Pakistan Entomologist

Journal homepage: www.pakentomol.com

SYNERGISTIC ACTIVITY OF A MIXTURE OF *PONGAMIA PINNATA* (KARANJ) AND *KIGELIA AFRICANA* (SAUSAGE TREE) LEAF EXTRACTS AGAINST YELLOW FEVER MOSQUITO, *AEDES AEGYPTI*

Savitri Poonia and *Rajender Kaushik

Department of Zoology, Center for Advanced Studies, University of Rajasthan, Jaipur, India

ARTICLE INFORMATION

Received: March 18, 2013
Received in revised form: June 8, 2013
Accepted: June 15, 2013

***Corresponding Author**:
Rajender Kaushik
Email: Rkaushik.jaipur@gmail.com

ABSTRACT

The present study was undertaken in Jaipur, Rajasthan, India during the year 2012. Laboratory bioassays were done to evaluate the effect of Karanj (*Pongamia pinnata*) and Sausage tree (*Kigelia africana)* for their individual and combined larvicidal activity against the larvae of yellow fever mosquito*, Aedes aegypti*. Efficacy of these plants was compared with neem *(Azadirachta indica*) which is a prominent biopesticide. Leaf extracts of both the plants were prepared by soxhlet extraction method. Extracts were evaluated against 3rd and 4th instar larvae using WHO protocol. To observe the synergistic effect, different concentrations of the plant extracts were mixed in 1:1, 1:2 and 2:1 ratios and tested against the larvae. Results showed that *P. pinnata* (PP) and *K. Africana* (KA) possess high larvicidal activity (LC_{50} = 63.24 ppm and LC_{90} = 112.2 ppm for both plants). The mixture of two plants in 2:1 ratio (PP 75 % : KA 25%) was found to be significantly more effective (LC_{50} = 25.12 ppm and LC_{90} = 57.68 ppm) than the *A. indica* (AI 100%) (LC_{50} 58.45ppm, LC_{90} 82.67ppm) extract. This combination exhibited maximum synergism (synergistic factor of 12.06 for 3rd instar) and was found to be 6 to 7 times more toxic than the *A. indica* and is thus recommended for field trials.

Keywords: *Aedes aegypti*, *Kigelia africana,* larvicidal, *Pongamia pinnata*, synergism

INTRODUCTION

Mosquitoes are responsible for transmitting the most important vector borne diseases (Hubalek and Halouzka, 1999) above all malaria, dengue, chikungunya and Japanese encephalitis. The high adaptability of mosquitoes makes no single strategy for their adequate control. Although synthetic insecticides are highly effective and fast acting yet their continuous use has resulted in serious problems such as environmental deterioration and development of resistance (Brown, 1986). Plants being rich source of bioactive chemicals (Rajkumar and Jebanesan, 2004) with insecticidal properties are now increasingly preferred as an ecofriendly alternative to chemical insecticides and so far there is no report of resistance to plant extracts due to presence of many active agents in plants (Sharma *et al*., 1992). However plant extracts are slower in action and are required in large amount because toxic agents in plants are present in small quantities. Mixing of plant extracts produces a synergistic effect which enhances their toxicity even at very low concentrations. Synergism improves the efficacy of plants and slows down the onset of resistance (Okumu *et al*., 2007). This approach has benefits of reducing the requirement of plant extracts and makes the application more effective, economical and comparatively less hazardous to the environment, an ideal ecofriendly option for management of mosquitoes. The synergistic activity of plant mixtures have been reported earlier against different pests (Rao and Dhingra, 1997; Vastrad *et al*., 2002) and vector mosquitoes (Kalyansundaram and Das, 1985; Thangam and Kathiresan, 1997; Shaalan *et al*., 2005; Youssifand Shaalan, 2011; Singha *et al*., 2011; Maurya *et al*., 2012; Rajkumar *et al.,* 2012 and Intirach *et al*., 2012). Combined formulations of different essential oils, which have more active substances than individual extracts have also been investigated as larvicides, and some mixtures were found to be more effective than neem extract (George and Vincent, 2005).

