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Effect of 25-azacholestane, 25-azacholesterol and N, N-dimethyldodecanamine on cholesterol incorporation in the tissues of *Locusta migratoria L*.

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Abstract
We determined the effect of 25-azacholestane, 25-azacholesterol and N, N dimethyldodecanamine on the incorporation of [^{3}H]-cholesterol in haemolymph, ovary, testis and fat body of *Locusta migratoria*. With 25-azacholestane treatment (0 to 10ppm) uptake of cholesterol by ovary decreased from 18.6% in control to 11.1% at 10 ppm and an increase was observed in fat body (26.5% to 35.8%). Similar trend was observed with 25-azacholesterol treatment. With an increase in 25-azacholesterol concentration (0 to 50 ppm), in case of female a decrease in the cholesterol uptake was observed in haemolymph (0.91% to 0.49%) and ovary (7.5% to 2.4%), while in fat body an increase from 3.3% to 5.8% was observed. In male locust also decrease in cholesterol uptake was observed in haemolymph (1% to 0.64%) but an increase is seen in testis (2.01% to 4%) and fat body (3.9% to 5.2%). However, with N, N-dimethyldodecanamine treatment, an increase in cholesterol uptake was observed both in ovary (17.6 % to 23.8%) and fat body (29.4% to 39.5%). The results were interpreted and discussed in the context of sterol metabolism inhibition as an effective strategy to control the locust population to a certain extent.

Keywords: *Locusta migratoria*, inhibitor, 25-azacholesterol, 25-azacholestane, N, N-dimethyldodecanamine, sterol metabolism, cholesterol

Introduction
Insects lack the ability to synthesize cholesterol, the most predominant sterol found in the insect body and its tissues (Goel and Agarwal, 1987a, Li and Jing 2020) [9, 24]. These phytophagous insects do not get cholesterol directly from their food but have to metabolize phytosterols to cholesterol which can then be utilized both for structural and physiological purpose (Rath *et al*., 1993, Jing and Behemer, 2020) [25, 17]. Certain steroidal compounds such as azasteroids which are structurally similar to cholesterol have been synthesized and may serve as model compounds in subsequent development of safe chemicals for pest management. These simple compounds have been shown to have an effect on the larval growth and development in insects and other arthropods (Goel and Agarwal, 1987b, Kuthiala *et al*., 1987, Svoboda and Weirich 1995, Rath *et al*., 2022) [10, 20, 32, 26]. The inhibitive effect of these compounds on the egg production and hatchability has also been reported by Al-Izzi and Hopkins (1982) [2]. 25-azacholesterol and 25-azacoprostane are highly active inhibitors of insect moulting and metamorphosis (Svoboda *et al*., 1972, Goel and Agarwal, 1987b, Agarwal *et al*., 1990, Rath *et al*., 2022, Saxena *et al*., 2022) [31, 10, 1, 26, 29]. Such effects were considered to be due to the limited amount of cholesterol available to the insect after azasteroid treatment. These compounds are considered to be potent inhibitors of Δ^{24}-sterol reductase, an enzyme involved in the dealkylation of phytosterols to cholesterol. Azasteroids are also reported to inhibit the phytosterol biosynthesis (Darnet *et al*., 2020) [5]. Azasteroids may hence, be directly or indirectly involved in the metabolic pathways other than those involving formation of cholesterol from phytosterols, such as ecdysone biosynthesis or interference with juvenile hormone titer (Svoboda & Weirich, 1995, Khalil *et al*.,1996) [32, 19].
The insects have evolved different metabolic pathways for sterol utilization and thus the mechanism of inhibition of the normal sterol metabolism in different species needs to be studied individually. Most of the studies done with these compounds relate their effect to the whole insect. Studies concerning the effect of these compounds on the development of tissues are virtually lacking.
Thus, it was considered desirable to study the effect of these compounds on the incorporation of [^{3}H]-cholesterol in the tissues of *Locusta*, an economically important polyphagous insect causing extensive damage to a large number of the field crops. In view of the above discussion, both *in vivo* and *in vitro* studies on the effect of 25-azacholestane, 25- azacholesterol and N, N dimethyldodecanamine, on cholesterol incorporation in *L. migratoria* were undertaken.

Materials and Methods

Locusts used in the present studies were obtained from the stock culture maintained in the laboratory in aluminium cages with plexi-glass in front, under optimum conditions of temperature (25-30^0 C), photoperiod regime (12L:12D) and humidity (60-80%.) The food consisted of green maize leaves and bran (Goel and Agarwal, 1987a) [9]. Water was supplied once a day in the form of soaked cotton wool pads. The cages were cleaned daily to remove the faeces and dead animals, and fresh food added.

