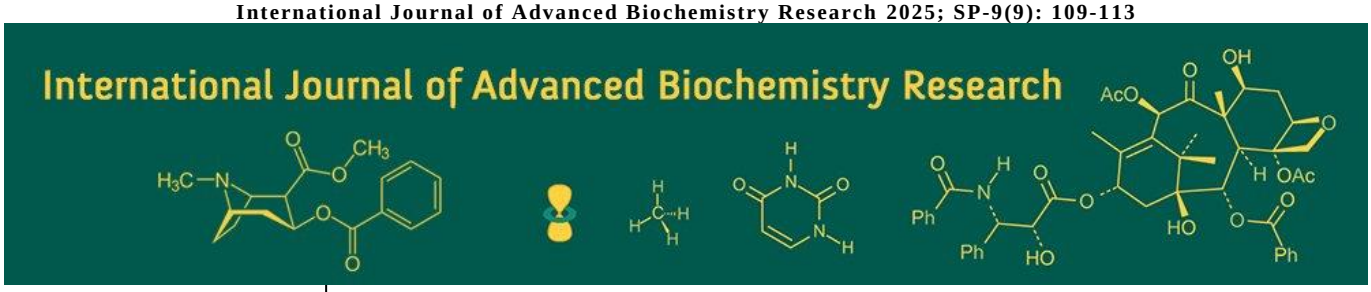

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Efficacy of plant leaf extracts and cow urine against brown citrus aphid, *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) of Assam lemon (*Citrus limon*) as a potential natural pesticide

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Abstract
A field trial was conducted in the experimental field of College of Horticulture & Farming System Research (CHFSR), Assam Agricultural University, Nalbari to assess the efficacy of two botanical insecticides and cow urine and their combinations against the Brown Citrus Aphid, *Toxoptera citricida* of Assam lemon. Plant extracts from *Lantana camara* and *Chromolaena odorata*, cow urine alone and in combination of all these were evaluated. The investigation showed that the leaf extract of *L. camara*, *C. odorata*, and cow urine has anti-insectidal activity against Brown Citrus Aphid, *T. citricida*. The treatment of combined mixture of *L. camara*, *C. odorata* and Cow Urine was the most effective in controlling the Brown Citrus Aphid, *T. citricida* in Assam lemon. These botanicals and cow urine have importance to the resource-poor farmers in many areas of developing countries.

Keywords: Field efficacy, cow-urine, *Lantana camara*, *Chromolaena odorata*, brown citrus aphid, Assam lemon

1. Introduction
The brown citrus aphid, *Toxoptera citricida* (Kirkaldy), is one of the most damaging insect pests of Assam lemon. Aphids damage citrus trees by sucking sap, causing leaf curling, deformation, and yellowing, which can stunt growth and affect fruit quality. They also transmit diseases like the Citrus tristeza virus. Several insecticides are found to be effective against citrus aphid populations in field. However, chemical pesticides can pose serious health risks to humans and animals over the long term and detrimental to the environment. One promising way to reduce the negative impact of synthetic insecticide is to replace them with botanical insecticides. Botanical insecticides contain biologically active plant extract components and are naturally degraded into harmless and non-toxic elements in a certain period; thus, they are considered safer for the environment and human health (Saleem *et al*., 2019; Shimira *et al*., 2021) [8, 14]. As a result, there is increasing social pressure to reduce chemical pesticide use and progressively replace it with biopesticides, which are safe for both humans and non-target creatures (Swamy *et al*., 2015) [15]. Plant like *Lantana camara* and *Chromolaena odorata*, particularly their leaf extracts, have shown promising result in wound healing and pest control with potential as natural alternatives to chemical pesticides and acaricides (Mvumi and Maunga. 2018) [10].
Lantana camara L. is the most widely distributed species of the Verbenaceae family and is commonly referred to as wild or orange sage. It is known as "*Raimuniya*" and "*Gu phul*" in Hindi and Assamese language, respectively. It is commonly utilized to treat different health issues in many traditional medical systems. Many human diseases are cured using various plant parts. The leaves of the plant have fungicidal, antibacterial and insecticidal activities (K, 2017). The chemical makeup and biological functions of the entire plant of *L. camara* was investigated by different scientist in recent years. *Chromolaena odorata* (Siam weed) extracts have also demonstrated insecticidal activity against various insects, with some studies showing significant mortality of larvae. The use of cow urine in combination with these plant extracts may further enhance their efficacy, but more research is needed to

