
Chemical composition and antifeedant activity of essential oils from *Eucalyptus camaldulensis* and *Callistemon viminalis* on *Tribolium confusum*

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Fatemeh Hamzavi and Saied Moharramipour (2017). Chemical composition and antifeedant activity of essential oils from *Eucalyptus camaldulensis* and *Callistemon viminalis* on *Tribolium confusum*. International Journal of Agriculture Technology 13(3): 413-424.

Abstract In order to assess the antifeedant activity of *Eucalyptus camaldulensis* (Dehnh) and *Callistemon viminalis* Gaertn the plant essential oil was extracted by hydrodistillation and then tested against the confused flour beetle, *Tribolium confusum* Jacquelin du Val. Several experiments were designed to measure the nutritional indices such as relative growth rate (RGR), relative consumption rate (RCR), efficiency of conversion of ingested food (ECI) and feeding deterrence index (FDI). Treatments experiments were evaluated by the method of flour disc bioassay in the dark, at 27±1 °C and 60±5 % R.H. Aliquots of 10 µl of acetone (controls) or an acetone solutions of essential oils (750 - 2500 ppm) were spread equally on the flour discs. The solvent was evaporated and after this stage 10 adult (1-3 days old) insects were involved into each treatment. After 72 h, nutritional indices were calculated. Results showed that essential oils concentrations had significant effect on nutritional indices. Essential oils of *C. viminalis* and *E. camaldulensis* decreased RGR, RCR and ECI activity significantly. Essential oils of *C. viminalis* and *E. camaldulensis* decreased RGR from the amount of 0.0347 and 0.0344 in control to 0.0067 and 0.0031 percentage respectively. Furthermore the amount of RCR decreased from 0.161 and 0.149 to 0.073 and 0.074 percentage in control treatment. ECI of control decreased from 21.62% to 9.34% and 23.10% to 4.12% in 2500 ppm concentration in comparison to control respectively. Moreover, essential oils of *C. viminalis* and *E. camaldulensis* increased FDI from 1.100 to 51.37 percent and 26.03 to 50.24 percent in highest concentration respectively. on the other hand,, GC/MS analysis of the oils showed that p-cymen (18.86%) and alpha-pinene (16.56%), alloaromadendrene (12.26%) and 1,8-cineole (11.79%) in *E. camaldulensis* oil, 1,8-cineole (41.26%), alpha-pinene (15.01%),Limonene (10.45%), and alpha-terpineol (10.30%) in *C. viminalis*. overallly, it was concluded that antifeedant activity is highly affected by feeding deterreny against *T. confusum*.

Keywords: *Eucalyptus camaldulensis*, *Callistemon viminalis*, *Tribolium confusum*, relative growth rate, relative consumption rate, efficiency of conversion of ingested food, feeding deterrence index.

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Introduction

Tribolium confusum Jacquelin du Val is one of the most important and destructive primary insect pests of stored cereals. *T. confusum* has been treated as one of the main targets in the control of stored product insect pests (Liu and Ho 1999). Post-harvest losses of food grain due to insect pests are a significant nutritional and economic burden to subsistence farmers in developing countries (Firdisa and Abraham 1999). Essential oils, naturally occurring substances, generally have a broad spectrum of bioactivity because of the presence of several active ingredients that work through several modes of action. The toxicity of individual oils or compounds often exerts differential effects depending on both the mode of action and the target pest. moreover, these materials are most considered because of fewer health hazards on both human and non-target organisms such as pollinators and natural enemies (Isman, 2000). Essential oils and their constituents have also been proven to be a potent source of botanical pesticides (Singh and Upadhyay 1993). An alternative method for control of insect pests of stored commodities is the use of plant oils as fumigants (Tayoub *et al.*, 2012). Taghizadeh and Nouri asses and compare nutritional indices and insecticidal activity of essential oils extracted from six medicinal plants against the fourth instar larvae and adults of Colorado potato beetle. Their results showed that RGR index was significantly reduced with increased concentrations of all essential oils. on the other hand, ECI was significantly reduced with increasing concentrations in all essential oils in the fourth instar larvae. Essential oils are selective to pests which have no or little harmful action against non-target organisms and the environment and act in many ways on various types of pest complexes and may be applied to the plant in the same way as conventional insecticides (Regnault-Roger 1997). Essential oils extracted from plants contain compounds that show ovicidal, repellent, antifeedant, sterilization and toxic effects on insects (Nawrot and Harmatha 1994., Isman 2006). Plants have developed for 400 million years and have acquired effective defense mechanisms that ensure survival under rough environmental conditions and in the presence of natural enemies. Recent chemical ecology studies have shown that many of these secondary compounds play an important role in plant-insect relations. Some compounds, either separately or synergically, makes up a chemical defense barrier in the plant against certain pests and diseases (Prakash and Rao 1997).

