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Chemical Composition And Acridicid Properties Of The Moroccan *Tanacetum Annuum* L. Essential Oils

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ABSTRACT: *The essential oil extracted from Tanacetum annuum L. by hydrodistillation was analyzed by GC and GC-MS. The main compounds were myrcene (13.67 %), camphor (12.67 %), sabinene (9.49 %), β-pinene (7.70 %), α-phellandrene (6.95 %) and chamazulene (5.87 %). The acridicid activity of this essential oil was studied. The essential oil obtained shows a toxic effect on the survival of adult locusts. The LC50 is 5 mg/l. The toxicity of the studied essential oil can be linked to one or some of these constituents. The obtained results can be exploited for the formulation of a product allowing as effective alternative natural way to control pest insects*

KEY WORDS: *Tanacetum annuum; essential oil; chemical composition; acridicid activity; natural biocides.*

I. INTRODUCTION

In Morocco, as in other countries in the world, the sector of aromatic and medicinal plant trade represents an important economic activity. Several species are exploited and used for various purposes: drug company, cosmetics, agro-alimentary, and alternative medicine. For Morocco, essential oils correspond to a significant marketing activity [1] in the program "Moroccan Green Plan", the goal is to promote agriculture for achieving environmental and economic sustainability over the fifteen years to come. Amongst other things, the project will develop the cultivation of several potentially important aromatic and medicinal plants, such as thyme, rosemary, saffron and sage. The characterization of essential oil activity is a very interesting and useful task, particularly for their phytotherapeutic properties. Thus, several essential oils showed their effectiveness against bacteria [2], phytopathogenic mushrooms [3], insects invading the foodstuffs [4.; 5] , larvae of mosquitos [6] and pest locusts [7] . It was shown that the extracts obtained of *Azadirachta indica* harbor a harmful effect on the insects. They cause a health deterioration related to an impossibility of emptying their intestines and a disturbance on the endoctrine system which controls metamorphosis [8]. In addition, [5] stressed that *Melaleuca quinquenervia* and *Ocimum gratissimum* present an insecticidal and anti-laying activity against *Callosobruchus maculatus* Fab. Other plants like *Eucalyptus gomphocephala* and *Schinus molle* [9], *Azadirachta indica*, *Melia azedarach* and *Eucalyptus globulus* [7; 10] , *Euphoria longana* and *Olea europaea* [11] as *Peganum harmala* [12] showed a repulsive effect on locusts.

The genus *Tanacetum* (Asteraceae) comprises approximately 150 to 200 species [13] of which some are largely used as additives in agro-alimentary sector, cosmetics and medicine [14]. Others are used as repellent substances against certain insects [15;16]. In spite of these data, no study was carried out on their antilocust effect. Annual blue tanaisy *Tanacetum annuum* L. is a Mediterranean species, largely widespread in the North of Morocco. It is an aromatic plant with the erected stems, pennatilobate leaves and yellow capitules made up of identical florets. In this work, we were interested to study the effect of essential oil of *Tanacetum annuum* L. on survival, food behavior, and body weight of the adults of the grasshopper *Paraeumigus parvulus*; the chemical composition of this essential oil was also given. This will enable us to highlight the potentialities of this plant as a biological acridicid, which would constitute another valorization of this very abundant plant in the north of Morocco.

II. MATERIAL AND METHODS

2.1. Plant material

The samples of *Tanacetum annuum* were collected when fully bloomed in mid-September 2007, in the area of Tangier at Sidi-El-Yamani (NW - Morocco).

2.2. Insect material

The larvae of the third stage of the grasshopper *Paraeumigus parvulus* (Bolívar, 1907) (Pamphagidae) were collected in Al-Azaghar station (Moyen-Atlas), a gregarigene area of the Moroccan locust *Dociostaurus maroccanus* (Thunberg, 1815) [17]. They were maintained in massive rearing at the laboratory in wooden cages (Figure 1) until the adult stage. Each cage of 64 L (38 X 38 X 45 cm), glazed on the three faces, is covered with a perforated metal plate and comprises a sliding door to facilitate handling. A metal plate in the bottom allows the separation of the cage in two compartments: the upper part reserved for breeding, and the bottom for the installation of ovipositors. One of the faces is latticed to ensure satisfactory ventilation. The rearing is maintained at a diurnal temperature of 28 ± 3°C, a night temperature of 24 ± 3°C and a relative humidity of 65 ± 2%, with a 12:12 photoperiod. They are fed with germinated corn *Triticum turgidum* L. cultivated at the laboratory, in order to avoid any negative effect of treatments which can take place in the field.

