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Field Efficacy of Biocapsules of Entomopathogenic Fungi for the Management of Aphis craccivora Koch (Homoptera: Aphididae)

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Many agro-ecosystems have been seriously undermined using synthetic pesticides, and are losing incentive in terms of productivity, with substantial loss of biodiversity. To reclaim ecosystem health and protect global health, there is an important need to put the ecology standards back at the core of crop protection. Biological control methods are favourable alternative methods to the chemical method. In the evaluation of the efficacy of biocapsules of *Lecanicillium lecanii* (Zimmermann) Zare and Gams and *L. saksenae* (Kushwaha) Kurihara and Sukarno for the management of cowpea aphid *Aphis craccivora* Koch (Homoptera: Aphididae), it was revealed that foliar application of *L. saksenae* and *L. lecanii* three capsules L^{-1} sprayed twice (at weekly intervals) were similarly effective in causing 94.38 and 92.28 per cent reduction in the population of *A. craccivora* and with lower dose at two and one capsules L^{-1} population noted was 37.51 to 63.96 per cent. The spore

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suspensions of both fungi were more effective than the lower doses (78.73 - 83.53 per cent reduction) in cowpeas. While Thiamethoxam 25 WG, was 95.79 per cent. Biocapsule treatment did not affect the natural enemy populations significantly. The yield recorded in the plots with *L. saksenae* capsules at two and three L^{-1} was higher than other treated and untreated plots. Therefore, the need-based creation of biocontrol formulations in capsules, tablets, powder, etc. should be popularized.

Keywords: Biocapsules; Lecanicillium lecanii; Lecanicillium saksenae; cowpea; Aphis craccivora.

1. INTRODUCTION

"Cowpea *Vigna unguiculata* (L.) Walp, is grown throughout the tropics and subtropics as a vegetable, pulse, fodder, and/ or cover crop. It is a nutritionally rich and highly priced vegetable and pulse in the domestic markets of Kerala. Though an array of pests attacks the crop, sucking pests predominantly the pea aphid *A. craccivora* is one of the most common pest species in the tropics with cosmopolitan and polyphagous occurrence leading to 20 to 40 per cent yield loss" [1]. "But the over reliance on synthetic chemical pesticides and their ultimate uninhibited utility has necessitated other possibilities mainly for environmental concerns. Although biopesticides cover about one per cent of the total plant protection products globally, their number and growth rate showed an increasing trend in the past two decades, about 175 biopesticides active ingredients and 700 products have been registered worldwide" [2].

"Entomopathogenic fungi (EPF), is a commercially available organic tool, especially in the case of vegetable production, which renders a cultivation practice free from pesticide residues. They constitute a group with over 800 species from 90 genera known to be entomopathogenic" [3]. "It was reported that more than 171 mycoinsecticides have been produced" [4]. Widely studied entomopathogenic fungi belong to 12 species with genera such as *Beauveria*, *Metarhizium*, *Lecanicillium*, *Hirsutella*, *Erynia* (Zoopththora), *Nomuraea*, *Aspergillus*, *Aschersonia*, *Paecilomyces*, *Tolypocladium*, *Leptolegnia*, *Culicinomyces*, *Coelomomyces*, and *Lagenidium* [5] of which, *Beauveria* spp., *Metarhizium* spp., *Lecanicillium* spp., and *Isaria* spp. have been developed as successful mycoinsecticides for various groups of insect pests [6]. The main benefit of EPF is its specificity in targeting pests, safety for non-target organisms, high virulence, persistence, and safety for the environment and human health.

The objective of the experiment was to evaluate the comparative efficacy of *L. saksenae* and *L. lecanii* capsules and standardize their dosage in managing aphids, the sucking insect pests of cowpea.

"With the rising attention of the eco-friendly approach of pest management, microbial control employing the application of entomopathogens particularly fungi is found to be promising. Formulations of microbial pesticides are largely talc-based which are bulky and difficult to transport and use. Furthermore, the chances of contamination and loss of viability are more in these formulations. Capsule is a stable formulation wherein the bioagent is encapsulated in coatings and thus protected from extreme environmental conditions such as UV radiation, rain, and temperature. The possibility of getting contaminated is also meager as the infective propagules are encapsulated in a protective covering. Capsules have more residual stability than spray formulations" [7].

