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Nano molecules and chemical approaches for effective management banded leaf and sheath blight in maize

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Abstract

The aim of this work was to evaluate efficacy of nano formulation and fungicides against Banded Leaf and Sheath Blight in maize to identify the effective management tools. For this purpose two field and lab experiments were conducted during 2020 and 2021. A significant reduction in the severity of the symptoms of *Rhizoctonia solani* was found after the use of Azoxystrobin + tabaconazole (100% reduction in Mycelial growth) and Azoxystrobin (100% reduction), control of BLSB of maize *In vitro* at 250, 500 and 750 PPM, whereas minimum mycelial growth inhibition recorded 68.89 percent in Fluxopyroxad + Pyraclostrobin followed by Sedexane with 67.50 percent respectively. Under artificially inoculated conditions in field in both tested seasons *kharif* 2020 and 2021, Azoxystrobin + tabaconazole and Azoxystrobin were provided best efficacy by minimum PDI and disease rating with maximum grain yield. However, this positive effect was still evident variations in effectiveness of fungicides were observed during the same treatment in repeated trials.

Keywords: *Rhizoctonia solani*, concentration, fungicides, management

Introduction

Maize (*Zea mays*) is prominent cereal worldwide after wheat and rice with 1211.64 m ton production and 5573 kg/ha. Productivity from 191.89 m ha area (Anno 2020). India stand 4th in terms of area and 7th in production among maize growing countries, which mean India has 4% of area and contributes 2% in production of maize, globally India stat.2018-19. Now widespread banded leaf blight and sheath blight are found in the provinces of Himachal Pradesh, Assam, Meghalaya, Uttar Pradesh, Nagaland, Jammu Kashmir, Haryana, Uttarakhand, Punjab, Sikkim, Madhya Pradesh, Delhi, Rajasthan, Orissa, Andhra Pradesh and West Bengal has also reached the stage of serious maize disease and Loss of grain yields can range from 20-40 percent, up to 100 percent (Madhavi *et al.*, 2011, Izhar and Chakraborty, 2013) [8, 5]. The BLSB symptoms are observed during post flowering and pre-harvest stage. Lal *et al.*, (1980) [6] reported that disease is predominantly found in Rice-Maize sequence of cropping. Disease spread required high humidity and temperature. Presently it is causing much more losses in yield comparing to earlier reports where 31.9% reduction in grain yield in popular maize cultivars with disease severity level up to 87.3% has been recorded. However, higher the severity, losses will be higher upto 100%. Lu *et al.*, (2012) [7] studied the BLSB pathogen extensively and reported that this pathogen is soil borne, it first infects and appears on leaves near to ground level and ultimately causes ear rot by extending itself to ears. The pathogen spreads rapidly causing cracking of ear sheath and premature drying. Finally plant debris and the cobs are impregnated by the fungus. Since the stalk rot of maize is a complex disease involving more than one organism, it is very difficult to manage the disease with single control measure. Beside them, Bioagents are alternative green approaches for effective and ecofriendly management strategies for soil borne pathogen propagules like sclerotia of *Rhizoctonia* species without any harmful residual effect on soil and human being. In present study, we tried to manage the BLSB using of biocontrol agents and organic ITK's formulations which applied to evaluate the efficacy against *Rhizoctonia* spp. *in vitro* as well as in field after artificial inoculations.

Materials and Methods

Chitosan oligomers were synthesized for use as nano formulation using 2g chitosan which was dissolved in 150 ml water followed by sonication. 38.46 ml of sonicated chitosan was mixed with 61.54 ml of acetate buffer (pH 4.8). Later on, 0.5% lipase was added to it and solution was incubated for 3hrs at 55 °C.

The supernatant so prepared were sucked out and stored in the room temperature for use in the experiments. Laboratory prepared nano formulation were used as 2,12.5,25 and 50% v/v as T-1, T-2, T-3 and T-4. The foliar spray was applied before 10 days of inoculation of pathogen i.e. BLSB and after appearing of disease symptoms.

