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Synergistic effects of insecticides and lactic acid bacterial formulation on *Plutella xylostella* (L.) and beneficial coccinellids in cauliflower

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Abstract

India, a leading producer of cauliflower, experiences up to 52 % yield loss due to defoliating caterpillars, particularly *Plutella xylostella* (L.). Epiphytic lactic acid bacteria (LAB), known for their role in plant microbiomes, are widely used in agriculture as biofertilizers, biocontrol agents and biostimulants. In this note, the present study focuses on the co-application of LAB formulation with insecticides emamectin benzoate 05 % SG, tolfenpyrad 15% EC, chlorantraniliprole 18.5 % SC on potted cauliflower plants in a screenhouse over two seasons to combat *P. xylostella* and assess the orientation behaviour of predators, particularly coccinellids. The results showed that leaf area damage by *P. xylostella* was significantly reduced in plants treated with chlorantraniliprole 18.5 % SC + LAB (5 %) (63.39-66.17 %), compared to chlorantraniliprole 18.5 % SC (60.82-63.08 %) and LAB (5 %) alone (25.45-29.05 %). Coccinellid abundance was higher in plants treated with LAB (5 %) alone (1.98-3.03 per four plants) than in those treated with insecticides and control plots. LAB density of both seasons showed a similar trend, with significantly higher values in plants treated with LAB alone (45.17 CFU/cm^2) and chlorantraniliprole 18.5 % SC (41.39 CFU/cm^2), compared to control plants (1.88 CFU/cm^2).

Keywords

coccinellids; diamond back moth; insecticides; lactic acid bacteria formulation

Introduction

India is a leading global producer of cruciferous vegetables and a primary producer of cauliflower (*Brassica oleracea* var. *botrytis)* productivity, ranking second in cabbage production worldwide. Despite the more significant cultivation, the productivity of these crops is low due to attacks by insect pests and diseases. These vegetables are ravaged by prominent defoliating caterpillars like the diamondback moth (DBM), *Plutella xylostella* (L.), leaf webber, *Crocidolomia binotalis*, Cabbage webworm, *Hellula undalis,* Cabbage butterfly, *Pieris brassicae*, Tobacco caterpillar, *Spodoptera litura*, Mustard sawfly, *Athalia lugens proxima* and sucking pests like aphids, *Brevicoryne brassicae* L. and painted bug, *Bagrada hilaris* resulting in significant losses in the yield. The diamondback moth (DBM) is the most notorious pest, often responsible for substantial yield losses. In India, 52 per cent of yield loss on cabbage is due to diamondback moth (DBM) on average and it was reported that the yield loss varied from 31 per cent to total loss (1). The overall management cost for diamondback moth is estimated at US $ 4-5 billion annually (2). The first sign of a diamondback moth infestation is skeletonization in the leaves. As the

infestation worsens, the larvae feed on the leaf, leaving only the veins, which deprives the plants of essential nutrients, hindering their development and overall productivity.

Lactic acid bacteria (LAB), traditionally used in food preservation and fermentation, have shown promising biological control agents against insect pests in agriculture. LAB, which are 'generally recognized as safe' (GRAS) bacteria, have emerged as promising allies in the fight against insect pests plaguing crops. These Gram-positive, facultative anaerobes from genera such as *Lactobacillus, Leuconostoc* and *Pediococcus* (3) produce antimicrobial compounds like lactic acid and bacteriocins. LAB disrupt pathogenic microorganisms and insect -microbe interactions, offering biocontrol potential by inhibiting pests through organic acids and antimicrobial peptides. LAB strains such as *Lactobacillus plantarum*, *L. casei* and *L. fermentum* have been extensively studied for various applications, but there is limited evidence supporting their use in controlling *P.xylostella*. LAB can induce systemic resistance in plants, enhancing their defence against pests. *L. plantarum* triggers defence responses in plants, making them less susceptible to insect attacks. LAB strains can colonize the insect gut, outcompete pests for resources and disrupt their development, effectively reducing pest populations. They have shown efficacy against various insect larvae, including caterpillars and beet armyworms and play a role in degrading pesticides (4). LAB produce bioactive compounds like organic acids and bacteriocin that deter pests and suppress pathogens development in the soil, thereby improving soil health and enhancing microbial diversity through using these bacterial cultures as fermented biocontrol sprays as an eco–friendly pest deterrent. Additionally, LAB prevents secondary infections that attract pests, which often attract pests like *P.* xylostella. Their use aligns with agroecological principles, promoting environmentally and socially responsible farming practices. Host plants treated with LAB produce semiochemicals that enhance biological control by guiding natural enemies of pests. These include volatile organic compounds (VOCs) such as methyl salicylate, which attracts predatory insects like lacewings and parasitoid wasps and indole, which signals herbivore presence to beneficial insects.

Terpenoids like limonene and β–caryophyllene serve as chemical cues for predators such as lady beetles and parasitic wasps, while green leaf volatiles (GLVs), including cis–3–hexanol, attract hoverflies and predatory mites to pest–infested plants. Additionally, the release of kairomones, triggered by LAB treatment, can draw parasitoids like *Cotesia* species to target pests such as *P. xylostella*. LAB amplifies these semiochemicals by inducing systemic changes in plant metabolism, enabling precise guidance of natural enemies to pest locations and significantly boosting biological control efforts.

Indiscriminate insecticide use in crop ecosystems necessitates sustainable measures. Applying LAB formulations can enhance the synergistic effects of insecticides, improving pest management in the crop matrix. The widely used insecticides in the cauliflower ecosystem include emamectin benzoate, chlorantraniliprole and tolfenpyrad. Emamectin, a derivative of abamectin, replaces an epi-amino-methyl group with a hydroxyl group at the 40-position, making it highly effective against crop pests and a safer alternative to toxic organophosphates. Commonly formulated as a benzoic acid salt, it poses potential endocrine risks, necessitating strict residue regulation. Chlorantraniliprole, an anthranilic diamide, targets ryanodine receptors, causing muscle paralysis in insects and is effective against lepidopterans and selective Coleoptera, Diptera and Hemiptera species. Tolfenpyrad, a pyrazolamide, inhibits mitochondrial complex I, making it effective against pests resistant to other insecticides (5, 6). Currently registered for controlling *P. xylostella* in leafy greens, it has been shown to induce chromosomal aberrations in cultured hamster cells and is classified as moderately toxic (7, 8). The prolonged use of pesticides has raised significant concerns regarding the residues present in leafy green vegetables and their impact on the environment, potentially posing threats to human health (9). Therefore, it is essential to explore alternative measures to enhance toxicity management. The application of LAB formulations emerges as a promising approach in this context. Considering this background, the present study aims to unravel bio-formulations complexity in enhancing the efficacy of crop-pest ecosystems. It seeks to explore the synergistic effects of co-applying LAB formulation with insecticides to combat insect pests while promoting predators' attraction to host plants. This approach could lead to more effective pest control strategies with increased biodiversity, healthier soil microbial communities, reduced pest resistance and a sustainable agricultural environment supporting crop productivity and ecosystem resilience of farming systems.

