Ecological engineering-based bhendi designer seeds enhanced biocontrol services of coccinellids on aphid, *Aphis gossypii*

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ABSTRACT

The ecologically-engineered bhendi seeds were developed with biologicals like azadirachtin 1%, *Beauveria bassiana*, *B bassiana* + *Azospirillum*, *B bassiana* + *Azotobacter*, humic acid, gibberellic acid, amino acid mixture, *Kappaphycus alvarezii* and *Sargassum wightii* and evaluated in the field with imidacloprid 600 FS-based designer seeds and conventional seeds on the biocontrol services of predatory coccinellids on aphids in RBD with three replications. Mean population of all coccinellids in all the treatments varied from 1.93 to 5.75 per plant. There was a remarkable increase in coccinellid population (5.75/plant) (*Cheilomenes sexmaculatus* F, *Coccinella transversalis* F, *Illeis cincta* F, *Propylea dissecta* M, *Brumoides suturalis* F and *Oenopia billieti* M) with an occurrence ratio (OR) of 2.99 due to designer seed plants treated with combination of *B bassiana* (10 g/kg of seeds) and *Azospirillum* (200 g/kg of seeds) which influenced for the maximum coccinellid population to 66.50 per cent when compared with control along with minimum population of aphids (1.70 per leaf) with preference ratio (PR) of 0.25. Designer seeds developed with seaweed extracts of *S wightii* (10%) and *K alvarezii* (7%) also influenced for higher coccinellid population of 59.97 and 56.18 per cent with OR of 2.50 and 2.28 and lesser population of aphids (3.10 and 3.05/plant) with PR of 0.46 and 0.46 respectively. However imidacloprid 600 FS-based designer seeds contributed for 38.39 per cent coccinellid population and 1.62 OR along with 4.39 aphids per plant with PR of 0.66 when compared to untreated bhendi plants (1.93 coccinellids/plant and 6.69 aphids/leaf). The behavioural bioassay by olfactometer showed that designer seed plants treated with combination of *B bassiana* (10 g/kg of seeds) and *Azospirillum* (200 g/kg of seeds) attracted more number of coccinellids (3.18) when compared to untreated bhendi seeds (0.83). The significance of designer seeds with biologicals in pest management is discussed.

Keywords: Bhendi; ecological engineering; designer seeds; coccinellids; aphids

INTRODUCTION

In India bhendi covers an area of 5.28 lakh ha and contributes for a production of 61.46 lakh MT with an average yield of 11.64 MT/ha (www.agricoop.co.in). Though the crop has gained more economic value, production is being hampered by insects, fungi, nematodes and viruses that results in yield loss (Nair et al 2017). The destructive aftermath of pesticide usage has led to an alternative strategy-ecological engineering. Ecologically-engineered seed is an integrated pre-sowing seed treatment (designer seeds) that involves addition of the nutrients, plant protectants and bio-inoculants for improved seed quality, emergence, establishment and yield attributes (Sujatha and Ambika 2016) and for early protection with synthetic insecticides like imidacloprid 600 FS (Sajjan et al 2010).

Usage of biologically-active products like microbial inoculants containing active strains of *Beauveria bassiana, Azospirillum, Azotobacter, Pseudomonas fluorescens,* seaweed extracts, humic acid, gibberellic acid and amino acid mixture seems to increase the plant growth including profused flowering. Usage of microorganism-treated seeds for sowing aids in settlement of microbial inoculates in soil which acts as an efficient mechanism for microorganism to colonize seedling roots or make contact with soil dwelling invertebrate pests that feed on plant roots

(Graham and Vance 2013). Colonization of several entomopathogenic fungi in plant tissues as endophytes can affect pests systemically via the plant (Vega et al 2012). Seaweeds contain plant growth regulators (Mooney and van Staden 1986), cytokinins (Featonby-Smith and van Staden 1984), IAA (Abe et al 1972), gibberellins and gibberellins-like substances. Similarly plant extracts exhibited antibacterial (Satish et al 1999), antifungal (Okigbo and Ogbonnaya 2006) and insecticidal properties (Shariff et al 2006).

Chemical insecticide (imidacloprid 600 FS like) with *P flourescens* or *Trichoderma viridi* are widely used in designer seed technology in many crops. However not much research is available to replace imidacloprid with biologicals such as plant and sea weed extracts, microbial consortia and plant growth regulators having insecticidal and plant growth promoting properties either alone or in combination. Recently Neethu and Muthukrishnan (2018) proved that ecologically engineering-based black gram seeds promoted services of coccinellids and suppressed *Aphis craccivora* on black gram through the maximum pest defender ratio (3.91), occurrence ratio (OR) of coccinellids (2.6), minimum *A craccivora* population (1.5 number/terminal shoot) and preference ratio (PR) of *A craccivora* (0.2). The present study was aimed at engineering bhendi seeds with gum Arabic polymer (2%), respective biologicals and *P fluorescence* and ecologically-engineered designer seed-based plants were field evaluated for their biocontrol services against aphids in comparison with imidacloprid-based designer seed plants and untreated plants.

