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Influence of environmental factors on stability and anti-feedant activity of biopesticide formulations of *Pachygone laurifolia* (DC.) L.Lian & Wei Wang (*Cocculus laurifolius*) against *Spodoptera litura* (Lepidoptera : Noctuidae)

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ABSTRACT

The efficacies of biopesticides are influenced by various storage conditions including environmental factors and stability is one of the key concerns. Emulsifiable concentrates (ECs) prepared from the bark crude extract of *Pachygone laurifolia* plant and its active fraction showed significant feeding deterrence on *Spodoptera litura,* a poly-phytophagous insect pest attacks many vegetables and economically important cultivators. Effect of various factors including storage duration, storage temperature, exposure to sunlight and ultraviolet light on the stability of the formulations prepared from two different concentrations (1 and 2.5%) were evaluated. The EC preparations of both crude extract and active fraction showed significantly high anti-feedant activity. In case of EC from crude extract, the accelerated storage conditions shown to decrease the anti-feedant activity at the lower concentration of 1% however no significant change was noted at 2.5% concentration except at 20^0C. The EC preparation from active fraction, up on long term storage at 30 ± 2^0C for different time intervals, significant loss of activity was noted at 12-month storage in both concentrations of crude extract EC and active fraction EC preparations. Exposure to direct sunlight has significantly reduced the activity in a time dependent manner. UV light exposure has significantly reduced the activity in low concentration (1%) EC formulation of both crude extract and active fraction however, the higher concentration preparations were stable up to 8 hrs. The study showed that, EC formulations of *P. laurifolia* crude extract and active fractions were sable at higher concentration and can be used for field applications.

Keywords: Biopesticide formulation, Shelf life, Thermal stability, Photodegradation, UV light degradation.

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INTRODUCTION

The crop yield around the World is reduced by 38% due to insect pest attack (Junaid *et al.,* 2024). Enhancement of crop yield using synthetic pesticides has been achieved nearly 100 per cent in USA and 70 per cent in Europe (Pretty, 2008). Various risks are associated with usage of chemical pesticides because of the adverse effects caused by them to ecosystem as a whole and human health in particular. The negative impacts caused by the synthetic pesticides has raised the demand for better greener alternatives and it led to the development of biopesticides. Biopesticides are a key element of sustainable agriculture, which contributes to achieve the goals of the United Nation's 2030 Agenda (Fenibo *et al*., 2021). These alternative methods offer valuable solutions and benefits for facing the increasing challenges

related to food security, environmental sustainability and human health. Over years, the biopesticide consumption has showed significant increase, with use of 8,647 metric tons in 2020–21 and 8,898 metric tons in 2021–22 (Hegde and Vijaykumar, 2022). However, this accounts only few percentage of all pesticides used for pest control. The problems with biopesticides are poor stability during storage, costly production techniques, efficacy issues, susceptibility to environmental conditions, etc (Gašić and Tanović, 2013). Many a times, field experiments do not produce the optimistic outcomes anticipated under practical circumstances. Though biodegradable, natural molecule-based products are typically less stable than synthetic pesticides often lead to efficacy issues (Gupta and Dikshit, 2010; Villaverde *et al*., 2014). Reduced effectiveness of plant-based products under outdoor conditions is primarily caused by the breakdown and volatile nature of bioactive chemicals. The product performances and effectiveness can be enhanced through improved formulations. Formulation is exceedingly challenging when it comes to improving and extending the activity of biopesticides. These goals could be accomplished by synthesizing bioagents in different ways. Higher concentrations of the botanicals or active compounds are reported to provide necessary efficacy. Similarly, botanicals or active compounds dissolved in a solvent along with emulsifiers and surfactants to create emulsifiable concentrates (ECs) are also reported to provide better efficacy. Several plant extracts were assessed for their pesticidal properties however, preparations of EC formulations and their stability studies under environmental conditions are limited (Waghmare *et al*., 2007; Patzke and Schieber, 2018).

Pachygone laurifolia (DC.) L.Lian & Wei Wang, is a shrub or medium sized tree, native to Indian subcontinent, Japan and Philippines, which is grow in seasonally dry tropical biome belonging to Menispermaceae (Lian *et al.,* 2020). It is one such plant which has got anti-insect properties against *Spodoptera litura* in our previous study (Paul and Jayaraj, 2020). *P. laurifolia* (DC.) locally referred to as "aadukolli," the name came from folk tradition because goats cannot survive on eating the leaves of this plant, maybe because of the presence of extremely active macromolecules. The bark and leaf of this plant showed remarkable amount of antimicrobial, antioxidant potential and insecticidal properties (Ajaib *et al*., 2017; Paul and Jayaraj, 2020). *Spodoptera litura* (Fab.) (Lepidoptera: Noctuidae) is a poly-phytophagous pest desecrate many economically important cultivars found in Asian countries. Plenty of crops are under the attack of this pest and several chemical agents are in use against this pest. In this regard, the current work aimed to explore the anti-feedant activity of *P. laurifolia* extract and its active fraction in order to develop an effective anti-insect formulation. The study focuses on the stability and efficacy of the formulations under different environmental as well as storage conditions to develop an effective anti-insect formulation.

