Available free online at www.medjchem.com

Mediterranean Journal of Chemistry **2012**, 1(6), 316-325

Biocidal effects of aqueous extract of the roots of Anacyclus pyrethrum (Asteraceae) on Callosobruchus maculatus (Fab.) (Coleoptera : Bruchidae)

Samira Khannouchi, Fatima Elhilali and Touriya Zair*

Laboratory of Chemistry of Bioactive Molecules and Environment, Moulay Ismail University, Faculty of Sciences, B.P 11201 Zitoune, 50000 Meknes, Morocco.

Abstract: In Morocco, *Callosobruchus maculatus* (Fab.) (Coleoptera : Bruchidae) causes very important damage on the seeds of stocked Leguminosae ; its control is carried out exclusively by synthetic insecticides which are harmful for both Man and Environment. Pesticides based on plants constitute an alternative solution in the synthetic insecticides. Many studies have focused on improving forms of using plants that enhance and leverage their insecticidal activity (alcoholic and aqueous extracts, essential oils, etc..). In this study, we sought to evaluate the potentialities of the aqueous extract of the roots of *Anacyclus pyrethrum* (L.) (Asteraceae) against adults, eggs and the emerging of *C. maculatus*. The tests were carried out on seeds of *Cicer arietinum* (L.) (Leguminosae) in the temperature of 28.0 ± 2.0°C, photoperiod 12/12 and relating humidity from 70 to 75%.

The results obtained showed that the aqueous extract of *A. pyrethrum* has an interesting insecticide effect against the adults of *C. maculatus*. It reduced longevity, fecundity, fertility, success rate and it increases the number of non-fertile eggs of these insects, especially for the high doses. This indicates that a durable protection and at low cost against this beetle is possible by using the aqueous extract of *A. pyrethrum*, which can represent an interesting alternative solution to chemical insecticides.

Keywords: Anacyclus pyrethrum, Callosobruchus maculatus, Cicer arietinum, insecticide of vegetable origin.

Introduction

One of the major concerns in the agricultural systems is the progressive replacement of the synthetic pesticides with less polluting, less toxic and less expensive natural substances for a better management of predatory of the agricultural food products. The product insects, mostly the Coleopters, can cause the total loss of a stock[1]. *Callosobruchus maculatus* (Fab.) (Coleoptera : Bruchidae) is an insect which is principal depredater of dry seeds of many Leguminosae and cereals[2, 3, 4]. The seeds of Leguminosae represent the principal source of proteins in many developing countries. They contain two to three times more proteins than cereals[5]. Unfortunately they undergo considerable losses during storage[6]. Between harvest and consumption, more than 30% of the production is lost[1].

During storage, the insects and especially certain kinds of Coleopters (Bruchidae and Curculionaidae) attack seeds[7, 8, 9, 10]. The Man seeks the useful means to fight against these ravageurs. The chemical fight, following its ease of use, its effectiveness, was the most used

**Corresponding author:*

E-mail address: touria.zair@yahoo.fr

DOI: http://dx.doi.org/10.13171/mjc.1.6.2012.06.07.17

method[11]. Among the products used in this method are insecticides such as the organochlorinated, the carbamates, etc.

Consequently, the synthetic products used in agriculture has been responsible for polluting most inhabitants, a loss of biodiversity and the depletion of clean water which is necessary for human life[12].

The adverse effects caused by the use of synthetic insecticides on human health, the environment and the high cost oblige farmers to use the traditional techniques of fight[13]. The use of natural insecticidal products, particularly those of vegetable origin, dates back to ancient times[14]. The first natural pesticides used are nicotine, rotenone, pyrethrum, neem, vegetable oils and essential oils.

Morocco has important potentialities in aromatic and medicinal plants[15]. This study seeks to evaluate the potentialities of *Anacyclus pyrethrum* (L.) roots (Asteraceae) as a botanical insecticide for the protection of *Cicer arietinum* (L.) (Leguminosae) against *C. maculatus*, the main pest of Leguminosae stored food.