Objectives: The present study investigated the effect of nutritional indices activity of essential oils from *E. camaldulensis* and *C. virninalis* on adults *T. confusum*.

Materials and methods

Insect rearing

Initial population of *T. confusum* introduced on whole wheat and cultures were maintained in the dark in growth chamber set at $27 \pm 1^\circ\text{C}$ and $65 \pm 5\%$ r.h. After three generations of rearing insects, all of adult insects were removed out; then emerged insects during 1-3 days were used for antifeedant test.

Plant materials

Aerial parts of *E. camaldulensis* and *C. viminalis* were collected at full flowering stage in September 2009 from Sistan and Namak abroad provinc, respectively in Iran. Plants were identified by the Research Institute of Forests and Rangelands, Tehran, Iran. The plant materials were dried naturally on laboratory benches at room temperature with suitable ventilation in the dark conditions until crisp. The dried material was stored in paper pockets until needed.

Extraction of essential oils

Plant materials were milled into fine powder using a milling machine. Fifty grams of the plant samples as well as 600 ml distilled water was put in a round bottom flask. Plant materials were subjected to hydrodistillation for 4 h using a modified Clevenger-type apparatus. The water of essential oils was removed using anhydrous sodium sulfate and the oils were stored in glass tubes at 4°C in refrigerator, until they were used.

Nutritional indices assay

This experiment for adults nutritional indices were calculated methods and formula and prepared of flour disks according to Huang *et al.* (2000). Aliquots of 200 ml of a suspension of flour wheat (without bran) in water (10 g flour in 50 ml water) were dropped onto a Petri dish to form the disks. The disks were left in the fume hood overnight to dry, after which they were equilibrated at $27 \pm 1^\circ\text{C}$ and $60 \pm 5\%$ r.h. for 12 h. Flour disks were treated with acetone solutions (10 ml) containing various concentrations (750, 1000,, 1500, 2000, 2500 ppm) of *E. camaldulensis* and *C. viminalis* oil, and acetone alone for the controls. After evaporation of the solvent, the disks were placed in glass vials (diameter 2.5 cm, high 5.5 cm). Ten groups weighed, unsexed adults

were added to each pre weighed vial containing the disks. For each concentration and control, two disks were given to the insects. Five replicates were prepared. After 72 h, the glass vials with disks and live insects were weighed again, and mortality of insects, if any, was recorded. Nutritional indices were calculated as follows.

Formula:

Relative Growth Rate (RGR): $RGR = (A - B)/(B \times \text{day})$

Where A – weight of live insect after experiment (mg to each insect); B – weight of insect before experiment (mg to each insect)

Relative Consumption Rate (RCR): $RCR = D/(B \times \text{day})$

Where D – dried weight of food consumed by insect (mg)

Efficacy of Conversion of Ingested Food (ECI): $ECI = RGR/RCR \times 100\%$

Feeding Deterrence Index (FDI): $FDI = [(C - T)/C] \times 100\%$

Where C – food consumed in control (mg); T – food consumed in treatment (mg)

Chemical analysis of the oils

Gas chromatography (GC) analysis was performed using a GC-MS model HP-6890 equipped with a capillary column (non-polar) HB-5 (30 m × 250 nm) with MS system HP-5973. Temperature was programmed to change from 60 °C to 220 °C at 6 °C/min. Amounts of 1 µl of per essential oils with Hexane injected. Injector and detector temperature were 250°C. Carrier gas was helium (99.999%) that used as at 1.1 ml/min. The ionization energy was 60 eV.

