



RESEARCH ARTICLE

Bio-efficacy of cow-urine-based neem, nochi and adhatoda extracts against sucking pests and impact on natural enemies in organic rice

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Abstract

To overcome the adverse effects of synthetic pesticides in rice, organic farming by utilizing botanicals is a viable alternative. The bio-efficacy of cow-urine-based extracts of neem (*Azadirachta indica*), nochi (*Vitex nigundo*) and adhatoda (*Adhatoda vasica*) was studied. The experiments targeted major sucking insect pests, brown plant hopper (BPH) and green leaf hopper (GLH), in wetland paddy fields, Tamil Nadu Agricultural University, Coimbatore, during Rabi 2023. The results showed that the cow-urine-based extracts of neem, nochi and adhatoda (NNA) leaves @15 % were most effective against BPH and GLH with 70.23 and 72.48 percent reduction over control (PROC), which was on par with NNA leaf extract @10 % and the PROC were 67.13 and 68.18, respectively. Individual 10 % leaf extracts of Neem, Nochi and Adhatoda recorded lower PROC values against BPH (53.68 %, 55.40 % and 53.68 %) and GLH (52.68 %, 55.75 % and 53.02 %) compared to the combined NNA extract at 15 % and 10 %. The neem, nochi and adhatoda (NNA) leaf extract @ 15 % has a moderate impact on beneficial arthropods, expressed as percent impact over control (PIOC) for natural enemies like spiders (27.70), coccinellids (32.99), rove beetles (10.88), mirid bugs (19.95) and ground beetles (43.99). Foliar application of NNA leaf extract at 10 % resulted in lower PIOC values (26.28 %, 31.33 %, 10.14 %, 18.74 % and 39.86 %, respectively, for spiders, coccinellids, rove beetles, mirid bugs and ground beetles), indicating its relative safety for beneficial arthropods. The eco-friendly, cost-effective and residue-free nature of the cow-urine-based neem, nochi and adhatoda leaf extract can be easily incorporated into the Integrated Pest Management programmes in the organic rice production system.

Keywords

Bio-efficacy testing; Cow-urine based botanicals; Integrated pest management; Organic rice production; Sucking insect pests,

Introduction

Rice (*Oryza sativa* L.) is a primary food crop for more than half the world's population. Globally, the crop is cultivated in 166.31 million hectares, producing 523.9 million tonnes. Asia contributes nearly 90 percent of the world's total production (1). India ranks first in rice cultivable area and second in production at the global level. Rice contributes over 43 % of India's food grain production (2). The production and productivity of rice is affected by various biotic and abiotic factors. Insect pests are a significant biotic factor contributing to yield reduction. More than 100 species of insects attack rice crops and 20 cause economic damage (3). Among them, brown planthopper (*Nilaparvata lugens* Stal.) and green leafhopper

(*Nephotettix virescens* Horvah) are considered as important sucking pests in rice not only for yield reduction but also as a vector for many viral diseases (4). Yield losses due to GLH and BPH range from 25-90% and 10-70%, respectively (5-6).

A Wide range of synthetic chemical pesticides is available for the management of sucking pest complex in rice (7). Continuous use of chemical pesticides leads to resistance development and pest resurgence. Additionally, it results in pesticide residues in food, feed and fodder. Organic rice farming is one of the methods to overcome these problems and it excludes the use of synthetic inputs. Botanicals are critical among the different components of pest management in organic rice due to their cost-effective, target-specific, biodegradable and environmentally friendly nature (8).

Around 2500 plant species from 235 families have been identified globally with pesticide properties (9). Of these, 350 species act as insecticidal agents and 800 are feeding deterrents and insect growth inhibitors (9). Neem is considered a universal botanical for pest management in organic farming due to the presence of effective active compounds, including Azadirachtin, Melantriol, Nimbinin, Nimbidin, Salanin, Nimbin, Nimbolin A and Nimbolin B. These active principles exhibit various bioactivities such as antifeedant, repellent, oviposition deterrent and insect growth regulation (10). Nochi (*Vitex nigundo*), commonly known as Vitex, contains active compounds such as vitexin and negundoside with pesticide and pest-repellent properties (11). Adhatoda (*Adhatoda vasica*) contains potent compounds such as vasicine, vasicinone and adhatodin and they also exhibit repellent and insecticidal properties (12-13). Given the importance of neem, nochi and adhatoda in pest management, this study aimed to evaluate their relative efficacy against BPH and GLH in organic rice production systems.

Materials and Methods

Laboratory experiments

Cow urine-based leaf extracts were prepared in the laboratory, Nammazhvar Organic Farming Research Centre, Tamil Nadu Agricultural University, Coimbatore. One kilogram of neem (*Azadirachta indica* L.), nochi (*Vitex negundo* L.), adhatoda (*Adhatoda vasica* N.) leaves was ground with two litres of fresh cow urine and fermented for 10 days with intermittent stirring. The resulting mixture was used as a stock solution for further experiments at varying concentrations of different treatments (Table 1). Preliminary evaluation of the cow urine-based neem, nochi and adhatoda extracts was conducted against BPH and GLH in a pot culture experiment at the Department of Rice, TNAU, Coimbatore. In the pot culture experiment, 15-day-old seedlings were transplanted into small mud pots (two seedlings per pot) and placed in plastic tubs containing water to simulate field conditions. Herbal extracts at varying concentrations were applied to the rice plants 15 days after transplanting (DAT) using an Aspee mini sprayer and the plants were covered with Mylar film cages. Mass-cultured nymphs of BPH and GLH were released at a density of 10 nymphs per pot through a slit provided in the cage. Observations on nymphal mortality, adult emergence and first-generation nymphal emergence were recorded daily for up to 15 days after spraying (DAS). Preliminary evaluation of the cow urine-based neem, nochi and adhatoda extracts was conducted against BPH and GLH in a pot culture experiment at the Department of Rice, TNAU, Coimbatore. In the pot culture experiment, 15-day-old seedlings were transplanted into small mud pots (two seedlings per pot) and placed in plastic tubs containing water to simulate field conditions. Herbal extracts at varying concentrations were applied to the rice plants 15 days after transplanting (DAT) using an Aspee mini sprayer and the plants were covered with Mylar film cages. Mass-cultured nymphs of BPH and GLH were released at a density of 10 nymphs per pot through a slit provided in the cage. Observations on nymphal mortality, adult emergence and first-generation nymphal emergence were recorded daily for up to 15 days after spraying (DAS).

