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Original Research Article

Biopesticide for controlling *Citrus limon* (L.) Burm f. (Assam lemon) pests and diseases

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Abstract: A biopesticide has been developed from *Azadirachta indica* A. Juss, *Polygonum glabrum* Willd., *Cyclosorus parasiticus* (L.) Farw., *Curcuma longa* L., *Capsicum annum* L. and cow urine to control insects and pests infestation on *Citrus limon* (L.) Burm f. (Assam lemon) grown at Kokrajhar district, BTR, Assam. Those five number of locally available medicinal plants selected for pesticide preparation were ground collectively into a fine paste using a mixer grinder along with distilled water. The paste was then sieved with a muslin cloth and squeezed to extract the solvent and diluted up to 200 ml. The resulting filtrate constitutes 50% stock solution later diluted into 1%, 5% and 10% and each solution has been mixed with 200 ml of cow urine before spray. The strength of aqueous extracts were evaluated by foliar spray on plants and their efficacy were determined for controlling the pests and diseases. Meanwhile four pest infested lemon trees were selected and tagged S_1, S_2, S_3, and S_4. Plant tagged as S_1, S_2, and S_3 were considered for applying biopesticide @ 1%, 5% and 10% while S_4 treated as a control plant. The said biopesticide of three different concentrations were sprayed once in a week from January 2024 to March 2025 with a hand sprayer (2L capacity). The biopesticide @10% (S_3) concentration showed the best result with reduction of *Dialeurodes citri* (white fly), *Anoplophora versteegi* (trunk borer), *Phyllocnistis citrella* (leaf miner), *Diaphorina citri* (Citrus psylla) and other insect population as compared to the lemon plants- S_1, S_2 and S_4.

Key words: Biopesticide, *Citrus limon* (L.) Burm f., Disease, Insect, Pest and IPM.

Introduction:

Citrus limon (L.) Burm f. (Assam lemon) is an indigenous citrus fruit of Assam belonging to the family- Rutaceae (Bhattacharya & Dutta, 1956; Gogoi, 2021). The agro-climatic conditions of Kokrajhar district as well as Assam is suitable to grow lemon at commercial scale (Gogoi *et al*., 2024). However, insect and pest infestation is a serious issue that is lowering the productivity as a whole. Therefore, a study has been undertaken to identify the lemon diseases and develop an effective biopesticide for controlling the same. Production of citrus crop is adversely affected by harmful insects and pests, which cause severe economic loss to the lemon cultivators. They are generally dependent on synthetic pesticides to control the plants from damage caused by insects (Rahman *et al*., 2007). The constituent biochemicals of synthetic pesticides have

been attributed to chronic human ailments either due to consumption or exposure (Kumari *et al*., 2014). On the other hand, excessive use of chemical pesticide creates many serious threats such as elimination of beneficial predators and insects, depletion in soil microbial diversity, resistance among the pests and diseases, deposition of toxic residues etc. (Gupta *et al.,* 1997). Meanwhile, management of pests without using harmful chemicals to human health and the environment is possible only by using biopesticides (Chandler *et al.,* 2011). It has been described by Dubey (2014) and Lengai *et al*. (2020) that biopesticide is prepared from naturally occurring substances or extracted from plant and animal species that exhibit pesticidal properties. On the other hand, biopesticides are environmentally less harmful than synthetic pesticides to control pests and they possess one or more useful properties, such as biodegradability, a broad spectrum of activity and ability to reduce insect resistance. According to Chaudhari (2014), synergistic effect due to the mixing of different plant species plays a key role in controlling pests. Preference has been given to the use of leaf, fruit, rhizome and stem from different plant species that have tested earlier by IBH, Science College, Kokrajhar during the preparation of biopesticide to manage lemon pests and diseases. Needless to mention that a large array of medicinal plant varieties grow naturally in Kokrajhar district out of that, 25 species have been initially considered for testing and finally five plant species have been selected to develop the biopesticide. The same has been applied in three different concentrations along with equal volume of cow urine on selected lemon tree and the findings are enumerated in the result and discussion section.