determine the exact mechanisms and optimal applications. *C. odorata*, commonly known as Siam weed and in Assamese as *Jarmani Bon*, is a perennial shrub forming dense tangled bushes 1.5-2.0 m in height, occasionally reaching 6m as a scrambler up trees. This weed plant parts are used by traditional practitioners for treatment of burns, wound healing, skin infections, post-natal wounds, and as an anti-malarial. It has also been reported to possess anti-inflammatory, hepatotropic activities. Both *L. camara* and *C. odorata* have demonstrated insecticidal properties and are used as natural insecticides in some regions.

Cow urine, a traditional agricultural input has natural pesticide properties that can help control pests and diseases in crops. Leaf extracts and cow urine can be effective when used together, especially in agricultural applications. Cow urine, with its antimicrobial and germicidal properties, acts as a biofertilizer and biopesticide. When combined with plant extracts, it can enhance the efficacy of these extracts in controlling pests and diseases, while also being eco-friendly.

Encouraged by the findings of earlier studies, we decided to conduct the current investigation of efficacy of leaf extracts of *L. Camara* and *C. odorata*along with cow urine against Brown Citrus Aphid, *T. citricida* (Kirkaldy) (Hemiptera: Aphididae) of Assam lemon as a Potential natural pesticide. This study became important for the need of alternative natural pesticide for management of this pest.

2. Materials and Methods

The experiment was carried out in the field of, College of Horticulture & Farming System Research (CHFSR), Assam Agricultural University, Nalbari during the year 2022-23, 2023-24 and 2024-25 in Randomized Block Design (RBD) with 6 treatments and 3 replications.

2.1 Collection of plant materials

Fresh leaves of *L. camara* and *C. odorata* were collected from the locality of CHFSR, AAU, Nalbari. Aqueous extracts of both the plants were obtained following standard technique given by Anjarwalla *et al*., (2016) [2]. Leaves were washed thoroughly with tap water and then dipped in sterile distilled water to remove the dust particles. After washing, leaves were oven dried at the temperature 100 °C for 45 minutes to 1 hour and finely powdered by grinding in pestle mortar and then strained with the help of strainer. The extracts were stored in a 10-L buckets and kept in the shade. Before application, the extracts were filtered twice through a fine cloth to remove the extra plant materials for the safety of sprayer.

2.2 Collection of Cow Urine

Fresh cow urine was collected in a sterile stainless-steel container from a local variety of cow. The urine was filtered through Whatman No. 1 filter paper to get rid of debris and precipitated material and was stored in airtight container at room temperature in dark place. This cow urine was directly applied in the trial.

2.3 Blending of urine with plant materials

The leaves of the two plants were mixed aT_1:1 ratio and crashed into paste. Then the paste of clean dirt-free fresh leaves was mixed with cow urine at the rate of 1.5 kg of paste per 20 litres of undiluted cow urine and was kept for 20 days. Then the mixture was strained and preserved at room temperature in dark place for further use in the trial.

2.4 Treatment layout

The two botanicals and cow urine separately; and combined mixture of all three were tested in this study. The treatment application was performed when the population of pests reached at the economic threshold level (ETL). The ETL for aphid is 5 adults or nymphs/leaf (Asif, *et al*. 2016) [3]. The application was carried out, using a 15-L knapsack sprayer. The sprayer was cleaned after application of each treatment.

