RESEARCH ARTICLE





Insecticide induced resurgence of *Scirtothrips dorsalis* Hood: Role of biochemical changes in chilli and natural enemies suppression

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Abstract

The improper use of insecticides poses risks to both human health and the environment, leading to issues such as insecticide residue, resistance, resurgence and increased management costs. The chilli thrips, *S. dorsalis* Hood (Thysanoptera: Thripidae) is a significant insect pest infesting vegetable crops and its management has become increasingly challenging due to its resurgence in response to commonly applied insecticides. A field experiment was conducted at UAS, Raichur, India, during the *Kharif* season of 2020 and the summer of 2021 to assess the effects of insecticides on the resurgence of *S. dorsalis*. The results demonstrated significant resurgence activity following the insecticide application. Fipronil, fenpropathrin and lambda-cyhalothrin led to high resurgence rates and a reduction in the populations of natural enemies of thrips. Subsequent treatments after the resurgence showed a decrease in defensive biochemicals such as flavonoids, tannins and phenols, while levels of total sugars, proteins and proline increased. Path coefficient analysis revealed a strong positive correlation between the insecticides and the increase in total sugars, proteins and proline, while negatively affecting flavonoids, tannins and phenols. The resurgence of *S. dorsalis* was closely associated with changes in plant biochemistry following insecticide application and the reduction of natural enemy populations.

Keywords: biochemical; chilli; insecticides; natural enemies; resurgence; thrips

Introduction

Globally, the concept of resurgence has been defined in various ways by different researchers. One of the earliest definitions, highlighted the role of pesticides and natural enemies explaining that resurgence refers to an unusually rapid increase in the population of a pest that was previously suppressed by pesticide treatments, which also harmed its natural enemies (1). The insecticide application induces a resurgence in the target pest population (primary pest resurgence) or minor pests grow into major pests (secondary pest resurgence) (2).

Chilli constitutes 31 % of the global spice trade and India is the world's largest producer of chilli, accounting for 36 % of world production (3). While insecticide-induced pest resurgence is a common and generally accepted consequence of pesticide use, it is often considered a negative impact on crop production (2). Managing *S. dorsalis* has become particularly challenging in

recent years due to the pest's ability to develop resurgence in response to commonly used insecticides (4). Insecticides should be applied judiciously to control population growth ensuring their use only when absolutely necessary to prevent unintended adverse effects. Given the difficulties in chilli cultivation, it is crucial to assess the current status of insecticide-induced resurgence to effectively address field control challenges. In this regard, insecticides commonly used by growers were selected for evaluation to assess their impact on *S. dorsalis* under field conditions.

Materials and Methods

Field experiment

A popular chilli variety, Byadagi kaddi and hybrid HPH-5531 were raised during *kharif* 2020 and summer 2021. Four continuous

sprays were applied at seven-day intervals and the experiment was set up in a Randomized Complete Block Design (RCBD) with eleven treatments, including an untreated control, each replicated thrice (Table 1).

Thrips populations (adults and nymphs) were observed on the top three leaves of ten randomly selected plants using a macro lens (12X) attached to a smartphone camera (One Plus Nord). Similarly, natural enemies (coccinellids, anthocorid bugs, green lacewings and big-eyed bugs) were recorded on ten marked plants one day before and seven days after each spray, including control, to calculate percent mortality. This observation was consistently followed for all subsequent sprays. The per cent resurgence was calculated using the formulae (5, 6);

$$Per cent resurgence = \left\{ \frac{T_{S} \times C_{F}}{C_{S} \times T_{F}} - 1 \right\} \times 100$$

Where,

 T_F = Infestation in the treated plot during the first count T_S = Infestation in the treated plot during subsequent count C_F = Infestation in the untreated check plot during the first count C_S = Infestation in the untreated check plot during subsequent count

The biochemical components from treated chilli plants were analyzed using the methods given in Table 2.

Data analysis

The data on the population of thrips, natural enemies and biochemical parameters were subjected to ANOVA (12, 13) and means were separated by Tukey's HSD (14) and interpretation by R software (15).

