

CU-CHITOSAN NANOPARTICLES AGAINST MAIZE POST FLOWERING STALK ROT DISEASE IN FIELD CONDITION

MANJU KUMARI CHOUDHARY^{1*} AND SAVITA BUDANIA²

¹Department of Molecular Biology and Biotechnology, Rajasthan College of Agriculture, Maharana Pratap University of Agriculture & Technology, Udaipur-313001 (Rajasthan), India

²Department of Veterinary Microbiology, Lala Lajpat Rai University of Veterinary and Animal Sciences, Hisar-125004 (Haryana), India

*(e-mail : manjuch44@gmail.com)

(Received : 15 December 2021; Accepted : 30 December 2021)

SUMMARY

In current context, there is an imperative need to conduct rigorous research on biopolymer-derived substances. In this study, we evaluated the effect of Cu-chitosan nanoparticles against Post Flowering Stalk Rot (PFSR) under field condition in maize. Cu-chitosan nanoparticles were prepared using ionic gelation technique. After disease inoculation inhibition effect of nanoparticles were analyzed to determine antifungal activities against PFSR. In field experiments it is proved that Cu-chitosan nanoparticles at the concentration of 0.06% expressed strong antifungal activity in controlling PFSR disease in 4hrs treated surya local variety. Thus, our study shows that Cu-chitosan nanoparticles could be a new generation for disease control in field condition.

Key words : Cu-Chitosan nanoparticles, Maize, PFSR, ionic gelation

Maize (*Zea mays* L.) is one of the highly demanding crops for food, livestock feed and energy sector. It is one of the most adaptable crop in diverse environment and third most important cereal crop after wheat and rice (Grassini *et al.*, 2011). Post flowering stalk rot (PFSR) is one of the most serious, destructive and widespread disease in maize. Pathogens and pests associated with plants are controlled by more than 5 million metric tons of pesticides annually worldwide (Shekhar *et al.*, 2010). Available immense sources of biomaterials can be researched and utilized in modulated way in plant disease control. Functionalized biomaterial based formulation would work in multi-dimensional mode to strengthen the plant defense system along with antimicrobial activity. Increasing use of synthetic fungicides has created serious problem of resistance development in fungal organism (Chen *et al.*, 2014; Sathiyabama and Manikandan, 2016). Chitosan, a biopolymer has been used to control plant disease (Photchanachai *et al.*, 2006). In present study, we evaluated the effect of Cu-chitosan nanoparticles against PFSR under field condition.

MATERIALS AND METHODS

Laboratory made Cu-Chitosan-nanoparticles (CuCH-NPs) were used in this research. Chitosan and Na- tri-polyphosphate (Na₃P₃O₁₀) were used to develop

CuCH-NPs. PFSR culture and varieties used in the present investigation were acquired from the Plant Pathology Unit, RCA, MPUAT, Udaipur, India.

Synthesis and characterization of Cu-chitosan NPs

Ionic gelation method was used for the synthesis of Cu-chitosan NPs. Mean hydrodynamic diameter, polydispersity index (PDI) and zeta potential on Particle size analyzer ZS 90 (Malvern, UK) at 25° C in triplicates were characterized as in prior reports (Choudhary *et al.*, 2017).

Field assay : Field experiments were performed to evaluate the effects of Cu-chitosan nanoparticles on pathogenic fungi at experimental field, Rajasthan College of Agriculture, Udaipur. Seeds of varieties (Pratap Hybrid-1 and Surya local) were treated with Cu-chitosan NPs with different concentrations of Cu-chitosan NPs (0.02, 0.06, 0.1, and 0.14% w/v) along with control-I (without water), control- II (without treatment), bulk chitosan and commercial fungicide in aqueous suspension for 4 hrs. The experiments were carried out on maize plants infected with PFSR caused by *Fusarium verticillioides* in field. Active culture of *F. verticillioides* was used as suspension in aqueous and inoculated at flowering stage by using toothpick method of inoculation (Young, 1943; Singh and Kaiser, 1989; Desai and Hegde, 1992). For

TABLE 1
Rating scale for PFSR disease

Description	Disease rating
Brownish and blackish discolouration only at the point of inoculation	1
<25% of the inoculated internode discoloured	2
25 to <50% of the inoculated internode discoloured	3
50 to <100% of the inoculated internode discoloured	4
Total of inoculated internodediscoloured and 25% of adjacent internode discoloured	5
½ discolouration of the adjacent internode	6
Discolouration of three internodes	7
Discolouration of four internodes	8
Discolouration of five internodes or plants prematurely killed	9

this, first an oblique hole about 1.5-2.0 mm was made in the second or third internode of the stalk from the ground level with the help of a zebber and the toothpick was inserted in the hole. Artificial inoculation was performed using toothpick inoculation as well as on already developed sick plot.

The observations for assessment of disease severity in field experiments were recorded. At the time of maturity, lower internodes of these plants were splited longitudinally to see the extent of pith damage. Disease severity was recorded on 1 to 9 standard disease rating scale (Table 1 and Fig. 1) as described (Young, 1943; Payak and Sharma, 1983; Singh and Kaiser, 1989; Desai and Hegde, 1992; Sobowale, 2011). Further, the disease severity and per cent efficacy of disease control (PEDC) was calculated by using formula given by Chester (1959) and Wheeler (1969). JMP software version 12 was used for statistical data analysis for determining significant differences among treatment at p = 0.05 level¹⁴. Experiments were repeated two times with minimum three replications (SAS, 2010).

