FARM-MADE PESTICIDES-DEGRADING LACTIC ACID BACTERIAL FORMULATION FOR PEST MANAGEMENT IN OKRA, *ABELMOSCHUS ESCULENTUS* (L.) MOENCH

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Abstract. The experiments were conducted to identify the potential lactic acid bacteria (LAB) by morphological and biochemical characterization; to screen pesticides for degradation by LAB in the laboratory and to assess the potential of LAB in residue biodegradation both in screen house and field at the Dept. of Agrl. Entomology, VOC Agricultural College and Research Institute, TNAU, Killikulam, Vallanadu, Tuticorin District, Tamil Nadu, India. In laboratory and field evaluations, a farm-made probiotic LAB formulation constituted from cane jaggery, milk powder and grape juice (*Cowine*) acted not only as an adjuvant that could be mixed with pesticide spray fluids but also as a reservoir of LAB, especially *Lactobacillus* and *Streptococcus* capable of degrading 78 per cent of insecticides in agar well diffusion method. Foliar sprays of *Cowine*, either alone or in combination with neem oil, imidacloprid 17.8 SL or profenofos 50 EC, significantly reduced the infestation of whitefly *Bemisia tabaci* (Gennadius), leafhopper *Amrasca devastans* Distant, aphids *Aphis gossypii* Glover, red spider mite *Tetranychus urticae* Koch, and shoot and fruit borer, *Earias* spp. in okra, *Abelmoschus esculentus* (L.) Moench.
Keywords: *characterization of LAB, pesticide degradation, cowine, insect pests, okra*

Introduction

Broad spectrum pesticides toxic to both pests and their natural enemies are widely used in crop protection. Overuse of such pesticides results in pest resurgence, outbreak of secondary pests, and resistance in insect populations (Khan et al., 2015), in addition to environmental pollution through residues, bound or conjugated (Quistad and Menn, 1983). Vegetables such as okra, *Abelmoschus esculentus* (L.) Moench often receive more rounds of pesticide sprays to manage pests (Rao et al., 2015), especially shoot and fruit borers (*Earias* spp.), fruit borer (*Helicoverpa armigera* Hubner) and sucking pests such as leafhopper (*Amrasca devastans* Distant), aphid (*Aphis gossypii* Glover), whitefly (*Bemisia tabaci* Gennadius), mealybug (*Phenacoccus solenopsis* Tansley) and red spider mite (*Tetranychus urticae* Koch). Although pesticides are indispensable in agriculture, their usage needs to be reduced in view of environmental pollution and health hazards (Pehkonen and Zhang, 2002; ICAR, 2015). Despite understanding the concept of integrated pest management, farmers continue to spray insecticides indiscriminately, ignorant of the toxicological principles such as maximum residual limit, waiting period, resistance, resurgence, residue, etc. (Bond et al., 2009).

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Consequently, pesticides continue to persist as residues on crops, especially on okra that receives more pesticides than others (Munawar et al., 2013). Therefore, alternative strategies like bioremediation and biodegradation are required to limit pesticide residues in situ (Aktar et al., 2009; Ghaffar et al., 2014).

Microbial degradation of pesticides is one of the components of bioremediation (Vogt and Richnow, 2014; Javaid et al., 2016) and it is a promising technology to remove pesticide residues from food and agricultural products as microorganisms use a variety of xenobiotic compounds, including pesticides, for their growth by mineralization and detoxification (Kanekar et al., 2004). A diverse group of soil bacteria, especially members of the genera *Alcaligenes, Flavobacterium, Pseudomonas* and *Rhodococcus* are able to degrade a variety of pesticides as they use pesticides as their carbon and energy sources (Aislabie and Lloyd-Jones, 1995). However, strains of lactic acid bacteria (LAB) that ferment hexoses to produce lactic acid (Rivka, 2013) also have the potential to decontaminate food stuffs by producing hydrolase enzyme degrading the pollutants as reported by Mansilla (2008) who categorized the LAB strains as biological control agents, especially bio-protective cultures. These probiotic LAB include *Lactobacillus, Leuconostoc, Pediococcus, Streptococcus, Aerococcus, Alloiococcus, Carnobacterium, Dolosigranulum, Enterococcus, Globicatella, Lactococcus, Micrococcus, Oenococcus, Tetragenococcus, Vagococcus,* and *Weissella* (Stiles and Holzapfel, 1997; Makarova et al., 2006; Khalid, 2011; Yu et al., 2020), *Lactobacilli, Carnobacteria* and some *Weissella* that are either rod-shaped or cocci. They are ubiquitous, occurring naturally in commodities such as milk and milk products (Martin et al., 2003), sugarcane juice (Sobrun et al., 2012), dried fruits (Askari et al., 2012), fruits and vegetables (Ni et al., 2015), wine (Moreno-Arribas et al., 2000), intestinal tract of animals (Hidalgo et al., 2022) and normal human gastrointestinal and vaginal flora (Raman et al., 2022). Their potential applications in agriculture as biofertilizers, biocontrol agents and biostimulants are increasingly now. LAB stimulates seed germination, increase soil fertility, aeration, mitigate various biotic stresses and neutralize toxic gases. In addition, they also control insect pests (Raman et al., 2022) on crops. LAB play a complex role in the food, agriculture and medicine sectors and has Generally Recognised as Safe (GRAS) position by the Food and Drug Administration (Bintsis, 2018). They are harmless for human and animal consumption and have become ideal for commercial development (Sadiq et al., 2019; Chen et al., 2021).

Lactic acid bacteria are gaining global attention, especially due to their role as a probiotic. They are increasingly being used as a flavoring agent and food preservative. Besides their role in food processing, lactic acid bacteria also have a significant role in degrading insecticide residues in the environment (Kiruthika et al., 2024). Recently, detoxification via microorganisms such as lactic acid bacteria and probiotics has been extensively studied and degradation of pesticides through hydrolytic enzymes has been introduced as the possible mechanism and it has been highlighted that some probiotics harbour pesticide-degrading genes (Mohammadi et al., 2021).

Sprayable formulations of LAB prepared by farmers themselves when mixed with market-purchased pesticide formulations can help reduce pesticide residues on crops. For instance, sugarcane is a good source of *Leuconostoc mesenteroides* (Sobrun et al., 2012). Milk and milk products have a score of LAB, especially *Lactobacillus acidophilus* (Rigotti et al., 2017). Species like *Oenococcus* and *Pediococcus* are rich in grape juice (Franques et al., 2017). The objective of this research was to evaluate the bio-ameliorant potential of an LAB formulation made from sugarcane jaggery, milk

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powder and grape juice that can be mixed with pesticide spray fluids as an adjuvant in pest management.

Materials and methods

The experiments were conducted to identify the potential lactic acid bacteria (LAB) by morphological and biochemical characterization; to screen pesticides for degradation by LAB in the laboratory and to assess the potential of LAB in residue biodegradation both in screen house and field at the Dept. of Agrl. Entomology, VOC Agricultural College and Research Institute, Tamil Nadu Agricultural University (TNAU), Killikulam, Vallanadu, Tuticorin District, Tamil Nadu, India.