Results

Field experiment, kharif 2020 and summer 2021

The field data from four consecutive insecticide sprays against chilli thrips demonstrated significant findings related to resurgence phenomenon. After the initial application of fipronil, fenpropathrin and lambda-cyhalothrin, the thrips population decreased. However, a slight increase in population was observed following the second spray, which subsequently escalated after the third and fourth sprays, ultimately leading to crop collapse. In the fipronil-treated plot, the chilli plants harbored a thrips population of 26.12, reflecting a notable resurgence of 88.50 %. Similarly, pyrethroid insecticides, fenpropathrin (21.72 thrips) and lambda-cyhalothrin (22.05 thrips), exhibited resurgences of 62.37 % and 62.60 % respectively (Table 3). The fourth fipronil application resulted in a higher thrips population of 102.37 thrips per 3 leaves, marking a maximum resurgence of 94.19 %, whereas fenpropathrin and lambda-cyhalothrin demonstrated resurgence rates of 72.04 % and 71.21 % respectively. These results indicate that the use of these insecticides in chilli cultivation contributes to the resurgence of *S. dorsalis*.

Insecticides effect on natural enemies

Three species of coccinellids, one species of predator in Phytoseiidae, Anthocoridae, Chrysopidae and Geocoridae and two species in the Phlaeothripidae families were observed during the study (Fig.1 A-I). A significant reduction in the populations of predatory thrips, mites, ladybird beetles, anthocorid bugs, green lacewings and big-eyed bugs was noted in fields treated with fipronil, fenpropathrin and lambdacyhalothrin during the Kharif season of 2020 and the summer of 2021. This reduction contributed significantly to the resurgence of S. dorsalis. Big-eyed bug mortality reached a peak of 83.33 % in fipronil and 80.56 % in ethion 50 EC at 750 g a.i./ha, followed by 77.78 % in fenpropathrin and 72.22 % in lambda-cyhalothrin. Mortality in other treatments ranged from 38.89 % to 47.22 % (Table 4). Similar reductions in the populations of predatory thrips, mites, ladybird beetles, anthocorid bugs and green lacewings were observed in plots treated with these insecticides.

Relationship between biochemical constituents and *S. dorsalis* under field condition

The chilli plants sprayed with spinetoram 11.7 SC, acetamiprid 20 SP, imidacloprid 17.8 SL, spinosad 45 SC, cyantraniliprole 10.26 OD, ethion 50 EC and emamectin benzoate 5 SG exhibited elevated levels of chlorophyll, flavonoids, tannins and phenols alongside lower concentration of total sugars, proteins and

Table 1. Insecticide treatments

Treatments	Insecticides and source	Dose (g a.i./ha)
T ₁	Spinetoram 11.7 SC (Delegate® Dow Agro Sciences Pvt. Ltd.)	60
T ₂	Acetamiprid 20 SP (Pride® Dow Agro Sciences Pvt. Ltd.)	20
T ₃	Imidacloprid 17.8 SL (Confidor® Bayer crop science Ltd.)	50
T ₄	Fipronil 5 SC (Regent [®] , Bayer crop science Ltd.)	50
T ₅	Spinosad 45 SC (Tracer® Dow Agro Sciences Pvt. Ltd.)	73
T ₆	Cyantraniliprole 10.26 OD (Benevia® FMC)	60
T ₇	Fenpropathrin 30 EC (Meothrin®, Sumitomo Chemicals India Ltd.)	75
T ₈	Ethion 50 EC (Fosmite® PI Industries Ltd.)	75
T ₉	Lambda cyhalothrin 5 EC (Karate®, Syngenta India Ltd.)	15
T ₁₀	Emamectin benzoate 5 SG (Proclaim®, Syngenta India Ltd.)	10
T ₁₁	Control	-

Table 2. Methods of analysis and equipment used for biochemical parameters in chilli leaves

Name of the biochemicals	Method of analysis	Equipment make and model
Chlorophyll & flavonoids	-	Hand-held device (DX13271 (force A company)
Total sugars	Dinitrosalicylate (DNS) method (7)	PerkinElmer UV/Visible spectrophotometer
i Otat Sugars	Diffict osaticytate (DNS) method (1)	(Lambda 365 N4100020)
Total protein content	Kjeldahl method (8)	Velp scientifica
rotat protein content	rgetdant method (6)	Automatic distillation and titration system (UDK 159)
Proline	Ninhydrin method (9)	UV/Visible spectrophotometer (Lambda 365 N4100020)
Total tannins	Vanillin reagent method (10)	PerkinElmer UV/Visible spectrophotometer
rotat tarriiris	vanittiir reagent method (10)	(Lambda 365 N4100020)
Total phenols	Folin Ciocalteu Reagent (FCR) (11)	UV/Visible spectrophotometer (Lambda 365 N4100020)

