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Research Article

Effect of Storage Duration on the Insecticidal Activity of Neem (*Azadirachta indica*) Seeds Against Fall Armyworm (*Spodoptera frugiperda*)

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Abstract

Neem (*Azadirachta indica*), native to India, thrives in tropical and sub-tropical climates. Its efficacy against economically significant pests is attributed to the pesticidal properties found in its leaves, bark, and roots. Neem seeds are always harvested by smallholder farmers and stored for use in crop-growing seasons when their availability is scarce. This study aimed to determine if storage duration can affect the toxicity of these neem seeds. Neem seed extracts were tested for toxicity after being stored at different durations, they were stored for 1-3 years. The objective of this study was to analyze the effects of storage time on the insecticidal activity of neem seeds. Fall armyworm larvae were used to test for the neem seed extracts' toxicity by feeding on maize plants which were sprayed with these extracts. The experimental design used was a complete randomized design in the laboratory. Extracts were made from neem seeds stored for 1-3 years which were harvested from year 2019, 2020, 2021 and 2022. Analysis of FAW larvae mortality at different concentrations (0.5%, 1.0%, 1.5%, and 2.0%) together with the control treatment under laboratory conditions was done. Damage severity rates of maize leaves in which the FAW larvae were allowed to feed were measured and FAW larvae mortality was calculated. The results showed a significant interaction between neem seed storage time and FAW mortality. The results showed that extracts from fresh neem seeds and those stored for one year had the lowest plant damage score. Also, the lowest mortality was obtained from seeds stored for 1 year and the fresh seeds because the larvae refrained from feeding. Following this study, we recommend the usage of fresh neem seeds and those stored for up to a year in the management of fall armyworms.

Keywords

Storage Duration, Neem Seeds, Fall Armyworm, Insecticidal Activity

1. Introduction

Agriculture serves as a crucial sector, providing essential resources like food, fibers, and biofuels, contributing to human well-being [1]. With the global population steadily rising, agriculture becomes even more pivotal, not only to meet the food demand but also to enhance a country's socio-economic standing [6]. However, due to the growing population and limited land resources, chemical fertilizers and pesticides are being increasingly employed to boost food pro-

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duction and crop yields. Regrettably, this practice is resulting in a decline in soil fertility and degradation, posing significant challenges [14]. Due to the detrimental consequences and repercussions of using synthetic chemical pesticides and the necessity to replace them, interest in developing pesticides of natural origin has surged in recent years [12].

Most farmers worldwide heavily depend on the usage of pesticides to protect the food and other commercial products they produce [6]. Although these chemical pesticides are believed to be genuine and effective against a variety of insect pests, their application comes with more issues and concerns than benefits [23]. Environmental harm, toxicity toward non-targeted animals, and the emergence of insect populations resistant to these synthetic chemical pesticides are some of the downsides associated with their use [9]. The utilization of chemical fertilizers and pesticides significantly impacts the environment, resulting in consequences such as water hardness, the emergence of insect resistance, genetic variations in plants, elevated toxicity levels within the food chain, and potential human health concerns including skin cancer [17]. These practices also contribute to water pollution due to the accumulation of nitrates stemming from the breakdown of nitrogen-based fertilizers [10]. Moreover, the excessive application of chemical fertilizers and pesticides in agriculture can progressively diminish the presence of beneficial microorganisms within the soil [5].

To establish a more environmentally friendly approach to plant protection, the introduction of alternatives to chemical fertilizers and pesticides becomes essential. This includes the adoption of biofertilizers and biopesticides as substitutes [13].

Under insect pest management, the focus has switched to the development of efficient biopesticides and those of natural origin to address the issues caused by the usage of chemical synthetic pesticides [11]. Biopesticides represent an eco-friendly and non-toxic class of natural pesticides sourced from elements like plants, bacteria, and minerals. They possess the capability to manage pests without causing harm to the environment [13]. Research indicates that certain plant components such as neem leaves, barks, and fruits exhibit robust effectiveness in managing a diverse array of insect pests [3]. Utilizing neem extracts and similar pesticidal plant materials offers advantages like reduced risk of pathogen resistance development, minimal toxicity to mammals, lower health concerns, and diminished environmental impact [4].

