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Studies on bioactivity of ethnopharmacological weed extract on storability of cowpea (*Vigna unguiculata* L.)

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Abstract
A laboratory study was conducted to evaluate the ethnopharmacological weed extract treatments on storability of cowpea seeds var. KBC-9. The seeds treated with weed powder @ 25 g/kg of seeds such as *Alternanthera sessilis*, *Cassia tora*, *Croton bonplondianum*, *Euphorbia hirta* and *Xanthium strumarium* and Bavistin and Spinetorium are also taken as check and treated seeds were stored up to 9 months. The results revealed that irrespective of treatments, the seed quality attributes reduced with increasing storage period due to seed deterioration. Among ethnopharmacological weed extract powder, seed treated with *Xanthium strumarium* @ 25g/kg of seeds can be stored up to 4 months with maintaining minimum seed germination standards.

Keywords: Ethnopharmacology, weed extract, germination, secondary metabolites

Introduction
Cowpea (*Vigna unguiculata* L.) is a versatile and resilient legume grown all over the world for its nutritional value and adaptability to various environments. Due to the high protein content of the seeds and leaves, it is frequently referred to as "poor man's meat". In addition to being a staple, it also used as fodder, vegetable and green manure crop. Cowpea is a widely cultivated legume crop, spanning approximately 14.5 million hectares worldwide with an annual production of 6.2 million metric tonnes. It holds great significance in India, particularly in the central and peninsular regions, where states like Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu, Madhya Pradesh and Rajasthan are the primary cultivators. In Karnataka alone, cowpea occupies around 0.0786 million hectares of land, yielding approximately 0.087 million tonnes with a productivity of 427.25 kg/ha (Anon., 2020) [2].

Storage of the seeds is the main problem in this crop due to potential infestation by postharvest pests. Insects, fungi, bacteria and rodents account for the majority of storage pests, which are accountable for grain loss and poor storage facility losses. Insect damage to stored grain alone accounts for around 10–50% of all pest damage (Mohapatra *et al*., 2015) [14] and the cowpea weevil, *Callosobruchus maculatus* L. among storage pests, can alone result in up to 100% yield loss of stored cowpea seeds in just a few months (Kang *et al.*, 2013) [10]. The use of chemical pesticides to control pests has failed because of insecticidal resistance and caused extensive damage of both health and environment.

The common methods for combating these insect pests are synthetic pesticides like phosphine and pyrethroids. But because of these chemical pesticides, concerns about insect resistance, food contamination from pesticide residues, human health risks and environmental pollution have grown (Daglish, 2008) [6]. These factors led to the exploration of new environmentally friendly strategies and innovations to improve seed performance both at filed level and storage period. As an alternative, plant-based extracts could be used for these purposes *i.e*., ethnopharmacological weed extracts, reducing the risks to the environment and human health.

The cross-cultural study of plants, animals, fungus or other naturally existing materials utilized as remedies by ethnic and cultural groups is known as ethnopharmacology. (Jalil *et al*., 2023) [9]. Ethnopharmacological weeds refer to wild plants or commonly considered as weeds which have been traditionally employed by different cultures for medicinal purposes. These weeds have been employed in conventional medical practices for generations and are recognized for their therapeutic properties. These weeds serve as valuable source of traditional remedies and natural compounds mainly secondary metabolites.

Given this context, the current investigation aims to assess the effectiveness of powdered ethnopharmacological weed extracts when applied to cowpea seeds during a storage period of 9 months.

Materials and Methods

Laboratory experiments were conducted to evaluate the effects of various ethnopharmacological weed extracts on the seed germination and seedling performance of maize seeds stored for 9 months. The weed extracts used were, *Alternanthera sessilis*, *Cassia tora*, *Croton bonplondianum*, *Euphorbia hirta* and *Xanthium strumarium*. The seeds of cowpea var. KBC-9 were dried to 8 per cent moisture content, having germination percent of 88 and nil infection and treated with these extracts @25 g/kg of seeds and stored in cloth bags for a period of 9 months. The experiment was formulated by adopting CRD with three replications.

