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Original Research Paper

Bio-efficacy of Annona formulation for the management of *Aphis gossypii* on vegetable crops

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ABSTRACT

Field experiments were conducted to optimize the dose and assess the efficacy of an Annona formulation against *Aphis gossypii* Glover on vegetable crops such as cucumber, bitter gourd, okra and dolichos. Based on LC_{50} values, different doses of the Annona formulation were evaluated specifically on bitter gourd and cucumber. The dose optimization study determined that a concentration of 3.5 ml/L of water of the Annona formulation effectively managed *A. gossypii* under field conditions. Subsequently, the efficacy of this dosage was further evaluated in field trials against *A. gossypii* in comparison with other botanicals and insecticides. The Annona formulation at 3.5 ml/L of water demonstrated significant effectiveness, reducing aphid populations by 97% compared to alternative botanicals and insecticides. This study suggests that the developed Annona formulation could serve as a promising eco-friendly approach for managing aphids on vegetable crops.

Keywords: Aphids, bitter gourd, botanicals, cucumber, IPM, vegetables

INTRODUCTION

Vegetable production in the world is severely hindered by sucking pests. Among them, aphids, *Apis gossypii* Glover (Aphididae: Hemiptera) pose a major threat to vegetable crops by infesting all stages, from planting to harvesting. Aphid feeding directly alters the plant metabolism through removal of phloem sap and induces many morphological and systemic changes in plants like crinkling, curling and stunting the plant growth (Dela et al., 2014). Under cold weather, aphid colonization, multiplication and infestation will be doubled due to favourable climate. The management of aphids mainly relies on use of insecticides, despite the use of many synthetic insecticides, managing them during outbreaks can be difficult due to their high reproductive potential, dispersal capacities, adaptability to local survival. Although, they are not very fecund (40–100 offspring/female), but have a high reproductive potential due to their long parthenogenesis, short life cycle and quick resistance development (Dedryver et al., 2010). Indiscriminate and over-application of synthetic insecticides lead to development of resurgence and resistance in the pest population which lead to search for alternative strategies to manage pest outbreaks (Pereira et al., 2006; Prasannakumar et al., 2021). Identification of sustainable and eco-friendly approaches will, therefore, help in managing aphids effectively. Botanicals with potent insecticidal activity are relatively safe to non-targeted organisms. Neem and neem-based insecticides are the most common botanical used worldwide (Isman, 2006). Due to its multiple modes of action, the development of resistance in pests is delayed and plant based pesticides are readily degraded in nature, resulting in low toxicity to mammals. As a result, the U.S. Food and Drug Administration (FDA) has acknowledged that botanical pesticides (essential oils) are safer than synthetic pesticides (Roger et al., 2012). The botanical based pesticides are therefore gaining a lot of interest due to organic farming (Prasannakumar et al., 2013; 2021b; Samada & Tambunan, 2020). We have developed an annona formulation to manage aphids on horticultural crops (Prasannakumar et al., 2021 & 2022). Demonstrations on field efficacy represent an important step in the development of botanical insecticides. A promising plant extract that shows high efficacy in the laboratory must be tested further in the greenhouse and/or in the field conditions to know its efficacy in a real crop protection context. In previous experiments, we have evaluated bio-efficacy of annona seed extract against *A. gosypii* with highest mortality (100%) under laboratory (Prasannakumar et al., 2023).

In continuation, the present experiment on field dose optimization and evaluation of the formulated annona extract against *A. gosyypii* was carried out. The results generated in the study thus would help in promoting the annona formulation in organic farming for sustainable pest management.

MATERIALS AND METHODS

The field experiments were carried out at ICAR-Indian Institute of Horticultural Research, Bengaluru to optimise the field dose of the annona formulation and evaluate its efficacy against *A. gossypii* on crops such as cucumber, bitter gourd, okra and dolichos. The annona extract was formulated at Botanical Formulation Laboratory, Division of Crop Protection, using carrier, adjuvant and sticker in an appropriate ratio (Prasannakumar et al., 2021).

