

meet its requirement and save foreign exchange but could also enter the international market. The samples of gum arabic collected during the present work and shown to the traders in Delhi appear to make the international grade. There is scope for about 20,000 tonnes of Indian gum arabic consumption in the markets of America and Europe<sup>1,8</sup>.

It is suggested that in the afforestation programmes for arid and semi-arid zones of India, *A. senegal* should be considered as a potential planting material. *A. senegal* is the only *Acacia* involved in afforestation of the recognized Sahelian gum belt. For many years, the Sudan gum traders even followed a policy of regenerating only *A. senegal* and eradicating other *Acacia* species<sup>2</sup>.

Gum arabic is a complex substance, with many unique features and properties. It is unlikely to be duplicated or reproduced by any viable man-made synthetics or modified natural substitutes. For many applications, the performance of gum arabic in a wide range of products is considered superior to other available substitutes<sup>2,6</sup>.

In Sudan, gum arabic is the third most important export item after livestock and cotton<sup>19</sup>. But *A. senegal* is more than an export earner. It is a vital source of income and employment to farmers who can tap the trees in the dry season when there are no other crops to tend. The deep tap root extends down the water table and the extensive lateral roots that reach ~ 100 m in length take the advantage of light showers, stabilize the soil and limit the risk of desertification. The tree also fixes nitrogen and encourages the growth of grasses around the trunk. The tree is a source of fodder and fuel. The naturally shed leaves and pods decompose and add nitrogen-rich organic matter to the soil<sup>7</sup>. Yields of crops increase when farmers intercrop or rotate *A. senegal* with other crops. Therefore, *A. senegal* has an economic value, significantly beyond that of the gum it produces. Raising *A. senegal* either in plantations for agroforestry or in small plots for social forestry can result in high rates of return, especially when the environmental benefits are added to the value of the gum.

The utilization of the initial finding reported here will require scaling-up of sample size, collection of data over longer periods, examination of wound-healing responses and estimation of yields of trees from different provenances.

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## $\alpha$ -Hexachlorocyclohexane: a potent insect growth regulator

V. S. Saxena and P. J. Rao

Division of Entomology, Indian Agricultural Research Institute, New Delhi 110 012, India

The inactive  $\alpha$ -isomer of hexachlorocyclohexane ( $\alpha$ -HCH or BHC) caused morphogenetic effects in fifth-instar hoppers of *Schistocerca gregaria* (Forsk.) when injected into haemolymph or administered through food. A dose as low as 2  $\mu$ g  $\alpha$ -HCH, when injected into 3-day-old fifth-instar hoppers, caused defective metamorphosis in 50% of the treated population. The fourth-instar hoppers moulted directly into adults when  $\alpha$ -HCH was either injected or administered topically or through food. Administration of cholesterol along with  $\alpha$ -HCH resulted in dose-dependent reduction in the deformities caused by  $\alpha$ -HCH.

AMONG synthetic insecticides, hexachlorocyclohexane (HCH or BHC) is the oldest of the organochlorine insecticides. It comprises  $\alpha$ ,  $\beta$ ,  $\gamma$ ,  $\delta$  and  $\epsilon$  isomers; only the  $\gamma$  is responsible for insecticidal properties. The other, inactive, isomers are by-products in the pesticide industry and cause disposal problems. While they are

toxic to some micro-organisms<sup>1</sup>, the  $\alpha$ ,  $\beta$ ,  $\delta$  and  $\epsilon$  isomers are practically non-toxic to insects<sup>2</sup>. Extensive work on the inactive isomers led to the finding that  $\alpha$ -HCH, which alone constitutes 60–70% of HCH technical, gives powerful synergistic effects with DDT against resistant pests (Indian Patent No. 153987)<sup>3</sup>.  $\alpha$ -HCH has also been evaluated for synergistic action with pyrethrins and carbamates<sup>4,5</sup>. In the present study, we demonstrate, for the first time, the role of  $\alpha$ -HCH as an insect growth regulator.

