

ISSN Print: 2664-844X
ISSN Online: 2664-8458
NAAS Rating (2026): 4.97
IJAFS 2026; 8(11): 423-426
www.agriculturaljournals.com
Received: 18-09-2026
Accepted: 31-10-2026
Published: 13-11-2026

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Evaluation of botanical and entomopathogenic fungal spray modules for the management of aphid (*Aphis gossypii* Glover) in okra

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DOI: https://www.doi.org/10.33545/2664844X.2026.v8.i11f.90756

Abstract

Seven spray modules were tested against the cotton aphid, *Aphis gossypii* Glover, on okra during the 2025 *kharif* season at the insectary farm of the Entomology Section, College of Agriculture, Nagpur, using a randomized block design with three replications. In modules M_1–M_3, azadirachtin 1.00% EC (2 ml l^{-1}) was sprayed at 30 days after sowing (DAS); this was followed at 40 DAS by *Metarhizium anisopliae* (M_1), *Beauveria bassiana* (M_2) or *Lecanicillium lecanii* (M_3), and at 50 DAS by a consortium of the three fungi. Modules M_4–M_6 repeated this sequence with neem oil (10 ml l^{-1}) in place of azadirachtin. Module M_7 was sprayed with water and served as the control. The pooled aphid count was significantly lower in M_1–M_6 (2.02–2.51 aphids leaf^{-1}) than in M_7 (6.40 aphids leaf^{-1}), although the six biopesticide modules did not differ significantly among themselves. M_3 had the lowest pooled count (2.02 aphids leaf^{-1}) and a 68.44% reduction over the control; reduction over the control increased with each spray and reached 70.13–78.03% nine days after the third spray. M_3 also gave the highest marketable fruit yield (61.96 q ha^{-1}), net incremental benefit (Rs. 1,41,374 ha^{-1}) and incremental cost-benefit ratio (1:18.73). These yield and economic figures describe the complete modules acting on the sucking-pest complex and cannot be credited to aphid suppression alone.

Keywords: *Aphis gossypii*, azadirachtin, biopesticides, *Lecanicillium lecanii*, neem oil, okra

Introduction

Okra [*Abelmoschus esculentus* (L.) Moench] is infested by more than seventy insect species (Rao *et al.*, 2003) [1]. Aphids, jassids and whiteflies make up the main sap-sucking complex of the crop, and together with the shoot and fruit borers they are its principal pests (Meena *et al.*, 2005) [2]. The cotton aphid, *Aphis gossypii* Glover, multiplies in colonies on tender shoots and the underside of leaves; nymphs and adults withdraw sap, so heavily infested plants lose vigour, and the honeydew they excrete favours sooty mould on the foliage. Repeated insecticide sprays against such pests have encouraged resistance, resurgence and residues, which has turned attention towards biopesticides.

Evidence for botanical and fungal control of aphids on okra comes largely from single-product trials. Neem-based formulations rotated with synthetic insecticides kept jassid and aphid numbers down (Bagade *et al.*, 2010) [3]. In organically grown okra, a neem formulation (3.5%) held aphids at 1.67–3.17 per three leaves, while *L. lecanii* at 2.5 g l^{-1} gave 2.53–6.67 (Naik *et al.*, 2012) [4]. Azadirachtin lowered aphid infestation progressively over successive sprays, with a 61.55% reduction after the fifth application (Kaushal *et al.*, 2021) [5], and *L. lecanii* used alone managed okra aphids while sparing ladybird beetles (Bade *et al.*, 2017) [6]. A combination of *Verticillium lecanii* and *M. anisopliae* also suppressed aphids on okra (Palthiya, 2014) [7]. Bioassays likewise show that *A. gossypii* is susceptible to entomopathogens: *L. lecanii* and *B. bassiana* at 0.25% caused complete mortality (Karthikeyan *et al.*, 2011) [8], an oil formulation of *L. lecanii* killed all aphids in vitro (Naik *et al.*, 2009) [9], *V. lecanii* was effective against the pest on cucumber under protected cultivation (Pinna, 1992) [10], and a virulent *B. bassiana* isolate at 1×10^8 conidia ml^{-1} killed 58–91% of cowpea aphids (Ekesi *et al.*, 2000) [11]. Little is known, however, about a programme in which a botanical is sprayed first, a single fungus second and a multi-fungus consortium last. The present study tested six such programmes against *A. gossypii* on okra.

