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LARVICIDAL EFFECT OF ALKALOIDS EXTRACTED FROM BITTER LUPIN SEEDS AGAINST MOSQUITOES (*CULEX PIPIENS*), FLIES (*MUSCA DOMESTICA*) AND FLEAS (*XENOPSYLLA CHEOPIS*) UNDER LABORATORY CONDITIONS IN EGYPT

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Abstract

The present work investigated the larvicidal efficacy of alkaloids extracted from Lupin seeds by water (aqueous extract) and methanol (methanol extract) against mosquitoes larvae (*Culex pipiens*), flies larvae (*Musca domestica*) and fleas larvae (*Xenopsylla cheopis)* under laboratory condition in Egypt. Alkaloids were analyzed by gas chromatography – mass spectrometry (GC-MS). Analysis revealed the presence of lupanine (100%), 13-OH-Lupanine (16.48%) and13α- Angeloyloxylupanine (1.3%) in methanol extract of *Lupinus luteus* and α- isolupanine (100%) only in aqueous extract of *lupinus luteus*. The lethal concentration LC_{50} and LC_{90} were obtained from the established regression log concentrate-response lines after 24 hours. Data indicated that the efficacy of methanol extract of alkaloids against *Culex pipiens* larvae was more effective than the effect of aqueous extract of alkaloids. The values of lethal concentration of methanol extract of alkaloids were 0.79 and 1.17 mg/ml for LC_{50} and LC_{90} respectively, while the values of lethal concentration of aqueous extract of alkaloids were 5.43 and 8.50 mg/ml for LC_{50} & LC_{90} respectively. Data also indicated that the efficacy of methanol extract of alkaloids against *Musca domestica* larvae was effective than the effect of aqueous extract of alkaloids. The values of lethal concentration of methanol extract of alkaloids were 6.40 and 19.18 mg/ml for LC_{50} &LC_{90} respectively, while the same values of aqueous extract of alkaloids were 11.20 and 34.00 mg/ml for LC_{50} and LC_{90} respectively. In addition, the efficacy of methanol extract of alkaloids against *Xenopsylla cheopis* larvae was more effective than the effect of aqueous extract of alkaloids. The lethal values concentration of alkaloids methanol extract were 10.56, 19.20 and mg/ml for LC_{50} &LC_{90} respectively, but alkaloids values of aqueous extract were 23.21 and 41.69 mg/ml for LC_{50} & LC_{90} respectively.

Key Words: Lupin seeds, Alkaloids, mosquitoes larvae, flies larvae, fleas larvae.

Introduction

A conventional method for the control of arthropod vectors of human tropical diseases (as *Culex pipiens, Musca domes-tica, and Xenopsylla cheopis*) is the uses of insecticides. The widespread and mass-ive applications of chemical insecticides frequently produce the risk of developing insect resistance and accumulation of residual insecticidal in the environment (Pohlit *et al*, 2011). Thus, many studies concerned the possibility of using plant extracts in the control of arthropod vectors of human tropical diseases (Alkafaji and Alzubaidi, 2014) instead of insecticides.

Bitter lupin (*Lupinus luteus*) seeds are used in animal and human nutrition. But, because of their high alkaloids content, the seeds could not be considered as a safe food component (Prusinski, 2015). Thus, they are used as potential sources of natu-ral insecticides (Herrera *et al*, 2009). The major alkaloids present in lupin are from the quinolizidine family, although some gramine alkaloids are found in *L. luteus*. Quinolizidine alkaloids received much att-ention, as they have a strong bitter taste and may be toxic in high doses; as lupanine, sparteine, lupinine, and some hydroxylated lupanine forms (Prusinski, 2017). The well identified alkaloids are from the lupanine group: sparteine, 5, 6-dehydro-α-isolupanine, angustifoline, α-isolupanine, 5,6-de-hydrolupanine, lupanine, 11, 12dehydrolu-panine,3αhydroxylupanine,17-oxolupanine,13α-hydroxylupanine, 13α-dihydroxylupanine,13α-angeloylolupanine,13α-tigloyloxylupanine,&4α-β-tigloyloxylupani-

ne (Michael *et al*, 2009; Frick *et al*, 2017).

