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Mithun M Naik

Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad, Karnataka, India

Bharamaraj Badiger

Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad, Karnataka, India

Jagadish Hosamani

Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad, Karnataka, India

Shivamurthy D

Agriculture Research Station, Hanumanamatti, UAS, Dharwad, Karnataka, India

Prasanna PM

Agriculture Research Station, Devihosur, UAS, Dharwad, Karnataka, India

Corresponding Author:**Mithun M Naik**

Department of Seed Science and Technology, University of Agricultural Sciences, Dharwad, Karnataka, India

Eco-friendly seed protectants for mitigating Bruchid infestation and maintaining seed longevity in green gram

Mithun M Naik, Bharamaraj Badiger, Jagadish Hosamani, Shivamurthy D and Prasanna PM

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Abstract

Green gram or Mung bean [*Vigna radiata* (L.) Wilczek] has major storage losses due to the infestation of pulse beetle (*Callosobruchus chinensis*), which causes deterioration of the seed quality. The effectiveness of entomopathogenic fungi (*Beauveria bassiana* and *Metarhizium anisopliae*) alone and in mixture with Diatomaceous earth, as eco-friendly seed protectant was studied during nine months of storage. Seeds of green gram variety DGGV-2 were used for the treatments and compared with spinosad 45% SC @ 2 mL/kg of seed and an untreated control. Periodic observations on insect infestation, egg laying potential, adult mortality, thousand seed weight, seed germination and vigour index of seeds were taken. Results revealed that the treatments significantly reduced the bruchid infestation when compared with the control. Spinosad provided maximum protection against storage infestation of seeds. Among the entomopathogenic formulations, *M. anisopliae* @ 20 g/kg + Diatomaceous earth @ 5g/kg and *B. bassiana* @ 20g/kg + Diatomaceous earth @ 5g/kg significantly maintained high seed quality. The findings of the present study indicated that entomopathogenic fungi based formulations incorporating an inert dust can be explored as efficient green and eco-friendly seed storage protectants for green gram.

Keywords: Green gram, bruchids, entomopathogenic fungi, diatomaceous earth, seed longevity, seed germination

Introduction

Green gram [*Vigna radiata* (L.) Wilczek], commonly known as mung bean, is a major pulse crop in India and other Asian countries and is widely cultivated for human consumption due to its high nutritional value. Its seed protein typically ranges from ~14.6 to 33.0 g per 100 g (commonly ~20-25% protein by dry weight), together with appreciable amounts of minerals and bioactive compounds, making it an important plant protein source for predominantly vegetarian diets (Dahiya *et al.*, 2015) [8]. Storage losses from harvest through marketing remain a serious problem for pulses in India and inadequate postharvest management contributes substantially to national pulse losses (Lal and Verma, 2007) [14]. Among storage pests, pulse beetles (*Callosobruchus* spp., especially *C. chinensis* and *C. maculatus*) are the most destructive. The impacts reported on stored legumes vary according to the intensity of the attack but include seed damage, weight loss and germination loss, often in the range of ~40 to 100% under infestations of extreme intensity, although most studies and reviews report typical heavy losses of about ~40 to 60% in weight or viability under unfavourable storage conditions (Singh, 2020) [24]. Chemical pesticides for stored-grain protection have led to insect resistance and residue contamination problems (Wakil *et al.*, 2023) [26]. Biological control using entomopathogenic fungi such as *Beauveria bassiana* and *Metarhizium anisopliae* is considered environmentally safe, with low mammalian toxicity and broad host range against stored-grain pests. Inert dusts like diatomaceous earth provide a physical mode of action by abrading the insect cuticle and causing desiccation, without leaving harmful chemical residues or fostering resistance (Ak, 2019) [3]. Combining entomopathogenic fungi with diatomaceous earth (or other inert dusts) has been shown in many studies to yield synergistic or additive effects, significantly increasing stored grain insect mortality and suppressing progeny production more effectively than either agent alone (Batta, 2005) [6].

