



Full Length Research Paper

EFFICACY OF *Tagetes minuta* and *Tephrosia vogelii* CRUDE LEAF EXTRACTS ON *Tetranychus urticae* (*Acari: Tetranychidae*) and *Aphis fabae* (*Homoptera: Aphididae*)

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ABSTRACT

Efforts in research are focused on the bioactivity, application methods, cost-effectiveness and sustainable use of botanical pesticides against insect pests. The insecticidal and repellent properties of *Tagetes minuta* L (Mexican marigold) and *Tephrosia vogelii* Hook (Fish poison bean) collected from KARI, Kitale botanical garden were evaluated for their efficacy and repellence against red spider mite (*Tetranychus urticae* Koch) and black bean aphids (*Aphis fabae* Scopoli) in the laboratory. Mortality potential of the plant was determined by spraying the crude extracts on the pests reared earlier in the screen house. *Tagetes minuta* crude extracts at concentrations of 50 g/L, 100 g/L, 150 g/L soaked for 24 hours and also 150 g/L soaked 48 hours in distilled water, *Tephrosia vogelii* crude leaf extract of 10 g/L, 30 g/L and 60 g/L soaked 12 hours and also 60 g/L soaked 48 hours in distilled water, were used to investigate treatment efficacy on mortality of the adult insects. Treatment using *T.minuta* at concentration 150 g/L soaked for 48 hours caused the highest mortality in aphids with $R^2=0.9908$ and slightly lower mortality in mites with $R^2=0.9505$. *T. vogelii* at concentration 60 g/L soaked 48 hours in water caused high mortality in the two pests with $R^2=0.9826$ in aphids and $R^2=0.9767$ in mites at the end of 24 hour exposure to the herb. Repellency tests were performed using filter- paper circles and extracts of *T.minuta* and *T. vogelii* were applied around the periphery of the filter papers. Percentage repellency (PR) was determined for each extract. *T.minuta* at concentration 150 g/L soaked 48 hours had the strongest PR on mites at 55% followed by that soaked 24 hours PR at 12%. *T. vogelii* at 60 g/L soaked 48 hours had a moderate repellent effect on mites at PR 17% while same concentration after 12 hours of soaking had PR of 8% on mites. However aphids were not repelled by any of the extracts used. The two botanicals are promising insecticides which can be used by subsistence farmers as alternatives to synthetic pesticides.

Keywords: *Tagetes minuta*, *Tephrosia vogelii*, *Tetranychus urticae*, *Aphis fabae* and Mortality

INTRODUCTION

Red spider mites and black bean aphids are some of the crop pests which are very problematic to many farmers in Kenya and further Sub-Saharan Africa. Although they can be effectively controlled by synthetic pesticides the majority of farmers in Africa are resource-poor and have neither the means nor the skills to obtain and handle

pesticides appropriately (Ogendo *et al.*, 2003). Plant derived products are increasingly being used to combat crop pests because they are natural and are often assumed to be safe for the environment (Kumar *et al.*, 2000). In recent years, research has focused on the bioactivity, application methods, cost-effectiveness and

sustainable use of botanical pesticides against insect pests (Ogendo *et al.*, 2003; Yallapa *et al.*, 2012).

The high costs of synthetic pesticides and associated toxicity risks (Mihale *et al.*, 2009), the increasing development of insect resistance to pesticides, (Ogendo *et al.*, 2003), the destruction of beneficial insects, pesticide residue magnification in humans and wildlife and disruption of ecosystem (Georghiou, 1986; Clergy and Mackean, 1994, Ruchika and Kumar, 2012), have increased the need to search for alternative insect control methods. Botanical insecticides have been reported to have a wide range of biological activities against insects. These include repellence and anti-feedant activities (Viglianco *et al.*, 2005), oviposition deterrence, toxicity, sterility, growth regulatory and fecundity reduction, molting and respiration inhibition, and cuticle disruption (Tinzaara *et al.*, 2006).

