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Effect of Nano-hexaconazole on the Phenotype and Pathogenicity of *Rhizoctonia solani* f. sp. *sasakii* causing Banded Leaf and Sheath Blight in Maize

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A study was carried out *in vitro* to know the effect of nano-hexaconazole and commercial hexaconazole on the phenotype of two highly virulent isolates *viz*., M25 and M16 of *Rhizoctonia solani* f.sp. *sasakii,* the incitant of banded leaf and sheath blight (BLSB) of maize. Nano-hexaconazole out performed in suppression of growth and sclerotial body initiation in both isolates at 0.1, 0.01 and 0.001 ppm concentrations. However, at 1ppm, both nano- and commercial hexaconazole inhibited growth and sclerotial body formation more efficiently. The effect on colony appearance, colony colour, sclerotial body colour, sclerotial aggregation, sclerotia formation on lid was not apparent. *In vivo* study under net house condition also revealed significant effect of nano-hexaconazole in restricting lesion formation on susceptible cultivar Vivek QPM -9. Disease rating (score 1-5) was also reduced upon inoculation with the fungus exposed to 0.1 and 0.01 ppm of nano-hexaconazole.

Key words: BLSB, *Rhizoctonia solani* f.sp. *sasakii*, nano-hexaconazole, phenotype, pathogenicity

Banded leaf and sheath blight (BLSB) incited by *Rhizoctonia solani* f. sp. *sasakii* Exner, was first recorded on maize in Sri Lanka[1]. It is one of the most widespread, destructive, and versatile pathogens. It is found in most parts of the world and continued devastation, causing epidemic outbreaks in maize growing countries like Bhutan, China, Indonesia, Philippines, Nepal, Vietnam, as well as in India, Africa and Latin America. The pathogen is capable of attacking a wide range of host plants, causing seed decay, damping-off, stem canker, root rot, aerial blight, and seed/ cob decay. It is the combination of its competitive saprophytic ability and high pathogenic potential that makes it persistent and destructive plant pathogen[2]. It caused losses in grain yield to the extent of 97 per cent[3]. A direct correlation with other yield parameters was exhibited in yield loss of 5 to 97.4 per cent[4].

In spite of advent of knowledge on etiology, epidemiology, host resistance, chemicals and bioagents, BLSB has still remained an Achille's heel for maize scientists all over the world. Very little progress has been achieved in development of resistant or tolerant cultivars. Chemicals, biocontrol approaches and cultural practices are still been the mainstay for minimizing the disease in South and South-east Asia. The effectiveness of fungicide hexaconazole against *R. solani* has been advocated in different parts of the world[5-10].

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But, the environmental problems associated with over use of the agrochemicals in management of disease have attracted a kin attention of the scientists. With the emergence and increase resistant to multiple antibiotics and continued emphasis on health care costs, many researchers have tried to develop new and effective antimicrobial reagents that do not stimulate resistance and are cost effective[11].

Currently, nanoparticles are thoroughly being explored and extensively investigated as potential antimicrobials. The antimicrobial activity of the nanoparticles is known to be a function of the wider surface area in contact with the microorganisms, the small size and the high surface to volume ratio i.e., large surface area of the nanoparticles enhances their interaction with the microbes to carry out a broad range of probable antimicrobial activities[12].

To overcome the adverse affect of the micronized formulations of pesticides, and to harness nanotechnology as potential green and efficient alternative, recently nano formulation of hexaconazole has been synthesized under NAIP project at IARI[13]. Keeping in view of lack of adequate study on effect of new molecules, the current study was undertaken to know the effect of novel molecule nano-hexaconazole on the phenotypic traits of highly virulent isolates of *R. solani* f. sp. *sasakii* causing BLSB in maize.

MATERIALS AND METHODS

Collection and maintenance of fungus culture

Two highly virulent isolates *R. solani* f. sp. *sasakii* M25 and M16 were collected Cereal Pathology lab., Division of Plant Pathology, IARI. These isolates were used in the present study. The hyphal tip cultures of the isolates were done on potato dextrose agar (PDA, potato-200g; dextrose-20g; agar-20g in 1000 ml distilled water at 6 to 6.5 pH) slants and incubated at 28±1°C for 6-7 days. The mother culture slants were preserved at 5°C in refrigerator. Further, these cultures were sub-cultured once in a month and used for future studies.

