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Safe Storage Grain Moisture of FABA Bean (*Vicia FABA l.*) for the Management of Acanthoscelides Obtectus say (Coleoptera: Bruchidae) in Amhara Region, Ethiopia

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Abstract

FABA bean is the earliest domesticated legume crop used as a source of protein. It is the main source of organic protein especially in developing countries. However, the crop is seriously damaged by bruchids, Acanthoscelides obtectus (Say) depending on grain moisture content. The experiment was conducted to determine the optimum grain moisture content of FABA bean grains during storage to manage bruchid infestation on FABA bean grains. For the study 5-5.5%, 9-9.5%, 11-11.5%, 14-14.5%, 16-16.5% and 20-20.5% grain moisture levels were considered. Twenty unsexed day one old bruchids were added to a two litter plastic pot contained each one kg treated grains. The number of bruchid eggs, number of emerged progenies, number of holed grains, grain weight loss, number of germinated grains, and developmental period of bruchids were recorded. There was no egg recorded at 5-5.5% grain moisture content, while 393±5.86 eggs were recorded at 20-20.5% grain moisture content. All grains were free from holes at 5-5.5% grain moisture content. Grains with 9-9.5% moisture content only had 12% grains with holes. The highest FABA bean grain weight loss was 18.6%±1.87 which was recorded at 20-20.5% grain moisture content. About 98.3% of the grains were germinated and developmental periods of the bruchids were extended for additional 17.5 days at 9-9.5% grain moisture content. From the current study, it can be concluded that grain moisture content ranging from 9% to 11.5% were safe for FABA bean grain storage from the point of view of bruchids' infestation.

Keywords: Bruchids; Egg; Progeny; Infestation; Grain; Moisture; Hole

Introduction

FABA bean, Vicia FABA L. is one of the earliest domesticated legume crops probably in the late Neolithic period [1]. Ethiopia is the secondary center of diversity for the crop [2]. The country is the second producer of the crop in the world next to China and the leading producer of the crop in Africa [1]. The crop is vital as a human food in Ethiopia and other developing countries and animal feed in industrialized countries [2]. It is used as a source of protein for peoples who can't afford animal proteins [3].

FABA bean is an important crop for various traditional dishes [3]. However, FABA bean storage is constrained by bruchids damage which causes both quantitative and qualitative losses in the store [4] Grain moisture content is a very critical factor for bruchid infestation in the storage [5]. The risk of bruchid damage is high on FABA bean grain stored at grain moisture content above 9% [5,6]. Hence, looking for optimum grain moisture content at which reproduction and development of bruchids are curtailed is crucial [5]. As FABA bean is very important crop in the Region while bruchids are damaging the grain started from the field to storage based on the availability of moisture contents in FABA bean grains. Therefore, the objective of the experiment was to look for optimum grain moisture content at the time of grain storage used to avoid or minimize bruchid infestation.

Method and Materials

Description of the study site

The study was conducted in Eastern Gojjam Zone, Debre Markose University which is located at 300km and 265km away from Addis Ababa and Bahir Dar, respectively. The average elevation of the study area was 2,400 meters above sea level (MASL). The rainfall pattern was Uni-modal with a mean annual rainfall of 1500 millimeter (mm). The daily average temperature was 24 ˚C. Major crops grown by the farmers were teff (Eragrostis tef Zucc. (Trotter)), wheat (Triticum aestivum L.), maize (Zea mays L.), FABA bean (Vicia FABA L.), chickpea (Cicer arietinum L.) and rough pea (Lathyrus hirsutus L.). Rearing of A. obtectus. Cultures of A. obtectus were established at Debre Markose University to obtain the same age group and required numbers of adult bruchids for the experiment. The bruchids used for rearing were collected from local farmer FABA bean stores together with infested grains and reared in three plastic pots having 5L capacity each. Each pot was field with three Kg FABA bean grains to serve as food for bruchids. Newly harvested FABA bean grains with no bruchid eggs were collected from local market. The grains were carefully examined by hand lens for the presence of bruchid eggs. The grains were washed with potable water to avoid any obscure sources of infestation and frozen at a temperature of -4±1 ºC for three weeks to protect fungal development and to ensure uniform moisture content of the grains [5,7]. Grains having 14% moisture contents were used as a substrate for A. obtectus rearing.

