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Effect of Two New Molecules as Seed Treatment against Storage Insect Pests, Seed Quality and Storability in Cowpea (*Vigna unguiculata* L.)

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Abstract

Pulse beetle (*Callosobruchus chinensis* L.) is a major insect pest commonly found in seed storage. As insects develops resistance to existing insecticides, it is crucial to explore new molecules with novel mode of action for effective insect pest management. Laboratory study was conducted in a completely randomised design to investigate the effect of new molecules as a seed treatment against storage insect pests, seed quality and storability of cowpea seeds. Cowpea seeds were treated with new molecules, broflanilide and dinotefuran along with check (emamectin benzoate and deltamethrin). The treated seeds were stored in gunny bags for ten months. The seed quality parameters and insect population were recorded in order to assess the efficacy of new molecules. The results revealed that treatment with broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed) recorded the highest seed quality parameters *viz.,* seed germination (82.67%), mean seedling length (39.95 cm), mean seedling dry weight (55.21 mg), seedling vigour index I and II (3302 and 4565). Whereas, broflanilide @ 3 ppm (300 SC @ 9.99 mg/kg seed) recorded least seed moisture content (9.64%), electrical conductivity (456.34 μScm^{-1}), seed damage (4.25%) after ten months of storage. A successful seed storage management strategy that kept seed quality above MSCS (Minimum Seed Certification Standards) level for up to ten months was the broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed) treatment.

Keywords : Storage pests, Pulse beetle, Broflanilide, Dinotefuran, Seed treatment, Seed damage

Cowpea (*Vigna unguiculata* L.) is a food and animal feed legume native from Africa. It is also known as black eyed pea or southern pea, which belong to the family Fabaceae. Cowpea contains 25 per cent protein and several vitamins and minerals. In India, it is grown in an area of 3.24 lakh ha with a production of 1.98 lakh tonnes and productivity of 612 kg/ha, where as in Karnataka, it is grown in an area of 0.78 lakh ha with a production of 0.36 lakh tonnes and productivity of 461 kg/ha (Anonymous, 2022).

After harvest, storage of seeds until the next cropping season without reducing their quality is crucial for successful seed production. Effective seed storage ensures that seeds remain viable, maintaining their germination potential and vigour. The challenge lies in preserving quality of seeds over time, which can be affected by various factors such as moisture, temperature and insect pests. Post-harvest damages by insect pests have been an increasingly important constraint to food legume supplies worldwide. Insects pose a significant threat to stored seeds, often leading to substantial losses.

The most important storage pest of cowpea is the weevil (Bruchid) called *Callosobruchus chinensis* (Order: Coleoptera, Family: Chrysomelidae). Its infestation starts either in the field on the maturing pod and is carried to the stores with the harvested crops or it originates in the storage itself (Nahdy *et al*., 1999 and Kedia *et al*., 2013). Severe infestation can lead to total grainloss in storage. It is a field-to-store pest; adult beetles lay eggs on pods (in the field) or seeds (in storage) (Theertha *et al*., 2023). It is known to be prolific and rapid in breeding and can swiftly cause a significant quantitative drop as well as diminish the nutritional value of stored grains (Seni and Mishra, 2022). Insecticides with various mode of action must be rotated often to maintain effective management of pest population. Emamectin benzoate, deltamethrin, spinosad and other regularly used pesticides are crucial in the management of pests. Insect pests are developing resistance to these pesticides after two three years of their application. Therefore, it is necessary to create and assess newer and safer chemical molecules (Dodiya *et al*., 2022).

Broflanilide is a powerful and versatile insecticide with a novel mode of action without known cross-resistance that delivers excellent efficacy in controlling problematic chewing insect pests, including Lepidopterans (Anonymous, 2018). It is a new meta-diamide insecticide that is strongly active against various insect pests, including lepidopteran, coleopteran and thysanopteran pests. (Katsuta *et al*., 2019). Broflanilide acts as an allosteric modulator of the GABA receptor. It binds with receptor and close the receptor site to pass out the chloride ion which is the main anion for inhibiting action potential.

