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Cowine, an Organic Adjuvant from Lactic Acid Bacteria-Rich Edible Commodities in Suppressing the Sucking Pests of Rice (*Oryza sativa*)

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ABSTRACT

Adjuvants are often mixed with insecticides during formulating or spraying. Resurgence of sucking pests follows pesticide application in rice. Increased tank mixing of most potent adjuvants such as organosilicone surfactants has been associated with declining health of honey bee population. Studies were carried out to investigate the influence of cowine, a safe lactic acid bacteria (LAB) fermented product made from cane jaggery, milk powder and grape juice/rice water that can be mixed with any spray fluid as an adjuvant. Cowine was evaluated against sucking pests in rice, especially in combination with neem oil, quinalphos, acephate and imidacloprid to reduce the probability of resurgence. LAB population was higher on plants sprayed with cowine and neem oil+cowine. Brown planthopper (BPH), *Nilaparvata lugens* (Stal.) adults were more numerous on plants treated with cowine. Neem oil + cowine reduced oviposition by BPH. Quinalphos, alone or in combination with cowine, increased egg laying by BPH. Egg parasitization was more on cowine and quinalphos + cowine treated plants, while quinalphos and neem oil+cowine decreased it. LAB increased both BPH oviposition and parasitoids activity. Rice mite, *Oligonychus oryzae* (Hirst) adults and eggs population were more abundant after cowine spray. The results recommended that cowine and cowine+neem oil increased paddy seed germination while plants grew faster with neem oil+cowine spray. Phenol content increased in rice following sprays with cowine, either alone or when sprayed with neem oil.

INTRODUCTION

Though insecticides are used need-based in IPM, farmers find it difficult to follow this principle in practice. Consequently, nearly 70 % of the chemical insecticides produced in India are used for pest control alone, from seed protection to grain protection (Gokila, 2017; Hertlein *et al*., 2011). However, chemical pesticides often lead to resistance in insects, leave pesticide residues in the trophic chain, kill natural enemies of the pests, upset the natural balance between insects and their natural enemies (Van den Bosch and Messenger, 1973) and cause pest outbreaks and resurgence (Doi *et al*., 2013; McClure, 1977). Adjuvants in pesticides comprise a large and heterogeneous group of substances (Valkenburg, 1982). They are defined as an ingredient in the pesticide formulation that which aids

Abbreviations
LAB, lactic acid bacteria; BPH, Brown planthopper.

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or modifies the action of the ingredient (Foy, 1987). They are of two types, formulation adjuvants and spray adjuvants (Krogh *et al*., 2003). Spray adjuvants are called tank mixing additives whereas the formulation adjuvants are called additives (Hochberg, 1996). They are designed to act as wetting agents, spreaders, stickers, emulsifiers, dispersing agents and drift-control agents (Cai and Starck, 1997). They enhance the adsorption, penetration and translocation of the active ingredients into the target plants (Foy, 1993). However, agrochemical spray adjuvants can also be toxic to the non-target organisms such as honey bees. Thus there is a need to have safer adjuvants as well.

Biological control envisages the use of natural enemies and microbial control uses bacteria (e.g. *Bacillus thuringiensis*), viruses (e.g. NPV), fungi (e.g. *Metarhizium anisopliae*) and protozoa (e.g. *Nosema locustae*) in pest management. As a bacteria, *B.t.* has been well exploited in agriculture especially in transgenics (Rao and Solanki, 2003). Lactic acid bacteria (LAB) are probiotic (pro = for, biotic = life), i.e. living bacteria that, when administered in adequate quantities, confer a health benefit on the host, capable of colonizing the intestines after being ingested with a positive effect on human and animal health (FAO/WHO, 2001; Sretenovic *et al*., 2008). LAB are generally recognized as safe (GRAS), G-positive, non-spore forming, immobile, catalase negative bacteria, which excrete lactic acid, acetic acid, hydrogen peroxide and bacteriocins (antimicrobial metabolites) and create an acidic environment that inhibits the harmful pathogens (Konings *et al*., 2000). LAB associated with foods include species of the genera *Carnobacterium, Enterococcus, Lactobacillus, Lactococcus, Leuconostoc, Oenococcus, Pediococcus, Streptococcus, Tetragenococcus, Vagococcus* and *Weissella* (Stiles and Holzapfel, 1997). They are either cocci or rods, subdivided into homo-fermentative or hetero-fermentative. They are able to survive in all extreme conditions. For instance, *Leuconostoc* and *Lactococcus* grow at a lower pH of 4.0-4.5 and stone Lactobacilli and Pedicocci even up to 3.5 (Steinkraus, 1983). LAB isolated from yogurt and milk are more resistant to stress conditions (Hamed *et al*., 2011). They are found even in insects like honey bees (Olofsson and Tobias, 2008). Jayanthi (2016) suggested that LAB can be used to manage sucking pests. Most plants have LAB too, especially on the oviposition sites (Harshini, 2016). Among the botanicals, neem is commonly used in IPM. However, products like neem oil need a wetting agent to prepare the spray fluid. The development a wetting agent from commonly available resources rich in LAB will exclude the dependence of farmers on chemical wetting agents. With this background, the present investigation was carried out to bring an ecofriendly adjuvant based on milk powder, grape juice or rice rinse water and stone-pressed cane jaggery.

