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Ecologically engineering based black gram seeds promotes services of Coccinellids and suppress *Aphis craccivora* (Koch)

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Abstract
We conducted an experiment on field evaluation of ecological engineering based designer seeds on the biocontrol services of predatory coccinellids on *Aphis craccivora* (Koch) in black gram (cv. VBN 6). Experiment was laid out randomized block design with 11 treatments and three replications. Ecologically engineered seed is an integrated seed treatment that involves addition of the nutrients, plant protectents and bio inoculants for improved emergence and establishment. Out of these 11 treatments coccinellids population determined range from 2.3 to 5.9 coccinellids/ plant. *Beauveria bassiana* with rhizobium treated black gram plot significantly recorded maximum coccinellids along with highest pest defender ratio (PDR) of 1: 3.91, occurrence ratio (OR) of coccinellids (2.6), minimum *A. craccivora* (1.5 numbers/terminal shoot) and preference ratio (PR) of *A. craccivora* (0.2). The treatment with other ecological engineering seeds also exerted a good impact against aphids compared to control. Ecologically engineered black gram seeds showed good impact in enhancing the population of natural enemies and pest suppression. Olfactometer studies revealed the preference of coccinellids towards leaves of *B. bassiana* with rhizobium treated black gram plot followed by *B. bassiana* treated black gram plot. Therefore, we conclude that farmers should use ecological engineering seeds instead of toxic chemical insecticides for controlling pests in the black gram field.

Keywords: Aphids, coccinellids, black gram, ecologically engineered seeds

1. Introduction
Black gram (*Vigna mungo* L.; Family: Fabaceae) (also known as urad dal or black lentil) is native of India and a rich source of protein (20.8 to 30.5%) and total carbohydrates (56.5 to 63.7%) and contributes 10% to the national pulse production (1.81 million tones) from an area of 13% (3.25 million ha) with the productivity of 463 kg ha-1 [1]. Black gram naturally owns plethora of entomophages (natural enemies).The major pests of black gram on Naraseepuram region are sucking pests mainly bean aphid (*Aphis craccivora* K.), whitefly (*Bemisia tabaci* G.), leaf hopper (*Empoasca* kerri L.), plant bugs, (*Nezara viridula* L.), and the pod bugs (*Riptortus pedestris* F. and *Clavigralla gibbosa* S.). Pod borers include spotted pod borer (*Maruca testulalis* G.) and spiny pod borer (*Etiella zinckenella* T.) were also observed. Plant produced many volatile compounds which guide them towards their host [2].

Biological seed treatments are made up of renewable resources and contain naturally occurring active ingredients targeting protection against soil-borne pathogens, crop pests, alleviate abiotic stress and increase plant growth. Seed treatment with microbial antagonists or fungicides protect the seed from infection by seed borne and soil borne pathogens, enables the seed to germinate and establish as a healthy seedling [3,4]. Application of beneficial microorganisms to seeds is an efficient mechanism for placement of microbial inoculam into soil where they will be well positioned to colonize seedling roots or make contact with soil dwelling invertebrate pests that feed on plant roots [5]. Several entomopathogenic fungi can also colonize plant tissues as endophytes and affect pests systemically via the plant [6]. Seaweed extract is a natural organic fertilizer which promotes faster seed germination and is highly nutritious to plants. The Seaweed extract contains regulators, plant growth hormones, carbohydrates, auxins, gibberellins and vitamins [7]. Seaweed extract has its wide applications as soil amendment in pest control [8].

The designer seed technology using chemical insecticide (imidacloprid) along with *Pseudomonas flouroscens* M. and *Trichoderma viridi* P. is widely used in many crops. Hitherto not much research is available to replace imidacloprid with biological materials such as plant extracts, sea weed extracts, microbial consortia and plant growth regulators having insecticidal and plant growth promoting properties either alone or in combination with bioinoculants in the development of ecological engineering based biological designer seeds. Therefore, this study aims at knowing the significances of "ecologically engineered seeds in the conservation biological control.

2. Materials and methods:

2.1 Screening of various biological materials to develop ecological engineering seeds in black gram

Aqueous CMC polymer (5%) was prepared and kept in magnetic stirrer over night. CMC polymer and black gram seeds were mixed at 5ml per kg of seeds. Then biological materials in varied concentrations were added and shade dried for 12 h prior to evaluation. Each biological material was evaluated at three different concentrations.

2.2 Developing ecological engineering seeds with suitable biological materials in black gram

Freshly harvested black gram seeds were treated with CMC polymer (5%) and further treated with best effective biological material and admixed with *P. flouroscens* to develop ecological engineering black gram seeds. These ecological engineering seeds were used in the field to study their sustainability in encouraging entomophages and pest's suppression.

