Chemistry for providing facilities for carrying out the work.

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EFFECTS OF NYMPHAL TREATMENT WITH TEPA ON REPRODUCTION OF *DYSDERCUS KOENIGII* F.

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ABSTRACT

Varying doses of tepa applied topically on the different nymphal instars of *Dysdercus koenigii* F. produced adults which exhibited a remarkable degree of sterility. The effects of this treatment on the mortality and the formation of abnormal stages of development have also been investigated. 100% sterility has been achieved with a minimum dose of 0·5 μg of tepa/nymph in the third and fourth instars.

INTRODUCTION

RED cotton-bug *Dysdercus koenigii* F. is an important pest of cotton and some vegetable crops in India. Control with insecticides in the fields has been reported but with limited success. The encouraging results of the use of chemosterilants in different groups of insects[1,2,7,9,10] have created an interest in using such chemicals as a possible means of control. Before determining the feasibility of the use of such chemicals in fields for a successful control, laboratory investigations are of utmost importance. Some information regarding the sterilization of a number of species of bugs is available in the literature. Economopoulos and Gordon (1969) have induced sterilization in male *Oncopeltus fasciatus* (Dallas) with tepa. Carcavallo and Carabajal (1971) carried out sterilization experiments on three species of *Triatoma*. Economopoulos (1971) has investigated the effects of tretamine on fourth and fifth instars of *O. fasciatus* male nymphs and female adults. Mustafa and Naidu (1964) demonstrated sterilization of male *D. cingulatus* with apholate as a surface-contact sterilant. Degeneration of the oocytes and inhibition of ovarian growth were induced in the adult female *D. cingulatus* with tepa injections[12]. In these investigations of chemosterilization of *Dysdercus*, the chemosterilant compound was administered either orally with the diet or by injection, and the insect was treated in the adult stage.

In our investigations on the effects of tepa on the fecundity and sterility of *Dysdercus*, we have treated both the male and female insects. The chemical has been administered topically. Attempts have been made to find out the most susceptible stage in the life-cycle with a minimum of mortality. Therefore, we selected the nymphal instars where the topical treatment in solution would least affect their general activity, with the result, we had to avoid first two instars because of their very small size. This paper, the first in a series, presents the results of the laboratory tests which give quantitative information on the toxic and sterilizing activity of tepa against *Dysdercus*.

MATERIALS AND METHODS

Stock culture of *Dysdercus* was maintained in the laboratory at 27 ± 2° C in 22 × 16 × 16 cm glass toffee-jars, covered with muslin held in place by rubber bands. The jars contained food, *i.e.*, dry cotton seeds (previously washed in running water and dried with clean filter-paper or cotton wool) and water (cotton wool dampened with distilled water) in small petri dishes. The experiments were performed on various nymphal stages picked up from the culture within 24 hours of their emergence. Before treatment the insects were immobilized by momentary cooling in the freezing chamber of a refrigerator. A stock solution of tepa, *Tris*(1-aziridinyl) phosphine oxide, was made in acetone. Agla all-glass syringe fitted with micro-

meter was used for delivering the chemical on all the stages which were treated topically with graded concentrations of tepa in 1·0 µl drops that were applied the ventral abdominal surface. First and second instar nymphs were not able to tolerate the drops of 1·0 µl. Therefore, the treatment was restricted to the third, fourth and fifth nymphal instars only, the stages when the maturation of the gonads actually takes place. The treated insects were removed to the jars containing cotton seeds and soaked cotton pads. They were observed daily for any mortalities or moults during development and these were regularly recorded. In the fifth nymphal stage they were sexed as far as possible and the males and females were separately placed. 3–4 days after adult emergence they were allowed to mate with treated or untreated insects of the opposite sex for different experimental designs. Daily examination for the eggs was made and the eggs from the jars were removed within 12–18 hrs of laying. After counting, the eggs were incubated in small vials (2·5 × 5 cm) covered with cotton plugs and kept in the humidity chamber at 27± 2° C. The number hatched was determined and recorded. In each test, six replicates of ten insects each, were taken at the start of the treatment, while a control replicate in each test was treated with solvent.