Materials and Methods

The laboratory experiment was conducted from November, 2024 to July, 2025 in the Department of Seed Science and Technology, College of Agriculture, Dharwad. Entomopathogens such as *B. bassiana* and *M. anisopliae* commercial products were purchased from Institute of Organic Farming, Dharwad. Inert Dust i.e., Diatomaceous earth was procured from Seed Unit, UAS, Dharwad and Spinosad 45 SC was purchased from a local agro center, Dharwad. The pulse beetle (*C. chinensis*) culture was mass reared on green gram seeds by confining 10-20 freshly

emerged adults with 500 g of seeds in a tightly secured cloth bag at room temperature. This culture was sustained throughout the study to ensure a continuous supply of adults for releasing onto treated seeds. Freshly harvested green gram seeds (cv. DGGV 2) were used. One kilogram of seed was packed in cloth bags for each treatment and replicated thrice. Seeds were treated with the entomopathogens and in combination with inert dust by mixing the prescribed quantity of seeds with the different formulations (Table 1). Treated seeds were stored under ambient conditions for a period of nine months.

Table 1: Treatment details of entomopathogens and inert dust seed treatment

T ₁	Control (Untreated)
T ₂	<i>Beauveria bassiana</i> @ 10 g/kg seed
T ₃	<i>Beauveria bassiana</i> @ 20 g/kg seed
T ₄	<i>Metarhizium anisopliae</i> @ 10 g/kg seed
T ₅	<i>Metarhizium anisopliae</i> @ 20 g/kg seed
T ₆	<i>Beauveria bassiana</i> @ 10 g/kg seed + Diatomaceous earth @ 5 g/kg seed
T ₇	<i>Beauveria bassiana</i> @ 20 g/kg seed + Diatomaceous earth @ 5 g/kg seed
T ₈	<i>Metarhizium anisopliae</i> @ 10 g/kg seed + Diatomaceous earth @ 5 g/kg seed
T ₉	<i>Metarhizium anisopliae</i> @ 20 g/kg seed + Diatomaceous earth @ 5 g/kg seed
T ₁₀	Spinosad 45% SC @ 2 mL/kg seed

Observations on insect infestation (%), egg laying capacity (%), adult mortality (%), thousand seed weight (g), seed germination (%) and seed vigour index I were recorded at three months intervals. For damage assessment, 400 seeds per treatment were examined for exit holes and egg counts and the data was used to calculate insect infestation and egg laying capacity following the procedures of Oni (2014)^[20] and Ileke (2014)^[11]. The observation on adult mortality was recorded at seven days after insect release from each treatment. Further data was used to calculate percentage of adult mortality by using formula.

$$\text{Adult mortality (\%)} = \frac{\text{Number of dead beetles}}{\text{Total No. of beetles released}} \times 100$$

Thousand seed weight was determined by weighing three independent samples randomly selected seeds per treatment using an analytical balance. The germination test was conducted as per ISTA procedures (ISTA., 2021)^[4]. Seed vigour indices were calculated following the method of Abdul-Baki and Anderson (1973)^[1]. All recorded data were subjected to statistical analysis following Gomez and Gomez (1984)^[9] applying appropriate transformations.

Results

Insect infestation gradually increased significantly with an advancement of storage period (Table 2). At three months after storage, Spinosad 45% SC @ 2 mL/kg seed (T₁₀) had no infestation (0.00%) followed by *M. anisopliae* @ 20 g/kg + Diatomaceous earth @ 5 g/kg (0.92%) and *M. anisopliae* @ 20 g/kg (1.67%) (T₉ and T₅ respectively), while the untreated control (T₁) had relatively higher infestation (9.58%). At six months after storage, there was an increase in infestation for all treatments. However, T₁₀ (3.58%) and T₃ (4.59%) were relatively effective, followed by T₉ (5.17%) and T₇ (7.00%), while in the control increased to 27.92 percent. At nine months after storage, infestation reached its highest level in untreated control (T₁) (72.50%) while T₁₀ still had relatively lower infestation (6.82%) followed by T₉ (9.49%) and T₇ (19.00%) (Fig. 1). Thousand seed weight also declined progressively with storage (Table

