ASSESMENT OF LAVENDER AND OREGANO ESSENTIAL OILS CAPACITY TO INHIBIT THE GROWTH OF POSTHARVAGE PATHOGENS *PENICILLIUM EXPANSUM* LINK. AND *BOTRYTIS CINEREA* PERS.

Otilia COTUNA [1], Mirela PARASCHIVU [2], Veronica SĂRĂȚEANU [1], Carmen Claudia DURĂU[1], Ilinca IMBREA [1]

[1]Banat`s University of Agricultural Sciences and Veterinary Medicine "King Michael I of Romania" Timisoara, Romania
[2]Agriculture Consulting Office, Craiova, Dolj county, Romania
University of Craiova, Agronomy Faculty

Corresponding author: e-mail: otiliacotuna@yahoo.com; e-mail: paraschivumirela@yahoo.com; e-mail: sch_carmen_1999@yahoo.com; vera_s_vera@yahoo.com

Abstract. *Research about antimicotic action of essential oils has been made for long time, but still little knowledge is available. Thus, there is particular interest especially for those postharvest pathogens that are difficult to be controlled. Some research emphasized high potential of essential oils to be used successfully against postharvest pathogens instead synthetic fungicides. Among the pathogens controlled by essential oils are known Botrytis cinerea, Penicillium italicum and Penicillium digitatum. The aim of this research was to assess the capacity of lavender and oregano essential oils to inhibit the mycelial growth of Penicillium expansum and Botrytis cinerea. Tests were made with oils in different concentrations and pathogens were isolated from the fruits skin and grown on specific medium being assessed the rate of mycelial growth. For both Penicillium expansum and Botrytis cinerea the rate of mycelial growth for control (no treated) was 0.29-0.30 mm/h. When 10 ml of essential oils were used it was observed that the rate of mycelial growth was very low for both pathogens, respectively 0,016 and 0,008 mm/h for Botrytis cinerea and 0,017 și 0,005 mm/h for Penicillium expansum. The inhibition rate was up to 90% for both pathogens, respectively 92,5% when lavender oil was applied, respectively 97.22% for oregano oil treatment. The results of the study emphasized that lavender and oregano essential oil had a significant impact on the inhibition on the growth of Botrytis cinerea și Penicillium expansum which can be an effective option for biological control of postharvest pathogens, substituting chemical fungicide control.*

Key words: *Botrytis cinerea, Penicillium expansum, lavender, oregano, essential oil.*

INTRODUCTION

Essential oils are concentrated, hydrophobic products containing volatile aromatic compounds extracted from plants, rich of bioactive chemicals with low impact on mammalian toxicity, environmentally friendly and wild public accepted. There are previous studies that reported the good impact of essential oils in plants due to their antifungal, antibacterial, insecticidal and nematicidal effects (DORMAN AND DEANS, 2000; ISMAN, 2000; PANDEY *ET AL.* 2000; SOLIMAN AND BADEAA, 2002).

Thus, the research about efficiency of essential oils on the control of postharvest pathogens is about particular interest worldwide. D. SPADARO ET AL. (2012) showed that treatments with essential oils are very effective against *Botrytis cinerea* and *Penicillium expansum* substituting successfully chemical fungicide. Previous studies showed the good impact of essential oils extracted from basil (*Ocimum basilicum*), fennel (*Foeniculum sativum*), lavender *(Lavandula officinalis*), marjoram

(*Origanum majorana*), oregano (*Origanum vulgare*), mint (*Mentha piperita*), rosemary (*Rosmarinus officinalis)*, sage (*Salvia officinalis*), thyme (*Satureja montana*), wild thyme (*Thymus vulgaris*) and wild mint (*Mentha arvensis*) in apples (Golden Delicious, Granny Smith, Red Chief, etc.) against both *Botrytis cinerea* and *Penicillium expansum* (Lopez - Reyes J. G. *et al*., 2010) Thus, tests showed that after 12 h the best results were recorded for thyme and oregano oils at 1%, respectively 10%. Also, BHASKARA *ET AL*., (1997) showed the efficency of thyme oil against *Botrytis cinere*a.

LEE *ET AL*. 2007, tasted 39 essential oils for their capacity to control five postharvest and soilborne pathogens. The test suggested that both *Eucalyptus citriodora* and *Cuminum cyminum* oils have a potential as antifungal preservatives for the control of storage diseases of different crops. Also the essential oil of *Thymus vulgaris* suppressed the mycelial growth of soilborne pathogens *Colletotrichum gloeosporioides, Fusarium oxysporum* and *Rizoctonia solani*.