Results and Discussion

Toxicity and Mortality

The toxicity pattern of four doses of tepa on third, fourth and fifth nymphal instars is given in Table I. The first and second instar nymphs showed a heavy mortality rate, even with 1·0 µl of the solvent. acetone/nymph. Therefore, the effectiveness of the chemosterilant on these stages is not considered at present. 0·5–1·0 µg tepa/nymph shows a comparatively low mortality rate but the higher doses of 1·5 µg and 2·0 µg/nymph are quite toxic, especially to the third and fourth instars. The mortality goes considerably high (98·2%) in the fourth instar. The mortality in the fifth instar treatment does not seem to be very high. This is possibly because it enters the adult stage sooner than the other two earlier instars. In all these cases, after the imaginal stage is reached, the mortality rate becomes normal and at par with that in control. The total mortality in the third instar treatment is highest because it takes a longer period to reach the adult stage. It is also possible that since same amount of tepa was applied on all the nymphs, the younger stages, being smaller in size, received a greater dose per unit body weight thus leading to higher mortality. X-irradiation of an instar of *D. koenigii* during

Table I

Mortality of D. koenigii *nymphs during development with varying doses of tepa*

Stage treated	Dosage of tepa/nymph (µg/µl acetone)	Mortality in various nymphal instars			Total mortality (out of 60)	Corrected total mortality* (%)
		3rd	4th	5th		
3rd instar	0·0 Control	2	1	0	3	..
	0·5	4	0	5	9	10·52
	1·0	1	0	9	10	12·32
	1·5	21	22	6	49	79·68
	2·0	15	26	10	51	84·21
4th instar	0·0 Control	..	2	0	2	..
	0·5	..	3	1	4	3·41
	1·0	..	15	6	21	32·71
	1·5	..	40	7	47	77·64
	2·0	..	51	8	59	98·24
5th instar	0·0 Control	..	0	0	0	..
	0·5	..	..	4	4	6·70
	1·0	..	..	20	20	33·33
	1·5	..	..	18	18	30·00
	2·0	..	..	26	26	40·00

* Using Abbot's formula.

its late phase has no effect on its next moult but irradiation during the early stages did affect the next moult[8]. It was therefore suggested that a treatment at a late stage is not very critical for an approaching moult. The present situation could also be explained in this context. Very little mortality is observed in 5th instar treatment but the nymphs treated earlier show more mortality. This is because of the toxic effect being carried over to the next instar. A similar explanation for higher mortality in *Oncopeltus* nymphs treated with tretamine in the early 4th instar than those in fifth, has been put forward[5]. Figure 1 gives a comparative account of corrected total mortality in the three stages treated with four different doses of tepa.

Abnormal Stages

As a result of this treatment in all the tests, some of the fifth instar nymphs, instead of moulting into adults, produced abnormal stages of development which were identified as sixth-instar nymphs and juvenile adults. Some nymphs struggled to moult, with old cuticle unsuccessfully cast off and crumpled wings. The results of such treatments are summarised in Table II. The fourth instar nymphs treated with 1·0 μg tepa/nymph produced maximum number of abnormal stages. All these stages died after a week's time, without any reaching maturity. These forms were also allowed to mate with untreated virgin males and females but were found incapable of reproduction. A variety of abnormal stages of development were observed in *D. fasciatus* by treating nymphal instars with juvenile hormone analogue, methyl fornesoate dihydrochloride, applied topically[4]. In the present study, the formation of abnormal stages could be attributed to some hormonal inbalance caused by the application of the chemosterilant.

