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CYANTRANILIPROLE-MEDIATED EFFECTS ON LOCOMOTORY BEHAVIOUR AND FOOD CONSUMPTION IN *Spodoptera litura* (LEPIDOPTERA: NOCTUIDAE)

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The tobacco caterpillar, *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae), is a highly destructive insect pest found Worldwide, including the Middle East, Asia, Oceania and the Pacific Islands (Shu *et al*., 2017; Yadav *et al*., 2023). *S. litura* is a polyphagous pest of 180 different species of cultivated plants (Shekhawat *et al*., 2018) including cabbage, cauliflower, tobacco, groundnut, potato, tomato and cotton (Garad *et al.,* 1984, Xu *et al*., 2016a). It completes five to six overlapping generations in a year and if not controlled, can lead to substantial damage (Yu *et al*., 2015; Islam *et al.,* 2022). The pest has the capacity to cause economic losses varying from 25.8 per cent to complete crop devastation (Sahu *et al*., 2020). The application of synthetic insecticides has been a widely preferred approach for managing *S. litura*. However, the excessive and indiscriminate use of these chemicals has contributed to the development of resistance in this pest (Liu *et al.,* 2012). *S. litura* has exhibited varying levels of resistance to multiple insecticides (Tong *et al*., 2013). These insecticides, which are newer and designed to target specific biochemical pathways in insects are gradually losing their effectiveness due to the pest's evolving resistance.

Cyantraniliprole is an anthranilic diamide insecticide, which targets ryanodine receptors of insect muscles, leading to excessive calcium release and eventual depletion (Lahm *et al*., 2009). It exhibits strong antifeedant properties, leading to a decrease in the relative growth rate, relative consumption rate, efficiency of conversion of ingested food, and efficiency of conversion of digested food (Xu *et al*., 2016b; Xu *et al*., 2017). Additionally, it lowers the respiration rate and alters the behavioural responses of insect pests (Rueda *et al*., 2019). Beyond metabolic, neurological, anatomical and physiological factors affecting pest control, cyantraniliprole also influences locomotory behaviour. The state of these systems in insects is often reflected in their movement patterns (Tooming *et al.,* 2014; Augusiak and Brink, 2016). Locomotion plays a key role in essential behaviours such as foraging, migration, predation, habitat selection, behavioural thermoregulation, and interactions within and between species. It also disrupts fundamental behavioural and physiological functions critical for insect fitness and survival, impacting both individuals and populations (Merivee *et al.*, 2015). Sublethal exposure to cyantraniliprole can induce a series of physiological, behavioural, and biochemical changes in pests, including modifications in survival rate, development duration, fecundity, and lifespan (Yang *et al*., 2019; Lin *et al*., 2020). In *S. litura*, exposure has been linked to abnormal developmental effects (Song *et al*., 2014), which result in impaired feeding, paralysis and ultimately death (Xu *et al*., 2016c; Dong *et al*., 2017). Moreover, this innovative insecticide offers the benefit of mammalian safety and high selectivity against non-target species (George *et al*., 2019). Therefore, our study aims to determine variations with respect to various behavioural parameters such as locomotion and feeding of *S. litura.*

Insect and insecticide

A population of *S. litura* was collected from cauliflower and cabbage fields at the screen house of the Entomological Research Farm, Punjab Agricultural University, Ludhiana, Punjab, India in 2023-2024. The collected larvae were then maintained in the Insect Physiology Laboratory, Department of Entomology, on cauliflower leaves under controlled conditions of 26±1°C temperature and 65±5 per cent relative humidity. Newly hatched *S. litura* caterpillars were kept in 25 mL plastic cups until reaching the second-instar, while adults were provided with a cotton swab soaked in 10 per cent honey solution, suspended from the muslin covering the jar's mouth. The formulation of cyantraniliprole 10.26 per cent w/w OD (Benivia 10.26 OD) was used in this experiment. Toxicity values (LC_{30} and LC_{50}) had already been determined using the standard leaf-disc dip method for bioassays. A total of 100 second-instar larvae (divided into 10 replications of 10 larvae each) were exposed to

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various concentrations of insecticide. Larvae treated with LC_{30} (0.000066 per cent) and LC_{50} (0.000080 per cent) were subsequently observed through their development into adult.