Other studies emphasized great results of treatments with essential oils extracted from *Satureja hortensis*, *Zataria multiflora*, și *Carum copticum* (200 ppm) in controlling postharvest pathogens in fruits and vegetables, such as *Aspergillus parasiticus* și *Aspergillus flavus* known for their capacity to produce aflatoxins (NEMAT ALAH ETEMADI *ET AL*., 2012).

Good results were also recorder by Nabigoo et al. 2007 in controlling previous pathogens in strawberry using essential oils extracted from *Salvia officinalis*, *Artemisia aucheri* și *Satureja sp*. KARAMI ET AL. 2010 showed that *Penicillium expansum* growth is inhibited by thymol (in oregano oil) and carvacaol (in fennel oil). Also, the use of *Ocimum basilicum, Rosmarinus officinalis* and *Thymus kotschyanus* essential oils in pears were effective against *Penicillium expansum* and *Botrytis cinerea.* RASUL JALILI MARANDI *ET AL*., 2011 reported that *Thymus kotschyanus* oil grows storage time in pears due to carvacol and thymol components.

There is high interest for controlling *Penicillium expansum* and *Botrytis cinerea* especially because they show resistance to tiabendazol. This is most important aspect knowing there is high risk for humans health due to the capability of *Penicillium expansum* to produce mycotoxines, especially patulines which might be found in fruits juice (J. HARWIG *ET AL*., 1973), thus the fruits used for juice will be carefully selected (BIRGITTE ANDERSEN ET AL., 2004). This mycotoxines can prodeces edems and bleeding in humans and also has carcinogenic effect (NOEL F. SOMMER *ET AL*., 1974; D. M. WILSON, G. J. NUOVO, 1973; P. W. BRIAN *ET AL*., 1956)

These alternative in control of postharvest pathogens are needed because of negative impact of chemical fungicides, resistance of fungicide among fungal pathogens and higher cost of pesticides.

For present study was tested the antifungal capacity of oregano and lavender oils through diffusion into the Sabouraud semisolid culture medium in Petri plates. Essential oils have been used through the incorporation into the culture medium in different doses.

MATERIAL AND METHOD

Biological material used consisted of fruits (apples and strawberries) attacked by fungi of the Penicillium expansum Link repository (Apple) and Botrytis cinerea pers. (Strawberry). For this purpose, 6 fruits samples (3 Apples and 3 Strawberries) were collected and were used for isolation of pathogens on the culture medium. Each sample had 100 fruit. Sampling was done from a storehouse in Sânnicolau Mare, Timiș County, Romania.

The essential oils tested in this experience has been obtained in the Fitotechnics laboratory using plant material (lavender and oregano plants). The plants have been grown on the experimental field station of the University of Agricultural Sciences and Veterinary Medicine of Banat "King Michael I of Romania in Timișoara.

The material used for the extraction of vegetable oils has been harvested at flowering stage when the content in oils is intense.

After harvest fresh plants were packed and left to dry in a cool place away from sunlight. Oils were obtained during distillation for 3 hours using equipment Clevenger. Essential oil was collected and stored at 4° C until use.

The culture medium used was Sabouraud Agar with chloramphenicol 4%. Isolation of fungi was made directly from the surface of the fruit. Fragments of mycelium were grown in Sabouraud medium with chloramphenicol (method of culture in Petri plates). After sowing, culture media were incubated for 7 days at a temperature of 200º C in the case of the fungus Penicillium expansum and Botrytis cinerea to 25ºC. Antifungal oils ability oregano and lavender was tested by diffusion into the culture medium in Petri plates Sabouraud semisolid.

Essential oils have been applied through incorporation in niches made on the surface of the culture medium in three doses: 1 microliter, 5 and 10 microliters microliters. Three doses of essential oils have been tested on different Petri plates and were covered with its lid immediately after pouring the medium and the incorporation of the oil (to prevent evaporation). For this purpose the Petri dish used had approximately 80 mm in diameter.

Inoculation of the culture media treated with essential oils was performed after the solidification of the culture environment by placing mycelial fragments into multiple points, using a sterilized needle flame sterilized before use. Incubation of Petri plates was done at a temperature of 25 ° C for 5 days. The plates without essential oil treatment have been considered control variants. Were given eight variants of the fungus *Penicillium expansum* and other eight for *Botrytis cinerea*.

