Vietnam Journal of Science and Technology ***57*** *(6) (2019) 657-664*

doi:10.15625/2525-2518/57/6/13013

EFFECT OF CALCIUM-ALGINATE AND ESSENTIAL OIL ON *Colletotrichum acutatum* AND THE SHELF LIFE OF THE GRAPE

Lieu My Dong[1, *], Nguyen Thi Truc Quyen[1], Le Thi Thu Thao[1], Tran Thi Thu Thao[1], Cu Thị Ngoc Quyen[1], Dang Thi Kim Thuy[2],

[1]*Faculty of Food Science and Technology, University of Food Industry, 140 Le Trong Tan, Ho Chi Minh City*

[2]*Institute of Tropical Biology,9/621 Ha Noi highway, Ho Chi Minh City*

[*]Email: *lieudong289@gmail.com*

Received: 24 August 2018; Accepted for publication: 7 October 2019

Abstract. This study aimed to investigate the efficacy of three kinds of essential oil, which are cassia oil, holy basil oil, and peppermint oil on inhibition of the plant pathogen *Colletotrichum acutatum* by the agar diffusion method. The selected essential oil was combined with the edible film (calcium-alginate 0.25 %; 0.5 %; and 1 % (w/v)) and applied in the green grape preservation (infected with *C. acutatum* 5 log spores/ml). The results showed that all essential oils have antifungal activity against *C. acutatum* with the Minimum Inhibitory Concentration (MIC) value of cassia oil was 2.5 µl/ml, whereas holy basil oil and peppermint oil was 20 µl/ml. The result of the grape preservation showed that control samples were completely rotten after ten days of storage that lower efficient than the edible film-coated samples. After 20 days of treatment by calcium-alginate (1 % w/v), the degree of damage was 30 %. The results also indicated that the essential oil at MIC value had a negative effect on the grape preservation process, whereas the combination of calcium-alginate (0.5 % w/v) and cassia oil (1.25 µl/ml) showed the best resistance against to *C. acutatum* infecting on the grape, which was not rotten after 20 days of storage.

Keywords: *Colletotrichum acutatum*, calcium-alginate, antifungal activity, cassia oil, essential oil.

Classification numbers: 1.4.5, 1.4.6.

1. INTRODUCTION

Grape fruit (*Vitis* spp.) isone of the widely grown fruits in the world. In Viet Nam, the green grape (*Vitis vinifera*) is an important grape variety and has high economic value. NinhThuanprovince is one of the few localities in Viet Nam that can grow this fruit. Grape is a non-climacteric fruit with a low rate of post-harvest physiological activity. Unlike many fresh fruits, the grape needs to harvest at a fully rip period. Therefore, the grape preservation time is shorter than other fruits such as apple, mango, and banana [1]. Grape ripe rot - a serious disease caused by *Colletotrichum*, was first reported in the United States in 1891 and found in most

grapes growing regions [2]. *Colletotrichum acutatum* has been reported as a causal agent of grape ripe rot that causes yield losses and decreases the quality of wines in the subtropical wine region [3]. Therefore, control of *C. acutatum* during preserving is necessary. To reduce the post-harvest losses, the grape is usually treated with common fungicides throughout the season and kept cold in the presence of sulfur dioxide [4]. However, these treatments are not permitted in organic agriculture [4]. In the last years, the applications of essential oils were carried out to control damage and extend the quality of the agricultural product, because of the natural safety as well as environment-friendly [5]. However, the main obstacle for using essential oils as food preservatives is that it is not strong enough as the single components and cause negative impacts when the supplement enough amount to provide an antibacterial effect [6]. A solution ideal to reduce the amount of essential oil while still maintaining the antifungal effectiveness of them that is to combines with other types of bioactive edible coatings [7]. These bioactive edible coatings were created from components capable of biological decomposition, is consumed as part of food products, acts as a respiratory barrier, help prevent and keep essential oil components on the surface. In the present study, cassia oil (*Cinnamomum cassia*), holy basil oil (*Ocimum gratissimum*) and peppermint oil (*Mentha arvensis*) derived from Viet Nam were evaluated for antifungal activity against to the plant pathogen *Colletotrichum acutatum* by the agar diffusion method. The essential oil having the best antifungal efficiency was combined with edible coatings calcium-alginate and applied in the green grape preservation (infected with *C. acutatum* 5 log CFU/ml) in 20 days at 30 ^{o}C.

