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BIOLOGICALLY ACTIVE PLANT EXTRACTS FOR CONTROL OF MOSQUITO LARVAE [1]

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ABSTRACT. Thirty-six plant samples collected from Central Kentucky or grown in a greenhouse were extracted with methanol, dried, weighed and diluted with water to contain 100, 500, and 1000 ppm of the dry weight. These were then tested against larvae of *Aedes aegypti*.

Eleven plant samples resulted in 53% or greater larval mortality at 1000 ppm; 4 at 500 ppm and 1 at 100 ppm.

Recently new restrictions on the use of chemicals for insect control have stimulated investigations of the insecticidal properties of plant materials. One of the first reports of the toxic effects of plant alkaloids on mosquito larvae was that of Campbell and Sullivan (1933). They found that the plant alkaloids, nicotine, anabasine, methyl anabasine and lupinine, killed larvae of *Culex pipiens* L., *C. territans* Walk. and *C. quinquefasciatus* Say. Haller (1940) noted the extracts of Amur corktree fruit (*Phellodendron amurense* Ruprecht) were toxic to mosquito larvae. Wilcoxon *et al.* (1940) reported that extracts of male fern (*Aspidium filix-mas* (L.) SW.) killed larvae of *C. quinquefasciatus*. Hartzell and Wilcoxon (1941) found that several of the 150 species and varieties of plants which they tested gave 90 to 100% kill of *C. quinquefasciatus*. Hartzell (1944) reported 11 acetone extracts from various plant species to be toxic to larvae of *C. quinquefasciatus*, but no mortality was observed with water extracts of these same plants. Later, Hartzell (1948) found acetone extracts of "Pinaceae," Cucurbitaceae, Labiatae, Liliaceae, Compositae, Umbelliferae, Leguminosae and Euphorbiaceae to be toxic to *C. quinquefasciatus*.

Recently, Amonkar and Reeves (1970) extracted minced dehydrated garlic, *Allium sativum* L. and found it to be active

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against third instar larvae of *Culex pcus* Speiser, *C. tarsalis* Coquillett, *Aedes aegypti* L., *A. triseriatus* Say, *A. sierrensis* Ludlow, and *A. nigromaculis* Ludlow.

This study was designed to determine which and to what degree certain plant products are capable of mitigating mosquito populations.

MATERIALS AND METHODS

This investigation of plant extracts included 36 plant species in 35 genera belonging to 17 families (Table 1). Plant materials were collected from the field in central Kentucky, and some were grown in a greenhouse. Seeds of plants not native to Kentucky were obtained from various sources and grown in field plots or in a greenhouse. The principal criterion in the selection of plant species was their reputation with respect to medicinal and insecticidal properties. Plants observed to be free from insect damage were also considered, and all parts of the plants were collected. Whole plant samples were placed in paper bags and brought to the laboratory for extraction. A part of each plant sample was saved and dried in an herbarium for subsequent identification. In the greenhouse the plants were grown in aluminum planting trays and were har-

TABLE 1. Plants investigated for biological activity against mosquito.

Family	Scientific name	Common name
Asclepiadaceae	*Asclepias syriaca* L.	Milkweed
Boraginaceae	*Borago officinalis* L.	Borage
	Lithospermum arvense	Gromwell
Caryophyllaceae	*Silene antirrhina* L.	Sleepy catchfly
	Lychnis alba, Mill.	White cockle
Chenopodiaceae	*Chenopodium ambrosioides* L.	Wormseed
Compositae	*Achillea millefolium* L.	Yarrow
	Erigeron annus (L.) Pers.	Daisy fleabane
	Ambrosia artemisiifolia L.	Ragweed
Cruciferae	*Barbarea vulgaris* R. Br.	Yellow rocket
	Brassica nigra (L.) Koch.	Black mustard
	Capsella bursa-pastoris (L.) Medic.	Shepherd's purse
	Raphanus sativus L.	Wild radish
	Lepidium campestre (L.) R. Br.	Field pepperweed
	Sisymbrium altissimum L.	Tumble mustard
	Thlaspi arvense L.	Fanweed
Euphorbiaceae	*Ricinus communis* L.	Castorbean
Geraniaceae	*Geranium* spp.	Geranium
Gramineae	*Agropyron repens* (L.) Beauv.	Quackgrass
Labiatae	*Hyptis sauveolens*	[1]
	Mentha arvensis L.	Field mint
	Ocimum basilicum L.	Sweet basil
	Origanum majorana	Sweet majoram
	Salvia officinalis L.	Sage
	Satuerja hortensis L.	Summer savory
	Thymus serphyllum L.	Thyme
Liliaceae	*Allium schoenoprasum* L.	Chives
	Allium vineale L.	Wild garlic
Moraceae	*Maclura pomifera* (Raf.) Schneid.	Osage orange
Plantaginaceae	*Plantago major* L.	Broadleaf plantain
Polygonaceae	*Rumex crispus* L.	Curly dock
Rubiaceae	*Galium aperine* L.	Bedstraw
Scrophulariaceae	*Verbascum blattaria* L.	Moth mullen
Umbelliferae	*Anethum graveolens* L.	Dill
	Conium maculatum L.	Spotted hemlock
	Pimpinella anisum L.	Anise

[1] No common name according to Gray's Manual of Botany, M. L. Fernald, American Book Company, 8th Edition, 1950.

vested for extraction prior to maturity, i.e., prior to flowering. All plant samples were cleaned with a cold-water wash, chopped into small pieces, wrapped in aluminum foil, frozen, and then dried in a bulk drying chamber of a lyophilizer. The dried samples were held at a controlled temperature of 76±2° F for subsequent extraction.