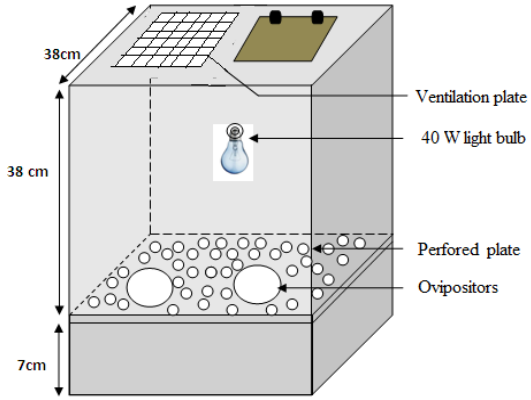


Fig. 1: Schematic view of the experimental apparatus used for rearing grasshoppers

2.3. Essential oil extraction

The samples used (leaves and flowers) were dried in darkness and the extraction of essential oils was carried out by hydrodistillation using a Clevenger-type apparatus [18]. In each experiment, 200g of dry plant material was treated. The duration of extraction is about 4:00. On the whole, four tests were carried out. Essential oil obtained is dehydrated by anhydrous sodium sulphate (approximately 20% of total volume).

2.4. Chromatographic methods of analysis

The chromatographic analyses were carried out on a chromatograph in gas phase with electronic regulation of pressure of the type Hewlett Packard (series HP 6890), equipped with a capillary tube HP-5 (30m X 0.25mm) with a thickness of film of 0.25 µm, with a detector FID regulated with 260°C and supplied with a mixture of gas H2/Air and a Split injector-splitless regulated at 275°C. The mode of injection is Split (report/ratio of escape: 1/50, flow: 66 ml/min). The gas used is the nitrogen with a flow of 1.7 ml/min. The temperature of the column is programmed from 50 to 250°C at a rate of 4°C/min. The apparatus is controlled by a computing system of the type "HP ChemStation", managing the operation of the apparatus and making it possible to follow the evolution of the chromatographic analyses.

The identification of the components was based on their indices of Kováts (IK) and on gas chromatography coupled with mass spectrometry (CG-SM). The latter is carried out on a chromatograph in gas phase of type Hewlett-Packard (series HP 6890) coupled with a mass spectrometer (series HP 5973). Fragmentation is carried out by electronic impact under a field of 70eV. The column used is a capillary tube HP-5MS (30 m x 0.25 mm), the thickness of film is of 0.25 µm. The temperature of the column is programmed of 50 with 250°C at a rate of 4°C/min. The carrier gas is the helium whose flow is fixed at 1.5ml/min. The mode of injection is Split (report/ratio of escape: 1/70 flow 112ml/min. The apparatus is connected to a computing system managing the NIST 98 library of mass spectrum. For the chromatographic analyses, essential oils were diluted in methanol (1/50 V/V). The identification of the components is based on the comparison of their respective mass spectra with the spectra of the library (NIST 98) and the bibliography [19] and on the calculation of the Kováts indices.

2.3.3. Insecticidal test

The methodology employed in this study is that adopted by [20], and consists in depositing 2µl of essential oil at the tested concentration on the membrane of the neck of the insect. The deposit of the product tested is renewed each day to avoid any evaporation. We used three dilutions (1/1000, 1/200 and 1/100) of essential oil in ethanol and the concentrations correspond to 0.8 mg/l, 4 mg/l, and 8 mg/l respectively. For each studied concentration, 30 individuals are treated. For the mock, we used distilled water and ethanol. We have 150 individuals on the whole. The parameters (number of deaths, body weight, daily consumption (food consumed in g by individual in 24:00) and the assimilation of food are recorded. The movement activity and the quality of faeces were surveyed every 24 hours during the 10 days of the experiment.

Death rate was calculated by using the Schneider formula:

$$Mc = (Mo - Me / 100 - Me) * 100$$

With, Mc = corrected deat rate (%)

Mo = death rate recorded in treated samples (%)

Me = death rate recorded in mock samples (%)

The comparisons between results were conducted using Student test (t) with a 5% threshold.

