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TWO INSECTICIDAL PRINCIPLES FROM MARIGOLD *(TAGETES SPP.)* ROOTS[1]

B. Morallo-Rejesus and L.C. Eroles[2]

The insecticidal activity of two purified principles (PA and PB) from both Tagetes erecta **(local and hybrid varieties) and** T. patula **were tested topically on diamondback moth, housefly and rice green leafhopper.**

Based on LD_{50}, the principles from T. patula **were more toxic than** T. erecta **against the housefly and diamondback moth but the reverse was true against the rice green leafhopper. The principles from** erecta **hybrid were more toxic than the local variety.**

In most cases, PA was more toxic than PB. Both principles from patula **are five times less toxic than malathion against the housefly, while acephate and isoprocab are equally toxic as PA** (patula) **and PB** (Erecta **hybrid), the most toxic principles against diamondback moth and rice green leafhopper, respectively.**

The mixture of the two principles produced either a potentaition or antagonistic action depending upon the source and the test insects.

Despite the current concern on the use of chemicals because of their persistence in the environment and resurgence of strains resistant to them, the agronomic and public health demands of the present will not permit the total elimination of chemical control. Clearly, however, the nature of the insecticides that need to be developed must pose the minimum risk for man and the environment. Furthermore, with the present energy crisis, there is a growing need to look for alternate sources or substitutes for the synthetic insecticides. The plant kingdom offers great possibilities as a source of naturally-occuring, non-persistent, and selective insecticides. Pyrethrum from flowers belonging to genus *Chrysanthemum* is one such insecticide.

The report of Uhlenbroek and Bijloo (1958) on the isolation of a nematocidal principle occuring in *Tagetes* roots and the observation of the senior author that marigold plants seem to be free from insect infestation initiated the research on the isolation of possible insecticidal principles from marigold. This paper reports on the isolation and insecticidal activity of two principles from *Tagetes* roots.

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[2]Associate Professor and Research Assistant, respectively, Department of Entomology, College of Agriculture, University of the Philippines at Los Banos.

MATERIALS AND METHODS

Isolation of the principles. The principles were isolated from the roots of one to two-week-old flowering African marigold, *Tagetes erecta* [hybrid (H)[3] and local (L)] and French marigold, *T. patula*[4] by slightly modifying the method of Uhlenbroek and Bijloo (1958, 1959). The French marigold (Fig. 1) can be easily differentiated from the African marigold. The former is a bushy, branched, one and ½ ft high plant with small, yellow-orange flowers having several petals with red markings. On the other hand, the African marigold is erect, stout and branching of more than two ft high with yellow or orange flowers (Fig. 2A & B) (Bailey 1960). The local *erecta* has small single-petalled flowers (Fig. 2C) while the hybrid has large and multi-petalled flowers (Fig. 2D).

Six hundred of clean, newly-harvested and air-dried roots from each *Tagetes* species or variety were chopped and extracted by soaking fortnight in 1.05 L of 95 percent ethanol. After filtration, the residue was extracted three times with 2.96 L of boiling ethanol for eight hours. The combined extracts were concentrated to a volume of about 250 ml by evaporation. The residue obtained was extracted three times with one L portion of petroleum ether (b.p. 40-50°C). The extracts were combined and the solvent was evaporated off.

The residue was refluxed fro three hours with a solution containing 3.5 M potassium hydroxide in six ml of water and 25 ml of ethanol. After cooling, 25 ml of water was added and the mixture was extracted three times with 12.5 ml of petroleum ether. The combined extracts were filtered and the top, oily, dark green layer was collected. The solvent was evaporated off and the residue was extracted three times with 12.5 ml of petroleum ether in a alparatory funnel. The oily, yellow top layer was collected. The crude concentrate was obtained by evaporating the solvent.

The crude concentrate (10 g) was dissolved in five ml of petroleum ether and fractionated on a column (60 x 5.5 cm) of aluminum oxide (Merck) using 50 ml of 10 percent petroleum ether in diethyl ether as eluent. Two ml fractions collected in vials were individually examined under ultraviolet (UV) light.

The fractions showing the same fluorescence were combined. Fractions 4 to 13 shoed ink-blue fluorescence (Principle A) and fractions 14 to 20 showed a somewhat greenish-gray fluorescence (Principle B). The solvent from each of these combined fractions was carefully removed by blowing

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[3] Seeds given to the senior author by Mrs. Hue Tze Chow, Dept. of Botany, National Chung Hsing University, Taichung, Taiwan.

[4] Secured by Miss Iluminada Novero, Instructor of the Dept. of Entomology, from Wageningen, Netherlands.