2. MATERIALS AND METHODS OF RESEARCH

2.1. Materials

Essential oils were used such as cassia oil (*Cinnamomum cassia*), holy basil oil (*Ocimum gratissimum*) and peppermint oil (*Mentha arvensis*) which derived from Viet Nam. The trees were hand-collected and immediately used to obtain essential oil by steam distillation. *Colletotrichum acutatum* Q1 was isolated from the grape ripe rot and cultured on Potato Dextrose Agar (PDA) medium. It was identified by the sequencing method at Nam Khoa company. The green grape (*Vitis vinifera*) which derived from Ninh Thuan province, was collected in the garden (at a fully rip period) and transported to the laboratoryin 24 hours. Essential oils were diluted in Tween 80 (0.3 % (v/v)) at different concentrations of 60; 40; 20;10; 5; 2.5; 1.25 µl/ml and were homogenized to the emulsion.

2.2. Methods

2.2.1. Determination of antifungal activity by the agar diffusion method.

The antifungal activity of essential oils was determined according to Lieu *et al.* [8]. Briefly, *C. acutatum* suspension spread on PDA medium plates (at a final concentration of 5 log CFU/ml). The essential oils in the emulsifying agent (Tween 80 (0.3 % v/v)) at different concentrations dripped on PDA media (15 µl) and the emulsifying agent was used at the control. The Petri dishes were incubated at 30 ^{o}C for 48 h and examined by inhibition zone. The MIC values determined as the lowest concentration of essential oil preventing visible growth of microorganisms. The essential oil having the best result was used for the next step.

2.2.2. Evaluation of the grape preservation efficiency by essential oil and calcium-alginate

The experiment was carried out according to Lieu *et al.* [9] with slight modifications. Briefly, grapes were washed with saline water for 2 minutes and then dried at ambient temperature. There were three treatment ways including spraying grapes by the essential oil, coating grapes by calcium-alginate, and coating grapes by the combination of calcium-alginate and the essential oil. In the essential oil spraying sample experiments, the grapes were sprayed by the selected essential oil at different concentrations (0.5; 1; 2 and 4 of MIC values). In the coating sample experiments, the grapes were dipped in sodium alginate solution (0.25 %; 0.5 %, and 1 % w/v) for two minutes then dipping in $CaCl_2$ solution for five minutes and dried at ambient temperature. In the experiment of the combination of calcium-alginate and the selected essential oil, a mixture of the essential oil and Tween 80 mixed with the film-forming solution. The grapes were dipped in the film-forming mixture for 5 minutes and dried at ambient temperature.

After that, all the samples were sprayed with *C. acutatum* spores at a concentration of 5 log CFU/ml. The grapes sprayed with *C. acutatum* that without any treatment were used as the control. All the samples were dried in ambient temperature. To avoiding the changing of the atmosphere around the fruit, the sample was kept in the bag containing the holes (7 mm) and then preserved at ambient temperature. The data were collected before treatment (day 0) and every 5-day of storage until 20 days of storage. Each experiment was carried out with 20 fruits in triplication. The grape was considered damage when the surface of the fruit has appeared black dots or the content of the grape was leaked out. The rotten grape was removed from the bag and considered as 5 % of the damaged sample.

2.2.3. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using Statgraphics 15 followed by the Student-Newman-Keuls t-test to compares all pairs of means, with a significance level of 5 %. All experiments were performed in triplicate for each condition and repeated at least twice, and the data expressed as means ± standard deviation.

3. RESULT AND DISCUSSION

3.1. The antifungalactivity of essential oils against *C. acutatum*

The antifungal activity of cassia oil, holy basil oil, and peppermint oil resists *C. acutatum* was presented in Figure 1. The results showed that three kinds of essential oils achieved the well antifungal effect with the diameter of the inhibition zone from 6.50 ± 0.58 mm to 27.75 ± 0.50 mm and the inhibition zone was not observed in the control sample. The antifungal efficacy of essential oils was different and depended on the concentration of essential oils (Figure 1). In this experiment, cassia oil has the best antifungal effect ($p < 0.05$) with a MIC value of 2.5 µl/ml compared to both holy basil and peppermint oils that were of 20 µl/ml, respectively.

