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Documenting resistance to modern insecticides and insect growth regulators in *Helicoverpa armigera* (Lepidoptera: Noctuidae) field populations from Karnataka, India

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ABSTRACT

This study in Karnataka, investigated insecticide resistance in *Helicoverpa armigera* and related farmer practices. The toxicity of IGR (novaluron) and newer insecticides (indoxacarb, rynaxypyr, spinosad, flubendiamide and emamectin benzoate) was assessed on field strains and farmers were surveyed regarding pesticide use and resistance management. Results revealed varying resistance levels, with the RCR strain showing higher resistance to newer insecticides and novaluron. A positive correlation was observed among tested insecticides, notably between indoxacarb and rynaxypyr. Morphometric analysis suggested that larger larval and pupal dimensions in some strains were linked to higher resistance. Farmer surveys indicated low awareness of recommended agrochemicals and widespread use of higher than recommended pesticide doses (81.78%). Farmers primarily relied on agrochemical vendors for advice (85.78%), rather than agricultural experts. Compliance with resistance management strategies like avoiding similar chemicals or using traps and refugia was very low. Conventional insecticides remained dominant (54.22%) in use. This study provides baseline data for newer insecticides and highlights early resistance development. It underscores the urgent need for enhanced farmer education and integrated pest management strategies, including judicious use of diverse mode of action insecticides like spinosad and IGRs, to combat escalating resistance in *H armigera*.

Keywords: *Helicoverpa armigera*; insecticide resistance; pesticide usage patterns; newer insecticides; resistance management

INTRODUCTION

Helicoverpa armigera (Hübner) is a worldwide insect obstacle. This pest has close to 360 plant species as preferred hosts, especially crop plants such as cotton, maize, sorghum, sunflower, tomato, okra and legumes (Singh and Singh 1975). Pawar et al (1986) identified 182 plant species as *H armigera* hosts, 56 of which suffered serious damage while the other 126 were infrequently affected. Worldwide, losses due to *Helicoverpa* in cotton, legumes, vegetables, cereals etc, exceed US$2 billion and the cost of insecticides used to control these pests estimates over US$1 billion annually (Reed and Pawar 1982). In India, *H armigera* reduced yields in a variety of crops by 20-30 per cent, occasionally reaching 75 per cent in chickpea (Rahman 1989) and 70-95 per cent in other crops (Prakash et al 2007). According to reports, 35-38 per cent of cotton is lost in Tamil Nadu and Karnataka. In India, insecticides cost 28,800 billion rupees a year, of which half are used on cotton alone (Rai et al 2009). The physiological, ethological and ecological features of *H armigera*, such as its high polyphagy, wide geographic range, mobility, migrating potential, facultative diapause, substantial fecundity and propensity to develop insecticide resistance, have significantly contributed to its pest status and enable it to support a variety of cropping systems. The vast majority of insecticide-resistant instances that were documented globally in the 1990s, developed resistance

to carbamates, pyrethroids and organophosphates, but more recently to toxins generated from *Bacillus thuringiensis*. However, widespread pesticide resistance is emerging in *H armigera* in India as a result of the intensive use of chemicals. According to Gunning et al (1984), Armes et al (1994) and McCaffery (1998), pests have been put through profound selection pressure and resistance to the majority of chemical families of insecticides has been observed. These consist of carbamates (methomyl, thiodicarb and carbaryl), organophosphates (monocrotophos, quinalphos and phoxim and to a lesser extent profenofos, methyl parathion, phosalone and chlorpyrifos) and especially pyrethroids (permethrin, fenvalerate, cypermethrin, deltamethrin and lambda-cyhalothrin). One of the primary causes of the rapid development of resistance is an excessive reliance on a certain class of compounds.

Containing *H armigera* has become crucial in many parts of the world after the development of insecticide resistance (Tabashnik et al 2014). Subject to the host crop and growing conditions, the resistance varies according to the patterns of insecticide usage (Kranthi et al 2001, Chaturvedi 2013, Hussain et al 2014, Ballari and Udikeri 2018). Different *H amigera* host crops in Karnataka, are primarily subject to varying agroclimatic conditions, which include varying soil types, patterns of precipitation and irrigation facilities. Thus optimum circumstances, with varying levels of resistance to every pest, prevail in Karnataka (Table 1). Therefore, to learn about the latest visibility of insecticide resistance to *H armigera* due to changes in pesticide usage patterns complying with the introduction of *Bt* cotton and an influential pest outbreak, as well as the inadequate comprehension of resistance in major cropping systems for *Helicoverpa* in different agro-ecological zones of Karnataka, this exercise has been undertaken.

MATERIAL and METHODS

The experiment was carried out in the laboratory at the Agriculture Research Station, Dharwad (Hebballi) farm of the University of Agricultural Sciences, Dharwad, Karnataka in 2017 to assess the situation of *H armigera* resistance in several agroclimatic zones in the state. *H armigera* larvae were collected from different locations of Karnataka (Fig 1), displaying different cropping patterns and agro-ecosystems in 2017.

Collection and rearing of *H armigera* populations: *H armigera* larvae were collected from various regions in Karnataka viz Raichur and Kalaburgi of northeastern dry zone, Vijayapur and Gadag of northern dry zone, Dharwad, Haveri and Belagavi of north transition zone, Shivamogga from southern transition zone and Kolar representing eastern dry zone, to have diverse populations belonging to different climatic conditions and cropping patterns of the state (Fig 1, Table 3). These populations were designated strain codes based on their locations and subsequently reared on an artificial diet for the next generations (F1) for bioassays.