Results and Discussion

Nutritional indices assay

Results showed that essential oils concentrations had significantly effect on nutritional indices. Essential oils of *C. viminalis* and *E. camaldulensis* decreased RGR, RCR and ECI activity significantly and athwart increased FDI (Figure. 1) which in this research showed a significant difference at all concentrations than the control. Furthermore, the effect of *C. viminalis* and *E. camaldulensis* essential oils on RGR of the adult of *T. confusum* at 750, 1000 and 2500 concentrations were significantly different; RGR index reduced with increased concentrations of two essential oils (Table. 1). Essential oils of *C. viminalis* and *E. camaldulensis* decreased RGR from 0.0347 and 0.0344 % in control to 0.0067 and 0.0031 % respectively. This result showed that 2500 ppm is the best concentration for forcing *T. confusum* to use less food and to have

less growth. At 2500 ppm Concentration, it was found that the essential oil of *E. camaldulensis* had the higher effect than *C. viminalis* on RGR in the adult of *T. confusum*. Also, RCR is significantly reduced with increasing concentrations in two essential oils on the *T. confusum*. Essential oils of *C. viminalis* and *E. camaldulensis* decreased RCR from control 0.161 and 0.149 decreased to 0.073 and 0.074 %. There is no significant different in RCR index (Table. 2) at the Highest concentration (2500 ppm) of the essential oils. on the other hand, effect of *C. viminalis* and *E. camaldulensis* essential oils on ECI of the adult of *T. confusum* at 750, 1000 and 2500 concentrations were significant ($P < 0.05$); ECI index reduced with increased concentrations of two essential oils (Table. 3) and ECI from 21.62% in control treatment decreased to 9.34% and the amount of this index from 23.10% in control treatment decreased to 4.12% in 2500 ppm concentration respectively. The lowest concentration (2500 ppm) of the essential oil of *C. viminalis* ECI (9.34) was more effective than essential oil of *E. camaldulensis* (4.12) ($P < 0.05$). With increasing concentrations of essential oils of *C. viminalis* and *E. camaldulensis* from the amount of 750 ppm to 2500 ppm FDI index in adult of *T. confusum* increased from 1.100, 51.37 % and 26.03, 50.24 %, respectively. FDI index was significant in two essential oils at 750 and 1000 ppm concentrations ($P < 0.05$) (table 4) overall, it was concluded that antifeedant activity is highly affected by feeding deterrence against *T. confusum*. The results of the current study are in line with a study on *Carum copticum* and *Vitex pseudo-negundo* on *Tribolium castaneum* by Sahaf and Moharramipour (2009). In fact, insects exposed to sub lethal doses of insecticides may display a variety of symptoms including reduction in growth rate, life span, pupa weight and adult fecundity and fertility (Ansari, et al., 2000., Bazzoni et al., 1997., Collart and Hink, 1986., Gurusubramanian and Krishma, 1996., Huang et al., 2000). Oils of *C. viminalis* and *E. camaldulensis* was toxic to *T. confusum* (Hamzavi et al., 2015., Negahban and Moharramipour, 2007). Our result showed that Cardamom oil did not affect the insects' growth (RGR), food consumption (RCR) and utilization (ECI) in both adults and larvae of *T. castaneum*. Therefore, little feeding deterrence was observed in *T. castaneum*. However, Cardamom oil significantly ($P < 0.05$) reduced RGR, RCR and ECI in the adults of *S. zeamais* at concentrations of 1.44 - 104 ppm only with slight feeding deterrence (up to 27%) (Huang et al., 2000). Essential oil of *Carum copticum* decreased RGR, RCR and ECI higher than that of *Vitex pseudo-negundo* essential oil. Both of plant essential oils, with the same activity, increased FDI as the oil concentration was increased, showing high feeding deterrence activity against *T. castaneum* (Sahaf and Moharramipour, 2009). Yang et al. (2015) by using eight compounds that were isolated from *Glycosmis lucida* and feeding deterrent activities concluded that

the sulphur-containing amides may have an important role in the feeding deterrent activity of the *G. lucida* extracts against the red flour beetles. Also the samples of four *Zanthoxylum* species antifeedant activities were assessed on the red flour beetle. That's results of their work demonstrate the potent antifeedant activities of six benzophenanthridines isolated from *Z. schinifolium* stem bark against *T. castaneum* adults (Wang *et al.*, 2015).