Collection of fungicide

Nano-hexaconazole synthesized at IARI encapsulated with poly ethylene glycol-400 having average particle size of 100nm was obtained from Division of Agricultural Chemicals IARI, New Delhi. Commercial formulation of hexaconazole was procured from market.

Effect of nano-hexaconazole on phenotypic characters

Two highly virulent isolates from above study were taken for *in vitro* study. Nano-hexaconazole and commercial-hexaconazole was tested against *R. solani* f. sp. *sasakii* by employing poisoned food technique[14]. The desired concentrations like 1ppm, 0.1ppm, 0.01ppm and 0.001ppm, were prepared by adding appropriate amount of stock solution of fungicides to PDA in Petri plates. Five replications were maintained in each treatment. PDA without fungicides served as control. Each plate was inoculated with a 5mm mycelial disc of the pathogen taken from 3 days old culture. The inoculated plates were incubated at 28±2°C. Some of the phenotypic characters were recorded from the culture. For example, colony colour was recorded using Munsell's soil colour Chart (Munsell' Colour Company, Inc., 1954), colony texture noted on the 7th day when growth of the colony completely covered the PDA plate. The radial growth of colony was recorded when maximum growth was observed in control plate and per cent inhibition was calculated by using the formula: I% = [(C - T)/C] x 100, where, I- Per cent inhibition, C- Radial growth of fungus in control and T- Radial growth of fungus in treatment[15]. Sclerotial characters *viz*., colour of the sclerotia, location of their formation and pattern of production, sclerotial number per plate were taken on the tenth day after inoculation. Sclerotial weight was taken after two months of incubation. The data obtained in the study was subjected to statistical analysis[16].

Infectivity of nano-hexaconazole treated pathogen on maize host

Virulent isolates of *R. solani* f. sp. *sasakii* were grown on PDA plates amended with nano-hexaconazole and commercial-hexaconazole at 0.1ppm and 0.01ppm by inoculating with a 5mm mycelial disc taken from 3 days old culture. After 3-5 days of incubation in BOD incubator at 28±2°C, the mycelia exposed to fungicide was cut with 8 mm cork borer and inoculated on 35 - 40 day old plant (using 2 mycelial discs) inserted between stalk and sheath at third internode level from soil. Mycelial discs taken from the PDA plate without

fungicide served as positive control, while the discs taken from plain agar for plant inoculation served as negative control. Record of disease intensity was made by 1-5 disease rating scale[17]. Observations on lesion length and rating of disease were recorded at 30 days post inoculation.

RESULTS

Effect of nano-hexaconazole on radial growth of *R. solani* f. sp. *sasakii*

The radial growth of *R. solani* f. sp. *sasakii* M25 was completely inhibited by both nano-hexaconazole and commercial-hexaconazole at the highest concentration 1 ppm as reflected inTable 1. The lowest concentration (0.001 ppm) provided maximum growth of *R. solani* f. sp. *sasakii* with an inhibition as minimum as 1.1% in nano-hexaconazole and no any inhibition in commercial-hexaconazole. Among the formulation of hexaconazole, significantly highest inhibition (42.7%) of *R. solani* f. sp. *sasakii* M25 was observed in nano-hexaconazole whereas least inhibition (36.5%) was observed in commercial formulation of hexaconazole. Per cent inhibition of the fungal growth was decreased with decrease in concentration from 1ppm (66.6%) to 0.001 ppm (0.3%) of fungicides.

Interaction effect of fungicides and their concentrations revealed that both nano and commercial formulation of hexaconazole recorded significantly higher inhibition (100.0%) of *R. solani* f. sp. *sasakii* at 1 ppm. In contrast, Commercial-hexaconazole at 0.001ppm did not show any inhibition (0.0%) of *R.solani* f. sp. *sasakii*, which was followed by 0.001 ppm of nano-hexaconazole showing 1.1% inhibition.