Different nymphal stages of *Schistocerca gregaria* (Forsk.) were taken from a laboratory colony maintained on cabbage and maize leaves<sup>6</sup>.  $\alpha$ -HCH (Hindustan Insecticides Limited, Delhi) was further purified by recrystallization in methanol until a single peak was obtained on gas-liquid chromatography (M.P. 157°C). It was administered to different nymphal stages either by injection or topical application or through food. A minimum of 20 insects of 3-day-old, 1st-, 2nd-, 3rd-, 4th- and 5th-instar nymphs were used for each treatment. Two concentrations of  $\alpha$ -HCH, 5 and 10  $\mu$ g in 5  $\mu$ l acetone, were topically applied on the ventral side of individual hoppers using a micro-applicator (Burkard, UK). An equal number of hoppers treated topically with only acetone served as control. Similarly 1, 2, 3 or 5  $\mu$ g  $\alpha$ -HCH in 5  $\mu$ l acetone was injected through the intersegmental membrane into the abdomen of 4th- and 5th-instar nymphs. Hoppers of similar age injected with 5  $\mu$ l acetone formed the control and untreated hoppers served as blank. For oral feeding, 1 ml of 0.3 and 0.1%  $\alpha$ -HCH in acetone were separately sprayed using Potter's tower (Burkard, UK) on both sides of cabbage leaf (7.5 cm  $\times$  2.5 cm) and the deposit of  $\alpha$ -HCH was calculated (61.53  $\mu$ g cm<sup>-2</sup>) according to ISI specifications for water-dispersable concentrate IS: 25-69; 1963. Each treated leaf was fed to individual nymphs previously starved for 8 h. Nymphs fed untreated leaves served as blank whereas those fed acetone-sprayed leaves formed the control. After overnight feeding, all individual hoppers were transferred to separate jars (15 cm  $\times$  10 cm) for daily observations and fed fresh leaves. All insects were kept at 30  $\pm$  1°C under 12 h dark and 12 h light conditions. Data were recorded as cumulative numbers till adult emergence based on the number of nymphs initially taken for the experiment.

The nymphs in control and blank survived and moulted normally. All 1st- and 2nd-instar nymphs topically treated with 2  $\mu$ g  $\alpha$ -HCH died before reaching 5th nymphal stage in 40 days. However, 3rd-, 4th- and 5th-instar nymphs treated with 5 and 10  $\mu$ g  $\alpha$ -HCH moulted periodically; adult emergence was 60 and 70% respectively. Interestingly, 4th-instar hoppers, irrespective of whether they were treated in 3rd or 4th nymphal stage, moulted directly into adults within the normal developmental period.

Individuals of 3rd- and 4th-instar hoppers fed 0.1

and 0.3%  $\alpha$ -HCH-treated leaves ingested 124 and 450  $\mu$ g  $\alpha$ -HCH respectively; 10% of the hoppers moulted incompletely. In these experiments, 60% of the hoppers emerged as healthy adults, compared to 70% in topically-treated ones. Though the food offered (cabbage leaf, 18.75 cm<sup>2</sup>) to both control and treated nymphs was the same, feeding in treated nymphs ranged from 3 to 13.5 cm<sup>2</sup> compared to total consumption in control. The consumption of  $\alpha$ -HCH by 5th-instar hoppers ranged from 375 to 1125  $\mu$ g, leading to 40% (0.1%  $\alpha$ -HCH) to 52.1% (0.3  $\alpha$ -HCH) emergence of deformed adults (Table 1). The deformities noticed were (i) incomplete ecdysis during the last moult, resulting in adults with exuviae attached on the ventral side; (ii) ill-developed wings; and (iii) crippled legs (Figure 1). The most significant observation was prolongation of the nymphal period. Some hoppers remained in 5th instar beyond 30 days.