Table 3. Insecticide effect on thrips in chilli under field conditions (Cumulative mean of Kharif 2020 and summer 2021)

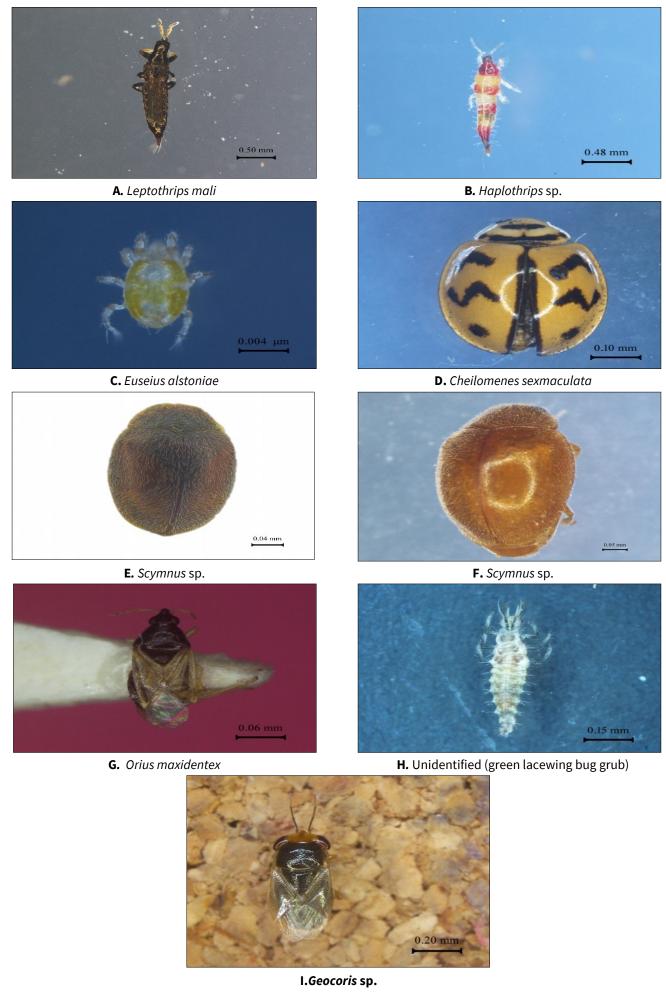
				10 m			buc			-			44	
Trootmont	Dose	*	*.	1" spray	ay		z spray	ay		3" spray	ly.		4 ^{···} spray	
li eatilieilt	(g a.i./ha)		Ts*/TF*	.* Cs*/ CF*	% Resurgence	${\rm T_S}^*/{\rm T_F}^*$	$C_{\rm S}^*/C_{\rm F}^*$	% Resurgence	$T_{S}^{\star}/T_{F}^{\star}$	C _S */ C _F *	% Resurgence	T_{S}^{*}/T_{F}^{*}	$\mathbf{C_S}^*/\mathbf{C_F}^*$	% Resurgence
Ļ	09	8.82 (3.05)	8.93 (3.07) ^b	q	-4.67	9.24 (3.12) ^b		-0.24	9.38 (3.14) ^d		-1.47	9.46 (3.16) ^d		-0.55
Ľ	20	8.61 (3.02)	8.79 (3.05) ^b	٩	-4.36	8.88 (3.06) ^b		-1.51	8.92 (3.07) ^d		-1.78	9.11 (3.10) ^d		-0.22
Ę	50	8.89	9.11 (3.10) ^b	٩	-4.06	9.18 (3.11) ^b		-1.52	8.55 (3.01) ^d		-5.24	8.67 (3.03) ^d		-0.53
4	20	8.77 (3.04)	6.85 (2.71) ^d	p	-15.58	6.72 (2.68) ^d		-2.92	26.12 (5.16) ^a		88.50	102.37 $(10.14)^{a}$		94.19
Ę	73	8.83 (3.05)	8.96 (3.08) ^b	٩	-4.36	8.82 (3.05) ^b		-2.79	8.76 (3.04) ^d		-2.42	8.69 (3.03) ^d		-1.51
£	09	8.91 (3.07)	8.87 (3.06) ^b	۵	-2.83	8.71 (3.03) ^b		-7.44	8.89 (3.06) ^d	9.74 (3.34) °	-1.26	8.93 (3.07) ^d	9.98 (3.38) °	-1.39
,	75	8.75 (3.04)	8.52 (2.87) °	v	-10.59	7.58 (2.84) ^c		-2.86	21.72 (4.71) ^b		62.37	66.74 (8.20) ^b		72.04
Ë	750	8.87 (3.06)	8.71 (3.03) ^b		-6.22	8.79 (3.05) ^b		-1.19	8.83 (3.05) ^d		-2.10	8.94 (3.07) ^d		-0.54
- °	15	8.71 (3. (3.03)	8.76 (3.04) 8.56 (2.90)	9.16 (3.21) ^a	-9.36	7.72 (2.86) °	9.55 (3.27) ^a	-3.19	22.05 (4.75) ^b		62.60	67.24 (8.23) ^b		71.21
T 10	10	8.81 (3.05)	8.90 (3.07) ^b	а	-4.67	9.21 (3.12) ^b		-0.24	9.38 (3.14) ^d		-1.47	9.47 (3.16) ^d		-0.55
T ₁₁ S. Em (±)		SN		0.04	a		0.04		0.05	25		90.0	9	
CD (P ≤0.05)			- - -	0.12	CD (P≤0.05) 0.12 0.16 0.19	-	0.13		0.16			0.19	6	