Growth rate of mycelium was calculated using the formula:

$$V = \frac{dc}{t}$$

where V = velocity of mycelium growth; dc = the diameter of the colony; t = time in hours.

The inhibition percentage of the mycelium growth on medium treated with different doses of essential oil of lavender and oregano was calculated with the formula:

$$\text{the percentage of inhibition} = (C/T) \times 100$$

where: C is the diameter of the colony and T is the diameter of the colony grown on the treated medium.

RESULTS AND DISCUSSIONS

The isolation of both two pathogens (*Penicillium expansum* and *Botrytis cinerea)* was made directly from the surface of the fruit. After sowing, culture medium were incubated for 7 days at a temperature of 200ºC (*Penicillium expansum*) and 25ºC (*Botrytis cinerea*). On culture medium have developed colonies of *Penicillium expansum* and *Botrytis cinerea* of various sizes. Microscopic examination has shown the presence of fructifications of two specific fungi (fig. 1 and 2).

In the context of the present study was tested the capacity of essential oils of lavender and origano to inhibit the growth of fungi *Penicillium expansum* and *Botrytis cinerea*. Culture medium

treated with essential oils of lavender and oregano was seeded with spores of fungi, resulting in 16 variants.

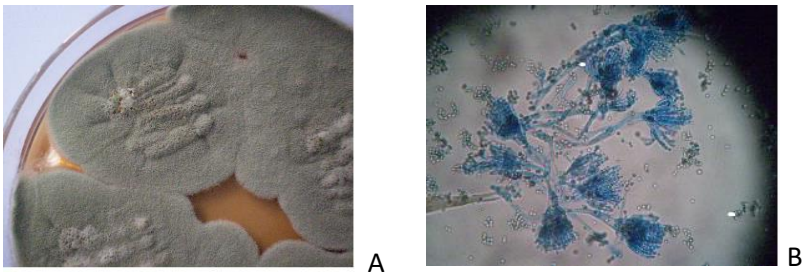

Fig. 1. A - Penicillium expansum mycelium grown on culture medium; B - Penicillium expansum spores under microscope (Otilia Cotuna, 2016)

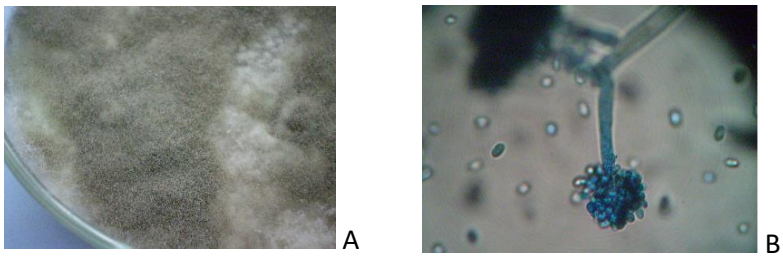

Fig. 2. A - *Botrytis cinerea* mycelium grown on culture medium; B - *Botrytis cinerea* conidiophores and conidia under microscope (Otilia Cotuna, 2016)

Petri plates were placed in an incubator at 230C ± 20 ° C for 7 days. After 7 days of incubation was made the assessment of mycelial growth into Petri plates. The culture medium used satisfied the nutritional requirements of fungi and was successfully used to increase studied pathogens.

In the case of the fungus *Penicillium expansum*, in control plates was noticed that the mycelium developed rapidly. After 7 days, around the four points where the seeding was carried out were formed more mycelial patches with different diameters. The diameter of the mycelial patches had dimensions of 37, 36, 37, 40 and 45 mm. On the culture medium treated with 1 microliter essential oil of lavender has grown a single fungal colony with a diameter of 3 mm. On the second plate, 4 microliters of essential oil favored growth of the mycelial patches with diameters of 1, 2 and 3 mm. In the third variant, treated with 10 microliters lavender essential oil, increased 8 mycelium colonies. The diameter of the colony ranged between 0.5 and 3 mm. On the culture medium with 1 microliter oregano oil were observed 7 mycelium colonies with diameters of 1, 3 and 4 mm. On the second plate treated with 5 microliters oregano oil favored increase of 12 colonies with diameters of 1 and 3 mm while the plate treated with 10 microliters oregano oil have developed 11 colonies of 1 and 2 mm.