In this study, Tween 80 (0.3 % (v/v)) was used to dilute the essential oil due to the hydrophobic character of the essential oil. The previous studies showed the antimicrobial activity of essential oil depending on the antimicrobial compounds which contain in the essential oil. The study of Viuda-Martos *et al.* [10] showed that orange oil had the best antifungal activity which resisted *A. niger*, whereas mandarin oil (*Citrus reticulata* L.) reduced the growth of *A. flavus*. Similar results, the antifungal activity of lemongrass oil against *A. niger* which achieved

the best effect with the MIC value of 10 µl/ml compared to citronella oil and cajeput oil with the MIC values was 20 µl/ml [9].

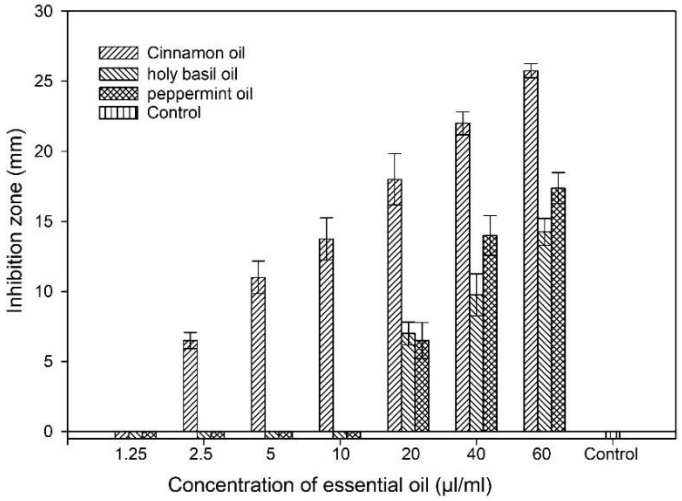

Figure 1. The antifungal activity of essential oilsresists *C. acutatum*
Control: emulsifying solution without essential oils.

In previous studies, Lopez *et al.* [11] reported that cassia oil showed the best antifungal efficiency on three species of the fungal (*Candida albicans*, *Penicillium islandicum*, and *A. flavus*). Besides, cassia oil also showed the ability to inhibit *A. niger, S. cerevisiae, E. coli*, and *S. aureus* in which the mold strains had the highest susceptible to the essential oil [8]. In the current study, cassia oil achieved the best antifungal activity compared to the other essential oil in the survey (Figure 1). Xing *et al.* [12] indicated that the main antifungal agent of the cassia oil was cinnamaldehyde. Cinnamaldehyde inhibited cell wall synthesis enzyme (β-(1,3)-glucan and chitin), causing the loss of cytoplasm, broken cell membrane, mitochondrion destroy, and loss of cell wall stability leading to affect the morphology and growth of the fungal [12]. The analysis of holy basil oil and its two aromatic components were (*Z*)-β-ocimene and eugenol that high toxicity with five species of the tested insects, which has the potential choice of synthetic fumigate drugs to control pests in sustainable agricultural products [13]. The main component determined in peppermint oil was menthol (71.40 %), *p*-menthone (8.04 %), iso-menthone (5.42 %) [14]. The result in the present study suggested that cinnamaldehyde and the other components in cassia oil had more effective inhibition of fungal compared to the other essential oils in this study (Figure 1). The cassia oil, which could inhibit *C. acutatum* at low concentration, was selected for the grape preservation that used alone or combined with edible films in the next step.

3.2. The storage of grapes by edible films combined with essential oil

The efficacy of thecassia oil and calcium-alginate films on grapes preservation was presented in Figures 2, 3, 4, 5, and 6. The results showed that the damage ratio of control samples was 30 % after five days of storage, and quickly increased in the following days with above 90 % damage ratio after ten days of storage and complete damage after 15 days of storage. In a similar condition, samples were treated by the edible coating decreasing significantly in the damage ratio of grapes ($p < 0.05$) compared to control samples (Figure