Materials and Methods

Plant materials

Screen house experiments were conducted in Insectary, Department of Agricultural Entomology, Tamil Nadu Agricultural University, Coimbatore, during Zaid (June 2024) and Kharif (August 2024). Seedlings of the local cauliflower variety (CFL 1522) were planted in pots containing a potting mixture made of soil, sand and farmyard manure (FYM) in a ratio of 2:1:1 and provided with recommended fertilizer application. Experimental trials of cauliflower potted plants are carried out with seven treatments with an untreated control replicated four times in a completely randomized design (CRD) (Table 1).

Insect culture

Leaves were collected from host plants during their active vegetative growth stage to rear diamondback moth (*P. xylostella*). The insect was reared on cauliflower leaves in an insect rearing cage at 27±1° C, 60-70 % RH and 10L:14D photoperiod at the Department of Agricultural Entomology, TNAU, Coimbatore, following the procedure described by Dong et al. (10) with slight modification. To avoid contamination, the insect-rearing cage is surface sterilized with 0.2 % sodium hypochlorite solution. The pupae of the DBM were collected and transferred to an oviposition cage for adult emergence and mating. A glass vial with a 10 % honey solution and fresh leaves in a conical flask was provided, with the setup covered by muslin cloth over the top of the cage. After 24-48 hours, the creamy white eggs laid on the fresh leaves by the adults were transferred to another insect-rearing cage and repeated the same procedure for further multiplication.

Table 1. List of treatments implicated in experiments with respective dosage

Treatments	Dosage (mL/L)
T_1-Emamectin Benzoate 5% SG	0.4 g/L
T_2-Tolfenpyrad 15% EC	2 mL/L
T_3- Chlorantraniliprole 18.5% SC	0.1mL/L
T_4-Emamectin Benzoate 5% SG + LAB (5%)	0.4 g/L + 5% LAB broth culture
T_5-Tolfenpyrad 15% EC + LAB (5%)	2 mL/L 5% +5% LAB broth culture
T_6-Chlorantraniliprole 18.5% SC + LAB (5%)	0.1mL/L +5% LAB broth culture
T_7- LAB (5%) alone	5% LAB broth culture
T_8- Untreated control	–

Assessment of compatibility between LAB and insecticides under in-vitro

The agar well diffusion method was employed to assess the compatibility of insecticide with LAB formulation incorporated into the agar wells. Following the preparation of the agar plates, approximately 100 µL of the LAB broth was evenly spread over the MRS medium using an L–rod. A sterilized cork borer (6 mm in diameter) was utilized to create 3-5 wells in the agar, into which various concentrations, precisely the X dose and 1.25X dose, of the insecticides emamectin benzoate, tolfenpyrad and chlorantraniliprole were introduced. After an incubation period of 24 hours, the presence/absence of the inhibition zones was recorded to evaluate the compatibility of LAB and insecticides.

Effect of LAB formulation and insecticides on pest and predators in screen house condition

The treatment of cauliflower plant includes emamectin benzoate 05 % SG, tolfenpyrad 15 % EC, chlorantraniliprole 18.5 % SC, emamectin benzoate 05 % SG + LAB (5 %), tolfenpyrad 15 % EC + LAB (5 %), chlorantraniliprole 18.5 % SC + LAB (5 %), LAB (5 %) and untreated control. The lactic acid bacterial formulation was made from different sources, including a semi-solid product of 100 g of milk powder, 1.0 kg of cane jaggery, 100 mL of one–day fermented grape juice and beaten egg in a selective fermentation process, curd and milk by performing serial dilutions (ranging from 10^4 to 10^6). This semi-solid product containing LAB was cultured in agar plates and inoculated in MRS (de Mann Rogosa Sharpe) broth after three days of incubation in petri plates (11). The cultured bacterial colonies were further studied for molecular identification from the total genomic DNA extracted in isolates using the standard protocol of cetyl hexadecyl - trimethyl ammonium bromide (CTAB) method (12) and published in NCBI to obtain accession numbers. The resulting microbial consortia of LAB in the formulation contains *Lactococcus lactis* strain LAB 1- PP474431, *L. lactis* strain LAB2-PP732186, *Lactobacillus paracasei* strain LAB3- PQ469952 and *Lactiplantibacillus plantarum* strain LAB4- PQ470018. The microbial consortia containing the LAB culture were sprayed with a hand sprayer in the potted cauliflower plants. After three days of LAB inoculation, the cultured broth is ready for immediate use at a 5% concentration (50 mL in 1L of spray fluid), which is necessary for spraying in plants. After the initial foliar spray at 35 days post-transplanting, a second foliar spray was carried out at two–week intervals. The control plants received no spray fluid. Leaf damage caused by *P. xylostella* was recorded as a percentage, with 20 larvae released per plant for all treatments, including the untreated control. Using the leaf area damage formula, observation is undertaken from 4 randomly selected plants at 7 days after spray in both seasons, season–1 and 2, following the fortnight observation in both the sprays as given in Equation 1(13).

$$\text{Leaf Area Damage } (\%) = \frac{(L_0\times0)+(L_1\times1)+(L_2\times2)+(L_3\times3)+(L_4\times4)}{T\times4}\times100 \quad \text{(Eqn. 1)}$$

where, L_0 = Number of undamaged leaves, L_1= Number of leaves with less than 10% damage, L_2 = Number of leaves with 10 -25 % damage, L_3 = Number of leaves with 25-50 % damage, L_4 = Number of leaves with more than 50% damage, T=Total number of leaves observed. The population of natural enemies, such as coccinellid beetles, was monitored on four plants at 7-day intervals following treatment (DAT).

Assessment of CFU from LAB colony

Cauliflower curd samples were collected from treated plots to conduct the leaf impression method to isolate and enumerate the LAB colony. A small piece of the cauliflower curd (1 cm^2) was pressed onto MRS agar medium. Cycloheximide was added at a concentration of 0.1 % before plating to inhibit fungal growth and prevent contamination. Additionally, calcium carbonate ($CaCO_3$) was incorporated at a concentration of 0.8 g per 100 mL to promote enhanced LAB growth (Fig. 1) (14, 15). LAB growth was evaluated 12 hours after the impression, noting differences between treated samples and untreated controls. Colony-forming units (CFUs) were manually counted and expressed as CFUs per cauliflower curd sample on the first- and second-day post-plating. The recorded CFUs from the cauliflower curd samples were tabulated and subjected to statistical analysis.

Statistical analysis

The *in vitro* experiments were analyzed using a completely randomized block design (CRBD). ANOVA was calculated for the experimental data using SPSS software, version 16.00 (SPSS Inc., USA), with means separated by the least significant difference (LSD) method. Data transformation techniques were applied: square root for coccinellid data, arc sine for *P. xylostella* data and logarithmic transformation for LAB counts. CFUs were manually counted after 24 hours to prevent slimy bacterial overgrowth.

Results and Discussion

Effect of compatibility assessment between LAB and insecticides under in-vitro

The agar well diffusion method to assess the compatibility of LAB with insecticides revealed positive interaction, as indicated by the absence of inhibition zones on agar plates for emamectin benzoate, tolfenpyrad and chlorantraniliprole at both standard (X) and elevated (1.25X) doses (Fig. 2). This suggests that the active ingredients in emamectin benzoate, tolfenpyrad and chlorantraniliprole do not exhibit antimicrobial activity against LAB or disrupt their growth. LAB inherent resilience to these chemical compounds may stem from their robust cell wall structure, enzymatic detoxification mechanisms, or lack of interaction between the insecticide mode of action and bacterial cellular processes. Consequently, this compatibility supports the potential for their co-application in integrated pest management strategies. The results are confirmed when experimented with in rice plants treated with the insecticide flubendiamide at five different doses in combination with LAB isolates (11).