Nirmala et al. [8] reported that VI1 isolate of *L. lecanii* @ 1×10^7 spores mL^{-1} caused 2 to 80.8 per cent mortality in *A. craccivora* nymphs within two days. In a laboratory study, Saranya et al. [9] reported that *L. lecanii* @ six different spore concentrations ($1x10^8$, $1x10^7$, $1x10^6$, $1x10^5$, $1x10^4$, $1x10^3$ spores mL^{-1}), resulted in 100, 100, 84, 60, 44, 28 per cent mortality respectively, in adult aphids within seven days of treatment. Abd et al. [10] reported that *L. lecanii* formulation Bio-Catch caused 100 per cent mortality to *A. craccivora* adults and nymphs @ 5.0 ml and 1.0 ml (5×10^6 spores mL^{-1}) respectively, over three days. El-Salam et al. [11] found that *L. lecanii* (Bio-Catch) @ 1×10^8 spores mL^{-1} caused an 80.7 per cent reduction within five days from the first spraying and then gradually decreased to 63.6 after the second spraying. Nithya [12] reported that *L. lecanii* @ 1×10^8 spores mL^{-1} caused 100 per cent mortality of *A. craccivora* within five days and @ 1×10^7 spores mL^{-1} it caused 100 per cent mortality within six days of treatment. In a bioassay study, [13] observed that toxicity of secondary metabolites of *L. saksenae* @ 1000 ppm (crude toxin) to *C.*

insolita revealed 95.98, 85.51 per cent mortality in the third instar nymphs and adults 48 h after treatment and 100 per cent mortality at 72 and 96 h after treatment on nymphs and adults brinjal mealybug, respectively.

2. MATERIALS AND METHODS

The study was carried out during 2018-2021 at the Biocontrol Laboratory for Crop Management, College of Agriculture, Vellayani, Kerala. The entomopathogenic fungi viz., *Lecanicillium lecanii* (Zimmermann) Zare and Gams and *Lecanicillium saksenae* (Kushwaha) Kurihara and Sukarno were sourced from National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru. These cultures maintained in the Biocontrol Laboratory, College of Agriculture, Vellayani, Kerala were used. The cultures were revived periodically by passing them through susceptible hosts *Aphis craccivora* Koch and *L. saksenae* were passed through *Leptocorisa* spp., so, as to maintain their virulence. Spore suspensions of each of the fungi, obtained from 21-day-old cultures were centrifuged in a Rotek centrifuge (REMI R 23) at 4000 rpm for 20 min to obtain the spore pellets. The spore pellets, after removing mycelial mats by gentle washing with sterile distilled water were mixed with an equal quantity of crude and powdered chitosan to obtain primary powder (10^{10} spores g^{-1}). The primary powder was then mixed with a carrier material (Industrial-grade talc/crude chitosan) in a ratio of 1:20 and loaded into empty capsules made of hard gelatin coating as standardized by Remya and Rani [14]. Loading the capsules was carried out using a hand-operated capsule-filling apparatus. The filled capsules were stored airtight in clean and dry food-grade plastic bottles made of PET (Polyethylene Terephthalate) under ambient temperature as well as refrigerated conditions.

Seeds of the KAU cowpea variety, Bhagyalakshmi which is a bush type and early yielding variety procured from the Department of Vegetable Science, College of Agriculture, Vellayani were used for the experiment. The experimental plot was laid out in Randomized Block Design (RBD) consisting of 10 treatments replicated thrice with a plot size of 2m x 2m. The treatments were as follows. T1 - 1 *L. lecanii* capsule L^{-1}, T2 – 2, *L. lecanii* L^{-1}, T3 - 3 *L. lecanii* capsule L^{-1}, T4 – 1 capsule *L. saksenae* L^{-1}, T5 - 2 *L. saksenae* capsule L^{-1}, T6 - 3 *L. saksenae* capsule L^{-1}, T7 - *L. lecanii* spore suspension @ 10^7 mL^{-1} - 20 mL L^{-1}, T8 - *L. saksenae* spore suspension @ 10^7 mL^{-1} - 20g L^{-1}, T9 - Chemical check - Thiamethoxam 25% WG (50 g a.i ha-1), T10 - Untreated control.

