# TOXICITY OF SOME COMPOUNDS ISOLATED FROM *ABRUS PRECATORIUS* L. SEEDS TOWARDS THE TWO-SPOTTED SPIDER MITE *TETRANYCHUS URTICAE* KOCH

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BIOLOGICAL COMPOUNDS AND SPIDER MITE CONTROL

ABSTRACT : Three compounds isolated from non-saponified fraction of crude petroleum ether extracts of *Abrus precatorius* L. seeds were tested for their acaricidal activity against the females and eggs of *Tetranychus urticae* Koch. The role of compounds II (β-amyrin) on the biological activity of this pest was taken in consideration.

The compounds isolated include compound I (may be coumarin), β-amyrin and mixture of sterols, all of which were toxic to both adult females and eggs of the two-spotted spider mite. B-amyrin proved to be more efficient than the other compounds tested for the toxicity of both stages.

Spraying the adult females with $LC_{25}$ of β-amyrin caused a high significant reduction in the fecundity in comparison to the control. Also the viability of the resulting eggs was greatly reduced while the durations of the immature stages were unaffected.

COMPOSÉS BIOLOGIQUES ET CONTRÔLE DE L'ARAIGNÉE ROUGE

RÉSUMÉ : Trois composés isolés d'une fraction non saponifiée d'extraits de graines d'*Abrus precatorius* L. à l'éther de pétrôle brut ont été testés du point de vue de leur activité acaricide sur les femelles et les œufs de *Tetranychus urticae* Koch. Le rôle du composé II (bêta amyrin) dans l'activité biologique de cette peste a été retenu.

Les composés isolés comprenaient un composé I (coumarin peut-être), le bêta amyrin, et un mélange de stérols, et tous ont été toxiques à la fois pour les femelles adultes et pour les œufs de l'acarien. Le bêta amyrin s'est montré plus efficace que les autres composés testés pour la toxicité à l'égard des deux stades. Un traitement de femelles adultes avec le bêta amyrin à LC 25 a provoqué une réduction significative de la fécondité par rapport au contrôle. La viabilité des œufs restant a été aussi grandement réduite tandis que les durées des stades immatures n'étaient pas affectées.

## INTRODUCTION

The two-spotted spider mite is a cosmopolitan pest. It has recently became a dangerous problem as a result of continuous use of pesticides. The rise of resistance among mite population implied the necessity for alternation of acaricides. The hypothesis that plant secondary coumpounds may serve a protective function against herbivory appears to be currently well accepted (RHOADES and CATES, 1976). Survey of plants possessing insecticidal and acaricidal properties have been reported during the last decade (REMBOLD *et al.*, 1980 ; WILLIAMS *et al.*, 1980 ; AHMED, 1983 and BARAKAT *et al.*, 1984-1985).

More recently, AMER *et al.*, 1989 and DIMETRY and ABDALLAH, (1988) have studied the effect of crude extracts of *Abrus precatorius* seeds with different organic solvents against the two-spotted spider mites and the cotton leafworm. They found that petroleum ether extracts of the above seeds were more toxic than the other extracts tested against the above mentioned pests.

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Because *A. precatorius* seeds is thus a potential source of naturally occuring pesticides, this paper reports the isolation procedures, acaricidal properties and biological activities of the most efficient compound isolated against the two-spotted spider mite *Tetranychus urticae*.

## Materials and methods

Mites were obtained from a laboratory culture of the two-spotted spider mite, *Tetranychus urticae* Koch on lima bean *Phaseolus vulgaris* L. under 20 ± 5° C and 65 ± 5 % R. H.

*Abrus precatorius* seeds were grounded to a powder. A known weight of the powdered seeds (250 gm.) was extracted with petroleum ether B. V. 40-60° in a continuous extraction apparatus till 5 ml. of the extract left almost nothing on evaporation. The extract was evaporated and the residue was subjected to steam distillation until no more odour could be detected in the distillate.

Ten grams of the petroleum ether extract was saponified by reflexing in 70 ml. 10 % alcoholic KOH and 20 ml. benzene on a boiling water bath for 6 hours. The mixture was distilled off to dryness, 100 ml. of water was added to the residue and the mixture was transferred to a separating funnel and shaken with ether (4 × 10 ml.).