Rearing *H armigera* larvae strains was done on semi-synthetic diet following procedures prescribed by Kranthi (2005) and Ahmad et al (2003). Neonates were transferred to plastic containers having artificial diet. Before reaching the second instar, the larvae were transferred to multi-well rearing (25 wells) trays and maintained until pupation. The larvae were moved to trays with fresh diet every third day.

Pupae from field-collected larvae were surface-sterilized with 0.5 to 1 per cent sodium hypochlorite, rinsed with distilled water, dried with tissue paper and air-dried briefly. A wooden cage (9.2" × 9.2" × 9.3"), featuring a glass front and three mesh sides, was used to house pupae for adult emergence. Pupae were placed in bread boxes containing a 1:1 mixture of moist sand and sawdust. Emerging adults were given an adult diet and one or two male-female pairs were then transferred to an egg cage for mating and oviposition. Emerging adults were fed a diet prepared by mixing 5 g each of sucrose and honey into 90 ml of sterile water and then boiling the solution for five minutes. After the mixture cooled completely, 0.2 g each of ascorbic acid and methyl hydroxy para benzoate were added and it was then stored at 4.0°C for 1-2 weeks (Kranthi 2005). After being soaked in the adult diet solution, sterile absorbent cotton swabs were put in an adult feeding cage which was changed on an alternate day. A fine black muslin cloth was draped over the cage. A camel hair brush was used to extricate the eggs off the muslin cloth and they were then dipped in a surface sterile solution of 0.1 per cent sodium hypochloride. For hatching, these eggs were put in little plastic jars. A photoperiod of 14D: 10L, a temperature of 27±1°C, and a relative humidity of 65±5 per cent were all maintained. Sterile absorbent cotton swabs, soaked in the adult diet solution, were placed in

the adult feeding cage and changed every other day. A fine black muslin cloth was draped over the cage collected eggs. Eggs were then carefully removed from the muslin cloth using a camel hair brush and surface-sterilized in a 0.1 per cent sodium hypochlorite solution. For hatching, these eggs were transferred to small plastic jars. A photoperiod of 14:10 (dark:light), a temperature of 27±1°C and a relative humidity of 65±5 per cent were maintained.

F_1 population maintenance: Larvae from each population were reared in groups (first instar only) in large plastic containers with mesh tops, feeding on an artificial diet. Additional larvae were individually housed in 24-well trays and maintained on the artificial diet until they reached the third instar, at which point bioassays were conducted.

Susceptible strain: A laboratory strain of *H armigera,* susceptible to insecticides, was obtained as gratis from Division of Entomology, Indian Agricultural Research Institute, New Delhi. This strain was maintained separately on the artificial diet as previously described.

Determination of insecticide resistance in *H armigera*

Test insecticides: Table 2 lists the pesticides and formulations utilized in the study. In 2017, all pesticides were purchased as commercial formulations available in the market. After precise weighing, appropriate quantities of the insecticides were dissolved in double-distilled water to create the desired test concentrations. These solutions were then refrigerated for later use. For probit, log-dose mortality analysis, those concentrations were used that caused between 10 and 90 per cent mortality. Five test concentrations were determined through preliminary pilot trials.

Bioassays: To assess insecticide resistance in various field strains of *H armigera* and a susceptible strain, the leaf disc dipping method was used, as suggested for specific insecticides. Five cm diameter discs were prepared from the center of fully expanded DCH-32 non-*Bt* cotton leaves, which were grown separately under insecticide-free conditions. These discs were dipped for 10 seconds in different pesticide concentrations and then air-dried for 30 minutes (Dastjerdi et al 2008). To prevent desiccation, the leaf discs were placed in plastic Petri dishes lined with damp filter paper. Each pesticide concentration was tested on ten third instar larvae (F1 generation), averaging 30±3.0 mg in weight, with the assay replicated four times. Control treatments involved leaf discs dipped in distilled water.

Data collection: Larval mortality was tracked at 24, 48 and 72 hours following treatment. The mortality percentage at 72 hours post-treatment was taken into account for estimating the toxicity of the test insecticides (Fisk and Wright 1992). Mortality instances were determined through the failure of insects to move after coordinated prodding with fine forceps.

Data analysis and interpretation of resistance levels: Larval mortality percentage at 72 hours post-treatment was used to assess the toxicity of test insecticides (Fisk and Wright 1992). The results were presented as per cent mortality using Abbott's formula. The lethal concentration (LC_{50}) data was calculated by applying probit analysis tools (Finney 1971). Statistical analysis was performed using the SPSS statistical computer programme. Furthermore, resistance ratios were assessed for each insecticide and strain using the formula provided below:

$$\text{Resistance ratio} = \frac{LC_{50} \text{ of field strain}}{LC_{50} \text{ of susceptible strain}} \times 100$$

Morphometric parameters of *H armigera* strains: Morphometric distinctions among larvae, pupae and adults of several *H armigera* strains were observed in the F1 population. To analyze larval morphometric features, the length and weight of 50 larvae per strain were recorded. The general body colours of the larval stage were noted through visual observation. Fifth instar larval length was measured on a centimeter scale and their weight was recorded using a microbalance. For pupal morphometrics, 20 *H armigera* pupae were randomly selected from each location and their weight and length were noted. Pupal length (mm) and weight (mg) were measured using a centimeter scale and microbalance methods respectively (Fakrudin et al 2007).