LAB formulation

The LAB formulation was prepared by thoroughly mixing crushed sugarcane jaggery 1.0 kg, milk powder (Nestle) 150 g, and 150 ml of grape juice extracted and filtered two days after crushing and microbial fermentation. This semisolid formulation, *Cowine* undergoing controlled fermentation primarily by sugar-tolerant LAB species, was diluted with water in 1: 4 ratio (*Cowine*: water) to facilitate rapid multiplication of LAB for a day before mixing it at the rate of 25 ml/L of water (1: 40) the next day as the spray fluid.

Isolation and characterization of LAB

Lactobacillus MRS (de Man, Rogosa and Sharpe) (Himedia) agar plates were used for the isolation of LAB (Guetouache and Guessas, 2015). The LAB formulation was serially diluted with sterile water up to the concentration of 10^{-5}, inoculated in MRS agar and left at 25-30°C overnight. Cycloheximide (0.1%) was added before plating to prevent other microbial contamination (Beukes et al., 2001). Calcium carbonate 1% was added to the MRS-agar plates for better growth and dissolution by LAB (Kimoto et al., 2004; Aween et al., 2012). Single colonies of LAB were counted after 12 h and expressed as colony forming units ($CFUml^{-1}$). To preserve the isolated LAB as slants, MRS agar was poured into test tubes and the LAB strain was inoculated after a day. The cultures thus obtained were preserved in the form of slants for further identification and storage (Harshini et al., 2018). A thin smear of each of the pure actively growing (log phase) bacterial culture was prepared on clean grease-free slides, fixed by passing over gentle flame. Each heat-fixed smear was stained by addition of crystal violet solution for 60 s and rinsed with water. The smears were again flooded with Lugol's iodine for 30 s and rinsed with water, decolourized with 70% alcohol for 15 s and rinsed with distilled water. They were then counter stained with safranin for 60 s and finally rinsed with water, then allowed to air dry. These smears were mounted on a binocular research microscope (Magnius MLX Plus) (40X) connected to a computer that uses an image analyzer software and observed under oil immersion objective lens (Acharya, 2015). The bacterial isolates were identified based on the morphological and biochemical characteristics according to the Bergey's Manual of Systematic Bacteriology (Clauss et al., 1986). The following tests were performed to identify the LAB: hot loop test, catalase test using hydrogen peroxide, and gas production from glucose. Hot-loop test detects the production of carbon dioxide from glucose which is a useful tool in identifying LAB (Sperber and Swan, 1976). When inserted into the culture broth tube,

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the inoculation needle produces effervescence. In catalase test, a few drops of 3% hydrogen peroxide were added to a Petri plate containing 24 h old LAB culture (Patil et al., 2010). Simultaneously, the LAB culture smeared slides were also tested with 3% hydrogen peroxide for the production of froth. Carbohydrate fermentation ability of the bacterial isolates were tested by inserting Durham tubes into the test tube containing glucose. All the tubes were sterilized for 15 min at 121°C. The tubes were inoculated with a single colony of the bacteria under study. The positive reaction of the bacteria was indicated by the changes in the colour of the phenol red medium.

Agar well diffusion method

A total of 23 pesticides viz., Acephate 75 SP, Acetamiprid 20 SP, Azadirachtin 0.03 EC, Buprofezin 25 SC, Carbofuran 3 G, Cartap hydrochloride 50 SP, Chlorantraniliprole 18.5 SC, Cypermethrin 25 EC, Dimethoate 30 EC, Fenazaquin 10 EC, Fipronil 5 SC, Fenpyroximate 5 EC, Flubendiamide 39.35 SC, Imidacloprid 17.8 SL, Malathion 50 EC, Novaluron 10 EC, Profenofos 50 EC, Phenthoate 50 EC, Phosalone 35 EC, Quinalphos 25 EC, Spinosad 45 SC, Thiacloprid 21.7 SC and Thiamethoxam 25 WG were used to determine the antimicrobial activity of pesticide residues against LAB by agar well diffusion method. Petri plates were filled with MRS agar and then 0.1 ml of the culture broth was smeared over it using an L-rod. Four to six, 5-10 mm diameter wells were made with a sterilized cork borer and filled with 0.1 ml pesticide spray fluid in different concentrations, *viz.*, 100, 200, 300, 400 and 500 ppm as per Bose (2016). The diameter of the zone of inhibition (ZOI) formed around the wells was measured in mm after 24 h (Ramalivhana et al., 2014) (*Plate 1*).

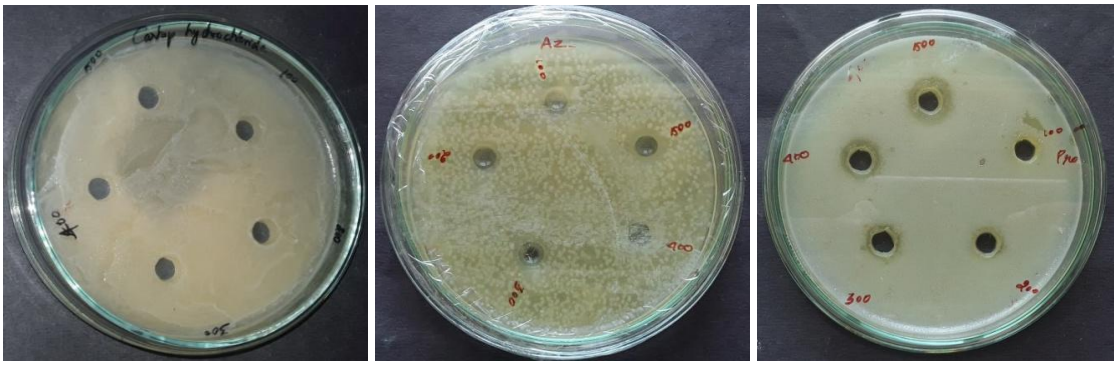

Plate 1. *Zero inhibition by cartap hydrochloride 50 SP (left) and inhibition by azadirachtin 0.03 EC (middle) and profenofos 50 EC (right) of LAB growth in agar-well diffusion method*

Screen house and field experiments

Two experiments in okra (cultivar COBhH 4), one in screen house and the other in field, comprising five treatments and four replications in completely/randomized block design, were conducted at VOC Agricultural College and Research Institute, TNAU, Killikulam, Vallanadu, Tuticorin district, Tamil Nadu, India. The ideal growth occurs between 75°F to 95°F (24°C to 35°C). Okra is sensitive to frost and prefers warm weather. Well-drained, loamy soil with a pH of 6.0 to 6.8 is optimal. It can tolerate a range of soil types but does not perform well in waterlogged conditions. Adequate spacing (60 × 45 cm) between plants allows for good air circulation and reduces the risk of disease.

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In screen house, plants were raised in pots (20 cm dia. × 18 cm ht.) while seeds were sown at 60 × 45 cm spacing in 20 cent plots in field. The seeds were treated with *Cowine*, neem oil + *Cowine* and imidacloprid and shade dried before sowing. Foliar sprays were given at weekly/fortnightly intervals using the spray fluid at 500 L/ha. The pesticides were sprayed in tandem: imidacloprid 17.8 SL at the rate of 0.6 ml/L one round 15 days after sowing, followed by profenofos 50 EC four rounds at fortnightly interval. *Cowine* was first diluted in water in 1:4 ratio, left overnight and sprayed the next day at 25 ml/L of water as the spray fluid. For neem oil + *Cowine*, neem oil was first mixed with *Cowine* in 1:2 ratio (neem oil to *Cowine*), diluted in water in 1:4 ratio before spraying this emulsion the next day at 25 ml/L of water as the spray fluid (*Plate 2*).