MATERIAL and METHODS

The most effective biologicals used for the development of ecologically-engineered blackgram designer seeds (Neethu and Muthukrishnan 2018) were used for the development of ecologically-engineered bhendi designer seeds. Freshly harvested untreated bhendi cv COBhH4 seeds served as the base material for the study. Aqueous gum arabic polymer, commercial formulation of azadirachtin and imidacloprid were purchased from Coimbatore market. Plant growth promoting *Rhizobacteria* (PGPR) such as *Azophos*, *Azospirillum* and *B bassiana* spore suspension and plant growth regulators like humic acid, gibberellic acid and amino acid mixture were obtained from the Department of Microbiology and *P flourescens* (*Pf* - 1 strain) talc-based formulation was received from the Department of Plant Pathology, TNAU, Coimbatore, Tamil Nadu. Seaweed extracts *Sargassum wightii* and *Kappaphycus alvarezii* were obtained from Marine Algal Research Station, CSIR- Central Salt and Marine Chemicals Research Institute, Mandapam Camp, Rameswaram, Tamil Nadu.

Developing bhendi designer seeds with biologicals

The methodology described for the development of ecologically-engineered blackgram designer seeds (Neethu and Muthukrishnan 2018) was followed for the development of ecologically-engineered bhendi designer seeds. Fresh bhendi seeds were treated with aqueous gum Arabic polymer (2%) at 5 ml per kg of seeds. Biological materials in varied concentrations such as azadirachtin (1%), *B bassiana (*15 g/kg), *B bassiana (*10 g/kg) + *Azospirillum* (200 g/kg), *B bassiana* (15 g/kg) +*Azotobacter* (200 g/kg), humic acid (0.3 g/kg), gibberellic acid (0.1 g/kg), amino acid mixture (0.5 g/kg), *K alvarezii* (7%), *S wightii* (10%) and imidacloprid 600 FS (6 m/kg) were then added separately and admixed with *P flourescens* (10 g/kg) individually to develop into ecologically-engineered bhendi designer seeds. The seeds were shade-dried for 12 h prior to evaluation. Treated seeds were evaluated in the laboratory for quality parameters such as germination percentage, root length, shoot length, dry matter content and vigour index in comparison with untreated seeds as per Anon (2013).

Field evaluation of ecologically-engineered bhendi designer seeds

One field experiment was laid out in field in the eastern block of TNAU, Coimbatore, Tamil Nadu from November 2018 to February 2019 in randomized block design (RBD) with three replications in an area of 0.37 acres in well prepared soil. Ecologically-engineered biologicals-based bhendi designer seeds including imidacloprid-based designer seeds and untreated bhendi seeds were sown separately at a spacing of 60 × 45 cm. The plot size for each treatment was 6 x 6 m^2.

Standard good agronomic practices as per the recommendations of TNAU except pest management strategies were adopted to maintain healthy bhendi plants. Application of chemical pesticides was neglected throughout the season.

In situ and sweep net observations were made on the population of grubs and adults of various species of coccinellids (number/plant) and population of nymphs

and adults of *Aphis gossypii* on bhendi from 10 randomly selected plants from each replication. Standard taxonomic keys were used for the identification of coccinellids. Observations were taken from 15 days after sowing (DAS) to 85 DAS at seven days interval during early morning hours. Based on the observations, occurrence ratio (OR) of coccinellids and preference ratio (PR) of aphids were estimated by using the formulae given by Muthukrishnan and Dhanasekaran (2014):

$$\text{Occurrence ratio} = \frac{\text{Population of coccinellids in treatment}}{\text{Population of coccinellids in control}}$$

$$\text{Preference ratio} = \frac{\text{Population of aphids in treatment}}{\text{Population of aphids in control}}$$

Behavioral bioassay of coccinellids towards flowers of biological designer seed-based bhendi plants using olfactometer

The behavioural bioassay was conducted using six-arm olfactometer and the methodology was conducted as per Neethu and Muthukrishnan (2018) and Lokesh (2017).

Statistical analysis

The data from laboratory and field experiments were evaluated by CRBD and RBD analysis of variance (ANOVA) respectively after getting transformed into $\sqrt{x + 0.5}$ and arc sine percentage values where appropriate using AGRES. Critical difference values were calculated at five per cent probability level and treatment mean values were compared using Latin square design (LSD) as per Gomez and Gomez (1984).

RESULTS and DISCUSSION

Coccinellid species observed

Coccinellid species like *Cheilomenes sexmaculatus* F, *Coccinella transversalis* F, *Illeis cincta* F, *Propylea dissecta* M, *Brumoides suturalis* F and *Oenopia billieti* M were observed.

Effect of ecologically-engineered designer seed-based bhendi ecosystems on the population of coccinellids (in situ observation)

The data on the population of coccinellids on ecologically-engineered designer seed-based bhendi ecosystems are given in Table 1 (Fig 1a). Combination of *B bassiana* (10 g/kg) + *Azospirillum* (200 g/kg)-treated designer seed-based plants most significantly influenced the maximum population of coccinellids (4.27, 4.87, 5.58, 5.98, 6.07, 6.10, 6.58, 6.77, 6.10, 5.62 and 5.33/plant at 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85 DAS respectively). The designer seed-based plants treated with seaweed extract of *S wightii* (10%) was the next best treatment and resulted in coccinellid population of 3.27, 3.87, 4.58, 4.98, 5.07, 5.10, 5.58, 5.77, 5.10, 5.30 and 4.33 per plant on bhendi at 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85 DAS respectively when compared to untreated bhendi plants (0.80, 1.33, 2.27, 1.80, 2.05, 2.17, 2.21, 2.33, 2.45, 2.09 and 1.70 coccinellids/plant at 15, 22, 29, 36, 43, 50, 57,64, 71, 78 and 85 DAS respectively).

