SOME PHYSICAL PROPERTIES OF ESSENTIAL OIL OF BARAKASEED *NIGELLA SATIVA* L. IMPACTED BY BAT GUANO *Otonycteris hemprichii* Camd AND SEAWEED EXTRACT

A. N. Al-mohammedi — Assist. Prof. — Field crops Dept. Coll. Agric. Uni. Tekrit,
A. F.Al-mehemdi — Assist. Prof. — Disst. Stud. University Al-anbar
O. H.Al-mehemdi — Assist. Prof. — Hort., Dept. Coll. Agric. Univ. of Anbar

ABSTRACT

Some fertilizers practices could be used to improve Baraka seed *Nigella sativa* L. as a medicinal crop like organic farmyard manure which efficiently applied to enhance growth and yield. Thus, a field experiment was conducted out at Research Station in Tikrit-Iraq during 2012/2013 season to estimate the impact of bat guano (0, 75, 125 and 175 kg.ha^{-1}), seaweed extract as kelpak (0, 1, 2 and 3ml.l^{-1}) on some physical and chemical essential oil components. Two factors were arranged in factorial experiment in randomized complete block design with three replicates. Thus, results revealed that application of bat guano at 125 kg.ha^{-1} was superior in enhancement of essential oil and its some physical and chemical properties as essential oil% of 1.53%, specific gravity of 0.95 g.cm^{-3}, refractive index of 1.82, essential oil density of 0.93, dithymohydroquinone of 37.44µg.µl^{-1} and thymol of 25.73 µg.µl^{-1}. Moreover, kelpak (3ml.l^{-1}) significantly improved physical and chemical properties of essential oil which were essential oil% of 1.52%, specific gravity of 0.93 g.cm^{-3}, refractive index of 1.72, and essential oil density of 0.94 and thymol of 25.92 µg.µl^{-1}. Whereas, the main component dithymohydroquinone effected by application of 2 ml.l^{-1}kelpak of 37.04 µg.µl^{-1}. It could be recommended to use bat guano as alternative to chemical fertilizer fortified with foliar application of seaweed extract as kelpak so as to improve physical and chemical properties of essential oil.

Keywords: bat guano, seaweed, kelpak, essential oil, Nigella sativa.

بعض الصفات الفيزيائية للزيت الاساسي لحبة البركة .*Nigella sativa* L بتاثير درق خفاش *Otonycteris hemprichii* Camd ومستخلص الاعشاب البحرية

عقيل نجم عبود المحمدي — أستاذ مساعد — قسم المحاصيل الحقلية –كلية الزراعة– جامعة تكريت
علي فدعم عبدالله المحمدي — أستاذ مساعد — مركز دراسات الصحراء. جامعة الانبار
عمر هاشم مصلح المحمدي — أستاذ مساعد — قسم البستنة وهندسة الحدائق. كلية الزراعة. جامعة الانبار