The Neem tree has received renewed interest as a result of the growing interest in biopesticides [12]. It has several benefits over most botanically active plants because of its wide diversity in secondary metabolites, which have a variety of pesticidal actions against different pests [3]. It is grown throughout the world's tropical and subtropical regions, despite being a native of South Eastern Asia [2]. It is also grown in Tanzania for a variety of uses, including plant protection, shade, and medicinal purposes. In recent years, the neem tree has drawn particular interest in pest control and plant protection, leading to its commercialization for optimal utilization [16]. Its extracts are poisonous, antifeedant, repulsive, and have an impact on growth regulators [12]. Although the neem tree can be used in all of its parts and has pesticidal properties, it is believed that the seeds contain the most significant active ingredient (azadirachtin) and do so in greater quantity than other sections of the tree [21]. Because of its short fruiting seasons, researchers and small-scale farmers that use these seeds gather and preserve them for use off-season or as needed [15]. There are factors which affect the botanical plants' efficacy in their insecticidal activities which are geographical location, climatic conditions, degradation of extracts in water or organic solvents, and also during storage where temperature, direct sunlight and microorganisms may trigger degradation [8].

Studies on the effect of time and storage methods on the efficacy of neem seeds indicated that the seed's efficacy deteriorates over time [18]. A study done in Sudan by Ahmed, 2010 focusing on controlling the red flour beetle (*Tribolium castaneum)* indicated significant losses in the efficacy of neem seeds with storage time from one to two years. It was observed that storage of neem seeds in jute sacks or exposed under room conditions can reduce the negative effect of storage on the efficacy of neem seeds [2]. The raw material of neem seeds as a candidate for biopesticide can be stored as powder or in its natural state (solid form) but storage methods that do not alter the content and activity of these compounds need to be addressed. The effectiveness of pesticidal plants in real-world settings, their economic viability, their effects on beneficial creatures, and their storage characteristics are all areas of research that have not yet been adequately addressed [25]. This study sought to unravel how the neem seeds' efficacy against fall armyworm (FAW) larvae is affected by storage time.

2. Methodology

Description of the Study Area

The study was conducted at The African Seed laboratory at Sokoine University of Agriculture. Neem seeds collected and stored by farmers from 2019 to 2022 were obtained from Mvomero district in Morogoro region. Mvomero district lies between 300m to 400m above sea level located between longitudes 37°20′ and 38°05′ east and between latitudes 05°80′ and 07°40′south.

3. Extract Toxicity Bioassay

3.1. Collection of Fall Armyworm (FAW) Larvae

Three distinct insecticide-free organic maize fields were used to gather the FAW larvae, and their offspring were then used in the experiment. Until they were pupated, the larvae were fed with pieces of insecticide-free maize leaves. Then, for aeration, they were transferred to 500 ml transparent containers covered in mesh. Adults that had just emerged were put into cages to mate, and maize seedlings were given to them as a substrate for oviposition. To prevent cannibalism, the newly hatched larvae were separated into different containers, and the larvae were used for bioassays. [22]

3.2. Preparation of Neem Extracts

Neem seed extract preparation followed Sidding's, (1991) method with few adjustments. Neem seeds were harvested over a four-year period (2019- 2022). They were dried in the shade, soaked in water for 24 hours, and then decorticated. A micro plant grinder was used to reduce the decorticated seeds to a fine powder. The needed amount of powder was weighed, combined with absolute ethanol (98%), swirled with a magnetic stirrer, and allowed to stand for 24 hours before filtering. Later four concentrations (0.5%, 1.0%, 1.5%, and 2.0%) were obtained from the solution.

3.3. Treatment Application

The FAW larvae were used to evaluate the toxicity of the neem seeds. The study was set up on a complete randomized design (CRD) and replicated three times. The larvae were put in plastic ventilated containers and given 24 hours to settle in and adapt. Neem seeds from all four years were used to make four concentrations (0.5%, 1.0%, 1.5%, and 2.0%) which were used together with one control (distilled water). Maize leaves were placed in each container and then sprayed with 20 ml of extracts for adequate and uniform coverage. These leaves were replaced every 48hrs then freshly sprayed. Insect mortality was assessed 36h, 48h and 60h after treatment application together with observational recordings of larval behavior or any morphological problems. The larvae were considered dead if they did not right themselves after being placed on the dorsal surface. The experiment was repeated two times to confirm the cause of mortality. This methodology was adapted from. [23]

