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Suppression of sucking insects in bhendi after spraying imidacloprid and milk-made lactic acid bacterial formulation

Elakkiya K, P Yasodha, CGL Justin, J Ejilane, S Somasundaram and PMM David

Elakkiya K
PG Scholar, Department of Plant Protection, Anbil Dharmalingam Agriculture College and Research Institute, Tamil Nadu Agricultural University, Trichy, Tamil Nadu, India

P Yasodha
Assistant Professor, Department of Plant Protection, Anbil Dharmalingam Agriculture College and Research Institute, Tamil Nadu Agricultural University, Trichy, Tamil Nadu, India

CGL Justin
Professor and Head, Department of Plant Protection, Anbil Dharmalingam Agriculture College and Research Institute, Tamil Nadu Agricultural University, Trichy, Tamil Nadu, India

J Ejilane
Assistant Professor, Department of Soil Science and Agricultural Chemistry, Anbil Dharmalingam Agriculture College and Research Institute, Tamil Nadu Agricultural University, Trichy, Tamil Nadu, India

S Somasundaram
Associate Professor, Department of Agronomy, Anbil Dharmalingam Agriculture College and Research Institute, Tamil Nadu Agricultural University, Trichy, Tamil Nadu, India

PMM David
Retired Professor, Tamil Nadu Agricultural University, Trichy, Tamil Nadu, India

Correspondence
P Yasodha
Assistant Professor, Department of Plant Protection, Anbil Dharmalingam Agriculture College and Research Institute, Tamil Nadu Agricultural University, Trichy, Tamil Nadu, India

Abstract
To manage sucking pests, imidacloprid is commonly sprayed on vegetable crops despite ecological implications, especially residues and declining bee colonies. With epiphytic lactic acid bacteria (LAB) degrading pesticide residues, spraying milk-made LAB formulations on crops is likely to reduce these risks as a bio-ameliorant. In this study, a milk-made colloidal formulation rich in sugar-tolerant LAB, referred to as Milkoid, was evaluated in bhendi in screen house and field, following imidacloprid spray, in comparison with the antimicrobial bleaching powder (calcium hypochlorite) against early sucking pests, namely, leafhopper *Amrasca biguttula biguttula*, whitefly *Bemisia tabaci* and aphid *Aphis gossypii*. The results indicated that the epiphytic LAB density on bhendi leaves was significantly higher on plants after spraying imidacloprid 17.8 SL at 0.02 ml / l and Milkoid at 2.0 % in tandem, followed by imidacloprid-treated and control plants, than on plants sprayed with bleaching powder 1.0 %, with or without imidacloprid. Whitefly-transmitted yellow mosaic viral infection, aphid and leafhopper infestations were significantly less on plants sprayed with imidacloprid with or without bleaching powder than on other plants with or without any sprays, including Milkoid, bleaching powder, and imidacloprid / Milkoid. The efficacy of Milkoid LAB in reducing the toxicity of imidacloprid and the potential of calcium hypochlorite as an antimicrobial agent in crop protection are discussed.

Keywords: Bhendi sucking pests, imidacloprid, lactic acid bacterial formulation

Introduction

An important vegetable crop, bhendi is susceptible right from the cotyledon stage to sucking pests like leaf hoppers, *Amrasca biguttula biguttula* (Ishida) (Hemiptera: Cicadellidae), whiteflies, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), and aphids, *Aphis gossypii* (Glover) (Hemiptera: Aphididae), which reduce the yield by more than half (Chaudhary and Dadheech, 1989) [5]. To make it good, toxic chemicals are sprayed on crops that carry residues harmful to the environment and non-target organisms. The largest selling pesticide in the world, imidacloprid persists much longer in the environment (Simon-Delso *et al*., 2015), thought to be one of the reasons for the colony collapse disorder (CCD) in honey bees (vanEngelsdrop, 2009), linked to changes in bee behaviour (Johnson *et al*., 2010) [19]. Described as 'mad bee disease', beekeepers in France blame it on imidacloprid (Rortais, 2005) [30] even as honey samples from all over the world have imidacloprid residues (Mitchel *et al*., 2017) [24]. However, honey bees survive this stress by harbouring probiotic LAB, which protect its host by producing antimicrobial metabolites as well as by modulating host immune response (Servin, 2004) [32]. Live microorganisms that confer health benefits on their hosts (Moritz *et al*., 2010) [26], probiotics are generally recognized as safe (GRAS) food grade microorganisms (Salminen *et al*. 1998) [31]. Probiotic lactic acid bacteria (LAB) from the genera *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* are widely used in fermentation and food industry (Stiles and Holzapfel, 1997; Beasley, 2004), with *Lactobacillus, Leuconostoc, Pediococcus, Lactococcus* and *Streptococcus* involved mainly in fermentations (Ouwehand *et al*., 2002) [28]. They have also been exploited in crop production as biofertilizers (effective microorganisms), biocontrol agents and biostimulants promoting plant growth (Lanton *et al*., 2017) [21]. They are able to degrade pesticides as well (Zhang *et al*., 2014) [39]. They occur in rhizosphere (Ekundayo, 2014) [12] and on plants as epiphytic (Harshini *et al*., 2018) [16] or even endophytic (Minervini *et al*., 2015) [23].