III. RESULTS AND DISCUSSION

3.1. Output and chemical composition of essential oil

The essential oil extracted from *Tanacetum annuum* L. presents an average output of about 1.2 %. This rate is relatively higher than the one obtained with two other species of the genus *Tanacetum*, namely *T. aucheranum* (0.15 %) and *T. chiliophyllum* (0.22 %) from Turkey [13] . The results of the chromatographic analyzes of studied oil are gathered in Table1

With a total percentage of 89%, forty components were detected. The majority compounds are myrcene (13.67 %), camphor (12.67 %), sabinene (9.49 %), β-pinene (7.70 %), α-phellandrene (6.95 %), and chamazulene (5.87%). Others compounds were identified with relatively important percentages, namely the borneol (4.79 %) and limonene (3.78 %). The terpinene-4-ol (2.89 %), the 3,6-dihydrochamazulene (2.18 %), the α-pinene (2.16 %), and the β-caryophyllene (2.13 %) have rates between 2 and 3 %. The percentages of the remaining components are lower than 2%. It should be noted that the essential oil of the same species collected in the area of Larache (NW-Morocco) was very low in myrcene (6 %) and chamazulene (2.8 %), but richer in sabinene (22.3 %), β-pinene (10.1 %), and p-cymene (8.9 %) [21] . These results also show that the chemical profile of studied essential oil is relatively different from that obtained with the essential oil extracted from the same species in Spain [22] . The latter is characterized by the principal components of myrcene and α-phellandrene (18 %), chamazulene (11 %), camphor (10 %), β-pinene (7.5 %) and the sabinene (5.5 %), and presents only 1.1 % of limonene and no borneol. The chemical profile of essential oils of *T. aucheranum* and *T. chiliophyllum* found by Salamci et al (2007) is however definitely different. In these oils, the prevailing compounds are the 1,8-cineole with rates respectively of 23.8 %,16.6 % and camphor with 11.6 % and 17.9 %. Moreover, the derivatives of the thujane and camphene (in particular, the thujone and camphor) with the 1,8-cineole are noted as major components in several species of the genus *Tanacetum* [14; 17; 23; 24; 25;26], but it is not the case for the species analyzed in this study. This intra and interspecific variability in the chemical composition of the species of the genus *Tanacetum* can be of geographic or genetic origin [27;28] .

Table I. Chemical composition of Moroccan *Tanacetum annuum* essential oil

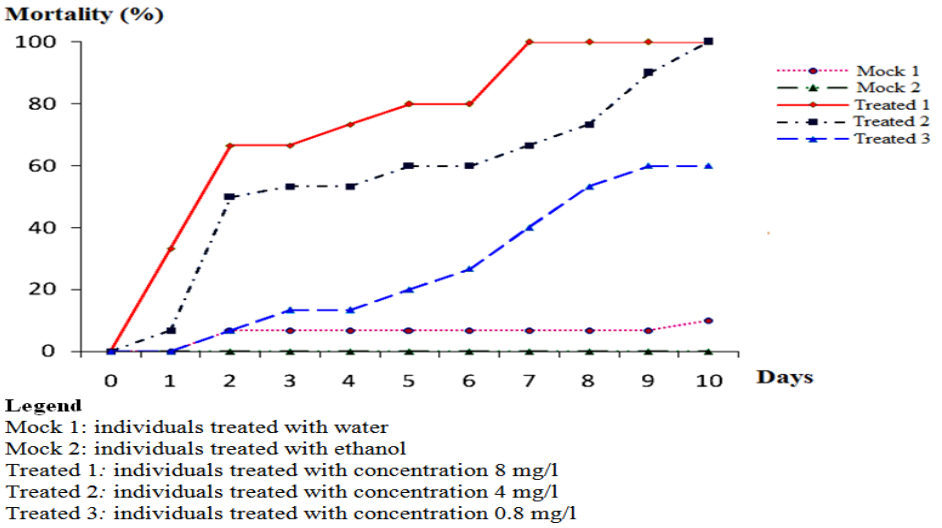
	IK	Constituant	%	Identification method
1	923	α- thujene	0.25	IK, SM
2	930	α–pinene	2.16	IK, SM
3	945	Camphene	1.01	IK, SM
4	970	**β-pinene**	**7.70**	IK, SM, Co
5	974	**Sabinene**	**9.49**	IK, SM
6	988	**Myrcene**	**13.67**	IK, SM
7	1003	**α-phellandrene**	**6.95**	IK, SM
8	1014	δ-3-carene	0.87	IK
9	1021	α-terpinene	0.79	IK
10	1025	o-cymene	0.42	IK
11	1029	Limonene	3.78	IK, SM
12	1054	γ-terpinene	1.65	IK, SM
13	1083	Camphenilone	0.70	IK, SM
14	1090	Terpinolene	0.28	IK, SM
15	1096	linalool	0.57	IK, SM
17	1113	Hydrate de trans-sabinene	0.34	IK, SM
18	1119	Trans pinan-2-ol	0.13	IK
19	1128	All-ocimene	0.49	IK, SM
20	1137	Cis β-dihydro terpineol	0.16	IK, SM
21	1142	**Camphre**	**12.67**	IK, SM, Co
22	1146	β-terpineol	0.17	IK, SM
23	**1163**	**Borneol**	**4.79**	IK, SM, Co
24	**1174**	**Terpinen-4-ol**	**2.89**	IK, SM
25	1188	α-terpineol	0.24	IK, SM
26	1238	Pulegone	0.19	IK, SM
27	1287	γ-terpinen-7-al	1.04	IK, SM
28	1385	Iso-longifollene	0.17	IK
29	1414	β-Caryophyllene	2.13	IK, SM
30	1438	β-farnesene	1.60	IK, SM
31	1452	α-humulene	0.23	IK, SM
32	1482	D-germacrene	1.38	IK, SM
33	1488	Valencene	0.50	IK, SM
34	1499	α-farnesene	0.34	IK, SM
35	1516	3,6-dihydrochamazulene	2.18	IK, SM
36	1520	δ-cadinene	0.23	IK, SM
37	1524	Elemol	0.46	IK, SM
38	1582	5,6-dihydrochamazulene	0.25	IK, SM
39	1647	β-eudesmol	0.45	IK, SM
40	**1726**	**Chamazulene**	**5.87**	IK, SM
		Total	89.19	