3.4. Assessment of Maize Leaves Damage

The assessment of maize leaves damage in all replications was done by using a rating scale as described by Williams and Davis, that 0 = no visible leaf damage, 1 = only pin-hole damage to the leaves, 2 = pin-hole and shot-hole damage to leaves, 3 = small elongated holes (5–10 mm) on 1–3 leaves, 4 = midsized holes (10–30 mm) on 4–7 leaves, 5 = large elongated holes (>30 mm) or small portions eaten on 3–5 leaves, 6 = elongated holes (>30 mm) and large portions eaten on 3–5 leaves, 7 = elongated holes (>30 cm) and 50% of leaf eaten, 8 = elongated holes (30 cm) and large portions eaten on 70% of leaves, 9 = most leaves have long holes [24].

3.5. Statistical Analysis

Data sets were checked for normality and then subjected to statistical analyses. GenStat, 22nd edition was used to tabulate and statistically analyze the data. To determine whether there is a statistically significant difference between the applied treatments on larvae mortality, analysis of variance (ANOVA) was performed. The Duncan's Multiple Range test was used to separate the means whenever significant differences were found $P < 0.05$.

4. Results

Effect of Neem Seed Extracts of Three Years (2019- 2022) on Mortality and Leaf Damage.

4.1. At 36 Hours of Exposure

Leaf damage score

The results showed that the interaction effect between the storage duration of neem seeds and per cent concentration had significantly influenced the leaf damage level inflicted by FAW (P=0.016) (Table 1). The seeds stored for one year had significantly the highest leaf damage while the fresh seeds had significantly the lowest damage score (Figure 1). However, no significant difference in FAW leaf damage was observed between trays treated with seeds stored for three years, two years and one year.

Table 1. *Analysis of variance (ANOVA) table showing the effect of neem seed extracts of 4 years on leaf damage score by FAW larvae after 36 hours of exposure.*

Df	Sum Sq	Mean Sq	F value	Pr (>F)	
Storage.Time	3	33.513	11.17	14.55	5.25E-07
Neam.Extract.Conc	1	0.04533	0.045	0.058	0.809
Storage.Time: Neam.Extract.Conc	3	8.597	2.864	3.7328	0.016
Residuals	52	39.90499	0.767		

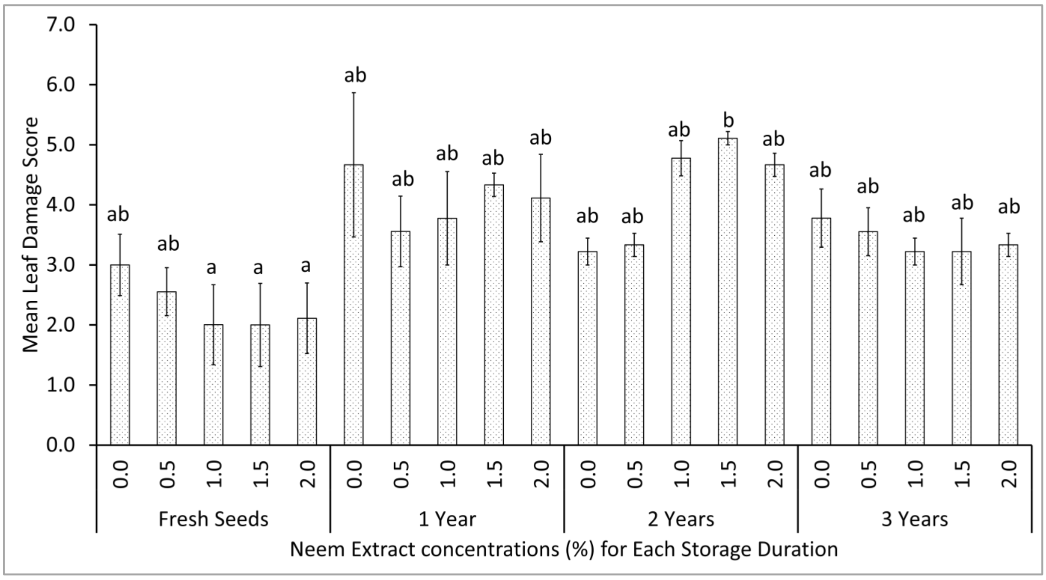

Figure 1. Main effect of year on leaf score damage on 36 hours.