المستخلص

قد تستخدم بعض العمليات الزراعية لتحسين اداء حبة البركة كمحصول طبي لاسيما التسميد العضوي كسماد المزرعة. اذ ان اضافته كفوءة في تحسين النمو وزيادة الحاصل. بذا نفذت تجربة حقلية في الموسم الشتوي (2012\2013) لمعرفة تاثير درق الخفاش بمستويات 0 و 75 و 125 و 175 كغم.هـ$^{-1}$ ومستخلص الاعشاب البحرية (Kelpak) بمستويات 0 و 1 و 2 و 3 مل.لتر$^{-1}$ في بعض الصفات الفيزيائية للزيت الاساسي ومكوناته الكيميائية في محطة الابحاث التابعة لجامعة تكريت العراق. اذ وزعت مستويات العاملين بترتيب التجارب العاملية وفق تصميم القطاعات الكاملة المعشاة بثلاثة مكررات. لقد اظهرت النتائج ان اضافة درق الخفاش بمقدار 125 كغم.هـ$^{-1}$ قد تفوقت معنويا في زيادة الزيت الطيار وتحسين صفاته الفيزيائية وبعض مكوناته الكيميائية بنسبة زيت اساسي 1.53% ووزن نوعي 0.95 غم.سم$^{-3}$ ومعامل انكسار 1.82° وكثافة الزيت الطيار 0.93 غم ومركب Dithymohydroquinone 37.44 مايكروغم.مايكرولتر$^{-1}$ ومركب thymol 25.73 مايكروغم.مايكرولتر$^{-1}$. حسن محلول kelpak معنويا بعض الصفات الفيزيائية و المكونات الكيميائية للزيت الطيار. اذ كانت نسبة الزيت الطيار 1.52% والوزن النوعي 0.93 غم.سم$^{-3}$ ومعامل انكسار 1.72° وكثافة الزيت الطيار 0.94 غم ومركب thymol 25.92 مايكروغم.مايكرولتر$^{-1}$. بيد ان المركب الرئيس dithymohydroquinone قد تاثر باضافة 2 مل.لتر$^{-1}$ من محلول kelpak بتركيز 37.04 مايكروغم.مايكرولتر$^{-1}$. يمكن الاستنتاج ان درق الخفاش ومحلول kelpak قد حسنا من بعض الصفات الفيزيائية للزيت الطيار ومكوناته الكيميائية. لذا يمكن الايصاء باستخدام درق الخفاش كبديل للسماد الكيميائي مدعما برش مستخلص الاعشاب البحرية كمحلول kelpak لتحسين بعض خواص الزيت الطيارالفيزيائية والكيميائية.

كلمات مفتاحية: درق الخفاش، اعشاب بحرية، محلول kelpak، الزيت الطيار ، حبة البركة.

INTRODUCTION

Barakaseed *Nigella sativa* L. (Black Cumin) is belonging to ranunuclaceae. This plant is represented as a small annual spice herb which considered as one of most important medicinal plant that widely sowed in Islamic countries because of being it classically used in treatment of various diseases as asthma, flatulence, polio, kidney stones, abdominal disorders (9,15), diarrhea, jaundice, helminthiasis and paralysis (5,7). Moreover, this herb was tested as antioxidant (7, 22), antibacterial (2), analgesic and anti-inflammatory drug (12).Barakaseed contains secondary compounds that caused prior curing properties. Where secondary compounds are such as essential oil (2,7,11), sterol (8), glycolipids (16, 17, 18) and diterpene alkaloids (15) and triterpene saponin (13). Therefore, recently many researchers focused on using agriculture pattern without chemicals to produce high quality and safety of medicinal and aromatic plant (namely organic farming) which organic manures from animals as sheep, cattle, camel, horses, poultry and bats are used to prepare liquid or solid fertilizer significantly meet nutrients requirements for various crops (10). In relation to bat guano, it could be applied as better alternative organic fertilizer compared to other organic fertilizers (14), who pointed that grain yield and biomass of maize significantly increased by application of bat guano and chicken manure. Thus, bat guano could be had nutritional contents that were similar to mineral fertilizers exploited in greenhouse cultivation (11). Similarly, Bhat et al. (6) showed that organic fertilizers which applied on soil were significantly effective such as desert bat guano and earth juice products. Some determinants retarded the availability of bat guano manures such as cave location of lived bat, quantity of guano, bat species and age of guano. However, immature information on bat guano for producing crops (21) who analyzed guano of bat *Hipposideros speoris* and so indicated that feces was contained high organic matter, carbon, nitrogen and phosphate. Consequently, the fortification of soil with guano at 20:1 gave highest shoot length, total dry matter, nitrogen content and nitrogen uptake in finger millet and black gram. From another study, Shetty et al., (19) concluded that the difference of location affected on nutrients content in guano of *Megaderma lyra* which Varanga guano was possessed highly nitrogen content than Yennehole guano. However, Yennehole guano was the best to improve plant growth of *Vigna radiate* L. amended the soil with small quantity of bat guano could induce plant growth and elevate crop production. Almohammedi et al. (3) showed that growth and yield of *Nigella sativa* L. were significantly increased using 125-175 kg bat guano and 3 ml kelpak per liter. In the course of this our farm observations, this study was conducted to investigate the effect of bat guano and kelpak (seaweed extract) on essential oil and its physical and chemical properties of barakaseed under Iraqi conditions.