Results and Discussion

For the aqueous extract, The normal dose D_n is 1g10 mL-1 of distilled water. The doses Dn/2 and D2n are respectively 0,5g10 mL-1 of distilled water and 2g10 mL-1 of distilled water. The longevity of *C. maculatus* varies depending on the dose and sex. The adults of *C. maculatus* put in contact with the various doses of aqueous extract of *A. pyrethrum* lived shorter than those in contact with untreated seeds. They have longevity varying from six to seven days, compared with 14 days for the control. The effect of the aqueous extract decreased the survival of the beetles of half compared to the control (Figure 1).

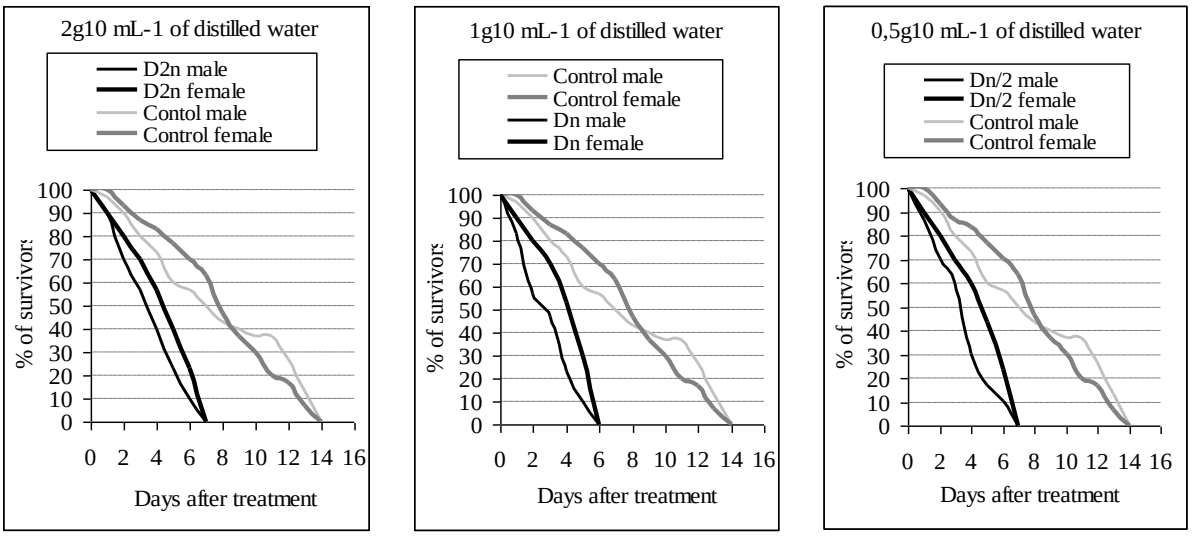

Figure 1. Survival curves of *C. maculatus* on seeds of *C. arietinum* treated by different concentrations of *A. pyrethrum* roots aqueous extract

Table 1. Longevity in days of *C. maculatus* on seeds of *C. arietinum* coated with *A. pyrethrum.*

Sexes	Dose (g/50 seeds)	Manpower	Average ± Standard deviation	Minimum	Maximum	Coefficient of variation (%)
Males	0	30	8,03±1,71	1	14	21,34
	0,5	30	3,73±0,83	1	7	22,28
	1	30	3,2±0,7	1	6	21,98
	2	30	3,9±0,87	1	6	22,35
Females	0	30	8,3±1,91	2	14	23,04
	0,5	30	4,66±1,06	1	7	22,92
	1	30	4,23±0,98	1	6	23,33
	2	30	4,6±1,05	2	6	23,74

Table 1 is used to display average longevity recorded for each batch. Males tend to live shorter than females. For the same sex, generally, the life decreases gradually as the aqueous extract dose increases. It is necessary, moreover, to note that there is a strong variability in the answer to the various concentrations tested. The coefficient of variation varies from 21,34% to 22,35% for males and from 22,92% to 23,74% for females (Table 1).