EXTRACTION. A modification of the extraction method described by Amonkar and Reeves (1970) was used. This consisted of mixing 20 gm of each dried plant sample with 300 ml of methanol in a Waring blender for 2 to 3 minutes. This blended mixture was then filtered through 24 cm Whatman No. 1 filter paper. One-half of the mixture was kept at 41° F, and the other half was placed on a rotary evaporator for 2 hours at 107.6° F to evaporate the methanol. The concentrated aqueous solution was collected, and the undissolved solid residue was discarded. The aqueous solution was held at 41° F until all samples were extracted. Then 25 ml of each solution was transferred into separate pre-weighed lyophilizer vials which were rotated in a mixture of dry ice and acetone until frozen. The frozen solution was immediately placed on a lyophilizer and dried for 24 hours. After drying, the vial and its content were re-weighed, and a 10,000 ppm water stock solution was prepared from each sample. This solution was stored at 41° F and served as the basic solution for various concentrations in the bioassays.

BIOLOGICAL ACTIVITY. *Aedes aegypti* L., which has been widely used in mosquito research (Fay, 1964), served as the test organism. A colony was established from eggs received from the University of Louisville, Louisville, Ky. and the rearing procedure was identical to that described by Gerberg (1970).

Two main steps were used in the bioassay. First, all plant extracts were screened using a concentration of 1,000 ppm. Second, those extracts that produced a high larval kill were further tested at lower concentrations of 500 and 100 ppm.

Twenty ml of each sample were drawn from the prepared solution and transferred into a 20 x 150 mm test tube. Treatments were replicated 4 times in a completely randomized design. Ten 4th instar *A. aegypti* were transferred by a pipette into a strainer and the excess water drained off before releasing them into each tube. No food was provided to the larvae during the test, and each tube was covered with a piece of fine mesh cloth to confine adults that emerged during the test period. The bioassay was conducted in a laboratory maintained at 76±2° F and 70±2% RH. Results were based on the number of dead larvae and pupae in each treatment at 1, 3, and 7-days.

The data collected were statistically analyzed corresponding to the test designs. Abbott's formula (Abbott, 1925) was applied to obtain the corrected mortality. Duncan's new multiple range test (Duncan, 1955) was employed to evaluate the significance of the treatments.

RESULTS

THE LETHAL EFFECT OF PLANT EXTRACTS TO 4TH INSTAR LARVAE. Eleven of 36 plant extracts tested at 1000 ppm resulted in a greater than 53% mortality at day-7 (Table 2). Five extracts (castorbean, mothmullen, yarrow, wild garlic and quackgrass) killed all larvae in 7 days. One of these, castorbean, killed all larvae in 3 days. Castorbean, mothmullen and yarrow produced the highest mortality at day-1, i.e., 74, 93 and 75%, respectively. As the plant extract concentration decreased, larval mortality also decreased. Quackgrass and gromwell were the only 2 extracts to retain a significant mortality at 500 ppm (100 and 95% at day-7, respectively) and only gromwell resulted in a significant mortality at 100 ppm (64% at day-7).

THE EFFECT OF PLANT EXTRACTS ON INHIBITION OF MOSQUITO DEVELOPMENT. The effectiveness of plant extracts against mosquitoes was measured by their ability to delay the mosquito's developmental time (Table 3).

TABLE 2. The effect of various plant extracts on mosquito larvae. Tested at 1,000 ppm.

Plant Name	Mean percent corrected mortality [a]		
	Day–1	Day–3	Day–7
Castorbean	74^{a}	100^{a}	100^{a}
Moth mullen	93^{a}	95^{ab}	100^{a}
Yarrow	75^{a}	84^{abc}	100^{a}
Wild garlic	33^{cdefg}	83^{abc}	100^{a}
Quackgrass	23^{efgh}	64^{bcdef}	100^{a}
Sleepy catchfly	43^{bcde}	56^{bcdef}	79^{b}
Curly dock	28^{defgh}	70^{bcde}	78^{b}
Black mustard	55^{b}	68^{bcde}	76^{b}
Gromwell	50^{bc}	70^{bcde}	73^{b}
Chives	0^{i}	75^{abcd}	73^{b}
Yellow rocket	53^{bc}	51^{defg}	53^{c}

[a] Mean percents followed by the same letter are not significantly different at the 5% level. All data was used in analyses but only that resulting in 51% corrected mortality or greater is shown above. Average % mortality of 3 controls for 1, 3 and 7 days were 0.8, 12.5, and 14.2 respectively.