Information on insecticide usage pattern and selection pressure against *H armigera*: A standardized schedule was used to interview farmers, gathering information on insecticide usage patterns within each cropping system and location. Discussions encompassed the frequency, doses and exposure levels to various pesticide types targeting *H armigera*,

Table 1. Rainfall, crop dominance, type of soil and usage of insecticides in agro-climatic zones of Karnataka

Agro-climatic zone	Annual rainfall (mm)	Soil type	Principal crops	Insecticide usage level
Northeastern transition zone	830-890	Major – Clay, Minor – Laterite	Pulses, jowar, oilseeds, bajra, cotton, sugarcane	High
Northeastern dry zone	633.2-806.6	Major – Deep black clay Minor – Medium black	Rabi jowar, bajra, pulses, oilseeds	High
Northern dry zone	464.5-785.7	Major – Shallow to deep black clay	Rabi jowar, maize, bajra, groundnut, cotton, wheat, sugarcane, tobacco	Moderate
Central dry zone	453.5-717.7	Major – Red sandy loam Minor – Shallow to deep black	Ragi, jowar, pulses, oilseeds	Low
Eastern dry zone	679.1-888.9	Major – Red loamy Minor – Lateritic	Ragi, rice, pulses, maize, oilseeds	Moderate
Southern dry zone	670.6-888.6	Major – Red sandy loam Minor – Red loamy	Rice, ragi, pulses, jowar, tobacco	Moderate
Southern transition zone	611.7-1053.9	Major – Red sandy loam Minor – Red loamy	Rice, ragi, pulses, jowar, tobacco	Low
Northern transition zone	619.4-1303.2	Black clay and red sandy loam	Rice, jowar, groundnut, pulses, sugarcane, tobacco	Low to moderate
Hill zone	904.4-3695.1	-	Rice, pulses	Low
Coastal zone	3010.9-4694.4	Red lateritic, coastal alluvial, black clay and red sandy loam	Rice, pulses, sugarcane	Low

(Ramachandra and Kamakshi 2005)

Table 2. Insecticides used for the determination of resistance in *H armigera*

Group	Chemical	Formulation	Trade name	Manufacturer
Insect growth regulator	Novaluron	10 EC	Rimon	Indofil Industries Limited
Spinosyn group	Spinasad	45 SC	Tracer	Dow AgroSciences
Oxadiazine group	Indoxacarb	14.8 SC	Avant	DuPont India Private Limited
Benzene dicarboxamide	Flubendiamide	480 SC	Fame	Bayer CropScience
Diamide	Rynaxypyr	20 SC	Coragen	DuPont India Private Limited

Table 3. *H armigera* collected from different locations

Location	Host	Date of collection	Geographic location
Haveri	Chilli, cotton, maize	July 2017	14°49’44.9" N 75°33’55.0" E
Vijayapura	Cotton, pigeonpea	August 2017	16°45’58.4" N 75°44’41.0" E
Dharwad	Cotton, chickpea	June 2017	16°15’59.5" N 74°52’32.4" E
Belagavi	Cotton, chilli, sorghum	August 2017	15°45’59.9" N 74°37’38.4" E
Shivamogga	Maize, chilli	October 2017	13°58’32.8" N 75°34’34.6" E
Kolar	Tomato	November 2017	13°20’07.6" N 78°04’57.1" E
Raichur	Pigeonpea, cotton, chickpea	September 2017	16°12’15.7" N 77°20’03.3" E
Kalaburgi	Pigeonpea, cotton, chickpea	September 2017	17°21’39.1" N 76°48’52.7" E
Gadag	Chickpea, cotton	November 2017	15°25’30.47" N 75°26’0.60" E

including refugia in *Bt* cotton. To obtain data on common pest management techniques, 50 farmers were selected for consultation from each district representing a specific region. Thus a total of 450 farmers were contacted during the survey. Data were collected using a yes-or-no questionnaire, designed around specific factors that could infer resistance to tested pesticides in various Karnataka sites. The gathered information was sorted, materialized and organized into an MS-Excel master worksheet. To interpret cross-resistance spectra among the tested insecticides, pair-wise correlation coefficients of LC_{50} values of the common populations for each insecticide were calculated using R Studio software (Ahmad et al 2006). Graphical interpretations were also based on the R programme.

RESULTS

IGR and newer insecticides resistance in field populations of *H armigera*

IGR (novaluron) resistance: With an LC_{50} value of 18.07 ppm, the RCR strain displayed greatest resistance, followed by the KBG (16.25 ppm) and HVR (13.42 ppm) strains. KLR had the lowest level of resistance (10.86 ppm). Accordingly, the KLR (1.17 fold) strain in novaluron had the lowest resistance ratio, whereas, the RCR (1.95 fold), KBG (1.75 fold) and HVR (1.45 fold) strains had the highest resistance ratio (Table 4).

Newer insecticides: The maximal resistance level was found with 6.96 ppm lethal concentration in RCR followed by KBG strain (6.7 ppm) to indoxacarb with 1.27 fold and 1.22 fold resistance levels respectively. Similarly, for rynaxypyr, 5.38 and 5.34 ppm resistance was noticed in RCR and KBG strains with resistance ratio of 1.09 and 1.08 fold respectively. For spinosad, KBG strain had 10.19 ppm LC_{50} followed by RCR with 9.90 ppm lethal concentration. The maximal lethal concentration of flubendiamide was tracked in the RCR strain at 3.82 ppm, showing a 1.07 fold resistance ratio, closely followed by the HVR strain with a 3.77 ppm lethal concentration and 1.06 fold resistance ratio.