In vitro* uptake of [^{3}H]-cholesterol by the ovary of *L. migratoria

In the present investigation, insects were used, 22 days after their last moult. Haemolymph of several locusts was collected in a small 3 ml stoppered conical glass tube. The insects were dissected, ovary removed and kept in cold buffered saline (40mM potassium chloride-8mMsodium phosphate buffer, pH-6.5). Ovary was kept frozen until used.

Incubation procedures: All incubations were conducted in 3 ml homeopathic vials at 30°c with constant shaking. The vials were previously silanised with 5% solution of dichlorodimethoxysilane in chloroform.

For standardization of haemolymph concentration to be used in the incubation media and the optimal time required for the maximum uptake of [^{3}H]-cholesterol by ovary having well developed oocytes, ovary was incubated in a media containing haemolymph to which [^{3}H]-cholesterol was added. The incubation medium contained varying amounts of haemolymph (0 µl to 100 µl) made up to 100 µl by buffered saline (0.05M sodium phosphate buffer containing 0.15M potassium chloride, pH-6.7) and 1.29 x 10 $^{-2}$ mµ moles of [^{3}H]- cholesterol (Sp. act. 8 Ci/mmol, Radiochemical Centre, Amerashan, U.K.) was added. Incubation was carried out for different time period (0 min. to 180 min.) at 30^{0}C. At the end of incubation, the tissue was removed from the medium and washed twice with cold buffered saline. Sterols were extracted by chloroform: methanol (2:1, v/v) and radioactivity estimated (Goel and Agarwal, 1987a; Goel *et al*., 2021) [9, 11]. An aliquot of 5 µl of the extract (dissolved in 250 µl hexane) was used for radioactivity estimation]. All the experiments were performed at least four times under identical condition.

Effect of 25-azacholestane and N, N-dimethyldodecanamine on incorporation of [^{3}H]- cholesterol in various tissues when incubated *in vitro*

The insects were injected with varying amounts of 25-azacholestane or N, N- dimethyldodecanamine (dissolved in 90% ethanol) in a volume of 1 to 5 µl in the abdomen of 22 days old adult female locust with a 10µl Hamilton micro-syringe. The insects were left on the normal food and dissected after 48 hours in cold buffered saline. The ovary and fat body were removed and incubated in the medium (haemolymph: buffered saline, 1:4, v/v) containing 1.33 x 10^{-2} mµ moles of [^{3}H]- cholesterol (Sp. act. 8 Ci/m mol) for 25-azacholestane and 2.06 x 10^{-2} mµ moles of [^{3}H]- cholesterol (Sp. act. 5.7 Ci/ m mol) for N, N- dimethyldodecanamine at 30°C for 2 hours. Each replicate contained a single ovary. Minimum of three replicates were used in each case. Sterols were extracted and amount of radioactivity incorporated estimated.

Effect of dietary 25-azacholesterol on incorporation of [^{3}H]- cholesterol in various tissues *In vivo*

L. migratoria were reared on the normal food till the fourth instar. Newly emerged fifth instar nymphs were thereafter reared on artificial diet containing 25-azacholesterol (3β-hydroxy-chol-5-en-24-dimethylamine) at concentration of 5 to 50 ppm of the wet weight of the diet. Diet without 25-azacholesterol served as control (Goel and Agarwal,1987b) [10]. The rearing conditions were otherwise same as for the stock culture. Diet was changed daily and the excreta removed. Twenty-two days old insects were then injected with 2.36 x 10^{-2} mµ moles of [^{3}H]-cholesterol (Sp. act. 5.7 Ci/m mol) dissolved in 5 µl of ethanol with a Hamilton micro-syringe. The insects were sacrificed after 16 hours and tissues processed. 20µl haemolymph was withdrawn from the insects by puncturing the hind leg region of the metathorax and transferred to the scintillation vials containing 100µl ethanol and radioactivity estimated after mixing with 10 ml of Scintillation fluid. Fat body, ovary and testes were removed and sterols extracted (Goel and Agarwal 1987a, Goel *et al*., 2021) [9,11]. A 10µl aliquot of the extract of the sterol sample was used for the estimation of total radioactivity. Minimum of three replicates were used in each case.

Results

Effect of haemolymph concentration: When the ovary was incubated in the media containing no haemolymph, only 13.5 % cholesterol was taken up by it (Fig.1). However, in the presence of 10% haemolymph, the uptake of cholesterol increased three folds to 40.3 % which remained the same even when the haemolymph concentration was raised up to 20 %. Further increase in the haemolymph concentration resulted in a decrease in the cholesterol uptake by the ovary. Hence, for all subsequent experiments 20 % haemolymph was used in the incubation media.