2.2.1 Treatment details

T1	Black gram + Carboxy Methyl Cellulose (CMC) + Azadirachtin 1% + *Pseudomonas flouroscens*
T2	Black gram+ CMC 5% + *Beauveria bassiana* 0.3% + *P. flouroscens*
T3	Black gram + CMC 5% + *B. bassiana* 0.3% + rhizobium 50g + *P. flouroscens*
T4	Black gram + CMC 5% + Mycorrhizae 0.4g + *P. flouroscens*
T5	Black gram+ CMC 5% + *Kappaphycus alvarezii* 5% + *P. flouroscens*
T6	Black gram + CMC 5% + *Sargassum wightii* 10% + *P. flouroscens*
T7	Black gram + CMC 5% + Humic acid 0.1g + *P. flouroscens*
T8	Black gram + CMC 5% + Gibberelic acid 2% + *P. flouroscens*
T9	Black gram + CMC 5% + Aminoacid 0.3% + *P. flouroscens*
T10	Black gram + CMC 5% + Imidacloprid 1% + *P. flouroscens*
T11	Control (Black gram alone)

2.3 Effect of ecological engineered black gram seeds to enhancing entomophages and pest management under field condition

Field experiment was laid out in farmer's holding at Narseepuram village, Thondamuthur block, Coimbatore during rabi season during 2016-17 with 11 treatments including imidacloprid based designer seeds and untreated black gram seeds for comparison. The experiment was laid out in randomized block design (RBD) with three replications in an area of 0.08 acres in well prepared soil. Black gram (cv. VBN 6) was used and the spacing adopted was 30 x 15 cm. Seed rate used was 8 kg per ha. The plot size for each treatment was 5 x 5m^2. Standard good agronomic practices as per the recommendations of TNAU except pest management strategies were adopted to maintain healthy black gram plants and the following observations were recorded during crop growth till the harvest.

In situ observations on the population of grubs and adults of various species of coccinellids (number/plant) and population of nymphs and adults of *A. craccivora* on black gram from 10 randomly selected plants from each replication were made. Observations were taken during early morning hours at seven day interval from 15 days after Sowing (DAS) to 64 DAS. Based on the observations, Occurrence ratio (OR) of coccinellids, preference ratio (PR) of aphids and the pest defender ratio (PDR) were estimated by using the formulae (PDR = Population of natural enemies on black gram/ population of *A. craccivora* on black gram; OR = Population of natural enemies on the treatment/ population of natural enemies on control; PR = Population of pests on the treatment/Population of pests on control) [9].

2.4 Behavioral bioassay of predators for host plant volatiles using olfactometer

Six arm olfactometer was used for this study. About 10g of black gram flowers from each treatment were kept in the arm and firmly closed with a lid. Out of the six arms one arm was treated as control and compared with other treatments. The inlet of the olfactometer on the top center place was connected to an aquarium pump (220- 240 volt AC) to release the pressure. The medical air was passed from aquarium pump at the rate of 4 lit/ min in to the olfactometer. After five minutes of saturation of host odor in the olfactometer, 10 coccinellids were released in the olfactometer through a centre hole, which also served as odor exit hole. Observations were made on number of coccinellids settled on each arm at 0, 5, 10, 15 and 20 minutes after release (MAR) to know their preference towards different biological designer seed based black gram plants. This experiment was replicated three times following the same methodology [10,11].

2.5 Statistical analysis

The data from various laboratory and field experiments were scrutinized by CRBD and RBD analysis of variance (ANOVA) respectively after getting transformed into $\sqrt{x+0.5}$ and arcsine 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 the methods of [12].

3. Results and discussion:

3.1 Coccinellid species observed

Coccinellid species like *Chielomenus sexmaculata*, *Coccinella septumpunctata* and *Brumoides suturalis* [13] were observed.

3.2 Behavioral bioassay of predators for host plant flowers using olfactometer

Semiochemicals or plant produced synomones played a major role in host or prey selection by natural enemies [14,15]. To verify these ideas and to confirm the results of a field experiment, olfactometer experiments were conducted using six arm olfactometer. There was significant difference between the arms. However designer seed treatment with combination of *B. bassiana* and rhizobium (2.3, 3.3, 4.0 and 3.7 at 5, 10, 15 and 20 minutes after release (MAR) respectively) followed by *B. bassiana* (2.3, 3.0, 3.0 and 3.3 at 5, 10, 15 and 20 MAR respectively), seaweed, *S. wighti* (2.3, 2.7, 3.0 and 2.7 at 5, 10, 15 and 20 MAR respectively), *K. alvarezii* (2.0, 2.3, 2.7 and 2.3 at 5, 10, 15 and 20 MAR respectively). Imidacloprid designer seed based plants (0.7, 0.7, 1.3 and 1.0 at 5, 10, 15 and 20 MAR respectively) and black gram alone (0.3, 0.7, 1.0 and 1.3 at 5, 10, 15 and 20 MAR respectively) attract less number of predators among the arms.