Materials & Methods

Experimental site

The agro-climatic conditions of Kokrajhar districtis suitable to grow lemon varieties but insects and pests attack at vegetative stage when new leaves are developed as well as flowering and fruiting stage which ultimately decrease lemon productivity as a whole. Aiming to develop an effective biopesticide to control the lemon diseases, a thorough study

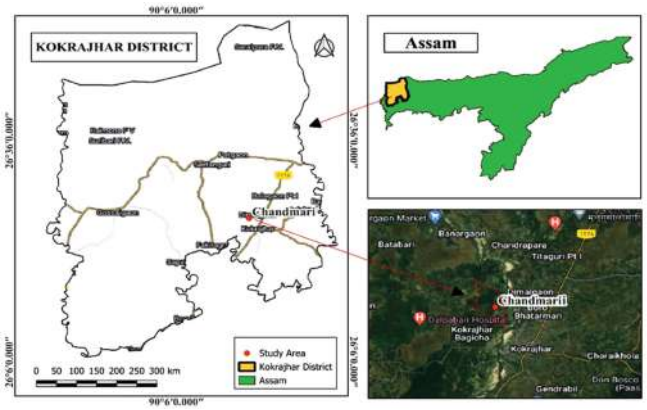

Fig. 1. Map showing the study area.

was conducted in Kokrajhar district during 2023-2025 for identification of the most prevalent lemon diseases in lemon orchards and their causal organisms that causes disease. Finally a study site (Fig. 1) was selected at Chandamari village (Lat: 26.424100N Long: 90.252649E) of Kokrajhar district for observation of the efficacy of biopesticide through foliar spray systematically.

Method of biopesticide formulation, dilution and spray

Plant species used to develop biopesticide were identified with the help of taxonomic literature including relevant book (Jain, 1968 and Sharma, 2009). Altogether five plant species viz. *Azadirachta indica* A. Juss, *Polygonum glabrum* Willd., *Cyclosorus parasiticus* (L.) Farw., *Curcuma longa* L., *Capsicum annum* L. have been selected on the basis of antioxidant availability as well as insecticidal properties found in leaf, stem, rhizome and fruit **(Table-1)** followed by collection, washing and weighing 20g of each plant parts in an electronic balance. The samples were ground collectively into a fine paste using a mixer grinder along with 200 ml of distilled water following the method of Chaudhari (1914) and Rehman *et al.* (2020). The paste was then sieved with a muslin cloth and squeezed to extract the liquid, while solid residue has been discarded. The final volume of the extract was made 200ml by adding distilled water. The aqueous extract harvested finally constitutes a 50% concentration, which was stored in a sterilized beaker as stock **(Fig. 2).** Further dilution of 1%, 5% and 10% has

Table. 1. List of plant species and their parts used with cow urine for biopesticide preparation.

Sl. No.	Vernacular name	Botanical name	Family	Plant part/ liquid byproduct of animal used	Pesticidal quality
1	Nim bilai (Bodo), Nim pat (Assamese) and Neem or Margosa (English)	*Azadirachta indica* A. Juss.	Meliaceae	Leaf	The leaf and seed has insecticidal, nematicidal, fungicidal and bactericidal property. (Schmutterer, 1995)
2	Bisongali (Bodo), Bihlongani (Assamese) and Dense flower knotgrass (English)	*Polygonum glabrum Willd.*	Polygonaceae	Leaf	Plant possesses high antimicrobial property. (Raja and Ramaya, 2016)
3	Saldaokhumwi (Bodo), Bihdhekiya (Assamese) and Parasitic maiden fern (English)	*Cyclosorus parasiticus (L.) Farw.*	Thelypteridaceae	Leaf	Flavanoids present in the plant are toxic to pests, microbes and viruses. (Gunawan *et al.,* 2016)
4	Haldwi (Bodo), Haldhi (Assamese) and Turmeric (English)	*Curcuma longa L.*	Zingiberaceae	Rhizome	Turmeric has insecticidal and anti-feedant property. (Saxena & Sayyed, 2018)
5	Banlu (Bodo), Jolokia (Assamese) and Red chili (English)	*Capsicum annum L.*	Solanaceae	Fruit	Red chili contains capsaicin which is an insecticide. (Koleva-Gudeva *et al.,* 2013)
6	Mwswo Hasudwi (Bodo), Gomutra Assamese) and Cow urine (English)	----------	----------	Cow urine	Cow urine possesses antifungal and antibacterial property. (Kgasudi & Mantswe, 2020)

