IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS)
e-ISSN: 2319-2380, p-ISSN: 2319-2372. Volume 8, Issue 12 Ver. II (Dec. 2015), PP 41-45
www.iosrjournals.org

Antibacterial activities of the essential oils from medicinal plants against the growth of *Pseudomonas savastanoi* pv. *savastanoi* causal agent of olive knot

Bouaichi A.[1,2], R. Benkirane [2], K. Habbadi [1,2], A. Benbouazza[1], Achbani E. H.[1]
[1]*Laboratory of Phytobacteriology and Biocontrol, National Institute of Agronomic Research. Meknes, Morocco.*
[2] *Laboratory of Laboratory of Botany, Biotechnology and Plant Protection, Faculty of Sciences, Kenitra, Morocco.*

Abstract: *In the present study antibacterial activities of essential oils obtained from aerial parts of aromatic plants were tested, thyme (Thymus ciliatus), oregano (Origanum compactum), rosemary (Romasmarinus officinalis) and wormwood (Artemisia vulgaris) oils were investigated against the Pseudomonas savastanoi pv. savastanoi (PSS 2064-10) the causal agent of olive knots. By using the paper disc diffusion assay, all essential oils have shown antibacterial activity. Essential oils used in the paper disc diffusion assay varied in their antibacterial activity. Essential oil from thyme was the most effective in inhibiting the growth of PSS 2064-10, followed by those obtained from oregano, rosemary and wormwood. Moreover, the thyme and the oregano exercise an antibacterial effect with percentages of inhibition of 56.25 % (v/v) and 50% (v/v) respectively. However, the MIC of the both of essential oils is 0.04 %(v/v) for the oregano and 0.08 % (v/v) for the thyme. Thus, the MBC was equal to 0.08 % (v/v) and 0.16 % (v/v) for the oregano and thyme respectively.*
Keywords*: Pseudomonas savastanoi pv. savastanoi, olive knot, antibacterial activities, essential oil.*

I. Introduction

Plant diseases caused by bacteria are one of the major problems in crop cultivation in several agricultural commodities. At present, rapid and effective management of plant disease and microbial contamination in several countries is generally achieved by the use of synthetic pesticides and antibiotics. Chemical control of plant disease relies upon the use of antibiotics (such as streptomycin) in USA or copper compounds in the rest of the world. Unfortunately, the frequent use of pesticides and antibiotics against plant and human pathogenic bacteria has led to the selection of resistant bacterial populations against antibiotics. The high cost of pesticides, development of pesticide/antibiotic resistant food-borne and plant pathogenic isolates, governmental restrictions on the use of antibiotics against plant pathogens in European countries, including Turkey, and the interest of environmental consideration raise the need to find alternative control methods. Combination of plant extracts or etheric oils from plants with copper and other chemical compounds can increase their effectiveness (Zeller, 2005) [1]. The chemistry of oils from plants including monoterpenes, sesquiterpenes and phenols is well documented (Bruneton, 1999) [2]. Some essential oils proved strong antifungal (Wang *et al*., 2005) [3], insecticide (Isman, 2000[4]; Pavela, 2005[5]) , and antibacterial activity (Burt, 2004[6]; El-Kamali *et al.*, 2005[7], Mahboubi *et al*., 2014[8]). *Pseudomonas savastanoi* pv. *savastanoi* (*PSS*), the causal agent of olive knot disease, is considered an important problem for olive crops because of its effect on vegetative growth (Quesada *et al*., 2010[9]; Wilson, 1935[10]), olive yield (Schroth *et al*., 1973[11]) and even on olive oil quality (Schroth *et al*., 1968[12]; Cayuela *et al*., 2006[13]). The disease can cause severe damages in olive groves, mainly when weather conditions favour the survival of epiphytic populations of the pathogen and their entry into the bark.

The aim of this study was to compare the effect of essential oils against *PSS 2064-10*, the best to be used in plant protection against olive knot disease in future.

II. Material and methods

1. Plant material and isolation of essential oils:

Medicinal and aromatic plants (MAPs) used for the extraction of essential oils (EO) were dried at room temperature in the shade. The distillation of MAPs was carried in the laboratory of "Plant Bacteriology and Bacteriological Control" RUPP CRRA - Meknes.