This study was undertaken to compare the individual and combined larvicidal efficacy of the leaf extracts of *Pongamia pinnata* (Fabaceae) and *Kigelia africana* (Bignoniaceae) to

Cite this article as: Poonia, S. and R. Kaushik, 2013. Synergistic activity of a mixture of *Pongamia pinnata* (Karanj) and *Kigelia africana* (Sausage tree) leaf extracts against yellow fever mosquito, *Aedes aegypti*. Pak. Entomol., 35(1): 1-4.

Azadirachta indica (Meliaceae) against vector mosquito *Aedes aegypti* under laboratory conditions.

MATERIALS AND METHODS

A laboratory culture of *A. aegypti* was maintained at controlled temperature of 28 ± 5 ºC and 70-80% RH (Sharma and Saxena, 1994) and photoperiod of 14L:10D light cycles. The larvae were fed on a powdered mixture of dog biscuits and yeast tablets in the ratio of 3:1. The emerged adults were fed with mice blood and with 10% glucose solution.

Leaves of *P. pinnata, K. africana* and *A. indica* were collected from University campus, Jaipur, shade dried at room temperature and powdered coarsely in mechanical grinder. A thirty gram leaf powder mixed with 10 times of solvent was extracted in soxhlet apparatus (Borosil Glass Works Ltd, India) for 8 hrs on a mantle heater at 50°C using petroleum ether as a solvent (WHO, 1996). The extract was then filtered and concentrated on water bath to evaporate the solvent. The filtrate was redissolved in petroleum ether to prepare 10% (w/v) standard stock solution. Different ppm concentrations were prepared by further dilution with distilled water. Tween-80 (Polyoxyethylene sorbitan monooleate) was used as an emulsifier at concentrations of 0.02% (v/v) in final test solution.

The synergistic effect of mixing *P. pinnata* and *Kigelia africana* leaf extracts in 1:1, 2:1 and 1:2 ratios was assessed by treating the 3rd and 4th instar larvae of *A. aegypti*. 1st and 2nd instar larvae being highly susceptibility were not treated. Test solutions of concentrations of 50, 100, 200, and 300 ppm were prepared. In each experiment, 25 larvae were released in glass beakers containing 100 ml of test formulation. Four replicates for each concentration were run. A control was also run in which solvent and Tween- 80 was used. Yeast powder was provided as food to the larvae. The experiment was carried out upto 72 hours without changing the treated solution. Mortality was recorded after 72 hours keeping in consideration the delayed biochemical effect (if any) of mixing the plant extracts. Lethal values were calculated according to Probit analysis (Finney, 1971). The larvae were subjected to various ppm concentrations of the extract of *P. pinnata* (PP 100%), *K. africana* (KA100%) independently and *P. pinnata* and *K. africana* combined in the ratios of PP 50%: KA 50 %; PP 75 %: KA 25 % and PP 25%: KA 75% respectively. The larvae were also subjected to the leaf extracts of *A. Indica* (AI 100%) for subsequent comparison of their efficacy. The *P. kigelia* combination extract with the maximum larvicidal potential was compared with the extract of *A. indica* (AI 100%) to study their effect on the larvae of *A. aegypti.*

The synergistic factor for different combinations of extracts was calculated according to the formula given by Kalyansundaram and Das (1985).

$$SF = \frac{LC_{50} \text{ value of the individual leaf extract}}{LC_{50} \text{ value of the leaf extract in combination}}$$

(value of SF > 1 indicate synergism and SF < 1 indicate antagonism)

