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Impact of Entomopathogens and Inert Dust Seed Treatment on Insect Pests and Seed Quality of Cowpea (*Vigna unguiculata* L.) during Storage

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Abstract

Bruchid, a pest that has survived from the field, is a severe problem with stored cowpea seeds. Although there are various approaches to addressing the issue, utilising biological agents is the most practical, cost-effective, and ecologically friendly choice. Laboratory study was conducted in a completely randomised design to investigate the effect of entomopathogens on the storability of cowpea seeds. Cowpea seeds were treated with the entomopathogens *Beauveria bassiana* and *Metarhizium anisopliae*, diatomaceous earth and a chemical check called deltamethrin @ 1 ppm. The seeds were then stored in HDPE bags for nine months, from July 2021 to April 2022. The parameters of insect population and seed quality were recorded in order to assess the efficacy of entomopathogens. The treatment with *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 20 g/ kg seed + Diatomaceous earth @ 5g/kg seeds recorded the highest seed quality parameters *viz.,* seed germination (82 %), mean seedling length (39.78 cm), mean seedling dry weight (55.09 mg), seedling vigour index I and II (3053 and 4244), lowest seed moisture content (9.64 %), electrical conductivity of seed leachate (1.56 mS cm^{-1}), insect infestation (1.42 %) after nine months of storage. A successful seed storage management strategy that kept seed quality above MSCS level up to nine months of storage was *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 20 g/kg seed + Diatomaceous earth @ 5g/kg seeds seed treatment.

Keywords : Entomopathogens, Cowpea storage, Inert dust treatment

Cowpea (*Vigna unguiculata* L.), often known as black-eyed pea or Southern pea, is a type of legume and it is one of the most important pulse crops farmed in India. Cowpea is an important part of the Indian diet. Furthermore, it is utilized as nutritional food, particularly for milch cows and green manure or cover crop. Cowpeas are nutrient-dense foods with a low energy density. It contains 23-32 per cent protein, 50-60 per cent carbohydrate and one per cent fat on a dry weight basis, as well as it also contains calcium (0.08-0.11%), iron (0.005%), essential amino acids (lysine, leucine, and phenylalanine) and phosphoric acid. It also serves as a cover crop and its thick root system prevents soil erosion. It occupies approximately 0.0786 m.ha. of land in Karnataka, producing 0.087 m.t. and yielding 427.25 kg/ha. (Anonymous, 2020).

Damage and losses to stored grain, especially cowpea by insect pests is very severe. *Losses* of up to 30-70 per cent have been recorded on *stored cowpea* in the absence of insect pest control measures (Yakubu *et al*., 2012). The most important storage pest of cowpea is the weevil (Bruchid) called *Callosobruchus chinensis*. Severe infestation can lead to total grain loss in storage. It is a field-to-store pest; adult beetles lay eggs on pods (in the field) or seeds (in storage). In India, various pulses have been infested by 17 species of bruchids from 11 genera (Arora, 1997).

Chemical pesticides like phosphine and pyrethroids are mostly used to control these insect pests. However, concerns regarding insect resistance, food contamination from pesticide residues, human health risks and environmental pollution have grown due to these chemical insecticides (Daglish, 2008). Inert dust, such as diatomaceous earth and kaolin were traditionally used as grain protectants. This is because, their formulations are nontoxic substances that can be mixed with seeds to control storage insect pests. Because inert dust does not decay or decompose, it offers long-term control of storage insect pests and is safe for humans and other mammals to consume.

Entomopathogenic fungi are a promising alternative to synthetic insecticides for the control of stored product insect pests. Their advantages include the absence of hazardous residues in products, as well as being safe for people and harmless to non-target creatures (Brower *et al*., 1996 and Athanassiou *et al*., 2008). Entomopathogenic fungi are well known for their capacity to kill insects, but they can also serve other purposes including enhancing nutrient intake or encouraging plant development. The ability of the entomopathogenic fungi in this division to kill insects by creating secondary metabolites that are of interest to industry and agriculture has led to their application as biopesticides (Ali *et al*., 2011 and Schulze *et al*., 2021).

