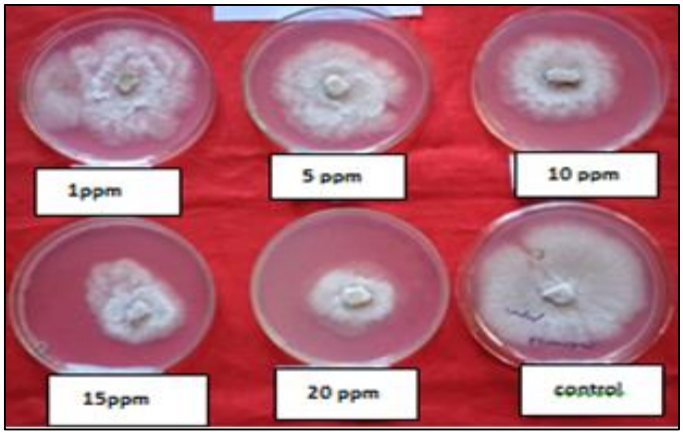


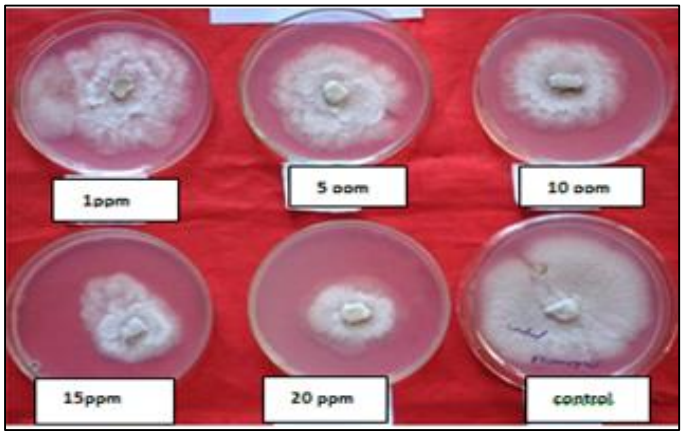
Fig 3: Potency of Nano and inducer chemicals against Leaf Spot causing *Phomosis vexans* pathogen on Brinjal under Net House Condition