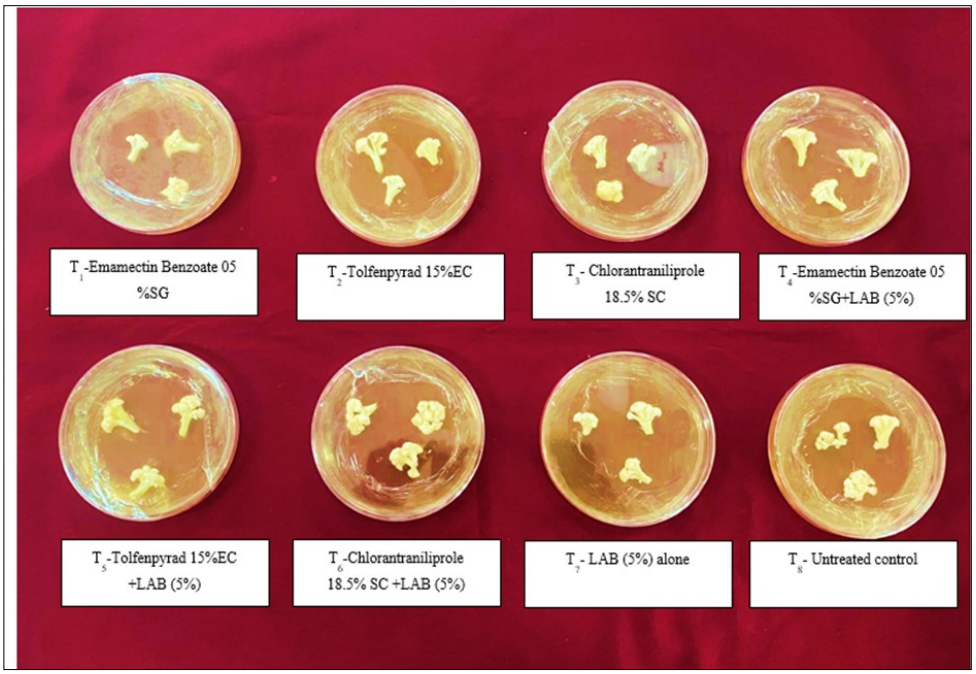

Fig. 1. LAB population density assessment in cauliflower after spraying insecticides with and without LAB formulation SG-Soluble granule. EC-Emulsifiable Concentrate. SC-Suspension concentrate. LAB-Lactic acid bacteria.

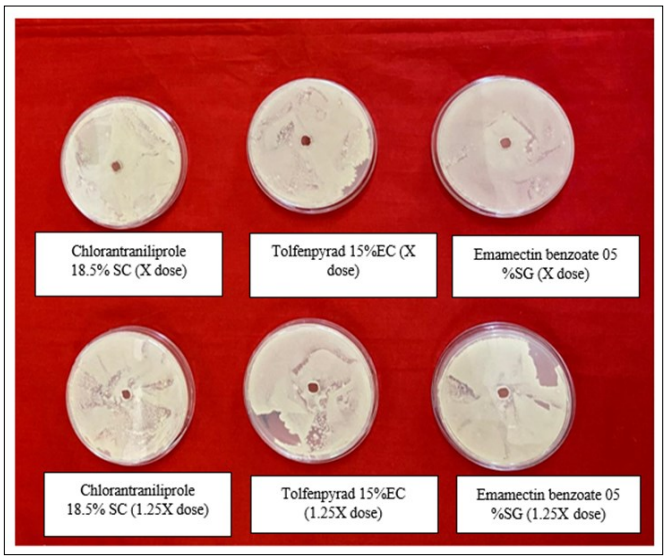

Fig. 2. Assessment of compatibility between LAB and insecticides in different *in vitro* concentrations. SG-Soluble granule. EC-Emulsifiable Concentrate. SC -Suspension concentrate. LAB-Lactic acid bacteria.

Impact of Insecticides and LAB Formulation on Leaf Area Damage Caused by P. xylostella

In both seasons, the extent of damage caused by *P. xylostella* was observed and analyzed using the leaf area damage (%) formula. The results varied significantly across treatments (Table 2-3). A pooled analysis of the two–season data showed that the damage caused by *P. xylostella* was substantially lower in all treated plots compared to the untreated control. Among the treatments, the lowest damage was observed in plants sprayed with chlorantraniliprole 18.5 % SC + LAB (5 %) (13.15 %), followed by chlorantraniliprole 18.5% SC (14.20%), tolfenpyrad 15% EC + LAB (5 %) (19.71 %), tolfenpyrad 15 % EC (23.69 %), emamectin benzoate 5 % SG + LAB (5 %) (27.14 %) and emamectin benzoate 5 % SG (31.07 %). The extent of damage in LAB-sprayed plots (35.02 %) was significantly lower than in the untreated control plots (37.27 %); however, it was higher than that observed in the other treated plots (Table 4). Although LAB

Table 2. Effect of insecticides and LAB on damage to leaves by *P.xylostella* (season-1)

Treatments	Per cent leaf damage#						Overall mean
	1st spray			2nd spray			
	7 DAS	14 DAS	Mean	7 DAS	14 DAS	Mean	
T_1–Emamectin Benzoate 5% SG	38.15 (38.14)^{b}	30.95 (33.80)^{c}	34.55 (35.99)^{c}	25.01 (30.00)^{c}	32.14 (34.53)^{d}	28.57 (32.30)^{d}	31.56 (34.17)^{c}
T_2–Tolfenpyrad 15% EC	23.91 (29.27)^{c}	32.89 (34.99)^{b}	28.40 (32.20)^{d}	22.36 (28.21)^{e}	25.01 (30.00)^{e}	23.68 (29.11)^{e}	26.04 (30.68)^{e}
T_3– Chlorantraniliprole 18.5% SC	10.01 (18.44)^{f}	13.23 (21.32)^{e}	11.62 (19.92)^{g}	15.27 (23.00)^{g}	22.50 (28.31)^{f}	18.88 (25.75)^{f}	15.25 (22.98)^{g}
T_4–Emamectin Benzoate 5% SG + LAB (5%)	23.75 (29.16)^{c}	32.50 (34.75)^{b}	28.12 (32.02)^{d}	23.61 (29.07)^{d}	36.25 (37.01)^{b}	29.93 (33.16)^{c}	29.02 (32.59)^{d}
T_5–Tolfenpyrad 15% EC + LAB (5%)	19.44 (26.15)^{d}	24.01 (29.34)^{d}	21.72 (27.77)^{e}	20.58 (26.97)^{f}	18.18 (25.23)^{g}	19.38 (26.11)^{f}	20.55 (26.95)^{f}
T_6–Chlorantraniliprole 18.5% SC + LAB (5%)	15.58 (23.46)^{e}	10.29 (18.70)^{f}	12.93 (21.07)^{f}	14.06 (22.02)^{h}	17.10 (24.42)^{h}	15.58 (23.24)^{g}	14.25 (22.17)^{h}
T_7– LAB (5%) alone	42.04 (40.41)^{a}	32.40 (34.69)^{b}	37.22 (37.59)^{b}	34.72 (36.10)^{b}	34.09 (35.72)^{c}	34.40 (35.90)^{b}	35.81 (36.75)^{b}
T_8– Untreated control	41.30 (39.98)^{a}	35.80 (36.75)^{a}	38.55 (38.38)^{a}	38.63 (38.42)^{a}	40.01 (39.23)^{a}	39.32 (38.83)^{a}	38.93 (38.60)^{a}
SE.d	0.240	0.270	0.100	0.210	0.180	0.260	0.120
CD (P=0.05)	0.520	0.580	0.220	0.450	0.380	0.560	0.270