An environmentally benign alternative to chemical inputs is seed treatment with biological control agents (Taylor and Harman, 1990). On the other hand, research into seed treatments to establish fungal entomopathogens as endophytes is just starting, with promising experimental outcomes for controlling insect pests. To get around the issue of inert dust which required large application rates to provide effective control of storage insect pests, binary mixtures of inert dust and fungi may be applied.

Therefore, due to the high susceptibility of cowpea seeds to seed deterioration brought on by insect pest infestations, novel inert dust like diatomaceous earth and entomopathogenic fungi like *Beauveria bassiana* and *Metarhizium anisopliae* have the potential to control storage insect pests and also to maintain seed quality throughout the storage period are the best alternatives. Taking into consideration of the need for the management of storage insect pests infestation and increase the storage life of cowpea seeds, present investigations were made to study the impact of entomopathogens and inert dust seed treatment on insect pests and seed quality of cowpea (*Vigna unguiculata* L.) during storage.

Material and Methods

Seeds of cowpea cv. C-152 with initial germination of 95 per cent and 8.15 per cent moisture were used for this study. Experiments were carried out at STR unit, NSP, AICRP on Seed (Crops), UAS, GKVK, Bengaluru during July 2021 to April 2022. One kg of freshly harvested certified seed with zero per cent insect pest infested seeds were taken for each treatment. The seeds were treated with the appropriate dose of entomopathogens and inert dust. Seeds were mixed manually for approximately two minutes to achieve uniform distribution of the conidial powder with the seed mass. Treated seeds were packed in HDPE bags and kept in a room under ambient temperature. The temperature and relative humidity of the room were recorded on a standard weekly basis.

Treatment Details

The effect of entomopathogens and inert dust with different treatments against storage insect pests of cowpea and their effect on seed quality parameters of cowpea were evaluated in this experiment. The experiment was carried out in Completely Randomized Design (CRD) with ten treatments and three replications. Details of treatments are described below.

Treatments

T_1 : *Beauveria bassiana* (CFU: 1.0×10^8) @ 10 g/kg seeds

T_2 : *Beauveria bassiana* (CFU: 1.0×10^8) @ 20 g/kg seeds

T_3 : *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 10 g/kg seeds

T_4 : *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 20 g/kg seeds

T_5 : *Beauveria bassiana* (CFU: 1.0×10^8) @ 10 g/kg seeds + Diatomaceous earth @ 5g/kg seeds

T_6 : *Beauveria bassiana* (CFU: 1.0×10^8) @ 20 g/kg seeds + Diatomaceous earth @ 5g/kg seeds

T_7 : *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 10 g/kg seeds + Diatomaceous earth @ 5g/kg seeds

T_8 : *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 20 g/kg seeds + Diatomaceous earth @ 5g/kg seeds

T_9 : Deltamethrin 2.8 EC @ 1.0 ppm (0.04 ml/kg seeds)

T_{10} : Control

Collection of Experimental Data

The seed samples were drawn at bimonthly intervals up to six months of storage and evaluated for the moisture content of the seed and seed germination were calculated and expressed as percentage (Anonymous, 1999). Mean seedling length and mean seedling dry weight were also measured at the end of eight days. Vigour index was calculated using the formula of Abdul-Baki and Anderson (1973). The observations of quality parameters and seed health were recorded bimonthly for up to six months and then monthly observations up to nine months of seed storage (July 2021 to April 2022) were recorded. (Change with actual parameters recorded at monthly and bimonthly intervals.).