Effect of time: The incorporation of [^{3}H]-cholesterol by the ovary from the incubation media was studied in relation to time which varied from 0 to 180 minutes. At zero time the ovary was able to pick up about 14 % of the cholesterol added to the media. As the time increased, the uptake of cholesterol also increased (27.3 % at 60

min). However, from 60 to 180 minutes the uptake was maximum and it remained more or less the same. Hence, for all subsequent studies an incubation period of 120 minutes was used (Fig.1).

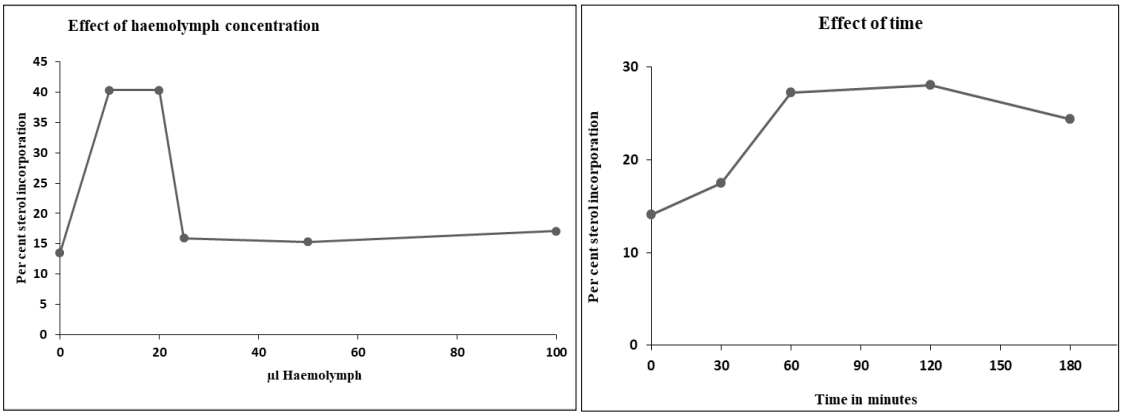

Fig 1: Effect of time and haemolymph concentration in the incubation medium on [^{3}H]-cholesterol incorporation by ovary.

***In vitro* studies**

Effect of 25-azacholestane on incorporation of [^{3}H]- cholesterol in tissues of female *L. migratoria.*

The incorporation of cholesterol in the ovary of locusts injected with 25-azacholestane decreased from 18.6% in control to 11.1% at 10 ppm. In fat body, however, cholesterol uptake per locust increased from 26.5 % in control to 35.8 % at a concentration of 10 µ g. However, when it was calculated per 10 mg fat body, not much difference was observed (Fig.2).

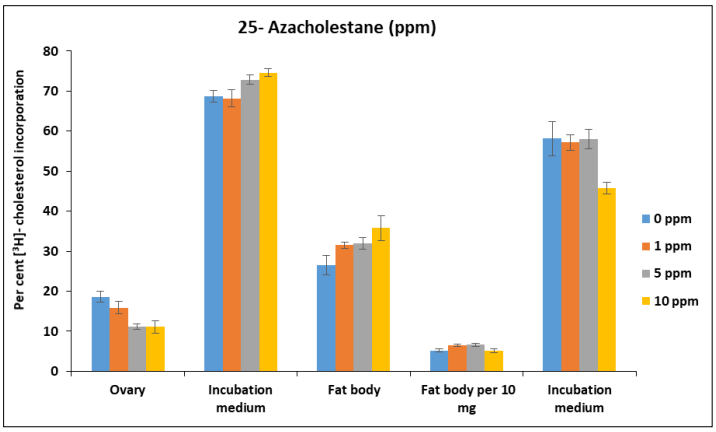

Fig 2: Per cent [^{3}H]-cholesterol uptake by the tissues of 22 days old female *L. migratoria* which were injected with 25-azacholestane.

Effect of N, N-dimethyldodecanamine on incorporation of [^{3}H]- cholesterol in tissues of female *L. migratoria.*

The uptake of cholesterol by ovary increased from 17.6 % in controls to 23.8 % at 10 ppm. In fat body, the uptake increased from 29.4 % in control to 39.5 % at 10 ppm . When this uptake was calculated on the basis of per 10 mg fat body, the uptake still showed an increased from 4.8 % to 9.2 % (Fig.3).

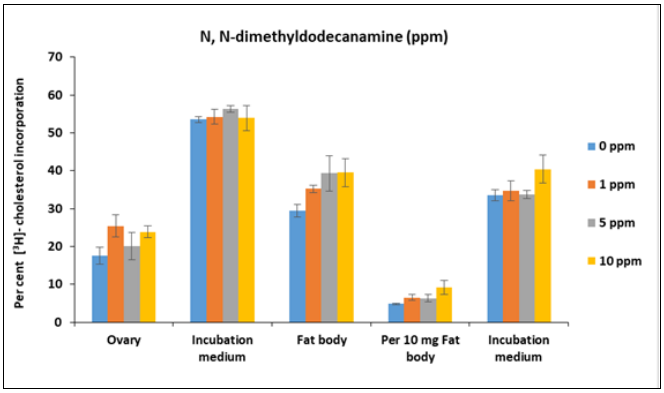

Fig 3: Per cent [^{3}H]-cholesterol uptake by the tissues of 22 days old female *L. migratoria* which were injected with N, N-dimethyldodecanamine.