The field experiment for disease assessment and plant growth, seeds of disease susceptible maize cultivar ‘Surya local’ were surface sterilized with 10% sodium hypochlorite for 10 min and further treated for 4h with different concentrations of nano-formulation (2, 12.5, 25 and 50% v/v) along with control (water). Treated seeds were dried and sown in field and afterwards following all standard agronomic practices to management of maize crop. The plants were subjected to first foliar spray of nano formulation (2, 12.5, 25 and 50%, v/v) until runoff at 35 days of sowing that is just 10 days before disease inoculation (Table 1). Inoculum of *R. solani*, prepared on barley seed medium, was inoculated on experiments plot. In brief, *R. solani* culture was seeded on medium papered using barley seeds and kept at 27±1 °C for 15 days to achieve adequate mycelial growth and sporulation. The inoculums of seeds (1-3) was kept benth of leaf sheath for disease development in maize plants. Further, disease assessment was performed after appearance of symptoms on leaves after 15 days of inoculation. Disease intensity was recorded on 1 to 9 standard disease rating scale and PDI (percent disease intensity) and percent efficacy of disease control (PEDC) were calculated by using the formula given by

$$PDI = \frac{\text{Sum of all individual disease ratings}}{\text{Total no. of plant access seed} \times \text{Maxi. rating}} \times 100$$

$$PEDC = \frac{\text{Disease severity in control} - \text{disease severity in treatment}}{\text{Disease severity in control}} \times 100$$

Observations to be recorded as Disease progress in treated and control, PDI in all treatments and PEDC of all treatments.

In vitro efficacy of fungicides against pathogen

In vitro efficacy of above eight mentioned fungicides were adjudged using ‘‘Poisoned Food Technique’’ given by Nene and Thapliyal, 1993. Concentrations of fungicides were maintained 250, 500 and 750 ppm. PDA was poisoned by calculated. Twenty ml of poisoned PDA media was poured in Petri plate having diameter of 90mm. After solidification of media 5mm mycelial disc were inoculated inverted down in center of Petri plate. Four replication of each concentration was taken and Petri plate inoculated with mycelial disc without none fungicides taken as Control. Inoculated Petri plates are incubated at 28 ± 2 °C. After 10 days of the inoculations diameter of the mycelia growth was measured in mm. Efficacy was calculated for every fungicide and percent

(%) inhibition was calculated by the formula according to Vincent’s formula (1947) as follows:

$$\text{Per cent inhibition (I)} = \frac{C - T}{C} \times 100$$

Where

C = Colony diameter in control

T = Colony diameter in treatment

In vivo efficacy of fungicides against pathogen

Field management trails were laid out during crop season *Kharif* 2020 and *Kharif* 2021 for assessment of fungicidal efficacy in RBD plot design. Local susceptible cultivar Surya was sown by dibbling method. Three rows were sown in one plot as one replication was taken and spacing was maintained 60cm × 30 cm. Randomized Block Design was used with three replications. All agronomical practices were followed as per recommended package of practices. After 30-35 days of for inoculation, inoculation was used just after flowering as per methods. After appearance of initial symptoms planned doses of fungicides were applied at various concentrations that are 0.2%, 0.3% and 0.5%. The disease scoring and calculations of PDI was recorded at drying stage for each treatment. Grain yield of the each treatment was taken at the time of crop harvesting.

In vivo efficacy of bio agents and organic ITK’s against pathogen

Field management trails were laid out during crop season *Kharif* 2020 and *Kharif* 2021 for assessment of bio agents and organic ITK’s efficacy in RBD plot design. Susceptible variety Surya was sown in four rows were sown in one plot as one replication was taken and spacing was maintained 60cm × 30cm. RBD was used with four replications. All field practices were also followed as per recommended package of practices. After 30-35 days of for artificial inoculations. Three bio control and six organic ITK’s received from Organic unit, Rajasthan College of Agriculture, Udaipur. After appearance of initial symptoms planned doses of bioagents (1% and 2%) and organic formulations (10% and 15%) were sprayed under field conditions in both *Kharif* seasons 2020 and 2021. The disease scoring and calculations of PDI was recorded at drying stage of crop for each treatment. Grain yield of the each treatment was taken at harvesting time of crop.

Disease rating score and calculate percent disease index (PDI): Observations were made on the appearance of disease symptoms as well as the severity of the disease and described the disease severity on a 0 to 9 standard disease rating scale. The table below contains information about the disease rating scale and the percentage of disease incidence.

Table 1: Rating scale

Disease Score	PDI	Symptom of disease	Reaction
0	0	No	I
1	0.1-2.0	Disease spots below 4 th sheath under ear	HR
2	2.1-4.0	Disease spots below 3 rd sheath under ear	R
3	4.1-5.0	Disease spots below 2 nd sheath under ear	MR
5	5.1-6.0	Disease spots below 1 st sheath under ear	MS
7	6.1-7.0	Disease spots upto ear	S
9	7.1 and above	Disease spots complete cover ear	HS

I = Immune, HR = highly resistant, R = resistant, MR = moderately resistant, MS = moderately susceptible, S = susceptible, HS = highly susceptible.