Capsules at the respective doses were dispersed in water with 0.1% tween 80. Spraying was carried out using a knapsack sprayer. The first spraying was given when 10 per cent of plants were infested and the second after one week of the first application. Incidence of aphids on cowpeas was recorded commencing from the seventh week after planting. The population was recorded from three plants selected at random from each replication. Aphid population on 5cm long terminal twigs were taken on the 3rd and 7th day after treatment. The incidence of other foliage and sap-feeding insects observed throughout the crop period was also recorded. The number of natural enemy $plots^{-1}$ was recorded by visual counting. Average yield $plot^{-1}$ was noted for comparison of treatments.

3. RESULTS AND DISCUSSION

3.1 Field Efficacy of Biocapsules against Aphids *A. craccivora* in Cowpea

First spraying: One week after spraying, the lowest population was noted in those plots treated with *L. saksenae* at 3 capsules L^{-1} (195.33) which was on par with that observed in *L. lecanii* at 3 capsules L^{-1} (215.33) as well as the chemical treatment with thiamethoxam 25WG (180.0). The mean population noted in plots treated with one and two capsules of *L. saksenae* and *L. lecanii* was significantly higher. It was 325 and 361.67 in the case of *L. saksenae* and *L. lecanii* sprayed at 2 capsules L^{-1}, respectively, while it was 331.33 and 382 respectively, when sprayed with single capsules of *L. saksenae* and *L. lecanii*. Population noted in plots treated with spore suspension of these fungi, were at par with each other, the values being 248.33 for *L. saksenae* and 247.33 for *L. lecanii.* The highest population was recorded in untreated plots (564.67) Table 1.

Second spraying: The population reduced significantly after the second spraying. Observations taken on the third day revealed that the plots treated with 3 capsules of *L. saksenae* and *L. lecanii* were on par with each other (83.33 and 72.67 respectively). Aphid population recorded from plots treated with 2 capsules L^{-1} of *L. saksenae* was 185.67 which were significantly lower than its corresponding dose of *L. lecanii* (225.67). When single capsule L^{-1} was sprayed the population noted was higher. It was 317 with *L. saksenae* and 315 with *L. lecanii* which did not vary significantly. The population noted in plots treated with spore suspension was higher than the dose 3 capsules L^{-1} but lower than those observed with 2 and 1 capsule L^{-1}.

At the end of the experimental period, the population reduced drastically to 20.01 in *L. saksenae* 3 capsules L^{-1} and 27.33 in *L. lecanii* @ 3 capsules L^{-1}, which were on par with each other. Lower doses were not effective in reducing the population to very low levels. It was 157.00 in *L. saksenae* and 177.67 in *L. lecanii* when 2 capsules were used, and the population was much higher i. e. 234.33 and 248.33 when single capsule L^{-1} was sprayed. *L. lecanii* spore suspension was better than *L. saksenae* spore suspension at 10^7 spores mL^{-1} (63.33 and 90.67). thiamethoxam 25 WG treated plots recorded the lowest population (15.33) Table 1.

Effect of capsules on natural enemy population in cowpea: The predatory spiders noted in the cowpea ecosystem were *Hermippus* sp., *Plexippus* sp. and *Xanthogramma* sp. and the coccinellid predators were *Coccinella septumpunctata* L. The total population encountered in the cowpea field is furnished in Table 2. Analysis of data revealed that, all through the field trial, population did not vary much among the treatments. The count varied from 0.83 to 5.0 $plot^{-1}$.

The population deviation noted between the count and last count revealed that thiamethoxam 25 % WG reduced the natural enemy population by 80.83 per cent while it was unaffected in *L. lecanii.*

Effect of biocapsules on the yield of cowpea: Comparison of yield obtained from 2 m^2 of cowpea plot revealed that there was significant variation in yield from plots sprayed with various treatments. The highest yield of 1.85 kg was recorded in plots treated with *L. saksenae* @ 2 capsules L^{-1} followed by that in thiamethoxam treated plots (1.65 kg). The yield obtained from plots with *L. saksenae* @ 3 capsules was 1.56 kg which was higher than its corresponding dose of *L. lecanii* (1.29 kg) but lower than the yield obtained from plots treated with its spore suspension @10^7 spores mL^{-1} (1.59 kg). Single dose application of *L. saksenae* was superior (1.45 kg) to that of *L. lecanii* (1.0 kg). In the untreated plot, the average yield recorded was significantly low (1.1kg) Table 3.