The mean population of predators was high towards combination of *B. bassiana* and rhizobium designer seed based plants (3.3) followed by *B. bassiana* (2.9), seaweed, *S. wightii* (2.6) and *K. alvarezii* (2.3). Less number of population observed in azadirachtin (1.2), imidacloprid (0.9) and untreated black gram seed based plants (0.8) (Table 1).

Table 1: Behavioral bioassay of coccinellids against biological designer seed based black gram flowers using olfactometer

Treatments	Number of coccinellids attracted at MAR *				Mean no. of predators
	5	10	15	20	
Black gram seeds + CMC (5%) + Azadirachtin (1%) + *Pseudomonas flouroscens*	1.0 [de]	1.0 [ef]	1.7 [ef]	1.0 [f]	1.2 [g]
Black gram seeds + CMC (5%) + *Beauveria bassiana* (0.3%) + *P. flouroscens*	2.3 [a]	3.0 [ab]	3.0 [b]	3.3 [a]	2.9 [ab]
Black gram seeds + CMC (5%) + *B. bassiana* (0.3%) + rhizobium (50g/kg) + *P. flouroscens*	2.3 [a]	3.3 [a]	4.0 [a]	3.7 [a]	3.3 [a]
Black gram seeds + CMC (5%) + Mycorrhiza (0.4g) + *P. flouroscens*	1.3 [cd]	1.0 [ef]	1.3 [fg]	1.7 [de]	1.3 [f]
Black gram seeds + CMC (5%) + *Kappaphycus alvarezii* (5%) + *P. flouroscens*	2.0 [ab]	2.3 [c]	2.7 [bc]	2.3 [bc]	2.3 [cd]
Black gram seeds + CMC (5%) + *Sargassum wightii* (10%)+ *P. flouroscens*	2.3 [a]	2.7 [bc]	3.0 [b]	2.7 [b]	2.6 [bc]
Black gram seeds + CMC (5%) + Humic acid (0.1g) + *P. flouroscens*	1.3 [cd]	1.7 [d]	2.3 [cd]	2.0 [cd]	1.8 [e]
Black gram seeds + CMC (5%) + Gibberellic acid (2%) + *P. flouroscens*	1.6 [bc]	1.7 [d]	2.0 [de]	2.3 [bc]	1.9 [de]
Black gram seeds + CMC (5%) + Amino acid (0.3%) + *P. flouroscens*	1.0 [de]	1.3 [de]	2.0 [de]	1.7 [de]	1.5 [ef]
Black gram seeds + CMC (5%) + Imidacloprid (1%) + *P. flouroscens*	0.7 [ef]	0.7 [f]	1.3 [fg]	1.0 [f]	0.9 [gh]
Black gram seeds alone	0.3 [f]	0.7 [f]	1.0 [g]	1.3 [ef]	0.8 [h]
SED	0.19	0.23	0.28	0.27	0.08
CD(0.05)	0.383	0.47	0.59	0.55	0.16

***Data are mean values of three replications
Figures were transformed by square root transformation and the original values are given
In a columns means followed by same letter(s) are not significantly different (P=0.05) by LSD
MAR- minutes after release

3.3 Effect of ecologically engineered seed based black gram ecosystems on the population of cocinellids (*In situ* count)

The data on population of coccinellids on black gram due to ecologically engineered designer seed treatment are given in (Table 2). The designer seed treatments were able to attract more number of coccinellids compared to other chemical treatment. Combination of *B. bassiana* and rhizobium treated designer seed based plants most significantly influenced for the maximum population (4.5, 4.8, 5.2, 6.7, 7.6, 6.8, 6.7 and 5.7/ plant at 15, 22, 29, 36, 43, 50, 57and 64 DAS respectively). The designer seed treatment with *B. bassiana* was next and resulted in a population of 4.0, 4.2, 4.5, 5.6, 6.3, 5.3, 5.3 and 4.1 per plant on black gram at 15, 22, 29, 36, 43, 50, 57and 64 DAS respectively followed by the seaweed, *S. wighti* (3.6, 3.9, 4.1, 5.2, 6.0, 5.7, 5.1 and 4.1/plant at 15, 22, 29, 36, 43, 50, 57 and 64 DAS respectively) and seaweed, *K. alvarezii* treated designer seed based plants (3.5, 3.8, 3.9, 5.1, 5.9, 5.5, 4.9 and 3.9/plant at 15, 22, 29, 36, 43, 50, 57 and 64 DAS respectively) (Table 2).

Imidacloprid treated designer seed based plants (1.9, 2.2, 2.7, 3.4, 4.4, 3.9, 3.7 and 2.5/plant at 15, 22, 29, 36, 43, 50, 57 and 64 DAS respectively) and black gram alone (1.3, 1.7, 1.7, 2.3, 3.3, 3.3, 2.7 and 1.7 per plant at 15, 22, 29, 36, 43, 50, 57 and 64 DAS respectively) contributed for the minimum population than other treatments (Table 2).