FAW mortality

The results showed that storage duration and extract concentration had a significant effect on FAW mortality (P<0.05) (Table 2). Trays treated with 1.5% and 2.0% had significantly the highest mortality count than the lower concentrations (Figure 2).

Table 2. Analysis of variance (ANOVA) table showing the effect of neem seed extracts of 4 years on mortality of FAW larvae after 36 hours of exposure.

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	4	6530.61	1632.65	17.14	<.001
Storage time	3	9860.54	3286.85	34.51	<.001
Concentration. Storage T	12	1142.86	95.24	1	0.466
Residual	40	3809.52	95.24		
Total	59	21343.54			

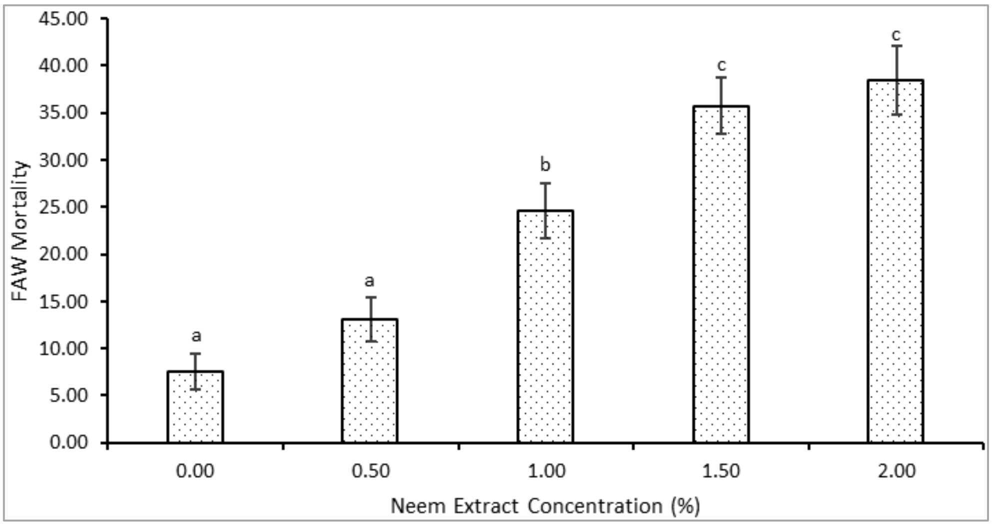

Figure 2. Main effect of concentration on FAW larvae Mortality in 36 hours.

Storage duration had a significant effect (p<0.001) on FAW larvae mortality. FAW mortality in trays treated with seeds stored for one year was significantly higher than the other seeds. While trays treated with extracts from fresh seeds had the lowest mortality (Figure 3).