Fig 1: The ethnopharmacological weeds used for the study

The following observations made at monthly interval (December, 2022 to September, 2023). The germination test was conducted by adopting between papers method as prescribed by ISTA 2021 (Anon, 2021) [1]. The seedling vigour index was calculated as per the formula given by Abdul-Baki and Anderson, 1973 [4].

SVI-I = Germination (%) × Mean seedling length (cm)

SVI-II = Germination (%) × Mean seedling dry weight (mg)

Seed infection: Seed infection was evaluated as per blotter paper method. Twenty-five seeds of four replications in each treatment were placed equidistantly in sterile glass petri plates with two moist blotter papers (Whatman No.1) and incubated at 20 ±1°C in a BOD incubator for eight days with alternate cycle of 12 hours light and for remaining 12 hours in dark. Then, on 8th day the seeds were examined for the presence of seed mycoflora. Species of fungus were identified by using stereo binocular microscope based on colony characters and expressed in percentage.

$$\text{Seed infection (\%)} = \frac{\text{Number of infected seeds}}{\text{Number of seeds incubated}} \times 100$$

Electrical conductivity (µS/cm/g): Twenty-five seeds were selected randomly from three replications of each treatment in a beaker. The selected seeds were soaked in 125 ml of distilled water for 24 hours at 25±1°C. The electrical conductivity (EC) of seed leachate was measured from the steeped water of soaked seeds in a digital conductivity meter (Model: Systronic conductivity meter 306). The actual EC of seed leachate was calculated by subtracting the EC of distilled water from the obtained values and expressed in µS/cm/g (Anon., 2021) [1].

$$\text{Electrical conductivity (µS/cm/g)} = \frac{\text{Conductivity reading(µS/cm) - Background reading}}{\text{Weight of the replicate (g)}}$$

Total dehydrogenase (TDH) activity (A_{480}): Ten seeds were randomly selected from seeds incubated for EC test from each treatment in three replications. The seed coat was carefully removed and made sure embryonic axis is soaked in a test tube containing 0.5 percent tetrazolium chloride solution and incubated at 25 ± 1°C in the dark for 4 hours. Further, seeds were washed thoroughly in distilled water, and the red colour formazan from stained embryos was eluted by soaking in 5 ml of 2- methoxy ethanol for 24 hours in an airtight container. The extract was decanted and colour intensity is measured using a spectrophotometer at 480 nm. The dehydrogenase activity is expressed in terms of optical density at A_{480} (Perl *et al*., 1978) [15].

Results and Discussions

The result of the present study as well as relevant discussion have been summarized under following heads:

Seed germination (%)

The seed germination of cowpea seeds differed significantly

among treatments. The highest seed germination of 78% noticed in seeds treated with Spinetoram 11.7% SC @ 8.5 mg/kg seed (T_8) followed by Carbendazim 50% WP @4 g/kg of seed (T_7) (71%) at the end of 9 months storage period. Among ethnopharmacological weed extract treatments, seeds primed with *Xanthium strumarium* L. @25 g/kg of seed (T_6) shown higher germination per cent (17%) and control having germination per cent of 9%. Whereas, lower germination (5%) seen in *Croton bonplandium* Baill. @25 g/kg of seed (T_4) and *Euphorbia hirta* L. @25 g/kg of seed (T_5). The data regarding germination presented in Table 1.

