Effect of alternative seed treatments on seed-borne fungal diseases in tomato

A. M Kasselaki[1], N. E. Malathrakis[1], D. E. Goumas[1], C. Leifert[2]

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Abstract

The fungus Didymella lycopersici infects tomato seed and results in great losses before and after germination. To control the disease, seed companies use thiram preventively, although human allergy problems have been reported. For this reason as well as to address needs in organic agriculture, this study has focused on the effects of alternative methods of control. Nitrite solutions and resistance inducers were tested in a growth chamber. Results showed that soaking the seed in a nitrite solution with a concentration of 300mM (in citric acid buffer, pH 2) for 10 minutes reduced losses due to low seed germination and disease incidence in the germinated seedlings completely. When applied for longer intervals sodium nitrite proved phytotoxic whereas in shorter intervals it was not as effective. The resistance inducer Tillecur (mustard seed extract) at the rate of 0.05g/ml was as much effective as sodium nitrite inhibiting disease incidence in germinated seedlings. None of the above treatments was significantly different to thiram and they could replace the fungicide in the control of seedborne D. lycopersici in tomato.

Introduction

Didymella lycopersici Kleb. is one of the most important seedborne diseases of tomato. *D. lycopersici* infections considerably affect seed germination while seedlings die within a week of infection appearance (Khulbe *et al.*, 1991). Currently, *D. lycopersici* can be controlled by seed treatment with the fungicide thiram, although some reports associate it with human allergies (Knox-Davis, 2001; Munkvold *et al.*,1999).

The aim of this study was to assess the efficacy of alternative seed treatments such as acid activated antimicrobial compounds (eg. nitrite) and resistance inducing agents against seed borne inocula of *D. lycopersici*.

Materials and methods

Seeds used were collected from inoculated and healthy fruits (uninoculated control). After application of treatments, seeds were sown individually in plastic pots (8 x 10cm) containing peat substrate (‘Favorit’, Germany) and placed in a walk-in growth chamber set at 19ºC and 12/12 photoperiod in a ‘Randomised block’ design with 4 blocks (replicates).

Fungicides (positive control treatments) were applied at the following rates: Kocide 101 (copper hydroxide 50% WP, Griffin LLC, USA) at 0.003 g/ ml and Thirasan (thiram

[1] Technological Educational Institute of Crete, Stavromenos 71004, Heraklio-Crete, Greece, E-mail: kaselaki@steg.teiher.gr

[2] Nafferton Ecological Farming group, Newcastle University, Stocksfield NE43 7XD, UK

80%WP, Bayer, Germany) at 0.001g per 0.25g seed.

Nitrite was used at the following concentrations: 30, 100 and 300mM and pH 2 and 2.5 in a citric acid buffer solution and at different exposure times (2.5-30 minutes).

Resistance inducing agents used were: a) Tillekur (‘Biofa’, Germany) at the rates of 0.0125, 0.025, 0.05, 0.1, 0.2 and 0 .4g /ml, b) Chitosan (deacetylated chitin, min 85%, Sigma, Germany) at the rates of 0.0025, 0.005, 0.01 and 0.05 g / ml of acetic acid (0.05%) and c) BABA (β-aminobutyric acid, min. 95%, Sigma, Germany) at the rates of 0.005, 0.01 and 0.05 g / ml.

Records of a) percentage of emerging seedlings and b) percentage of disease incidence in seedlings were taken thirty days after sowing for all of the experiments. Data were logit transformed (logit = ln (%incidence+0.1)/ (100.1-% incidence)) assuming an asymptote of 100%.

Data were analyzed by Univariate Analysis of Variance (ANOVA). Individual treatments means were compared by Tukey’s HSD test of homogenous subsets ($P \leq 0.05$). The statistical package SPSS ed. 11 for Windows was used.

Eight experiments were performed in total: five with the nitrite solutions and three with the resistance inducers. The most effective treatments tested in the experiments applied on the seeds for ten minutes are listed in table 1.

Table 1: The most effective treatments tested, in all of the experiments

	Nitrite (300mM, pH2)	**Tillecur** (0.05g/ml)	**Chitosan** (0.005g/ml)	**BABA** (0.005g/ml)	**Copper hydroxide** (0.003g/ml)	**Thiram** (0.001g/ 0.25g seed)
Exp. 1	*				*	*
Exp. 2	*					
Exp. 3	*					
Exp. 4	*					
Exp. 5	*				*	*
Exp. 6			*	*		*
Exp. 7		*	*			*
Exp. 8		*	*			*

Results and Discussion

Acidified nitrite, Tillecur and the fungicide thiram, at the concentrations presented in tables 1 and 2, significantly reduced disease incidence (100% inhibition of disease appearance) in young seedlings in all of the experiments. The efficacy of the above treatments to control the disease before emergence of seedlings was inconsistent and differences to untreated control were not always significant (table 2). Copper hydroxide and BABA did not provide adequate control against the disease, while Tillecur at high concentrations (0.1, 0.2 and 0.4/ml) and nitrite at times of exposure longer than ten minutes proved to be toxic to the tomato seed (individual results not shown). Chitosan at the concentration 0.005g/ml significantly reduced disease incidence in young seedlings, in all of the three experiments that it was tested. In the two out of three experiments it controlled disease completely (95-100%).

Table 2: Results of the most effective treatments tested compared to the untreated control by Tukey's HSD test of homogenous subsets (P ≤ 0.05), in all of the experiments, for the control of tomato seedborne *Didymella lycopersici*, in the growth chamber

Exposure time: 10 minutes	**% Inhibition of the disease**		
Treatment	Before germination*	After germination**	Toxicity
Nitrite (300mM, pH2)	0-24	100	Non-toxic
Tillecur (0.05g/ml)	0	100	Non-toxic
Chitosan (0.005g/ml)	0	51-100	Non-toxic
BABA (0.005g/ml)	0	17	Non-toxic
Copper hydroxide (0.003g/ml)	0-10	15	Non-toxic
Thiram (0.001g / 0.25g seed)	7-21	100	Non-toxic

* Significantly different to the untreated control: nitrite in two out of five experiments and thiram in one out of five experiments
** Significantly different to the untreated control in all of the experiments tested

Conclusions

Given the above results, both nitrite and the inducing agent Tillecur could be used as alternatives to the fungicide thiram since they provided equal levels of disease control.

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