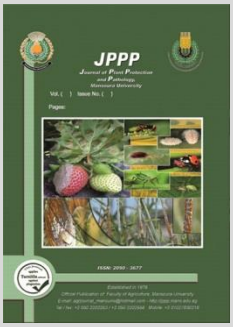
Journal of Plant Protection and Pathology

Journal homepage: www.jppp.mans.edu.eg
Available online at: www.jppp.journals.ekb.eg

The Use of Some Bio-Agents in The Control of Varroa Mite, *Varroa destructor* Infected Honey Bee Colonies

Karima A. Elsaied[2]; R. E. Sanad[1*]; H. E. Elsharkawy[2] and N. A .Omar[2]

[1]Plant Protection Res. Institute, Agric. Research Centre, Dokki, Giza, 12618 Egypt
[2]Plant Production Dept. , Fac. Technology &Development Zagazig University ,Egypt



ABSTRACT

This work was conducted in the experimental laboratory and apiary of the Plant Production Department of the Faculty of Technology and Development Zagazig University , Egypt. The main purpose of this work was to throw the light on the control of varroa mite, *Varroa destructor* infected honey bee colonies using some biological control agents. Six essential oils were used: Cinnamon oil**,** Clove oil, Jasmine oil, Eucalyptus oil, Peppermint oil, in addition to Formic acid 65% , Oxalic acid and Di stop. The obtained results reported that the increase percentages of dropped varroa numbers, comparing with check , were the highest with the treatments of Cinnamon oil 78% ,while the least percentages was 61 % at Oxalic acid treatment. The decrease percentages of infected sealed broods per 100 cell , comparing with check , were the highest with Distop 38 % , while the least percentages was 25 % at Cinnamon oil. The highest increase percentages of infested bee workers , comparing with control treatment , was the highest with the treatment of Oxalic acid 41 % , while the least percentages was recorded with Di stop giving 22%. The highest increases in sealed brood area were noticed with Di stop 28 % , while the least increase percentages were recorded with jasmine **.** The highest increase percentages in honey bee production , in comparison with control hives, were registered with the treatments of Clove oils giving 171.4 %.

Keywords: Varroa, Honeybee, Essential oil, Bio- control agents, parasitic mite

INTRODUCTION

The honey bee (*Apis mellifera* L.) insects is considered as one of the most important activities in the industry and agriculture in Egypt and all over the world. Honey bee colonies is susceptible to some pests and diseases that cause severe losses in the production of honey and may destroy bee hives. *Varroa destructor*, Acari : Varriodae (Anderson and Trueman 2000) former known as *Varroa jacobsoni* (Oudmans, 1904) has become one of the most serious threats to managed beekeeping and considered the most damaging for the colonies of honeybee (*A. mellifera*), it acts as ectoparasitic. Most commercial colonies usually die following 1-2 years of consecutive infestation with varroa if colonies are left untreated (Martin *et al*., 1998; Downy and Winston, 2001). Incidence of mites in honeybee colonies increases the infection by different diseases because they act as vectors of honeybee pathogens (Ball,1994) . Consequently, without mite control, bee colonies usually perish within a short period infestation with varroa (Ritter, 1981) .Beekeepers rely heavily on synthetic acaricides to reduce varroa population to non-damaging levels (Caron ,1999; Melathopoulos and Farney , 2002). Varroa populations were successfully controlled by acaricides such as the pyrethroid pesticide fluvalinate has developed resistance to multiple class of synthetic acaricides in many geographic areas (Elzen *et al*., 2000; Spreafico *et al*., 2001). Furthermore ,acaricide residues have been detected in honey and bees wax products (Wallner , 1999) .Biocide activity of several compounds of plants origin such as plant oils have been investigated as possible means to control varroa mites and have been considered more environment friendly acaricides (Imdorf *et al*., 1999). Of these compounds, oils such as origanum and thyme oil (Sammataro, *et al* ., 1998), essential oils from the plant family Lamiacaea as labiatae (Colin,1990) and plant derivatives, such as thymol and menthol (Imdorf *et al*.,1995), are possible materials of biocide effects , in program of mites control.