2).The damage ratio was decreased in the samples using calcium-alginate 0.25 % (w/v). However, the damage ratio still observed and completed damage after 15 days of storage. The samples using calcium-alginate (0.5 % or 1 % (w/v)) showed well of storage effect. But there was not significantly different ($p > 0.05$) between 0.5 % (w/v) and 1 % (w/v) of the calcium-alginate concentration, that had the damage ratio lower than 40 % until the day 20th. However, calcium-alginate 1 % (w/v) affect significantly to the organoleptic properties of grapes, whereas calcium-alginate 0.5 % (w/v) and 0.25 % (w/v) were no difference (data not shown) in the sensory evaluation. In the essential oil sprayed samples, the cassia oil reduced the damage ratio of grapes compared to control samples. The treatment samples by the cassia oil at the MIC value were not damaged after ten days of storage and damaged by 30 % after 20 days of storage (Figure 3). The same results were observed in the samples using calcium-alginate 0.5 % (w/v) and 1 % (w/v) (Figure 4). At the lower concentration of 1.25 (µl/ml), the grapes were rotten significantly. The reason could be due to the too low cassia oil concentration that was evaporated quickly and decreases the antifungal activity against *C. acutatum*, leading to fruit damage. The results also showed that the grapes were more rotten quickly at high concentration (higher than the MIC value) (Figure 3), due to the high cassia oil concentration affecting the fruit peel, leading to fruit damage [6, 9].

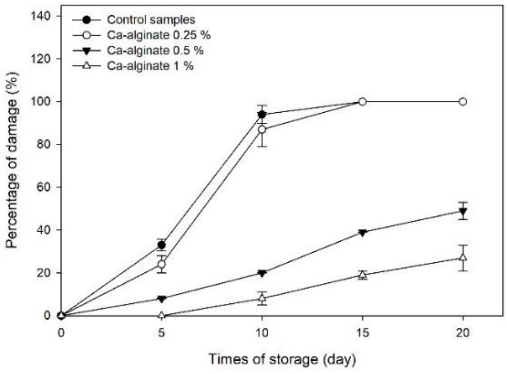

Figure 2. The damage ratio (%) of grapes treating by calcium-alginate coatings.

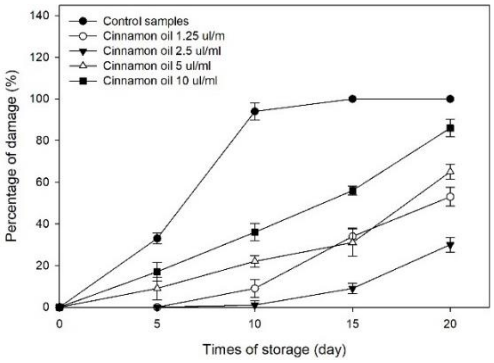

Figure 3. The damage ratio (%) of grapes treating by spray cassia oil.

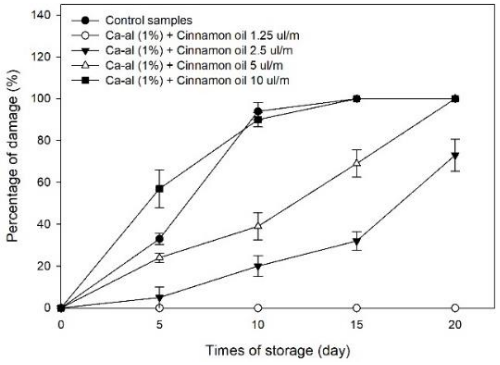

Figure 4. The damage ratio (%) of grapes treating by calcium-alginate 1 % combined with cassia oil.

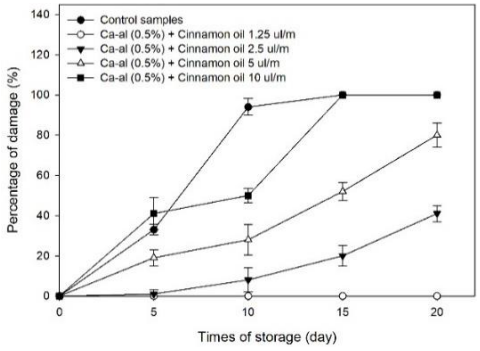

Figure 5. The damage ratio (%) of grapes treating by calcium-alginate 0.5 % combined with cassia oil.

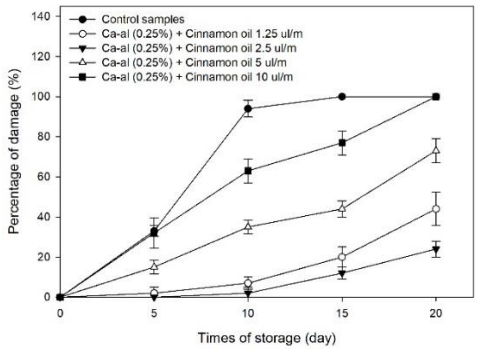

Figure 6. The damage ratio (%) of grapes treated calcium-alginate 0.25 % combined with cassia oil.

