BENEFIC EFFECTS OF ESSENTIAL OILS TREATMENTS IN HEALTHY AND POTATO VIRUS *Y* INFECTED PLANTS OF *SOLANUM TUBEROSUM* L. (cv. ROCLAS) AND *NICOTIANA TABACUM* (cv.WHITE BURLEY)

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ABSTRACT

Antioxidants such as rosmarinic acid, chlorogenic acid and poliphenols, present in essential oils extracted from *Lamiaceae* family plants, as well as many other compounds like hydrogen peroxide and ascorbic acid are implicated in the stress signalling process. The effects of treatments with essential oils from *Rosmarinus officinalis* (for potato) and from *Thymus serpyllum* or *Lavandula officinalis* (for tobacco) on pigments content, *minituber* yield (in potato) and on antiviral activity (in tobacco) were evaluated after virus mechanical inoculation. The treatments of positive potato plants significantly reduced the number of minitubers, increasing their weight, while leaf pigment content also increased. Concerning the antiviral effect of the *Thymus serpyllum* and *Lavandula officinalis* oils, all the treated tobacco plants presented after PVY infection values of absorbance at 405nm significantly lower than the untreated and inoculated controls. This research demonstrates potential benefits of *Rosmarinus officinalis* oils in enhancing the potato yield and quality of tubers and of *Thymus serpyllum* or *Lavandula officinalis* oils on the immunization of *Nicotiana tabacum.*

Key words: potato virus, essential oils, *Rosmarinus officinalis, Thymus serpyllum*, *Lavandula officinalis*, tobacco.

Abbreviations: AA - ascorbic acid; ROS reactive oxygen species; RA rosmarinic acid; RO *Rosmarinus officinalis*; PVY potato virus *Y*; OD optic density; SD standard deviation.

INTRODUCTION

Obtaining healthy and safe food imposes the improvement of techniques used for control the pathogen agents, choosing new opportunities, methods, players and natural resources.

Potato virus (PVY) (*Potyviride*) is one of the most important viruses of potato (*Solanum tuberosum* L.) (Ragsdale et al., 2001). High PVY level can cause stand loss, reduced yields, undersized tubers and reduced quality (Beemsteret al., 1987). Over the past 20 years, PVY has become an increasingly serious constraint to seed potato production in the world (Davis et al., 2008; Lorenzen, 2006). Thus, efforts to control PVY are essential when producing potatoes for market or seed (Bădărău et al., 2010a, b; Bădărău et al., 2009; Beemster et al., 1987; Lorenzen et al., 2006).

Being very susceptible to potyvirus infection, *Nicotiana tabacum (*family *Solanaceae)* is used usually as test plant for potato virus *Y* (Beemster et al., 1987). Phenolic compounds and other well-known constituents of *Rosmarinus officinalis, Thymus serpyllum, Lavandula officinalis* plants (Family *Lamiaceae,* order *Lamiales)* have antioxidant activity and pharmaceutical properties (Petersen and Simmonds, 2001). They are also antimicrobial and antiviral, which protects the plants. Oils extracted from *Rosmarinus officinalis, Thymus serpyllum, Lavandula officinalis* introduced in healthy and infected potato plants could be implicated in the stress signalling process (Triantaphyllou, 2001).

Plant cells have defensive responses to pathogen attack associated with changes in oxidative metabolism (Hammerschmidt, 2005).

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One of the consequences of stress is an increase in the cellular concentration of reactive oxygen species (ROS), which are subsequently converted to hydrogen peroxide (H_2O_2). These ROS, particularly H_2O_2, play versatile roles in normal plant physiological processes and in resistance to stresses. H_2O_2 produced in excess is harmful, but lower concentrations are beneficial (Quan et al., 2005).

For example, exogenous application of H2O2 induced tolerance to high temperature (López-Delgado et al., 1998) and to chilling (Mora-Herrera et al., 2005) in microplants of Solanum tuberosum. Genetic and physiological evidence suggests that H_2O_2 acts as a signalling second messenger, mediating the acquisition of tolerance to both biotic and abiotic stresses and providing information about changes in the external environment (Apostol et al., 1998; Quan et al., 2008).