Quinolizidine alkaloids (QAs) are toxic secondary metabolites that occur in the lupine. They are produced in large amounts and serve as chemical defense against pathogens and herbivores (Wink, 1988). QAs show a wide range of biological activities; they can inhibit the multiplication of viruses (Wink, 1987), the proliferation of bacteria (Tyski *et al*, 1988) and the growth of certain fungi (Wippich and Wink, 1985). Some allelopathic (phytotoxic) effects of QAs were described, including the inhibition of the growth of competing plants (Wink 1983; El Hella *et al*, 2013). They also detect a number of herbivores (nematodes, caterpillars, beetles, aphids, locusts, snails, rabbits and cows); effect on house fly (Alkafaji and Alzubaidi, 2014), as well as mosquitoes, fleas and other insect-vectors (Kullu *et al*, 2015).

The acetylcholinesterase enzyme (AChE) is known to be located on the acetylcholine receptor (AChR), which is also bound by such Quinolizidine alkaloids as lupanine. These alkaloids can activate AChE or inhibit it by influence of enzyme AChE. As in cases of Amaryllidaceae alkaloids, AChE can be inhibited. Thus, acetylcholine activity increases needed for human brain function. QA such as lupanine and sparteine inhibit Na^+ & K^+ channels, thus blocking the signal transduction in nerve cells at a second critical point (Wink and Twardowski, 1992; Wink, 1993).

The present work investigated the larvicidal efficacy of alkaloids extracted from Lupin seeds (aqueous extract, and metha-nol against *Culex pipiens* larvae, *Musca domestica* larvae and *Xenopsylla cheopis* larvae under laboratory conditions.

Materials and Methods

Preparation of *Lupinus luteus* extract: Completely dried seeds were blended into powdered using electrical blender and sieved to get fine powder. The powder was put in airtight dark vials for further analysis. The seeds powder (50g) was extracted with methanol (250ml) or water (250ml). Seeds powder was soaked separately in water or methanol in a sealed container twice at room temperature for 24hrs and filtered through Whitman filter paper No 102. Filtrate was concentrated to dryness by rotary evaporation at 40°C. After complete evaporation, concentrated extract was collected and sto-red in glass vials at 4°C in refrigerator for experimentations. Concentrated extract was dissolved in 50ml water, kept as a stock solution. Stock solution was used to prepare concentrations for mosquitoes, flies & fleas exposure (Dhawan and Gupta 2017).

Characterization of alkaloids: Alkaloids were analyzed by Gas Chromatography Mass Spectrometry Apparatus. GC-MS (Agilent Technologies) was equipped with gas chromatography (7890B) & mass spectrometer detector (5977A). Column used was HP-5MS (30mx, 250µm x 0.25µm). Flow rate of carrier gas (He) was 1.2mL /min. Samples (1µL) were injected in the split-less mode injector and detector were held at 250ºC & 280ºC, respectively. Oven temperature was used as: 100ºC for 0.5min; raised at 5ºCmin to 250ºC and held for 5min; raised at 10ºC min to 280ºC, and held for10min. Electron impact mass spectra were recorded at 70 eV. Spectral rang was from 50-550m/z. Identification of alkaloids was confirmed by comparing mass spectral data with those of authentic compounds according to literature's data (Wink, 1983; Mei-ßner *et al*, 1995; Muzquiz *et al*, 1994; 2011). More identification was done by comparison of the mass spectra with those stored in Wiley & NIST Mass Spectral libraries. The retention indices (RI) values were measured on HP-5MS column for all identified alkaloids.

Culex pipiens larvae used were obtained from Research Institute of Medical Entomology, Giza, maintained in water of their biotopes under laboratory conditions (75% R.H. & 25°C). All 3rd instars larvae selected for larvicidal activity was from the same generation and same breading sites.