Results and Discussion

Impact of Entomopathogens and Inert Dust on Seed Quality of Cowpea During Storage

In the present study the seed moisture content (Table 1) varied from 8.15 to 12.11 per cent. *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 20 g/kg seeds + Diatomaceous earth @ 5 g/kg seeds (T_8) had the lowest seed moisture content of 9.64 per cent, while the other entomopathogens and inert dust treatments had higher seed moisture content in proportion to insect population after nine months of

Table 1

Effect of entomopathogens and inert dust on seed moisture content (%) of cowpea cv. C-152 seeds stored under ambient condition

Treatments	Seed moisture(%)					
	Storage period					
	2 MAS	4 MAS	6 MAS	7 MAS	8 MAS	9 MAS
T_1	8.53	9.37	9.41	10.34	10.46	11.10
T_2	8.48	8.94	9.29	9.57	10.08	10.88
T_3	8.58	8.99	9.48	10.16	10.39	11.04
T_4	8.43	8.87	9.18	9.53	10.07	10.71
T_5	8.40	8.51	8.90	9.33	9.89	10.49
T_6	8.29	8.42	8.63	9.16	9.37	9.96
T_7	8.41	8.63	9.04	9.87	9.95	10.51
T_8	8.25	8.28	8.54	9.13	9.23	9.64
T_9	8.36	8.43	8.72	9.21	9.67	10.28
T_{10}	8.58	9.38	9.63	10.35	10.68	12.11
Mean	8.43	8.78	9.08	9.66	9.98	10.67
SEm±	0.26	0.25	0.24	0.28	0.30	0.32
CD at 5%	NS	0.74	0.70	0.84	0.88	0.95
CV (%)	5.40	4.92	4.55	5.08	5.18	5.20

MAS: Months After Storage NS: Non-Significant Initial moisture content- 8.15 per cent

TABLE 2

Effect of entomopathogens and inert dust on seed germination (%) of cowpea cv.C-152 seeds stored under ambient condition

Treatments	Seed germination (%)					
	Storage period					
	2 MAS	4 MAS	6 MAS	7 MAS	8 MAS	9 MAS
T_1	92	86	82	80	77	71
T_2	92	87	85	82	78	73
T_3	91	87	82	81	77	72
T_4	92	88	86	82	78	73
T_5	93	91	85	84	80	74
T_6	94	92	89	87	84	80
T_7	93	90	86	85	81	75
T_8	94	93	93	88	85	82
T_9	93	92	88	86	82	76
T_{10}	90	82	81	78	72	65
Mean	92	89	86	83	79	74
SEm±	2.12	2.15	2.43	2.45	2.56	2.47
CD at 5%	NS	6.33	7.18	7.21	7.55	7.28
CV (%)	3.98	4.19	4.92	5.09	5.58	5.77

MAS: Months After Storage NS: Non-Significant Initial seed germination - 95 per cent

storage. Whereas, untreated seeds recorded the lowest hundred seed weight (8.29 g) and highest seed moisture content (12.11%). The decrease in the seed weight and increase in seed moisture content was mainly due to an increase in the insect population. The results obtained in the current study are similar to Abdelgaleil *et al*. (2021) in cowpea and Iqbal *et al*. (2019) in chickpea where they observed that the minimal increase of seed moisture content of cowpea seeds was in the entomopathogenic treatment containing inert dust compared to treatments containing entomopathogens alone.

During the storage period, *Beauveria bassiana* (CFU: 1.0×10^8) @ 20 g/kg seeds + Diatomaceous earth at 5 g/kg seeds (T_6) treated seeds (80%) and *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 20 g/kg seeds+Diatomaceous earth at 5 g/kg seeds (T_8) treated seeds (82%) both exhibited satisfactory germination (Table 2). According to this, it could be stated that higher dosages of entomopathogens along with inert dust maintains germination rates compared to lesser levels. The direct feeding of storage insect pests on storage materials and the natural aging of cowpea seeds caused the germination rates to vary from 95 to 65 per cent. After eight months of storage, seed germination drastically decreased. The primary cause of this was pathogen development and seed mortality caused by seed damage brought on by insect infestation on cowpea seeds. The results obtained in the experiment are concurrent with Abdelgaleil *et al*. (2021) in cowpea, Nithya and Geetha (2017) in rice, Jyothi *et al.* (2018) in maize.

