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Evaluation of mosquitocidal activities of *Lepidium sativum* L. and *Millettia ferruginea* (Hochst) Baker essential oils against the malaria vector, *Anopheles gambiae sensu lato* (Diptera: Culicidae)

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Abstract
Anopheles gambiae sensu lato is one of the important mosquito species transmitting *Plasmodium falciparum* in several parts of Ethiopia. In order to find out eco-friendly vector control sources, the present study was initiated to isolate essential oils from *Lepidium sativum, Millettia ferruginea* and their bio-potential against immature and adult *Anopheles gambiae*. This plant was indigenous to Ethiopia and traditionally used by local people to control pest and vectors. The mosquitocidal properties of the essential oils were tested following World Health Organization (WHO) recommended a protocol. After 72 hr exposure period, 96.00 and 100.00% IInd instar larva mortality was observed at 100 ppm concentration of *L. sativum* and *M. ferruginea* respectively. In IVth instar larva, 91.00 and 96.00% mortality was recorded at 100 ppm concentration of *L. sativum* and *M. ferruginea* respectively after 72 hr. The pupal mortality was 99 and 100% in *L. sativum* and *M. ferrugiena* essential oil respectively at 100 ppm concentration after 72 hr. After 1 hr exposure period, median knockdown time of 7.34 minute was recorded at 10% concentration of *M. ferruginea* essential oil impregnated filter paper. The percentage of adult mortality was 95.66% and 82.66% exposed to 10% concentration of *M. ferruginea* and *L. sativum* essential oil respectively. Results clearly demonstrate the strong mosquitocidal potential of *L. sativum* and *M. ferruginea* essential oils against *An. gambiae*. However, further formulation and field evaluation are important for large scale field application to control the population of *An. gambiae.*

Keywords: *Anopheles gambiae,* essential oil, knockdown, larvicidal, *Lepidium sativum, Millettia ferruginea,* pupicidal

1. Introduction
Plants with bio-potential secondary metabolites have played a significant role in traditional medicine and insect pest management since the origin of human life. From medicinal plants, several novel compounds have been isolated, commercialized and utilized for various purposes globally. *Lepidium sativum* an annual spice crop, native to Egypt and West Asia belongs to the family Brassicaceae and commonly referred to as garden cress [1]. This plant is cultivated in different agro-ecological zones in the highlands of Ethiopia along with cereal crops for medicinal purposes [2]. The plant is used to treat abdominal ache, diarrhea, scorbutic disease, hepatic complaints, skin and other internal problems in livestock [2, 3]. In addition to the medicinal uses, bioactivity of the aqueous extracts has been reported against early-stage nymph of the agricultural pest *Bemisia tabaci* and also to prevent adult emergence from the pupae [4]. The seeds contain several bioactive secondary metabolites such as flavonoids, coumarins, sulphur, glycosides, triterpenes, sterols and imidazole alkaloids [5-7]. According to published work in Ethiopia, mosquitocidal properties of *L. sativum* essential oil was inadequate. Therefore, this plant was selected for isolation of essential oil and to evaluate against *An. gambiae* vector mosquitoes.
Millettia ferruginea commonly called Birbira belonging to the family Leguminosae subfamily Papilionoidea is endemic, indigenous, and a nitrogen-fixing tree species planted with other crops and coffee plants in Ethiopia. The seed and root extracts contain rotenone used as

insecticide or pesticide [8, 9]. The water extract of the seed powder was reported to kill 93 to 100% adult *Macrotermes* termites and 45-65% sorghum chaffer, *Pachnoda interruptua* [10]. The toxic effect of polar solvent seed extracts was confirmed against *Sitophilus zeamais* by filter paper bioassay method [11]. The faba bean treated with essential oil of the seeds to prevent damage caused by Adzuki bean beetle, *Callosobruchus chinensis* [12, 13]. The aqueous extract of seed powder was reported to be highly toxic to macroinvertebrates [14]. The maize plants are grown in the plot sprayed with 5% concentration of aqueous crude extract reduce the damage caused by the larvae of maize stem borer *Busseola fusca* [15]. The methanol extract of the seeds reported to kill the larvae, pupae, and adults of laboratory-reared and field strains of *Anopheles arabeinsis* [16]. The acetone extract of the seed caused 100% larval and adult mortality of the tick *Boophilus decoloratus* [17]. Therefore, this plant essential oil was selected to evaluate immature and adult mosquitocidal properties against *An. gambiae.*

