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Larvicidal efficacy of *Jatropha curcas* L. (*Euphorbiaceae*) leaf and seed aqueous extracts against *Culex pipiens* L.

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Culex **mosquitoes play a vital role in transmitting pathogens which continue to have a harmful impact on human beings. Indeed, Innovative vector control strategy like use of plant extracts as alternative sources of insecticidal and larvicidal agents against vector-borne diseases has become unavoidable. In this context, the purpose of the present search was to explore the larvicidal properties of *Jatropha curcas* L. leaf and seed extracts against *Culex pipiens* L. The larvicidal activity was evaluated in eight different provenances recently introduced in Tunisia (Tanzania (ARU), Mozambique (MOZ), Surinam (SUR) and Brazil represented by five provenances: Paraná (PAR), Norte de Minas (NMB), Mato Grosso (MGB), Regiao Sureste (RSB) and Vale do Fequitinhonha (VFB). The assessment of larval activity showed after 24 h of exposure, 100% mortality for aqueous seed extract and between 60 and 100% for aqueous leaf extract according to provenances. Highest mortality is observed at 1 mg/ml against *C. pipiens* L with LC_{50} values of 0.49 and 0.5% for aqueous seed extract and leaf extract, respectively. Commonly, the mortality increase with the increase in concentration of each extract. However, the inhibitory effect of seeds extract on *C. pipiens* was more pronounced than that of leaves. These results suggest that the aqueous seed and leaf extracts of *J. curcas* have the potential to be used as an ideal eco-friendly compound for the control of hurtful mosquito larvae.**

Key words: Aqueous extract, *Culex pipiens* L., *Jatropha curcas* L., larvicidal activity.

INTRODUCTION

The species *Jatropha curcas* L. is a drought resistant shrub of the family *Euphorbiaceae*, which is predominant in Central America and today is found throughout the world in the tropics (Chavan et al., 2014). Different extracts of *J. curcas* seeds, leaves, stem and bark were used as an antiseptic, diuretic, purgative, larvicide as well as for treating cancer, gout, and skin diseases (Dalziel, 1955; Duke, 1985, 1988; Kaushik and Kumar, 2004). Many studies have been conducted on the genus *Jatropha* covering various aspects as bioactivity to insect pests of stored products (Silva et al., 2012), this property was confirmed after toxicity tests that were realized on

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the pests of the stocks of corn and bean seeds and the results have been spectacular, since damages in seeds were reduced to 10% (Solsoloy and Solsoloy, 1997). The aqueous extracts of bark and leaves have a larvicidal activity against mosquito species *Aedes aegypti* L. and *Culex quinquefasciatus* (Rahuman et al., 2008). The linoleic acid present in the composition of the seed oil is used in the treatment of eczema and other skin diseases (Heller, 1996). The leaf juice is an inhibitor of watermelon mosaic virus (Tewari and Shukla, 1982) and also has a human blood coagulant activity (Osoniyi and Onajobi, 2003). Roots showed an anti-inflammatory activity, the application of the powder paste spread over the inflamed part or the injection of methanolic extracts by oral or cutaneous way reduced enormously the inflammation (Mujumdar and Misar, 2004). In addition, the curcin, which is a main component of crude oil, is used in very low concentrations, as a cytotoxic agent in cancer cells; the protein that is responsible can inhibit the spread of tumor cells (Luo et al., 2006). Hence natural plant products may be a possible option to synthetic substances, as they are efficient and friendly with environment (Shaalan et al., 2005). Due to the favorable conditions for the cultivation of *J. curcas* in Tunisia, there is an increasing interest in the study of this plant and it becomes attractive as alternative mosquito larvae control agent because *J. curcas* extracts show no harmful sequel on the environment. Therefore, this encourages us to undertake a study of the larvicidal activity of seeds and leaves aqueous extracts of *J. curcas*.