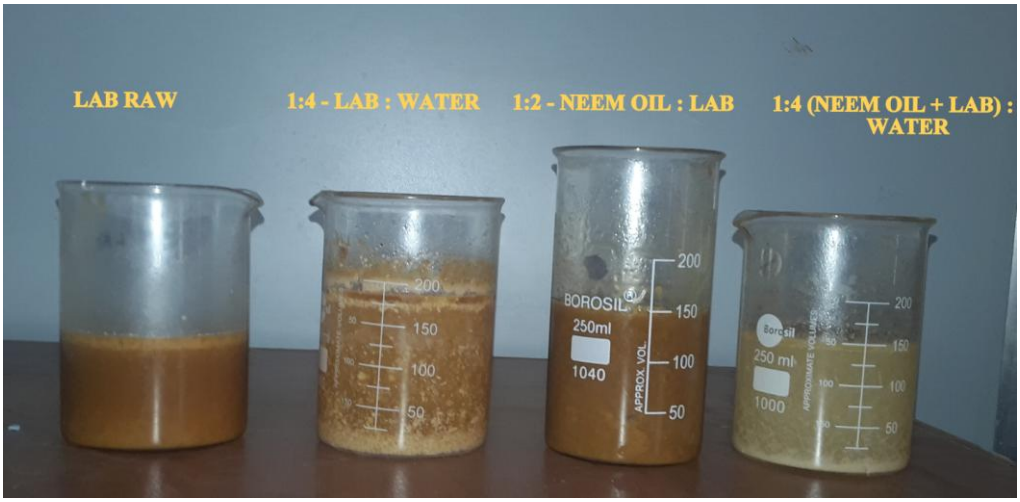

Plate 2. *Preparation of semisolid formulation, Cowine*

Assessment of LAB colonization

Leaf impression technique (Priya, 2016) was adopted to assess LAB populations on plant samples collected post-spray in sterile polythene cover or Petri plates (Harshini et al., 2018). Leaf samples were pressed on MRS agar medium in Petri plates and left overnight before assessing the LAB populations the next morning and expressed as CFU/cotyledon (*Plate 3*).

Assessment of pest populations

Pest counts were made at cotyledon, vegetative, flowering and fruiting stages. *B. tabaci* and *T. tabaci* were recorded at cotyledon stage; *A. devastans, P. solenopsis, Earias* spp. and *T. urticae* at vegetative stage; *A. devastans, Earias* spp. and *T. urticae* at fruiting stage. Nymphs and adults of *B. tabaci, T. tabaci,* and *A. gossypii* were counted from top, middle and bottom leaves (Manju et al., 2018; Ahmad et al., 2019). Plants infested by *A. devastans* and *T. urticae* and fruits damaged by *Earias* spp. were expressed in percentage.

The analysis of variance (ANOVA) was done with the pooled data from both screen house and field experiments for each parameter and the means were separated by LSD using the AGRES software. Log, square root and arc sine transformations were adopted for LAB counts, insect counts, and damage caused by insects, respectively.

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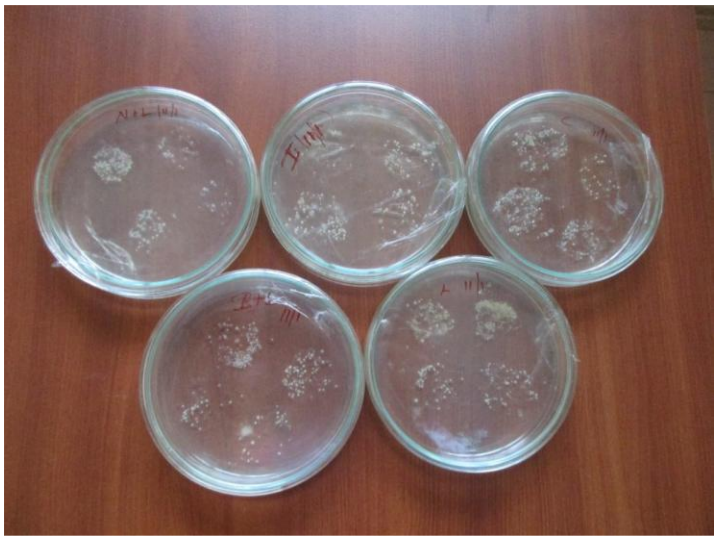

Plate 3. *Impression method used to count the LAB population as colony forming units (CFU) on MRS agar medium*

Results

Characterization of LAB isolates

The lactic acid bacterial isolates were selected and characterized based on their colony morphology and bio-chemical characteristics, the LAB isolates 1 and 2 as *Streptococcus* sp. and the LAB isolate 3 as *Lactobacillus* sp. (*Table 1*; *Plate 4*).

Table 1. *Morphological and biochemical characteristics of the LAB isolated from Cowine*

Isolates	Colony morphology	Cell characteristics	Catalase test	Gas production test	Hot loop test	Species
LAB 1	Dull brown, irregular shape, flat, margin undulated	Cocci in singles and pairs; gram-positive	+	+	+	*Streptococcus*
LAB 2	Dull white, irregular shape, flat, margin undulated	Cocci in pairs and chains; gram-positive	+	+	+	*Streptococcus*
LAB 3	Creamy white, circular and irregular shape, raised, entire and undulated margin	Long rods in singles and pairs; gram-positive	+	+	+	*Lactobacillus*

LAB, lactic acid bacteria

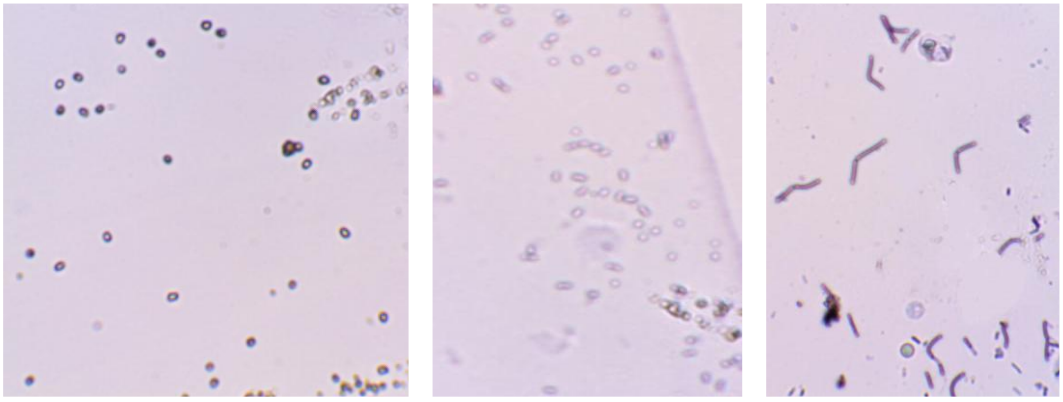

Plate 4. *LAB isolate 1, 2, 3 from Cowine*

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Inhibition of LAB by insecticides

Most pesticides (18 out of 23) did not inhibit LAB growth in the agar well diffusion method. However, azadirachtin, carbofuran, fenazaquin, imidacloprid and profenofos at 100 - 500 ppm were found to inhibit LAB growth, the inhibition significantly increasing (P = < 0.05) with increase in concentration (*Tables 2* and *3*).