From the previous points of view the purpose of this work was to throw the light on the efficacy of six essential plant oils, two acids and Di stop against varroa mite, *Varroa destructor* infested honeybee colonies under field conditions as dropped numbers ,number of mites infested brood cells, the numbers of honeybee workers infested with Varroa destructor mites, effect on the area of brood in honeybee colonies , honey yield per colony after treating with essential oils in comparison with untreated colonies.

MATERIALS AND METHODS

This experimental work was carried out at the Plant Production Department of the Faculty of Technology and Development Zagazig University, Egypt. Twenty seven of *Apis mellifera* honey bee Carniolan colonies nearly equal in their strength and headed with local Carniolan queens at the same age were chosen. Each Langestroth hives housing the colonies, contained 4 combs of adult bees and 3 combs of sealed brood contained honey and pollen (bee bread). The tested colonies were arranged at random in 9 groups, 8 groups were assigned to the treatments and one group was used as a control treatment. Each group contained 3 colonies.

* **Corresponding author.**
E-mail address: reda_eliwa@yahoo.com
DOI: 10.21608/jppp.2021.207495

To study the efficacy of some plant essential oils against varroa mite, *Varroa destructor* infested honeybee colonies under field conditions ,six botanical volatile oils were used: Cinnamon oil**,** Clove oil, Jasmine oil, Eucalyptus oil, Peppermint oil, in addition to Formic acid 65% , Oxalic acid and Di stop.

The tested oils were mixed with sunflower oil as a carrier at the rate of 1: 9 resulting 10 % liquid materials. Cotton stripes (2 cm × 20 cm) were absorbed each with 10 ml of this mixture and were inserted between the brood combs, one stripe per / hive . This treatment was carried out weekly for two times (Grant and Jackson , 2004).

As for Formic acid and Oxalic acid it was used at the rate of 5 cm3/colony which treated on a paper sheet and put on the upper direction of infested frame, while Di-stop (local product) which is consisted of different physical and chemical materials at the rate of 4 cm3/colony which treated on a paper sheet and put in between the frame of brood .

The effect of these materials as controlling agents was tested for 35 days. Infestation levels of mites in the untreated colonies were determined before and after treatments for each colony and treatment. Data were recorded every 7 days and continued for 5 weeks. The obtained results were compared using the standard analysis of variance (ANOVA) according to Little and Hills (1978). Sendecor and Cochran, (1989) , and Costat 22 (1998). Reduction percentages were counted according to the formula of Henderson and Tilton (1955) :

$$\text{Reduction \%} = \left[1 - \left(\frac{\text{Treatment after}}{\text{Treatment before}} \times \frac{\text{Control before}}{\text{Control after}}\right)\right] \times 100$$

RESULTS AND DISCUSSION

Results in Table (1) showed that effect of treating honeybee hives with essential oils and formic & oxalic acids in comparison with Di stop on the numbers of dropped *V*arroa *destructor* mites infested honeybee stages , in addition to the increase percentages of dropped varroa mites per colony after treating with tested materials in comparison with untreated colonies.

Data indicated that the numbers of dropped *varroa mites were* increased by increasing the period of treatments, where the highest numbers of dropped varroa mites after five weeks of treatment were recorded with Peppermint oil giving 252 individual /5 weeks comparing with 56 individuals in the control treatment, followed by the treatment of jasmine oil 250 individual, while Di stop , Eucalyptus oil and Clove oil recorded the least numbers 205 individual for each. The increase percentages of dropped varroa numbers, comparing with control , were the highest with the treatments of Cinnamon oil (78%), Di stop (77%) , and peppermint oil (76 %) , while the least percentages was 61 % at Oxalic acid treatment.

Results presented in Table (2) cleared that the effect of treating honeybee hives with essential oils and tested acids on the decrease of infested sealed brood with varroa mites per 100 cell, in addition to the decrease percentages of infested sealed brood with mites per colony after treating with tested agents in comparison with untreated colonies.

Data revealed that the least mean number of infested brood cells with varroa mites was recorded at the treatment of Clove oil giving 5.0 infested cells , in comparison with control 15.2 infested cells.

Statistical analysis of data in Table (2) indicated that there were significant differences in the number of infested brood cells with varroa mites between all treatments and control treatment , while there were no significant differences among treatments of all tested oils.

In conclusion, the decrease percentages of infected sealed broods per 100 cell, comparing with control treatment , were the highest with the treatments of Di stop 38 % , followed by the treatments of peppermint oil and clove oil giving 36 , and 34 % , while the least percentages was 25 % at cinnamon treatment.

