International Journal of Research Studies in Biosciences (IJRSB)
Volume 7, Issue 10, 2019, PP 26-30
ISSN No. (Online) 2349-0365
DOI: http://dx.doi.org/10.20431/2349-0365.0710004
www.arcjournals.org

"*Habak Al-Madinah Al-Munawarah*"; (*Ocimum Basilicum*) Endophytic-ability against Pathogenic-bacteria Proven Success-use-constantly in Saudi-society, Taif, KSA

Sherifa Mostafa M. Sabra[1*], Afaf Bushara M. Ismail[2]

[1]*Corresponding Author, Senior Const., Asst. Prof., Dr., Specialty Microbiology*

[2]*Asst. Prof., Analytical Chemistry Specialty*

[1]*Microbiology Br., Biology Dept., Science College, Taif University, KSA*

[2]*Med. Laboratory Dept., App. Med. Sci. College, Taif University, KSA*

[1]*Serology Unit and Bacterial Strains Bank, [AHRI], Dokki, [ARC], Giza, Egypt*

[2]*Chemistry Dept., Sci. College, Al-Dalanj University, Sudan*

[*]**Corresponding Author:** ***Sherifa Mostafa M. Sabra,*** *Senior Const., Asst. Prof., Dr., Specialty Microbiology, Micro. Br., Biology Dept., Sci. College, Taif University, KSA, Egypt.*

Abstract: *This work was first time for "Habak Al-Madinah Al-Munawarah"; (Ocimum basilicum) (OB) endophytic-ability against pathogenic-bacteria (PB) proven success-use-constantly (SUC) in Saudi-society (SS), Taif, KSA". Through in-vitro, experiment had found Fungi endophytic crude extracts (FECEs)had eliminated Gram-positive (GP-PB), and Gram-negative (GN-PB). Actinomyces endophytic crude extracts (AECEs)were stronger, eliminating GP-PB and GN-PBthan FECEs. Colony Forming Unit (CFU)/ml found through in-vitroexperiment the growth colonies number were more effective by AECEs than FECEs to kill PB that could lead to SSinfectious diseases. The characteristics of OBis a plant preserves endophyte microorganisms (MOs) and kills PB in a very vital bio-bactericidal way. It is available and cheap price. The results confirm OBproven SUC in SS and athigh altitude area (HAA).As the use in food and beverages because of their medical importance in maintaining public health (PH).That decided endophytes present in OBare of medicinal and therapeutic significance as they included Fungi and Actinomycetes. They are secreting substances capable of killing PB by bio-bactericidal way. Therefore, it is using in KSA, but the current in-vitroexperiment has used at HAA and proved highly effective to kill PB that might lead to infection disease, which maintains PH.This concluded OBendophytes proved its bio-bactericidal effects to kill PB. AECEs were stronger thanFECEs at HAA. OBrecommended using in beverages and foods without boiling prevent killingendophytes MOs and kept its bio-bactericidal effects to kill PB. This provenSUC in SS and at HAA helps maintain PH.*

Keywords: *Habak Al-Madinah Al-Munawarah, Ocimum basilicum, Endophytic-ability, Pathogenic-bacteria, Saudi-society, In-vitro experiment.*

ABBREVIATIONS

Actinomyces endophytic crude extracts: AECEs	*Fungi endophytic crude extracts: FECEs*	*High-altitude-area: HAA*	*Ocimum basilicum: OB*	*Public-health: PH*	*Streptococcal pyogenes: SP*
Colony Forming Unit: CFU	*Gram-negative: GN*	*Klebseilla pneumonia: KP*	*Pathogenic-bacteria: PB*	*Staphylococcous aureus: SA*	*Saudi-society: SS*
Escherichia coli: EC	*Gram-positive: GP*	*Microorganisms: MOs*	*Pseudomonas aeruginosa: PA*	*Staphylococcous epidermidis: SE*	*Success-use-constantly: SUC*

1. INTRODUCTION

"*Habak Al-Madinah Al-Munawarah*"; *OB* used to SUC in SS; leaves possess strong endophytes have antibacterial activities [1].That considered bio-antibacterial potential materials, there are two famous common fundamental MOs groups asendophytes, are include *Fungi* and *Actinomyces*; have antibacterial activity against more PB, which induce infectious diseases [2].