Mean population of coccinellids varied from 1.93 to 5.75 per plant due to various designer seed treatments. Combination of *B bassiana* and *Azospirillum*-treated designer seed-based plants was significantly superior in maximizing the population of coccinellids to 5.75 per plant with 66.50 per cent increase over control with occurrence ratio of 2.99 as compared to 1.93 coccinellids per plant on bhendi alone (Table 1) (Fig 1b).

The increased population of coccinellids might be due to profuse flowering of *B bassiana* (10 g/kg) + *Azospirillum* (200 g/kg), *S wightii* (10%) and *K alvarezii* (7%)-treated designer seed-based plants. These flowering plants might have provided alternate resources like pollen, nectar, alternate preys and lekking sites to the coccinellids which made coccinellids for the effective attraction and conservation in the above biological-treated designer seed-based plants. Kopta et al (2012) stated that presence of coccinellids on *A graveolens* and *F vulgare* before flowering was due to aphids foraging on these plants. Similar results were obtained in blackgram (Neethu and Muthukrishnan 2018) and cabbage (Thaiyalnayagi et al 2019) when seeds were treated with *B bassiana*.

Table 1. Effect of biological designer seed-based bhendi ecosystems on the coccinellid population (in situ method)

Treatment	Mean number of adult coccinellids/plant on DAS											Mean/ plant	% increase over control	OR
	15	22	29	36	43	50	57	64	71	78	85			
T1	1.13^{h}	2.53^{e}	2.67^{f}	3.13^{f}	4.33^{c}	5.27^{c}	5.99^{b}	6.18^{b}	4.14^{de}	3.61^{de}	3.13^{e}	3.83^{c}	49.67	1.99
T2	2.27^{d}	2.47^{e}	2.68^{f}	2.98^{fg}	3.27^{e}	3.40^{g}	3.68^{g}	4.40^{ef}	4.20^{d}	3.62^{de}	3.43^{d}	3.31^{de}	41.78	1.72
T3	4.27^{a}	4.87^{a}	5.58^{a}	5.98^{a}	6.07^{a}	6.10^{a}	6.58^{a}	6.77^{a}	6.10^{a}	5.62^{a}	5.33^{a}	5.75^{a}	66.50	2.99
T4	1.53^{f}	2.73^{d}	2.73^{f}	3.60^{d}	3.27^{e}	4.56^{d}	5.84^{b}	5.32^{d}	3.87^{f}	3.53^{de}	2.23^{h}	3.56^{cd}	45.95	1.85
T5	1.47^{f}	2.33^{fg}	3.07^{e}	3.33^{e}	3.40^{e}	3.90^{f}	4.17^{f}	4.60^{e}	3.52^{g}	3.65^{d}	2.92^{f}	3.31^{de}	41.71	1.72
T6	1.00^{i}	2.05^{h}	3.00^{e}	3.67^{d}	3.83^{d}	3.86^{f}	4.05^{f}	4.16^{g}	3.61^{g}	2.87^{f}	2.79^{g}	3.17^{de}	39.26	1.65
T7	1.27^{g}	2.33^{fg}	3.24^{d}	3.60^{d}	3.93^{d}	4.29^{e}	4.63^{e}	4.33^{fg}	3.35^{h}	3.05^{f}	2.73^{g}	3.34^{de}	42.33	1.73
T8	3.07^{c}	3.27^{c}	4.08^{c}	4.28^{c}	4.97^{b}	5.00^{c}	5.18^{d}	5.37^{d}	4.60^{c}	4.42^{c}	4.13^{c}	4.40^{b}	56.18	2.28
T9	3.27^{b}	3.87^{b}	4.58^{b}	4.98^{b}	5.07^{b}	5.10^{bc}	5.58^{c}	5.77^{c}	5.10^{b}	5.30^{b}	4.33^{b}	4.81^{b}	59.97	2.50
T10	2.07^{e}	2.27^{g}	2.48^{g}	2.88^{g}	3.07^{f}	3.20^{h}	3.58^{g}	4.30^{fg}	4.00^{ef}	3.42^{e}	3.13^{e}	3.13^{e}	38.39	1.62
T11	0.80^{j}	1.33^{i}	2.27^{h}	1.80^{h}	2.05^{g}	2.17^{j}	2.21^{h}	2.33^{h}	2.45^{i}	2.09^{g}	1.70^{i}	1.93^{f}	-	-
SEd	0.012	0.023	0.016	0.022	0.022	0.018	0.022	0.023	0.018	0.026	0.014	0.050	-	-
$CD_{0.05}$	0.026	0.048	0.035	0.046	0.046	0.038	0.047	0.048	0.038	0.055	0.030	0.100	-	-

T1: Bhendi seeds + gum Arabic (2%) + azadirachtin (1%) + *Pseudomonas flourescens* (10 g/kg), T2; Bhendi seeds + Gum Arabic (2%) + *Beauveria bassiana* (15 g/kg) + *P flourescens* (10 g/kg), T3: Bhendi seeds + gum Arabic (2%) + *B bassiana* (10 g/kg) + *Azospirillum* (200 g/kg) + *P flourescens* (10 g/kg), T4: Bhendi seeds + gum Arabic (2%) + *B bassiana* (15 g/kg) + *Azotobacter* (200 g/kg) + *P flourescens* (10 g/kg), T5: Bhendi seeds + gum Arabic (2%) + humic acid (0.3 g/kg) + *P flourescens* (10 g/kg), T6: Bhendi seeds + gum Arabic (2%) + gibberellic acid (0.1 g/kg) + *P flourescens* (10 g/kg), T7: Bhendi seeds + gum Arabic (2%) + amino acid mixture (0.5 g/kg) + *P flourescens* (10 g/kg), T8: Bhendi seeds + gum Arabic (2%) + *Kappaphycus alvarezii* (7%) + *P flourescens* (10 g/kg), T9: Bhendi seeds + gum Arabic (2%) + *Sargassum wightii* (10%) + *P flourescens* (10 g/kg), T10: Bhendi seeds + gum Arabic (2%) + imidacloprid 600 FS (6 ml/kg) + *P flourescens* (10g/kg), T11: Untreated bhendi seeds