2). At three months after storage, T₉ (56.89 g) and T₁₀ (55.98 g) were superior to T₁ (51.18 g). By six months after storage, in control seed weight declined sharply to 41.38 g, while T₁₀ and T₉ retained 53.53 g and 52.80 g, respectively. At nine months after storage, thousand seed weight reduced drastically among all the treatments (Fig. 1). In T₁ (32 g) lowest thousand seed weight was recorded, whereas Spinosad 45% SC (T₁₀) retained the highest value (50.67 g), followed by T₉ (49.53 g) indicating better storability under treated conditions.

Egg laying capacity also increased with storage duration, though the treatments differed significantly (Table 3). Across all nine months, Spinosad 45% SC @ 2 mL/kg seed (T₁₀) consistently recorded the lowest oviposition, followed by T₉ and T₇. At three months after storage T₁₀ and T₉ recorded no egg laying while T₇ recorded 1.42 percent. At six months after storage T₁₀ maintained lower egg laying (1.25%) followed by T₉ (4.00%), while control recorded 37.75 percent. At nine months after storage, spinosad treatment recorded lower egg laying capacity (10%) compared to other treatments. Among entomopathogenic treatments, T₉ recorded lower egg laying capacity (17.75%) followed by T₇ (27.33%). In contrast, the control reached a peak of 86.25 percent. Although all treatments showed gradual increase over time, T₁₀ and T₉ remained the most effective. Adult mortality showed distinct trends across key storage intervals (Table 3). At three months after storage, Spinosad 45% SC @ 2 mL/kg (T₁₀) and *M. anisopliae* @ 20 g/kg + Diatomaceous earth @ 5 g/kg (T₉) maintained 100 percent mortality, whereas the control showed only 6.67 percent. At six months after storage, a gradual reduction in adult mortality was observed in all the treatments, yet T₁₀ (93.33%) and T₉ (86.67%) remained superior, followed by T₇ (80.00%), while the control recorded just 15.00 percent, indicating sustained pest survival. By nine months, adult mortality further declined across all treatments. However, Spinosad (T₁₀) still recorded the highest mortality (68.33%), closely followed by T₉ (65.00%) and T₃ (61.67%). In contrast, the untreated control (T₁) displayed only 25.00 percent mortality.

Seed germination exhibited clear treatment wise differences across storage intervals (Table 4). At three months after storage, all treated seeds maintained higher germination, with Spinosad 45% SC @ 2 mL/kg seed (T₁₀) recording the highest germination (94.00%), closely followed by *M. anisopliae* @ 20 g/kg (T₅) (93.33%) and combination of *M. anisopliae* and *B. bassiana* @ 20 g/kg with Diatomaceous earth (T₉ and T₇ respectively with 93.00%), whereas the untreated control (T₁) recorded a lower value of 87.33 percent. By six months after storage, a gradual decline in germination was evident across treatments. However, T₁₀ (90.67%) and T₉ (87.67%) remained superior followed by T₇ (87.00%) and T₅ (87.33%), while the control showed a sharp reduction to 71.00 percent. At nine months after storage, seed germination declined markedly in all treatments, indicating the adverse effect of prolonged storage. Nevertheless, Spinosad (T₁₀) continued to maintain the highest germination (77.00%), followed by *M. anisopliae* @ 20 g/kg + Diatomaceous earth @ 5 g/kg (75.67%) and *B. bassiana* @ 20 g/kg + Diatomaceous earth @ 5 g/kg (73.67%). In contrast, the untreated control (T₁) recorded only 27.00 percent germination, highlighting severe deterioration of seed quality under ambient storage conditions.