SG-Soluble granule; EC-Emulsifiable Concentrate; SC-Suspension concentrate; LAB-Lactic acid bacteria; DAS-days after spray. Values in parentheses are *arc sine* values. #Mean of four replications

Table 3. Effect of insecticides and LAB on damage to leaves by *P. xylostella* season-1 & 2 (pooled)

Treatments	Per cent leaf damage#						Overall mean
	1st spray			2nd spray			
	7 DAS	14 DAS	Mean	7 DAS	14 DAS	Mean	
T_1–Emamectin Benzoate 5% SG	30.26 (33.37)^c	32.15 (34.54)^c	31.20 (33.95)^c	32.56 (34.79)^c	27.38 (31.51)^c	29.97 (33.19)^b	30.58 (33.57)^c
T_2–Tolfenpyrad 15% EC	19.04 (25.87)^f	26.25 (30.82)^e	22.64 (28.41)^e	22.61 (28.39)^d	17.50 (24.72)^f	20.05 (26.60)^d	21.34 (27.51)^e
T_3– Chlorantraniliprole 18.5% SC	13.23 (21.32)^g	11.90 (20.18)^h	12.56 (20.75)^g	16.25 (23.73)^f	11.25 (19.59)^h	13.75 (21.76)^f	13.15 (21.26)^g
T_4–Emamectin Benzoate 5% SG + LAB (5%)	28.14 (32.03)^d	28.75 (32.42)^d	28.44 (32.22)^d	22.61 (28.38)^d	21.59 (27.68)^d	22.10 (28.04)^c	25.27 (30.17)^d
T_5–Tolfenpyrad 15% EC + LAB (5%)	20.58 (26.97)^e	18.42 (25.41)^f	19.50 (26.20)^f	17.51 (24.73)^e	19.04 (25.86)^e	18.27 (25.30)^e	18.88 (25.75)^f
T_6–Chlorantraniliprole 18.5% SC + LAB (5%)	10.12 (18.54)^h	13.75 (21.76)^g	11.93 (20.20)^h	11.25 (19.59)^g	13.09 (21.21)^g	12.17 (20.41)^g	12.05 (20.31)^h
T_7– LAB (5%) alone	34.21 (35.79)^b	33.69 (35.48)^b	33.95 (35.48)^b	36.90 (37.40)^a	32.14 (34.53)^b	34.52 (35.98)^a	34.23 (35.80)^b
T_8– Untreated control	37.50 (37.76)^a	35.86 (36.78)^a	36.68 (36.78)^a	34.78 (36.13)^b	34.37 (35.89)^a	34.57 (35.89)^a	35.62 (36.64)^a
SE.d	0.220	0.160	0.140	0.210	0.180	0.210	0.220
CD (P=0.05)	0.470	0.340	0.310	0.450	0.390	0.460	0.480

SG-Soluble granule; EC-Emulsifiable Concentrate; SC-Suspension concentrate; LAB-Lactic acid bacteria; DAS-days after spray. Values in parentheses are *arc sine* values. #Mean of four replications

Table 4. Effect of insecticides and LAB on damage to leaves by *P. xylostella* (season-2)

Treatments	Per cent leaf damage#						Overall mean
	1st spray			2nd spray			
	7 DAS	14 DAS	Mean	7 DAS	14 DAS	Mean	
T_1–Emamectin Benzoate 5% SG	34.20 (35.78)^c	31.55 (34.17)^c	32.8 (34.98)^c	28.79 (32.44)^c	29.76 (33.05)^c	29.27 (32.75)^c	31.08 (33.88)^c
T_2–Tolfenpyrad 15% EC	21.47 (27.60)^e	29.57 (32.94)^e	25.52 (30.34)^e	22.49 (28.30)^d	21.26 (27.45)^e	21.87 (27.88)^e	23.70 (29.13)^e
T_3– Chlorantraniliprole 18.5% SC	11.62 (19.93)^h	12.56 (20.75)^g	12.09 (20.34)^g	15.76 (23.38)^f	16.88 (24.25)^g	16.32 (23.82)^g	14.20 (22.13)^g
T_4–Emamectin Benzoate 5% SG + LAB (5%)	25.94 (30.61)^d	30.62 (33.59)^d	28.28 (32.12)^d	23.11 (28.73)^d	28.92 (32.53)^d	26.02 (30.67)^d	27.15 (31.40)^d
T_5–Tolfenpyrad 15% EC + LAB (5%)	20.01 (26.57)^f	21.21 (27.42)^f	20.61 (26.99)^f	19.05 (25.87)^e	18.61 (25.55)^f	18.83 (25.71)^f	19.72 (26.36)^f
T_6–Chlorantraniliprole 18.5% SC + LAB (5%)	12.85 (21.00)^g	12.02 (20.28)^g	12.44 (20.65)^f	12.66 (20.84)^g	15.10 (22.86)^h	13.88 (21.87)^h	13.16 (36.28)^b
T_7– LAB (5%) alone	38.13 (38.12)^b	33.04 (35.08)^b	35.58 (36.61)^b	35.81 (36.75)^b	33.12 (35.13)^b	34.46 (35.94)^b	35.02 (36.28)^b
T_8– Untreated control	39.40 (38.88)^a	35.83 (26.76)^a	37.62 (37.83)^a	36.71 (37.29)^a	37.19 (37.57)^a	36.95 (37.43)^a	37.28 (37.62)^a
SE.d	0.220	0.230	0.160	0.220	0.200	0.300	0.290
CD (P=0.05)	0.480	0.490	0.340	0.470	0.420	0.640	0.620

SG-Soluble granule; EC-Emulsifiable Concentrate; SC-Suspension concentrate; LAB-Lactic acid bacteria; DAS-days after spray. Values in parentheses are *arc sine* values. #Mean of four replications

was effective when applied alone, its performance was inferior to the other three insecticide formulations, with or without LAB, which exhibited lower damage levels (34.23-35.81 %). Comparatively, the damage percentage was progressively reduced in plants treated with different insecticide formulations. The highest reduction was observed in plants sprayed with chlorantraniliprole 18.5% SC + LAB (5 %) (63.39-66.17 %), followed by chlorantraniliprole 18.5 % SC alone (60.82-63.08 %), tolfenpyrad 15 % EC + LAB (5 %) (46.98-47.21 %), tolfenpyrad 15 % EC alone (33.11-40.08 %), emamectin benzoate 5 % SG + LAB (5 %) (25.45-29.05 %) and emamectin benzoate 5 % SG alone (14.14-18.93 %) (Fig. 3). The obtained results are in corroborates with *Scirpophaga incertulas*. *Cnaphalocrocis medinalis* (the rice leaf folder) can be more effectively managed by applying flubendiamide 20 WG at 25 or 50 g a.i./ha, combined with formulated LAB ferments at 2.5 % (19). This approach is safer for egg parasitoids, *Trichogramma schoenobii* and *Telenomus* spp (12). The results following the application of cow's milk as a spray fluid, though not an insecticide, led to a reduction in sucking pests on rose plants (16). Milk is a key component of "panchakavya," a traditional pest management solution