Malaria is one of the major public health problem in Ethiopia and it is estimated that 68% of the population is at the risk of disease [18, 19]. The anuual deaths of people with malaria infection in Ethiopia was decresed from 2.1 to 1.1/100 000 between 2011 to 2016 due to various intervention including the distribution of insecticides treated net (ITN), long lasting insecticides spray (LLINS) and insecticide residual spray (IRS) [20]. Eventhough, significant malaria morbidity and mortality was reported in the county till many districts malaria incidence is not under control. *Anopheles gambiae sensu lato* is one of the important mosquito species transmitting *Plasmodium falciparum* in several parts of Ethiopia. These mosquito species breeds at the river and stream edges, temporary rain pools, ponds, dams, drainage ditches, burrow pits, rice fields, swamp margins and roadside puddles and tree holes [21]. In order to find out eco-friendly vector control sources, the present study was initiated to isolate essential oils from *Lepidium sativum, Millettia ferruginea* and their bio-potential against immature and adult *Anopheles gambiae*. This plant was indigenous to Ethiopia and traditionally used by local people to control pest and vectors.

2. Materials and Methods

2.1 Laboratory rearing of *Anopheles gambiae*

Anopheles gambiae immature stages were collected from suspected breeding sites at Kolla Diba district of Northwest Ethiopia due to successive malaria epidemic with high mortality in the past years. The immature mosquitoes were collected during September and October 2016 from stagnant water in hoof print, temporary flood accumulated places around the selected area. The immature stages were collected using kitchen strainer and transferred to wide- mouthed 1 L capacity plastic container ¾th filled with water. The container mouth was covered with muslin cloth for aeration and brought to General Entomology laboratory, Maraki campus, University of Gondar. The four larval instar stages such as I, II, III, and IV was separated and allowed to acclimatize in deionized water half-filled in a tray (25 cm X 12 cm X 12 cm). The larval instar stages were identified based on larvae size and morphological features as described by Walker and Lynch [22]. *Anophele gambiae s.l.* with other *Anopheles* species were confirmed based on morphological character identification key for adults and larvae [23]. In the laboratory, larvae were provided with powdered dog biscuit with yeast powder (3:1 ratio) as a feed. Once the larva reached the pupal stage, they were collected separately and kept in an adult emergence cage. After adult emergence, 10% sucrose solution (w/v) soaked cotton was provided *ad libitum* access to adult mosquitoes and kept in Bugdorm cages (30 cm x 30 cm x 30 cm; Mega View Science, Taiwan). The colony was maintained at 27 ± 1 °C, 65–70% relative humidity and 12:12 hr light: dark photoperiod cycle. Throughout the experiment, a sufficient number of larvae were collected from the field, maintained in the laboratory and used for bioassay.

2.2 Essential oil extraction

Fresh seeds of *Lepidium sativum* and *Millettia ferruginea* were collected in and around Gondar. The seeds were thoroughly washed with distilled water and shade dried by spreading on a clean and well-ventilated surface in the laboratory. After complete drying, seeds were ground into fine powder using an electric blender. The powdered seeds were sieved through a kitchen strainer to obtain a fine powder for oil extraction. Two hundred grams of seed powder from each plant was mixed with 1000 mL of distilled water individually in a conical flask. The plant parts were subjected to essential oil extraction by the hydro-distillation method at 100 °C using a Clevenger apparatus for 3 hrs. The extracted oil was passed through cotton wool containing anhydrous sodium sulphate to remove moisture and kept in light insensitive glass bottles and stored at 4 °C [24]. The stock oil was used to prepare different concentrations and utilized for toxicity bioassay against *An. gambiae*.