The air-dried aerial parts of plants collected to water distillation for 3 h using a Clevenger-type apparatus. Oils recovered in a dark sterile glass and stored at +4°C until it was used.

Percentage yield of oil which was calculated as the percent of the ratio of weight of the oil to weight of the plant

2. *In vitro* antibacterial effect of essential oils against plant pathogenic bacteria

2.1. Microorganism

One strain of *P. savastanoi* pv. *savastanoi* (*PSS* 2064-10) was tested.This strain isolated by laboratory of "Plant Bacteriology and Bacteriological Control" RUPP CRRA – Meknes, was cultured in King B medium (King *et al*., 1954) [14] containing (20g proteose peptone; 1, 5g $MgSO_4$; 1, 5g K_2HPO_4 ; 10 ml glycerol; 20g agar; distilled water to 1.0 L) and incubated at 26°C for 48 h. Inoculum was prepared by adjusting the concentration at 10^7 colony forming units/ml (cfu/ml).

2.2. Determination of antibacterial activity of the essential oils

The antibacterial activity of the essential oils was determined by using the paper disc diffusion technique. Briefly, the test was performed in YPGA medium containing (5g yeast extract; 5g bacto peptone; 10g glucose; 20g agar; distilled water to 1.0 L). The surface of Petri plates (80 mm of diameter) with appropriate media was flooded with 200 µl of bacterial suspension prepared as described previously.

Sterile filter paper discs (6 mm in diameter) containing 5 µl of the tested essential oils were placed in the center of the agar surface. The sterile filter paper soaked whit 2µl of sterile distilled water (SDW) was considered as a negative control. However, the positive control was a sterile filter paper soaked with 2µl of streptomycin antibiotic (0.02%; w/v). The lid of each individual Petri dish was replaced immediately to prevent eventual evaporation. After allowing 1h at room temperatures for the essential oils to diffuse across the surface, the plates were sealed with sterile parafilm and incubated at 25°C for 48 hours. The antibacterial activity of oils and antibiotics was demonstrated by a clear zone of inhibition around the disc and the diameter of these zones was measured in millimetres. All the tests were performed three replicates.
The percentage of inhibition was calculated using the formula:

Percentage of inhibition = [(D1- D2)/ D1] x 100
D1: diameter of the bacterial load with treatment by sterile distilled water (SDW).
D2: diameter of the bacterial load with treatment by essential oil.

2.3. Determination of the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC)

The determinations of minimum inhibitory concentrations (MICs) of essential oils were determined on the dilution technique (Remmal *et al.,* 1993) [15]. All tests were performed in YPG broth supplemented with bacteriological agar (0.15% (w/v)). Serial twofold dilutions, ranging from 0.32 to 0.0025% (v/v) of the essential oils dissolved in 10% Dimethyl Sulfoxide (DMSO) were prepared in a 8 tubes, Then inoculated with Inoculum tested was prepared by adjusting the concentration at 10^7 cfu/ml and final volume is 4ml (Table 1). The tubes were incubated at 25°C for 24 h. At that time, 50 µl of resazurin (indicator of microorganism growth) at a concentration of 0.5 mg/ml, dissolved in sterile water, were added to the tubes. After incubation at appropriate temperature for 2 h, the MIC was then determined as the lowest essential oil concentration prevented change of colouring of resazurin from blue to pink (Bouhdid *et al 2008*) [16]. For MBC determination, by spreading 50µl of each tube, the concentration is greater than or equal to the MIC on YPGA medium and incubated at 25 ° C for 48 h. The lowest concentration that yielded no growth after this sub-culturing was taken as the MBC, indicating that superior than 99.9% of the original inoculum was killed. The determination of MIC and MBC values was properly replicated three times. Each MIC and MBC value was obtained from three independent experiments. To determine the nature of antibacterial effect of EOs, the MBC/MIC ratio was used; when the ratio was lower than 4, the EO was considered as a bactericidal EO and when the ratio was higher than 4, it was considered as a bacteriostatic EO (Levison, 2004) [17].

III. Results and discussion

1. Yield of essential oils

Essential oils obtained by steam distillation of the samples (rosemary, wormwood, thyme and oregano), are light yellow to brown, each with a characteristic odor. Their yields vary with the species.