The aqueous extract affects the longevity of beetles significantly ; the same was observed at *Trialeurodes vaporariorum*[16] and in *Schistocerca gregaria* treated with extracts of two Meliaceae *Azadirachta indica* (Juss) and *Melia voklensii* (Gurke)[17]. These results are similar to those of[18], which showed that the aqueous extracted of leaves of the ricinus *Ricinus communis* (L.) and the wood of thuja *Tetraclinis articulata* (Vahl) Mast. decreased the longevity of four Culicidae mosquitos: *Culex pipiens* (Linne), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) and *Anopheles maculipennis* (Meigen).

The fecundity depends on the longevity of the females put in contact with seeds treated with various doses of aqueous extract, which decreases itself as the concentration in aqueous extract increases ; that lets suppose that the extract tested exerts a toxic effect.

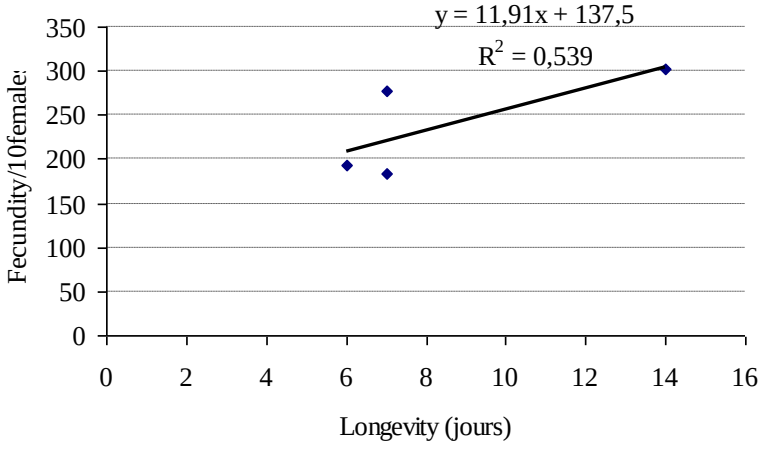

Figure 2. Curve of the fecundity according to the longevity of the adults of *C. maculatus* on seeds of *C. arietinum* treated by different concentrations of *A. pyrethrum* roots aqueous extract

The fecundity of the beetles on chickpea seeds treated with the aqueous extract, with various doses was always lower than these raised on untreated seeds, particularly when the doses increased. The average number of eggs laid varies from 278 to 183 eggs/10 females against 302 eggs with the control (Figure 2).

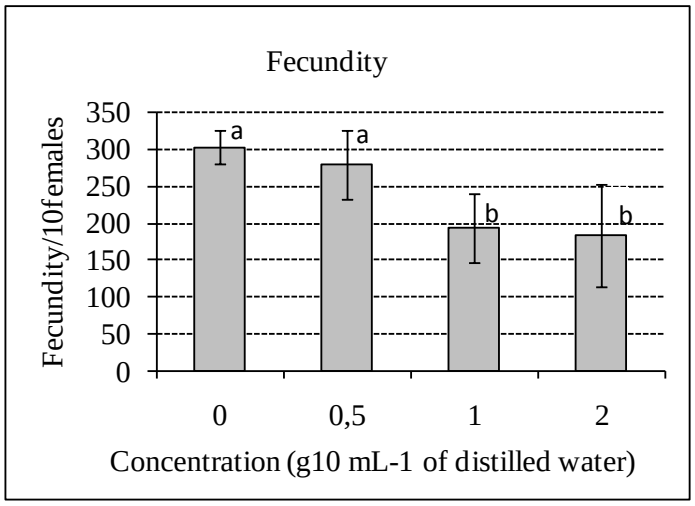

Figure 3. Histograms showing the fecundity of *C. maculatus* on seeds of *C. arietinum* treated by different concentrations of *A. pyrethrum* roots aqueous extract (The histograms affected by the same letter do not differ statistically between them, test of student with the threshold of 5%).