The standard formula was used to calculate the percent disease incidence (PDI) and percent efficacy of disease control (PEDC) (Wheeler, 1969) [9].

$$\text{Percent disease incidence} = \frac{\text{No. of infected plants}}{\text{Total no. of plants assessed}} \times 100$$

Results and Discussion

In vitro Efficacy of bio agents against the *Rhizoctonia* spp.

The tested fungicides were computed for percent inhibition of growth over the control and selected fungicides showed percent inhibition of mycelia growth. The maximum inhibition of mycelia growth was recorded 100 percent at 500 ad 750 ppm concentration whereas, least was 58.89 percent at the 250 ppm concentration. Among the tested fungicides maximum percent inhibition of mycelium growth was recorded Azoxystrobin 11% + tabuconazole 18.30% with 100 percent followed by Azoxystrobin 23% SC with 100 percent inhibition at 500 and 750 PPM concentrations. Minimum mycelial growth inhibition recorded 58.89 percent in Mancozeb 75% WP followed by carbendazim 50% WP and Mancozeb + pyraclostrobin with 63.00 percent and 66.67 percent at 250 PPM concentration, respectively.

Manage the disease through Nano formulation and Fungicides in field under artificial inoculations

Disease data were recorded after visible appearance of symptoms (~20 days after inoculation) using 0 to 9 standard disease rating scale. Two years (*Kharif* 2020 and *Kharif* 2021) field experiments were conducted, and two years data were pooled to explicit the results. Pooled data revealed that maximum PDI (75.8%) was observed in control plants, followed by 68.7% PDI in 2% nano formulation (Table 2). The minimum PDI (5.68%) was recorded in 50% nano formulation treatment, which was significantly lower to control as well as with other concentrations of nano formulation. Amongst the treatments, significant control on disease i.e. PEDC (25.57%) was recorded in 50% nano-formulation application. Pooled data of two years further revealed that; yield/plot was significantly higher in all nano formulation application compared to control. Maximum yield/plot was recorded 1195.9 gm/plot in 50% nano formulation followed by 1148 gm/plot in 25% nano formulation treatment. Whereas in control minimum yield/plot, 766.36 gm/plot was observed.

Seven fungicides namely Azoxystrobin + tabuconazole, Mancozeb + pyraclostrobin, Mancozeb 75% WP, Carbendazim 50% WP, Azoxystrobin 23% SC, Sedexane, Fluxopyroxad + pyraclostrobin were tested under epiphytotic conditions (*in vivo*) at three different concentrations *viz.* 0.2%, 0.3% and 0.5%. susceptible maize cultivar Surya was sown at 30X60 cm spacing and 4 replication of the each treatment was maintained under field conditions. Maize plants at flowering stage (55-65 days) were inoculated with different isolates of *R. solani*. After initiation of the BLSB symptoms, above dosages of the fungicides applied at twice at 15 days interval. The inoculated control showed 78.00 percent disease index, while all the treatments showed significantly less disease that ranged from 13.30 to 32.5 percent.

Results for the first tested season (*Kharif* 2020) showed that

(Table 3, 4, 5 and 6) disease rating for each fungicidal concentration differed significantly. Among the individual treatments in *Kharif* 2020, T-1 (Azoxystrobin 11% + tabuconazole 18.30%) showed minimum Disease rating 1.25, 1.00 and 0.50 with PDI 24.25, 22.22 and 13.33 percent in susceptible variety surya with the yield 1850gm, 1875gm and 1890 gm per plot, at 0.2, 0.3 and 0.5% concentration, respectively. T-5 (Azoxystrobin 23% SC) was next best fungicide with lower disease rating 2.00, 1.50 and 0.50 with 28.88, 25.66 and 15.30 percent PDI with the yield in variety surya 1778gm, 1805gm and 1838gm per plot at 0.2, 0.3 and 0.5 percent concentration, respectively followed by T-7 (Fluxopyroxad + Pyraclostrobin) and T-6 (Sedexane 700MF.) with disease rating 2.50, 1.50 and 1.00 with the mean PDI 32.32, 27.57 and 18.50 percent and 2.50, 2.00 and 1.00 with the mean PDI that was 33.33, 31.65 and 22.50 percent at 0.2, 0.3 and 0.5 percent concentration, respectively with the yield 1605gm, 1640gm and 1700gm and 1475gm, 1508gm and 1590gm per plot, respectively T-2 (Mancozeb + Pyraclostrobin) showed 3.00, 2.50 and 2.00 disease rating with mean PDI 41.11, 35.00 and 24.60 and yield 1390gm, 1402gm and 1480gm at different concentrations i.e. 0.2, 0.3 and 0.5 percent, respectively followed by T-4 (carbendazim 50% WP) with disease rating 4.00, 3.00 and 2.00 with mean PDI 43.33, 34.00 and 28.25 percent at 0.2, 0.3 and 0.5% concentration, respectively. Yield were obtained minimum in Mencozeb 75% WP treatment that was 1055gm, 1082gm and 1110 gm per plot at 0.2, 0.3 and 0.5% concentration, respectively (Plate-14).