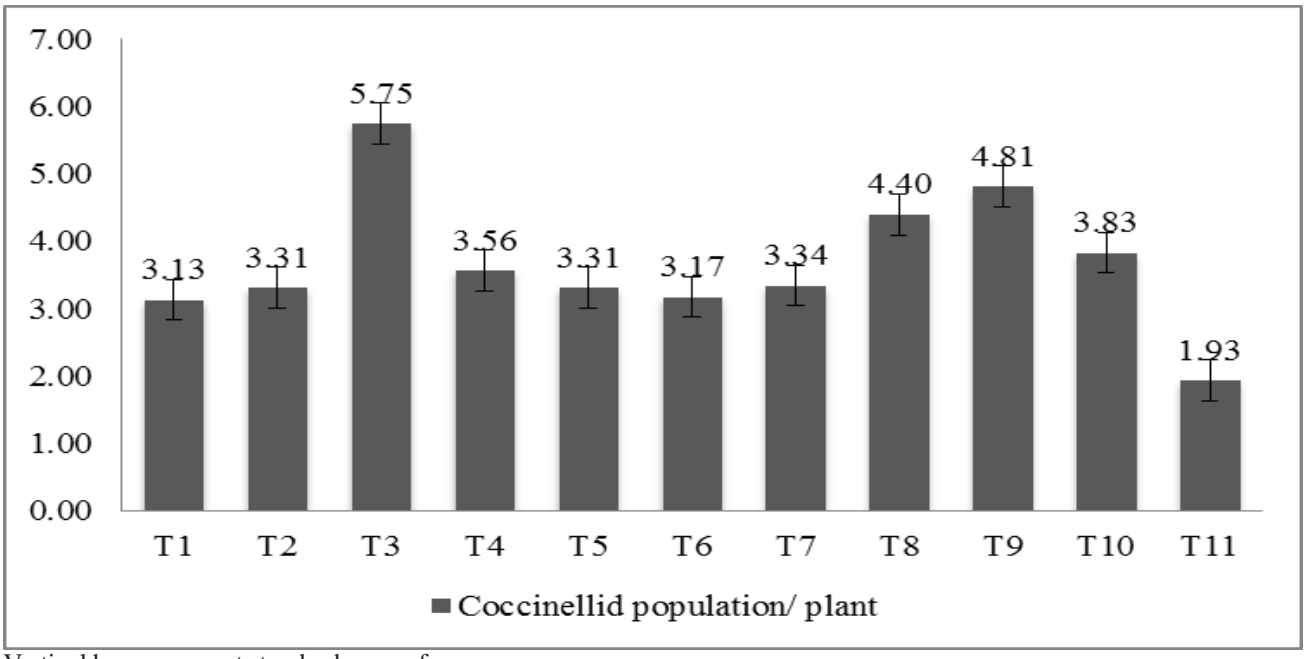
Figures are mean values of three replications; Figures transformed by square root transformation and the original values are given; In a column means followed by same letter(s) are not significantly different at 5% level; OR: Occurrence ratio

Table 2. Effect of biological designer seed-based bhendi ecosystems on the aphid population (in situ method)

Treatment	Mean number of aphids/leaf on DAS											Mean/ leaf	% reduction over control	PR
	15	22	29	36	43	50	57	64	71	78	85			
T1	2.00^{e}	3.50^{g}	3.80^{f}	4.80^{hi}	5.00^{gh}	5.80^{g}	6.20^{gh}	6.50^{g}	6.20^{g}	5.00^{h}	4.50^{i}	4.85^{f}	27.58	0.72
T2	2.20^{f}	3.30^{f}	3.50^{e}	3.80^{e}	3.80^{e}	4.90^{e}	5.30^{ef}	5.40^{e}	5.00^{e}	3.20^{e}	3.00^{f}	3.95^{d}	41.03	0.59
T3	0.50^{a}	0.90^{a}	1.10^{a}	1.70^{a}	1.90^{a}	2.20^{a}	2.70^{a}	2.90^{a}	2.60^{a}	1.20^{a}	1.00^{a}	1.70^{a}	74.59	0.25
T4	0.90^{b}	1.30^{b}	1.70^{b}	2.30^{b}	2.70^{b}	3.10^{b}	3.30^{b}	4.00^{b}	3.40^{b}	1.50^{b}	1.30^{b}	2.32^{b}	65.35	0.35
T5	1.80^{d}	2.90^{e}	3.40^{e}	4.00^{f}	4.10^{f}	4.80^{e}	5.10^{e}	5.30^{e}	4.70^{d}	3.10^{e}	2.50^{e}	3.79^{d}	43.34	0.57
T6	3.00^{i}	3.90^{h}	4.40^{h}	4.90^{i}	5.20^{h}	6.30^{h}	6.40^{h}	6.90^{h}	6.40^{g}	5.30^{i}	4.90^{j}	5.24^{g}	21.74	0.78
T7	2.60^{g}	3.50^{g}	4.20^{g}	4.70^{h}	4.80^{g}	5.80^{g}	6.00^{g}	6.30^{g}	6.20^{g}	4.70^{g}	4.30^{h}	4.83^{f}	27.85	0.72
T8	1.80^{d}	2.50^{d}	2.90^{d}	2.90^{c}	2.90^{c}	3.70^{c}	4.10^{c}	4.30^{c}	4.20^{c}	2.20^{c}	2.10^{c}	3.05^{c}	54.35	0.46
T9	1.10^{c}	1.70^{c}	2.20^{c}	3.40^{d}	3.20^{d}	4.20^{d}	4.50^{d}	4.80^{d}	4.20^{c}	2.50^{d}	2.30^{d}	3.10^{c}	53.67	0.46
T10	2.80^{h}	3.40^{fg}	3.50^{e}	4.50^{g}	4.80^{g}	5.20^{f}	5.50^{f}	5.80^{f}	5.50^{f}	3.80^{f}	3.50^{g}	4.39^{e}	34.38	0.66
T11	3.00^{i}	4.30^{i}	4.80^{i}	7.00^{j}	7.30^{i}	8.10^{i}	8.40^{i}	9.20^{i}	9.00^{h}	7.00^{j}	5.50^{k}	6.69^{h}	-	-
SEd	0.011	0.017	0.016	0.018	0.024	0.014	0.023	0.027	0.028	0.019	0.021	0.038	-	-
$CD_{0.05}$	0.023	0.036	0.034	0.038	0.050	0.030	0.048	0.057	0.058	0.039	0.044	0.076	-	-

