Sterility Induced by Apholate, Tepa and Hempa in *Locusta migratoria* (L.). Chander SHEIKHER, P. K. MITTAL and Vishwa NATH (Department of Zoology, Panjab University, Chandigarh-160014, India) Received September 6, 1977. *Botyu-Kagaku*, 42, 171, 1977.

26. *Locusta migratoria* (L.) に対する apholate, tepa および hempa の不妊作用

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Locusta migratoria の成虫雌雄に apholate, tepa および hempa を注射し，1) 処理雄×無処理雌，2) 処理雌×無処理雄，3) 処理雄×処理雌の交配をして，その産卵数，ふ化率を調べて，これら不妊剤の不妊作用を検討した．その結果3種の不妊剤はいずれも雌雄共に不妊効果があった．Hempa は雌に対する不妊作用が強く，哺乳動物に対する毒性が弱いので有望である．

Introduction

Apholate and tepa (alkylating agents) are established chemosterilants for both the sexes of insects whereas the activity of hempa (analogue of tepa) is uncertain as insect chemosterilant[1,2,3]. This paper deals with the study of the effect of these three chemosterilants on the fertility of *Locusta migratoria* (L.), an important pest all over the world.

Material and Methods

The specimens of *L. migratoria* were collected from the sandy areas of Bikaner (Rajasthan, India), and were reared in the laboratory in the aluminium cages of the size of 1 cubic foot each at the temperature 28° to 32° C.

All the three chemosterilants were dissolved in double distilled water, and different doses viz., 0.01, 0.05, 0.10 and 0.20mg of apholate per insect; 0.01 and 0.05mg of tepa per insect; and 0.01, 0.05 and 0.10mg of hempa per insect, were injected into the haemocoele of the insects. Male locusts were, in addition, also injected with 0.20 and 0.50mg of hempa per insect. The control animals received an equivalent amount of distilled water.

The treated insects (with individual chemosterilants) were kept under three different sets of experiments as below:

1. Treated males with normal females
2. Treated females with normal males
3. Treated males with treated females

The control insects of both sexes were also kept under identical sets of experiments.

The egg pods were collected up to one and a half months; the number of the hatched instars, and recovery of fertility were observed.

Results and Discussion

The sterility effects of the three chemosterilants in *L. migratoria*, measured as the hatchability of eggs, are summarized in Tables 1, 2 and 3, and all the three chemosterilants seem to be potent sterilants in the present insect.

A. *Alkylating Agents (Apholate and Tepa).* The present studies revealed that the various doses of the alkylating agents (apholate and tepa) had no detriemental effect on the normal activity of the *L. migratoria*, measured as longevity and the sexual behaviour of the insects. The chemosterilized locusts showed an active sexual behaviour inasmuch as they mated and laid eggs. Also the chemosterilized locusts did not show any high rate of mortality. Various other authors[4~7] have also observed that sexual behaviour, competitiveness and longevity are not affected by various alkylating agents in insects. On the contrary Economopoulos and Gordon[8], and Klassen *et al.*[9] have reported a reduced life span and sexual competitiveness in sterile insects. However, with higher doses of the chemosterilants an adverse effect on the longevity and sexual competitiveness is bound to happen as the chemosterilants adversely interfere with various metabolic activities.

A dose of 0.01 mg of apholate caused 42% sterility in males (Table 1a) and 50% in females

(Table 1b) initially which increased to about 90% and 92% respectively in males and females by the 12th day after treatment. However, complete sterility was achieved in males after 6 days of a single injection treatment with 0.05 and 0.10mg doses (Table 1a). In females also practically co mplete sterility was produced after 6 days of post-treatment period with 0.05 and 0.10mg doses (Table 1b). 0.20mg dose also caused complete sterility in both the sexes, but the frequency of egg laying decreased (Tables 1a, b). Similarly with tepa complete sterility was achieved with 0.01mg dose 12th day post-treatment period onwards in males (Table 2a), and 6th day onwards in females (Table 2b). 0.05mg dose induced complete sterility 6th day onwards following treatment in both the sexes of *L. migratoria* (Tables 2a, b).