The increase in yield varied from 30.63 to 66.66 per cent in *L. saksenae* treatments, while it was 16.21 to 30.63 in *L. lecanii* treatments and 48.64 per cent in chemical treatment.

It is notable that there was an increase in yield over all other treatments, which may be attributed to the endophytic nature of the isolate as reported by Divyasree [15]. The insecticidal, nematicidal and antimicrobial metabolites produced by this fungus as reported by Sreeja and Reji [13] might have contributed to the yield.

This geographical isolate of *L. saksenae*, ITCC 7714 from soils of Vellayani was reported to be pathogenic to various sucking pests of vegetables [16] and rice [17]. It was proved to be an ideal candidate in IPM as it was compatible with many of the new generation insecticides [18]. The same isolate has been reported to be effective to *A. craccivora* causing a seven per cent increase in the yield of cowpeas [19].

Pathogenicity of *L. lecanii* is a well known fact across the world. It is the most widely used species of *Lecanicillium* reported to be effective to sucking pests of various crop pests. In a laboratory study by Nirmala et al. [8] *L. lecanii* VI-I reported a high mortality of 80.80 per cent in *A. craccivora* @ $1x10^7$ spores mL^{-1}. Vu et al. [20] found that *L. lecanii* 41185 @ $1x10^7$ conidial mL^{-1} showed the highest pathogenicity for *M. persicae* and *Aphis gossypii* Glover, with 100 per cent, reduction five and two days after treatment, respectively. Suresh et al. [21] reported 71.62 per cent suppression of *A. craccivora* under field conditions when the isolate VL-3 isolate @ $1x10^9$ spores mL^{-1} was given as foliar spray.

Ramanujam et al. [22] reported that VI 8 was more effective than Vl 12 and Vl32, as their oil formulations @ $1×10^8$ spores mL^{-1} given as foliar spray suppressed *A. craccivora* population by 80.05, 65.88 and 66.83 per cent respectively. Nithya and Rani [23] while testing the oil formulation of *L. lecanni* @ 1 x 10^8 spores mL^{-1} reported that spraying chitin-based oil formulation of Vl8 could result in a 99 per cent reduction in the population of *A. craccivora* in cowpea field, after two sprayings carried out at fortnight intervals. In a laboratory study by Reddy and Sahotra [24], *L. lecanii* @ higher concentration of 10^9 spores mL^{-1} caused 93.33 per cent mortality of *A. craccivora* within eight days of treatment.

Thiamethoxam is a neonicotinoid insecticide belonging to thianicotinyl compounds and is recommended for sucking pests with moderate levels of toxicity (blue labelled). It is effective in sucking pests such as aphids, rice hoppers, rice bugs and mealy bugs in laboratory as well as field trials Senn et al. [25].

In the present study, the percentage reduction noticed in the aphid population was 95.79 when sprayed with thiamethoxam 25 WG (50 a. i ha $^{-1}$) @ 2g L^{-1}. Its efficacy in managing aphids was reported by several workers [26,27,28,29] worldwide, causing 60 to 100 per cent mortality. Due to its high effectiveness, it has been broadly used by growers and therefore various studies have indicated the developed resistance in a wide range of insect pests.