Table 2. *Effect of insecticides on the in-vitro growth of LAB*

Insecticide	Inhibition (100-500 ppm)
Acephate (Asataf 75 SP)	-
Acetamiprid (Manik 20 SP)	-
Azadirachtin (Vijayneem 0.03 EC)	+
Buprofezin (Applaud 25 SC)	-
Carbofuran (VC Furan 3G)	+
Cartap hydrochloride (Swift SP 50 SP)	-
Chlorantraniliprole (Coragen18.5 SC)	-
Cypermethrin (Hilcyperin 25 EC)	-
Dimethoate (Tafgor 30 EC)	-
Fenazaquin (Magister10 EC)	+
Fipronil (Regent 5 SC)	-
Fenpyroximate (Neon 5 EC)	-
Flubendiamide (Asset 39.35 SC)	-
Imidacloprid (Hilmida17.8 SL)	+
Malathion (Hilmala 50 EC)	-
Novaluron (Rimon10 EC)	-
Profenofos (Profex 50 EC)	+
Phenthoate (Phendal 50 EC)	-
Phosalone (Zolone 35 EC)	-
Quinalphos (Ekalux 25 EC)	-
Spinosad (Tracer 45 SC)	-
Thiacloprid (Alanto 21.7 SC)	-
Thiamethoxam (Tagzone 25 WG)	-

LAB, lactic acid bacteria

Table 3. *Inhibitory activity of pesticides against LAB*

Treatments	Zone of inhibition (mm)				
	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm
Azadirachtin 0.03 EC	0.00 ± 0.00 (0.70)	0.00 ± 0.00 (0.70)	0.00 ± 0.00 (0.70)	0.10 ± 0.10 (0.80)	0.80 ± 1.30 (1.10)
Carbofuran 3 G	0.00 ± 0.00 (0.70)	0.10 ± 0.00 (0.80)	0.00 ± 0.00 (0.70)	0.00 ± 0.00 (0.70)	1.80 ± 0.10 (1.50)
Fenazaquin 10 EC	0.00 ± 0.00 (0.70)	0.00 ± 0.00 (0.70)	0.10 ± 0.17 (0.80)	0.50 ± 0.20 (1.00)	1.30 ± 0.10 (1.30)
Imidacloprid 17.8 SL	0.00 ± 0.00 (0.70)	0.00 ± 0.00 (0.70)	0.00 ± 0.00 (0.7)	0.10 ± 0.10 (0.80)	1.20 ± 0.20 (1.30)
Profenofos 50 EC	0.70 ± 0.10 (1.10)	1.40 ± 0.10 (1.40)	2.00 ± 0.28 (1.60)	3.20 ± 0.30 (1.90)	4.20 ± 0.30 (2.20)
CD (P = < 0.05)	0.17**	0.17**	0.21**	0.39**	0.30**
SEd	0.08	0.08	0.11	0.19	0.15

N = 3; figures in parentheses are transformed values; LAB, lactic acid bacteria

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LAB population in Cowine and spray fluids (leaf impression technique)

The LAB population density was 36.1 × 10^5 CFU ml^{-1} in the undiluted semisolid *Cowine*, increased to 56.6 × 10^5 CFU ml^{-1} after 1: 4 dilution, and to 88.8 × 10^5 CFU ml^{-1} after 1: 40 dilution in the final spray fluid (*Table 4*; *Fig. 1*). When mixed with neem oil, imidacloprid or profenofos the second day, its density in the spray fluid increased further, ranging from 123.0 ± 5.7 to 129.0 ± 12.7 × 10^5 CFU ml^{-1}.

Table 4. *LAB population in Cowine and spray fluids*

Treatments	LAB population (× 10^5 CFu ml^{-1})
Cowine	36.1 ± 7.8
Cowine + water (1: 4)	56.6 ± 1.0
Cowine + water (1: 40)	88.8 ± 5.5
Cowine + water	108.3 ± 9.9
Neem oil + *Cowine* + water	126.0 ± 3.6
Imidacloprid + *Cowine* + water	123.0 ± 5.7
Profenofos + *Cowine* + water	129.0 ± 12.7
Chlorantraniliprole + *Cowine* + water	94.3 ± 4.1
Imidacloprid + water	Nil
Profenofos + water	Nil
Chlorantraniliprole + water	Nil

Mean ± SE, 3 replicates; LAB, lactic acid bacteria; CFU, colony forming unit

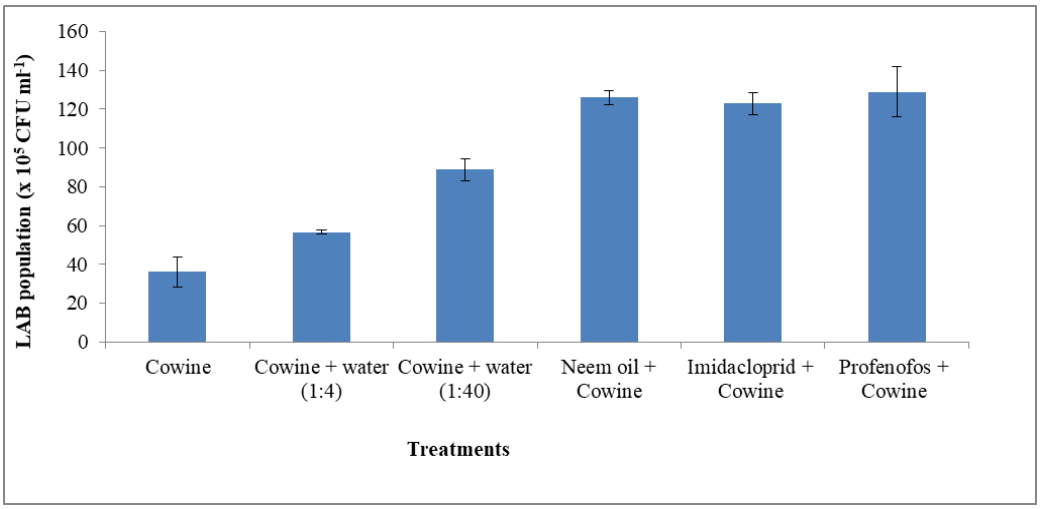

Figure 1. *LAB population in Cowine and spray fluids. Mean of 3 replicates. Vertical bars indicate the standard error. LAB, lactic acid bacteria; CFU, colony forming unit*

Effect of Cowine on pests

There was no significant difference between treatments in whitefly, *B. tabaci* populations and the yellow vein mosaic disease transmitted by them (*Table 5*).

Thrips, *T. tabaci* population was significantly lower on cotyledons of plants whose seeds were treated with *Cowine* alone or in combination with neem oil, and control plants (0.93-1.18/cotyledon) than on cotyledons of plants that received seed treatment with

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imidacloprid alone or imidacloprid + *Cowine* (1.68-3.65/cotyledon) (*Table 6*). *A. devastans* was significantly less numerous on *Cowine*, and neem oil + *Cowine* treated plants as in control (4.38-6.42/3 leaves) (*Table 6*) while they were significantly more abundant on plants which received imidacloprid spray, either alone or in combination with *Cowine* (7.80-10.39/3 leaves). Neem oil + *Cowine* was on par with imidacloprid in hopper population density (6.42-7.80/3 leaves). There was no significant difference in mealy bug, *P. solenopsis* infested plants between the treatments including control following *Cowine* spray, alone or in mixture with neem oil and imidacloprid (33.33 - 58.33%) (*Table 6*).