With a 2.07-day half-life (Pandit, 2016) [29], imidacloprid is commonly sprayed on bhendi (Kumar *et al*., 2017) [20], often with a chemical wetting agent for better efficacy. This calls for remedial measures right on crops being sprayed to reduce the hazards. Milk is a rich source of probiotic LAB, especially *Lactobacillus acidophilus* (Wang *et al*., 2016) [35]. Thus an LAB formulation made from milk may help reduce these risks if sprayed on crops as adjuvant that performs specific functions including wetting, spreading, sticking and spray drifting (Green, 2000) [13]. However, how they influence pest infestations is not known, for microorganisms like yeasts attract insects by producing volatiles (Becher *et al.*, 2012) [2]. This study was undertaken to assess the status of sucking insects in bhendi after spraying imidacloprid and a milk-made LAB formulation in comparison with bleaching powder (calcium hypochlorite), a commercially available chlorine compound with antimicrobial properties (Dychdala, 1991) [11].

Materials and methods

The experiments were conducted at Anbil Dharmalingam Agricultural College and Research Institute, Tiruchirappalli, Tamil Nadu, India during 2018-19. In screenhouse, bhendi plants (hybrid Jaani) raised in pots (23 cm high, 22 cm diameter) were arranged in a completely randomized design (CRD) with seven treatments and three replications. In the field the same treatments were evaluated in a randomized block design (RBD) with three replications where the hybrid was raised in 5 x 4’ plots adopting a spacing of 45 x 30 cm. Imidacloprid 17.8% SL (Nagarjuna Agrichem Limited) was sprayed at 0.2 ml per litre of water. The lactic acid bacterial formulation, called Milkoid, was prepared through a process of controlled fermentation by mixing bovine milk (1.0 litre) and table sugar (1.0 kg), again fortified with table sugar (0.5 kg) and beaten egg (1 number) 15 days later (David *et al.*, 2018) [9]. To prepare the spray fluid, Milkoid was first diluted in water (3 parts), kept overnight and sprayed the next day at the rate of 20 ml per litre of water (2.0 %). There were three controls. Bleaching powder (calcium hypochlorite) was purchased from the local market and sprayed at 1.0 % as the antimicrobial control while water-spray and no-spray were the other controls. Two rounds of foliar sprays at fortnightly interval were made early in the morning at the vegetative stage of the crop growth using a 1-litre capacity hand-operated sprayer, separate one for each treatment to avoid contamination. Pre and post-treatment counts of the sucking pests, namely, leafhopper *A. biguttula biguttula*, whitefly *B. tabaci* and aphid *A. gossypii*, were recorded at weekly interval by recording the mean number of hoppers present on the lower side of a leaf, the mean percentage of aphid-infested plants and the mean percentage of whitefly-transmitted yellow vein mosaic virus (YMV) infected plants. The population density of the LAB present on the leaves was assessed at weekly interval by the leaf impression method wherein leaf bit (1 cm^2) samples were placed on Lactobacillus MRS (de Mann Rogosa Sharpe Agar)
(16.78 g/250 ml of distilled water) (Himedia), a specific medium for LAB growth. $CaCO_3$ (2.0 g) was added to the medium to induce better LAB growth (Wright and Klaenhammer, 1981; Aween *et al.*, 2012) while cycloheximide (0.1 %) was added to prevent contamination by other microbes. Leaf bit samples (0.5 cm diameter) cut from each treatment plants were collected in the evening using a sterilized cork borer to have uniform sized colony growth. The colony forming units (CFU) were counted manually after 12 h to avoid the slimy over growth of the bacteria. The experimental data were subjected to analysis of variance (ANOVA) and the means separated by least significant difference (LSD). Square root transformation was adopted for the data on leafhoppers, arc sin for the data on whitefly and aphid and log for the data on LAB counts.