been prepared from the stock adding distilled water and mixed with 200 ml of cow urine with each extract. Prior to the formulation of biopesticide, four pest infested lemon tree were selected and tagged S_1, S_2, S_3, and S_4. Lemon tree S_1, S_2, and S_3 were considered for biopesticide application of three different concentrations, while S_4 was treated as a control plant without any application. The pesticide efficacy was assessed through foliar spraying of biopesticide of three concentrations, where 1% applied on S_1, 5% on S_2 and 10% on S_3 of three branches of each plant on the same date. Biopesticide was sprayed once in a week four times in a month from January, 2024 to March, 2025 with a hand sprayer (2L capacity). Data were recorded for the comparison of the population of insects before and after pesticide spray and presented in the result section.

Result

Field trials indicate a significant control in the lemon insects, pests and diseases. It was revealed that the mean variation (S_3) of curled leaf (16.3 ± 4.63), chewed leaf (16 ± 2.51), leaf miner (11.3 ± 2.96), white fungus (12.6 ± 3.48), spider (5.33 ± 1.45), black spot on leaf (3.33 ± 0.66), white fly (8.66 ± 0.33), black fly (7 ± 2.88), black sooty mould (6.33 ± 1.20), trunk borer (1.66 ± 0.33), aphids (10 ± 2.64), caterpillar (0.66 ± 0.66) and gummosis (1 ± 0.57) disease before spray the biopesticide (Table-2 & Fig. 3). While mean variation (S_3) of curled leaf (1 ± 0.57), chewed leaf (4.66 ± 1.45), leaf miner (0 ± 0), white fungus (0.66 ± 0.33), spider (1 ± 0.57), black spot on leaf (0 ± 0), white fly (1.33 ± 1.33), black fly (0 ± 0), black sooty mould (0 ± 0), trunk borer (0 ± 0), aphids (0.33 ± 0.33), caterpillar (0 ± 0) and gummosis (0 ± 0) disease after spray the biopesticide (Table-3 & Fig. 4).

The said biopesticide was found to have deterrent action against *Diaphorina citri, Phyllocnistis citrella, Aleurocanthus woglumi, Dialeurodes citri, Papilio demoleus, Anoplophora versteegi* and other pests, gradually reduced the damage to lemon plants. On the other hand, a significant change in the percentage of disease infestation in S_2 and S_3 plants sprayed with 5% and 10% biopesticide observed while the untreated S_4 plant remains the same. The S_2 plant showed

Fig. 2: A. Leaf of *Azadirachta indica* A. Juss, **B**. Branch of *Polygonum glabrum* Willd., **C.** Frond of *Cyclosorus parasiticus* (L.) Farw., **D**. Rhizome of *Curcuma longa* L., **E**. Ripe fruit of *Capsicum annum* L., **F**. Aqueous extract (concentration-50%), **G.** Dilution at 10%, 5%, 1%, **H**. 1% pesticide applied on lemon plant, **I.** 5% pesticide applied on lemon plant and **J**. 10% pesticide applied on lemon plant.