The results showed that both oils used in this experiment have very high capacity of inhibition of the mycelial growth of *Penicillium expansum*. Comparatively with the untreated control, where were noticed mycelium colonies of large diameter, on culture media treated with the essential oil in different

doses, the number of colonies formed was very small and below 4 mm in diameter. It was also found that pathogens mycelium stopped from growing keeping the same dimensions. The speed of mycelium growth in the control was 0.238-0.3 mm/h. In the variants with different doses of lavender and oregano essential oils, mycelium growth rate was very low in both variants (0.017 and 0.005 mm/h and in variants treated with essential oil 10 microliters). It was observed that in these variants have formed many colonies, but of a smaller size, compared to the other versions. The growth of a greater number of mycelium colonies may be due to uneven dissemination of essential oils into the culture medium.

The inhibition percentage of the mycelium growth was very high, over 90%. Thus, in treated variants with lavender oil the inhibition percentage was 92.5%. In the case of oregano oil treatment at a dose of 10 microliters, the inhibition percentage was 96% (table 1).

Table 1

The capacity of essential oils of lavender and oregano to inhibit the growth of *Penicillium expansum* pathogen

Variant	**No. of mycelium colonies**		**Diameter of mycelium colonies treated with essential oils**		**Growth rate of mycelium mm/h (168 h) for essential oil treated variants**		**Inhibition percentage of mycelial growth**	
	M_L	M_O	Lavender	Oregano	Lavender	Oregano	Lavender	Oregano
Control	4	5	36 - 40 mm	35 - 50 mm	0,238	0,3	-	-
V_1 - 1ml	1	7	3 mm	1 - 4 mm	0,017	0,023	92,5%	92%
V_2 - 5ml	4	12	1 - 3 mm	1 - 3 mm	0,006 - 0,017	0,006 - 0,017	92,5%	94%
V_3 - 10ml	8	11	0,5 - 3 mm	1 - 2 mm	0,003 - 0,017	0,006 - 0,005	92,5%	96%

Table 2

The capacity of essential oils of lavender and oregano to inhibit the growth of *Botrytis cinerea* pathogen

Variant	**No. of mycelium colonies**		**Diameter of mycelium colonies treated with essential oils**		**Growth rate of mycelium mm/h (120 h) for essential oil treated variants**		**Inhibition percentage of mycelial growth**	
	M_L	M_O	Lavender	Oregano	Lavender	Oregano	Lavender	Oregano
Control	28	4	20 - 35 mm	27 - 36 mm	0,29	0,3	-	-
V_1 - 1ml	2	4	1 mm	0,5 - 2 mm	0,008	0,016	97,14%	94,44%
V_2 - 5ml	4	7	2 - 3 mm	1 - 4 mm	0,016 - 0,025	0,033	91,42%	88,88%
V_3 - 10ml	4	2	2 - 3 mm	1 mm	0,016 - 0,025	0,008	91,42%	97,22%

In control plate the mycelium of *Botrytis cinerea* began to develop rapidly. After 5 days in the four points where the seeding was carried out, were formed several mycelium colonies that confluent occupying the entire surface of the Petri dish with a diameter of 80 mm. Initially, the diameter of the mycelium colonies had sizes between 20 and 36 mm. On the plates treated with 1 microliter of oregano essential oil have developed four mycelium colonies with a diameter of between 0.5 and 2 mm. On the plate treated with 5 microliter of oregano oil have developed 7 mycelium colonies with diameters from

1 to 4 mm. In the third plate, treated with 10 microliters of oregano essential oil, grew only two mycelium colonies. The diameter of the colony has not exceeded 1 mm.

In the case of variants treated with lavender oil, have been developed mycelium colonies of Botrytis cinerea which have not exceeded the diameter of 3 mm (fig. 2). Mycelium colonies diameter ranged between 2 (first variant) and 4 in the other variants (table 2).

The results obtained in the case of the fungus Botrytis cinerea are similar to those obtained previously from Penicillium expansum. Compared with the untreated control plate, where they grew mycelium colonies of large diameter, on plates treated with the essential oil in different doses, the number of colonies formed was very small and below 4 mm in diameter. It was also found that the fungi mycelium stopped from growing. The speed of growth of the mycelium in the control was 0.29-0.3 mm/h. In the variants treated with different doses of essential oils of lavender and oregano, growth rate of mycelium was very low in both variants (0.016 and 0.008 mm/h and in those treated with 10 microliters essential oil). It appears that in these variants have formed many small size colonies compared to the other versions. The development of a greater number of mycelium colonies may be due to uneven dissemination of essential oils into the culture medium.