Result and discussion

Sucking pests

B. tabaci starts infesting bhendi even at the cotyledon stage, transmitting YMV. In the screenhouse, its infection was significantly lowest (P = 0.05) when the plants were treated with imidacloprid 17.8% SL 0.2 ml/l either alone or in tandem with bleaching powder 1.0% (11.00%) (Table 1). The infection was of a moderate level after spraying imidacloprid / Milkoid 2.0% (38.67%), on par with bleaching powder (41.33%) and Milkoid 2% (58.0%). All the control plants, with or without water spray, had viral infection (100.0 %). In the field, the diseased plants were significantly less numerous in plots treated with imidacloprid / bleaching powder (14.17%), on par with imidacloprid (16.67%) (Table 1). The disease incidence was significantly moderate in plots treated with imidacloprid / Milkoid (24.58%), followed by bleaching powder 1.0% (41.46%) and Milkoid 2.0% (66.04%). The injury level was highest in control plots, water-sprayed and untreated (89.17 - 92.50%). Pooled analysis of the data from both screenhouse and field experiments also indicated imidacloprid / bleaching powder to suppress the disease infection significantly most (12.58 %), on par with imidacloprid (13.83%), followed by imidacloprid / Milkoid (31.63%) (Table1, Fig.1). Bleaching powder 1.0% reduced the disease infection by 42.73 per cent, on par with Milkoid 2.0% (63.35%), significantly less than that in control plots (96.25 – 94.58%).

Table 1: *B. tabaci*-transmitted YMV-infected plants following imidacloprid, Milkoid and bleaching powder spray in bhendi.

Treatments	YMV-infected plants (%)		Pooled mean (%)	YMV Reduction from untreated control (%)
	Screenhouse	Field		
Imidacloprid 17.8 SL 0.2 ml/l	11.00 (11.68)a	16.67 23.72)a	13.83(17.83)a	85.63
Imidacloprid 17.8 SL 0.2 ml/l /Milkoid 2.0 %	38.67 (38.37)b	24.58 (29.34)b	31.63(33.86)b	67.13
Imidacloprid 17.8 SL 0.2 ml/l / Bleaching powder 1.0 %	11.00 (11.68)a	14.17 (21.50)a	12.58(16.73)a	86.92
Milkoid 2.0 %	58.00 (49.67)b	66.04 (54.39)d	63.35(52.75)c	34.18
Bleaching powder 1.0 %	41.33 (39.93)b	41.46 (40.03)c	42.73(39.98)bc	55.60
Water spray	100.00 (90.00)c	89.17 (71.06)e	94.58(80.33)d	1.73
Untreated control	100.00 (90.00)c	92.50 (74.42)e	96.25(82.01)d	
Mean	11.00 (11.68)a	16.67 (23.72)a	50.71 (46.10)	
CD (P = 0.05)	19.58	4.48	13.37	
SEd	9.56	2.17	6.67	

(Mean of 3 replications; Figures in parenthesis are arc sin transformed values; Means followed by the same letter are not significantly different; YMV, yellow vein mosaic virus)

The aphid, *Aphis gossypii* infestation was noticed only in the field and the average infested plants were significantly fewer (P = 0.05) in plots treated with imidacloprid / bleaching powder (11.08%), or imidacloprid (12.92%) alone, on par with imidacloprid / Milkoid (21.93%) (Table 2, Fig. 1). With 27.50 per cent infested plants, bleaching powder was also on par with imidacloprid / Milkoid. Aphid infestation was significantly less after spraying Milkoid (53.54%) than that in control plots, water-sprayed (75.0%) or untreated (78.96%).