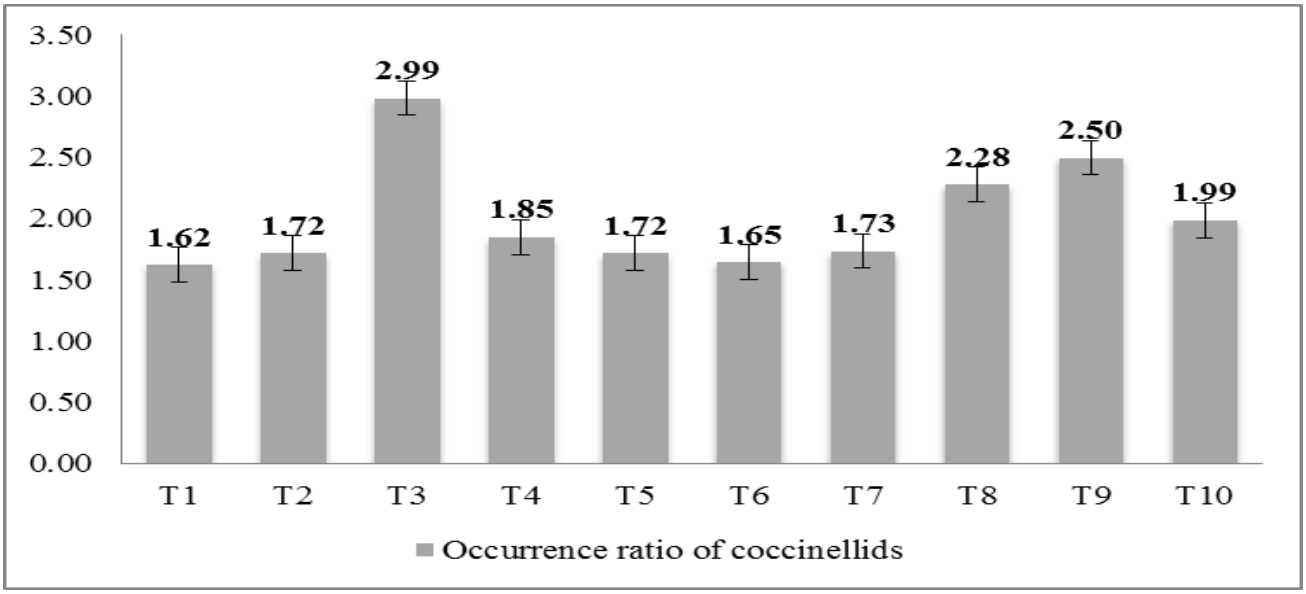
T1: Bhendi seeds + gum Arabic (2%) + azadirachtin (1%) + *Pseudomonas flourescens* (10 g/kg), T2; Bhendi seeds + Gum Arabic (2%) + *Beauveria bassiana* (15 g/kg) + *P flourescens* (10 g/kg), T3: Bhendi seeds + gum Arabic (2%) + *B bassiana* (10 g/kg) + *Azospirillum* (200 g/kg) + *P flourescens* (10 g/kg), T4: Bhendi seeds + gum Arabic (2%) + *B bassiana* (15 g/kg) + *Azotobacter* (200 g/kg) + *P flourescens* (10 g/kg), T5: Bhendi seeds + gum Arabic (2%) + humic acid (0.3 g/kg) + *P flourescens* (10 g/kg), T6: Bhendi seeds + gum Arabic (2%) + gibberellic acid (0.1 g/kg) + *P flourescens* (10 g/kg), T7: Bhendi seeds + gum Arabic (2%) + amino acid mixture (0.5 g/kg) + *P flourescens* (10 g/kg), T8: Bhendi seeds + gum Arabic (2%) + *Kappaphycus alvarezii* (7%) + *P flourescens* (10 g/kg), T9: Bhendi seeds + gum Arabic (2%) + *Sargassum wightii* (10%) + *P flourescens* (10 g/kg), T10: Bhendi seeds + gum Arabic (2%) + imidacloprid 600 FS (6 ml/kg) + *P flourescens* (10g/kg), T11: Untreated bhendi seeds