MATERIAL AND METHODS

Experimental field: Field study was applied during 2013 season at Experiment Station College of agriculture / University of Tikrit on gypsiferous soil. Rodivator was used to plough and prepare field to be sown. To estimate some chemical and physical characteristics, Soil samples were taken before tillage, which are shown in Table (1).

Table 1.Some chemical and physical properties of the soil (Tikrit Univ.).

Soil properties	Value
Texture	Sandy clay loam
Soil separations	
Sand%	60
Clay%	20
Silt%	20
O.M.%	1.5
Gypsum $g.kg^{-1}$	7.5
pH	7.55
EC dsm^{-1}	3.31
Porosity%	56
Calcite $g.kg^{-1}$	4.06
CEC $cmol.kg^{-1}$	5.5

Factorial experiment using RCBD with three replicates was applied to arrange the experimental treatments to study two factors: one is bat guano with 0, 75, 125 and 175 $kg.ha^{-1}$ and other one is seaweed extract (kelpak, KPK) with 0, 1, 2 and 3 $ml.l^{-1}$ those were applied in experimental unit area of 12 m^{-2}. The geometrical dimensions of each two plants and each two rows were 20 x 50 cm. the separator limit between each two units was one misused meter. Consequently, each treatment unit was fortified with urea of 80 $kg.ha^{-1}$, trisuper phosphate of 60 $kg.ha^{-1}$ and potassium sulphate of 80 $kg.ha^{-1}$ which the last two fertilizers were added before sowing. While, half dose of urea was incorporated in soil and the second half dose applied 45 day after sowing. Where, The seeds were sown in 20/ 11/ 2013. Kelpak was sprayed on foliage using hand sprayer which above-ground parts were completely wetted. The kelpak solution was partitioned in twice; the first partition was sprayed 4 weeks after germination. Whereas, the second one was applied at flowering. Surfactant material was mixed into extract solution of 0.15 $cm^3.l^{-1}$, whereas the spraying quantity was 100 $L.ha^{-1}$(1). Irrigation and weed control was done as it is necessary. Bat Guano *Otonycteris hemprichii* Camd was worked out from cave which located in Albaghdady town (city belongs to Anbar province) 250 km North West of Baghdad. Where, cave is located near to Euphrates river bank which bat was lived there. Table 2 represented the chemical analysis of bat guano.

Table 2. Some chemical properties of bat guano extract analyzed in soil analysis lab (Center of Desert Studies, Anbar Un)

Bat guano property	Value
Ca (ppm)	**0.3**
Cl (ppm)	**0.4**
HCO3 (ppm)	**0.45**
CO3 (ppm)	**Null**
K (ppm)	**23.208**
Mg (ppm)	**1.5**
Na (ppm)	**33.486**
SO4 (ppm)	**741.86**
EC($\mu S.L^{-1}$)	**133.7**
pH	**7.14**

The data parameters that were recorded are on essential oil%. Essential oil was extracted as following .Ground seeds of black cumin (200g) were placed in round bottom flask (2L),water was added (1:6 v/v) and heated in Hydro Distillation Apparatus for 4 hours at 100°C. The essential oil collected on water surface was decanted. The moisture content from the oil was removed with anhydrous sodium sulfate. Yield of essential oil was determined and stored at 4°C for further use.

Specific gravity:

Specific gravity of oil sample was determined at 25◦C with specific gravity bottle (Pycnometer; Sigma-Aldrich) with capillary bored stopper (4).

Refractive index:

The refractive index of the oil samples was recorded using Abbe’s Refractometer following the protocol No. Cc 7-25 as described in AOCS (4) and essential oil density.