Seed vigour index I (SVI-I) declined progressively with advancement of storage period, with significant variation among treatments (Table 4). At three months after storage, Spinosad 45% SC @ 2 mL/kg seed (T₁₀) recorded the highest SVI-I (3763), followed by *M. anisopliae* @ 20 g/kg + Diatomaceous earth @ 5 g/kg (3677) and *B. bassiana* @ 20 g/kg + Diatomaceous earth @ 5 g/kg (3490), whereas the untreated control (T₁) recorded the lowest value (2812). By six months after storage, SVI-I declined across all treatments, however, T₁₀ (3210) and T₉ (3042) remained superior, followed by T₇ (2802) and T₅ (2716), while the control dropped sharply to 1574. At nine months after storage, a marked reduction in SVI-I was evident, Spinosad (T₁₀) continued to maintain the highest seed vigour (2288), closely followed by T₉ (2043) and T₇ (1888). In contrast, the untreated control (T₁) exhibited severe deterioration, recording the lowest SVI-I (463) indicating substantial loss of seed vigour under ambient storage conditions.

Discussion

Among the entomopathogenic treatments, T₉-*M. anisopliae* @ 20 g/kg + Diatomaceous earth @ 5 g/kg emerged as the most effective treatment, recording lower egg laying capacity (17.75%), lower insect infestation (9.49%) and a higher adult mortality (65%). In contrast, untreated control (T₁) exhibited higher egg laying (86.25%), severe insect infestation (72.50%) and lower adult mortality (25%). The mortality observed in *M. anisopliae* treated insects is largely attributed to the production of immune suppressive metabolites such as destruxin E, which paralyzes haemocytes and disrupts tissue formation (Huxham *et al.*, 1989) ^[10]. Entomopathogenic fungi infect insects primarily through cuticular penetration, ultimately reducing egg laying and shortening adult lifespan. These results corroborate the findings of Cherry *et al.* (2007) ^[7] in cowpea, Vassilakos *et al.* (2006) ^[25] in wheat and Khashaveh *et al.* (2013) ^[13]. Diatomaceous earth complements the fungal action by abrading and damaging the insect epicuticle, causing desiccation and mortality prior to oviposition (Lord, 2001; Athanassiou *et al.*, 2005; Batta,

2005; Pedrini *et al.*, 2010) ^[15, 5, 6, 23]. The reduced infestation levels observed in *B. bassiana* and *M. anisopliae* treated seeds can be attributed to eventual death of insects due to toxin production as reported by Huxham *et al.* (1989) ^[10], Kannan *et al.* (2008) ^[12] and Mahdneshin *et al.* (2009) ^[16]. By the end of the ninth month of storage, T₁₀-Spinosad 45% SC @ 2 mL/kg seed consistently provided the best protection, recording the highest thousand seed weight (50.67 g) compared to other treatments because it effectively suppressed bruchid infestation throughout storage. Treatments with entomopathogens, particularly *M. anisopliae* + Diatomaceous earth (T₉) and *B. bassiana* + Diatomaceous earth (T₇), significantly reduced infestation and consequently maintained higher thousand seed weight than the control. Although Spinosad (T₁₀) remained the most effective, entomopathogens still offered strong protection by limiting bruchid damage. The present findings are in agreement with Mishra *et al.* (2018) ^[18] and Mondal *et al.* (2018) ^[19] in pulses. Among the entomopathogens, T₉-*M. anisopliae* @ 20 g/kg + Diatomaceous earth @ 5 g/kg was the most effective, maintaining a higher thousand seed weight while the untreated control (T₁) suffered severe deterioration, with lower thousand seed weight. The entomopathogens inhibit oviposition and larval development, thus reducing internal grain feeding and weight loss. The combination of fungi with diatomaceous earth further enhances protection, as diatomaceous earth disrupts the insect cuticle and accelerates desiccation, facilitating fungal infection and prolonging residual activity (Michalaki *et al.*, 2006; Mahdneshin *et al.*, 2009) ^[17, 16]. Similar findings were reported by Padin *et al.* (2002) ^[21] in wheat and bean and Cherry *et al.* (2007) ^[7] in cowpea.