The seeds treated with *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 20 g/kg seeds + Diatomaceous earth at 5 g/kg seeds (T_8) recorded highest (Table 3 and Plate 1) mean seedling length (49.88 and 39.78 cm), (Table 4) mean seedling dry weight (67.48 and 55.09mg) (Fig.1) vigour index I(4662 and 3053) and (Fig. 2) vigour index II (6346 and 4244) from initial to end of storage period. Overall, the entomopathogens with inert dust treatment at higher concentrations (20 g) except at lower concentrations (10 g) showed

Plate 1 : Effect of entomopathogens and inert dust on mean seedling length (cm) in cowpea cv. C-152 after nine months of storage.

a beneficial effect on seed germination and seedling vigour index s when compared to untreated seeds. With the passage of time, a decrease in vigour index was observed due to insect infestation (%) which has led to reduction in germination, mean seedling length and mean seedling dry weight, which in turn resulted in reduced seed vigour. Current findings are contemporaneous with Jagadeesh *et al*. (2017) in pigeon pea and Raghu (2014) in cowpea, Thirumalaraju *et al.,* (2015) in maize.

The electrical conductivity (Table 5) of seed leachate gradually increased over time. It differed from 0.595 to 2.28 mS cm^{-1}. Least electrical conductivity of seed leachate among the treatments was observed in *Metarhizium anisopliae* (CFU: 1.0×10^{8}) @ 20 g/kg seeds + Diatomaceous earth @ 5 g/kg seeds (T_8)

Plate 2 : Comparison of T8 and T10 on Callosobruchus chinensis infestation (%) on cowpea cv. C-152 after nine months of storage

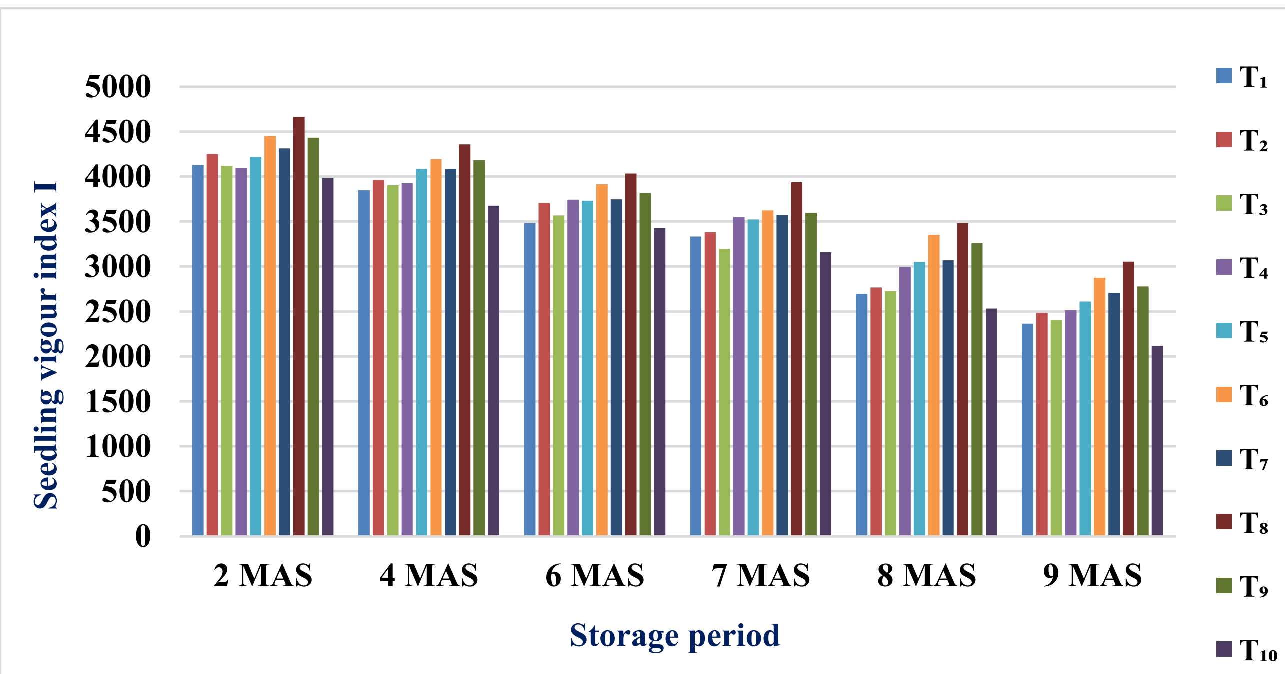

Fig. 1 : Effect of entomopathogens and inert dust on mean seedling vigour index I of cowpea cv. C-152 seeds stored under ambient condition (MAS: Months after storage)