MATERIALS AND METHODS

Investigations on the influence of the adjuvant cowine on sucking pests of rice were carried out under screenhouse conditions at AC and RI, Killikulam, Tamil Nadu, India.

Cowine

The adjuvant cowine was prepared by mixing milk powder, grape juice or rice rinse water and stone-pressed cane jaggery (Gokila, 2007).This fermenting cowine stock was kept in a wide mouthed container with its lid loosely closed. It stirred up twice a day before it was ready for spraying in a week.

Spray fluids and seed treatments

Cowine emulsion was prepared by mixing 1 part of it with 4 parts of water in the first step. This milky emulsion was kept for one day for LAB multiplication. The next day 25 ml of this milky solution was mixed with one liter of water before spraying on crop with hand operated hydraulic sprayer using 500 L of spray fluid/ ha. Neem oil+cowine emulsion was prepared by mixing 2 parts of cowine with one part of neem oil before mixing it with 4 parts of water and stirred well. This milky emulsion was then mixed with 2 ml of water at the rate of 20 ml per L quinalphos (Ekalux, 25 EC) 2 ml was thoroughly mixed with one liter of water to get 0.2 % emulsion. Imidacloprid 0.6 ml (Confidor 17.8 SL) was mixed in one liter of water. Acephate (Astaf 75 SP) was mixed at the rate of 2 g per liter of water. Paddy seeds (ASD 16) (50 g) were soaked in water overnight, drained the next day, mixed well with cowine at the rate of 25 ml per kg of seed and shade dried for 1 h before sowing in the nursery bed. ASD16 seeds (50 g) were mixed well with 0.5 ml of imidacloprid 48 FS (Gaucho), allowed to shade dry for 1 h before sowing. To treat the seeds with both imidacloprid and cowine, first paddy seeds (50 g) were mixed with 0.5ml of imidacloprid 48 FS (Gaucho). A few minutes later 1.25 ml of cowine was added and mixed well before shade drying and sowing.

Screenhouse experiments

Screenhouse experiments were conducted with zinc trays (90 x 45 cm) for rice. In each tray 20 plants in tube pots (15 x 10 cm) were arranged in rows. In rice, the influence of cowine on *N. lugens* and *O. oryzae* was assessed at different stages of rice crop growth. Seed treatment preceded spraying at fortnightly interval with cowine, neem oil+cowine, quinalphos (Ekalux, 25 EC) + cowine, quinalphos in comparison with untreated control. A total of four sprays were applied in both experiments.

Both BPH nymphs and adults were counted at 15 day interval from all the 20 plants in each treatment. To assess the BPH population, 20 old leaf sheaths were collected at random in petriplates one from each plant. They were examined in the laboratory under a stereo zoom binocular microscope (MOTICAM 1000 1.3 M PIEXEL USB 2.0) for the presence of BPH eggs and parasitization of eggs at fortnightly interval (Plate 2). *O. oryzae* population was assessed in all the 20 plants in each treatment by counting its adults, nymphs and eggs per leaf. Unidentified predatory thrips were recorded from the entire leaf area on 20 leaves, one from each hill in each treatment. All observations were recorded at fortnightly interval after each spray.

Statistical analysis

The software Agres was used to analyse the data on all parameters with suitable transformations, if needed. Data on the percentage of germination were analysed after arcsine transformation. Number of BPH adults, BPH eggs, rice mite were analysed after square root (x+0.5) transformation. The data on LAB were log transformation before analysis of variance.

RESULTS

BPH eggs

In experiment 1, BPH laid significantly fewer eggs on leaf sheaths of plants sprayed with neem oil+cowine (2.36/ leaf sheath), on a par with that on control plant leaves (2.50/leaf sheath), quinalphos (3.05/leaf sheath) and LAB (3.38/leaf sheath) (Table I). Significantly highest egg density was found on leaf sheaths of plants treated with quinalphos+cowine (3.81/leaf sheath), comparable to that on leaves that received LAB and quinalphos+cowine. The rate significantly increased from 1.07 per sheath at spray 1 to 4.67 per sheath at spray 4.

Table I. Oviposition by BPH in rice leaf sheaths after cowine spray in experiment 1 and 2 (CFU/leaf bit).