The seed quality observations recorded by the end of the nine month storage period revealed marked differences among the seed treatments. Treatment with spinosad 45% SC @ 2 mL/kg recorded highest seed germination and seed vigour as compared to other treatments. Among the entomopathogenic treatments, T₉-*M. anisopliae* @ 20 g/kg + Diatomaceous earth @ 5 g/kg recorded higher seed germination and seed vigour as compared to control (T₁). This may be attributed to the lower seed damage by the insects as reported by Aishwarya *et al.* (2024) in mung bean and Patil (2011) ^[22] in pigeon pea.

Table 2: Effect of entomopathogens and inert dust seed treatment on insect infestation and thousand seed weight in green gram cv. DGGV-2 during storage

Treatments	Storage period (November 2024-July 2025)								
	Insect infestation (%)			Thousand seed weight (g)					
	3 MAS	6 MAS	9 MAS	3 MAS	6 MAS	9 MAS			
T ₁	9.58	27.92	72.50	51.18	41.38	32.00			
T ₂	4.92	12.75	32.92	54.71	48.53	42.88			
T ₃	1.83	4.59	21.50	55.90	50.36	45.40			
T ₄	4.17	9.04	26.08	54.80	48.57	43.10			
T ₅	1.67	8.33	23.74	55.91	50.80	46.45			
T ₆	3.92	9.08	21.55	55.18	49.33	45.12			
T ₇	2.50	7.00	19.00	55.71	51.13	47.95			
T ₈	4.75	10.36	25.08	55.39	49.84	45.56			
T ₉	0.92	5.17	9.49	56.89	52.80	49.53			
T ₁₀	0.00	3.58	6.82	55.98	53.53	50.67			
Mean	3.43	9.78	25.87	55.17	49.63	44.87			
S.E m (±)	0.07	0.10	0.071	0.38	0.54	0.60			
CD @ 1 %	0.28	0.39	0.284	1.51	2.16	2.40			

Note: Initial Insect infestation (%): Nil

Initial thousand seed weight (g): 55 g

Table 3: Effect of entomopathogens and inert dust seed treatment on egg laying capacity and adult mortality in green gram cv. DGGV-2 during storage

Treatments	Storage period (November 2024-July 2025)					
	Egg laying capacity (%)			Adult mortality (%)		
	3 MAS	6 MAS	9 MAS	3 MAS	6 MAS	9 MAS
T ₁	19.00	37.75	86.25	6.67	15.00	25.00
T ₂	8.08	17.33	48.33	90.00	78.33	58.33
T ₃	3.00	11.33	31.67	96.67	81.67	61.67
T ₄	5.50	13.58	42.50	91.67	75.00	56.67
T ₅	2.42	11.00	30.00	98.33	81.67	60.00
T ₆	3.83	12.75	33.00	91.67	76.67	56.67
T ₇	1.42	9.00	27.33	96.67	80.00	60.00
T ₈	2.33	10.67	30.25	88.33	75.00	56.67
T ₉	0.00	4.00	17.75	100.00	86.67	65.00
T ₁₀	0.00	1.25	10.00	100.00	93.33	68.33
Mean	4.56	12.87	35.71	86.00	74.33	56.83
S.E m (\pm)	0.05	0.03	0.02	0.12	0.07	0.08
CD @ 1 %	0.19	0.12	0.06	0.46	0.28	0.33