Endophytic *Fungi*yield special stimulated metabolites [3], include terpenoids [4], alkaloids [5], 4-Chloro-benzenesulfonamide and N-methyl, that all have antibacterial properties [6] Fourteen Fungal

endophytes were isolated from *OB* leaves, included *Aspergillus, Ascochyta, Nigrospora, Blastomyces, Colletotrichum, Exidia, Clitopilus* and *Nomuraea*. FECEs had tested against PB were included *SA, SE, EC,KP*and*PA.* All FECEs showed grades of antibacterial activity, *Nigrospora* MFLUCC16-0605 showed broad-spectrum, indicated endophyticbio-active power that has a strong bio-antibacterial activity [7].

Endophytic *Actinomyces* has specially *Streptomyces flavovirdis* A3WK as famous [8], their products include phenols, carboxylic acid and alkanes have potent antibacterial properties [9].The bioactivity power of AECEs had antibacterial activity against hospital PB. Antibiotic creating having broad-spectrum bactericidal activity [10].1H-pyrazole-3-carboxylic acid-5- methyl [11], pyrazol-5-carboxylic acid-3-methyl, tri-fluro-acetoxypentadecane, propionic acid, 3-(maminobenzoyl)-2-methyl-, 2,6-octadiene,2,6-dimethyl and 1Methyl-3-nitro-5[4-nitropyrazole-1-yl]has double antibacterial activity [12]. AECEs were basis of antibacterial activity that gives plant medicinal price. Eleven endophytic *Actinomycetes* were isolated, 12 compounds revealed *Streptomyces flavovirdis* A3WK has more antibacterial action. AECEs had tested against PB included extremely PB revealed as antibacterial inhibitory activity [13].

This research had carried out in an aim of Taif region experiment, which is one of HAA in KSA and has characteristics distinct from the normal area. An*in-vitro*experiment had made to "*Habak Al-Madinah Al-Munawarah*", *OB* endophytic-ability against PB proven SUC in SS, Taif, KSA. Endophytes is natural products in plants, can kill PB. FECEs and AECEs had tested against more PB. The results were followed relationship between them and PB for 11 hours. That might be proving the ability of ECEs to capable until which extent to kill PB. This could help to maintain PH at HAA, which by using natural plants. *OB* contains the capabilities to kill more PB in a very vital and very cheap ways and is still a habit of SS since long ancients.

2. METHODOLOGY

2.1. Sample Collections: Farms-owners agreement on research had obtained.*OB* plant leaves had collected from organic farms. The leaves had collected from medium sized healthy plants in closed sterile polythene bag. That had labeled and had brought to "Bacteriological Laboratory" within 24 hours [14].

2.2. Samples Preparation: Leaves had washed gently under running tap water, had cut into parts (0.5-1cm), had dipped in 70% ethanol for 5 seconds then by 4% sodium hypochlorite for 90 seconds. Then they had rinsed in sterile distilled water for 10 seconds. The excess moisture had tarnished in sterile filter paper [15].

2.3. Extracts Preparation: The surface sterilized parts had placed in *Fungi* medium, had incubated at 28ºC for 3 days [14].FECEs had obtained by Ethyl Acetate [16]. On the other side the surface sterilized segments had placed in *Actinomyces* medium, had incubated at 28°C for (3-4) weeks [17].AECEs had obtained by Ethyl Acetate [18]

2.4. *In-Vitro* Experiment: Obtained classified PB isolates had got from "Research Center"; appropriate environments had improved for PB growth. PB had tested into GP included (*SA, SE* and *SP*); GN included (*EC, PA* and *KP*) [19]. Sterile screw capped tubes had used; put 5ml ECE + 5ml of PB suspension (10^3– 10^4 /ml), then had mixed for each ECE separately. Tubes had incubated at (35-37) ºC, and the experiment had followed up. One ml had taken from each tube in the following time (0, 1, 3, 5, 7, 9 and 11) hours. They were cultured on PB appropriate media; they had incubated for 48 hours at (35-37)º C. Growth colonies had recorded [20]. Developed colonies mean had calculated by "Law Equation: CFU / ml = (Colony count / 300) X 100". The mean rates of reducing growth colonies amount had followed-up [21].