***In vivo* studies**

a. Effect of dietary 25-azacholesterol on the weight of adult *L. migratoria* and its tissues

Addition of 25-azacholesterol to the diet of fifth instar nymph and the adults, did not seem to have any effect on the weight of the 22 days old adult females or males, although at a concentration of 50 ppm, no male nymphs reached the adult stage. However, addition of 25-azacholesterol did affect the weights of the tissues (Table 1). This effect was very marked in the case of ovary, where weight declined sharply from 163.4 mg in controls to 54 mg at 5 ppm and did not show much change thereafter. Similar trend was observed in case of fat body of the female locust where the weight decreased from 90.6 mg in controls to 79.5 mg at 5 ppm, remaining almost stationary, thereafter.

In contrast to the female tissues, the tissues from the male locust (testes and fat body) showed an increase in weight with an increase in azasteroid concentration from 0 to 25 ppm, from 38.9 mg to 50.1 mg in fat body and from 41 mg to 60.3 mg in testes. However, the increase in weight was pronounced as compared to controls only at a concentration of 10 ppm of this compound (Table 1).

Table 1: Effect of dietary 25-azacholesterol on the weight of *L. migratoria* and its tissues.

25-Azacholesterol (ppm)	Female			Male		
	Adult (gm)	Fat body (mg)	Ovary (mg)	Adult (gm)	Fat body (mg)	Testis (mg)
0	1.4±0.07	90.6±16.99	163.4±26.45	0.97±0.07	38.9±5.13	41.0±2.72
5	1.4±0.03	79.5±24.17	54.1±22.21	0.88±0.07	23.1±5.97	44.1±6.64
10	1.2±0.14	101.4±51.19	73.0±11.89	0.94±0.06	50.8±8.98	53.5±0.21
25	1.4±0.17	68.6±18.17	50.8±3.74	1.10±0.05	50.1±18.58	60.3±8.01
50*	1.4±0.08	77.9±12.3	40.8±2.97	-	-	-

*No male nymph was able to complete development and become adult.

b. (b) Effect of dietary 25-azacholesterol on cholesterol incorporation in tissues of adult *L. migratoria.*

Females:

It was observed that in the haemolymph, the concentration of cholesterol decreases from 0.91 % in controls to 0.49 % at 50 ppm of the azasteroid. Uptake of cholesterol by the ovary also showed a decrease from 7.5 % in controls to 2.4 % at 50 ppm of 25-azacholesterol. Addition of 25-azacholesterol to the diet at 5 ppm had almost no effect on the per cent uptake of cholesterol by the fat body. However, an increase in the uptake occurred from 3.3 % at 0 ppm to 5.8 % at 10 ppm, which remained almost same even with an increasing concentration of 25 ppm and 50 ppm (Fig. 4).

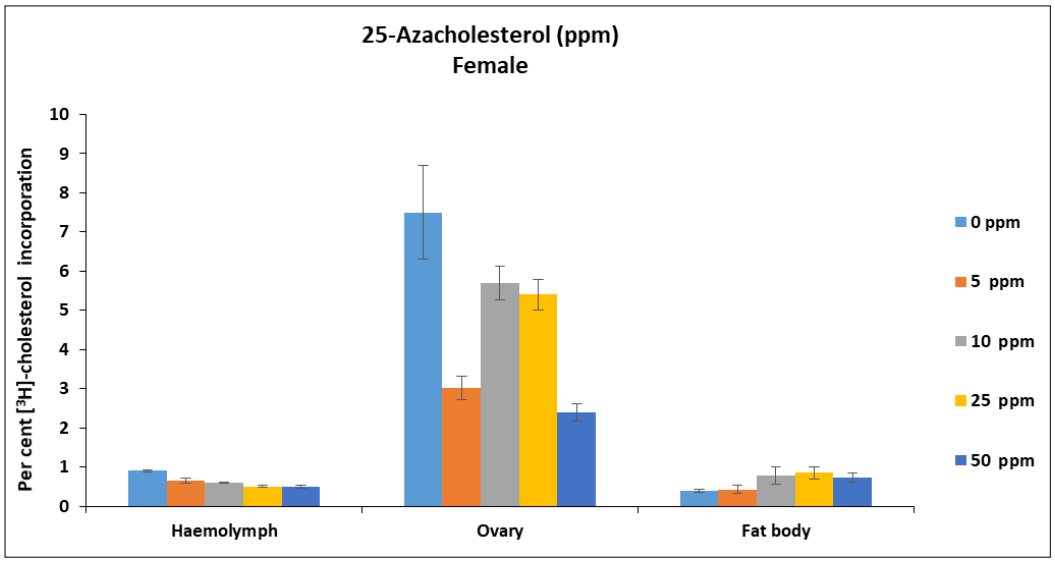

Fig 4: Effect of dietary 25-azacholesrterol on per cent incorporation of cholesterol in tissues of female *L. migratoria.*

