ISSN 0494-3244

Тутун/Tobacco,Vol.64, N^{0}1-6, 37-45, 2014 UDC: 633.71-248.843:631.811.98

Original Scientific paper

THE EFFECT OF PLANT HORMONE KINETIN ON REDUCING THE INTENSITY OF BROWN SPOT DISEASE IN TOBACCO

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ABSTRACT

Brown spot disease is economically important disease which has a particular impact on the reduction of tobacco leaves quality and, hence, on the total economic effect. The main factors for its occurrence are the climate and irrational use of agrotechnical practices. Ontogenetic age of leaves has also a big influence on disease attack, i.e. its intensity increases with aging of the leaves. A number of fungicides are applied in the control of this disease.

The aim of integral protection, however, is to include preventive measures and to reduce the number of treatments. Also, the biointensive model of integral protection aims to replace them by natural resources. Our objective was to study the effect of plant hormone kinetin on the intensity of attack of brown spot disease.

Two concentrations of kinetin were applied (30 mg/l and 60 mg/l), with one and two treatments of tobacco plants. They were inoculated with a suspension of pure culture of the pathogenic fungus *Alternaria alternata* - the causing agent of the disease.

It was concluded that kinetin treatment has a positive effect on reducing the disease intensity. The lowest intensity in the two-year investigation was recorded in plants with a single treatment of kinetin -60 mg/l. Among treatments with 30 mg/l, lower intensity was recorded when two treatments were applied. Histological investigations of tobacco leaves confirmed the effect of kinetin in reduction of the possibilities for infection.

The investigations point out to the possibility for application of the plant hormone kinetin in tobacco as a biological and preventive measure in the control of brown spot disease.

Keywords: brown spot disease, intensity of attack, kinetin, reduction, preventive measure

ВЛИЈАНИЕ НА РАСТИТЕЛНИОТ ХОРМОН КИНТЕТИН ВРЗ НАМАЛУВАЊЕТО НА ИНТЕНЗИТЕТОТ НА БОЛЕСТА КАФЕНА ДАМКАВОСТ КАЈ ТУТУНОТ

Болеста кафена дамкавост е една од економските значајни болести, со особено влијание врз намалување на квалитетот на тутунските листови, а оттука, на вкупниот економски ефект. Како главни предиспонирачки фактори за нејзината појава се климатските услови и нерационалната примена на агротехничките мерки. Исто така, онтогенетската возраст на листовите има големо влијание врз интензитетот на напад, односно, истиот се зголемува со стареење на листовите.

За заштита од оваа болест се применуваат повеќе фунгициди. Но, една од целите на интегралната заштита на тутунот е најпрвин вклучување на превентивни мерки и намалување на бројот на третирања.

Исто така, биоинтензивниот модел на интегрална заштита се повеќе се насочува кон намалување на употребата на хемиски средства за заштита и користење на природните ресурси.

Оттука, нашата цел беше да го проучиме дејството на растителниот хормон кинетин врз интензитетот на напад од болеста кафена дамкавост.

Беа применети две концентрации на кинетин (30 mg / *l* и 60 mg / *l*)со едно и две третирања на тутунските растенија. Истите беа инокулирани со суспензија од чиста култура на предизвикувачот на болеста-патогената габа *Alternaria alternata*.

Беше констатирано дека третирањето со кинетин има позитивно дејство врз намалувањето на интензитетот на болеста. Во двете години на истражување, најмал интензитет беше утврден кај растенијата со едно третирање со кинетин -60 mg / *l*. Помеѓу третирањата со 30 mg / *l*, помал интензитет е утврден при две третирања.

Хистолошките испитувања на тутунските листови го потврдуваат влијанието на кинетинот во намалувањето на можноста за инфекција.

Овие истражувања укажуваат на можноста за примена на растителниот хормон кинетин кај тутунот како биолошка и превентивна мерка за заштита од болеста кафена дамкавост.

Клучни зборови: кафена дамкавост, интензитет на напад, кинетин, редукција, превентивна мерка

INTRODUCTION

Brown spot is one of the economically important diseases on tobacco. It attacks all tobacco types but achieves the highest intensity in large-leaf tobaccos. Its occurrence largely depends on climate conditions, variety and modes of tobacco production. Its intensity is increased in areas with warm climate and higher relative humidity or heavy rainfalls (Gveroska, 2006 a). Occurrence of the disease is greatly affected by the undue harvesting of tobacco leaves. In recent years, the intensive mode of production and efforts to obtain higher yields resulted in application of some cultural practices (excessive irrigation, low planting density, over-fertilization, etc.) which indirectly increase the disease incidence.