Today, researchers are seeking new classes of naturally occurring insecticides that might be compatible with newer pest control approaches (Rajashekar *et al.*, 2012). *Tagetes minuta* L have several compounds in their tissue which have biological activity against a range of organisms (Vasudevan *et al.*, 1997). One such compound, thiophene, demonstrates antiviral, antibacterial, antifungal, nematocidal, and insecticidal properties (Marles *et al.*, 1992, Margl *et al.*, 2001, Riga *et al.*, 2005). *Tephrosia vogelii* Hook crude extracts have been used for wound dressings and other medicinal purposes (Dzenda *et al.*, 2007), for arrow poisons, and for insect and fish poisons (Ekanem *et al.*, 2004). The objective of the current study was to investigate the potential of these two plant species as sources of sustainable alternatives to synthetic insecticides for use in agricultural crop protection using methods compatible with small scale farmers in Africa.

MATERIALS AND METHODS

The study was conducted at Kenya Agricultural Research institute- KARI, Kitale center. The average annual rainfall in the area is 1143 mm per annum which is fairly evenly distributed but with peaks in May and August and relatively dry season from December to March. The average maximum air temperature is 27.8°C and the minimum is 10°C.

Rearing of the black bean aphid and the red spider mites

The two pests were reared on potted broad bean plants (*Vicia faba*) in a screen house in separate compartments to obtain a bigger population that was used in the laboratory experiment. After two weeks of germination; the shoots were infested with 200 red spider mites'

colonies and 50 black bean aphids collected elsewhere and were left to multiply.

Preparation of crude leaf botanical extracts

Crude leaf plant extracts evaluated were obtained from *Tagetes minuta* L and *Tephrosia vogelii* Hook collected from KARI botanical garden. *T. minuta* leaves, at flowering time were collected and weighed to get 50 g, 100 g, and 150 g respectively, Leaves of *T. vogelii* at flowering time were collected and weighed to get 10 g, 30 g and 60 g respectively and were separately crushed using a mortar and a pestle then put in separate containers. 1 litre of distilled water was added in each container and sealed tightly. *T. minuta* leaves were soaked for 24 hours while *T. vogelii* soaked for 12 hours under ambient condition. At the end of soaking period, the extracts were filtered using a sieve to remove the large pieces of leaf material and the filtrates were then each mixed with 3ml of Teepol detergent per liter (potash based soap) to assist in dispersion of the spray on the plant and pest surface since it acts as a sticking agent (Viglianco *et al.*, 2008). Another extract of 150 g/L *T. minuta* was soaked for 48 hours and 60 g/L *T. vogelii* soaked for 24 hours was also used on the two pests (60 g/L means an extract of 60g soaked in 1 litre of water). The filtrates were then put separately into a 1 liter plastic hand sprayer and used immediately on the pests.

Application of botanical extracts on the two pests

The black bean aphids and red spider mites that had been reared earlier in the screen house were collected 30 minutes of the beginning of experiment and taken to the laboratory. The two pests on the bean leaves were each sprayed with different concentrations of *T. minuta* and *T. vogelii* which had been prepared earlier and placed in plastic containers and covered with a well-ventilated lid. A total of 50 aphids per leaf was used and replicated four times while 100 mites were used per leaf and replicated four times

Distilled water was used as a negative control. Observations were made at 1,2,4,6,8,10, and 24 hours after spraying the pests and numbers of dead pests were recorded at each observation. The pests were considered alive if they exhibited normal behavior when breathed upon or physically stimulated with a wooden stick. For each time point, if the pests were incapable of moving, maintaining a normal posture, leg coordination, or any signs of life they were considered dead (Panella *et al.*, 2005).

In vitro repellency bioassay

Two filter papers (Whatman No. 1) were placed inside a petri dish with an inner diameter of 9.5 cm (Thorsel *et al.*,

