The Response of the Mediterranean Fruit Fly, *Ceratitis capitata* (Wied.) (Diptera:Tephritidae) Males to Trimedlure Diluted with Some Fixed Oils

Sherihan M. Al-amin[1,2*], Moustafa M. El-metwally[1], Nashat A. Ali[1] and Abdel-all A. Abdel-all[2]

[1]Plant Protection Rsearch Institute, Agricultural Research Center, Cairo, Egypt
[2]Faculty of Science, Assuit University, Assuit, Egypt

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Abstract

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) has a high economic importance because of its rapid development and wide host range in the world. The chemical production of synthesized sex pheromone, trimedlure (TML) is more expensive so there are new trends to reduce the cost of TML. In this study, some of the fixed oils (sunflower oil, corn oil, and paraffin oil) were used to dilute TML in two concentrations, 75% and 50% and tested in the field by Jackson trap for one month in two areas (Saheil-saliem and Elbadary) in Assuit Governorate. In the first district (Saheil-saliem), no significant differences were found along the exposure of all treatments under normal field conditions except at TML-C 50% which was the lowest response (relative attractiveness of 11.92%). In the second district (Elbadary), TML-PF 75%, TML-SF 75% and TML-SF 50% showed highly significant differences when compared with other treatments but no significant differences among these three treatments were appeared. Infrared analysis (IR) and GC analysis were achieved on TML before and after dilution. According to IR analysis, it was found that there were no new function groups appeared after dilution with any fixed oils. Besides, no changes in retention time of TML after dilution were detectedin GC analysis.

Keywords: Medfly, MFF, trimedlure, TML, fixed oil, effectiveness, Jackson trap
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1. Introduction

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) is the dangerous fruit pest because the diversity of its hosts and its ability to tolerate cooler climates are better than most other species of tropical fruit flies. Its life cycle is short and has heavy populations. Early detection of incipient infestations of *C. capitata* is essential because it allows both the delimitation of the outbreak and the implantation of control and eradication measures while the pest population is still small.A rapid response not only limits crop damage but also reduces the programmatic costs incurred in the eradication effort [1, 2]. Alternatives were investigated to reduce production costs and volatility of trimedlure (TML) and capilure (CPL) containing TML plus extenders and enriched ginger root oil (EGRO) containing the male attractant α-copaene, have been compared with TML[3].

*Corresponding author. E-mail: tamersherihan@gmail.com

TML was used as sex pheromone to attract males of *C. capitata*. Regarding the latter, TML is now the standard male medfly attractant in USA detection programs and is deployed in solid dispensers (polymeric plugs) containing 2 g of the lure (and no toxicant) that are placed in Jackson traps, which in turn are suspended within the canopy of host trees [4].

TML provides a successful monitoring program by timing pesticide sprays more accurately. As TML is itself fairly expensive to be produced and also has high volatility, thus the objective of this work is to reduce the high price of this synthetic pheromones by diluting pure crude material by 50% with fixed oils. Three fixed oils were used in this experiment; corn oil, sunflower oil, and paraffin oil. TML was diluted with these oils into two concentrations, 75% and 50%. Comparison among different concentrations of TML was investigated under field conditions and detection response of adult's males to these treatments was also recorded.

2. Materials and Methods

2.1 Study area and orchards

The experiment was carried out in Assuit Governorate, Egypt in two locations (with high infestation by *Ceratitis capitata,* Mediterranean fruit fly or medfly or MFF): Saheil-saliem and Elbadary in about two feddans in mandarin orchards.

2.2 Experimentation

Three fixed oils, i.e. sunflower oil, corn oil and paraffin oil, were mixed with crude TML in two concentrations: 75% and 50%. A 2 cm-long wick was saturated with 2 ml of the treatments and hanged in Jackson trap. Jackson traps were prepared to hang on the tree at the height of 1.5-2 m. Three replicates were achieved for each treatment. The replicates were distributed randomly in the field and the distance between two adjacent was about 35 m to avoid interactions among treatment. A survey was done weekly for 5 weeks. A captured fly per trap per day (CTD) was weekly calculated.