The combined ethereal extract was washed with water till it became free from alkalinity, dried over anhydrous sodium sulphate and the ether was evaporated to dryness. The unsaponified residue obtained was yellowish brown, semi-solid, and gave positive test for steroids. The unsaponified fraction was dissolved in chloroform and chromatographed on preparative silica gel G plates together with authentic samples using the following system : Benzene + ethyl acetate 86 : 14. The spots were located by sulphuric acid reagent 50 % then subjected to ultra violet rays at wave length 254 mu. The bands corresponding to each spot was removed from the plates and elluted with methyl alcohol. The methyl alcohol extract of each spot was concentrated to give three compounds.

From each of the three compounds isolated, four concentrations were made serially. The different solutions were applied to the adult *T. urticae* by the slide dip method technique according to Dittrich (1962). Five replicates for each concentration were adopted. Every replicate contains twenty adult females. Mortality counts were recorded after 24, 48, 72 and 96 hours.

The different compounds were tested also for their ovicidal effect. For this purpose, ten females were placed on lima bean discs and left to deposit their eggs for 24 hours and removed thereafter. The accumulated eggs were sprayed with the different concentrations by a glass atomizer. Five replicates, each containing twenty eggs were treated. Six days after treatments, unhatched eggs were counted.

Mortality counts for adults and eggs were corrected using Abbott's formula (1925). Results were statistically analysed according to the method of Finney (1952) and plotted on log dosage probit paper.

To investigate the possible effect of the sublethal dose of the most promising compound isolated on reproduction of the mites, females were sprayed by means of an atomizer with $LC_{25}$ of compound II

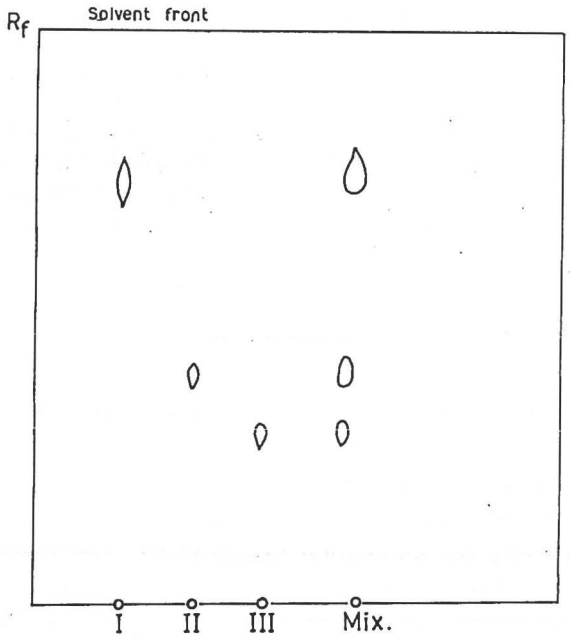


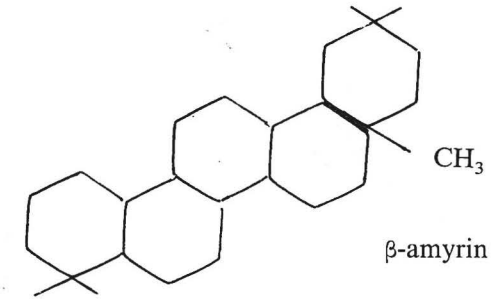

Fig. 1 : Chromatogram (TLC) of crude pretroleum ether extracts of *Abrus precatorius* seeds and isolated compounds.Mix. : Crude petroleum ether extracts. I : may be coumarin. II : β-amyrin. III : mixture of sterols.

and then transferred to untreated lima bean discs and the different biological aspects of both the females and the resulting progeny were followed up.

## Results

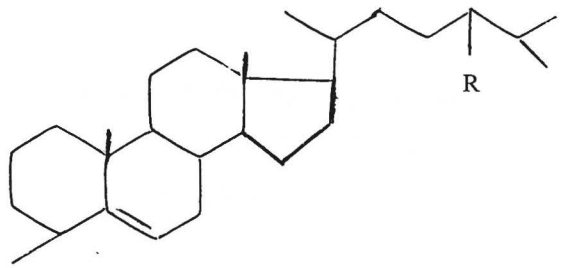
In TLC analysis (Fig. 1), the purified extract shows 3 compounds when it was examined under U. V. light after spraying with sulphuric acid. The three had $R_f$ values of 0.73, 0.40 and 0.30. Compounds No. II and III were identical to β-amyrin and sterols mixture (β-sitosterol, campesterol and stigmasterol), while compound I may be Coumarin but needs further investigation. The compounds II and III have been shown to have the following structures :