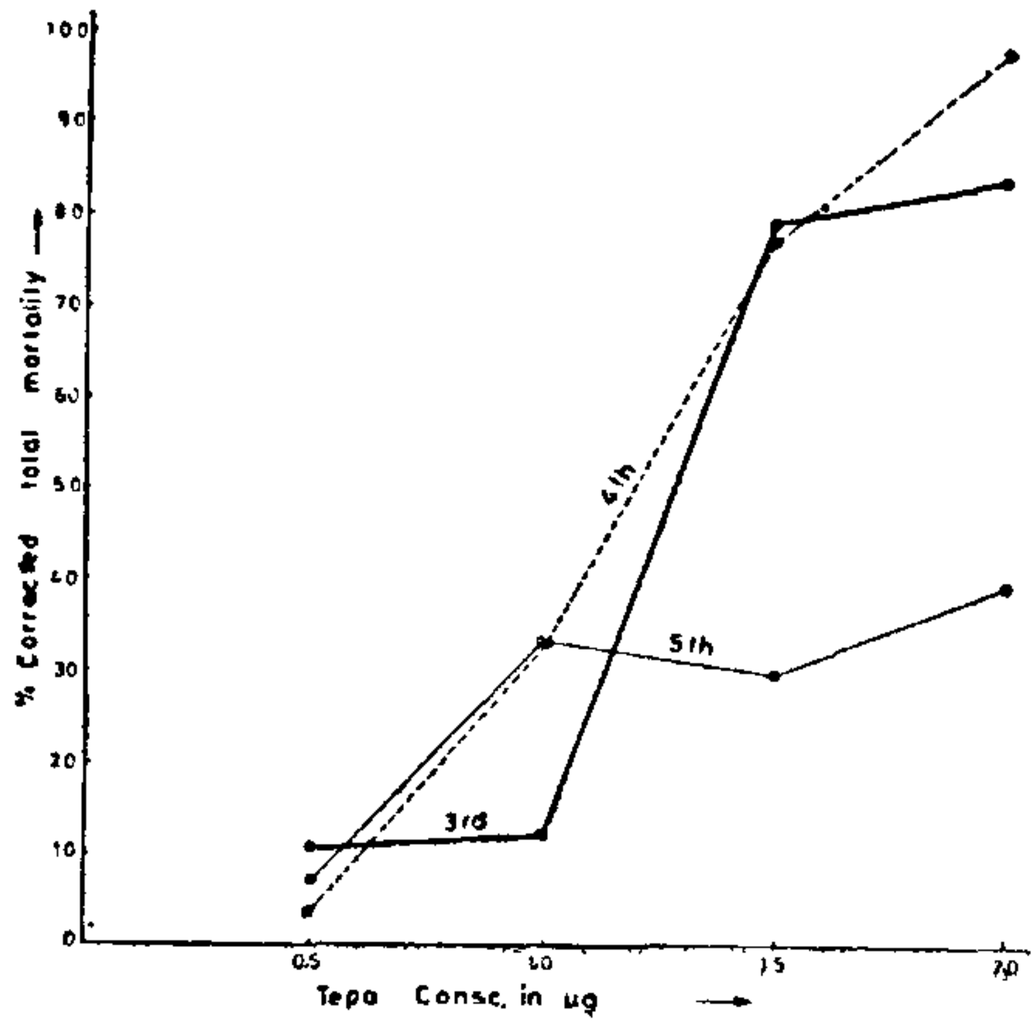

FIG. 1. Mortality curves of third, fourth and fifth nymphal instars treated topically with different concentrations of tepa.

TABLE II

Abnormal stages of development produced in Dysdercus *by tepa treatment*

Stage Treated	Dosage of tepa/ nymph (μg/μl acetone)	No. of 6th instar/Juvenile; adult produced; (out of 60)	Per cent
3rd instar	0·5	..	..
	1·0	4	6·67
	1·5	4	6·67
	2·0	2	3·34
4th instar	0·5	2	3·34
	1·0	10	16·67
	1·5	6	10·0
	2·0	..	..
5th instar	0·5	..	..
	1·0	3	5·0
	1·5	2	3·34
	2·0	5	8·33

Fecundity and Sterility

Because of high mortality in the third instar with dosages of 1·5 and 2·0 μg/nymph, the mating experiments were not done. The adults, surviving from the third instar nymphs treated with 0·5 and 1·0 μg/nymph, were allowed to mate both with untreated and treated virgins of the opposite sex. The data presented in Table III indicates that both the doses cause no hatch in the eggs laid with any of the three crosses, *i.e.*, treated females × normal males, treated females × treated males and normal females × treated males. 0·5 μg dose had also caused a significant reduction in oviposition, which was still more pronounced with 1·0 μg dose. A female treated with 0·5 μg laid an average of 58 eggs which was further reduced to 14·5 eggs female with 1·0 μg dosage. In the control the number of eggs laid was 500/female. A similar reduction in the fecundity was obtained in the adults which emerged from the fourth instar nymphs treated with same doses (Table IV). 0·5 μg of tepa applied to fourth instar nymphs caused 95% sterility if crosses were made between treated male and normal/treated female. In a reciprocal cross between treated females and normal males, the per cent hatch is much higher, showing that tepa induces more sterility in males. A dose of 1·0 μg/ nymph shows a similar result. A treated female crossed with a normal male produces on the average 5·4% viable eggs while 100% sterility is achieved in crosses between treated males and normal/treated females,