Dinotefuran is furanicotinyl insecticide which belongs to the third generation of neonicotinoids with a broad spectrum and systemic insecticidal activity. Dinotefuran provides a tetrahydrofuran (THF) moiety distinct from other neonicotinoids with a chloropyridine or chlorothiazole ring, which is considered to be an essential structural element for the neonicotinoid action. The unique chemical and excellent biological properties and favourable toxicological profile make dinotefuran available for pest management in wide range of crops with a variety of application methods (Wakita, 2011).

Therefore, to manage the insect pests of cowpea during storage, new molecules with a novel mode of action are needed. Due to the significant vulnerability of cowpea seeds to deterioration caused by insect pest infestation, new molecules like broflanilide and dinotefuran are used along with check. Taking into consideration the need for the management of storage insect pest infestation and increase the storage life of cowpea seeds, present investigations were made to study the effect of new molecules as seed treatment against storage insect pests, seed quality and storability in cowpea (*Vigna unguiculata* L.).

Material and Methods

Seeds of cowpea cv. PGCP-6 with initial germination of 95 per cent and 8.52 per cent moisture were used for this study. Experiments were carried out at Seed Technology Research Centre, All India Coordinated Research Project on Seed (Crops), National Seed Project, University of Agricultural Sciences, GKVK, Bengaluru during August, 2023 to June, 2024. The seeds were treated with appropriate dose of insecticides by diluting in water to make total volume of five ml for treating one kg of seeds. After drying in shade, treated seeds and the control were packed in gunny bags and kept in a room under ambient condition (Plate 1).

The research was carried out in Completely Randomized Design (CRD) with nine treatments and four replications (Table 1-5).

Collection of Experimental Data

The seed samples were drawn at bimonthly intervals up to ten months of storage and evaluated for the seed moisture and seed germination were calculated and expressed as percentage (Anonymous, 2021). Mean seedling length and mean seedling dry weight were also measured at the end of eight days. Seedling vigour index was calculated using the formula of Abdul Baki and Anderson (1973). The observations of quality parameters and seed damage were recorded bimonthly for up to eight months and then monthly

Plate 1 : General view of cowpea seeds stored under ambient condition

T_1	Broflanilide @ 1 ppm (300 SC @ 3.33 mg/kg seed)
T_2	Broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed)
T_3	Broflanilide @ 3 ppm (300 SC @ 9.99 mg/kg seed)
T_4	Dinotefuran @ 1 ppm (20 SG @ 5 mg/kg seed)
T_5	Dinotefuran @ 2 ppm (20 SG @ 10 mg/kg seed)
T_6	Dinotefuran @ 3 ppm (20 SG @ 15 mg/kg seed)
T_7	Emamectin benzoate @ 2 ppm (5 SG @ 40.0 mg/kg seed)
T_8	Deltamethrin @ 1.0 ppm (2.8 EC @ 0.04 ml/kg seed)
T_9	Control

observations up to ten months of seed storage (August, 2023 to June, 2024) were recorded.

Results and Discussion

Effectiveness of New Molecules on Cowpea Seed Quality

In the present study, the seed moisture content (Table 1) varied from 8.52 to 12.11 per cent. broflanilide @ 3 ppm (300 SC @ 9.99 mg/kg seed) (T_3) had the least seed moisture content of 9.64 per cent which is on par with broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed) (T_2), while the other insecticidal treatments had higher seed moisture in proportion to insect population. Whereas, untreated seeds recorded the highest seed moisture content (12.11%). The mean values varied from 8.76 to 10.65 per cent during ten months of storage. Increase in seed moisture content is due to insect biological activity (Lamani and Deshpande, 2017). Current findings are similar with Yogitha (2017) in cowpea and Nishad *et al*. (2017) in chickpea.

During the storage period, broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed) (T_2) and broflanilide @ 1 ppm (300 SC @ 3.33 mg/kg seed) (T_1) treated seeds both exhibited satisfactory germination (82.67% and 80.33%) (Table 2). While, broflanilide @ 3 ppm (300 SC @ 9.99 mg/kg seed) (T_3) recorded less germination (77.67 %) compared to treatments broflanilide @ 2 ppm (T_2) and broflanilide @ 1 ppm (T_1). The mean values varied from 92.44 to 74.63 per cent during ten months of storage. Decrease in germination percentage with increase in concentration of broflanilide can be attributed to its detrimental effect at higher dose as observed in current studies and are similar with Sharma *et al*. (2016) in pigeon pea and Kadam *et al*. (2013) in chickpea.