Determination of essential oil components

Essential oil was subjected to GC–MS analysis using a Gas Chromatograph (Model: 14-A, Shimadzu, Japan) attached with quadruple Mass Spectrometer (Model HP 5973) with a capillary column of HP-5MS (5% phenylmethylsiloxane, length = 30m, inner diameter = 0.25mm and film thickness = 0.25µm). The injector, GC–MS interface, ion source and mass selective detector temperatures were maintained at 280, 280, 230 and 150°C, respectively. The oven temperature was programmed linearly as follows: from 60 to 185°C at 1.5◦C/min, held for 1min, then from 185 to 275°C at 9°C/min and held for 2min. Components were identified on the basis of comparisons of their retention indices and mass spectra. Computer matching was done with the Wiley 275 and National Institute of Standards Technology (NIST 3.0) libraries provided with the computer controlling the GC–MS System (Singh *et al*., 2005).

These data were analyzed using GENSTAT 10.3. Means were compared according to LSD ($P< 0.05$).

RESULTS AND DISCUSSION

Essential oil %(v/w):

Table 3 refers to results of this chemical parameter of barakaseed fruits that was affected by different batguano and kelpak levels. thus, results in the same table indicated that bat guano application was significantly efficient to increase seed essential oil thereby application bat guano of (125 $Kg.ha^{-1}$), where was superior in increase essential oil % of barakaseed of 1.53% followed by bat guano (175 $Kg.ha^{-1}$) of 1.48%, and the level 75 Kg $bg.ha^{-1}$ of 1.34%, control was the last one of 1.27%. It seems that the applied bat guano treatments possessed potential to improve barakaseed plant growth characteristics which stimulated effect is resulted from nutrients of guano of bat (3, 22, 26), in general, each factor that influenced on the photosynthesis could effect on the production of essential oil. These affects could be due to the nutrient content of the organic matter as well as to the improvement of soil physical properties. Effect trend of Kelpak application is similar to bat guano on essential oil% of barakaseed which level 3ml.l^{-1} possesses maximum increment in this chemical property was of 1.52% consequenced by level 2ml.l^{-1} of 1.45% and level 1ml.l^{-1} of 1.34% ended by control of 1.3%. Thus kelpak 3ml.l^{-1} application had the superior effect. Consequently, the improved effect is in relative mainly to the capability of the added kelpak to increase photosynthesis products via increase of nutrients absorption by raising root growth due to kelpak content of plant growth regulators and the polyamines (3), led to induce the physiological growth components (3), and from this study essential oil. Essential oil percentages are significantly changed with various combinations of interaction of bat guano with kelpak levels. The interactive combination of bat guano (125Kg. h^{-1}) with kelpak (3mml.l^{-1}) gave the highest essential oil% in barakaseed of 1.8%. While the control (0X0) had the lowest one of 1.25%.

Table3. Effect of bat guano and seaweed extract on essential oil% (v/w) of barakaseed

Bat guano Kg.ha^{-1}	Seaweed extract(kelpak, ml.l^{-1})				Means
	0	1	2	3	
0	1.25	1.27	1.28	1.27	1.27
75	1.28	1.32	1.36	1.39	1.34
125	1.34	1.41	1.58	1.80	1.53
175	1.31	1.38	1.58	1.63	1.48
LSD 0.05	0.05				0.03
Means	1.30	1.34	1.45	1.52	
LSD 0.05	0.03				

Specific gravity

Results presented in Table 4 showed that specific gravity (g.cm^{-3}) significantly increased due to increase bat guano with increase of levels in comparison to the control. Application of 125 Kg.ha^{-1} Bat guano gave significantly the highest increase of specific gravity of 0.95 followed by level 175 Kg BG.ha^{-1}of 0.94 and level 75 Kg BG.ha^{-1}of 0.88 terminated by control of 0.83. These improved effects could be occurred due to bat guano had ability to trigger Baraka seed growth via production of photosynthetic metabolites (3) and enhancement of nutrients absorption. Thus, in turn, it results in increase essential oil components as specific gravity. This particular property associated with other components of essential oil

Table4. Effect of bat guano and seaweed extract on specific gravity (g.cm^{-3}) of barakaseed