RESULTS AND DISCUSSION

In the present investigation petroleum ether extracts of *P. pinnata* and *K. africana* were mixed in 1:1, 2:1 and 1:2 ratios and their larvicidal effect was evaluated against 3rd and 4th instar larvae of *A. aegypti* after 72 hrs of treatment. Results obtained from bioassay studies are presented in Table 1. The individual extract of PP (100%) showed slightly higher toxicity as is evident by its lower lethal values compared to KA 100%. The LC_{50} values obtained with PP (100%) were 49.09 ppm and 59.02 ppm for 3rd and 4th instars respectively. Among their combined extracts the mixture in 2:1 ratio (PP 75%: KA 25 %) was found to be more toxic than the other combinations and thus showed strong synergism. The LC_{50} values were lowered to 4.07 ppm and 25.12 ppm for 3rd and 4th instars respectively indicating manifold increase in efficacy. Mixture of extracts in 1:1 ratio (PP 50 %: KA 50 %) also showed increased toxicity. However, in 1:2 ratio (PP 25 % : KA 75%) the efficacy of mixture was reduced (LC_{50} 53.83 ppm for 3rd instar and 66.22 ppm for 4th instar) and mixture exhibited antagonistic effect . This might be due to lower concentration of *P. pinnata* in 1:2 ratio which probably reduced its synergistic effect. Similar antagonistic effect was observed when thymol oil isolated from the plant *Thymus capitatus* was mixed with permethrin although it synergized the toxicity of malathion (Mansour *et al* ., 2000).

In the present study the mixture of two plants in 2:1 ratio (PP 75 %: KA 25%) was found to be significantly more effective (LC_{50}=25.12ppm, LC_{90}=57.68ppm) than the *A. indica* (AI 100%) (LC_{50} 58.45ppm, LC_{90} 82.67ppm) extract (Table 1). It is evident from the present study that the mixture of *P. pinnata* and *K. africana* extracts showed very high toxicity in 2:1 ratio (synergistic factor of 12.06 for 3rd instar) and could be used in the control of mosquito larvae along with *A. indica* in integrated mosquito control program.

Plants when used in mixtures have shown higher toxicity compared to their individual extracts. Combined effect or synergistic effect of plant extracts has proved very advantageous in the control of various pests (Narasimhan *et al.,* 1998; George and Vincent, 2005). Thangam and Kathiresan (1997) stated that synergistic activity shown by plant mixtures may be due to their inhibitory effect on some factors such as detoxifying enzymes in mosquito larvae. In the present study it was reported that efficacy of mixture of *P. pinnata* and *K. africana* increases manifold when their extracts are mixed in 2:1 ratio. From the results it becomes evident that the *P. pinnata* extract has acted as a powerful synergist with *K. africana*. Synergistic property of *P. pinnata* has been reported earlier by several workers (Narasimhan *et al.*, 1998; Shanmugasundaram *et al.,* 2008). Parmar and Dutta (1987) and Rao and Dhingra (1997) have reported that karanj oil extracted from *P. pinnata* is a good synergist. The synergistic effect of mixing *P. pinnata* and *K. africana* exhibited in the present study is probably due to the presence of several toxic compounds in mixture which enhances their insecticidal activity.The results of the present work are in conformation to the observations of Shanmugasundaram *et al.* (2008) who reported that a mixture of neem and karanj oil showed strong synergism.

George and Vincent (2005) evaluated the 1:1, 2:1 and 1:2 mixtures of *Annona squamosa* and *P. glabra* against

Table 1
Combined effect of petroleum ether leaf extracts of *Pongamia pinnata* with *Kigelia africana* against *Aedes aegypti* after 72 hours of treatment .