The seeds treated with broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed) (T_2) recorded highest mean seedling length (39.95 cm) (Table 3 and Plate 2), mean seedling dry weight (55.21 mg) (Table 4), seedling vigour index I (3302) (Fig. 1) and seedling vigour index II (4565) (Fig. 2) from initial to end of storage period. The mean values varied from 46.81 to

Table 1

Effect of new molecules on seed moisture of cowpea seeds cv. PGCP-6 stored under ambient condition

Treatments	Seed moisture content (%) Storage period (August 2023 to June 2024)					
	2 MAS	4 MAS	6 MAS	8 MAS	9 MAS	10 MAS
T_1	8.70	8.79	8.85	9.32	9.71	10.28
T_2	8.69	8.76	8.82	9.28	9.42	9.96
T_3	8.62	8.67	8.79	9.21	9.36	9.64
T_4	8.82	9.12	9.20	10.19	10.53	11.04
T_5	8.79	9.02	9.14	9.62	10.04	10.71
T_6	8.73	8.85	8.90	9.39	9.86	10.49
T_7	8.78	8.96	9.07	9.58	9.97	10.71
T_8	8.81	9.08	9.18	9.87	10.12	10.88
T_9	8.88	9.41	9.65	10.52	10.75	12.11
Mean	8.76	8.96	9.07	9.66	9.97	10.65
SEm±	0.12	0.13	0.11	0.16	0.15	0.21
CD (P=0.05)	NS	0.38	0.34	0.49	0.45	0.62
CV (%)	2.46	2.48	2.17	2.93	2.65	3.41

MAS: Months After Storage NS: Non-Significant Initial seed moisture (%) – 8.52

Table 2

Effect of new molecules on seed germination of cowpea seeds cv. PGCP-6 stored under ambient condition

Treatments	Seed germination (%) Storage period (August 2023 to June 2024)					
	2 MAS	4 MAS	6 MAS	8 MAS	9 MAS	10 MAS
T_1	93.67	92.67	90.67	87.00	84.00	80.33
T_2	94.33	93.00	92.00	89.33	85.67	82.67
T_3	93.33	92.33	89.00	86.00	82.33	77.67
T_4	91.00	86.67	83.00	81.00	77.00	70.00
T_5	92.00	88.67	85.67	82.33	78.00	73.33
T_6	93.00	90.67	87.33	85.00	81.00	75.00
T_7	92.67	90.00	87.33	83.67	80.33	74.33
T_8	91.67	87.67	85.00	82.00	78.00	72.00
T_9	90.33	84.33	81.33	78.67	72.00	66.33
Mean	92.44	89.56	86.81	83.89	79.81	74.63
SEm±	1.30	1.29	1.56	1.53	1.57	1.51
CD (P=0.05)	NS	3.85	4.63	4.55	4.68	4.47
CV (%)	2.45	2.51	3.11	3.16	3.42	3.49

MAS: Months After Storage NS: Non-Significant Initial seed germination (%) – 95.00

Seeds treated with broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed) (T_2)

Untreated seeds (T_9)

Plate 2 : Effect of new molecules on mean seedling length in cowpea cv. PGCP-6 after ten months of storage

Table 3

Effect of new molecules on mean seedling length of seedling of cowpea seeds cv. PGCP-6 stored under ambient condition

Treatments	Mean seedling length (cm)					
	Storage period (August 2023 to June 2024)					
	2 MAS	4 MAS	6 MAS	8 MAS	9 MAS	10 MAS
T_1	50.00	47.50	45.62	43.41	40.81	38.63
T_2	51.04	49.01	46.58	45.29	41.82	39.95
T_3	48.49	47.20	44.77	42.40	39.97	38.16
T_4	44.26	43.54	41.57	40.11	36.83	35.59
T_5	45.51	44.93	42.81	41.59	38.43	36.48
T_6	47.19	46.56	44.18	42.55	39.54	37.95
T_7	46.16	45.50	43.27	41.79	38.87	37.04
T_8	45.08	43.78	42.62	40.98	38.00	36.05
T_9	43.54	42.98	40.33	38.16	35.02	33.64
Mean	46.81	45.67	43.53	41.81	38.81	37.05
SEm±	0.79	0.78	0.72	0.58	0.46	0.70
CD (P=0.05)	2.31	2.33	2.14	1.72	1.36	2.07
CV (%)	2.88	2.98	2.87	2.39	2.04	3.26

