Scholars Academic Journal of Biosciences (SAJB) **ISSN 2321-6883 (Online)**
Sch. Acad. J. Biosci., 2016; 4(11):1032-1037 **ISSN 2347-9515 (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI : 10.36347/sajb.2016.v04i11.010

Original Research Article

Antimycotic activity of zinc and manganese nanoparticles on commercially important phytopathogens of soybean (*Glycine max* (L.) Merril)

Shraddha M. Dhoble[1], Nikhilesh S. Kulkarni[2]
[1]Microbiology Research Laboratory, Department of Microbiology, R. A. College, Washim-444505 (M.S), India.
[2]Head, Department of Microbiology, Microbiology Research Laboratory, R. A. College, Washim-444505 (M.S), India.

***Corresponding author**
Shraddha M. Dhoble
Email: shraddhamdhoble@gmail.com

Abstract: Soybean (*Glycine max* (L.) Merril) is a most important oil crop. Soybean is frequently infected by plant pathogenic fungi belong to *Rhizoctonia*, *Fusarium*, *Sclerotium*,*Collectotrichum*,*Alternaria* and *Macrophomina sp.* The present research is concerned with the fungicidal properties of metal nanoparticles used as an agent for antifungal treatment of these plant pathogenic fungi. We used Zinc (ZnNPs) and Manganese (MnNPs) nanoparticles at concentrations of 25, 50, 75 and 100 ppm. Six different Soybean plant pathogenic fungi viz., *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Collectotrichum truncatum*, *Alternaria alternate* and *Macrophomina phaseolina* were used as test pathogens and treated with both the nanoparticles in potato dextrose agar (PDA) by food poisoning technique. We calculated fungal inhibition in order to evaluate the antifungal efficacy of Zinc and Manganese nanoparticles against fungal pathogens. The results indicated that both nanoparticles possess antifungal properties against these plant pathogens at various levels. Treatment with 100 ppm concentration of both nanoparticles resulted in maximum inhibition of most fungi. Results also showed that the inhibition of plant pathogenic fungi was increased with increased in concentration of metal nanoparticles. In summary, ZnNPs and MnNPs exerted potent antifungal effects on test fungi *in vitro*. Thus, it can be effectively used against plant phytopathogenic fungi to protect the various crop plants and their products, instead of using the commercially available synthetic fungicides, which show higher toxicity to humans.
Keywords: Antifungal effect, Pathogenic fungi, Zinc nanoparticles (ZnNPs), Manganese nanoparticles (MnNPs).

INTRODUCTION

Soybean (*Glycine max* (L.) Merril) is an Asiatic leguminous plant cultivated in several parts of the world for its oil and protein, which are extensively used in the manufacture of animal and human food stuffs. Soybean is the fourth largest grain crop in India. In addition, soybean is an important oil crop with high nutritional value. Soybean is extremely rich in soy proteins, which have greater nutritional value than cereal proteins, and it is therefore an important source of plant protein for human consumption [1-3]. Furthermore, soybean also contains active substances that are beneficial for human health, such as isoflavones, saponins and oligosaccharides [4-6]. The most commercially important of pathogenic fungal isolates of soybean belong to six fungal group's viz., *Fusarium*, *Rhizoctonia*, *Sclerotium*, *Colletotrichum*, *Alternaria* and *Macrophomina* specie. All the pathogenic fungal isolates were isolated from diseased soybean plant. Results supported the work of [7], isolated *Alternaria*, *Aspergillus*, *Cladosporium*, *Colletotrichum*, *Fusarium*, *Paecilomyces* and *Penicillium* from soybean leaves and seeds. *Rhizoctonia solani*, one of the sclerotia forming microbe is widespread in the world and causes many important diseases in a wide host range of plants. Also, *Alternaria alternata* can have a certain pathogenic effect; it has been recorded as a saprophytic or a weak pathogen causing so-called "indefinite or opportunistic disease" on a number of crops [8]. Soybean is frequently infected by *Fusarium* sp. and responsible for root rot disease in soybean [9, 10], obtain four *Fusarium* sp. from diseased soybean plant viz., *Fusarium oxysporum*, *Fusarium avenaceum*, *Fusarium poae and Fusarium equiseti* respectively. Another soil-born fungal pathogen, *Sclerotium rolfsii* identified by [11], responsible for root rot and damping-off of crop were isolated from diseased soybean plant [12], also reported that, soybean is an important host plant for *Macrophomina* phaseolina which is responsible for Charcoal rot, seedling blight, dry root rot, dry weather wilt disease and ashy stem blight [13], reported that the *Macrophomina phaseolina* is a another soil-born fungal pathogen having wild range of host plant including soybean crop and widely distributed and responsible for charcoal rot of soybean resulting in great economic loss [14].