Treatments	BPH eggs (Numbers/leaf sheath)				Mean	% increase/decrease over control
	Spray 1	Spray 2	Spray 3	Spray 4		
Experiment 1						
Cowine* @ 25 ml/l	0.55 (1.00)	2.10 (1.36)	6.40 (2.55)	4.50 (2.22)	3.38 (1.78)	+35.20
Neem oil 1% + cowine* @ 25 ml/l	0.65 (1.02)	1.80 (1.41)	4.95 (2.25)	2.05 (1.59)	2.36 (1.57)	- 5.60
Quinalphos 25 EC 0.2% + cowine* @ 25ml/l	1.40 (1.34)	4.20 (2.09)	4.90 (2.25)	4.75 (2.26)	3.81 (1.99)	+52.40
Quinalphos 25 EC 0.2%	1.30 (1.29)	0.80 (1.07)	1.55 (1.39)	8.55 (2.95)	3.05 (1.68)	+22.00
Control	1.45 (1.34)	1.00 (1.20)	4.05 (2.11)	3.50 (1.99)	2.50 (1.66)	-
Mean	1.07 (1.20)	1.98 (1.42)	4.37 (2.11)	4.67 (2.20)	-	-
Experiment 2						
Cowine* @ 25 ml/l	1.25 (1.21)	1.75 (1.49)	4.85 (2.31)	6.30 (2.81)	3.53 (1.95)	+52.81
Neem oil 1% + cowine* @ 25 ml/l	0.75 (1.10)	1.55 (1.39)	1.30 (1.26)	2.60 (1.75)	1.55 (1.38)	- 32.90
Quinalphos 25 EC 0.2% + cowine* @ 25ml/l	0.70 (1.06)	3.15 (1.89)	3.40 (1.92)	5.15 (2.15)	3.10 (1.76)	+34.19
Quinalphos 25 EC 0.2%	0.65 (1.00)	1.15 (1.21)	2.70 (1.69)	3.81 (2.03)	2.07 (1.48)	- 10.38
Control	1.95 (1.47)	1.70 (1.47)	2.30 (1.64)	3.30 (1.91)	2.31 (1.62)	-
Mean	1.06 (1.17)	1.86 (1.49)	2.91 (1.77)	4.22 (2.13)	-	-
Experiment 1 + 2						
Cowine* @ 25 ml/l	0.90 (1.11)	1.92 (1.42)	5.62 (2.43)	5.40 (2.51)	3.46 (1.87)	+44.16
Neem oil 1% + cowine* @ 25 ml/l	0.70 (1.06)	1.67 (1.40)	3.12 (1.76)	2.32 (1.67)	1.95 (1.47)	- 18.75
Quinalphos 25 EC 0.2% + cowine* @ 25ml/l	1.05 (1.20)	3.67 (1.99)	4.15 (2.09)	4.95 (2.20)	3.49 (1.87)	+45.40
Quinalphos 25 EC 0.2%	0.97 (1.14)	0.97 (1.14)	2.12 (1.54)	6.18 (2.49)	2.56 (1.58)	+6.66
Control	1.70 (1.40)	1.35 (1.33)	3.17 (1.88)	3.40 (1.95)	2.40 (1.64)	-
Mean	1.06 (1.18)	1.91 (1.46)	3.63 (1.94)	4.45 (2.17)	-	-

*Cowine + water (1:4) fermented overnight, (Mean of 5 observations. Figures in parenthesis are square root x + 0.5 transformed values. LAB, lactic acid bacteria), CD ($P < 0.05$). BPH, brown plant hopper.

In experiment 2 also BPH laid significantly fewer eggs on leaf sheaths of plants sprayed with neem oil+cowine (1.55/leaf sheath), quinalphos (2.07/leaf sheath) and on control leaves (2.31/leaf sheath), all on par with each other, followed by quinalphos+cowine (3.10/leaf sheath) (Table I). The eggs were more numerous on leaf sheaths of plants treated with LAB (3.53/leaf sheath) as well as quinalphos+cowine (3.10-3.53/leaf sheath). Quinalphos+cowine and cowine were on a par with each other in egg density. The oviposition rate increased significantly after spray 1(1.06/sheath) to spray 4 (4.22/sheath). Results of both the experiments combined indicated that BPH preferred to lay significantly more eggs on leaves treated with quinalphos+cowine (3.49/leaf sheath) and cowine (3.46/leaf sheath), then on other leaves (1.95/sheath) in neem oil+cowine 2.5/sheath in quinalphos (Fig. 1) or no spray control (2.4/sheath) (Table I).

Parasitized BPH eggs

The number of parasitized BPH eggs in experiment 1 were significantly larger on leaf sheaths of plants sprayed with quinalphos+cowine (1.25/leaf sheath), than on other leaves including control (0.23/leaf sheath) in control to 0.62/leaf sheath in LAB (Table II). The rate of parasitization was significantly more after sprays 1-2 (0.11–0.48/sheath) than after spray 3 (1.38/sheath). Statistically, there was no significant difference in parasitization among the treatments in experiment 2, indicating that the none of the treatments had any significant influence on egg parasitization (Table II). Pooled analysis of the experiment data highlighted that parasitized BPH eggs were significantly more on leaf sheaths sprayed with quinalphos+cowine and LAB (0.69-0.37/leaf sheath), compared to other treatments (0.17-0.20/leaf sheath) (Table II).

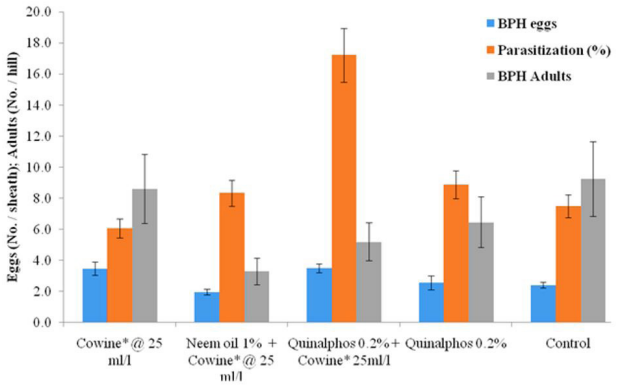

Fig. 1. BPH adults, eggs and parasitization following cowine spray in rice. Vertical bars indicate the standard error. * cowine+water (1:4) fermented over night.

Table II. Parasitization of BPH eggs after spraying with cowine in experiment 1 and 2.