Males

As seen in case of female insects, hemolymph of the males also show a gradual decrease in the amount of cholesterol incorporation with an increase in 25-azacholesterol concentration in the diet (Fig.5). When no azasteroid was added to the diet, [^{3}H]-cholesterol was 1.0 % in haemolymph, decreasing to 0.64 % at 25 ppm of the azasteroid. Unlike ovary, the testis shows an increase in per cent [^{3}H] incorporation from 2.01 per cent at 0 ppm to 4.0 per cent at 25 ppm of 25-aascholesterol. Cholesterol uptake by the fat body increased from 3.9 per cent to 5.2 per cent as the concentration of azasteroid increased from 0 ppm to 10 ppm as in females. At 25 ppm it again decreased to 3.1 per cent (Fig. 5).

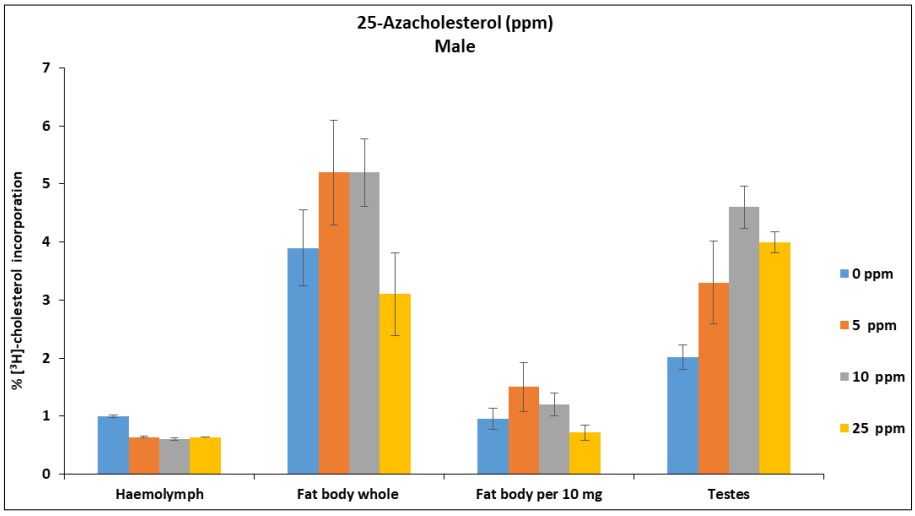

Fig 5: Effect of dietary 25-azacholesrterol on per cent incorporation of cholesterol in tissues of male *L. migratoria.*