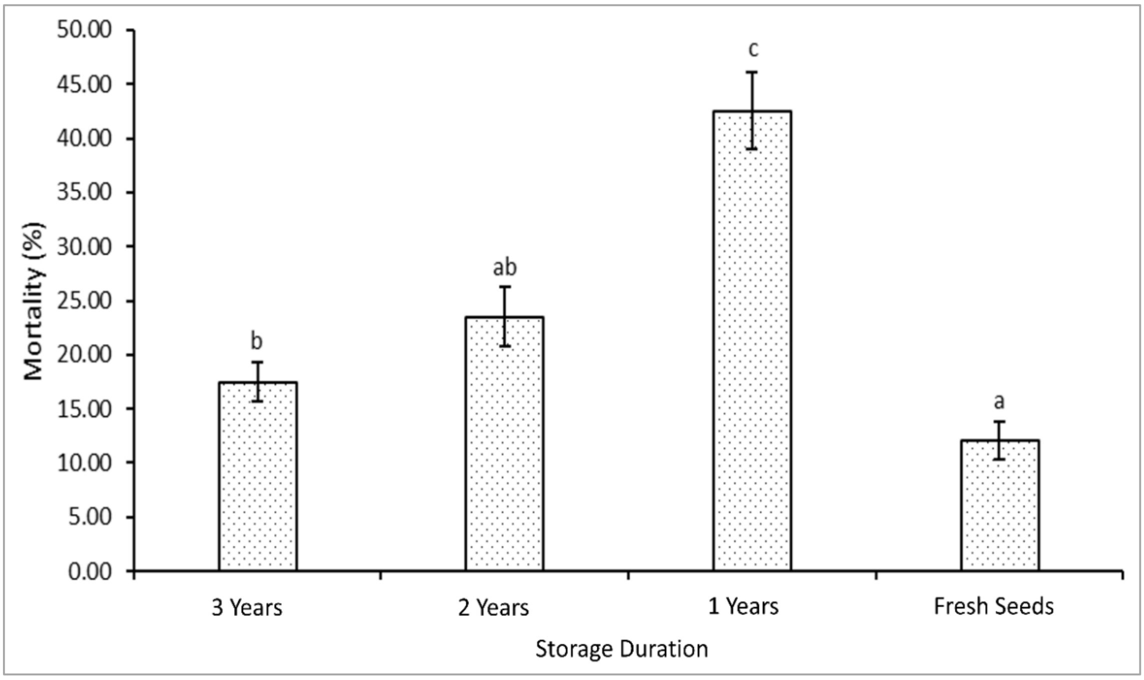

Figure 3. *Main effect of year on FAW larvae Mortality in 36 hours.*

4.2. At 48 Hours of Exposure

Leaf damage score

At 48 hours of exposure, storage duration had a significant effect (p<0.001) on leaf damage (Table 3). Significant higher leaf damage was observed when trays treated with seeds stored for 1 year and 2 years compared with the trays treated with seeds stored for 3 years and the fresh seeds (Figure 4).

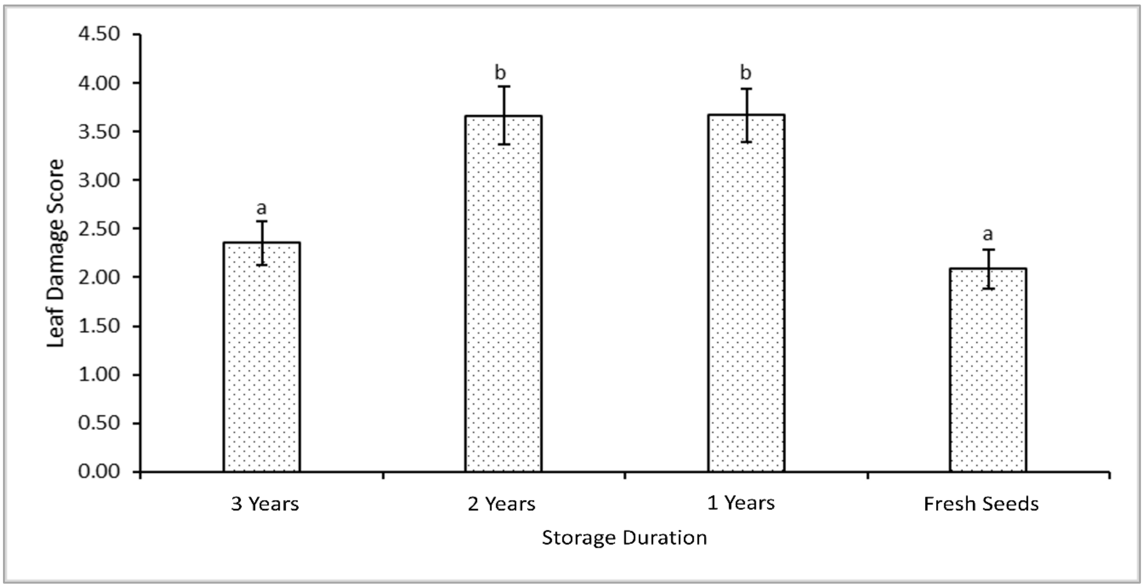

Figure 4. *Main effect of year on leaf score damage in 48 hours.*

Table 3. *Analysis of variance (ANOVA) table showing the effect of neem seed extracts of 4 years on leaf damage score at 48 hours of exposure.*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	4	5.0741	1.2685	1.35	0.269
Storage time	3	31.8296	10.6099	11.28	<.001
Concentration. Storage T	12	11.0593	0.9216	0.98	0.484
Residual	40	37.6296	0.9407		
Total	59	85.5926			

FAW larvae mortality

Results showed that the interaction between storage duration and extract concentrations had a significant effect on causing FAW mortality (Table 4). The analysis of main effects also had a significant difference (p<0.001). A significantly higher FAW mortality count was observed when trays treated with seeds stored for 1 and 2 years (s) compared with the trays treated with seeds stored for 3 years and the fresh seeds. However, the differences were not significant when seeds stored for 3 years were compared to the fresh seeds but those stored for 2 years when compared to those stored for 1 year had a significant difference (Figure 5).