Another molecule that participates in response to both biotic and abiotic stresses is ascorbic acid (AA), which acts as an antioxidant, protecting the cell against oxidative stress caused by environmental factors and pathogens. As a direct scavenger of ROS, protecting or regenerating carotenoids or tocopherols, AA is the major redox buffer in plants, and is present at high concentrations in most plant cell compartments, including the apoplast (Noctor, 2006).

Changes in AA content can modulate PR gene expression and systemic acquired resistance, acting as a signal transducing molecule (Noctor, 2006; Pastori et al., 2003). Moreover, AA is a regulator of cell division, cell elongation and growth (Kerk and Feldman, 1995). Considering that compounds from *Lamiaceae* plants oils have antiviral and antioxidant activity (Bedoux et al., 2010; Triantaphyllou et al., 2001) and that H_2O_2 and AA have been implicated in signalling gene expression against biotic and abiotic stresses (Foyer and Noctor, 2005; Noctor, 2006), the objectives of this work were to evaluate the effects of treatments with *Rosmarinus officinalis* oils, hydrogen peroxide and AA on photosynthetic pigments in healthy and mechanical inoculated potato plants with potato virus Y (PVY). Another aim of our researches was to study the antiviral activity of *Thymus serpyllum, Lavandula officinalis* oils treatments on *Nicotiana tabacum* plants inoculated with PVY.

MATERIAL AND METHODS

Plant material

Solanum tuberosum L. microplants cv. Roclas, tested virusfree, were obtained from the Biotechnology Department of National Institute of Research and Development for Potato and Sugar Beet Brasov. Single node cuttings were propagated in test tubes on Murashige and Skoog (Murashige and Skoog, 1962) medium, at 20 ± 1°C under a 16 h photoperiod (fluorescent lights, 400-700 nm), in sterile conditions. The microplants were transferred to greenhouse conditions 30 days after the single-node subculture step. For obtaining positive material, a part of these plants were mechanically inoculated, using a PVY secondary infected plant from Record variety (Bădărău et al., 2010a, b). The infection of the material was confirmed by ELISA tests. *Nicotiana tabacum* plants cv. Whyte Burley were obtained by the classical technology. The tobacco plants were inoculated mechanically in the 4 leaves phase and leaves were harvested 4 weeks after inoculation.

ELISA test

A press with smooth roles was used for preparation of leaf samples. The antiserum and conjugated used for viruses detection were obtained in our laboratory (Cojocaru et al., 2009). The analysis was performed following essentially the protocol described by Clark and Adams (1977) (100 µl from each reactive solution). Microplates were filled with substrate solution (p-nitrophenyl-phosphate) incubated 1 hour and the absorbance values were estimated at 405 nm (A_{405}) on PR1100 reader. The samples having A_{405} values exceeding the cut-off (two times the average of healthy controls) were considered virus infected.

Chemical treatments

Solanum tuberosum L. microplants were transplanted to pots and after 10, 20 and 30 days. All plants (excepting the controls) were injected with *Rosmarinus officinalis* oil (dilution 1/1000) 100 µl each plant. Before inoculation, in the 4 leaves phase, tobacco plants were injected with *Thymus serpyllum* or *Lavandula officinalis* oils (dilution 1/100 and 1/1000) 100 µl each /plant. For 7 days after the first injection, the plants were sprayed twice weekly with 10 mL per plant of either 1 mM H_2O_2 or 3 mM AA at pH 5.6 (Bădărău et al., 2010a, b). Controls and plants treated only with natural oil were sprayed with distilled water. Four virus infected (positive) and healthy (negative) plants were sprayed in randomised arrays for each chemical treatment, and each treatment was performed in four independent experiments.

