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BIOLOGICAL CHARACTERISTICS OF *ALTERNARIA ALTERNATA* IN TOBACCO

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ABSTRACT

Brown spot disease causes severe economic losses on tobacco in R. Macedonia, mainly because of the impairment of quality of tobacco raw. Investigations must be made on pathogenic fungus *Alternaria alternata*, the causing agent of this disease, in order to find solutions to the problems.

Due to the wide range of host-plants and the important role of ecological factors on occurrence and distribution of this disease, our aim was to study their influence on biology of the pathogen.

Estimations were made on nutrient medium, temperature and light effects on percentage of germinated conidia and on the way of conidia germination. It can be stated that germination rate was higher in media with better supply of nutrient matters. The highest rate of conidia germination was noticed in tobacco juice and the highest speed of germination in tomato juice.

Temperature increase had a positive effect on conidia germination and the optimal temperature was 28^{0}C. We also found that germination was higher in dark conditions than in light.

We believe that these investigations will contribute to understand the pathology of brown spot disease and to take preventive measures for its control.

Key words: *Alternaria alternata*, tobacco

БИОЛОШКИ ОСОБИНИ НА *ALTERNARIA ALTERNATA* КАЈ ТУТУНОТ

Болеста кафена дамкавост на тутунот во Р. Македонија предизвикува штетни економски ефекти главно поради нарушување на квалитетот на тутунската суровина. Поради тоа, за решавање на проблемите поврзани со оваа болест во наши услови, неопходно е проучување на предизвикувачот - патогената габа *A. alternata.*

Поради широкиот круг на домаќини и големата улога на еколошките фактори врз појавата и ширењето на болеста, со овие истражувања сакавме да испитаме какво е влијанието на истите врз биологијата на овој патоген.

Проучувано е влијанието на хранливата средина, температурата и светлината врз процентот на из'ртени конидии и начинот на 'ртење на конидиите. Притоа беше констатирано дека тие 'ртат подобро во средините побогати со хранливи материи. Конидиите из'ртеле во најголем процент во сок од тутун, а најбрзо во сок од домат.

Зголемувањето на температурата позитивно влијае врз 'ртењето на конидиите, а оптималната температура изнесува 28^{o}C. Исто така, конидиите ртат подобро во темни услови, отколку на светлина.

Бидејќи проучувањето на одредена болест се заснова врз првичниот пристап кон патогенот и, поконкретно, неговите биолошки особини, сметаме дека овие истражувања се од особен интерес за проучување на патологијата на болеста кафена дамкавост и дека ќе придонесат за преземање на превентивни мерки за заштита од неа.

Клучни зборови: *Alternaria alternata*, тутун

INTRODUCTION

The occurrence of plant diseases is greatly affected not only by he specific plant-pathogen relations, but also by ecological factors. They have a direct impact on biological properties of the pathogen, making possibilities for development of further stages in disease pathogenesis.

Sometimes even the microclimate conditions can enable the occurrence of disease and its further development.

Environmental factors are so influential that some investigations point out to differences of their effect in certain stages of pathogen's biology.

In the case of brown spot disease on tobacco, conidia germination of its causing agent - the pathogenic fungus *A. alternata* highly depends on environmental factors, i.e. nutrition media, temperature and light.

Conidia germination occurs in media rich with nutrient elements, especially with sugars (Stavely and Main, 1970). They stimulate conidia germination and spread of infections (Pearson and Hall, 1975).

According to Rotem (1994), the rich inoculum is of particular importance for necrotrophic parasites as *Alternaria sp*.

Waggoner and Parlange (1975), Rotem (1994) and Jovancev (1997) determined the optimal temperature for conidia germination and its effect on germination duration. According to Norse (1973, cit. by Rotem 1994), conidia germination and growth of germ tubes are differently affected by temperature, especially in suboptimal conditions. Germination occurs in considerably wider temperature range than germ tubes growth.