Figures are mean values of three replications; Figures transformed by square root transformation and the original values are given; In a column means followed by same letter(s) are not significantly different at 5% level; PR: Preference ratio



Vertical bars represent standard error of mean

(a)

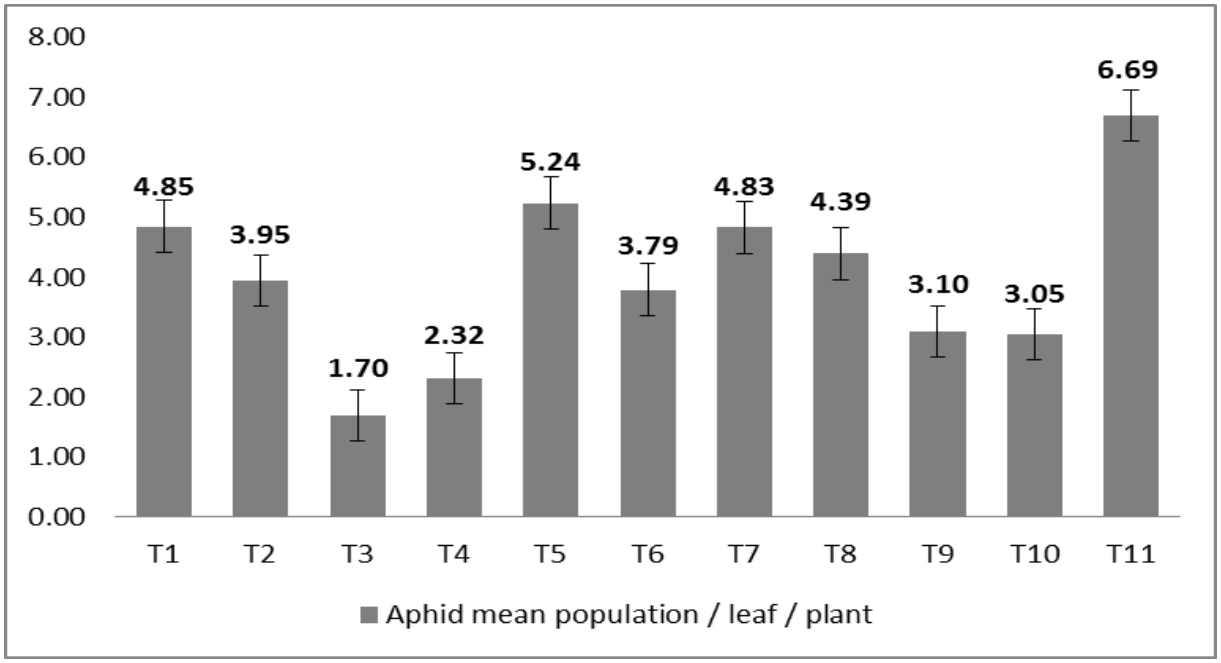


Vertical bars represent standard error of mean

(b)

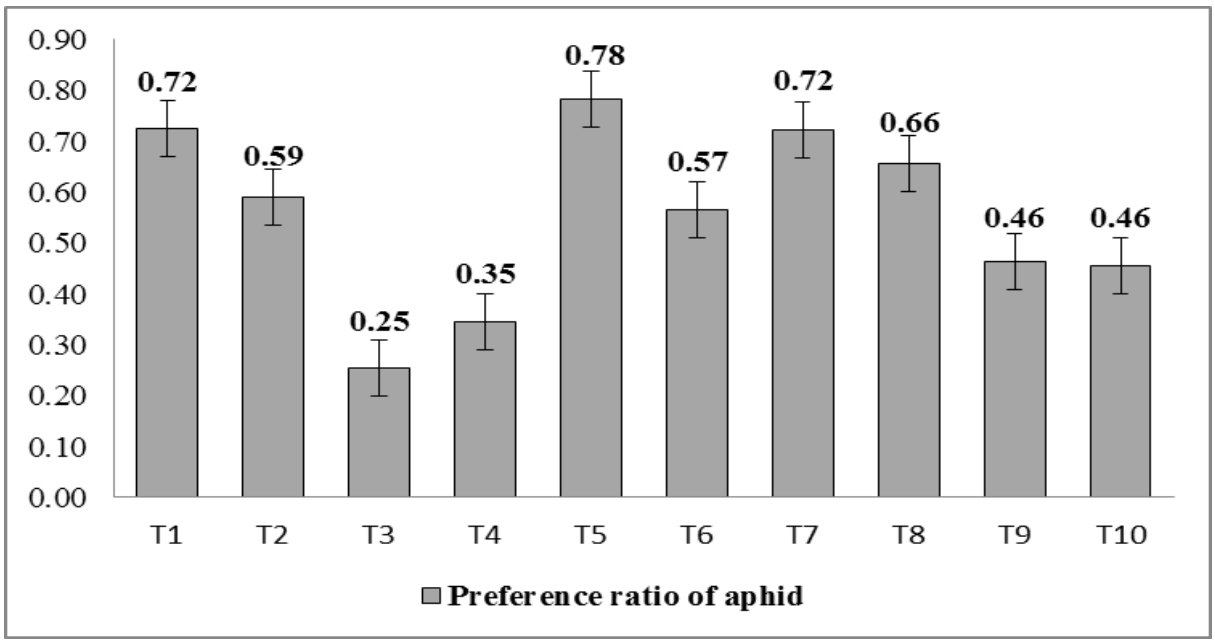
T1: Bhendi seeds + gum Arabic (2%) + azadirachtin (1%) + *Pseudomonas flourescens* (10 g/kg), T2; Bhendi seeds + Gum Arabic (2%) + *Beauveria bassiana* (15 g/kg) + *P flourescens* (10 g/kg), T3: Bhendi seeds + gum Arabic (2%) + *B bassiana* (10 g/kg) + *Azospirillum* (200 g/kg) + *P flourescens* (10 g/kg), T4: Bhendi seeds + gum Arabic (2%) + *B bassiana* (15 g/kg) + *Azotobacter* (200 g/kg) + *P flourescens* (10 g/kg), T5: Bhendi seeds + gum Arabic (2%) + humic acid (0.3 g/kg) + *P flourescens* (10 g/kg), T6: Bhendi seeds + gum Arabic (2%) + gibberellic acid (0.1 g/kg) + *P flourescens* (10 g/kg), T7: Bhendi seeds + gum Arabic (2%) + amino acid mixture (0.5 g/kg) + *P flourescens* (10 g/kg), T8: Bhendi seeds + gum Arabic (2%) + *Kappaphycus alvarezii* (7%) + *P flourescens* (10 g/kg), T9: Bhendi seeds + gum Arabic (2%) + *Sargassum wightii* (10%) + *P flourescens* (10 g/kg), T10: Bhendi seeds + gum Arabic (2%) + imidacloprid 600 FS (6 ml/kg) + *P flourescens* (10g/kg), T11: Untreated bhendi seeds