plot during subsequent count. Values in the column followed by common letters are non-significant at p ≤ 0.05 as per Tuckey's HSD (14). Figures in the parenthesis indicate √x+0.5 transformed values. *Number of thrips/top three leaves. Tr: Infestation in the treated plot during first count, Ts: Infestation in the treated plot during subsequent count, Cr: Infestation in the untreated check plot during first count, Cs: Infestation in the untreated check

 Table 4.
 Influence of insecticide application on natural enemies under field conditions in chilli (Kharif 2020 and summer 2021)

Predatory mite Per cent (nymphs and mortality adults)/ plant
54.63
56.91
55.84
100.00
56.78
55.70
97.45
93.42
96.91
55.30
•

Values in the column followed by common letters are non-significant at p ≤ 0.05 as per Tuckey's HSD (14). Figures in the parenthesis indicate √x+0.5 transformed values.



 $\textbf{Fig. 1 (A-I).} \ \ \text{Natural enemies recorded in chilli crop during the study period.}$

proline. The biochemical parameters did not differ significantly among the mentioned treatments. In contrast, chilli plants treated with fipronil, fenpropathrin and lambda-cyhalothrin, which showed higher pest resurgence, were characterized by increased levels of total sugars, proteins and proline along with decreased levels of chlorophyll, flavonoids, tannins and phenols. Among these, fipronil-treated plots showed significantly elevated biochemical compound concentrations (Table 5). The path coefficient analysis (Fig. 2 A-C) further revealed that fipronil spray had a strong positive direct effect on total sugars ($\beta = 0.342$), proteins ($\beta = -0.553$) and proline ($\beta = -0.364$) correlating with thrips occurrence. Conversely, it exerted a negative direct effect on chlorophyll (β = -0.293), flavonoids (β = -0.171), tannins (β = -0.481) and phenols (β = -0.589). A similar trend was observed with lambda-cyhalothrin, which showed a strong positive influence on sugars ($\beta = 0.738$), proteins ($\beta = 0.535$) and proline $(\beta = 0.491)$ along with a negative influence on chlorophyll $(\beta = -$ 0.267), flavonoids ($\beta = -0.133$), tannins ($\beta = -0.359$) and phenols $(\beta = -0.468)$. Likewise, fenpropathrin exhibited positive effects on sugars ($\beta = 0.345$), proteins ($\beta = 0.720$) and proline ($\beta = 0.353$) while negatively affecting chlorophyll ($\beta = -0.277$), flavonoids (β = -0.108), tannins (β = -0.368) and phenols (β = -0.321).