The fecundity of the beetles was significantly affected by the concentration 1g10 mL-1 of distilled water and 2g10 mL-1, while for the concentration 0,5g10 mL-1, there is no effect, it is comparable to that of the control

Thus, the aqueous extract of *A. pyrethrum* reduced fecundity of *C. maculatus*. Like that was observed by[19], in the adults of *Bemisia tabaci* treated by the ethereal and aqueous compounds extracts of *Capsicum frutescens* (L.).

Table 2. Fecundity of *C. maculatus* on seeds of *C. arietinum* treated by different concentrations of aqueous extract of the roots of *A. pyrethrum*.

Concentration (g/50 seeds)	**Fecundity/10♀ ± Standard deviation**	**Minimum**	**Maximum**	**Coefficient of variation (%)**
0	302± 19,52	283	322	6,46
0,5	278 ± 40,84	232	310	14,79
1	192,33 ± 40,77	147	226	21,19
2	183 ± 61,88	128	250	33,81

The individual answers of the females of the insect are however variable, the coefficients of variation range between 6,46% and 33,81% (Table 2).

The fertility of eggs laid by *C. maculatus* on chickpea seeds passes from 82,93% to 67,31% in the batches treated against 96,36% in the control (Figure 3).

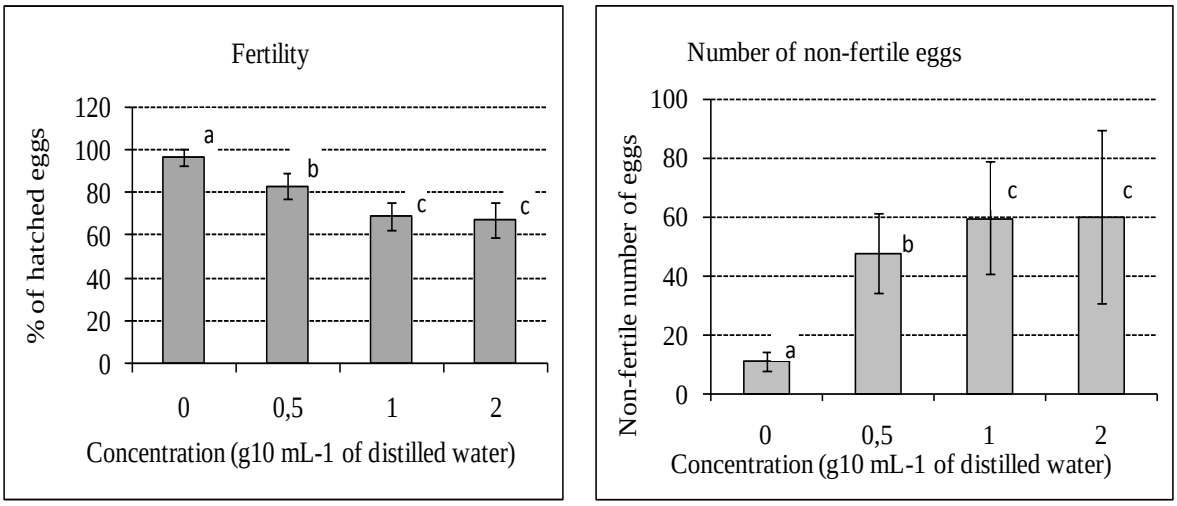

Figure 4. Histograms presenting the fertility and the non-fertile number of eggs of *C. maculatus* on seeds of *C. arietinum* treated by different concentrations of *A. pyrethrum* roots aqueous extract (The histograms affected by the same letter do not differ statistically between them, test of student with the threshold of 5%).

The individual variability is low; the coefficients of variation range between 0,10% to 10,51% (Table 3). The fertility is relatively influenced by the aqueous extract of *A. pyrethrum*. These results are in conformity with those of[20], which shows that aqueous extracts of neem displayed harmful effects on the fertility of certain Diptera. Also, the extract of *Peganum harmala* in bloom reduced the fertility of the females treated in the imaginale state to the desert locust[21].

Table 3. Percentage of hatched eggs of *C. maculatus* raised on seeds of *C. arietinum* treated by different concentrations of aqueous extract of the roots of *A. pyrethrum*.

