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Larvicidal activities of five *Kotschya* species against *Culex quinquefasciatus* Say (Culicidae: Diptera)

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ABSTRACT

Resistance of mosquito vectors to the commonly used chemical insecticides is posing threats to human health. It is becoming necessary to identify safe, eco-friendly and effective alternative sources of larvicides in order to reduce mosquito menace. HPLC profiling of the chemical constituents in the stem and root bark ethanol extracts had similar pattern of chemical constituents except for *K. aeschynomenoides* which indicated to have large quantity of polar compounds compared to others. In this study, the ethanolic extracts and powders of five *Kotschya* species were tested against *Culex quinquefasciatus* Say. Chemical profiling of the stem and root bark ethanol extracts from *K. speciosa, K. thymodora, K. platyphylla, K. aeschynomenoides* and *K. strigosa* did not show any major differences in terms of their chemical composition. At 0.5 mg/ml, the root and stem ethanol extracts from *K. speciosa, K. thymodora* and *K. strigosa* exhibited high larvicidal activity (≥ 70%) on the 8th day post treatment. Stem powder of *K. thymodora* and root powder of *K. speciosa* and *K. strigosa* had activity comparable to their respective extracts at 0.2% w/v and 0.4% w/v at the same exposure time. This suggests that *Kotschya* species contain same or related compounds in varying quantities that are responsible for larvicidal activity.

Keywords: *Fabaceae, Kotschya strigosa, K. speciosa, K. thymodora, K. platyphylla, K. aeschynomenoides, Larvicidal, Culex quinquefasciatus.*

INTRODUCTION

Lymphatic filariasis is a mosquito-borne parasitic disease which is transmitted by *Culex quinquefasciatus.* The disease is endemic throughout the United Republic of Tanzania with an estimated 6 million people having debilitating manifestations in the form of lymphoedema, genital pathology (especially hydroceles) (Malecela et al., 2009). Besides, being an old disease, its control has been difficult, probably because the communities are not aware of the vector's ecology and full developmental stages, therefore living with them in their home environment unknowingly hence accelerates disease incidence. The uses of chemicals

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insecticides in larviciding mosquito breeding sites in order to alleviate mosquito-borne diseases is one of the methods recommended by WHO (Barat, 2006). But there have been problems of non-biodegradability and resistance to most of the commonly used chemical insecticides used for mosquito control (WHO, 1996). Therefore, it has been necessary to identify safe, eco-friendly and effective alternative sources of larvicides in order to reduce disease incidences and mosquito menace (Chavase et al., 1995; Chowdhury et al., 2007; Coleman et al., 2007). Larviciding mosquito breeding sites using plant materials may be the best alternative way if deployed rationally and effectively. Previous studies showed long term exposure of *Anopheles gambiae s.s* larvae to methanol extracts and plant powders from *Kotschya uguenensis* to disrupt larvae growth and eventually caused death (Innocent et al., 2008, 2009). This study therefore aimed at examining mosquito larvicidal activity of ethanol extracts and powders from *K. speciosa, K. thymodora, K. platyphylla, K. aeschynomenoides* and *K. strigosa* against *Culex quinquefasciatus* Say larvae.

MATERIALS AND METHODS

Plant material

The plant materials were collected from Mufindi and Mafinga District in Iringa region in Central Tanzania (Table 1). The plant materials were air dried, pulverized and sieved to obtain 60 mesh powders and then stored at 25 ^{o}C until time of analysis. The plant specimens were identified and deposited at the Department of Botany Herbarium, University of Dar es Salaam.

Extraction

For each plant species tested, 300 g of whole root, whole stem, root bark or stem bark (Table 1) powders were soaked in ethanol (96.4%) for 48 hours and then filtered and re-soaked for another 48 h. The crude filtrates were concentrated using a rotary evaporator. The percentage yields are indicated in Table 1.