2.3 Preparation of stock and experimental concentrations

From the extracted oil, a stock concentration of 10,000 ppm was prepared by adding 1 mL of pure essential oil mixed with 1 mL of acetone and made up to 100 mL in 250 mL conical flask by adding distilled water. Four experimental concentrations viz., 100, 75, 50, 25 ppm was selected based on preliminary wide range tolerance test conducted at 10, 100, 1000 ppm concentration. For adult mosquitoes, mortality and knockdown effect of essential oil was conducted by using the impregnated filter paper bioassay method. Four concentrations viz., 0.5, 2.5, 5.0 and 10.0% ppm was selected for filter paper impregnation. In addition, control contains 1mL of acetone and the amount of distilled water varied according to parallel concentration prepared [25].

2.4 Evaluation of the larvicidal activity of the essential oil

Larvicidal activity of essential oils extracted from the seeds of *L. sativum* and *M. ferruginea* was evaluated by using the standard World Health Organization method [26]. Twenty-five IInd and IVth instar larva were selected from the culture and released into 250 mL plastic beaker individually. In each beaker, a concentration of essential oil was maintained at 0.00 (control), 25, 50, 75 and 100 ppm by adjusting the final water volume of 200 mL. The experimental condition was 27±1 °C temperature and 65-70% relative humidity. Each concentration replicated four times and an equal number of mosquito larvae were released. The larval mortality was recorded after 24, 48 and 72 hr post-exposure period [27].

2.5 Evaluation of the pupicidal activity of the essential oil

The pupicidal activity of selected essential oils was tested against freshly emerged pupae collected from the culture. The concentration of plant essential oils and methods were

followed as explained in larvicidal activity. For each concentration, 25 freshly emerged pupae were released into the plastic beaker and covered with nylon mesh to prevent adult dispersal. The number of dead pupa from each concentration was recorded continuously from all the four replications after 24, 48 and 72 hr post-exposure period. The pupal mortality was calculated and corrected as per Abbott's formula.

2.6 Evaluation of the adulticidal activity of the essential oil

The knock-down activity of selected plant essential oil was performed against un- blood fed adult mosquitoes following the protocol of World Health Organization [26]. Based on the preliminary screening results four different concentrations viz., 0.5, 2.5, 5.0 and 10.0% ppm was prepared and impregnated with Whatman no. 1 filter paper. The Whatman filter paper treated with bendiocarb (0.1%) was used as a positive control and acetone was used as negative control. The toxic and knock-down bioassay was conducted by WHO adult test tubes. Each test tube, three to five days old sugar fed adult mosquitoes were exposed and live, knocked down mosquitoes were recorded continuously 1 hr with every 5-minute interval. After 1 hr exposure period, mosquitoes were transferred into recovery test tubes and observed for 24 hr. During the recovery period, 10% sucrose solution imbibed cotton was placed on the mesh screen of the tubes as adult feed. Throughout experimental period temperature was maintained at 27±1 °C and relative humidity of 65-70%. The number of knockdown and dead mosquitoes were recorded from four replication and percentage mortality was corrected and calculated by using Abbott's formula.

2.7 Statistical analysis

The number of dead immature and mature mosquitoes from the experiments and control groups were converted into percentage mortality. The percentage mortality in control exceeds 5% was corrected as per Abbott's formula [28]. Corrected% mortality = (% mortality in test -% mortality in control)/ (100-% mortality in control) X 100. One way ANOVA followed by post hoc test LSD (Least Significant Difference) was used to compare mean percentage mortality within the concentration tested. Probit analysis was carried out to calculate LC_{50} and LC_{90} concentrations and lower confidence limit (LCL) and upper confidence limit (UCL). *Chi*-square ($\chi 2$) analysis was used to confirm statistical significance within the concentrations of plant essential oil tested. All levels of statistical significance were determined at 5% level ($P< 0.05$). The SPSS version 20 software for Windows 7 was used to carry out the statistical analysis.