The effect of light was investigated from the aspect of activity spectra, especially the ultraviolet and infrared light. It was reported that the lifespan of conidia is 6 to 30 times shorter when exposed to direct sunlight than in laboratory conditions. The long wave UV radiation is the principal factor for spores mortality (Rotem et al., 1985).

Simmons (1992) reported that exposure of cultures to UV light induces changes in pigmentation and structure of conidia walls, which makes them different from those grown in natural conditions.

Leach (1975) reported that a number of fungi, including *A. tenuis*, intensively release spores when relative humidity is reduced from saturation state in the presence of IR light.

Rotem (1994) explains the effect of light and activity spectra on genus *Alternaria*.

The aim of our investigations was to study biological characteristics of pathogenic fungus *Alternaria alternata* in various environmental conditions. Results obtained in our investigations should contribute to tobacco protection from brown spot disease.

MATERIAL AND METHODS

Conidia germination was investigated by application of hanging drop method in Van Tieghem cell.

Percentage of germinated conidia was estimated by the method of Ko et al. (1973).

Germ tube length was determined with an ocular micrometer. The effect of liquid medium on germination and germ tube length was investigated on conidia from naturally infested material, with application of tap water, distilled water, solution of 1% glucose, tobacco juice and ripe tomato juice. The conidia suspensions

prepared in adequate media were incubated in a thermostat at 28^{0}C.

Monitoring of germination was made 30-40 min after the start of incubation up to 6 hours, at 1-hour intervals. Experiments for each liquid medium were made in three replications, monitoring 5 preparations for each interval.

Measurements of germ tube length started two hours after incubation. At least 200 samples of each liquid medium, randomly chosen in the preparations, were measured. Mean value of the measurements was used for size determination.

Investigations on the effects of temperature and light were made in conidia from pure fungus culture, obtained from isolates of several tobacco varieties and localities.

To study the temperature effect on germination, conidia suspensions in distilled water were placed in Van Tieghem cells and incubated at 5, 10, 15, 20, 25, 28, 30 and 32^0 C. The experiment was made in three replications, monitoring 5 microscopic preparations for each temperature. Percentage of germinated conidia was determined 4-5 hours after the start of incubation.

The effect of light on conidia germination was investigated in laboratory conditions, in two variants: in light and in dark. Van Tieghem cells with prepared suspensions in distilled water were placed in light and in dark place. The experiment was replicated three times, with 3 microscopic preparations for each time interval and each variant. Control of conidia germination was made at 3-40 minutes up to 5 hours.

RESULTS AND DISCUSSION

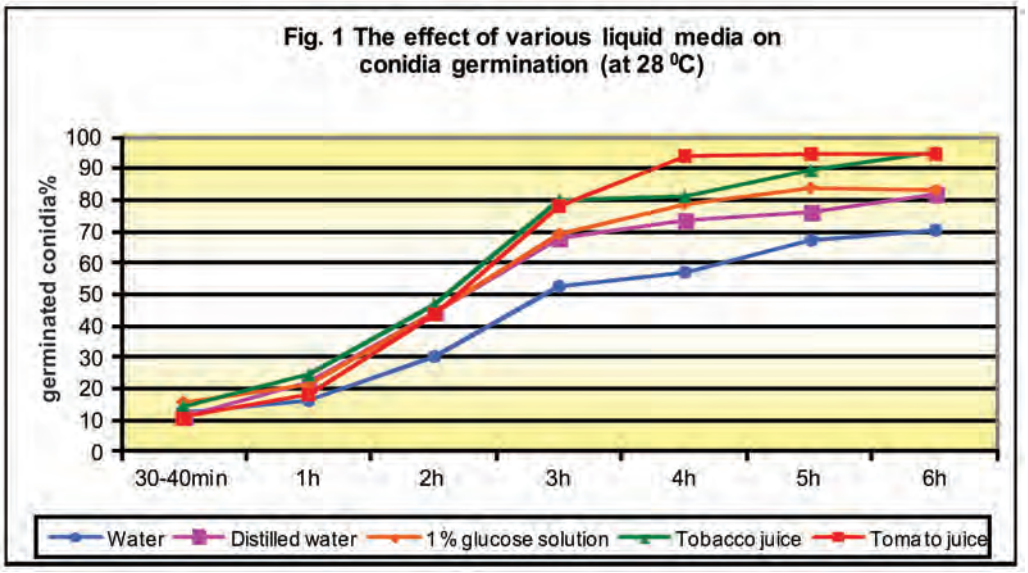
The highest rate of conidia germinate in the interval of 3-4 hours, with over 50% of germinated conidia in all investigated media (Table 1).