Toxicity methodology was based on standardized sensitivity tests (WHO, 1970a; 1981

b). Using aqueous extract and methanol extract of alkaloids of *L. luteus*, series of methanol extract concentrations (0.5, 0.75, 0.85, 1.0 & 1.5mg/ml) and aqueous extract (3.0, 5.0, 6.0, 8.0 & 9.0mg/ml) were prepared. Twenty five 3rd instar larvae were put into a 500ml beaker containing the test solution of each concentration. Four replicates were done for each concentration. In control experiments, larvae were placed into distilled water. Larval mortalities were determined 24hr post-treatment. A larva was considered dead if did not move when prodded with a fine wooden dowel (Perumalsamy *et al*, 2010). Lethal concentrations were determined by mortality rates after 24hr exposure.

Musca domestica larvae used were obtained from Research Institute of Medical Entomology, Giza. All 3rd instars larvae for alkaloids activity were from the same generation and breading sites.

Toxicity methodology was based on standardized sensitivity tests (WHO, 1970b). A concentrations of methanol extract of alkaloids (2.5, 5.0, 10.0, 12.5 & 15.0mg/ml) and aqueous extract (4.0, 8.0, 12.0, 16.0 & 20.0 mg/ml) were prepared.

Twenty 3rd instar larvae were put into media mixed with different extract concentrations (Kristensen and Jespersen 2003). Four replicates were used for each test, and incubated under controlled conditions of 27°C, R.H. 70-75% & 12-hr light-dark regime. In control experiments, larvae were placed into normal media without treatment. Larval mortalities were determined 24hr post treatment. Lethal concentrations were determined by mortality rates after 24hr exposure.

Xenopsylla cheopis larvae used were obtained from Research Institute of Medical Entomology, Giza, maintained in water of their medium under laboratory conditions (80% R.H. &26°C). All 3rd instars larvae for alkaloids activity was from same generation and same breading sites.

Toxicity methodology was based on standardized sensitivity tests (WHO 1970c; Rust *et al*, 2014). Using aqueous and methanol extracts of alkaloids of *L. luteus*, methanol extract concentrations (4.0, 8.0, 12.0, 16.0 & 20.0mg/ml) and aqueous extract (8.0, 16.0, 24.0, 32.0 & 40.0mg/ml) were added to media of fleas larvae, in Pyrex Petri dish (2gm/ vial). Ten 3rd instar larvae were put in a glass Petri dish (60x15mm). Four replica-tes were done for each concentration. In control experiments, larvae were placed into normal media without treatment (Van Natta *et al*, 1972). Larval mortalities were determined 24hr post-treatment. Lethal concentrations were mortality rates after 24hr exposure.

Statistical analysis: Probit analysis (Finney, 1971) was used for lethal concentrations and obtaining slope values using Polo-PC & v.3.1 statistical software. Data were corrected for control mortality using Abbott's formula (1925). Lethal concentrations data was significant when corresponding confidence limits (CLs) didn't overlap (El-Sheikh *et al*, 2015).

Results

The results showed that total of 3 different alkaloids was identified (Tab.1, Fig.1) for methanol extract among which lupani ne (100%), 13-OH-Lupanine(16.48%) and 13α Angeloyloxylupanine (1.3%) were the most abundant, but only one alkaloid was identified (Tab. 2, Fig. 2) for aqueous extract which was α isolupanine (100%). Some alkaloids were not identified due to lack of reference and lack of mass spectra libraries.

Table 1: Mass spectra data of alkaloids identified in methanol extract of *Lupinus luteus*

Alkaloid	Molecular formula	RI	Area	Area sum%
Lupanine	C15H24N2O	24.544	730109496.9	100
13-OH-Lupanine	C15H24N2O2	28.853	120348395.4	16.48
13α- Angeloyloxylupanine	C20H30N2O3	34.094	9483339.87	1.3

Table 2: Mass spectra data of alkaloids identified in aqueous extract of *lupinus luteus*

Alkaloid	Molecular formula	RI	Area	Area sum%
alpha- isolupanine	C15H24N2O	24.229	19628685	100

Table 3**:** Response of *Culex pipiens* larvae to different concentrations of alkaloids.