Concentration (g/50 seeds)	Average fertility ± Standard deviation	Minimum	Maximum	Coefficient of variation (%)
0	96,36 ± 0,10	96,27	96,47	0,10
0,5	82,93 ± 2,49	80,32	85,27	2,99
1	68,97 ± 6,02	62,25	73,89	8,73
2	67,31 ± 7,08	62,50	75,44	10,51

The remarkable effect is especially with the number of non-fertile eggs which varies between 11 and 60 and increases with dose (Figure 3). The individual variability is average; the coefficients of variation oscillate between 9,09% and 43,58 % (Table 4).

Table 4. Number of non-fertile eggs of *C. maculatus* raised on seeds of *C. arietinum* treated by different concentrations of aqueous extract of the roots of *A. pyrethrum*.

Concentration (g/50 seeds)	Average non-fertile number of eggs ± Standard deviation	Minimum	Maximum	Coefficient of variation (%)
0	11 ± 1	10	12	9,09
0,5	47,67 ± 11,72	39	61	24,58
1	59,67 ± 17,01	43	77	28,5
2	60 ± 26,15	42	90	43,58

Concerning the fertility and the non-fertile number of eggs, the histograms affected by the same letter do not differ statistically between them. But, by comparing those affected by different letters, the answers are significant.

Thus, the aqueous extract of *A. pyrethrum* strongly acts on eggs. It decreased the number of fertile eggs and increases the number of non-fertile eggs. The latter remain translucent in the batches treated like those of the control batches, but differ by their desiccated aspect contrary to the control. The same was observed by[22], in the adults of *C. maculatus* treated by the extracts of *Striga hermonthica*.

Concerning the success rate obtained on chickpea seeds treated by the aqueous extract of the roots of *A. pyrethrum*, the downward staff are lower than those stemming from seeds control. It varies from 95,14% with the control until a value of 45,15% with the dose 2g10 mL-1 of distilled water (Figure 4).

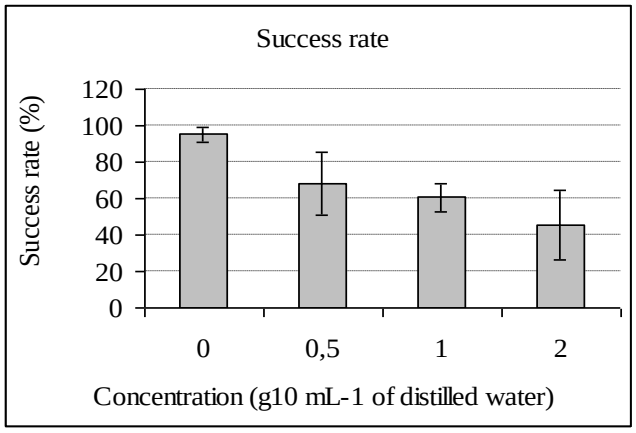

Figure 5. Histograms presenting the success rate of *C. maculatus* on seeds of *C. arietinum* treated by different concentrations of *A. pyrethrum* roots aqueous extract

The individual variability is average ; the coefficients of variation pass from 0,72% to 37,39% (Table 5). The success rate is significantly affected, These results are similar to those of[23], in which it is observed that the extracts of garlic reduce significantly the descendants of *Tribolium castaneum* and *Sitophilus zeamais*.

Table 5. Success rate in percentage of *C. maculatus* on seeds of *C. arietinum* treated by different concentrations of aqueous extract of the roots of *A. pyrethrum*.

Concentration (g/50 seeds)	Average descendants ± Standard deviation	Minimum	Maximum	Coefficient of variation (%)
0	95,14 ± 0,69	94,35	95,68	0,72
0,5	68,25 ± 15,39	50,65	79,11	22,54
1	60,47 ± 7,20	52,45	66,37	11,90
2	45,15 ± 16,89	26	57,89	37,39