The highest percentage of germinated conidia in the initial intervals was recorded in the solution of glucose and tobacco juice. But in the interval of 5 hours, this percentage was the highest in tomato juice, where maximum germination of 94.47% was achieved.

In the last interval (6 hours after incubation), germination percentage ranged from 70.09% in water to 95.06% in tobacco juice. Accordingly, best results of germination are achieved in tobacco and tomato juices. Although in tomato juice germination is not markedly expressed in the initial interval, the maximum value of germinated conidia in this medium is achieved two hours earlier than in tobacco juice.

Table 1. The effect of various liquid media on conidia germination (at 28^{0}C)

Liquid media	Germinated conidia %						
	Incubation period						
	30-40 ‘	1h	2h	3h	4h	5h	6h
Water	12,50	16,29	30,30	52,51	57,01	67,41	70,09
Distilled water	11,09	21,56	44,00	67,55	73,40	76,02	81,76
1% glucose solution	15,80	21,18	44,05	69,20	78,71	83,82	83.04
Tobacco juice	14,28	24,52	46,59	79,67	81,06	89,28	**95,06**
Tomato juice	10,71	17,91	43,44	78,00	**93,66**	94,47	**94,52**



The lowest rate of germinated conidia from the start to the end of incubation was recorded in tap water (Table 1, Fig. 1).

Accordingly, higher rate of germination was recorded on nutrient-rich media (tomato and tobacco juice, glucose solution). Jovancev (1997) also reported highest germination of conidia in the juice of ripe or green tomato.

The above results were comparable to those of Stavely and Main (1970), who reported that addition of sugars increases conidia germination. According to Pearson and Hall (1975), glucose and fructose solution in exudates of ripe tomatoes stimulate the conidia germination and thereby favor the spread of infections. Rotem (1994) found that in necrotrophic parasites like *Alternaria sp*. the addition of sugar strengthens the inoculum and accelerates its penetration in physiologically weakened tissue.

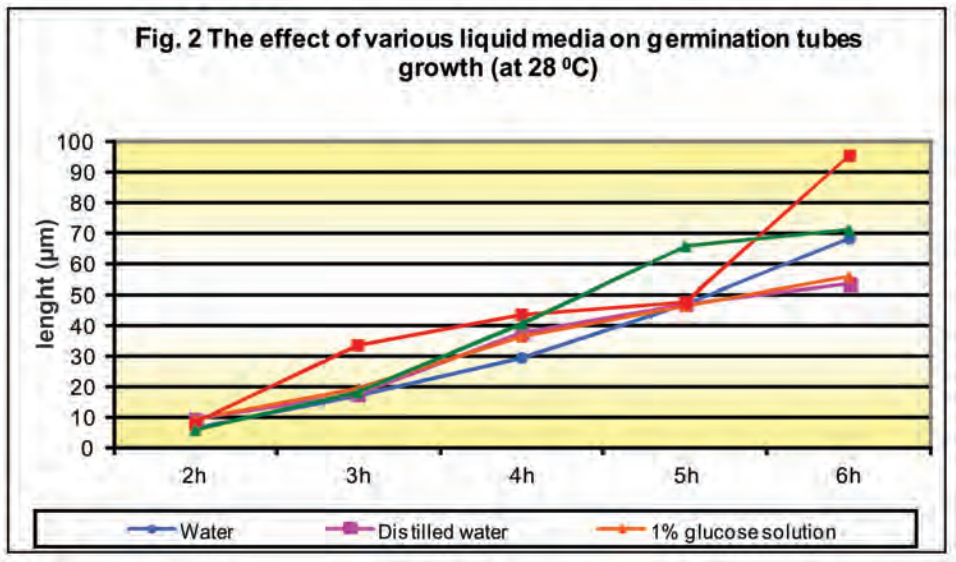
After conidia germination, germ tubes continue to grow (Photo 1, 2). In the starting interval (2 hours), their length ranges from 5.91 µm in tomato juice to 9.46 i.e. 9.23 µm in 1% solution of glucose and distilled water, respectively. The highest length is achieved in tomato juice in the 5-hours interval, when in all other media they have almost equal lengths (Table 2).