Dose optimization of Annona formulation

For dose optimization, the field bio assays were carried out with six doses of annona extract *viz.*, 2.5, 3.5, 4.5, 5.5, 6.5 and 7.5 ml/L based on LC_{50} values (Prasannakumar et. al., 2022) and untreated control. Cucumber 'Rani F_1 hybrid' and Bitter gourd var. 'Palee', were raised in the field in a randomised complete block design (RCBD) with three replications, in an area of 500 m^2 and further divided in 21 plots each measuring 3x5 m^2. Before initiating the treatments, a pre-count of aphids was conducted by randomly selecting five plants from each bed. From each selected plant, five top leaves were examined, and the average number of aphids per plant was calculated. The first spray was imposed after the aphid infestation noticed. Observation on aphids were recorded at 2 days intervals up to 20th day after the treatments. The experiment was repeated on another crop (cucumber) for confirmation.

Efficacy of Annona formulation

Comparative efficacy of annona formulation with botanicals like neem oil formulation (10 g/L) and neem seed powder formulation (30 g/L), as well as insecticides such as imidacloprid 17.8SL and dimethoate 30EC, against *A. gossypii* on okra and *A. craccivora* on dolichos. The okra var. Arka anamika was raised in RCBD in an area of 550 m^2 which further divided into smaller plots each measuring 3 x 5 m^2. The treatment consists of annona formulation 3.5 ml/L, imidacloprid 17.8SL 0.5 ml/L, dimethoate 30EC 1.7 ml/L, rotation of imidacloprid 17.8SL 0.5 ml/L, dimethoate 30EC 1.7 ml/L and untreated control with 14 days interval for rotation; except in dolichos, the rotation was made at 20 days interval (based on pest load). Each treatment was replicated four times. Before treatment imposition the pre-count on aphid population was documented on randomly selected five plants from each replication. Once the treatments were imposed, the observation on aphids were recorded on randomly selected five plants per replication on different days (1st day after treatment upto 20th day). The cumulative mean number of aphids per plant in each treatment was calculated and compared using two- way ANOVA using SPSS software and DMRT.

Table 1 : Optimization of field dose for annona formulation against *A. gossypii* on cucumber

Treatment	Mean number of aphids/plant				
	Pre-count	Day 1	Day 3	Day 7	Day 14
2.5 ml/L	43.33	2.00 (1.42±0.7)	2.00 (1.48±0.45)	2.00 (1.32±1.13)	1.33 (1.26±0.39)
3.5 ml/L	47.33	0 (0.7±0)	1.67 (1.35±0.51)	5.33 (2.16±1.71)	2.67 (1.56±1.11)
4.5 ml/L	60.33	5.00 (2.03±2.0)	1.67 (1.39±0.36)	0 (0.7±0)	3.00 (1.78±0.47)
5.5 ml/L	118.67	3.67 (1.86±1.0)	1.00 (1.17±0.19)	0 (0.7±0)	2.00 (1.32±1.13)
6.5 ml/L*	103.33	6.67 (2.32±2.6)	0 (0.7±0)	0 (0.7±0)	0 (0.7±0)
7.5 ml/L*	190	8.00 (2.59±2.68)	0 (0.7±0)	0 (0.7±0)	0 (0.7±0)
No spray	75.33	112.00 (10.60±0.14)	233.00 (15.23±1.9)	170.67 (13.01±2.63)	159.00 (12.48±5.57)
CD (5%)	-	4.20	1.80	0.60	1.00
SEM	-	1.00	1.30	2.10	1.70
F	-	28.36	158.30	99.98	60.67
P (0.05)	-	0.00001	0.00001	0.00001	0.00001

Table 2 : Optimization of field dose for annona formulation against *A. gossypii* on bitter gourd