Twelve extracts (fanweed, sweet basil, yellow rocket, wild radish, black mustard, sweet marjoram, geranium, poison hemlock, *Hyptis sauveoleus,* gromwell, chives, and curly dock) significantly reduced adult emergence at day-7. All but the last 4 extracts plus extracts of sage, dill, field pepperweed, borage, wormseed, thyme and milkweed significantly inhibited pupal development. A few extracts also affected larval development. Sage for instance significantly delayed larval development whereas 10 extracts (summer savory, anise, field pepperweed, white cockle, borage, wormseed, sleepy catchfly, *Hyptis sauveoleus,* gromwell, chives, and curly dock) significantly increased larval development time.

DISCUSSION. The toxic effect of various plant extracts to mosquito larvae may play an important future role in mosquito control. However, the active ingredient of each extract and the minimum amount needed has yet to be determined. Of the 36 tested here, it appears that 11 of them (Table 2) warrant further study.

Just as important as the toxic effect that these plant extracts had on the mosquitoes was the delay of development, especially within the pupal stage. The reason for this phenomenon is not known, but previous reports by Russell (1971), and Russell *et al.* (1972), suggested that certain plants contain an insect hormone-like substance which may inhibit insect development. Similar work was also reported by Jacobson (1971). The results of these tests also indicate that some plants used in the tests may have contained a similar substance. The possibliity that a nutritional factor influenced the results was considered, and is not ruled out completely. However, due to the fact that only the last instar was utilized in the test the nutritional factor was considered minimal.

Those interested in research in this area may obtain additional data, not presented here, by writing to either of the junior authors.

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TABLE 3. Effect of the plant extracts on the development of *A. aegypti* larvae at day-7.

Plant Name	Percent survival	Percent remaining alive in indicated stages[a b]		
		Larvae	Pupae	Adult
Test No. 1				
Sage	72.5	10.0^{a}	2.5^{c}	60.0^{ab}
Fanweed	60.0	2.5^{b}	5.0^{b}	52.5^{bc}
Sweet basil	52.5	0^{b}	12.5^{b}	40.0^{bcd}
Yellow rocket	42.5	0^{b}	12.5^{b}	30.0^{cd}
Wild radish	60.0	2.5^{b}	30.0^{a}	27.5^{cd}
Black mustard	22.5	0^{b}	10.0^{b}	12.5^{d}
Control	87.5	2.5^{b}	0^{c}	85.0^{a}
Test No. 2				
Summer savory	82.5	2.5^{b}	17.5^{abc}	62.5^{a}
Dill	87.5	5.0^{a}	22.5^{ab}	60.0^{a}
Anise	55.0	0^{b}	5.0^{bc}	50.0^{a}
Sweet marjoram		7.5^{a}	32.5^{a}	12.5^{b}
Control	85.0	12.5^{a}	0^{c}	72.5^{a}
Test No. 3				
Field pepperweed	95.0	5.0^{cd}	37.5^{ab}	52.5^{a}
White cockle	50.0	0^{d}	7.5^{cde}	42.5^{ab}
Borage	87.5	0^{d}	47.5^{a}	40.0^{ab}
Shepherd's purse	100.0	50.0^{a}	12.5^{bcde}	37.5^{abc}
Daisy fleabane	92.5	32.5^{ab}	22.5^{abcde}	37.5^{abc}
Field mint	85.0	27.5^{abc}	22.5^{abcde}	35.0^{abc}
Wormseed	82.5	20.0^{bcd}	27.5^{abcd}	35.0^{abc}
Broadleaf plantain	92.5	45.0^{ab}	17.5^{bcde}	30.0^{bcd}
Osage orange	85.0	37.5^{ab}	17.5^{bcde}	30.0^{bcd}
Thyme	97.5	47.5^{a}	30.0^{abc}	20.0^{cde}
Bedstraw	87.5	50.0^{a}	17.5^{bcde}	20.0^{cde}
Milkweed	77.5	25.0^{abcd}	32.5^{abc}	20.0^{cde}
Ragweed	77.5	32.5^{ab}	27.5^{abcd}	17.5^{cde}
Sleepy catchfly	17.5	2.5^{cd}	0^{e}	15.0^{cde}
Tumble mustard	67.5	32.5^{ab}	20.0^{bcde}	15.0^{cde}
Geranium	70.0	35.0^{ab}	27.5^{abcd}	7.5^{e}
Poison hemlock	70.0	27.5^{abc}	35.0^{ab}	7.5^{e}
Hyptis sauveolens	65.0	40.0^{ab}	25.0^{abcde}	0^{e}
Gromwell	22.5	20.0^{bcd}	2.5^{de}	0^{e}
Chives	5.0	5.0^{cd}	0^{e}	0^{e}
Curly dock	17.5	17.5^{bcd}	0^{e}	0^{e}
Control	85.0	47.5^{a}	0^{e}	37.5^{abc}

[a] Mean percents followed by the same letter are not significantly different at the 5% level.
[b] Analysis based on the actual number remaining alive in each treatment.

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