Recommended techniques for protection from the disease include application of preventive measures, resistant tobacco varieties and use of chemicals. The biointensive model for integral protection, however, is inclined towards reduced number of treatments and the use of natural resources.

The long-term use of chemicals increases both resistance to the pathogen and the costs. It also causes harmful effects to human health and the environment. Therefore, the scientific approach is focused o finding alternative modes for control of the disease.

The influence of ontogenetic age of tobacco on the intensity of disease (also known as "disease of weakness and old age") imposed investigations in another field - physiological basis of reducing the intensity of the attack.

Physiologically active substances are regularly used in agriculture. Plant hormones or phytohormones are substances produced by plants that are used for regulation of growth and development. As in many other cultures, they can cause physiological changes in tobacco (Miceska et al., 1999). They find practical application in increase of productivity and improvement of technological properties of plants (Petrova and Iliev, 1990), but they also have their role in the adaptive response of plants to various stressful situations (Atanasova et al., 1996).

According to Seilaniantz et al. (2007), plant hormones play an important role in growth regulation and in creation of a network of signals involved in plant response to a wide range of biotic and abiotic stresses.

Cytokinins promote a wide range of diverse biological activities that appear in the regulation of growth, formation of organs, aging process and dormation period. These substances, especially kinetin, have an influence on cell division, elongation and differentiation of cells, growth of the root tip and its initiation, initiation and growth of vegetative cone and on suspension of dormancy. Among many other functions, they increase plant tolerance to low and high temperatures and to fungal infection (Sarić et al., 1987).

Cytokinins affect the susceptiblity of leaves and other organs and increases the resistance to pathogens and extreme temperatures (Annonimus, 2011). It was stated that plants which have an ability to survive in suboptimal conditions contain increased amount of cytokinin. Such is the case with the beans exposed to AL toxin (Massos et al., 1994 , loc cit Atanassova et al., 1996).

No data have been found on the influence of plant hormones on tobacco reaction to certain diseases, but according to Haberlach et al. (1978), the resistance of tobacco in tissue culture can be controlled by regulation of the hormone regimen.

With reference to the effect of phytohormones on *Alternaria alternata*, Perez et al. (1995) report that the brown spot disease on fruits treated with giberelin acid is manifested with much lower severity.

Data on the effect of phytohormones on plant reaction to pathogens has raised the interest for investigation of their impact on tobacco and its reaction to brown spot disease. The aim of this paper was to study the effect of the plant hormone kinetin on the intensity of brown spot disease caused by the pathogenic fungus.

MATERIAL AND METHOD

Two-year investigations (2009 and 2010) were carried out in the biological laboratory of the Scientific Tobacco Institute - Prilep. Pots filled with 2:1 ratio of soil and sterilized manure were planted with 50 tobacco plants of the large-leaf variety B 2/93 for each variant. They were grown in traditional way, with the following variants:

1	30 mg /l kinetin	1 treatment
2		2 treatments
3	60 mg /l kinetin	1 treatment
4		2 treatments
5	Ø (control)	untreated plants

The first treatment of plants with prepared solutions containing appropriate concentrations of kinetin was made about a month after planting. The second treatment was made on half of the plants, a month after the first treatment.

Plants were inoculated with a suspension of fresh material, i.e. brown spot from infected leaves. The material was blended in a ratio 10 spots per 100 ml of distilled water and then filtered through a three-layer gauze. The leaves were wounded with karborundum, sprayed with 1 % solution of glucose, and then whole plants were sprayed with the prepared suspension. Inoculated tobacco plants were covered with polyethylene and stored in biological laboratory for 10 days.

Small deviation was made in 2010, when the period of humidification was 13 days, because of the very low temperatures that followed after inoculation.

Intensity of attack was assessed using a six-grade scale (0-5). The leaves were classified in appropriate category :0) healthy leaves ; 1) 1 spot on a leaf 2) 2-5 spots on a leaf 3) 6-10 spots on a leaf 4) 11-25 spots on a leaf 5) more than 1/2 of leafy area infected. Disease index was calculated according to the formula of Mc-Kiney:

$$i = \frac{\sum n \cdot k}{N \cdot K} x100$$

where:

n - number of leaves in the appropriate category
k - category
N - total number of analyzed leaves
K-Total number of categories

Before inoculation, leaf samples were taken from the investigated variants and they were used for making permanent preparations.