Chemical profiling

The chromatograms of ethanol extract of the root and stem barks from five species and the blank solution (MeOH, 99.7%) were produced by using HPLC. This was done on an analytical Hewlett-Packard (HP) 1090 liquid chromatography fitted with a reverse phase column (HI-5C8-2610 column, manufactured by Hichrom Ltd; 250 mm x 4.6 mm, 5 µm particle size). The mobile phase consisted of a mixture of water (solvent A, 85%) and acetonitrile (solvent B, 15%), run in an isocratic condition for 35 min at a rate of 1 ml/min and pressure of 2.9 mmHg. Compounds in the eluent were detected using an ultra violet (UV) dual-array detector (HP 1040M) set at 380 nm and the percentage abundance analyzed using an HP Chemstation software on a PC.

Mosquito larvae

Third instar larvae of *Culex quinquefasciatus* were obtained from a colony maintained at the Institute of Traditional Medicine, Muhimbili University of Health and Allied Sciences, Dar es Salaam. The strain originated from wild and has been reared since 2007. Eggs were allowed to hatch in plastic containers filled with distilled water. The larvae were reared in a room where the temperature was maintained at 32-36 ^{o}C, and fed on TetraMin® food (Tetra GmbH, Germany) each day. Larvicidal experimental room temperatures and relative humidity were maintained at 26±2 ^{o}C and 70-80%, respectively.

Larvicidal assay

Larvicidal activities were carried out according to WHO (1996) protocol by exposing ten late third instar larvae of *Culex quinquefasciatus* in stem and root ethanol extracts from *K. speciosa, K. thymodora, K. platyphylla, K. aeschynomenoides* and *Kotschya strigosa*. Various concentrations (0.1, 0.25, 0.5 mg/ml) were made by adding known volume of the stock solution in beakers to make up 50 ml of water sample solution. Dimethyl sulfoxide (DMSO) was used in preparation of stock solution and

hence as a blank in the control experiment. The ten powder samples were also put in beakers containing 50 ml of distilled waters to obtain 0.1, 0.2 and 0.4% w/v concentrations and the control contained distilled water only. Experiments were done for eight days with observation of mortalities been done cumulatively every 24 h. During the experiment, larvae were fed on Tetramin® fish food (Tetra GmbH, Germany) at 1 mg per beaker per day.

Data analysis

Mean larval mortality and Standard error was performed by using SAS system (SAS, 2000). Graphs were drawn using Microsoft office Excell, 2003.

RESULTS

Ethno-botanical information (Table 1) collected from Hehe and Bena Tribes of Iringa region, central Tanzania indicated no specific uses of the five plant species except for *Kotschya uguenensis* as previously reported (Innocent et al., 2008, 2009). However, *Kotschya* species whose heights were higher than 2 metres tall were having vernacular names (Table 1). Upon extraction with ethanol, the yield was higher for *K. thymodora* stem bark (17.6%), *K. speciosa* root (7.4%) and *K. strigosa* root (4.8%) (Table 1). HPLC profiling of the chemical constituents in the stem and root bark ethanol extracts had similar pattern of chemical constituents except for *K. aeschynomenoides* which indicated to have large quantity of polar compounds compared to others (Figures 1 and 2).

The cumulative mean percentage mortality of larvae due to the effect of exposure to different concentrations of the ethanol extracts and powder materials showed an increase in activity with exposure time except for *K. platyphylla* and *K. aeschynomenoides* which were not active. At 0.5 mg/ml, ethanol extracts from the root and stem of *K. speciosa*, *K. thymodora* and *K. strigosa* attained ≥ 70% mortality on the 8th day (Figures 3-5). The powder materials of *K. thymodora* stem barks, *K. speciosa* roots and *K. strigosa* roots also gave high larvicidal activity at 0.2 w/v and 0.4 w/v upon exposure for 8 days (Figures 6-8).