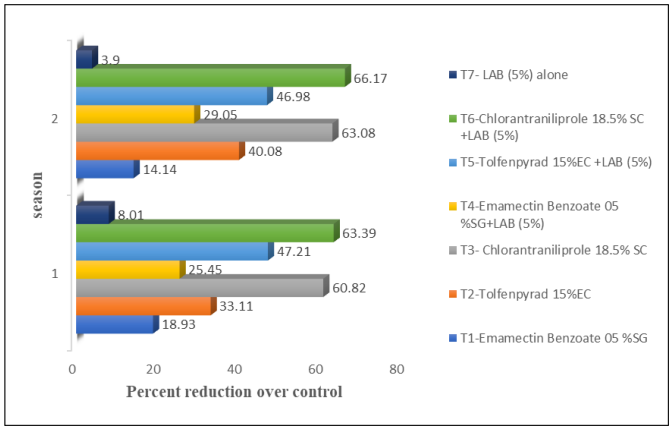

Fig. 3. Percent reduction in leaf area damage caused by *P. xylostella* (compared to the control).

fermented by a mix of microbial species (17), highlighting its potential in sustainable agricultural practices.

Effect of insecticides and LAB formulation on coccinellids

In both seasons, populations of natural enemies, particularly predatory coccinellids, were significantly lower on plants treated with chlorantraniliprole 18.5 % SC, tolfenpyrad 15 % EC and emamectin benzoate 05 % SG, both with and without LAB formulations, compared to plants sprayed solely with LAB. Across the two seasons, coccinellid abundance was lowest on plants treated with emamectin benzoate 05 % SG (0.68-0.85 coccinellids per four plants in season 1 and 1.16 -1.41 in season 2), followed by tolfenpyrad 15 % EC (1.10-1.30 in season 1 and 1.60-1.84 in season 2). In contrast, plants treated only with LAB exhibited the highest coccinellid populations (1.98 in season 1 and 3.03 in season 2), followed by chlorantraniliprole 18.5 % SC (1.87 in season 1 and 2.66 in season 2) and the control group (Table 5, 6). The declining range on percent reduction over control observed in the treatments with chlorantraniliprole 18.5% SC + LAB (5 %) and LAB alone indicates a significant increase in the coccinellid population under these treatments (Fig. 4). The pooled analysis confirmed higher coccinellid numbers in LAB-treated plants (2.33 per four plants) compared to insecticide treatments, with or without LAB and untreated control (Table 7). The results were the following reports: spraying flubendiamide 20 WG @ 25 or 50 g a.i can be more effectively managed with formulated LAB at 2.5 %. This combination is safer for natural enemies, including *Oxyopes javanus,* coccinellids and *Paederus fusipes* (18). Research on *P. xylostella* shows that plants under attack release volatile organic compounds (VOCs) as a defence mechanism. These VOCs attract natural enemies like coccinellids and are drawn to infested

Table 5. Effect of insecticides and LAB on coccinellids in cauliflower (season-1)

Treatments	No. of coccinellids per four plants#						Overall mean	PRC
	1st spray			2nd spray				
	7 DAS	14 DAS	Mean	7 DAS	14 DAS	Mean		
T_1–Emamectin Benzoate 5% SG	0.34 (0.58)^b	0.53 (0.72)^c	0.43 (0.65)^b	0.88 (0.93)^a	1.01 (1.00)^b	0.94 (0.97)^b	0.68 (0.82)^c	58.53
T_2–Tolfenpyrad 15% EC	0.95 (0.97)^f	1.02 (1.01)^f	0.98 (0.99)^f	1.10 (1.04)^f	1.36 (1.16)^f	1.23 (1.10)^f	1.10 (1.04)^f	32.92
T_3– Chlorantraniliprole 18.5% SC	1.24 (1.11)^d	1.36 (1.16)^d	1.30 (1.14)^d	1.52 (1.23)^d	1.64 (1.28)^d	1.58 (1.25)^d	1.44 (1.20)^d	12.19
T_4–Emamectin Benzoate 5% SG + LAB (5%)	0.56 (0.74)^g	0.66 (0.81)^g	0.61 (0.78)^g	0.97 (0.98)^g	1.21 (1.10)^g	1.09 (1.04)^g	0.85 (0.92)^g	48.17
T_5–Tolfenpyrad 15% EC + LAB (5%)	1.12 (1.05)^e	1.21 (1.09)^e	1.16 (1.07)^e	1.33 (1.15)^f	1.56 (1.25)^e	1.44 (1.20)^e	1.30 (1.14)^e	20.73
T_6–Chlorantraniliprole 18.5% SC + LAB (5%)	1.51 (1.22)^c	2.01 (1.41)^c	1.76 (1.32)^c	2.06 (1.43)^h	1.93 (1.39)^h	1.99 (1.41)^c	1.87 (1.36)^c	–14.02
T_7– LAB (5%) alone	1.73 (1.31)^a	2.11 (1.45)^a	1.92 (1.38)^a	1.96 (1.40)^b	2.12 (1.45)^a	2.04 (1.42)^a	1.98 (1.40)^a	–20.73
T_8– Untreated control	1.36 (1.16)^b	1.81 (1.34)^b	1.58 (1.25)^b	1.63 (1.27)^a	1.78 (1.33)^b	1.70 (1.30)^c	1.64 (1.28)^h	–
SE. d	0.005	0.007	0.005	0.010	0.010	0.007	0.007	
CD (P=0.05)	0.012	0.016	0.012	0.019	0.021	0.016	0.016	

SG-Soluble granule; EC-Emulsifiable Concentrate; SC-Suspension concentrate; LAB-Lactic acid bacteria; DAS-days after spray. Values in parentheses are *square root (x + 0.5)* transformed values. #Mean of four replications

Table 6. Effect of insecticides and LAB on coccinellids in cauliflower (season-2)