Present studies revealed the dominance of pathogenic fungi in soybean root system. The study indicates the serious sickness in these regions. Many studies have shown the biological effects of different nanoparticles against human pathogen: However, the effects of different nanoparticles against fungal pathogens of plant have not yet been fully studied. Hence, in the present study, the antimycotic activity of different nanoparticles against phytopathogens of soybean will be investigated.

MATERIAL AND METHODS

In vitro Assay for antimycotic activity of metal nanoparticles

The two metal nanoparticles viz., Zinc and Manganese nanoparticles were screened for their *in vitro* antimycotic activity against the isolated fungal pathogens of Soybean. The isolated fungal pathogens viz., *Rhizoctonia solani, Fusarium oxysporum, Sclerotium rolfsii, Collectotrichum truncatum, Alternaria alternate* and *Macrophomina phaseolina* were used as test pathogens. The antifungal assays were performed by food poisoning method. The plates containing PDA with different concentrations, viz., 25, 50, 75 and 100 ppm of Zinc and Manganese nanoparticles were prepared separately. The PDA plats were punchered at the center by using cork borer and inoculated with 5 mm agar plug containing cultured pathogenic fungus and incubated at 25°C for 7 days. This procedure was replicated trice. The medium with 5 mm agar plug containing pathogenic fungus without metal nanoparticles as control was maintained. After 7 days of incubation the mycelial growth were measured using ruler and expressed in mm [15, 16].

The inhibition rate (%) was calculated by using the following formula:

$$\text{Inhibition Rate (\%)} = \frac{\mathrm{R} - \mathrm{r}}{\mathrm{R}} \times 100$$

Where,

R = Redial growth of fungi in control

r = Redial growth of fungi in test

RESULTS AND DISCUSSION

In vitro Assay for antimycotic activity of Zinc and Manganese nanoparticles

Effect of graded doses of Zinc nanoparticles on growth of fungal pathogens in soybean

In present study, the effect of different concentration of Zinc (Zn) nanoparticles viz; 25, 50, 75 & 100 ppm respectively on the per cent inhibition rates of isolated pathogenic fungi viz; *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Colletotrichum truncatum*, *Alternaria alternate* and *Macrophomina phaseolina* were examined by food poison technique and the results are summarized in Table (1) and graphically represented in Fig. (1).

From the result it was observed that, the per cent inhibition rates were increased with an increase in concentration of zinc nanoparticle. In case of the per cent inhibition rates, it was observed that the growth inhibition of *Rhizoctonia solani* was found to be 11.58% at 25 ppm, 32.93% at 50 ppm, 70.00% at 75 ppm and 85.06% at 100 ppm concentrations of zinc nanoparticles respectively. Whereas comparatively in case of untreated control no inhibition was recorded, this indicates the statistically significant antimycotic property of ZnNP against *Rhizoctonia solani* over untreated control. In case of *Fusarium oxysporum,* from the result it was observed that, the diameter of fungal mycelium decreased in development, with an increase in concentration of zinc nanoparticle. The per cent inhibition rates of *Fusarium oxysporum* was found to be 23.67, 31.44, 52.18 and 71.75 per cent at 25 ppm, 50 ppm, 75 ppm and 100 ppm concentrations of zinc nanoparticles respectively. Whereas in case of untreated control no inhibition was recorded, statistically analysis revealed the significant antimycotic property of ZnNP against *Fusarium oxysporum* over untreated control.