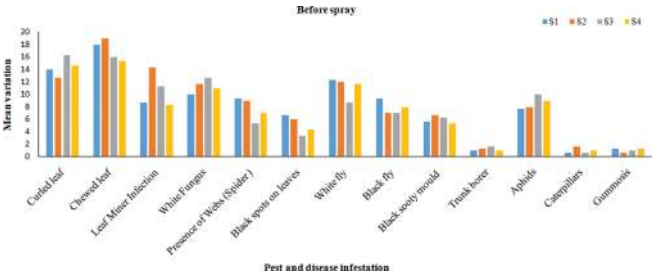

Fig. 3. Mean variation of pest and disease occurrence before spray with biopesticide.

highest infestation 57% chewed leaves before, reduced to 17% after spraying and in S_3 plant from 48% to 14%. Before spraying (Fig. 5), leaf miner infestation was observed 43% in S_2 plant which reduced to 5% and 30-38% white fungus infection reduced to 2-4% in treated plants. The spider webs also reduced to 3% when sprayed with 10% pesticide solution. The black sooty mould population has been wiped out in all treated lemon plants. Aphid population reduced to 1-3% after the spray. Initially, there was moderate infection by white fly and black fly & the same has also been decreased to 4% when sprayed with 10% pesticide solution. On the other hand, black sooty mould was around 16-20% across different samples but it was later observed between 0-2% infections. Another two harmful insects, trunk borer and caterpillar population were observed almost zero population when sprayed three rounds in the same concentration (10%). Gradual decrease of aphid population can be considered as another convincing indication of the biopesticide efficacy (Fig. 6). The biopesticide with @10% concentration was the best in terms of efficacy to

Table. 2. Before treatment.

Sl. No.	PARAMETER	MEAN ± SE (S1)	MEAN ± SE (S2)	MEAN ± SE (S3)	MEAN ± SE (S4)
1	Curled leaf	14 ± 2.08	12.6 ± 2.33	16.3 ± 4.63	14.6 ± 2.72
2	Chewed leaf	18 ± 1.73	19 ± 1.15	16 ± 2.51	15.3 ± 0.88
3	Leaf miner infection	8.66 ± 0.88	14.3 ± 4.3	11.3 ± 2.96	8.33 ± 1.45
4	White fungus	10 ± 1.15	11.6 ± 1.76	12.6 ± 3.48	11 ± 2.08
5	Spider webs	9.33 ± 3.48	9 ± 2.51	5.33 ± 1.45	7 ± 2.88
6	Black spots on leaves	6.66 ± 1.66	6 ± 1	3.33 ± 0.66	4.33 ± 2.18
7	White fly	12.3 ± 2.02	12 ± 1.73	8.66 ± 0.33	11.6 ± 0.33
8	Black fly	9.33 ± 2.02	7 ± 2.08	7 ± 2.88	8 ± 1.52
9	Black sooty mould	5.66 ± 1.20	6.66 ± 1.20	6.33 ± 1.20	5.33 ± 2.40
10	Trunk borer	1 ± 1	1.33 ± 0.88	1.66 ± 0.33	1 ± 1
11	Aphids	7.66 ± 1.76	8 ± 1.52	10 ± 2.64	9 ± 1.52
12	Caterpillars	0.66 ± 0.66	1.66 ± 0.88	0.66 ± 0.66	1 ± 0.57
13	Gummosis	1.33 ± 0.88	0.66 ± 0.33	1 ± 0.57	1.33 ± 0.66

Table. 3. After treatment.

Sl. No.	PARAMETER	MEAN ± SE (S1)	MEAN ± SE (S2)	MEAN ± SE (S3)	MEAN ± SE (S4)
1	Curled leaf	9.66 ± 2.60	3 ± 2.08	1 ± 0.57	14.66 ± 1.20
2	Chewed Leaf	7.66 ± 0.66	5.66 ± 2.02	4.66 ± 1.45	10.6 ± 2.6
3	Leaf miner infection	1.66 ± 1.20	0.33 ± 0.33	0 ± 0	5.66 ± 1.20
4	White fungus	1.33 ± 1.33	0.66 ± 0.33	0.66 ± 0.33	3.33 ± 0.88
5	Spider webs	4 ± 2	3 ± 0.57	1 ± 0.57	5.66 ± 1.20
6	Black spots on leaves	0.33 ± 0.33	0 ± 0	0 ± 0	2.66 ± 1.20
7	White fly	4 ± 0.57	3 ± 0.57	1.33 ± 1.33	6.33 ± 1.45
8	Black fly	2 ± 1	1.66 ± 0.88	0 ± 0	4.66 ± 1.45
9	Black sooty mould	0.33 ± 0.33	0.33 ± 0.33	0 ± 0	3 ± 0.57
10	Trunk borer	0 ± 0	0 ± 0	0 ± 0	0.33 ± 0.33
1	Aphids	1 ± 0.57	1 ± 0.57	0.33 ± 0.33	3.66 ± 0.88
12	Caterpillars	0 ± 0	0 ± 0	0 ± 0	0 ± 0
13	Gummosis	0.33 ± 0.33	0.33 ± 0.33	0 ± 0	0.66 ± 0.66