2.5. Data Analysis: "Simple Basic Excel Formulas" had cast-off method the results data [22].

3. RESULTS AND DISCUSSION

Table1 and graph1: Mean of bacterial growth based on inoculation time

Items			0 Hour	1 Hour	3 Hour	5 Hour	7 Hour	9 Hour	11 Hour
*GP	*SA	*FECEs	100%	80%	50%	30%	10%	00%	
		*AECEs	100%	70%	40%	10%	00%		
	*SE	FECEs	100%	90%	80%	60%	30%	10%	00%
		AECEs	100%	70%	40%	10%	00%		
	*SP	FECEs	100%	80%	60%	30%	10%	00%	
		AECEs	100%	70%	40%	10%	00%		
*GN	*EC	FECEs	100%	80%	60%	40%	10%	00%	
		AECEs	100%	70%	50%	10%	00%		
	*PA	FECEs	100%	90%	70%	60%	30%	10%	00%
		AECEs	100%	80%	70%	40%	10%	00%	
	*KP	FECEs	100%	90%	80%	50%	30%	10%	00%
		AECEs	100%	70%	60%	40%	10%	00%	

**GP: Gram-positive, ^SA: Staphylococcous aureus, *SE: Staphylococcous epidermidis, *SP: Streptococcal pyogenes, *GN: Gram-negative, *EC: Escherichia coli, *PA: Pseudomonas aeruginosa, *KP: Klebseilla pneumonia, *FECES:Fungi endophytic crude extracts, *AECEs: Actinomyces endophytic crude extracts*

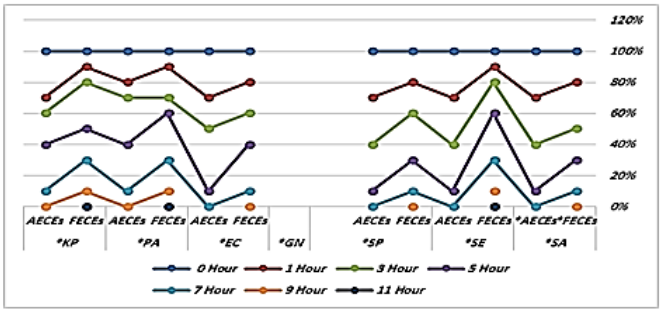

Table 1 and graph 1 revealed mean of bacterial growth based on inoculation time, because of an *in-vitro* experiment, it had found products of endophytes MOs were able to kill PB by varying degrees. It had found that FECEs had eliminated the GP-PB, and GN-PB [3-7]. Nevertheless, AECEs were stronger, eliminating the GP-PB in less time than FECEs, also in the GN-PB. That indicated the ability of AECEs stronger than FECEs [8-13]. That all had indication the effect of endophytes MOs on PB, which revealed protection role, that was sign in proven SUC in SS at HAA, Taif, KSA [1-2].

Table2 and graph2: *Mean of bacterial *CFU/ml based on inoculation time*

Items			0 Hour	1 Hour	3 Hour	5 Hour	7 Hour	9 Hour	11 Hour
*GP	*SA	*FECEs	300	240	150	90	30	00	
		*AECEs	300	210	120	30	00		
	*SE	FECEs	300	270	240	180	90	30	00
		AECEs	300	210	120	30	00		
	*SP	FECEs	300	240	180	90	30	00	
		AECEs	300	210	120	30	00		
*GN	*EC	FECEs	300	240	180	120	30	00	
		AECEs	300	210	150	30	00		
	*PA	FECEs	300	270	210	180	90	30	00
		AECEs	300	240	210	120	30	00	
	*KP	FECEs	300	270	240	150	90	30	00
		AECEs	300	210	180	120	30	00	

**CFU: Colony Forming Unite, *GP: Gram-positive, ^SA: Staphylococcous aureus, *SE: Staphylococcous epidermidis, *SP: Streptococcal pyogenes, *GN: Gram-negative, *EC: Escherichia coli, *PA: Pseudomonas aeruginosa, *KP: Klebseilla pneumonia, *FECES:Fungi endophytic crude extracts, *AECEs: Actinomyces endophytic crude extracts*