2.3 Data analysis

Statistical analysis was done by one-way ANOVA. Two types of chemical analysis were achieved on TML and its dilutions. By using FTIR and GC analyses, a change of basic structure of TML is recorded. The infrared absorption spectrum of TML (before dilution), TML with corn oil (1:1), TML with sunflower oil (1:1) and TML with paraffin oil (1:1) was carried out.

3. Results and Discussion

3.1 The efficacy of TML and its response in the field before and after its dilution

3.1.1 The first district

Data presented in Tables 1 and 2 showed the response of MFF males to different TML concentrations. In Table 1, the result was recorded throughout the period of exposure in the field, by using L. S. D. test at probability 5% significant differences among all treatments in all concentrations. The efficiency of MFF males attracted to traps was observed during each weeks of exposure. When comparing TML as crude substance (98%) with TML diluted with paraffin oil (TML-PF 50% and TML-PF 75%), TML diluted with sunflower oil (TML-SF 50% and TML-SF 75%), and TML diluted with corn oil (TML-C 50% and TML-C 75%) on the first week, there were significance differences among TML 98% and other diluted TML with TML-SF 50% showed the

lowest result (2.35). There were no significant differences among the exposure period on the 2nd week. The response in the 5th week was generally higher than other weeks. On an average, no significant differences were recorded among the exposure of all treatments under normal field conditions, except at TML-C 50% which was significantly lower than other exposures (3.62).

Among all concentrations used throughout the first three weeks of study, no significant differences on the efficiency of MFF males attracted to traps were observed except for 98% concentration in the 1st week, which was significantly higher (7.74) than other tested concentrations. In the 4th week of study, no significant differences on the efficiency of MFF males attracted to traps were observed among all concentrations, except at TML-C 50%, which was significantly lower (2.39) than other tested concentrations. In the fifth week of study, TML-C 75% was superior (8.92).

In Table 2, it shows the efficiency of TML-baited Jackson traps and its different concentrations for five weeks. For TML aged one week, the attractiveness to MFF males was 31.63% but the efficiency gradually decreased to 14.12% after five weeks of exposure under normal field conditions. For TML-PF 75%, it showed more or less the same percentage of efficiency along the experiment procedure. However, the relative attractiveness of TML-PF 50% was higher when compared to week one and reached the highest efficiency in the 4th week at 19.55%. The high efficiency of TML-SF 75% was in the 2nd week at 21.85% while the efficiency of TML-SF 50% was 17.01% when the age of lure was in the 3rd week.

General means of males' number of CTD over the tested period of study (5 weeks) were 5.51 for 98%, 4.04 for 75% and 4.77 for 50% (diluted by paraffin oil), 4.79 for 75% and 4.11 for 50% (diluted by sun flower oil), 5.67 for 75% and 3.62 for 50% (diluted by corn oil) (Table 1). Values of average percentages of attracted males through 5 weeks were 18.06%, 13.05%, 14.61%, 15.88, 12.81, 14.83 and 10.93% for concentrations at 98%, TMC-PF 75%, TMC-PF 50%, TML-SF 75%, TML-SF 50%, TML-C 75% and TML-C 50%, respectively (Table2). These results indicated that there was semi-equal of the efficacy of each treatment towards adult males of MFF along the experiment and TML-C 50% was the lowest.

Table 1. Responses of MFF males to different concentrations of TML under field conditions in mandarin orchards in Saheil-saliem district.

Concentration of TML	Oil	Weeks of inspection*					General mean*
		1st	2nd	3rd	4th	5th	
98%	-	7.74[a]	2.93[a]	5.70[a]	4.50[a]	6.57[a]	**5.51[a]**
75%	TML-PF	3.46[b]	3.07[a]	3.82[a]	4.54[a]	5.28[b]	**4.04[a]**
50%		2.21[b]	2.75[a]	6.00[a]	6.39[a]	6.50[a]	**4.77[a]**
75%	TML-SF	4.57[b]	4.64[a]	4.03[a]	3.92[a]	6.78[a]	**4.79[a]**
50%		1.75[b]	3.21[a]	5.71[a]	4.61[a]	5.25[b]	**4.11[a]**
75%	TML-C	2.39[b]	2.03[a]	5.42[a]	6.32[a]	8.92[a]	**5.67[a]**
50%		2.35[b]	2.61[a]	2.89[a]	2.39[b]	7.85[a]	**3.62[b]**
L.S.D 5%		**3.13**	**2.82**	**2.51**	**2.63**	**2.98**	**1.55**

Note: TML=Trimedlure PF=paraffin oil SF= sunflower C= Corn oil p value < 0.05
*Different superscript letters in the same column means significant different.