Several studies proved that the roots of some plants are endowed with insecticidal properties[24, 25]. The experiments carried out show that the aqueous extract of *A. pyrethrum* roots decreased longevity, fecundity, fertility and success rate of the beetles in the three doses compared to the negative control. So, it exerts a negative effect on these insects. The reduction in these parameters would be the fact of the early death of the beetles. These results are in conformity with those of[24, 25], in which it is observed that the garlic extract respectively causes the mortality of the various stages of *Bemisia argentifolii* and of *Myzus persicae*. These results are similar to those of[18], which showed that the aqueous extracts of leaves of the

ricinus *Ricinus communis* (L.) and the wood of thuja *Tetraclinis articulata* (Vahl) Mast. present a significant insecticidal activity on the larvae of four Culicidae mosquitos: *Culex pipiens* (Linne), *Aedes caspius* (Pallas), *Culiseta longiareolata* (Aitken) and *Anopheles maculipennis* (Meigen).

Analysis and Identification of the compounds of the extracts of *A. Pyrethrum*

The analysis of the chemical composition of the essential oil or the hydrolat is realized by CPG coupled with the mass spectrometry. The identification of the compounds of the essential oil or the hydrolat is based on the comparison of their retention index (IR) on columns, determined with regard to the retention index of a range standard of alkanes; with those of the reference compounds. The chemical analysis of the essential oil allowed to identify 29 compounds (Table 6). It is necessary to note that there is a predominance of sesquiterpenoides compounds (88%) of which the majority, these are sesquiterpenols.

Table 6. Chemical composition of the essential oil of *A. pyrethrum*

N°	TR (Retention Time)	%	IK (Kovats index)	Identified compound
1	24.00	0.20	1475	α-Neocallitropsene
2	25.04	0.15	1515	cubebol
3	25.34	0.40	1535	10-epi-Cubebol
4	26.18	2 .20	1552	Occidentatol
5	26.50	2.83	1572	1α,10α-Epoxy-amorph-4-ene
6	26.84	16.90	1578	sptahulenol
7	26.96	7.11	1583	Caryphylene oxide
8	27.27	4.66	1594	Salvial-4(14)-en-1-one
9	27.65	2.90	1608	Beta-Atlantol
10	27.82	5.16	1613	Beta-Biotol
11	28.64	2.58	1637	Beta-Acorenol
12	28.79	1.54	1643	tujopsanone
13	28.97	9.24	1653	***Eudesmol- alpha***
14	29.21	0.42	1662	7-epi-α-eudesmol
15	9.45	1.86	1670.	5-Isocedranol
16	29.70	3.29	1679.	khusimol
17	29.86	12.89	1685.	4(15),5,10(14)-Germacratrien-1-ol
18	29.86	12.89	1699	4(15),5,10(14)-Germacratrien-1-ol
19	30.24	0.12	1705	juniper camphor
20	30.39	0.62	1717	mayurone
21	31.27	1.41	1738	Eremophilone
22	31.75	2.88	1757	Z-beta-Curcumen-12-ol
23	31.91	1.58	1763	Beta-acoradienol
24	32.10	1.49	1770	squamulosone
25	32.30	0.86	1778	14- hydroxy-alpha-muurolene
26	32.95	1.26	1803	14-Hydroxy-δ-cadinene
27	33.29	2.18	1817	Coumarin-6-ol, 3,4-dihydro-4,4,5,7,8-pentamethyl-
28	33.73	0.59	1847	8-hydroxyeremphilone
29	36.18	5.21	1934	8-Diethyl-3,6-diazahomoadamantan-9-ol

According to Bellakhdar[26], and Hmamouchi[27], *A. pyrethrum* is known for its insecticidal properties. This activity would be due to the richness of this plant in pyrethrins. These results are similar to those described by Glynne-Jones[28], who showed that pyrethrum of Dalmatie is already included in most lists of approved organic insecticides in the world with these pyrethrins. Similar results were reported by Rabezandrina[29], who affirmed that pyrethrins act by contact on all kinds of insects (caterpillar, coleopter, fly, mosquito, bug, etc.) by causing their fast paralysis ; and that the total safety of pyrethrins for the man and the animals make the best produced to treat stored foodstuffs.