Pigment analysis

Measurements were performed for each experiment on plants, 80 days after transplanting. Five leaf discs (about 1.5 cm diameter) per plant were taken from mid-shoot leaves of three plants per treatment. Samples for each assay comprised 15 discs, homogenized in 4 mL of 80% acetone at 4°C. Insoluble materials were removed by centrifugation at 2500 rpm for 10 min. Chlorophylls *a* and *b*, and carotenoids, were analysed spectrophotometrically according to the method of Lichtenthaler and Wellburn (1983).

Statistical analysis

Data were analysed by ANOVA and Duncan's Multiple Range Test and scored as significant if $P<0.05$. To illustrate the precision of the mean we used the confidence interval (CI).

RESULTS

Researches on *Solanum tuberosum* L.

Effects of treatments with Rosmarinus officinalis oil and H_2O_2 or AA, were compared on pigment contents and tuber harvest parameters of both healthy and virus infected (PVY) plants cv. Roclas.

Changes in photosynthetic pigment contents were evaluated 80 days after transplanting (Figure 1 and Figure 2).

Without chemical treatments, the positive leaves showed significant reductions, compared to uninfected leaves, in chlorophyll a (by 29%), chlorophyll b (44%), total chlorophyll (30%), and carotenoids (57%). Treatments with RO (*Rosmarinus officinalis* oil) and H_2O_2 or AA significantly increased pigment contents of virus PVY infected plant leaves to levels similar to uninfected plants (with the exception of the oil treatments on chlorophyll a and AA effects on carotenoids). No significant differences were induced by these treatments in the uninfected plants (Figure 1 and Figure 2).

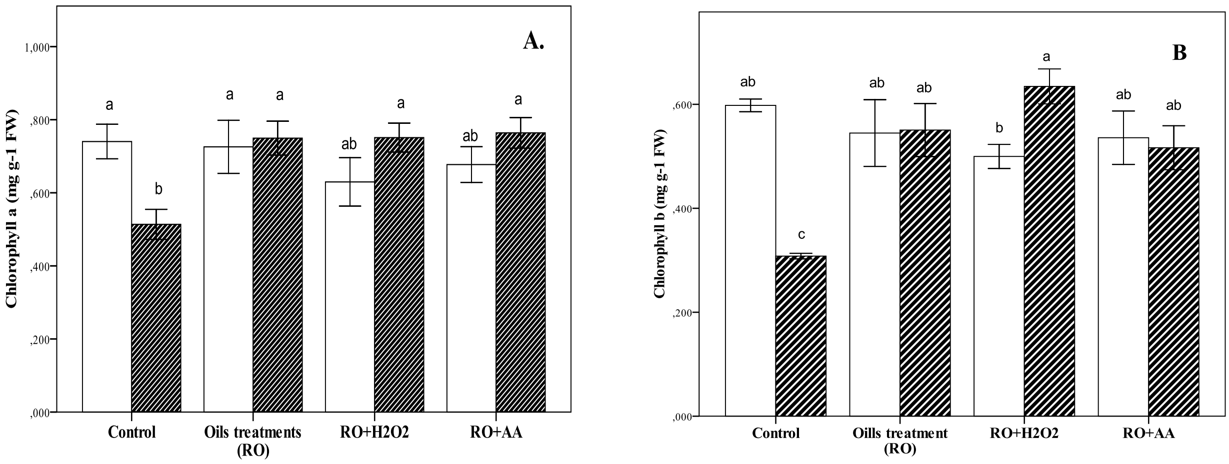

Figure 1. Chlorophyll *a* (A) and chlorophyll *b* (B) of leaves of healthy plants (□) and potato virus Y (PVY) infected plants (■), following treatments with *Rosmarinus officinalis* oil (RO) and spray with H_2O_2 (1 mM) or AA (3 mM) or water (controls)
[Data are means ± SD of four experiments (n=4).
Columns with different letters differ significantly by ANOVA and Duncan's test (P<0.05)]