Treatments	Parasitization of BPH eggs (Numbers/leaf sheath)			Mean	% increase/decrease over control
	Spray 1	Spray 2	Spray 3		
Experiment 1					
Cowine* 25 ml/l	0.45 (0.90)	0.3 (0.87)	1.12 (1.18)	0.62 (0.99)	+520
Neem oil 1% + cowine * @ 25 ml/l	0.00 (0.70)	0.15 (0.79)	2.80 (1.08)	0.98 (0.86)	+880
Quinalphos 25 EC 0.2%+cowine *@ 25ml/l	1.50 (1.31)	0.00 (0.70)	2.25 (1.65)	1.25 (1.22)	+1150
Quinalphos 25 EC 0.2%	0.40 (0.89)	0 (0.70)	0.60 (1.02)	0.33 (0.87)	+230
Control	0.05 (0.77)	0.10 (0.76)	0.15 (0.79)	0.10 (0.84)	-
Mean	0.48 (0.91)	0.11 (0.80)	1.38 (1.16)	--	-
Experiment 2					
Cowine * @ 25 ml/l	0.15 (0.79)	0.05 (0.73)	0.20 (0.82)	0.13 (0.90)	- 18.75
Neem oil 1% + cowine * @ 25 ml/l	0.10 (0.77)	0.05 (0.73)	0.15 (0.79)	0.10 (0.76)	-37.50
Quinalphos 25 EC 0.2% +cowine*@ 25ml/l	0.15 (0.79)	0.00 (0.70)	0.30 (0.86)	0.15 (0.79)	- 6.25
Quinalphos 25 EC 0.2%	0.05 (0.73)	0.00 (0.70)	0.00 (0.70)	0.01 (0.71)	- 93.75
Control	0.05 (0.73)	0.30 (0.89)	0.15 (0.79)	0.16 (0.78)	-
Mean	0.10 (0.76)	0.08 (0.75)	0.16 (0.79)	-	-
Experiment 1+2					
Cowine * @ 25 ml/l	0.30 (0.85)	0.17 (0.80)	0.66 (1.00)	0.17 (0.79)	+30.76
Neem oil 1% + cowine * @ 25 ml/l	0.05 (0.73)	0.10 (0.76)	1.47 (0.93)	0.54 (0.81)	+315.38
Quinalphos 25 EC 0.2%+cowine *@ 25ml/l	0.82 (1.05)	0 (0.70)	1.27 (1.25)	0.69 (1.00)	+430.76
Quinalphos 25 EC 0.2%	0.22 (0.81)	0 (0.70)	0.30 (0.86)	0.17 (0.79)	+30.76
Control	0.05 (0.73)	0.20 (0.91)	0.15 (0.83)	0.13 (0.82)	-
Mean	0.28 (0.84)	0.09 (0.78)	0.77 (0.98)	-	-

For statistical details and abbreviations, see Table I.

Table III. BPH adult population density in rice following cowine spray in experiment 1 and 2.

Treatments	BPH adults (Numbers/hill)				Mean	% increase/decrease over control
	Spray 1	Spray 2	Spray 3	Spray 4		
Experiment 1						
Cowine* @ 25 ml/l	0.50 (0.98)	5.00 (2.26)	6.75 (2.60)	18.75 (4.25)	7.75 (2.52)	- 4.90
Neem oil 1 % + cowine* @ 25 ml/l	0.35 (0.91)	6.60 (2.57)	3.55 (1.98)	3.65 (2.02)	3.53 (1.87)	- 56.68
Quinalphos 0.2% 25 EC + cowine 25 ml/l	3.00 (1.73)	1.15 (1.23)	6.70 (2.67)	13.05 (3.66)	5.97 (2.32)	+26.74
Quinalphos 0.2% 25 EC	0.50 (0.97)	1.20 (1.29)	3.25 (1.92)	18.35 (4.26)	5.82 (2.11)	- 28.58
Control	1.05 (1.23)	4.95 (2.29)	5.05 (2.30)	21.55 (4.65)	8.15 (2.62)	-
Mean	1.08 (1.16)	3.78 (1.93)	5.06 (2.29)	15.07 (3.77)	-	-
Experiment 2						
Cowine* @ 25 ml/l	6.85 (2.67)	12.95 (3.63)	7.30 (2.77)	10.85 (3.35)	9.48 (3.11)	- 8.49
Neem oil 1% + cowine* @ 25 ml/l	3.75 (1.97)	4.00 (2.07)	2.75 (1.77)	1.60 (1.41)	3.02 (1.80)	- 70.84
Quinalphos 25 EC 0.2% +cowine*@ 25 ml/l	1.50 (1.33)	3.45 (1.94)	3.15 (1.90)	9.55 (3.14)	4.41 (2.08)	-57.43
Quinalphos 25 EC 0.2%	0.80 (1.01)	1.50 (1.36)	7.45 (2.76)	18.35 (4.35)	7.07 (2.37)	-31.75
Control	8.45 (2.94)	15.35 (3.94)	8.95 (3.05)	8.70 (3.01)	10.36 (3.24)	-
Mean	4.27 (1.98)	7.45 (2.59)	5.92 (2.45)	8.11 (3.05)	-	-
Experiment 1+2						
Cowine* @ 25 ml/l	3.76 (1.82)	8.97 (2.95)	7.02 (2.69)	14.80 (3.80)	8.61 (2.81)	- 6.91
Neem oil 1% + cowine* @ 25 ml/l	2.05 (1.44)	5.30 (2.32)	3.15 (1.87)	2.62 (1.71)	3.28 (1.84)	- 64.50
Quinalphos 25 EC 0.2% + cowine* @ 25ml/l	2.25 (1.53)	2.30 (1.58)	4.92 (2.28)	11.30 (3.40)	5.19 (2.20)	- 43. 89
Quinalphos 25 EC 0.2%	0.65 (0.99)	1.35 (1.32)	5.35 (2.34)	18.45 (4.30)	6.45 (2.24)	-30.27
Control	4.75 (2.09)	10.15 (3.61)	7.00 (2.68)	15.12 (3.83)	9.25 (3.05)	-
Mean	2.67 (1.57)	5.61 (2.36)	5.48 (2.37)	12.45 (3.41)	-	-