Table 2: *A. gossypii*-infested plants following imidacloprid, Milkoid and bleaching powder spray in field bhendi

Treatments	Aphid-infested plants (%)	Reduction from untreated control (%)
Imidacloprid 17.8 SL 0.2 ml/l	12.92 (19.78)[a]	83.63
Imidacloprid 17.8 SL 0.2 ml/l /Milkoid 2.0 %	21.93 (27.41)[b]	72.22
Imidacloprid 17.8 SL 0.2 ml/l / Bleaching powder 1.0 %	11.08 (18.28)[a]	85.96
Milkoid 2.0 %	53.54 (47.16)[c]	32.19
Bleaching powder 1.0 %	27.50 (31.18)[b]	65.17
Water spray	75.00 (61.38)[d]	5.01
Untreated control	78.96 (64.30)[d]	
Mean	12.92 (12.92)[a]	
CD (P = 0.05)	7.99	
SEd	3.89	

(Mean of three replications; Figures in parenthesis are arc sin transformed values; Means followed by the same letter are not significantly different).

The leafhopper, *A. biguttula biguttula* occurred in both screenhouse and field experiments. In screenhouse, compared to the water-sprayed and untreated control plants, significantly fewer leafhoppers (P = 0.05) were found on plants treated with imidacloprid / bleaching powder (0.25 / leaf), and with imidacloprid (0.33 / leaf) than on plants sprayed with imidacloprid / Milkoid (0.92 / leaf) and bleaching powder 1.0% (1.00 / leaf) (Table 3). Leafhopper density was significantly highest on plants treated with Milkoid 2.0% as on control plants (2.08 - 2.58 / leaf). In the field trial too, the leafhopper infestation was significantly lowest after imidacloprid spray, with or without bleaching powder (0.51 - 0.63 / leaf) (Table 3). Bleaching powder was inferior to imidacloprid / Milkoid but superior to Milkoid 2.0% (1.87/ leaf) in reducing leafhopper numbers. All the other control plants had highest leafhopper density (2.32 - 2.37 / leaf). The pooled data from both screenhouse and field experiments also indicated significantly lowest (P = 0.05) leafhopper density following spray with imidacloprid / bleaching powder (0.38 / leaf), and imidacloprid alone (0.48 / leaf) (Table 3, Fig. 1), the latter on par with imidacloprid / Milkoid (0.87 / leaf). Bleaching powder 1.0% (1.17 / leaf) was on par with imidacloprid / Milkoid but superior to Milkoid 2.0% (1.97 / leaf). Leafhoppers were most abundant on control plants (2.39 – 2.45 / leaf).

Table 3: Population density of *A. biguttula biguttula* following imidacloprid, Milkoid and bleaching powder spray in bhendi

Treatments	Leafhoppers (No. / leaf)		Pooled mean (No. / leaf)	Reduction from untreated control (%)
	Screenhouse	Field		
Imidacloprid 17.8 SL 0.2 ml/l	0.33 (0.90)[a]	0.63 (1.06)[a]	0.48 (0.98)[a]	79.90
Imidacloprid 17.8 SL 0.2 ml/l /Milkoid 2.0 %	0.83 (1.14)[b]	0.92 (1.18)[b]	0.87 (1.16)[b]	63.59
Imidacloprid 17.8 SL 0.2 ml/l / Bleaching powder 1.0 %	0.25(0.86)[a]	0.51(1.00)[a]	0.38 (0.92)[a]	84.10
Milkoid 2.0 %	2.08(1.61)[c]	1.87 (1.54)[d]	1.97 (1.57)[d]	17.57
Bleaching powder 1.0 %	1.00(1.22)[b]	1.34 (1.36)[c]	1.17 (1.29)[c]	51.04
Water spray	2.58 (1.75)[c]	2.32 (1.67)[e]	2.45 (1.72)[e]	- 2.51
Untreated control	2.42 (1.70)[c]	2.37 (1.69)[e]	2.39 (1.70)[e]	
Mean	1.35 (1.31)	1.42 (1.36)	1.38 (1.33)	
CD (P = 0.05)	0.27	0.10	0.20	
SEd	0.13	0.05	0.10	

(Mean of three replications; Figures in parenthesis are square root transformed values; Means followed by the same letter are not significantly different)