MATERIALS AND METHODS

Plant material

The leaves and seeds of *J.* curcas L. were collected from the region of Nabeul (Tunisia) between April and September 2010. Plant material belongs to eight different origins. The sources are Tanzania (ARU), Mozambique (MOZ), Surinam (SUR) and five Brazilien provenances: Paraná (PAR), Norte de Minas (NMB), Mato Grosso (MGB), Regiao Sureste (SNR) and Vale do Fequitinhonha (VFB).

Animal material

Larvae subjected to toxicity tests were from larval habitat of mosquitoes untreated, located in rural areas of Mjez el bab (North West Tunisia). They were identified by Dr. Bejaoui Mustafa in Faculty of Sciences of Bizerta, according to Brunches et al. (2000). They were maintained at ambient rearing conditions in the National Institute for Research in Rural Engineering Water and Forestry. For the bioassays, only larval stage 4 was used and all tests were conducted at room temperature.

Preparation of aqueous extracts

Leaves were chapped, dried and powdered. Seeds were peeled and crushed using a mortar until having a kind of paste. Then, 100 mg of plant material was diluted in 1000 ml of distillated water previously heated to boiling. The aqueous solution was placed under magnetic stirring for 30 min. Finally, the mixture was filtered using a Whatman paper (3 MM). The recovered filtrate represents an initial stock solution with a concentration equal to 0.1 g/1000 ml.

Estimated quantity of dry residue

In order to give a more significance to the quantities of plant material soluble in aqueous extracts, they were concentrated under reduced pressure at 40°C using a rotary evaporator for 48 h, until a dry residue was obtained which quantity is expressed in mg. This helps to express the lethal concentrations of soluble dry residues in water in mg / ml.

Toxicity tests

From the initial extract (stock solution) of *J. curcas* L. seeds and leaves, and water of larvae sites, concentrations of 0.1, 0.2, 0.5 and 1 mg/ml were prepared. The tests were performed in 9 cm Petri dishes diameter, each containing 20 ml of solution and 10 mosquito larvae of *C. pipiens* of the same caliber (L4 stage). The same number of larvae was placed in a control Petri dish containing 20 ml of larvae water breeding sites. Three repetitions were performed for each concentration as well as for the control. The larvae were considered dead if they were immobile and unable to reach the water surface (Macedo et al., 1997). Mortality response was noted after exposure of 1, 2, 4, 6, 12, and 24 h, and the mortality percentage was reported from the average of three replicates.

Determination of lethal concentrations (LC_{50})

The estimates of LC_{50} were determined after 24 h of exposure using the software Spearman- Kaber (Hamilton et al., 1977).

Statistical analysis

A general linear model (ANOVA) analysis was used to determine the effect of seeds and leaves origin and the concentration of the aqueous extracts on mortality data recorded after 1, 2, 6, 12 and 24 h of treatment of the larvae. Differences between mean values were compared using the Duncan Multiple Range Test (5%) by SAS (1990), version 6.12.

RESULTS AND DISCUSSION

Evaluation of the mortality of larvae of *C. pipiens* L. exposed to aqueous seed extract of *J. curcas* L.

After 12 h of exposure, most provenances exhibited 100% mortality at 1 mg /ml except the provenances of Vale do Fequitinhonha (Brazil) and Surinam with a mortality of 76.7 and 80% respectively (Figure 1). After 24 h, the totality of provenances showed 100% mortality at 1 mg / ml and also at a concentration of 0.5 mg /ml (Figure 2). It should be noted that the Mozambique population showed a high toxicity since we obtained 100% mortality after 12 h of exposure to only a concentration of 0.2 mg /ml, while the two populations Vale do Fequitinhonha (Brazil) and Suriname showed a low toxicity, since the mortality rate after 24 h was about 96.7% for 1 mg /ml. Furthermore, the results of the