3. Results

3.1 Mortality of immature *Anopheles gambiae* exposed to *Lepidium sativum* essential oil

The mean percentage mortality of IInd and IVth instar larvae and pupae of *An.gambiae* exposed to different concentrations of *L. sativum* essential oil after 24, 48 and 72 hr exposure periods were presented in Table 1. Among the concentrations tested, IInd instar larvae exposed to 75 ppm concentration observed 96.00% mortality after 48 hr. After 72 hr exposure period 97.00% mortality was recorded at 100 ppm concentration. The calculated LC_{50} and LC_{90} values for 24 hr exposure period were 317.8 and 437.1 ppm respectively. The calculated 95% LCL and UCL of LC_{50} concentration was 136.6 and 182.7 ppm respectively for 72 hr exposure period. The $\chi 2$ analysis results showed statistically significant difference within the concentration tested ($\chi 2 = 122.57$; $P = 0.000$ for 24 hr; $\chi 2 = 62.43$; $P=0.000$ for 48 hr and $\chi 2=204.94$; $P=0.000$ for 72 hr). The percentage mortality of IVth instar larvae exposed to 100 ppm concentration was 91.00% after 72 hr. The calculated LC_{50} and LC_{90} concentration was 337.3 and 466.4 ppm respectively after 72 hr exposure period. The calculated 95% LCL and UCL of LC_{50} was 210.1 and 456.5 ppm respectively after 72 hr exposure period. The $\chi 2$ analysis results showed statistically significant difference within the concentration tested ($\chi 2 = 44.93$; $P = 0.000$ for 24 hr; $\chi 2 = 100.19$; $P=0.000$ for 48 hr and $\chi 2=1372.59$; $P=0.000$ for 72 hr). The pupal mortality was 99.00% and 98.00% at100 ppm and 75 ppm concentration respectively after 72 hr exposure period. The calculated LC_{50} and LC_{90} concentrations was 245.3 and 344.2 ppm respectively after 72 hr exposure period. The calculated 95% LCL and UCL of LC_{50} concentration was 186.8 and 297.3 ppm respectively after 72 hr exposure period. The $\chi 2$ analysis results showed statistically significant difference within the concentration tested ($\chi 2 = 133.53$; $P= 0.000$ for 24 hr; $\chi 2= 566.53$; $P=0.000$ for 48 hr; $\chi 2=792.39$; $P = 0.000$ for 72 hr).

3.2 Mortality of immature *Anopheles gambiae* exposed to *Millettia ferruginea* essential oil

The mean percentage mortality of IInd and IVth instar larvae and pupae of *An. gambiae* exposed to different concentrations of *M. ferruginea* essential oil after 24, 48 and 72 hr exposure periods were presented in Table 2. Among the concentrations tested, the IInd instar larvae exposed to 75 and 100 ppm 100.00% concentration showed 100.00% mortality after 72 hr exposure period. The calculated LC_{50} and LC_{90} concentration was 327.5 and 408.2 ppm respectively for 24 hr exposure period. The calculated 95% LCL and UCL of LC_{50} concentration was 317.1 and 337.9 ppm respectively for 24 hr. The $\chi 2$ analysis results showed statistically significant difference within the concentration tested ($\chi 2 = 52.78$; $P = 0.000$ for 24 hr; $\chi 2 = 36.75$; $P=0.000$ for 48 hr and $\chi 2=2183.21$; $P=0.000$ for 72 hr). The IVth instar larvae exposed to 100 ppm concentration after 72 hr observed 96.00% mortality. The calculated LC_{50} and LC_{90} concentration was 284.2 and 457.7 ppm respectively for 72 hr. The calculated 95% LCL and UCL of LC_{50} concentration was 265.5 and 302.4 ppm respectively after 72 hr. The $\chi 2$ analysis results showed statistically significant difference at 5% level within the concentration tested ($\chi 2 = 37.9$; $P = 0.000$ for 24 hr; $\chi 2 = 56.47$; $P=0.000$ for 48 hr and $\chi 2=80.09$; $P=0.000$ for 72 hr). The pupae exposed to 100 ppm and 75 ppm concentration, the percent mortality was 100% and 98.00% respectively after 72 hr exposure perior. The calculated LC_{50} and LC_{90} concentration was 351.8 and 446.6 ppm respectively for 24 hr. The calculated 95% LCL and UCL of LC_{50} concentration were 322.0 and 380.9 ppm respectively after 24 hr exposure period. The $\chi 2$ analysis results showed statistically significant difference within the concentration tested ($\chi 2 = 298.87$; $P= 0.000$ for 24 hr; $\chi 2= 6388.67$; $P=0.000$ for 48 hr; $\chi 2=3178.78$; $P = 0.000$ for 72 hr).