Table 1. Effect of cow-urine-based leaf extracts of neem, nochi and adhatoda on BPH and GLH under laboratory condition

Treatment details	Brown plant hopper (BPH)			Green leafhopper (GLH)		
	Nymphal mortality (%)	Adult emergence (%)	Nymphal emergence/adult	Nymphal mortality (%)	Adult emergence (%)	Nymphal emergence/adult
T ₁ - Neem leaf extract 10 %	60.33 (50.96) ^{abc}	40.67 (39.62) ^{cde}	129.66 (11.37) ^f	63.33 (52.73) ^{bc}	36.67 (37.26) ^{bcd}	204.33 (14.32) ^e
T ₂ - Nochi leaf extract 10 %	53.33 (46.92) ^{bc}	46.33 (41.15) ^{cd}	147.66 (12.13) ^e	70.67 (56.95) ^{ab}	29.33 (31.33) ^{de}	179.66 (13.42) ⁱ
T ₃ - Adhatoda leaf extract 10 %	50.67 (45.13) ^c	49.33 (44.03) ^{cde}	168.66 (13.00) ^d	60.66 (51.76) ^c	39.34 (38.13) ^{bc}	215.33 (14.69) ^d
T ₄ - Neem + Nochi + Adhatoda leaf extract 5 %	46.66 (43.08) ^c	53.33 (43.09) ^c	199.66 (14.14) ^c	43.33 (41.16) ^d	56.67 (48.83) ^c	231.66 (15.23) ^c
T ₅ - Neem + Nochi + Adhatoda leaf extract 10 %	70.66 (56.78) ^b	29.66 (30.99) ^{ef}	107.33 (10.38) ^h	76.66 (61.11) ^b	23.33 (28.88) ^{ef}	158.33 (12.60) ^j
T ₆ - Neem + Nochi + Adhatoda leaf extract 15 %	76.66 (61.21) ^a	23.66 (26.07) ^f	78.66 (8.89) ⁱ	83.33 (65.90) ^a	16.67 (24.09) ^f	135.33 (11.65) ^k
T ₇ - Cow urine alone 10 %	26.66 (30.99) ^d	73.33 (56.99) ^b	294.33 (17.19) ^b	33.33 (35.26) ^e	66.67 (54.73) ^b	254.33 (15.96) ^b
T ₈ - Neem seed kernel extract 5 % (standard check)	63.66 (52.92) ^{ab}	37.33 (36.26) ^{def}	114.66 (10.73) ^g	66.66 (54.73) ^{abc}	33.33 (35.26) ^{cde}	185.66 (13.64) ^f
T ₉ - Control (untreated check)	6.66	93.33	379.66	13.33	86.67	289.66
S Ed	1.338	1.432	4.246	1.023	0.913	4.400
CD (P=0.05)	2.812	3.009	8.921	2.169	1.918	9.244

*Figures in parentheses are arcsine transformation values

Field experiment

A field experiment was conducted at wetland paddy fields (11.002 °N latitude and 76.923 °E longitude), TNAU, Coimbatore during *Rabi* 2023. The experimental field was laid out in a Randomized Block Design (RBD) replicated thrice with a plot size of 5 m × 4 m. Rice variety CO 55 was selected for the study. Seeds were sown in the nursery and transplanted into the main field using the System of Rice Intensification (SRI) with a 25 × 25 cm spacing. Before transplanting, the green manure crop *Sesbania aculeate* was raised in the main field and in-situ incorporation was done at 50 % flowering 15 days before transplanting. The organic rice production package developed by the TNAU was followed throughout the cropping period.

Cow-urine-based neem, nochi and adhatoda extracts were prepared separately, with one part of the leaves and two parts of fresh cow urine. The mixture was fermented for 10 days with intermittent stirring, filtered through a muslin cloth and utilized in a study similar to the laboratory experiment.

Pre-treatment pest counts on BPH and GLH were recorded from 10 randomly selected hills. Treatments were applied at 40 and 60 DAT and post-treatment counts were recorded at 1, 3, 5, 7, 10 and 15 DAS. Data were analyzed statistically using the standard method (14). Treatment variations were tested for significance using mean standard error (SE) and critical difference (CD) at the 5 % significance level.