Rosemary has recorded the largest yield with 1.65% (w/w) followed by wormwood with 0.55% (w/w), oregano with 0.5% (w/w) and thyme with 0.4% (w/w).

The average yield of *T. ciliatus* recorded at this part of work isn't consistent with those obtained by Amarti *et al.*, 2009[18]. Concerned *O. compactum* our results are the same with those shown by Benjilali *et al*., 1986[19]

The yield of essential oils extracted plants varies greatly according to the species used, the part of the plant used, and the stage of development of the plant, the harvest area, the harvest time, climate, mode of extraction and the age of the tree (Zrira *et al*., 2004) [20]

2. Evaluation of the antibacterial activity of the essential oils

The antibacterial activity of the essential oils from each aromatic plant under study was estimated by using the paper disc diffusion technique and the response of *PSS 2064-10* to the essential oils is presented in Table 2.

Figures 1 and 2 show that the thyme (*Thymus ciliatus*) and oregano (*Origanum compactum*) have a strong inhibitory effect on bacterial growth with an inhibition zone of 45 mm and 40 mm respectively, while, it's 30 mm for rosemary and 17 mm for wormwood.

The determination of the MIC and MBC values was based on the high zone inhibition values of the tow essential oils (Thymus ciliatus and Origanum compactum). Thus, this determination was evaluated by using the macro-dilution method. The lowest MIC was obtained against *PSS* 2064-10 at 0.04% (v/v) for oregano and 0.08% (v/v) for thyme oils. However, the oregano and thyme MBCs were 0.08% (v/v) and 0.16% (v/v) respectively. There of indicated the high activity of these essentials oils against the *PSS* 2064-10 strain (Table2). The report of MBC and MIC was equal to 2, indicating the bactericidal effect of two essential oils.

Many studies were showed the antibacterial effect of MAPS the essential oils extract. Thus, others works were showed, the ability of the different essential oils of the oregano and thyme specie to retard and inhibit the growth of the different phytopathogenic bacteria; *Agrobacterium tumefaciens*, *Clavibacter michiganensis* subsp. *Michiganensis, Erwinia amylovora, E. herbicola, Pseudomonas syringae, Pseudomonas viridiflava* and *Xanthomonas axonopodis* pv. *vesicatoria* (Daferera et al., 2003[21]; Yegen et al., 1998[22], Scortichini and Rossi ., 1993[23]) . Otherwise, the *Lawsonia inermis* leaves extract antibacterial activity was proved against *Pseudomonas savastanoi* pv. *savastanoi* and *Agrobacterium tumefaciens* (Trigui et al., 2013) [24]. However, the active antibacterial substance of the oregano and thyme are the carvacrol and thymol respectively (Zaika and Kissinger 1981) [25]. The action mode of this actives substance is the dissolve of the bacteria membrane, which enables to entre on the cell and integrated to the cellular mechanism (Judis, 1963[26]; Juven et al., 1972[27]; Oussalah et al., 2006[28]), increasing the plasmatic membrane permeability, who depolarized it potential (Xu et al., 2008) [29].

IV. Figures and Tables

Table 1 : The concentration of essential oils onto YPG medium containing the *PSS 2064-10* suspension.

	Tube	Bacterial suspension (µl)	volume of EO (µl)	Concentration (%)
- **Thyme** (*Thymus ciliatus*)	1	3872	128	0.32
	2	3936	64	0.16
	3	3968	32	0.16
	4	3984	16	0.04
	5	3992	8	0.02
- **Oregano**(*Origanumcompactum*)	6	3996	4	0.01
	7	3998	2	0.005
	8	3999	1	0.0025
T+	**9**	4000	200	10
T-	**10**	4000	-	0

Table 2: The inhibition percentage, MIC and MBC of essential oils against *PSS 2064-10* growth

Essential oil	**Inhibition zone (mm)** **Repetition**			**% of inhibition**	**MIC** (%)	**MBC** (%)
Thyme	40	43	52	56.25	0.08	0.16
Oregano	38	41	41	50	0.04	0.08
Rosemary	27	32.5	30.5	37.50	ND	ND
wormwood	16	17.5	17.5	21.25	ND	ND
T+ (streptomycin)	20	21	19	25	ND	ND
T- (SDW)	0	0	0	0,00	ND	ND