Similar trend of the observations also found for the next tested season *viz.* *Kharif* 2021. Disease rating was minimum 1.50, 1.00 and 0.50 for T-1 (Azoxystrobin 11% + Tabuconazole 18.30%) in all tested concentrations 0.2%, 0.3% and 0.5% respectively with lowest PDI 26.66%, 24.44% and 13.30% with the grain yield 1840gm, 1870gm and 1892gm respectively. Second best fungicide was T-5 (Azoxystrobin 23% SC) at all three tested concentrations 0.2, 0.3 and 0.5% that showed minimum disease rating 2.00, 1.25 and 0.50 with mean PDI 27.30, 25.60 and 15.10 percent with grain yield 1780 gm, 1803gm and 1840 gm per plot respectively. Whereas, T-7 and T-6 were least effective at various concentrations in field in *kharif* 2021. T-3 (Mancozeb 75% WP) showed least effective with highest disease rating 4.50, 3.50 and 2.00 at different tested concentrations *viz.* 0.2, 0.3 and 0.5% respectively with highest 48.88, 45.55 and 32.50 with grain yield 1050, 1080.01gm and 1113gm per plot followed by T-4 (carbendazim 50% WP) with disease rating 4.00, 3.50 and 2.00 with mean PDI 44.44, 38.88 and 28.00 percent with the grain yield, 1260gm, 1290gm and 1355gm per plot at 0.2, 0.3 and 0.5 percent concentration.

T-1 (Azoxystrobin 11% + Tabuconazole 18.30%) and T-5 (Azoxystrobin 23% SC) was found best suitable fungicides in *kharif* 2020 ad 2021 for the field management which gave lowest PDI 13.30% and 15.20% PDI with lower disease rating 0.50 and 0.50 at higher tested concentration (0.5%) with maximum grain yield i.e. 1892gm and 1840gm per plot, respectively.

Pooled analysis for both tested season indicated that T-1 (Azoxystrobin 11% + Tabuconazole 18.30%) gave best results in all aspects. Azoxystrobin 11% + Tabuconazole 18.30% gave disease score of 2.35, 2.15 and 1.05 with 25.46%, 23.33% and 13.32% PDI at 0.2%, 0.3% and 0.5% concentration respectively. Grain yield was significantly

higher than other fungicides viz. 1845gm, 1872.52 and 1891.00gm per plot. Disease rating for all fungicides ranges

between 0.05 to 8.00. PDI ranged between 13.30% -78.00% and range of grain yield was from 1891gm-745 gm per plot.

Table 2: Efficacy of different fungicides on *Rhizoctonia solani* causing BLSB in maize against at varying concentrations using poisoned food technique

Treatments	Mycelial growth (mm) at different conc.			Percent mycelial growth inhibition at different conc.		
	250 ppm	500 ppm	750 ppm	250 ppm	500 ppm	750 ppm
T 1 Azoxystrobin 11% + tabuconazole 18.30%	13.00	0.00	0.00	75.56 (60.37)	90.00 (71.57)	90.00 (71.57)
T 2 Mancozeb 60%+Pyraclostrobin 5%WG	21.00	17.00	0.00	66.67 (54.74)	71.11 (57.49)	90.00 (71.57)
T 3 Mancozeb 75%WP	28.00	21.50	17.00	58.89 (50.12)	66.11 (54.40)	71.11 (57.49)
T 4 carbendazim 50% WP	24.30	19.00	13.00	63.00 (52.54)	68.89 (56.10)	75.56 (60.3)
T 5 Azoxystrobin 23% SC	17.25	0.00	0.00	70.83 (57.31)	90.00 (71.57)	90.00 (71.57)
T 6 Sedexane 700MF	20.25	14.30	0.00	67.50 (55.25)	74.11 (59.42)	90.00 (71.57)
T 7 Fluxopyroxad + Pyraclostrobin	19.00	13.50	0.00	68.89 (56.15)	75.00 (60.00)	90.00 (71.57)
T8 control	90.00	90.00	90.00	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)
	Conc.	Fungicide	Con x Fungicide	Conc.	Fungicide	Con x Fungicide
S.Em±	0.2193	0.6244	0.8369	0.151	0.247	0.427
CD at 5%	0.3582	1.0196	1.3666	0.430	0.703	1.216