MATERIALS AND METHODS

Chemicals and reagents

All the chemicals were purchased from M/s. Merck Specialties Private Limited, Mumbai, India. Whatman No #3 paper was procured from M/s. GE Healthcare Life Sciences. Anionic emulsifier purchased from Organic Dews, Lucca Retail Pvt. Ltd. Dindigul, Tamil Nadu, and Vegetable (coconut) oil purchased from local market.

Plant materials and extraction

Plant material *P. laurifolia* bark was collected from Mattupetti, India (N 10º 07’11.0” E 077º 10’24.4”) which falls in the foot hills of southern Western Ghats. Voucher specimens were deposited in Kerala Forest Research Institute Herbarium (KFRI), India with accession number 18026. Thoroughly cleaned, shade dried and powdered samples (10gm) were hot extracted with 200 ml methanol using soxhlet apparatus for 6–8 hr (4-5 repeat refluxes). The extracts were concentrated with rotavapor and kept at -20°C for future use.

Fractionation of the samples were carried out using a glass column packed with 100 - 200 mesh silica gel. The samples were eluted using different solvents as mobile phase in the sequence, hexane (H), chloroform (C), methanol (M) and water (W) with the flow rate of 1 mL min^{-1}. Six major fractions were collected. Fraction - I (H-100%), Fraction - II (H 50%: C 50%), Fraction - III (C - 100 %), Fraction - IV (C 50%: M 50%), Fraction - V (M-100%) and Fraction - VI (W 100%). Collected fractions were evaporated and the dry weights were recorded. Fraction - IV (C 50%: M 50%), Fraction -V (M-100%) were found to have anti-feedant activities. The emulsifiable concentrate (EC) formulations were prepared from the crude extract and the active fractions.

Preparation of Emulsifiable concentrates (EC)

Biopesticide - Emulsifiable concentrates were prepared by dissolving different combinations of extract or active fractions with emulsifiers in a solvent. The maximum amount of crude extract or active fraction allowed in the EC formulation is up to 70 % (Patzke and Schieber, 2018). Different combinations were attempted for the preparation of an effective EC and the final EC stock formulations were prepared by mixing crude extract or active fraction, anionic emulsifier and solvent in the ratio 7:1:2. Our earlier studies indicated the anti-insect property of crude bark methanol extract of *P. laurifolia* in terms of feeding deterrence at its lower concentration (0.5 %) itself (Paul and Jayaraj, 2020) as well as its fractions (Fraction IV-Chloroform : Methanol 1:1 and V-Methanol). Hence, these extract/active fraction with different concentrations were used for the preparation of EC. The final spraying formulations for application in insects were prepared in such a way that the concentration of the crude extract or active fraction becomes 0.5, 1.0, 2.5 and 5% after the dilution with water.

Insect rearing and bioactivity assay

Culture and maintenance of *Spodoptera litura*

Larvae of *S. litura* were captured from banana plantations of central Kerala, India (N 9º58'35'' E 76º26'20'') and their successive generations were kept in a condition of 25°C ± 2°C and relative humidity of 60 ± 5% with a 14:10 h (L:D) photoperiod. It was moved to jars with wet sterilized sand wrapped in filter paper during the pupation stage. When the adults emerged, they were placed in oviposition jars and given a solution of honey along with some drops of multivitamin. After emerging from the egg, the newborns were placed in glass jars (20 cm × 15 cm × 10 cm) filled with freshly harvested, well-washed wild variety of *Ricinus communis* L. (castor) leaves. Throughout the duration of the study, this procedure was continued and the insect culture was kept up.

Anti-feedant activity

The efficacy of the preparations from *P. laurifolia* (a) crude extract of bark (b) EC prepared from crude extract, (c) active fraction and (d) EC prepared from active fraction; were assayed for the anti-feedant activity by no-choice method (Isman *et al*., 1990). The efficacy of EC at different concentrations (0.5, 1.0, 2.5 and 5.0%) were attempted in *S. litura* larvae at its third instar stage (length - 11.5 to 12.5 mm and weight - 0.10 to 0.13 g). *Ricinus communis* leaf disc without extract was used as control. Three replicates were maintained per concentration. The anti-feedant activity in per cent was calculated after 24 h of exposure (Singh and Pant, 1980). The efficacy of crude extracts and active fraction from previous studies and formulated samples were compared in terms of their feeding deterrence.