Results and Discussion

Effect of botanical extracts on brown planthoppers under laboratory condition

The highest nymphal mortality of BPH (76.66 %) was observed with NNA leaf extract applied at 15 %, followed by NNA extract at 10 % (70.66 %), which was on par with NSKE at 5 % (63.66 %). NSKE at 5 % was comparable to neem leaf extract at 10 %, which achieved a nymphal mortality of 60.33 %. The untreated control recorded the lowest nymphal mortality (6.66 %). The higher BPH mortality observed with NNA and NSKE extracts could be attributed to their insecticidal properties and juvenile hormone mimic activities inherent in neem, nochi and adhatoda. These results showed high nymphal mortality of mustard aphids at 70.82 %, 69.40 % and 55.81 % for neem, nochi and adhatoda leaf extracts under laboratory conditions on mustard (15). The lowest adult emergence (23.66 %) was recorded with NNA extract at 15 %, followed by NNA extract at 10 % (29.66 %), which was comparable to NSKE at 5 % (37.33 %). The untreated control recorded the highest adult emergence (93.33 %). The lowest first-generation nymphal emergence per adult BPH (78.66) was observed with NNA leaf extract at 15 %. This was followed by NNA extract at 10 % and NSKE at 5 % (114.66). In contrast, the untreated control recorded the highest nymphal emergence (379.66 per adult; Table 1). These results showed similar outcomes for BPH under pot culture experiments on rice (16).

Effect of botanical extracts on green leafhoppers under laboratory condition

The highest GLH nymphal mortality (83.33 %) was observed with NNA leaf extract at 15 %, followed by NNA extract at 10 % (76.66 %) and nochi leaf extract alone at 10 % (70.67 %), which was comparable to NSKE at 5 % (66.66 %). The high mortality of the

GLH may be due to the key active principles present in neem, nochi and adhatoda plant parts. The results follow the earlier findings on the effect of neem, nochi and adhatoda (17-19). The lowest adult emergence (16.67 %) was recorded with NNA leaf extract at 15 %, followed by NNA at 10 % (23.33 %) and nochi leaf extract at 10 % (29.33 %), which was comparable to NSKE at 5 % (33.33 %) (Table 1). The lowest emergence of first-generation nymphs per adult was observed with NNA extract at 15 % (135.33), followed by NNA at 10 % (158.33) and NSKE at 5 % (185.66). The untreated control recorded the highest emergence (289.66). The reduced emergence in NNA leaf extracts might be due to the combined effect of active principles in neem, nochi and adhatoda leaves. Vitex leaf extracts have previously been reported to be effective against plant hoppers, resulting in higher pupal mortality and reduced adult emergence (20, 21). NSKE 5 % and Vitex leaf extracts were effective on the hopper population (22).

Bio-efficacy of botanical extracts against BPH under field condition

The differences in the population of BPH recorded in the pre-treatment count of the first spray were non-significant among treatments ranging from 8.33 to 9.98 nos./hill. They indicated that the initial BPH population across the experiment was homogenous. Post-treatment, the BPH population decreased to 7 DAS but increased from 10 DAS onwards in all treatments except untreated control (Table 2). The results are corroborated by the researchers who found the persistent control of plant hoppers up to 10 days post-treatment with botanical extracts in rice crops (23). On the first day after spraying, the 15 % neem, nochi and adhatoda leaf extract (NNA) recorded the lowest BPH population of 5.05 nos./hill, followed by NNA 10 % (5.69 nos./hill), which was similar to NSKE 5 % (5.56 nos./hill), compared to the untreated control (8.62 nos./hill). At 3 DAS, the BPH population among the treatments ranged from 8.33 to 8.96 compared to the untreated check. The BPH population at 3 DAS was the lowest (1.66 nos./hill) in NNA 15 %, followed by NNA 10 % (2.01 nos./hill), which was at par with 5 % NSKE (2.12 nos./hill) when compared with the untreated control (8.73 nos./hill). At 5 DAS, the PROC ranged from 22.89 to 82.23 among the treatments and the lowest BPH population of 1.56 nos. / hill was registered in NNA 15 %, which was on par with NNA 10 % (1.66 nos./hill) compared to the untreated check (8.78 nos./hill). The PROC of mulberry mealybug ranged from 67.81 % to 69.11 % due to Vitex leaf extract 10 % application (24). The PROC for mealybug is 69.03 % for 5 % NSKE application in mulberry (25).

At 7 DAS, the PROC for BPH ranged from 20.16 to 79.06 with the lowest BPH population of 1.68 nos./hill in NSKE 5 % application, which was at par with NNA 15 % (1.87 nos./hill). The subsequent best treatment was NNA 10 % with a BPH population of 1.98 nos./hill. The highest BPH population of 8.93 nos./hill was registered in the untreated control. At 10 DAS, the PROC ranged from 19.46 to 75.24 %, with the lowest population of 2.29 nos./hill in NNA 15 %, followed by NNA 10 % (2.47) and NSKE 5 % (2.89). At 15 DAS, the BPH reduction over control ranged from 11.86 % to 70.14 %. The lowest population of 2.92 nos./hill was registered in NNA at 15 %, which was on par with NNA at 10 % (3.06 nos./hill) compared to the control treatment (9.78 nos./hill). The mean PROC for BPH was the highest (71.09 %) in neem, nochi and adhatoda leaf extract 15 % spray treatment, followed by NNA at

Table 2. Effect of cow-urine-based leaf extracts of neem, nochi and adhatoda against brown planthoppers in organic rice