It is interesting to record that 1, 2, 3 and 5  $\mu$ g  $\alpha$ -HCH, when injected into the haemocoel of 4th- and 5th-instar nymphs, caused morphogenetic effects in 33.3 to 65% of the insects (Table 2). On the basis of per cent abnormal moulters, effective dose (ED<sub>50</sub>) for 5th instar was 2.0  $\mu$ g. Developmental abnormalities, prolongation of the nymphal period, and reduced food intake observed in  $\alpha$ -HCH-treated nymphs are similar to the effects of azadirachtin on *S. gregaria*<sup>7</sup> and *Locusta migratoria*<sup>8</sup>. This is the first report that inactive  $\alpha$ -HCH causes morphogenetic effects in *S. gregaria* hoppers.

Ecdysis in exopterous phytophagous insects is largely dependent on the intake of plant sterols, which are converted into cholesterol for the synthesis of ecdysone in the prothoracic glands<sup>9</sup> in final-instar hoppers<sup>10</sup>. Therefore, to enhance biosynthesis of ecdysone, 5  $\mu$ g of cholesterol was injected along with 1, 2, 3 or 5  $\mu$ g  $\alpha$ -HCH into 5th-instar hoppers. As usual, untreated blanks and acetone-treated controls were maintained for comparison. All the hoppers treated with different

Table 1. Effect of  $\alpha$ -HCH feeding on 5th-instar hoppers of *S. gregaria*.

	Days				
	5	10	15	20	25
Nymphs alive	70.0* (71.8)	45.0 (34.2)	35.0 (16.8)	20.0 (5.8)	5.0 (2.4)
Nymphs dead	10.0 (10.4)	20.0 (14.1)	25.0 (18.1)	25.0 (23.3)	35.0 (23.3)
Abnormal moulters/dead	20.0 (16.5)	30.0 (38.9)	30.0 (47.6)	35.0 (50.3)	40.0 (52.1)
Normal adults alive	0.0 (1.3)	0.0 (12.8)	5.0 (13.5)	10.0 (14.2)	5.0 (14.2)
Normal adults dead	0.0 (0.0)	5.0 (0.0)	5.0 (4.0)	10.0 (6.0)	15.0 (8.1)

\*Numbers are per cent insects (mean of five replicates) in each category for treatment with 0.1%  $\alpha$ -HCH ( $n=20$ ); numbers in parenthesis are mean per cent insects for treatment with 0.3%  $\alpha$ -HCH ( $n=24$ ).



Figure 1. Morphogenetic effects due to  $\alpha$ -HCH.

Table 2. Effect of injected  $\alpha$ -HCH on developmental changes in 4th- and 5th-instar hoppers of *S. gregaria*.

		4th instar				5th instar			
		$\alpha$ -HCH ( $\mu$ g)				$\alpha$ -HCH ( $\mu$ g)			
		1	2	3	5	1	2	3	5
Nymphs alive	a	90.0	90.0	80.0	80.0	46.7	46.7	60.0	65.0
	b	50.0	50.0	60.0	30.0	33.3	6.6	13.3	20.0
	c	20.0	20.0	30.0	10.0	6.6	—	6.6	15.0
	d	—	—	—	—	—	—	—	—
Nymphs dead	a	10.0	10.0	20.0	10.0	20.0	26.7	20.0	15.0
	b	40.0	40.0	30.0	40.0	20.0	26.7	26.7	15.0
	c	50.0	40.0	40.0	40.0	26.7	26.7	26.7	20.0
	d	50.0	50.0	40.0	40.0	36.7	26.7	26.7	20.0
Abnormal moults/dead	a	—	—	—	10.0	20.0	20.0	20.0	10.0
	b	10.0	10.0	10.0	30.0	20.0	40.0	60.0	45.0
	c	20.0	20.0	10.0	30.0	26.7	46.7	60.0	45.0
	d	20.0	30.0	30.0	30.0	33.3	46.7	60.0	65.0
Normal adults alive	a	—	—	—	—	13.3	6.6	—	10.0
	b	—	—	—	—	26.7	20.0	—	20.0
	c	10.0	20.0	20.0	20.0	40.0	20.0	6.6	15.0
	d	20.0	10.0	20.0	20.0	40.0	20.0	—	10.0
Normal adults	a	—	—	—	—	—	—	—	—
	b	—	—	—	—	—	6.6	—	—
	c	—	—	—	—	—	6.6	—	—
	d	10.0	10.0	10.0	10.0	—	6.6	6.6	5.0
n		20	20	20	20	30	20	30	40