Whereas in case of *Sclerotium rolfsii,* it was observed that the growth inhibition of *Sclerotium rolfsii* was found to be 14.10% at 25 ppm, 37.29% at 50 ppm, 52.28% at 75 ppm and 77.60% at 100 ppm concentrations of zinc nanoparticles respectively. Comparatively no statistical inhibition was recorded in untreated control set. This indicates the statistically significant antimycotic property of CuNP against *Sclerotium rolfsii* over untreated control. Similarly, in case of the per cent inhibition rates, it was observed that the growth inhibition of *Colletotrichum truncatum* was found to be 31.40%, 49.29%, 76.94% and 88.87% at 25 ppm, 50 ppm, 75 ppm and 100 ppm concentrations of zinc nanoparticles respectively. At all concentration significant antimycotic property of ZnNP against *Collectotrichum truncatum* were recorded as compared untreated control at which no inhibition was recorded.

In *Alternaria alternate*, it was observed that, the growth inhibition of *Alternaria alternate* was found to be 27.82% at 25 ppm, 52.74% at 50 ppm, 66.37% at 75 ppm and 82.92% at 100 ppm concentrations of zinc nanoparticles respectively. Whereas in case of untreated control no inhibition was recorded, this indicates the statistically significant antimycotic property of ZnNP against *Alternaria alternate* over untreated control. In case of *Macrophomina phaseolina*, from the result it was observed that, the growth inhibition of *Macrophomina phaseolina* was found to be 11.66% at 25 ppm, 27.06% at 50 ppm, 57.74% at 75 ppm and 79.83% at 100 ppm concentrations of zinc nanoparticles respectively. Whereas in case of untreated control no inhibition was recorded, this indicates the statistically significant antimycotic property of ZnNP against the mycelium development of *Macrophomina phaseolina* over untreated control.

From the studies on effect of zinc nanoparticles on the fungal growth, it was revealed that inhibitory effect was initiated at lowest concentration under study and increased with an increase in concentration of nanoparticle viz., 25 ppm up to 100 ppm. The results also revealed that the maximum antimycotic activity of zinc nanoparticle was against *Colletotrichum truncatum* 88.46 followed by *Rhizoctonia solani* 85.06, *Alternaria alternate* 82.92, *Macrophomina phaseolina* 79.83, *Sclerotium rolfsii* 77.60 and *Fusarium oxysporum* 71.75per cent respectively. The present study indicates zinc nanoparticles may be used as effective fungicides against *Colletotrichum truncatum*.

Table-1: Effect of graded doses of Zinc nanoparticles on in vitro growth inhibition of fungal pathogens in soybean.

	Inhibition rate (%)					
Treatment (ppm)	***R. solani***	***F. oxysporum***	***S. rolfsii***	***C. truncatum***	***A. alternate***	***M. phaseolina***
00(Control)	00	00	00	00	00	00
25	11.58	23.67	14.10	31.40	27.82	11.66
50	32.93	31.44	37.29	49.29	52.74	27.06
75	70.00	52.18	52.28	76.94	66.37	57.74
100	85.06	71.75	77.60	88.87	82.92	79.83
F-test	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**
SE.(m) ±	1.110111	3.112401	1.051747	1.374408	2.295993	2.074811
C.D.(P=0.05)	3.497812	9.806764	3.313915	4.330577	7.234372	6.537455