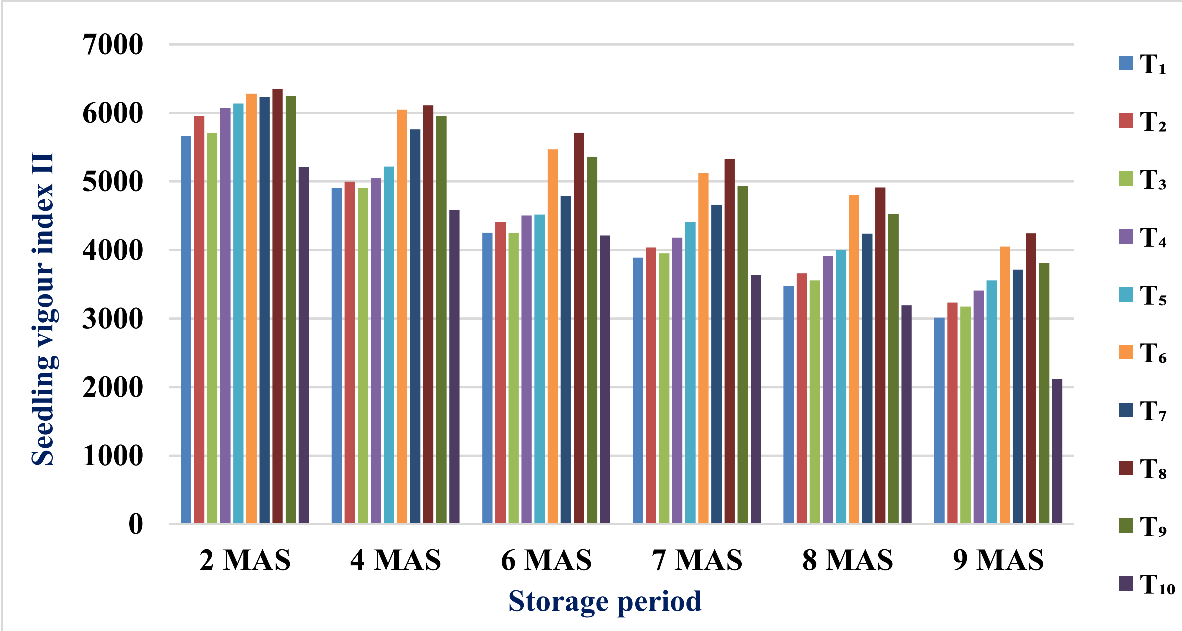

Fig. 2. Effect of entomopathogens and inert dust on seedling vigour index a! of cowpea cv. C-152 seeds stored under ambient condition
MAS: Months After Storage

TABLE 3

Effect of entomopathogens and inert dust on mean seedling length (cm) of cowpea cv. C-152 seeds stored under ambient condition

Treatments	Mean seedling length (cm)					
	Storage period					
	2 MAS	4 MAS	6 MAS	7 MAS	8 MAS	9 MAS
T_1	44.66	43.49	41.15	40.34	36.59	34.65
T_2	46.37	45.13	43.61	42.01	37.52	36.09
T_3	44.92	44.22	42.43	39.42	36.98	35.57
T_4	44.23	44.16	43.60	41.98	38.53	36.47
T_5	45.43	45.40	44.04	42.85	39.07	36.97
T_6	48.98	46.56	45.21	43.34	40.36	38.60
T_7	46.55	45.97	43.60	42.52	39.01	37.85
T_8	49.88	48.90	45.61	44.06	41.47	39.78
T_9	47.85	46.42	44.13	42.68	39.77	38.13
T_{10}	44.16	42.74	40.69	38.31	36.36	33.54
Mean	46.30	45.30	43.41	41.75	38.57	36.77
SEm±	1.47	1.15	1.02	1.18	0.94	1.22
CD at 5%	NS	3.39	3.01	3.47	2.78	3.61
CV (%)	5.49	4.40	4.08	4.88	4.23	5.77