Toxicity assay

The toxicity of crude extract of *P. laurifolia* and active fraction based EC formulations were - evaluated on *S. litura* 3rd instar (length - 11.5 to 12.5 mm and weight - 0.10-0.13g) by topical application method (Rani *et al.,* 2015) with few modifications. Various concentrations (10 – 140 mg/mL) of extracts (20 µL) were applied to the larvae at the dorsal side of the thoracic region using a micro-applicator (Gilson, USA). For each treatment 20 larvae were used in three replicates (i.e., n = 60 per treatment). After treatment larvae were placed in plastic dishes and allowed to feed

on natural diet (castor leaves) at 25 ± 2°C, 60 ± 5 % for *S. litura*. The percent mortality for *S. litura* was measured at the end of 24 h and corrected mortality was calculated using Abbott's formula (Abbott, 1925). Acute toxicity based on LC_{50} value was calculated using Probits with Polo plus 2.0 software (Version 2007). The dosages were estimated as mg/larva based on the exposure of a larva to each concentration for LC_{50} calculations.

Stability studies

Influence of different storage as well as exposure conditions on stability of *P. laurifolia* crude extract and active fraction-based EC formulations were assessed in order to identify the best conditions for storage and handling. The *P. laurifolia* crude extract and its active fraction-based EC formulations were taken at 1.0 and 2.5 % from stock solution in a clear bottle. Effects of environmental conditions on formulations were evaluated according to CIPAC method (CIPAC handbook J, 2000). All the EC formulations were kept at following conditions for the specified time periods.

1. Accelerated temperature storage: At 30 ± 2°C (RT) for zero time, 72 ± 2°C for 3 days, 54 ± 2°C for 14 days, 35 ± 2°C for 12 weeks, and 20 ± 2°C for 24 weeks (CIPAC M46.1. (1995)). The samples were kept in temperature controlled incubators.
2. Shelf life: Samples were kept away from direct sunlight for 1 year in an incubator with temperature 30 ± 2°C.
3. Photolysis: All the samples were exposed to direct sunlight for 1, 2, 4, 8 and 24 hr (Temperature range - 36 ± 2°C).
4. Photo degradation by UV light: The stability of samples under UV exposure were evaluated (UV light - G13T8 tube, 30 W, 254 nm) for 1, 2, 4, 8 and 24 hr.

After specific time period or exposure, the activity of the EC formulations were measured by anti-feedant bioassay as mentioned above (Isman *et al*., 1990).

RESULTS

Preparations of Emulsifiable concentrate (EC)

In an ideal EC formulation, after water dilution it should be initially stable and after 24 hours, creaming and oil separation should not be noticed (Kala *et al*., 2020). After attempting different combinations, an effective EC was prepared by mixing "crude extract (CE) and or active fraction (AF)", "anionic emulsifier" and "solvent" in the ratio 7:1:2. This combination found to satisfy the condition of an ideal EC formulation as mentioned above. Hence this preparation was used for the activity studies against *S. litura.* The EC formulation for final application was prepared out of this concentrate formulation, leading to concentration of active ingredient/fraction as specified.

Anti-feedant activity of EC formulations

Comparing the anti-feedant activity of only crude extract with emulsifiable concentrates of *P. laurifolia* on *S. litura,* had shown enhanced feeding deterrence effect (Fig. 1a). The percentage of feeding deterrence of crude *P. laurifolia* extract was 41.30 ± 1.17, 47.29 ± 0.88, 74.47 ± 4.32, and 92.18 ± 1.36 % at its concentrations of 0.5, 1.0, 2.5 and 5.0 % respectively. In case of tested EC formulation on *S. litura,* showed anti-feedant activity of 74.07 ± 1.32, 84.83 ± 0.72, 92.88 ± 2.19 and 96.51 ± 1.06 % in the dose of 0.5, 1.0, 2.5 and 5.0 % respectively. EC formulations dose dependent feeding deterrence was recorded.

The effects of the active fraction of *P. laurifolia* extract and its EC formulation against the pest *S. litura* were studied (Fig. 1b). The anti-feedant activity of fraction at different concentrations showed significantly high feeding deterrence compared to control. The EC prepared with fraction also showed a similar trend. The feeding deterrence was less in prepared EC formulations compared to its active fraction alone. The feeding deterrence was maximum observed in prepared EC at highest concentration of 5.0 % (96.46 ± 0.43 %).

Toxicity analysis

The toxicity studies of emulsifiable concentrates based *P. laurifolia* crude extract and active fraction were carried out. The LC_{50} of emulsifiable concentrate based *P. laurifolia* crude extract was

Table 1. Thermostability of tested EC formulations under different temperature storage condition. *Indicates the significant difference at $p \leq 0.05$ by Dunnet's test in anti-feedant activity compared with control (30 ± 2°C, 0 days). (+) indicates enhancement of anti-feedant activity compared with the control.