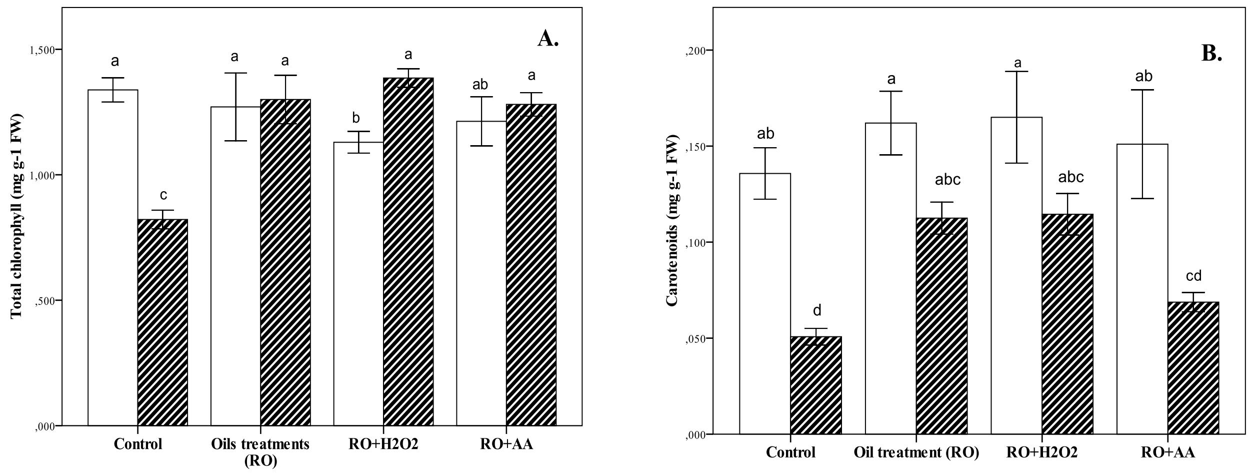

Figure 2. Photosynthetic pigments. A) total chlorophyll, and B) carotenoids of leaves of healthy plants (□) and PVY infected plants (■), following *Rosmarinus officinalis* oil (RO) and H_2O_2 (1 mM) or AA (3 mM) treatments or water (controls), twice weekly
[Data are means ± SD of four experiments (n=4). Errors bars are 95% CI of means. Columns with different letters differ significantly by ANOVA and Duncan's test (P<0.05)]

Final harvests were carried out at 60 or 90 days after transplanting. At 60 days no significant differences were observed in the number of tubers in positive or uninfected control-treatments (Figure 3A). However, at the same date, positive plants treated only with *Rosmarinus officinalis* oil produced significantly more tubers (by 47%) than the positive controls. None of the treatments induced significant differences in the number of tubers in negative plants (Figure 3A). At 90 days after transplanting, the number of tubers produced by positive control plants was significantly higher than the uninfected control (by 65%) (Figure 3B). In uninfected plants no significant differences were obtained by the treatments relative to their controls (Figure 3B). However, all the treatments significantly reduced the number of tubers produced per plant (by 25, 29 and 25% respectively) in the positive plants compared to their control (Figure 3B).

Interestingly, this reduced number of tubers was similar to that produced in uninfected plants subjected to any of the treatments (Figure 3B).