In case of second isolate *R. solani* f. sp. *sasakii* M16 also, significantly least inhibition (36.6%) was observed in commercial-hexaconazole and highest inhibition (48.4%) in nano-hexaconazole as observed in Table 2. Mean per cent inhibition of isolate M16 was also increased with the increase in concentration of both formulations of hexaconazole from 0.001ppm (12.3%) to 1ppm (66.7%)

It was evident from the interaction of fungicides and their concentrations that, the highest concentration 1ppm of both nano - and commercial -hexaconazole completely inhibited *R. solani* f. sp. *sasakii* (100%), which was followed by 0.1 ppm of nano-hexaconazole (52.9%). Significantly lowest inhibition (10.2%) was recorded in commercial molecule at 0.001ppm, followed by nano fungicide molecule (12.2%) at the same concentration.

Effect of nano-hexaconazole on the development of sclerotia of *R. solani* f. sp .*sasakii*

R. solani f. sp. *sasakii* M25 produced significantly lesser number of sclerotia (29.8) in presence of nano-hexaconazole as compared to the commercial formulation (67.7), while the highest number of sclerotia (155.4) was recorded in control without chemicals were shown in Table 3. Mean sclerotial number of the isolate M25 was increased with the decrease in concentrations of both fungicides from 1 ppm (51.8) to 0.001 ppm (120.1).

Interaction effect of fungicides and their concentrations on sclerotia production revealed that there was no any development of sclerotia upon treatment with both the fungicides at 1 ppm. However, significantly highest number of sclerotia (129.8) was observed in 0.001ppm of commercial-hexaconazole followed by 0.01ppm (87.4) of the same chemical.

The second isolate *R. solani* f. sp. *sasakii* M16 could produce maximum number of sclerotia (63.2) in commercial-hexaconazole treatment, while it was significantly least (31.2) in nano-hexaconazole amended medium. Control without any chemical treatment recorded highest (141.8) sclerotial numbers as reflected in Table 4. Production of the sclerotia of both fungicides was observed to be decreased with the increase in concentration of both fungicides from 0.001ppm (115.9) to 1 ppm (47.3).

Interaction of fungicides and concentrations revealed that both the fungicides under the study completely checked sclerotia production in *R. solani* f. sp. *sasakii* at 1ppm. Highest sclerotial number was recorded in commercial-hexaconazole at 0.001ppm (127.2) followed by 0.01 ppm (82.8) of same chemical, whereas least count (13.4) was found in nano-hexaconazole at 0.1 ppm.

Effect of nano-hexaconazole on sclerotial weight of *R. solani* f. sp .*sasakii*

Sclerotial weight (mg) per culture plate of *R. solani* f. sp. *sasakii* M25 treated with 1.0, 0.1, 0.01, and 0.001 ppm of nano-hexaconazole and

Table 1. Effect of nano-hexaconazole on radial growth of *Rhizoctonia solani* f.sp. *sasakii* M25

Fungicides	Concentrations (ppm)								Mean
	1.0		0.1		0.01		0.001		
	Growth* (mm)	% inhibition*	Growth (mm)	% inhibition	Growth (mm)	% inhibition	Growth (mm)	% inhibition	Percent Inhibition
Nano-hexaconazole	0.0	100.0 (89.6) **	38.2	57.6 (49.3)	78.8	12.4 (20.6)	89.0	1.1 (4.7)	42.7 (41.1)
Commercial-hexaconazole	0.0	100.0 (89.6)	52.0	42.2 (40.5)	86.4	4.0 (11.4)	90.0	0.0 (0.3)	36.5 (35.4)
Control	90.0	0.0 (0.0)	90.0	0.0 (0.0)	90.0	0.0 (0.0)	90.0	0.0 (0.3)	0.0 (0.3)
Mean	30.0	66.6 (59.9)	60.0	33.2 (30.0)	85.0	5.4 (10.7)	89.6	0.3 (1.7)	26.4 (25.6)

	C.D. at 5%	S. Em
Fungicide (F)	0.96	0.33
Concentration (C)	1.11	0.39
F x C	1.93	0.67

* Mean of five replications ** Figures in parentheses are arcsine transformed values

Table 2. Effect of nano-hexaconazole on radial growth of *Rhizoctonia solani* f.sp. *sasakii* M16