For statistical details and abbreviations, see Table I.

BPH adults

In experiment 1, the mean number of BPH adults was significantly minimum on plants sprayed with neem oil+cowine (3.53/hill) and quinalphos (5.28/hill) (Table III). A moderate level of population was found on plants sprayed with quinalphos+cowine (5.97/hill), LAB (7.75/hill). BPH adults were more numerous on control plants as on cowine sprayed (7.75-8.15/hill). In experiment 2 also, neem oil+cowine spray resulted in significantly fewer BPH adults (3.02/hill), on par with quinalphos+cowine (4.41/hill) (Table III). Control and cowine sprayed plants had significantly more adults than other leaves (9.48-10.36/hill). From the pooled data analysis too, neem oil+cowine was found to support significantly minimum number of BPH adults (3.28/hill) (Fig. 1) (Table III). Both control plants and those treated with cowine had maximum numbers (8.61-9.25/hill) whereas then population was of moderate numbers on plants sprayed with quinalphos or quinalphos+cowine spray (5.19/hill).

O. oryzae *eggs*

In experiment 1, the rice mite *O. oryzae* laid significantly fewer eggs on leaves sprayed with cowine in combination with quinalphos or neem oil (4.65-4.95/leaf) and more on control and cowine sprayed leaves (4.62-6.62/leaf). A moderate density of 5.33 per leaf was observed on quinalphos-treated leaves (Table IV). In experiment 2, the mites laid significantly fewer eggs on leaves sprayed with neem oil+cowine (3.40/leaf), quinalphos (4.35/leaf) and on control leaves (4.55/leaf) (Table IV). The eggs were more numerous on leaves treated with cowine (9.28/leaf). The density in control was in similar to that of quinalphos+cowine (4.55-5.81/leaf). From both the experiments, the eggs density was found minimal on leaves sprayed with a neem oil+cowine, quinalphos, quinalphos+LAB and control (4.17-5.58/leaf) (Fig. 2). The density was more on cowine treated leaves (9.26/leaf), on a par with that on control (5.58/leaf) (Table IV).

Table IV. *O. oryzae* eggs on rice leaves following cowine spray in experiment 1 and 2.

Treatments	Mite eggs (Numbers/leaf)			Mean	% increase/decrease over control
	Spray 1	Spray 2	Spray 3		
Experiment 1					
Cowine* @ 25 ml/l	2.35 (2.73)	2.00 (1.57)	9.50 (3.14)	4.62 (2.48)	- 30.21
Neem oil 1% + cowine* @ 25 ml/l	4.95 (2.22)	7.70 (2.85)	2.20 (1.62)	4.95 (2.23)	- 25.22
Quinalphos 25 EC 0.2% + cowine* @ 25ml/l	9.25 (3.03)	2.05 (1.56)	2.65 (1.76)	4.65 (2.12)	- 29.75
Quinalphos 25 EC 0.2%	5.25 (2.38)	7.9 (2.85)	2.85 (1.82)	5.33 (2.35)	- 19.48
Control	9.35 (3.11)	8.15 (2.91)	2.35 (1.68)	6.62 (2.57)	-
Mean	4.38 (2.69)	5.56 (2.35)	3.91 (2.00)	-	-
Experiment 2					
Cowine* @ 25 ml/l	9.05 (3.05)	18.2 (4.31)	0.60 (1.02)	9.28 (2.79)	+103.95
Neem oil 1% + cowine* @ 25 ml/l	2.00 (1.50)	7.05 (2.73)	1.15 (1.26)	3.40 (1.83)	- 25.27
Quinalphos 25 EC 0.2% + cowine* @ 25ml/l	5.65 (2.45)	10.40 (3.27)	1.40 (1.30)	5.81 (2.34)	27.96
Quinalphos 25 EC 0.2%	3.75 (2.02)	8.70 (2.97)	0.60 (0.99)	4.35 (1.99)	- 4.39
Control	2.45 (1.66)	9.85 (3.19)	1.35 (1.35)	4.55 (2.07)	-
Mean	4.58 (2.14)	10.84 (3.29)	1.02 (1.19)	-	-
Experiment 1+2					
Cowine* @ 25 ml/l	5.70 (2.89)	10.10 (2.94)	5.05 (2.08)	9.26 (2.64)	+65.94
Neem oil 1% + cowine* @ 25 ml/l	3.47 (1.86)	7.37 (2.79)	1.67 (1.44)	4.17 (2.03)	- 25.26
Quinalphos 25 EC 0.2% + cowine * @ 25ml/l	7.45 (2.74)	6.22 (2.41)	2.02 (1.53)	5.23 (2.23)	- 6.27
Quinalphos 25 EC 0.2%	4.50 (2.20)	8.30 (2.91)	1.72 (1.40)	4.84 (2.17)	- 13.26
Control	5.90 (2.38)	9.00 (3.05)	1.85 (1.52)	5.58 (2.32)	-
Mean	5.40 (2.42)	8.19 (2.82)	2.46 (1.59)	-	-

For statistical details and abbreviations, see Table I.