Extract	Concentration (mg/ml)	mosquito larvae tested	Died	Alive	Mortality%
Methanol	0.5	100	16	84	16
	0.75	100	32	68	32
	0.85	100	64	36	64
	1.0	100	88	12	88
	1.5	100	96	4	96
Aqueous	3.0	100	27	73	27
	5.0	100	32	68	32
	6.0	100	52	48	52
	8.0	100	90	10	90
	9.0	100	95	5	95

Table 4: Efficacy of different concentrations of alkaloids against *Culex pipiens* larvae.

Extract	LC values (mg /ml)		LC_{90}/LC_{50}	Slope ± SE	Toxicity index		Folds based on	
	LC_{50} (lower-upper)	LC_{90} (lower-upper)			LC_{50}	LC_{90}	LC_{50}	LC_{90}
Methanol	0.79 (0.60-0.97)	1.17 (0.98-1.83)	1.48	6.52 ± 0.54	100	100	6.86	7.29
Aqueous	5.43 (4.31- 6.46)	8.50 (7.29 -11.17)	1.56	4.58 ± 0.40	14.56	13.71	1.0	1.0

Efficacy of alkaloids extracted by methanol and water against *C. pipiens* larvae was evaluated by levels of LC_{50}/LC_{90} and slope of log-concentration probit response lines after 24hrs. Efficacy of alkaloids methanol extract against mosquito larvae was more than aqueous extract ones. Levels of LC_{50} & LC_{90} for alkaloids methanol extract were 0.79±0.60 & 0.97mg/ml & 1.17±0.98 & 1.83mg/ml, respectively. LC_{50} & LC_{90} levels for alkaloids aqueous extract were 5.43±4.31 &6.46mg/ml & 8.50±7.29 & 11.17mg/ml respectively. LC_{90}/LC_{50} ratio was steepness of log-concentration probit lines in reversal way to slope value. Lower LC_{90}/LC_{50} of slope value of ratio showed high efficacy & vice versa. Slope of efficacy regression line of alkaloids methanol extract was high than aqueous one, recorded 6.52±0.54 for methanol extract & 4.58±0.40 for aqueous extract, & LC_{90}/LC_{50} ratio for alkaloids methanol extract and aqueous extract were 1.48 & 1.56 respectively. Relative efficacy for alkaloid aqueous extract was 14.56 & 13.71, respectively, and for methanol extract was 6.86 &7.29, respectively.

Table 5: Response of *Musca domestica* larvae to different alkaloids concentrations.

Extract	Concentration (mg/ml)	Fleas larvae tested	Died	Alive	Mortality%
Methanol	2.5	80	16	64	20
	5.0	80	24	56	30
	10.0	80	50	30	62.5
	12.5	80	60	20	75
	15.0	80	76	4	95
Aqueous	4.0	80	15	65	18.75
	8.0	80	20	60	25
	12.0	80	38	42	47.5
	16.0	80	48	32	60
	20.0	80	70	10	87.5

Table 6: Efficacy of different concentrations of alkaloids against flies larvae (*Musca domestica*).

Extract	Lc values (mg /ml)		LC_{90}/LC_{50}	Slope ± SE	Toxicity index		Folds based on	
	LC_{50} (lower-upper)	LC_{90} (lower-upper)			LC_{50}	LC_{90}	LC_{50}	LC_{90}
Methanol	6.40(4.15-8.89)	19.18 (12.66 - 55.53)	2.99	2.69 ± 0.26	100	100	1.51	1.77
Aqueous	11.20(7.55-17.10)	34.00 (20.54-224.13)	3.04	2.65 ± 0.29	66.07	56.41	1.0	1.0

Larvicidal efficacy of alkaloids extracted by methanol and water showed efficacy of methanol extract of alkaloids against flies' larvae were more effective than alkaloids aqueous extract. LC_{50} & LC_{90} levels for alkaloids methanol extract were 6.40±4.15 & 8.89mg/ml & 19.18±12.66 & 55.53mg/ml, alkaloids aqueous extract were 11.20±7.55 &17.10mg/ml & 34.00±20.54 & 224.13mg/ml respectively. Slope of methanol extract of alkaloids showed slightly higher than that of the alkaloids aqueous extract; 2.69±0.26 & 2.65±0.29 for methanol and aqueous extract, respectively. LC_{50} & LC_{90} ratio for methanol was slightly lower than aqueous, 2.99 & 3.03, respectively. Methanol gave high efficacy than aqueous ones. Relative efficacy of alkaloids aqueous extract was 66.07 & 56.41 respectively and for methanol extract was 1.51 &1.77, respectively.