The seeds treated with ethnopharmacological weed extracts initially showed promising effect, but as the storage period progressed, a noticeable decline in seed germination emerged, potentially suggesting that these weed powders were ineffective in preventing insect infestation. In contrast, seeds treated with *Xanthium strumarium* L. @ 25 g per kilogram of seed (T_6) displayed remarkable resilience. They maintained a robust germination rate of 75%, meeting the stringent minimum seed certification standards even after four months of storage compared to control. The decline in germination per cent observed in all treatments with the advancement of storage period may be attributed to the phenomenon of decline in enzyme activity and degradation of seed coat which resulted in leaching constituents (Abdul-Baki and Anderson, 1973) [4]. The increased insect infestation by bruchid (*Callosobruchus chinensis*) and natural ageing of the seeds caused the germination per cent of seeds varied from 88 to 9 per cent. The lower germination per cent (5%) seen in *Croton bonplandium* Baill. @25 g/kg of seed (T_4) and *Euphorbia hirta* L. @25 g/kg of seed (T_5). The observed reduction in germination percentages across all treatments as the storage period advanced could potentially be attributed to aging effects, depletion of seed food reserves and deterioration of the seed coat, leading to the leaching of its components, as documented by the findings of Chandrasenan (1996) [5]. Similar findings reported by Rana *et al*. (2014) [16] in pea, Ibrahim *et al*. (2010) [8] in forage crops and Khatun *et al*. (2011) [11] in lentil.

Tabel 1: Effect of ethnopharmacological weed extract on seed germination (%) of cowpea var. KBC-9 during storage

Treatments	Seedling vigour index-I								
	Storage period (December 2022- September 2023)								
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS
T_1	2670	2455	2250	1939	1612	1129	832	244	90
T_2	2765	2557	2350	2145	1874	1464	800	168	71
T_3	2658	2530	2150	1987	1641	1039	398	117	59
T_4	2640	2455	2100	1844	1567	830	183	84	43
T_5	2691	2479	2187	1977	1695	926	417	139	49
T_6	2733	2622	2424	2207	1966	1612	857	374	175
T_7	2842	2713	2533	2364	2191	1980	1841	1671	1475
T_8	2847	2791	2656	2600	2422	2310	2182	2029	1920
S.Em±	54.46	44.16	54.66	48.35	43.54	26.27	24.07	9.33	13.86
CD (P=0.05)	NS	182.41	225.76	199.72	179.85	108.49	99.42	38.56	57.23
CV (%)	3.45	2.97	4.06	3.93	4.03	3.22	4.44	2.68	4.95

MAS: Months After Storage NS: Non-Significant Initial seedling vigour index-I– 3075

Treatment details

T_1	: Control		T_5	: *Euphorbia hirta* L. – 25g/kg,
T_2	: *Alternanthera sessilis* (L.) R.Br. ex.DC – 25g/kg,		T_6	: *Xanthium strumarium* L. – 25g/kg,
T_3	: *Cassia tora* L. – 25g/kg,		T_7	: Carbendazim 50% WP- 4g/kg,
T_4	: *Croton bonplandium* Baill. – 25g/kg,		T_8	: Spinetoram 11.7% SC @ 8.5 mg/kg seed

Electrical conductivity of seed leachate ($\mu Scm^{-1}g^{-1}$)

The data pertaining to electrical conductivity of seed leachate as influenced by ethnopharmacological weed extract during storage are presented in Fig 2. The electrical conductivity differed significantly among treatments during storage period. The seeds treated with Spinetoram 11.7% SC @ 8.5 mg/kg seed (T_8) recorded lower electrical conductivity (1452 $\mu Scm^{-1}g^{-1}$) followed by Carbendazim 50% WP-@ 4 g/kg of seed (T_7) (1689 $\mu Scm^{-1}g^{-1}$) at the end of storage period. Among ethnopharmacological weed extract, *Xanthium strumarium* L. @25g/kg of seed (T_2) shown lower electrical conductivity (1835.6 $\mu Scm^{-1}g^{-1}$) and observed significant difference compared to control (2216.7 $\mu Scm^{-1}g^{-1}$) after 9 months of storage. In contrast, higher electrical conductivity (2285.4 $\mu Scm^{-1}g^{-1}$) observed in seeds treated with *Croton bonplandium* Baill. @25 g/kg of seed (T_4) at the end of storage period. The significant increase in electrical conductivity of the seed leachate observed between treatments with increasing storage period because increase in cellular membrane degradation and decrease in compactness of seed lead to increased electrolytes leachate, soluble sugars as noticed with advancement of the storage period leading to an increased seed leachate from 245.6 to 2216.7 $\mu Scm^{-1}g^{-1}$. Similar findings were also noticed by Ibrahim *et al*. (2010) [8] in forage crops and El-Dahab *et al*. (2016) [7] in sorghum.