The KLR strain for indoxacarb had a lethal concentration of 6.09 ppm and a resistance ratio of 1.11 fold, while the KLR strain for rynaxypyr had a resistance level of 5.01 ppm and a resistance ratio of 1.01 fold. Similarly, for flubendiamide, significant subordinate resistance was observed in HVR, SMG and GDG strains (Table 4).

Data in Table 5 depict that all the tested insecticides showed a positive correlation in pair-wise comparisons of their log LC_{50} values within the same populations. Specifically, only indoxacarb and rynaxypyr exhibited a significant positive correlation (0.701).

Variations in morphometric parameters of insecticide resistant and susceptible strains of *H armigera*

Table 6 shows that in laboratory susceptible strain, larval length ranged 1.85-2.50 cm with a mean of 2.48±0.41 cm and larval weight varied 0.395-0.500 g with a mean of 0.423±0.15 g. Likewise, pupal length and weight ranged 1.30-1.85 cm and 0.17-0.300 g with a mean of 1.58±0.14 cm and 0.225±0.03 g. RCR strain (Raichur) thrived in all morphometric criteria. The average larval length (2.00-3.25 cm and 2.75±0.48 cm), weight (0.460-0.600 g and 0.511±0.04 g), pupal length (1.50-2.00 cm and 1.76±0.18 cm) and weight (0.222-0.391 g and 0.309±0.05 g) were noted.

The KBG strain from Kalaburgi had the highest range and mean of larval length (2.00-3.20 cm and 2.71±0.42 cm), larval weight (0.470-0.590 g and 0.462±0.17 g), pupal length (1.50-2.00 cm and 1.72±0.20 cm) and pupal weight (0.235-0.383 g and 0.286±0.05 g) respectively.

Apart from the susceptible strain, the minimal morphometric dimensions were marked out in SMG strain with range and mean larval length (1.90-2.95 cm and 2.52±0.49 cm) larval weight (0.400-0.520 g and 0.454±0.04 g), pupal length (1.35-1.90 cm and 1.62±0.16 cm) and pupal weight (0.185-0.302 g and 0.237±0.03 g). Further, KLR strain (Kolar) appeared to be next in order with a range of larval length and weight (1.90-2.95 cm and 0.400-0.550 g) and mean larval length and weight (2.58±0.47 cm and 0.462±0.06 g) and also a range of pupal length and weight (1.35-1.90 cm and 0.183-0.301 g) and mean of pupal length and weight (1.63±0.15 cm and 0.239±0.03 g).

The remaining *H armigera* strains showed no significant disparity, falling within the previously defined superior and inferior groups. Notably, morphometric parameters of the two insecticide groups (IGR, specifically novaluron and newer insecticides)

Table 4. Toxicity of insect growth regulator, *Bacillus thuringiensis kurstaki* and newer insecticides against different strains of *Helicoverpa armigera* from various localities in Karnataka during 2017-18

Chemical	Strain	LC_{50} (ppm)	FL 95%	LC_{90} (ppm)	Slope±SE	RR
Novaluron	SUS-L	9.27	6.4-12.02	48.26	1.78±0.28	-
	HVR	13.42	9.05-18.31	114.98	1.37±0.23	1.45
	VJP	13.02	8.65-17.87	115.89	1.35±0.23	1.40
	DWD	13.25	8.74-18.30	124.34	1.31±0.23	1.43
	BLG	12.85	8.33-17.84	125.52	1.29±0.23	1.39
	SMG	11.75	7.82-15.95	92.77	1.42±0.24	1.27
	KLR	10.86	7.05-14.84	87.3	1.41±0.23	1.17
	RCR	18.07	13.28-24.2	127.57	1.51±0.24	1.95
	KBG	16.25	11.95-21.44	105.68	1.57±0.24	1.75
	GDG	12.62	8.25-17.42	116.8	1.32±0.23	1.36
Spinosad	SUS-L	9.03	6.51-11.46	42.41	1.9±0.30	-
	HVR	9.59	6.44-12.73	64.28	1.55±0.27	1.06
	VJP	9.39	2.39-16.56	52.02	1.72±0.27	1.04
	DWD	9.78	6.58-12.98	66.35	1.54±0.27	1.08
	BLG	9.56	6.7-12.41	53.58	1.71±0.27	1.06
	SMG	9.67	6.72-12.62	56.73	1.66±0.27	1.07
	KLR	9.85	6.86-12.86	58.45	1.65±0.27	1.09
	RCR	9.9	6.6-13.22	70.76	1.50±0.26	1.1
	KBG	10.19	6.91-13.53	70.45	1.52±0.26	1.13
	GDG	9.15	6.36-11.91	50.95	1.71±0.27	1.01
Indoxacarb	SUS-L	5.64	3.41-8.11	57.06	1.27±0.19	-
	HVR	6.14	3.7-8.90	69.76	1.18±0.19	1.12
	VJP	6.31	3.88-9.01	65.48	1.26±0.19	1.15
	DWD	6.58	3.95-9.60	81.93	1.17±0.19	1.20
	BLG	6.63	4.02-9.62	79.51	1.18±0.19	1.21
	SMG	5.94	3.53-8.64	68.75	1.2±0.19	1.08
	KLR	6.09	3.63-8.87	71.76	1.19±0.19	1.11
	RCR	6.96	4.07-10.35	103.83	1.09±0.18	1.27
	KBG	6.70	3.99-9.83	88.59	1.14±0.19	1.22
	GDG	6.51	3.99-9.4	73.77	1.21±0.19	1.18
Flubendiamide	SUS-L	3.57	2.60-4.47	15.17	2.04±0.33	-
	HVR	3.77	2.82-4.65	14.92	2.14±0.33	1.06
	VJP	3.64	2.60-4.59	16.66	1.93±0.32	1.02
	DWD	3.74	2.73-4.68	16.44	1.99±0.33	1.05
	BLG	3.72	2.69-4.67	16.74	1.96±0.30	1.04
	SMG	3.63	2.65-4.54	15.51	2.03±0.33	1.02
	KLR	3.66	2.64-4.6	16.37	1.97±0.33	1.03
	RCR	3.82	2.82-4.77	16.51	2.01±0.33	1.07
	KBG	3.66	2.64-4.6	16.37	1.97±0.33	1.03
	GDG	3.65	2.68-4.55	15.25	2.06±0.33	1.02
Rynaxypyr	SUS-L	4.95	2.97-7.11	51.19	1.26±0.20	-
	HVR	5.26	3.12-7.64	61.12	1.2±0.20	1.06
	VJP	5.13	3.13-7.32	51.69	1.27±0.20	1.04
	DWD	5.31	3.28-7.53	52.18	1.29±0.20	1.07
	BLG	5.23	3.17-7.5	54.97	1.25±0.20	1.06
	SMG	5.19	3.12-7.47	56.15	1.24±0.20	1.05
	KLR	5.01	2.96-7.26	55.66	1.22±0.22	1.01
	RCR	5.38	3.29-7.69	56.62	1.25±0.20	1.09
	KBG	5.34	3.33-7.55	51.14	1.3±0.20	1.08
	GDG	5.16	3.18-7.34	50.65	1.29±0.20	1.04