Compound II




β-amyrin

Compound III




Mixture of stereol found in *Abrus* seeds

R = $CH_3$ campesterol
R = $CH_2CH_3$ β-sitosterol
R = $CH_2CH_3$ $\Delta^{22}$, stimagsterol

Table 1 shows mortality data for the three compounds isolated from petroleum ether extracts of *A. precatorius* seeds against adult females. Increased mortality resulted over the four day test period. Fig. 2 shows the probit regression line for 24-96 hrs. data. The steepness of the lines indicates that *T. urticae* females were quite susceptible to the three compounds isolated. β-amyrin was the most effective compound. The $LC_{50}$ values after 96 hours were 1.056, 0.295 and 1.153 mg/ml. for compound I, β-amyrin and sterol mixture, respectively. At $LC_{90}$ levels similar trend was obtained as in $LC_{50}$.

β-amyrin is considered the standard compound isolated in calculating the toxicity index at $LC_{50}$ level. Relative potency levels at the $LC_{50}$ indicated that this compound was the most toxic compound isolated from petroleum ether extract of *A. precatorius* seeds in comparison to the other two compounds isolated under the different periods of the experimental time (24-96 hrs.). The toxicity of β-amyrin for the adult females *T. urticae* increased gradualllly after application reached its maximum value after 72 hr. where its relative potency level was 5.72 and 4.27 times more toxic than sterols mixture and compound I, respectively. After 96 hrs., its potency decreased again to 3.91 and 3.58 times more toxic than sterols mixture and compound I, respectively.

Table 1 : Relative susceptibility of the adult females. *T. urticae* to the different compounds isolated from petroleum ether extracts of *A. precatorius* seeds.

| Compouds isolated | $LC_{50}$ mg/ml | | | | No. of folds compared with mixture of sterols at | | | |
|---|---|---|---|---|---|---|---|---|
| | hr. | | | | hr. | | | |
| | 24 | 48 | 72 | 96 | 24 | 48 | 72 | 96 |
| Compound I | 4.240 | 3.6110 | 2.463 | 1.056 | 1.03 | 1.19 | 1.34 | 1.09 |
| β-amyrin | 2.511 | 0.9003 | 0.577 | 0.295 | 1.74 | 4.76 | 5.72 | 3.91 |
| Mixture of sterols | 4.377 | 4.2870 | 3.303 | 1.153 | 1.00 | 1.00 | 1.00 | 1.00 |

Ovicidal action . — Table 2 shows that β-amyrin was the most effective compound at $LC_{50}$ and $LC_{90}$ levels.

At $LC_{50}$ level, the number of folds shown in Table 2 indicate that β-amyrin was 4.86 and 1.04 more toxic than sterols mixture and compound I, while at $LC_{90}$ level, it was 2.40 and 1.97 times more toxic respectively. At LC25, compound I was 11.2

and 1.59 more toxic than sterols mixture and β-amyrin respectively.

TABLE 2 : Relative susceptibility of the egg stage of adult females *T. urticae* to the different compounds isolated from petroleum ether extracts of *A. precatorius* seeds.

| Compounds isolated | Conc. mg/ml | | | Slope | No. of folds compared with mixture of sterols at | | |
|---|---|---|---|---|---|---|---|
| | *$LC_{25}$ | **$LC_{50}$ | ***$LC_{90}$ | | $LC_{25}$ | $LC_{50}$ | $LC_{90}$ |
| Compounds I | 0.300 | 1.204 | 12.260 | 1.27 | 11.20 | 4.66 | 1.22 |
| β-amyrin | 0.478 | 1.155 | 6.248 | 1.75 | 7.03 | 4.86 | 2.40 |
| Mixture of sterols | 3.360 | 5.615 | 14.970 | 3.01 | 1.00 | 1.00 | 1.00 |

* $LC_{25}$ = Lethal concentration for 25 % of the population.
** $LC_{50}$ = Lethal concentration for 50 % of the population.
*** $LC_{90}$ = Lethal copncentration for 90 % of the population.

From the foregoing results, it can be concluded that β-amyrin which is triterpenoid, is the most potent compound isolated from petroleum ether extract of *A. precatorius* seeds against both the adult females and the eggs of *T. urticae*, followed by compound I. Sterols mixture, even at high concentrations, was the least effective one. Toxicity data show that the females were more susceptible to the different compounds tested than the eggs.