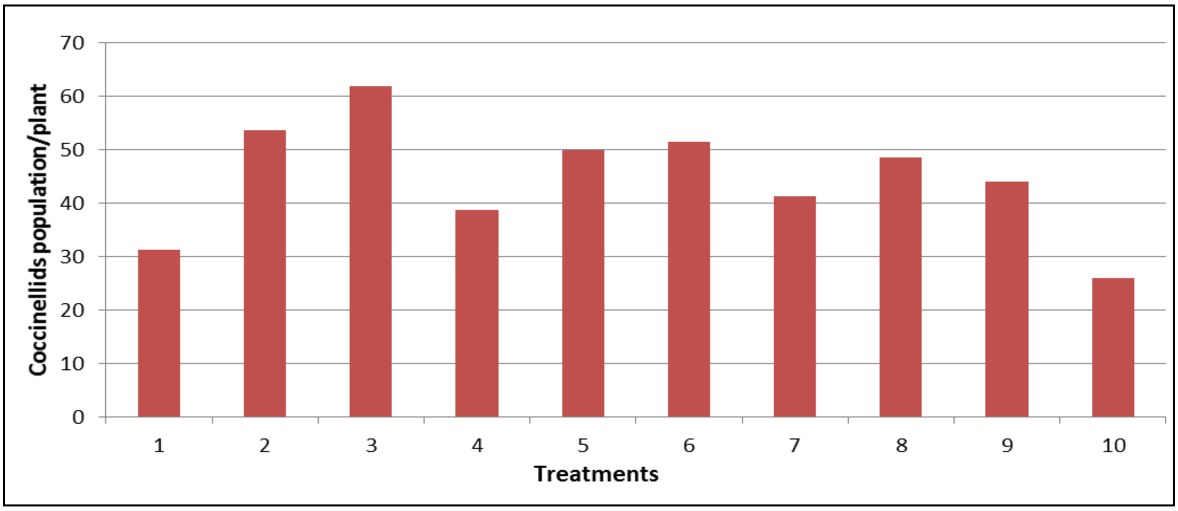

Fig 1: Effect of ecologically engineered seed based black gram ecosystems on the population of coccinellids per cent increased over control.

Mean population of varied from 2.3 to 5.9 per plant due to various designer seed treatments. Combination of *B. bassiana* and rhizobium treated designer seed based plants was significantly superior in maximizing the population to 5.9 per plant on black gram along with 61 per cent increase over control with occurrence ratio of 2.6 is present in (figure 1 and figure 2) when compared to 2.3 per plant on black gram alone. *B. bassiana* treated designer seed based black gram ecosystem was the next best which achieved population of 4.9 per plant with 53 per cent increase over control with occurrence ratio of 2.2. Azadirachtin and imidacloprid designer seed treatments however achieved less population of 3.3 and 3.1 per plant and registered with 31 and 25 per cent increase over control with occurrence ratio of 1.5 and 1.4 respectively (Table 2).