Materials and Methods

The trial was laid out at the insectary field of the Entomology Section, College of Agriculture, Nagpur (21°10′ N, 79°19′ E; 321.26 m above mean sea level) in the 2025 *kharif* season. Okra cultivar NOL-303 (Julie) was dibbled on 15 July 2025 at a spacing of 60 cm between rows and 45 cm between plants. Net plots

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measured 2.4 m × 3.1 m, fertiliser was applied at 100:50:50 kg N:P:K ha^{-1}, and all other operations followed standard recommendations. Seven modules (Table 1) were allotted in a randomised block design with three replications. Each spray liquid was prepared in the field just before use, and sprays were given at 30, 40 and 50 days after sowing (DAS), i.e. on 14 August, 24 August and 3 September 2025; the control plots received plain water. Marketable fruits were harvested plot-wise and yield was converted to q ha^{-1}. Yield and treatment-cost data from the same trial were used to work out net incremental benefit and the incremental cost-benefit ratio (ICBR) at an okra price of Rs. 4,000 q^{-1}. Treatment cost comprised the price of the biopesticides and spraying labour, which came to Rs. 7,546 ha^{-1} for the azadirachtin modules and Rs. 13,300 ha^{-1} for the neem oil modules. The value of the additional yield over the control was calculated at the same price; deducting treatment cost from it gave the net incremental benefit, and ICBR was expressed as treatment cost: net incremental benefit.

Nymphs and adults of aphid were counted on three leaves per plant (one each from the upper, middle and lower canopy) on five tagged plants in every plot. Counts were taken one day before the first spray (1 DBS) and nine days after the first, second and third sprays (9 DAFS, 9 DASS and 9 DATS). The cumulative value for a plot was the mean of the three post-spray counts. Before analysis of variance the counts were transformed as $\sqrt{x+0.5}$, and treatment means were compared using SE(m)± and critical difference (CD) at the 5% level. Reduction over control (%) was calculated from untransformed means as $\frac{C-T}{C} \times 100$, where C is the control mean and T the treatment mean.

Results

Before the first spray, aphid density in the plots ranged from 3.76 to 4.13 per leaf and did not differ significantly, so the infestation was uniform (Table 2). Nine days after the first spray (9 DAFS), every treated plot held fewer aphids than the control (5.60 per leaf). M_3 had the fewest (2.47 per leaf), followed by M_2 (2.53), M_1 (2.60), M_6 (2.73), M_5 (2.80) and M_4 (2.87), and these six modules did not differ significantly from one another. After the second spray the control rose to 6.00 per leaf, whereas M_3 (1.93), M_6 (2.11), M_2 (2.20), M_5 (2.27), M_1 (2.33) and M_4 (2.40) stayed significantly lower. Nine days after the third spray (9 DATS), M_3 again recorded the lowest count (1.67), followed by M_6 (1.80), M_2 (1.93), M_5 (2.00), M_1 (2.20) and M_4 (2.27), against 7.60 in the control.

Pooled over the three sprays, M_3 had the lowest density (2.02 per leaf), followed by M_6 (2.21), M_2 (2.22), M_5 (2.36), M_1 (2.38) and M_4 (2.51), compared with 6.40 in the control. All six biopesticide modules were significantly better than the control (CD at 5% = 0.22), but none differed significantly from another. Reduction over control (Fig 1) was highest in M_3 (68.44%), followed by M_6 (65.47%) and M_2 (65.31%), and lowest in M_4 (60.78%). It increased with each successive spray, from 48.75–55.89% at 9 DAFS to 70.13–78.03% at 9 DATS.