The experiment was carried out in the experimental field of College of Horticulture & Farming System Research (CHFSR), Assam Agricultural University, Nalbari (Latitude N26.398548, Longitude E91.252308) during the year 2022-23, 2023-24 and 2024-25. Treatments were applied during active period (May-June and August-September**)** of the pest twice at 15 days interval. The aphid infestation in Assam lemon is shown in Fig 1.

2.5 Determination of percent population reduction

Pre-and post-treatment counts were done by counting the number of aphid-infested terminals out of 20 randomly selected terminals from around each of the trees. The first spray was given with the onset of pest incidence after recording pretreatment count and subsequent sprays were repeated after 15 days by using a high-volume sprayer with required concentrations. The posttreatment counts on pests were recorded on 1, 3, 7, 14 days after each application. Both adults and nymphs were counted from both sides of the leaf by visual observation.

2.6 Statistical analysis

The data generated from the field experiments were analyzed for ANOVA. The data on aphid populations were transformed into square root transformation and analyzed using SPSS (version 22) (IBM Crop Released 2013) software to identify the most effective treatments and their means were compared by significant difference at $p \leq 0.05$.

3. Results and Discussion

The present investigation shows that the leaf extract of *L. camara*, *C. odorata*, and cow urine has anti-insectidal activity against Brown Citrus Aphid, *T. citricida*. The findings of the insecticidal activity of different treatments are shown in Table (1-3). The lowest population of *T. citricida* in the treatment of combined mixture of *L. camara*, *C. odorata* and Cow Urine (0.70/terminal) was recorded followed by *L. camara* (0.97/terminal), cow urine (1.65/terminal) and the highest number of *T. citricida* was observed in the control (13.68/terminal). Further, the order of efficacy of the treatments was the control (13.68 aphid/terminal) <Local check (Spinosad 45% SC @ 0.3mL/L (2.93/terminal) <*C. odorata* (2.12) <Cow urine (1.65)<L. camara (0.97) < Combined mixture of *L. camara*, *C. odorata*, Cow Urine (0.70/terminal).The treatment of combined mixture of leaf extract of *L. camara*, *C. odorata* and Cow urine (T_4)showed the most efficacious effect on Brown Citrus Aphid, *T. citricida* in Assam lemon(mean population level 0.70). The least was recorded when no extract treatment (distilled water) was applied

Table 1: Efficacy of plant extracts and cow urine (Natural pesticides) tested against Brown Citrus Aphid, *Toxoptera citricida* infesting Assam lemon

Treatments	Aphid Population/terminal								
	2022-23			2023-24			2024-25		
	3 DAT	7 DAT	14DAT	3 DAT	7 DAT	14DAT	3 DAT	7 DAT	14DAT
T_1: *Leaf extract of L. camara*	2.41 (1.82)	1.85 (1.66)	0.40 (1.18)	2.53 (1.74)	1.60 (1.45)	1.22 (1.31)	4.53 (2.24)	3.30 (1.95)	1.30 (1.34)
T_2: *Leaf extract of C. odorata*	2.14 (1.76)	1.16 (1.46)	0.46 (1.20)	2.77 (1.81)	2.00 (1.58)	1.46 (1.40)	6.46 (2.64)	6.00 (2.55)	4.46 (2.23)
T_3: Cow urine	3.15 (1.91)	3.79 (2.07)	1.95 (1.57)	4.87 (2.32)	2.03 (1.59)	1.95 (1.57)	2.81 (1.94)	2.14 (1.76)	1.05 (1.24)
T_4: *Leaf extract of L. camara* + *C. odorata* + Cow Urine	0.93 (1.38)	0.73 (1.03)	0.13 (1.00)	0.97 (1.21)	1.05 (1.24)	0.93 (1.19)	3.97 (2.11)	2.15 (1.63)	1.05 (1.24)
T_5: Local check (Spinosad 45% SC @ 0.3mL/L)	2.81 (1.94)	3.16 (2.03)	1.74 (1.64)	2.91 (1.84)	3.87 (2.09)	2.03 (1.59)	8.91 (3.07)	8.00 (2.92)	5.03 (2.35)
T_6: Control (Distilled water)	10.27 (3.28)	9.60 (3.25)	9.72 (3.15)	10.07 (3.25)	9.00 (3.08)	8.67 (3.03)	20.00 (4.53)	21.00 (4.64)	22.67 (4.81)
SE (m±)	0.09	0.08	0.10	0.13	0.04	0.16	0.23	0.21	0.14
CD at 5%	0.31	0.52	0.28	0.21	0.09	0.23	0.41	0.39	0.27
CV%	6.85	7.51	5.12	1.03	1.32	1.50	1.05	1.02	0.70