Fig. 1. French marigold, *Tagetes patula* is a bushy, branched, less than 1 1/2 ft high plant with yellow-orange, multi-petalled flowers having red markings.

with a stream of air. Purified crystals were obtained. The percentage crystals recovered per roots and the solubility of the crystals in organic solvents and water were determined.

Toxicity tests. The insecticidal activity of the crude extract, the purified principles (PA and PB) and the mixture (M) of the principles were tested on diamondback moth (*Plutella xylostella* L.), housefly (*Musca domestica* L.), and rice green leafhopper (*Nephotettix virescens* Dist) which were reared according to the method of Barroga (1974), Baldos (1974), and Huynh (1974), respectively.

The crystals were dissolved in petroleum ether and diluted with acetone-petroleum solvent (4:1) to prepare the solution for topical application except when applied on the rice green leafhopper (RGL) where pure acetone was used for dilution. Preliminary test showed that the mixture of acetone-petroleum ether at 4 : 1 or 4 : 6 was toxic to RGL.

Each test insect was topically applied with one microliter (UL) of each concentration of the biologically-active principles on the thorax using an automatic microapplicator with a calibrated microsyringe. Five concentrations per principle per variety were tested.

Twenty-five third instar larvae of the diamondback moth (DBM) and twenty, 3-day old adult houseflies (HF) and 4-to-6-day old adult RGL were used per treatment. Each treatment was replicated. The controls were treated with the solvent system.

The treated DBM were placed in petri dishes provided with pieces of pechay leaves, the HF were transferred to a ball jar provided with sugar, while the RGL were caged on potted rice plants. These were kept at 24°C. All mortality counts were made 24 hours after treatment.

Fig. 2A-B. African marigold *Tagetes erecta* is a stout, erect, more than 2 ft high, branching annuals with yellow or orange flowers.
A — local, small, single-petalled flowers
B — hybrid, large, multi-petalled flowers

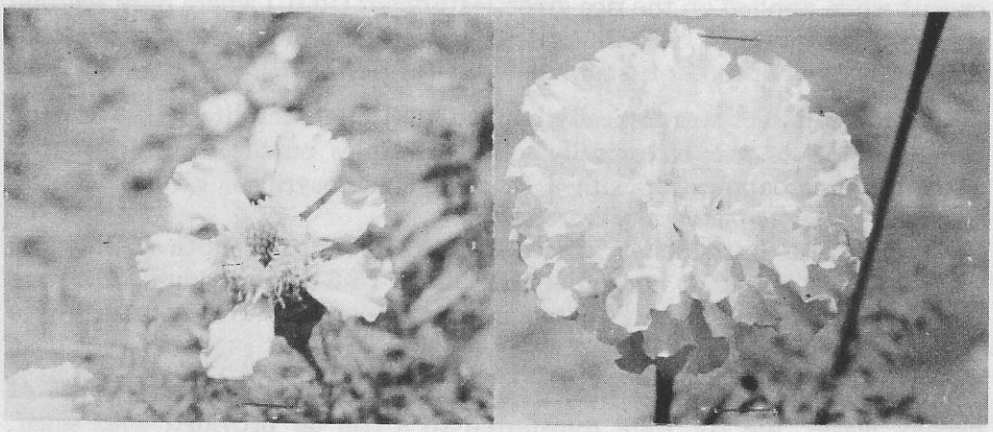

Fig. 2C-D. Close-up of *Tagetes erecta* flowers. C - local variety, single-petalled. D - hybrid variety, multi-petalled.

All mortality data were corrected for natural mortality using Abbott's formula (1925) and analyzed by probit analysis (Finney, 1971). To test the joint toxic action of PA and PB in the mixture (M), the potentiation factor and co-toxicity coefficient of M were computed based on the formula of Bliss (1939) and Sun and Hohnson (1960), respectively.

RESULTS AND DISCUSSION

The isolated principles. The crystals obtained from the ink-blue and greenish-gray fluorescent fractions were designated as Principle A (PA) and Principle B (PB), respectively. In the absence of a standard compound, the identification of the principles isolated in the present study was not carried out. Probably they are polythienyls which have been reported to be the common fluorescing components of the yellow petals of the lemon variety of *T. erecta* (Zechmeister and Sease, 1947). The principles are suspected to be similar if not the same as the nematicidal compounds isolated by Uhlenbroek and Bijloo (1958, 1959) from *Tagetes* roots whose method of isolation was followed with slight modification in this present study.

The crystals recovered from the different species varied in color and size. The crystals of Principles A and B from both *erecta* varieties are yellow, oily, thin, short and clumped together (Fig. 3 and 4). The crystals from *patula* are orange, oily, big and long; however, the PA crystals are darker and loosely arranged than PB crystals (Fig. 5).