At the end of investigation, the length of germ tubes was 53.33 µm in distilled water, 71.02 µm in tomato juice and 95.06 µm in tobacco juice.

Table 2. The effect of various liquid media on germ tubes growth (at 28^{0}C)

Liquid media	Germ tubes length (µm)				
	Incubation period				
	2h	3h	4h	5h	6h
Water	6,29	16,92	29,52	47,08	68,25
Distilled water	9,23	17,46	37,77	47,02	53,33
1% glucose solution	9,46	19,38	36,24	46,59	55,67
Tobacco juice	7,80	33,52	43,33	47,50	**95,06**
Tomato juice	5,91	18,14	40,43	66,60	71,02

According to Table 2 and Graph 2, the length of germ tubes in the investigated time intervals was increasing and the highest rates were achieved in the 5- and 6-hour intervals in tomato and tobacco juices respectively.



From the aspect of nutrient media, the highest length of germ-tubes was recorded in tobacco juice and in distilled water.

The results of investigation reveal that nutrient rich media enable faster conidia germination and germ tubes growth.

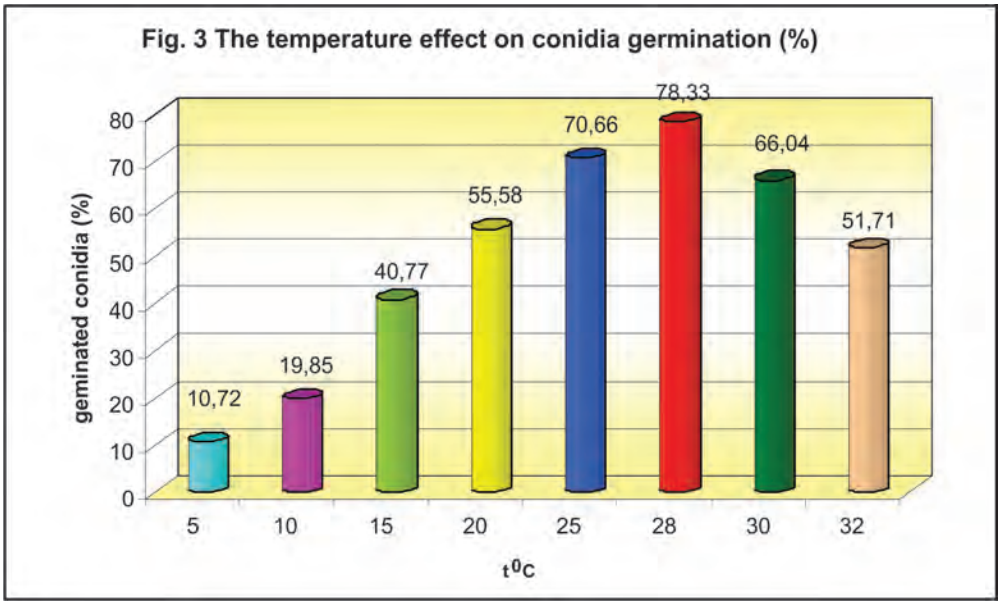
Photo 1. *A. alternata* – conidia

Photo 2. *A. alternata* - Germ tubes growth

At 5^{0}C, the range of germinated conidia is from 8.88% to 12.08% in the isolates MB 179 and IP 163 respectively. At higher temperatures, however, the percentage of germinated conidia increases. Thus, at 15^{0}C, the percentage of germinated conidia is almost doubled, raising from 26.74% to56.35%, and also at 20^{0}C (from 42.75% to 63.44%). (Table 3)