Treatment	First spray									Second spray									Pooled mean	Pooled Proc
	PT	1 DAS	3 DAS	5 DAS	7 DAS	10 DAS	15 DAS	Mean	PROC	PT	1 DAS	3 DAS	5 DAS	7 DAS	10 DAS	15 DAS	Mean	PROC		
T ₁	9.98 (3.23)	7.66 (2.85)	5.33 (2.41)	2.33 (1.68)	2.67 (1.78)	3.66 (2.03)	5.33 (2.41)	4.50	50.12	5.33 (2.41)	4.33 (2.19)	3.36 (1.96)	2.73 (1.79)	2.93 (1.85)	3.56 (2.01)	4.45 (2.22)	3.56	57.52	4.03	53.68
T ₂	9.11 (3.10)	7.33 (2.79)	4.87 (2.31)	2.62 (1.76)	2.71 (1.79)	3.73 (2.05)	4.66	4.32	52.08	5.16 (2.37)	4.07 (2.13)	3.32 (1.95)	2.82 (1.82)	2.97 (1.86)	3.52 (2.00)	3.86 (2.08)	3.43	59.07	3.88	55.40
T ₃	9.53 (3.16)	7.86 (2.89)	5.04 (2.35)	2.97 (1.86)	2.35 (1.68)	3.12 (1.90)	5.69 (2.48)	4.51	50.03	5.69 (2.48)	4.43 (2.22)	3.45 (1.98)	2.87 (1.83)	2.95 (1.85)	3.58 (2.01)	3.94 (2.10)	3.54	57.76	4.03	53.68
T ₄	8.66 (3.02)	7.01 (2.74)	5.63 (2.47)	3.19 (1.92)	2.26 (1.66)	3.33 (1.95)	6.01 (2.55)	4.57	49.29	6.04 (2.55)	5.23 (2.39)	4.15 (2.15)	3.62 (2.02)	3.76 (2.06)	3.88 (2.09)	4.21 (2.17)	4.14	50.60	4.36	49.89
T ₅	8.98 (3.07)	5.69 (2.48)	2.01	1.66 (1.46)	1.98 (1.57)	2.47 (1.72)	3.06 (1.88)	2.81	68.81	4.96 (2.33)	4.02 (2.12)	3.09 (1.89)	2.63 (1.76)	2.08 (1.60)	2.65 (1.77)	2.95 (1.85)	2.90	65.39	2.86	67.13
T ₆	9.66 (3.18)	5.05 (2.35)	1.66 (1.46)	1.56 (1.43)	1.87 (1.53)	2.29 (1.67)	2.92 (1.84)	2.56	71.62	4.62 (2.26)	3.21 (1.92)	3.03 (1.87)	2.47 (1.72)	2.03 (1.59)	2.37 (1.69)	2.59 (1.75)	2.62	68.74	2.59	70.23
T ₇	9.33 (3.13)	9.05 (3.09)	7.95	6.77 (2.69)	7.13 (2.76)	7.45 (2.81)	8.62 (3.01)	7.83	13.16	6.12 (2.57)	5.97 (2.54)	5.84 (2.51)	5.32 (2.41)	5.55 (2.45)	5.86 (2.52)	6.03 (2.55)	5.76	31.26	6.80	21.84
T ₈	9.12 (3.10)	5.56 (2.46)	2.12 (1.61)	1.87	1.68 (1.47)	2.89 (1.84)	3.75 (2.06)	2.98	66.96	4.75 (2.29)	3.91 (2.10)	3.23 (1.93)	2.67 (1.78)	2.12 (1.61)	2.78 (1.81)	3.06 (1.88)	2.96	64.68	2.97	65.86
T ₉	8.33 (2.97)	8.62 (3.01)	8.73 (3.03)	8.78 (3.04)	8.93 (3.07)	9.25 (3.12)	9.78	9.02	0.00	8.89 (3.06)	8.03 (2.92)	8.12 (2.93)	8.35 (2.97)	8.43 (2.98)	8.62 (3.01)	8.71 (3.03)	8.38	0.00	8.70	0.00
S Ed	NS	0.132	0.100	0.108	0.084	0.076	0.149	-	-	NS	0.196	0.089	0.092	0.114	0.070	0.080	-	-	-	-
CD (P=0.05)	NS	0.279	0.213	0.228	0.179	0.163	0.316	-	-	NS	0.415	0.188	0.195	0.241	0.148	0.170	-	-	-	-

T₁ - Neem leaf extract 10 %, T₂ - Nochi leaf extract 10 %, T₃ - Adhatoda leaf extract 10 %, T₄ - Neem + Nochi + Adhatoda leaf extract 5 %, T₅ - Neem + Nochi + Adhatoda leaf extract 10 %, T₆ - Neem + Nochi + Adhatoda leaf extract 15 %, T₇ - Cow urine alone 10 %, T₈ - Neem seed kernel extract 5 % (standard check), T₉ - Control (untreated check), *Figures in parentheses are square root transformation values, PT - Pre-treatment, DAT - Days after treatment, PROC - Percent reduction over control

10 % (68.35 %) and NSKE at 5 % application (64.93). Among individual plant leaf extracts, the PROC was more (53.03 %) in adhatoda leaf extract applied at 10 % followed by 10 % nochi leaf extract (52.08) and neem leaf extract at 10 % (50.12 %). The cow's urine registered the lowest PROC at 13.16 %. The nymphal mortality of plant hopper was 73.87 and 74.77 percent, respectively, for neem and nochi leaf extracts.

In the second spray, the overall BPH population was less in all the treatments when compared to the first spray. In the pre-treatment count, the BPH population ranged from 4.62 to 8.89 nos./hill. In contrast, the range of PROC for BPH in post-treatment counts was 25.65–60.02, 28.08–62.68, 36.29–70.42, 34.16–75.92, 32.02–72.51 and 30.77–70.26 for 1, 3, 5, 7, 10 and 15 DAS, respectively. At 1 DAS, the lowest BPH population of 3.21 nos./hill was recorded in NNA leaf extract at 15 % spray, which was on par with NSKE at 5 % application (3.91 nos./hill). The subsequent best treatment at 1 DAS was NNA at 10 % (4.02 nos./hill), which was also on par with nochi leaf extract at 10 % application (4.07 nos./hill). At 3 DAS, the same trend as 1 DAS was observed with the lowest BPH population (3.03 nos./hill) in NNA leaf extract 15 % spray. At 5 DAS, the lowest BPH number of 2.47/hill was observed in NNA at 15 % application, followed by NNA 10 % (2.63), which was at par with NSKE 5 % spray (2.67). 10 and 15 % concentrations of botanical extracts resulted in better control of BPH than lower concentrations (27).