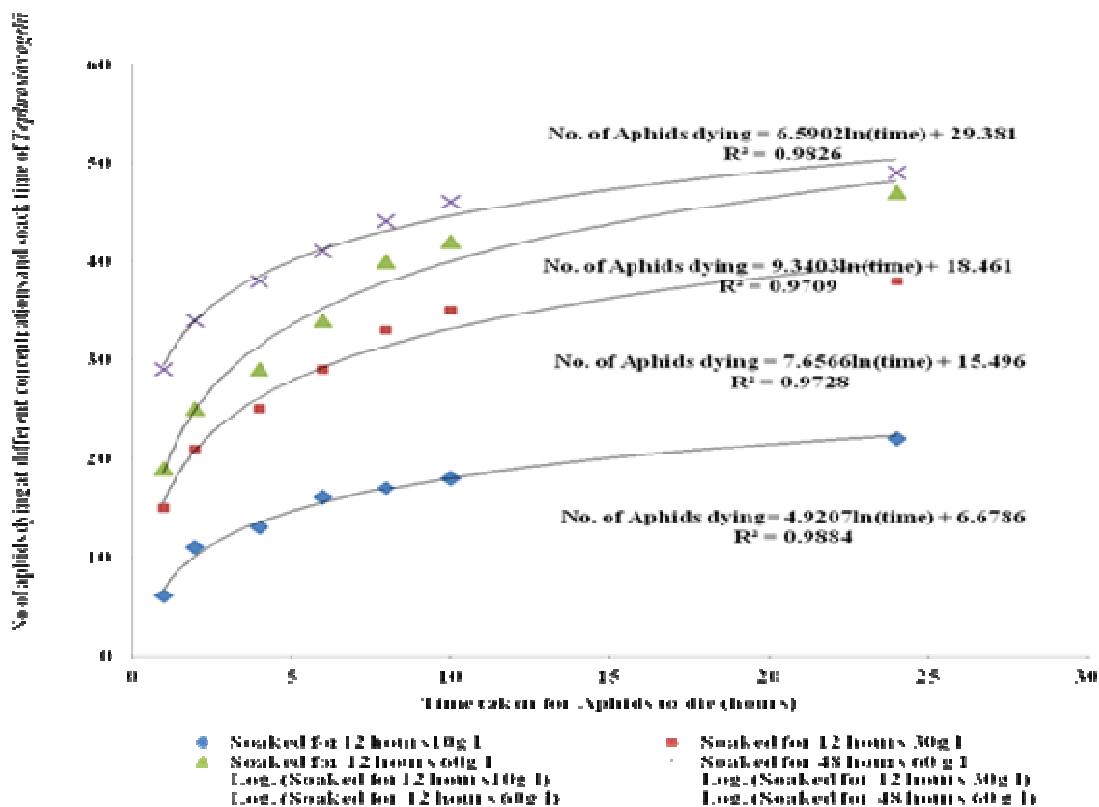


Figure 1. Model equation that relates the number of black bean aphids dying when sprayed with different concentrations and soak period of *Tephrosia vogelii* crude leaf extract

2005). The treatments of 10 g/L, 30 g/L, 60 g/L of *T. vogelii* crude leaf extracts soaked 12 hours and 60 g/L extract soaked 48 hours, *T. minuta* crude leaf extracts of 50 g/L, 100 g/L, 150 g/L soaked 24 hours and 150 g/L soaked 48 hours were applied along the periphery of the filter papers. Each treatment test was replicated three times.

Ten adults of each pest were placed respectively at the center of each of the treated filter papers and their movement with regard to avoiding the treated area was physically observed. The pest that continued its motion beyond the periphery of the treated area within 5 minutes was indicated as non-repelled, and the pest that reversed its direction before reaching the periphery of the treated area was considered as repelled.

The repellency was expressed as number of red spider mites or black bean aphids avoiding the treated area to the total number of red spider mites or black bean aphids at every observation. Thus 10 pests avoiding out of a total of 10 was recorded as 100% repellency. The Mean class repellency rate percentage values were classified in the same similitude as described by Adriana and Mauricio,(2008).

Statistical analysis

Data analysis was done using descriptive statistics

software MINITAB version 14. Modeling was done using logarithmic curve graphs for the number of *T. urticae* and *A. fabae* dying at different times for 24 hours of exposure to different concentration of *T. minuta* and *T. vogelii* crude leaf extract. R² in the graphs represents the relationships between different concentrations of the two botanicals and the two pests used in the experiment.

The *in vitro* repellency bioassay was calculated using the method described by Thorsel *et al.*, (2005);

$$R = a/n \times 100$$

Where: **R** is the repellency

a is the number of adults of each pest avoiding treated area

n is the total number of aphids or red spider mites put in the center of filter paper

The data was then subjected to one-way ANOVA for the significance test and further, Fisher's LSD test for mean comparison test for separation was applied.