		Lethal values after 72 hours of treatment							
		3rd instar		4th instar		Synergistic factor		Effect	
Name of plant extracts	Combination of extract	LC_{50} (95% FL)	LC_{90} (95% FL)	LC_{50} (95% FL)	LC_{90} (95% FL)	III	IV	III	IV
Pongamia pinnata(PP)	PP 100%	49.09 (47.4-50.78)	81.47 (79.56-83.38)	59.02 (57.25-60.79)	97.95 (95.96-99.94)	–	–	–	–
Kigelia africana (KA)	KA 100%	49.09 (47.4-50.78)	104.7 (102.7-106.7)	63.24 (61.44-65.04)	112.2 (110.15-114.25)	–	–	–	–
Pongamia pinnata +*Kigelia africana*	PP 50% : KA50%	18.62 (17.35-19.89)	38.90 (37.31-40.49)	43.65 (42.01-45.29)	100 (98-102)	2.63	1.35	S	S
Pongamia pinnata +*Kigelia africana*	PP 75% : KA25%	4.07 (3.46-4.68)	23.99 (22.61-25.37)	25.12 (23.72-26.52)	57.68 (55.92-59.44)	12.06	2.34	S	S
Pongamia pinnata +*Kigelia africana*	PP 25% : KA75%	53.83 (50.78-56.13)	199.5 (197.2-201.8)	66.22 (64.4-68.04)	223.9 (221.5-226.2)	0.91	0.89	A	A
Azadirachta indica (AI)	AI 100%	31.46 (28.38-34.46)	59.34 (56.23-62.36)	58.45 (55.87-61.33)	82.67 (78.22-85.88)	–	–	–	–

All values are in ppm (Figures in parenthesis are 95% fiducial limits) ; S: Synergism, A: antagonism

Culex quinquefasciatus and compared their efficacy with the efficacy of *A. indica*. Their results indicated that the mixture of the plants in 1:1 ratio showed highest toxicity as evident by their highest synergistic factor (15.1) and was comparable to the toxicity of *A. indica*. Results obtained in our study also showed that synergism shown by the plants in a mixture differed with their proportion in a mixture.

Synergism shown by insecticides in mixture could be of great benefit both economically and ecologically as it reduces the cost and increases toxicity (Metcalf, 1992). Bernard and Philogene (1993) recommended the use of synergists for diagnosing resistance mechanisms in insects. The role of phytoproducts for synergistic activity along with synthetic pyrethroids is well known against different pests (Rao and Dhingra, 1997; Vastrad *et al*., 2002). Mixtures of two or more insecticides with different modes of actions are proving to be effective and recommended for integrated resistance management in some insect pests (Shen and Wu, 1995; Zhang *et al.*, 1995; Ru *et al.*, 1998). Synergistic effect of botanical mixtures with and without synthetic insecticides was reported against vector mosquitoes by Shallan *et al*. (2005), Youssif and Shaalan (2011), Singha *et al.* (2011), Maurya *et al.* (2012) Rajkumar *et al.* (2012) and Intirach *et al.* (2012).

Toxicity of *Nerium* (Indian oleander) and fenugreek extracts increases on combining and the mortality rate increased steadily with increasing concentration and duration of treatment (Lokesh *et al.*, 2010). Mixed formulation of plant extracts which act as synergists is being increasingly recognized in mosquito management (Mohan *et al.*, 2010). Similarly larvicidal activity of plant extracts used alone and in combination with known synthetic larvicidal agents was reported by Harve and Kamath (2004) against *A. aegypti*. Renugadevi *et al.* (2012) demonstrated that combined efficacy of four mangrove plant species was higher compared to the efficacy of two plants and about 75% reduction in LC_{50} values was observed on mixing the extracts. This may be due to qualitative and quantitative increase in the active toxic compounds in the mixture.

Although neem has been fully acknowledged for its use as an insecticide but if used indiscriminately it is bound to develop resistance in due course of time. This necessitates the search for an alternative effective biopesticide . The results of present study indicate that the mixture of *P. pinnata* and *K. africana* in 2:1 ratio could be an effective substitute to the *A. indica* extract.

ACKNOWLEDGMENT

We acknowledge the financial support of University Grants Commission, New Delhi, in conduction of this study.