At 7 DAS, the BPH population in NNA is 15 %, NNA 10 % and NSKE 5 % are on par with each other and their respective values were 2.03, 2.08 and 2.12 nos./hill. At 10 DAS, the BPH number was the lowest (2.37 nos./hill) in NNA leaf extract at 15 %, followed by NNA at 10 % (2.65 nos./hill) and NSKE at 5 % spray (2.78 nos./hill). At 15 DAS, the same trend was observed as that of 10 DAS. The PROC for the second spray was higher (68.74 %) in neem, nochi and adhatoda leaf extract at 15 % application, followed by NNA at 10 % spray (65.39 %) and NSKE at 5 % as a foliar spray (64.68 %). The pooled PROC of two sprays was also

higher (70.23 %) in neem, nochi and adhatoda leaf extract 15 % spray followed by neem, nochi and adhatoda leaf extract 10 % application (67.13 %) and NSKE at 5 % (65.86 %). The combined effect of nochi leaf and adhatoda leaf extracts was better against BPH when compared to the neem-based extract alone (28).

Bio-efficacy of botanical extracts against GLH under field condition

The GLH population in the pre-treatment count of the first spray ranged from 4.66 to 5.67 nos./hill, statistically non-significant with each other. In the post-treatment count, the population levels showed a decreasing trend up to 5 DAS and an increasing trend from 7 DAS. On the first day after treatment, the lowest GLH population of 2.32 nos./hill was recorded in NNA leaf extract at 15 % (2.32), followed by NNA extract at 10 % (2.67) and NSKE at 10 % spray (1.96) when compared with the untreated control (5.36). The same trend was observed at 3, 5, 7, 10 and 15 DAS. At 3 DAS, the NNA at 15 % treated plants registered the lowest GLH population of 1.33, followed by NNA at 10 % (1.66) and NSKE at 5 % (1.96) and the NSKE at 5 % was at par with nochi leaf extract at 10 % spray (1.99) and neem leaf extract at 10 % (2.01). At 5 DAS, the GLH population was ever less in all the treatments except the untreated check than the previous day counts (Table 3).

At 7 DAS, the GLH population slightly increased except for T₆ (0.66 nos./hill). At 10 DAS, the lowest GLH population of 1.67 nos./hill was recorded in NNA 15 %, which was at par with NNA 10 % (1.68) and NSKE 5 % (1.71) and the next best treatment was neem leaf extract at 10 % (2.46). At 15 DAS count, the lowest GLH count of 1.86 nos./hill was registered in NNA leaf extract 15 % spray, followed by NNA at 10 % (1.96) and NSKE at 5 % spray (2.15). The PROC was higher (69.88 %) in neem, nochi and adhatoda leaf extract at 15 % spray, followed by NNA at 10 % application (67.31) and NSKE at 5 % as a foliar spray (66.08 %). This study followed researchers' findings in which a combination of adathoda leaf extract and cow urine recorded 44.00 % mortality of GLH nymphs in rice (29).

Table 3. Effect of cow-urine-based leaf extracts of neem, nochi and adhatoda against green leafhoppers in organic rice