Temperature /Time	EC Formulations - Anti-feedant activity (%)							
	P. laurifolia (Crude extract)				*P. laurifolia* (Active fraction)			
	1.0 % Extract	Loss (%)	2.5 % Extract	Loss (%)	1.0 % Extract	Loss (%)	2.5 % Extract	Loss (%)
30 ± 2°C 0 days	84.83 ± 0.72	0	92.88 ± 2.19	0	54.91 ± 1.26	0	81.93 ± 0.49	0
20 ± 2°C 18 weeks	46.20 ± 4.14*	45.54*	79.02 ± 0.67*	14.92*	29.94 ± 0.87*	45.47*	74.10 ± 0.91*	9.56*
35 ± 2°C 12 weeks	73.34 ± 2.42*	13.54*	88.49 ± 2.15	4.73	55.38 ± 1.65	0.86 (+)	90.67 ± 1.03*	10.66* (+)
54 ± 2°C 14 days	65.16 ± 3.63*	23.19*	89.28 ± 0.47	3.88	7.63 ± 0.26*	86.11*	51.29 ± 1.59*	37.4*
72 ± 2°C 3 days	76.42 ± 1.05	9.92	93.06 ± 1.02	0.19 (+)	38.83 ± 0.77*	29.28*	81.92 ± 3.13	0.01

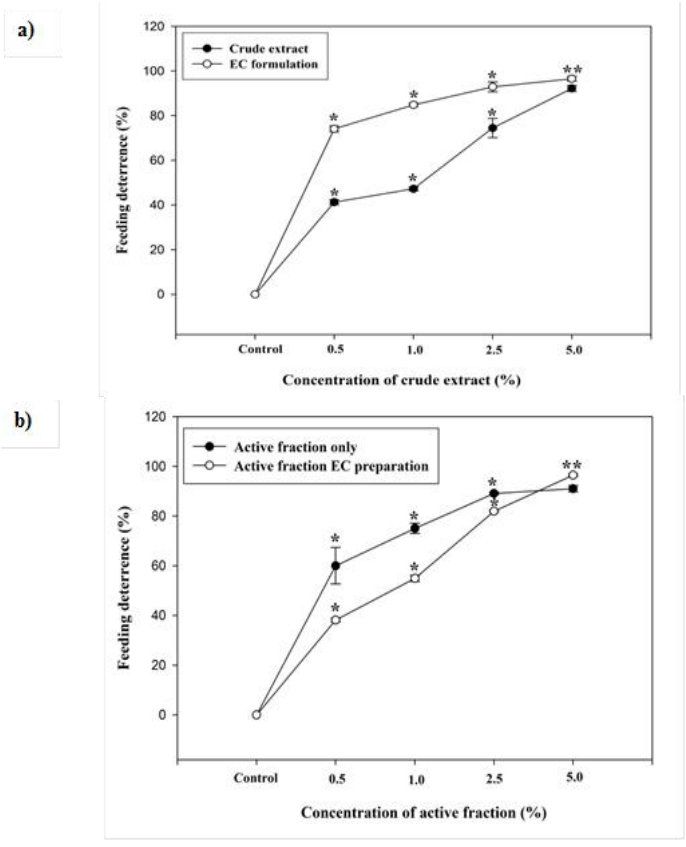

Fig. 1. Anti-feedant activity of crude extract (a) and active fraction (b) with corresponding emulsifiable concentrates (EC) of *P. laurifolia* against third instar larvae of *Spodoptera litura.* The results are mean of three replicates per experiment and expressed as mean ± SE. *Significantly different from respective controls at $p \leq 0.05$ by Dunnet's test.

found to be 1.594 mg/larva and emulsifiable concentrate based on active fraction was 5.383 mg/larva respectively. The study indicated that, on topical application the emulsifiable concentrates does not have any significant toxicity to target organism *S. litura.*

Environmental factors on efficacy and stability of EC formulations

Thermostability at accelerated storage conditions

The effect of storage of ECs prepared from *P. laurifolia* bark methanolic extract and its active fraction on the feeding activity of *S. litura* was evaluated under conditions 30 ± 2°C (RT) for 0 days, 20 ± 2°C for 24 weeks, 35 ± 2°C for 12 weeks, 54 ± 2°C for 14 days and 72 ± 2°C for 3 days (Table 1). The loss of feeding deterrence activity after accelerated storage conditions is presented in Table 1. The result indicated that lower concentrations of preparations were susceptible for loss of activity under different storage conditions compared to higher concentration of 2.5%. The crude EC formulations were kept at 72 ± 2°C for 3 days shown

Table 2. Effect of storage at ambient conditions for 12 months on stability of the tested EC formulations. *Significant difference at $p \leq 0.05$ by Dunnet's test in the anti-feedant activity compared with control. (+) indicates enhancement of anti-feedant activity compared with control.