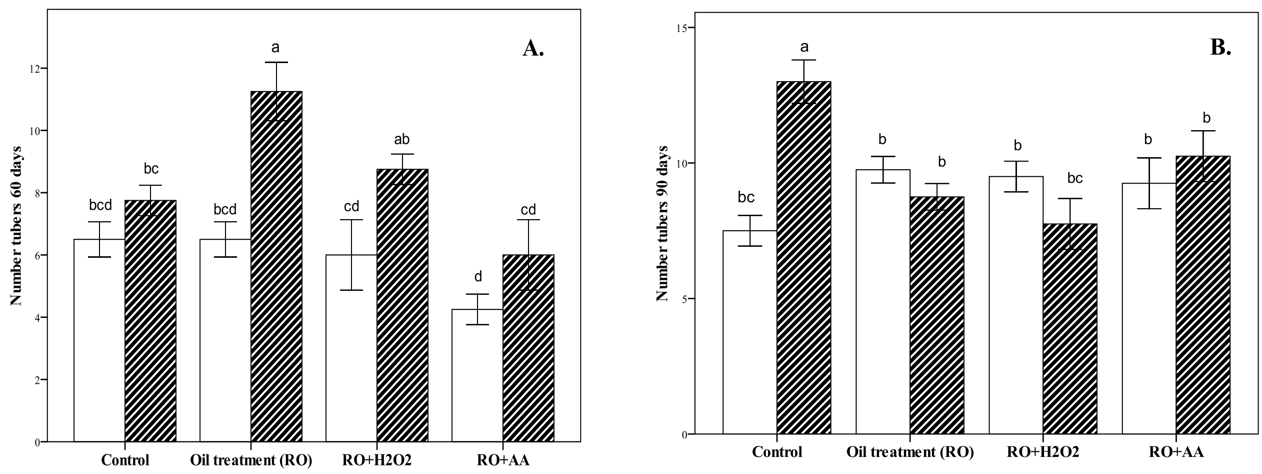

Figure 3. Number of tubers produced by plants healthy (□) or positive-infected plants with potato virus Y(PVY) (■), following injections with *Rosmarinus officinalis* oil (RO) and spray treatments with H_2O_2 (1 mM), AA (3 mM) or water (controls), twice weekly for 60 days
[Data are means ± SD of four experiments (n=4). Columns with different letters differ significantly by ANOVA and Duncan's test (P<0.05)]

Tuber weights of the uninfected control plants were significantly higher (by 80 and 64%) than the positive control at 60 and 90 days respectively (Figure 4). However, H_2O_2 and *Rosmarinus officinalis* oil treatments significantly increased the weight of tubers at 60 days (by 95% and 116% respectively) in the positive plants compared to their control (Figure 4A). Furthermore, this response was maintained at 90 days after transplanting (107% and 78% respectively), when the AA treatment also registered a significant (47%) increase (Figure 4B).

The chemical treatments of positive plants resulted in tuber weights that were either not significantly different to, or greater than (in the H_2O_2 treatment at 90 days), those of uninfected controls (Figure 4).

Significant reduction by the chemical treatments of the weight of tubers harvested was observed in the uninfected plants compared with their control at 60 days, this effect remaining significant at 90 days for the plants treated only with *Rosmarinus officinalis* oil (Figure 4).