3.3 Knock downtime (KDT_{50}) and toxic effect of essential oil against adult *An. gambiae*

The median knock downtime (KDT_{50}) observed for adult non-blood fed female *An. gambiae* after a 1hr exposure period to

essential oils of *L. sativum* and *M. ferruginea* is presented in Table 3. After the exposure period, KDT_{50} was 7.34 minute at 10% concentration of *M. ferruginea*. However, KDT_{50} for *L. sativum* oil at the same concentration was 10.05 minute. The KDT_{50} observed at 2.5% concentration of *M. ferruginea*; 5 and 10% concentration of *L. sativum* and *M. ferruginea* were comparatively better than bendiocarb 0.1% impregnated filter paper.

Percentage mortality (X-axis) of adult *An. gambiae* exposed to 10% concentration of essential oils of *L. sativum* and *M. ferruginea* (Y-axis) is depicted in Figure 1. Results revealed that 100% of mortality was observed in positive control bendiocarb. However, *M. ferruginea* and *L. sativum* percent mortality were 95.66 ± 2.4 and 82.66 ± 3.48% respectively compared to negative control (7.33 ± 0.88).

4. Discussion

Eco-friendly vector control strategies are currently needed to prevent environmental pollution caused by synthetic chemicals, negative consequences to non-target organisms and also to control insecticide resistance mosquito species. Mosquito control in the larval stage is worthwhile to minimize the emergence of the adult population and thereby reduce the risk of spreading vector-borne diseases. The mosquitoes breeding in small habitats such as small ponds, marshes, ditches, pools, drains, water containers and any other utensils holding water can easily be managed with locally available resources. In this study, essential oils of *L. sativum* and *M. ferrugina* was tried in the laboratory against immature and adult stages of *An. gambiae* mosquitoes to collect baseline data for the development of eco-products.

The present results confirmed toxic activities of essential oils extracted from *L. sativum* and *M. ferruginea*. The mortality rate of IInd and IVth instar larvae and pupae varied significantly based on a concentration of the plant extracts tested and period of exposure. The results are incomparable with an earlier report of Singh *et al.* [29] who have observed the larvicidal activity of *Ocimum canum* oil against different species of vector mosquitoes. The high percentage mortality observed in the *L. sativum* oil may be associated with the accumulation of secondary metabolites in seeds. Those chemicals may be blocking the respiratory activity and also alter various physiological mechanisms in treated mosquito larvae thereby mortality rate increased. Earlier authors reported seed extracts of *L. sativum* to contain flavonoids, coumarins, sulphur, glycosides, triterpenes, sterols and imidazole alkaloids [5-7]. Among the various compounds reported earlier either anyone or in the group may be toxic to kill the larvae. In addition, the toxic effect of aqueous extract of *M. ferruginea* was also reported against potato whitefly *Bemisia tabaci*. The percentage mortality observed with this plant extracts and Imidocloprid was statistically not significant [4].

The essential oil of *M. ferruginea* at 75 and 100 ppm concentration after 72 hr exposure period 100% mortality rate was observed. The percentage mortality of IInd and IVth instar larvae showed that as the concentration of the plant extract increased, percentage mortality was also increased. The oil of *M. ferruginea* may be toxic to mosquito larvae. The present findings are in corroborate with earlier reports of Andemo *et al.* [16] who have have observed 100% mortality of *An. arabiensis* larvae exposed to *M. ferruginea* methanol extract. In addition, toxic principles of this plant extracts were confirmed by several authors against pest and vectors [10, 11, 13, 15-17]. Toxic effect of botanicals was confirmed by several researchers, for example, *Jatropha curcus* [30] and *Tribulus Terrestris* [31] against *An. arabiensis* larvae. The result of *Dianthus caryophyllus, Lepidium sativum, Pimpinella anisum*, and *Illicium verum* and their major component to control West Nile vector *Culex pipiens* reported by Kimbaris *et al.* [32] was also comparable with present findings for the utilization of *M. ferruginea* essential oil in a vector control program.