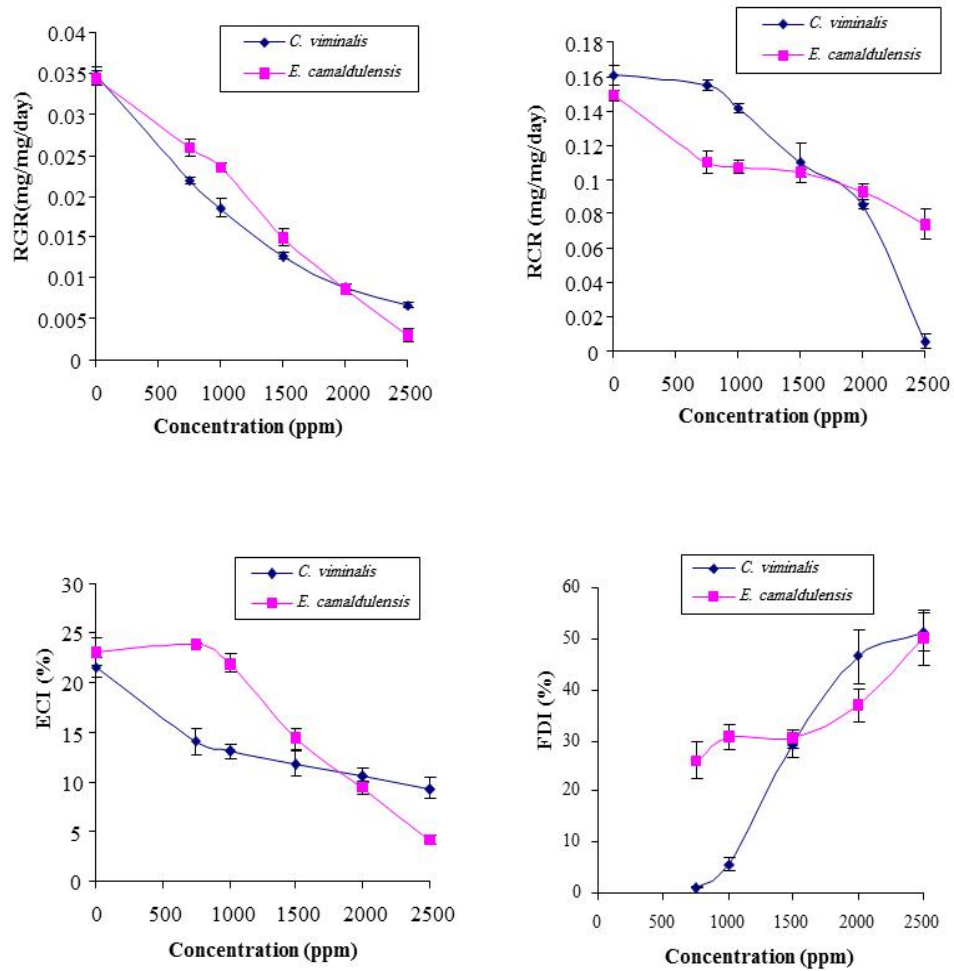


Figure 1. Efficacy of different concentration of essential oils of *Eucalyptus camaldulensis* and *Callistemon viminalis* on nutritional indices of *Tribolium confusum*

Table 1: The effects of essential oils of *Eucalyptus camaldulensis* and *Callistemon viminalis* on relative growth rate (RGR) (mean \pm SE) of the adults of *Tribolium confusum*

Concentrate(ppm)	RGR(%) (Mean \pm 1)		t-student(df) ²	p-value
	<i>E. camaldulensis</i>	<i>C. viminalis</i>		
0	0.0009 ^a \pm 0.0344	0.0011 ^a \pm 0.0347	-0.209(8)	0.840
750	0.0010 ^b \pm 0.260	0.0004 ^b \pm 0.0219	3.66(8)	0.006
1000	0.0006 ^b \pm 0.0235	0.0011 ^c \pm 0.0185	3.78(8)	0.006
1500	0.0011 ^c \pm 0.0149	0.0004 ^d \pm 0.0126	1.88(8)	0.096
2000	0.0006 ^d \pm 0.0088	0.0004 ^e \pm 0.0089	-0.293(8)	0.777
2500	0.0008 ^e \pm 0.0031	0.0002 ^e \pm 0.0067	-4.236(8)	0.003

1-Different letters in the same column indicate significant differences ($P \leq 0.05$) between treatments according to ANOVA and Tukey's Multiple Range Test

2-Means were compared pair wise for each concentration between essential oils of *Eucalyptus camaldulensis* and *Callistemon viminalis* by Student's *t*-test.