Treatment	Mean number of aphids/plant						
	Pre-count	Day 1	Day 2	Day 3	Day 5	Day 7	Day 10
2.5 ml/L	876.6	147.30 (9.5±0.03)	8.30 (2.9±0.13)	4.00 (1.64±2.6)	0 (0.70±0)	1.60 (1.40±0.06)	3.33 (1.93±0.14)
3.5 ml/L	678.33	94.00 (8.23±0.414)	16.66 (4.02±1.3)	3.66 (1.977±0.42)	0 (0.70±0)	2.60 (2.16±8.3)	2.00 (1.46±.50)
4.5 ml/L	810	55.00 (7.44±2.02)	3.00 (1.6±1.33)	0.66 (0.99±0.25)	0.33 (0.87±0.08)	0 (0.70±0)	2.60 (1.64±0.7)
5.5 ml/L	759.3	55.66667 (5.63±2.40)	0 (0.7±0)	1.66 (1.38±0.38)	1.00 (1.17±0.19)	0 (0.70±0)	0.33 (0.87±0.089)
6.5 ml/L	550.66	50.00 (13.89±29.23)	1.00 (1.09±0.45)	0.33 (0.87±0.08)	0 (0.70±0)	0 (0.70±0)	0.33 (0.87±0.089)
NSPP (10 g/L)	739	311.33 (17.63±0.58)	183.3 (13.23±0.06)	158.33 (21.03±2.28)	443.33 (7.83±0.16)	371.33 (19.18±5.94)	427.33 (20.66±0.77)
Control	831.6	835.00 (28.75±10.05)	837.00 (28.7±10.78)	842.00 (28.87±10.95)	820.00 (28.55±7.96)	790.00 (28.06±5.11)	901.60 (29.97±5.09)
CD at 5%	-	4.14	5.80	6.77	1.90	2.20	1.53
SEM	-	3.02	3.90	4.43	4.50	4.20	4.53
F	-	40.70	84	172.78	300	99.29	446.115
P (0.05)	-	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

Table 3 : Comparative efficacy of annona formulation with botanicals and insecticides against aphids in dolichos

Treatment	Mean number of aphids/plant				
	Pre-count	Day 4	Day 6	Day 11	Day 20
Annona formulation 3.5 ml/L	393.75	10.00 (2.8±3.2)	0.75 (0.99±0.33)	1.00 (0.998±0.338)	27.50 (4.94±4.0)
Formulation with spreader + emulsifier 3.5 ml/L	912.50	47.50 (4.5±0.373)	0 (0.70±0)	0 (0.70±0)	0 (0.70±0)
Formulation with annona oil 3.5 ml/L	590.00	135.75 (10.16±0.90)	11.25 (3.06±3.12)	26.33 (4.13±5.18)	10.00 (2.88±2.91)
Formulation without spreader 3.5 ml/L	1277.50	129.50 (16.48±6.76)	57.75 (6.39±23.21)	75.00 (6.39±23.215)	24.50 (4.45±6.83)
Neem oil formulation 10 g/L	1228.75	510.75 (22.5±6.18)	497.00 (22.28±1.18)	690.50 (26.20±5.87)	785.00 (28.01±1.10)
Imidacloprid 0.5 ml/L	563.75	36.25 (5.8±3.9)	257.75 (15.9±6.8)	475.01 (21.77±1.97)	263.75 (1.7±1.58)
Neem seed powder formulation 30 g/l Control	286.25	86.75 (8.126±26.32)	64.00 (7.98±1.06)	161.33 (12.69±0.30)	330.25 (15.9±7.79)
	807.5	927.01 (30.38±6.09)	1049.10 (32.34±5.03)	1065.06 (31.84±3.21)	1024.20 (31.97±2.18)
CD at 5%	-	3.28	14.67	4.30	5.28
SEM	-	3.39	4.02	4.27	4.20
F	-	24.60	101.44	114.0	112
P (0.05)	-	0.00001	0.00001	0.00001	0.0001