The combination of calcium-alginate 0.25 % (w/v) and the different concentrations of the cassia oil improved the antifungal efficacy but, the rotten grapes still observed. However, there was no significant difference ($p > 0.05$) which compared to cassia oil sprayed samples (Figures 3 and 6). Besides, the combination ofcassia oil and calcium-alginate 0.5 - 1.0 % (w/v) gave an outstanding effect (Figures 3, 5). In previous studies, the combination of cassia oil and calcium-alginate film extended the shelf life of cucumber slices was extended over 21 days at 5 ^{o}C [15]. In the study of Azarakhsh *et al.* [16], the bioactive edible coatings formed by alginate combined with lemongrass oil 0.3 % (w/v) which had potential to prolong the shelf life and maintain the quality of fresh pineapple. The study of Maqbool *et al.* [17] showed that gum arabic (10 % w/v) did not give the effect on antifungal, but the combination of gum arabic (10 % w/v) and cassia oil gave the antifungal effect against *C. musae* and *C. gloeosporioides* in the process of mycelium growth with 73.4 % and 70 % as well as in inhibiting spore germination with 88 % and 85 % of these two fungal. The combination of calcium-alginate (0.5 % and 1 % w/v) and cassia oil (1.25 µl/ml) gave high efficiency on the grape storage with no damage after 20 days of storage, and there was no significant difference ($p > 0.05$) between calcium-alginate 0.5 % and 1 % (w/v) (Figures 4, 5). However, when the concentration of cassia oil reached to the MIC value (2.5 µl/ml), the combination of calcium-alginate (0.5 % or 1 % w/v) and cassia oil had a significant difference with 40 % and 70 % rotten grapes, respectively. And this was not effective comparing to the samples of cassia oil spraying (Figures 3, 4, 5). This could be explained by the synergy of essential oil and the edible coating having the ability to reduce the respiration rate of fresh fruits, slowed down the dehydration process, leading to extending the shelf life of fruits [18]. The advantage of edible coating combined with essential oil was to keep antimicrobial agents on the surface of the product (where the contaminated phenomenon often occurs) in long storage time [7]. This method was more effective than the essential oil spraying method to reduce the degree of microorganism contamination [7]. However, the combination of essential oil at high concentrations and edible coating would affect the fruit peel [9, 14], leading to damage fruit as soon as treatment. In the present study, there was no significant difference between calcium-alginate 1 % and 0.5 % (w/v) (Figure 4, 5). Comparing to calcium-alginate of 0.5 % (w/v), calcium-alginate 1 % (w/v) was a high viscosity mixture which impacted on the process of creating the coating membrane, organoleptic properties of the product and economic problems. Therefore, calcium-alginate 0.5 % (w/v) combined with cassia oil 1.25 µl/ml is the most effective formula for the grape preservation application.

4. CONCLUSION

The results showed that *Cinnamomum acutatum* was more sensitive to cassia oil than to holy basil essential oil and peppermint essential oil. The cassia oil had antifungal efficiency at concentrations 2.5 µl/ml to 60 µl/ml, respectively, for the diameter of the antifungal zone from 6.50 ± 0.58 mm to 25.75 ± 0.50 mm. In the grape preservation study, the control samples were completely rotten after 15 days of storage, whereas the samples treated with cassia oil or coated with calcium-alginate which extended the shelf life of grapes, significantly. When increasing the concentration of cassia oil, the antifungal efficiency will be enhanced. However, when applying to the process preservation combined with edible coatings, a high concentration of cassia oil not only increased the storage efficiency but also cause faster decay, as the edible coatings restricted the evaporation of the cassia oil. The concentration of cassia oil increased that affected the fruit peel. The combination of calcium-alginate (0.5 % w/v) and cassia oil (1.25 µl/ml) for effect in inhibiting the growth of *C. acutatum*, grapes were not rotten after 20 days stored at ambient temperature.

Conflict of Interest. The authors declare that they have no conflict of interest.