ND: No Determinated, SDW: sterile distilled water

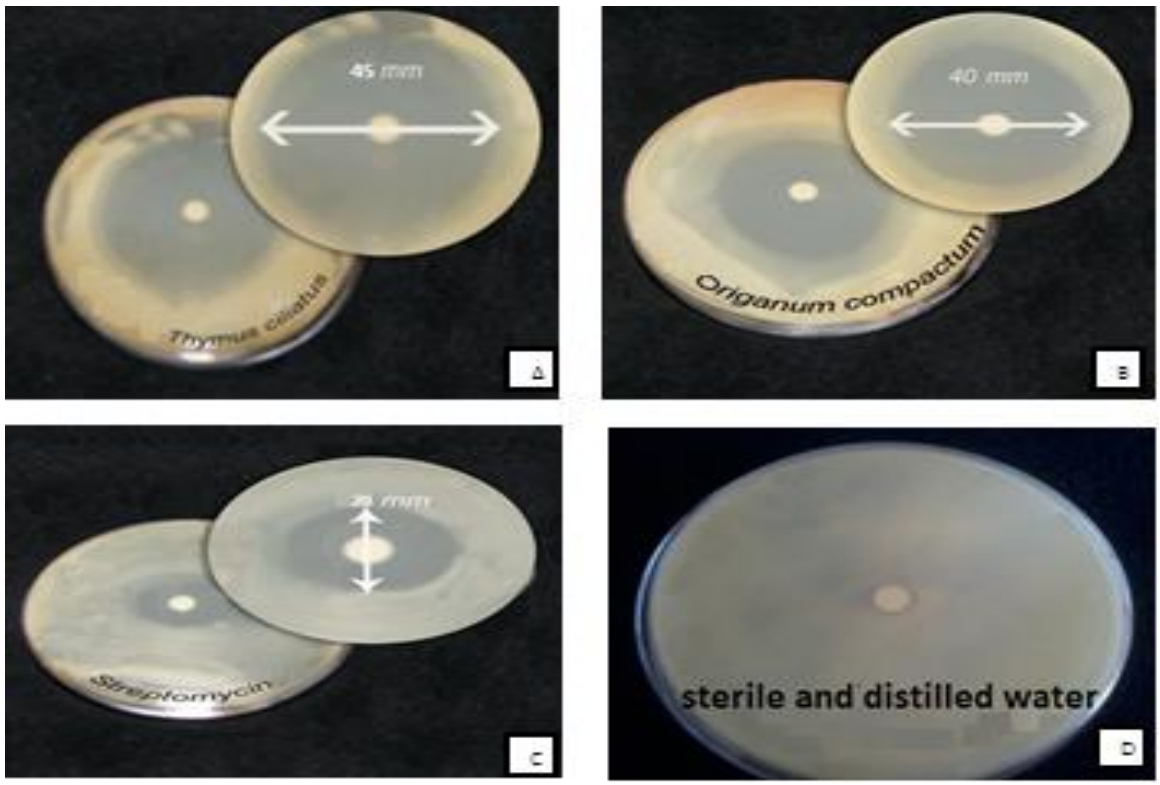

Fig1. Inhibition zone diameters obtained with the essential oils against *PSS* 2064-10 (Inhibition zone of the thyme (A), oregano (B), positive control (streptomycin) (C) and negative control (SDW) (D))