Treatment	First spray							Second spray							Pooled					
	PT	1 DAS	3 DAS	5 DAS	7 DAS	10 DAS	15 DAS	Mean	PROC	PT	1 DAS	3 DAS	5 DAS	7 DAS	10 DAS	15 DAS	Mean	PROC	mean	d proc
T ₁	4.86 (2.31)	3.33 (1.95)	2.01 (1.58)	2.03 (1.59)	2.33 (1.68)	2.46 (1.72)	3.33 (1.95)	2.58	55.57	3.67 (2.04)	3.45 (1.98)	2.68 (1.78)	2.01 (1.58)	2.42 (1.70)	2.76 (1.80)	2.83 (1.82)	2.69	51.68	2.74	52.63
T ₂	4.93 (2.33)	2.92 (1.84)	1.99 (1.57)	1.51 (1.41)	2.12 (1.61)	2.52 (1.73)	3.48 (1.99)	2.42	58.29	3.62 (2.02)	3.36 (1.96)	2.71 (1.79)	1.99 (1.57)	2.48 (1.72)	2.54 (1.74)	2.56 (1.74)	2.61	53.20	2.52	55.75
T ₃	5.21 (2.38)	3.13 (1.91)	2.16 (1.63)	1.62 (1.45)	1.94 (1.56)	2.68 (1.78)	3.58 (2.01)	2.52	56.66	3.97 (2.11)	3.34 (1.95)	3.01 (1.87)	2.66 (1.77)	2.07 (1.60)	2.82 (1.82)	3.02 (1.87)	2.82	49.37	2.67	53.02
T ₄	5.33 (2.41)	4.12 (2.14)	2.37 (1.69)	1.67 (1.47)	2.03 (1.59)	3.12 (1.9)	3.99 (2.11)	2.88	50.37	4.12 (2.14)	3.99 (2.11)	3.22 (1.92)	2.76 (1.80)	2.33 (1.68)	3.19 (1.92)	3.43 (1.98)	3.15	43.39	3.02	46.88
T ₅	5.11 (2.36)	2.66 (1.77)	1.66 (1.46)	0.37 (0.93)	1.15 (1.28)	1.68 (1.47)	1.86 (1.53)	1.55	71.09	4.35 (2.2)	3.13 (1.9)	2.21 (1.64)	1.63 (1.45)	1.56 (1.43)	1.77 (1.50)	1.98 (1.57)	2.05	63.26	1.80	68.18
T ₆	5.66 (2.48)	2.32 (1.67)	1.33 (1.35)	0.88 (1.17)	0.66 (1.07)	1.67 (1.47)	1.96 (1.56)	1.47	74.70	3.39 (1.97)	2.87 (1.83)	2.13 (1.62)	1.49 (1.41)	1.45 (1.39)	1.58 (1.44)	1.76 (1.50)	1.88	66.25	1.68	72.48
T ₇	4.66 (2.27)	4.62 (2.26)	4.67 (2.27)	4.65 (2.26)	4.71 (2.28)	4.85 (2.31)	5.33 (2.41)	4.81	17.30	4.16 (2.15)	4.02 (2.12)	3.95 (2.10)	3.86 (2.08)	3.89 (2.09)	3.97 (2.11)	3.99 (2.12)	3.95	29.14	4.38	23.22
T ₈	5.48 (2.44)	2.76 (1.81)	1.96 (1.56)	1.12 (1.27)	1.33 (1.35)	1.71 (1.48)	2.15 (1.62)	1.84	68.36	3.43 (1.98)	3.22 (1.92)	2.57 (1.75)	1.73 (1.49)	1.63 (1.45)	1.83 (1.52)	2.27 (1.66)	2.21	60.35	2.03	64.36
T ₉	5.33 (2.41)	5.36 (2.42)	5.45 (2.43)	5.52 (2.45)	5.65 (2.47)	6.23 (2.59)	6.65 (2.67)	5.81	0.00	5.15 (2.37)	5.23 (2.39)	5.36 (2.42)	5.48 (2.44)	5.62 (2.47)	5.81 (2.51)	5.92 (2.53)	5.57	0.00	5.69	0.00
S Ed	NS	0.053	0.084	0.037	0.090	0.071	0.077	-	-	NS	0.078	0.080	0.085	0.067	0.063	0.076	-	-	-	-
CD (P=0.05)	NS	0.112	0.179	0.078	0.190	0.150	0.164	-	-	NS	0.165	0.170	0.180	0.143	0.133	0.162	-	-	-	-

T₁ - Neem leaf extract 10 %, T₂ - Nochi leaf extract 10 %, T₃ - Adhatoda leaf extract 10 %, T₄ - Neem + Nochi + Adhatoda leaf extract 5 %, T₅ - Neem + Nochi + Adhatoda leaf extract 10 %, T₆ - Neem + Nochi + Adhatoda leaf extract 15 %, T₇ - Cow urine alone 10 %, T₈ - Neem seed kernel extract 5 % (standard check), T₉ - Control (untreated check), *Figures in parentheses are square root transformation values, PT - Pre-treatment, DAT - Days after treatment, PROC - Percent reduction over control

In the second spray, the GLH population in the pre-treatment count ranged from 4.62 to 5.15 nos./hill. Whereas in post-treatment counts, the range of PROC among different treatments ranged between 15.08 – 54.34 %, 17.98 - 57.44 %, 27.62 - 66.39 %, 23.10 - 66.27 % and 21.79 - 61.22 % at 1, 3, 5, 7, 10 and 15 DAS, respectively. On the first day after spraying, the lowest GLH population of 2.87 nos./hill was recorded in NNA leaf extract at 15 % application, followed by NNA 10 % (3.13 nos./hill) and NSKE at 5 % (5.23 nos./hill). At 3 DAS, the same trend was observed at 1 DAS, but the GLH population was lower than the 1 DAS. The lowest GLH population of 2.13 was recorded in NNA at 15 % and the highest was with the untreated control (5.36 nos./hill). At 5 DAS, a further decrease in the GLH population was observed irrespective of treatments except the control plot. The lowest GLH number of 1.69 was registered in NNA leaf extract at 15 % spray, followed by NNA at 10 % (1.83) and NSKE at 5 % (1.93).

At 7 DAS, the GLH population trends were as that of the earlier days counts, but the numbers further reduced with the lowest (1.45) in NNA at 15 % followed by NNA at 10 % (1.66), NSKE at 5 % (1.73) with the highest at the number at of 5.62 in control. At 10 DAS, the increase in GLH population was registered irrespective of the treatments except for untreated control. The foliar spray of NNA leaf extract at 15 % recorded the lowest GLH of 1.68, followed by NNA at 10 % (1.87) and NSKE at 5 % (2.13). At 15 DAS, a further slight increase in GLH population was observed in all the treatments except untreated control. The highest mean PROC of 62.45 % was registered in NNA leaf extract at 15 % application, followed by NNA 10 % spray (60.56 %), NSKE 5 % (59.71 %) and nochi leaf extract at 10 % foliar spray (55.63 %). Nochi leaf extract demonstrated a notable reduction in the pest

population, with a percent reduction over control ranging between 48.7 % to 91.5 % for GLH (29). The pooled PROC for GLH was the highest (72.48 %) in the NNA 15 % spray, followed by NNA 10 % (68.18 %) and NSKE 5 % (64.36 %).