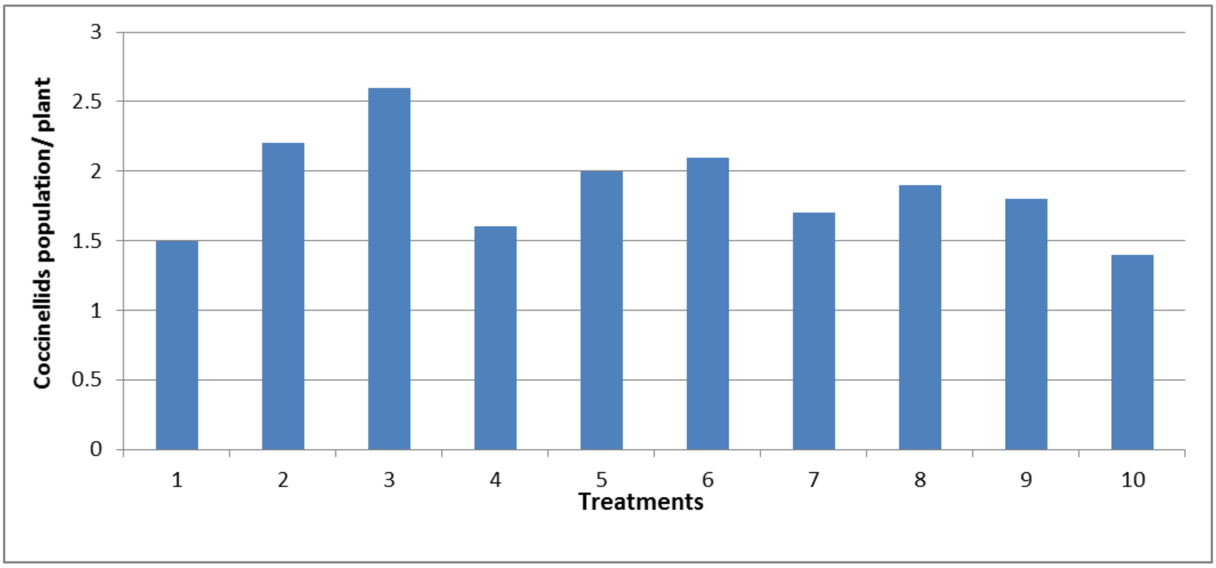

Fig 2: Coccinellids occurrence ratio on ecologically engineered seed based black gram

Table 2: Effect of biological designer seed based black gram ecosystems on the population of coccinellids (*In situ* method)

Treatments	Mean no. of coccinellids/ plant on DAS								Mean / plant	Percent increase over control	OR
	15	22	29	36	43	50	57	64			
Black gram seeds + CMC (5%) + Azadirachtin (1%) + *Pseudomonas flouroscens* (1g/kg)	2.3 e	2.6 ef	2.7 e	3.9 de	4.5 c	4.3 cd	3.7 d	2.5 e	3.3 ef	31.3	1.5
Black gram seeds + CMC (5%) + *Beauveria bassiana* (0.3%) + *P. flouroscens* (1g/kg)	4.0 ab	4.2 ab	4.5 ab	5.6 ab	6.3 ab	5.3 bc	5.3 b	4.1 b	4.9 b	53.6	2.2
Black gram seeds + CMC (5%) + *B. bassiana* (0.3%) + rhizobium (50g) + *P. flouroscens* (1g/kg)	4.5 a	4.8 a	5.2 a	6.7 a	7.6 a	6.8 a	6.7 a	5.7 a	5.9 a	61.8	2.6
Black gram seeds + CMC (5%) + Mycorrhiza (0.4g) + *P. flouroscens* (1g/kg)	2.6 de	2.9 de	3.0 de	4.1 cd	5.2 bc	4.7 bc	4.1 cd	3.1 cd	3.7 e	38.7	1.6
Black gram seeds + CMC (5%) + *Kappaphycus alvarezii* (5%) + *P. flouroscens* (1g/kg)	3.5 bc	3.8 bc	3.9 bc	5.1 b	5.9 b	5.5 b	4.9 bc	3.9 bc	4.5 bcd	49.8	2.0
Black gram seeds + CMC (5%) + *Sargassum wightii* (10%) + *P. flouroscens* (1g/kg)	3.6 bc	3.9 bc	4.1 bc	5.2 b	6.0 b	5.7 a	5.1 b	4.1 b	4.7 bc	51.4	2.1
Black gram seeds + CMC 5%+ Humic acid (0.1g) + *P. flouroscens* (1g/kg)	3.0 cd	3.3 cd	3.1 de	4.1 cd	5.3 bc	5.1 bc	4.1 cd	3.1 de	3.9 e	41.3	1.7
Black gram seeds + CMC (3g/l) + Gibberellic acid (2%) + *P. flouroscens* (1g/kg)	3.2 cd	3.7 bc	3.8 bc	5.0 bc	5.9 bc	5.3 bc	4.8 cd	3.8 bc	4.4 cd	48.6	1.9
Black gram seeds + CMC (3g/l) + Amino acid (0.3%) + *P. flouroscens* (1g/kg)	3.0 cd	3.4 cd	3.5 cd	4.7 bc	5.2 bc	5.0 bc	4.4bcd	3.4 cd	4.1 de	44.1	1.8
Black gram seeds + CMC (3g/l) + Imidacloprid (1%) + *P. flouroscens* (1g/kg)	1.9 f	2.2 fg	2.7 e	3.4 e	4.4 c	3.9 de	3.7 d	2.5 e	3.1 f	25.9	1.4
Black gram seeds alone	1.3 g	1.7 g	1.7 f	2.3 f	3.3 d	3.3 e	2.7 e	1.7 f	2.3 g	-	-
SED	0.09	0.09	0.15	0.11	0.12	0.14	0.12	0.09	0.10	-	-
CD (0.05)	0.18	0.19	0.28	0.23	0.25	0.28	0.24	0.19	0.22	-	-

*****Data are mean values of three replications
Figures were transformed by square root transformation and the original values are given
In a columns means followed by same letter(s) are not significantly different (P=0.05) by LSD
OR – Occurrence ratio