REFERENCES

Bernard, C.B. and B.J. Philogene, 1993. Insecticide synergists: role, importance and perspectives. J. Toxicol. Environ. Health, 38: 199-223.

Brown, A.W.A., 1986. Insecticide resistance in mosquitoes: pragmatic review. J.Am. Mosq. Control Assoc., 2: 123-40.

Finney, D.J., 1971. Probit Analysis (3rd ed.), Cambridge University Press, London, UK. P. 38.

George, S. and S. Vincent, 2005. Comparative efficacy of *Annona squamosa* Linn and *Pongamia glabra* Vent. to *Azadirachta indica* A. Juss against mosquitoes. J. Vector Borne Dis., 42: 159-163.

Harve, G. and V. Kamath, 2004. Larvicidal activity of plant extracts used alone and in combination with known synthetic larvicidal agents against *Aedes aegypti*. Indian J. Exp. Biol., 42: 12161219.

Hubalek, Z. and J. Halouzka, 1999. West Nile Fever- A reemerging mosquito-borne viral disease in Europe. Emerg. Inf. Dis., 2: 519-529.

Intirach, J., A. Junkum, B. Tuetun, W. Choochote, U. Chaithong, A. Jitpakdi, D. Riyong, D. Champakaew and B. Pitasawat, 2012. Chemical constituents and combined larvicidal effects of selected essential oils against *Anopheles cracens*. (Diptera:Culicidae). Hindawi Publishing Corporation Psyche, p. 1155.

Kalyanasundaram, M. and P.K. Das, 1985. Larvicidal and synergistic activity of plant extracts for mosquito control. Indian J. Med. Res., 82: 1921.

Lokesh, R.E., L. Barnabas, P. Madhuri, K. Saurav and K. Sunder, 2010. Larvicidal activity of *Trigonella foenum* and *Nerium oleander* leaves against Mosquito larvae found in Vellore city, India. Curr. Res. J. Biol. Sci., 2(3):154-160.

Mansour, S.A., S.S. Messeha and S.E. Gengaihi, 2000. Botanical biocides. Mosquitocidal activity of certain *Thymus capitatus* constituents. J. Nat. Toxins, 9: 49-62.

Maurya, P., P. Sharma, L. Mohan, M.M. Verma and C.N. Srivastava, 2012. Larvicidal efficacy of *Ocimum basilicum* extracts and its synergistic effect with neonicotinoid in the management of *Anopheles stephensi.* Asian Pac. J. Trop. Dis., 2(2): 110-116.

Metcalf, R.L., 1992. The synergistic activity of various selected chemical on the insecticides carbofuran and malathion against brine shrimp larvae [B.Sc. (Hons.) Thesis]. UK: Department of Agricultural and Environmental Science, University of Newcastleupon-Tyne., p.53.

Mohan, L., P. Sharma and C.N. Srivastava, 2010. Combination larvicidal action of *Solanum xanthocarpum* extract and certain synthetic insecticides against filarial vector, *Culex quinquefasciatus* (Say). Southeast Asian J. Trop. Med. Public Health, 41(2): 311-319.

Narasimhan, V., K. Rajappan, C. Ushamalini and A.A. Kareem, 1998. Efficacy of new EC formulations of neem oil and pongam oil for the management of sheath rot disease of rice. Phytoparasitica, 26(4): 301-306.

Okumu, E.O., B.G.J. Knols and U. Fillinger, 2007. Larvicidal effects of a neem (*Azadirachta indica*) oil formulation on the malaria vector *Anopheles gambiae*. Malaria J., 6:.63.

Parmer, B.S. and S. Dutta, 1987. Evaluation of some oils as malathion synergists. Int. J. Trop. Agric., 5: 223-226.

Rajkumar S. and A. Jebanesan, 2004. Mosquitocidal activities of octacosane from *Moschosma polystachyum* (Linn.) (Lamiaceae). J. Ethnopharmacol., 90: 87-89.