Fifty unsexed adult A. obtectus were released to each jar assuming that 50% of the adults are females and the rest are males as the sex ratio was 1:1 [7]. The jars were covered with muslin cloths held in place by rubber bands. Rearing was conducted at 28±1.5 ºC and 65±5% RH [7,8]. The same age group newly emerged day one old bruchids were used for the experiment. The temperature used for rearing was adjusted by electric power, while the follow up record was taken by Thermo-Hygro (≠TH-439) and Thermometer (≠DET6Q.MFR≠12410). Frequent inspection of the culture for emergence of the progeny was carried out daily starting from twenty two days after parent bruchids introduction [9]. The newly emerged one day old adult F_1 progenies were used for the experiment [7].

Treatment preparation

FABA bean grain moisture contents were prepared to approve optimum moisture levels of FABA bean grains for bruchids infestation. The treatments used for the experiment were 5-5.5%, 9-9.5%, 11-11.5%, 14-14.5%, 16-16.5% and 20-20.5% grain moisture contents. Higher moisture levels such as 16-16.5% and 20-20.5% were obtained by immersing moistened grains in water. After the addition of water grains were shaken six times a day for five minutes and left for three weeks in a refrigerator at -4±1°C to protect mold growth and to enable the moisture to be distributed uniformly throughout the grains [5]. The required amounts of water to be added were determined using [5,10] methods as follows:

$$Water\ to\ be\ added\,(g) = \frac{Final\ moisture - Actual\ moisture}{100 - Final\ moisture} X\ measured\ weight$$

Farmers around the experimental site store their bean grains at 14-14.5% moisture content. Hence, 14-14.5% grain moisture content was set as a control. The rest of the treatments like 5-5.5%, 9-9.5% and 11-11.5% moisture contents of FABA bean grains were adjusted by oven drying chamber [11]. Each treatment was dried to the pre-determined moisture content by placing grains on drying trays. Trays with grains were placed in an oven set at 103 ˚C for 14, 32 and 52 hours for 11-11.5%, 9-9.5% and 5-5.5% moisture levels, respectively. The amount of water removed from each treatment was calculated based on [10] formula.

$$W2(g) = W1 - \frac{W1(M1 - M2)}{(100 - M2)}$$

Where: W1 = Initial grain weight before drying

M1 = Moisture content of undried grain (%); W_2 = Final grain weight after drying; M2 = Moisture content of dried grain (%)

The initial and final bean grain moisture content was measured by moisture testing device (LDS-1H 20122032 7-5). Electronic sensitive balance (WT-G, SST-160mm) with ±0.01 gram accuracy was used to measure weight of the grains before and after moisture adjustments. The treatments were placed in laboratory for one week to ensure moisture homogeneity and to adapt the experimental room [12]. The experiment was conducted in two litters plastic pots contained one kg bean grains. The pots were covered with muslin cloth held in place by rubber bands. The research was carried out for two years in 2013 and 2014. The experiment was arranged in a Completely Randomized Design (CRD) with three replications. Twenty unsexed day one F_1 adult A. obtectus were added to each pot having grains of different moisture contents.

Data collection methods

Data on the number of eggs laid were collected at two, four, seven and ten days after parent bruchids introduction. All dead and alive bruchids were removed ten days after treatment application. First progeny adult emergence was expected and frequent follow up was continued starting from 22 days after treatment application [9]. The number of eggs and holes, grains with and without eggs per twenty grains and total number of emerged adult progenies were recorded for three months in ten days interval. The suitability of the treatments for bruchids containing different moisture levels were evaluated based on 'Growth Index' used by [13] as follows:

$$Growth\ Index = \frac{Log\ F}{D} \times 100$$

Where, F is emerged adults in percent, while D is the median developmental period in days determined by the biology of the insect from the middle of oviposition to 50% of F_1 progeny adult emergence. If no bruchids were emerged over the test period, the growth index value was given as zero [13]. FABA bean grains with high growth indexes were considered suitable and those with low growth indexes were non suitable treatments [8]. This was based on the assumption that a few insect progenies would emerge out and developmental period would take longer time to reach an adult stage in non-suitable treatments [8,13].