Sublethal effects of cyantraniliprole on locomotory behaviour

Test insecticide was uniformly applied using an atomizer and allowed to dry upon the surface of wax paper in room temperature (27±1°C). After application, a second-instar larva was placed on the treated wax paper and its movement was recorded using a video tracking software, EthoVision (Panasonic WV-CP480/G) including 30 mm arena and CS 1/3, which analyses and records behavioural pattern including activity and movement. Various movement parameters were documented over a 5-minute period. Each treatment was replicated six times to ensure a comprehensive analysis of *S. litura* larval locomotory behaviour. Observations were recorded from the test-larvae per unit time and path shape, *i.e*., distance travelled, speed and turn angle.

Determination of food consumption and its utilization in *S. litura*

A cabbage leaf disc was carefully cut along the mid vein to obtain two equal halves; each were individually weighed. One half was treated with the insecticide, while the other remained untreated and both were placed in separate Petri dishes. Ten second-instar *S. litura* larvae from each population were introduced onto one half of the leaf disc and allowed to feed for four days. Before being released, the initial weight of the larvae was recorded using an electronic balance. After the feeding period, the larvae's weight gain was measured and the weight of each half-leaf disc was reassessed to determine the amount of leaf consumed. The corrected weight of the consumed leaf material was then calculated. Additionally, excretal pellets were collected at the end of the four-day period and their weight was recorded. Feeding indices were calculated using standard formulae (Waldbauer, 1968) and included following: relative consumption rate (RCR) = F/TA; relative growth rate (RGR) = G/TA; efficiency of conversion of ingested food to body substance (ECI) = 100 G/F; efficiency of conversion of digested food to body substance (ECD) = 100 G/(F-E); approximate digestibility (ADI) = 100 (F-E)/F, where F= fresh weight (mg) of food consumed; T= duration of feeding period; A= geometric mean of fresh larval weight (mg) during feeding point; and G = fresh weight (mg) gain of larvae.

Effect of cyantraniliprole on locomotory behaviour

The locomotory behaviour of *S. litura* exhibited significant alterations following surface exposure to insecticides. The larva travelled the longest distance of 271.30± 5.64 cm on the surface treated with LC_{50}, which was significantly greater than the 240.30± 5.72 cm at LC_{30}. Both distances were significantly different from the control ones (115.10± 2.8 cm). The larvae exhibited the highest recorded speed at LC_{50} with 0.92± 0.011 cm/s, followed by LC_{30} with 0.75 ± 0.008 cm/s and control with 0.50 ± 0.007 cm/s (Table 1). Moreover, at LC_{50}, the larvae demonstrated the highest recorded turn angle of 118.76 ± 0.308°, which was followed by 109.25± 2.274° at LC_{30} and 75.43 ± 0.864° in control. When exposed to a treated surface, insects may demonstrate heightened locomotion as an avoidance response. This behaviour suggests that the insects detect the presence of the treatment and actively move away from it to minimize contact or exposure.

This study aligns with findings by Chandi and Suri (2019), which reported that larvae exhibited shorter movement distances, reduced speed and fewer directional changes on untreated surfaces compared to insecticide-treated ones. Among the studied populations, larvae from Amritsar demonstrated the most pronounced behavioural modifications, characterized by the widest turn

Table 1. Behavioural response (locomotion) of *S. litura* caused by sublethal dose of cyantraniliprole

Treatment	Distance (cm)	Speed (cm/s)	Turning angle (°)
Control	115.10 ± 2.8	0.50 ± 0.007	75.43 ± 0.864
LC_{30}	240.64 ± 5.72	0.75 ± 0.008	109.25 ± 2.274
LC_{50}	271.30 ± 5.64	0.92 ± 0.011	118.76 ± 0.308
CD (*p=0.05*)	*17.35*	*0.03*	*5.00*