Table 3. The temperature effect on conidia germination (%)

Isolate \ t ºC	5	10	15	20	25	28	30	32
MB 179	8,88	21,89	41,24	55,42	72,73	75,75	64,10	48,81
MB 157	10,52	13,98	56,35	60,73	74,83	75,88	59,35	47,20
KP 166	11,39	19,11	26,74	42,75	62,89	82,33	68,44	51,54
IP 163	12,08	24,45	38,74	63,44	72,18	79,35	72,26	59,28
Average	10,72	19,85	40,77	55,58	70,66	78,33	66,04	51,71



The highest percentages of germinated conidia among the isolates were obtained at 28^{0}C, and the differences between them were smaller than at other temperatures investigated (ranging from 75.75% for the lowest and 82.33% for the highest). (Table 3).

The average results for all investigated isolates reveal that the lowest percentage of germinated conidia was obtained at 5^{0}C and the highest at 28^{0}C, which indicates that the optimal temperature for germination is 28^{0}C Table 3, Fig. 3).

Other research workers also reported 25^{0}C - 30^{0}C as optimal temperature range for conidia germination, which is in accordance with our results.

Rotem (1994), investigating several species of *Alternaria* found that the optimal germination temperature was 25^{0}C, and the maximum about35^{0}C. For *A. alternata* the minimum temperature is below 5^{0}C, the optimum is 22.5 - 29^{0}C and the maximum 35-40^{0}C. Investigations of Jovancev (1997) also confirmed that the highest conidia germination is achieved at 20 - 28^{0}C.

With temperature increase, conidia germination in all isolates decreases. Percentage of germinated conidia at 32^{0}C is almost one-third lower compared to those at 28^{0}C (Table 3). The above results are confirmed by Waggoner and Parlange (1974), who investigated the dependence of germination and temperatures. They reported that germination increases linearly at temperatures up to 30^{0}C, while at temperatures above 30-40 ^{0}C the germination decreases. They also found that higher temperatures lead to increased time of germination.

The results on conidia germination in investigated isolates show that at almost all temperatures the highest percentage of germinated conidia was achieved in the isolate IP 163. In isolates where lowest percentage of germinated conidia was obtained at lower temperatures (MB 157 and MB 179), the values were significantly higher at optimal temperatures. When temperature increased above the optimum, however, conidia germination was lower comoared to the other isolates.

Accordingly, the isolate IP 163 shows better adaptability to temperature changes, while the other isolates required higher and optimal temperatures for conidia germination.

Germination of *A. alternata* conidia is highly affected by light (Table 4).

In the starting intervals of incubation, the rate of conidia germinated in light was only 5.26 - 13.63%. An hour later it increased from 8.10% in IP 163 isolate to 24.61% in MB 179. At the end of incubation, the percentage of germinated conidia ranged from 58.46% to 85.71%.

In dark conditions, all isolates even in the starting intervals showed higher conidia germination than in light, ranging from 54.41% to 88.33% (Table 4).

Table 4. The light effect on conidia germination (%)