Table 3: Effect of ethnopharmacological weed extract on seedling vigour index-II cowpea var. KBC-9 during storage.

Treatments	Seedling vigour index-II								
	Storage period (December 2022- September 2023)								
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS
T_1	4224	4040	3625	3171	2687	2151	1306	422	173
T_2	4390	4156	3861	3477	3180	2734	1428	327	135
T_3	4273	4115	3500	3276	2771	2034	632	200	114
T_4	4220	3997	3379	3036	2646	1652	342	169	89
T_5	4303	4073	3574	3209	2871	1827	763	259	102
T_6	4360	4200	3991	3571	3252	2897	1639	693	333
T_7	4485	4343	4108	3927	3808	3602	3321	2889	2652
T_8	4575	4471	4353	4233	4092	3819	3633	3368	3183
S.Em±	94.01	71.75	79.04	81.16	74.75	37.17	36.94	23.67	14.58
CD (P=0.05)	NS	296.39	326.50	335.22	308.76	153.55	152.59	97.79	60.23
CV (%)	3.74	2.98	3.60	4.03	4.09	2.49	3.92	3.94	2.98

MAS: Months After Storage NS: Non-Significant Initial seedling vigour index--II - 4765

Treatment details:

T_1	: Control		T_5	: *Euphorbia hirta* L. – 25g/kg,
T_2	: *Alternanthera sessilis* (L.) R.Br. ex.DC – 25g/kg,		T_6	: *Xanthium strumarium* L. – 25g/kg,
T_3	: *Cassia tora* L. – 25g/kg,		T_7	: Carbendazim 50% WP- 4g/kg,
T_4	: *Croton bonplandium* Baill. – 25g/kg,		T_8	: Spinetoram 11.7% SC @ 8.5 mg/kg seed

Total dehydrogenase activity (OD value@A_{480})

The data pertaining to total dehydrogenase activity as influenced by ethnopharmacological weed extract during storage are presented in Fig 3. The dehydrogenase activity differed significantly among treatments during storage period. The seeds treated with Spinetoram 11.7% SC @ 8.5 mg/kg seed (T_8) recorded higher dehydrogenase activity (1.027) followed by Carbendazim 50% WP-@ 4 g/kg of (T_7) (0.996) at the end of storage period. Among ethnopharmacological weed extract, *Xanthium strumarium* L. @25 g/kg of seed (T_6) have shown higher dehydrogenase activity (0.769) differed significantly and control shown (0.619) after 9 months of storage. In contrast, lower dehydrogenase activity observed in seeds treated with *Croton bonplandium* Baill.@25 g/kg of seed (T_4) (0.612) at the end of storage period.

The decrease in dehydrogenase activity with increasing storage period mainly due to increase in denaturation enzyme activity resulting in 56.65% decrease in dehydrogenase activity. These findings similar with those reported by Manjula (2021) [13] for cowpea.

The seed infection differed significantly among treatments during storage period. The seeds treated with Spinetoram 11.7% SC @ 8.5 mg/kg seed (T_8) recorded lower seed infection (20) followed by Carbendazim 50% WP-@ 4g/kg of seed (T_7) (36) at the end of storage period. Among ethnopharmacological weed extract, *Alternanthera sessilis* @25g/kg of seed (T_2) shown lower infection rate (64%) and observed significant difference compared to control (68%) after 9 months of storage. In contrast, higher infection (72%) observed in seeds treated with *Croton bonplandium* Baill. @25g/kg of seed (T_4) at the end of storage period. The data pertaining to seed infection% presented in Table 4.