Table 5. *Effect of Cowine on Bemisia tabaci and yellow vein mosaic disease on okra*

Treatments	Whitefly (No./3 leaves)	Yellow vein mosaic diseased plants (%)
Cowine @ 25 ml/L	0.54 ± 0.16 (0.99)	15.14 ± 4.61 (17.89)
Neem oil + *Cowine* @ 25 ml/L	0.33 ± 0.12 (0.89)	12.78 ± 4.69 (15.84)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	0.55 ± 0.00 (0.99)	12.14 ± 3.14 (18.10)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	0.36 ± 0.01 (0.91)	9.17 ± 2.94 (15.59)
Control	0.35 ± 0.10 (0.90)	12.16 ± 5.60 (15.90)
Mean	0.43 ± 0.03 (0.93)	12.28 ± 4.20 (16.67)
CD (P < 0.05)	0.07^{NS}	12.40^{NS}

Mean of 5 observations for whitefly; n = 20 for YVMV; figures in parenthesis are transformed values; NS, not significant

Table 6. *Effect of Cowine on Thrips tabaci, Amrasca devastans and Phenacoccus solenopsis on okra*

Treatments	*T. tabaci* (No./cotyledon)	*A. devastans* (No./3 leaves)	*P. solenopsis* infested plants (%)
Cowine @ 25 ml/L	0.93 ± 0.18 (1.14)	4.38 ± 0.22 (1.97)	54.17 ± 18.75 (49.32)
Neem oil + *Cowine* @ 25 ml/L	1.18 ± 0.30 (1.25)	6.42 ± 1.01 (2.30)	33.33 ± 8.33 (31.47)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	1.68 ± 0.12 (1.43)	10.39 ± 1.93 (2.79)	58.33 ± 8.33 (51.76)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	3.65 ± 0.34 (1.90)	7.80 ± 1.23 (2.55)	50.00 ± 12.50 (43.10)
Control	0.98 ± 0.12 (1.16)	5.01 ± 0.53 (1.98)	45.83 ± 18.75 (40.67)
Mean	1.68 ± 0.14 (1.38)	6.80 ± 0.98 (2.32)	48.33 ± 13.33 (43.26)
CD (P < 0.05)	0.19**	0.40**	22.14^{NS}

Mean of 5 observations; figures in paranthesis are transformed values; NS, non-significant

Plants protected with imidacloprid, either alone or in combination with *Cowine*, supported fewer aphids, *A. gossypii* (0.61-0.75/3 leaves) than plants sprayed with *Cowine* or neem oil + *Cowine*, on par with control (1.52-4.42/3 leaves) (*Table 7*). The

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mite, *T. urticae* infestation was significantly more on plants that received *Cowine* spray, alone or in combination with imidacloprid (18.75-33.69%) (*Table 7*). However, the intensity of mite attack was significantly less in control, neem oil + *Cowine* and imidacloprid plants (6.25-17.31%), on par with *Cowine* (18.75%). The shoot and fruit borer, *Earias* spp. damage was significantly less in imidacloprid, *Cowine*, neem oil + *Cowine* (54.63-58.50%) treated plots, on par with imidacloprid and *Cowine* (60.62%) than in control plots (66.61%) (*Table 7*).

Table 7. *Effect of Cowine on Aphis gossypii, Tetranychus urticae and Earias spp. on okra*

Treatments	***A. gossypii* (No./3 leaves)**	***T. urticae* infested plants (%)**	***Earias* infested fruits (%)**
Cowine @ 25 ml/L	4.42 ± 1.52 (1.81)	18.75 ± 7.00 (28.68)	57.83 ± 6.37 (49.48)
Neem oil + *Cowine* @ 25 ml/L	2.25 ± 0.68 (1.46)	10.57 ± 3.96 (20.45)	58.50 ± 5.21 (49.99)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	0.75 ± 0.06 (0.99)	33.69 ± 3.98 (24.73)	60.62 ± 4.24 (51.24)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	0.61 ± 0.24 (0.90)	17.31 ± 3.99 (21.85)	54.63 ± 6.19 (47.86)
Control	1.52 ± 0.38 (1.27)	6.25 ± 4.21 (21.66)	66.61 ± 3.94 (54.87)
Mean	1.90 ± 0.71 (1.29)	20.11 ± 4.63 (23.47)	59.64 ± 5.19 (50.69)
CD (P < 0.05)	0.52**	6.26 **	5.65**

Mean ± SE; n = 5 for aphids and red spider mites; n = 20 for Earias; figures in parentheses are arc sine transformed values

Discussion

In the present study, the results revealed that lactic acid bacteria (LAB) can used to mitigate the problem of pesticide residue on crops under field conditions by applying LAB as a spray along with pesticides. As a first step, a natural product was developed from LAB-rich commodities, namely, sugarcane jaggery, milk powder and grape juice. As it embodies the qualities of an adjuvant, i.e. a non-pesticidal material added to a pesticide product or pesticide spray mixture to improve the pesticide's performance and alter the physical properties of the spray mixture, it is called here Adjuvant-Lactic Acid Bacteria, or A-LAB in short. Agricultural adjuvants perform specific functions including wetting, spreading, sticking and spray drifting (Green, 2000). A-LAB not only serves as a medium for LAB growth also helps the target pesticides to spread and stick better. It is a reservoir of LAB, especially *Lactobacillus* and *Streptococcus* as identified by phenotypic and biochemical characterization. Casein of milk powder contributes to the emulsifying properties of the product. When mixed with water, A-LAB yields an emulsion stable at least for 3-6 h. When diluted with water @1:4 ration (A-LAB: water), LAB multiply two times the next day i.e. 56.60×10^5 CFU/ ml from 36.10×10^5 CFU/ml. This fermented emulsion is again diluted in water @ 25-30 ml/L before it is sprayed on crops where it not only adds LAB but also spreads as a wetting agent. This is especially proven in combination with neem oil. For better results, neem oil + A-Lab need to be mixed @ 1:2 ration (neem oil: A-LAB) to get a milky emulsion and this is

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mixed with water at the rate of 25-30 ml/L of water on the day itself. In neem oil + A-LAB, the LAB load is 3-fold higher, i.e. 108.30 × 10^5 CFU/ml, that that in A-LAB. They multiplied on MRS medium in mixture with imidacloprid and profenofos in the laboratory and the pesticide + A-LAB emulsion was stable for more than a day when their population multiplied to 94.30 × 10^5 CFU/ml in chlorantraniliprole, 129.00 × 10^5 CFU/ml in profenofos + A-LAB, more than that in insecticide + water emulsion (90.70 10^5 CFU/ml). Because it is rich in sugar, it may also have other sugar-loving microbes. Safety-wise, these probiotic LAB positively influence the composition of gut microflora as well (Herich and Levkut, 2002). In insects, LAB occur in honeybee's environment, including its stomach, honey, bee bread, bee pollen, royal jelly and heads of nurse bees (Mathialagan, 2014). They occur on plants (Priya, 2016), especially on the oviposition sites of many pests (Priya, 2016).

When A-LAB was tried on okra from seed to fruiting stages, an assessment of insect population revealed that, except leafhopper, *A. devastans* and thrips, *T. tabaci*, either alone or in combination with neem oil, plants had significantly lower population of mealybug, *P. solenopsis*, aphid, *A. gossypii* and red spider mite, *T. urticae*. When mixed with imidacloprid, it caused *P. solenopsis*, *A. gossypii* and *T. urticae* to increase in population. However, insects like whitefly, *B. tabaci* and *Earias* spp. were fewer when it is mixed with insecticides.