MAS: Months After Storage Initial mean seedling length – 53.77 cm

37.05 cm in mean seedling length and 65.16 to 49.83 mg in mean seedling dry weight. Overall, the broflanilide treatment at a moderate concentration tested (1-3 ppm) registered a beneficial effect on seed germination, seedling vigour indices when compared to untreated seeds. With the passage of time, 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 least seed vigour. Current

Table 4

Effect of new molecules on mean seedling dry weight of cowpea seeds cv. PGCP-6 stored under ambient condition

Treatments	Mean seedling dry weight (mg)					
	Storage period (August 2023 to June 2024)					
	2 MAS	4 MAS	6 MAS	8 MAS	9 MAS	10 MAS
T_1	67.28	65.52	62.78	60.81	57.94	54.45
T_2	67.41	65.68	63.46	61.39	58.56	55.21
T_3	67.13	64.47	61.81	58.33	55.21	53.19
T_4	62.16	58.73	52.88	49.82	47.87	44.78
T_5	65.35	61.37	58.47	56.23	51.33	49.65
T_6	66.87	62.59	61.09	57.45	54.82	51.87
T_7	66.38	62.09	60.87	57.22	53.49	50.46
T_8	65.14	60.19	55.28	51.14	49.73	47.21
T_9	58.71	55.65	49.88	47.51	45.66	41.68
Mean	65.16	61.81	58.5	55.55	52.73	49.83
SEm±	0.89	0.86	0.92	0.79	0.67	0.77
CD (P=0.05)	2.65	2.56	2.74	2.34	1.98	2.28
CV (%)	2.37	2.42	2.73	2.46	2.19	2.66

MAS: Months After Storage Initial mean seedling dry weight – 69.12 mg

findings are contemporaneous with Jagadeesh *et al*. (2017) in pigeon pea, Raghu (2014) in cowpea and Thirumalaraju *et al*. (2015) in maize.

The electrical conductivity of seed leachate gradually increased over time. It varied from 244.63 to 612.24 μScm^{-1} (Table 5). Least electrical conductivity of seed leachate among the treatments was observed in Broflanilide @ 3 ppm (300 SC @ 9.99 mg/kg seed) (T_3) treated seeds about 456.34 μScm^{-1}. The highest conductivity of seed leachate (612.24 μScm^{-1}) was

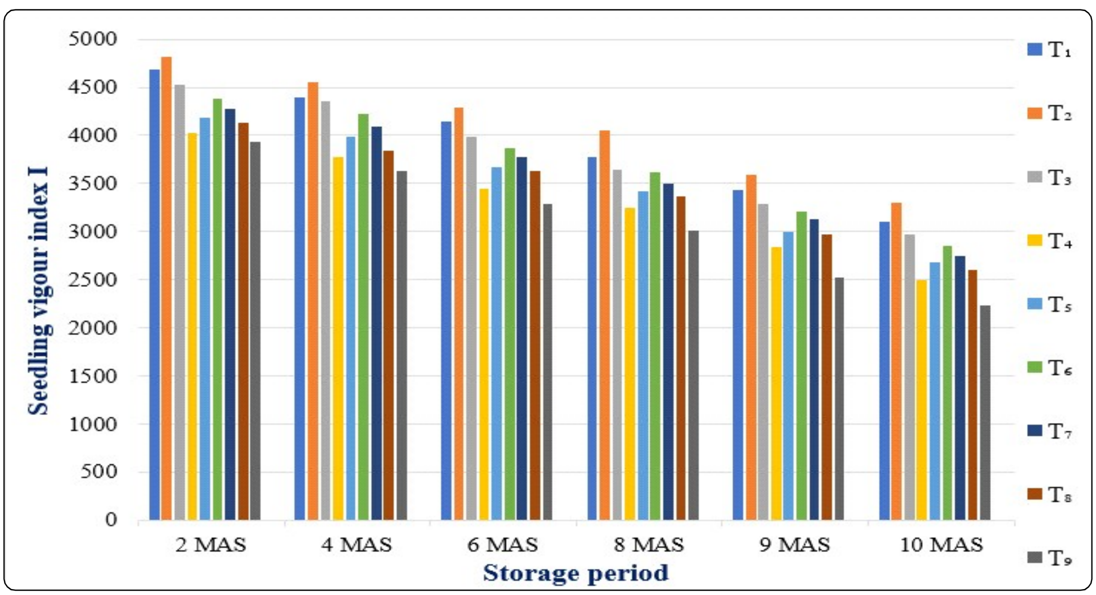

Fig. 1 : Effect of new molecules on seedling vigour index I of cowpea seeds cv. PGCP-6 stored under ambient condition