SUS-L: Susceptible strain, HVR: Haveri, VJP: Vijayapura, DWD: Dharwad, BLG: Belagavi, SMG: Shivamogga: KLR: Kolar, RCR: Raichur, KBG: Kalaburgi, GDG: Gadag, RR: Resistance ratio

Table 5. Pair-wise correlation of insecticide toxicity (LC_{50}) against *H armigera* field populations

Insecticide	Novaluron	Spinasad	Indoxacarb	Flubendiamide	Rynaxypyr
Novaluron	1				
Spinasad	0.488	1			
Indoxacarb	0.805	0.266	1		
Flubendiamide	0.598	0.242	0.533	1	
Rynaxypyr	0.847	0.440	0.701*	0.648	1

*Correlation significant at 5% LoS (2-tailed) and correlation coefficient values (r)

Table 6. Geographic variation in larval and pupal morphometric traits of *H armigera* populations in Karnataka and their correlation with insecticide susceptibility

Strain	Larval length (cm)		Larval weight (g)		Pupal length (cm)		Pupal weight (g)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
SUS-L	1.85-2.50	2.48±0.41	0.395-0.500	0.423±0.15	1.30-1.85	1.58±0.14	0.170-0.300	0.225±0.03
HVR	1.90-3.10	2.66±0.38	0.410-0.580	0.501±0.06	1.40-1.95	1.64±0.19	0.201-0.352	0.271±0.05
VJP	2.00-3.00	2.61±0.39	0.410-0.570	0.479±0.06	1.40-1.95	1.64±0.19	0.181-0.350	0.268±0.05
DWD	1.90-3.00	2.56±0.41	0.410-0.570	0.479±0.06	1.40-1.90	1.63±0.18	0.180-0.320	0.254±0.04
BLG	1.90-3.00	2.62±0.50	0.410-0.570	0.479±0.06	1.40-1.90	1.63±0.16	0.182-0.321	0.252±0.04
SMG	1.90-2.95	2.52±0.49	0.400-0.520	0.454±0.04	1.35-1.90	1.62±0.16	0.185-0.302	0.237±0.03
KLR	1.90-2.95	2.58±0.47	0.400-0.550	0.462±0.06	1.35-1.90	1.63±0.15	0.183-0.301	0.239±0.03
RCR	2.00-3.25	2.75±0.48	0.460-0.600	0.511±0.04	1.50-2.00	1.76±0.18	0.222-0.391	0.309±0.05
KBG	2.00-3.20	2.71±0.42	0.470-0.590	0.469±0.17	1.50-2.00	1.72±0.20	0.235-0.383	0.286±0.05
GDG	2.00-3.10	2.66±0.45	0.400-0.585	0.487±0.06	1.50-1.95	1.63± 0.15	0.201-0.352	0.271±0.05
SEm	-	0.01	-	0.02	-	0.01	-	0.01
$CD_{0.05}$	-	0.03	-	0.06	-	0.03	-	0.03
CV (%)	-	3.60	-	1.27	-	2.25	-	1.23

Relationship between morphometric parameters and insecticide resistance (pair-wise correlation)

	Larval length	Larval weight	Pupal length	Pupal weight
Insect growth regulator	0.781*	0.502	0.951	0.488
Newer insecticides	0.681*	0.359	0.839	0.397

SUS-L: Susceptible strain, HVR: Haveri, VJP: Vijayapura, DWD: Dharwad, BLG: Belagavi, SMG: Shivamogga: KLR: Kolar, RCR: Raichur, KBG: Kalaburgi, GDG: Gadag; Superscripted letters in a column with similar case are not significantly different (Tukey's HSD test, Alpha = 0.05), *Correlation is significant at the 0.05 level (2-tailed) and correlation coefficient values (r)

correlated positively. Within both insecticide groups, larval length, larval weight, pupal length and pupal weight all demonstrated a significant positive correlation (0.781 and 0.681 respectively).