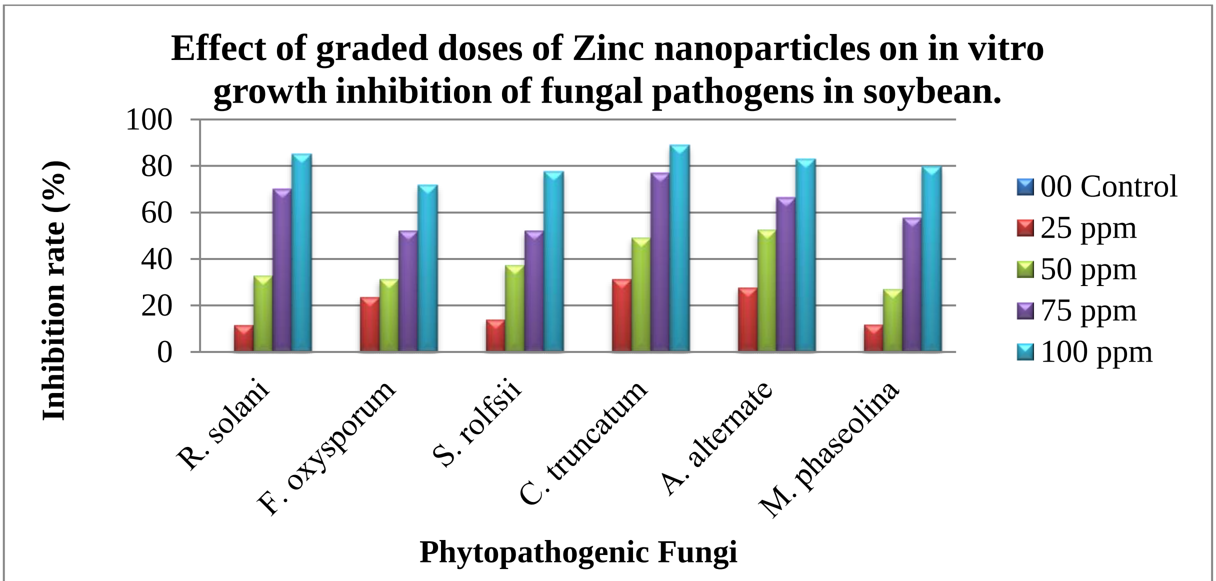

Fig-1: Effect of graded doses of Zinc nanoparticles on in vitro growth inhibition of fungal pathogens in soybean.

Our results on antifungal activity of manganese nanoparticles were also in accordance with [17], study the efficacy of different concentrations different concentration of nanoparticles of MgO, and ZnO on the spore germination of fungi such as *Alternaria alternate*, *Fusarium oxysporum*, *Rhizopus stolonifer and Mucor plumbeus*. They observed that nanoparticles caused significant inhibition in the spore germination as compared to control.

Effect of graded doses of Manganese nanoparticles on growth of fungal pathogens in soybean

In present study, the effect of different concentration of manganese (Mn) nanoparticles viz; 25, 50, 75 & 100 ppm respectively on the per cent inhibition rates of isolated pathogenic fungi viz; *Rhizoctonia solani*, *Fusarium oxysporum*, *Sclerotium rolfsii*, *Colletotrichum truncatum*, *Alternaria alternate* and *Macrophomina phaseolina* were examined by food poison technique and the results are summarized in Table (2) and graphically represented in Fig. (2).

From the result it was observed that, the per cent inhibition rate was increases with an increase in concentration of manganese nanoparticle. The per cent inhibition rate in case of *Rhizoctonia solani* was found to be 11.78% at 25 ppm, 32.21% at 50 ppm, 50.55% at 75 ppm and 80.67% at 100 ppm concentrations of manganese nanoparticles respectively. Whereas comparatively in case of untreated control no inhibition was recorded, this indicates the statistically significant antimycotic property of MnNP against *Rhizoctonia solani* over untreated control. In case of *Fusarium oxysporum*, it was observed that the growth inhibition rate of *Fusarium oxysporum* was found to be 16.01, 28.06, 47.12 and 64.00 at 25 ppm, 50 ppm, 75 ppm and 100 ppm concentrations of manganese nanoparticles respectively. Whereas in case of untreated control no inhibition was recorded, statistically analysis revealed the significant antimycotic property of MnNP against *Fusarium oxysporum* over untreated control.