Adult non-blood fed female *An. gambiae* after 1 hr exposure period knockdown time to kill 50% adult exposed population was 7.34 min at 10% concentration of *M. ferruginea* followed by *L. sativum* (10.05 min.). In general, KDT_{50} values recorded for 5 and 10% concentration of *L. sativum* and 2.5, 5 and 10% concentration of *M. ferruginea* was comparatively less than bendiocarb 0.1% impregnated filter paper. Present results regarding the percentage of adult mortality of *An. gambiae* showed 100% in bendiocarb. However, *M. ferruginea* and *L. sativum* treatment was 95.6 and 82.6% respectively after 24 hr at 10% concentration. In the negative control, by using 1% acetone impregnated filter paper the percentage mortality was 0. The adult percentage mortality may be associated with the toxic nature of the compound. Positive control showed 100% mortality because of pure and proved chemical impregnated filter paper and the concentration was recommended by WHO. In the present findings, even though the compounds are not isolated but the oil may have a mixture of different bioactive molecules that may responsible to kill the adult mosquitoes. The present results are in agreement with the report of Andemo *et al.* [16] they have reported 100% adult mortality of *An. arabiensis* treated with methanol extract of *M. ferruginea* seeds. Present study percentage mortality of *An. gambiae* exposed to *M. ferruginea* and *L. sativum* essential oil may be associated with the nature of phytochemicals and dissolving nature of those chemicals in respective solvents. Earlier the toxic compound rotenone was isolated from the seeds of *M. ferruginea* and reported [33]. The rotenoids have been used to prepare insecticides since 1848 [34]. The present findings also agreed with the earlier reports that the percentage mortality of *M. ferruginea* may be associated with rotenone.

Table 1: Mean percentage mortality of immature *Anopheles gambiae* exposed to *Lepidium sativum* essential oil

***An.gambiae* stages**	**Concentration in ppm**	**Exposure period in time**		
		24 hr	**48 hr**	**72 hr**
IInd instar	Control	0.00 ± 0.00	1.00 ± 1.00	5.00 ± 1.91
	25 ppm	10.00 ± 2.58	19.00 ± 2.88	83.75 ± 2.01
	50 ppm	26.00 ± 2.16	58.50 ± 3.30	94.00 ± 2.58
	75 ppm	91.00 ± 2.00	96.00 ± 3.26	96.00 ± 1.63
	100 ppm	96.00 ± 1.62	97.00 ± 3.82	97.00 ± 1.91
	LC_{50} (LCL - UCL)	317.8 (297.8 - 337.0)	267.7 (253.7 – 281.3)	160.4 (136.6 – 182.7)
	LC_{90} (LCL - UCL)	437.1 (404.5 – 491.0)	389.9 (365.9 – 423.9)	261.7 (226.6 – 325.1)

	$\chi 2$	122.57	62.43	204.94
	P value	P = 0.000	P = 0.000	P = 0.000
IVth instar	Control	0.000 ± 0.00	1.00 ± 1.00	2.00 ± 1.15
	25 ppm	5.00 ± 1.91	7.00 ± 1.91	8.00 ±1.63
	50 ppm	20.00 ± 4.32	29.5.50 ± 6.50	11.00 ± 1.91
	75 ppm	47.00 ± 5.18	60.00 ± 1.63	86.50 ± 5.50
	100 ppm	74.00 ± 2.58	88.00 ± 1.63	91.00 ± 3.26
	LC_{50} (LCL - UCL)	403.3 (385.5 - 423.5)	354.2 (331.0 - 378.7)	337.3 (210.1 – 456.5)
	LC_{90} (LCL – UCL)	653.7 (597.1 - 740.5)	564.0 (507.0 - 684.0)	466.4(379.0– 2830.4)
	$\chi 2$	44.93	100.19	1371.59
	P value	P = 0.000	P = 0.000	P = 0.000
Pupae	Control	0.00 ± 0.00	2.00 ± 1.91	6.00 ± 2.58
	25 ppm	3.00 ± 1.91	6.00 ± 2.58	7.00 ± 1.91
	50 ppm	33.50 ± 2.98	46.50 ± 3.86	85.00 ± 1.91
	75 ppm	91.00 ± 2.51	96.00 ± 1.63	98.00 ± 1.15
	100 ppm	93.00 ± 4.43	94.00 ± 3.82	99.00 ± 1.00
	LC_{50} (LCL – UCL)	321.5 (301.7 - 340.7)	292.9 (241.0 – 340.2)	245.3 (186.8 – 297.3)
	LC_{90} (LCL – UCL)	429.1 (398.0 – 481.5)	224.0 (360.6 – 635.1)	344.2 (286.5 – 600.1)
	$\chi 2$	133.53	566.53	792.39
	P value	P = 0.000	P = 0.000	P = 0.000