Pesticide usage patterns and selection pressure on *H armigera* in various Karnataka locations: In 2017, across various regions of Karnataka, 31.56 per cent of interviewed farmers were aware of the recommended agrochemicals for *H armigera* control, a finding that considered their social status (Table 7). Farmers growing the main crops for the *H armigera* experiment were found across various locations, with 62.44 per cent being illiterate and 41.56 per cent having some education.

Only a small fraction of farmers (2.44%) appreciated pesticide classification based on toxicity, though 75.11 per cent were familiar with pesticide mixtures. A mere 18.22 per cent of farmers applied pesticides at recommended dosages, with the vast majority (81.78%) using higher than recommended

Table 7. Usage pattern of insecticides to manage *H armigera* in Karnataka

Component	Respondents (n = 450)			
	Frequency		Percentage	
	Yes	No	Yes	No
Level of education of farmers				
Illiterate	281	169	62.44	37.56
Literate	187	263	41.56	58.44
Awareness about recommended pesticides against *H armigera*	142	308	31.56	68.44
Awareness about the pesticide classification based on toxicity	11	439	2.44	97.56
Whether pesticide mixtures used	338	112	75.11	24.89
Whether the adequate quantity of pesticides used	41	409	9.11	90.89
Recommended dose	82	368	18.22	81.78
More than recommended dose (double or more than double dose)	368	82	81.78	18.22
Whether high pesticide dose gives higher yield	424	26	94.22	5.78
How to measure the chemical				
Bottle cap	150	300	33.33	66.67
Approximation	300	150	66.67	33.33
Whether consecutive applications of products from the same chemical group used	319	131	70.89	29.11
Whether pest levels monitored	71	379	15.78	84.22
Whether NPV/*Bt* used	82	368	18.22	81.78
Refugia in *Bt* cotton	105	345	23.33	76.67
Contact person for pesticide recommendations				
Agricultural officer	56	394	12.44	87.56
Dealer	386	64	85.78	14.22
Scientist	8	442	1.78	98.22
Types of pesticides used by farmers				
Novaluron 10 EC	133	317	29.56	70.44
Spinosad 45 SC	94	356	20.89	79.11
Indoxacarb 14.8 SC	61	389	13.56	86.44
Flubendiamide 480 SC	75	375	16.67	83.33
Rynaxypyr 20 SC	124	326	27.56	72.44
Emamectin benzoate 5SG	94	356	20.89	79.11
Conventional insecticides	244	206	54.22	45.78
Bacillus thuringiensis kurstaki	44	406	9.78	90.22

doses. This was largely driven by the belief held by 94.22 per cent of farmers that higher pesticide doses lead to increased yields. When seeking pesticide recommendations, 85.78 per cent of farmers consulted agrochemical vendors, significantly more than those who approached agriculture officers (12.44%) or scientists (1.78%). The method of chemical measurement also varied, with approximately 66.67 per cent of farmers estimating dosages and 33.33 per cent using a bottle cap for measurement, likely influenced by their education levels. The survey revealed that 62.44 per cent of farmers were illiterate, while 37.56 per cent were literate, ranging from primary education to graduation.

Farmer Awareness: Compliance with resistance management advisories was low among farmers; only 29.11 per cent avoided using similar or same chemicals. Furthermore, the adoption of specific strategies was minimal as merely 15.78 per cent of farmers monitored pest levels with sex pheromone traps, 18.44 per cent utilized NPV/*Bt* and 23.33 per cent employed refugial strategies. Among the various classes of pesticides farmers used against *H armigera* (Table 7), conventional insecticides constituted the majority at 54.22 per cent. The application of newer insecticides was limited, with spinosad at 20.89 per cent, indoxacarb at 13.56 per cent, flubendiamide at 16.67 per cent, rynaxypyr at 27.56 per cent, emamectin benzoate at

20.89 per cent, IGR (novaluron) at 29.56 per cent and *Btk* formulations at 9.78 per cent.

DISCUSSION

In pest management, novel insecticides with distinct modes of action are important, yet the *H armigera* strains in this study exhibited varied responses. Among the nine locations, the RCR strain showed greater resistance, even to recent insecticides like indoxacarb (1.27 fold), flubendiamide (1.07 fold), and rynaxypyr (1.09 fold). The KBG strain demonstrated 1.13 fold resistance to spinosad.

As Fig 2 illustrates, newer molecules generally encountered less resistance in several *H armigera* strains from Karnataka compared to novaluron. The strains exhibiting higher resistance to novaluron were RCR (1.95%), KBG (1.75%) and HVR (1.45%), with KLR (1.17%) and other strains such as SMG and GDG showing the least resistance.