*Data are average of 4 replications **Figures in parentheses are arcsine $\sqrt{\text{percent angular transformed values}}$

Table 3: Evaluation of different fungicides to control BLSB under artificial inoculations in field (Two Way Mean Table)

Treatments	Rating 2020			Rating 2021			Pooled		
	0.20%	0.30%	0.50%	0.20%	0.30%	0.50%	0.20%	0.30%	0.50%
T Azoxystrobin 11% + Tabuconazole 18.30%	1.25	1.00	0.50	1.50	1.00	0.50	2.35	2.15	1.05
T ₂ Mancozeb 60%+ Pyraclostrobin 5%WG	3.00	2.50	2.00	3.50	3.00	1.50	3.60	3.35	3.05
T ₃ Mancozeb 75% WP	5.00	4.00	2.50	4.50	3.50	2.00	5.10	4.90	4.05
T ₄ Carbendazim 50% WP	4.00	3.00	2.00	4.00	3.50	2.00	4.20	4.05	3.45
T ₅ Azoxystrobin 23% SC	2.00	1.50	0.50	2.00	1.25	0.50	2.60	2.40	1.45
T ₆ Sedexane 700MF	2.50	2.00	1.50	3.00	2.50	1.50	3.18	3.00	2.10
T ₇ Fluxopyroxad + Pyraclostrobin	2.50	1.50	1.00	2.50	2.00	1.00	3.05	2.75	1.81
T ₈ control	7.00	7.50	7.00	7.00	7.25	8.00	7.6	7.40	7.40
	Conc.	Fungi.	Con x Fungi.	Conc.	Fungicide	Con x Fungicide	Conc.	Fungicide	Con x Fungicide
S.Em±	0.05	0.03	0.09	0.07	0.04	0.12	0.04	0.02	0.07
CD at 5%	0.14	0.09	0.25	0.19	0.12	0.34	0.12	0.07	0.22

Table 4: Evaluation of different fungicides to control BLSB under artificial inoculations in field (Two Way Mean Table)

Treatments	PDI 2020			PDI 2021			Pooled		
	0.20%	0.30%	0.50%	0.20%	0.30%	0.50%	0.20%	0.30%	0.50%
T ₁ Azoxystrobin 11% + Tabuconazole 18.30%	24.25 (29.49)	22.22 (28.12)	13.33 (21.41)	26.66 (31.07)	24.44 (29.55)	13.30 (21.39)	25.46 (30.28)	23.33 (28.84)	13.32 (21.40)
T ₂ Mancozeb 60%+ Pyraclostrobin 5%WG	41.11 (39.79)	35.00 (36.27)	24.60 (29.73)	43.33 (41.17)	36.63 (37.13)	24.80 (29.87)	42.22 (40.48)	35.81 (36.70)	24.70 (29.80)
T ₃ Mancozeb 75%WP	45.50 (42.42)	43.00 (40.97)	30.27 (33.37)	48.88 (44.36)	45.55 (42.45)	32.50 (34.76)	47.19 (43.39)	44.28 (41.71)	31.39 (34.06)
T ₄ Carbendazim 50% WP	43.33 (41.17)	34.00 (35.66)	28.25 (32.10)	44.44 (41.81)	38.88 (38.57)	28.00 (31.95)	43.89 (41.49)	36.44 (37.11)	28.13 (32.02)
T ₅ Azoxystrobin 23% SC	28.88 (32.50)	26.66 (31.08)	15.30 (23.02)	28.88 (32.50)	26.00 (30.66)	15.20 (22.94)	28.88 (32.50)	26.33 (30.87)	15.25 (22.98)
T ₆ Sedexane 700MF	33.33 (35.26)	31.65 (34.23)	22.50 (28.31)	35.55 (36.60)	33.33 (35.26)	22.80 (28.52)	34.44 (35.93)	32.49 (34.75)	22.65 (28.42)
T ₇ Fluxopyroxad + Pyraclostrobin	32.32 (34.64)	27.57 (31.67)	18.50 (25.47)	33.33 (35.26)	30.00 (33.21)	18.00 (25.10)	32.83 (34.95)	28.79 (32.44)	18.25 (25.29)
T ₈ control	77.50 (61.72)	75.00 (60.09)	75.00 (60.04)	74.00 (59.37)	78.00 (62.15)	78.00 (62.08)	75.75 (60.54)	76.50 (61.12)	76.50 (61.06)
	Conc.	Fungi.	Con x Fungi.	Conc.	Fungicide	Con x Fungicide	Conc.	Fungicide	Con x Fungicide
S.Em±	0.95	0.58	1.64	0.97	0.59	1.69	0.70	0.43	1.22
CD at 5%	2.71	1.66	4.69	2.77	1.69	4.80	1.97	1.21	3.43