Whereas in case of *Sclerotium rolfsii,* the growth inhibition rate of *Sclerotium rolfsii* was found to be 14.22% at 25 ppm, 36.44% at 50 ppm, 55.98% at 75 ppm and 77.63% at 100 ppm concentrations of manganese nanoparticles respectively. Comparatively no statistical inhibition was recorded in untreated control set. This indicates the statistically significant antimycotic property of MnNP against *Sclerotium rolfsii* over untreated control. Similarly the growth inhibition rate of *Colletotrichum truncatum* was found to be 16.25%, 29.06%, 53.00% and 79.71% at 25 ppm, 50 ppm, 75 ppm and 100 ppm concentrations of manganese nanoparticles respectively. At all concentration significant antimycotic property of MnNP against *Colletotrichum truncatum* were recorded as compared untreated control at which no inhibition was recorded.

In *Alternaria alternate,* it was observed that the growth inhibition of *Alternaria alternate* was found to be 26.18% at 25 ppm, 32.51% at 50 ppm, 53.13% at 75 ppm and 70.80% at 100 ppm concentrations of manganese nanoparticles respectively. Whereas in case of untreated control no inhibition was recorded, this indicates the statistically significant antimycotic property of MnNP against *Alternaria alternate* over untreated control. In case of *Macrophomina phaseolina*, from the result it was observed that, the growth inhibition of *Macrophomina phaseolina* was found to be 11.96% at 25 ppm, 27.25% at 50 ppm, 65.05% at 75 ppm and 85.50% at 100 ppm concentrations of manganese nanoparticles respectively. Whereas in case of untreated control no inhibition was recorded, this indicates the statistically significant antimycotic property of MnNP against *Macrophomina phaseolina* over untreated control.

From the studies on effect of manganese nanoparticles on the fungal growth, it was revealed that inhibitory effect was initiated at lowest concentration under study and increased with an increase in concentration of nanoparticle viz., 25 ppm up to 100 ppm. The results also revealed that the maximum antimycotic activity of zinc nanoparticle was against *Macrophomina phaseolina* 85.50 followed by *Rhizoctonia solani* 80.67, *Collectotrichum truncatum* 79.71, *Sclerotium rolfsii* 77.63, *Alternaria alternate* 70.80 and *Fusarium oxysporum* 64 cent respectively. The present study indicates manganese nanoparticles may be used as effective fungicides against *Macrophomina phaseolina*.

Our results on antifungal activity of manganese nanoparticles were also in accordance with [18], who observed the antifungal activity of MnNP against four funguses Viz *Candida albicans*, *Curvularia lunata*, *Aspergillus niger* and *Trichophyton simii*. Whereas, inhibitory activity against two gram-positive bacteria viz. *Staphylococcus aureus* and *Bacillus subtilis* and two gram negative bacteria Viz. *Escherichia coli* and *Staphylococcus bacillus.*

Table-2: Effect of graded doses of Manganese nanoparticles on in vitro growth inhibition of fungal pathogens in soybean.

	Inhibition rate (%)					
Treatment (ppm)	***R. solani***	***F. oxysporum***	***S. rolfsii***	***C. truncatum***	***A. alternate***	***M. phaseolina***
00(Control)	00	00	00	00	00	00
25	11.78	16.01	14.22	16.25	26.18	11.96
50	32.21	28.06	36.44	29.06	32.51	27.25
75	50.55	47.12	55.98	53.00	53.13	65.05
100	80.67	64.00	77.63	79.71	70.80	85.50
F-test	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**	**Sig**
SE.(m) ±	2.080913	2.395446	5.611886	1.414656	1.887251	2.708343
C.D.(P=0.05)	6.556681	7.547733	17.68231	4.457394	5.946479	8.53363