A host of workers have recorded partial to complete sterility produced by various alkylating agents in a variety of male and female insects [4,5,10,11]. Some alkylating agents have been reported to be more effective on males than on females, e. g., tepa[12] and thiotepa[7,10]. However, the present observations revealed that the sterilizing capabilities of both apholate and tepa do not differ markedly in the two sexes of the locusts (Tables 1 and 2), but critically speaking they are slightly more effective in females. In our earlier histopathological studies of the effects of tepa and apholate on the gonads of *L. migratoria*, it was observed that apholate was more effective on males because a dose of 0.01mg, though causing necrosis in testes, failed to do so in ovaries[13,14]. However, the present studies undoubtedly show that 0.01mg dose of apholate, though caused no histopathological effects in ovaries, produces sterility parallel to, and in fact, slightly

Table 1.
APHOLATE

a. Treated males crossed with untreated females:

No.	Dose in mg/ insect	Average hatching per egg pod laid between		
		1-6 days	7-12 days	12 days onwards
1.	0.01	30±5	15±3	5±2
2.	0.05	13±2	Zero	Zero
3.	0.10	6±1	Zero	Zero
4.	0.20	Zero*	No egg laying	No egg laying
5.	Control	52±3		

b. Treated females crossed with untreated males:

No.	Dose in mg/ insect	Average hatching per egg pod laid between		
		1-6 days	7-12 days	12 days onwards
1.	0.01	26±3	14±2	4±1
2.	0.05	9±3	2**	Zero
3.	0.10	3±1	Zero	Zero
4.	0.20	Zero	Zero	No egg laying
5.	Control	52±3		

c. Treated males crossed with treated females:

No.	Dose in mg/insect		Average hatching per egg pod laid between		
	Males	Females	1-6 days	7-12 days	12 days onwards
1.	0.01	0.01	8±2	Zero	Zero
2.	0.05	0.05	2±1	Zero	Zero
3.	0.10	0.10	Zero	Zero	Zero
4.	0.20	0.20	Zero	No egg laying	No egg laying
5.	Control		52±3		

* Only 2 egg pods were laid which showed no hatching.
** Only 1 egg pod hatched with 2 hatch number. However, both the nymphs died.

more than that in males (Table 1).

We have earlier concluded that in males apparently the germ cells in active phase of proliferation are affected first, and it takes quite a time before the sperms are affected. In females also the maturing oocytes are demaged[13,14]. However, the present studies revealed that the egg hatchability decreased drastically in the first batch of egg pods laid, a time when apparently the mature sperms and eggs are histologically unaffected. It implies therefore, that the mature sperms and eggs, though apparently normal looking, are affected inasmuch as lethal mutations develop in them which express themselves during embryogenesis resulting in reduced egg viability. It was also observed that the egg hatchability reduced still further if both the mating partners were treated (Tables 1c and 2c), suggestive of the expression of some lethal mutations in zygote which were not expressed when only one sex was treated and the other was not.

The fertility in *L. migratoria*, measured as egg hatchability, registered a progressive decline as the post-treatment period was increased. The egg pods laid immediately after treatment showed comparatively high hatchability which decreased progressively as the post-treatment period was increased. The number of egg pods laid by the locusts involving one or both chemosterilized mating partners, also showed a decline with an increase in the post-treatment period, especially when the higher doses of the chemosterilants were administered, thereby recording a reduced fecundity. It also implies that the testes and ovaries atrophy when the post-treatment period increases. These observations find support from the work of Lindquist and House[15] who reported that the percentage hatch during the first 24 hours when the female *Anthonomus grandis* had mated with apholate-treated males was much higher than the percentage hatch of eggs laid subsequently. They also observed that longer the treated males were denied mating after treatment, the lower was the egg hatch number. Similarly a progressive decrease in the number of eggs laid had been observed in tretamine-

Table 2.

TEPA

a. Treated males crossed with untreated females:

No.	Dose in mg/ insect	Average hatching per egg pod laid between		
		1-6 days	7-12 days	12 days onwards
1.	0.01	25±5	7±3	Zero*
2.	0.05	9±2**	Zero	Zero
3.	Control	52±3		

b. Treated females crossed with untreated males:

No.	Dose in mg/ insect	Average hatching per egg pod laid between		
		1-6 days	7-12 days	12 days onwards
1.	0.01	23±4	Zero	Zero
2.	0.05	6±4***	Zero	Zero
3.	Control	52±3		

c. Treated males crossed with treated females:

No.	Dose in mg/ insect	Average hatching per egg pod laid between		
		1-6 days	7-12 days	12 days onwards
1.	0.01	5±2	Zero	Zero
2.	0.05	4±1	Zero	Zero
3.	Control	52±3		

* In one case 1 egg pod hatched with hatch number 2.