Conclusion

C. maculatus is the main pest of Leguminosae stored food. In order to preserve the environment as well as human and animal health, the pesticides at base of the plants constitute an alternative solution with insecticides of synthesis. This study shows that the aqueous extract of *A. pyrethrum* has an unquestionable action in the control of *C. maculates*. This work could be continued in order to find a practical use of these formulations in protection of store of leguminous in general and store chickpea in particular.

In conclusion, the aqueous extract of *A. pyrethrum* shows an interesting insecticide effect against the adults of *C. maculatus*. It reduced longevity, fecundity, fertility, success rate and increases number of non-fertile eggs of these insects especially for the high doses. This shows that a durable protection against *C. maculatus* is possible by using the aqueous extract of *A. pyrethrum* which can represent an interesting alternative solution to chemical insecticides. This might be due to the pyrethrins as what has been shown to pyrethrum of Dalmatie, by[30], who have proven that properties insecticidal were due to the presence of two esters, that they have called pyrethrin I and pyrethrin II.

Experimental Section

The plant material used are the roots of *A. pyrethrum* which were collected in the fall, in the Middle Atlas of Morocco, in the area of Timahdite (120 km far from Meknes), identified at the National Center of Rabat. The seeds of *C. arietinum* healthy, coming from a farm in Moulay Idriss constitute the substrate spawning and development for the beetles.

The animal material used are the adults of *C. maculatus* which were derived from a strain of mass in the laboratory of plant biology in the Science College of Meknes, at a temperature of 28°C, relative humidity between 70% and 75% and a photoperiod 12/12.

The *A. pyrethrum* roots were cleaned by distilled water, dried and crushed. 20g of the broyat of the roots are brought to reflux in 100ml of distilled water during 1h, the extract was filtered then evaporated to dry.

The output in dry weight is 10,03%. While basing itself on the output obtained, we calculate the doses (Dn), (Dn/2) and (D2n). The various doses of the aqueous extract were expressed in g10 mL-1 of distilled water. The biological tests were made in petri dishes. Each petri dish receives 50 chickpea seeds coated by the aqueous extract by dose study and 10 pairs of *C. maculatus* newly emerged. A control is carried out under the same conditions but without aqueous extract. For each dose, three repetitions were carried out. Various parameters were studied : longevity, fecundity, fertility, number of non-fertile eggs and success rate. After 24h, the content of each petri dish is recovered.

The longevity of the adults of *C. maculatus* was followed each day until the total death of the beetles. For fecundity, the eggs laid by the females in each petri dish were counted under binocular magnifying glass. After that, the emerging adults were followed daily until the total stopping of emerging and they were counted. Finally, the statistical analyses were done by Excel.

For the calculation methods, fecundity is the number of eggs laid by ten females. Fertility is the percentage of eggs hatched by the total number of eggs laid and the success rate indicates the percentage of adults emerged compared to the total number of laid eggs. Then, the average, the standard deviation and the reliable interval of all these parameters were calculated by using the functions of Excel.