Discussion

The investigation of thrips resurgence in chilli identified several contributing factors, including enhanced pest fecundity due to physiological stimulation, modifications in host-plant nutritional profiles and increased mortality of natural enemies, with the latter emerging as the predominant cause. Notably, repeated applications, particularly following the third and fourth sprays of fipronil 5 SC at 50 g a.i./ha, fenpropathrin 30 EC at 75 g a.i./ha and lambda-cyhalothrin 5 EC at 15 g a.i./ha were associated with elevated natural enemy mortality. This reduction in biocontrol agents likely facilitated an increase in thrips populations. Additionally, chilli plants treated with these insecticides exhibited enhanced nutritional quality compared to those treated with spinetoram 11.7 SC, acetamiprid 20 SP, imidacloprid 17.8 SL, spinosad 45 SC, cyantraniliprole 10.26 OD, ethion 50 EC and emamectin benzoate 5 SG. These findings support the hypothesis that the suppression of natural enemies, coupled with improved host-plant nutritional status, plays a critical role in promoting thrips resurgence.

Applications of imidacloprid and spinosad have been reported to be relatively safe for key natural enemies such as predatory mites and coccinellids (16). Similarly, spinetoram and cyantraniliprole demonstrated only mild toxicity to chrysopids and were considerably less harmful compared to fipronil (17-19). In contrast, ethion was found to negatively impact predatory mites in chilli ecosystems (20). The substantial mortality of natural enemies observed following treatments with fipronil, fenpropathrin and lambda-cyhalothrin and to a lesser extent, ethion likely contributed to ecological imbalance, consistent with the predator-prey dynamics outlined in the Lotka-Volterra model.

Our findings suggest that the suppression of natural enemy populations combined with the high specificity and rapid knockdown action of these insecticides may have facilitated thrips resurgence. This is further supported by similar instances in rice ecosystems where the elimination of mirid bugs and spider predators led to increased populations of brown planthopper (21, 22). Notably, while ethion did negatively affect beneficial arthropods it did not induce thrips resurgence, possibly due to its persistence and limited influence on host-plant nutritional status (23).

Our observations align with earlier research documenting the resurgence of cotton leafhopper following the use of synthetic pyrethroids, particularly after the third spray, as well as similar resurgence trends observed in rice due to repeated applications of these compounds (24). In the present study, chilli thrips are phloem-feeders and feed more on phloem sap with higher sugar and protein levels caused by continuous insecticide use (25). Symbiotic microorganisms present in the gut of the thrips together with higher phloem sugar levels may have helped them better tolerate and break down insecticides (26).

In the current study, Type II pyrethroids such as lambdacyhalothrin and fenpropathrin, which possess a cyano functional group in their chemical structure, were associated with insecticide induced resurgence likely through their alterations of host plant biochemical profiles (27). These results align with earlier reports on whiteflies where repeated applications of fipronil and Type II pyrethroids such as lambda-cyhalothrin and fenpropathrin led to insecticide-induced resurgence (28, 29). Furthermore, the long-term use of these insecticides spanning over two decades may have contributed to resistance development, thereby enhancing the potential for pest resurgence, which was as similarly noted in *Helicoverpa armigera* (30).

The present study further demonstrated that insecticide treatments associated with thrips resurgence in chilli were characterized by elevated levels of total sugars, proteins and proline accompanied by reduced concentrations of chlorophyll, flavonoids, tannins and total phenols. These alterations in the plant's biochemical profile are likely to have facilitated increased thrips populations. In particular, the reduced levels of defensive metabolites such as flavonoids and tannins may have rendered the plants more vulnerable to *S. dorsalis* infestation (31, 32).

The substantial increase in thrips populations observed in plots treated with fipronil, fenpropathrin and lambda-cyhalothrin can be attributed to the plant's biotic stress response. Under such stress, plants tend to accumulate higher levels of proline as an adaptive mechanism, while elevated protein content has been positively correlated with increased thrips abundance (33, 34). Moreover, reduced chlorophyll content has been associated with feeding damage caused by thrips (35, 36). Laboratory studies conducted under controlled environmental conditions (25±1 °C and 65±5 % RH) have shown that the life cycle of *S. dorsalis* is shortened and fecundity enhanced when reared on plants treated with these insecticides (37).

Our results highlight a strong correlation between chilli thrips population dynamics and insecticide-induced changes in plant biochemistry. Specifically, treatments with fipronil, fenpropathrin and lambda-cyhalothrin led to significant alterations in biochemical composition in contrast to insecticides that did not induce resurgence. Consequently, these insecticides proved ineffective in field conditions and contributed to thrips resurgence through both direct and indirect effects on host plant physiology.