Treatments	No. of coccinellids per four plants#						Overall mean	PRC
	1st spray			2nd spray				
	7 DAS	14 DAS	Mean	7 DAS	14 DAS	Mean		
T_1–Emamectin Benzoate 5% SG	0.97 (0.98)^h	1.14 (1.07)^h	1.04 (1.02)^g	1.2 (1.10)^g	1.36 (1.16)^h	1.29 (1.13)^g	1.16 (1.07)^h	51.46
T_2–Tolfenpyrad 15% EC	1.36 (1.16)^f	1.57 (1.25)^g	1.46 (1.20)^f	1.9 (1.38)^e	1.56 (1.24)^f	1.74 (1.31)^f	1.60 (1.26)^f	33.05
T_3– Chlorantraniliprole 18.5% SC	1.92 (1.38)^d	2.14 (1.47)^d	2.02 (1.42)^d	2.3 (1.52)^d	2.37 (1.53)^d	2.34 (1.53)^d	2.18 (1.47)^d	8.78
T_4–Emamectin Benzoate 5% SG + LAB (5%)	1.17 (1.11)^g	1.74 (1.31)^f	1.43 (1.19)^f	1.3 (1.16)^f	1.45 (1.20)^g	1.40 (1.18)^f	1.41 (1.18)^g	41.04
T_5–Tolfenpyrad 15% EC + LAB (5%)	1.49 (1.22)^e	1.83 (1.35)^e	1.64 (1.29)^e	1.9 (1.40)^e	2.16 (1.12)^e	2.04 (1.42)^e	1.84 (1.35)^e	23.01
T_6–Chlorantraniliprole 18.5% SC + LAB (5%)	2.37 (1.53)^b	2.84 (1.68)^b	2.60 (1.61)^g	2.5 (1.60)^b	2.90 (1.70)^b	2.73 (1.65)^b	2.66 (1.63)^b	-11.29
T_7– LAB (5%) alone	2.69 (1.64)^a	2.94m (1.71)^a	2.81 (1.67)^a	2.8 (1.69)^a	3.25 (1.80)^a	3.05 (1.74)^a	3.03 (1.74)^a	-26.77
T_8– Untreated control	2.15 (1.46)^c	2.32 (1.52)^b	2.22 (1.49)^b	2.4 (1.57)^c	2.69 (1.64)^c	2.56 (1.60)^c	2.39 (1.54)^c	-
SE.d	0.007	0.010	0.010	0.010	0.011	0.010	0.007	
CD (P=0.05)	0.015	0.020	0.020	0.023	0.024	0.021	0.015	

SG-Soluble granule; EC-Emulsifiable Concentrate; SC-Suspension concentrate; LAB-Lactic acid bacteria; DAS-days after spray. Values in parentheses are *square root (x + 0.5)* transformed values. #Mean of four replications

Table 7. Effect of insecticides and LAB on coccinellids in cauliflower season-1 & 2 (pooled)

Treatments	No. of coccinellids per four plants#						Overall mean	PRC
	1st spray			2nd spray				
	7 DAS	14 DAS	Mean	7 DAS	14 DAS	Mean		
T_1–Emamectin Benzoate 5% SG	0.65 (0.80)^h	0.83 (0.91)^h	0.73 (0.85)^h	1.05 (1.02)^h	1.18 (1.08)^h	1.11 (1.05)^h	0.92 (0.95)^f	54.22
T_2–Tolfenpyrad 15% EC	1.15 (1.07)^f	1.29 (1.13)^a	1.28 (1.31)^f	1.51 (1.22)^e	1.46 (1.20)^f	1.48 (1.21)^f	1.35 (1.16)^e	32.83
T_3– Chlorantraniliprole 18.5% SC	1.58 (1.25)^d	1.72 (1.31)^d	1.66 (1.28)^d	1.92 (1.38)^c	2.01 (1.41)^d	1.96 (1.40)^d	1.81 (1.34)^c	9.90
T_4–Emamectin Benzoate 5% SG + LAB (5%)	0.84 (0.91)^g	1.17 (1.08)^g	1.02 (1.01)g	1.16 (1.07)^g	1.33 (1.15)^g	1.24 (1.11)^g	1.51 (1.22)^d	24.87
T_5–Tolfenpyrad 15% EC + LAB (5%)	1.30 (1.14)^e	1.45 (1.20)^e	1.40 (1.18)^e	1.65 (1.28)^d	1.85 (1.36)^e	1.74 (1.31)^e	1.32 (1.15)^e	34.32
T_6–Chlorantraniliprole 18.5% SC + LAB (5%)	1.94 (1.39)^b	2.01 (1.41)^b	2.18 (1.47)^b	2.31 (1.52)^a	2.41 (1.55)^b	2.36 (1.53)^b	2.26 (1.49)^b	–12.43
T_7– LAB (5%) alone	2.21 (1.48)^a	2.43 (1.55)^a	2.36 (1.53)^a	2.41 (1.18)^f	2.69 (1.63)^a	2.54 (1.59)^a	2.33 (1.52)^a	–15.92
T_8– Untreated control	1.74 (1.31)^c	1.95 (1.39)^c	1.90 (1.37)^c	2.03 (1.42)^b	2.23 (1.49)^c	2.13 (1.45)^c	2.01 (1.41)^b	–
SE. d	0.010	0.010	0.004	0.010	0.011	0.010	0.004	
CD (P=0.05)	0.022	0.020	0.010	0.023	0.024	0.020	0.010	

SG-Soluble granule; EC-Emulsifiable Concentrate; SC-Suspension concentrate; LAB-Lactic acid bacteria; DAS-days after spray. Values in parentheses are *square root (x + 0.5)* transformed values. #Mean of four replications

plants (19). *Heydenia. unica* are prone to attack their host in plants by producing VOCs upon infestation with the fungi genera such as Ophiostoma or closely related fungi (20).

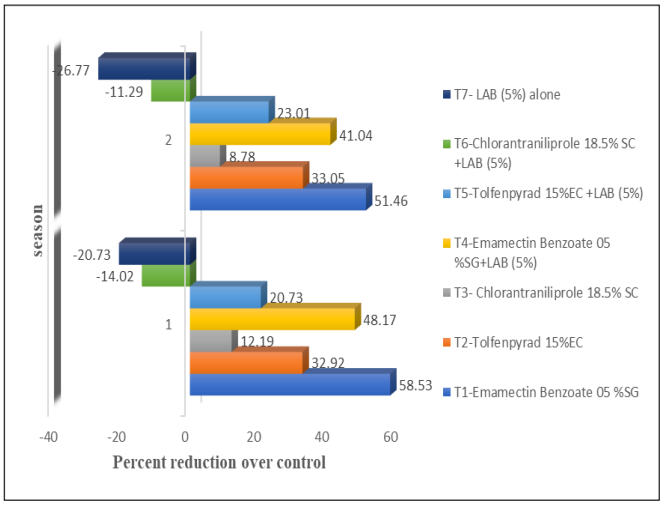

Fig. 4. Percent reduction over control in coccinellids influenced by insecticides with or without LAB.