Fungicides	Concentrations (ppm)								Mean
	1.0		0.1		0.01		0.001		
	Growth* (mm)	% inhibition*	Growth (mm)	% inhibition	Growth (mm)	% inhibition	Growth (mm)	% inhibition	Percent Inhibition
Nano-hexaconazole	0.0	100.0 (89.6)	42.4	52.9 (46.7)	64.4	28.4 (32.2)	79.0	12.2 (20.4)	48.4 (47.2)
Commercial-hexaconazole	0.0	100.0 (89.6)	70.6	21.6 (27.6)	77.0	14.4 (22.3)	80.8	10.2 (16.2)	36.6 (38.9)
Control	90.0	0.0 (0.3)	90.0	0.0 (0.3)	90.0	0.0 (0.3)	90.0	0.0 (0.3)	0.0 (0.3)
Mean	30.0	66.7 (59.8)	67.7	24.8 (24.8)	77.1	14.3 (18.2)	83.3	7.5 (12.3)	28.3 (28.8)

	C.D. at 5%	S. Em
Fungicide (F)	2.79	0.97
Concentration (C)	3.22	1.13
F x C	5.58	1.95

* Mean of five replications ** Figures in parentheses are arcsine transformed values

Table 3. Effect of Nano-hexaconazole on development of sclerotia (number) and sclerotial weight of *Rhizoctonia solani* f.sp. *sasakii* M25

Fungicides	Sclerotial number* Concentrations (ppm)				Mean sclerotial	Sclerotial weight(mg)* Concentrations (ppm)				Mean sclerotial
	1.0	0.1	0.01	0.001	No.	1.0	0.1	0.01	0.001	weight
Nano-hexaconazole	0.0 (1.0) **	13.8 (3.8)	30.2 (5.5)	75.2 (8.7)	29.8 (4.7)	0.0	9.4	38.6	68.2	29.0
	0.0 (1.0)	53.6 (7.3)	87.4 (9.4)	129.8 (11.4)	67.7 (7.3)	0.0	42.8	69.4	98.4	52.6
Commercial-hexaconazole										
Control	155.4 (12.5)	155.4 (12.5)	155.4 (12.5)	155.4 (12.5)	155.4 (12.5)	115.8	115.8	115.8	115.8	115.8
Mean	51.8 (4.8)	74.3 (7.9)	91.0 (9.1)	120.1 (10.8)	84.3 (8.1)	38.6	56.0	74.6	94.1	65.8
			C.D.@ 5%	S. Em±				C.D. @ 5%	S. Em±	
		Fungicide (F)	0.09	0.03			Fungicide (F)	3.7	1.3	
		Conc. (C)	0.10	0.03			Conc. (C)	4.3	1.5	
		F x C	0.18	0.06			F x C	7.5	2.6	

* Mean of five replications ** Figures in parentheses are square root transformed values

Table 4. Effect of Nano-hexaconazole on development of sclerotia (number) and sclerotial weight of *Rhizoctonia solani* f.*sp. sasakii* M16

Fungicides	Sclerotial number* Concentrations (ppm)				Mean sclerotial	Sclerotial weight(mg)* Concentrations (ppm)				Mean sclerotial
	1.0	0.1	0.01	0.001	No.	1.0	0.1	0.01	0.001	weight
Nano-hexaconazole	0.0 (1.0) **	13.4 (3.7)	32.4 (5.7)	78.8 (8.9)	31.2 (4.8)	0.0	9.8	34.2	58.8	25.7
	0.0 (1.0)	42.6 (6.5)	82.8 (9.1)	127.2 (11.3)	63.2 (7.0)	0.0	37.8	65.6	95.8	49.8
Commercial-hexaconazole Control	141.8 (11.9)	141.8 (11.9)	141.8 (11.9)	141.8 (11.9)	141.8 (11.9)	108.8	108.8	108.8	108.8	108.8
Mean	47.3 (4.6)	65.9 (7.4)	85.7 (8.9)	115.9 (10.7)	78.7 (7.9)	36.2	52.1	69.5	87.8	61.4
	C.D@ 5%	S. Em	C.D@5%			S. Em				
	Fungicide (F)	0.14	0.04			Fungicide (F)	3.8	1.3		
	Conc. (C)	0.16	0.05			Conc. (C)	4.3	1.5		
	F x C	0.28	0.09			F x C	7.6	2.6		

* Mean of five replications ** Figures in parentheses are square root transformed values

Table 5. Effect of nano-hexaconazole on cultural and sclerotial characters of *Rhizoctonia solani* f.sp. *sasakii*