Effect of β-amyrin on the different biological aspects of *T. urticae* :

Table 3 shows that adult females treated with $LC_{25}$ level of β-amyrin reduced significantly the fecundity of the females and the percentage of egg hatchability. The percentage of reduction in the number of eggs laid reaches 51.9 %, where as the pre-oviposition period is highly significantly elon-

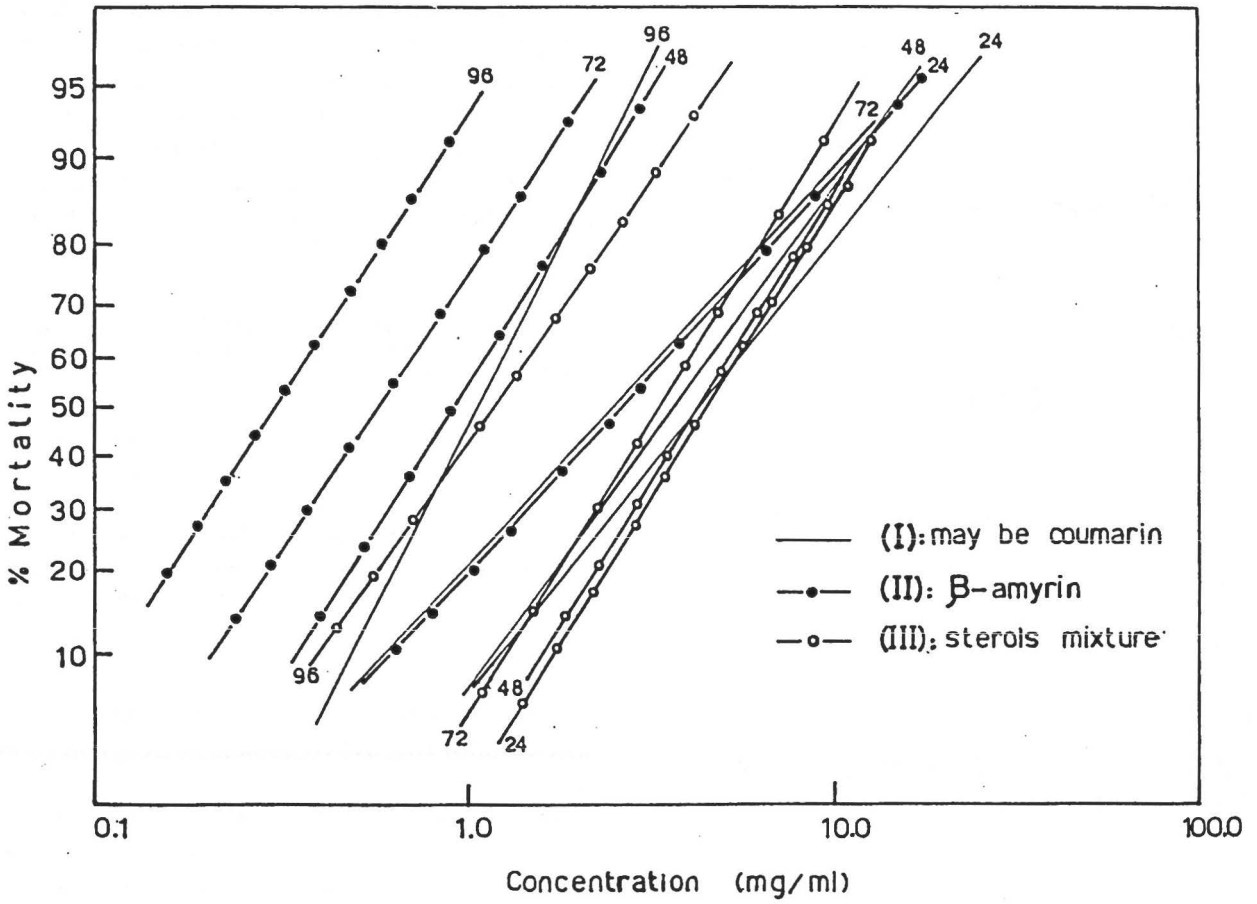



FIG. 2 : Relative susceptibility of adult females of *T. urticae* to the 3 compounds isolated from petroleum ether extract of *Abrus precatorius* seeds during the period from 24-96 hours.