Table 5. Changes in biochemical components of chilli after insecticide applications under field conditions (Kharif 2020 and summer 2021)

Treatment Libose Chlorophyll (µg/cm²) (µg/cm²) Flavonoids (µg/cm²) T ₁ 60 20.23° 1.56° T ₂ 20 20.26° 1.56° T ₃ 50 20.24° 1.56° T ₅ 73 20.25° 1.12° T ₇ 75 16.33° 1.55° T ₅ 750 20.21° 1.26° T ₈ 750 20.27° 1.52° T ₉ 15 16.31° 1.22° T ₁₀ 10 20.28° 1.56°	Flavonoids (μg/cm²) Total sugars (mg/g) 1.56° 23.77° 1.56° 24.22° 1.12° 39.16° 1.56° 23.99° 1.56° 23.99°	(%) II	4.39 ^d 4.47 ^d 4.42 ^d 8.52 ^a	(mg/g) 16.84° 16.85° 16.82° 10.21° 16.83°	(mg/g) 18.28° 18.21° 18.25° 13.32d
60 20.23° 20 20.26° 50 20.24° 51 14.72° 62 20.25° 73 20.25° 75 16.33° 75 16.33° 15 16.31° 10 20.28°		1.62 (7.32) d 1.67 (7.42) d 1.67 (7.43) d 1.95 (8.03) a 1.63 (7.34) d	4.39 ^d 4.47 ^d 4.42 ^d 8.52 ^a 4.38 ^d	16.85° 16.82° 10.21° 16.83°	18.28^{a} 18.21^{a} 18.25^{a} 13.32^{d}
20 20.26° 50 20.24° 50 14.72° 60 20.25° 73 20.25° 75 16.33° 75 16.33° 10 20.27°		1.67 (7.42) d 1.67 (7.43) d 1.95 (8.03) ³ 1.63 (7.34) ^d	4.47 ^d 4.42 ^d 8.52 ^a 4.38 ^d	16.85° 16.82° 10.21 ^d	18.21^{a} 18.25^{a} 13.32^{d}
50 20.24 ^a 50 14.72 ^d 73 20.25 ^a 60 20.21 ^a 75 16.33 ^c 750 20.27 ^a 15 16.31 ^c		1.67 (7.43) ^d 1.95 (8.03) ^a 1.63 (7.34) ^d	4.42 ^d 8.52 ^a 4.38 ^d	16.82ª 10.21 ^d 16.83ª	18.25° 13.32°
50 14.72 ^d 73 20.25 ^a 60 20.21 ^a 75 16.33 ^c 750 20.27 ^a 16.31 ^c 10 20.28 ^a		1.95 (8.03) a 1.63 (7.34) d	8.52° 4.38°	10.21 ^d	13.32 ^d
73 20.25° 60 20.21° 75 16.33° 750 20.27° 15 16.31°		1.63 (7.34) ^d	4.38d	16.83ª	10 278
60 20.21 ^a 75 16.33 ^c 750 20.27 ^a 15 16.31 ^c		!			18.27
75 16.33° 750 20.27° 15 16.31°	1.55ª 24.13⁴	1.65 (7.38) ^d	4.45 ^d	16.84ª	18.22ª
750 20.27^{a} 15 16.31^{c} 10 20.28^{a}	1.26° 31.04 ^b	1.86 (7.84) ^b	7.21 ^b	12.25°	15.41°
15 16.31° 10 20.28°	1.52ª 24.21 ^d	1.68 (7.45) ^d	4.4 8 ^d	16.81ª	18.23ª
$10 20.28^{a}$	1.22 ^c 31.37 ^b	1.84 (7.79) ^b	7.27 ^b	12.27°	15.49°
	1.56ª 23.95⁴	1.64 (7.36) ^d	4.46°	16.86ª	18.27ª
${\bf T}_{11}$ - 18.59^{b} 1.45 b	1.45 ^b 28.50 ^c	1.74 (7.57) ^c	6.24¢	14.36 ^b	17.11 ^b
S. Em (±) 0.02 CD (P ≤ 0.05) 1.24 0.05	0.02 0.55 0.05 1.52	0.04	0.23	0.54	0.31

'Values in the column followed by common letters are non-significant at p ≤ 0.05 as per Tuckey's HSD (14). Figures in the parenthesis indicate arcsine transformed values.