Table 2. The relative attractiveness of MFF males captured into traps loaded with different TML concentrations in Saheil-saliem district.

Concentration of TML	Oil	Weeks of inspection					General mean
		1st	2nd	3rd	4th	5th	
98%	-	31.63%	13.79%	16.98%	13.77%	14.12%	**18.06%**
75%	TML-PF	14.14%	14.45%	11.41%	13.89%	11.35%	**13.05%**
50%		9.43%	12.95%	17.87%	19.55%	13.27%	**14.61%**
75%	TML-SF	18.78%	21.85%	12.00%	11.99%	14.76%	**15.88%**
50%		6.42%	15.11%	17.01%	14.11%	11.41%	**12.81%**
75%	TML-C	9.96%	9.56%	16.15%	19.34%	19.16%	**14.83%**
50%		9.61%	12.37%	8.61%	7.32%	16.76%	**10.92%**
Total		100.0%	100.0%	100.0%	100.0%	100.0%	**100.0%**

3.1.2 The second district

The results for the second district was presented in Table 3. In the first week, there were no significant differences among all different concentrations in captured *C. capitata* with L.S.D 5% of 2.90 except at TML-PF 50% with the lowest response of 1.17 whereas at TML-SF 50% gave the highest response of 9.46 in comparation with other treatments (Table 3). For lures aged 2 weeks, TML-SF 75% baits resulted in significantly higher trap captures (9.85) than others. In the 3rd week, TML-PF 75% had highly significant trap captures (5.21) whereas TML-C 50% had the lowest significant in the number of male medflies (2.28). In the 4th week, there were no significant differences among TML-SF 50%, TML-C 75% and TML-C 50%. Also, there were no significant differences among these three concentrations in the 5th week. In general, along with the exposure of all treatments under normal field condition, TML-PF 75%, TML-SF 75% and TML-SF 50% had highly significant differences in comparison with other treatments followed by crude substance at 98%.

Table 4 showed the percentage of the attractiveness of each treatment. Within the experiment, the percentage of attracting of adult males of MFF varied from week to week. For the lure aged 1 week, TML-SF 75% and TML-SF 50% gave higher efficiency than other lures with the efficiency of 20.61% and 32.13%, respectively. In the 2nd week, TML-SF 75% still showed higher efficiency than other lures (19.63%). For lures aged 3 weeks, TML-PF 75% and TML-SF 75% had higher efficiency at 21.37% and 19.77% in capture males of *C. capitata*. In the 4th week, TML (98%) and TML-PF 75% were superior than other treatments with the efficiency of 32.63% and 26.30%, respectively. In the 5th week, TML-PF 75% and TML-PF 50% had the higher efficiency at 28.17% and 20.26%, respectively. A high percentage of attracting the pest was TML 98% in the 4th week with 32.63% whereas TML-C 75% and TML-C 50% showed the lowest attractive along the exposure. Generally, TML-PF 75%, and TML-SF 75% were higher attractiveness to MFF adult males with 20.95% and 17.69%, respectively and TML-C 75% and TML-C 50% had the lowest attraction of 7.10% and 8.51%, respectively.

Table 3. Responses of MFF males to different concentrations of TML under field conditions in mandarin orchards in Elbadary district.

Concentration of TML	Oil	Week of inspection*					General mean*
		1st	2nd	3rd	4th	5th	
98%	-	2.82^{bc}	7.82^{abc}	3.18^{abc}	3.76^{a}	0.46^{b}	**3.59^{ab}**
75%	TML-PF	3.78^{bc}	7.92^{ab}	5.21^{a}	3.03^{ab}	1.53^{a}	**4.30^{a}**
50%		1.17^{c}	5.32^{b}	3.50^{abc}	1.42^{bc}	1.10^{ab}	**2.51^{b}**
75%	TML-SF	6.07^{b}	9.85^{a}	4.82^{ab}	1.75^{bc}	0.75^{ab}	**4.56^{a}**
50%		9.46^{a}	8.00^{ab}	2.67^{bc}	0.86^{c}	0.53^{b}	**4.31^{a}**
75%	TML-C	3.32^{bc}	5.82^{ab}	2.71^{bc}	0.35^{c}	0.53^{b}	**2.35^{b}**
50%		2.82^{bc}	5.43^{b}	2.28^{c}	0.35^{c}	0.53^{b}	**2.39^{b}**
L.S.D 5%		**2.90**	**2.89**	**1.98**	**1.80**	**0.76**	**1.20**