Epiphytic LAB

The LAB population density was significantly highest (P = 0.05) on bhendi leaves after spraying imidacloprid / Milkoid (12.83 – 16.17 CFU/ cm^2) and Milkoid 2% (10.72 – 16.06 CFU / cm^2), both in screenhouse and field trials (Table 4). The density was second highest on leaves after spraying imidacloprid (6.61 – 8.67 CFU/ cm^2) as on control plants (6.33 – 8.11 CFU / cm^2). Bleaching powder 1.0%, with or without imidacloprid 17.8% SL, suppressed the LAB density significantly most (2.94 – 3.61 CFU/ cm^2) (Table 4). The pooled means from both experiments also indicated highest LAB density after spraying imidacloprid / Milkoid (14.50 CFU/ cm^2), on par with Milkoid 2.0% (13.39 CFU/ cm^2), moderately high density on leaves sprayed with imidacloprid on par with control plant leaves (7.22 - 7.64 CFU/ cm^2), and lowest after spraying imidacloprid/ bleaching powder, or bleaching powder alone (3.25 - 3.31 CFU/ cm^2) (Table 4, Fig. 2).

Table 4: LAB population density following imidacloprid, Milkoid and bleaching powder spray in bhendi

Treatments	LAB (CFU/cm²)		Pooled mean (CFU/cm²)	Increase over / Reduction from untreated control (%)
	Screenhouse	Field		
Imidacloprid 17.8 SL 0.2 ml/l	6.61 (0.81)^{c}	8.67 (0.94)^{b}	7.64 (0.88)^{b}	5.37
Imidacloprid 17.8 SL 0.2 ml/l /Milkoid 2.0 %	12.83 (1.11)^{a}	16.17 (1.21)^{a}	14.50 (1.16)^{a}	100.0
Imidacloprid 17.8 SL 0.2 ml/l / Bleaching powder 1.0 %	2.94 (0.46)^{d}	3.56 (0.49)^{c}	3.25 (0.48)^{c}	- 55.17
Milkoid 2.0 %	10.72(1.02)^{b}	16.06 (1.20)^{a}	13.39 (1.11)^{a}	84.68
Bleaching powder 1.0 %	3.00 (0.47)^{d}	3.61 (0.53)^{c}	3.31 (0.49)^{c}	- 54.34
Water spray	6.33 (0.79)^{c}	8.11 (0.91)^{b}	7.22 (0.85)^{b}	- 0.41
Untreated control	6.45 (0.80)^{c}	8.06 (0.90)^{b}	7.25 (0.85)^{b}	
Mean	6.98 (0.78)	9.18 (0.88)	8.05 (0.83)	
CD (P = 0.05)	0.08	0.14	0.11	
SEd	0.04	0.07	0.06	

(Mean of three replications; Figures in the parentheses are log transformed values; Means followed by the same letter are not significantly different; LAB, lactic acid bacteria; CFU, colony forming units).