Residue-wise, LAB degraded most insecticides in the laboratory in agar well diffusion method. Only five insecticides viz., azadirachtin, carbofuran, fenazaquin, imidacloprid and profenofos appeared to inhibit its growth. However, as LAB consume the residues of most insecticides in the laboratory with no inhibition zone. It can reduce the residues of even imidacloprid and profenofos when sprayed on crops in the field as evidenced by the results.

Thus, the primary objective of this investigation that LAB can mitigate pesticide residues under field conditions. Though many factors cause pesticide residues to dissipate under field conditions, among the microbial agents, consuming LAB is good for health as they are probiotics. This investigation thus demonstrates that A-LAB can be mixed with pesticides to reduce pesticide residues on vegetables.

Conclusion

In conclusion, this study highlights the potential of a farm-made fermenting LAB formulation constituted from sugarcane jaggery, milk powder and grape juice as *Cowine* that can be mixed with pesticide formulations not only as an effective wetting agent but also as a bacterial culture to address the problem of pesticide residues on vegetables. However, the dissipation rate of pesticide residues on crops after spraying *Cowine* needs to be studied in future through residue analysis under controlled conditions. This formulation also has vast scope for improvement with table sugar, milk, curd, yogurt, rice rinse water, etc. as LAB strains improve nutrient availability, reduce biotic and abiotic stresses, and stimulate plant growth.

REFERENCES

[1] Acharya, T. (2015): Bacteriology, staining techniques in microbiology. – Microbiology Online. http://microbeonline.com/gram-staining-principle-procedure-results/.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_17171733

[2] Ahmad, S., Safia, B., Khan, T., Shah, N., Abdal, M., Uddin, J. (2019): Management of *Bemisia tabaci* (Hemiptera: Aleyrodidae) through plant-based derivatives on *Abelmoschus esculentus* under field conditions in District Mardan, Khyber Pakhunkhwa. – Pakistan Int. J. Biosci.14: 293-299.
[3] Aislabie, J., Lloyd-Jones, G. (1995): A review of bacterial degradation of pesticides. – Aust. J. Soil Res. 33(6): 925-942.
[4] Aktar, W., Sengupta, D., Chowdhary, A. (2009): Impact of pesticides use in agriculture: their benefits and hazards. – Toxicology 2(1): 1-2.
[5] Askari, G. A., Kahouadji, A., Khedid, K., Charof, R., Mennane, Z. (2012): Screenings of lactic acid bacteria isolated from dried fruits and study of their antibacterial activity. – Middle-East J. Sci. Res. 11(2): 209-215.
[6] Aween, M. M., Hassan, Z., Muhialdin, B. J., Noor, H. M., Eljamel, Y. A. (2012): Evaluation on antibacterial activity of *Lactobacillus acidophilus* strains isolated from honey. – Americ. J. Appl. Sci. 9(6): 807-817.
[7] Beukes, E. M., Bester, B. H., Mostert, J. F. (2001): The microbiology of South African traditional fermented milks. – Int. J. Food Microbiol. 63(3): 189-197.
[8] Bintsis, T. (2018): Lactic acid bacteria as starter cultures: an update in their metabolism and genetics. – Aims Microbiol. 4: 665-684.
[9] Bond, J. L., Kriesemer, S. K., Emborg, J. E., Chadha, M. L. (2009): Understanding farmers' pesticide use in Jharkhand India. – Extension Farming Systems Journal 5(1): 53-62.
[10] Bose, S. C. (2016): Detecting pesticide residues on-farm and managing them with lactic acid bacteria (LAB). – Unpublished M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University. Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli.
[11] Chen, H., Yan, X., Du, G., Guo, Q., Shi, Y., Chang, J., Wang, X., Yuan, Y., Yue, T. (2021): Recent developments in antifungal lactic acid bacteria: application, screening methods, separation, purification of antifungal compounds and antifungal mechanisms. – Crit. Rev. Food Sci. Nutr. 15: 1-15.
[12] Claus, D., Berkeley, R. C. W. (1986): Genus Bacillus Cohn, 1872. – In: Sneath, P. H. A., Mair, N. S., Sharpe, M. E., Holt. J. G. (eds.) Bergey's Manual of Systematic Bacteriology. The Williams & Wilkins Co., Baltimore.
[13] Franques, J., Araque, I., Palahi, E., del C. Portillo, M., Reguant, C., Bordons, A. (2017): Presence of *Oenococcus oeni* and other lactic acid bacteria in grapes and wines from Priorat (Catalonia, Spain). – Food Sci. Technol. 81: 326-334.
[14] Ghaffar, T., Irshad, M., Anwar, Z., Aqil, T., Zulifqar, Z., Tariq, A., Kamran, M., Ehsan, N., Mehmood, S. (2014): Recent trends in lactic acid biotechnology: a brief review on production to purification. – J. Radiat. Res. Appl. Sci. 222-229.
[15] Green, J. M. (2000): Adjuvant outlook for pesticides. – Pesticides Outlook 11(5): 196-199.
[16] Guetouache, M., Guessas, B. (2015): Characterization and identification of lactic acid bacteriaisolated from traditional cheese (*Klila*) prepared from cow's milk. – Afr. J. Microbiol. Res. 9(2): 71-77.
[17] Harshini, R., Yasodha, P., Sabarinathan, K. G., Ambethgar, V., David, P. M. M. (2018): Diversity of epiphytic lactic acid bacteria (LAB) on insect oviposition sites. – Int. J. Curr. Microbiol. App. Sci. 7(07): 607-621. DOI: https://doi.org/10.20546/ ijcmas.2018. 707.074.
[18] Herich, R., Levkut, M. (2002): Lactic acid bacteria, probiotics and immune system. – Med. Czech. 47(6): 169-180.
[19] Hidalgo, D., Corona, F., Martín-Marroquin, J. (2022): Manure biostabilization by effective microorganisms as a way to improve its agronomic value. – Biomass Convers. Bioref. 12: 4649-4664.
[20] ICAR (2015): Vision 2050. – www.icar.in.
[21] Javaid, M. K., Ashiq, M., Tahir, M. (2016): Potential of biological agents in decontamination of agricultural soil. – Scientifica 2016: 1-10.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_17171733