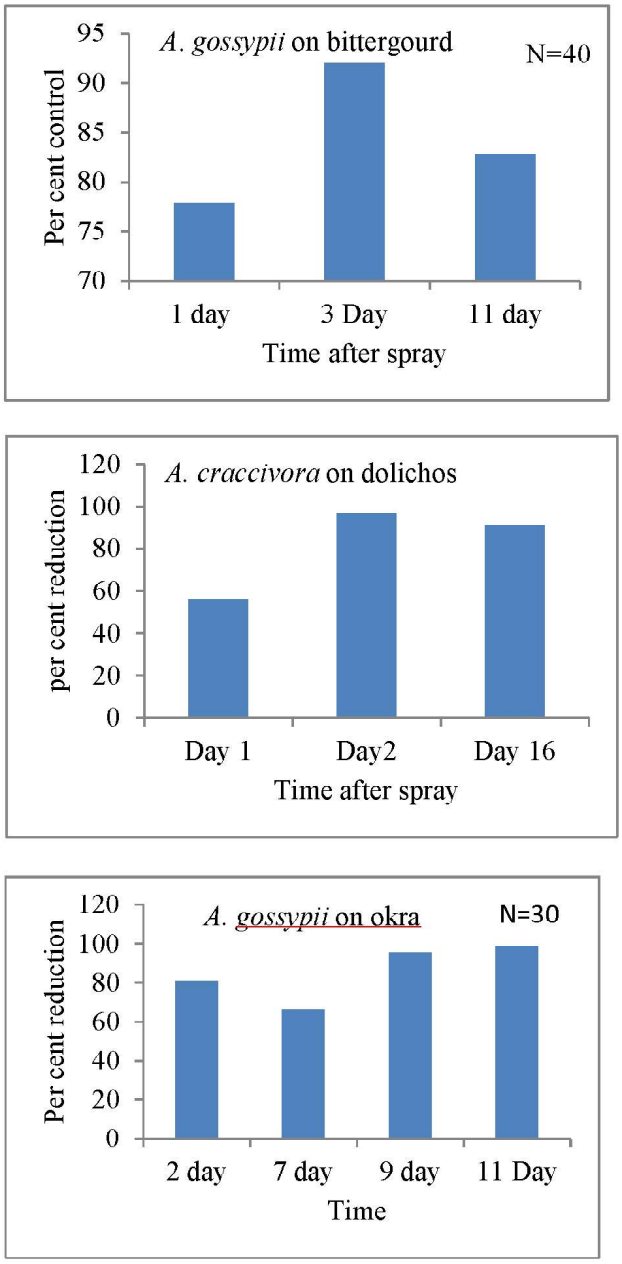

Fig. 1 : Efficacy of Annona formulation on *Aphis gossyypii* on bitter gourd, dolichos and okra

RESULTS AND DISCUSSION

The results from dose optimisation experiment showed significant reduction of *A. gosypii* population on cucumber and bitter gourd from 1st, 3rd, 7th and 14th day after spray of annona extract under field conditions. On the 1st day after the treatment, about 97% reduction of *A. gossypii* was recorded in 2.5 ml/L with mean number 2.00 aphids/plant and 100% reduction at 3.5 ml on cucumber. After 14 days of treatment, 2.67 aphids/plant were recorded at dosage of 3.5 ml/L, whereas, in untreated control the population was 195.00 aphids/plant (Table 1). Similarly, in bitter gourd, *A. gossyppii* was reduced by 84% (147.3 aphids/pant) in 2.5 ml/L and 86% (94 aphids/plant) in 3.5 ml/L. After ten days of the treatment, the mean number of *A. gosypii* was 2.67/plant in 3.5 ml was observed (Table 2).

The results from the field efficacy of annona formulation against the different insecticide experiment revealed that the formulated annona had caused significant mortality of *A. gossypii*. Around 97% of *A. craccivora* population was reduced after the 4 days of treatment with an average of 10 aphids per plant on dolichos compared to other botanicals and insecticides (Neem oil based formulation, 50%; neem powder based, 71.0% and imidacloprid, 94.0%) (Table 3 & Fig. 1).