MAS: Months After Storage NS: Non-Significant Initial mean seedling- 50.44 cm

TABLE 4

Effect of entomopathogens and inert dust on mean seedling dry weight (mg) of cowpea cv. C-152 seeds stored under ambient condition

Treatments	Mean seedling dry weight (mg)					
	Storage period					
	2 MAS	4 MAS	6 MAS	7 MAS	8 MAS	9 MAS
T_1	62.00	56.35	51.25	49.17	47.19	43.16
T_2	65.03	57.03	51.71	50.04	49.62	47.20
T_3	62.07	56.17	51.19	49.37	48.29	44.56
T_4	65.26	57.39	52.40	51.31	50.37	49.43
T_5	66.28	58.01	53.15	53.28	51.42	50.32
T_6	67.31	65.46	62.08	60.74	57.90	54.30
T_7	67.06	59.30	55.71	55.20	53.82	51.94
T_8	67.45	65.71	63.29	61.28	58.45	55.09
T_9	66.99	63.42	61.55	58.28	55.08	52.27
T_{10}	58.06	54.39	49.18	47.21	45.81	41.54
Mean	64.75	59.32	55.15	53.59	51.80	48.98
SEm±	1.86	1.77	1.55	1.55	1.69	1.43
CD at 5%	5.49	5.23	4.57	4.58	4.98	4.23
CV (%)	4.98	5.18	4.86	5.02	5.64	5.07

MAS: Months After Storage Initial mean seedling dry weight- 68.02 mg

treated seeds about 1.65 mS cm^{-1} which was on par with *Beauveria bassiana* (CFU: 1.0×10^8) @ 20 g/kg seeds + Diatomaceous earth @ 5 g/kg seeds (T_6) (1.65 mS cm^{-1}) and a chemical check *i.e.*, deltamethrin 2.8 EC @ 1.0 ppm (0.04 ml/kg seeds) (T_9) treated seeds (1.70 mS cm^{-1}) after nine months of storage. The conductivity of seed leachate, which increased as storage time increased, is inversely proportional to seed quality. The enhanced electrical conductivity of seed leachate is owing to the increased permeability of the cell membrane and decreased compactness of the seed coat, which allow sugars, organic acids and amino acids to escape in the presence of water (Basra, 2000). The current study's seed leachate conductivity results agreed with those of Jagadeesh *et al*. (2017) in pigeon pea, Babu *et al*. (2008) in soybean and Amrutha *et al*. (2015) in black gram.

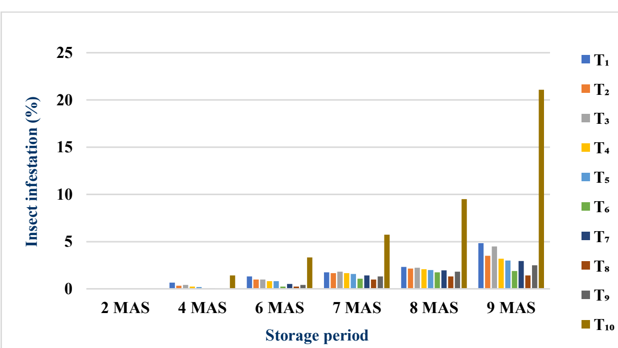

Fig. 3: Effect of entomopathogens and inert dust on insect infestation (%) in cowpea cv. C-152 seeds stored under ambient condition (MAS: Months After Storage)

Impact of Entomopathogens and Inert Dust on Storage Insect Pests of Cowpea During Storage

The percentage of insect infestation (Fig. 3) ranged from 0.00 to 21.08. The *Callosobruchus chinensis* infestation increased e as the storage time advanced. After nine months of storage, seeds treated with *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 20 g/kg seeds + Diatomaceous earth @ 5 g/kg seeds (T_8) (Plate 2) were least infested (1.42%), while the