Regarding to the results on the effect of essential oils on the numbers of honeybee workers infested with *Varroa destructor* mites at honeybee colonies (Table 3) , the least numbers of infested bee workers were recorded at the treatment of clove oil, eucalyptus oil and Di stop (about 205 dead mites / 500 worker) , without significant differences among them, in comparison with control treatment where 56 dead mites / 500 worker were counted .

There were significant differences in the numbers of dead mites / 100 worker between all tested agents and control treatment.

Table 1. Effect of essential oils on the numbers and increase percentages of dropped *Varroa destructor* mites infested honeybee

Treatments	Average number and increase percentages of dropped mites / colony											
	Pre treatment	Post treatment										
		1 week		2 week		3 week		4 week		5 week		Total	
	D	D	%	D	%	D	%	D	%	D	%	D	%
Cinnamon oil	8 b	16 def	50	46 a	83	64 d	88	47 a	83	51 d	84	224 bc	78
Clove oil	9 b	13 f	30.8	41 b	78	65 d	86	29 e	69	57 c	84	205 c	70
Jasmine oil	14 a	23 ab	39.1	50 a	72	74 c	81	35 d	60	68 a	79	250 a	66
Eucalyptus oil	10 b	20 bcd	50	35 c	71	58 e	83	45 ab	60	49 d	87	207 c	70
Pepper mint oil	10 b	22 abc	54.5	48 a	79	78 b	87	42 bc	76	62 bc	84	252 a	76
Formic acid	12 ab	18 d	30	42 b	71	66 d	81	45 ab	73	65 ab	82	236 ab	68
Oxalic acid	15 a	25 a	40	35 c	57	82 a	82	32 de	53	58 c	74	232 ab	61
Di stop	7 b	14 ef	50	32 c	78	68 d	90	39 c	82	52 d	87	205 c	77
Control	10 b	8 g	-	12 d	-	9 f	-	14 f	-	13 e	-	56 d	-
LSD 5%	3.4	3.4		3.4		3.4		3.4		4.2		15.2	

D = dropped mites

Means in each column followed by different letter(s) are significantly different

Table 2. Effect of essential oils on the numbers of infested brood cells of honeybee with *Varroa destructor* mite and reduction percentages

Treatments	Number of infested brood cells and reduction % of infested area / 100 sealed brood											
	Pre treatment	Post treatment										
		1 week		2 week		3 week		4 week		5 week		Grand mean	
	B	B	%	B	%	B	%	B	%	B	%	B	%
Cinnamon oil	12	10	10.7	7	38.8	6	14	4	0.0	2	63	5.8 c	25
Clove oil	10	11	0.0	6	52.3	5	17	2	40	1	63	5.0 c	34
Jasmine oil	14	12	8.2	10	27.1	8	20	5	6.3	2	70	7.4 c	26
Eucalyptus oil	16	18	0.0	12	41.7	10	17	9	35	4	67	10.6b	32
Pepper mint oil	12	13	0.0	6	59.6	5	17	4	20	1	81	5.8 c	36
Formic acid	10	10	0.0	8	30.0	6	25	5	25	2	70	6.2 c	30
Oxalic acid	16	12	19.6	10	27.1	9	10	4	33	2	62	7.4 c	31
Di stop	18	16	4.8	8	56.3	7	13	7	50	3	68	8.2 bc	38
Control	15	14	0.0	16	30.0	18	25	12	25	16	70	15.2 a	-
LSD 5%												2.7	

Means in column followed by different letter(s) are significantly different B= Infested brood cells

Results in Table (3) recorded the reduction percentages in *Varroa destructor* mites infested workers as affected by tested agents in honeybee colonies in comparison with control treatment.

The highest increase percentages of infested bee workers , comparing with control treatment , was the highest with the treatment of Oxalic acid 41 % while the least was recorded with Di stop (22 %) , and 26.0 % for both of eucalyptus and cinnamon oils .

The obtained data in Table (4) revealed that the effect of essential oils and other agents treated in bee hives to control *Varroa destructor* mites on the area brood in honeybee colonies.