The inhibition percentage of the mycelium of *Botrytis cinerea* was very high, exceeding 90% while in treated with lavender oil, inhibition percentage ranged between 91.42% and 97%. In the case of oregano oil the variant treated with 10 microliters oil emphasized inhibition percentage of 97,22%.

Compared with lavender oil, oregano oil inhibitor effect could be noticed in the first days following inoculation, in all three doses used. The highest percentage of inhibition was observed in the third variant treated with 10 microliters oregano essential oil (97,22%)-table 2.

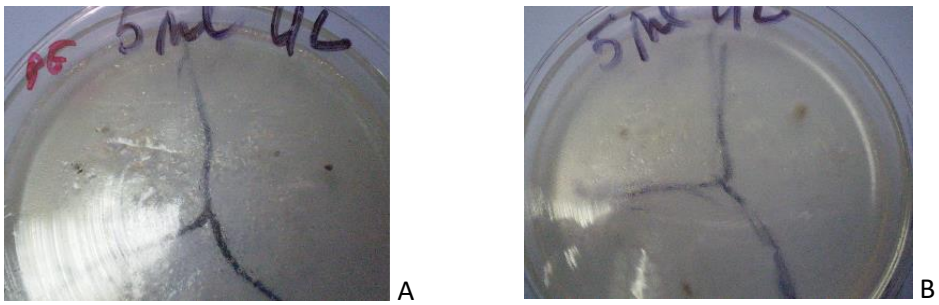

Fig. 3. *Penicillium expansum* (A) and *Botrytis cinerea* (B) mycelium colonies grown on medium treated with 5 microliters of lavender essential oil (Otilia Cotuna, 2016)

ARCHBOLD ET AL., 1997 consider that antifungal effect refers to the property of certain substances to inhibit the growth of pathogenic fungi and to kill pathogenic fungi.

Research in this topic showed that the essential plant oils have a real potential to replace synthetic fungicides in controlling of infection fungi installed on the fruit. Along with other plants oils, oregano oil was tested during time against postharvest pathogens such as *Botrytis cinerea, Penicillium italicum*, and *Penicillium digitatum*.

Previous studies reported that this oregano oil has proved to be very effective in the control of grey mold (*Botrytis cinerea*) on tomatoes, strawberries and cucumbers. Also, ANDREW VITORATOS ET AL. (2013) showed that essential plant oils, produced following a proper formulation can be used successfully in controlling diseases that occur during storage of fruits and vegetables.

The results of the present study joins existing ones in and emphasized the postharvest inhibition capacity of lavender and oregano oils against growth of *Botrytis cinerea* and *Penicillium expansum*.

CONCLUSIONS

Lavender and origano oils used in present study showed very high inhibition capacity of mycelium growth of *Penicillium expansum* and *Botrytis cinerea* fungi. Compared with the untreated control, where they grew mycelium colonies of large diameter, on culture medium treated with essential oils in different doses, the number of colonies formed was very small and the diameter was below 4 mm. Also, it was found that on treated variants mycelium growth have been stopped.

The inhibition percentage of the mycelium growth was very high, exceeding 90% while in treated with lavender oil, inhibition percentage ranged between 91.42% and 97%. In the case of oregano oil the variant treated with 10 microliters oil emphasized inhibition percentage of 97,22%.

For *Botrytis cinerea* fungus comparatively the variant treated with lavender oil, the inhibitor effect of oregano could be noticed since the first days following inoculation for all three doses used.

BIBLIOGRAPHY

1. ANDERSEN BIRGITTE, SMEDEGARD J., FRISVAD J. C., 2004 – *Penicillium expansum*: *Consistent production of Patulin, Chaetoglobosins and other secondary metabolites in culture and their natural occurence in fruit products.* J. Agric. Food Chem., 52(8), 2421 – 2428*;*

2. ARCHBOLD D. D., HAMILTON - KEMP T. R., BARTH M. M., LANGLOIS B. E., 1997 - I*dentifying natural volatile compounds that control gray mold (Botrytis cinerea) during postharvest storage of strawberry, blackberry and grape,* Journal of Agriculture and Food Chemistry, 45 (10), 4032 - 4037;

3. BRIAN P. W., ELSON G. W., LOWE D., 1956 – *Production of patulin in apple fruits by Penicillium expansum*, Nature 178, 263 – 264;

4. BHASKARA REDDY M.V.B, P. ANGERS, A. GOSSELINT, AND I. ARUL., 1998 - *Characterization and use of essential oil from Thymus vulgaris against Botrytis cinerea and Rhizopus stolonifer in strawberry fruits*. Phytochemistry, 47:1515-1520;

5. DORMAN H.J.D. AND DEANS S.G. 2000 **–** *Antimicrobial agents from plants: antibacterial activity of plant volatile oils.* J.Appl.Mocrobiol.88:308-316.