These investigations indicate that *H armigera* strains show low-profile resistance and variability to newer compounds. Similarly, Qayyum et al (2015) found that these novel compounds had lower lethal concentrations than organophosphates (OPs) and pyrethroids, which had higher LC_{50} values for *H armigera*. Furthermore, Kranthi et al (2000), Rao (2008), Cook et al (2005) and Gupta et al (2005) observed minor variance in sensitivity but minimal lethal dose requirements against *H armigera* compared to spinosad and indoxacarb.

Ahmad and Mehmood (2015) also reported very low resistance to growth regulators due to their novel modes of action. In India, IGR resistance is rarely addressed. The use of such newer chemicals would effectively manage pyrethroid resistance as reported by Bhatti et al (2013). Thus these insecticides could be considered highly effective and safe in *H armigera* management throughout Karnataka and in any agroclimatic region similar to the present study.

These LC_{50} results represent initial efforts to create baseline data for rynaxypyr, flubendiamide, spinosad and indoxacarb, as no such studies have been conducted previously. Although leaf disc method bioassays may not provide the optimal scale of toxicity for all insecticides used here, the procedure proved effective for those requiring ingestion for toxicity. This baseline data could help to monitor changes in susceptibility to these novel insecticides as their use becomes more widespread across various host crops in Karnataka or elsewhere with similar cropping patterns or agroclimatic conditions.

The correlation analysis of resistance patterns for several insecticides clearly shows a substantial relationship between novaluron and the newer insecticides evaluated. A significant positive association exists between rynaxypyr and indoxacarb. Interestingly, spinosad showed a sound correlation with other tested insecticides (Fig 3) and the network corrplot revealed a distinct network pathway with reduced colour intensity away from the other insecticides. Thus among newer insecticides, spinosad undoubtedly offers promise for rotation in managing insecticide resistance against *H armigera* despite many other options. Rotation of still-effective conventional chemicals with new chemicals such as emamectin benzoate and insect growth regulators (IGRs), especially novaluron, has been suggested previously (Ahmad et al 2006). However, the choice of insecticide for rotation in resistance management should have a sound experimental base as evidenced in this study.

The RCH strain of *H armigera* exhibited the best morphometric dimensions, followed by the KBG strain, which indicated the biological fitness cost associated with pesticide resistance. According to Parmar and Patel (2018), field populations of *H armigera* in Vadodara and Ahmedabad had considerably larger larval and pupal lengths and weights than susceptible strains maintained in laboratories. Morphometric correlation (Table 6) with insecticidal resistance was significantly positive for larval length in novaluron (IGR). Higher phenotypic traits, such as longer larvae, may have elevated the body's enzymatic activity, enabling larvae to withstand larger pesticide dosages or titers (Fakrudin et al 2004). Similar correlations between larval weight and cypermethrin resistance were explained by Firko and Hayes (1990) concerning the tobacco budworm, *Heliothis virescens* (F). Improved resistance predictions are possible by considering weight variation in treated populations, which supports this study. Even small-scale weight variation mediates tolerance expression and tolerance dynamics within populations. Relationships between tolerance and weight depend on the dose.

In addition to certain good agricultural practices, the use of insecticides to control *H armigera* in large cropping systems is related to resistance