Duration	EC Formulations - Anti-feedant activity (%)							
	P. laurifolia (Crude extract)				*P. laurifolia* (Active fraction)			
	1.0 % Extract	Loss (%)	2.5 % Extract	Loss (%)	1.0 % Extract	Loss (%)	2.5 % Extract	Loss (%)
Zero time	84.83 ± 0.72	0	92.88 ± 2.19	0	54.91 ± 1.26	0	81.93 ± 0.49	0
3 months	82.06 ± 0.64	3.27	92.72 ± 0.22	0.17	62.29 ± 1.86*	13.44*(+)	81.15 ± 0.43	0.95
6 months	79.43 ± 1.98	6.36	94.49 ± 0.95	1.73 (+)	71.62 ± 2.03*	30.43*(+)	81.15 ± 0.12	0.95
12 months	6.32 ± 0.03*	92.55*	71.01 ± 3.35*	23.55*	6.72 ± 3.17*	87.76*	33.95 ± 2.06*	58.56*

significantly more stable. This result suggests that the crude *P. laurifolia* EC can withstand high temperature up to 72 ± 2°C for few days. Contradictory, the preparation kept at 20 ± 2°C for 24 weeks were not much stable, and showed an efficacy loss of 14.92 %. The result indicated that the optimum and stable condition for storage of EC formulations is 35 ± 2°C for 12 weeks. The loss percentage was 3.88, 4.73, and 14.92 % after storage at 54 ± 2°C –14 days, 35 ± 2°C – 12 weeks, and 20 ± 2°C – 18 weeks respectively, for crude EC solution.

Likewise, EC formulation from active fraction of *P. laurifolia* was also evaluated for its stability on the feeding deterrence activity at different storage conditions. Lower concentration (1.0%) of fraction-based EC preparations were less stable compared to its higher concentrations. Similar to crude EC preparations, this formulation was also temperature stable at 72 ± 2°C for 3 days with no significant stability loss at 2.5% extract concentration compared to control. The fraction was stable at 35 ± 2°C for 12 weeks on its 2.5% dose. The maximum efficacy loss was noticed at 54 ± 2°C with an activity loss of 86.11 %. In general, the crude extract as well as its fraction were stable at a temperature of 35 ± 2°C for up to 12 weeks.

Effect of storage

The efficacy of EC formulations from crude as well as active fraction of *P. laurifolia* were evaluated on third instar larvae of *S. litura* after

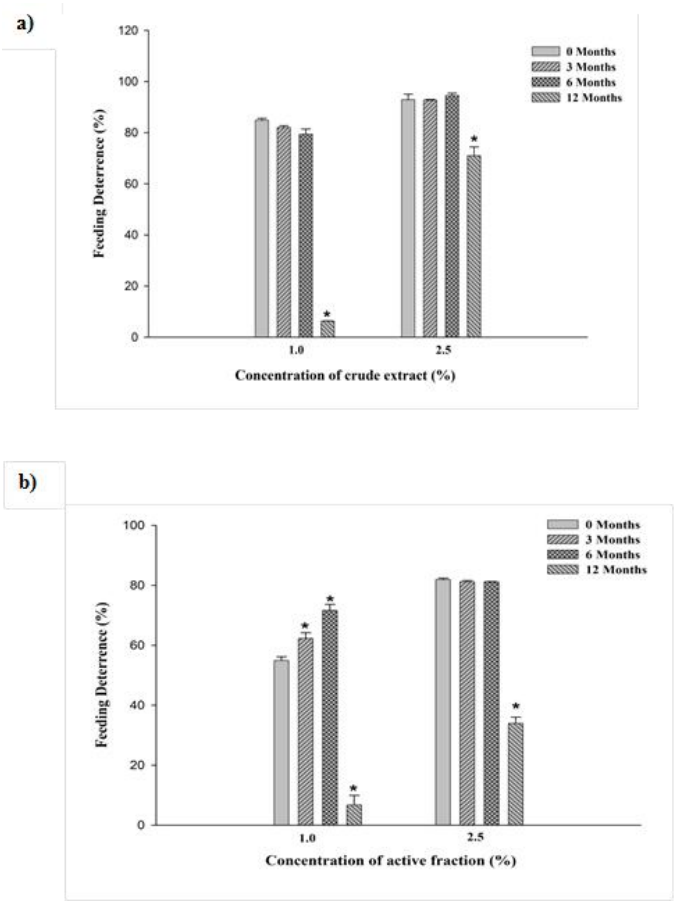

Fig. 2. Effect of storage of EC formulations of (a) crude (b) active fraction of *P. laurifolia* crude extract at ambient conditions for up to 12 months. The feeding deterrence of *Spodoptera litura* larvae after the stipulated time points were evaluated. The results are mean of three replicates per experiment and expressed as mean ± SE. * Significantly different from respective controls at $p \leq 0.05$ by Dunnet's test.

storage at ambient conditions for a period of 3 months, 6 months and 12 months (Table 2). The EC formulations prepared from crude extract concentrations of 1% and 2.5%, the feeding deterrence activity was found to be stable up to 06 months, then a significant decrease in activity was noted on 12 months' storage (Fig. 2a). In case of EC formulations prepared from active fraction, similar trend was noted, a significant loss in activity was noted on 12 months' storage. In case of formulation prepared from 1% active fraction, significant increase in activity compared to control was noted after 3 and 6 months' storage (Fig. 2b).