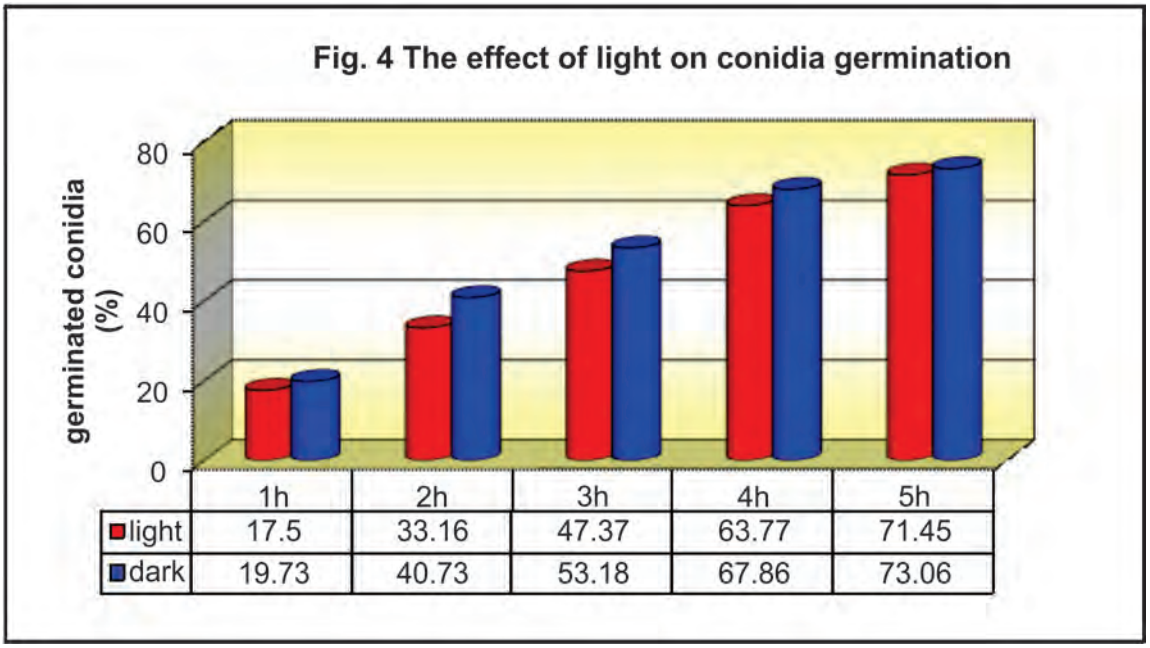
Isolate	LIGHT						DARK					
	incubation period						incubation period					
	30 ’	1 h	2 h	3 h	4 h	5 h	30 ’	1 h	2 h	3 h	4 h	5 h
MB 157	9,47	19,78	42,40	70,86	80,85	85,71	18,86	22,87	54,31	76,65	82,38	88,33
MB 179	13,63	24,61	27,07	34,96	64,63	70,17	10,71	27,58	35,78	44,14	71,87	76,43
IP 163	5,26	8,10	30,00	36,19	45,83	58,46	6,66	8,75	32,11	38,76	49,32	54,41
Average	9,45	17,50	33,16	47,37	63,77	71,45	12,08	19,73	40,73	53,18	67,86	73,06

Significant increase of germinated conidia in light conditions was noticed 3 hours after the start of incubation in MB 157 and 4 hours after in IP 163 and MB 179 isolates.

In dark, the highest percentage of germinated conidia in MB 157 was noticed after 3 hours (just like in light), indicating that this isolate has better conidia germination compared to all others. In dark conditions, too, MB 179 the percentage of germinated conidia increased after 4 hours, while in IP 163 the increase was gradual.

It can be stated that environmental factors have a great impact on biological properties of the pathogen, but they also depend on the isolate itself.

Lacey (1992) recommended alteration of light and dark periods. However, investigations of the three isolates reveal higher average rate of germinated conidia in dark compared to light conditions. This difference was more noticeable up to 3 hours after the start of incubation, and then it became smaller. At the end of incubation, the rate of germinated conidia was 73.06% in dark and 71.45% in light (Table 4, Fig.4).



CONCLUSIONS

- Various liquid media are suitable for conidia germination.
- The highest percentage of conidia (more than 50%) is germinating in the interval of 3-4 hours.
- Conidia germination percentage is the highest in tobacco and tomato juices. The maximum percentage of germinated conidia in tomato juice is achieved two hours earlier than in tobacco juice.
- The highest length of germination tubes is achieved also in the above media (tobacco and tomato).
- The temperature affects the conidia germination. The highest percentage of germinated conidia was observed at 28^{0}C, which is denoted as an optimal temperature for this process.

The percentage of germinated conidia is higher in dark conditions than in light.

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