Table 4. *Analysis of variance (ANOVA) table showing the effect of neem seed extracts of 4 years on FAW larvae mortality at 48 hours of exposure.*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	4	9605.4	2401.4	19.61	<.001
Storage time	3	9047.6	3015.9	24.63	<.001
Concentration. Storage T	12	3265.3	272.1	20.52	0.029
Residual	40	4898	122.4		
Total	59	26816.3			

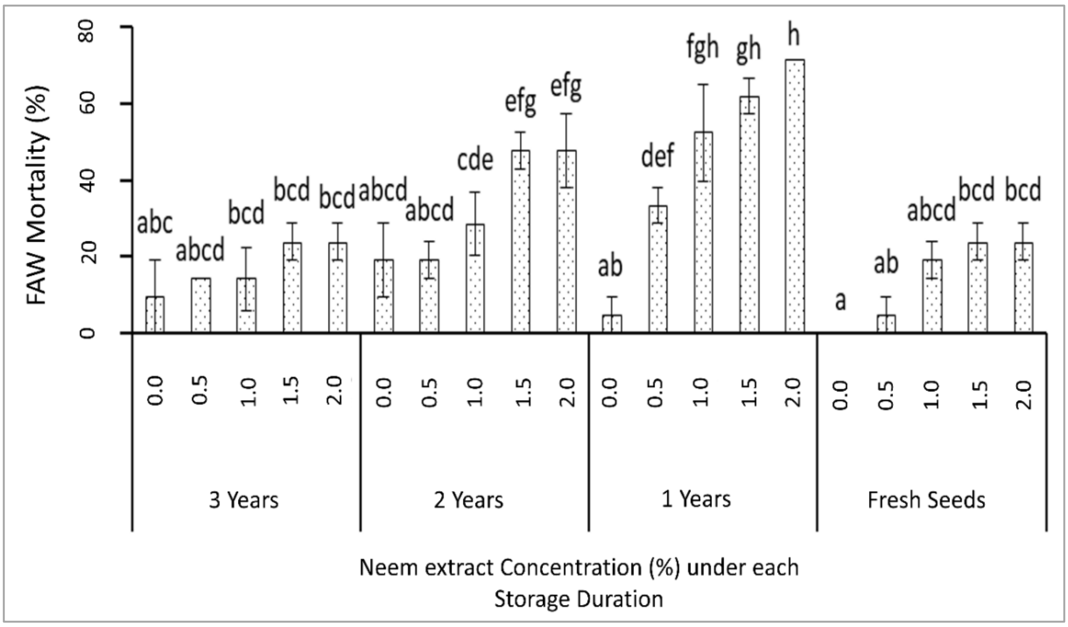

Figure 5. *Interaction effects of concentration and year on FAW larvae Mortality at 48 hours.*

4.3. At 60 Hours of Exposure

Leaf damage score

The results showed no significance effect on the interaction between storage duration and extracts concentration on leaf damage but storage duration as a main effect had a significant difference (p<0.001) on leaf damage (Table 5). The highest leaf damage score was recorded from trays sprayed with neem seed extracts from seeds stored for 2 years and the lowest damage score rate was recorded from trays sprayed with neem seed extracts from fresh neem seeds. There was a significant difference among the storage durations (Figure 6).