Isolate	Fungicide	Conc.(ppm)	Colony		Sclerotia				
			Appearance	Colour	Formation time (hrs)	Formation pattern	Formation on lid	Aggrigation	Colour
M25	Nano-hexaconazole	1.0	NIL	NA	NIL	NA	NA	NA	NA
		0.1	Cottony	Light brown	96	Irregular	+	+	Light brown
		0.01	Cottony	Light brown	96	Sub central and irregular	+	+	Light brown
		0.001	Cottony	Light brown	72	Irregular	+	+	Light brown
	Commercial-hexaconazole	1.0	NIL	NA	NIL	NA	NA	NA	NA
		0.1	Cottony	Light brown	96	Irregular	+	+	Light brown
		0.01	Cottony	Light brown	72	Sub central and scattered	+	+	Light brown
		0.001	Cottony	Light brown	72	Irregular	+	+	Light brown
	Control		Cottony	Light brown	72	Irregular	+	+	Light brown
M16	Nano-hexaconazole	1.0	NIL	NA	NIL	NA	NA	NA	NA
		0.1	Cottony	Light brown	96	Central and irregular	+	+	Light brown
		0.01	Cottony	Light brown	96	Irregular	+	+	Light brown
		0.001	Cottony	Light brown	96	Irregular	+	+	Light brown
	Commercial-hexaconazole	1.0	NIL	NA	NIL	NA	NA	NA	NA
		0.1	Cottony	Light brown	96	Sub-central /Irregular	+	+	Light brown
		0.01	Cottony	Light brown	72	Irregular	+	+	Light brown
		0.001	Cottony	Light brown	72	Irregular	+	+	Light brown
	Control		Cottony	Light brown	72	Irregular	+	+	Light brown

Table 6. Effect of nano-hexaconazole on the pathogenicity of *Rhizoctonia solani* f.sp. *sasakii* under net house condition

Treatment of R. solani f.sp. *sasakii*	Isolate M25						Isolate M16					
	Lesion length (cm)			Rating (1-5 scale)			Lesion length (cm)			Rating (1-5 scale)		
	0.1 ppm	0.01 ppm	Mean	0.1 ppm	0.01 ppm	Mean	0.1 ppm	0.01 ppm	Mean	0.1 ppm	0.01 ppm	Mean
Nano-hexaconazole	28.4*	37.8	33.1	2.4	3.0	2.7	31.4	38.6	35.0	2.0	2.9	2.4
Commercial-hexaconazole	39.4	51.4	45.4	2.7	3.3	3.0	41.8	45.2	43.5	3.0	3.2	3.1
Control (without fungicide)	55.4	55.4	55.4	3.7	3.7	3.7	50.4	50.4	50.4	3.7	3.7	3.7
Plain agar disc	0	0	0	0	0	0	0	0	0	0	0	0
Mean	30.8	36.1	33.4	2.9	3.3	3.1	30.9	33.5	32.2	2.9	3.2	3.0
		CD at 5%	S. Em±					CD at 5%	S. Em±			
		F	6.6	2.2				F	5.6	1.9		
		C	4.6	1.6				C	NS	1.3		
		Fx C	NS	3.2				Fx C	NS	2.7		

* Mean of five replications; +, present; -, absent; F,Fungicide; C,Concentration

commercial-hexaconazole was measured after 2 months of inoculation. Significantly least sclerotial weight (29.0) was recorded with treatment of nano-hexaconazole and comparatively higher sclerotial weight (52.6) was recorded in commercial-hexaconazole. In control (without fungicide), highest sclerotial weight was recorded (115.8) as shown in Table 3. Mean sclerotial weight of isolate M25 increased with decrease in concentration of fungicide from 1ppm (38.6) to 0.001(94.1).

Interaction effect of both fungicide and concentration revealed that at 1 ppm of both the fungicides totally suppressed sclerotia formation, and, sclerotial weight was significantly less (9.4 mg) in 0.1 ppm of nano-hexaconazole. In contrast, commercial-hexaconazole at 0.001ppm contributed significantly higher sclerotial weight (98.4 mg) which was followed by the same chemical at 0.001ppm (69.4 mg).

