

INHIBITORY EFFECT OF QUATERNARY AMMONIUM SALTS ON THE MYCELIUM OF *ALTERNARIA PORRI* F. SP. *SOLANI*, CAUSING EARLY BLIGHT DISEASE ON TOMATOES

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Abstract

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By amination of polyvinyl alcohol with N, N-dimethyl-4-amine-benzaldehyde and quaternization of the amine polymer obtained with dimethylsulphate, the bioactive aminepolymeric salt GN was obtained. In the period 2006-2007 GN in concentration 3 g/kg and 2 g/kg) as well as the alkyl ammonium salt Horti Desin (2 g/kg), were tested in two years experiment in field conditions against *Alternaria porri* f. sp. *solani*, the casual agent of early blight disease on tomatoes. The results from the experiment with the tested pathogen at the beginning of vegetation at middle-early tomatoes, cv. "Ideal" show a preventive effect of GN (3 g/kg) – 39.3% and at GN (2 g/kg) – 33.2%, in comparison with Dithane 45M (2 g/kg), used as etalon – 31.1%. At the end of vegetation, the preventive effect of GN (3 g/kg) was 27.1%, in comparison with Dithane M 45 /2 g/kg – 29.5%. The preventive effect of preparation Horti Desin was 24.8% at the beginning and 20.3% at the end of vegetation.

Key words: amine polymeric salt, Horti Desin, early blight disease, tomatoes cv. "Ideal"

Abbreviations: PVA – polyvinyl alcohol, SAS – surface-active substances, DMS - dimethyl sulphate, DAB – N, N-dimethyl-4-amine-benzaldehyde, QAS - quaternary ammonia salt, DMSO – dimethyl sulphoxide

Introduction

In the world plant protection practice according to Nakov et al. (1998) in agreement with Fetvadzieva et al. (1994) is used a wide range of fungicides. Some of them according to Stoyanov et al. (1999) are highly toxic to warm-blooded organisms, including the man. Residues of fungicides according to Daly et al. (2007) in agreement with Komárek et al. (2010) remain in the soil, as some of them accumulate in the plants, passing into the fruits. Studies on the widely used in practice dithiocarbamate fungicides as maneb, mancozeb and propineb according to Larson et al. (2005) show that they exhibit a teratogenic effect. Furthermore, by their degradation according to Hewitt (1998) the highly toxic metabolite ethylene thiourea is excreted. That is the reason the scientists working in this area to seek alternative ways of conducting effective and environmentally friendly plant protection, in order to reduce the harmful consequences.

Matthew et al. (1999) studied the inhibitory effect of salicylic acid on the mycelium of *Alternaria solani* in hydroponically grown tomatoes. In last decades according to Ioffe et al. (1988) in agreement with Merianos (1991) the quaternary alkyl ammonium and amine polymeric salts (QAS) have found a wide application for micro biological control in food industry and agriculture, thanks to they major advantages in comparison with the traditional disinfectants. They are lower toxic against warm-blooded animals, biodegradable, possess unlimited water solubility, surface activity and wide spectrum of microbiological action, because they cause disruption of intermolecular interactions and coagulation of cellular proteins. One of the most used cation SAS are Horti Desin, presenting didecyldimethylammonium chloride and benzalkonium chloride (alkyldimethylbenzylammonium chloride). Vanachter et al. (1991) have used successfully Horti Desin for phytosanitary control in horticulture.

Alternaria porri f. sp. *solani*, the causal agent of early blight disease in tomato according to Vloutoglou et al. (2000) is responsible for significant economic losses sustained by tomato producers each year.

The purpose of the present study was to be synthesized amine polymeric salt (GN) and to study the possibility for its use, together with the preparation Horti Desin, for protection of middle early tomatoes, cv. Ideal grown under field conditions against *Alternaria porri* f. sp. *solani* causing early blight disease on tomatoes.

Materials and Methods

Synthesis of amine polymeric salt GN

For synthesis of the salt, the following compounds were used: PVA (75 000, Merck) and DAB, extra pure (GFS Chemicals, Inc.) as an aminating agent. As quaternizing agent was used DMS (AD "Neohin") with purity 99.4%, as well as hydrochloric acid and sodium hydroxide, p.a. (Merck)

To 100 g of PVA (72 000 Merck), dissolved in 400 ml of distilled water add 10 ml of hydrochloric acid 37%. The mixture was heated to 323 K, and then adds 20.4 g DAB, dissolved in 50 ml ethanol (absolute alcohol) for 1 h. Synthesis continued for a further 4 h at the same temperature. Then the reaction mixture was cooled and to it adds 150 ml of absolute alcohol and 5 ml of ammonia to pH 8-9. Amine polymer (G1) obtained precipitated by adding 200 ml of acetone (p.a.). Aqueous phase containing unreacted DAB, acetone and salts decant. Amine polymer dried at 378 K for 2 h. and after that was quaternized, using DMS by ratio G1: DMS = 1:3. Quaternization occurs at temperatures 343-354 K in the presence of sodium hydroxide at an amount equivalent to releasing hydrogen chloride. DMS and the aqueous solution of sodium hydroxide was dosed to G1 for 1 h. Reaction mixture allowed to stand 24 h. Aqueous phase with the salts decant and the amine polymeric salt GN was extracted with diethyl ether. The solvent was distilled and the quaternary salt is diluted to 200 g/kg aqueous solution and refrigerate.