Marketable fruit yield and the economics of the complete sequential modules are given in Table 3. M_3 gave the highest yield (61.96 q ha^{-1}), followed by M_6 (59.54 q ha^{-1}), while the control yielded 24.73 q ha^{-1}. With a yield CD of 9.00 q ha^{-1} at 5%, M_3 was statistically at par with M_6, M_2 (55.78 q ha^{-1}) and M_5 (53.36 q ha^{-1}). M_3 also recorded the highest net incremental benefit (Rs. 1,41,374 ha^{-1}) and ICBR (1:18.73), whereas M_4 had the lowest ICBR (1:5.71). Because the modules also acted on other sucking pests, these are module-level outcomes and cannot be attributed to aphid suppression alone.

Discussion

The lower aphid counts in every sprayed plot after the first spray show that opening the schedule with a botanical checked the early growth of colonies. Azadirachtin and neem oil act as antifeedants and growth disruptors, so nymphs feed poorly and moult abnormally, and colonies expand slowly. Comparable suppression of okra aphids by neem-based products and azadirachtin has been recorded earlier (Bagade *et al.*, 2010; Naik *et al.*, 2012; Kaushal *et al.*, 2021) [3, 4, 5]. At 9 DAFS the azadirachtin modules (M_1–M_3; 2.47–2.60 aphids leaf^{-1}) were numerically ahead of the neem oil modules (M_4–M_6; 2.73–2.87 aphids leaf^{-1}), but the difference was not significant. Since botanicals and fungal agents have been found compatible against sucking pests (Halder *et al.*, 2021) [12], following a botanical with fungal sprays is also biologically sound.

From the second spray onward the modules containing *L. lecanii* (M_3 and M_6) carried the fewest aphids, and they were also the two best on the pooled mean. Aphids have a thin, soft cuticle that germinating conidia can penetrate readily, which probably explains the strong showing of this fungus. The result agrees with the complete mortality obtained in vitro with an *L. lecanii* oil formulation (Naik *et al.*, 2009) [9], with the control of okra aphids by *L. lecanii* alone and its safety to ladybird beetles (Bade *et al.*, 2017) [6], and with the effect of *V. lecanii* reported by (Palthiya) [7]. Even so, the *B. bassiana* (M_2, M_5) and *M. anisopliae* (M_1, M_4) modules were also significantly better than the control and statistically at par with M_3, so all three fungi seem useful after a botanical. The ranking of fungi is not constant across studies: in Bt cotton, *M. anisopliae* gave fewer aphids than *L. lecanii* and *B. bassiana* (Nazir, 2008) [13], whereas the *M. anisopliae* modules ranked last here, which suggests that performance depends on crop, isolate, dose and formulation. The fall in aphid numbers continued through the third spray, which carried the consortium, and reduction over control reached 70.13–78.03% at 9 DATS. This rise reflects the accumulated effect of all three sprays together with the growth of the control population (5.60 to 7.60 aphids leaf^{-1}), so the separate contribution of the consortium cannot be judged from this design.

Yield and economics assess the modules as a whole. M_3 gave the highest yield and ICBR, and its yield was statistically at par with M_2, M_5 and M_6. The cheaper azadirachtin schedules (Rs. 7,546 ha^{-1} against Rs. 13,300 ha^{-1} for neem oil) raised ICBR: M_3 and M_6 had statistically similar yields, yet their ICBR values were 1:18.73 and 1:9.47, respectively. Azadirachtin was likewise profitable on okra in earlier work (yield 59.63 q ha^{-1}; ICBR 1:5.72) (Kaushal *et al.*, 2021) [5], and *L. lecanii* gave the best yield and ICBR in Bt cotton (Raghunandan *et al.*, 2018) [14]. As the modules also acted on other sucking pests, these figures show combined module performance, and the present trial does not isolate the part played by aphid suppression.