Table 7: Response of *Xenopsylla cheopis* larvaeto different concentrations of alkaloids.

Extract	Concentration (mg/ml)	Fleas larvae tested	Died	Alive	Mortality%
Methanol	4.0	40	10	30	25
	8.0	40	16	24	40
	12.0	40	22	18	55
	16.0	40	29	11	72.5
	20.0	40	38	2	95
Aqueous	8.0	40	9	31	22.5
	16.0	40	11	29	27.5
	24.0	40	23	17	57.5
	32.0	40	29	11	72.5
	40.0	40	34	6	85

Table 8: Efficacy of different concentrations of alkaloids against *Xenopsylla cheopis* larvae.

Extract	Lc values (mg /ml)		LC_{90}/LC_{50}	Slope ± SE	Toxicity index		Folds based on	
	LC_{50} (lower-upper)	LC_{90} (lower-upper)			LC_{50}	LC_{90}	LC_{50}	LC_{90}
Methanol	10.56 (9.28 - 11.86)	19.20 (17.23 - 22.06)	1.81	2.68 ± 0.41	100	100	2.19	2.17
Aqueous	23.21 (20.52 - 26.03)	41.69 (37.32 - 48.19)	1.80	2.63 ± 0.41	45.49	46.05	1.0	1.0

Larvicidal efficacy of alkaloids extracted by methanol and water against *Xenopsylla cheopis* larvae showed that the efficacy of methanol extract of alkaloids larvae was more effective than aqueous extract. LC_{50} & LC_{90} levels for alkaloids methanol extract were 10.56±9.28 & 11.86mg/ml and 19.20± 17.23 & 22.0 mg/ml, respectively. For aqueous extract were 23.21±20.52 &26.03mg/ml and 41.69±37.32 & 48.19mg/ml, respectively. Slope of alkaloids methanol extract was slightly higher than aqueous one; 2.68±0.41 & 2.63±0.41, respectively. Ratio for methanol showed slightly higher than aqueous; 1.81 & 1.80, respectively. Methanol gave high efficacy than aqueous one. Taking LC_{50} & LC_{90} levels of alkaloids methanol extract as efficacy index for comparison, relative efficacy of aqueous extract was 45.49 & 46.05 respectively, and alkaloids aqueous extract as based fold efficacy, methanol was 2.19 &2.17, respectively.

Discussion

Lupin (*Lupinus* spp.) is an economically and agriculturally valuable plant (Sujak *et al*, 2006; Gulewicz *et al*, 2008). Its seeds are employed as a protein source for animal and human nutrition in various parts worldwide, not only for their nutritional value, but also for their adaptability to marginal soils and climates. Human consumption of lupins has increasable used (De Cortes-Sanchez *et al*, 2005). *Lupinus* is a diverse genus, though only four species have been domesticated and are agriculturally significant: *L. angustifolius* (NLL), *L. albus* (white lupin), *L. luteus* (yellow lupin), and *L. mutabilis* (pearl lupin) (Petterson *et al*, 1998). Most lupin species are toxic due to their high content of quinolizidine alkaloids and each lupin species has a characteristic quinolizidine alkaloids profile (Butler *et al*, 1996). The levels

of alkaloids in seeds or meal can be reduced through a de-bittering process involving soaking or washing with water. This is practice in Europe where high alkaloid lupins, so-called 'bitter lupins', are grown (Al-Harbi *et al*, 2014).