Chemical analysis of the amine polymer G1

For characterization of the intermediate amine polymer G1, ¹H-NMR, spectroscopic analysis was carried out, using Bruker DRX - 250 MHz ¹H-NMR Spectrophotometer. As solvent was used DMSO.

The content of bount nitrogen was determined by the method of Kjeldhal by apparatus VELP UDK 132 (Jones, 1991).

Field experiment

Experience was based on experimental field of Plovdiv Agricultural University during the period June - September

2006-2007, at middle early tomatoes cv. Ideal. The plants were formed to one steam. There were made all required agricultural activities. Feeding was done three times, at a dose 15 g for a plant, corresponding to a rate 60 kg NH₄NO₃/da. The first feeding was done one week after the interception, the second - two weeks after the first and the third - at the appearance of the first red tomatoes. Scheme of experience includes six variants containing 12 tomato plants in separate rows. To establish the inhibitory activity against the causative agent of early blight disease on tomato *Alternaria porri* f. sp. *solani*, there were tested the following preparations: amine polymeric salt GN, Horti Desin and fungicide preparation Dithane M45, used as etalon. Control variants include a negative control (-) - plants grown under natural infective background and positive (+) - plants inoculated with 14 daily mycelia spore suspension of the pathogen.

Scheme of experience included the following variants.

- Variant 1 – treated tomato plants with GN in concentration 30 g/kg,
- Variant 2 - treated tomato plants with GN (20 g/kg),
- Variant 3 - treated tomato plants with Horti Desin (20 g/kg)
- Variant 4 - treated tomato plants with Dithane M 45 (20 g/kg)
- Variant 5 - Control (+) - artificially infected tomato plants
- Variant 6 - Control (-) - uninfected tomato plants

Tomato plants of the experimental variants in phase but-toning were treated preventive at 13th of July with the tested antifungal preparations. During the treatment, the plants in the different variants were separated by polyethylene barrier. After that was done infection by mycelia spore suspension of *Alternaria porri* f. sp. *solani*. Plants of the control variants were insulated with polyethylene barrier, which was maintained until the end of vegetation period.

Reporting of attack of the disease in the two years studying was made twice - at 20th of July and at 11th of October. A 5-point scale was used:

- Ball 0 - healthy plants
- Ball 1 - 5% attacked leaf surface
- Ball 2 - 6 to 25% attacked leaf surface
- Ball 3 - 26 to 50% attacked leaf surface
- Ball 4 - more than 51% attacked leaf surface.

The index of the attack was calculated by the formula of Mc Kinney:

$$I = \frac{\sum /k.n./100}{K.N}$$

where: k - degree assault in score

n - Number of leaves infested with the respective ball

K - the highest score

N - total number of recorded sheets

The program "BIOSTAT" made the statistical processing of the experimental data obtained.

Results and Discussion

Characterization of GN

¹H-NMR spectrum of the intermediate amine polymer G1 is illustrated on Figure 1. The analysis shows a presence of the following peaks:

Peak at 9.7 ppm corresponds to protons of unreacted aldehyde DAB, and that of 7.4-7.3 ppm - aromatic protons attached to the polymer by DAB. Peaks at 7.7 and 6.8 ppm are satellite and that of 5.5 ppm corresponds to the acetal proton (DAB connected to PVA) At 3.8 ppm - CH-OH protons of PVA + water (DMSO-d6).

Peak at 3.0 corresponds to ppm - methyl groups of DAB. Peaks at 2.48-2.5 ppm - solvent DMSO-d6 (deuterium containing) Peaks at 1.0-1.9 ppm - CH₂ of PVA.

From the integrals of the acetal proton (5.5 ppm) and CH₂ of PVA (1.0-1.9 ppm) was obtained containing of DAB-acetal groups - 5.3 mol %.

The content of associated nitrogen in the intermediate amine polymer (G1) calculated by the Kjeldahl method was 6.8 g/kg.