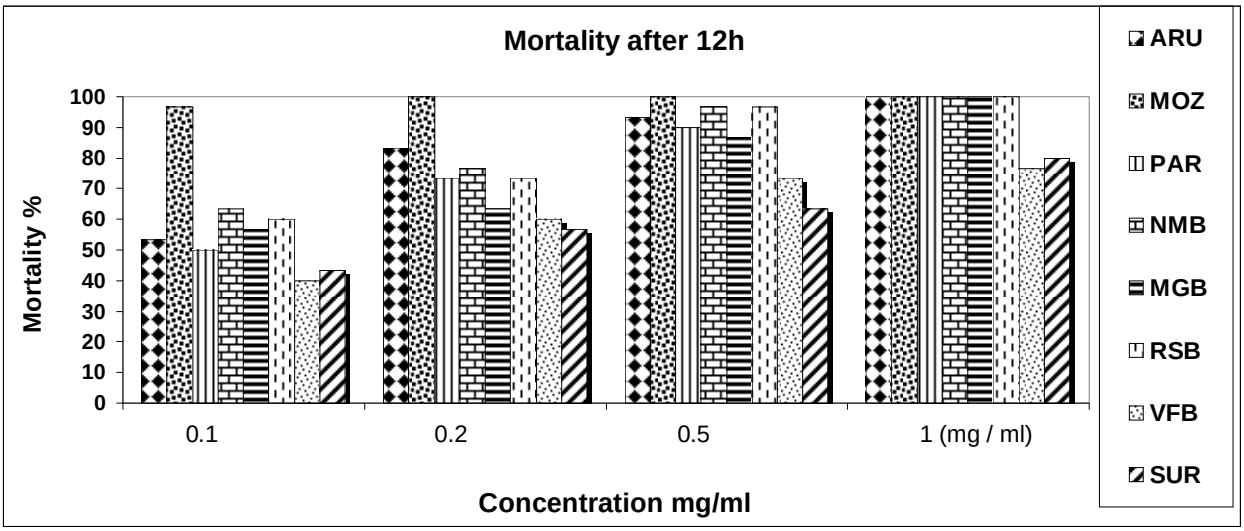

Figure 1. Mortality after 12 h of exposure of larvae to different concentrations (1 to 10%) of seed aqueous extracts.

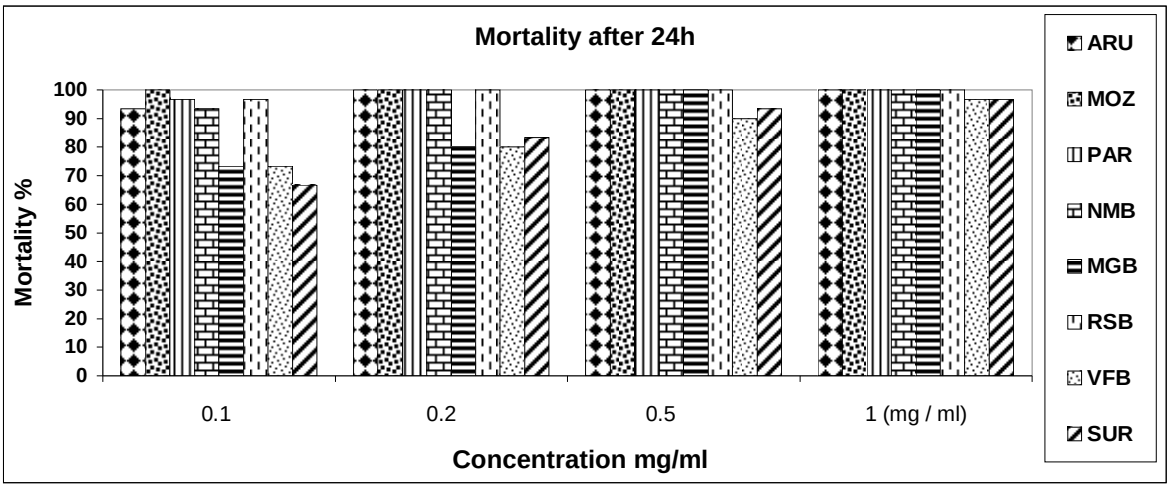

Figure 2. Mortality after 24 h of exposure of larvae to different concentrations (1 to 10%) of seed aqueous extracts.