* Mean±SE, based on five replications each comprising ten larvae

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angles, slowest movement speeds, and shortest distances travelled, irrespective of insecticide exposure. Exposure to deltamethrin on *Spodoptera frugiperda* showed less resting time and walking distance. Both distance travelled and resting time was higher in control (Vinha *et al*., 2021). Moreover, *S. oryzae* showed changes in locomotion patterns and fewer food approaches at sublethal concentrations (LC_{10} and LC_{30}) of chlorantraniliprole, while *S. zeamais* exhibited longer walking durations and shorter immobility periods by Kavallieratos *et al.* (2024). Chandi *et al.* (2023) further documented significant alterations in the movement patterns of *S. litura* on cyantraniliprole-treated surfaces. Specifically, larvae from the Amritsar population exhibited reduced mobility, covering shorter distances at lower speeds and displaying greater turn angles compared to larvae from Malerkotla, Kapurthala and Ludhiana populations. In contrast, the highest movement speeds were observed in treated larvae from Ludhiana, followed by Malerkotla, Kapurthala and Amritsar. Similarly, untreated larvae exhibited less pronounced turning behaviour, with the highest turn angles recorded in Amritsar (control) and the lowest in Ludhiana (control). Additionally, Chandi and Kaur (2017) reported that *Plutella xylostella* populations travelled greater distances at increased speeds on insecticide-treated surfaces compared to untreated ones. These behavioural modifications may be attributed to neurophysiological alterations induced by insecticide exposure.

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Effect of cyantraniliprole on food consumption and its utilization

Sublethal concentrations of cyantraniliprole had a significant impact on the feeding indices of *S. litura* larvae. Compared to the control group, the treated larvae exhibited a significant decrease in RGR, RCR, ECI and ECD. However, exposure to sublethal concentrations of cyantraniliprole led to an increase in AD (Table 2). RCR was lower at 2.74 mg/mg/day at LC_{50} (0.000080 per cent), while at LC_{30} (0.000066 per cent), it measured 3.25 mg/mg/day. Similarly, RGR declined to 0.42 mg/mg/day at LC_{50} and 0.55 mg/mg/day at LC_{30}, both significantly lower than the untreated control (1.08 mg/mg/day). The ECI was lowest at LC_{50} (8.85 per cent) and LC_{30} (16.58 per cent), compared to 21.06 per cent in the control group. Likewise, the ECD was lowest at LC_{50} (21.33 per cent) and LC_{30} (25.69 per cent), while the control group recorded 44.02 per cent. In contrast, AD increased with treatment, reaching 81 per cent at LC_{50} and 78.74 per cent at LC_{30}, significantly higher than the control (48.73 per cent). These findings indicate that cyantraniliprole exposure reduces food utilization efficiency while increasing digestibility in *S. litura* larvae.

Feeding efficiency is a crucial aspect of feeding ecology, as it quantifies an insect's ability to convert the ingested food into biomass. This parameter plays a key role in understanding insect growth, development and overall energy utilization within an ecosystem (Yazdani *et al*., 2013). Insecticides and plant extracts have been found to impact negatively in the feeding efficiency of target insect species, also reducing their ability to convert ingested food into biomass (Liu *et al*., 2008; Xu *et al*., 2016a). The findings of Lahm *et al.* (2012) align closely with our study, demonstrating that cyantraniliprole effectively reduces the RCR of *Helicoverpa armigera* and exhibited a significant decrease in leaf consumption, with a 45 per cent reduction in RCR compared to untreated controls. Similarly, Selby *et al*. (2013) reported that *S. frugiperda* larvae exposed to 0.2 mg/L of cyantraniliprole experienced a 50 per cent reduction in consumption, while *P. xylostella* larvae treated with 0.05 mg/L showed a 35 per cent decrease. Teixeira *et al.* (2015) also observed a decline in the RCR of *Helicoverpa zea* larvae exposed to 0.15 mg/L of cyantraniliprole. Additionally, Xu *et*