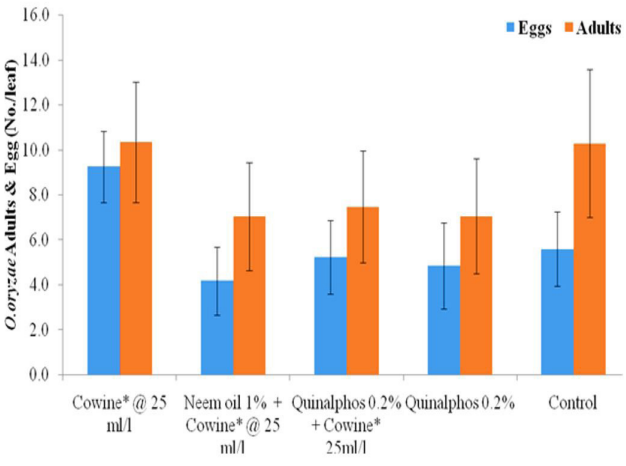

Fig. 2. Rice mite, *O. oryzae*eggs and adults following cowine application in rice. Vertical bars indicate the standard error. *Cowine+water (1:4) fermented over night.

O. oryzae *adults*

In experiment 1, *O. oryzae* mite population was significantly lower in on leaves sprayed with quinalphos, either alone or in combination with cowine (5.31-5.76/ leaf), followed by cowine and neem oil+cowine (8.07-9.32/ leaf) (Table V) and the mites population were significantly more abundant on control leaves (16.5/leaf). In experiment 2, the mite population density was significantly as less on control leaves (4.03/leaf) on leaves treated with neem oil+cowine (4.78/leaf) (Table V). The density was highest on leaves treated with cowine (12.61/leaf) and moderate on leaves sprayed with quinalphos, either alone or in mixture with cowine (8.80–9.2/leaf). The pooled data indicated that the mite population was significantly more on leaves sprayed with quinalphos, quinalphos+cowine and neem oil+cowine (7.05-7.47/leaf) (Fig. 2) and more numerous on control leaves and cowine treated (10.29-10.34/leaf) (Table V). The mites increased significantly in number after the spray 1, reached peaks during sprays 2-3 before declining after the spray 4.

Table V. *O. oryzae* adults on rice leaves following spray in experiment 1 and 2.

Treatments	Adult mite (Numbers/leaf)				Mean	% increase/decrease over control
	Spray 1	Spray 2	Spray 3	Spray 4		
Experiment 1						
Cowine* @ 25 ml/l	2.55(1.62)	9.30(3.03)	14.75(3.82)	6.00(2.54)	8.07 (2.75)	- 51.09
Neem oil 1% + cowine* @ 25 ml/l	2.90(1.83)	16.00(3.91)	12.30(3.55)	6.10(2.55)	9.32 (2.96)	- 43.51
Quinalphos 25 EC 0.2%+cowine* @ 25ml/l	0.45(0.94)	4.80(2.28)	14.70(3.77)	3.10(1.84)	5.76 (2.20)	- 65.09
Quinalphos 25 EC 0.2%	0.85(1.14)	5.45(2.39)	12.35(3.57)	2.60(1.74)	5.31 (2.21)	- 67.81
Control	8.05(2.90)	22.2(4.68)	27.20(4.76)	8.75(3.03)	16.50 (3.84)	-
Mean	2.90(1.69)	11.55(3.26)	16.26(3.89)	5.31(2.34)	-	-
Experiment 2						
Cowine* @ 25 ml/l	12.85(3.63)	20.25 (4.52)	14.95 (3.92)	2.40 (1.68)	12.61 (3.44)	+212.90
Neem oil 1% + cowine* @ 25 ml/l	2.00 (1.53)	7.15 (2.72)	9.35 (3.09)	0.65 (1.04)	4.78 (2.09)	+18.61
Quinalphos 25 EC 0.2% + cowine* @ 25ml/l	9.65 (3.13)	16.55 (4.05)	10.25 (3.21)	0.35 (0.90)	9.20 (2.82)	+113.95
Quinalphos 25 EC 0.2%	7.55 (2.76)	17.95 (4.28)	9.85 (3.20)	0.25 (085)	8.80 (2.77)	+104.65
Control	1.20 (1.27)	4.25 (2.05)	9.20 (3.09)	1.50 (1.34)	4.03 (1.94)	-
Mean	6.57 (2.46)	13.23 (3.52)	10.72 (3.30)	1.03 (1.68)	-	-
Experiment 1+2						
Cowine* @ 25 ml/l	7.55 (2.62)	14.77 (3.77)	14.85 (2.43)	4.20 (3.87)	10.34 (3.09)	+0.004
Neem oil1% + cowine* @ 25 ml/l	2.45 (1.68)	11.57 (3.31)	10.82 (3.32)	3.37 (1.79)	7.05 (2.53)	- 31. 48
Quinalphos 25 EC 0.2% + cowine* @ 25 ml/l	5.05 (2.04)	10.67 (3.17)	12.47 (3.49)	1.72 (1.37)	7.47 (2.52)	- 27.40
Quinalphos 25 EC 0.2 % 0.2%	4.00 (1.95)	11.70 (3.33)	11.10 (3.38)	1.42 (1.30)	7.05 (2.49)	- 31.48
Control	4.62 (2.08)	13.22 (3.36)	18.20 (3.93)	5.12 (2.19)	10.29 (2.89)	-
Mean	4.73 (2.07)	12.38 (3.39)	13.48 (3.60)	3.16 (1.75)	-	-

For statistical details and abbreviations, see Table I.