Numbers are per cent insects (mean of five replicates) in each category; a, b, c, d give data recorded after 5, 10, 15 and 20 days respectively.

In none of the treatments did 4th-instar hoppers moulted into 5th instar, but adults emerged.

combinations of  $\alpha$ -HCH and cholesterol, and the controls moulted in 7 to 13 days. The percentage of abnormal moults in 1:5, 2:5, 3:5 and 5:5  $\alpha$ -HCH:cholesterol combinations was 25, 35, 53.3 and 60 (Table 3), compared to 33.3, 46.7, 60.0 and 65.0% respectively in the  $\alpha$ -HCH-treated insects (Table 2). The different theories on mode of action of HCH and its isomers do not have an explanation for morphogenetic effects. The increased percentage of normal

Table 3. Effect of cholesterol in combination with  $\alpha$ -HCH on metamorphosis of 5th-instar hoppers of *S. gregaria*.

		$\alpha$ -HCH + cholesterol ( $\mu$ g)				Cholesterol alone
		1:5	2:5	3:5	5:5	
Nymphs alive	a	50.0	35.0	46.7	44.0	80.0
	b	100.0	—	—	—	—
	c	—	—	—	—	—
Nymphs dead	a	5.0	10.0	—	8.0	—
	b	5.0	10.0	—	8.0	—
	c	5.0	10.0	—	8.0	—
Abnormal moults/dead	a	15.0	20.0	40.0	36.0	—
	b	25.0	35.0	53.3	60.0	—
	c	25.0	35.0	53.3	60.0	—
Normal adults alive	a	10.0	25.0	13.3	12.0	20.0
	b	30.0	40.0	46.7	32.0	100.0
	c	40.0	35.0	46.7	32.0	—
Normal adults dead	a	20.0	10.0	—	—	—
	b	30.0	15.0	—	—	—
	c	30.0	40.0	—	—	—
n		20	20	30	25	20

Numbers are per cent insects (mean of five replicates) in each category; a, b, c give data recorded after 5, 10 and 15 days respectively.

adults when cholesterol was injected with  $\alpha$ -HCH suggests interference of  $\alpha$ -HCH in biosynthesis of moulting hormone resulting in abnormal adults in paurometabola. An explanation for delayed moulting and emergence of adults from 4th-instar hoppers of *S. gregaria* on administration of  $\alpha$ -HCH is possible only after a detailed study.

Earlier, a combination of  $\alpha$ -HCH and DDT was shown to be highly toxic against various DDT-resistant pests of medical and agricultural importance, including grasshoppers and locusts<sup>3, 11-13</sup>, and to counteract cross-resistance<sup>14, 15</sup>. The present work points to the necessity of detailed studies on the inactive  $\alpha$ -isomer of HCH for exploitation in plant protection.