DAT: Days After Treatment; Figures in parentheses are x + 0.5 transformed values.

Table 2: Efficacy of plant extracts and cow urine (natural pesticides) tested against Brown Citrus Aphid, *T.citricida* infesting Assam lemon

Treatments	Aphid Population/terminal											
	3 DAT				7 DAT				14DAT			
	2022-23	2023-24	2024-25	Mean	2022-23	2023-24	2024-25	Mean	2022-23	2023-24	2024-25	Mean
T_1	2.41 (1.82)	2.53 (1.74)	4.53 (2.24)	3.15 (1.91)	1.85 (1.66)	1.60 (1.45)	3.30 (1.95)	2.25 (1.66)	0.40 (1.18)	1.22 (1.31)	1.30 (1.34)	0.97 (1.21)
T_2	2.14 (1.76)	2.77 (1.81)	6.46 (2.64)	3.79 (2.07)	1.16 (1.46)	2.00 (1.58)	6.00 (2.55)	3.05 (1.88)	0.46 (1.20)	1.46 (1.40)	4.46 (2.23)	2.12 (1.62)
T_3	3.15 (1.91)	4.87 (2.32)	2.81 (1.94)	3.61 (2.02)	3.79 (2.07)	2.03 (1.59)	2.14 (1.76)	2.65 (1.18)	1.95 (1.57)	1.95 (1.57)	1.05 (1.24)	1.65 (1.55)
T_4	0.93 (1.38)	0.97 (1.21)	3.97 (2.11)	1.95 (1.57)	0.73 (1.03)	1.05 (1.24)	2.15 (1.63)	1.31 (1.35)	0.13 (1.00)	0.93 (1.19)	1.05 (1.24)	0.70 (1.10)
T_5	2.81 (1.94)	2.91 (1.84)	8.91 (3.07)	4.87 (2.32)	3.16 (2.03)	3.87 (2.09)	8.00 (2.92)	5.01 (2.35)	1.74 (1.64)	2.03 (1.59)	5.03 (2.35)	2.93 (1.85)
T_6	10.27 (3.28)	10.07 (3.25)	20.00 (4.53)	13.44 (3.73)	9.60 (3.25)	9.00 (3.08)	21.00 (4.64)	13.2 (3.70)	9.72 (3.15)	8.67 (3.03)	22.67 (4.81)	13.68 (3.77)
SE (m±)	0.09	0.13	0.23	0.32	0.08	0.04	0.21	0.45	0.10	0.16	0.14	0.26
CD at 5%	0.31	0.21	0.41	0.63	0.52	0.09	0.39	0.86	0.28	0.23	0.27	0.53
CV%	6.85	1.03	1.05	1.32	7.51	1.32	1.02	1.37	5.12	1.50	0.70	1.21

T_1: Leaf extract of L. camara, T_2: Leaf extract of *C. odorata*, T_3: Cow urine, T_4: Leaf extract of L. camara + *C. odorata* + Cow Urine
T_5: Local check (Spinosad 45% SC @ 0.3mL/L), T_6: Control (Distilled water)