Predatory thrips

In experiment 1, unidentified predatory thrips were significantly more on leaves treated with cowine (0.27/leaf) than on leaves in control (0.06-0.11/leaf) and neem oil+cowine sprayed (Table VI). They were nil on leaves sprayed with quinalphos alone and quinalphos+cowine. There was no significant difference in predatory thrips population among the treatments including control post spray (Table VI). They were not observed in quinalphos and quinalphos+cowine treatments while 0.1-0.17 thrips were found on other leaves. In the experiment 2 data analysis, more predatory thrips were found on leaves treated with LAB on par with that on control leaves (0.10-0.20/leaf) (Table VI), which was on par with neem oil+cowine (0.11/leaf). No predatory thrips were observed on leaves treated with quinalphos + cowine or quinalphos.

DISCUSSION

An adjuvant is a non-pesticide 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. Agricultural adjuvants perform specific functions including wetting, spreading, sticking, and spray drifting (Cai *et al*., 1997). Generally, farmers use wetting agents (e.g. Spreadmax, Sandowit, Teepol) while spraying insecticides in the field to improve their wettability and efficacy (Stock, 1998). This is also applicable to botanicals, especially neem products. It may cause phytotoxicity (e.g. chlorosis) after repeated usage. Other than the commercial formulations, neem products are mixed with one of the wetting agents before spray. As an alternative non-chemical, soap-free wetting agent, the adjuvant-cowine was developed from three naturally available edible materials rich in LAB, a group of beneficial bacteria called probiotics (Kelin *et al*., 1998). Sugarcane jaggery, milk powder and grapewine /rice water are reservoirs of LAB (Wollowski *et al*., 2001). They serve as a growth medium for LAB, improves the physical properties of a pesticide spray fluid and may reduce their residues postspray (Gokila, 2017) as they aid in microbial

Table VI. Unidentified predastory thrips population on rice leaves following cowine spray in experiment 1 and 2.

Treatments	Thrips (Number/leaf)				Mean	% increase/de-crease over control
	Spray 1	Spray 2	Spray 3	Spray 4		
Experiment 1						
Cowine* @ 25 ml/l	0.00 (0.70)	0.30 (0.86)	0.45 (0.95)	0.35 (0.90)	0.27 (0.86)	+118. 18
Neem oil 1% + cowine* @ 25 ml/l	0.00 (0.70)	0.20 (0.81)	0.05 (0.73)	0.00 (0.70)	0.06 (0.74)	- 45.45
Quinalphos 25 EC 0.2% + cowine* @ 25ml/l	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	- 100. 0
Quinalphos 25 EC 0.2%	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	- 100. 0
Control	0.10 (0.77)	0.10 (0.76)	0.15 (0.79)	0.10 (0.77)	0.11 (0.77)	-
Mean	0.02 (0.72)	0.12 (0.77)	0.13 0.78)	0.09 (0.76)	-	-
Experiment 2						
Cowine* @ 25 ml/l	0.10 (0.77)	0.10 (0.77)	0.30 (0.87)	0.05 (0.73)	0.13 (0.79)	+30.00
Neem oil 1% + cowine* @ 25 ml/l	0.00 (0.70)	0.05 (0.73)	0.60 (1.00)	0.05 (0.73)	0.17 (0.79)	+70.00
Quinalphos 25 EC 0.2% + cowine* @ 25ml/l	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	- 100. 0
Quinalphos 25 EC 0.2%	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	-100.0
Control	0.05 (0.73)	0.05 (0.73)	0.05 (0.73)	0.25 (0.83)	0.10 (0.76)	-
Mean	0.03 (0.72)	0.04 (0.73)	0.19 (0.80)	0.07 (0.74)	-	-
Experiment 1+2						
Cowine* @ 25 ml/l	0.05 (0.73)	0.20 (0.82)	0.37 (0.91)	0.20 (0.82)	0.20 (0.82)	+100.0
Neem oil 1% + cowine* @ 25 ml/l	0 .00 (0.70)	0.12 (0.77)	0.32 (0.87)	0.02 (0.72)	0.11 (0.76)	+10.00
Quinalphos 25 EC 0.2% + cowine* @ 25ml/l	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	-100.0
Quinalphos 25 EC 0.2%	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	0.00 (0.70)	-100.0
Control	0.07 (0.75)	0.07 (0.75)	0.10 (0.76)	0.17 (0.80)	0.10 (0.77)	-
Mean	0.02 (0.72)	0.07 (0.75)	0.15 (0.79)	0.07 (0.75)	-	-

For statistical details and abbreviations, see Table I.