larvicidal activity of seeds aqueous extract were subjected to analysis of variance which highlighted significant differences of larvae mortality rate between the provenances and among concentrations (Table 1). This reveals that the seeds aqueous extract is responsible for the susceptibility of *Culex pipiens* larvae. Goel et al. (2007) and Makkar et al. (1997) have shown that the higher larvicidal activity of *J. curcas* seeds extract indicate that toxic phorbol esters and other compounds are highly accumulated in seeds, rather than in other parts of the plant including the leaves.

Evaluation of the mortality of larvae of *C. pipiens* L. exposed to aqueous leaf extract of *J. curcas* L.

After the exposure of *C. pipiens* larvae to leaves aqueous extracts, it was found that after 1 h, majority of provenances present a low mortality didn't exceed 3.33% in the case of Vale do Fequitinhonha (Brazil) and Surinam, and 13.33% in the case of Paraná (Brazil) and Mato Grosso (Brazil). In both the provenances of Mozambique and Norte de Minas (Brazil), we recorded a high mortality rate which reaches 40% (Figure 3). On the other hand, after 12 h of exposure, the mortality rate reached 100% for 1 mg /ml concentration in both the provenances of Mozambique and Norte de Minas (Brazil), whereas in other provenances didn't exceed 90% (Figure 4). After 24 h, the mortality rate reached 100% only in the Mozambique, Norte de Minas (Brazil) and Regiao Sureste (Brazil) provenances. Other provenances: Tanzania, Paraná (Brazil), Mato Grosso (Brazil) and Suriname showed a low toxicity. The population of Vale do Fequitinhonha (Brazil) showed the

Table 1. Variance analyses results of larvicidal activity of seed and leaf aqueous extracts of *Jatropha curcas* L. (F and P values, for the significance of the differences)

Time (h)	Variation	Seed		Leaf	
		F	P	F	P
1	Provenance	3.57	0.0022	26.38	<0.0001
	Concentration	30.22	<0.0001	67.84	<0.0001
	Prov x Conc	2.08	0.006	6.26	<0.0001
2	Provenance	40.51	<0.0001	33.73	<0.0001
	Concentration	94.06	<0.0001	112.83	<0.0001
	Prov x Conc	4.22	<0.0001	3.06	<0.0001
4	Provenance	29.00	<0.0001	49.38	<0.0001
	Concentration	205.59	<0.0001	151.24	<0.0001
	Prov x Conc	2.76	0.002	3.98	<0.0001
6	Provenance	14.37	<0.0001	71.57	<0.0001
	Concentration	217.98	<0.0001	243.7	<0.0001
	Prov x Conc	3.14	<0.0001	5.01	<0.0001
12	Provenance	34.29	<0.0001	109.93	<0.0001
	Concentration	804.59	<0.0001	476.32	<0.0001
	Prov x Conc	5.02	<0.0001	7.59	<0.0001
24	Provenance	20.90	<0.0001	134.69	<0.0001
	Concentration	1803.36	<0.0001	716.4	<0.0001
	Prov x Conc	4.83	<0.0001	10.35	<0.0001

$F_{theoric}$ = 1.83 (5%); 2.32 (1%).