*Data are average of 4 replications **Figures in parentheses are arcsine $\sqrt{\text{percent angular transformed values}}$

Table 5: Evaluation of different fungicides on grain yield under artificial inoculations in field (Two Way Mean Table)

Treatments	Yield (gm/plot) 2020			Yield (gm/plot) 2021			Pooled		
	0.20%	0.30%	0.50%	0.20%	0.30%	0.50%	0.20%	0.30%	0.50%
T Azoxystrobin 11% + Tabaconazole 18.30%	1850.00	1875.03	1890.00	1840.00	1870.00	1892.00	1845.00	1872.52	1891.00
T ₂ Mancozeb 60%+ Pyraclostrobin 5% WG	1390.00	1402.00	1480.00	1383.00	1410.00	1481.67	1386.50	1406.00	1480.84
T ₃ Mancozeb 75% WP	1055.00	1082.00	1110.00	1050.00	1080.01	1113.00	1052.50	1081.01	1111.50
T ₄ Carbendazim 50% WP	1270.00	1300.00	1360.00	1260.00	1290.00	1355.00	1265.00	1295.00	1357.50
T ₅ Azoxystrobin 23% SC	1778.00	1805.00	1838.00	1780.00	1803.00	1840.00	1779.00	1804.00	1839.00
T ₆ Sedexane 700MF	1475.00	1508.00	1590.00	1468.00	1500.00	1587.00	1471.50	1504.00	1588.50
T ₇ Fluxopyroxad + Pyraclostrobin	1605.00	1640.00	1700.00	1610.00	1638.00	1698.00	1607.50	1639.00	1699.00
T ₈ control	760.00	755.00	750.00	745.00	760.00	765.00	752.50	757.50	757.50
	Conc.	Fungi.	Con x Fungi.	Conc.	Fungicide	Con x Fungicide	Conc.	Fungicide	Con x Fungicide
S.Em±	44.12	27.02	76.42	33.28	20.38	57.64	27.28	17.43	48.9
CD at 5%	125.6	76.9	217.56	94.44	57.83	163.58	79.31	48.64	137.5

*Data are average of 4 replications **Figures in parentheses are arcsine $\sqrt{\text{percent angular transformed values}}$

Table 6: Effect of different concentration of Nano formulation

Treatment (%)	PDI (%)			PEDC (%)			Yield (gm/plot)		
	2020	2021	Pooled	2020	2021	Pooled	2020	2021	Pooled
Control	75.13±0.5	76.63±1.6	75.88±0.7	0.00±0.00	0.00±0.00	0.00±0.00	765.22±15.0	767.50±22.1	766.36±16.5
Nano formulation (%)									
2	68.49±4.43	68.94±4.92	68.71±4.59	8.63±5.88	11.63±6.3	10.13±5.9	976.17±105.4	977.33±110.5	976.75±107.7
12.5	62.68±6.08	63.11±6.04	62.89±6.01	16.36±8.08	15.84±8.0	16.10±8.0	1076.83±162.4	1057.00±157.6	1066.9±159.1
25	59.12±8.14	59.30±8.14	59.21±8.07	21.65±10.7	21.41±10.6	21.53±10.6	1152.54±196.4	1145.00±181.7	1148.77±188.2
50	56.22±9.28	57.49±10.1	56.85±9.70	25.03±12.3	26.30±13.0	25.67±12.6	1209.15±210.2	1182.67±195.8	1195.91±210.9
S.Em±	0.59	0.66	0.44	0.860	0.694	0.552	17.814	16.450	12.124
CD at 5%	1.70	1.89	1.25	2.447	1.976	1.552	50.710	46.827	34.052

*Data are average of 5 replications **Figures in parentheses are arcsine $\sqrt{\text{percent angular transformed values}}$

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