Effect of Natural Extracts During Storage Conditions on Seed Quality and Yield Performance of Soybean Cultivar *Giza 22*

ABSTRACT

Aims: The present study aimed to investigate the effects of Diatomaceous Earth (DE) at 20 g/kg, Neem seed extract (NSE) at 25 g/kg, and Silica gel (SG) at 25 g/kg on seed quality, field performance, and seed yield of *Giza 22* soybean seeds duringvarious of storage durations and environmental conditions.

Study design: Three experiments investigated storage, germinability, and field performance of seeds using a FCRD with four replicates. The seed storage trial assessed open and cool storagesconditions across durations of 1, 4, 8, and 12 months, with seed treatments of DE, NSE, and SG. Germinability and field performance were evaluated under the same storage conditions and treatments. Data were analyzed using analysis of variance (ANOVA) at P = 0.05.

Place and Duration of Study: The storage and germination trials were conducted at the preservation labs of the Seed Technology Research Unit, Mansoura City, FCRI, ARC, Egypt. While, the field experiment took place on a private farm in the village at geoical location 30°53'39"N 31°20'51"E.

Methodology: The seeds were tested under open storage at ambient room conditions and cool storage at 5°C and 30% RH, and storage periods at four levels with two months interval. **Results:** Isolated data clarify applying cool storageorseed treatments (DE, NSE, and SG) over all storage periods significantly (P = 0.05) exceed open storage or the untreated control seeds, respectively. Cool storage combined with DE, NSE, and SG seed treatments significantly yielded the highest stored seed quality indices, field performance and seed yield compared to the untreated control seeds stored at open or cool storage conditions. **Conclusion:**It could be concluded that, the application of diatomaceous earth, neem seed extract, and silica gel on soybean seeds during storage has been shown to provide several benefits *i.e.*, extend seed storability, reduce insect damage, improve seed germination and seedling vigor, and increasing seed yield.

Keywords: Soybean seed,Storage conditions,Natural extracts, Seed quality,Field performance, Seed yield

INTRODUCTION

A major challenge in soybean production in arid and semi-arid regions is the rapid decline in seed quality during storage. Farmers in these areas often store seeds under suboptimal conditions, negatively impacting seed quality and germination. Enhanced packaging can help maintain storage quality. Assessing seed quality and vigor is crucial for selecting seed lots for the planting season, emphasizing the need for reliable laboratory methods and vigor tests to accurately evaluate seed viability. Maintaining seed quality during storage is crucial, as seeds experience physiological deterioration over time. This underscores the necessity for controlled storage conditions to preserve germination and vigor, especially for short-lived seeds like soybeans and onions [1]. While seed storability is largely genetic, factors such as maturation, seed shape, moisture, pre-storage conditions, environmental influences, rapid deterioration, fungal infections, and storage duration also significantly affect seed metabolism and physiological quality [2&3]. High storage quality is vital for successful sowing, as it influences the maintenance or deterioration of seed quality, impacting planting suitability. For example, storing soybean seeds in air-conditioned environments at varying temperatures for six months can improve seed quality. Rapid deterioration in soybeans is linked to lipid peroxidation, which reduces viability [4]. Fungal infections can cause substantial quality losses, including seed abortion, rot, necrosis, reduced germination capacity, and lower nutritional value [5]. Optimal storage conditions, particularly temperature, are essential for preserving seed quality. Elevated storage temperatures can drastically diminish soybean seed viability, leading to metabolic deterioration. High storage temperatures can also induce physiological stress in soybean seeds, adversely affecting germination potential and seedling vigor [6]. Conversely, cooler temperatures enhance soybean seed longevity by slowing metabolic decline, making artificial cooling critical [7]. Seeds stored in cold conditions maintain better vigor and germination rates, while those at room temperature degrade more rapidly [8]. Lower temperatures help reduce biochemical reactions, preserving initial seed characteristics for a longer period [9]. Thus, maintaining appropriate storage temperatures is vital for ensuring the viability and germination potential of soybean seeds. Recent studies emphasize the detrimental effects of high temperatures on seed quality and germination rates, highlighting the importance of effective temperature management during storage.

Diatomaceous earth (DE), a naturally occurring compound, serves as an effective pest control agent. DE typically has a particle size of 10.3 µm and is composed mainly of 85.3% amorphous silicon dioxide (SiO_2), with traces of other minerals including aluminum, calcium, iron, magnesium, and sodium [10]. The silica in DE is derived from fossilized diatoms and may release additional trace minerals during extraction. Studies have found that applying DE extract helps maintain soybean seed viability and improve germination rates during storage, also protect against pests and pathogens, and support overall seed health. Additionally, adsorbed water in DE ensures optimal moisture levels in stored soybean seeds, preventing excess drying or moisture accumulation, thereby helping to preserve seed viability [11], and providing a protective barrier against pests and moisture [12]. Neem extract (*Azadirachtaindica* A. Juss), known for its insecticidal properties, improves plant growth parameters like height, stem girth, leaf area, and branching. Neem oil nanoemulsions effectively control fungal pathogens in soybean seeds without causing phytotoxicity, thus enhancing seedling quality and yields [13]. Research indicates that neem seed extract maintains seed viability and boosts germination rates during storage [14]. The bioactive compound azadirachtin is particularly effective against pests and pathogens, safeguarding seed quality and vigor [15]. Neem-based pesticides are well-suited for organic farming and developing countries due to their simple preparation methods.Silica gel extract, primarily composed of silica (SiO_2), enhances seed viability and vigor by improving water absorption and germination while protecting against pests [16]. The silica gel retains water during extraction, helping maintain seed moisture content. It may also contain trace minerals like calcium, magnesium, and potassium, which benefit seed health [17]. Additionally, organic compounds in silica gel may offer antioxidant and antimicrobial properties, further protecting soybean seeds [18]. Studies show that silica gel effectively maintains low moisture levels crucial for seed preservation, leading to higher germination rates and seedling vigor when used for storage [12].