REFERENCES

1. Kanellis. A and Roubelakis. K. Angelakis - "Grape," in Biochemistry of fruit ripening, Ed: Springer, 1993, pp. 189-234.
2. Pearson R. C. and Goheen A. C - Compendium of grape diseases: Aps Press, 1988.
3. Whitelaw - Weckert. M. A., Curtin. S. J., Huang, R., Steel. C. C., Blanchard. C. L., and Roffey. P. E. - Phylogenetic relationships and pathogenicity of *Colletotrichum acutatum* isolates from grape in subtropical Australia, Plant Pathology **56** (3) (2017) 448-463.
4. Servili A., Feliziani E., and Romanazzi G. - Exposure to volatiles of essential oils alone or under hypobaric treatment to control postharvest gray mold of table grapes, Postharvest Biology and Technology **133** (2017) 36-40.
5. Abdolahi A., Hassani A., Ghosta Y., Bernousi I., and Meshkatalsadat M. - Study on the potential use of essential oils for decay control and quality preservation of Tabarzeh table grape, Journal of Plant Protection Research **50** (2010) 45-52.
6. Hyldgaard M., Mygind T., and Meyer R. L. - Essential oils in food preservation: mode of action, synergies, and interactions with food matrix components, Frontiers in microbiology **3** (2012) 12.
7. Sánchez-González L., Vargas M., González-Martínez C., Chiralt A., Cháfer M. - Use of essential oils in bioactive edible coatings: a review, Food Engineering Reviews**3** (2011) 1-16.
8. Lieu M. Dong, Dang T. K. Thuy, and Nguyen T. Huong - Enhance the antimicroorganismactivity of cinnamon oil by xanthan gum as emulsifying agent, In AIPConference Proceedings **1954** (1) p. 040017. AIP Publishing, 2018.
9. Lieu M. D., Ngo N. N. H., Lieu T. L., Nguyen K.T., and Dang T. K. T - The efficacy of combined application of edible coatings and essential oi in mango preservation, Vietnam Journal of Science and Technology **56** (4) (2018) 458-467.

10. Viuda-Martos M., Ruiz-Navajas Y., Fernández-López J., and Pérez-Álvarez J. - Antifungal activity of lemon (*Citrus lemon* L.), mandarin (*Citrus reticulata* L.), grapefruit (*Citrus paradisi* L.) and orange (*Citrus sinensis* L.) essential oils, Food control **19** (2008) 1130-1138.
11. Lopez S. C. P., Batlle R., Nerin C. - Solid-and vapor-phase antimicrobial activities of six essential oils: Susceptibility of selected foodborne bacterial and fungal strains, Journal of Agricultural and Food Chemistry **53** (2005) 6939-6946.
12. Xing F., Hua H., Selvaraj J. N., Zhao Y., Zhou L., Liu X., Liu Y. - Growth inhibition and morphological alterations of Fusarium verticillioides by cinnamon oil and cinnamaldehyde, Food Control **46** (2014) 343-350.
13. Ogendo J. O., Kostyukovsky M., Ravid U., Matasyoh J. C., Deng A. L., Omolo E. O., Kariuki S. T., and Shaaya E. - Bioactivity of *Ocimum gratissimum* L. oil and two of its constituents against five insect pests attacking stored food products, Journal of Stored Products Research **44** (2008) 328-334.
14. Pandey A., Rai M., and Acharya D. - Chemical composition and antimycotic activity of the essential oils of corn mint (*Mentha arvensis*) and lemon grass (*Cymbopogon flexuosus*) against human pathogenic fungi, Pharmaceutical Biology **41** (2003) 421-425.
15. Raybaudi-Massilia R. M., Mosqueda-Melgar J., and Martín-Belloso O. - Edible alginate-based coating as carrier of antimicrobials to improve shelf-life and safety of fresh-cut melon, International Journal of Food Microbiology **121** (2008) 313-327.
16. Azarakhsh N., Osman A., Ghazali H. M., Tan C. P., and Adzahan N. M. - Lemongrass essential oil incorporated into alginate-based edible coating for shelf-life extension and quality retention of fresh-cut pineapple, Postharvest Biology and Technology **88** (2014) 1-7.
17. Maqbool M., Ali A., Alderson P. G., Mohamed M. T. M., Siddiqui Y., and Zahid N. - Postharvest application of gum arabic and essential oils for controlling anthracnose and quality of banana and papaya during cold storage, Postharvest biology and technology **62** (2011) 71-76.
18. Šuput D. Z., Lazić V. L., Popović S. Z., and Hromiš N. M. - Edible films and coatings: Sources, properties and application, Food and Feed Research **42** (2015) 11-22.