Rajkumar, S., A. Jebanesan and R. Nagarajan, 2012. Synergistic effect of *Andrographis echioides* and *Cadaba trifliata l*eaf extracts against larvae of dengue mosquito Aedes aegypti L. Asian Pacific J. Trop.l Biomed., 5:1588-1591.

Rao, G.R. and S. Dhingra, 1997. Synergistic activity of some vegetable oils in mixed formulations with cypermethrin against different instars of *Spodoptera litura* (Fabricius). J. Entomol. Res., 21(2): 153-160.

Renugadevi, G., T. Ramanathan, R. Shanmuga and P. Thirunavukkarasu, 2012. Studies on combined effect of mangrove plants against three dangerous mosquitoes. Int. J. Pharmaceut. Biol. Archives, 3(2): 357-362.

Ru , L.J., C.H. Rui, X.L. Fan, J.Z. Zhao and C. Wei, 1998. Realized heritability analysis of resistance to single and multiple insecticides in *Lipaphis erysimi* (Kaltenbach) and *Helicoverpa armigera* (Hubner). Acta Entomol. Sinica, 41(3): 243-249.

Shaalan, E. A., D.V.Canyon , M.W. Younes, H. Abdel-Wahab and A.H. Mansour, 2005. Synergistic efficacy of botanical blends with and without synthetic insecticides against *Aedes aegypti* and *Culex annulirostris* mosquitoes. J. Vector Ecol., 30(2): 284-288.

Shanmugasundaram, R., T. Jeyalakshmi, M.S. Dutt and P.B. Murthy, 2008. Larvicidal activity of neem and karanj oil cakes against mosquito vector, *Culex quinquefasciatus* (Say), *Aedes aegypti* (L.) and *Anopheles stephensi* (L.). J. Envir. Biol., 29(1): 43-45.

Sharma , R.N., A.S. Gupta, S.A. Patwardhan, D.S. Hebbalkar, V. Tare and S.B. Bhonde, 1992. Bioactivity of Lamiaceae plants against insects. Indian J. Exper. Biol., 30: 244-246.

Sharma, M. and R.C. Saxena, 1994. Phytotoxicological evaluation of *Tagetes erectes* on aquatic stages of *Anopheles stephensi*. Indian J. Malariol., 31(1): 21-26.

Shen , J.L. and Y.D. Wu, 1995. Insecticide resistance and resistance management in *Helicoverpa armigera*, China Agric Press, Beijing, China.

Singha, S., S. Banerjee and G. Chandra, 2011. Synergistic effect of *Croton caudatus* (fruit) and *Tiliacora acuminate* (flower) extracts against filarial vector *Culex quinquefasciatus*, Asian Pac. J. Trop. Biomed., 1(2): 159-S164.

Thangam, T.S. and K. Kathiresan, 1997. Mosquito larvicidal activity of mangrove plant extracts and synergistic activity of *Rhizophora apiculata* extract mixed with pyrethrum against *Culex quinquefasciatus*. Int. J. Pharmacog., 35: 69-71.

Vastrad , A.S., S. Lingappa and K. Basavanagoud, 2002. Vegetable oils as synergists of synthetic pyrethroids against diamondback moth, *Plutella xylostella* L. (Yponomeutidae:Lepidoptera). J. Entomol. Res., 26: 285-290.

W.H.O., 1996. Report of the WHO informal consultation on the evaluation and testing of insecticides.CTD/WHOPES/IC/96.I. Control of Tropical Diseases Division. Geneva, WHO..

Youssif, R.S. and E.A. Shaalan, 2011. Mosquitocidal activity of some volatile oils against *Aedes caspius* mosquito. J. Vector Borne Dis., 48: 113-115.

Zhang ,W.J., X.L. Hen, X.F. Li and Z.A. Yuan, 1995. The effect of mixture of phoxim and deltamethrin on development of resistance in houseflies. Acta Entomol. *S*inica, 38(14): 25-29.