Table 2: The effects of essential oils of *Eucalyptus camaldulensis* and *Callistemon viminalis* on relative consumption rate (RCR) (mean \pm SE) of the adults of *Tribolium confusum*

Concentrate(ppm)	RCR(%) (Mean \pm 1)		t-student(df) ²	p-value
	<i>E. camaldulensis</i>	<i>C. viminalis</i>		
0	0.0037 ^a \pm 0.149	0.0056 ^a \pm 0.161	-1.832(8)	0.104
750	0.0065 ^b \pm 0.110	0.0033 ^a \pm 0.155	-6.224(8)	0.0001
1000	0.0036 ^b \pm 0.107	0.0028 ^a \pm 0.142	-7.520(8)	0.0001
1500	0.0024 ^b \pm 0.104	0.0113 ^b \pm 0.110	-0.507(8)	0.626
2000	0.005b ^c \pm 0.093	0.0061 ^c \pm 0.086	-0.97(8)	0.359
2500	0.0091 ^c \pm 0.074	0.0041 ^c \pm 0.073	-0.18(8)	0.860

1-Different letters in the same column indicate significant differences ($P \leq 0.05$) between treatments according to ANOVA and Tukey's Multiple Range Test

2-Means were compared pair wise for each concentration between essential oils of *Eucalyptus camaldulensis* and *Callistemon viminalis* by Student's *t*-test.

Table 3: The effects of essential oils of *Eucalyptus camaldulensis* and *Callistemon viminalis* on efficiency of conversion of ingested food (ECI) (mean \pm SE) of the adults of *Tribolium confusum*

Concentrate(ppm)	ECI(%) (Mean \pm 1)		t-student(df) ²	p-value
	<i>E. camaldulensis</i>	<i>C. viminalis</i>		
0	1.00 ^a \pm 23.10	1.37 ^a \pm 21.62	0.869(8)	0.410
750	1.30 ^a \pm 23.79	0.32 ^b \pm 14.12	3.664(8)	0.006
1000	0.79 ^a \pm 21.89	0.91 ^b \pm 13.04	7.308(8)	0.0001
1500	1.29 ^b \pm 14.35	1.12 ^{bc} \pm 11.85	1.457(8)	0.183
2000	0.76 ^b \pm 9.46	0.67 ^{bc} \pm 10.65	-1.164(8)	0.278
2500	1.12 ^c \pm 4.12	0.46 ^c \pm 9.34	-4.28(8)	0.003

1-Different letters in the same column indicate significant differences ($P \leq 0.05$) between treatments according to ANOVA and Tukey's Multiple Range Test

2-Means were compared pair wise for each concentration between essential oils of *Eucalyptus camaldulensis* and *Callistemon viminalis* by Student's *t*-test.

Table 4: The effects of essential oils of *Eucalyptus camaldulensis* and *Callistemon viminalis* on Feeding Deterrence Index (FDI %) (mean \pm SE) of the adults of *Tribolium confusum*

Concentrate(ppm)	FDI(%) (Mean \pm 1)		t-student(df) ²	p-value
	<i>E. camaldulensis</i>	<i>C. viminalis</i>		
750	3.78 ^b \pm 26.03	0.18 ^d \pm 1.100	6.586(8)	0.0001
1000	2.53 ^b \pm 30.68	1.16 ^d \pm 5.50	9.028(8)	0.0001
1500	1.91 ^b \pm 30.34	2.69 ^c \pm 29.35	0.299(8)	0.773
2000	3.2 ^{ab} \pm 36.89	5.36 ^b \pm 46.60	-1.55(8)	0.159
2500	5.46 ^a \pm 50.24	3.83 ^a \pm 51.37	-0.15(8)	0.881

1-Different letters in the same column indicate significant differences ($P \leq 0.05$) between treatments according to ANOVA and Tukey's Multiple Range Test

2-Means were compared pair wise for each concentration between essential oils of *Eucalyptus camaldulensis* and *Callistemon viminalis* by Student's *t*-test.