TABLE III

Effect on fecundity, egg hatchability and sterility of Dysdercus *third instar nymphs topically treated with varying doses of tepa*

Treatment dose (μg/μl acetone/ nymph)	Sex treated	No. of pairs mated	Total Nos. of eggs laid	Eggs laid/ female	Total Nos. of eggs hatched	Per cent hatch	Corrected sterility (%)
0·5	Female	5	290	58	0	0	100
	Male	5	1,635	327	0	0	100
	Both	4	190	475	0	0	100
1·0	Female	4	70	175	0	0	100
	Male	5	575	115	0	0	100
	Both	4	58	145	2	3·45	95·73
	None (Control)	5	2,620	524	2,120	80·92	..

TABLE IV

Effect on fecundity, egg hatchability and sterility of Dysdercus *fourth instar nymphs topically treated with varying doses of tepa*

Treatment dose (μg/μl acetone/ nymph)	Sex treated	No. of pairs mated	Total Nos. of eggs laid	Eggs laid/ female	Total Nos. of eggs hatched	Per cent hatch	Corrected sterility (%)
0·5	Female	6	1,376	229·3	612	44·47	47·43
	Male	5	2,128	425·6	251	11·79	86·06
	Both	6	338	56·3	42	12·5	85·22
1·0	Female	5	1245	249	67	5·38	93·64
	Male	4	1,174	293·5	0	0	100
	Both	5	240	48	0	0	100
	None (Control)	5	2,540	508	2,160	84·60	...

TABLE V

Effect on fecundity, egg hatchability and sterility of Dysdercus *fifth instar nymphs topically treated with varying doses of tepa*

Treatment dose (μg/μl acetone/ nymph)	Sex treated	No. of pairs mated	Total Nos. of eggs laid	Eggs laid/ female	Total Nos. of eggs hatched	Per cent hatch	Corrected sterility (%)
0·5	Female	5	1,430	286	534	37·29	53·38
	Male	4	1,144	286	77	6·76	91·55
	Both	4	534	113·5	14	2·6	96·75
1·0	Female	6	654	109	202	30·88	61·4
	Male	5	875	175	0	0	100
	Both	5	410	82	0	0	100
1·5	Female	5	165	33	30	18·18	77·27
	Male	5	1,980	396	40	2·02	97·47
	Both	6	240	40	0	0	100
2·0	Female	4	208	52	16	7·68	90·4
	Male	4	884	221	0	0	100
	Both	5	90	18	0	0	100
	None (Control)	5	2,715	543	2,172	80	..