The significant increase in seed infection observed between treatments with increasing storage period because with the advancement in the storage period infection rate increased from 0 to 68%. The increase in seed infection with increasing storage period of seeds can be attributed to several factors such as increase in moisture content over a storage period creating more favourable environment for pathogens, thus results in increased infection period over a storage period. Similar results obtained by El-Dahab *et al*. (2016) [7] in sorghum and Kandhare, 2019 [12] in pigeon pea.

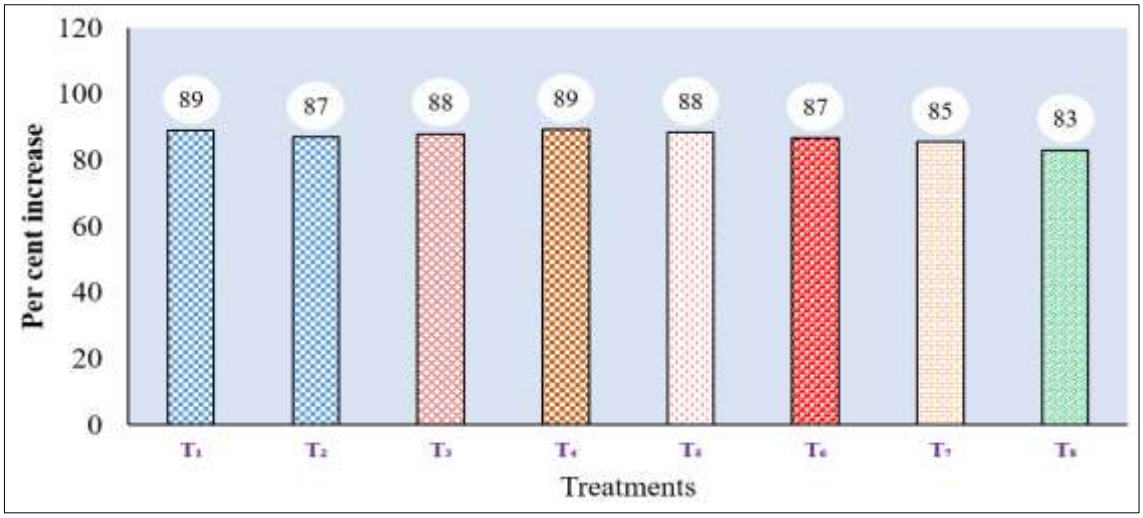

Fig 2: Per cent increase in electrical conductivity at the end of storage period over the initial electrical conductivity as influenced by ethnopharmacological weed extract in cowpea var. KBC-9