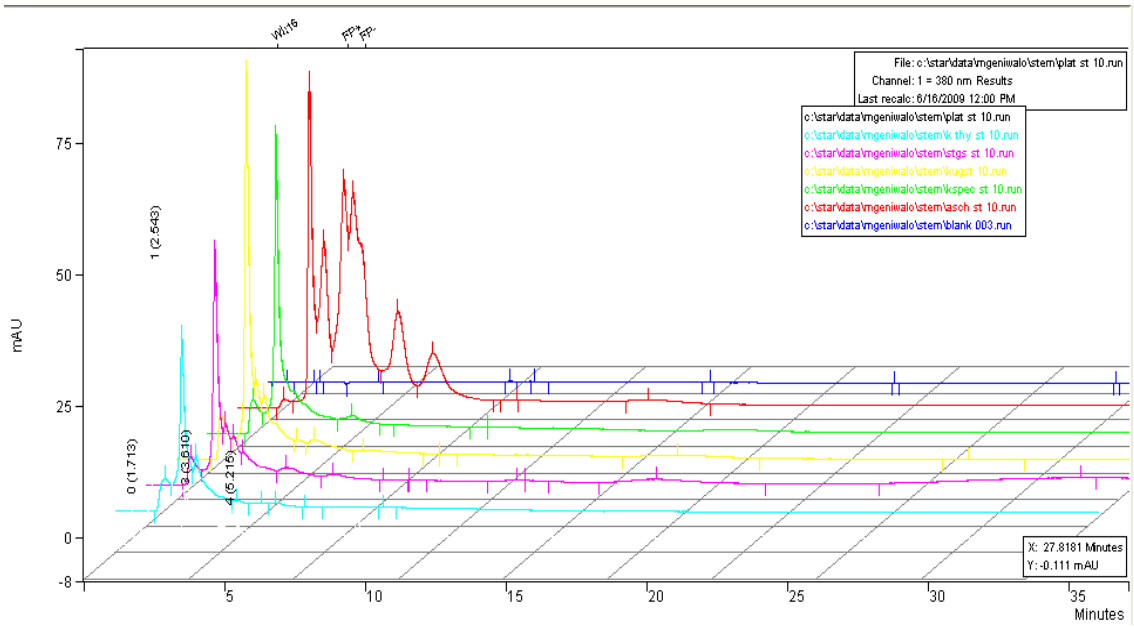

Figure 1: 3D Superimposed HPLC profiles of the blank solvent; *K. speciosa* stem*; K. uguenensis* stem; *K. strigosa* stem;*K. thymodora* stem; and *K. platyphylla* stem arranged along z-axis from right to zero intercept.