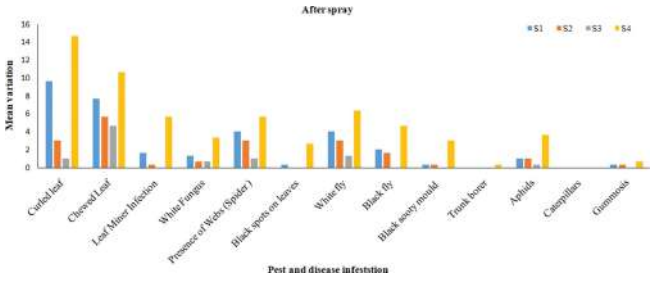

Fig. 4. Mean variation of pest and disease occurrence after spray with biopesticide.

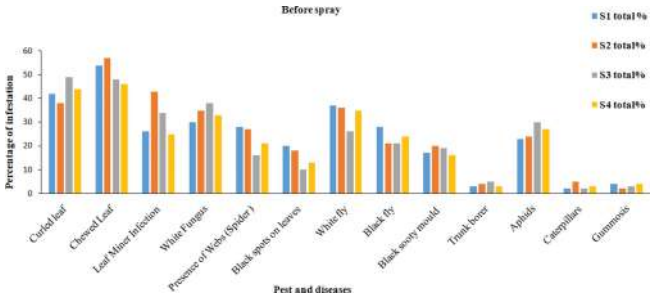

Fig. 5. Data recorded before foliar spray.

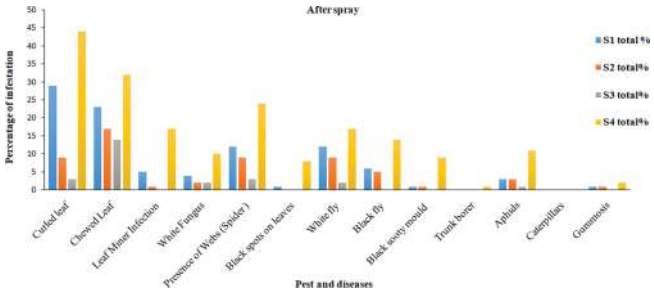

Fig. 6. Data recorded after foliar spray.

control over *Dialeurodes citri* (white fly), *Anoplophora versteegi* (trunk borer), *Phyllocnistis citrella* (leaf miner), (*Diaphorina citri* (Citrus psylla) and other insect population as compared to 1% and 5% bioesticide.

Discussion

Being a host plant to many insects, the young leaf, branch and fruit of *Citrus limon* (L.) Burm f. (Assam lemon) is attacked by insects and pests resulting in reduced productivity as a whole. Technology intervention in citrus industry is necessary for increasing its production but organic cultivation is essential for ecosystem restoration (Cheremisinoff & Rosenfeld, 2010) by using biopesticide (Gullan & Cranston, 2014). Needless to mention that Geographical Indication (GI) has been awarded to Assam lemon in 2019 by RGI, Govt. of India and the Govt. of Assam has notified the Assam lemon as the state fruit of Assam on 13th February, 2024 (Gogoi *et al.,* 2024). There is still limited research information on lemon cultivation as well as disease management. It is a demand of the hour to standardize integrated pest management method to control insects and pests attack on lemon plant through organic process. The use of biopesticide in lemon plants either biochemical or microbial pesticides have many advantages. The result shows positive effect against numerous insect populations as presented in Fig. 4 & Fig. 6. Earlier studies