6. HARWIG J., CHEN Y. K., KENNEDY B. P. C., SCOTT P. M., 1973 – *Ocurrence of patulin and patulin producting strains of Penicillium expansum in natural rate of apple in Canada.* Can. Inst. Food Sci. Technol. J. 6: 22 – 25;

7. ISMAN M.B. 2000 **–** *Plant essential oils fpor pest and disease management.* Crop Prot. 19:603-608.

8. LOPEZ-REYES, J. G., SPADARO, D., GULLINO, M. L. AND GARIBALDI, A., 2010 - *Efficacy of plant essential oils on postharvest control of rot caused by fungi on four cultivars of apples in vivo.* Flavour Fragr. J., 25: 171–177. doi: 10.1002/ffj.1989;

9. KARAMI M., ALIMON A. R., GOH Y. M., AWIS Q. S., IVAN M., 2010 - *Effects of dietary herbal antioxidants supplemented on feedlot growth performance and carcass composition of male goats.* Am. J. Anim. Vet. Sci. 2010;5:33–39;

10. NEMAT ALAH ETEMADI, MARYAM BEHDAD, HOSSEIN ZEINALI, 2012 - *Antifungal Effects of Three Plant Essential Oils Against Botrytis cinerea The : Cause of Gray Mold on Strawberry,* Journal of Research in Agricultural Science Vol. 8, No. 2 (2012), Pages: 165-170;

11. NABIGOO A. AND M. FARZANEH. 2007 - *Antifungal potential of essential oils lavender and anise plants against the fungus Rhizopus stolonifer.* Third Symposium on Medicinal Plants. Shahed University. Page 302;

12. PANDEY R. KALRA A, TANDON S., MEHROTRA N., SINGH H.N., KUMAR S., 2000 **–** *Esential oils as potent sources of nematicidal compounds.* J. Phytopathol 148:501-502.

13. RASUL JALILI MARANDI, ABBAS HASSANI, YOUBERT GHOSTA, ALI ABDOLLAHI, ALIREZA PIRZAD AND FATEMEH SEFIDKON, 2011 - *Control of Penicillium expansum and Botrytis cinerea on pear with Thymus kotschyanus, Ocimum basilicum* and *Rosmarinus officinalis essential oils*, Journal of Medicinal Plants Research Vol. 5(4), pp. 626-634, 18 February, 2011 Available online at http://www.academicjournals.org/JMPR ISSN 1996-0875 ©2011 Academic Journals;

14. SOLIMAN K.M. AND BADEAA R.I., 2002 – *Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi*.Food Chem. Toxicol. 440:1669-1675.

15. SPADARO D., G. LOPEZ, A. GARIBALDI, M. GULINO, 2012 - *Use of Lamiaceae essential oils to control postharvest rots caused by Botrytis cinerea and Penicillium expansum on four cultivars of apple,* The American Phytopathological Society;

16. SOMMER F. NOEL, BUCHANAN J. R., FORTLAGE R. J., 1974 – *Production of patulin by Penicillium expansum*, Applied Microbiology, vol. 28, No. 4, p. 589 – 593;

17. LEE S.O., CHOI G.J., JANG K.S., LIM H.K., CHO K.Y. AND KIM J.C. 2007 – *Antifungal activity of five plant esential oils as fumigant against postharvest and soilborne plant pathogenic fungi*. Plant Pathol. J. 23(2):97-102.

18. VITORATOS A., BILALIS D., KARKANIS A., ASPASIA EFTHIMIADOU ASPASIA**,** 2013 - *Antifungal Activity of Plant Essential Oils Against Botrytis cinerea, Penicillium italicum and Penicillium digitatum, Academic Press,* Not Bot Horti Agrobo, 2013, 41(1):86-92, Available online at www.notulaebotanicae.ro.;

19. WILSON D. M., NUOVO G. J., 1973 – *Patulin production in apples decayed by Penicillium expansum.* Appl. Microbiol. 25: 124 – 125.