IK : Kováts indices ; (%) : Percentage, **MS** : mass from NIST98 data bank, **Co** : Co-injection of authentic standard.

3.2. Study of the acridicid activity of the essential oil of *Tanacetum annuum*

3.2.1. Effects on mortality

The results obtained show that, whatever its concentration, essential oil has as a negative effect on survival of the individuals tested: the higher the concentration, the higher the mortality. Thus, the total mortality of individuals is obtained after 7 days of treatment with the highest concentration (8 mg/l) and 10 days with a lower one (4 mg/l) (Figure 2). There is a significant effect of the treatment on daily mortality according to the concentration used (Figure 2), compared to the mock ($p< 0.05$). At the end of the first day, the mortalities observed in the pilot batches treated with the highest concentration were significantly different ($p<0.05$) from the mock. In contrast, the effect of the lowest concentration (0.8 mg/l) was not significant until the fifth day of the treatment. The regression line of the percentages of mortality after two days of exposure according to the concentrations used thus enabled to us calculating the CL50, around 5 mg/l.



Legend
Mock 1: individuals treated with water
Mock 2: individuals treated with ethanol
Treated 1: individuals treated with concentration 8 mg/l
Treated 2: individuals treated with concentration 4 mg/l
Treated 3: individuals treated with concentration 0.8 mg/l

Fig. 2 : *Tanacetum annuum* essential oil effect on the survival adult of *Paraeumigus parvulus* as a function of time

3.2.2. Action on the weight of the individuals

The study of the body weight (figure 3) shows that the individuals treated with concentrations of 8 mg/l and 4 mg/l present the largest progressive reduction. Their averages are significantly different from those of the mock ($p<0.05$).

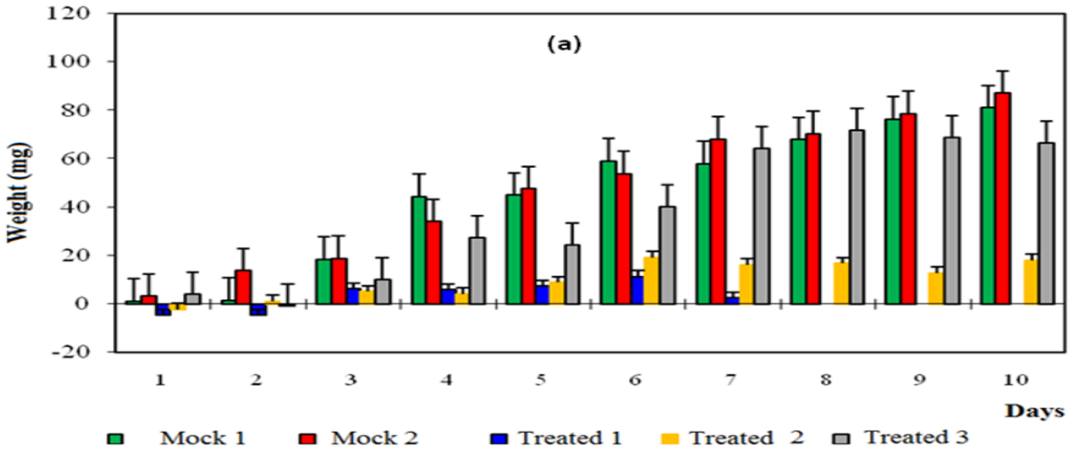


Fig. 3: Variation in the average weight of studied individuals according to time (n = 30 for each lot)