RESULTS

Results showed significant differences ($p < 0.05$) among treatments and over time in the cumulative number of adult pests' mortality. The most concentrated botanical extracts and soaked for longer period had the highest mortality at the end of 24 hour exposure of the two pests to the extract. No mortality was recorded from pests

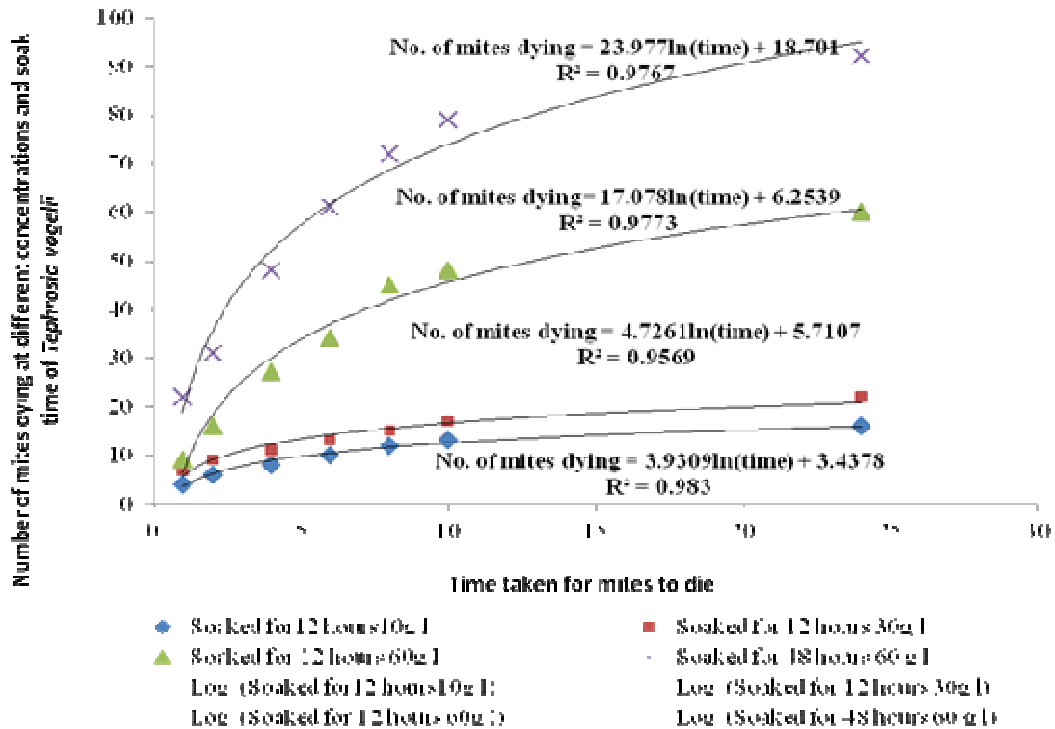


Figure 2. Model equation that relates the number of red spider mites dying when sprayed with different concentrations and soak period of *Tephrosia vogelii* Hook crude leaf extract