Bat guano Kg.ha^{-1}	Seaweed extract(kelpak, ml.l^{-1})				means
	0	1	2	3	
0	0.81	0.83	0.83	0.84	0.83
75	0.83	0.89	0.89	0.90	0.88
125	0.87	0.93	0.98	1.00	0.95
175	0.89	0.91	0.97	0.98	0.94
LSD 0.05	0.007				0.003
Means	0.85	0.90	0.92	0.93	
LSD 0.05	1.03				

It observed from same Table 4, kelpak application had significant enhancement in the specific gravity. Thus, application of kelpak (3ml.l^{-1}) produced the highest specific gravity of 0.93 consequenced by kelpak (2ml.l^{-1}) of 0.92 and kelpak (1ml.l^{-1}) of 0.90, control gave the lowest of 0.85. That meant that kalpak had similar as trend as bat guano. Therefore, kelpak could increase carbohydrates production resulted in improvement of yield components (3). Kelpak could be as distributer and transporter of nutritional chemicals from biosynthesis source to active zones (sink). Where, kelpak contains some plant growth regulators and polyamines that caused synergistic effects on overall plant growth because of containing cytokinins, auxins, putrescine and spermine (1,3). Finally, it improves essential oil properties. Furthermore, Table 4 contains the interactive combinations of bat guano and kelpak levels which showed results that these combinations significantly influenced on specific gravity. Where, the interactive combination of bat guano (125 Kg.h^{-1}) with kelpak (3ml.l^{-1}) gave the highest specific gravity of 1 as compared to control which caused the lowest one of 0.81.

Refractive index

Refractive index is the function of essential oil upon the light. Table 5 reflected various bat guano levels which significantly effected on refractive indices. Thus, the application of 125 Kg BG.ha^{-1} achieved highest degree of refractive index was of 1.82, followed by 175 Kg BG.ha^{-1} of 1.77 and 75 Kg BG.ha^{-1} of 1.54. It also was seen that the control possessed the lowest one of 1.23. These significant differences could physiologically be to improve the physiological components (3) as biochemicals by bat guano treatments which these components could be represented as source of fortified nutrients to biosynthesize essential oil that resulted in physical and chemical components of essential oil.

Table5. Effect of bat guano and seaweed extract on refractive index of barakaseed

Bat guano Kg.ha^{-1}	Seaweed extract(kelpak, ml.l^{-1})				Means
	0	1	2	3	
0	1.22	1.23	1.23	1.24	1.23
75	1.24	1.57	1.58	1.77	1.54
125	1.49	1.89	1.90	1.98	1.82
175	1.49	1.81	1.89	1.89	1.77
LSD 0.05	0.013				0.006
Means	1.36	1.63	1.65	1.72	
LSD 0.05	0.006				

Refractive indices were significantly varied with the increase change in spraying level of kelpak (table 5) similar pattern of effect on refractive index as effect as bat guano levels. Thus, it was showed that the foliar application of 3ml KPK.l^{-1}barakaseed plants possessed the highest degree of refractive index of 1.72and then kelpak (2ml.l^{-1}) gave the second value of this physical property of essential oil of 1.65 and kelpak (1ml.l^{-1}) of 1.63. The lowest value 1.36 was caused by control. The enhancement of increased levels of kelpak could result from improving the photosynthesis (3) thereby biochemicals that transferred to action zones such as seeds. Thus essential oil constructed. Like these active effects of kelpak could be interpreted its content of polyamines. Such that amines are synergistically integrating with other components of this seaweed extract as auxin (1,3). These physiological and biochemical processes resulted in improvement of physical and chemical components of essential oil.