3.2.3. Action on nutrition and its assimilation

Figure 4 (a and b) illustrates the variations in consumption and daily assimilation of *Paraeumigus parvulus* during time. There is a strong reduction in the assimilation, following a decrease in consumption in the treated individuals. These differences of consumption and assimilation are highly significant between the mock and the individuals treated with concentrations of 8 mg/l and 4 mg/l. We deduce that the essential oil of *T. annuum* could contain active substances which affect digestion function in the adult individuals of *Paraeumigus parvulus*. During all the period of the treatment, we paid attention to the behavior of the individuals and we noticed that the treated insects present a deceleration in their moving activity, followed by a reduction in food uptake which could be due to an anti-appetant effect of the essential oil of *Tanacetum annuum*. Moreover, the faeces appeared wetter compared to the mock, which could be explained by digestive disturbances and a dysfunction of the process of hydrous regulation, as a result of re-uptake inhibition of water by rectum. The same phenomenon was often noted in *Locusta migratoria* exposed to synthetic insecticides [29; 30] and in *Schistocerca gregaria* fed with *Peganum harmala* [2] . The toxicity of the essential oil of *Tanacetum annuum* on the treated grasshoppers seems to be due primarily to the action of one or several toxic compounds of this essential oil. Interestingly, it was shown that the monoterpene β-pinene has an insecticide activity [31], moreover, the toxicity of mycene was demonstrated on *Sitophilus oryzae* [32].

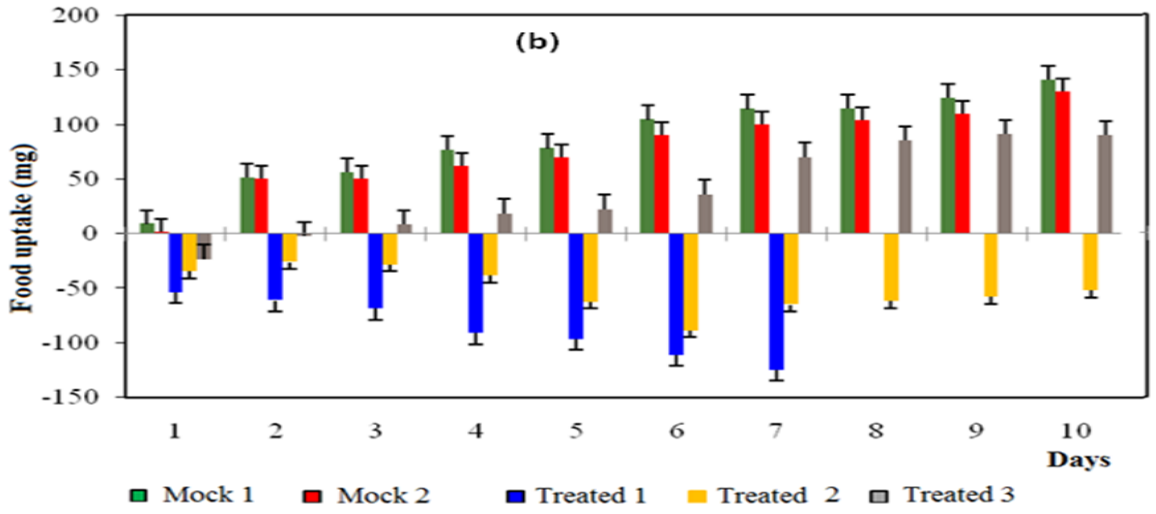


Fig. 4 : Variation in food consumption (a) and assimilation (b) for *P. parvulus* according to time (n = 30 for each lot)

VI. CONCLUSION

The control of pest grasshoppers remains until now a national problem, and even international, because of the limited availability of insecticides without negative effects on the environment. The search for new effective molecules having acridicid activity and less polluting effect for man and his environment is an crucial issue. Thus, the use of essential oils extracted from aromatic and medicinal plants of Morocco, and in particular from *Tanacetum annuum* could play a significant role in the fight against devastating insects. The chromatographic analysis of the essential oil of *Tanacetum annuum* made it possible to identify 40 different components, with contents varying between 0.13 % to 13.67 %, of which myrcene is the prevalent compound. The remarkable toxicity of the essential oil of *Tanacetum annuum* with respect to the individuals tested seems to be induced by myrcene itself or by the synergy with the other components detected. These natural compounds could constitute basic elements for the synthesis of molecules respectful toward the environment, of which the use would be useful for the fight against devastating grasshoppers.

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