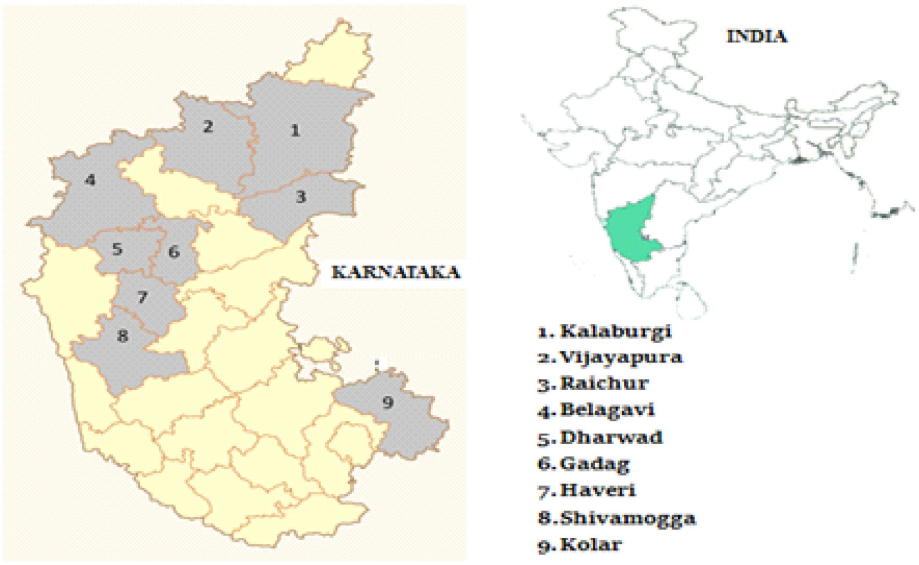

Fig 1. Sampling locations for *H armigera* resistance monitoring in Karnataka

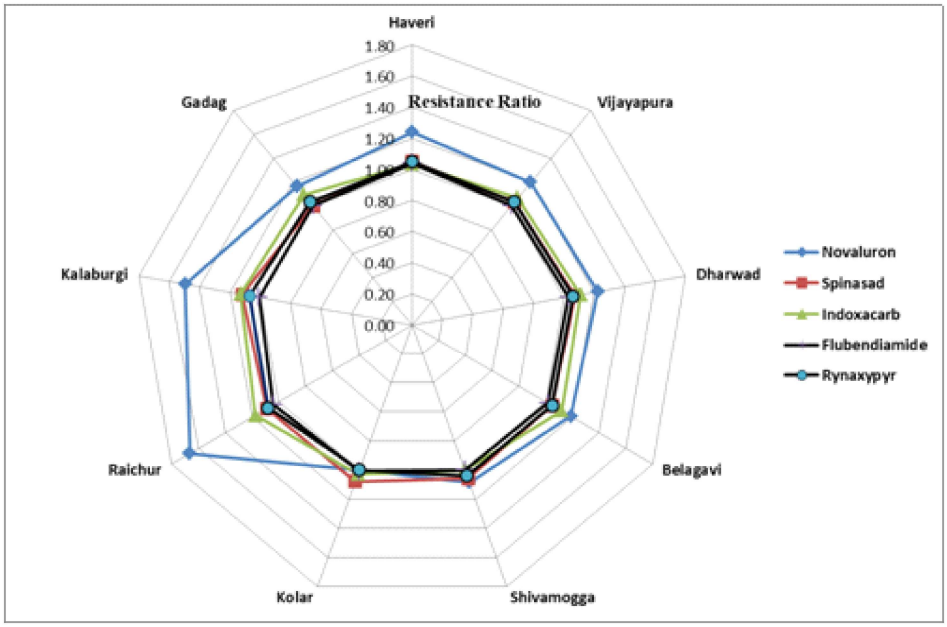

Fig 2. Insecticide resistance levels in field populations of *H armigera* from Karnataka

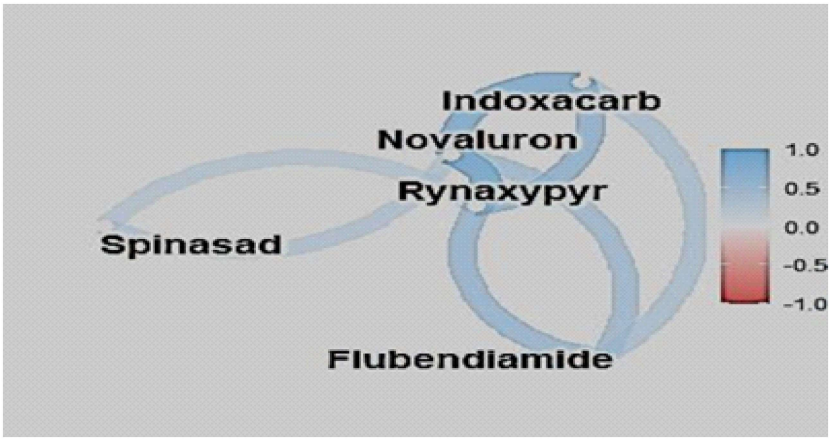

Fig 3. Network correlation plot of insecticide efficacy against *H armigera* field populations

patterns (Ballari and Udikeri 2018). The current study revealed that most farmers lacked understanding about toxicity categorization and insecticide recommendations made under the Insecticide Act. In rural regions, most farmers contacted pesticide dealers for spraying advice. Over 60 per cent of farmers have very little education, leading them to spray pesticides more frequently at double or more than twice the prescribed dosage and fail to follow directions given in insecticide leaflets or bottles, which could explain the high volume of insecticides used for spraying. Increased insecticide application for crop protection against insect pests poses critical challenges as it may accelerate widespread resistance in strains (Ranson et al 2011).

It's evident here that only 15.78 per cent of farmers monitored the pest and 18.44 per cent used the refugia method. This indicates limited resistance management compliance among most farmers, such as monitoring pest levels by maintaining sex pheromone traps, which provide clues for initiating spray against pests (Table 7). In the past, cotton seed firms offered non-*Bt* cotton and pigeonpea seeds along with *Bt* cotton in an attempt to restore susceptible strains of *Bt*-resistant insect populations. However, farmers believed that cultivating non-*Bt* seeds would not be yield-worthy. Using bioagents like NPV and *Bt* is a crucial part of IPM and IRM advisories and should not be neglected when managing resistance. This will clearly discern the pattern of resistance level found in *H armigera* from distinct agroclimatic locations of Karnataka among different groups of tested insecticides (Ballari and Udikeri 2022).

CONCLUSION

This study provides critical baseline data on the susceptibility of *Helicoverpa armigera* populations to newer insecticides and novaluron across diverse agroclimatic zones in Karnataka. While current resistance levels to these newer chemicals remain relatively low, significant variability was observed among strains, notably with the RCR strain showing higher resistance. The findings also suggest a potential link between larger insect morphometric dimensions and increased resistance, indicating possible biological fitness costs.

Crucially, the farmer survey revealed alarming practices that contribute to the escalation of insecticide resistance. A substantial majority of farmers apply pesticides at doses exceeding recommendations, rely heavily on agrochemical vendors for advice and exhibit limited adoption of fundamental resistance management strategies like pest monitoring, avoiding chemical rotations or utilizing refugia and bioagents.

In summary, the prevailing low compliance with responsible pesticide use and resistance management advisories creates a strong selection pressure that could rapidly accelerate widespread resistance in *H armigera*. Therefore, sustainable management of this polyphagous pest necessitates immediate and intensive efforts focusing on farmer education regarding judicious pesticide application, promoting the adoption of integrated pest management (IPM) and insecticide resistance management (IRM) strategies and continuous systematic monitoring of insecticide susceptibility using the baseline data established herein.

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