Results in the same table showed that the interactive combination of bat guano (125 Kg. h^{-1}) with kelpak (3 ml. l^{-1}) significantly increased the refractive index of 1.98 that was consequenced by interactive combination of bat guano (125 Kg. h^{-1}) with kelpak (2 ml. l^{-1}) of 1.9, as compared to the interaction combination of control, This interaction of bat guano with kelpak application could synergistically enhance some physiological components (3) and then in turn to essential oil and its components via some biochemicals in kelpak associated with nutritional chemicals of bat guano that released into rhizosphere solution. Consequently, they are taken in roots to transfer into action zones. Spraying of seaweed (kelpak) fortified plants that underwent from deficit of nutrients such P and N, because it enhances uptaken these minerals that released from decomposed bat guano in soil solution. Finally, physical and chemical properties of essential were improved.

Essential oil density

Results that tabulated in Table 6, referred to significant impacts of applied bat guano levels. Where, applied 125 kg.ha^{-1} of bat guano had the highest mean of essential oil density of 0.94. Similarly, the application 175 kg.BG.ha^{-1} achieved same mean of 0.94 then by 75 kg.BG.ha^{-1} of 0.88 and control achieved lowest one of 0.83.

Table6. Effect of bat guano and seaweed extract on essential oil density of barakaseed

Bat guano $Kg.ha^{-1}$	Seaweed extract(kelpak, $ml.l^{-1}$)				Means
	0	1	2	3	
0	0.82	0.83	0.84	0.84	0.83
75	0.83	0.89	0.90	0.90	0.88
125	0.89	0.94	0.96	0.99	0.94
175	0.89	0.90	0.98	0.99	0.94
LSD 0.05	0.03				0.01
Means	0.86	0.89	0.92	0.93	
LSD 0.05	0.01				

From the same above table, the application of kelpak on foliage of barakaseed had clear significance. So, the application the foliage with $3ml.l^{-1}$ produced the highest density of essential oil of 0.93 sequenced by kelpak ($2ml.l^{-1}$) of 0.92 and kelpak ($1ml.l^{-1}$) of 0.89. Whereas, the control treatment gave the smallest one of 0.86.

In respect of the same table, its tabulated results showed significant differences among interactive combinations of the two factors levels which Interaction of bat guano (125 $Kg.h^{-1}$) with kelpak ($3ml.l^{-1}$) achieved the highest essential oil density of 0.99. Likely, the interactive combination of bat guano (175 kg. $BG.ha^{-1}$) with kelpak ($3mml.l^{-1}$) had 0.99 as compared to combination of control (0X0) which gave the lowest average of 0.82. Thus, bat guano could have synergistic action with application of kelpak. Where soil supplemented with bat guano would fortified with nutrients such that chemicals could be absorbed via developed roots thereby application of kelpak which this organic contains some growth regulators that improved root development and plant growth and finally increased the taking in insoluble nutrients in the rhizosphere zone (3, 19).

Essential oil components

Six constituents identified by GC–MS analysis are histogrammly presented in Fig.1 and Fig.2. The oil was dominated by dithymohydroquinone and thymol which they represented the two major components of essential oil of black cumin seed. Those two constituents were increased as applied bat guano was increased. Thus, bat guano (125kg.BG ha^{-1}) gave the highest amount of two constituents of 37.44 and $26.44µg.µl^{-1}$, respectively. Whereas, the lowest constituents effected by bat guano were thymoquinone and τ-anethole of 20.03 and 19.56 $µg.µl^{-1}$, respectively (Fig.1).