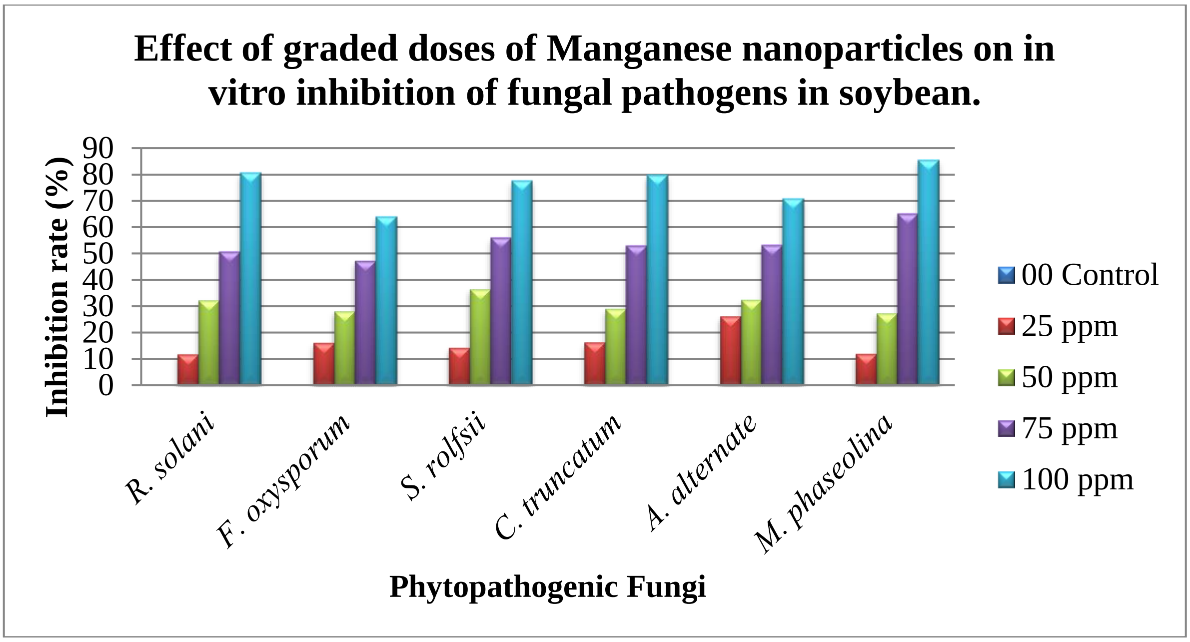

Fig-2: Effect of graded doses of Manganese nanoparticles on in vitro growth inhibition of fungal pathogens in soybean.

DISCUSSION

Management of fungal diseases of Soybean crops is economically important. Recently, a greater effort has been given to development of safe management methods that pose less danger to humans and animals, with a focus on overcoming deficiencies of synthetic fungicides. Findings from the current investigation demonstrated that Zinc and Manganese nanoparticles were very effective against plant phytopathogenic fungi. However, the current study is based on *in vitro* Petri dish evaluation. Still, data from this study provide valuable preliminary efficacy data on ZnNPs and MnNPs for use in control of plant pathogens. In this study, we analyzed the inhibition effect of two different viz., ZnNPs and MnNPs against Soybean plant pathogenic fungi viz., *Fusarium*, *Rhizoctonia*, *Sclerotium*, *Colletotrichum*, *Alternaria* and *Macrophomina* specie *in vitro*. The results suggest that both nanoparticles are capable of inhibiting these pathogens. Most fungi showed a high inhibition effect at a 100 ppm concentration of both metal nanoparticles. In addition, results indicate that the inhibition increased as the concentration of nanoparticles increased. This could be due to the high density at which the solution was able to saturate and cohere to fungal hyphe and to deactivate plant pathogenic fungi. In summary, ZnNPs and MnNPs exerted potent antifungal effects on fungi tested *in vitro*, probably through destruction of membrane integrity; therefore, it was concluded that ZnNPs and MnNPs have considerable antifungal activity. Thus, it can be effectively used against plant phytopathogenic fungi to protect the various crop plants and their products, instead of using the commercially available synthetic fungicides, which show higher toxicity to humans. Moreover, this report opens up for further research, the area of mode of action of ZnNPs and MnNPs on the phytopathogenic fungi. Further investigation for field applications is needed.