RESULTS AND DISCUSSION

Brown spot disease is manifested by the appearance of small brown spots which number is gradually increasing. At the same time, the spots enlarge and coalesce, spreading over the largest part of the leaf (Fig. 1). They become necrotic, with concentric rings surrounded by chlorotic area. In case of severe attack, irregular angular areas are formed and the infected tissue becomes stiff and falls off. These symptoms indicate that the tissue has undergone biochemical changes which deteriorate the quality of raw tobacco.

Fig 1. Symptoms of brown spot disease in large-leaf tobacco

The causing agent of this tobacco disease in R. Macedonia is the pathogenic fungus *A. alternata* (Fig. 2), subdivision *Deuteromycotina* (Fungi Imperfecti) - imperfect fungi, class *Hyphomycetes*, order *Hyphomycetales*, family *Dematiaceae* (Ivanović, 1992).

A. alternata – pure culture

This pathogen has a wide range of hosts. It attacks almost all types of tobacco and all the varieties are, more or less, susceptible. Still, the large-leaf varieties are subject to greater intensity of attack than the oriental and semi-oriental varieties. Among the large-leaf varieties, higher intensity of attack was observed in B 2/93 than in MV 1 (Gveroska, 2006b).

The symptoms of brown spot in this variety in conditions of natural and artificial inoculation are presented in Fig. 3 and 4.

Fig. 3 Brown spot in B2/93 -natural infestation

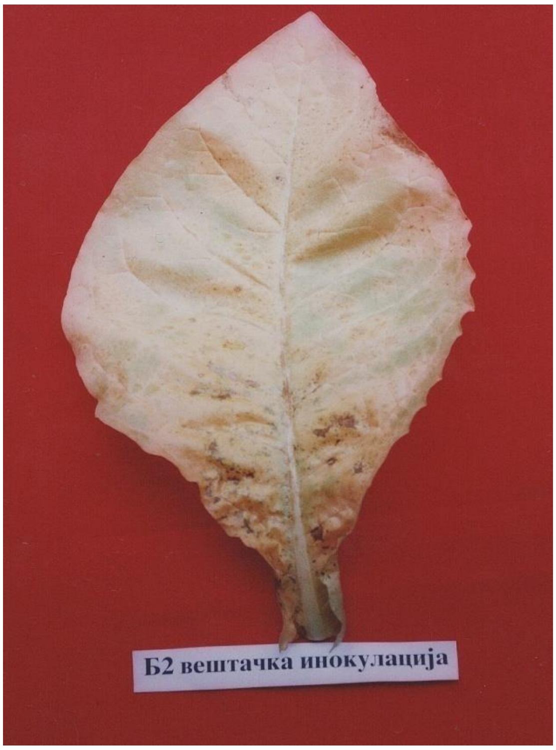


Fig.4 Brown spot in B2/93 – artificial infestation

In 2009, the intensity of disease attack ranged from 14.39% in plants treated with 60 mg/l kinetin (1 treatment) to 26.73% in plants treated with 30 mg/l kinetin (1 treatment). Between the two variants of treatment with 60 mg/l kinetin, higher intensity of attack was observed in those where two treatments were applied (20.85%). The highest intensity of 40.13% was observed in the check variant.

Table 1. The intensity of brown spot disease in 2009

Variant	Total number of analyzed leaves	Percentage of infected leaves	Intensity of attack (%)
30 mg / *l* kinetin 1 treatment	270	70,00	26,73
30 mg / *l* kinetin 2 treatments	165	61,82	26,67
60 mg / *l* kinetin 1 treatment	161	50,31	14,39
60 mg / *l* kinetin 2 treatments	227	51,10	20,85
Ø Check	179	89,38	40,13

In 2010, the intensity of attack in the check variant was much higher compared to 2009. The same situation was observed in the other variants investigated. It was reflected in the percentage of infected leaves (Table 2). Such a high percentage of infected leaves was due to the longer period of moistening. The temperature decrease in this case had no negative influence, because the infection was possible even at very low temperatures, which are not typical for tobacco growing season.