Table 1. Efficacy of biocapsules in managing *Aphis craccivora* in cowpea

Treatments (L^{-1})	*Number of aphids 5cm shoot^{-1}				
	First spraying			Second spraying	
	Pre count	3 DAT	7 DAT	3 DAT	7 DAT
1 *L. lecanii* capsule	449.33 (21.19)	391.33 (19.77)	382.00 ^{b} (19.53)	315.00 ^{b} (17.70)	248.33 ^{b} (15.72)
2 *L. lecanii* capsules	493.00 (22.18)	469.67 (21.64)	361.67 ^{b} (18.97)	225.67 ^{c} (15.01)	177.67 ^{c} (13.32)
3 *L. lecanii* capsules	354.33 (18.78)	337.00 (18.31)	215.33 ^{d} (14.64)	72.67 ^{g} (8.50)	27.33 ^{f} (5.20)
1 *L. saksenae* capsule	375.00 (19.38)	345.67 (18.59)	331.33 ^{bc} (18.20)	317.00 ^{b} (17.81)	234.33 ^{b} (15.28)
2 *L. saksenae* capsules	369.33 (19.18)	355.67 (18.80)	325.00 ^{bc} (17.95)	185.67 ^{cd} (13.57)	157.00 ^{c} (12.50)
3 *L. saksenae* capsules	368.33 (19.10)	347.33 (18.56)	195.33 ^{d} (13.94)	83.33 ^{g} (9.12)	20.01 ^{f} (4.16)
L. lecanii spore suspension @ 10^7 spores mL^{-1} - 20 mL L^{-1}	384.67 (19.44)	359.67 (18.73)	247.33 ^{cd} (15.60)	137.33 ^{e} (11.70)	63.33 ^{e} (7.87)
L. saksenae spore suspension @ 10^7 spores mL^{-1}- 20 g L^{-1}	426.33 (20.64)	397.00 (19.90)	248.33 ^{cd} (15.65)	153.67 ^{de} (12.36)	90.67 ^{d} (9.52)
Thiamethoxam 25 % WG (50 g a. i ha^{-1})	364.33 (19.02)	342.67 (18.43)	180.00 ^{d} (13.40)	55.00 ^{g} (7.40)	15.33 ^{g} (3.88)
Untreated control	500.67 (22.38)	484.67 (22.01)	564.67 ^{a} (23.74)	549.00 ^{a} (23.43)	555.67 ^{a} (23.57)
CD (0.05)	NS	NS	2.71	1.61	1.04

NS - Not Significant. Values in the parentheses are square root transformed
*DAT - Days after treatment, * Mean of three replications*

Table 2. Effect of biocapsules on natural enemy population in Cowpea

Treatments (L^{-1})	No. of natural enemies plot^{-1}*				
	First spraying			Second spraying	
	Pre count	3 DAT	7 DAT	3 DAT	7 DAT
1 *L. lecanii* capsule	4.17 (2.27)	5.00 (2.23)	3.67 (1.88)	3.67 (1.88)	4.00 (1.96)
2 *L. lecanii* capsules	3.67 (1.88)	3.33 (1.81)	3.33 (1.72)	3.33 (1.72)	3.00 (1.65)
3 *L. lecanii* capsules	4.00 (1.96)	3.00 (1.65)	4.33 (2.08)	3.67 (1.88)	4.00 (1.96)
1 *L. saksenae* capsule	4.00 (1.98)	4.00 (1.96)	2.67 (1.55)	3.00 (1.67)	2.67 (1.55)
2 *L. saksenae* capsules	3.33 (1.74)	3.00 (1.65)	4.33 (2.07)	2.67 (1.55)	4.33 (2.07)
3 *L. saksenae* capsules	3.67 (1.90)	3.33 (1.74)	2.67 (1.55)	3.00 (1.71)	2.67 (1.55)
L. lecanii spore suspension @ 10^7 spores mL^{-1} - 20 mL L^{-1}	5.00 (2.23)	3.00 (1.62)	4.67 (2.13)	3.67 (1.81)	3.67 (1.88)
L. saksenae spore suspension @ 10^7 spores mL^{-1} - 20 g L^{-1}	3.33 (1.79)	3.33 (1.82)	4.33 (2.07)	3.33 (1.79)	3.00 (1.71)
Thiamethoxam 25 % WG (50 g a.i ha^{-1})	4.33 (2.08)	3.50 (1.87)	2.00 (1.41)	1.00 (1.00)	0.83 (0.93)
Untreated control	2.67 (1.55)	3.00 (1.65)	3.67 (1.81)	3.67 (1.88)	4.67 (2.13)
CD (0.05)	NS	NS	NS	NS	NS

**Plot size 2 x 2m. Mean of three replications. Figures in parentheses are square root transformed values. DAT - Days after treatment. NS - Non significant*