Identification of Compounds

It is well known that variation in the composition of essential oils depends on genetics, type and age of leaf source, environment and oil analysis (Lee et al., 2016). The *E. camaldulensis* and *C. viminalis* oils yielded 1.89 and 0.78% w/w, respectively on a dry weight basis. GC and GC-MS analysis resulted in the identification of a total of 17 and 36 constituents in essential oils of *C. viminalis* and *E. camaldulensis* of representing 99.25% and 99.60%, respectively and oils constituent showed that p-cymen (18.86%) and alpha-pinene (16.56%), alloaromadendrene (12.26%) and 1,8-cineole (11.79%) in *E.*

camaldulensis oil, 1,8-cineole (41.26%), alpha-pinene (15.01%), Limonene (10.45%), and alpha-terpineol (10.30%) in *C. viminalis*. The relative concentrations of the volatile components are presented in Table 5. The major constituents in the oil from the *C. viminalis* of northern India of total 42 constituent were 1,8-cineole (61.7%), α pinene (24.2%) and menthyl acetate (5.3%) (Srivastava., 2003). The major constituents in the oil from the *E. camaldulensis* of khoozestan (South East of Iran) were 1,8-cineole (74.7%), α pinene (8.35%) and viridiflorol (7.17%) (Ghasemi et al., 2016). The essential oil of a plant may contain hundreds of different constituents but certain components will be present in larger quantities. For example, 1,8-cineole is predominant in the essential oil of *Eucalyptus* spp., linalool in *Ocimum* spp., limonene in *Citrus* spp (Rajendran and Sriranjini, 2008). *C. viminalis* and *E. camaldulensis* are from myrtaceae family and major constituents in two essential oils were 1,8-cineole. 1,8-cineole was accepted as food flavourings in a number of countries (Ash and Ash, 1995). Finally, there was 7 uniform constituent in *E. camaldulensis* and *C. viminalis* essential oils such as α -pinene (16.56%) and (15.01), β -pinene (3.65%) and (2.33%), p-cymene (18.86%) and (4.10%), 1,8-cineol (11.79%) and (41.26%), γ -terpinene (5.52%) and (1.29%), 4-terpineol (0.72%) and (2.44%), α -terpineol (0.33%) and (10.3%) respectively.

Table 5: Chemical constituents of the essential oils from *Eucalyptus camaldulensis* and *Callistemon viminalis*

Compounds	Retention Index	<i>Eucalyptus camaldulensis</i>	<i>Callistemon viminalis</i>
		%Composition	%Composition
α -pinene	939	16.56	15.01
β -pinene	990	3.68	2.33
p-cymene	1033	18.86	4.1
1,8-cineole	1039	11.79	41.26
γ -terpinene	1074	5.52	1.29
4-terpineol	1179	0.72	2.44
α -terpineol	1207	0.33	10.3
allo-ocimene	1129	1.06	-
Trans-pinocarveol	1141	1.02	-
pinocarvone	1168	0.74	-
2-hexanal	854	6.02	-
o-cymene	1082	2.15	-
carvone	1243	0.33	-
cuminal	1262	0.46	-
thymol	1298	0.68	-

carvacrol	1299	0.46	-
Isolatedene	1373	0.58	-
isolongifolene	1402	0.42	-
caryophyllene	1404	0.62	-
α -guaiene	1442	1.59	-
α -himachalene	1447	0.36	-
α -acoradiene	1463	0.41	-
calerene	1470	0.99	-
alloaromadendrene	1478	12.26	-
germacerene	1480	3.44	-
α -gurjunene	1506	0.84	-
γ -cadinene	1512	1.19	-
β -selinene	1525	2.08	-
epiglobulol	1532	0.29	-
α -amorphene	1546	0.84	-
Ledol	1565	0.21	-
viridiflorol	1590	2.08	-
hinesol	1638	0.51	-
aromadendrene	1654	0.29	-
B-Eudesmol	1694	0.24	-
α -thunjene	934	-	1.12
myrcene	994	-	1.87
α - phellandrene	1005	-	1.84
limonene	1036	-	10.45
linalool	1107	-	1.11
β -caryophyllene	1467	-	1.03
spathulenol	1598	-	1.7
caryophyllene oxide	16 06	-	1.63
2-Amino-3,5-dicyano- 6-(4-methoxyphenoxy)- pyridine	2087	-	1.78
unknown	unknown	0.4	0.75

Conclusion

The results show that essential oils from *E. camaldulensis* and *C. viminalis* may potentially provide novel antifeedant that can effectively control *T. confusum* along with minimum hazards for human. Anyway, further studies will be necessary to determine important role constituent of *E. camaldulensis*

and *C. viminalis* essential oil to confirm the effectiveness of these eco-friendly materials as organic insecticide for the treatments of *Tribolium* (spp).

Acknowledgement

The authors would like to offer particular thanks to Mr. S.M.Mazlounzadeh, responsible for guide and support.

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(Received 16 February 2017, accepted 30 April 2017)