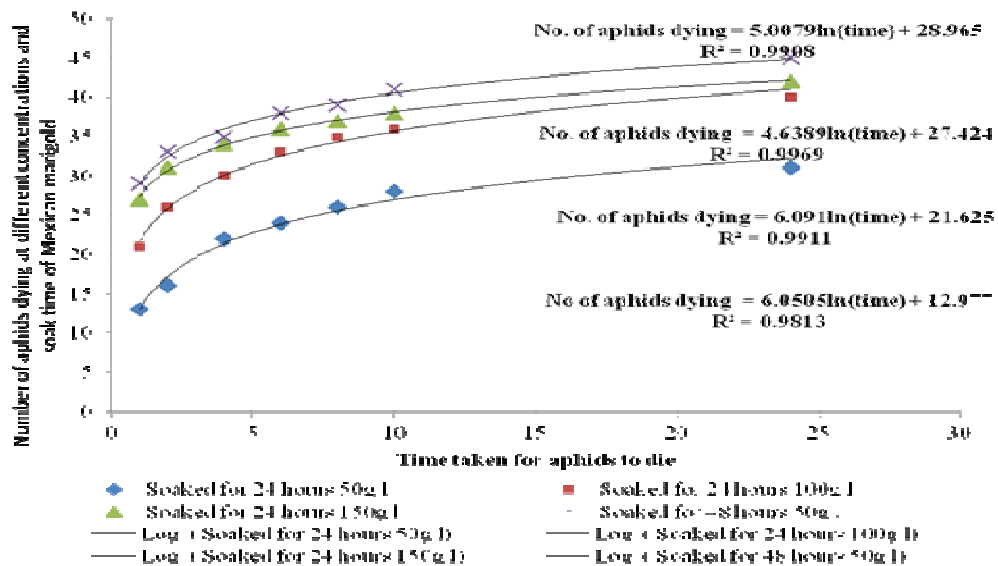


Figure 3. Model equation that relates the number of aphids dying when sprayed with different concentrations and soak period of *Tagetes minuta* crude leaf extract

sprayed with distilled water. Using logarithmic curve graphs for analysis, the extracts used against the pests showed that R was more than 90% (Figures 1, 2, 3, and 4) meaning that the botanicals showed significant results. The initial death of the pests was quite significant, extract of *T.vogelii* at 60 g/L and soaked for 48 hours, had an initial death of 29 aphid and 18 red spider mites (Figures

1 and 2) while *T. minuta* at 150 g/L soaked for 48 hours had an initial death of 29 aphids and 27 red spider mites (Figures 3 and 4).

The filter paper treated with *T. minuta* and *T. vogelii* was significantly repellent against red spider mites but not black bean aphids (Fisher's LSD test $p < 0.05$). The repellency duration was 45 minutes for *T. minuta* at 150

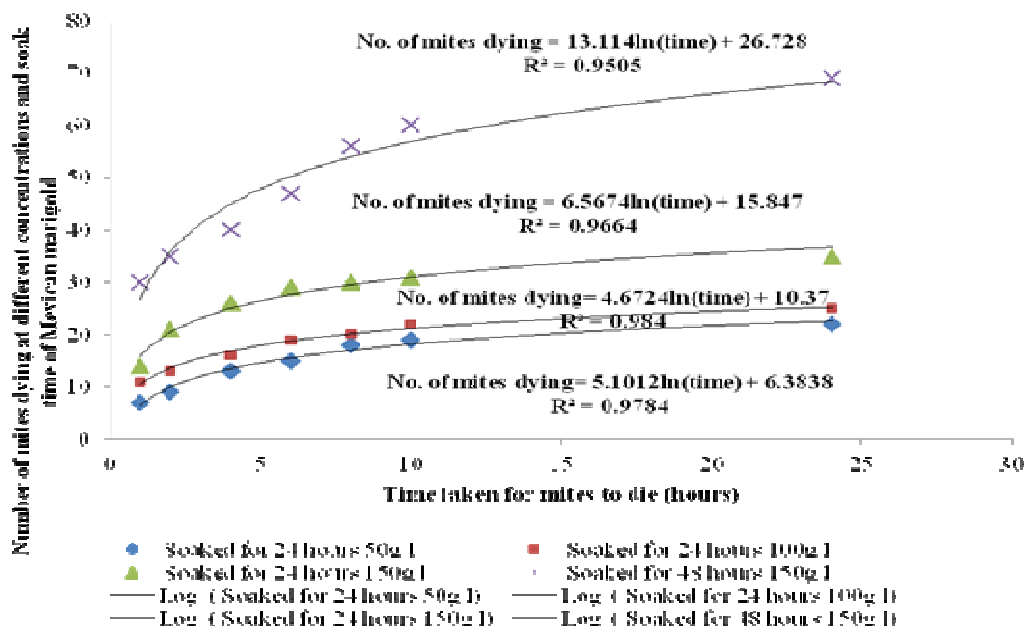


Figure 4. Model equation that relates the number of red spider mites dying when sprayed with different concentrations and soak period of *Tagetes minuta* crude leaf extract.