Mortality values are mean ± standard error of four replications. LC_{50} indicates 50% mortality of exposed immature mosquitoes. LCL –UCL- lower confidence limit and upper confidence limit concentration of the essential oils.

Table 2: Mean percentage mortality of immature *Anopheles gambiae* exposed to *Millettia ferruginea* essential oil.

***An.gambiae* stages**	**Concentration in ppm**	**Exposure period in time**		
		24hr	**48hr**	**72hr**
IInd instar	Control	0.00 ± 0.00	0.00 ± 0.00	5.00 ± 1.91
	25 ppm	2.00 ± 1.15	4.00 ± 1.63	7.00 ± 1.91
	50 ppm	26.00 ± 2.16	58.50 ± 6.60	94.00 ± 2.58
	75 ppm	91.00 ± 2.00	99.00 ± 2.00	100.00 ± 0.00
	100 ppm	98.00 ± 2.30	100.00 ± 0.00	100.00 ± 0.00
	LC_{50} (LCL - UCL)	327.5(317.1- 337.9)	285.6 (277.1 - 293.5)	Not calculated
	LC_{90} (LCL - UCL)	408.2(391.1- 431.8)	353.0 (340.9 -369.1)	Not calculated
	$\chi 2$	52.78	36.75	2183.21
	P-value	P = 0.000	P = 0.000	P = 0.000
IVth instar	Control	0.000 ± 0.00	1.00 ± 1.00	2.00 ± 1.15
	25 ppm	5.00 ± 1.91	18.00 ± 2.58	19.50 ±2.62
	50 ppm	23.00 ± 3.41	34.50 ± 2.98	45.50 ± 3.30
	75 ppm	47.50 ± 5.18	60.00 ± 3.26	86.50 ± 2.75
	100 ppm	79.00 ± 2.58	88.00 ± 1.63	96.00 ± 1.63
	LC_{50} (LCL - UCL)	400.0(383.3- 418.7)	334.7 (315.3 - 355.4)	284.2(265.6- 302.4)
	LC_{90} (LCL – UCL)	659.6(604.9- 740.6)	609.8 (548.8 - 704.4)	457.7(419.2- 516.3)
	$\chi 2$	37.9	56.47	80.09
	P-value	P = 0.000	P = 0.000	P = 0.000
Pupae	Control	0.00 ± 0.00	3.00 ± 1.91	6.00 ± 2.58
	25 ppm	3.00 ± 1.91	6.00 ± 2.58	7.00 ± 1.91
	50 ppm	8.00 ± 1.63	11.00 ± 1.91	15.00 ± 1.91
	75 ppm	91.00 ± 2.51	96.00 ± 1.63	98.00 ± 1.15
	100 ppm	97.00 ± 3.82	99.00 ± 1.91	100.00 ± 0.00
	LC_{50} (LCL – UCL)	351.8(322.0- 380.9)	Not calculated	Not calculated
	LC_{90} (LCL – UCL)	446.6(407.6- 534.0)	Not calculated	Not calculated
	$\chi 2$	298.87	6388.67	3178.78
	P-value	P = 0.000	P = 0.000	P = 0.000

Mortality values are mean ± standard error of four replications. LC_{50} indicates 50% mortality of exposed immature mosquitoes. LCL –UCL- lower confidence limit and upper confidence limit concentration of the essential oil.