In summary, using diatomaceous earth, neem extract, and silica gel for seed treatments significantly impacts the viability and vigor of soybean seeds during storage, preserving seed quality and enhancing overall yield. These practices are valuable for promoting food security and sustainability.

MATERIALS AND METHODS

Materials and location

Freshly harvested seeds of the soybean (*Glycine max* L.) cultivar Giza 22 were obtained from Food Legumes Research Department, Field Crops Research Institute, Agricultural Research Center, Giza, Egypt. Seed treatments such as diatomaceous earth, neem seed extract, and silica gel were chosen for seed storage purposes. The storage and laboratory germination trials were conducted at the preservation labs of the Seed Technology Research Unit, Mansoura, Dakahlia Governorate, FCRI, ARC, Egypt. While, the field experiment took place on a private farm in the village at geoical location 30°53'39"N 31°20'51"E, Mait al-Amel, Aga Center, Dakahlia Governorate.

Seed sampling

The recently harvested soybean seeds underwent a cleaning process to eliminate impurities, gravel, debris, and foreign matter. These seeds were then dried in silos using radial airflow (Retsch TG100 model, 230V&50Hz) set to 1m/s, 40°C air temperature, and 20% air humidity. Moisture content was checked every thirty minutes with a digital grain moisture meter (Multi Grain) until reaching a stable 7% level. Subsequently, before implementing storage procedures, a seed sample was subjected to tests for electrical conductivity, cold test, and germination percentage. The sample exhibited electrolyte leakage of 27 µs/cm^{-1}/g^{-1} seed weight, a cold germination rate of 86%, and an overall germination percentage of 91%. Following these tests, the seed sample was partitioned into three groups for experimenting with natural extract treatments at recommended concentrations.

Treatments details

The process of treating diatomaceous earth (DE) or neem seed extract (NSE) involved blending the designated quantity of seeds with pure DE or NSE powder. The rate for DE was 20 g per kg of dry seeds, and for NSE, it was 25 g per kg of dry seeds. The blend was carefully stirred in a clean bowl to guarantee uniform distribution and coating of the seeds, aligning with the recommended procedures outlined by Subramanyam and Roesli (2000) [19].

The silica gel extract (SG) was made using food-grade silica gel granules. Weighed SG extract was put in a clean glass container and combined with distilled water at a 1:5 ratio by weight. After thorough stirring for complete wetting, the mixture sat for 24 hours to extract the active compounds. The mix was then filtered through a mesh sieve to separate the extract from the solids. The resulting SG extract was ready for use at a concentration of 2-3% by weight. Around 25g/kg seed was applied to soybean seeds by coating them in a clean container, then drying them to the initial moisture level. The soybean seeds were stored in dual-layer kraft bags in dry, well-ventilated areas at ambient room varying temperatures and relative humidity and in airtight plastic bags at 5 °C & 30% RH to maintain their quality in both room temperature and cool storage environments.

Experiments characterization

1. Storage experiment

The seed storage trial involved a 2 x 4 x 4 factorial setup in a Randomized Complete Block Design (RCBD) with four replications. **The first factor** examined storage environments under two conditions: (1) Open storage under ambient room conditions with varying temperatures and relative humidity, and (2) Cool storage at 5°C & 30% RH.**The second factor** studied storage periods of 1, 4, 8, and 12 months. **The third factor** included seed treatments induced by some natural extract materials: (1) Diatomaceous Earth (DE) at 20 g/kg^{1} dry seeds, (2) Neem seed extract (NSE) at 25 g/kg^{1} dry seeds, (3) Silica gel (SG) at 25 g/kg^{1} dry seeds, and (4) untreated seeds as the control treatment.

During storage, seed storage quality was assessed every four months (at 1, 4, 8, and 12 months) through tests for seed moisture content, electrical conductivity, cold test, and fungal disease contamination tests, according to the guideline of ISTA (2024) [20], as follow;