Table 2. Feeding indices of *S. litura* at sublethal doses of cyantraniliprole

Treatment	RCR (mg/mg/day)	RGR (mg/mg/day)	ECI (per cent)	ECD (per cent)	AD (per cent)
Control	6.04± 0.146	1.08± 0.007	21.06± 0.504	44.02± 0.366	48.73± 0.735
LC_{30}	3.25± 0.039	0.55± 0.012	16.58± 0.086	25.69± 0.08	78.72± 1.598
LC_{50}	2.74± 0.51	0.42± 0.002	8.85± 0.184	21.33± 0.400	81.42± 1.313
CD (*p=0.05*)	*0.19*	*0.03*	*0.58*	*1.17*	*1.46*

* Mean±SE, based on five replications each comprising ten larvae
RCR= Relative consumption rate; RGR= Relative growth rate; ECI= Efficiency of conversion of ingested food to body substance; ECD= Efficiency of food digestion; AD= Approximate digestibility.

al. (2016a) found that cyantraniliprole significantly suppressed the feeding activity of *Agrotis ipsilon* larvae, with increasing concentrations (LC_5, LC_{20}, and LC_{40}) leading to progressively lower food intake. Moreover, RGR is an index which is related to insect weight gain. Lower RGR indicates the unsuitability of cyantraniliprole treated food source (Xu *et al*., 2017). In our study, LC_{30} showed a higher RGR than LC_{50}, which was the lowest. The results align with Ding *et al.* (2017), who reported that cyantraniliprole affected the relative growth rate (1.2 mg/day) of *A. ipsilon*. Xu *et al.* (2016a) reported a decrease in larval growth rate following exposure to cyantraniliprole. Larvae treated with sublethal concentrations (LC_5, LC_{20}, and LC_{40}) exhibited significantly lower relative growth rates of 0.28, 0.18, and 0.11 mg/mg/day, respectively, while the control group had a rate of 1.41 mg/mg/day. Our study concludes that ECI and ECD has decreased, while increase in AD at LC_{30} and LC_{50} compared to the control. Gupta *et al.* (2018) also reported a significant decline in ECI in *Spodoptera exigua* following cyantraniliprole exposure. Larvae treated with 0.1 mg/L of the insecticide exhibited a 30 and 14.5 per cent reduction in ECI and ECD, respectively compared to untreated controls, with treated larvae showing a conversion efficiency of 12.5 per cent, whereas the control group had 18.0 per cent. These findings suggest that cyantraniliprole affects digestive efficiency, possibly influencing nutrient utilization and metabolism. The increased approximate digestibility may result from prolonged food retention in the digestive tract and reduced excretion rates, similar to observations in *S. litura*.

Cyantraniliprole affects the locomotory behaviour of larvae of *S. litura* showing effects like larvae travelled more distance with higher speed and turn angle. The low relative growth rate, relative consumption rate, efficiency of conversion of ingested food and efficiency of conversion of digested food indicated that the greater portion of food may be utilized for energy, which may be required for detoxification, rather than being converted into body mass. This results in a decline in fitness for *S. litura* due to starvation. The decrease in feeding indices indicates that insecticides have caused chronic toxicity in *S. litura*. Cyantraniliprole exhibits anti-feeding properties, preventing insects from consuming food, which may result in nutrient deficiencies that hinder normal growth and metabolic functions. With its low mammalian toxicity, safety for non-target organisms and effects in response to behaviour and feeding indices, cyantraniliprole has the potential to be an effective tool for managing *S. litura*. Also, future studies should incorporate extended temporal monitoring to more comprehensively capture variations in behaviour and feeding over time. Future studies incorporating multiple or continuous time-point assessments would provide a more comprehensive understanding of these dynamic responses.

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Authors' contribution

Conceptualization of research work and designing of experiments (DS, AKC); Execution of field/lab experiments and data collection (DS); Analysis of data and interpretation (DS, AKC); Preparation of manuscript (SC).

Conflicts of interest

The authors declare that there are no conflicts of interest.

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