References

1 - L.S.T. Ngamo, Th. Hance, Tropicultura, **2007**, *15*, 215-220.
2 - J. M. Adams, J. Stored Prod. Res., **1976**, *12*, 269-272.
3 - L.J. Bamaiyi, I. Onu, C.I. Amatobi, M.C. Dike, Archives of Phytopathology and Plant Protection, **2006**, *39*, 119-127.
4 - L. Matos, B. Mikolo, D. Massamba, V. Mamonekene, T. Miller, Ann. Univ. M. Ngouabi., **2009**, 10, 42-48.
5 - D. Soltner, Les bases de la production végétale - Tome 3, La plante et son amélioration; ed. by D. Soltner; Collection Sciences et Techniques agricoles: Angers, **1999**, pp. 252-257.
6 - A. Kellouche, N. Soltani, International Journal of Tropical Insect Science, **2004**, *24*, 184-191.
7 - A. Delobel, M. Tran, Les coléoptères des denrées alimentaires entreposées dans les régions chaudes; ed. by ORSTOM/CTA; Faune Tropicale XXXII: Paris, **1993**, 424 p.
8 - A. Bell, O. Mück, H. Schneider, La protection intégrée des denrées stockées est une affaire rentable!; ed. by G. Quiter; GTZ, Eschborn: Germany, **1998**, 42 p.
9 - A. Kouakap Nguéché, Diversité des ravageurs des denrées au cours du stockage au Cameroun; ed. by A. Kouakap Nguéché; Ibis Press: Ngaoundéré, **2002**, pp. 21-28.
10 - L.A. Tapondjou, C. Adler, H. Bouda, D.A. Fontem, Journal of Stored Products Research, **2002**, *38*, 395-402.
11 - R.L. Metcalf, W.H Luckman, Introduction to Insect pest Management, 3rd Edition: New York, **1994**.
12 - B.J.R. Philogène, C. Regnault-Roger, C. Vincent, Biopesticides d'origine végétale; ed. by C. Regnault-Roger; Tec et Doc - Lavoisier: Paris, **2002**, pp. 1-17.
13 - B.P. Seri-kouassi, C. Kanko, L.R. Nondenot aboua, K.A. Bekon, A.I. Glitho, G. Koukoua, Y.T. N'Guessan, C. R. chimie., **2004**, *7*, 1043-1046.
14 - M. Seck, M. Ndiaye, Effet de l'extrait aqueux des feuilles de neem (Azadrachta Indica A. JUSS) sur la population de thrips et le rendement du niébé (Vigna Unguiculata); ed. by M. Seck; Ibis Press: Bambey, **1997**, 78 P.
15 - S. Marouane, N. Oumam, A. Abourriche, A. Bennamara, M. Charrouf, Mediterr. J. Chem., **2012**, 1(5), 210-220.
16 - WI. Choi, EH. Lee, BR. Choi, HM. Park, YJ. Ahn, J. Econ. Entomol., **2003**, *96*, 1479-1484.
17 - H. Wilps, C.M. Habib kane, International Journal of Tropical Insect Science, **2003**, *25*, 72-89.
18 - B. Aouinty, S. Oufara, F. Mellouki, S. Mahari, Biotechnol. Agron. Soc. Environ., **2006**, *10*, 67-71.
19 - A. Bouchelta , A. Blenzar , A.J.P. Beavougui, T. Lakhlifi, Rev. Méd. Pharm. Afr., **2003**, *17*, 19-28.

20 - S. Shivendra, Journal of Applied Entomology, **2003**, *127*, 540-547.
21 - K. Abbassi, L. Mergaoui, Z. Atay-Kadiri, S. Ghaout, A. Stambouli, Zool. Baetica., 2005, *16*, 31-46.
22 - M. Kiendrebeogo, A.P. Ouedraogo, O.G. Nacoulma, Biotechnol. Agron. Soc. Environ., **2006**, *10*, 17-23.
23 - S.H. Ho, L. Koh, Y. Ma, Y. Huang, K.Y. Sim, Postharvest Biol. Technol., **1996**, *9*, 41-48.
24 - HM. Flint, NJ. Parks, Southwestern Entimologist, **1995**, *20*, 137-150.
25 - M. Hori, Appl, Entomol, Zool., **1996**, *31*, 605.
26 - J. Bellakhdar, la pharmacopée marocaine traditionnelle : Médecine arabe ancienne et savoirs populaires; Ibis Press: paris, **1997**, 50 p.
27 - M. Hmamouchi, Les plantes médicinales et aromatiques marocaines : utilisation, biologie, écologie, chimie, pharmacologie, toxicologie, lexiques; Imprimerie de Fédala: Mohammedia, **1999**, 177 p.
28 - A. Glynne-Jones, The Royal Society of Chemistry, 2001, 195-198.
29 - R. Rabezandrina, Manuel de pedologie malagasy, cours à l'essa Universite d'antananarivo; ed. by R. Rabezandrina; Collection Sciences et Techniques agricoles: Madagascar, **1998**, pp. 54-69.
30 - H. Staudinger, L. Ruzicka, Insektenttende stoffe. Helvetica Chimica, **1924**, *7*, 177-458.