TABLE 5
Effect of entomopathogens and inert dust on electrical conductivity (mS cm^{-1}) of cowpea cv. C-152 seeds stored under ambient condition

Treatments	Electrical conductivity (mS cm^{-1})					
	Storage period					
	2 MAS	4 MAS	6 MAS	7 MAS	8 MAS	9 MAS
T_1	0.76	1.15	1.56	1.74	1.86	2.10
T_2	0.75	1.11	1.47	1.67	1.86	1.98
T_3	0.76	1.14	1.52	1.71	1.81	2.08
T_4	0.74	1.10	1.44	1.60	1.77	1.90
T_5	0.69	1.09	1.39	1.57	1.73	1.85
T_6	0.62	0.89	1.18	1.35	1.51	1.65
T_7	0.65	0.99	1.29	1.44	1.60	1.76
T_8	0.60	0.83	1.12	1.27	1.43	1.56
T_9	0.64	0.91	1.20	1.39	1.58	1.70
T_{10}	0.83	1.24	1.62	1.81	1.91	2.28
Mean	0.70	1.05	1.38	1.56	1.71	1.89
SEm±	0.02	0.02	0.04	0.05	0.06	0.05
CD at 5%	0.06	0.06	0.13	0.14	0.17	0.16
CV (%)	4.77	3.56	5.62	5.23	5.77	4.96

MAS: Months After Storage Initial electrical conductivity- 0.59 mS cm^{-1}

maximum insect infestation (21.08%) was recorded in untreated seeds (T_{10}).

As time advances, the percentage of damaged seeds increased. This increased seed damage or exit holes, is caused by an increase in insect population levels of *C. chinensis* that soar with favourable temperature conditions, which may further be caused by a decrease in insecticide effectiveness as they are exposed to storage conditions. When compared to untreated

Seeds treated with *Metarhizium anisopliae* (CFU:1.0×10^8) @ 20 g/kg seed + Diatomaceous earth @ 5g/kg seeds (T_8) (9 MAS)

Untreated seeds (T_{10}) (9 MAS)

seeds, the majority of entomopathogens and inert dust treatments demonstrated potent lethal activity on *C. chinensis*. These observations are similar to that of Abdelgaleil *et al*. (2021) in cowpea, Iqbal *et al*. (2019) in chickpea and Manjula (2021) in cowpea. The neurotoxic mechanism of action, the antifeeding property of entomopathogens and the dehydrating property of inert dust, these factors contribute to the reduced quantity of bruchids in treated seeds. The persistence of entomopathogenic fungi for longer duration are due to the presence of an inert dust, which leads control of storage insect infestation (Athanassiou *et al*., 2008). At the lowest fungal rates, the addition of DE did not increase the fungal efficacy and at highest fungal rates, an additive effect was more often recorded (Storm *et al*., 2016).

Entomopathogenic fungi and inert dust are a promising alternative to synthetic insecticides for the control of stored product insect pests and to maintain seed quality during storage of cowpea seeds. The results from the present study revealed that cowpea seeds treated with entomopathogens along with inert dust *i.e., Metarhizium anisopliae* (CFU: 1.0×10^8) @ 20 g/kg seeds + Diatomaceous earth @ 5 g/kg seeds (T_8) was effective in maintaining seed moisture content (%), seed germination (%), mean seedling length (cm), mean seedling dry weight (mg), seedling vigour index I and II and also there was very minimal increase in the electrical conductivity of seed leachate attributing towards its effectiveness in maintaining seed quality throughout the storage period. Similarly, the same treatment combination was effective in keeping *Callosobruchus chinensis* under control which in turn reduced the insect infestation (%) or seed damage (%).

Therefore, from the study it is evident that seed treatment with *Metarhizium anisopliae* (CFU: 1.0×10^8) @ 20 g/kg seeds + Diatomaceous earth @ 5 g/kg seeds could be effectively maintained the seed quality and controlled the storage pest damage in cowpea during storage.

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