Discussion

It has been reported that certain azasteroids inhibit the growth and development in insects (Goel and Agarwal,1987 b; Kuthiala *et al.*,1987; Agarwal *et al*., 1990; Rath *et al*., 2022; Saxena *et al*, 2022) [10,20,1,26,29]. This disruption in insect growth and development was largely attributed to the reduction in the cholesterol formation caused by the inhibition of Δ^{24}- and $\Delta^{22,24}$- sterol reductase, an enzyme involved in the conversion of the phytosterols to cholesterol (Al-Izzi and Hopkins, 1982; Svoboda and Weirich 1995) [2,32]. One of the other possibility is that the cholesterol transport and its absorption by the insect tissues actively involved in ecdysone synthesis may be affected and hence the reported effect on moulting and metamorphosis. Incorporation studies with 25-azacholesterol, when added to the diet of freshly moulted fifth instar nymphs of *Locusta* and [^{3}H]-cholesterol injected in 22 days old female adults, showed that [^{3}H]-cholesterol uptake by the ovaries was inhibited as the concentration of 25-azachloesterol increased in the diet along with decline in the weight of the ovary. However, in the fat body of these female there was an increase in the weight with concomitant increase in the uptake of [^{3}H]- cholesterol. A slight reduction in cholesterol uptake by the ovary was also observed in the presence of 25-azacholestane. The uptake by the fat body, however, did not seem to be affected in this case. The decrease in the weight of the ovary is correlated with the reduced ovarian development in which oocyte growth (vitellogenesis) is also inhibited and hence reduction in the uptake of [^{3}H]-cholesterol. Our earlier studies on *L. migratoria* have shown that cholesterol incorporation is maximum in the ovary of 22 days old adult, in which oocyte development has completed (Goel *et al*., 2021) [11]. Cholesterol incorporation by the organs depend on active metabolism and/or membrane properties (Gujar and Pillai,2016, Entringer *et al.*, 2021) [12,6]. In the present study also in control locust in which ovary was almost completely developed, cholesterol uptake was maximum. Ovary of mature *L. migratoria* females have the capacity of synthesizing ecdysteroid from the precursor cholesterol (Lagueux *et al*., 1977) [21]. Thus, in the treated females in which the ovaries are not completely developed, the [^{3}H] - cholesterol uptake is also reduced. Similar results were reported in *Helicoverpa armigera* (Rath and Goel, 2022) [27]. It has been shown that maternal cholesterol is the major source of sterols in embryo accumulated during oogenesis. Hence, for successful reproduction, transport of cholesterol to oocytes is essential (Behemer and Grebenok, 1998; Jing and Behmer, 2020) [4,17]. Studies also suggested that the azasteroids might be inhibiting some steps in the transport and incorporation of cholesterol (Rath and Goel, 2022) [27]. In lubber Grasshopper, ecdysteroids may be involved in egg production but are not necessary for vitellogenin production (Hatle *et al.*, 2003) [13]. Juvenile Hormone (JH) is the main gonadotropin hormone controlling the reproduction in the female orthopterans (Gijbels *et al.*, (2019) [8]. The present study suggests that the reduction in both the weight of the ovary and the uptake of cholesterol from the hemolymph might be due to some effect caused by the azasteroids on the neuroendocrine system i.e. ecdysteroids and JH level. In hemimetabolous insects such as *L. migratoria* and German cockroach, *Blattella germanica*, ovarian development and vitellogenin synthesis in the fat body are dependent on JH (Song and Zhou, 2020; Wu *et al*., 2021) [30, 34]. Large amounts of ecdysteroids are synthesized at the end of the oocyte maturation only after vitellogenesis is almost completed (Lagueux *et al*., 1981, Lenarts *et al.*,2019) [22, 23]. Hatle *et al* (2003) [13] suggested that in lubber grasshoppers, ovary is the site for ecdysteroid synthesis. Role of azasteroids in reducing the juvenile hormone titre and hence causing a delay of vitellogenesis has been suggested in *Argas hermanni* (Khalil *et al*., 1996) [19]. It is thus probable that 25-azacholesterol might also have some inhibitory effect on the JH receptors (Gujar and Pillai, 2016) [12] and hence reduction in ovarian development along with the reduced cholesterol incorporation which is an important precursor of ecdysteroids in insects. The effects of some other compounds such as azadirachtin on moulting in several insects have been shown to be due to interference with the ecdysone and juvenile hormone levels (Barnaby and Klocke 1990, Garcia *et al.*, 1991) [3, 7].

The results of the present study show that the cholesterol uptake by the fat body increases with the increase in 25-azacholesterol concentration in the diet. A similar result was observed in fat body after N, N dimethyledodecanamine treatment however unlike with azasteroids the cholesterol incorporation in ovary also increased with increase in the N, N dimethyledodecanamine concentration. The reason for this needs to be investigated. Our previous study has shown that the fat body and ovary behave opposite to each other as far as the cholesterol incorporation is concerned (Goel *et al.,* 2021) [11]. This effect may probably be due to the inhibitory effect of 25-azacholesterol on corpora allata i.e. JH release or synthesis which in turn is responsible for increase in the weight of fat body and also increase in the uptake of cholesterol. This is also evidenced by the fact that there is a simultaneous decrease in the ovarian development due to decreased vitellogenin transport from the fat body as also suggested by Wu *et al*. (2021) [34]. It is known that vitellogenins are majorly synthesized in fat body (Roy *et al*., 2018; Lenaerts *et al.,* 2019; Swevers, 2019) [28,23,33] and transported to the developing oocytes. Khalid *et al.* (2021) [18] also showed that JH are involved with the fat body changes.
In male locust an increase in the weight as well as uptake of the [^{3}H]-cholesterol by both the fat body and testes was observed with an increase in the concentration of the 25-azacholesterol. It seems that 25- azacholesterol might affect the JH level in the insect body by inhibiting JH receptors as reported in *Schistocerca gregaria* by Holtof (2021) [16] and hence the slight increase in the weight and cholesterol uptake is seen. Heming (2003) [14] showed that ecdysteroids promote spermatogenesis whereas JH depress it. Studies with JH mimic (NC-1840) on fifth instar nymph of *S. gregaria* have shown underdevelopment of accessory reproductive glands, seminal vesicles and impaired moulting to adult stage indicating a complex role of JH in development of reproductive organs (Hiroyoshi *et al.,* 2019) [15]. Further detailed studies are, however, required to understand the complex mechanism and interaction of these compounds with the JH receptors and neuroendocrine system in *Locusta*. Such studies can prove to have great potential in regulating the population of this polyphagous agricultural pest, devastating several crops and that has been the cause of famines and human migration.