Influence of LAB Formulation

As a pure culture, several bacterial strains other than LAB are capable of transforming neonicotinoids, especially imidacloprid, in the presence of an additional carbon source (Hussain *et al*., 2016) [17]. A key component in sustainable agriculture, LAB are easy to culture without sophisticated equipment and expertise as Lanton *et al*. (2017) [21] review how LAB relieve the plants of (i) stress from pests by producing antimicrobial metabolites and through pre-emptive colonization, (ii) environmental stress through systemic acquired resistance and abiotic stress alleviation, (iii) nutrient limitations through biofertilization. Imidacloprid is both a systemic and contact poison (Yamamoto, 1999) [38], effective against all the three sucking insects as observed in this investigation on bhendi in both screenhouse and field experiments. However, its efficacy after spraying was influenced by the LAB in Milkoid and by the bleaching powder as well. For example, the epiphytic LAB density, when compared to that on unsprayed control, got increased by 84.68 per cent after spraying Milkoid and by 100.0 per cent after spraying imdiacloprid / Milkoid (Table 4, Fig. 2 - 3), compared to 54.34 – 55.17 per cent reduction after spraying bleaching powder, with or without imidacloprid. Pest-wise, imidacloprid, with or without bleaching powder, decreased the overall injury due to *B. tabaci* by 85.63-86.92 per cent, *A. gossypii* infestation by 83.63 – 85.96 per cent, and *A. biguttula biguttula* numbers by 79.9 – 84.1 per cent, compared to that on control plants (Table 1 - 3, Fig. 3). In tandem with Milkoid, imidacloprid was second most effective, suppressing the *B. tabaci*-transmitted YMV infection by 67.13 per cent, *A. gossypii* infested plants by 72.22 per cent and *A. biguttula biguttula* population by 63.59 per cent when compared to leafhoppers in control. On the one hand, this indicates that the reduction in bioefficacy of imidacloprid was due to the LAB in Milkoid, the density of which was 100.0 per cent higher on these plants than on untreated control plants. On the other hand, bleaching powder, which reduced the LAB density by 54.34 – 55.17 per cent with or without imidacloprid, caused YVM injury to decrease by 55.60 per cent, aphid infestation by 65.1 per cent and leafhoppers by 51.04 per cent (Table 1-3, Fig. 3). Usually, it is used as a disinfectant for sanitizing the sprouting seeds (Damron *et al*., 2005) [8], rice seeds (Miche *et al*., 2001) [22] and tissue culture plants (Oyebanji *et al*., 2009) [27]. Alone, Milkoid was able to increase the phyllosphere LAB by 84.68 per cent and reduce YVM infected plants by 34.18 per cent, aphid-infested plants by 32.19 per cent and leafhoppers by 17.57 per cent (Table 1-3, Fig.3). That is though it caused significantly marginal reduction in insects or their damage, it made imidacloprid significantly less effective or less hazardous, probably through biodegradation which needs to be studied further by assessing the residue levels. There are reports that LAB degrade pesticides not only in products like kimchi (Cho *et al.*, 2009) [7] and skimmed milk (Zhou and Zhao, 2014) [40] but also on plants as epiphytic (Islam, 2010) [18]. Though not an insecticide, Gupta *et al.* (2015) [14] observed reduction in sucking pests on rose plants after spraying cow's milk. Milk is one of the five components of a traditional preparation cow-five (panchakavya) in pest management, fermented by mixed species of microbes (Belina *et al.*, 2005a; Belina *et al.*, 2005b) [3, 4]. Milkoid is only sugar-preserved, egg-nourished milk undergoing controlled fermentation only by sugar-tolerant LAB. It is in the form of a colloid, probably due to both calcium caseinate and calcium phosphate in mixture (Gaucheron, 2005) [15]. However, Milkoid is better than milk as it acts as an adjuvant, improving the spray fluid efficiency, especially its wettability and stickiness so that it can be used as an alternative to chemical wetting agents on edible crops. Once mixed in water, with or without imidacloprid, its LAB density increases. Not a pure culture, the strains of LAB in Milkoid need to be identified. When consumed, bhendi fruits sprayed with Milkoid would probably have more such LAB with probiotic properties but less of imidacloprid residues, probably protecting the non-target organisms like honey bees from man-made xenobiotics. Moreover, both LAB and bleaching powder are likely to influence host-finding by insects as well as microbial volatiles attract or repel insects by eliciting behavioural changes (Davis *et al.*, 2013) [10] as demonstrated by Venu *et al*. (2014) [34] that volatiles derived from microbes are responsible for long distance attraction of fruit flies to their food sources. More research is needed in this area in crop protection in future. In conclusion, the milk-made sugar-preserved LAB formulation Milkoid could be used as an adjuvant bio-remedy to limit the toxicity of pesticides like imidacloprid in view of their environmental hazards to the consumers and non-target organisms like honey bees, which need further study. Whether it could be mixed directly with insecticide-mixed spray fluids also needs further investigations. Its interaction with other phyllosphere microbes is also not known. It is also worth exploring the potential of calcium hypochlorite in crop protection as it suppressed the population density of not only the epiphytic LAB but also insects when sprayed alone or in tandem with imidacloprid.