The highest increases in sealed brood areas, comparing to control (348 cm ^{2}), was recorded with the treatments of Clove oil and Oxalic acid 516.2 , 510 cm ^{2} / colony ,while the least values were recorded with peppermint oil, jasmine oil 409 , 423 cm ^{2} respectively, comparing with control treatment which produce only 348 cm^2.

Table 3. Effect of essential oils and other agents on the numbers and reduction percentages of honeybee workers infested with *V. destructor* mites at honeybee colonies

Treatments	Average numbers and reduction percentages of dead mites / 100 worker											
	Pre treatment	Post treatment										
		1 week		2 week		3 week		4 week		5 week		Total	
	IW	IW	%	IW	%	IW	%	IW	%	IW	%	IW	%
Cinnamon oil	8 b	16 def	2.7	46 a	49	64 d	41	47 a	5.9	51 d	33	224 bc	26
Clove oil	9 b	13 f	22	41 b	40	65 d	61	29 e	47	57 c	11	205 c	36
Jasmine oil	14 a	23 ab	6.7	50 a	33	74 c	29	35 d	21	68 a	55	250 a	29
Eucalyptus oil	10 b	20 bcd	12	35 c	23	58 e	49	45 ab	21	49 d	25	207 c	26
Pepper mint oil	10 b	22 abc	14	48 a	49	78 b	41	42 bc	21	62 bc	40	252 a	33
Formic acid	12 ab	18 d	33	42 b	34	66 d	37	45 ab	27	65 ab	55	236 ab	37
Oxalic acid	15 a	25 a	13	35 c	60	82 a	41	32 de	21	58 c	70	232 ab	41
Di stop	7 b	14 ef	13	32 c	25	68 d	26	39 c	15	52 d	33	205 c	22
Control	10 b	8 g		12 d		9 f		14 f		13 e		56 d	
LSD 5%	3.4	3.4		3.4		3.4		3.4		4.2		15.2	

Means in each column followed by different letter(s) are significantly different IW= infested workers

Table 4. Effect of essential oils and other agents to control *Varroa destructor* on the area and increase percentages of brood in honeybee colonies

Treatments	Sealed brood area cm ^{2} and increase percentage/ colony												
	Pre treatment	Post treatment											
		1 week		2 week		3 week		4 week		5 week		Grand mean	
	B	B	%	B	%	B	%	B	%	B	%	B	%
Cinnamon oil	390 c	420 b	11	456 b	21	459 b	23	487 d	28	521 b	33	469 c	23
Clove oil	432 a	458 a	9.5	490 a	19	521 a	25	543 a	29	569 a	32	516 a	23
Jasmine oil	364 g	384 c	9.1	397 d	16	420 cd	22	432 f	25	480 d	32.4	423 e	21
Eucalyptus oil	378 f	395 c	8.2	421 c	17	435 c	21	455 e	26	498 c	32	441 d	21
Pepper mint oil	350 h	354 d	5.2	368 e	12	412 d	23	451 e	31	460 e	32	409 e	21
Formic acid	410 b	436 b	9.7	462 b	18	498 a	25	510 c	28	540 b	32	489 b	23
Oxalic acid	410 b	462 a	15	480 a	21	510 a	27	532 ab	31	564 a	35	510 a	26
Di stop	384 d	436 b	16	458 b	22.8	501 a	30.5	521 bc	34	533 b	36	490 b	28
Control	380 e	365 d	-	350 f	-	345 e	-	341 g	-	339 f	-	348 f	-
LSD 5%	1.72	17.2		17.2		17.2		17.2		17.2		16.8	

Means in each column followed by different letter(s) are significantly different B = Area brood cm^2

Statistical analysis of the obtained results indicated that there were significant differences in the area of sealed brood per hive , between all tested agents and that of the control. Furthermore , there were significant differences in the area of sealed brood per hive between the treatments of Clove oil and Oxalic acid and all other treatments.

As shown in the obtained results in Table (4) the treatments of Di stop and Oxalic acid registered the highest increases in sealed brood area giving 28 and 26 % , while the least increase percentages were recorded with jasmine , peppermint and eucalyptus oils , 21 % , for each.

Regarding to the effect of tested materials on the yield of honey bee , results in Table (5) indicated that the highest yields in honey bee production , in comparison with untreated hives (2.1 kg per hive) , were registered with the treatments of Clove and cinnamon oils giving 5.7 and 5.4 kg per hive , recording 171.4 % and 157.1 % increase percentages ,respectively , in comparison with control

treatment, while the least increase percentage was recorded at the treatment of Di stop 66.6 %.