Effect of LAB population density after spraying insecticides and LAB

The pooled data analysis from season-1 of the screen house experiment showed that the mean density of epiphytic LAB on cauliflower plants was significantly highest when sprayed with LAB alone (45.71 CFU/cm^2). This was statistically on par with the combination treatment of chlorantraniliprole 18.5% SC + LAB (5%) (41.58 CFU/cm^2), followed closely by tolfenpyrad 15 % EC + LAB (5 %) (41.39 CFU/cm^2) (Table 8). The LAB density was moderate on cauliflower plants treated with chlorantraniliprole 18.5 % SC (3.77 CFU/cm^2), tolfenpyrad 15 % EC (3.25 CFU/cm^2) and emamectin benzoate 05 % SG (3.12 CFU/cm^2), while the control plants had the lowest LAB density (1.88 CFU/cm^2). This trend was consistent after both the first and second sprays. Season-wise data also reflected similar findings, with the highest

Table 8. LAB population density after spraying insecticides and LAB in cauliflower(season-1)

Treatments	LAB density (CFU/ cm^2)#						Overall mean	% Increase over control
	1st spray			2nd spray				
	7 DAS	14 DAS	Mean	7 DAS	14 DAS	Mean		
T_1–Emamectin Benzoate 5% SG	1.58 (0.19)^g	1.32 (0.12)^g	1.45 (0.16)^g	4.35 (0.67)^f	5.36 (0.72)^f	4.85 (0.68)^f	3.15 (0.49)^f	14.28
T_2–Tolfenpyrad 15% EC	4.66 (0.66)^a	2.73 (0.43)^f	3.69 (0.56)^c	3.89 (0.59)^b	2.26 (0.35)^g	3.07 (0.48)^e	3.38 (0.52)^d	20.11
T_3– Chlorantraniliprole 18.5% SC	2.67 (0.42)^e	3.50 (0.54)^e	3.08 (0.48)^e	5.96 (0.77)^e	6.32 (0.80)^e	6.14 (0.78)^e	4.61 (0.99)^e	41.43
T_4–Emamectin Benzoate 5% SG + LAB (5%)	35.44 (1.55)^d	5.42 (0.73)^d	20.43 (1.31)^d	21.76 (1.33)^d	18.39 (1.26)^d	20.07 (1.30)^d	20.25 (1.39)^d	86.66
T_5–Tolfenpyrad 15% EC + LAB (5%)	42.34 (1.62)^c	9.37 (0.97)^c	25.85 (1.41)^c	39.67 (1.59)^c	32.32 (1.50)^c	35.99 (1.55)^c	30.92 (1.40)^c	91.26
T_6–Chlorantraniliprole 18.5% SC + LAB (5%)	57.93 (1.76)^b	20.69 (1.31)^b	39.31 (1.59)^b	59.36 (1.77)^a	49.38 (1.69)^a	54.37 (1.73)^a	42.84 (1.62)^b	94.23
T_7– LAB (5%) alone	61.38 (1.78)^a	32.54 (1.51)^a	46.96 (1.67)^a	56.46 (1.75)^b	43.21 (1.65)^b	49.83 (1.69)^b	48.39 (1.78)^a	94.44
T_8– Untreated control	2.12 (0.32)^f	2.54m (0.40)^f	2.33 (0.36)^f	3.21 (0.50)^h	2.95 (0.47)^h	3.08 (0.48)^h	2.70 (0.43)^g	–
SE. d	0.050	0.060	0.050	0.060	0.050	0.050	0.070	
CD (P=0.05)	0.120	0.110	0.120	0.110	0.120	0.120	0.140	

SG-Soluble granule; EC-Emulsifiable Concentrate; SC-Suspension concentrate; LAB-Lactic acid bacteria; DAS-days after spray. Values in parentheses are log-transformed values. #Mean of four replications. CFU-colony forming unit.

LAB densities observed on plants sprayed with LAB (5 %) alone or in combination with chlorantraniliprole 18.5 % SC + LAB (5 %) and the lowest densities recorded on control plants. Similarly, during the second season, LAB on treated samples showed significant variation following two applications of chlorantraniliprole 18.5 % SC, with or without LAB (Table 9). The pooled analysis revealed that the LAB (lactic acid bacteria) treatment achieved the highest population density at 45.71 CFU/ cm^2, closely followed by chlorantraniliprole 18.5 % SC, which recorded a population density of 41.58 CFU/cm^2. Both treatments outperformed other insecticide treatments, with or without LAB and the untreated control (Table 10). The combination of chlorantraniliprole 18.5 % SC with LAB (5 %) resulted in a much higher LAB density than when chlorantraniliprole was applied alone. The percent increase in lactic acid bacteria (LAB) population density in the LAB (5 %) treatment and the chlorantraniliprole 18.5 % SC + LAB (5 %) treatment is significantly more significant compared to all other treatments and the control (Fig. 5). The obtained results are in close agreement with the previous studies when milkoid at a 2 % concentration significantly enhanced the phyllosphere LAB population on okra plants, showing an 84.68 % increase. Additionally, it effectively reduced the incidence of yellow vein mosaic virus (YVMV) by 34.18 %, aphid (*Aphis gossypii*) infestations by 32.19 % and leafhopper (*Amrasca biguttula*) populations by 17.57 %. Although it caused only a slight reduction in insect damage, it notably reduced the toxicity of imidacloprid, likely due to biodegradation, which warrants further investigation through residue analysis (21). LAB have been shown to degrade pesticides not only in fermented food products like kimchi and skimmed milk, but also when present as epiphytes on plants (22-24). LAB and bleaching powder are likely to influence insect host-finding behaviour through microbial volatiles, which can attract or repel insects by altering their behaviour. Studies have shown that microbial volatiles are critical in shaping insect responses. For instance, Acetoin and 2,3 -butanediol volatiles, emitted by microbes, can serve as long-distance attractants for fruit flies, guiding them to their food

Table 9. LAB population density after spraying insecticides and LAB in cauliflower (season-2)

Treatments	LAB density (CFU/ cm^2)#						Overall mean	% Increase over control
	1st spray			2nd spray				
	7 DAS	14 DAS	Mean	7 DAS	14 DAS	Mean		
T_1–Emamectin Benzoate 5% SG	2.36 (0.37)^f	1.95 (0.28)^f	2.15 (0.33)^e	2.15 (0.33)^f	1.65 (0.21)^f	1.9 (0.27)^f	3.10 (0.49)^d	65.48
T_2–Tolfenpyrad 15% EC	3.68 (0.56)^a	2.83 (0.45)^e	3.25 (0.51)^b	3.22 (0.50)^b	2.75 (0.43)^e	2.98 (0.47)^d	3.11 (0.49)^c	65.59
T_3– Chlorantraniliprole 18.5% SC	3.69 (0.56)^e	2.84 (0.45)^e	3.25 (0.51)^d	3.15 (0.49)^e	2.12 (0.32)^e	2.63 (0.42)^e	2.94 (0.46)^e	63.60
T_4–Emamectin Benzoate 5% SG + LAB (5%)	29.65 (1.47)^d	7.35 (0.86)^d	18.5 (1.26)^c	19.65 (1.29)^d	6.39 (0.80)^d	13.02 (1.11)^d	15.76 (1.19)^c	93.21
T_5-Tolfenpyrad 15% EC + LAB (5%)	40.45 (1.59)^c	8.45 (0.86)^c	24.85 (1.38)^b	34.65 (1.54)^c	8.65 (0.93)^c	21.65 (1.33)^c	23.05 (1.34)^b	95.49
T_6–Chlorantraniliprole 18.5% SC + LAB (5%)	63.45 (1.80)^b	25.86 (1.41)^a	44.65 (1.65)^a	59.37 (1.77)^b	19.36 (1.28)^b	38.36 (1.58)^b	40.32 (1.60)^a	97.45
T_7- LAB (5%) alone	72.35 (1.85)^a	15.96 (1.20)^b	44.15 (1.64)^a	63.21 (1.80)^a	20.65 (1.31)^a	41.93 (1.62)^a	43.04 (1.63)^a	97.51
T_8- Untreated control	1.12 (0.04)^h	1.05 (0.02)^h	1.08 (0.03)^g	1.10 (0.04)^h	1.03 (0.01)^h	1.06 (0.02)^h	1.07 (0.02)^g	–
SE. d	0.070	0.050	0.070	0.040	0.040	0.050	0.060	
CD (P=0.05)	0.140	0.120	0.140	0.100	0.100	0.120	0.130	