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Table 1. Details of the sequential biopesticide modules tested against *Aphis gossypii* on okra

Module	Spray 1 (30 DAS)	Spray 2 (40 DAS)	Spray 3 (50 DAS)
M_1	Azadirachtin 1.00% EC (2 ml l^{-1})	*Metarhizium anisopliae* 1.15% WP, 1 × 10^8 CFU g^{-1} (5 g l^{-1})	Bioinoculant (*Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium lecanii*) 2 × 10^8 CFU ml^{-1} (10 ml l^{-1})
M_2	Azadirachtin 1.00% EC (2 ml l^{-1})	*Beauveria bassiana* 1.15% WP, 1 × 10^8 CFU g^{-1} (5 g l^{-1})	Bioinoculant (*Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium lecanii*) 2 × 10^8 CFU ml^{-1} (10 ml l^{-1})
M_3	Azadirachtin 1.00% EC (2 ml l^{-1})	*Lecanicillium lecanii* 1.15% WP, 2 × 10^8 CFU g^{-1} (5 g l^{-1})	Bioinoculant (*Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium lecanii*) 2 × 10^8 CFU ml^{-1} (10 ml l^{-1})
M_4	Neem oil (10 ml l^{-1})	*Metarhizium anisopliae* 1.15% WP, 1 × 10^8 CFU g^{-1} (5 g l^{-1})	Bioinoculant (*Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium lecanii*) 2 × 10^8 CFU ml^{-1} (10 ml l^{-1})
M_5	Neem oil (10 ml l^{-1})	*Beauveria bassiana* 1.15% WP, 1 × 10^8 CFU g^{-1} (5 g l^{-1})	Bioinoculant (*Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium lecanii*) 2 × 10^8 CFU ml^{-1} (10 ml l^{-1})
M_6	Neem oil (10 ml l^{-1})	*Lecanicillium lecanii* 1.15% WP, 2 × 10^8 CFU g^{-1} (5 g l^{-1})	Bioinoculant (*Metarhizium anisopliae*, *Beauveria bassiana*, *Lecanicillium lecanii*) 2 × 10^8 CFU ml^{-1} (10 ml l^{-1})
M_7 (control)	Water spray		

DAS: days after sowing; EC: emulsifiable concentrate; WP: wettable powder; CFU: colony-forming units.

Table 2. Mean aphid population per leaf under different biopesticide modules at each spray interval

Module	1 DBS	9 DAFS	9 DASS	9 DATS	Cumulative
M_1	3.87 (2.07)	2.60 (1.74)	2.33 (1.67)	2.20 (1.64)	2.38 (1.69)
M_2	3.91 (2.09)	2.53 (1.74)	2.20 (1.63)	1.93 (1.55)	2.22 (1.64)
M_3	4.00 (2.11)	2.47 (1.71)	1.93 (1.54)	1.67 (1.46)	2.02 (1.58)
M_4	4.13 (2.14)	2.87 (1.83)	2.40 (1.69)	2.27 (1.66)	2.51 (1.73)
M_5	3.76 (2.06)	2.80 (1.80)	2.27 (1.65)	2.00 (1.57)	2.36 (1.69)
M_6	4.11 (2.14)	2.73 (1.79)	2.11 (1.60)	1.80 (1.51)	2.21 (1.64)
M_7 (control)	3.91 (2.09)	5.60 (2.46)	6.00 (2.55)	7.60 (2.84)	6.40 (2.62)
F test	NS	Sig.	Sig.	Sig.	Sig.
SE(m)±	0.15	0.12	0.15	0.10	0.07
CD at 5%	–	0.38	0.47	0.30	0.22
CV (%)	11.98	11.25	14.73	9.37	6.70

DBS: days before spray; DAFS, DASS, DATS: days after first, second and third spray; NS: non-significant; Sig.: significant at 5%. Data in parentheses are square-root transformed [$\sqrt{x+0.5}$] values.