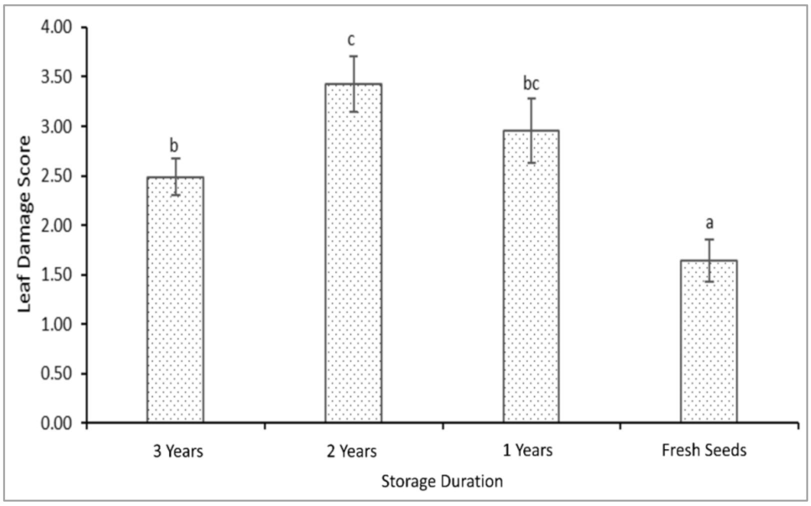

Figure 6. *Main effect of storage duration (years) on Leaf damage score at 60 hours.*

Table 5. *Analysis of variance (ANOVA) table showing the effect of neem seed extracts of 4 years on leaf damage score at 48 hours of exposure.*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	4	9.456	2.364	2.35	0.071
Year	3	25.872	8.624	8.56	<.001
Concentration. Year	12	6.619	0.552	0.55	0.87
Residual	40	40.296	1.007		
Total	59	82.243			

FAW larvae mortality

Results showed that the interaction between storage duration and extracts concentrations had a significant effect on causing FAW mortality (Table 6). The analysis of main effects also had a significant difference (p<0.001). Significant higher FAW mortality count was observed in trays treated with seeds stored for 2 years and 1 year compared with the trays treated with seeds stored for 3 years and the fresh seeds. There was a significant difference across all trays treated with seeds stored for different durations (Figure 6).

Table 6. *Analysis of variance (ANOVA) table showing effect of neem seed extracts of 4 years on FAW larvae mortality at 60 hours of exposure.*

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration	4	10972.8	2743.2	20.68	<.001
Year	3	6381	2127	16.03	<.001

Source of variation	d.f.	s.s.	m.s.	v.r.	F pr.
Concentration. Year	12	3993.2	332.8	2.51	0.015
Residual	40	5306. and 1	132.7		
Total	59	26653.1			

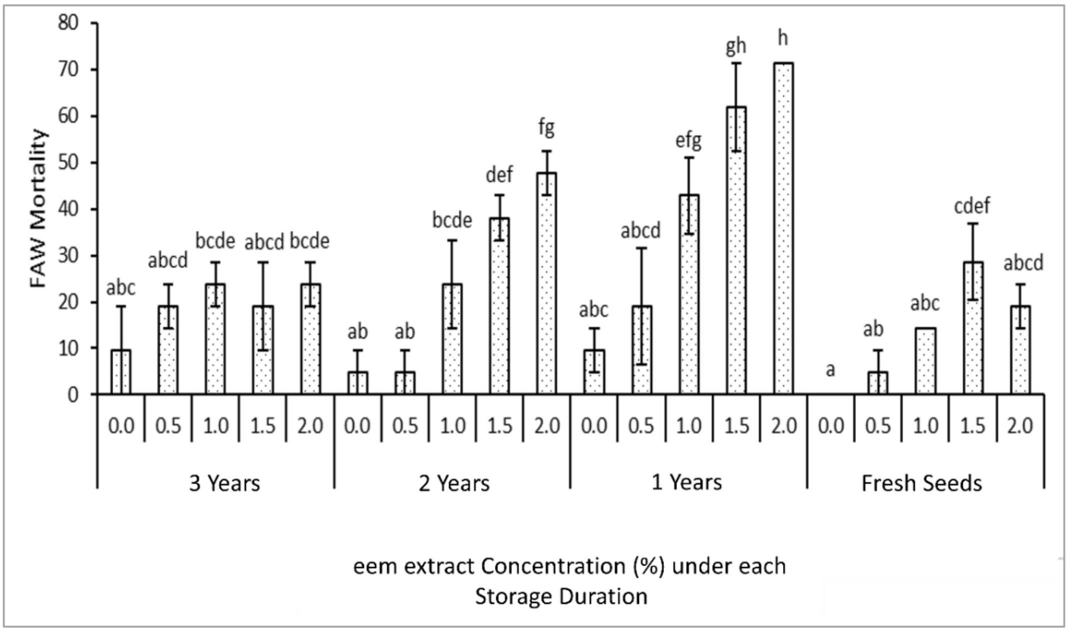

Figure 7. *Interaction effects of concentration and storage duration (years) on FAW Mortality on 60 hours.*

The highest mortality level was recorded from trays treated with 1.5%, 2.0% concentrations from seeds stored for 2 years and seeds stored for 1. The lowest level of mortality was recorded from trays sprayed with 0.5%, 1.0% concentrations made from seeds stored for 3 years and from fresh seeds (Figure 7).