** In one case no hatching was observed.

*** In one case the hatch number was 24.

treated *Dysdercus* by Sukumar and Naidu[16].

B. *Analogue of Alkylating Agents* (*Hempa*). After 0.01 and 0.05 mg doses of hempa no effect on male sterility in *L. migratoria* could be observed. With 0.10mg dose 23% to 38% sterility was achieved (Table 3a). After treatment with 0.2mg dose, the hatch number decreased from initial average of 23±3% to final 15±3% thereby recording sterility up to the tune of 23.8%. However, complete sterility in males could be achieved with 0.50mg dose 12 days after treatment (Table 3a). However, in females complete sterility was observed with 0.05 and 0.10mg doses 12 days after treatment. Even a dose of 0.01mg could induce 92% sterility in female locusts 12 days after treatment (Table 3b).

From these observations it can safely be concluded that hempa is more effective chemosterilant in female locusts. Hempa has been known to have a variable sterilizing activity in various insects. It successfully sterilises *Aedes aegypti*[17], *Culex pipiens fatigans*[18], *Musca sorbens*[19], *M. domestica*[20], *M. autumnalis*[21]. On the other hand, hempa is completely ineffective against boll-weevil, *Anthonomus grandis*[22, 23], the Japanese beetle, *Popillia japanica*[24], and two-spotted spider

Table 3.

HEMPA

a. Treated males crossed with untreated females:

No.	Dose in mg/ insect	Average hatching per egg pod laid between		
		1-6 days	7-12 days	12 days onwards
1.	0.01	50±3	53±3	50±3
2.	0.05	48±3	51±3	52±3
3.	0.10	32±3	40±3	33±3
4.	0.20	28±3	24±3	15±3
5.	0.50	16±4	4±2	No egg laying
6.	Control	52±3		

b. Treated females crossed with untreated males:

No.	Dose in mg/ insect	Average hatching per egg pod laid between		
		1-6 days	7-12 days	12 days onwards
1.	0.01	28±4	9±2	4±1
2.	0.05	20±3	4±2	Zero
3.	0.10	15±3	3±1	Zero
4.	Control	52±3		

c. Treated males crossed with treated females:

No.	Dose in mg/insect		Average hatching per egg pod laid between		
	Male	Female	1-6 days	7-12 days	12-days onwards
1.	0.10	0.10	12±3	3±2	Zero
2.	0.10	0.05	19±3	7±3	Zero
3.	0.10	0.01	28±4	9±3	4±1
4.	0.20	0.10	12±4	3*	Zero
5.	0.20	0.05	16±3	5±2	Zero
6.	0.20	0.01	25±5	4±1**	2
7.	0.50	0.10	19±2	3±1	Zero
8.	0.50	0.05	12±2	3±1	Zero
9.	0.50	0.01	16±4	4±1	Zero
10.	Control		52±3		

* In one egg pod no hatching took place.

** In 2 egg pods no hatching took place.

mite[25]. The explanation of Chang *et al.*[26] for the low activity of hempa in comparison with that of tepa in house flies appears to be most convincing. They suggest that the low activity of hempa is due to its slow action and by its rapid metabolism within the organism. The complete inactivity of hempa in other insects may, therefore, be the result of even faster rate of its degeneration.

The present studies confirm our earlier findings that hempa is more effective on ovary than on testes in locusts[27], and finds support from the work of Hafez *et al.*[19] in *Musca sorbens*.

The importance of hempa and its derivatives as insect chemosterilants lie in the fact that they are less toxic orally in mammals. In rats the LD_{50} in case of hempa is 2,500mg/kg as compared to 37mg/kg in case of tepa[28]. Similarly Shott *et al.*[29] failed to observe toxicological effect or effect on reproduction in rats. So hempa is a safer chemosterilant, and Sacca *et al.*[30] have successfully controlled the house fly population by spraying city dumps with 1.25-3.75% hempa.

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