- **Seed moisture content (SMC%):** The standardized method accurately determines the moisture level in seed samples, a crucial factor for seed quality, storage, and viability. Seeds are first weighed and then dried in an oven at 103°C for 17 hours. After drying, they are weighed again. The change in weight is used to calculate moisture content as a percentage of the original weight with the formula; Moisture content (%) = [(Initial weight - Dried weight) / Initial weight] × 100.
- **Electrical conductivity test**: - Electrical conductivity test: Four seed samples of 5 grams each treatment was selected, weighed, and surface sterilized with 0.1% sodium hypochlorite for 10 minutes. They were then thoroughly washed in distilled water, placed in a glass beaker with 100 mL of distilled water, and incubated at 25°C for 24 hours. Following imbibition, the seeds were extracted, the electrical conductivity of distilled water was measured. After cleaning the electrode with tissue paper, the conductance of the leachate was recorded. The leachate's EC was calculated by subtracting the distilled water reading from the sample reading. The result was expressed as mmhos/cm/g of seed, as follow; Electrolyte leakage (µs. cm^{-1}. g^{-1} seed weight) = (leachate solution electrical conductivity – distilled water electrical conductivity) / seed sample weight (gram).
- **Cold test**: Four replications of 50 seeds for each treatment were sown on two sheets of paper, enclosed by a third sheet, rolled up, and stored in plastic bags at 10°C for seven days. After this period, the bags were unsealed, and the rolls were moved to the incubator at 25°C for four days and evaluated similar to the germination test. The results are reported as a percentage of viable seedlings.
- **Fungal diseases contamination**: Four samples of each treatment containing 100 intact seeds were used. Seeds were soaked in a 1% sodium hypochlorite (NaOCl) solution for 10 minutes, then drain and rinse thoroughly with sterile water before draining again. Following this, 10 seeds were placed on the agar surface in each Petri dish and incubate for 7 days at 20 °C in darkness. After incubation, visually inspect each seed for abundant white mycelium that may cover infected seeds. Percentage of infected seeds = (Number of infected seeds / Total number of seeds tested) × 100.

2. Laboratory germination experiment

An experiment testing germination was carried out on the seed samples stored for 8 months, which closely mimics the duration of natural seed storage until the next planting season. The seed germination trial was conducted in a 2 x 4 factorial experiment following a

Randomized Complete Block Design (RCBD) with four replications. **The first factor** investigated two storage conditions: Open storage under ambient room conditions with varying temperatures and relative humidity and cool storage at 5°C & 30% RH. **The second factor**involved seed treatments induced by natural extract materials: DE at 20 g/kg^{1} dry seeds, NSE at 25 g/kg^{1} dry seeds, SG at 25 g/kg^{1} dry seeds, with untreated seeds used as the control treatment.

Before initiating the germination process for soybean seeds, a suitable quantity of treated seeds was selected. The seeds were sterilized using a diluted solution of sodium hypochlorite at 0.1% [21], washed multiple times with distilled water. Physiological seed quality (vigor and viability tests) was conducted following ISTA (2024) [20]guidelines. Four sets of 100 seeds per treatment were placed between moistened Whatman No.1 filter papers in 15 cm diameter Petri dishes. Each dish contained 20 seeds, with four dishes grouped as one replication and placed in an incubator room at 25 °C and 80% relative humidity. Filter papers were periodically moistened with 10 ml of distilled water to prevent drying. Physiological seed quality parameters were monitored daily for 7 days after sowing to track seedgerminabilitymeasurements.

- Germination percentage (G%): It was determined by counting only viable seedlings 7 days after sowing using the following formula;G% = (Number of germinated seeds / Total seeds evaluated) × 100.
- Mean germination time (MGT, day): It was determined using the formula; MGT = Σ (**D** × **n**) / Σ**n**,where n is the number of seeds germinated on day D, and D is the number of days from the start of the test [22].
- Seedling dry weight was measured by selecting 30 seedlings randomly from all replications (10 per replicate) seven days post-sowing, expressed in mg seedling^{-1}[23].
- Seedling vigor index (SVI-II) was calculated using the formula;SVI-II = Seedling dry weight (mg) × Germination percentage [24].

3. Field experiment

The field trial was conducted in a 2 x 4 factorial experiment following a Randomized Complete Block Design (RCBD) with four replications. **The first factor** consisted of two storage environments: (1) Open storage under ambient room conditions with varying temperature and relative humidity, and (2) Cool storage at 5°C & 30% RH. **The second factor** included three natural extract materials: (1) Diatomaceous Earth (DE) at 20 g/kg^{1} dry seeds, (2) Neem seed extract (NSE) at 25 g/kg^{1} dry seeds, (3) Silica gel (SG) at 25 g/kg^{1} dry seeds, with untreated seeds as the control group. Seeds were sown on 1st June (2021/2022 and 2022/2023) at 2.5 cm depths with a density of 25Pln/m. Each plot (10.5 m^2) consisted of 5 rows, 4 meters in length, spaced 50 cm apart. Soil moisture was maintained for germination, and irrigation was performed as needed. Weeds were manually controlled during crop growth. Field performance and yield parameters were evaluated as follow;

- Field emergence was monitored daily until no further emergences were observed and percentages of seedling emergence were calculated; FE% = (Number of emerged seeds / Total seeds evaluated) × 100.

At maturity, plants from the middle meter of each plot were harvested, and seed yields per unit area were recorded, including the number of pods per plant, seeds per plant, seed weight per plant (g), 100 seed weight (g), plot seed yield, and seed yield (ton/fed). Seed yield (ton/fed) was measured in kilograms from each plot and converted to kilograms per feddan (1 fed = 10.5 m² × 400). The results from the two growing seasons were combined and presented.