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Seed weight loss determination

On the 90th day of treatment application, 1000 grains were randomly taken from each treatment. The grains were separated and categorized into damaged with exit holes and undamaged grains. Grains with and without exit holes were counted and weighed separately. The data were used to calculate the percentage weight loss. Percentage weight losses were determined by the count and weigh method used by [14] as follows:

$$Percent\ weight\ loss = \frac{(W\mu \times Nd) - (Wd \times N\mu)}{W\mu \times (Nd + N\mu)} \times 100$$

Where

Wμ = weight of undamaged grains; Nμ = number of undamaged grains; Wd = weight of damaged grains; Nd = number of damaged grains

Germination test of treated grain

The effects of various moisture levels on FABA bean grain germination were evaluated three months after treatment application. Fifty grains were randomly selected from each treatment and separately treated with sodium hypo-chlorite (Chlorox10%) for one minute to avoid fungal contamination [13]. Grains treated with sodium hypo-chlorite were washed by potable water for one minute to avoid physical damage. The seeds were placed on moist filter paper in petri dish for seven days. The number of sprouted seeds was noted seven days after incubation. Subsequently, germination percentage was determined using [14] formula as follows:

$$Germination\ (\%) = \frac{Total\ No.germinated\ bean\ grains}{Total\ No.of\ bean\ grains\ added\ in\ petridish} \times 100$$

1.1. Statistical analysis

Analysis of variance (ANOVA) was done based on [15] procedures. All data were transformed using square root and logarithmic transformations before the analysis. SAS (SAS 9.2) and MS Excel 07 soft wares were used for the analyses. Tukey's studentized range test (HSD) at P=0.05 was used to separate significant means. Results were reported using back transformed values.

Result

Effect of FABA bean grain moisture levels on egg laying by parent female adult A. obtectus

Parent A. obtectus laid significantly lower number of eggs (0.67±0.33) at 5-5.5% grain moisture content and significantly higher number of eggs (4±1.47) was recorded at 20-20.5% moisture contents two days after treatment application. Four days after treatment application significantly higher number of bruchid eggs (10.83±3.4) was recorded at 20-20.5% grain moisture content (Table 1). Bruchids laid significantly lower number of eggs (3.83±1.11) at 5-5.5% grain moisture content as compared to the highest number of eggs (19.3±4.39) laid on grains with 20-20.5% moisture content seven days after treatment application. Parent adult female laid the highest number of eggs on grains having 20-20.5% moisture content 10 days after treatment application. The current result indicated that FABA bean grains with low moisture contents of less than 11.5% were not suitable for Adult female A. obtectus to lay eggs, while grain moisture content ranging from 14% to 20.5% found to be suitable grain moisture content for A. obtectus egg laying.

Table 1: Eggs of Acanthoscelides obtectus infested on moisture treated FABA bean grains.

Moisture Levels	No of Eggs Laid by Parent Bruchids/20 Grains*			
	2dat	4dat	7dat	10dat
5-5.5%	0.67±0.33^{c}	1.67±0.63^{c}	3.83±1.11^{d}	5.17±1.88^{e}
9-9.5%	1.16±0.48b^{c}	4.5±0.67^{b}	6.0±1.78^{cd}	6.3±1.33^{de}
11-11.5%	2.17±0.48^{ab}	5.8±0.95^{b}	6.67±0.98^{cd}	9.3±1.45^{cd}
14-14.5%	2.33±0.52^{ab}	6.0±0.58^{b}	7.50±1.34^{c}	10.8±0.49^{c}
16-16.5%	3.0±0.91^{a}	10.67±0.78^{a}	14.5±0.97^{b}	17.8±1.11^{b}
20-20.5%	4.0±1.47^{a}	10.83±3.4^{a}	19.3±4.39^{a}	22.6±5.28^{a}

Columns within the same letter are not significantly different at α ≤ 5% Tukey's studentized range test (HSD). Means presented in the table are square root (*) back transformed values. Data from both years pooled together. D at= days after treatment application. This result is in line with the findings of Hyma [16] who reported that 8-11% grain moisture content inhibited egg laying by storage insect pests such as A. obtectus. Similarly, Mason [17] reported that grains with low moisture contents significantly reduced egg laying by parent bruchids and minimize adult emergence after storage. Previous study by Astuti [13] indicated that parent Rhizopertha dominica (F.) laid lower number of eggs on milled rice at lower moisture contents. Ekechukwu [18] reported that stored grains below 10% moisture content were not suitable for egg lying by adult females. Weinberg [12] reported that cereal stored grain insect pests were unable to develop and reproduce under cool and well dried grains.