Quinolizidine alkaloids are called due to quinolizidine ring structure and divided into major structural classes: lupanine, angustifoline, lupinine, sparteine, multiflorine, aphylline, anagyrine and cytosine **(**Wink 1987a; Boschin and Resta, 2013; Karen *et al*, 2017). The present quinolizidine alkaloids in *L. luteus* seeds were analyzed and showed 100%lupanine, 13-OH-Lupanine (16.5%) & 13α-Angeloyloxylupanine (1.3%) in methanol extracts of *L. luteus* and 100%α- isolupanine only in aqueous extract of *L. luteus*. Some alkaloids were unidentified due to lack of reference substances and mass spectra libraries (Meibner *et al*, 1995; Muzquiz *et al*, 2011; Karen *et al*, 2017).

The toxic effect of quinolizidine alkaloids such as lupanine, 13-OH-Lupanine & 13α-angeloyloxylupanine due to acetylcholine receptors and Na+/K+ channels modulated them (Paolisso *et al*, 1985; Korcz *et al*. 1987; Wink *et al*,1992; 1993), so QAs offer protection against insect pests (Philippi *et al*, 2015).

In the present work, the alkaloids methanol extract were more efficacy than aqueous one, since the LC_{50} recorded 0.79mg/ml for methanol extract and 5.43mg/ml for aqueous extract against *C. pipiens*, and 6.40mg/ml for methanol extract and 11.20mg/ml for aqueous extract against *M. domestica* and 10.56mg/ml for methanol extract and 23.21 mg/ml for aqueous extract against *X. cheopis*, respectively. Isman (2006) reported that phytochemicals such as alkaloids, phenols and terpenoids, alone or in combination, contribute to acute toxicity toward various arthropod-vectors. Alkafaji and Alzubaidi (2014) evaluated alkaloids extract effect from *Amaranthus gracilis* (L.) on eggs of *M. domestica*, and reported that highest mortality rate 83.3% at 10.0mg/ml and followed by 44.4% & 31.1% at 7.5mg/ml & 5mg/ml, respectively. Also, there were mortality rates of eggs, larvae and pupae that increased with increasing concentration. Khatter and Abuldahab (2012) reported that *Calotropis procera* (Family: Asclepidaceae) contains alkaloids, methanol extract with potential control of *M. domestica* larvae that caused reduction in total protein, increased the mean total carbohydrate, and increased lipid contents. Toxic *C. procera* groups increase conversion rate of carbohydrates to lipids and stored in fat tissues. Suresh *et al.* (2017) reported that *Couroupita guianensis* contains phenol as major component along with alkaloids, methanol extract exhibited larvicidal action for *M. domestica*, as well as larvicidal effects of the leaf extract on the early 3rd instar larvae were active with concentration of 62.5 to 500ppm. Among the extracts ethyl acetate extract of *C. guianensis* gave good larvicidal activity with LC_{50} & LC_{90} of 479.137 & 1969.851ppm against *M. domestica* larvae, respectively. Ogbalu *et al.* (2014) reported that nicotine and related alkaloids nornicotine and anabasine from aqueous extract of tobacco, induced highly insecticidal effects as they synaptic poisons that mimic the neurotransmitter acetylcholine with poisoning symptoms similar to those with organophosphate and carbamate insecticides (El Bahmasawy *et al*, 2014; 2015). They tested the tobacco leaf which grounded and mixed in distilled water for its larvicidal effect on the third instars of *M. domestica*, using 1mg/l, 2mg/l & 3mg/l were 11.05%, 72.6% & 97.8%, respectively. Pavela and Govindarajam (2017) reported that the benzenoids, triterpenes, and alkaloid identified in the essential oil of *Zanthoxylum monophyllum* had potent larvicidal activity with LC_{50} & LC_{90} of 41.50 and 82.19µg/ml to toward *Anopheles subpictus*, and for *Aedes albopictus;* 45,35 & 88.07µg/ml and for *Culex tritaeniorhynchus* gave 49.01 & 92.08 µg/ml. Komalamisra *et al*. (2005) found that methanol extracts from *Rhinacanthus nasutus, Derris elliptica, Trigonostemon reidioides,*