Statistical analysis of the obtained results indicated that there were significant differences in the honey bee per hive , between that of Clove oil and cinnamon oils and that of all other tested oils. Furthermore , there were significant differences in the yield of honey per hive between all treatments and control treatment.

Table 5. Increase percentages of honey per colony after treating with essential oils in comparison with untreated colonies

Treatments	Weight of honey (kg)	% of increase
Cinnamon oil	5.4 ab	157.1
Clove oil	5.7 a	171.4
Jasmine oil	3.9 c	85.7
Eucalyptus oil	4.2 c	100
Peppermint oil	4.0 c	90.5
Formic acid	4.3 c	104.7
Oxalic acid	5.1 b	142.8
Di stop	3.5 d	66.6
Control	2.1 e	-
LSD 5%	3.28	17.31

Means in each column followed by different letter(s) are significantly different

The obtained results are in agreement with those obtained by Sammataro *et al.* (1998) , Rosenkranz *et al.* (2010) , and Abd El-Wahab *et al.* (2012) .

REFERENCES

Abd El-Wahab, T. E. , Ebadah, I.M.A. and Zidan, E.W. (2012). Control of Varroa Mite by Essential Oils and Formic Acid with Their Effects on Grooming Behaviour of Honey Bee Colonies. Journal of Basic and Applied Scientific Research. 2, 7674-7680.

Anderson, D.L. and T.W.H. Trueman (2000). *Varroa jacobsoni* (Acari: varroidae) is more than one species. Experimental and. Applied Acarology, 24 : 165 -189.

Ball, B.V. (1994). Host- parasite pathogen interactions . In Matheson A (ed) New Perspectives on Varroa .IBRA ; Cardiff , UK; p5 – 11 .

Caron, D. M. (1999a) . IPM for beekeeepers. American Bee Journal 139 (5): 363 – 365.

Colin , M. E. (1990). Essential oils of labiates for controlling honey bee varrosis , J. App. Ent., 110: 19 – 25 .

Costat 22 (1998). A software computer program for statistical analysis.

Downey , D. and M. L. W. Winston (2001). Honey bee colony mortality and productivity with single and dual infestations of parasitic mite species . Apidologie, 32 : 567 – 575 .

Elzen, P.J. ; J.R. Baxter ; G.W. Elzen ; R. Rivera and W.T. Wilson (2000). Evaluation of grapefruit essential oils for controlling *Varroa jacobsoni* and *Acarpis woodi*. American Bee Journal, 140 (8): 666 - 668.

Grant, F. C. and M .O. Gillard–Jackson (2004) . An experiment in the efficacy of fogged mineral oil . American Bee Journal, 146 (7): 860 – 863.

Henderson, C. F. and W. Tilton (1955): Tests with acaricides against the brown wheat mite. Journal of Econ. Ent. 48: 157 – 161.

Imdorf, A.; S. Bogdanov ; V. Kilchenmann, and C. Maquelin (1994). Apilife –VAR a Varroa treatment substance with thymol as the active ingredient. Schweizerische Bienen Zeitung, 117 (6) 326-333.

Imdorf, A. ; S.Bogdanov ; V. Kilchenmann, and C. Maquelin (1995). Apilife -VAR : a new varroacide with thymol as main ingredient. Bee World 76 (2) :77 – 83.

Imdorf, A. ; S. Bogdanov ; R .I. Ochoa and N.W. Calderone (1999). Use of essential oils for the control of *Varroa jacobsoni* in honey bee colonies Apidologie, 30 (23): 209 – 228 .

Little, T.M. and F. J. Hills (1978). Agricultural and experimentation design and analysis. John Wiley and Sons. New York, Chichester, Brisbane, Toronto.

Martin, S. J. ; A. Hogarth ; van Breda and J. Perrett (1998). A scientific note on *Varroa jacobsoni* Oud. and the collapse of *Apis mellifera* L. colonies in the United Kingdom. Apidologie, 29: 369-370.

Melathopoulos, A.P. and W. J. Farney (2002). The Canadian beekeeper pest management survey ,Hivelights, 15 : 13- 16 .

Ritter, W.(1981). Varroa disease of the honey bee (*Apis mellifera).* Bee World, 62 (4) :141 – 153 .