Sclerotial weight of *R. solani* f. sp. *sasakii* M16 upon treatment with fungicides recorded highest mean sclerotial weight (49.8 mg) in commercial-hexaconazole and lowest sclerotial weight (25.7 mg) in nano-hexaconzole, while control without any fungicide treatment recorded significantly highest sclerotial weight of 108.8 mg as shown in Table 4. Like M25, mean sclerotial weight of the isolate M16 was also increased with the reduction of fungicidal concentration from 1ppm (36.2 mg) to 0.001ppm (87.8 mg).

The interaction effect of both fungicides and their concentrations revealed that there was no sclerotia formation at 1ppm in any of the fungicides. Significantly least sclerotial weight (9.8 mg) was recorded in with nano-hexaconazole at 0.1ppm and higher sclerotial weight (95.8 mg) was observed at 0.001ppm of commercial-hexaconazole.

Effect of nano-hexaconazole on cultural and sclerotial characters of *R. solani* f. sp. *sasakii*

Both the isolates (M25 and M16) of *R. solani* f. sp. *sasakii* were failed to grow at 1ppm concentration of nano-hexaconazole and commercial-hexaconazole as reflected in Table 5. In case of isolate M25, irrespective of fungicides and their three concentrations (0.1, 0.01 and 0.001ppm), cottony growth of the colony with light brown colour was observed. Sclerotia formation was observed after 72h in control, 0.001ppm and 0.01ppm of commercial- hexaconazole and also in 0.001ppm of nano-hexaconazole. Sclerotia were

initiated after 96h in 0.01ppm and 0.1ppm of nano-hexaconazole and in 0.1ppm of commercial-hexaconazole. In most of the concentrations of both fungicides, sclerotia formation pattern was observed to be irregular except in 0.01ppm commercial-hexaconazole where it was sub-central and scattered. In 0.01ppm of nano-hexaconazole, it was sub- central with irregular pattern. Formations of sclerotia in aggregated manner and on the undersurface of lid were observed in all the treatments.

Simililarly, the colony of second isolate M16, appeared as cottony with light brown colour in both fungicides at the concentrations, 0.1, 0.01 and 0.001ppm were reflected in Fig 1. The sclerotia were formed at 72h post inoculation at 0.001 and 0.01ppm of commercial-hexaconazole treated plates and in untreated control. But, nano-hexaconazole at all the concentrations resulted in 24h delayed sclerotia formation, i.e. 96h post inoculation and, such effect was also observed in commercial-hexaconazole at 0.1ppm. The colour of scleotia was light brown in all the treatments. The sclerotia formation pattern was irregular in all the treatments except in 0.1 ppm of nano-fungicide and commercial hexaconazole where it was recorded central and irregular and sub-central and irregular, respectively. The isolate M16 also formed sclerotia in aggregation and on the undersurface of lid in all the treatments.

Fig. 1. Effect of hexaconazole 0.1ppm (Commercial-hexaconazole, B) and nano-hexaconazole 0.1ppm (C) on the growth of *Rhizoctonia solani* f. sp. *sasakii* M16 at 7 DPI

Infectivity of nano-hexaconazole treated *R.solani* f. sp .*sasakii* in maize under net house condition

In the isolate M25, significantly least lesion length (33.1 cm) was observed in nano-hexaconazole, while commercial-hexaconazole recorded higher lesion length (45.4 cm) were shown in Table 6. Control without fungicide recorded highest lesion length (55.4 cm). However, there was decrease in mean lesion with the increase in fungicide concentrations from 0.01 (36.1 cm) to 0.1 (30.8 cm). But, there was no significant difference between treatments and their concentrations interaction. The rating (1-5 scale) of disease was 2.4 and 3.0 at 0.1 and 0.01ppm nano-hexaconazole, respectively, while it was 2.7 and 3.3 at 0.1 and 0.01ppm of commercial-hexaconazole, respectively. Disease rating of 3.7 was recorded in control without treatment.

The isolate M16 caused least mean lesion length of 35.0 cm in nano-hexaconazole, while it was 43.5 cm in commercial-hexaconazole. Control without fungicide recorded highest lesion length (50.4 cm). No significant difference was observed in lesion length among various concentrations and their interaction effect in both fungicides. The mean disease rating of 2.0 and 2.9 recorded in nano-hexaconazole at 0.1 and 0.01ppm, respectively, while commercial-hexaconazole resulted 3.0 and 3.2 at 0.1 and 0.01ppm, respectively.