Table 1. Red spider mites repelling activity to *T. minuta* and *T. vogelii* at different concentrations and different soak period. Mite Repellency (%) at:

Name of Soaking extract time	5 min	10 mins	15 mins	20 mins	25 mins	30 mins	35 mins	40 mins	45 mins	Mean (%)	Repellency Class	repellency
<i>T. minuta</i> (150g/l) 48 hours	100	97	77	63	57	47	30	17	3	55	3b	
<i>T. minuta</i> (150g/l) 24 hours	60	40	7	0	0	0	0	0	0	12	1a	
<i>T. vogelii</i> (60g/l) 48 hours	97	40	20	0	0	0	0	0	0	17	1a	
<i>T. vogelii</i> (60g/l) 12 hours	50	20	0	0	0	0	0	0	0	8	1a	
<i>T. vogelii</i> (30g/l) 12 hours	20	0	0	0	0	0	0	0	0	2	1a	

Values followed by the same letter are not significantly different according to the Fisher's LSD test ($p < 0.05$)

g/L soaked for 48 hours, while same concentration soaked for 24 hours repelled the mites up to 15 minutes only. *T. vogelii* at concentration 60 g/L soaked for 48 hours repelled the mites up to 15 minutes while same concentration at 12 hour soaking repelled the mites up to 5 minutes only (Table 1).

DISCUSSION

T. minuta and *T. vogelii* at high concentration and longer soak period resulted to higher mortalities in black bean aphids and red spider mites while a lower concentration and shorter soaking period resulted in lower mortalities in

especially red spider mites than black bean aphids. According to Gaskin *et al.*, (1972), Lungu (1987), Ibrahim *et al.*, 2000 and Obongoya *et al.*, (2010), *T. vogelii* has a pesticidal property that is capable of killing vegetable pests due to the isoflavonoid rotenone present in its leaves explaining its traditional use as a fish poison as well as its efficacy against a range of pests Ogendo *et al.*, (2003). Several studies have been written on the use of *T. minuta* (Keita *et al.*, 2000; Boeke, 2004), and its reported to have a high seasonable level of effectiveness. Whole plant extracts of *T. minuta* contain a phototoxin alphaterthieenyl, which is even highly insecticidal (Philogene *et al.*, 1985). The degree of repellence of extracts on the pests was greatly influenced by the

concentration of the crude leaf extract, the soaking period of the extract, and the exposure time. *T. minuta* repelled the red spider mites for slightly longer time than *T. vogelii*. Ogendo *et al.*, (2003), in their study, found out that maize treated with *T. vogelii* had higher percent repulsion values of upto 87%.

CONCLUSION

The insect mortality results of the current study suggests that there exists good potential for the two local plants, *T. minuta* and *T. vogelii*, to be effectively used as pesticide on vegetable crops especially in the green houses, in the traditional resource-poor farming communities in sub-Saharan Africa. The repellency results however, repelled for a short period in red spider mites. The use of botanical pesticide will boost food security in those environments where investment in synthetic pest control is uneconomical.