Table 5

Effect of new molecules on electrical conductivity of cowpea seeds cv. PGCP-6 stored under ambient condition

Treatments	Electrical conductivity (μScm^{-1})					
	Storage period (August 2023 to June 2024)					
	2 MAS	4 MAS	6 MAS	8 MAS	9 MAS	10 MAS
T_1	269.17	291.18	342.15	385.22	422.51	488.23
T_2	261.28	285.15	334.58	378.11	416.25	471.18
T_3	257.49	281.86	329.19	375.35	409.64	456.34
T_4	293.62	327.48	394.26	438.91	483.16	562.65
T_5	284.12	311.61	373.11	417.68	459.08	521.29
T_6	275.09	299.99	359.55	392.71	433.55	497.62
T_7	278.22	305.41	368.43	402.41	441.72	509.11
T_8	289.33	321.72	382.59	429.57	472.48	538.73
T_9	301.28	343.56	419.64	489.24	537.63	612.24
Mean	278.84	307.55	367.06	412.13	452.89	517.49
SEm±	4.09	4.15	6.15	7.79	8.06	9.95
CD (P=0.05)	12.16	12.33	18.28	23.14	23.94	29.56
CV (%)	2.54	2.34	2.90	3.27	3.08	3.33

MAS: Months After Storage Initial electrical conductivity - 244.63 μScm^{-1}

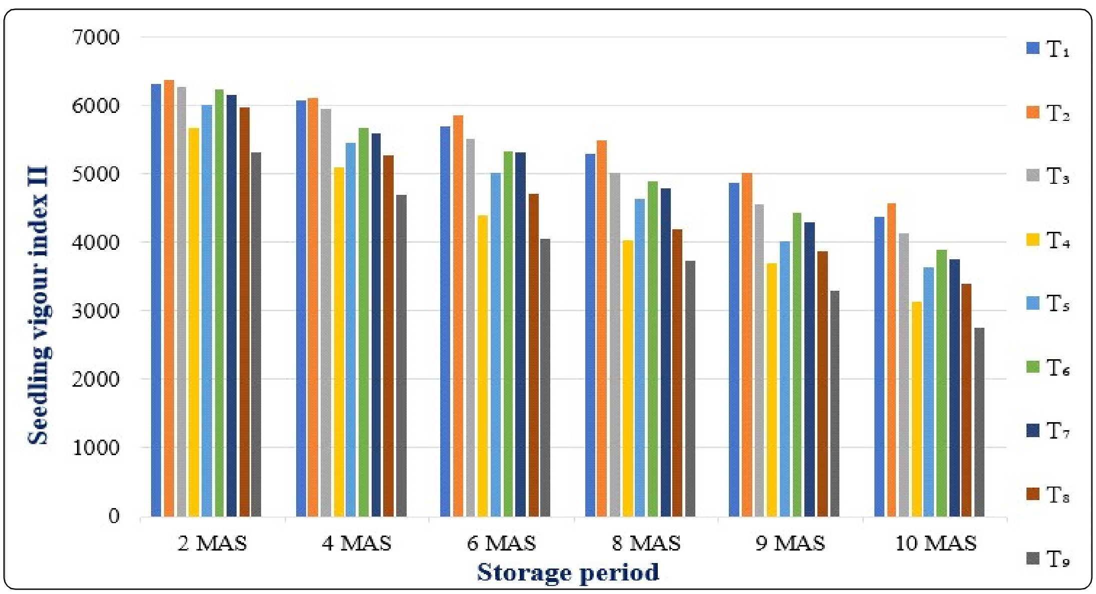

Fig. 2 : Effect of new molecules on seedling vigour index II of cowpea seeds cv. PGCP-6 stored under ambient condition

observed in untreated seeds (T_9). The electrical conductivity, 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 *et al*., 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.