Table 3. Photolysis of tested biopesticides under direct sunlight. *Significant difference at $p \leq 0.05$ by Dunnet's test in the anti-feedant activity and loss percentage in the treatments compared with control. (+) indicates enhancement of anti-feedant activity compared with control.

Time	EC Formulations - Anti-feedant activity (%)							
	P. laurifolia (Crude extract)				*P. laurifolia* (Active fraction)			
	1.0 % Extract	Loss (%)	2.5 % Extract	Loss (%)	1.0 % Extract	Loss (%)	2.5 % Extract	Loss (%)
Zero time	84.83 ± 0.72	0	92.88 ± 2.19	0	54.91± 1.26	0	81.93 ± 0.49	0
1 Hour	85.05 ± 0.62	0.26 (+)	94.16 ± 0.89	1.38 (+)	50.51 ± 0.42	8.01	86.93 ± 0.97*	6.10* (+)
2 Hour	83.04 ± 0.96	2.11	88.95 ± 0.11	4.23	35.62 ± 3.47*	35.13*	84.46 ± 1.6	3.09 (+)
4 Hour	74.51 ± 0.92*	12.16*	86.97 ± 0.73*	6.36*	27.20 ± 1.68*	50.46*	33.89 ± 0.76*	58.64*
8 Hour	74.63 ± 0.96*	12.02*	82.97 ± 1.22*	10.67*	26.06 ± 2.0*	52.54*	31.54 ± 1.85*	61.50*
24 Hour	21.60 ± 2.28*	74.54*	60.47 ± 0.72*	34.89*	18.72 ± 0.27*	65.91*	21.13 ± 0.74*	74.21*

Effect of direct sunlight

The study revealed that, the stability of EC formulations under direct sunlight has reduced significantly after 4 hours of exposure. At 1.0% crude extract of *P. laurifolia* EC formulation on third instar of *S. litura* showed a significant decline in feeding deterrence (%) after 4, 8 and 24 hours of exposure respectively. EC formulation with 2.5 % crude extract significant decrease in anti-feedant activity was observed at 4 hr onwards upon exposure of direct sunlight. After 4, 8 and 24 hours of direct exposure of sunlight, feeding deterrence was 86.97 ± 0.73 % (6.36 % loss), 82.97 ± 1.22 % (10.67 % loss) and 60.47 ± 0.72 % (34.89 % loss) respectively (Fig. 3a and Table 3). The result suggested that the crude *P. laurifolia* extract based EC formulation was only stable up to 4 hours of direct sunlight exposure.

In case of EC formulations from active fraction of *P. laurifolia* extract, the direct exposure of sunlight has reduced the feeding deterrence activity of preparations from 1 % as well as 2.5 % concentrations of active fractions. In case of EC formulation from 1 % active fraction, significant activity loss was noted from 1 hr exposure onwards, leading to almost 65.91 % loss in 24 hrs exposure. The formulation from 2.5 % concentration of active fraction was stable up to 2 hrs and showed a significant decrease in activity after 4 hrs of exposure and showed 74.21 % loss activity in 24 hrs exposure (Fig. 3b and Table 3). The phytochemical constituents are less stable on sunlight exposure. Sunlight can trigger chemical processes that might change the structure of molecules or degrade them. This may lead to reduction in concentration and potential bioactivity of the active ingredient. The result also confirms that the formulations are less stable in direct sunlight exposure and suggest that the preparation must be stored away from sunlight.