Impact of botanicals on natural enemies in organic rice production system

Impact on spiders: In the first spray, the pre-treatment population of a spider among the treatments ranged from 1.51 to 1.96 per hill (Fig. 1). A minor decline in spider numbers was observed one week after spraying and a steady increase in their numbers was noticed during the second week. The percent impact over control (PIOC) on spiders in the organic rice production system was considered safe, remaining below 30 % across all treatments, ranging from 16.88 to 27.08. The lowest PIOC (16.88 %) was observed with 10 % cow urine spray, followed by 5 % neem, nochi and adhatoda (NNA) leaf extract (17.14 %) and 10 % adhatoda leaf extract (18.29 %), compared to the control treatment. The cow urine alone treated plots registered the highest PIOC of 27.08. In the second spray, the PIOC ranges were more (19.36-32.58) when compared to the first spray PIOC. The lowest PIOC in the second spray was observed with 10 % cow urine spray (19.36 %), followed by 5 % NNA leaf extract (19.43 %). The pooled mean PIOC was less (18.12) in neem, nochi and adhatoda leaf extract 15 % application followed by neem, nochi and adhatoda leaf extract 5 % spray (18.29) and nochi leaf extract 10 % spray (22.46). The NSKE sprayed at 5 % concentration showed the highest pooled PIOC of 29.83. NSKE 5 % impacted 10-20 % against spiders in the rice ecosystem (30). Neem products such as neem leaf extract (10 %) and NSKE (5 %) were relatively safe for the spiders.

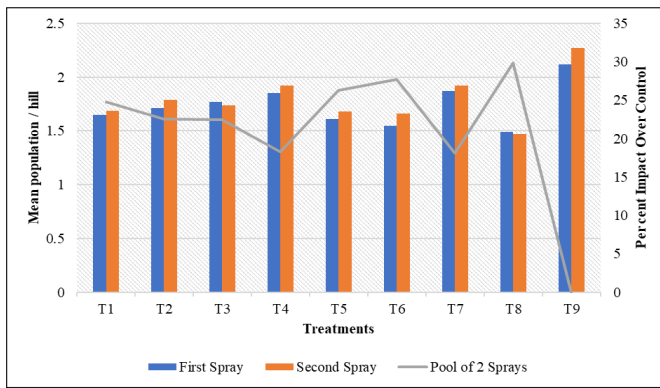


Fig. 1. Impact of botanical extracts on spiders in organic rice.

Impact on coccinellid beetles: The mean population of coccinellid beetles in the first spray ranges from 1.55 to 1.95 nos./hill (Fig. 2). The beetle numbers at 7 and 15 DAS were in the range between 1.43 - 1.86 and 1.67 - 2.16, respectively. The lowest PIOC of 10.75 was recorded in spraying of cow urine alone at 10 % (1.55), followed by neem, nochi and adhatoda leaf extract at 5 % spray. The highest PIOC of 20.46 % was registered in the NSKE 5 % application. In the second spray, the coccinellid beetle population was less than in the first spray, irrespective of the treatments and the mean population ranged from 0.60 to 1.20 nos./hill. The PIOC of the second spray was higher than that of the first spray, which ranged from 15.34 % to 49.51 %. The overall pooled mean of coccinellid beetles ranges from 1.08 to 1.57. Meanwhile, the overall pooled PIOC ranged from 13.05 % to 34.99 %. Neem seed kernel extract impacted natural enemies with a 25-35 % reduction in rice production (32).

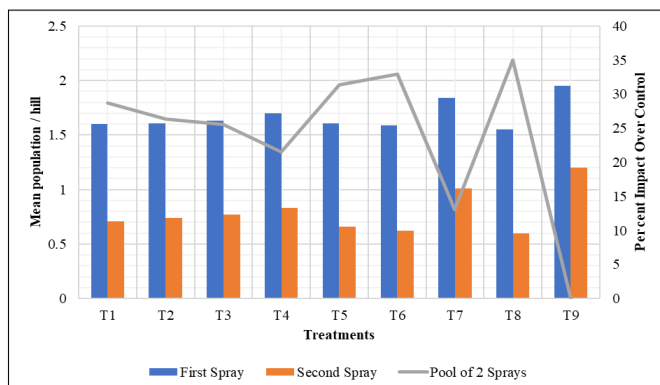


Fig. 2. Impact of botanical extracts on coccinellids in organic rice.

Impact on rove beetles: The mean rove beetle population ranges were 3.42-3.94 and 3.41-4.01 nos./hill, respectively, for the first and second sprays (Fig. 3). The PIOC were much less in both the sprays when compared to all other natural enemies of the experimental field. For the first and second sprays, the PIOC ranges were 3.08 %-8.51 % and 7.51 %-15.39 %. The pooled mean of rove beetles/hill was more (3.97) in untreated control, followed by cow urine alone at 1 % spray (3.74) and neem, nochi and adhatoda leaf extract at 5 % application (3.65). The pooled PIOC of the experiment ranges from 5.30 % to 11.95 %, which was lower (5.30 %) in cow urine alone application, followed by neem, nochi and adhatoda leaf extract at 5 % application (6.64 %) and adhatoda leaf extract at 10 % as a foliar spray (6.64 %). The findings showed that the PIOC range of 13 % - 17 % for rove beetles in rice (33).

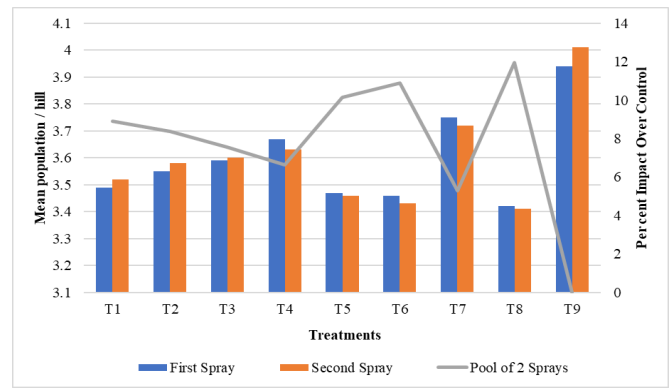


Fig. 3. Impact of botanical extracts on rove beetles in organic rice.