TABLE 3 : The effect of β-amyrin on the biological aspects of *T. urticae* Koch at $LC_{25}$ level.

| Treatment | Duration in day | | | | | | | | | | | % | N° |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Preovipo-sition period | Ovipo-sition period | Female longevity | Incuba-tion period | Larval stage | Larval quiescent | Proto-nymphal stage | Proto-nymphal quies. stage | Deuto-nymphal stage | Deuto-nymphal quies. stage | Fecundity ♀ | Reduc-tion in fecund. | Unhat-ched eggs |
| Control | 1.4 | 14.8 | 25.6 | 9.7 | 2.2 | 3.4 | 2.8 | 3.6 | 4.0 | 3.7 | 38.6 | Zero | 0.4 |
| -Amyrin | 6.6 | 12.1 | 20.6 | 12.4 | 3.0 | 3.0 | 1.8 | 2.3 | 2.0 | 3.2 | 12.2 | 51.9 | 2.0 |
| L.S.D. level at : | | | | | | | | | | | | | |
| 0.05 | 2.45 | 4.42 | 11.53 | 3.22 | 1.03 | 0.64 | 0.74 | 1.05 | 1.08 | 1.55 | 9.11 | — | 1.15 |
| 0.01 | 3.36 | 6.06 | 15.79 | 4.41 | 1.41 | 0.88 | 1.02 | 1.44 | 1.47 | 2.13 | 12.48 | — | 1.57 |

gated. The resulting progeny, when offered untreated discs, is not affected, except in case of protonymph, quiescent protonymph and deutonymph, where their durations are significantly shortened in comparison to the control.

## DISCUSSION

In the light of the results obtained during the experimental work with the bead tree (*Abrus precatorius*) seeds, the compounds isolated from its petroleum ether extract, however, suggest that it has got an acaricidal property against the two-spotted spider mite. The toxicity of each compound isolated, i.e. β-amyrin, compound I (may be coumarin) and mixture of sterols, was highly significant ($LC_{50}$ values were 0.9003, 3.611 and 4.287 mg/ml. for each compound, respectively) in comparison to the results obtained by AMER *et al.*, 1989 ($LC_{50}$ values of the crude petroleum ether extract was 174 mg/ml. against adult stage of *T. urticae*).

Compound I (Coumarin) is less toxic in comparison to β-amyrin against both the adult females and eggs of *T. urticae*. The toxicity of Coumarin against the two-spotted spider mite is confirmed also by REDA and EL-BANHAWY (1986), who found that contacting treated leaves with coumarin at 250 ppm. caused mortality and low reproduction of *T. urticae* when the time of exposure increased gradually from 2 to 144 hr. β-amyrin, on the other hand, which is triterpenoid, was found to be the most active compound against both the adult and egg stages of the two-spotted spider mite.

The present study is in agreement with previous studies done for other triterpenoids extracted from the neem tree, which is effective against *Locusta* sp. (PRADHAN and JOTWANI, 1968) but only moderately so against *Pieris brassicae* and *Plutella xylostella* (BUTTERWORTH and MORGAN, 1971 ; GILL, 1972). Also, NAWROT *et al.* (1984) coincided with the present findings and reported that three sesquiterpene lactones isolated form plants when topically applied on the larvae, pupae and adults of *Tribolium confusum* Duv. and *Trogoderma granarium* and on adults of *Sitophilus granarius* were toxic and caused morphogenetic changes in pupae.

Assays of the toxicity of the sterols mixture in fact is apparently less active than the other two compounds but when applied at a concentration of 1.153 mg/ml. it gave mortality for 50 % of the population. This findings confirm the previous work done by NAYAR and FRAENKEL (1962) who found that the biting response of *Bombyx* larvae on agar diet discs was inhibited by cholesterol, β-sitosterol and stigmasterol.

Adults *T. urticae* treated with β-amyrin at the $LC_{25}$ level caused a high significant reduction in the number of eggs laid and the fertility of the resulting eggs. There would be some reasons explaining this phenomenon. It is direct influence of the substance on the female ovaries or it is possible also that after contact application of any substance on the cuticle of the pest the production of pheromones is disturbed. SLAMA (1969) and HEROUT (1970) gave many examples of disturbing insect development process by natural products. In agreement with

present results, fresh extract of *Ajuga remota*, which included allelochemics, caused high morphological abnormalities and low rate of oviposition in *T. urticae* (SCHAUER and SCHMUTTERER, 1981). Nevertheless, further studies are urgently needed such as field trials, mammalian toxicity and its persistance over long time intervals. However, our studies to determine its deterrency on the mite are still in progress.

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*Paru en Février 1991.*