Conclusion:
Our studies with 25-azacholestane, 25-azacholesterol and N, N-dimethyldodecanamine show that there was a decrease in the cholesterol incorporation in the ovary and haemolymph in contrast to the fat body and testes where an increase in cholesterol uptake was observed. They may be directly or indirectly involved in the sterol metabolic pathways other than those involving formation of cholesterol from phytosterols. The reasons for the increase in the uptake of [^{3}H]- cholesterol by the testes are not known and need to be investigated. Further studies are necessary in order to learn the exact mechanism by which these compounds effect the uptake of cholesterol by different tissues/organs especially ovary and testes of *Locusta* and perhaps other insects as well.

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Conflict of interest
VG, RR and RG hereby declare that there is no conflict of interest.

References
1. Agarwal HC, Gupta R, Rath R, Goel V. Sterol inhibition in *Chilo partellus*. International Journal of Tropical Insect Science,1990:11(4-5):583-591. https://doi.org/10.1017/s1742758400021159
2. Al-Izzi MAJ, Hopkins TL. Effects of 25-azasteroids on development and reproduction of southwestern corn borer, *Diatraea grandiosella* Dyar. J Insect Physiol, 1982:28:267-271. https://doi.org/10.1016/0022-1910(82)90086-5
3. Barnaby MA, Klocke JA. Effects of azadirachtin on levels of ecdysteroids and prothoracicotropic hormone-like activity in *Heliothis virescens* (Fabr.) Larvae. J Insect Physiol,1990:36:125-132.
4. Behmer ST, Grebenok RJ. Impact of dietary sterols on life-history traits of a caterpillar. Physiol Entomol,1998:23:165-175. https://doi.org/10.1046/j.1365-3032.1998.232074.x
5. Darnet S, Martin LBB, Mercier P, Bracher F, Geoffroy P, Schaller H. Inhibition of Phytosterol Biosynthesis by Azasterols. Molecules,2020:25:1111:1-14. doi:10.3390/molecules25051111
6. Entringer PF, Majerowicz D, Gondim KC. The Fate of Dietary Cholesterol in the Kissing Bug *Rhodnius prolixus*. Frontiers in Physiology,2021:12:1-10. https://doi.org/10.3389/fphys.2021.654565
7. Garcia ES, Gomes JEPL, Azambuja P De. Head transplantation in *Rhodnius prolixus* larvae. A tool for studying depression of brain neurohormone release induced by natural compounds. Cienc Cult (Sao Paulo),1991:43:447-450.
8. Gijbels M, Lenaerts C, Vanden Broeck J, Marchal E. Juvenile Hormone receptor Met is essential for ovarian maturation in the Desert Locust, *Schistocerca gregaria*. Sci Rep,2019:9:1-12. https://doi.org/10.1038/s41598-019-47253-x

