Carbohydrate Polymers 133 (2015) 400–407

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Fungal cell wall polymer based nanoparticles in protection of tomato plants from wilt disease caused by *Fusarium oxysporum* f.sp. *lycopersici*

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ARTICLE INFO

Article history:
Received 6 April 2015
Received in revised form 3 July 2015
Accepted 19 July 2015
Available online 26 July 2015

Keywords:
Nanoparticle
Tomato
Fusarium wilt
Protection
Yield

ABSTRACT

Cell wall polymer (chitosan) was isolated from *Fusarium oxysporum* f.sp. *lycopersici*. They were cross linked with sodium tripolyphosphate (TPP) to synthesize nanoparticles (CWP-NP). The nanoparticles were characterized by FTIR, DLS, SEM, XRD and NMR analyses. The isolated CWP-NP exhibit antifungal activity under in vitro condition. The foliar application of the CWP-NP to tomato plants challenged with *F. oxysporum* f. sp. *lycopersici* showed delay in wilt disease symptom expression and reduce the wilt disease severity. Treated plants also showed enhanced yield. These results suggested the role of the CWP-NP in protecting tomato plants from *F. oxysporum* f.sp. *lycopersici* infection.

1. Introduction

Fusarium wilt of tomato caused *by Fusarium oxysporum* f. sp. *Lycopersici* (Sacc.) W.C. Snyder and Hansen is a widespread disease of the cultivated tomato (*Solanum lycopersicum* L.). It is one of the most economically important diseases which are highly destructive to tomatoes grown in glass-house and in the field all over the world, and causes up to 50% yield loss (Borrero, Trillas, Ordales, Tello, & Aviles, 2004; Larkin & Fravel, 1998). Currently fungicide applications are employed as control measures (Sudhamoy, Nitupama, & Adinpunya, 2009), however repeated application of fungicides may lead to microbial resistance, environmental pollution (Meng, Yang, Kennedy, & Tian, 2010), thus giving a strong reason for alternative eco-friendly methods of disease control.

As promising alternatives, Chitosan a natural polymer has been reported to effectively protect hosts from fungal infection (Meng et al., 2010). It has been reported that chitosan could boost the activity of defence-related enzymes in Arabidopsis, tomato (Cabrera, Messiaen, Cambier, & Van Cutsen, 2006; Liu, Tian, Meng, & Xu, 2007) and upregulate gene expression of β-1,3 glucanase and chitinase (Lin, Hu, Zhang, Rogers, & Cai, 2005). Furthermore, it has been shown that chitosan has broad antifungal activity (Sathiyabama & Balasubramanian, 1998; Xu, Zhao, Wang, Zhao, & Du, 2007). However, the insolubility of bulk chitosan in aqueous media limits its wide spectrum application as an antifungal agent. More ever, it has relatively low antifungal property in bulk form. Hence alternate methods have been employed to enhance its antifungal activity (Li et al., 2013; Qin et al., 2013). The chelating property of chitosan towards various organic and inorganic compounds makes it a suitable biopolymer for improvement in stability, solubility and antifungal activity (Shukla, Mishra, Arotiba, & Mamba, 2013).

Nanotechnology offers an important role in improving crop management techniques (Khot, Sankaran, Maja, Ehsani, & Schuster, 2012). Increasing agricultural productivity is necessary, and so new approaches need to be considered without causing damage to the ecosystem. Khodakovskaya et al. (2009) reported that mutiwalled carbon nanotubes (MWCNTs) increased the germination rate of tomato seeds by increasing the seed water uptake. The use of nanobiopesticide is more acceptable since they are safe for plants and cause less environmental pollution in comparison to conventional pesticides (Nair et al., 2010). Hence, the use of bio-based nanomaterials for crop protection is gaining interest. They are also known for growth promotion (Srilatha, 2011) and induction of systemic acquired resistance (SAR). SAR is a promising approach for controlling plant diseases (Houssien, Ahmed, & Ismail, 2010) which is effective against diverse pathogens, including viruses, bacteria and fungi. Hence, the utilization of plants (tomato) own defence mechanism through application of cell wall polymer based nanoparticle is the area of current interest in the management of Fusarium wilt of tomato. Therefore, an attempt has been made to isolate polymer from mycelium of *F. oxysporum* f.sp. *lycopersici*

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http://dx.doi.org/10.1016/j.carbpol.2015.07.066