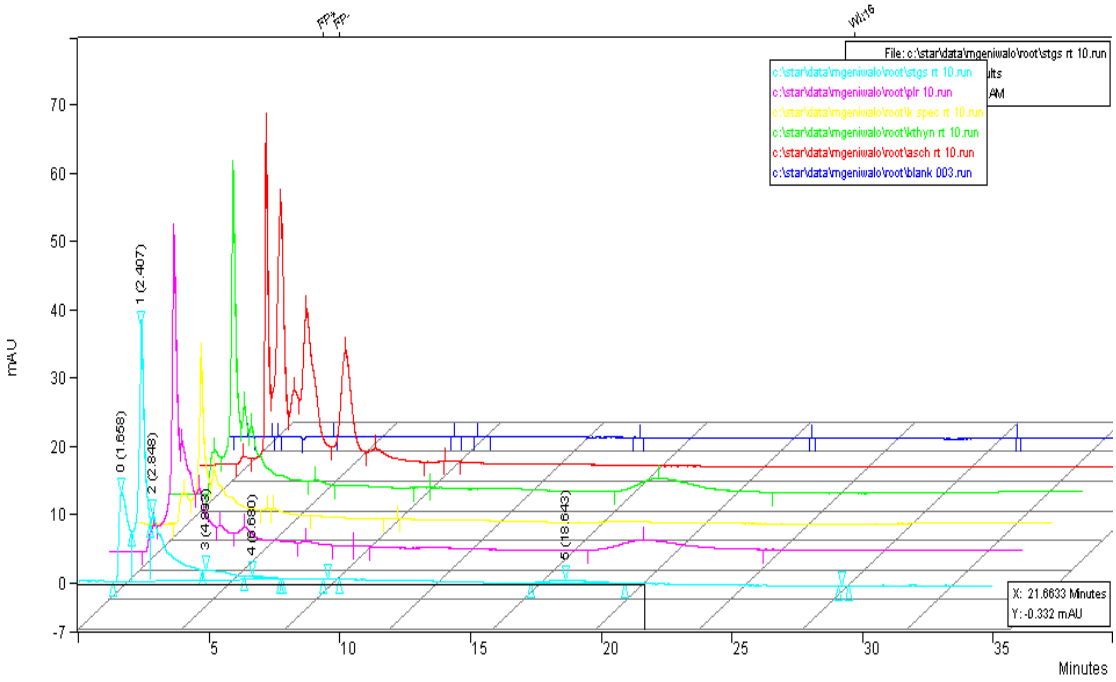

Figure 2: 3D Superimposed HPLC profiles of the blank solvent; *Kotschya aeschynomenoides* root; *K. thymodora* root; *K. speciosa* root; *K. platyphylla* root; *K. strigosa* root arranged along z-axis from right to zero intercept.

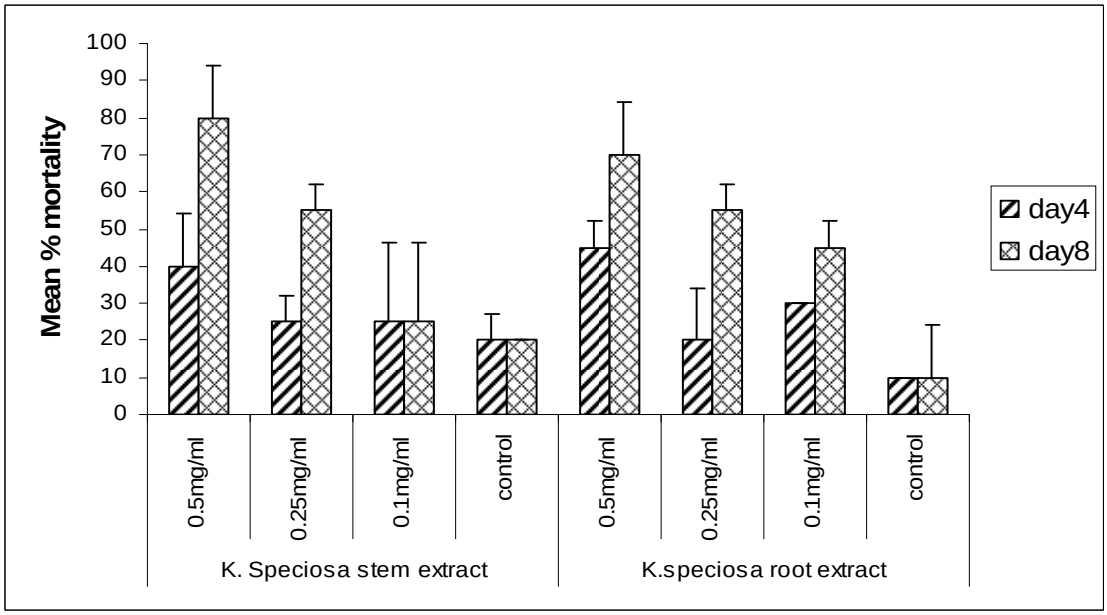

Figure 3: Percentage mortality ± SE of *Culex quinquefascintus* larvae exposed to stem and root extracts of *Kotschya speciosa* species on day 4 and 8 post-treatment.