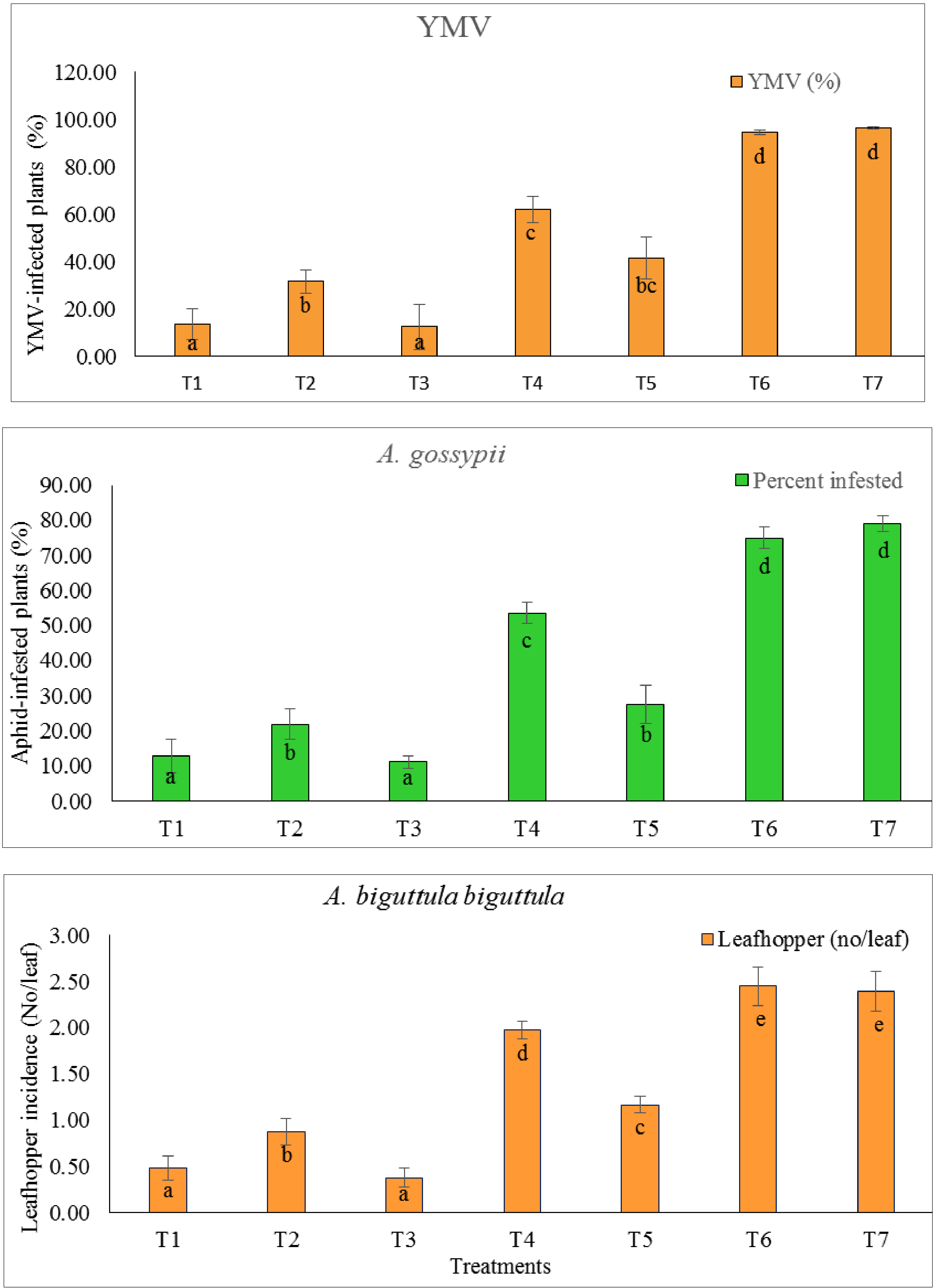

Fig 1: Suppression of sucking pests in bhendi. *B. tabaci*-transmitted YMV-infected plants (%), *A. gossypii*-infested plants and *A. biguttula biguttula* density (No./ leaf) after spraying imidacloprid, Milkoid and bleaching powder in bhendi. T1, imidacloprid 17.8 SL 0.2 ml/l; T2, imidacloprid 17.8 SL 0.2 ml/l / Milkoid 2.0 %; T3, imidacloprid 17.8 SL 0.2 ml/l / bleaching powder 1.0 %; T4, Milkoid 2.0 %; T5, bleaching powder 1.0 %; T6, water spray; T7, untreated control; Mean of three replications; Means followed by the same letter are not significantly different. Vertical bars indicate the standard error.