Statistical analysis

A factorial experiment was conducted using a Randomized Complete Block Design (RCBD). The averages from two growing seasons were calculated for the field experiment. Data were statistically analyzed using analysis of variance (ANOVA) at a 0.05 significance level, with Tukey HSD applied to identify significant differences among means. Co-Stat (version 6.400) software was used for the analysis, and ical statistics were created with Microsoft Excel (2021).

RESULTS

The data in **Table 1** indicate significant differences (P = 0.05) in stored soybean seed indices, indicating that cool storage, along with DE, NSE, and SG treatments over a one-month storage period, outperformed the other treatments studied.Cold storage treatments outperformed open storage, showing improvements of approximately 8% for SMC, 4.3% for G, 10% for EC, 7% for CT, and 75% for infected seeds. The highest storage indicators were recorded after the one-month storage treatment compared to other periods in the experiment. All seed treatments significantly outperformed the untreated control seeds, with DE showing the highest improvement rates with approximately 13% for G and 19% for CT. SG treatment achieved the best results with 8.8% for SMC and 30% for EC. Additionally, all treatments reduced infected seeds by around 62% compared to the untreated control seeds.

Table 1: Means of germination percentage (G%), electric conductivity test (EC, μs cm^{-1} g^{-1} dry seed), cold test (CT, %), and fungal diseases contamination (infected seeds, %)as affected by storage environments conditions, storage periods and seed treatments and their interactions on soybean stored seed quality indices.

Treatments / Traits	SMC (%)	G (%)	EC(μs cm^{-1} g^{-1})	CT (%)	Infected seeds (%)
Main factors					
I: Storage conditions (SC)					
- Open storage (Variable ˚C & RH)	7.94	82.12	44.28	73.12	6.26
- Cool storage (5 °C & 30% RH)	7.30	85.63	39.79	78.18	2.63
HSD 0.05	0.02	0.39	0.96	0.39	0.15
II: Storage periods (SP)					
- 1 Month	7.20	89.76	32.43	84.73	3.98
- 4 Months	7.47	85.80	37.97	80.41	3.28
- 8 Months	7.81	82.33	46.83	71.27	4.10
- 12 Months	8.00	77.62	50.93	66.19	6.43
HSD 0.05	0.04	0.72	1.80	0.74	0.29
III: Seed treatments (ST)					
- Untreated seed (Control)	8.01	76.99	52.07	66.58	8.36
- DE (20 g/kg^{1} dry seeds)	7.40	86.95	38.42	79.43	3.20
- NSE (25 g/kg^{1} dry seeds)	7.78	86.16	41.20	76.15	3.11
- SG (25 g/kg^{1} dry seeds)	7.30	85.41	36.47	78.45	3.12
HSD 0.05	0.04	0.72	1.80	0.74	0.29
Grand Means	7.61	83.87	42.03	75.65	4.44
CV	0.80	1.14	5.63	1.28	8.60

*Comparison methods; Tukey HSD at 0.05 level of probability, Grand Means, and coefficient of variation (CV)

Table 2: Pairwise comparisons test as affected by the interaction between storage environment conditions, storage periods, and seed treatments on soybean stored seed quality indices.