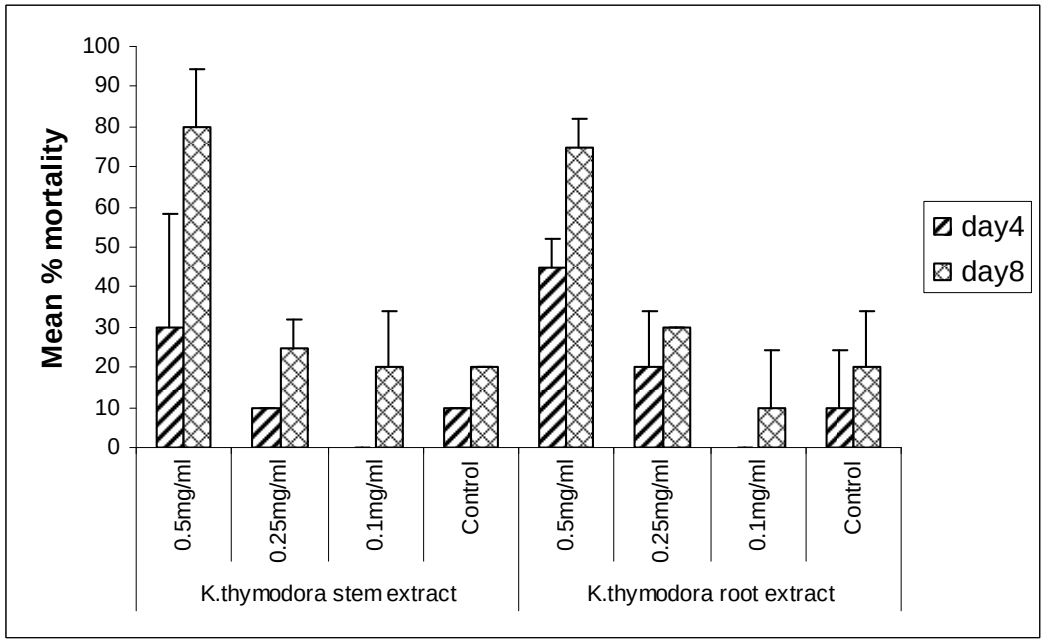

Figure 4: Percentage mortality ± SE of *Culex quinquefascintus* larvae exposed to stem and root extracts of *Kotschya thymodora* species on day 4 and 8 post-treatment.

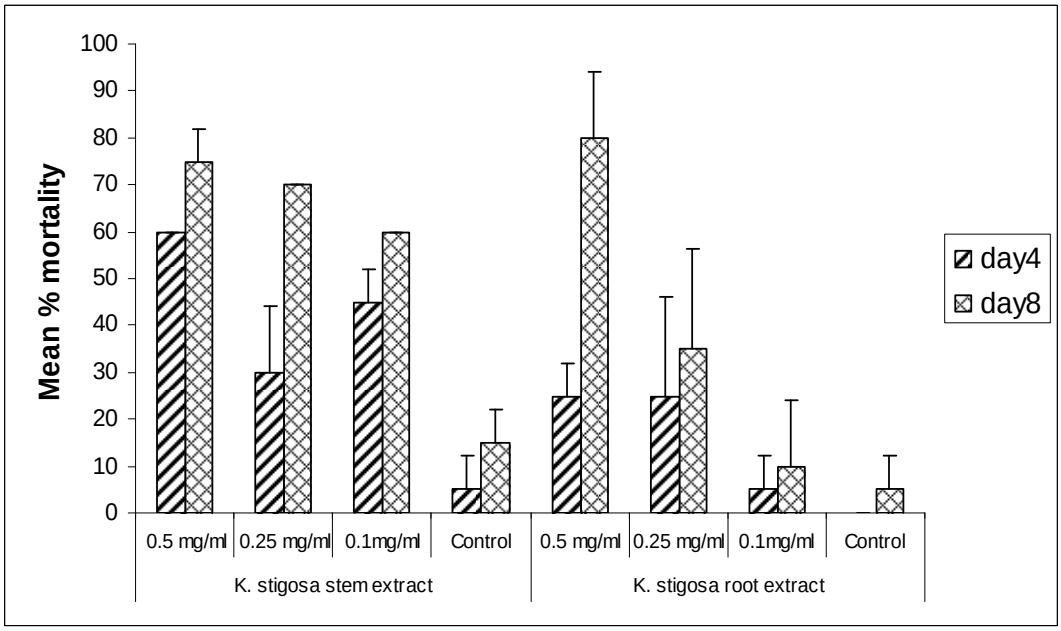

Figure 5: Percentage mortality ± SE of *Culex quinquefascintus* larvae exposed to stem and root extracts of *Kotschya strigosa* species on day 4 and 8 post-treatment.