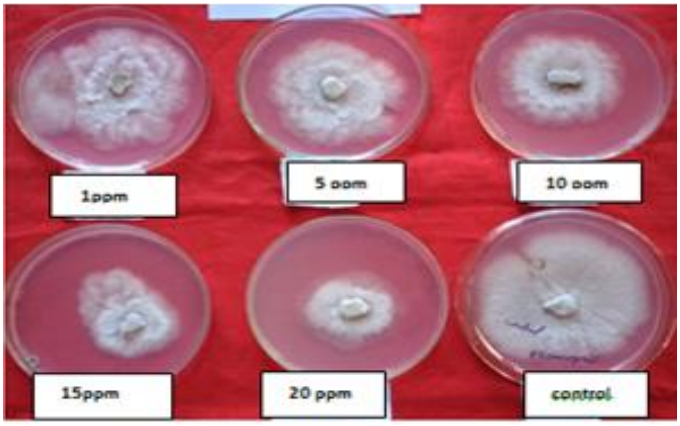
In-vitro effect of Chitosan



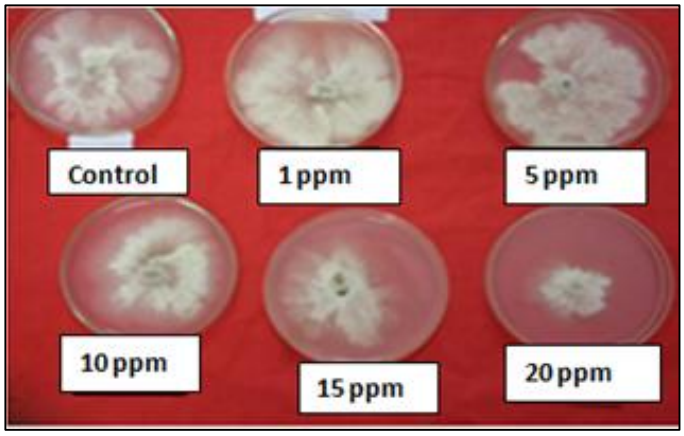
In-vitro effect of AgNPs



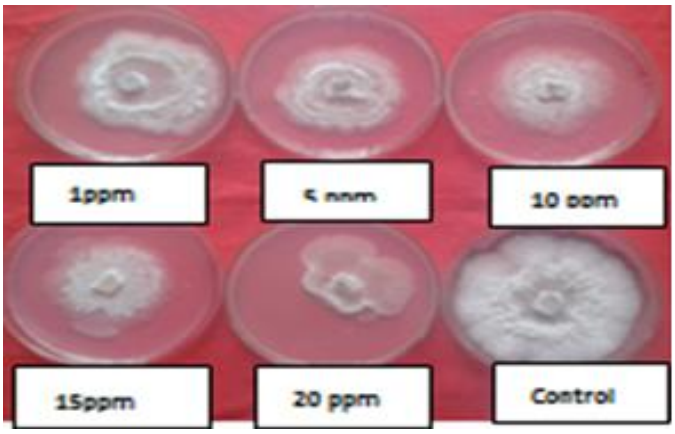
In-vitro effect of Silvox



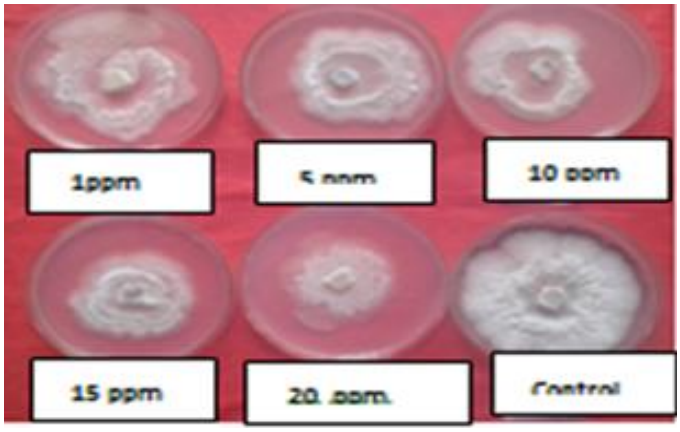
In-vitro effect of $AgNO_3$



In-vitro effect of H_2O_2



In-vitro effect of Salicylic acid



In-vitro effect of Isoprothionale

Plate 1: *In-Vitro* Study of responsiveness of *Phomopsis vexans* against 7 Different Chemicals

***In-vitro* effect of Isoprothionale**

The figure (3) indicated that curative application (spraying 1 day after pathogen inoculation) of mentioned nano-formulation (Silver Nanoparticle, Silvox, Chitosan, Silver nitrate)manifest significant disease suppression in compared to prophylatic application (spraying 1 day before pathogen inoculation).Application of nano formulation as curative measure gave significant result at 50 ppm (Silver Nanoparticle-(AgNPs)-76.48%,$AgNO_3$-63.66%,Silvox-68.45% and Chitosan-52.62%) applied after onset of disease.

From the figure (3) at was indicated that inducer chemical (salicylic acid, Hydrogen peroxide) inhibited growth of pathogen only due to prophylatic way rather than curative ones. All the chemical give good result than check chemical Isoprothiolane.

Conclusion

From the above discussion it is concluded that out of seven chemical considered for the *in-vitro* study, $AgNO_3$ @ 4.89 ppm found to be most effective in respect of mycelia growth inhibitation against the pathogen *Phomopsis vexans* .The second best result was found in case of H_2O_2(7.24ppm) followed by Silvox(12.95ppm) as compared to check chemical Isoprothiolane.Under net house condition it recorded that curative application of nano-formulation resulted significant disease suppression at 50ppm (Silver Nanoparticle-(AgNPs)-76.48%,$AgNO_3$-63.66%,Silvox-68.45% and Chitosan-52.62%) as compared to check chemical Isoprothiolane(54.54%)at 25ppm.

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