Table 3. Effect of biocapsules on yield of cowpea

Treatments (L^{-1})	Yield (kg $plot^{-1}$) *
1 *L. lecanii* capsule	1.00^{e}
2 *L. lecanii* capsules	1.29^{cde}
3 *L. lecanii* capsules	1.45^{bcd}
1 *L. saksenae* capsule	1.45^{bcd}
2 *L. saksenae* capsules	1.85^{a}
3 *L. saksenae* capsules	1.56^{abc}
L. lecanii spore suspension @ 10^7 spores mL^{-1} - 20 mL L^{-1}	1.41^{bcd}
L. saksenae spore suspension @ 10^7 spores mL^{-1} - 20 g L^{-1}	1.59^{abc}
Thiamethoxam 25 % WG (50 g a.i ha^{-1})	1.65^{ab}
Untreated control	1.11^{e}
CD (0.05)	0.37

**Plot size 2m x 2m. Mean of three replications. Values sharing same alphabets in superscript are statistically on par based on ANOVA*

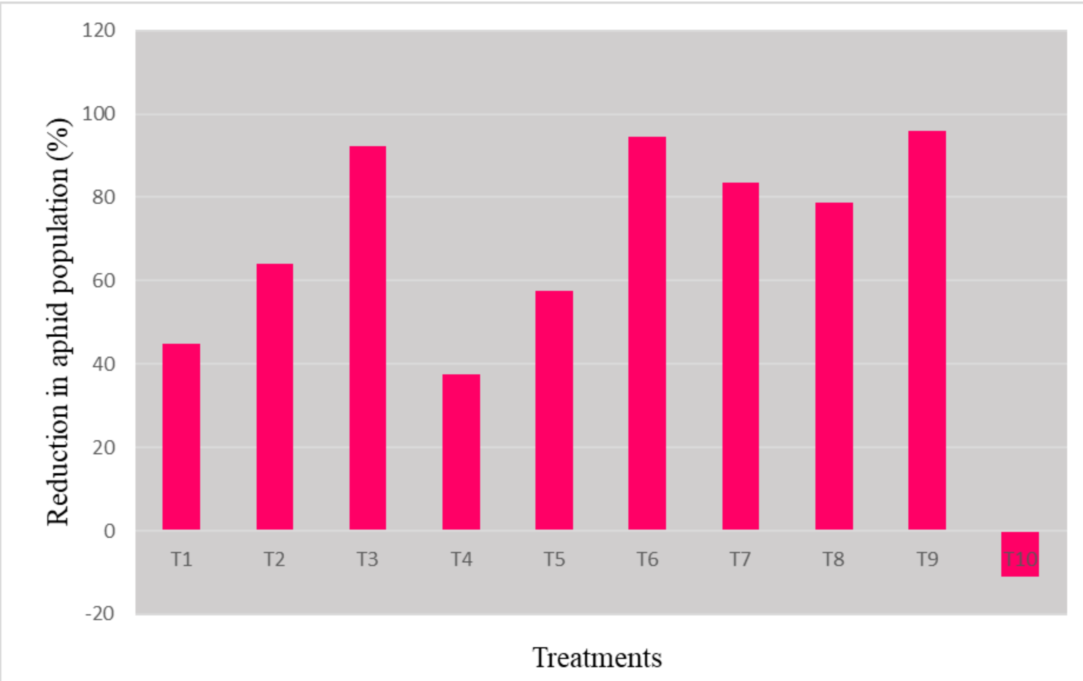

Fig. 1. Effect of biocapsules in managing *Aphis craccivora* in cowpea

T1 - L. lecanii capsule @ 1 L^{-1}
T2 - L. lecanii capsule @ 2 L^{-1}
T3 - L. lecanii capsule @ 3 L^{-1}
T4 - L. saksenae capsule @ 1 L^{-1}
T5 - L. saksenae capsule @ 2 L^{-1}
T6 - L. saksenae capsule @ 3 L^{-1}
T7 - L. lecanii spore suspension @ 10^7 mL^{-1}
T8 - L. saksenae spore suspension @ 10^7 mL^{-1}
T9 - Thiamethoxam 25 WG (50 g a.i ha^{-1})
T10 - Untreated control

4. CONCLUSION

Biocapsules of *L. lecanii* and *L. saksenae* @ 3 capsules L^{-1} were effective in managing cowpea aphid *A. craccivora.* None of the biocaspule treatments had any adverse effect on natural enemy population. *L. saksenae* treatment increased the yield in cowpea significantly.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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