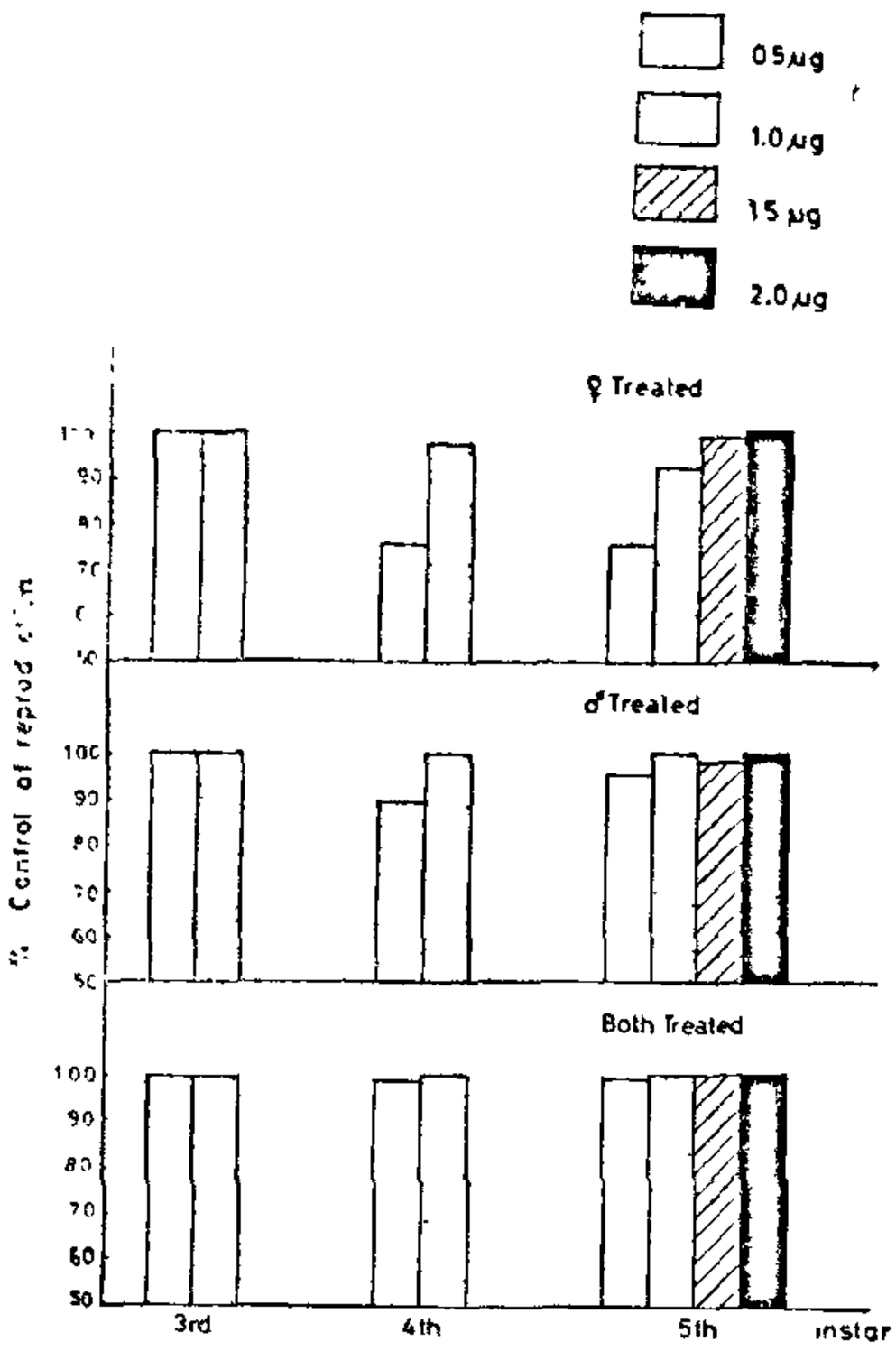

FIG. 2. Results showing per cent control of reproduction in different crosses between the adults derived from the nymphs treated with varying doses of tepa.

The effects of four doses of tepa on the fecundity and egg hatchability from different crosses of adults emerged from the nymph treated in fifth instar are given in Table V. Treated females with 0·5 µg and 1·0 µg/nymph crossed with normal males show 53·4% and 61·1% of sterility respectively. Similar crosses treated with 1·5 µg and 2·0 µg/nymph show 90% sterility. 100% sterility, however, was achieved with 1·0–2·0 µg doses in reciprocal crosses between treated males and normal/treated females. The oviposition rate was also considerably lowered in the crosses between both the treated sexes.

Figure 2 gives a comparative result of the per cent control of reproduction of the adults derived from the stock of nymphs administered with different doses of tepa in different instars after appropriate crosses. Third nymphal instar treatment gives 100% sterility with tepa dosage as low as 0·5 µg/nymph of either sex. In fourth instar a dose of 1·0 µg/nymph causes 100% sterility in male but slightly less in females. Further 100% sterility in males is produced in the fifth instar treatment with 1·0% µg/nymph. In adult *D. cingulatus* a contact-dose as high as 1·4 mg of apholate/sq inch with an exposure time of four hours could induce 0% hatch[11]. Similarly 94·6% sterility was induced in some insects injected with 5·0 µg of tepa/adult female[12]. It is, therefore, suggested that a low dose of tepa administered to the third and fourth nymphal instars is better to induce successfully a 100% sterility in *Dysdercus* than a very high dosage to the adult.

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