Treatments / Traits		SMC (%)	G (%)	EC (μs cm^{-1} g^{-1})	CT (%)	Infected seeds (%)
SC × SP	Open storage × 1 Month	7.27^{ef}	89.32^{a}	31.87^{f}	84.17^{ab}	4.96^{c}
	Open storage × 4 Month	7.72^{c}	84.77^{c}	40.61^{d}	77.41^{c}	4.17^{d}
	Open storage × 8 Month	8.31^{b}	79.46^{d}	48.64^{b}	67.89^{f}	5.72^{b}
	Open storage × 12 Month	8.45^{a}	74.90^{e}	56.00^{a}	63.00^{g}	10.19^{a}
	Cool storage× 1 Month	7.13^{g}	90.18^{a}	32.99^{ef}	85.27^{a}	2.99^{e}
	Cool storage× 4 Month	7.21^{f}	86.82^{b}	35.32$_{e}$	83.41^{b}	2.39^{f}
	Cool storage× 8 Month	7.29^{e}	85.18^{c}	45.00^{c}	74.65^{d}	2.47^{f}
	Cool storage× 12 Month	7.52^{d}	80.33^{d}	45.85^{bc}	69.37^{e}	2.67^{ef}
HSD 0.05		0.07	1.22	3.02	1.24	0.48
SC × ST	Open storage × Cont.	8.59^{a}	74.72^{e}	57.20^{a}	64.04^{f}	12.64^{a}
	Open storage × DE	7.54^{c}	85.11^{c}	40.19^{cd}	76.83^{c}	4.22^{b}
	Open storage × NSE	8.22^{b}	84.58^{c}	42.46^{c}	75.58^{d}	4.02^{b}
	Open storage × SG	7.41^{d}	84.04^{c}	37.26^{de}	76.02^{cd}	4.16^{b}
	Cool storage × Cont.	7.42^{d}	79.24^{d}	46.92^{b}	69.10^{e}	4.07^{b}
	Cool storage × DE	7.24^{f}	88.77^{a}	36.64^{e}	82.03^{a}	2.18^{c}
	Cool storage × NSE	7.32^{e}	87.74^{ab}	39.93^{cd}	80.68^{b}	2.20^{c}
	Cool storage × SG	7.17^{f}	86.77^{b}	35.68^{e}	80.88^{ab}	2.06^{c}
HSD 0.05		0.07	1.22	3.02	1.24	0.48
SP × ST	1 Month × Cont.	7.28^{gh}	88.89^{ab}	33.16^{i}	84.257^{a}	4.18^{d}
	1 Month × DE	7.17^{hi}	89.51^{ab}	32.29^{i}	85.17^{a}	3.99^{de}
	1 Month × NSE	7.24^{gh}	90.12^{ab}	31.74^{i}	85.12^{a}	3.89^{de}
	1 Month × SG	7.09^{i}	90.50^{a}	32.52^{i}	84.36^{a}	3.85^{de}
	4 Month × Cont.	7.70^{d}	79.15^{f}	42.83^{def}	71.22^{cd}	5.76^{c}
	4 Month × DE	7.33^{g}	89.46^{ab}	35.88^{ghi}	83.69^{a}	2.53^{g}
	4 Month × NSE	7.58^{de}	88.40^{b}	38.71^{fgh}	83.53^{a}	2.53^{g}
	4 Month × SG	7.25^{gh}	86.17^{c}	34.43^{hi}	83.19^{a}	2.31^{g}
	8 Month × Cont.	8.32^{b}	75.06^{g}	60.88^{b}	59.83^{e}	8.68^{b}
	8 Month × DE	7.48^{e}	86.00^{c}	41.41^{def}	77.00^{b}	2.51^{g}
	8 Month × NSE	8.07^{c}	84.48^{cd}	45.69^{cd}	72.76^{c}	2.70^{fg}
	8 Month × SG	7.35^{fg}	83.75^{d}	39.31^{efgh}	75.49^{b}	2.49^{g}
	12 Month × Cont.	8.72^{a}	64.83^{h}	71.37^{a}	50.99^{f}	14.81^{a}
	12 Month × DE	7.57^{e}	82.80^{de}	44.07^{cde}	71.86^{cd}	3.78^{de}
	12 Month × NSE	8.20^{b}	81.64^{e}	48.65^{c}	71.12^{cd}	3.32^{ef}
	12 Month × SG	7.46^{ef}	81.19^{e}	39.62^{efg}	70.76^{d}	3.81^{de}
HSD 0.05		0.12	1.96	4.87	1.99	0.78

- SC: Storage conditions, ST: Seed treatments, DE: Diatomaceous Earth at 20 g/kg^{1} dry seeds, NSE: Neem seed extract at 25 g/kg^{1} dry seeds, SG: Silica gel at 25 g/kg^{1} dry seeds. Comparison methods; Tukey HSD at 0.05 level of probability.

Regarding the pairwise comparisons test, the interaction between storage environment conditions, storage periods, and seed treatments significantly (P = 0.05) affected all studied seed storage indices (**Table 2**). The interaction treatment (cool storage x one month of storage) resulted in the highest seed vigor indices compared to other treatments involving storage conditions and periods. The best rates were 7.13% for SMC, 90.18% for G, 32.99 µs cm^{-1} g^{-1} for EC, 85.27% for CT, and 2.99% for infected seed. Additionally, the combinations of DE, NSE, or SG with storage periods achieved the highest seed vigor indices compared to the untreated control seeds across all duration periods. Furthermore, the cool storage combined with DE resulted in simple increases in stored seed quality indices compared to the other combined treatments.

Table 3 indicates significant differences (P = 0.05) in various soybean seed germinability tests. The results showed that cool storage conditions outperformed open storage, showing improvements of approximately 7.2% for G, 8.3% for SDW, and 19% for SVI (II). Additionally, all seed treatments (DE, NSE, and SG) demonstrated significantly higher rates than untreated control seeds, with increases of 14.6%, 12.5%, and 11.6% for G; 8.7% for SDW; and 24.2%, 21.4%, and 17.2% for SVI(II), respectively.

Table 3: Means of germination percentage (G%), mean germination time (day), seedling dry weight (mg), and seedling vigor index (II) as affected by storage environments conditions and seed treatmentson soybean seeds germinability indices.

Treatments / Traits	G(%)	MGT (day)	SDW(mg)	SVI (II)
<u>**Main factors**</u>				
I: Storage conditions (SC)				
- Open storage (Variable ˚C & RH)	79.46	3.88	0.24	18.89
- Cool storage (5°C & 30% RH)	85.19	3.75	0.26	21.91
HSD 0.05	0.59	0.06	2.40	0.16
II: Seed treatments (ST)				
- Untreated seed (Control)	75.07	4.01	0.23	17.63
- DE (20 g/kg^1 dry seeds)	86.01	3.77	0.25	21.90
- NSE (25 g/kg^1 dry seeds)	84.48	3.78	0.25	21.41
- SG (25 g/kg^1 dry seeds)	83.75	3.70	0.25	20.66
HSD 0.05	1.13	0.11	4.60	0.31
Grand means	82.32	3.81	0.24	20.40
CV	0.82	1.69	1.11	0.91

*Comparison methods; Tukey HSD at 0.05 level of probability, Grand Means, and coefficient of variation (CV)

Regarding the pairwise comparisons test, the combination between storage environment conditions and seed treatments significantly (P = 0.05) affected all studied seed germinability indices (**Table 4**). Cool storage combined with DE, NSE, and SG seed treatments significantly yielded the highest seed germination indices compared to the untreated control seeds stored at open or cool storage conditions. The combination of cool storage with DE, followed by cool storage with NSE and SG, produced the highest seed vigor indices compared to the untreated control, with increases of approximately 25%, 23%, and 22% for G; 23%, 18%, and 14% for SDW; and 51.5%, 46%, and 40% for SVI(II), respectively.