degradation of pesticides. *Streptococcus*, *Pediococcus*, *Leuconostoc micrococcus*, *Lactobacillus plantarum* and *Leuconostoc mesenteroides* are dominant LAB in palm juice. *Lactococcus, Enterococcus, Streptococcus, Lactobacillus* and *Leuconostoc* are found in milk, milk powder and dairy products (Harzallah and Belhadj, 2013). *Lactobacillus acidophilus* and *L. planetarium* are good dairy starter cultures (Wouters *et al*., 2000). Species of *Lactobacillus, Enterococcus, Lactococcus* and *Weissella* were detected in grapewine (Yanagida *et al*., 2008). LAB such as *Oenococcus* and *Pediococcus* are involved in the malo-lacto fermentation of grapewine. Several species of *Lactobacillus* such as *L. johnsonii* and *L. plantarum* occur in fermented rice bran (Doi *et al*., 2013; Yokoyama *et al*., 2002). Rice rinse water is also a good source of LAB (Ikeda *et al*., 2013). Cowine of this investigation was found to have LAB, namely *Streptococcus* and *Lactobacillus* with a population density (28.3 x 10^5 CFU/ml^{-1}), identified by phenotypic and biochemical characterization. They are used in bio-preservation (Khandakar *et al*., 2014).

LAB produce antimicrobials (Konings *et al*., 2000). They also produce exopolysaccarides (EPS) and many other products. The efficacy of cowine was evaluated in rice, especially in combination with neem oil, quinalphos, acephate, imidacloprid, potential resurgence causing agents. Quinalphos and acephate cause resurgence of *N. lugens* in rice (Heinrichs *et al*., 1982) and *P. latus* in chilli (Ashokan *et al*., 1992), respectively. Neem oil not only controls BPH but also causes resurgence. When mixed with water cowine gives an emulsion stable atleast for 3-6 h. The first day it needs to be diluted in water in 1:4 ratio to allow the suppressed LAB to multiply (109.3 x 10^5 CFU/ml^{-1}). The next day this diluted cowine water is mixed with insecticide spray fluid @ 25-30 ml/l and sprayed on crop where it acts as a wetting and sticking agent. It has proved particularly effective in combination with neem oil. However, neem oil+cowine need to be mixed with water first in 1:2 ratio (neem oil:cowine) when it gives milky solution. When sprayed either alone or in mixture with quinalphos on rice, it causes BPH to lay more eggs on leaf sheath. Neem oil+cowine and quinalphos suppressed oviposition. This indicates that both cowine

and quinalphos+cowine promote oviposition by BPH but not in combination with neem oil. Neem oil is a potential antifeedant and oviposition deterrent for the control of *N. lugens*. In contrast, egg parasitization was more on cowine and quinalphos+cowine.

On the other hand, BPH adult density was lower on neem oil+cowine treated plants than moderate on quinalphos or quinalphos+cowine sprayed plants. Probably, cowine attract not only BPH but also parasitoids even in screenhouse where BPH culture is maintained all round the year. If this is probably in field too, the problem of BPH resurgence may not occur, especially due to increased egg parasitism as natural enemy destruction is cited as a major reason for its outbreak (McClure, 1977). The effect of neem products on BPH is well known (Krishnaianh *et al*., 1985). Quinalphos also suppressed all these three. However, in quinalphos+cowine both BPH oviposition and its parasitization increased, leaving a moderate BPH density, probably preventing resurgence, which occurs commonly in rice after spraying quinalphos and other insecticides (methyl parathion, fenthion, imidacloprid). More *O. oryzae* were attracted to cowine sprayed and control leaves for egg laying than other leaves (quinalphos+cowine, neem oil+cowine and quinalphos), probably under screenhouse conditions where their natural enemies are less common and the condition itself is favourable to mites. The data on mites also confirm that LAB can cause both insects and mites to lay more number of eggs. However, in combination with pesticides, especially neem oil, it suppressed both egg laying and population density. Resurgence of mite, *P. latus* follows application of neem products on chilli (David, 1991). Predatory thrips can feed on mite eggs. They were killed by quinalphos with or without cowine. Cowine or even neem oil+cowine were safe to natural enemies. As seed treatment, both cowine and neem oil+cowine were better than imidacloprid or imidacloprid+cowine. LAB has been reported to promote the growth in several crops.

Earlier, *L. josonii* in fermented rice bran was reported to promote plant growth. They promoted the growth of the crop as well BPH and mites to lay more eggs. Compared to control, the phenol content was also higher after cowine and neem oil+cowine sprays, probably due to more stress from increased oviposition rate. Both high phenol profile and parasitism may not lead to resurgence (Doi *et al*., 2013). Similar to phenol, LAB colonization was also more on leaves sprayed with cowine and neem oil+cowine than on control, quinalphos+cowine leaves. Thus in the presence of these three factors, namely, high growth rate, phenol content and LAB density, BPH is not likely to resurge in rice as demonstrated in this research. Phenol content in rice and BPH oviposition negatively correlated (Suri and Singh, 2010).

CONCLUSION

As an adjuvant, cowine had significant influence on BPH, *O. oryzae* mites and crop growth. Cowine, may be used as a potential reservoir of proboitic LAB, similar to panchakavya, can be used on crop plants, either alone or mixed with botanicals and pesticides towards the goal of reducing pesticides usage.

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