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## Identification of a unique factor in quail foam, and its effect on motility and fertilizing ability of cock spermatozoa

R. P. Moudgal and Jagmohan

Division of Physiology and Reproduction, Central Avian Research Institute, Izatnagar 243 122, India

**A factor in aqueous extract of quail foam caused complete blockage of motility of cock spermatozoa. On dilution of treated semen samples, sperm motility was recovered up to about 70%. The active principle was present in the water-soluble ash component. The factor did not affect the fertilizing ability of cock spermatozoa.**

QUAIL foam, produced by cloacal glands of sexually active male Japanese quail, is a unique feature among bird species. There have been conflicting reports about the role of quail foam. It was reported to act as an impediment to satisfactory fertility in this species<sup>1</sup>. On the other hand, insemination of females after mixing semen with quail foam showed improved fertility<sup>2</sup>. Inseminating males whose foam glands had been cauterized achieved low fertility when competing with normal males<sup>3</sup>. No conclusive evidence on the functions of foam has been presented. We have observed that a water-soluble component of quail foam causes reversible immobility of cock spermatozoa. This observation encouraged us to characterize quail foam further and to isolate the component of foam that has the active principle. The effect of this active principle on fertility was also tested to evaluate the possibility of using it to extend the life of spermatozoa by arresting their metabolic activity even at room temperature.

Foam from sexually active adult male quail was collected by pressing the cloacal gland and squeezing either side of the vent. It was dried in an oven at 105°C overnight. Dried foam was homogenized (2 min) with saline (0.9% w/v NaCl) using a glass-Teflon homogenizer and then centrifuged for 15 min at about 2000 g. The extract was separated from the residue and immediately used for experiments. Cock semen was preferred for this study as quail foam is likely to get mixed with semen during collection of semen from male quail. Semen samples from six healthy white leghorn cocks maintained under standard husbandry practices were collected by

massage<sup>4</sup> on alternate days and samples were pooled. Semen samples were incubated at 37°C after mixing with an equal volume of 5% or 10% quail foam extract (1:1). Simultaneously control semen samples were incubated with saline alone. The samples were tested for sperm motility<sup>5</sup> immediately after mixing the extract or saline with semen and after 15 and 30 min of mixing. After 30 min of incubation the treated samples were further diluted with saline, to 1:1, 1:2, 1:3 and 1:4, and the diluted semen samples were evaluated for sperm motility.

In another experiment, 5 or 10% aqueous extract was heated in a test tube placed in boiling water for 10 min. After cooling, the filtrate was tested for effect on sperm motility. Finally the ash component equivalent to 5 or 10% foam was dissolved in saline, centrifuged (2000 g for 10 min), and the supernatant bearing water-soluble ash was tested.

Hens, 12 for each sample, were inseminated with semen from the control, ash component-treated (after 30 min of incubation with ash equivalent to 10% foam), or diluted (1:3) ash-treated semen samples. Insemination was carried out between 4 p.m. and 5 p.m., and about 60 million spermatozoa were introduced into each hen. The insemination was repeated after two days. Eggs were collected on seven subsequent days and fertility was evaluated by incubating and testing the eggs of each group. A second fertility check was conducted.

Addition of 10% aqueous extract of oven-dried quail foam to semen caused a drastic reduction in sperm motility immediately; a smaller reduction was noted in the case of 5% extract (Table 1). In both cases, motility was zero after 30 min of incubation. The filtrate of heated foam extract retained sperm-immobilizing activity. The active principle was present in the soluble ash component of foam. Motility was regained (about 70%) by the sperm on dilution of the samples with saline (Table 2). The fertility trials suggested that the active

**Table 1.** Immobilization of cock spermatozoa by quail foam.

Addition to semen	Per cent motile spermatozoa at		
	0 min	15 min	30 min
Saline (control)	86.67 (80-90)	83.33 (80-90)	78.33 (70-80)
5% Aqueous extract of oven-dried quail foam	70.00 (60-80)	02.50 (00-10)	0.0
10% Aqueous extract	4.17 (0-10)	0.0	0.0
Filtrate of heated 5% aqueous extract	68.33 (60-80)	3.33 (0-10)	1.67 (0-5)
Filtrate of heated 10% aqueous extract	3.33 (0-10)	0.0	0.0
Soluble ash of foam in saline:	5% 66.66 (60-80)	2.50 (0-10)	0.0
	10% 0.0	0.0	0.0