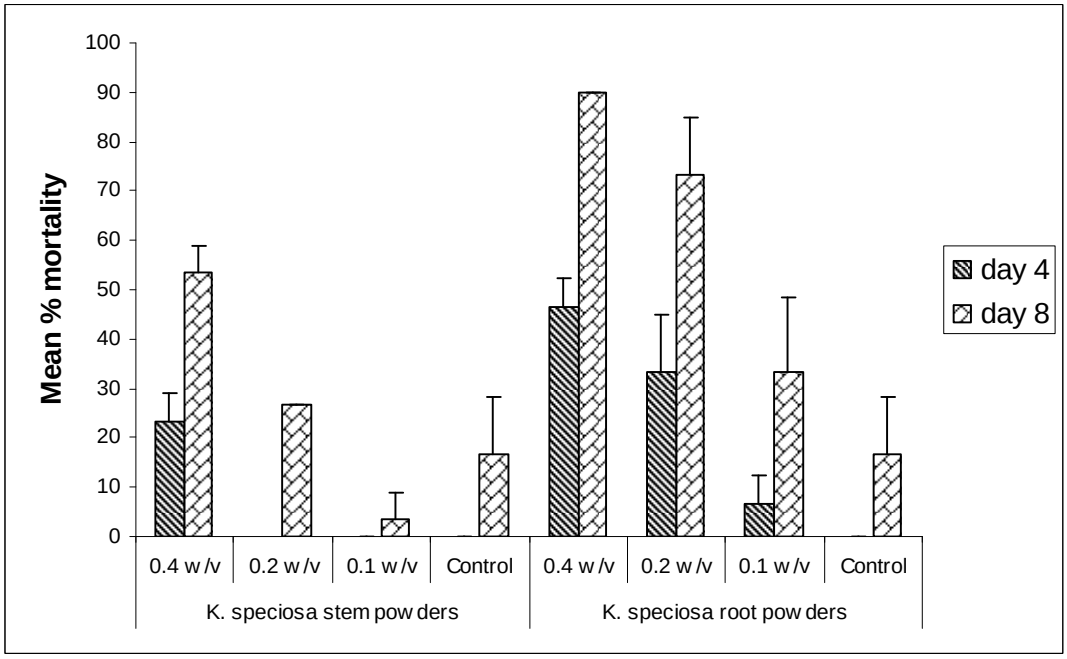

Figure 6: Percentage mortality ± SE of *Culex quinquefascintus* larvae exposed to stem and root powders of *Kotschya speciosa* species on day 4 and 8 post-treatment.