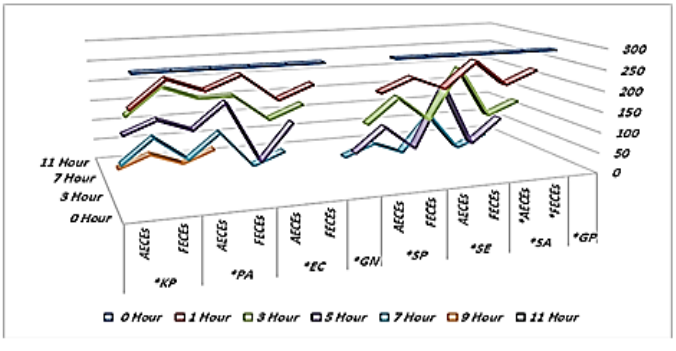

Table 2 and graph 2 revealed mean of bacterial CFU/ml based on inoculation time, it was found through *in-vitro* experiment that, the number of growth colonies were more effective by AECEs, because were more powerful than FECEs to kill PB that could lead to infectious diseases in SS [3-13]. The characteristics of *OB* are a plant that preserves endophytes MOs and kill PB in a very vital bio-bactericidal way. It is available and cheap price. The results confirm *OB* proven SUC in SS at HAA, as the use in food and beverages. That because of their medical importance in maintaining PH at HAA [3-13].The present simple research decided endophytes present in *OB*are of medicinal and therapeutic significance as they included *Fungi* and *Actinomycetes*, which secreting substances capable of killing PB by bio-bactericidal way [1-2]. Therefore, that used to adding in drinks and foods in KSA, but the current *in-vitro* experiment had used at HAA and proved highly effective to kill PB, that might lead to infection. That work which maintained PH and was a sign in proven SUC in SS at HAA, Taif, KSA [1-2].

4. CONCLUSION

This present applied effort decided that, *OB* endophytes MOs showed its bio-bactericidal properties to kill PB. As well, AECEs were stronger than FECEs at HAA.

RECOMMENDATION

OB recommended using in beverages and foods without boiling to stop killing endophytes MOs and reserved its bio-bactericidal belongings to kill PB. This had established SUC in SS and at HAA, which assistances to uphold PH.

ACKNOWLEDGMENT

Many thanks directed for all people aided in crop this paper.

REFERENCES

[1] Tilebeni, G., Review to basil medicinal plant. Int. J. Agronomy and Plant Production, 2011;2(1):5-7.

[2] Alvin, A., Miller, I. and Neilan, A.,Exploring the potential of endophytes from medicinal plants as sources of antimycobacterial compounds. Micro. Res., 2014; 169:483-495.

[3] Stierle, A., Strobel, A. and Eugenia D., Taxol and taxane production by *Taxomycesandreanae*, an endophytic fungus of *Pacic Yew*. Science, 1993; 260:214-216.

[4] Alexei, B., Joseph, S. and Olga, F.,Endogenous cardiotonic steroid: Physiology, pharmacology and novel therapeutic targets. Pharm. Rev., 2009; 61:9-38.

[5] Benbott, A., Yahyia, A. andBelaidi, A.,Assessment of the antibacterial activity of crudealkaloids extracted from seeds and roots of the plant *Peganumharmala* L.J. Nat. Prod. PlantResour.,2012; 2:568-573.

[6] Lei, W., Peng, Q.,Xiu-Feng, L. and Shuai, Z., Comparative analysis of chemical constituents, antimicrobial and antioxidant activities of ethyl acetate extracts of *Polygonumcuspidatum* and its endophytic *Actinomycetes*, *Streptomyces* sp. As 0916. Chi. J. Nat. Med.,2016; 14(2):0117-0123.

[7] Piyamas, A.,Sakon, M.,Eleni, G.,Siraprapa, B. and Patcharee, P., Antibacterial and antioxidant constituents of extracts of endophytic *Fungi* Isolated from *Ocimum basilicum* var. *thyrsiflora* leaves. Curr.Micro.,2017; 74:1185–1193.