Table 3: Knock down time (KDT_{50}) taken for female adult *Anopheles gambiae* exposed to *Lepidium sativum* and *Millettia ferruginea* essential oils after 1 hr.

Essential oil	**Concentration in percentage**			
	0.5%	**2.5%**	**5%**	**10%**
Lepidium sativum	31.13 ± 0.6	23.05 ± 0.2	13.25 ± 0.1	10.05 ± 0.5
Millettia ferruginea	35.15 ± 0.4	19.7 ± 0.6	11.15 ± 0.2	7.34 ± 0.2
Conrol (Bendocarb 0.1%)	22.75			

KDT_{50} = Knock down time required to kill 50% of the exposed population. Values are mean ± standard error of four replications.

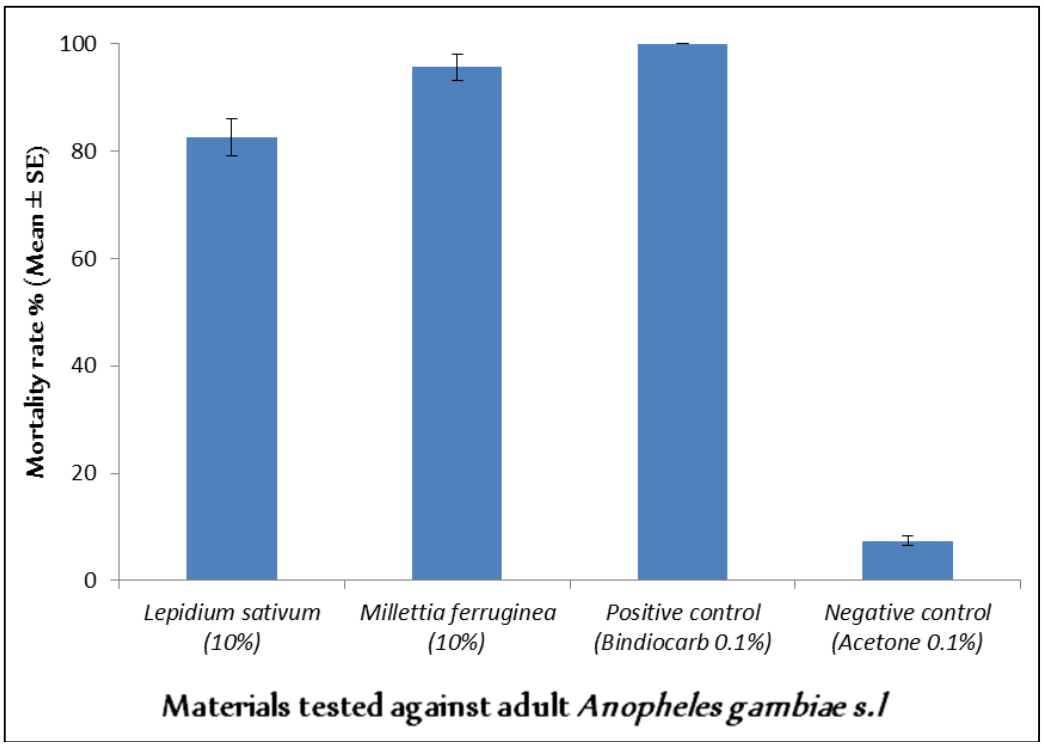

Fig 1: Percent adult mortality (X-axis) of *Anopheles gambiae* after 24 hr recovery period exposed to *Lepidium sativum* and *Millettia ferruginea* essential oil (Y-axis).

5. Conclusion

In conclusion, the present study revealed that essential oils of *L. sativum* and *M. ferruginea* showed strong mosquitocidal potential against malaria vector *An. gambiae.* However, formulation and field evaluation of this essential oil is important for large scale field application to control *An. gambiae* thereby to reduce the risk of malaria transmission in the developing country like Ethiopia.

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