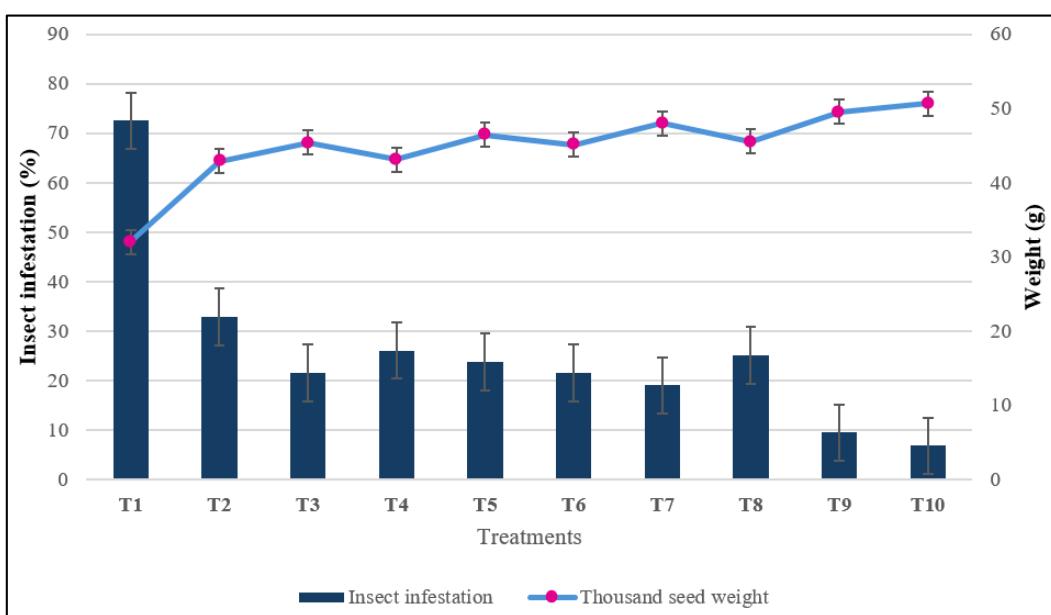
Note: Initial egg laying capacity and adult mortality (%): Nil

Table 4: Effect of entomopathogens and inert dust seed treatment on seed germination and seed vigour index I in green gram cv. DGGV-2 during storage

Treatments	Storage period (November 2024-July 2025)					
	Seed germination (%)			Seed vigour index I		
	3 MAS	6 MAS	9 MAS	3 MAS	6 MAS	9 MAS
T ₁	87.33	71.00	27.00	2812	1574	463
T ₂	90.67	84.33	67.00	3258	2522	1510
T ₃	92.33	86.00	71.33	3447	2723	1674
T ₄	90.67	83.67	69.33	3234	2482	1509
T ₅	93.33	87.33	72.67	3407	2716	1681
T ₆	91.00	85.67	71.67	3309	2610	1741
T ₇	93.00	87.00	73.67	3490	2802	1888
T ₈	91.00	84.67	72.33	3292	2740	1757
T ₉	93.00	87.67	75.67	3677	3042	2043
T ₁₀	94.00	90.67	77.00	3763	3210	2288
Mean	91.63	84.80	67.77	3369	2653	1647
S.E m (\pm)	0.51	0.45	0.57	41	37	27
CD @ 1 %	2.04	1.80	2.28	164	147	107

Note: Initial seed germination: 95%

Initial seed vigour Index I: 3971

**Fig 1:** Graphical representation of effect of entomopathogens and inert dust seed treatment on insect infestation and thousand seed weight of seeds in green gram cv. DGGV-2 after nine months of storage

Conclusion

The present investigation underlines the necessity to develop sustainable and eco-friendly strategies for the management of *Callosobruchus chinensis* in stored legumes. Among these, entomopathogens especially *Metarhizium anisopliae* and *Beauveria bassiana* have emerged as very promising alternatives to chemical insecticides owing to their biological specificity, environmental safety, and compatibility with integrated pest management approaches. Their ability to offer long term protection without hazardous residues makes them valuable for enhancing storability and supporting safety in seed storage. Future perspectives are the isolation and evaluation of native entomopathogenic strains from diverse agro-climatic zones to identify highly virulent, storage stable isolates effective against multiple storage pests, formulation in different crops and application methods in order to increase persistence. Inclusion with inert dusts or botanical products is likely to further enhance efficacy and stability under realistic storage conditions. Such approaches may be usefully complemented by a

deeper exploitation of the molecular and physiological interactions between fungi, insects, and stored seeds, leading to more focused and efficient biocontrol systems. Overall, entomopathogens hold strong potential for sustainable and scalable bruchid management in pulse seeds storage systems.

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