have shown that plant active compounds are effective against various insect pests (Ingle *et al*., 2017), fungi (Salhi *et al*., 2017), bacteria (Chatterjee *et al*., 2007), nematodes (Neeraj *et al.,* 2017) and viruses (Elbeshehy *et al*., 2015). The bioactive compounds that are present in plant extract are mainly secondary metabolites such as steroids, alkaloids, tannins, terpenes, phenols, flavonoids and resins that possess antifungal, antibacterial, antioxidant or insecticidal properties (Lengai *et al*., 2020). Among different locally available medicinal plants, Neem products alone can prevent many harmful insects and pests. Both leaves and fruit of neem plant are known to have bitter taste having fungicidal, insecticidal and nematicidal properties (Schmutterer, 1995). Similarly, *Polygonum glabrum* is another plant species which possess high antimicrobial property. The plant contains many flavonoids including diosmetin, rutin, hyperin and quercitrin, act as antimicrobial and defensive to pests and diseases (Raja and Ramaya, 2016). It has been reported that *Cyclosorus parasiticus* contains several secondary metabolites, among them are flavonoids and alkaloids found in the leaves (Tangavelou and Viswanathan, 2017). The flavonoids acts as defensive chemicals against pests, microbes, and viruses, whereas the secondary metabolite alkaloid served as toxins to protect plants from pests and diseases (Gunawan *et al*., 2016). The red chilli is used as a source of capsaicin that has repellent and insecticidal properties (Koleva-Gudeva*et al.,* 2013). Research studies reveal that hot chili extract is effective in organic farming as a natural biopesticide and it has tremendous scope as sustainable alternative to synthetic pesticides. Similarly, *Curcuma longa* L. extracts have shown potential as repellents, insecticides, and anti-feedants on different insects. The extracts of *Azadirachta indica and C. longa* exhibited potent insecticidal and repellent effects on *C. maculates* (Saxena & Sayyed, 2018).

Plant extracts mixed with cattle urine has been studied to check their efficacy to protect crops from pests. Cow urine contains 95% water, 2.5% urea, and the remaining 2.5% a mixture of salts, hormones, enzymes, and minerals (Ramani, *et al.,* 2012). Many studies in India found that cow urine enhances soil fertility, growth of plant and control of insect pest and diseases. Cow urine ingredients are capable of treating many diseases as it has several medicinal properties and it is the best remedy to cure fungal and bacterial diseases (Kgasudi and Mantswe, 2020). A mixture of cow urine and water extract of neem leaves protected soybean crop from the pest (Purwar and Yadav, 2003). Various combinations of cow urine and plant parts and neem-based commercial products have shown significant synergistic effect to enhance product toxicity resulting in pest mortality (Gahukar, 2013). Cow urine is used by the farmers as an effective indigenous method to control crop pests (Banjo *et al*., 2003) and spraying of the cow urine has been recommended to minimize the harmful effects of synthetic pesticides (Chauhan and Singhal, 2006). From the above discussion it has become clear that applying herbal pesticide in recommended dose with cow urine can prevent insects and pests infestation on lemon plants. However, it will work more efficiently when Integrated Pest Management (IPM) method is used in a scientific and systematic manner with application of biopesticide regularly.

Conclusion

The biopesticide has shown deterrent action against *Diaphorina citri, Phyllocnistis citrella, Aleurocanthus woglumi, Dialeurodes citri, Papilio demoleus, Anoplophora versteegi* and other pests, reducing the damage of lemon plants and the fruits. Among three different dilutions, biopesticide @10% concentration spraying at one week interval is the best solution to control white fly, trunk borer, leaf miner and other insect population as compared to 1% and 5% and untreated plant. Therefore, lemon cultivators are urged to adopt the use of biopesticide for controlling lemon diseases instead of chemical pesticide.

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