Effect of grain moisture content on progeny emergence and oviposition of A. obtectus

Bruchid progenies were not totally emerged from 5-5.5% grain moisture content. In contrary, higher number of progenies (366.7±9.38) was recorded from 20-20.5% grain moisture content (Table 2). FABA bean grains were highly infested and damaged by bruchids at grain moisture contents of 20-20.5%, 16-16.5% and 14-14.5% than grains with low moisture contents such as 5-5.5% (Table 2). Bruchids were not emerged from grains having 5-5.5% moisture content. Hence, there is no egg laid at this grain moisture content. Significantly higher number of eggs (393±586) was recorded at 20-20.5% grain moisture content (Table 2). Table 2 demonstrated that FABA bean grains having 5-5.5% moisture contents were free from holes done by bruchids. Higher number of holed grains (53.7±4.29) was recorded on grains having 20-20.5% moisture content. The current results demonstrated that there was no progeny emergence at lower grain moisture content, while large number of progenies was emerged from grains having high moisture contents which is proportional to the number of eggs

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laid. Moreover, number of holes done by bruchids and total number of damaged grains by bruchids was higher on FABA bean grains having high moisture contents. Columns within the same letter are not significantly different at α ≤ 5% Tukey's studentized range test (HSD). Means presented in the table are back square root (*) and log (**) transformed values. Data from both years pooled together. The current findings are in line with investigation of a number of scientists. For instance, Hyma [16] who noted that lower number of progenies was emerged from grains stored at lower moisture contents. Brook [6] also reported that higher moisture contents of stored grains were conducive to higher insect population buildup during storage. Similarly Ekechukwu [18] reported that storage insects face a problem to sustain and reproduce in pulse grains below 10% moisture contents. As Weinberg [12], comparable result was recorded that in cereal grains decreasing moisture contents from 14.38% to 12.89% proportionally decrease stored grain insect pest infestation and increase storage period from 30 to 120 days. Mason [17] also noted that bruchids were unable to lay eggs on well dried pulse grains.

Table 2: Emergence of Acanthoscelides obtectus progenies and eggs laid on moisture treated FABA bean grains.

Treatments	Total Numbers of Bruchids (**)	Total Numbers of Eggs (**)	Total no of Holes/20 Grains (*)	Free from Bruchid Eggs/20 Grains (*)	Free from Holes/20 Grains (*)
5-5.5%	0±0^{d}	0±0^{e}	0±0^{d}	17.8±2.41^{a}	20±0^{a}
9-9.5%	13±0.55^{cd}	37.7±0.84^{ed}	2.3±1.72^{d}	13.3±1.75^{b}	17.2±0.48^{a}
11-11.5%	51.7±1.28^{c}	76.2±6.33^{d}	8.5±2.07^{c}	3±1.27c	12.2±1.3^{b}
14-14.5%	246.7±17.51^{b}	273.5±14.44^{c}	20.3±4.22^{b}	1±2.82^{d}	7.5±1.17^{c}
16-16.5%	321.8±17.26^{ab}	342.5±29.92^{b}	45±5.7^{a}	0±0^{d}	4.8±1.3^{d}
20-20.5%	366.7±9.38^{a}	393±5.86^{a}	53.7±4.29^{a}	0±0d	3.7±1.35^{d}

Effects of grain moisture content on bean grain weight loss due to A. obtectus

Bean grain weight loss was not recorded at grain moisture content of 5-5.5%. Significantly higher weight losses (18.6%±1.87) were recorded on grains having 20-20.5% grain moisture content (Table 3). The result obtained on bean grain weight loss clearly demonstrated that bean grain that are stored at low moisture content suffer less from A. obtectus grain damage, while grains stored at high moisture content are susceptible to A. obtectus damage which eventually lead to high losses. The current findings are in line with Mason [17] who reported that dry grains stored in a dry storage environment suffer less from losses due to stored grain insect pests. Astuti [13] indicated that insects develop easily and cause remarkable weight losses in grains with high moisture content. Mason [18] reported that grains stored at low moisture contents showed minimum weight losses from damage by stored grain insect pest. Columns within the same letter are not significantly different at α ≤ 5% Tukey's studentized range test (HSD). Means presented in the table are square root (*) back transformed values. Data from both years pooled together.