Table 2. The intensity of brown spot disease in 2010

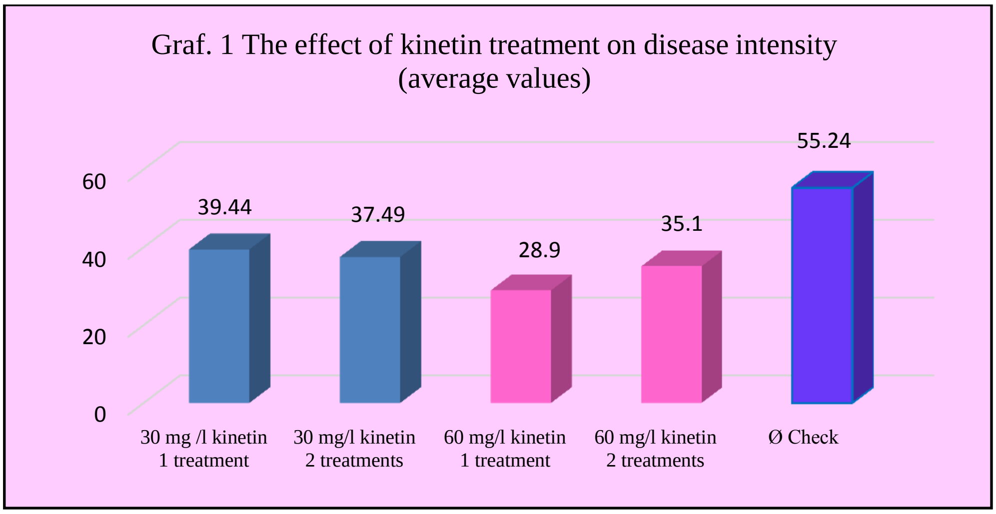
Variant	Total number of analyzed leaves	Percentage of infected leaves	Intensity of attack (%)
30 mg / *l* kinetin 1 treatment	132	94,69	52,15
30 mg / *l* kinetin 2 treatments	138	96,38	48,31
60 mg / *l* kinetin 1 treatment	138	96,37	43,42
60 mg / *l* kinetin 2 treatments	128	93,87	49,35
Ø Check	223	95,54	70,25

Percentage of infected leaves in the check variant is almost equal to that in the investigated variants. However, the number of analyzed leaves is higher and the infected leaves belong to higher categories in the six-grade scale, which has surely influenced the value of disease index in the check variants (Table 2).

In treated plants, the highest intensity of attack was observed with 30 mg/l kinetin - 52,15 %. In plants where two treatments with the same concentration of kinetin were applied, the intensity was slightly lower - 48.31%.

The lowest intensity of disease attack (43.42 %) was recorded in plants with a single treatment of 60 mg /l kinetin. In variants treated twice with the same concentration of kinetin, the intensity of attack in this year was higher than the previously mentioned variant (49.35 %) (Table 2).

According to the average results on influence of the plant hormone kinetin on intensity of brown spot disease in the two-year investigation (Graph 1), the highest intensity of attack was recorded in plants treated with 30 mg /l kinetin (one treatment) and the lowest intensity of in plants treated with 60 mg / l kinetin (two treatments) (Graph 1).



Such an effect was determined in tobacco variety that was the most susceptible to this disease. Accordingly, the treatment with kinetin has a positive effect on reducing the intensity of brown spot attack in tobacco. Little is known about the interaction between plant hormones and the response to various pathogens.

Seilaniantz et al. (2007) investigated the interaction between the virulence of the pathogen and signaling networks involved in the response to various pathogens. The authors reported that the hormones can influence disease outcomes through their effect on SA (salicylic acid) or JA (jasmonic acid) signaling.

Similar statements were reported by Perez et al. (1995) in the treatment with giberellic acid. They claim that there is no direct effect on the pathogen, but rather physiological one, delaying senescence or healing wounded tissue.