The amount of PA crystals recovered were higher than the PB crystals in both species (Table 1). The crystals obtained were relatively pure but the recovery was lower than that in Uhlenbroek and Bijloo (1958, 1959). The crystals were highly soluble in petroleum ether, acetone and diethyl ether but insoluble in water. The solubilities obtained were that of 1-terthienyl and 5-(3-buten-1-ynyl-2,2'-bithienyl) as reported by Uhlenbroek and Bijloo (1958, 1959).

The elucidation on the properties, characterization and identification of the principles will be made in another study.

Insecticidal activity. The crude extracts from *patula* at 0.01 ug/insect killed 15 percent of the RGL but not the other test insects. The crude extracts from *erecta* showed toxic activity at a does higher than one ug/insect in all test insects except in red flour beetles which were tolerant to the extract even at 10 ug/insect. This insect was therefore no longer used as a screening organism.

Based on the LD_{50}'s (mg/g body weight), shown in Table 2, the order of decreasing toxicity of PA and PB isolated from the different species or varieties and mixtures of the two principles (M) are:

Fig. 3. Photomicrograph of crystals obtained from local *T. erecta* A - Principle A B - Principle B. (Magnification: 6.3 x 102)

Fig. 4. Photomicrograph of crystals obtained from hybrid *T. erecta* A - Principle A B - Principle B. (Magnification: 6.3 x 102)

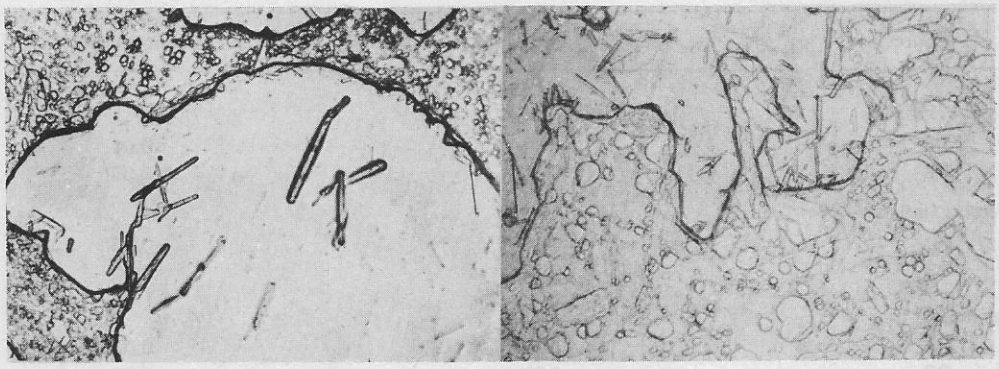

Fig. 5. Photomicrograph of crystals obtaind from *T. patula* A - Principle A, B Principle B. (Magnification: 6.3 x 10^2)

Housefly: *patula* PA = *patula* PB > *erecta* HM > *erecta* HPA *erecta* LM = *erecta* LPA = *erecta* HPB > *erecta* LPB = *patula* M.

Diamond backmoth: *patula* PA > *erecta* LPA > *erecta* LM = *erecta* HM = *patula* M > *erecta* HPA = *erecta* HPB > *erecta* LPB = *patula* PB.

Rice green leafhopper: *erecta* HPB = *patula* M > *patula* PA = *erecta* HM = *erecta* LPA = *erecta* LPB > *erecta* HPA = *patula* PB > *erecta* LM.

It is seen that the toxicity of the same principle differs in the two *Tagetes* species or in different varieties of same species. The toxicity also varied with the test insects. The principles seem to be most toxic to the RGL and least toxic to the HF.

In most cases, PA was more toxic than PB. The two principles from *erecta* H were equally to DBM; from *erecta* L to RGL and from *patula* to HF. The *erecta* hybrids were more toxic than the local variety.

Principles A and B from *patula* were the most toxic against the HF; however, the standard insecticide malathion was five times more toxic than both fractions. PA from *patula* and PB from *erecta* H were the most toxic fractions against DBM and RGL, respectively. The former principle is as toxic as acephate (Orthene) and the latter as toxic as isoprocarb (Mipcin) to the test insects.

TABLE 1. **Crystals recovered from 100 g of** Tagetes **roots.**

Sources	Weight (mg)	Percent recovery
erecta hybrid		
PA	400	0.04
PB	200	0.02
erecta native		
PA	300	0.03
PB	200	0.02
patula		
PA	200	0.03
PB	100	0.01

The potentiation factor (PF indicates whether there is a positive synergistic effect (PF $>$ 1), negative joint action or antagonism (PF $<$ 1) and unchanged joint action (PF = 1). On the other hand, the co-toxicity coefficient indicates whether the joint action is similar (act on the same site) or dissimilar (they act on different sites) to produce the response. According to Sun and Johnson (1960), a coefficient near 100 indicates probability of similar action; less than but near 100 indicates dissimilar activity or independent joint action. In both types of action, the combination results in very little gain or loss of insecticidal action. A coefficient significantly above 100 indicates synergism or potentiation while a coefficient significantly less than 100 indicates antagonism if the LD_{50} of the mixture is greater than the strongest toxicant in the mixture.