Note: TML=trimedlure PF=paraffin oil SF=sunflower oil C=corn oil p value< 0.05
***** Different superscript letters in the same column means significant different.

Table 4. The relative attractiveness of MFF males captured into traps loaded with different concentrations of TML in Elbadary district.

Concentration of TML	Oil	Weeks of inspection					General mean
		1st	2nd	3rd	4th	5th	
98%		9.66%	15.59%	13.04%	32.63%	8.47%	**15.88%**
75%		13.14%	15.78%	21.37%	26.30%	28.17%	**20.95%**
50%	TML-PF	3.97%	10.61%	14.36%	12.32%	20.26%	**12.30%**
75%		20.61%	19.63%	19.77%	15.19%	13.28%	**17.69%**
50%	TML-SF	32.13%	15.95%	10.95%	7.46%	9.76%	**15.23%**
75%		11.27%	11.61%	11.12%	3.03%	9.76%	**7.10%**
50%	TML-C	9.57%	10.82%	9.36%	3.03%	9.76%	**8.51%**
Total		100%	100%	100%	100%	100%	**100%**

3.2 The chemical structure of TML before and after dilution with some fixed oils

From IR and GC analysis, it was found that the infrared absorption spectrum of each solution showed the same characteristic of maximum absorption bands as presented in Figures 1-4 and there were no new function groups appeared after dilution.

The characteristic band at 2,957.16 cm^{-1} was corresponding to aliphatic cyclohexane, ester group forming 1,780.73 cm^{-1} was corresponding to carbonyl group band and C-O group at 1,280.21 cm^{-1}, methyl group band at 1,457.20 cm^{-1} and chlorine group occurred at 845.70 cm^{-1}. All these functional groups occurred after dilution with any oil used in this study.