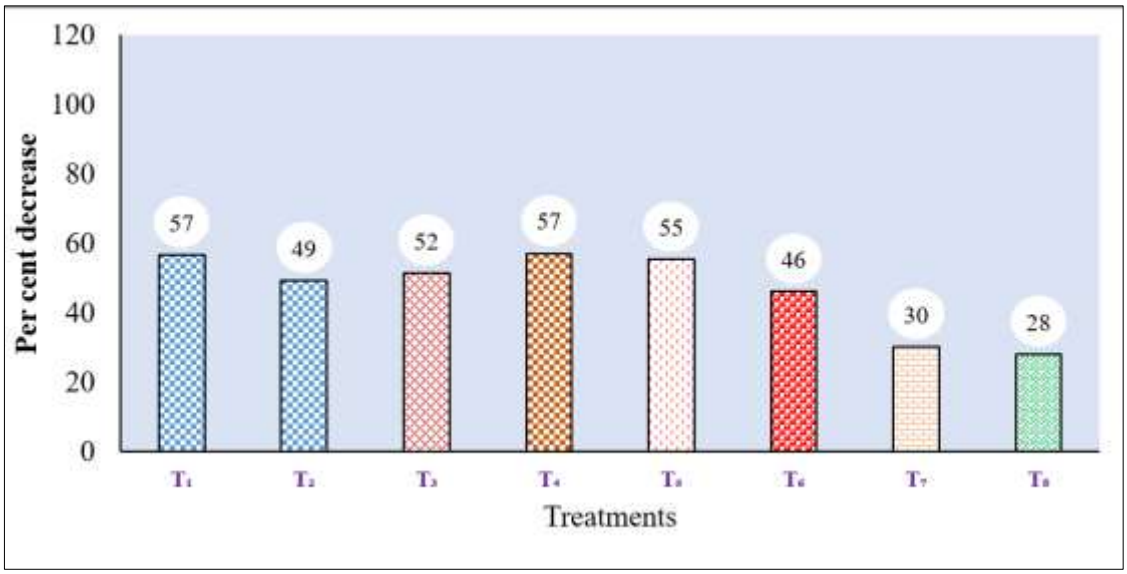

Fig 3: Per cent decrease in total dehydrogenase activity at the end of storage period over the initial total dehydrogenase activity as influenced by ethnopharmacological weed extract in cowpea var. KBC-9

Tabel 4: Effect of ethnopharmacological weed extract on seed infection of cowpea var. KBC-9 during storage

Treatments	Seed infection (%)								
	Storage period (December 2022- September 2023)								
	1 MAS	2 MAS	3 MAS	4 MAS	5 MAS	6 MAS	7 MAS	8 MAS	9 MAS
T_1	0.00	8 (2.92)	16 (4.06)	20 (4.53)	26 (5.15)	40 (6.36)	48 (6.96)	56 (7.52)	68 (8.28)
T_2	0.00	8 (2.92)	12 (3.54)	16 (4.06)	20 (4.53)	32 (5.70)	38 (6.20)	52 (7.25)	64 (8.03)
T_3	0.00	4 (2.12)	16 (4.06)	24 (4.95)	26 (5.15)	36 (6.04)	40 (6.36)	54 (7.38)	68 (8.28)
T_4	0.00	12 (3.54)	20 (4.53)	24 (4.95)	28 (5.34)	44 (6.67)	52 (7.25)	60 (7.78)	72 (8.51)
T_5	0.00	12 (3.54)	16 (4.06)	20 (4.53)	24 (4.95)	36 (6.04)	44 (6.67)	56 (7.52)	68 (8.28)
T_6	0.00	8 (2.92)	12 (3.54)	16 (4.06)	20 (4.53)	28 (5.34)	36 (6.04)	48 (6.96)	68 (7.78)
T_7	0.00	4 (2.12)	4 (2.12)	8 (2.92)	12 (3.54)	20 (4.53)	24 (4.95)	28 (5.34)	36 (6.04)
T_8	0.00	0 (0.71)	0 (0.71)	4 (2.12)	8 (2.92)	12 (3.54)	16 (4.06)	16 (4.06)	20 (4.53)
S.Em±	NA	0.02	0.03	0.03	0.03	0.04	0.04	0.05	0.04
CD (P=0.05)	NA	0.07	0.11	0.12	0.14	0.16	0.19	0.19	0.18
CV (%)	NA	1.10	1.42	1.23	1.31	1.25	1.28	1.18	1.02

*Values in the parenthesis are square root transformed values
MAS: Months After Storage NS: Non-Significant NA: Not analysed Initial seed infection - Nil

Treatment details

T_1	: Control		T_5	: *Euphorbia hirta* L. – 25g/kg
T_2	: *Alternanthera sessilis* (L.) R.Br. ex DC – 25g/kg		T_6	: *Xanthium strumarium* L. – 25g/kg
T_3	: *Cassia tora* L. – 25g/kg		T_7	: Carbendazim 50% WP- 4g/kg
T_4	: *Croton bonplandium* Baill. – 25g/kg		T_8	: Spinetoram 11.7% SC @ 8.5 mg/kg seed

Conclusion

The ethnopharmacological weed extract *Xanthium strumarium* L. @25 g/kg of seed have demonstrated notable potential in extending seed germination viability for up to four months. This research suggests a novel approach to preserving seed quality and agricultural sustainability. However, further investigation is required to understand the long-term effects and practical applications of these extracts. Their promising initial results highlight the need for continued research and development in this area. Ultimately, harnessing the full potential of ethnopharmacological weed extracts could significantly benefit global agriculture and contribute to food security efforts.

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