RESULTS AND DISSCUSION

Effect of Cu-chitosan NPs on PFSR in Maize plant : The inhibition effect of Cu-chitosan nanoparticles was analyzed against PFSR in both maize plant varieties at field. In Surya local variety (4 hrs treated seed), control plants (water treated + inoculation) showed average disease severity 58.3 %. All plants treated with 0.02 to 0.14% Cu chitosan NP showed significant antifungal activity and express lower disease severity 30.6% to 44.6%. Bulk chitosan, commercial fungicide Bavistin (1%) and CuSO₄ were used as positive control, showed 44.8%, 50.3% and 52.8% disease severity respectively. PEDC was found maximum (33.9%) at 0.06% of Cu-chitosan NP (Table 2). In Pratap hybrid-1 variety (4 hrs treated seed),

TABLE 2
Effect of Cu-chitosan NPs on control of PFSR disease of maize variety (Surya local) after 4 hrs seed treatment in field condition (Data recorded at the end of physiological maturity)

Treatment	Disease severity (%) ^A	PEDC (%) ^A
Control ^B	58.3 ± 0.00 a	0.0 ± 0.0 g
Bulk chitosan ^C	44.8 ± 0.31 d	23.1 ± 0.54 d
Fungicide ^D	50.3 ± 0.01 c	13.8 ± 0.02 e
CuSO ₄ ^E	52.8 ± 0.26 b	9.45 ± 0.45 f
Cu chitosan NPs		
0.02%	41.7 ± 0.15 f	28.5 ± 0.25 b
0.06%	30.6 ± 0.01 g	33.9 ± 0.01 a
0.1%	42.8 ± 0.25 e	26.6 ± 0.43 c
0.14%	44.6 ± 0.08 d	23.5 ± 0.14 d

^AEach value is mean of 3 replicates from 2 experiments. Mean ± SE followed by same letter in column of each treatment are not significant different at p = 0.05 by Tukey - Kramer HSD, ^BControl with water. ^CChitosan dissolved in 0.1% acetic acid. ^DFor the positive control, Bavistin (1%) was used. ^ECuSO₄ dissolved in water. PEDC = Percentage efficacy of disease control was calculated compare to control.

control plants (water treated + inoculation) showed average disease severity 47.8 %. All plants treated with 0.02 to 0.14% Cu chitosan NP showed significant antifungal activity and express lower disease severity 32.9% to 47.4%. Bulk chitosan, commercial fungicide Bavistin (1%) and CuSO₄ were used as positive control, showed 40.4%, 46.6% and 45.7% disease severity respectively. PEDC was found maximum (31.2%) at 0.1% of Cu-chitosan NP (Table 3). In field experiments, Cu-chitosan NPs at the concentration of 0.06% found effective in controlling PFSR disease. Significant disease control of PFSR was observed (33.9%) at 0.06% Cu-chitosan NPs in Surya local (4 hrs treated seeds).

CONCLUSION

Overall in present study, Cu-chitosan NPs has

TABLE 3

Effect of Cu-chitosan NPs on control of PFSR disease of maize variety (Pratap Hybrid-1) after 4 hrs seed treatment in field condition (Data recorded at the end of physiological maturity)

Treatment	Disease severity (%) ^A	PEDC (%) ^A
Control ^B	47.8 ± 0.00 a	0.0 ± 0.0 f
Bulk chitosan ^C	40.4 ± 0.16 e	15.5 ± 0.34 b
Fungicide ^D	46.6 ± 0.05 ab	2.43 ± 0.11 ef
CuSO ₄ ^E	45.7 ± 0.11 bc	4.41 ± 0.23 de
Cu chitosan NPs		
0.02%	47.4 ± 0.16 ab	0.80 ± 0.34 ef
0.06%	42.7 ± 0.23 d	10.4 ± 0.49 c
0.1%	32.9 ± 0.70 f	31.2 ± 1.46 a
0.14%	43.9 ± 0.44 cd	7.90 ± 0.93 cd

AEach value is mean of 3 replicates from 2 experiments. Mean ± SE followed by same letter in column of each treatment are not significant different at p = 0.05 by Tukey - Kramer HSD, ^BControl with water. ^CChitosan dissolved in 0.1% acetic acid. ^DFor the positive control, Bavistin (1%) was used. ^ECuSO₄ dissolved in water. PEDC = Percentage efficacy of disease control was calculated compare to control.



Fig. 1. Disease scale of PFSR disease.

established as very effective antifungal agent against PFSR of maize in field conditions. Cu-chitosan NP developed in this study showed growth promotory effect in maize plants and also effective in controlling disease. The potential of Cu-chitosan NP in this study anticipated that developed NP could be further exploited in field experiments for its efficacy.

ACKNOWLEDGEMENT

This study was financially supported by the RKVY Project, MBBT, RCA, Udaipur (Rajasthan).

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