Fig 1. a) Effect of ecologically-engineered seed-based bhendi ecosystems on the coccinellid population (% increase over control), b) Coccinellids occurrence ratio on ecologically-engineered seed-based bhendi ecosystems



Vertical bars represent standard error of mean

(a)



Vertical bars represent standard error of mean

(b)

T1: Bhendi seeds + gum Arabic (2%) + azadirachtin (1%) + *Pseudomonas flourescens* (10 g/kg), T2; Bhendi seeds + Gum Arabic (2%) + *Beauveria bassiana* (15 g/kg) + *P flourescens* (10 g/kg), T3: Bhendi seeds + gum Arabic (2%) + *B bassiana* (10 g/kg) + *Azospirillum* (200 g/kg) + *P flourescens* (10 g/kg), T4: Bhendi seeds + gum Arabic (2%) + *B bassiana* (15 g/kg) + *Azotobacter* (200 g/kg) + *P flourescens* (10 g/kg), T5: Bhendi seeds + gum Arabic (2%) + humic acid (0.3 g/kg) + *P flourescens* (10 g/kg), T6: Bhendi seeds + gum Arabic (2%) + gibberellic acid (0.1 g/kg) + *P flourescens* (10 g/kg), T7: Bhendi seeds + gum Arabic (2%) + amino acid mixture (0.5 g/kg) + *P flourescens* (10 g/kg), T8: Bhendi seeds + gum Arabic (2%) + *Kappaphycus alvarezii* (7%) + *P flourescens* (10 g/kg), T9: Bhendi seeds + gum Arabic (2%) + *Sargassum wightii* (10%) + *P flourescens* (10 g/kg), T10: Bhendi seeds + gum Arabic (2%) + imidacloprid 600 FS (6 ml/kg) + *P flourescens* (10g/kg), T11: Untreated bhendi seeds

Fig 2. a) Effect of ecologically-engineered seed-based bhendi ecosystems on the aphid population (% reduction over control), b) Preference ratio of aphid in ecoclogical engineering-based bhendi ecosystems

Effect of ecologically-engineered seed-based bhendi ecosystems on the aphids population (in situ count)

Aphid population observed on bhendi is presented in Table 2. The combination of *B bassiana* with *Azospirillum*-treated designer seed-based plants most significantly influenced for the minimum population on bhendi (0.50, 0.90, 1.10, 1.70, 1.90, 2.20, 2.70, 2.90, 2.60, 1.20, 1.00 and 1.70 per leaf at 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85 DAS respectively). The next best treatment was *B bassiana* with *Azotobacter* designer seed-based plants which resulted in population of 0.90, 1.30, 1.70, 2.30, 2.70, 3.10, 3.30, 4.00, 3.40, 1.50 and 1.30/leaf at 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85 DAS respectively. The maximum population was observed on untreated bhendi plants which registered 3.00, 4.30, 4.80, 7.00, 7.30, 8.10, 8.40, 9.20, 9.00, 7.00 and 5.50/leaf at 15, 22, 29, 36, 43, 50, 57, 64, 71, 78 and 85 DAS respectively. Mean population of aphids ranged from 1.70 to 6.69/leaf due to various designer seed treatments. The combination of *B bassiana* with *Azospirillum*-treated designer seed-based plants was significantly superior in minimizing the population to 1.70/leaf along with 74.59 per cent reduction over control with preference ratio of 0.25 (Figs 2a, 2b) as compared to 6.69/leaf on bhendi alone. *B bassiana,* imidacloprid, amino acid mixture, azadiractin and gibberellic acid-treated designer seed plant showed minimum mean population next to control of 3.95, 4.39, 4.83, 4.85 and 5.24 respectively with 41.03, 34.38, 27.85, 27.58 and 21.74 per cent respectively (Table 2) reduction over control with a preference ratio of 0.59, 0.66, 0.72, 0.72 and 0.78 respectively.