Table 3: Mean population of Brown Citrus Aphid, *T. citricida* infesting Assam lemon

Treatments	Aphid Population/terminal (Pooled data of 2022-23, 2023-24 and 2024-25)		
	3 DAT	7 DAT	14 DAT
T_1	3.15 (1.91)	2.25 (1.66)	0.97 (1.21)
T_2	3.79 (2.07)	3.05 (1.88)	2.12 (1.62)
T_3	3.61 (2.02)	2.65 (1.18)	1.65 (1.55)
T_4	1.95 (1.57)	1.31 (1.35)	0.70 (1.10)
T_5	4.87 (2.32)	5.01 (2.35)	2.93 (1.85)
T_6	13.44 (3.73)	13.2 (3.70)	13.68 (3.77)
SE (m±)	0.32	0.45	0.26
CD at 5%	0.63	0.86	0.53
CV at 5%	1.32	1.37	1.21

T_1: Leaf extract of L. camara, T_2: Leaf extract of *C. odorata*, T_3: Cow urine, T_4: Leaf extract of L. camara + *C. odorata* + Cow Urine
T_5: Local check (Spinosad 45% SC @ 0.3mL/L), T_6: Control (Distilled water)

There are numerous studies that used the potential of *L. camara*, *C. odorata*, and cow urine as insecticide and can confirm to the result obtained. In our study, the biorational insecticides tested were found to be significantly effective and lowered the infestation of *T. citricida*. These findings are in accordance with Amin *et al*. (2017) [1] who observed the reduction of *Aphis gossypii* population and infestation in a bitter gourd with sequential application of bio pesticide, botanical insecticides.

L. camara and *C. odorata* extracts, along with cow urine, have shown varying levels of efficacy against insect pests in agricultural crops. *L. camara* extracts have shown larvicidal, pupicidal, insecticidal and mosquito repellent activities (Kumar *et al*., 2024) [7]. *L. camara* extracts can effectively control aphids on rapeseed, with higher concentrations and longer exposure times resulting in higher mortality (Mvumi and Maunga. 2018) [10]. Samuel *et al*. (2019) [11] reported the insecticidal effect of *C. odorata* leaves and stems which significantly controls beans weevil. *C. odorata* extracts also exhibit insecticidal and allelopathic effects, suppressing the growth of other plants (Julio *et al*. 2019) [5].

Many studies support cow urine as effective insecticides. In traditional Indian agriculture, cow urine has been used as a natural pesticide and fertilizer for centuries. Cow urine is rich in nitrogen, potassium and other nutrients essential for plant growth. In addition to its nutrient content, cow urine also contains beneficial microorganisms that can help to enhance soil health and reduce plant diseases (Boraiah *et al*., 2017) [4]. Jandaik *et al*. (2015) [13] reported that the cow urine can be used as effective biopesticide. It provides essential nutrients for plant growth, improve soil structure and water retention and help control pests and diseases naturally (Patra and Nalin, 2024) [12].

The findings of the present study therefore indicate that Combining leaf extracts with cow urine have some toxic properties and therefore could be considered as potential source of natural pesticide for economical and environmentally friendly pest control strategies against aphids in Assam lemon.

Fig 1: Infestation of Brown Citrus Aphid, *Toxoptera citricida* (Kirkaldy) (Hemiptera: Aphididae) in Assam lemon

4. Conclusion

The present investigation showed that the leaf extract of *L. camara*, *C. odorata*, and cow urine has anti-insectidal activity against plant damaging insects like Brown Citrus Aphid, *T. citricida*. Cow urine, when combined with extracts of *L. camara and C. odorata*, can be an effective natural solution for pest control. The eco-friendly, cost-effective and residue-free nature of the cow-urine-based leaf extract can be easily incorporated into the natural farming system. These botanical options including cow urine will help the farmers for opting wide range of biopesticides instead of schedule chemical sprayings.

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