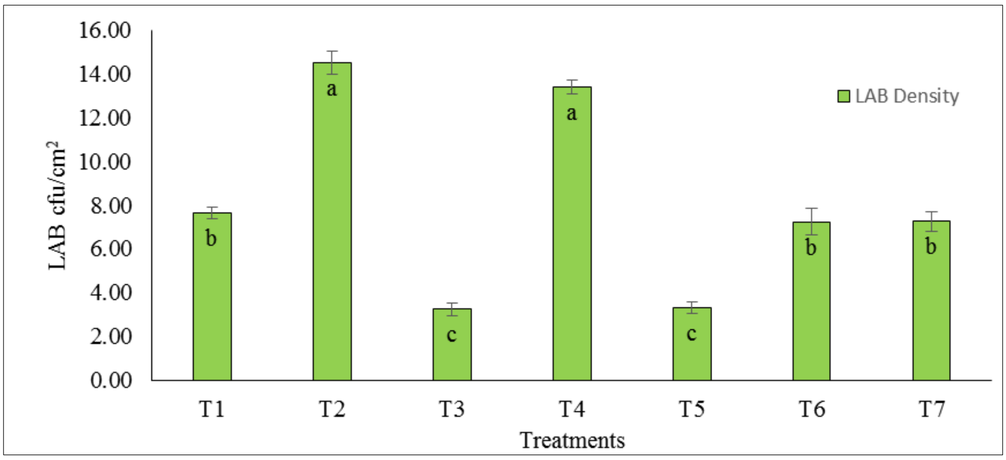

Fig 2: LAB population density after spaying imidacloprid, Milkoid and bleaching powder in bhendi. T1, Imidacloprid 17.8 SL 0.2 ml/l; T2, Imidacloprid 17.8 SL 0.2 ml/l / Milkoid 2.0 %; T3, Imidacloprid 17.8 SL 0.2 ml/l / Bleaching powder 1.0 %; T4, Milkoid 2.0 %; T5, Bleaching powder 1.0 %; T6, Water spray; T7, Untreated control; Mean of three replications; Means followed by the same letter are not significantly different; CFU, colony forming units; Figures in the parenthesis are log transformed values. Vertical bars indicate the standard error.

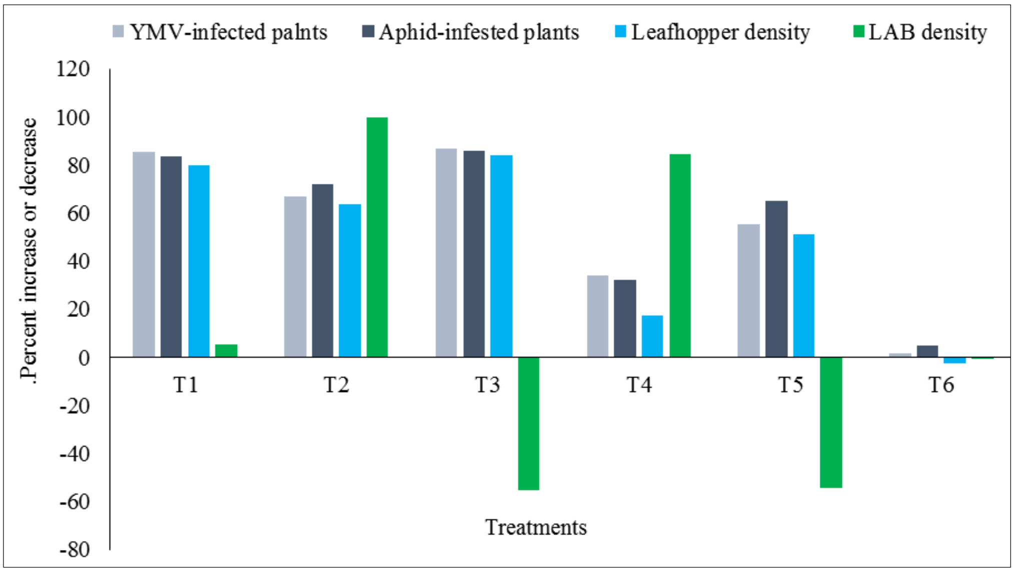

Fig 3: Percent reduction from or increase over control in pests and LAB density after spraying imidacloprid, Milkoid and bleaching powder in bhendi. T1, Imidacloprid 17.8 SL 0.2 ml/l; T2, Imidacloprid 17.8 SL 0.2 ml/l / Milkoid 2.0 %; T3, Imidacloprid 17.8 SL 0.2 ml/l /Bleaching powder 1.0 %; T4, Milkoid 2.0 %; T5, Bleaching powder 1.0 %; T6, Water spray.

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