Impact on mirid bugs: The mirid bug population, which is an exclusive predator of hoppers, were steady throughout the crop period irrespective of the treatments and sprays (Fig. 4). The mean population ranges were 2.34-3.48 and 2.47-3.11, respectively, for the first and second sprays. The PIOC ranges for the first and second sprays were 11.31 %-23.69 % and 10.87 %-22.31 %, respectively. The overall pooled mean of mirid bugs was lowest (2.41) in NSKE and highest (3.29) in the untreated control. The pooled PIOC ranges from 11.09 % to 23.00 %, with the lowest impact in cow urine alone at 10 % (11.09), followed by neem, nochi and adhatoda leaf extract at 5 % application (14.28 %) and adhatoda leaf extract alone at 10 % as foliar spray (16.06 %). A combination of nochi and adhatoda leaf extracts showed minimal adverse effects (10 %-20 %) for mirid bugs in rice (34).

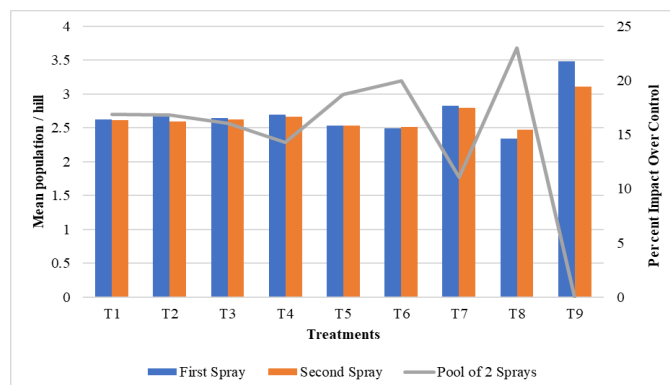


Fig. 4. Impact of botanical extracts on mirid bugs in organic rice.

Impact on ground beetles: The ground beetle population of the experiment was less than 1.00 no./hill throughout the cropping season, irrespective of the treatments. The mean population ranges from 0.61-1.28 in the first spray and 0.51-0.95 in the second spray (Fig. 5). The PIOC for the first spray was in the ranges from 26.42 % to 50.67 %, which was lesser (26.42 %) in cow urine alone at 10, followed by neem, nochi and adhatoda leaf extracts applied at 5 % (35.90 %). Meanwhile, the PIOC for the second spray ranges from 22.89 % to 45.95 %, with the highest in cow urine at 10 % alone and the highest in the NSKE at 5 % as a foliar spray. The range of the pooled mean of ground beetles was 0.56-1.11 and the pooled PIOC ranges were 24.65 %-48.31 %. Hemantha et al. (35) also registered a minimal PIOC range of 25 %-28 % for nochi and adhatoda leaf extracts on ground beetles in rice.

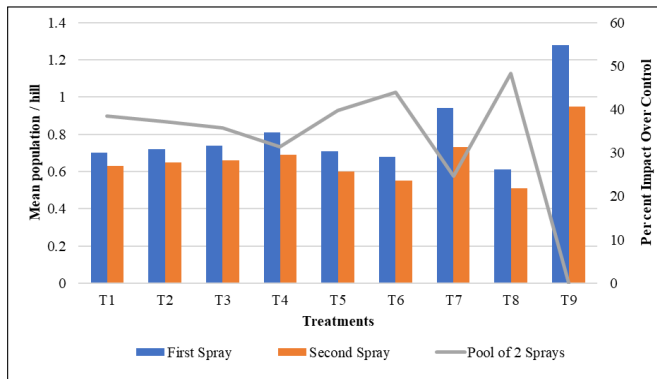


Fig. 5. Impact of botanical extracts on ground beetles in organic rice.

Conclusion

The present study demonstrated effective management of *Nilaparvata lugens* and *Nephotettix virescens* through foliar application of cow-urine-based extracts of neem, nochi and adhatoda leaves at 10 % and 15 % concentrations. The results revealed that cow urine-based extracts of neem, nochi and adhatoda (NNA) leaves at 15 % concentration were the most effective against BPH and GLH, achieving 70.23 % and 72.48 % reduction over control (PROC), respectively. The efficacy of NNA extracts was better than NSKE @ 5 % (standard check) under laboratory and field conditions. A gradual increase in their efficacy was observed across two consecutive sprays. This led to detrimental effects on BPH's survival, growth, development, reproduction and GLH, likely due to multiple active compounds affecting various metabolic pathways. The use of cow urine in herbal extraction is thought to enhance the solubility and efficacy of phytochemicals, potentially resulting in more potent therapeutic formulations. Using these eco-friendly botanical extracts in pest management reduces the total cost of cultivation and provides residue-free produce for consumers. In the near future, there may be a chance for the development of cow urine based on three-leaf botanical formulations with neem, nochi and adhatoda. This botanical formulation could serve as a superior alternative to NSKE for managing various sucking pests, not only in rice but also in other crops cultivated under organic production systems.

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Authors' contributions

BAK conducted the original experiment under the guidance of KG, wrote the original manuscript & did the data analysis. MS, SV and VM helped prepare extracts and conducted pot culture and field experiments. KG, RP S, MM and SVS were involved in correcting and supervising the process. All authors read and prepared the final manuscript.

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Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interest to declare.

Ethical issues: None

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