The joint toxic action of the principles was calculated from the LD_{50}'s of PA, PB and their mixtures (Table 2). Results of the calculations of potentiation factor (PF) and co-toxicity coefficient (Table 3) show that the combination of the two principles from *erecta* increased the toxicity to the HF and DBM but the mixture from *patula* was antagonistic to HF but although highly potent to DBM and RGL. As shown by the co-toxicity coefficient, the antagonistic action of the mixture from *erecta* local and *patula* to RGL and HF, respectively, is due to the dissimilar action of the principles. This indicates that PA and PB act on different sites which modify the physiological action of the other. The coeficient of the principles from *erecta* hybrid against RGL indicates similar action. Their combination against RGL show little loss of toxicity as shown by the LD_{50} of the mixture (Table 2).

The analysis of the joint toxic action indicates that the principles from *patula* need not be isolated separately if they are to be applied against DBM and RGL. The same is true of the principles from *erecta* against the DBM and HF, thus, lowering the cost of isolating the active material.

TABLE 2. **Median lethal dosages (mg/gm body weight) of the different** Tagetes **principles and mixtures to three insects.**

Treatments	Housefly			Diamond backmoth			Rice green leafhopper		
	LD_{50}	Fiducial Limits (P = .05)		LD_{50}	Fiducial Limits (P = .05)		LD_{50}	Fiducial Limits (P = .05)	
T. erecta hybrid									
PA	0.184	0.173 -	0.196	4.149	3.895 -	4.421	0.010	0.009 -	0.011
PB	0.260	0.235 -	0.287	4.316	4.053 -	4.605	0.003	0.002 -	0.005
M	0.161	0.151 -	0.171	2.754	2.640 -	2.886	0.007	0.006 -	0.008
T. erecta local									
PA	0.238	0.227 -	0.249	1.684	1.605 -	1.763	0.009	0.008 -	0.011
PB	12.100	11.866 -	12.318 -	12.318	12.200	11.731 -	12.688	0.008 -	0.009
M	0.227	0.213 -	0.242 -	2.500	2.289 -	1.737	0.300	0.250 -	0.362
T. patula									
PA	0.107	0.100 -	0.114	1.228	1.088 -	1.386	0.007	0.006 -	0.007
PB	0.108	0.101 -	0.117	12.522	10.859 -	14.449	0.012	0.010 -	0.014
M	14.501	11.221 -	17.500	2.860	2.784 -	3.053	0.0038	0.0034 -	0.0043
Standard Insecticide									
Malathion	0.019	0.016 -	0.024						
Orthene				0.974	0.877 -	1.079			
Mipcin							0.0028	0.0025 -	0.0031

PA – Principle A.
PB – Principle B.
M – Mixture of PA and PB.

TABLE 3. **Analysis of the joint toxic action of the principles in a mixture**[a]

Source of Principles and Insects Treated[b]	Pontentiation Factor[c] of M	Co-toxicity Coefficient[d] of M
T. erecta hybrid		
HF	1.37	519
DBM	1.53	3559
RGL	0.92	218.46
T. erecta local		
HF	17.18	5870
DBM	2.78	970
RGL	0.03	33.3
T. Patula		
HF	.007	67
DBM	4.45	482
RGL	1571	36800

[a]Mixture (M) of Principles A and B.

[b]HF - housefly; DBM - diamond backmoth; RGL - rice green leafhopper.

[c]Computed according to Bliss (1939).

[d]Computed according to Sun and Johnson (1960).

This study clearly shows that *Tagetes* is a potential source of insecticides against the three test insects especially against HF and RGL. It is interesting to note that the PA from the local variety of *erecta* was toxic against the three test insects especially to the RGL. Because of its wide distribution and ease of cultivation, the marigold offer great promise of being a cheap source of insecticides and nematicides. The local marigold, however, needs much improvement by selecting and breeding with other species like *patula* to increase its insecticidal content and potency. The imported *erecta* hybrid and *patula* could probably be planted extensively in the Philippines as shown by the good growth of the varities in our experimental fields.

However, before these principles can be used for field control, it is necessary to know their identity, toxicity to other insects and to vertebrates, persistence in the environment, and effectiveness under field conditions.

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