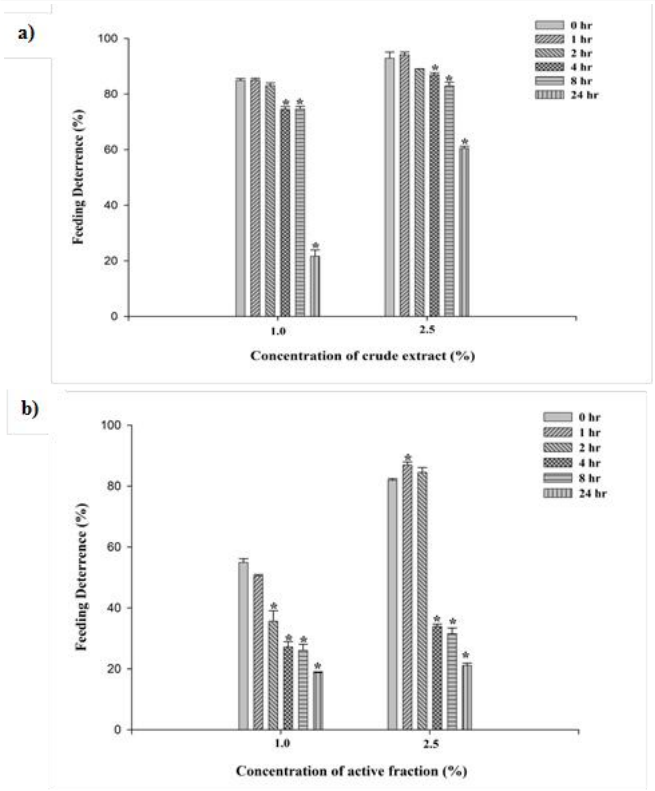

Fig. 3. Photolysis of EC formulations of (a) crude extract (b) active fraction of *P. laurifolia* extract at different time duration under direct sunlight on third instar larvae of *Spodoptera litura*. The results are mean of three replicates per experiment and expressed as mean ± SE. * Significantly different from respective controls at $p \leq 0.05$ by Dunnet's test.

Degradation under UV light

The impact of subjecting both EC formulations to ultraviolet radiation was shown in Fig. 4. The outcome showed that the tested formulations prepared from lower concentration (1%) of both extract and active fraction was degraded very rapidly under UV light. A significant decline in the feeding deterrence was observed even at 1 hour of exposure on both the formulations. But in formulation with higher concentration of crude extract (2.5%) significant loss in feeding deterrence was noted in 1 hr, 8 hr and 24 hr. In case of formulation with active fraction at 2.5 % concentration, no decline in feeding deterrence was noted even up to 24 hr exposure (Fig. 4 and Table 4)

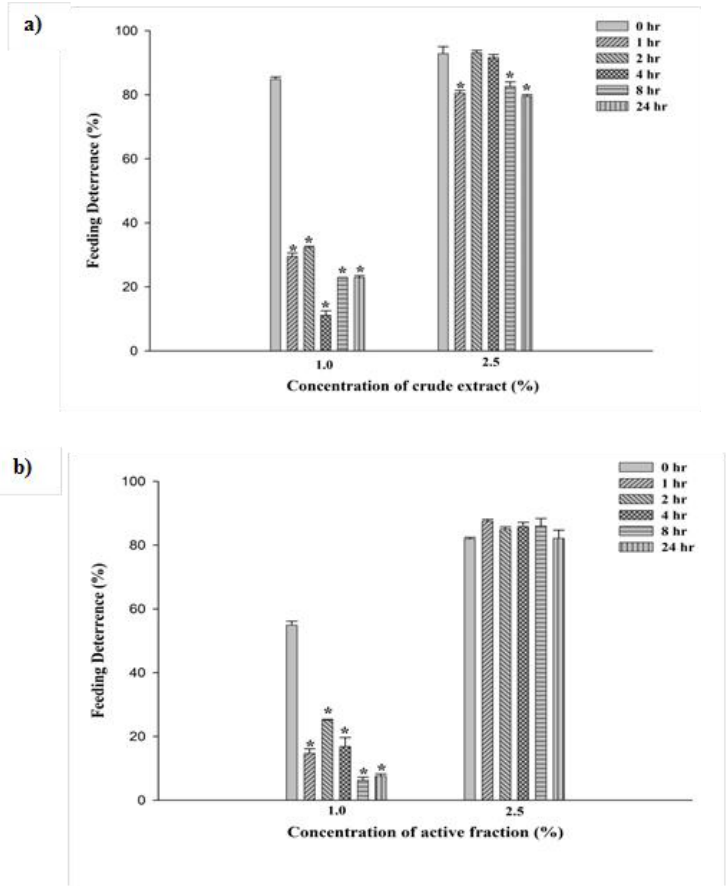

Fig. 4. Effect of EC formulations of (a) crude (b) active fraction of *P. laurifolia* crude extract at UV radiations on *Spodoptera litura*. The results are mean of three replicates per experiment and expressed as mean ± SE. * Significantly different from respective controls at $p \leq 0.05$ by Dunnet's test.

DISCUSSION

Plant derived biopesticides are natural products that are effective against pests and has biodegradability, diverse mode of action, less harmful to non-target organisms including humans (Neeraj *et al*., 2017). Factors such as biological and chemical degradation, formulation interactions, storage conditions and regulatory challenges are contributing to the stability issues of biopesticide formulations. This problem is illustrated well by researchers that, biopesticides are rapidly deteriorated by environmental conditions; hence frequent application is required for effectiveness (Casida and Quistad, 1995). The

Table 4. Photolysis of tested biopesticides under UV radiation.*Significant difference at $p \leq 0.05$ by Dunnet's test in the anti-feedant activity and loss percentage in the treatments compared with control. (+) indicates enhancement of anti-feedant activity compared with control.