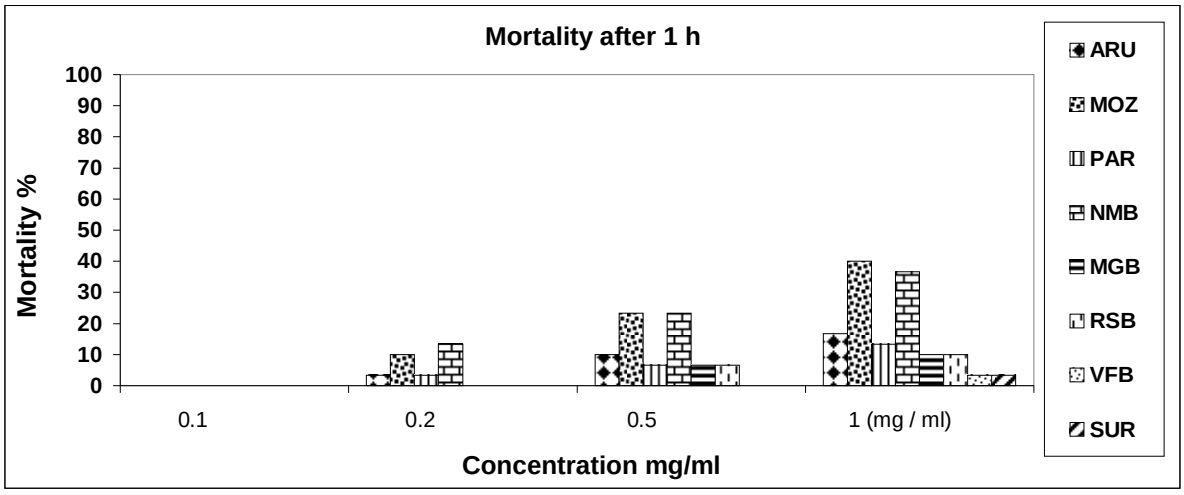

Figure 3. Mortality after 1 h of exposure of larvae to different concentrations (1 to 10%) of leaf aqueous extracts.

lowest toxicity because after 24 h and at a high concentration (10%), the mortality rate did not exceed 56% (Figure 5). Furthermore, the results of the larvicidal activity of leaves aqueous extract were subjected to

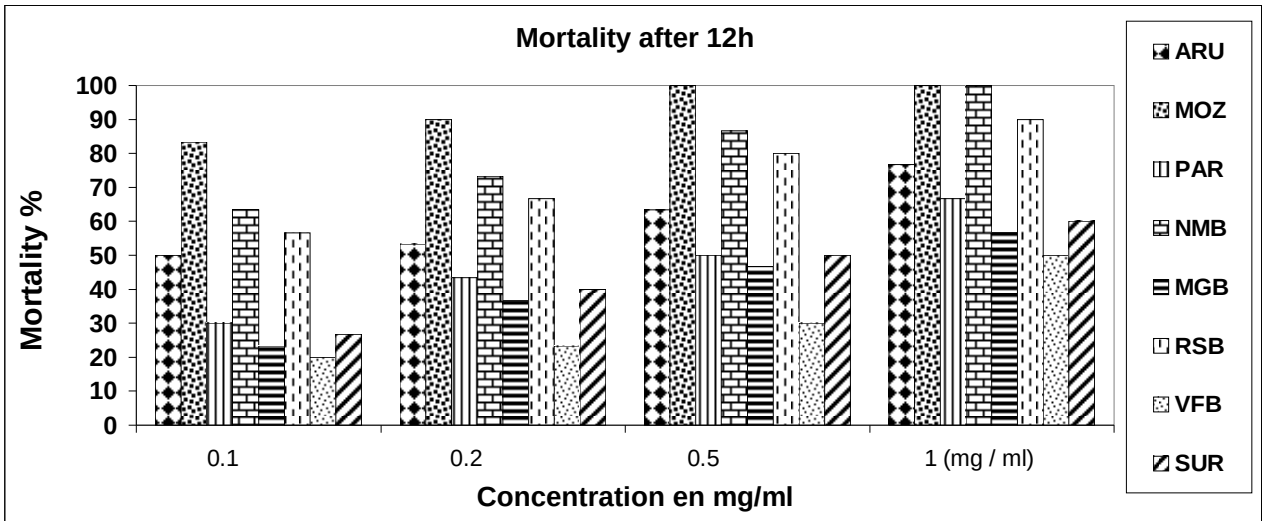

Figure 4. Mortality after 12 h of exposure of larvae to different concentrations (1 to 10%) of leaf aqueous extracts.