The reduction in population of aphid might be due to endophytic action of *B bassiana (*10 g/kg) + *Azospirillum* (200 g/kg) (Santos et al 2014). The authors reported that *Azospirillum* inoculated in maize seeds was able to resist the attack of *Diabrotica* larvae by producing sesquiterpenes systematically which were deterrent to the larval feeding. It has been reported by Pineda et al (2012) that plants primed with *Rhizobacteria* would have stronger expression of JA-responsive genes upon aphid attack. Similar results of negative effect on aphid population were observed in cotton seeds when inoculated with *B bassiana* (Lopez et al 2014). Parallel results were obtained when seeds of blackgram (Neethu and Muthukrishnan 2018) and cabbage (Thaiyalnayagi et al 2019) were treated with endophyte *B bassiana.*

Behavioral bioassay of predators for designer seed flowers using olfactometer

Six-arm olfactometer was used to study the behavioral bioassay of coccinellids towards flowers of bhendi designer seed plants. Designer seed flowers obtained with combination of *B bassiana* and *Azospirillum* attracted 3.4, 3.2, 3.1 and 3.0 of coccinellids at 5, 10, 15 and 20 minutes after release (MAR) respectively followed by *K alvarezii* 3.2, 2.8, 2.8 and 2.6 at 5, 10, 15 and 20 MAR respectively. Azadirachtin-treated designer seed (1.4, 1.4, 1.2 and 1.6 of coccinellids at 5, 10, 15 and 20 MAR respectively) and bhendi alone (0.3, 0.7, 1.0 and 1.3 of coccinellids at 5, 10, 15 and 20 MAR respectively) attracted less number of predators among the arms (Table 3). Arthur (1981) reported that semiochemicals or plant-produced synomones played a major role in host or prey selection by natural enemies. Heimpel and Jerwis (2005) stated that volatiles from flowers act as a medium for attraction of natural enemies towards plant.

CONCLUSION

Seed, a living entity and the basic material for any crop production has to be taken more care in its protection. Seed treatment such as seed priming, seed pelleting and seed coating are some of the methodologies employed to these services. But ecological-engineered designer seeds not only play an important role in enhancing seed quality parameters but also in biocontrol services by enhancing the entomophagous insect population. This makes ecological engineering-based designer seeds more reliable by reducing the usage of chemicals in the field. An ecologically-sound and improvised environment can be brought out using these seeds which will stabilize and extemporize the naturally occurring natural enemies which in turn may decrease the burden of farmers.

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Table 3. Behavioral bioassay of coccinellids against biological designer seed-based bhendi flowers using olfactometer

Treatment	Number of coccinellids attracted minutes after release				Mean number of predators
	5	10	15	20	
T1	1.4^{g}	1.4^{h}	1.2^{g}	1.6^{e}	1.40^{e}
T2	1.2^{h}	1.6^{g}	1.8^{f}	2.0^{d}	1.65^{de}
T3	3.4^{a}	3.2^{a}	3.1^{a}	3.0^{a}	3.18^{a}
T4	3.2^{b}	2.8^{c}	2.6^{c}	2.4^{c}	2.75^{ab}
T5	2.2^{e}	2.0^{e}	2.4^{d}	2.6^{b}	2.30^{bc}
T6	2.4^{d}	2.6^{d}	2.8^{b}	2.0^{d}	2.45^{b}
T7	1.6^{f}	1.8^{f}	2.0^{e}	1.4^{f}	1.70^{de}
T8	3.2^{b}	2.8^{c}	2.8^{b}	2.6^{b}	2.85^{ab}
T9	3.0^{c}	3.0^{b}	2.6^{c}	2.4^{c}	2.75^{ab}
T10	1.6^{f}	1.8^{f}	2.0^{e}	2.0^{d}	1.85^{cd}
T11	0.3^{i}	0.7^{i}	1.0^{h}	1.3^{g}	0.83^{f}
SEd	0.017	0.012	0.012	0.016	0.084
$CD_{0.05}$	0.035	0.025	0.025	0.034	0.173

T1: Bhendi seeds + gum Arabic (2%) + azadirachtin (1%) + *Pseudomonas flourescens* (10 g/kg), T2; Bhendi seeds + Gum Arabic (2%) + *Beauveria bassiana* (15 g/kg) + *P flourescens* (10 g/kg), T3: Bhendi seeds + gum Arabic (2%) + *B bassiana* (10 g/kg) + *Azospirillum* (200 g/kg) + *P flourescens* (10 g/kg), T4: Bhendi seeds + gum Arabic (2%) + *B bassiana* (15 g/kg) + *Azotobacter* (200 g/kg) + *P flourescens* (10 g/kg), T5: Bhendi seeds + gum Arabic (2%) + humic acid (0.3 g/kg) + *P flourescens* (10 g/kg), T6: Bhendi seeds + gum Arabic (2%) + gibberellic acid (0.1 g/kg) + *P flourescens* (10 g/kg), T7: Bhendi seeds + gum Arabic (2%) + amino acid mixture (0.5 g/kg) + *P flourescens* (10 g/kg), T8: Bhendi seeds + gum Arabic (2%) + *Kappaphycus alvarezii* (7%) + *P flourescens* (10 g/kg), T9: Bhendi seeds + gum Arabic (2%) + *Sargassum wightii* (10%) + *P flourescens* (10 g/kg), T10: Bhendi seeds + gum Arabic (2%) + imidacloprid 600 FS (6 ml/kg) + *P flourescens* (10g/kg), T11: Untreated bhendi seeds

Figures are mean values of three replications; Figures transformed by square root transformation and the original values are given; In a column means followed by same letter(s) are not significantly different at 5% level

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