Time	EC Formulations - Anti-feedant activity (%)							
	P. laurifolia (Crude extract)				*P. laurifolia* (Active fraction)			
	1.0 % Extract	Loss (%)	2.5 % Extract	Loss (%)	1.0 % Extract	Loss (%)	2.5 % Extract	Loss (%)
Zero time	84.83 ± 0.72	0	92.88 ± 2.19	0	54.91 ± 1.26	0	81.93 ± 0.49	0
1 Hour	29.54 ± 1.01*	65.18*	80.53 ± 0.87*	13.30*	14.71 ± 1.4*	73.21*	87.53 ± 0.56	6.84 (+)
2 Hour	32.25 ± 0.43*	61.98*	93.09 ± 0.76	0.23 (+)	25.11 ± 0.28*	54.27*	85.03 ± 0.66	3.78 (+)
4 Hour	11.12 ± 1.4*	86.89*	91.61 ± 0.96	1.37	16.83 ± 2.8*	69.35*	85.87 ± 1.25	4.81 (+)
8 Hour	22.84 ± 0.1*	73.08*	82.63 ± 1.35*	11.04*	6.27 ± 0.96*	88.58*	85.98 ± 2.35	4.94 (+)
24 Hour	22.93 ± 0.58*	72.97*	79.52 ± 0.52*	14.38*	7.56 ± 0.69*	86.23*	82.14 ± 2.56	0.26 (+)

shelf life of biopesticides can be significantly prolonged with proper storage and transportation under regulated conditions. Even though, the loss percentage of many biopesticides after 2 years of storage were reported to be above the acceptable limit of FAO specifications (Kandil *et al*., 2018). Researchers were successful in preparing novel combinations of an effective biopesticides with plant based constituents (Purkait *et al*., 2019; Ghongade and Sangha, 2021; Homayoonzadeh *et al*., 2022).

In the current investigation, we have prepared two emulsifiable concentrate (EC) based preparations, and compared the efficacy with its corresponding original extract. The results showed a significant enhancement in the efficacy in their highest concentration on both formulations compared to the extracts. Outcomes of researches have shown that formulations can enhance the activity of biopesticides (Wilson *et al*., 2020; Šunjka and Mechora, 2022). Under field conditions, bioactive constituents in extract can deteriorate and volatilize quickly in absence of stabilizing agents (Dhifi *et al*., 2016; Borges *et al*., 2018). Many studies are carried out for the preparation and efficacy evaluation of biopesticides, but stability studies were limited. Our results showed that the biopesticides based on methanolic extract of *P. laurifolia* and its active fraction exhibits good efficacy for the control of tobacco cutworm *S. litura*. Under different storage conditions, the present emulsifiable concentrate (EC) based preparations were shown good stability compared to the original extract. The loss of activity was minimal in emulsifiable concentrate (EC) preparation with increased concentration (2.5%). Hence higher concentrations of preparations might be suitable for EC formulations which are stable at different temperature conditions.

As per the WHO specifications, the maximum allowed limit for loss of stored bio-insecticide is up to 16 % (World Health Organization, 2012). Among the preparations, lower concentration (1.0 %) of crude EC showed highest efficacy loss percentage of 6.36 % after 6 months of storage (Table 2). All the preparations have minimal percentage loss, which is less than the permissible limit of active ingredients. Most of the preparations were stable at room temperature up to 6 months and they are to be used for field applications. The stability of bioinsecticides like Tracer were investigated using High Performance Liquid Chromatography followed by bioassays with *Spodoptera littoralis,* revealed that all preparations lost their efficacy as per the permissible limit of FAO specifications after 2 years of storage (Kandil *et al*., 2018). In order to control insect pests on crops, *Bacillus*

thuringiensis (Bt) has been employed extensively in agriculture for many years. However, the effectiveness of Bt can be influenced by several environmental factors. Loss of activity of *Bacillus thuringiensis* in *S. litura* after 2 years of storage at optimum temperature above the acceptable level of WHO specifications was reported (Moustafa *et al*., 2018). Temperature, exposure of sunlight and UV irradiations are much influenced on the stability of bioactive compounds. The concentration of active ingredient/s in the preparation has a role in maintaining the activity under different conditions. Stability of natural components of biopesticides are affected by the UV irradiation at its lower concentration; however no significant effect was noted at higher extract concentrations (Moustafa *et al*., 2018). The findings of this study showed that the stability loss was minimal in emulsifiable concentrates with higher extract concentration (both with crude extract and active fraction) compared to lower concentrations at varying environmental conditions. However, the stability of the product was declined at different environmental conditions like temperature, direct sunlight and UV exposure and it can be only maintained when stored under ambient conditions. Ensuring stability is important to guarantee that the products will remain effective when they reach the end-users, whether farmers or pest control operators. This study shows the stability of EC formulations from *P. laurifolia* plant and its potential in effective insect control.

Author Contribution

Alina Paul - Conceived presented study, carried out the experiments, verified the analytical methods. Carried out the implementation, performed the calculations. Wrote the manuscript with input from all authors.

R. Jayaraj - Conceived idea of the presented study, developed the theory and verified the analytical methods. Encouraged A to investigate [a specific aspect] and supervised the findings of this work. Wrote the manuscript with support from A. Both authors discussed the results and contributed to the final manuscript.

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