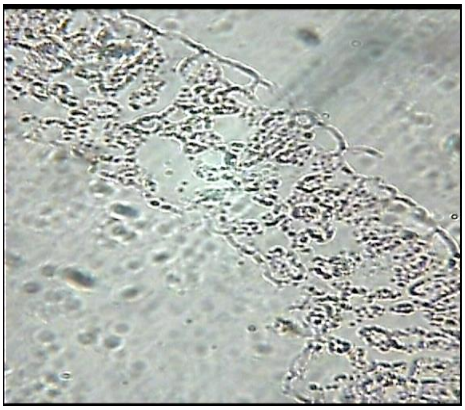


Fig. 5 Tobacco leaf – untreated plants

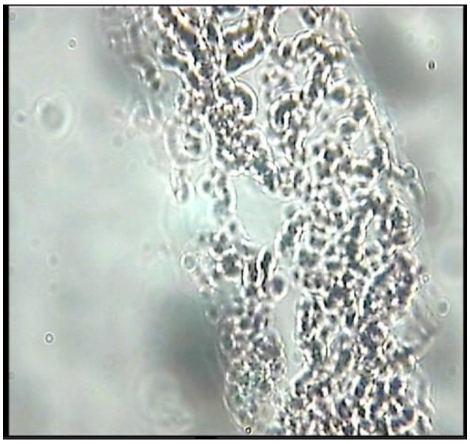


Fig. 6 Treatment with 30 mg/l kinetin

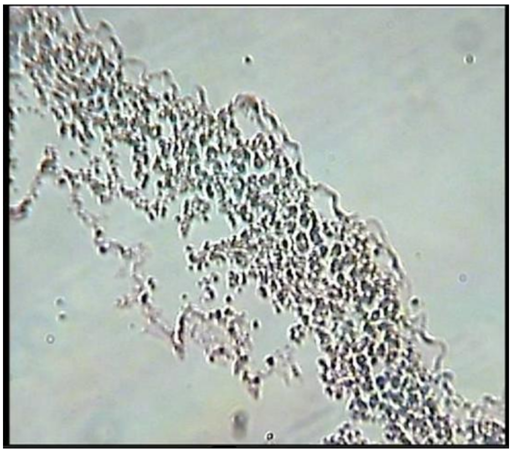


Fig. 7 Treatment with 60 mg/l kinetin

According to Geraats (2003), ethylene can induce resistance to plant diseases. In his experiments, tobacco plants that do not response to ethylene become susceptible to diseases. Ethylene plays important role in maturation of plants and ripening of fruits (Sarić et al., 1987). Although it is a hormone with completely different role, such information can contribute to explaining the role of cytokinins. The pathogen usually attacks the plant in a stage of turgor and uses its nutrients. This plant disease, however, is known as a disease of oldness and weakness and it attacks the old, overmature leaves. In this case cytokinins increase the activity of plants (slowing down the aging process) and thereby reduce the intensity of the disease.

In our investigation, higher intensity of disease was observed in the variant with two treatments of 60 mg/l kinetin, compared to the variant with one treatment. Petrova and Iliev (1990) reported that the increased concentration of cytokinins leads to reduced content of sugars. Brown spot is a disease that attacks the tissues with low content of sugars (Rotem, 1994). Hence, such treatment can lead to decrease of sugar content in tobacco leaves, which on the other hand will increase the susceptibility of the tissue to the pathogen. In support of this statement is the fact that the investigated tobacco variety is characterized by a low content of sugars and highest susceptibility to the disease causing agent.

Haberlach et al. (1978) reported that the increased concentration of cytokinin in tissue culture can have an influence on tobacco resistance. Hypersensitive reaction of the resistant varieties to *Phytophtora parasitica var. nicotianae* is lost and the tissue is poorly colonized with zoospores. However, in that case, too, when kinetin concentration was increased over 2 μм, the resistance was lost.

According to Turner (1986, loc cit. Atanassova et al., 1996), plants have the ability to respond to the pathogen through morphological and physiological changes, by which they can adapt to the changed conditions. In our study, the treatment with kinetin resulted in increase of length of the parenchymal tissue compared to that of untreated plants (Figure 5-7), which certainly reduced the possibility of infection and development of the disease.

Due to the role of cytokinins in plant response to diseases, their application may be useful in inducing resistance to pathogens and extreme temperatures (Atanassova, 1996; Annonimus, 2011). Therefore, the positive effect of treatment with kinetin enables its application in protection from brown spot disease.

CONCLUSIONS

1. The plant hormone kinetin affects the intensity of attack of brown spot disease in tobacco.
2. The highest intensity of attack was observed in plants treated with 30 mg/lkinetin (one treatment). In variant with two treaments with the same concentration of kinetin, the intensity of disease attack was lower.
3. Brown spot disease achieved the lowest intensity of attack in plants with a single treatment of 60 mg/l kinetin. However, higher intensity of attack was observed in plants where two treatments with 60 mg/l kinetin were applied.
4. In both years of investigation, the highest intensity of disease was recorded in plants treated with 30 mg/l kinetin (one treatment), and the lowest intensity was observed in plants treated with 60 mg/l kinetin (one treatment).
5. Treatment with kinetin induces histological changes in tobacco leaves, which leads to reducing the possibility

of infection and development of disease.

6. The positive effect of treatment with kinetin is a good reason for its use in protection from brown spot disease on tobacco.
7. The application of biochemical - physiological products in reducing the intensity of disease attack is a modern and environmentally acceptable measure in protection of tobacco crop.

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