3.4 Effect of ecologically engineered seed based black gram ecosystems on the population of aphids (*In situ* count)

The population of aphids observed on black gram is presented in (Table 3). The combination of *B. bassiana* with rhizobium treated designer seed based plants most significantly influenced for the minimum population on black gram (0.6, 1.2, 1.4, 1.7, 2.2, 2.4, 2.1 and 0.7 / terminal shoot at 15, 22, 29, 36, 43, 50, 57 and 64 DAS respectively). Fungal endophytes play an important role in protecting plants against herbivorous insects [16] and plant pathogens [17]. The combination of *B. bassiana* and *P. fluorescens* treated plots had lower population of cowpea aphids, and could be used as potential biocontrol agent for the management of cowpea aphid [18]. It has been also reported that inoculation of Rhizobium with PGPR (*Beauveria* sp.) enhanced the growth, nodulation and yield of black gram as microbial inoculants applied as seed treatments deliver microorganisms directly to the plant rhizosphere the narrow zone of soil that surrounds the roots where plants interact directly with microorganisms [19].

The next best was *B. bassiana* designer seed treatment which resulted in population of 1.2, 1.8, 2.2, 2.6, 2.8, 3.5, 2.9 and 1.0 / terminal shoot at 15, 22, 29, 36, 43, 50, 57 and 64 DAS respectively. *Beauveria bassiana* has been reported as an endophyte in a variety of plants, including maize [20]. [21] demonstrated that *B. bassiana* is able to invade maize plants via the epidermis, thereafter persisting in the plant though the entire growing season and reducing tunneling by *Ostrinia nubilalis* (Lepidoptera: Pyralidae). There was reasonable population due to imidacloprid designer seed treatment (1.7, 2.9, 2.7, 3.7, 4.0, 4.3, 3.7 and 2.0 / terminal shoot at 15, 22, 29, 36, 43, 50, 57 and 64 DAS respectively). The maximum population was observed on untreated black gram plants, which registered 4.3, 6.5, 6.8, 7.6, 7.9, 8.7, 8.5 and 6.5 / terminal shoot at 15, 22, 29, 36, 43, 50, 57 and 64 DAS respectively (Table 3).

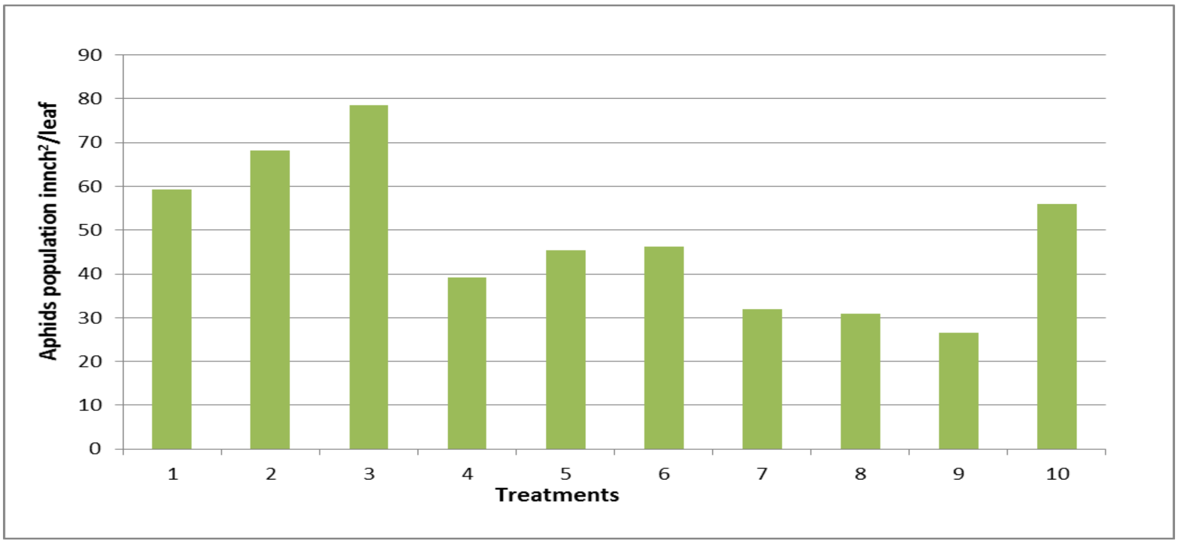

Fig 3: Effect of ecologically engineered seed based black gram ecosystems on the population of aphids per cent reduction over control.

Mean population of ranged from 1.5 to 7.1 per inch^2/ leaf due to various biological designer seed treatments. The combination of *B. bassiana* with rhizobium treated designer seed based plants was significantly superior in minimizing the population to 1.5/ terminal shoot along with 78.4 per cent reduction over control with preference ratio of 0.2 is in is in (figure. 3 and figure. 4) when compared to 7.1 terminal shoot on black gram alone. *B. bassiana* treated designer seed based plants was the next best and achieved population of 2.3 terminal shoot with 68.1 per cent reduction over control and preference ratio of 0.3. Azadirachtin and imidacloprid ranked next, which registered population of 2.9 and 3.1 / terminal shoot with 59.2 and 56 per cent increase reduction over control with a preference ratio of 0.4 and 0.5. Humic acid, gibberellic acid and amino acid resulted in highest population of 4.8, 4.9 and 5.2 /terminal shoot and registered with 32, 30 and 26 per cent reduction over control with a preference ratio of 0.7, 0.7 and 0.7 respectively (Table 3).