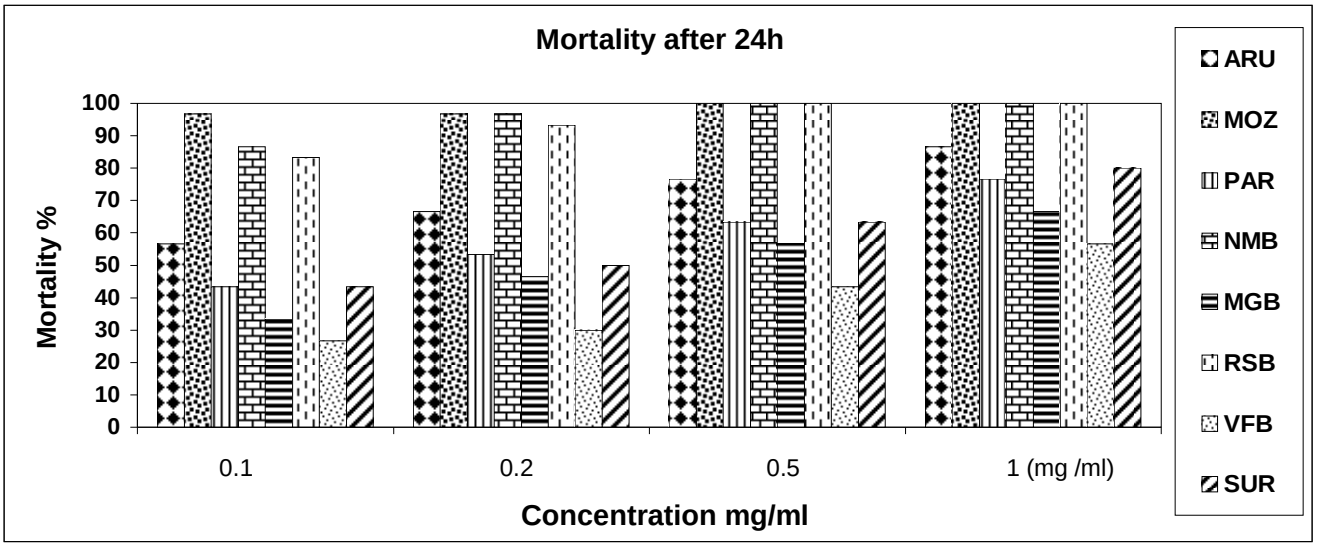

Figure 5. Mortality after 24 h of exposure of larvae to different concentrations (1 to 10%) of leaf aqueous extracts.

analysis of variance which highlighted significant differences of death percentage of the treated larvae between the provenances and among concentrations (Table 2). This reveals that the leaves aqueous extract is responsible for the susceptibility of *C. pipiens* larvae. In fact, Juliet et al. (2012) confirmed the presence of flavones like apigenin, orientin, vitexin, in *J. curcas* leaves. Hence, the efficacy of the leaf extract could be attributed to the presence of the flavones which can cause the toxicity to larvae.

It is noted that the percentage mortality increased with increasing concentrations of the leaves and seeds extracts. This effect is attributed to some well known toxic compounds such as alkaloids, steroids, flavonoids in *J. curcas* leaves extract, and oleic acid and linoleic acid in seeds extract of the same species, which are known to have insecticidal activities (Gutierrez et al., 2014; Elsayed Edriss et al., 2013; Khani et al., 2012). Moreover, the mortality of mosquito larvae was also increased in relation to the time exposure from 1 to 24 h (Figures 1 and 2) which confirmed what has been determined by several authors, who showed a progressive increase in mortalities in relation to time (Elsayed Edriss et al., 2013; Okeniyi et al., 2013; Shirvani Farsani et al., 2011; Adegbite and Adesiya, 2005). Indeed, the larvicidal activity of the highest concentration

Table 2. LC_{50} for seed and leaf aqueous extracts of eight provenances of *Jatropha curcas* L. against *Culex pipiens* L.