Table 3. Marketable fruit yield and economic performance of the sequential biopesticide modules

Module	Yield (q ha^{-1})	Yield increase over control (q ha^{-1})	Total treatment cost (Rs. ha^{-1})	Net incremental benefit (Rs. ha^{-1})	ICBR (cost:benefit)
M_1	49.59	24.86	7,546	91,894	1:12.18
M_2	55.78	31.05	7,546	1,16,654	1:15.46
M_3	61.96	37.23	7,546	1,41,374	1:18.73
M_4	47.04	22.31	13,300	75,940	1:5.71
M_5	53.36	28.63	13,300	1,01,220	1:7.61
M_6	59.54	34.81	13,300	1,25,940	1:9.47
M_7 (control)	24.73	—	—	—	—

Note: Okra price = Rs. 4,000 q^{-1}. Gross value of yield increase = yield increase over control × market price; net incremental benefit = gross value of yield increase − total treatment cost. ICBR is expressed as treatment cost: net incremental benefit (1:x). Yield CD at 5% = 9.00 q ha^{-1}.

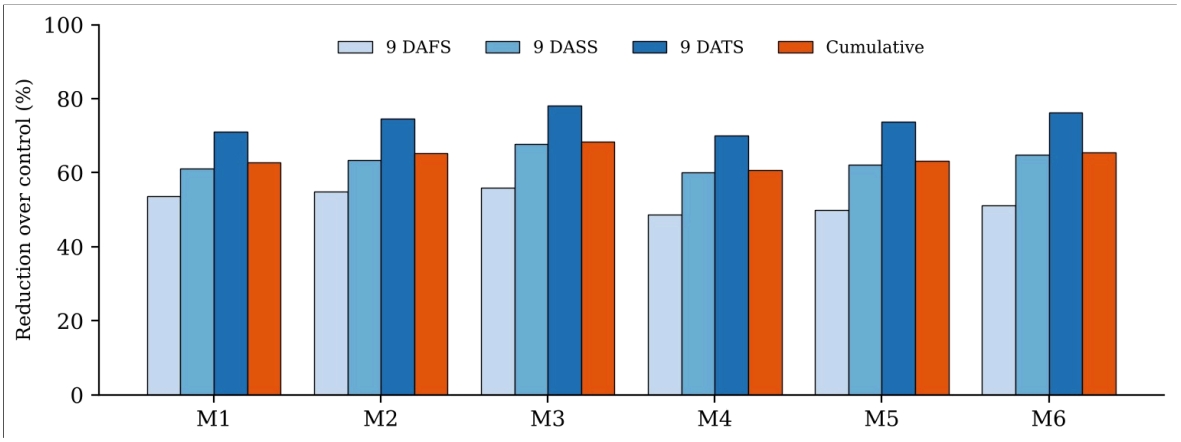


Figure 1. Reduction (%) in aphid population over the untreated control (water-spray) after each spray and for the cumulative mean under different biopesticide modules.

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Conclusion

All six sequential biopesticide programmes reduced aphid density on okra significantly relative to the water-sprayed control. The azadirachtin–*L. lecanii*–consortium programme (M_3) recorded the lowest pooled count (2.02 aphids leaf^{-1} against 6.40 in the control) and the greatest reduction over control (68.44%); however, the six programmes did not differ significantly from one another. M_3 also gave the highest marketable fruit yield (61.96 q ha^{-1}) and ICBR (1:18.73), but these figures reflect the performance of the full modules on the sucking-pest complex and not aphid control alone. Multi-season and multi-location trials are needed to confirm these programmes.

Acknowledgments

The authors are grateful to the Head of the Entomology Section and the Associate Dean of the College of Agriculture, Nagpur, for providing field and laboratory facilities.

Conflict of interest

The authors declare no conflict of interest.

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How to cite this article: Nannaware SV, Panchbhai P, Wadaskar R, Chaudhari B, Pillai T, Goramnagar H. Evaluation of botanical and entomopathogenic fungal spray modules for the management of aphid (*Aphis gossypii* Glover) in okra. *International Journal of Agriculture and Food Science*. 2026;8(11):423-426. doi: https://www.doi.org/10.33545/2664844X.2026.v8.i11f.90756

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