Table 3: Weight loss, developmental period and Growth index of FABA bean grains infested by Acanthoscelides obtectus.

Treatments	% Weight Loss (*)	Develop-mental Period (days) (*)	Growth Index (*)	% Germination (*)
5-5.5%	0±0d	0±0d	0±0^{c}	70.8±8.71^{d}
9-9.5%	1±0.59^{c}	48.7±1.15^{a}	0.3±0.13^{c}	98.3±0.76^{a}
11-11.5%	2.2±1.98^{c}	41.7±1.35^{b}	1.5±0.29^{b}	93.2±4.68^{ab}
14-14.5%	12.9±1.06^{b}	32±0.71^{c}	2.4±0.50^{a}	87.8±2.86^{bc}
16-16.5%	16.5±2.88^{a}	31.2±0.46^{c}	2.8±0.19^{a}	84.3±6.43^{bc}
20-20.5%	18.6±1.87^{a}	31.3±0.57^{c}	2.8±0.11^{a}	80.7±5.22^{c}

Effects of grain moisture content on germination capacity of bean grains

Germination percentage of FABA bean grains stored at various moisture contents are shown in Table 3. Grains with moisture contents of 9-9.5%, 11-1.5% and14-14.5% showed high germination percentage capacity than the other grain moisture contents. Germination percentages of FABA bean grains with 5-5.5%, 9-9.5% and 20-20.5% moisture contents were 70.8%±8.71, 98.3%±0.76 and 80.7%±5.22, respectively. The current findings indicated that intermediate grain moisture content enhanced the germination capacity of FABA bean grains. The result is in line with Astuti [13] who reported that germination percentage of maize grains dropped from 84.3% to 28.6% after 75 days of storage at lower (14%) and higher (22%) grain moisture contents. Obopile [4] demonstrated that pulse grains stored at higher moisture contents have low viability and germination.

Effects of grain moisture content on developmental period of A. obtectus

The effect of FABA bean grain moisture content on developmental period of A. obtectus is shown in Table 3. According to the Table FABA bean grains having 9-9.5% and 11-11.5% moisture contents took longer periods of 48.7±1.15 and 41±1.35 days, respectively for development. As there was no egg laid on grains having 5-5.5% moisture content no investigation was conducted on developmental period. FABA bean grains with higher moisture contents were significantly shortened the developmental period of A. obtectus to 31.2±0.46 to 32±0.71 days. The present result demonstrated that medium moisture contents were extended the developmental periods of A. obtectus than high moisture contents. The result was in agreement with Astuti [13] who reported that grains stored at high moisture contents shorten the developmental periods of the insects involved in grain infestation. Similar result was reported by Thakur [9] that the developmental periods of A. obtectus ranged

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from 31.42±0.364 to 46.71±2.02 days when grains were stored at higher and lower moisture contents, respectively. This result is in line with the findings of Ekechukwu [12] who reported that grains stored below 10% moisture content extended the developmental periods of insects. Obopile [5] indicated that the developmental periods of C. maculatus were ranged from 28.25±7.84 to 43.3±1.67 days in higher and lower moisture contents, respectively. Previous study by Weinberg [12] indicated that insects develop quickly and cause significant grain damage in a higher moisture contents.

Relationship between Developmental period and Growth index of A. obtectus

In the present experiment 9-9.5% and 11-11.5% FABA bean grain moisture contents were extended the developmental periods of the bruchids from 10.5 to 17.5 days. Grains with higher moisture contents led to higher growth index and short developmental periods of A. obtectus. Longer developmental periods of bruchids led to lower growth index and the relationship was strongly negative with r^2 = 94% (Figure 1). The present result indicated that higher moisture contents of FABA bean grains increased growth index and decrease developmental periods of A. obtectus. Similarly, Astuti [13] reported that grains stored at low moisture contents increased the development periods and reduced the growth index of the insects. Thakur [9] report was also confirmed that bruchids needed high moisture contents for short developmental period and high growth index and the two parameters were negatively correlated. Suitable grain moisture contents were increased the value of growth index and reduced developmental periods of insects Obopile [4].