Table 4: Pairwise comparison test for the impact of storage environment and seed treatments interaction on soybean seed germination indices.

Treatments (SC × ST) / Traits	G(%)	MGT (day)	SDW(mg)	SVI (II)
- Open (Variable ˚C & RH) × Cont.	70.76^{d}	4.14^{a}	0.22^{e}	15.52^{f}
- Open (Variable ˚C & RH)× DE	83.57^{b}	3.82^{bc}	0.24^{d}	20.28^{d}
- Open (Variable ˚C & RH)× NSE	82.10^{b}	3.79^{bc}	0.25^{cd}	20.19^{de}
- Open (Variable ˚C & RH)× SG	81.42^{bc}	3.76^{bc}	0.24^{d}	19.53^{d}
- Cool (5°C & 30% RH)× Cont.	79.37^{c}	3.87^{b}	0.25^{cd}	19.72^{de}
- Cool(5°C & 30% RH) × DE	88.44^{a}	3.72^{bc}	0.27^{a}	23.52^{a}
- Cool (5°C & 30% RH)× NSE	86.86^{a}	3.76^{bc}	0.26^{ab}	22.63^{b}
- Cool (5°C & 30% RH)× SG	86.07^{a}	3.63^{c}	0.25^{bc}	21.78^{c}
HSD 0.05	1.94	0.18	7.897E-03	0.53

- SC: Storage conditions, ST: Seed treatments, DE: Diatomaceous Earth, NSE: Neem seed extract, SG: Silica gel. Comparison methods; Tukey HSD at 0.05 level of probability, Uppercase letters; Scheffe All-Pairwise Comparisons Test

Figure 1 demonstrates the significant increase in G% and SVI percentages due to seed treatments compared to the untreated control seedsunder varying storage conditions for soybean seeds. All seed treatments*i.e.*, DE, NSE, and SG outperformed the control treatment regardless of storage conditions, with the combination of cool storage and seed treatments showing the highest improvement. The most notable enhancements as indicated by DE were seen in G% (18.10% for open storage and 24.99% for cool storage) and in SVI (23.44% for open storage and 51.48% for cool storage).

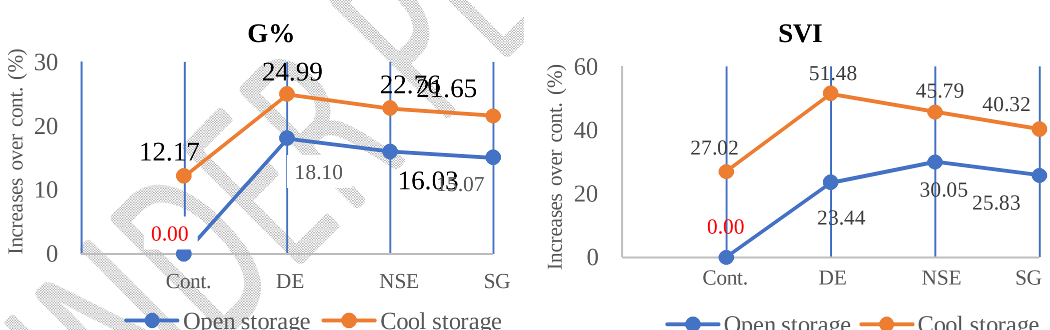

Fig 1: Relative increasesover control treatment of germination (G%) and seedling vigor index (SVI) induced by seed treatments under open and cool storage environments.

Table 5 indicates significant differences (P = 0.05) in various soybean field establishment parametersinfluenced by storage conditions and seed treatments. The results indicate that storing seeds at 5°C & 30% RH and treating them with 20 g/kg^{1} of DE resulted in superior outcomes compared to other methods.

Calculated the percentage of increases according to data in the **Table 5** showed that, cool storage at 5°C & 30% RH showed significant improvements over open storage, with increases of about 9.9% in FE%, 27.7% in No. pods/plant, 45.1% in No. seed/plant, and 10.8% in 100 seed wt. (g), and 45.3% in seed yield (ton/fed). All seed treatments (DE, NSE, and SG) displayed significantly higher rates than the untreated control seeds.Specifically, DE increased FE% by approximately 12.3%, pods per plant by 19.0%, seed weight per plant by 26.6 g, 100-seed weight by 21.1 g, and seed yield by 27.0 ton/fed. NSE and SG also had beneficial effects, though less pronounced than those of DE.

Table 5: Means of the two growing seasons of field emergence (FE%), No. pods/plant, No. seed/plant, seed weight/plant (g), 100 seed weight (g), plot yield (kg),and seed yield (ton/fed)as affected by storage environments conditions, and seed treatments and their interactions on soybean seed yield parameters, across the two growing seasons (2021/2022 and 2022/2023).