Biotests

The average data of experimental results from the both years studied are illustrated in Table 1 and Figure 2. The results obtained under field conditions show that the polymeric salt GN exhibited high inhibitory effect on the development of pathogenic fungus in both tested concentrations - 30 g/kg and 20 g/kg. The reported inhibitory effect of GN (30 g/kg) in the first reading (39.3%) was even higher, than that in variant 4, treated with the etalon preparation Dithane M45 - 31.1%. The tested quaternary salt Horti Desin in concentration 20 g/kg (variant 3) also exhibits good inhibitory effect

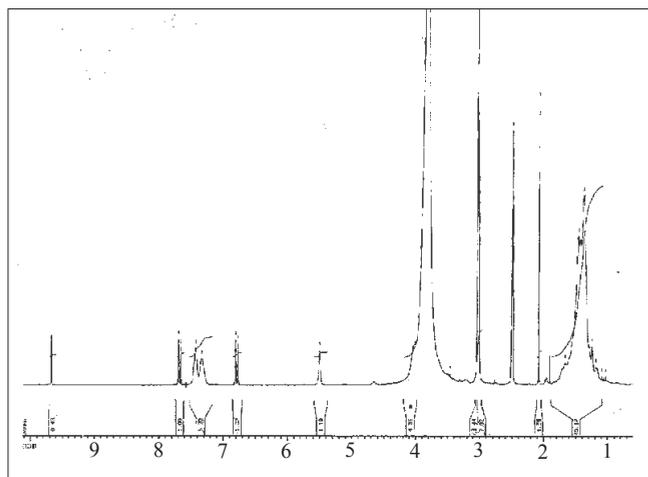


Fig. 1. ¹H-NMR spectrum of the amine polymer G1

against the tested pathogen - 24.8% in the first and 20.3% in the second reading (Figure 2).

Analysis of the results after the first reading indicated that under natural conditions there was a high infective background of early blight at the beginning of vegetation - 23.2%. At the end of vegetation index of attack increased to 68.3%. When set high infectious background - artificially infected control, index of attack started from 34.8%, reaching 93.7%, until indices of attack at variant 3 (Horti Desin) were 13.9% and 37.8% in the second reading. In the variants with GN they were as follows: For variant 1 (30 g/kg) in the range 13.2% - 19.4% and by variant 2 (20 g/kg) slightly higher - 17.9% and 24.8% (Table 1).

Inhibitory effect (Y) of GN (variant 1) was moving in the range 39.3% at the beginning to 27.1% at the end of vegetation (Figure 2). For variant 2 (GN 20 g/kg) these values were several units lower - from 33.2% to 22.2%. At the etalon preparation Dithane M 45, index of infestation were in the range 16.8% - 18.2%, indicating a good inhibitory effect in both readings, which is in close range 31.1% - 29.5%.

Table 1
Index of attack of *Alternaria porri* f. sp *solani* at the tested experienced variants

№	Variants	Conc., %	Number of plants	Index of attack (I), %	
				20.07	11.10
1	1. GN	0.3	12	13.2	19.4
2	2. GN	0.2	12	17.9	24.8
3	3. Horti Desin	0.2	12	13.9	37.8
4	4. Dithane M 45	0.2	12	16.8	18.2
5	5. Control (+)		12	34.8	93.7
6	6. Control (-)		12	23.2	68.1

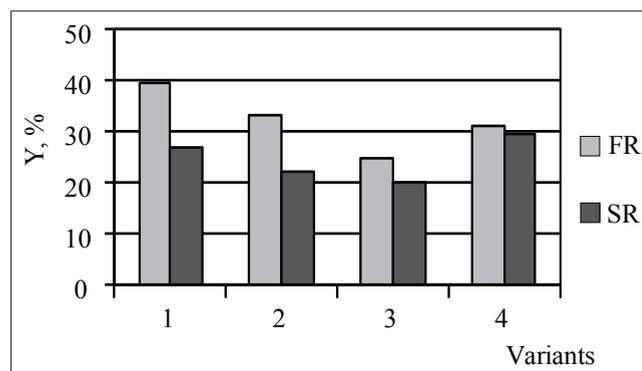


Fig. 2. Inhibitory effect of preparations GN (1, 2), Horti Desin (3) and Dithane M 45 (4) against *Alternaria solani*
Legend: Y – Inhibitory effect (%), FR- first reading (20.07), SR - second reading (11.10).

The differences between the tested variants were proved at a level GD 5%. Compared to Control (+) at the first reading they were 15.3% and 11.4% respectively at the second reading. Regarding the negative control - C(-), warranted at the first reading was 20.1% and 16.2% in the second reading. During the field experiment, no symptoms of phytotoxic action were noticed on the prevented tomato plants, treated with the both amine compounds GN and Horti Desin.

Conclusions

Amine polymeric salt "GN" was synthesized on the base of PVA, DAB and DMS. GN was tested together with preparation Horti Desin against early blight disease of tomato cv. Ideal. The both amine compounds showed a high fungistatic effect against micellium of *Alternaria porri* f. sp. *solani*, the causal agent of early blight disease of tomato. The tested preparations GN in concentration 30 g/kg and Horti Desin (20 g/kg) can be applied for preventive purposes against the agent of early blight disease of tomatoes. There were noticed no phytotoxic manifestations on the protected with the preparations GN and Horti Desin tomato plants.

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