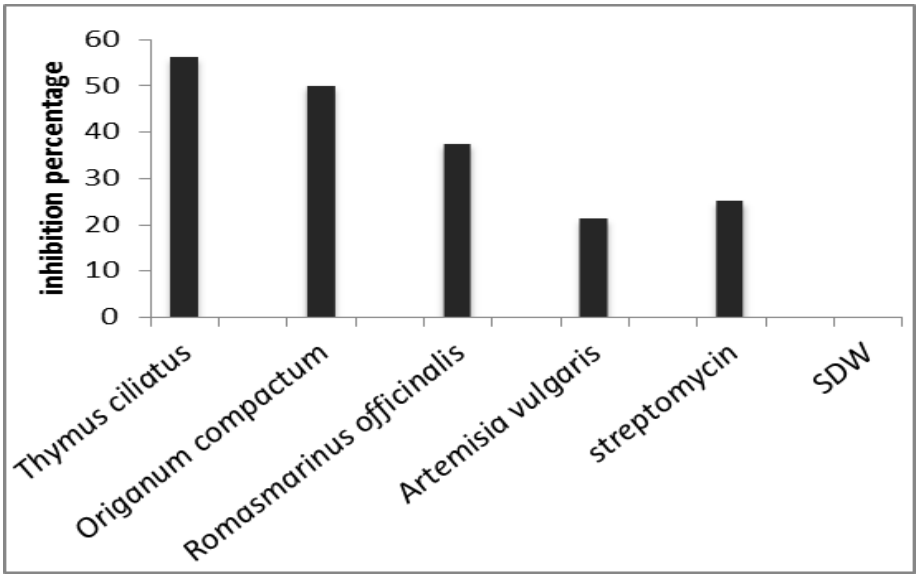

Fig2. The antibacterial activity of the essential oils the different plant species against *PSS2064-10* with positive control (streptomycin) and negative control (SDW)

V. Conclusion

In conclusion, we show an interesting antibacterial activity of some essential oils against *Pseudomonas savastanoi* pv. *savastanoi*, particularly *Thymus ciliatus* and *Origanum compactum* essential oils but we need further investigations to evaluate bactericidal properties for their application to bio-control due to their evaporation and the decrease their effectiveness in open fields. Further researches are needed in order to determine if they could substitute efficiently copper compounds or, perhaps, be used in combination.

Acknowledgments

We gratefully acknowledge the reception of the Laboratory of Phytobacteriology and Biocontrol, National Institute of Agronomic Research, Meknes- Morocco and Laboratory of Botany, Biotechnology and Plant Protection, Kenitra,Morocco.

References:

[1] Zeller, W. 2005. Status of biocontrol methods against fire blight. Internal Conference on: Biological and pro-ecological methods for control of diseases in orchards and small fruit plantations. J. of Skierniewice. 21: 29- 31.
[2] Bruneton, J. 1999. Pharmacognosy phytochemistry medicinal plants. Second Edition Technique & Documentation Lavoisier, Paris, France. 11-19.
[3] Wang, S.W., Chen, P.F. and Chang, S.T., 2005. Antifungal activities of essential oils and their constituents from indigenous cinnamon (Cinnamomum osmophloeum) leaves against wood decay fungi. J. Bioresour. Techno. 96: 813-818.
[4] Isman, M. B. 2000. Plant essential oils for pest and disease management. J. Crop Prot. 19: 603- 608.
[5] Pavela, R. 2006. Insecticidal activity of some essential oils against larvae of Spodoptera littoralis. Fitoterapia. 76: 691-696.
[6] Burt, S. 2004. Essential oils: their antibacterial properties and potential applications in foods - a review. INT. J. FOOD MICROBIOL. 94: 223-253.
[7] EL-Kamali, H.H., Hamza, M.A, and El-Amir, M.Y. 2005. Antibacterial activity of the essential oil from Cymbopogon nervatus inflorescence. Fitoterapia. 76: 446-449.
[8] Mahboubi, M., Kazempour, N. and Taghizadeh, M. 2014. The antibacterial activity of some essential oils against clinical isolates of Acinetobacter baumannii. Songklanakarin J. of Sci. and Technol. 36 (5): 513-519.
[9] Quesada, J.M., Penyalver, R., Pérez-Panadés, J., Salcedo, C. I., Carbonell, E.A. and López , M. M. 2010. Dissemination of Pseudomonas savastanoi pv. savastanoi populations and subsequent appearance of olive knot disease. J. Plant. Pathol. 59: 262-269.
[10] Wilson, E.E. 1935. The olive knot disease: Its inception, development, and control. J. of Hilgardia. 9: 231-264.
[11] Schroth, M.N, Osgood, J.W and Miller,T.D. 1973. Quantitative assessment of the effect of the olive knot disease on olive yield and quality. Phytopathol. 63: 1064-1065.
[12] Schroth, M.N., Hildebrand, D.C. and O'Reilly, H.J. 1968. Off-flavor of olives from trees with olive knot tumors. Phytopathol. 58: 524-525.
[13] Cayuela, J.A., Rada, M., Rios, J.J., Albi, T. and Guinda, A. 2006. Changes in phenolic composition induced by Pseudomonas savastanoi pv. savastanoi infection in olive tree: presence of large amounts of verbascoside in nodules of tuberculosis disease. J. AGR. FOOD CHEM. 54(15): 5363-5368.
[14] King E.O., Ward M.K., Raney D.E. 1954. Two simple media for the demonstration of pyocyanin and fluorescein. J. Lab. Clin. Med. 44: 301-307.
[15] Remmal, A, Tantaoui Elaraki, A., Bouchikhi, T., Rhayour, K. and Ettayebi, M. 1993. Improved method for the determinatin of antimicrobial activity of essential oils in agar medium. J. ESSENT. OIL. RES. 1993a, 5: 179-184.
[16] Bouhdid S, Skali SN, Idaomar M, Zhiri A, Baudoux D, Amensour M, Abrini J. Antibacterial and antioxidant activities of Origanum compactum essential oil. Afr. J. Biotechnol. 2008;7(10):1563-1570.
[17] Levison, M.E. 2004. Pharmacodynamics of antimicrobial drugs. Infectious disease clinics of North America. 18: 451-465.
[18] Amarti F., Satrani B., Ghanmi M., Farah A., Aafi A., El Ajjouri M., et ChaouchA., (2009). Composition chimique et activité antimicrobienne des huiles essentielles de Thymus algeriensis Boiss & Reut et Thymus ciliatus (Dest.) Benth. Du maroc.
[19] Benjilali B, Richard H MJ, Baritaux O. Etude des huiles essentielles de deux espèces d'Origan du Maroc: Origanum compactum Benth et Origanum elongatum Emb. Et Maire. Lebensm. wiss. Technol. 1986; 19:22-26.
[20] Zrira, S., Bessiere, J. M., Menut, C., Elamrani, A. and Benjilali, B. 2004. Chemical composition of the essential oil of nine eucalyptus species growing in Morocco.J. FLAVOUR FRAG. 192: 172-175.
[21] Daferera, D.J., Basil, N., Ziogas, N. and Polissiou, M.G. 2003. The effectiveness of plant essential oils on Botrytis cinerea, Fusarium sp. and Clavibacter michiganensis subsp. michiganensis. J. Crop. Prot. 22: 39–44.
[22] Yegen, O., Ünlü, A., Berger .and BM.1998. Use and side effects on the sil microbial activity of the essential oil from Thymbra spicata to control pepper blight Phytophthora capsici. J. PLANT. DIS. PROTECT. 105: 602-610.
[23] Scortichini, M. and Rossi, M.P. 1993. In vitro behavior of Erwinia amylovora towards some natural products showing bactericidal activity. Acta Hort. 338: 191-198.
[24] Trigui ,M., Ben Hsouna,A ., Hammami, I., Culioli,G., Ksantini,M. , Tounsi, S. and Jaoua. S. 2013. Efficacy of Lawsonia inermis leaves extract and its phenolic compounds against olive knot and crown gall diseases, J. Crop Prot. 45: 83-88.
[25] Zaika, L.L. and Kissinger, J.C. 1981. Inhibitory and stimulatory effects of oregano on Lactobacillus plantarum and Pediococcus cerevisiae. J. of Food Sci. 46: 1205–1210.
[26] Judis, J. 1963. Studies on the mechanism of action of phenolic disinfectants: II. Patterns of release of radioactivity from Escherichia coli labeled by growth on various compounds. J. Pharm. Sci. 52: 261–264.
[27] Juven, B., Henis, J. and Jakoby, B. 1972. Studies on the mechanism of the antimicrobial action of oleuropein. J. APPL. BACTERIOL. 35: 559–567.
[28] Oussalah, M., Caillet. S, Saucier L. and Lacroix, M. 2006. Antimicrobial effects of selected plant essential oils on the growth of a Pseudomonas putida strain isolated from meat. J. Meat Sci. 73: 236-244.
[29] Xu,J., Zhou ,F., Ji, B.P., Pei, R.S. and Xu, N. 2008. The antibacterial mechanism of carvacrol and thymol against Escherichia coli. J.LETT. APPL. MICROBIOL. 47: 174-179.