Rosenkranz, P. ; P. Aumeier and B. Ziegelmann (2010). Biology and control of *Varroa destructor*. Journal of Invertebrate Pathology. 103 : S96-S119.

Sammataro, D. ; H.G. Degrandi ; G. Needham and G. Warell (1998) .Some volatile oils potential control agents for varroa mites (Acari : Varroidae) in honey bee colonies (Hymenoptera Apidae) Am. Bee .J., 138 (9) : 681 – 685 .

Snedecor, G.W. and W.G. Cochran (1989). Statistical Methods, 8 th Ed . Iowa State Univ. Ames, Iowa, U.S.A.

Spreafico, M. ; F.R. Eordegh ; I. Bernardinelli and M. Colombo (2001) . First detection of strains of *Varroa destructor* resistant to camphos. Results of laboratory tests and field trials. Apidologie , 32 : 235 – 248 .

Wallner, K .(1999). Varroacides and their residues in bee products . Apidologie , 30 : 235 – 248 .

إستخدام بعض المواد الحيوية فى مكافحة حلم الفاروا الذى يصيب طوائف نحل العسل

كريمة عدنان السعيد[2] ، رضا عليوة سند[1] ، حمزة السيد الشرقاوى[2] ونبيل عبدالله عمر[2]

[1]معهد بحوث وقاية النبات – مركز البحوث الزراعية – الجيزة – الدقى - مصر

[2]قسم الانتاج النباتى – كلية التكنولوجيا والتنمية – جامعة الزقازيق – مصر

أجرى هذا البحث فى المعامل البحثية والمنحل البحثى التابع لقسم الانتاج النباتى بكلية التكنولوجيا والتنمية – جامعة الزقازيق وكان الهدف هو تقييم إستخدام ستة زيوت طبيعية نباتية (القرفة – القرنفل - الياسمين - الكافور - النعناع) وحمضى الاوكساليك والفورميك و الداى ستوب لدراسة تاثيرهم على اكاروس الفاروا الذى يصيب طوائف النحل الكرنيولى . تم تطبيق المواد المختبرة على الطوائف المصابة وعد الاكاروس قبل المعاملة وبعد 1 ، 2 ، 3 ، 4 ، 5 أسبوع من بدء المعاملة وتم حساب نسبة الموت بإستخدام معادلة هاندرسون وتيليتون . أظهرت النتائج إرتفاع النسبة المئوية لحلم الفاروا المتساقط عند المكافحة بالزيوت الأساسية والمواد المختبرة حيث زاد معدل التساقط خلال فترة التجربة (5 أسبوع) في حالة إستخدام زيت القرفة والداى ستوب وزيت النعناع بمتوسط 78، 77 ، 76 ٪ على التوالي ، بينما كانت أقل النتائج عند إستخدام حمض الاوكساليك بمتوسط 61 ٪ . تناقصت النسبة المئوية للحضنة المصابة بدرجات متفاوتة حيث أعطى الداي ستوب أعلى نسبة 38 ٪ ، يليها زيت النعناع وزيت القرنفل بنسب 36 ٪ ،34٪ على التوالي ، وكانت اقل النتائج مع زيت القرفة 25 ٪ ، الياسمين 26٪. إنخفضت نسبة الاصابة بحلم الفاروا الذي يصيب الشغالات البالغة الى 41 ٪ مع حمض الاوكساليك ، كما بلغت النسبة 37 ٪ ، 36 ٪ لكل من حمض الفورميك وزيت القرنفل. أدى إستخدام المواد المختبرة الى زيادة في مساحة الحضنة فى معاملات داي ستوب وحمض الاوكساليك بنسبة 28 ، 26٪ على التوالي بينما تراوحت النسبة بين 21 – 23 ٪ فى باقى المعاملات . زاد إنتاج العسل في الطوائف المعالجة بزيت القرنفل وزيت القرفة بنسبة 171.4٪ ، 157.1٪ على التوالي بينما كانت أقل زيادة عند إستخدام الداي ستوب بنسبة 66.6٪ . أظهرالتحليل الإحصائي فروقا معنوية للصفات تحت الدراسة بين كل المعاملات تقريبا بإستثناء معاملات زيت القرنفل وزيت القرفة لم تظهر أي فروق معنوية.