Treatments / Traits	FE (%)	No. pod/plant	No. seed/plant	Seed Wt./plant (g)	100 Seed Wt. (g)	Plot yield (kg)	Seed yield (t/fed)
Main factors							
I: Storage conditions (SC)							
- Open storage (Variable ˚C & RH)	76.40	19.25	43.45	8.61	17.74	2.71	1.08
- Cool storage (5°C & 30% RH)	84.00	24.59	60.02	12.50	19.66	3.93	1.57
HSD 0.05	0.70	0.85	1.06	0.43	0.58	0.13	0.05
II: Seed treatments (ST)							
- Untreated seed (Control)	74.02	19.73	46.44	8.85	16.16	2.79	1.11
- DE (20 g/kg[1] dry seeds)	83.13	23.48	54.21	11.21	19.58	3.53	1.41
- NSE (25 g/kg[1] dry seeds)	82.22	22.15	52.78	11.01	19.43	3.47	1.38
- SG (25 g/kg[1] dry seeds)	81.35	22.32	53.50	11.14	19.62	3.51	1.40
HSD 0.05	0.99	1.20	1.49	0.61	0.83	0.19	0.07
Grand means	80.18	21.92	51.73	10.55	18.70	3.32	1.33
CV	1.03	4.49	2.36	4.74	3.62	4.74	4.75

*Comparison methods; Tukey HSD at 0.05 level of probability, Grand Means, and CV

Table 6: Pairwise comparisons of soybean seed yield parameters impacted by the interaction between storage conditions and seed treatments across the two growing seasons (2021/2022 and 2022/2023).

Treatments (SC × ST) / Traits	FE (%)	No. pod/plant	No. seed/plant	Seed Wt./plant (g)	100 Seed Wt. (g)	Plot yield (kg)	Seed yield (t/fed)
- Open (Variable ˚C & RH)× Cont.	67.97^{d}	17.25^{e}	40.78^{d}	7.56^{c}	15.19^{e}	2.38^{c}	0.95^{c}
- Open (Variable ˚C & RH)× DE	80.56^{b}	20.58^{cd}	45.53^{c}	9.04^{b}	18.64^{bcd}	2.85^{b}	1.14^{b}
- Open (Variable ˚C & RH)× NSE	79.52^{bc}	19.13^{de}	43.05^{cd}	8.87^{bc}	18.50^{cd}	2.79^{bc}	1.11^{bc}
- Open (Variable ˚C & RH)× SG	77.53^{c}	20.06^{cd}	44.43^{c}	8.98^{bc}	18.62^{bcd}	2.83^{b}	1.13^{b}
- Cool (5°C & 30% RH)× Cont.	80.07^{b}	22.22^{bc}	52.11^{b}	10.15^{b}	17.14^{d}	3.20^{b}	1.27^{b}
- Cool (5°C & 30% RH)× DE	85.70^{a}	26.39^{a}	62.89^{a}	13.38^{a}	20.52^{ab}	4.21^{a}	1.68^{a}
- Cool (5°C & 30% RH)× NSE	84.91^{a}	25.18^{a}	62.52^{a}	13.15^{a}	20.36^{abc}	4.14^{a}	1.65^{a}
- Cool (5°C & 30% RH)× SG	85.17^{a}	24.58^{ab}	62.57^{a}	13.31^{a}	20.62^{a}	4.19^{a}	1.67^{a}
HSD 0.05	1.40	1.70	2.12	0.86	1.17	0.27	0.11

- SC: Storage conditions, ST: Seed treatments, DE: Diatomaceous Earth, NSE: Neem seed extract, SG: Silica gel. Comparison methods; Tukey HSD at 0.05 level of probability, Uppercase letters; Scheffe All-Pairwise Comparisons Test.

In the pairwise comparisons test, it was found that the combination of storage environment conditions and seed treatments significantly (P = 0.05) affected all studied seed field indices (**Table 6**). Specifically, cool storage in combination with DE, NSE, and SG seed treatments achieved the highest seed yield indices compared to other storage and treatment methods, with the best results recorded by the combination of cool storage and DE seed treatment.

Figure 2 in the experiment illustrates that applying seed treatments led to significant increases in seed weight per plant (g) and seed yield (ton/fed) over the control treatment. All DE, NSE, and SG seed treatments outperformed the control across different storage conditions for soybean seeds, with the most relative increases observed in the combination treatment of (cool storage × DE). The highest increases over control treatment in seed weight per plant were 76.98%, and the seed yield reached 76.84% (ton/fed).