DISCUSSION

Management of disease through chemical, biological and cultural practices are still the mainstay as till date there is no availability of true resistant varieties in maize for BLSB disease. The widespread use of agrochemicals has certainly decreased the outbreak of fungal diseases, but at the same time has lead to the development of resistant pathogens[11], causing threat to non-target organisms and the environment due to their overuse[18]. Recently, efforts have been made to develop safe management methods that pose less danger to humans and animals, and focused on overcoming the deficiencies of synthetic fungicides. In this effort nano-particles showed possibilities for more efficient and effective control of disease because of their properties like larger surface to volume ratio they increase in contact with microbes, and thereby enhanced permeability

into cell. The current investigation showed that nano-hexaconazole could be potential and very effective fungicide for reducing sheath blight pathogen. The results obtained are discussed as below.

Among the two fungicides, higher radial growth inhibition of both isolates M25 and M16 of *R. solani* f. sp. *sasakii* was observed with nano-hexaconazole, and comparatively least inhibition was observed with commercial-hexaconazole (Table 1 and 2). This implies that nanomolecule is more effective in inhibiting the fungal growth than the micronized form of commercial molecule. The effect of hexaconazole on inhibition of mycelial growth of *R. solani* were earlier reported by many workers. Recently, it was observed toxic effect of hexaconazole EC_{50} 0.18 ppm a.i. on *R. solani*[19]. In addition, the present results followed the findings that nano molecules of hexaconazole as more effective than the commercial molecules against rice sheath blight fungus *R. solani* [20].

The inhibition of the isolates M25 and M16 decreased with decrease in fungicide concentration. Interaction of fungicides with their concentrations also recorded complete inhibition of both the isolates at 1ppm. It was observed that commercial-hexaconazole at 0.001ppm could not inhibit the growth of M25, while M16 was inhibited by 10.2 per cent. In case of nano-hehaconazole also per cent inhibition was higher in M16 than the isolate M25. Hence, the isolate M16 seemed to be more sensitive to the tested fungicides than M25. It was further showed the action of nano- and commercial hexaconazole varies with isolate to isolate and inhibition of growth is dose dependent. This is in conformity with previous reports that, antimicrobial activity of silver nano particles was variable depending on microbial species[21]. Like the present study silver nanoparticles also significantly delayed mycelial growth of *R. solani*, *Sclerotinia sclerotiorum* and *S. minor* in dose dependent manner *in vitro*[22] .

Effect of nano-hexaconazole and commercial-hexaconazole on sclerotial production of isolates M25 and M16 of *R. solani* f. sp. *sasakii* revealed that significantly least mean number was recorded with nano-hexaconazole and higher number of sclerotia formed in commercial-hexaconazole, while the control without fungicides produced significantly highest sclerotia (Table 3 and 4). Sclerotial weight (mg) per culture plate of *R. solani* f. sp. *sasakii* treated with 1.0, 0.1, 0.01, and 0.001ppm of nano-hexaconazole and commercial-hexaconazole was measured two months of post inoculation when it was completely dried. In both the isolates, least sclerotial weight recorded in the treatment of nano-hexaconazole and significantly higher sclerotial weight observed in commercial-hexaconazole. Mean sclerotial number and weight of both isolates increased with decreasing concentration of fungicides from 1ppm to 0.001ppm. Interaction effect of fungicides and their concentration on sclerotia development of both isolates also revealed complete restriction on sclerotia development at 1ppm of both the fungicides. However, significantly higher number of sclerotia and sclerotial weight were observed at 0.001ppm of commercial-hexaconazole. Thus, nano-hexaconazole outperformed in suppression of sclerotia formation. It is obvious from the study that chemical treatment was inhibitory to the sclerotia formation, particularly the nano-hexaconazole as compared to commercial hexaconazole. Previously hexaconazole was found to be highly effective in inhibiting sclerotia formation in *Rhizoctonia solani*[23]. And considerable variation in the toxicity of fungicides to *R. solani* and complete inhibition of sclerotia formation at 2.0 ppm a.i. of hexaconazole[19]. Significant inhibition of sclerotial germination was achieved using silver nanoparticles[22].