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REFERENCES

- Adriana YM, Mauricio UV(2008). Repellent, Antifeedant and Isecticidal Effects of Neem oil on *Microthecapunctigera*. An International Journal. 51(6): 1121-1126
- Boeke SJ(2004). Efficacy of plant extract against the cowpea beetle, *Collosobruchomaculatus*. International Journal of Pest Management 50:251-258
- Clergy CJ, Mackean DG(1994). Advanced Biology: Principles and Applications. Murray. London
- Dzenda T, Ayo JO, Adelaiye AB, Auda AO(2007). Ethnomedical and Veterinary uses of *Tephrosia vogelii* Hook. f.: A review. 28(3):24-39
- Ekanem AP, Meinelt T, Kloas W, Knopf K(2004). Early life stages toxicity of extract from the African fish poison plant *Tephrosia vogelii* Hook and *Asystasiavogelians* Benth on Zebra fish embryos, 65(2):489-494
- Gaskins MH, White GA, Martin FW, Delfel NE, Rupel EG, Barnes DK(1972). *Tephrosiavogelii*: A source of Rotenoids for insecticidal and pesticidal use. Technical Bulletin No.1445. Agricultural Research Service- United States. Department of Agriculture.
- Georghiou GP(1986). The magnitude of the resistance problem. In pesticide resistance, Strategies and tactics for management. National Academy press. Washington D.C pp 11-44
- Ibrahim B, M'batchi B, Mounzeo H, Bouroubou HPB, Posso P(2000). Effects of *Tephrosia vogelii* and *Justiciaextensa* on *Tilapia nilotica* in vivo. *J. Ethnopharmacol.* 69, 99-104.
- Keita SM, Vincent C, Schmit JP, Ramaswamy S, Belenger A(2000). Effect of various essential oils on *Callosobruchusmaculatus* (F). (Coleoptera: Bruchidae). Journal of Stored Products Research 36:355-364
- Kumar A, Dunkel FV, Broughton MJ, Sriharan, S(2000). The effect of root extract Of Mexican marigold, *Tagetes minuta* (Asterales: Asteraceae) on Non target aquatic Macro invertebrates Environmental Entomology 29(2): 140-149
- Lungu S(1987). An Ecological Evaluation of Zambian Trees and shrubs in Relation to Their Potential uses for Agro forestry in Zambia. Master's Thesis, University of Zambia
- Margl L, Tei A, Gyurján I, Wink M(2001). GLC and GLC-MS Analysis of ThiopheneDerivates in Plants and in vitro Cultures of *Tagetes patula* L. (Asteraceae). *Z. Naturforsch Section C*, 57:63-71.
- Marles RJ, Hudson JB, Graham EA, Soucy-Breau C, Morand P, Compadre RL, Compadre CM, Towers G, JT Arnason(1992). Structure-activity studies of photoactivated antiviral and cytotoxic tricyclic thiophenes. *Photochemical Photobiology*, 56:479-487
- Mihale MJ, Deng AL, Selemani HO, Mugisha-Kamatenesi M, Kidukuli AW, Ogendo JO (2009). Use of Indigenous knowledge in the management of field and storage pests around Lake Victoria basin in Tanzania. *African Journal of Environmental Science and Technology*, 3(9): 251-259.
- Ogendo JO, Belmain SR, Deng AL, Walker DJ(2003). Comparison of toxic and repellent effects of *Lantana camara*L. With *Tephrosia vogelii* Hook on a synthetic pesticide against *Sitophiluszeamais* Motsch.(Coleoptera: Curculionidae) in stored maize grain. *Insect Science Applied* 23(2):127-135
- Obongoya BO, Wagai SO, Odhiambo G(2010). Phytotoxic effect of selected crude Plant extracts on soil-borne Fungi of common bean. *Crop Science*.18 (1): 15-22.
- Panella NA, Dolan MC, Karchesy JJ, Xiong Y, Peralta-Cruz J, Khasawneh M, Montener JA, Maupin GO(2005). Use of novel Compounds for pest control, Insecticidal and Acaricidal activity of Essential Oil Components from Heartwood of Alaska Yellow Cedar. *Journal of Medical Entomology*, 42:352-358
- Philogene BJR, Arnason JT, Berg CW, Duval F, Morand P(1985). Efficacy of Plant phototoxin alphaterthienyl against *Aedesintrudens* and effects on nontarget Organisms. *Journal of Chemical Ecology* 12:893-898
- Rajashekar Y, Bakthavatsalam N, Shivanandappa T(2012). Botanicals as Grain Protectants. doi:10.1155/2012/646740
- Riga E, Potter J, Hooper C(2005). In vitro effect of marigold exudates on plant parasitic nematodes. *Phytoprotection*, 86:31-35
- Ruchika K, Kumar D(2012). Occurrence and Infestation Level of Sucking Pests: Aphids on various host Plants in Agricultural Fields of Vadora, Gujarat (India). *International Journal of Science and Research Publications* vol.2, ISSN2250-3153
- Tinzaara W, Tushemereire W, Nankinga CK, Gold CS, Kashajja I(2006). The potential of using botanical insecticides for control of the banana weevil, *Cosmopolites sordidus* (Coleoptera: Curculionidae). *African Journal of Biotechnology* 5 (20):1994-1998
- Thorsell W, Mikiver A, Tunon H(2005). Repelling properties of some plants materials on the tick *Ixodesricinus*L. *Phytomedicine*, 13: 132-134.
- Vasudevan P, Kashyap S, Sharma S(1997). *Tagetes*: a multipurpose plant. *Bio-resource Technology*, 62: 29-35
- Viglianco AI, Novo RJ, Clara I, Cragnolini Mirta NE, Alicia Cavallo(2008). Antifeedant and Repellent Effects of Extracts of Three Plants from Córdoba (Argentina) Against *Sitophilusoryzae*(L.) (Coleoptera: Curculionidae) *BioAssay*3:4
- Yallapa R, Nandagopal B, Thimmappa S(2012). Botanicals as Grain Protectants. *A Journal of Entomology*. 2012:2012

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