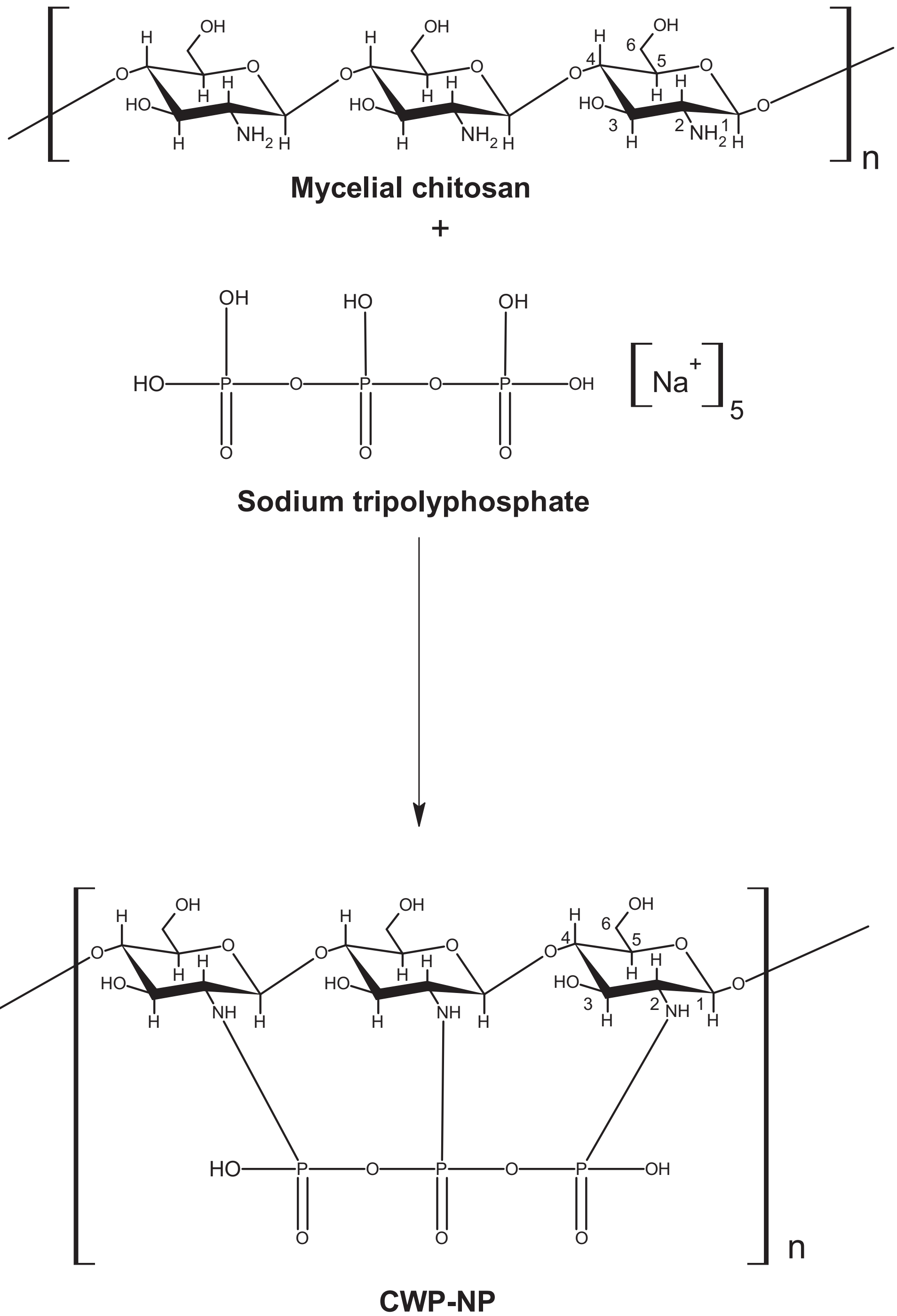

Fig. 1. Schematic representation of cross linking of chitosan and sodium tripolyphosphate.

(FOL), synthesize nanoparticle using the polymer and to investigate the efficacy of cell wall polymer based nanoparticles (CWP-NPs) in suppressing Fusarium wilt of tomato under glass house condition.

2. Materials and methods

2.1. Biological material

F. oxysporum f.sp. *lycopersici* isolate was obtained from ITCC (Indian Type Culture Collection), IARI, New Delhi, India and maintained in PDA slants at 27 °C. Seeds of tomato (PKM1) were obtained from Tamil Nadu Agricultural University, Coimbatore. Plants were raised in earthen pots (3 plants/pot) containing soil, manure and maintained under glasshouse condition.

2.2. Preparation of cell wall polymer

Spores of *F. oxysporum* f.sp. *lycopersici* (1×10^5 spores/ml) was inoculated into (5 ml/50 ml) potato dextrose broth and incubated at 27 °C for 10 days. The mycelium was harvested after 10 days, rinsed with sterile distilled water several times. Polymer from mycelium of FOL was prepared according to the procedure of