[8] Waheeda, K. and Shyam, V., Formulation of novel surface sterilization method and culture media for the isolation of endophytic *Actinomycetes* from medicinal plants and its antibacterial activity. J. Plant Pathol.Micro.,2017; 8:2-7.

[9] Narendhran, S., Rajiv, P., Vanathi, P. and Rajeshwari, S., *Spectroscopic* analysis of bioactive compounds from *Streptomyces cavouresis*kuv39: Evaluation of antioxidant and cytotoxicity activity. Int. J. Pharm. Sci.,2014; 6(7):319-322.

[10] Golinska, P., Wypij, M., Agarkar, G. and Rathod, D., Endophytic *Actinobacteria*of medicinal plants: Diversity and bioactivity. Ant. Van. Leeuw., 2015; 108:267–289.

[11] Puri, C., Verma, V., Amna, T., Qazi, N. and Spiteller, J., An endophytic fungus from *Nothapodytesfoetida* that produces camptothecin. J. Nat. Prod.,2005; 68:1717-1719.

[12] Silva, H., Teles, H., Zanardi, M. and Young, C., *Cadinanesesquiterpenoids* of *Phomopsiscassiae* an endophytic fungus associated with *Cassia spectabilis* (Leguminosae). Phytochemistry, 2007; 67:1969-1976.

[13] Waheeda, K. and Shyam, V., Comparative study of *Ocimum basilicum* and its endophytic *Actinomycetes StreptomycesFlavoviridis* A3WK: Evaluation of antioxidant, anti-inflammatory and antimicrobial activity. IJPSR., 2018; 9(3):1023-1034.

[14] Lakshman, C. and Jayshree, K., Diversity of the endophytic fungi isolated from *Ocimum basilicum* Linn. A Promising Medicinal Plant. PARIPEX, Indian J. Res., 2014; 3(4):204-206.

[15] Dobranic, K., Johanson, A. and Alikhan, R.,.Isolation of endophytic fungi from eastern larch (*Larixlancina*) leaves from New Brunswick, Canada. Can. J. Micro.,1995; 41(2):194-198.

[16] Tomsheck, R., Strobel, A., Booth, E., Geary, B., Spakowicz, D., Knighton, B., Floerchinger, C., Sears, J., Liarzi, O. and Ezra, D., *Hypoxylon* sp., an endophyte of *Perseaindica*, producing 1,8-cineole and other bioactive volatiles with fuel potential. Micro. Ecol., 2010; 60:903-914.

[17] Waheeda, K. and Shyam, V., Formulation of novel surface sterilization method and culture media for the isolation of endophytic *Actinomycetes* from medicinal plants and its antibacterial activity. J. Plant Pathol.Micro.,2017; 8:2-7.

[18] Lei, W., Peng, Q., Xiu-Feng, L. and Shuai, Z., Comparative analysis of chemical constituents, antimicrobial and antioxidant activities of ethyl acetate extracts of *Polygonumcuspidatum* and its endophytic *Actinomycetes*, *Streptomyces* sp. As 0916. Chi. J. Nat. Med., 2016; 14(2):0117-0123.

[19] Panel-Prem, B., Parajulia-Kyle, R. and Mankinb-Philip, B.,Source specific fecal bacteria modeling using soil and water assessment tool model. Bioresource Technology,2009; 100(2):953-963.

[20] https://quizlet.com/41741253/microbiology-lab-final-study-guide-flash-cards/2019

[21] https://courses.lumenlearning.com/boundless-microbiology/chapter/counting-bacteria/2019

[22] https://people.umass.edu/evagold/excel.html2019

Citation: *Sherifa Mostafa M. Sabra & Afaf Bushara M. Ismail, ""Habak Al-Madinah Al-Munawarah"; (Ocimum Basilicum) Endophytic-ability against Pathogenic-bacteria Proven Success-use-constantly in Saudi-society, Taif, KSA". International Journal of Research Studies in Biosciences (IJRSB). 7(10), pp. 26-30. DOI: http://dx.doi.org/10.20431/2349-4050.0710004*