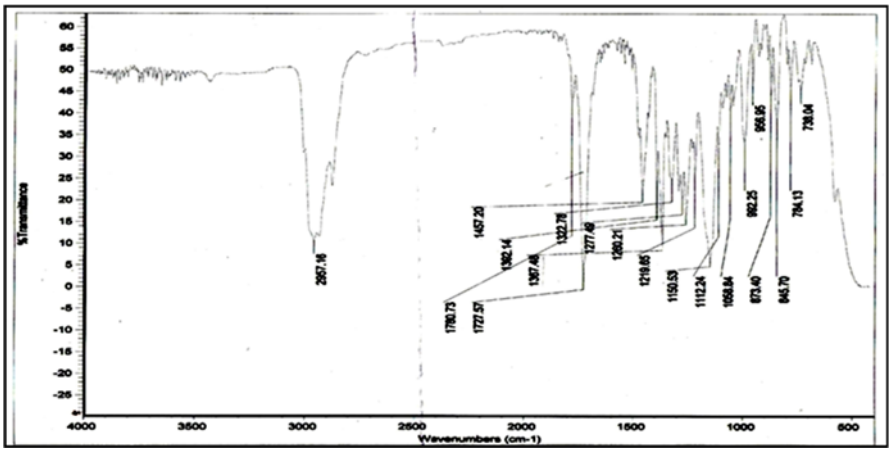

Figure 1. IR spectrum of TML before dilution

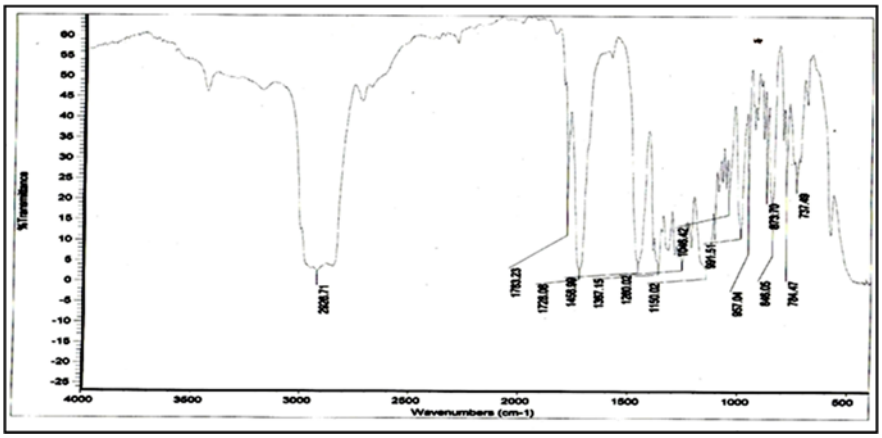

Figure 2. IR spectrum of TML after dilution with paraffin (1:1) showing no new function group

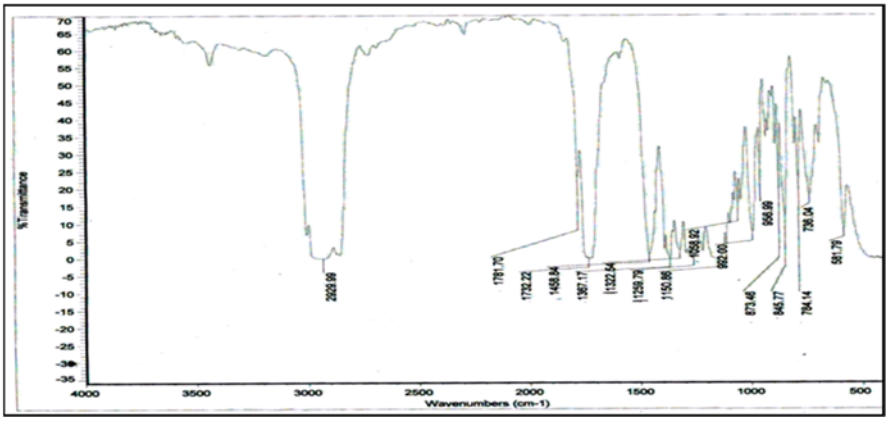

Figure 3. IR spectrum of TML after dilution with corn oil (1:1) showing no function group appeared compared to reference

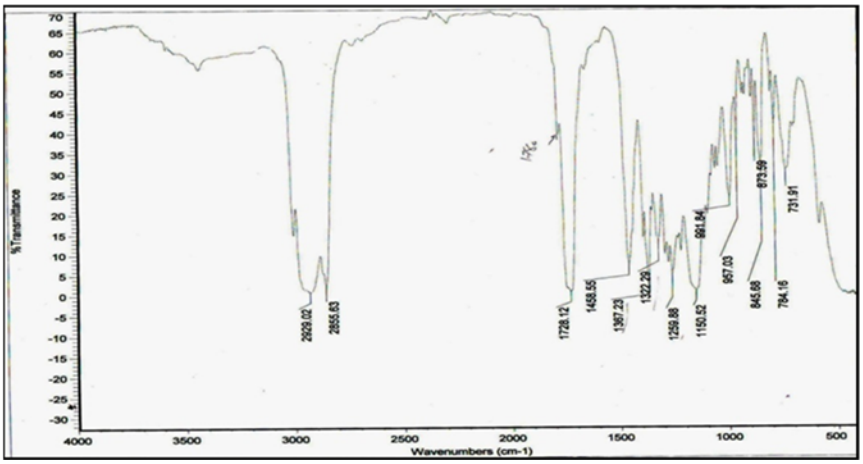

Figure 4. IR spectrum of TML after dilution with sunflower oil (1:1) showing no function groups appeared compared to reference

Another chemical analysis was achieved through GC analysis of each solution; TML before and after dilution, and analysis of each oil. For TML after dilution with each oil, it was found that there was no change in retention time approximately to any diluted solution that appeared at 0.395, 1.415, 1.400, 1.400, 1.395, 1.400 and 1.395 min of TML, sunflower oil, corn oil, paraffin oil, TML-PF 50%, TML-SF 50%, and TML-C 50%.