9. Goel V, Agarwal HC. Sterols of *Locusta migratoria* Linn. reared on two different host plants, corn and sorghum. J of Enotomol Res,1987a:11(1):90-98.
10. Goel V, Agarwal HC. Effect of 25- azacholestrol on development in *Locusta migratoria* (Orthoptera: acrididae). Entomon,1987b:12(2):127-129.
11. Goel V, Rath R, Saxena R. Incorporation of [^{3}H]-cholesterol in various tissues during ovarian development of *Locusta migratoria* L. (Orthoptera:Acrididae). International Journal of Entomology Research,2021:6(6):17-22.
12. Gujar H, Palli SR. Juvenile hormone regulation of metamorphosis in the common bed bug, *Cimex lectularius*. Sci Rep,2016:6:1-10 https://doi.org/10.1038/srep35546
13. Hatle JD, Juliano SA, Borst DW. Hemolymph ecdysteroids do not affect vitellogenesis in the lubber grasshopper. Archives of Insect Biochemistry and Physiology,2003:52(1):45–57. https://doi.org/10.1002/arch.10067
14. Heming BS. "Chapter 1. The Male Reproductive System and Spermatogenesis". Insect Development and Evolution, Ithaca, NY: Cornell University Press, 2018, 6-28. https://doi.org/10.7591/9781501720758-004
15. Hiroyoshi S, Kokwaro E, Mettupalli S, Mitsunaga T, Yagi S, Reddy GVP. Effects of the juvenile hormone mimic NC-184 on the development of the reproductive organs and mating behaviour of nymphs of the desert locust, *Schistocerca gregaria* (Orthoptera: Acrididae). Eur J Entomol,2019:116:477–485. https://doi.org/10.14411/eje.2019.049
16. Holtof M, Lommel JV, Gijbels M, Dekempeneer E, Nicolai B, Broeck JV, Marchal E. Crucial Role of Juvenile Hormone Receptor Components Methoprene-Tolerant and Taiman in Sexual Maturation of Adult Male Desert Locusts. Biomolecules,2021:11:244:1-21. https://doi.org/10.3390/biom11020244
17. Jing X, Behmer ST, Insect sterol nutrition: physiological mechanisms. Ecology, and Applications. *Annu Rev Entomol*,2020:65:251-271. https://doi.org/10.1146/annurev-ento-011019-025017
18. Khalid MZ, Ahmad S, Ngegba PM, Zhong G. Role of Endocrine System in the Regulation of Female Insect Reproduction. Biology,2021:10(7):614:1-12. https://doi.org/10.3390/biology10070614
19. Khalil GM, Marzouk AS, Mohamed FSA, Darwish, ZEA. Effect of 25-Azacholestane on *Argas (Argas) hermanni* (Ixodoidea: Argasidae). J Med Entomol,1996:33(5):783-789. doi:10.1093/jmedent/33.5.783
20. Kuthiala A, Agarwal HC, Thompson MJ, Svoboda JA. 25- Azasteroid inhibition of development and conversion of C28 and C29 phytosterols to cholesterol in *Spodoptera litura* (F). Archives of Insect Biochemistry and Physiology,1987:4**:**57-66. https://doi.org/10.1146/annurev-ento-011019-025017
21. Laguex M, Hirn M, Hoffmann JA. Ecdysone during ovarian development in *Locusta migratoria*. J Insect Physiol,1977:23:109-119. https://doi.org/10.1016/0022-1910(77)90116-0
22. Lagueux M, Sall C, Hofmann JA. Ecdysteroids During Embryogenesis in *Locusta migratoria*. AMER ZOOL,1981:21:715-726.
23. Lenaerts C, Marchal E, Peeters P, Broeck J. The ecdysone receptor complex is essential for the reproductive success in the female desert locust, *Schistocerca gregaria*. Scientific reports,2019:9(15):1-12 DOI:10.1038/s41598-018-36763-9.
24. Li S, Jing X. Fates of dietary sterols in the insect alimentary canal. Curr Opin Insect Sci,2020:41:106-111. https://doi.org/10.1016/j.cois.2020.08.001
25. Rath R, Singhal R, Goel V, Agarwal HC. In vivo and in vitro metabolism of sitosterol in *Heliothis armigera* (Hubner) and *Locusta migratoria* L. Indian J Exp Biol,1993:31:699-703.
26. Rath R, Saxena R, Goel V, Gupta, R. Effect of dietary azasteroids on the growth and development of the gram pod borer, *Helicoverpa armigera* (Hubner). Indian Journal of Entomology, 2022. Online published Ref. No. e21237 DoI.: 10.5958/IJE.2022.180
27. Rath R, Goel V. *In vivo* and *in vitro* studies on the uptake of [^{3}H]-cholesterol by different tissues of the gram pod borer, *Helicoverpa armigera* (Hubner) in the presence of sterol metabolism inhibitors- the azasteroids. International Journal of Entomology Research,2022:7(2):193-202.
28. Roy S, Saha TT, Zou Z, Raikhel AS. Regulatory pathway controlling female insect reproduction. Ann Rev Entomol,2018:63:489-511.
29. Saxena R, Rath R, Goel V, Gupta, R. Efficacy of 25-azacoprostane and N, N dimethyldodecanamine as potential inhibitors of growth and development in *Spodoptera litura* (F.) Journal of Contemporary Research and Communications,2022:5(1):1-12.
30. Song J, Zhou S. Post-transcriptional regulation of insect metamorphosis and oogenesis. Cell Mol Life Sci,2020:77:1893-1909. doi: 10.1007/s00018-019-03361-5
31. Svoboda JA, Thompson MJ, Robbins WE. Azasteroids, potent inhibitors of insect molting and metamorphosis. Lipids,1972:7:(8):553-556. https://doi.org/10.1007/bf02533023
32. Svoboda JA, Weirich GF. Sterol metabolism in tobacco hornworm, *Manduca sexta*-A review. Lipids,1995:30(3):263-267. https://doi.org/10.1007/bf02537831
33. Swevers L. An update on ecdysone signalling during insect oogenesis. Curr Opin Insect Sc,2019:31:8-13. https://doi.org/10.1016/j.cois.2018.07.003
34. Wu Z, Yang L, He Q, Zhou S. Regulatory mechanisms of vitellogenesis in insects. Frontiers in Cell Developmental Biology,2021:8:593-613. https://doi.org/10.3389/fcell.2020.593613