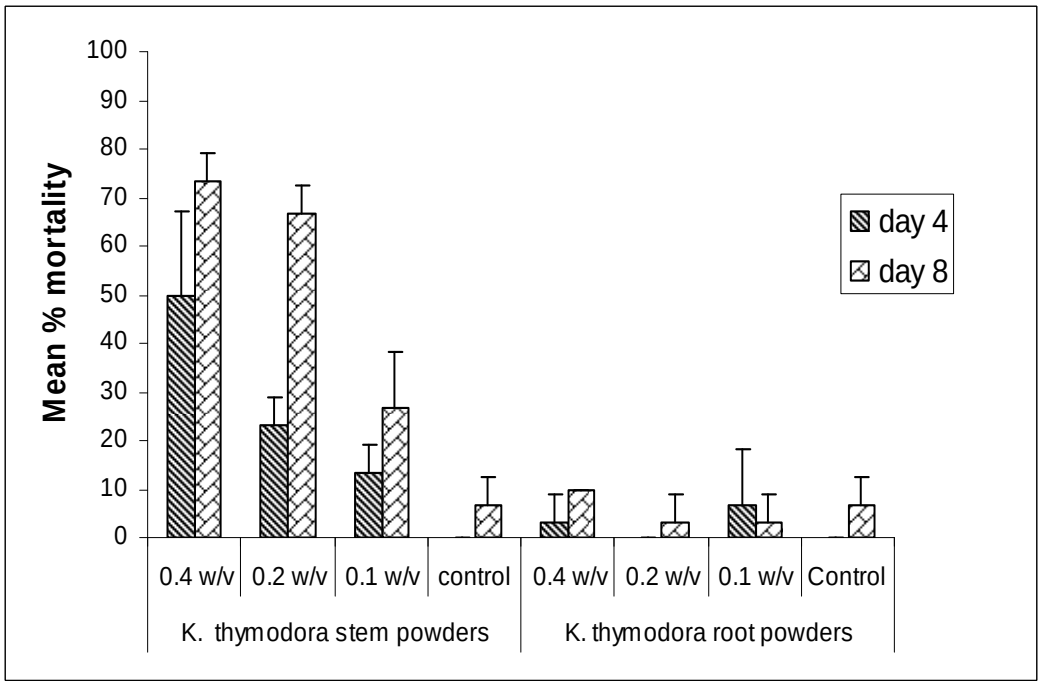

Figure 7: Percentage mortality ± SE of *Culex quinquefascintus* larvae exposed to stem and root powders of *Kotschya thymodora* species on day 4 and 8 post-treatment.

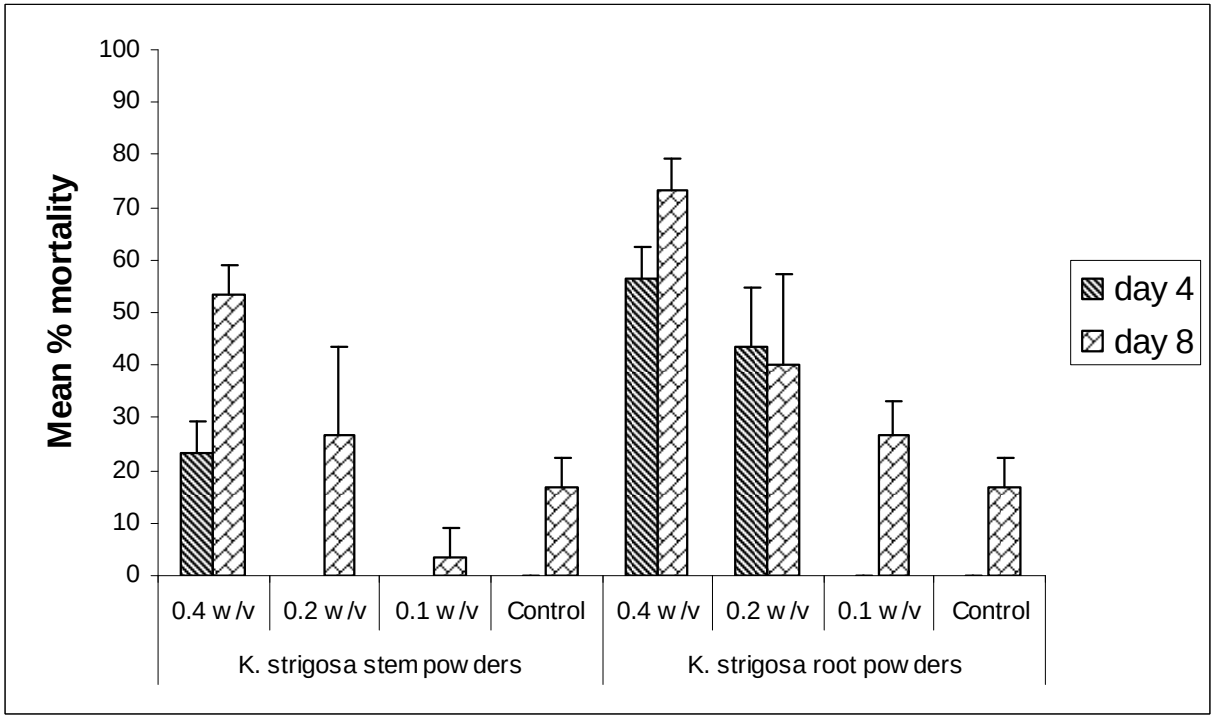

Figure 8: Percentage mortality ± SE of *Culex quinquefascintus* larvae exposed to stem and root powders of *Kotschya strigosa* species on day 4 and 8 post-treatment.