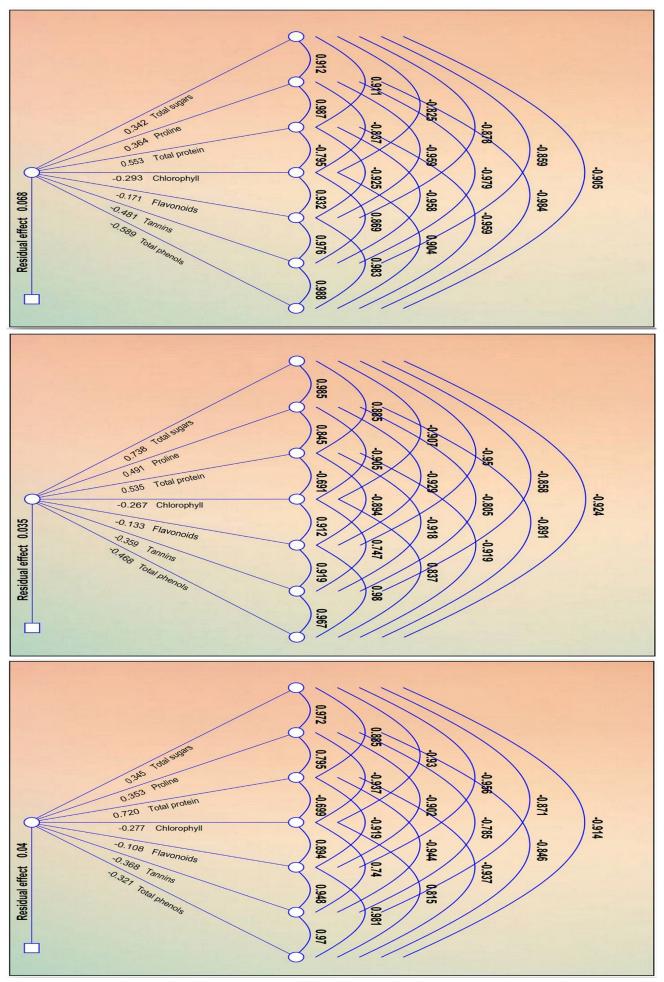


Fig. 2(A-C). Path diagram for relation of *S. dorsalis* and biochemical parameters in chilli sprayed with resurgence occurred insecticides under field condition during *kharif* 2020 and summer 2021.

These findings provide a foundational basis for developing improved pest management strategies that take into account both the pest-promoting effects of certain insecticides and their influence on plant biochemical profiles. This study presents the first documented evidence of *S. dorsalis* resurgence in chilli following the application of fipronil, fenpropathrin and lambdacyhalothrin. The identified drivers of resurgence in order of significance include mortality of natural enemies, insecticide-induced biochemical alterations in the host plant and a shortened pest life cycle as demonstrated under controlled laboratory conditions.

Accordingly, it is essential to undertake comprehensive evaluations of insecticides against the full spectrum of major insect pests affecting chilli before recommending them for thrips management. This approach is critical to minimize the risk of pest resurgence and ensure sustainable pest control.

Conclusion

Our study provides clear empirical evidence demonstrating that thrips resurgence was initiated following the third insecticide application and reached its peak after the fourth spray. Treatments with fipronil 5 SC at 50 g a.i./ha, fenpropathrin 30 EC at 75 g a.i./ha and lambda-cyhalothrin 5 EC at 15 g a.i./ha were associated with significant mortality of natural enemies and notable alterations in plant nutritional composition, both of which contributed to the escalation of thrips populations. An additional contributing factor to this resurgence was the observed reduction in the life cycle duration and increased reproductive potential of *S. dorsalis* under these treatments.

As chilli growers often spray insecticides 20-22 times per season, these findings highlight the need for thorough evaluation of insecticides against all major pests in the chilli agro ecosystem before making recommendations. Such an approach is critical for preventing both the resurgence of primary pests and the emergence of secondary pest outbreaks, thereby supporting more sustainable pest management practices.

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Authors' contributions

TH, MB, AH, RHN, SNR, NMN designed the study, conducted the experiment and collected the data. DSR, LRC performed the statistical analysis and MCK, BLM contributed to the revision of the manuscript. All authors contributed to the interpretation of the results and critically reviewed the manuscript. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no potential conflict of interest.

Ethical issues: None

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