Soluble granule; EC-Emulsifiable Concentrate; SC-Suspension concentrate; LAB-Lactic acid bacteria; DAS-days after spray. Values in parentheses are log-transformed values. #Mean of four replications. CFU-colony forming unit

Table 10. LAB population density after spraying insecticides and LAB in cauliflower season -1&2 (pooled)

Treatments	LAB density (CFU/ cm^2)#						Overall mean	% Increase over control
	1st spray			2nd spray				
	7 DAS	14 DAS	Mean	7 DAS	14 DAS	Mean		
T_1–Emamectin Benzoate 05% SG	1.97 (0.29)^f	1.63 (0.21)^g	1.80 (0.25)^f	3.25 (0.51)^e	3.50 (0.54)^f	3.37 (0.52)^e	3.12 (0.48)^f	39.74
T_2–Tolfenpyrad 15% EC	4.17 (0.62)^a	2.78 (0.44)^e	3.47 (0.54)^b	3.56 (0.55)^b	2.51 (0.40)^f	3.03 (0.48)^d	3.25 (0.51)^c	42.15
T_3– Chlorantraniliprole 18.5% SC	3.18 (0.50)^e	3.17 (0.50)^e	3.16 (0.50)^e	4.5 (0.65)^d	4.22 (0.62)^e	4.38 (0.64)^d	3.77 (0.57)^e	50.13
T_4–Emamectin Benzoate 05% SG+LAB (5%)	32.54 (1.51)^d	6.38 (0.80)^d	19.46 (1.28)^d	20.70 (1.31)^c	12.39 (1.09)^d	16.54 (1.21)^c	18.01 (1.25)^d	89.56
T_5–Tolfenpyrad 15% EC +LAB (5%)	41.39 (1.61)^c	8.91 (0.95)^c	25.35 (1.40)^c	37.16 (1.57)^b	20.48 (1.31)^c	28.82 (1.46)^b	26.83 (1.42)^c	92.99
T_6–Chlorantraniliprole 18.5% SC +LAB (5%)	60.69 (1.78)^b	23.27 (1.36)^b	41.98 (1.62)^b	53.36 (1.45)^a	34.37 (1.53)^b	46.86 (1.67)^a	41.58 (1.61)^b	95.47
T_7– LAB (5%) alone	66.86 (1.82)^a	24.25 (1.38)^a	45.55 (1.65)^a	59.83 (1.77)^a	31.93 (1.50)^a	45.88 (1.66)^a	45.71 (1.66)^a	95.88
T_8– Untreated control	1.62 (0.20)^g	1.79 (0.25)^f	1.70 (0.23)^g	2.15 (0.33)^g	1.99 (0.29)^h	2.07 (0.31)^g	1.88 (0.27)^h	–
SE. d	0.070	0.040	0.070	0.040	0.040	0.050	0.060	
CD (P=0.05)	0.140	0.100	0.140	0.100	0.100	0.120	0.130	

SG-Soluble granule; EC-Emulsifiable Concentrate; SC-Suspension concentrate; LAB-Lactic acid bacteria; DAS-days after spray. Values in parentheses are log-transformed values. #Mean of four replications. CFU-colony forming unit.

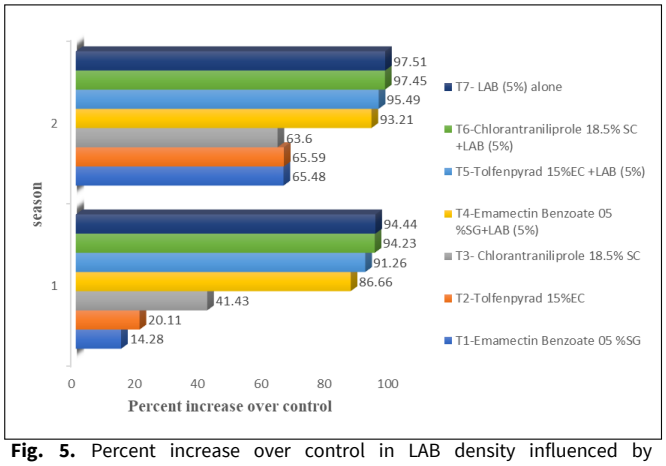

Fig. 5. Percent increase over control in LAB density influenced by insecticides with or without LAB.

sources (25, 26). This highlights the significance of microbial emissions in mediating insect-plan interactions and pest behaviour.

Conclusion

In conclusion, excessive insecticide use harms both the environment and human health. Sustainable strategies must be undertaken to establish environment-friendly integrated pest management in the cauliflower ecosystem. The study results demonstrate that co-applying LAB at a 5 % concentration with insecticides like chlorantraniliprole, tolfenpyrad and emamectin benzoate is adequate and compatible. Specifically, the combination of chlorantraniliprole 18.5 % SC + LAB (5 %) significantly reduced the damage caused by *P. xylostella*. Additionally, higher populations of beneficial coccinellids were attracted to plants treated with LAB alone or combined with chlorantraniliprole + LAB (5 %). Future research could explore the co -application of lactic acid bacteria (LAB) formulations with insecticides as an innovative strategy for crop protection. LAB strains, such as *L. brevis* and *L. plantarum*, produce bioactive volatiles that suppress harmful microorganisms, potentially creating a more hospitable environment for beneficial microbes while reducing reliance on chemical pesticides. Combining LAB with insecticides might mitigate pesticide residue levels through microbial degradation and enhance pest control efficacy through synergistic effects. Investigating optimal formulations, application methods and compatibility with diverse crop systems will be key to realizing the potential of this integrated approach in sustainable agriculture. Further studies on LAB-associated volatiles and metabolites in priming plants to withstand abiotic stresses like drought and salinity must be explored to pave a new way into farming. Research into microbe-induced plant volatiles (MIPVs) and herbivore-induced plant volatiles (HIPVs), mainly through inoculating LAB on plants, is essential. These studies will help to strengthen the integration of LAB formulations with insecticides, reinforcing their potential as a sustainable pest management strategy. Highlighting these research areas can contribute significantly to advancing environmentally friendly agricultural practices.

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Authors' contributions

MJ contributed to the conceptualization, methodology, data curation, visualization, and writing of the original manuscript and provided lab study resources. JTE assisted with guidance in conducting lab experiments, contributed to the methodology, corrected the conceptual aspects, edited and assisted with data analysis. AS was involved in the method, writing, resource collection, revision and editing of the overall manuscript. MK contributed to the methodology visualization and assisted in manuscript revision. RA provided guidance on bacterial species and supervised the study. RK offered supervision, contributed to the writing and revision of the manuscript and assisted with data analysis. TR assisted in writing and resource collection.

Compliance with ethical standards

Conflict of interest: The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper for the publication of this work.

Ethical issues: None

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