Table 1: General information of *Kotschya* species which were collected in Iringa Region, Tanzania.

	Botanical name (Vernacular name)	Place of collection	Plant nature and its habitat	Plant part used	Wt of extract (%)
1.	*Kotschya strigosa*	Lake Ngwazi-Mufindi District	Shrub of ca. 2 m height/Miombo woodland	Whole roots Whole stem	14.3 (4.8) 9.0 (3.0)
2.	*Kotschya aeschynomenoides*	Ngwazi wet meadow- Mufindi District	Shrub of 1.5-2 m high, flowers are pale blue and leaves sticky/Wet meadow glassland	Root bark Stem bark	7.5 (2.5) 7.5 (2.5)
3.	*Kotschya speciosa*	Lwihomelo village-Mufindi District	A wood shrub 1.5-2 m high, stem are black and roots pale brown/Woodland	Whole root Whole root	22.2 (7.4) 15.5 (5.2)
4.	*Kotschya thymodora* (Mtenga in Hehe)	Ludewa village-Njombe District	A small tree of 4-5 m high/ bushland with red soil and dominated with *Dodomea angustifolia*	Root bark Stem bark	12.9 (4.3) 53.0 (17.6)
5.	*Kotschya platyphylla* (Melezi in Bena)	Along Uwemba-Lugalawa road - Njombe District	Shrub of about 3-4 m high tall/ bushland with *Dodomea angustifolia* dominated	Root bark Stem bark	10.3 (3.4) 16.2 (5.4)

*Calculations based on 300 g of plant powders soaked from each plant species.

DISCUSSION

Previous studies reported the potential of *Kotschya uguenensis* extract as source of growth inhibitory of immature *Anopheles gambiae s.s.* mosquitoes upon a prolonged exposure time (Innocent et al., 2008, 2009). The major physiological disruption observed was elongated mid-gut which caused larval death (Innocent et al., 2008). The present study suggests the presence of active compounds in crude ethanol extracts from the roots and stem of *K. thymodora*, *K. speciosa* and *K. strigosa* that are effective against *Culex quinquefasciatus*. However, poor larvicidal activity of plant powders of *K. thymodora* root barks, *K. speciosa* stem and *K. strigosa* stem (Figures 6-8), may have been contributed to lower extractability of active ingredient by the solvent used or else, lower amount of active compound in these extracts. Nevertheless, these results call for efforts to isolate, identify and quantify compounds responsible for larvicidal activity in *Kotschya* species. This is due to the fact that, prominent insecticidal plants like, *Chrysanthemum cinerariafolium* (Matsui and Yamamoto, 1971; Casida, 1983; Saxena, 1990) and *Azadrichta indica* (Schmutterrer, 1990; Siddiqui et al., 2000; Moore et al., 2003) have so far yielded many useful compounds and some chemical analogies have been synthesized and formulated for the purpose of controlling pests and insect disease vectors (Casida, 1983; Moore et al., 2003). Also, extracts from plant species from *Tagetes minuta, Polyalthia longifolia, Solanum nigrum, Ageratum conyzoides, Cleome icosandra* and *Tridax procumbens* have demonstrated their ability to inhibit growth of immature stages of mosquitoes by causing deformities and death (ICMR, 2003). This indicates how plants are potential source of chemical agents available for mosquito control.

Conclusion

Plants from the genus *Kotschya* may be potential source of safe and eco-friendly mosquito larvicidal agents. Chemical studies may impart some knowledge as to whether the larvicidal activity is caused by the same compound which is present in varying quantities or related compounds from the five species investigated and whether the compounds work individually or in a synergism.

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