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EFFECT OF ALFALFA MOSAIC VIRUS (AMV) ON THE CONTENT OF SOME MACRO- AND MICRONUTRIENTS IN ALFALFA

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Summary

Leaves showing mosaic virus symptoms were collected for analyses from alfalfa growing areas in the Isparta region. Alfalfa mosaic virus (AMV) was detected in the plant samples by using mechanical inoculation of test plants and serological tests. The macro- and microelements were assayed in the infected plant samples in order to determine the effects of AMV on the nutrient content of alfalfa. The quantity of P, Fe, Cu, Zn and Mn decreased and N increased in the infected alfalfa leaves as compared to the healthy plant samples, while K did not change. The forage containing virus infected alfalfa could not be harmful for the domestic animals, and the nutritious value is even improved because of the increased protein contents.

Key words: *alfalfa mosaic virus, alfalfa, nutrient contents.*

Introduction

Alfalfa (*Medicago sativa*, *Fabaceae*) is a very important forage legume having high nutrient value as compared to other plants. Protein content of alfalfa is about 35 %. Alfalfa fixes air free nitrogen into the soil, providing a potential source of natural fertilization for plants [4].

Alfalfa is rich in protein, mineral elements, trace elements and vitamins, and it is more valuable as a forage grass compared to the rest of the legumes, the *Poaceae* grasses and forage plants of other families. The optimum nutrient contents of *M. sativa* are shown in Table 2 [10].

Some plant diseases, pests, parasites and weeds affect the production of alfalfa. A significant part of the most common alfalfa diseases is comprised of viral infections. Alfalfa has been reported to be attacked by more than 28 plant viruses [7].

Alfalfa mosaic virus (AMV) is one of the most important and wide spread plant viruses, and it is found to infect 599 species belonging to 245 genera of 68 families, most of which are of the *Fabaceae* family. AMV can be transferred from infected to healthy alfalfa plants in various ways: through mechanical inoculation by plant sap, by seeds, by aphids in a non-persistent manner, by dodder (*Cuscuta*), and by weed seeds [7, 9, 13]. The typical symptoms of AMV infection of alfalfa are bright green and yellow plant color, chlorotic mottle between the lateral veins of leaves, vein banding, and leaf and petiole distortion. Additionally, root necrosis and plant death may appear in susceptible varieties. Severe stunting and dwarfing occur in alfalfa plants infected with AMV. Deformation, curling, chlorotic banding and mosaic are also seen in early growing leaves [17].

AMV infection in alfalfa fields reduces fodder yield by 14.8 to 22.8 % and by 15 to 18.1 % on a fresh and on a dry weight basis, respectively. In the field, AMV infection rates

appear between 53 and 76 %, and the yield losses comprise 11 to 17 %, respectively. The amount of fresh grass decreases significantly with AMV [3]. In Czechoslovakia, the death rate of plants infected with AMV ranges between 26.6 and 80 % depending on growing areas [11]. The virus also impedes the growth of alfalfa and reduces re-shooting rate after cutting [12].

Many researchers report physiological and biochemical disorders due to pathologic changes in plants. Protein content of bean leaves grows up with *Pseudomonas syringae* infection. Respiration rate increases in bean plants with bean common mosaic virus disease on the 9th and the 15th day after inoculation. The leaves of cotyledon plants infected by cucumber specific mosaic virus cause high chlorophyllase enzyme activities and chlorophyll demolition [14]. Another research on the same virus reports that the sugar content decreases, but free amino acids increase and protein level is considerably high in the infected cucumber leaves [1, 8]. Watermelon mosaic virus infection increases the protein content, but depresses other physiological and biochemical activities such as respiration rate, and reduces starch, sugars and total nitrogen contents in diseased compared with healthy plants [8]. Green part, seed and nitrogen fixation decrease with viral infection. Occurrences of nodules on the plant roots are reduced by AMV infections [7, 15].

The aim of this research was to determine the effects of AMV on the nutrient content of alfalfa plants.

Materials and Methods

Leaf samples from alfalfa plants exhibiting AMV-like symptoms were collected in the Isparta region. The alfalfa leaves were kept frozen at –20 ^{o}C in sterile polyethylene bags.

Alfalfa samples were tested for presence of the virus by an AMV specific ELISA detection kit (Agdia Company, Elkhart, USA) using the previously reported DAS-ELISA method [6]. Absorbance values were measured at 405 nm with a microplate reader (EL X 800 Universal Microplate Reader, Bio-Tek Instruments, Inc. B-2610 Wilrijk, Belgium).

Inoculums from AMV infected alfalfa leaves were prepared in phosphate buffer (0.01 M, pH 7.2, 1 ml per 1 g of leaf material) and were applied to test plants. The inoculated test plants were grown in greenhouse conditions at 18-20 ^{o}C until the disease symptoms were observed [9]. No application was done to the control plants.

Alfalfa leaves (young and elder, mix) with viral symptoms were used for analyses. The samples were washed in tap water to remove surface residues and soaked in 0.2 N HCl for 20 s. Following 4 or 5 rinses with distilled water, samples were dried at 65 ^{o}C for 48 h to a constant weight. Dried samples were ground by using a mortar and pestle, and were stored in polyethylene bottles.