TML has retention time at 0.395 min. Disappearance of TML retention time after dilution may be due to the composition of new unstable hydrogen bonds between the oil and TML that make them miscible together. However, no change in the main chemical structure of TML or TML was broken at the beginning of injection at boiling point temperature (104-134˚C) lower than that used in diluted solution (200-250˚C).

The reasons of dilution of TML with these oils can be summarized as follows:

1) Paraffin oil, corn oil and sunflower oil were fixed oils and used as carrier oil. Unlike essential oils, they do not contain concentrated aroma, have mild distinctive smell and do not evaporate. The use of carrier oils should be as natural and clear as possible. Carrier oils can be easily blended to combine their properties of viscosity, acceptability, lubrication, absorption, and aroma and so forth [5].

2) TML has ester group and the tested oils also have ester group, that means high miscibility without any change in basic structure of TML, as shown in Figures 1-4.

3) Each oil used in this study is cheap and available in markets.

3.3 Discussion

The pheromone is a complex medley having three to five major components that attract females [6, 7] plus at least 50 minor components whose function has not been elucidated [8]. Gas chromatograph and IR spectrum analyses indicated that there are changes in the main chemical structure of TML. Because of its high cost and its high volatility, recent researches focused attention on several alternatives that were effective male lures towards the medfly. Ceralure, an iodinated analogue of TML, was shown to be 4-9 times as attractive to male medflies as TML [9, 10]. Capilure, that replaces a portion of TML with proprietary extenders that evaporate [11, 12], was used for *C. capitata* detection [3, 4, 13-15]. In addition to these TML-based alternatives,

enriched ginger root oil (*Zingeber officinale* L.), which contained α-copaene, was used as attractant to male medflies [3, 16-19] that may be more superior than TML.

The results of our experiments confirm the finding of El - Abbassi and El - Metwally [20] who tested the efficiency of different concentrations of sex attractant trimedlure (TML) diluted with paraffin oil on attractant males of the Mediterranean fruit fly, *C. capitata* (Wiedemann). They found that 75% concentration of TML was superior to 98% concentration throughout 8 weeks and especially in guava orchards. Data obtained from these trials are in agreement with results reported by Jang *et al.* [10] who found that doses of (-) ceralure B1 of 87.5% and 75% were as effective as 98% concentration in attracting med fly. Jang *et al*. [9] also found that in outdoor olfactometer cage and in field tests, there were no significant differences in medfly male capture into traps loaded with 10.0 µg and 1.0 µg of TML. Moreover, in the second week captures at traps baited with 10 mg TML decreased drastically and they were not significantly greater than control. Our results were also in alignment with data obtained by Khrimian *et al*. [21] and Khrimian [22] who developed an easier synthesis of the racemic ceralure B1, and subsequent studies showed that >75% optically pure (-)-ceralure B1 could be as effective as the 98% (-)-ceralure B1, and the racemic ceralure B1 was also attractive. The results of our experiments confirm the finding of Nabih [23] who mentioned that no significant differences between ginger oil of purity 100% and ginger oil 75% in attracting MFF males.

Early detection of incipient infestations of *C. capitata* is essential because it allows both the delimitation of the outbreak and the implantation of control and eradication measures while the pest population is still small. A rapid response of *C. capitata* to TML not only limits crop damage but also reduces the programmatic costs incurred in the eradication effort [1, 2].

The chemical structures of trimedlure or TML before and after dilution do not change. There are no change in chemical structures of main compound (TML) in comparison to TML before dilution and no new function groups appear in any treatment comparative to target material (TML). Thus, the efficacy of TML after dilutions was not affected. This work is the first time of applying diluted TML which indicates the economic importance for the application in the field because it can give similar result as crude TML (98%).

4. Conclusions

In conclusion, dilution of trimedlure or TML with some of the fixed oils as carriers to avoid the interaction between TML and the diluted substances can also be used for attracting adult males of MFF besides the use of pure TML. This procedure will reduce the cost of TML by diluting 1l of TML to 2l with fixed oils like paraffin oil, sunflower oil and corn oil. These oils are cheap, available and not volatile for a longer period that enable TML to keep its effectiveness in the field for a long time without renewal preparations.

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