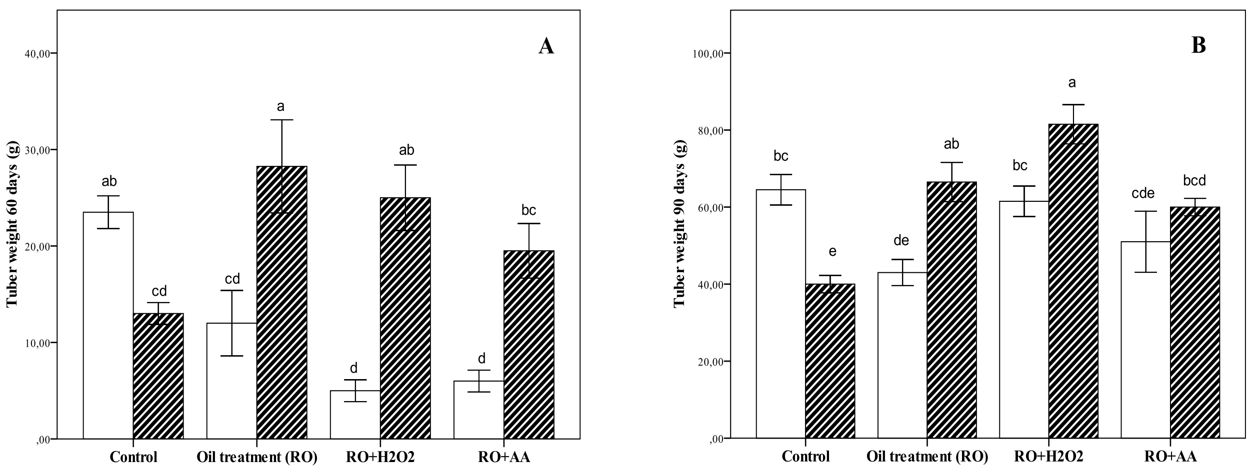

Figure 4. Weight of tubers produced by healthy plants (□), or positive-infected plants with potato virus Y(PVY) (■), following injections with *Rosmarinus officinalis* oil (RO) and spray treatments with H_2O_2 (1 mM), AA (3 mM) or water (controls), twice weekly for 60 days.
[Data are means ± SD of four experiments (n=4). Columns with different letters differ significantly by ANOVA and Duncan's test (P<0.05)]

Researches on *Nicotiana tabacum*

Effects of treatments with *Thymus serpyllum* or *Lavandula officinalis* oils and H_2O_2 or AA, were compared on absorbance values obtained after testing (by DAS ELISA technique) healthy and inoculated plants (cv. Whyte Burley) with potato virus Y(PVY).

The antiviral activity of treatments was as evaluated 40 days after the last transplanting (Figure 5). Compared with their positive controls, with chemical treatments, the inoculated plants showed significant decreases of the absorbance values (Figure 5). Treatments with *Thymus serpillum* oils and H_2O_2 or AA significantly decreased DO_{405nm} of samples prelevated from virus PVY infected plant leaves to levels similar to uninfected. The best results were obtained using the 1/100 oil dilution. No significant differences were induced by these treatments in the uninfected plants (Figure 5A). The *Lavandula officinalis* oils had lower effect on plants immunity compared with *Thymus serpillum* (Figure 5B).

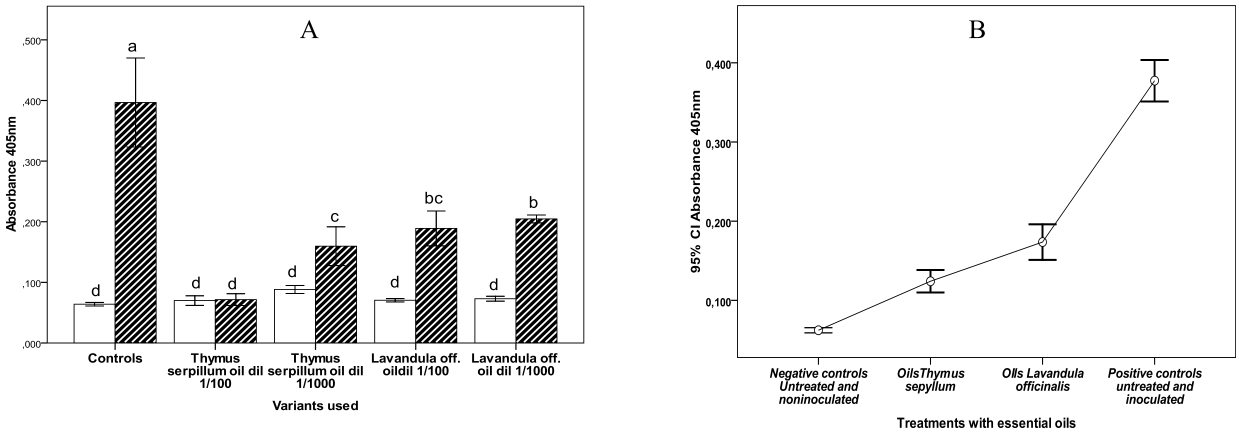

Figure 5. A. Absorbance (optic density) values at 405 nm of healthy (□) and infected (■) *Nicotiana tabacum* plants with potato virus Y(PVY), following treatments with *Thymus serpillum* and *Lavandula officinalis* oil (dilution 1/100; 1/1000) and H_2O_2 (1 mM), AA (3 mM) or water (controls)
[Data are means ± SD of four experiments (n=4). Columns with different letters differ significantly by ANOVA and Duncan's test (P<0.05)
B. Influence of treatments of healthy and inoculated *Nicotiana tabacum* plants with potato virus *Y* (PVY) on optic density (absorbance) values at 405 nm. 95% CI - 95% confidence interval of the difference

DISCUSSION

Our data demonstrate potential benefits of *Thymus serpyllum* oils on the immunization of *Nicotiana tabacum*, a close relative of *Solanum tuberosum*. The effects of these oils were amazing, the OD_{405nm} values being significantly decreased.