Nitrogen content in samples was determined according to the Kjeldahl method [5]. For this purpose, 0.5 g of the ground sample were digested using a block digesting system (KB 8 S Kjeldatherm, Gerhardt) in digesting tube with 6 ml of concentrated H_2SO_4 in the presence of 5 g of a catalyst (K_2SO_4 + $CuSO_4$). After 40 % NaOH (w/w) was added, the sample was distilled using an automated unit (VAP20, Gerhardt). The ammonium N was fixed in 2 % H_3BO_3 and was titrated with 0.1 N H_2SO_4 in the presence of an indicator (bromocresol green and methyl red in 95 % ethanol). The N content was calculated according to the following equation:

$$N\ (\%) = \frac{(\text{ml } H_2SO_4 \text{ for sample titration} - \text{ml } H_2SO_4 \text{ for blank titration}) \times \text{Normality of } H_2SO_4 \times 1.4}{\text{sample dry weight in grams}}$$

The protein content in the plant was determined by multiplying the N amount by 6.25 factor [5].

For determining the P, K, Fe, Cu, Zn and Mn content in plant tissues, 0.5 g samples were dry-ashed at 500 ± 50 ^{o}C for 6 h. The residue was dissolved in 5 ml of 4 N HNO_3, filtrated and was then filled up to 100 ml with distilled water. Phosphorus content in the filtrate was determined with a spectrophotometer at 430 nm according to the vanadomolybdophosphoric acid yellow color method. The other elements were measured by an atomic absorption spectrophotometer [16].

Results and Discussion

AMV infection in the plant samples of diseased alfalfa was proved by DAS-ELISA. The reaction of the test plants is presented in Table 1. All the AMV infected samples induced similar symptoms. *Chenopodium amaranticolor* and *Ch. quinoa* displayed local lesions. On the other hand, *Nicotiana tabacum* cvs Samsun NN, White Burley and Xanthii, and *N. glutinosa* showed systemic infection as well as local reaction [9].

The alfalfa samples in which the AMV infection was detected by serological test and by mechanical inoculation of indicator plants were used in the next study.

Table 1. Symptoms of test plants induced after inoculation with AMV infected alfalfa samples.

Test plants	Symptoms
Ch. amaranticolor	Necrotic local lesions
Ch. quinoa	Necrotic local lesions
Datura stramonium	Necrotic local lesions, deformation
N. tabacum cv. Xanthii	Systemic mosaic, deformation, necrotic and chlorotic local lesions
N. tabacum cv. Samsun NN	Systemic mosaic, deformation, chlorotic local lesions
N. tabacum cv. White Burley	Systemic mosaic, deformation, chlorotic local lesions
N. rustica	Systemic mosaic, deformation, necrotic local lesions
N. glutinosa	Systemic mosaic, deformation, chlorotic local lesions
Gomphrena globosa	No symptoms

The quantities of N, P, K, Fe, Zn, Mn and Cu in the infected and healthy control alfalfa samples are presented in Table 2. The protein content was 35.31 % in healthy plants and 37.25 % in AMV infected plants, respectively.

The obtained data showed that the contents of Fe, Cu, Zn and Mn in leaves markedly decreased with AMV infection, whereas the amount of N slightly increased. No significant changes were observed in leave K content. These results could be due to the possible adverse effects and alterations in plant metabolism and cell integrity induced by viral infections.

Table 2. Changes of some macro- and micronutrients in infected with AMV and healthy alfalfa plants.

Nutrients	Infected plants	Healthy plants	Optimum ranges of nutrients
N (%)	5.96	5.65	4.50 - 5.00
P (%)	0.31	0.40	0.26 - 0.70
K (%)	2.70	2.68	2.00 - 3.50
Fe (ppm)	15.0	60.0	30 - 250
Cu (ppm)	23.50	48.50	7 - 30
Zn (ppm)	15.0	40.0	21 - 70
Mn (ppm)	25.0	35.0	31 - 100

N, P and K are mobile elements in plants, and they are actively transported to the young tissues when needed. The disease resistance and plant growth can adversely be affected by P deficiencies. Fe is a less mobile element; young growing leaves in particular are more susceptible to Fe deficiencies. Cu, Zn and Mn are immobile elements in the plant tissues. Zn

and Mn directly constitute chlorophyll. Cu is also reported to increase the chlorophyll formation [16].

In previous studies, the virus infected plants were reported to contain more N than control plants by analytic calculations. Agrios [2] observed in the diseased plants that their own protein level decreased, but the virus specific protein increased. Consequently, the established higher total protein content was more likely to be due to the increased level of viral proteins in the plant.

In conclusion, the presented investigation demonstrated that AMV infected alfalfa accumulated lower amounts of heavy metals. Hence, the forage containing virus infected alfalfa could not be harmful for the domestic animals. At the same time, the nutritious value is not decreased and is even improved because of the increased protein contents.

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ЕФЕКТ НА ЛЮЦЕРНОВОМОЗАИЧНИЯ ВИРУС (AMV) ВЪРХУ СЪДЪРЖАНИЕТО НА НЯКОИ МАКРО- И МИКРОЕЛЕМЕНТИ ПРИ ЛЮЦЕРНАТА

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Резюме

От люцернови площи в района на Испарта са събрани и анализирани листа с мозаични вирусни симптоми. В растителните проби е открит люцерновомозаичния вирус (AMV) чрез прилагане на механично инокулиране на индикаторни растения и серологични тестове. В заразените листа са изследвани макро- и микроелементите, за да се определи ефектът на AMV върху хранителния състав на люцерната. Количеството на P, Fe, Cu, Zn и Mn намалява, а на N се увеличава в сравнение със здрави люцернови листа. Съдържанието на K не се променя. Фуражът, съдържащ вирусно болна люцерна няма да е вреден за селскостопанските животни, а хранителната му стойност дори нараства, поради увеличеното белтъчно съдържание.