Homalomena aromatica, Stemona tuberosa and Acorus calamus possessed high larvicidal activity against *Ae. aegypti, C. quinquefasciatus, An. dirus and Mansonia uniformis*. Larvicidal activity with LC_{50} < 50mg/l was active, LC_{50} between 50mg/l & 100mg/l was moderately active, LC_{50} between100mg/l & 750mg/l was effective, and LC_{50} > 750mg/l was inactive. Talontsi *et al*. (2011) reported that four alkaloids were isolated from *Zanthoxylum lemairei* (Rutaceae) was active against *Anopheles gambiae* larvae, with complete mortalities within 24hr. The larvicidal action showed that compound 10-o-dmethyl-17-o-methyli-soarnottianamid and compound 6- acetonl-N-methyl-dihydrodecarine were best potent with mortality rates of 96.7% & 98.3% at 250mg/l, respectively. Sung-Eun Lee (2002) showed that a methanol extract of *Piper longum* fruit was active against larvae of *C. pipiens* at 10ug/ml after 24hr. A piperidine alkaloid, pipernonaline, was responsible for this activity, with 24-hr LC_{50} value of 0.21 mg/liter. Abo El-Mahasen and Mahmoud (2016) found that essential oils from *linseed, Linum usitatissimum, watercress, Nasturtium officinale* and black seed *Nigella sativa* gave larvicidal activity against 3rd instar larvae *C. pipiens.* since the results showed that all the three tested oils induced larval mortality, watercress oil was more effective followed by linseed and black seed oil and with dose dependent and time of exposure, and has phytochemical, as isofalvonoids, essential oils, saponin, steroids, alkaloids and tannins with larvicidal action. Several essential oils have been reported to possess insecticidal action against mosquitoes. Simon-Oke *et al*. (2015) reported that the phytochemical screening of the perennial herb, *Solanum xanthocarpum* revealed saponin and alkaloids in fruits. The extracts result showed after 24hrs exposure on Culicine species mosquito larvae at 1ml concentration, mortality of 86.67%, at 5ml concentration, mortality of 90.0%, & 100% with 1ml-5ml concentration after 48 hrs. Guerrini and Kriticos (1998) found that azadirachtin-containing seed extract has a powerful insect growth regulator, a feeding deterrent and repellent with low toxicity, since methanolic extracts with 200, 1000 or 2400 ppm azadirachtin reduced fleas in a dose-dependent manner in contaminated environments. Marc *et al.* (2007) evaluated the biocidal activity of three steam distilled wood essential oils, incense cedar (*Calocedrus decurrens*) Florin, Port-Orford-cedar (*Chamaecyparis lawsoniana*) Parl, and western juniper (*Juniperus occidentalis*), heartwood against *Aedes aegypti* (L.) *Ixodes scapularis* and *Xenopsylla cheopis*, and that wood-derived essential oils such as incense and Port-Orford-cedar and western juniper were useful in controlling mosquitoes, ticks, and fleas. Since Incense cedar heartwood was the most toxic to all three vector species followed in order of activity by western juniper and Port-Orford-cedar based on LC_{50} & LC_{90} values. *Ae. aegypti* were more susceptible to oils than either *I. scapularis* or *X. cheopis*.

Conclusion

Alkaloids extracted from lupin seeds by water & methanol proved to good larvicidal agent against larvae *C. pipiens*, *M. domestica* and *X. cheopis* under laboratory condition. They are relatively harmless to non-target organism and present little risks to users and consumers as plant-products are culturally acceptable, economical and locally available.

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Explanation of figures

Fig. 1:- GC-MS chromatogram obtained from alkaloids isolated from methanol extract of *Lupinus luteus*, abundance of Lupanine (RT: 24.544), 13-OH-Lupanine (RT: 28.853), and13α- Angeloyloxylupanine (RT: 34.094).

Fig. 2:- GC-MS chromatogram obtained from alkaloids isolated from aqueous extract of *Lupinus luteus*. abundance of a-Isolupanine (RT:24.229), other substance not alkaloid such as Octasiloxane,1,1,3,3,5,5,7,7,9,9,11,11,13,13,15,15-hexadecamethyl (RT:39.238).