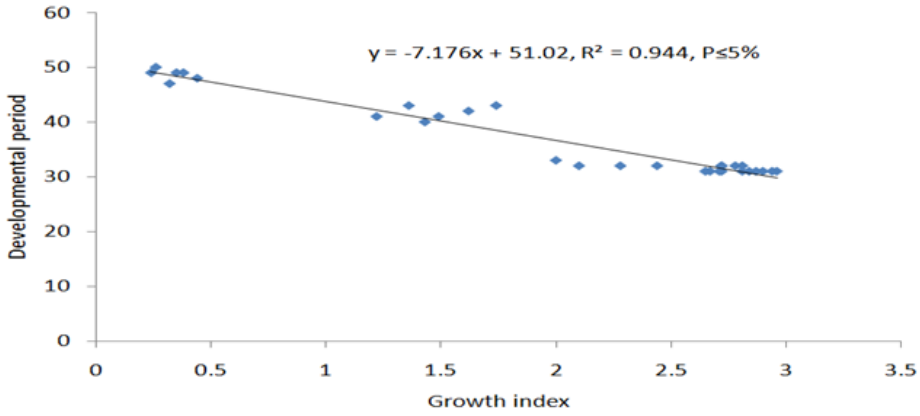

Figure 1: Relationship between developmental period and growth index of Acanthoscelides obtectus in Faba bean grains with various moisture contents.

Relationship between FABA bean grain weight loss and developmental period

FABA bean grain moisture contents that reduced grain weight losses were extended the developmental periods of bruchids and the two parameters were negatively correlated (Figure 2). Shorter developmental periods and higher FABA bean grain weight losses were recorded at 20-20.5%, 16-16.5% and 14-14.5% grain moisture content. There was no weight loss and development lower grain moisture content of 5-5.5%.

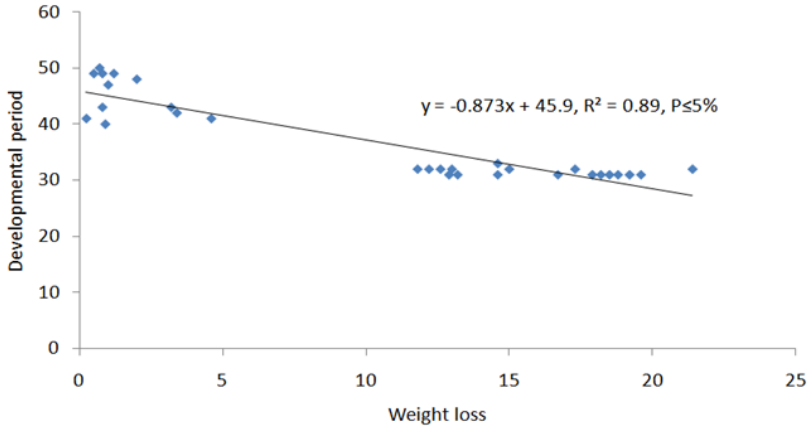

Figure 2: Relationship between Acanthoscelides obtectus developmental period and faba bean grain weight loss in grains of different moisture content.

The current study demonstrated that high moisture content of the grains were conducive for A. obtectus to cause high FABA bean grain weight losses and to reduced bruchid developmental periods. Weinberg [12] report was in line with the current finding in that insect infestation at a higher grain moisture content caused substantial weight losses and the developmental period was short.

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Mason and McDonough [17] added that grains stored at lower moisture contents face little or no grain weight losses and the developmental period was longer. Grains stored at safe moisture content lead to extended developmental periods to the associated insects and the grain weight loss by the insect was ultimately low Astuti [13].

Conclusion

FABA bean grain moisture content highly influenced the infestation of A. obtectus. The lower grain moisture content below 11% led to low number of eggs laid by adult female, low number of progeny emergence, low grain weight loss, high germination percentage and long developmental period. Hence, from the current experiment it can be concluded that if FABA bean grain before storage dry to less than 10% or 11% grain moisture content it can be stored for long period of time without significant problem posed by A. obtectus.

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