[22] Kanekar, P. K., Bhadbhade, B. J., Deshpande, N. M., Sarnaik, S. S. (2004): Biodegradation of organophosphorus pesticides. – Proceeding of Indian National Science Academy 70(1): 56-70.
[23] Khalid, K. (2011): An overview of lactic acid bacteria. – Int. J. Biosciences. 1(3): 1-13.
[24] Khan, S. Z., Ullah, F., Khan, S., Khan, M. A., Khan, M. A. (2015): Residual effect of pesticides against different stages of green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae). – J. Entomol. Zool. Stud. 3(4): 114-119.
[25] Kimoto, H., Nomura, M., Kobayashi, M., Okamoto, T., Ohmomo, S. (2004): Identification and probiotic characteristics of *Lactococcus* strains from plant materials. – JPN. Agr. Res. Q. 38(2): 111-117.
[26] Kiruthika, K., Suganthi, A., Johnson Thangaraj Edward, Y. S., Anandham, R., Renukadevi, P., Murugan, M., Bimal Kumar Sahoo, Mohammad Ikram, Kavitha, P. G., Jayakanthan, M. (2024): Role of lactic acid bacteria in insecticide residue degradation. – Probiotics and Antimicrobial Proteins. https://doi.org/10.1007/s12602-024-10298-0.
[27] Makarova, K., Slesarev, A., Wolf, Y., Sorokin, A., Mirkin, B., Koonin, E., Pavlov, A., Pavlova, N., Karamychev, V., Polouchine, N., Shakhova, V. (2006): Comparative genomics of the lactic acid bacteria. – Proceeding in National Academy Science 103(42): 15611-15616.
[28] Manju, K. P., Vijaya Lakshmi, K., Sarath Babu, B. and Anitha, K. (2018): Management of whitefly, Bemisia tabaci and whitefly transmitted Okra Yellow Vein Mosaic Virus (OYVMV) in Okra. – Int. J. Curr. Microbio. App. Sci. SP(6): 1676-1681.
[29] Mansilla, R. T. (2008): Lactic Acid Bacteria as bioprotective agents against food borne pathogens and spoilage microorganisms in freshfruits and vegetables. – Ph. D. Thesis. Institute of Food and Agricultural Technology, University of Girona, Spain, pp. 6-20.
[30] Martín, R., Langa, S., Reviriego, C., Jimínez, E., Marín, M. L., Xaus, J., Fernández, L., Rodríguez, J. M. (2003): Human milk is a source of lactic acid bacteria for the infant gut. – J. Pediatr. 143(6): 754-758.
[31] Mathialagan, M. (2014): Probiotic lactic acid bacteria (LAB) for *Varroa*-associated stress management in honeybees. – Unpublished M. Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore.
[32] Mohammadi, M., Shadnoush, M., Sohrabvandi, S., Yousefi, M., Khorshidian, N., Mortazavian, A. M. (2021): Probiotics as potential detoxification tools for mitigation of pesticides: a mini review. – Int. J. Food Sci. Technol. 56(5): 2078-2087. https://doi.org/10.1111/ijfs.14880.
[33] Moreno-Arribas, V., Torlois, S., Joyeux, A., Bertrand, A., Lonvaud-Funel, A. (2000): Isolation, properties and behaviour of tyramine-producing lactic acid bacteria from wine. – J. Appl. Microbiol. 88: 584-593.
[34] Munawar, A., Hameed, S. W., Sarwar, M., Wasim, M., Hashmi, A. S., Imram, M. (2013): Identification of pesticide residues in different vegetables collected from market of Lahore, – Pakistan. J. Agroaliment. Processes Technol. 19(4): 392-398.
[35] Ni, K., Wang, Y., Li, D., Cai, Y., Pang, H. (2015): Characterization, identification and application of lactic acid bacteria isolated from forage paddy rice silage. – PLoS ONE 10(3): e0121967.
[36] Patil, M. M., Pal, A., Anand, T., Ramana, K. V. (2010): Isolation and characterization of Lactic Acid Bacteria from curd and cucumber. – Indian J. Biotechnol. 9: 166-172.
[37] Pehkonen, S. O., Zhang, Q. (2002): The degradation of organophosphorus pesticides in natural waters. – Crit. Rev. Environ. Sci. Technol. 32: 17-72.
[38] Priya, H. (2016): Exploring the diversity of epiphytic Lactic Acid Bacteria (LAB) on oviposition sites for pest management. – Unpublished M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University. Anbil Dharamalingam Agricultural College and Research Institute, Tiruchirappalli.
[39] Quistad, G. B., Menn, J. J. (1983): The disposition of pesticides in higher plants. – Residue Reviews. 85: 173-197.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/2302_17171733

[40] Ramalivhana, J. N., Obi, C. L., Samie, A., Iweriebor, B. C., Uaboi-Egbenni, P., Idiaghe, J. E., Momba, M. N. (2014): Antibacterial activity of honey and medicinal plant extracts against gram negative microorganisms. – Afr. J. Biotechnol. 13(4): 616-625.
[41] Raman, J., Kim, J. S., Choi, K. R., Eun, H., Yang, D., Ko, Y. J., Kim, S. J. (2022): Application of lactic acid bacteria (LAB) in sustainable agriculture: advantages and limitations. – Int. J. Mol. Sci. 23: 7784. https://doi.org/10.3390/ijms23147784.
[42] Rigotti, R. T., Feijo, C. A., Maia, N. J. L., Cesaro, G., Rosa, E. A. R., Freitas de Macedo, R. E., Luciano, F. B. (2017): Combination of natural antimicrobials and sodium dodecyl sulfate for disruption of biofilms formed by contaminant bacteria isolated from sugarcane mills. – Innov. Food Sci. Emerg. Technol. 41: 26-33.
[43] Rivka, C. (2013): Bioremediation of toxic pollutants. – http://www.ariel.ac.il/ research/ btp.
[44] Sadiq, F. A., Yan, B., Tian, F., Zhao, J., Zhang, H., Chen,W. (2019): Lactic acid bacteria as antifungal and anti-mycotoxigenic agents: a comprehensive review. – Compr. Rev. Food Sci. Food Saf. 18: 1403-1436.
[45] Sobrun, Y., Bhaw-Luximon, A., Jhurry, D., Puchooa, D. (2012): Isolation of lactic acid bacteria from sugar cane juice and production of lactic acid from selected improved strains. – Adv. Biosci. Biotechnol. 3: 398-407.
[46] Sperber, H. W., Swan, J. (1976): Hot-loop test for the determination of carbon dioxide production from glucose by lactic acid bacteria. – Appl. Environ. Microbiol. 31(6): 990-991.
[47] Stiles, M. E., Holzapfel, W. H. (1997): Lactic acid bacteria of foods and their current taxonomy. – Int. J. Food Microbiol. 36(1): 1-29.
[48] Vogt, C., Richnow, H. H. (2014): Bioremediation via in situ microbial degradation of organic pollutants. – Adv. Biochem. Eng. Biotechnol. 123: 123-146.
[49] Yu, A. O., Leveau, J. H. J., Marco, M.L. (2020): Abundance, diversity and plant-specific adaptations of plant associated lactic acid bacteria. – Environ. Miocrobiol. Rep. 12: 16-29.