Effect of nano-hexaconazole and commercial-hexaconazole on cultural and sclerotial characters of isolates M25 and M16 of *R. solani* f. sp. *sasakii* was also studied *in vitro* (Table 5). Both the isolates could grow at lower concentrations of the fungicides, that is 0.1ppm or less. The colonies were cottony with light brown colour in all the concentrations of fungicides (Fig 1). Previously it was reported that, *R. solani* isolates had brownish pigmentation of the mycelial growth[7] . On the other hand, most of the isolates of *R. solani* were sparse and light brown in colour[23]. As against 72h time required for sclerotia formation in control, longer time was required for this event in presence of both the fungicides. It was prominent that at 0.01ppm, nano-hexaconazole delayed sclerotia formation by one day more than commercial-hexaconazole. The sclerotia formation pattern in most of the treatments observed to be

irregular except at 0.01ppm it was sub-central and irregular in nano-hexaconazole, and sub-central and scattered in commercial-hexaconazole in isolate M25 and at 0.1ppm, it was central and irregular in nano-hexaconazole, and sub-central and irregular in commercial-hexaconazole in isolate M25. This variation in sclerotia formation could not be reasoned out. The isolate wise variability in sclerotial characters reported as light brown, brown, dark brown and black in colour and formation pattern as central, sub-central ring, peripheral ring, scattered and irregular[24]. Formation of sclerotia in aggregated manner and the undersurface of the lids of Petri plates were observed in all the treatments. Although, nano-hexaconazole was found to play definite role in delaying sclerotial body formation as compared to commercial-hexaconazole, it did not exhibit a clear role in colony colour, colony appearance, sclerotial colour, sclerotial aggregation and sclerotia formation pattern of the test isolates of *R. solani* f. sp. *sasakii*.

Our study has established that nano-hexaconazole is superior to the commercial hexaconazole in controlling *R. solani* f. sp. *sasakii*. However, another study was carried out within a confined net house condition to know if any alteration occurred in pathogenicity of *R. solani* f. sp. *sasakii* under the influence of nano-hexaconazole. Vivek QPM-9, a susceptible cultivar was inoculated with both isolates of *R. solani* f. sp. *sasakii* previously exposed to sub lethal dose of nano- and commercial hexaconazole.

Significantly least lesion length was recorded in nano-hexaconazole compared to commercial-hexaconazole in both the isolates whereas highest lesion lengths were measured in control (without fungicide) (Table 6). Moreover, there was decrease in mean lesion formation with the increase in fungicide concentrations from 0.01 to 0.1ppm in isolate M25. But, it was not significant in case of M16 and also no significant difference was observed in the interaction between fungicides and their concentrations. The disease score in nano-hexaconazole was less than commercial-hexaconazole in both isolates of pathogen. Lessening in the lesion length in nano-hexaconazole poisoned mycelium might be attributed by more damage on hyphal walls caused by nano-particles that resulted into aggravated plasmolysis[22]. The outstanding protectant, curative, translaminar and systemic properties of hexaconazole enable it to be used effectively against various diseases at very low application rates[25]. The redistribution of fungicide, hexaconazole within the leaf and other tissue enhanced sheath blight disease control in untreated parts remote from the sprayed tissue, thus increasing overall effectiveness against *R. solani*[26]. Previous studies indicate that isolates also differ when fungicides are evaluated under field and greenhouse conditions[27]. However, there is a need of additional field and green house studies to establish the full potentiality of nano-hexaconazole.

CONCLUSION

To conclude, little is known about application of nanotechnology in the management of crop diseases and the most of studies have so far been focused on the control of bacterial and viral pathogens in animals. Here, we evaluated efficacy of nano-hexaconazole *in vitro* and *in vivo* against of *R. solani* f. sp. *sasakii* causing BLSB disease in maize. Our data demonstrated that nano-hexaconazole as compared to commercial-hexaconazole has strong inhibitory activity on mycelia growth, sclerotial body formation, reduced sclerotial weight, delayed initiation of sclerotia, reduced lesion development on host plant *etc*. The result also suggest that bioactivity of nano-hexaconazole is many fold higher as compared to conventional formulations. Therefore, nano-hexaconazole could be a potential double edged weapon for managing disease in the coming days.

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