5. Discussion

In this study extracts from seeds stored for 1 year showed the highest mortality count compared to fresh seeds and the oldest seeds stored for 3 years. This demonstrates that the toxicity of the neem seeds lasted for up to one year. It was further observed that neem seeds stored for more than two years tend to lose their efficacy. The low mortalities recorded in trays sprayed with extracts from fresh seeds may be due to repellent effect emitted by fresh neem seeds preventing the larvae from feeding hence not being affected by the toxicity of neem extracts. Similar trend of results were reported in a study whereby water extracts from seeds stored for a short time had the best results when compared to water extracts of the oldest seeds with longer time of storage in causing mortality of insects pests of okra crop in Sudan [18].

Low level of leaf damage was observed from trays treated with extracts from fresh seeds. This can be explained by the fact that fresh neem fruits have a higher level of repellent and antifeedant effect when compared to older seeds [21]. Larvae were observed crawling away from the treated maize leaves in trays treated with extracts from fresh seeds, but this was not observed in treatments containing extracts from older neem seeds. The characteristics of antifeedant and repellent effect in fresh neem seeds can be used in preventing the FAW larvae from causing plant damage. It was reported that the antifeedant and repellent of neem seeds is reduced with one year of storage where the effects of storage conditions and duration of potency of Neem as a homemade insecticide was studied against the red flour beetle [7].

Significant differences were observed in trays treated with extracts from neem seeds stored for up to two years and they all caused mortality significantly when compared with controls. Therefore, it can be concluded that the toxicity of neem seeds can be maintained for relatively long up to two years before it starts deteriorating. It was also observed that, low level of mortality was recorded in treatments with lower level of concentrations but physiological effects like prolonged larval stage in non-dead larvae were observed. In reference to the non-dead larvae from controls trays it was concluded that the larval stage was prolonged suggesting that larvae which survived lower doses suffered physiological effects.

Studies investigating the storage conditions of neem seeds are notably limited in the country. The findings obtained in this study correspond with results from other researchers who examined various insect pests, yielding comparable outcomes. The impact of time and storage methods on neem seed potency has been studied and highlighted that the peak insecticidal effect of neem occurred within a two-year storage period.

Moreover, another study demonstrated that azadirachtin, the most potent limonoid, exhibits toxic activity against arthropods, inducing mortality and disrupting hormonal processes within the insects' bodies, ultimately affecting hormonal activities and causing mortality [21].

Neem products tend to show delayed mortalities in insect pests which increase with time and concentration as reported in this study. It was reported that the toxicity of neem extracts acts through systemic action rather than contact action [20], which aligns with the results reported by this study. Proper storage of neem seeds is important for them to retain their toxicity against pests. In a report by [19] it was insisted that it is important to store neem seeds away from high temperatures, direct UV light and rainfall to slow down their degeneration. Also, there is a similar report that when neem seeds are stored in jute sacks under room conditions, the effects of storage on the efficacy of these seeds can be reduced hence retaining their active ingredient's efficiency [7]. More studies can be done to determine the effects of storage durations of other botanical plants used by smallholder farmers in pest management. Even though old seeds caused the highest mortality of FAW, fresh seeds are recommended to be used because they succeeded in preventing plant damage and maintaining it to a relatively low level when a low level of damage was scored unlike in older seeds.

6. Conclusion

According to the results of this study, fresh neem seeds and neem seeds stored for one year or less exhibit high repellant and antifeedant effects on FAW larvae. However, as storage time increases, these effects lessen even though the toxicity is maintained for a longer period, up to two years. This study recommends that farmers use fresh neem seeds and seeds maintained in jute sacks for one year in a dark room to manage FAW based on the local environmental conditions in Morogoro, Tanzania.

Abbreviations

FAW	Fall Armyworm
CRD	Complete Randomized Design
ANOVA	Analysis of Variance

Conflicts of Interest

The author declares no conflicts of interest.

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