Similarly, on okra, significant reduction of *A. gosypii* population treated with annona formulation was observed (Table 4 & Fig. 2). Mean number of *A. gossypii* in annona treated crop was 11.6 aphids/ plant compared to untreated control (100.4 aphids/ plant). The population of aphids were reduced by 98.3% on 11th day after spraying of annona formulation.

Pest management is one of the essential components in the vegetable production. Amongst sucking pests, aphids are also major problem in vegetable production, management is mainly depends on use of synthetic pesticides but use of pesticides will leave lot of residue on fresh produce and also create environmental issue. Recent days, pest management using eco-friendly approaches has become a growing trend for researchers who prioritize safety to environment and non-target organisms (Chakraborty et al., 2023). Discovery of plant derived compounds and their application as pesticide is undoubtedly an efficient way to check the pest population without disturbing the ecological balance (Souto et al., 2021). In present study, the annona seed extract was formulated, optimized the field dose and evaluated against *A. gossypii* under field conditions. The formulation at 3.5 ml/L exhibited significant reduction of aphids on cucumber and bitter gourd. The field dose optimization of the developed formulation was important for the right use of the formulation in management of the pest (Durairaj et al., 2023). Annonaceae are empirically known to elicit insecticidal activities. Plant species in this family contain an array of toxic compounds such

Table 4 : Field efficacy of anonna seed extract formulation against *Aphis gossyypii* on Okra

Treatment	Cumulative mean aphids/plant (No.)
T_1: Annona formulation 3.5 ml/L	11.6[a]
T_2: Neem oil formulation 10 g/L	21.1[b]
T_3: Neem seed podwer formulation 30 g/L	20.3[ab]
T_4: Imidacloprid 17.8SL 1 ml/L	12.4[ab]
T_5: Dimethoate 30EC 1.7 ml/L	16.1[ab]
T_6 : IR (Rotation imidacloprid & dimethoate)	12.2[ab]
T_7 : Control	100.4[c]
CD at 5%	9.0
CV %	18.2

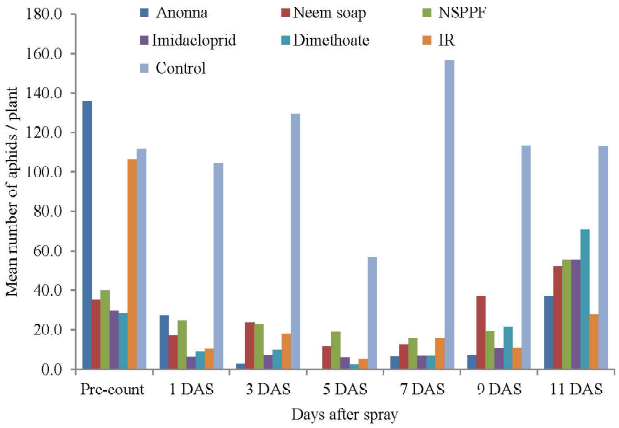

Fig 2 : Efficacy of annona formulation on *Aphis gossyypii* in okra over time

as acetogenins, alkaloids, flavonoids that confer to these plants their insecticidal proprieties. The aphicidal action of annona in different crops at various time intervals may be due to presence of compounds like octadecenoic acid (Z) 2,3 dihydroyprophyl ester 7-hexadecenoic acid and acetogenins which are lethal to insects (Prasannakumar et al., 2022; Mondal et al., 2018; De Anita et al., 2012 and Baldin et al., 2023). The bioactivity of anonna formulation may be related to several compounds in its composition, which can act separately or synergistically in causing insect mortality. Alkaloids, triglycerides and, notably, ACGs are the most common classes of compounds found on *A. mucosa* extracts (Ribeiro, 2014; Ansante et al., 2015). During the entire cropping period of okra, cucumber, bitter gourd and dolichos vegetable crops, only 2 sprays of annona formulation was enough to manage the aphid outbreaks indicating reduction of spray numbers thus contributing towards reduction of cost of protection (Tudi et al., 2021). The developed annona formulation therefore will be additional botanicals to strengthen the pest management strategies in organic farming systems.

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