Copper heptagluconate as ecofriendly compound enhancing the plant immune system of *Solanum lycopersicum* against *Pseudomonas syringae*, causal agent of bacterial speck

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Abstract

*The use of organic and natural compounds enhancing the plant immune system are among the most ecofriendly management strategies currently available in crop protection. Development of new formulations of liquid Copper (Cu) complexed with heptagluconic acid could be able to induce plant innate defences against pathogens, being a potential alternative to the classical treatments based in inorganic Cu. In this study, we evaluated the systemic effect of Cu heptagluconate in tomato (*Solanum lycopersicum*) against* Pseudomonas syringae *to control bacterial speck. The efficacy of this compound was evaluated by preventive treatments in soil drench. Applications of Cu heptagluconate were performed when seedlings of tomato were at 3-4 true leaf stage by irrigating 20 ml per plant of a product solution at 6 ml l^{-1}, 72 h before inoculation. Pathogen inoculation was performed by dipping the third and fourth leaves into a bacterial suspension for 3 sec. Additionally, lots of plants treated with Cu heptagluconate and non-inoculated, non-treated and inoculated, and non-treated and non-inoculated were included for comparative purposes. Infection development and physiological parameters such as plant growth, and shoot and root dry weight were evaluated. Parameters involved in plant resistance were monitored through determination and quantification of reactive oxygen species (ROS) response (i.e. H_2O_2), and evaluation of hormones and phenolic compounds levels by chromatographic analysis. Preventive soil drench treatments with Cu heptagluconate reduced the incidence of* P. syringae *by more than 50%. Concerning parameters involved in plant resistance, ROS response and accumulation of phenolic compounds were observed. Treatments with Cu heptaglunote decreases markedly the levels of H_2O_2 in treated plants, and induced accumulation of caffeic and chlorogenic acids. Therefore, the activation of plant innate immunity combined with the effect of Cu could prevent bacteria speck in tomato.*

Keywords: Copper heptagluconate, induced resistance, *Pseudomonas syringae*, *Solanum lycopersicum*

Introduction

The study of new compounds based on organic formulations of Cu and its double effect as an inductor of the plant innate defences would provide a valuable alternative to farmers to control pathogens such as *Pseudomonas syringae*. Cu heptagluconate is a compound characterized by the great Cu absorption and diffusion over the plant, and it is safer for the environment compared with others traditional inorganic Cu-based fungicides (Zhang et al., 2013). The main goal of this study was to elucidate the efficacy of Cu enhancing the plant immune system in tomato against *P. syringae* pathovar tomato.

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Material and Methods

Applications of Cu heptagluconate were performed when seedlings of tomato were at 3-4 true leaf stage by irrigating 20 ml per plant of a product solution at 6 ml l^{-1}, 72 h before inoculation. Pathogen inoculation was performed by dipping the third and fourth leaves into a bacterial suspension for 3 sec. Additionally, lots of plants treated with Cu heptagluconate and non-inoculated, non-treated and inoculated, and non-treated and non-inoculated were included for comparative purposes. Infection development and physiological parameters such as plant growth, and shoot and root dry weight were evaluated. Parameters involved in plant resistance were monitored through determination and quantification of reactive oxygen species (ROS) response (i.e. H_2O_2), and evaluation of hormones and phenolic compounds levels by chromatographic analysis. Evaluation methods described in Llorens et al. 2013.

Results and Conclusions

In this study, the effectiveness of a formulation of Cu complexed with heptagluconic acid, which is characterized by high assimilability by the plant, was evaluated. The results obtained showed that, although the formulation of this compound is highly uptake by the plant, there are no symptoms of phytotoxicity, since plants showed the same physiological parameters than those observed in untreated plants. A single preventive soil drench treatment with Cu heptagluconate reduced the incidence of *P. syringae* by more than 50% and the number of viable bacteria in leaves. Concerning parameters involved in plant resistance, ROS response and accumulation of phenolic compounds were observed. Treatments with Cu heptagluconate decreases markedly the levels of H_2O_2 in treated plants, and induced accumulation of salicylic, caffeic and chlorogenic acids (Table 1). Our results suggested that the activation of plant innate immunity combined with the effect of Cu could prevent bacteria speck in tomato.

Table 1: Effect of treatment with Cu heptagluconate on parameters involved in plant resistance. The data show the average of three independent experiments. Different letters represent statistically significant differences ($P < 0.05$ least-significant difference test).

Treatment	Peroxide (% pixels per image)	SA (ng/g)	Caffeic acid (ng/g)	Chlorogenic acid (ng/g)
Control non-inoculated	12.2 ± 0.6 c	507.7 ± 187.5 b	9405.1 ± 788.6 a	205.9 ± 197.8 a
Control inoculated	24.0 ± 1.6 a	820.9 ± 281.2 ab	17011.2 ± 2277.1 b	0 ± 0
Soil non-inoculated	7.8 ± 0.3 d	998.7 ± 388.5 ab	14798.5 ± 3042.6 ab	5721.0 ± 5431.9 b
Soil inoculated	17.3 ± 0.9 b	1206.3 ± 416.9 a	18160.9 ± 2184.9 b	5802.4 ± 3515.5 b

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