Provenance	Exposure period (h)	CL50 of seed aqueous extracts (%) ± SD	CL50 of leaf aqueous extracts (%) ± SD
ARU	24	0.52 ± 0.02	1.00 ± 0.06
MOZ	24	0.49 ± 0.01	0.50 ± 0.01
PAR	24	0.49 ± 0.04	1.41 ± 0.02
NMB	24	0.52 ± 0.04	0.50 ± 0.02
MGB	24	0.65 ± 0.06	2.90 ± 0.03
RSB	24	0.51 ± 0.02	0.50 ± 0.03
VFB	24	0.65 ± 0.02	4.72 ± 0.05
SUR	24	0.75 ± 0.05	1.00 ± 0.07

Mean of 3 repetitions; SD = Standard deviation.

(1 mg/ml) of the leaves and seeds extracts on *C*. *pipiens* L. larvae within 24 h of exposure increased the larvae mortality.

Lethal concentrations LC_{50}

The susceptibility of *C. pipiens* L. larvae to leaves and seeds extracts of *J. curcas* L. is illustrated in Table 2, showing the LC_{50} (Lethal concentration) values obtained after 24 h of treatment. The LC_{50} confirmed that seeds extracts are more effective than leaves extracts for most provenances. This potent activity is shown by lowest LC_{50}. Analyses showed that the seeds extract of Mozambique and Paraná (Brazil) provenances are the most effective against *C. pipiens* L. larvae and showed the lowest LC_{50} value (0.49%). However, the seeds extract of Surinam provenance showed the lowest toxicity against larvae. This low toxicity is confirmed by the highest LC_{50} value (0.75%). This toxicological study revealed that the leaves extract of Mozambique and Norte de Minas (Brazil) provenances were the most toxic with a LC_{50} (0.5%) while the leaves extract of Vale do Fequitinhonha (Brazil) provenance was the less toxic one (LC_{50} = 4.72%). These results are in agreement with other studies such as the one carried out by Tomass et al. (2011). This study affirms the larvicidal impact of crude methanol leaf extract of *J. curcas* and its column chromatographic fractions against the late third instar larvae of *Anopheles arabiensis*, the major vector of malaria in Ethiopia.

In addition, a study elaborated by Ojha and Pattabhiramaiah (2013), showed that the seed oil extract of *J.curcas* can be effectively used against *Aedes aegypti* and can be considered for eco-friendly vector control programs. Gutierrez et al. (2014) unveiled that larvicidal activity of *J. curcas* leaf is supported by the abundance of phytochemicals which show synergestic effects in terms of larvicidal action to mosquito larvae. Indeed, they determined the presence of alkaloids, steroids and flavonoids in *J. curcas* leaves. Alkaloids are known to possess medicinal and pesticidal properties. These compounds can be found in the whole *J. curcas* L. plant, but are more abundant in its seeds (Haas and Mittelbach, 2000). However, several studies have shown that the major factor responsible for *J. curcas* toxicity is the high concentration in the seeds of phorbolesters (tetracyclic diterpenoids) with known tumour promoting activity (Goel et al., 2007; Makkar et al., 1997). Other toxic compounds and anti-nutritional factors in the kernel and the seed cake include flavonoids, vitexine and isovitexine and 12-deoxyl-16-hydroxyphorbol (Aregheore et al., 2003). This larvicidal activity differ based on the plant species and the part used. The presence of several bioactive chemicals like alkaloids, steroids and flavonoids can be attributed to the susceptibility of the plant extracts as killing agent against mosquito larvae. The results reported here open the possibility for further investigation on the efficacy of larvicidal properties of natural product extracts.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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