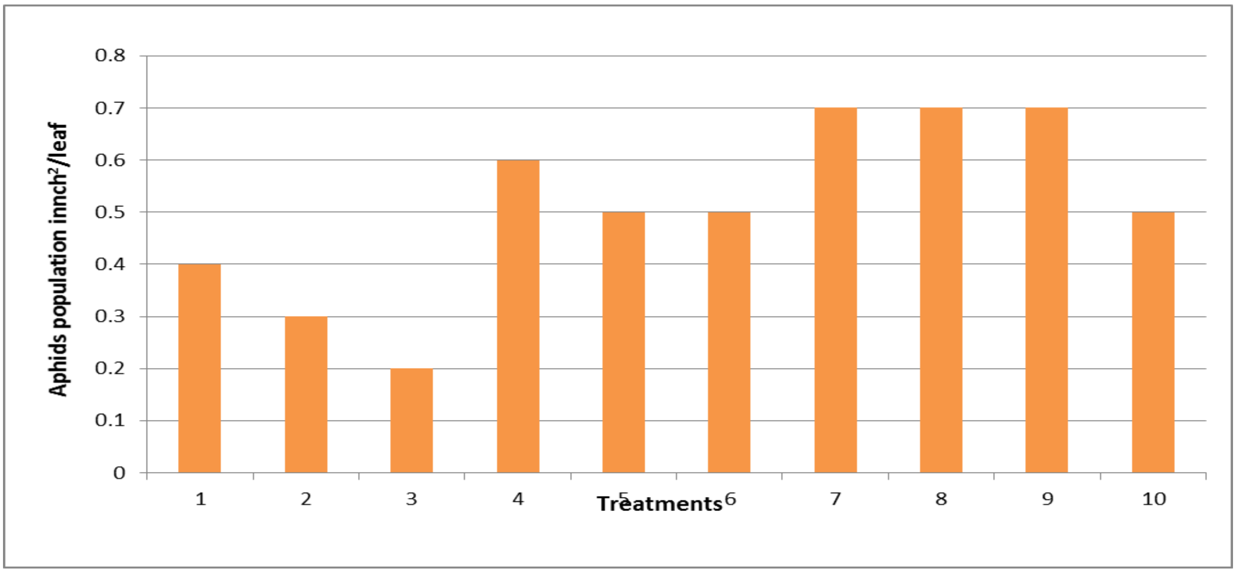

Fig 4: Preference ratio of aphids on ecologically engineered seed based black gram

In the present study, increased availability of grubs and adults of coccinellids due to *Beauveria bassiana* and rhizobium treatment might be reason for the less occurrence of aphids on blackgram. It was attributed that aphids infesting black gram might have provided highly preferred prey to coccinellids for their survival and multiplication. Similar study was conducted to examine the effects of pulse border crops on the biocontrol services of predatory coccinellids on *Aphis gossypii* (Glover) in black gram [22]. Similar results were also obtained in rice + cowpea border cropping system which registered maximum population coccinellids and rove beetle on rice and border crops and minimum population of plant hoppers and leafhoppers on rice [23]. Okra + cowpea border cropping system registered a maximum population of dragonflies, damselflies, wasps, predatory pentatomid bugs and coccinellids on okra and border crops, and reduced the population of *Bemisia tabaci* and *Helicoverpa armigera* on okra. These conditions resulted in higher occurrence ratio of natural enemies, higher pest defender ratio, higher yield and cost benefit ratio [24].

Table 3: Effect of biological designer seed based black gram ecosystems on the population of aphid (*In situ* method)