Hydrogen peroxide is a diffusible signal-transducing molecule and its accumulation is perceived by the plant as a signal of environmental change, alerting the cell of both biotic and abiotic threats (Noctor, 2006). It also alters the concentrations and redox status of intracellular antioxidants, such as ascorbate (Foyer and Noctor, 2005). The role of H_2O_2 in the induction of tolerance to stresses in potato plants has been demonstrated. Wu et al. (1995 and 1997) observed that transgenic potato plants expressing a fungal gene encoding glucose oxidase, which generates H_2O_2 when glucose is oxidized, exhibited strong resistance to *Erwinia carotovora* subsp. *carotovora*, and to *Phytophthora infestans*. This resistance to soft rot and to potato late blight was apparently mediated by elevated levels of H_2O_2.

The results of the present study demonstrated that plants mechanical infected with potato virus *Y* (PVY) suffered significantly harmful effects on pigment contents and on the number and weight of tubers produced. In general, these effects were reduced by injecting the plants with *Rosmarinus officinalis* oil and spraying H_2O_2 or AA. Concerning the changes in the leaves pigment contents, foliar mosaic (alternative pale green and dark green areas) represents a common symptom of primary infection with potato virus *Y* (PVY). Our results show that the presence of PVY in potato plants significantly reduced the content of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids. *Rosmarinus officinalis* oil injections and H_2O_2 or AA treatments of mechanical infected plants with PVY significantly increased the levels of chlorophylls compared with positive control plants, while similarly treated uninfected plants did not show significant differences in these pigments after spraying.

Under greenhouse conditions, 90 days after transplanting, the infected plants produced a higher number of tubers than the uninfected controls, relative to uninfected controls. Increased number and reduced weight of tubers is a characteristic response to stress in potato. The virus also causes an array of symptoms suggestive of disturbances in the normal balance of plant hormones such as cytokinins and auxins (Dermastia, 1995). Increased number of tubers could be due to disturbance of plant hormones involved in tuber formation (Fernie and Willnitzer, 2001).

It has been suggested that a physiological balance of antioxidant components is necessary in order to obtain protection to generalized stress; however, antioxidants are not always accessible to some of the sites where they are most needed in times of stress (Foyer et al., 1994). Our results agree with this statement, since the *Rosmarinus officinalis* oil injections and AA/ H_2O_2 treatments induced significant anti-stress effects only in the tubers from positive plants.

This research presents a novel potential approach for overcoming the most common damage in tubers of potato virus *Y (*PVY) infected material, using natural compounds that offer the possibility of reduction of biocide usage.

CONCLUSIONS

The results of the present study demonstrated that potato plants mechanical infected with PVY suffered significantly harmful effects on pigment contents and on the number and weight of tubers produced. These effects were reduced by injecting the plants with *Rosmarinus officinalis* oil and spraying with H_2O_2 or AA. The treatments of mechanically infected plants with potato virus *Y* significantly increased the levels of chlorophylls compared with positive control plants, while similarly treated uninfected plants did not show significant differences in these pigments.

Thymus serpyllum and *Lavandula officinalis* oils demonstrated a strong antiviral effect as tobacco plants mechanically inoculated with PVY and injected with these oils presented absorbance values at 405nm significantly lower than the untreated and inoculated controls.

The elucidation of the precise role played by *Rosmarinus officinalis, Thymus serpyllum* and *Lavandula officinalis* oil treatments in addition with H_2O_2, AA on potato virus *Y* infected and healthy plants awaits further investigation.

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