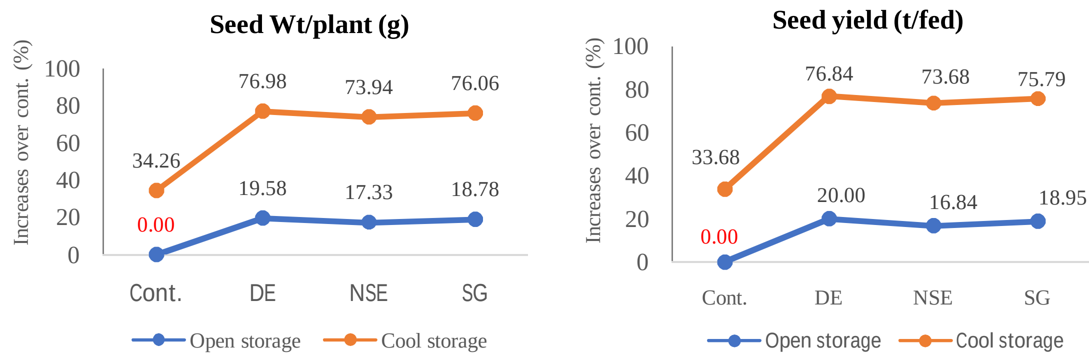

Figure 2: Relative increases in seed weight per plant (g) and seed yield per feddan (ton/fed) from seed treatments in open and cool storage conditions over the two growing seasons (2021/2022 and 2022/2023).

DISSCUSSIONS

Adverse storage conditions, including temperature and humidity, can degrade seed quality, irrespective of the seeds' initial state. Our findings indicate that cold storage significantly enhances the storability and quality of soybean seeds compared to room temperature storage [6&25). Research shows that soybean seeds stored at cold temperatures (around 4°C) sustain higher germination rates and better physiological quality than those stored at room temperature (approximately 25°C). For instance, vacuum-packed soybean seeds in cold storage maintained a germination rate above 91% after 12 months, while those at room temperature saw a significant decline in viability [26]. Storage duration is also crucial; seeds in cold conditions exhibited minimal deterioration over time, whereas seeds at room temperature experienced rapid declines due to increased metabolic activity and susceptibility to fungal infections [27]. Thus, lower temperatures and controlled humidity in cold storage effectively preserve the viability and vigor of soybean seeds, making this method preferred for long-term storage. [28] noted a significant increase in seed conductivity after 180 days, indicating cellular structure changes. Soybean seed quality is sensitive to storage duration and conditions, with vigor declining before standard germination rates. Deterioration correlates with storage time, leading to decreased food reserves, heightened enzyme activity, increased fat acidity, and greater membrane permeability. As seeds age, their germination capacity diminishes due to catabolic processes [29]. Vigor significantly drops after six months; seeds lose 23% of vigor after three months and 71% after six months compared to fresh seeds. This deterioration impacts quality, performance, and establishment rates [30]. However, low temperatures and controlled humidity can slow biochemical and metabolic processes, thereby extending seed quality [31]. Our findings align with Da Silva *et al*. (2022) [9], who identified temperature and storage duration as key predictors of soybean seed quality over time.

Diatomaceous earth (DE) benefits soybean seed storage in several ways: (1) Enhanced germination and vigor: Treating soybean seeds with DE improves germination rates and seedling vigor. Bern *et al.* (2018) [32] found DE-treated seeds reached 90-95% germination after 12 months, compared to 60-70% for untreated seeds. DE's desiccant properties help keep seeds dry, preventing fungal growth and insect issues. (2) Reduced insect damage: DE acts as a physical insecticide, damaging insect exoskeletons[33]. The DE significantly reduces infestations by pests and lessens seed damage and weight loss. (3) Longer storability: DE-treated seeds retain quality for 12-18 months, while untreated ones deteriorate after 6-9 months, due to DE maintaining low moisture and protecting against pests [33].

Neem oil is known to possess acaricidal, antibacterial, antifungal, antimalarial, antiparasitic, and anti-inflammatory properties. Azadirachtin, a biodegradable tetranortriterpenoid derived from neem seeds, has gained recognition as a natural biopesticide due to its effectiveness and low side effects [34]. Neem seed powder is highly effective, requiring only small amounts at low concentrations to eliminate fungal diseases from groundnut seeds, making it an economical solution. Neem seed powder contains biologically active compounds that are effective against certain pathogenic fungi, positioning it as a natural option for controlling these pathogens[35].As a result, neem seed extract enhances seed viability and vigor, leading to better seedling performance and increased seed yield.

Silica gel enhances soybean seed storage in several key ways: (1) Moisture control and seed viability: Bern *et al.* (2019) [36] demonstrated that using silica gel packets helps maintain optimal moisture levels, preventing both excessive drying and moisture absorption. Soybean seeds stored with silica gel achieved over 90% germination rates after 12 months, compared to around 70% for those without desiccants. (2) Fungal growth and mycotoxin prevention: Silica gel's moisture-regulating properties inhibit fungal growth and mycotoxin accumulation. Battilani*et al.* (2016)[37] found that soybean seeds stored with silica gel had significantly lower incidences of Aspergillus flavus and aflatoxin B1 over a six-month period. (3) Insect pest management: Silica gel also helps deter insect infestations. Kavallieratos *et al.* (2018) [38] found that incorporating silica gel packets significantly reduced populations and damage from pests like the Cowpea weevil and Maize weevil in stored soybean seeds.

CONCLUSION

Natural extracts are preferred for their minimal environmental impact and lower risks compared to synthetic options. The application of diatomaceous earth, neem seed extract, and silica gel on soybean seeds during storage has been shown to provide several benefits. These natural treatments can improve seed germination and vigor, reduce insect damage, and extend the storability of the seeds. This integrated approach using multiple natural protectants offers a sustainable and environmentally-friendly solution for maintaining soybean seed quality during prolonged storage periods.

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