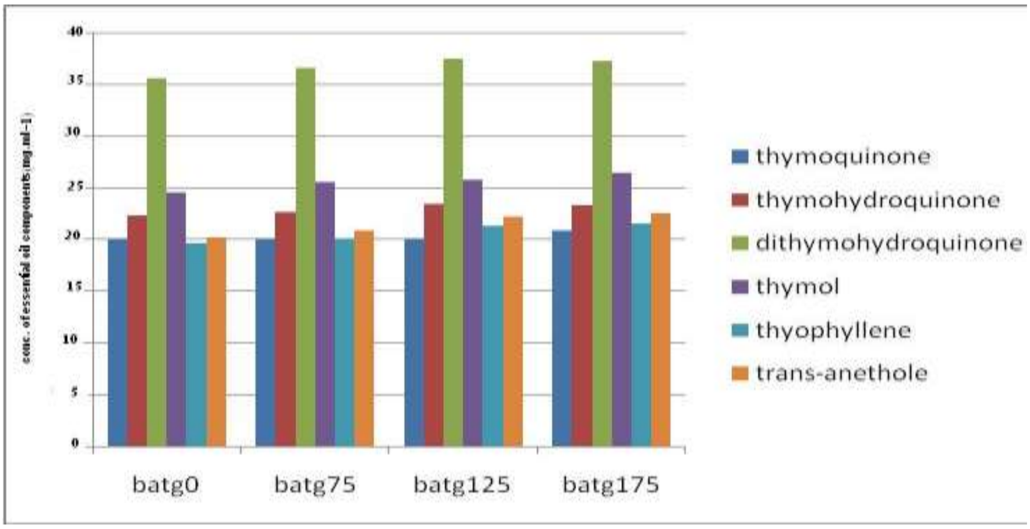

Fig.1. Essential oil content as effected by bat guano application

Barakaseed plants sprayed with $2ml.l^{-1}$ resulted in the highest quantities of essential oil constituents arranaged as dithymohydroquinone, thymol, thymohydroquinone, thyophyllene and thymoquinone of 37.04, 25.75, 23.13, 20.92 and 20.59 $µg.µl^{-1}$, respectively. Whereas, t-anethole had biggest amount of 22.20 $µg.µl^{-1}$ as kelpak was applied with 3 $ml.l^{-1}$ (Fig.2). Finally, Bat guano and seaweed extract as kelpak were applied to achieve this vital priority. Thus, essential oil enhancement was effectively triggered by above organic fertilizers. Physical and chemical properties were correlated together. Thus, it could be resulted in improvement of essential oil. On-going investigations are essential to formulate mixtures from these manures and assess over field environments trials to achieve satisfactory and aspired results.

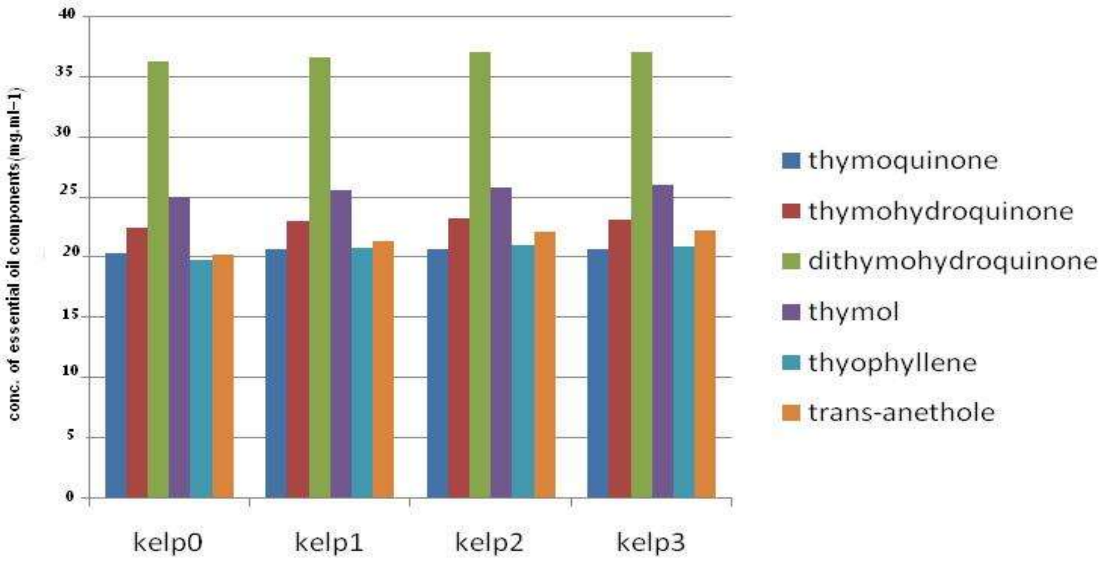

Fig.2. Essential oil content as effected by kelpak application

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