REFERENCES

1. Jin J, Liu X, Wang G, Mi L, Shen Z, Chen X, Herbert SJ. Agronomic and physiological contributions to the yield improvement of soybean cultivars released from 1950 to 2006 in Northeast China. Field Crops Research. 2010 Jan 4;115(1):116-23.
2. Hou J, Zhang P, Yuan X, Zheng Y. Life cycle assessment of biodiesel from soybean, jatropha and microalgae in China conditions. Renewable and Sustainable Energy Reviews. 2011 Dec 31;15(9):5081-91.
3. Liu X, Li Y, Han B, Zhang Q, Zhou K, Zhang X, Hashemi M. Yield response of continuous soybean to one-season crop disturbance in a previous continuous soybean field in Northeast China. Field Crops Research. 2012 Oct 25;138:52-6.
4. Shao S, Duncan AM, Yang R, Marcone MF, Rajcan I, Tsao R. Tracking isoflavones: From soybean to soy flour, soy protein isolates to functional soy bread. Journal of Functional Foods. 2009 Jan 31;1(1):119-27.
5. Tantry MA, Khan IA. Saponins from Glycine max Merrill (soybean). Fitoterapia. 2013 Jun 30;87:49-56.
6. Wang Q, Ying T, Jahangir MM, Jiang T. Study on removal of coloured impurity in soybean oligosaccharides extracted from sweet slurry by

adsorption resins. Journal of food engineering. 2012 Jul 31;111(2):386-93.
7. Miller WA, Roy KW. Mycoflora of soybean leaves, pods, and seeds in Mississippi. Canadian Journal of Botany. 1982 Dec 1;60(12):2716-23.
8. NISHIMURA S. Host-specific toxins from alternaria alternata problems and prospects. Proceedings of the Japan Academy, Series B. 1980;56(6):362-6.
9. Gordon TR, Martyn RD. The evolutionary biology of Fusarium oxysporum. Annual review of phytopathology. 1997 Sep;35(1):111-28.
10. Jasnic SM, Vidic MB, Bagi FF, Djordjević VB. Pathogenicity of Fusarium species in soybean. Matica Srpska Proceedings For Natural Sciences. 2005:113.
11. Punja ZK. The biology, ecology, and control of sclerotium rolfsh. Annual review of Phytopathology. 1985 Sep;23(1):97-127.
12. Mengistu A, Reddy KN, Bellaloui N, Walker ER, Kelly HM. Effect of glyphosate on Macrophomina phaseolina in vitro and its effect on disease severity of soybean in the field. Crop Protection. 2013 Dec 31;54:23-8.
13. Smith GS, Wyllie TD. Charcoal rot. Compendium of Soybean Disease. 1999;4:29-31.
14. Dubey RC, Kumar H, Pandey RR. Fungitoxic effect of neem extracts on growth and sclerotial survival of Macrophomina phaseolina in vitro. J. Am. Sci. 2009;5:17-24.
15. Singh A, Jain D, Upadhyay MK, Khandelwal N, Verma HN. Green synthesis of silver nanoparticles using Argemone mexicana leaf extract and evaluation of their antimicrobial activities. Dig J Nanomater Bios. 2010 Jul;5(2):483-9.
16. Chauhan S, Upadhyay MK. Fruit based synthesis of silver nanoparticles-an effect of temperature on the size of particles. Recent Research in Science and Technology. 2012 Jul 5;4(5).
17. Wani AH, Shah MA. A unique and profound effect of MgO and ZnO nanoparticles on some plant pathogenic fungi. Journal of Applied Pharmaceutical Science. 2012 Mar 1;2(3):4.
18. Jayandran M, Haneefa MM, Balasubramanian V. Green synthesis and characterization of Manganese nanoparticles using natural plant extracts and its evaluation of antimicrobial activity. Journal of Applied Pharmaceutical Science Vol. 2015 Dec;5(12):105-10.