Efficacy of New Molecules against Storage Insect Pests

The percentage of seed damage ranged from 0.00 to 24.25 (Fig. 3). *Callosobruchus chinensis* infestation rose as storage time increased. After ten months of storage, seeds treated with broflanilide @ 3 ppm (300 SC @ 9.99 mg/kg seed) (T_3) recorded least seed damage (4.25%) which is on par with broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed) (T_2), while the maximum seed damage (24.25%) was observed in untreated seeds (T_9) (Plate 3).

As time went on, the percentage of damaged seeds increased. This increased seed damage or exit holes, was caused by an increase in insect population levels

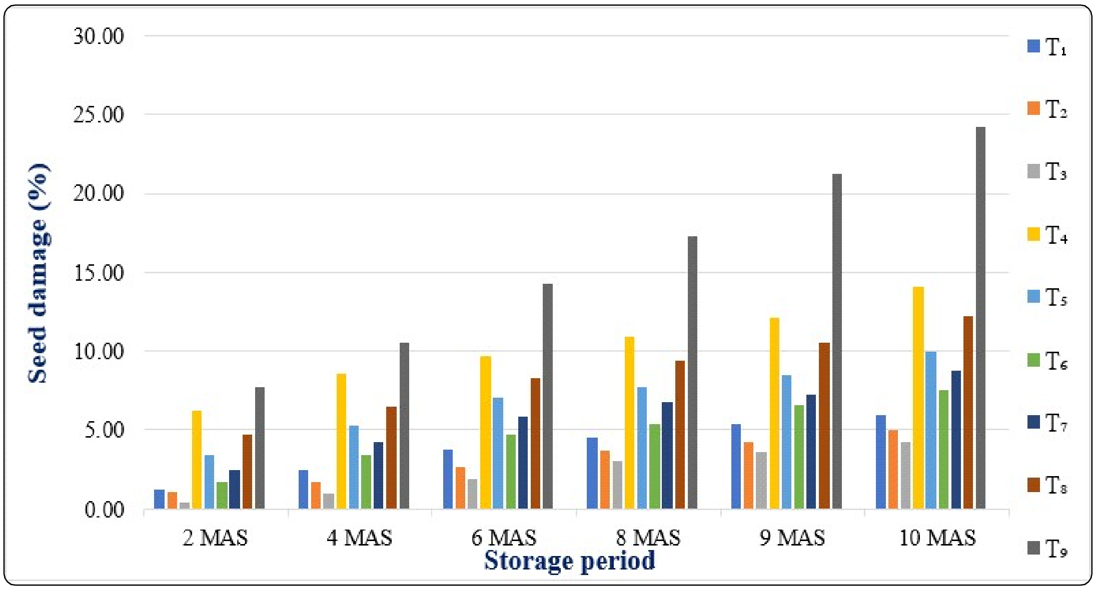

Fig. 3 : Effect of new molecules on seed damage of cowpea seeds cv. PGCP-6 stored under ambient condition

Seeds treated with broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed) (T_2)

Untreated seeds (T_9)

Plate 3 : Comparison of T_2 and T_9 on *Callosobruchus chinensis* infestation in cowpea cv. PGCP-6 after ten months of storage

of *C. chinensis* that soar with favourable storage conditions. When compared to untreated seeds, the majority of broflanilide treatments demonstrated potent lethal activity on *C. chinensis*. These observations are similar to that of Manjula *et al*. (2021) in cowpea and Yogitha (2017) in cowpea.

New molecules with novel mode of action are effective in controlling stored product insect pests and also maintained seed quality during storage. The results revealed that cowpea seeds treated with broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed) (T_2) was effective in maintaining seed moisture (%), seed germination (%), mean seedling length (cm), mean seedling dry weight (mg), seedling vigour index I and II, least electrical conductivity and seed damage (%) attributing towards its effectiveness in maintaining seed quality throughout the storage period. Whereas, broflanilide @ 3 ppm (300 SC @ 9.99 mg/kg seed) inhibited seed quality parameters because of its toxic effect at higher concentration.

From the findings it can be concluded that, broflanilide @ 2 ppm (300 SC @ 6.66 mg/kg seed) can be used effectively for preserving cowpea seeds ensuring quality by managing storage insect pest, *Callosobruchus chinensis* for up to ten months of storage.

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