Treatments	Mean no. of aphid/ terminal shoot on DAS								Mean / plant	Percent reduction over control	PR
	15	22	29	36	43	50	57	64			
Black gram seeds + CMC (5%) + Azadirachtin (1%) + *Pseudomonas flouroscens* (1g/kg)	2.4 d	2.4 bc	2.4 b	3.2 bc	3.6 bc	3.8 bc	3.7 bc	1.7 bc	2.9 bc	59.2	0.4
Black gram seeds + CMC (5%) + *Beauveria bassiana* (0.3%) + *P. flouroscens* (1g/kg)	1.2 b	1.8 b	2.2 b	2.6 b	2.8 b	3.5 b	2.9 b	1.0 ab	2.3 b	68.1	0.3
Black gram seeds + CMC (5%) + *B. bassiana* (0.3%) + rhizobium (50g) + *P. flouroscens* (1g/kg)	0.6 a	1.2 a	1.4 a	1.7 a	2.2 a	2.4 a	2.1 a	0.7 a	1.5 a	78.4	0.2
Black gram seeds + CMC (5%) + Mycorrhiza (0.4g) + *P. flouroscens* (1g/kg)	3.0 e	4.0 e	4.3 de	4.7 ef	5.0 ef	5.3 de	5.0 d	3.3 ef	4.3 ef	39.1	0.6
Black gram seeds + CMC (5%) + *Kappaphycus alvarezii* (5%) + *P. flouroscens* (1g/kg)	3.0 e	3.3 de	3.3 cd	4.4 ef	4.8 dc	4.9 cd	4.5 cd	2.7 de	3.9 de	45.4	0.5
Black gram seeds + CMC (5%) + *Sargassum wightii* (10%) + *P. flouroscens* (1g/kg)	2.9 de	3.5 de	3.6 de	4.3 de	4.6 de	4.8 cd	4.2 cd	2.6 d	3.8 de	46.3	0.5
Black gram seeds + CMC (5%) + Humic acid (0.1g) + *P. flouroscens* (1g/kg)	3.7 fg	4.2 f	4.3 ef	5.3 fg	5.5 ef	5.8 e	5.7 e	4.2 fg	4.8 f	32.0	0.7
Black gram seeds + CMC (5%) + Gibberellic acid (2%) + *P. flouroscens* (1g/kg)	3.3 ef	4.3 f	4.5 ef	5.3 gh	5.7 fg	6.0 e	5.7 e	4.5 g	4.9 f	30.8	0.7
Black gram seeds + CMC (5%) + Amino acid (0.3%) + *P. flouroscens* (1g/kg)	3.9 fg	4.4 f	4.7 f	5.8 h	5.9 g	6.4 e	5.9 e	4.8 g	5.2 f	26.5	0.7
Black gram seeds + CMC (5%) + Imidacloprid (1%) + *P. flouroscens* (1g/kg)	1.7 c	2.9 d	2.7 bc	3.7 cd	4.0 d	4.3 c	3.7 c	2.0 cd	3.1 d	56.0	0.5
Black gram seeds alone	4.3 g	6.5 g	6.8 g	7.6 i	7.9 h	8.7 f	8.5 f	6.5 h	7.1 g	-	-
SED	0.09	0.08	0.11	0.11	0.11	0.17	0.13	0.12	0.10	-	-
CD (0.05)	0.22	0.18	0.24	0.23	0.23	0.24	0.22	0.29	0.21	-	-

*****Data are mean values of three replications
Figures were transformed by square root transformation and the original values are given
In a columns means followed by same letter(s) are not significantly different (P=0.05) by LSD
PR –Preference ratio

3.5 Pest defender ratio for ecologically engineered seed based black gram ecosystems:

Pest defender ratio was 3.91, 3.1, 2.89, 2.77, 1.49, 1.44, 1.40, 1.39, 1.34 and 1.28 due to combination of *B. bassiana* and rhizobium, *B. bassiana*, *S. wightii*, *K. alvarezii*, azadirachtin, humic acid, gibberellic acid, mycorrhiza, amino acid mixture and imidacloprid treated designer seed based plants respectively when compared to untreated black gram plants (0.12) (Table 4).

Table 4: Pest defender ratio for biological designer seed based black gram ecosystems

Treatments	PD ratio
Black gram seeds + CMC 5% + Azadirachtin 1% + *Pseudomonas flouroscens* 1g/kg seeds	1:1.49
Black gram + CMC 5% + *Beauveria bassiana 0.3%* + *P. flouroscens*	1:3.10
Black gram + CMC 5% + *Beauveria bassiana 0.3%* + rhizobium (50g) + *P. flouroscens*	1:3.91
Black gram + CMC 5% + Mycorrhizae 0.4g + *P. flouroscens*	1:1.39
Black gram + CMC 5% + *Kappaphycus alvarezii* 5% + *P. flouroscens*	1:2.77
Black gram + CMC 5% + *Sargassum wightii* 10% + *P. flouroscens*	1:1.89
Black gram + CMC 5% + Humic acid 0.1g + *P. flouroscens*	1:1.44
Black gram + CMC 5% + Gibberelic acid 2% + *P. flouroscens*	1:1.40
Black gram + CMC 5% + Aminoacid 0.3% + *P. flouroscens*	1:1.34
Black gram + CMC 5% + Imidacloprid 1% + *P. flouroscens*	1:1.28
Control (Black gram alone)	1:0.12

4. Conclusion

This experiment concluded that Ecological engineering based designer seed treatment with *Beauveria bassiana* and rhizobium could be a better choice for conserving the coccinellids species, which would in turn facilitate for the natural suppression of blackgram aphids.

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