APPENDIX

ANOVA tables

Table A1. *Influence of Cowine on Bemisia tabaci on okra*

Treatments	Whitefly population (No./3 leaves)		Mean
	Screenhouse	Field	
Cowine @ 25 ml/L	0.38 ± 0.08 (0.90)	0.70 ± 0.12 (1.09)	0.54 ± 0.16 (0.99)
Neem oil + *Cowine* @ 25 ml/L	0.21 ± 0.16 (0.81)		0.33 ± 0.12 (0.89)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	0.54 ± 0.13 (0.98)	0.45 ± 0.09 (0.97)	0.55 ± 0.00 (0.99)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	0.38 ± 0.17 (0.90)	0.55 ± 0.20 (1.00)	0.36 ± 0.01 (0.91)
Control	0.46 ± 0.10 (0.95)	0.35 ± 0.05 (0.92)	0.35 ± 0.10 (0.90)
Mean	0.39 ± 0.13 (0.88)	0.25 ± 0.09 (0.86)	0.43 ± 0.03 (0.93)

Mean of 5 observations; figures in parenthesis are square root x + 0.5 transformed values

	CD (P < 0.05)
Treatment	0.07^{NS}
Location	0.11*
Treatment × location	0.16^{NS}

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Table A2. *Influence of Cowine on Okra yellow vein mosaic disease (YVMD)*

Treatments	Diseased plants (%)		Mean
	Screenhouse	Field	
Cowine @ 25 ml/L	3.13 ± 1.99 (5.58)	27.16 ± 7.24 (30.21)	15.14 ± 4.61 (17.89)
Neem oil + *Cowine* @ 25 ml/L	2.08 ± 2.08 (4.67)	23.49 ± 7.30 (27.01)	12.78 ± 4.69 (15.84)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	5.21 ± 2.44 (10.72)	19.07 ± 4.03 (25.49)	12.14 ± 3.14 (18.10)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	6.25 ± 2.90 (11.64)	12.10 ± 2.98 (19.55)	9.17 ± 2.94 (15.59)
Control	4.17 ± 3.03 (10.53)	20.15 ± 8.18 (24.20)	12.16 ± 5.60 (15.90)
Mean	4.17 ± 2.42 (8.63)	20.45 ± 5.94 (25.29)	12.28 ± 4.20 (16.67)
CD (P < 0.05)			12.40^{NS}

Mean ± SE, n = 20; figures in parentheses are square root x + 0.5 transformed values; NS, Non-significant; YVMD, okra yellow vein mosaic disease

Table A3. *Influence of Cowine on Thrips tabaci at cotyledonary stage on okra*

Treatments	Thrips (No./cotyledon)		Mean
	Count 1	Count 2	
Cowine @ 25 ml/L	1.70 ± 0.26 (1.47)	0.15 ± 0.10 (0.80)	0.93 ± 0.18 (1.14)
Neem oil + *Cowine* @ 25 ml/L	1.80 ± 0.28 (1.50)	0.55 ± 0.31 (0.99)	1.18 ± 0.30 (1.25)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	2.60 ± 0.14 (1.75)	0.75 ± 0.10 (1.12)	1.68 ± 0.12 (1.43)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	6.20 ± 0.28 (2.58)	1.10 ± 0.39 (1.22)	3.65 ± 0.34 (1.90)
Control	1.75 ± 0.10 (1.50)	0.20 ± 0.14 (0.82)	0.98 ± 0.12 (1.16)
Mean	2.81 ± 0.14 (1.77)	0.55 ± 0.15 (0.99)	1.68 ± 0.14 (1.38)

Mean of 5 observations; figures in parenthesis are square root x + 0.5 transformed values

	CD (P < 0.05)
Treatment	0.19**
Location	0.12**
Treatment × location	0.27**

Table A4. *Influence of Cowine on Amrasca devastans in Okra field*

Treatments	Mean (No. of hoppers/3 leaves)
Cowine @ 25 ml/L	4.38 ± 0.22 (1.97)
Neem oil + *Cowine* @ 25 ml/L	6.42 ± 1.01 (2.30)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	10.39 ± 1.93 (2.79)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	7.80 ± 1.23 (2.55)
Control	5.01 ± 0.53 (1.98)
Mean	6.80 ± 0.98 (2.32)

Mean ± SE, n = 20; figures in parenthesis are arc sine transformed values; NS - Non-significant

	CD (P < 0.05)
Treatment	0.40**
Location	0.25**
Treatment × location	0.57**

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Table A5. *Influence of Cowine on Phenacoccus solenopsis on okra*

Treatments	Mealy bug infested plants (%)
Cowine @ 25 ml/L	54.17 ± 18.75 (49.32)
Neem oil + *Cowine* @ 25 ml/L	33.33 ± 8.33 (31.47)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	58.33 ± 8.33 (51.76)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	50.00 ± 12.50 (43.10)
Control	45.83 ± 18.75 (40.67)
Mean	48.33 ± 13.33 (43.26)

Mean ± SE, n = 5; figures in parenthesis are arc sine transformed values; NS - Non-significant

	CD (P < 0.05)
Treatment	22.14**
Location	14.00**
Treatment × location	31.31^{NS}

Table A6. *Influence of Cowine on Aphis gossypii on okra*

Treatments	No. of aphids/3 leaves)		Mean
	Screenhouse	Field	
Cowine @ 25 ml/L	8.29 ± 1.52 (2.63)	0.55 ± 0.34 (0.98)	4.42 ± 1.52 (1.81)
Neem oil + *Cowine* @ 25 ml/L	4.00 ± 0.68 (1.94)	0.50 ± 0.20 (0.98)	2.25 ± 0.68 (1.46)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	0.96 ± 0.06 (0.97)	0.55 ± 0.12 (1.01)	0.75 ± 0.06 (0.99)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	0.83 ± 0.24 (0.88)	0.40 ± 0.24 (0.92)	0.61 ± 0.24 (0.90)
Control	1.96 ± 0.38 (1.29)	1.10 ± 0.10 (1.26)	1.52 ± 0.38 (1.27)
Mean	3.21 ± 1.10 (1.54)	0.62 ± 0.20 (1.03)	1.90 ± 0.71 (1.29)

Mean ± SE, n = 20; figures in parenthesis are arc sine transformed values; NS - Non-significant

	CD (P < 0.05)
Treatment	0.52**
Location	0.32**
Treatment × location	0.73**

Table A7. *Influence of Cowine on Tetranychus urticae on okra*

Treatments	Infested plants (%)		Mean
	Screenhouse	Field	
Cowine @ 25 ml/L	11.46 ± 3.70 (17.14)	44.97 ± 10.30 (47.52)	18.75 ± 7.00 (28.68)
Neem oil + *Cowine* @ 25 ml/L	8.33 ± 1.70 (12.19)	26.30 ± 6.22 (28.43)	10.57 ± 3.96 (20.45)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	15.63 ± 2.74 (21.54)	25.09 ± 5.21 (27.06)	33.69 ± 3.98 (24.73)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	12.50 ± 3.99 (17.88)	22.13 ± 3.99 (24.06)	17.31 ± 3.99 (21.85)
Control	9.38 ± 3.20 (15.31)	25.38 ± 2.93 (27.41)	6.25 ± 4.21 (21.66)
Mean	11.46 ± 3.07 (16.80)	28.77 ± 6.19 (30.89)	20.11 ± 4.63

Mean ± SE, n = 5; figures in parenthesis are arc sine transformed values; NS - Non-significant

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	CD (P < 0.05)
Treatment	6.26**
Location	7.00**
Treatment × location	14.00**

Table A8. *Influence of Cowine on damage to okra fruit by Earias spp. in field*

Treatments	**Infested fruits (%)**
Cowine @ 25 ml/L	57.83 ± 6.37 (49.48)
Neem oil + *Cowine* @ 25 ml/L	58.50 ± 5.21 (49.99)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2% + *Cowine* @ 25 ml/L	60.62 ± 4.24 (51.24)
Imidacloprid 48 FS 1.6%/Profenofos 50 EC 0.2%	54.63 ± 6.19 (47.86)
Control	66.61 ± 3.94 (54.87)
Mean	59.64 ± 5.19 (50.69)

Mean ± SE, n = 20; figures in parenthesis are arc sine transformed values; NS - Non-significant

	CD (P < 0.05)
Treatment	5.65**
Location	3.57^{NS}
Treatment × Location	8.00*

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