Assessment on The Efficacy of The Leaf Powders of Neem *Azadriachta indica* A. Juss, Eucalyptus *Eucalyptus globulus*Labill and Sugar Apple *Annona squamosa* L. on *Callosobruchus maculatus* (Fab.) (Coleoptera: Bruchidae) that Infested Stored Butter Beans

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Abstract

Present study was to evaluate the effect of leaf powders of three plants namely neem (*Azadriachta indica* A.juss), Eucalyptus (*Eucalyptus globulus* Labill) and sugar apple (*Annona squamosa* L.) as stored butter beans protectant against *Callosobruchus maculatus* adult mortality, weight loss and seed damaged was conducted. The leaf pounder of each plant was tested at 2.5g, 5.0g and 10g/100g of sound butter beans. Each treatment was replicated three times. The highest mean mortality (100%) was observed in case of *Annona squamosa* followed by *Eucalyptus globulus* and *Azadriachta indica*. The highest mean seed damage and weight loss was found in control treatment, followed by *Azadriachta indica, Eucalyptus globulus* and *Annona squamosa* respectively. All the leaf powders showed significant effect on the above parameters.

Keyword: Plant leaf powder, mortality, seed damage and weight loss, pulse beetle, Butter bean seeds

Introduction

Ensuring the safety of stored products is one of the crucial steps for food security, especially in developing countries. However, pulses become infected by many insect pests both in the field and during post-harvest storage. The damage caused by insect pests in stored beans and pulses may account to 10-40% in developing countries (Shaaya *et al*., 1997) (cited by Hasan *et al*., 2013).

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To overcome this problem, different conventional synthetic chemicals have been used. However, these insecticides have created a number of problems (Saleem, 2014).

Nowadays various products of plants have been tried recently by researchers with a high degree of success as grain protectants against pulse beetle to reduce infestation in storage (Umrao and Verma, 2002; Epidi *et al*., 2008; Mahadi and Rahman, 2008) (cited by Hammed *et al*., 2012).

Therefore, this research was carried out

- to evaluate the efficacy of the three plant leaf powders on stored pulse against the infestation of *C. maculatus*
- to assess the effectiveness of the three plant leaf powders of different concentrations on the physical and biological parameters (percent adult mortality, percent seed damage, percent weight loss) of the pulse beetle that infested the beans

Materials and Methods

Study Sites

Meiktila University situated between latitude 20º51'55"- 20º52'18"N and longitude 95º50'09"- 95º50'27" E (Plate 1).

Study Period

Study period was from September, 2015 to February, 2016.

Preparation of Seeds

The butter bean seeds were obtained from Meiktila Market. Healthy and fresh seeds were used to avoid any pre-storage infestation or egg lying of bruchids.

Insect Rearing

Adult bruchids, *Callosobruchus maculatus* were collected from previously infested butter bean seeds. Approximately 300 unsex adults were introduced into plastic jars (20 cm length, 20 cm width and 35 cm high) containing 1 kg of butter bean seeds samples and maintained at room temperature 28º – 34º C and relative humidity 64% – 84%. The jars covered by a fine mesh cloth and fastened with rubber bands to allow free air

circulation and also to prevent insects from escaping. The insects were allowed to lay eggs for seven days as outlined by (Hammed *et al*, 2012).

After which the bruchids in the butter bean seeds were sieved out and the butter bean seeds containing eggs were left undisturbed until the new adults emerge (about 23 – 30 days). The subsequent F1 progenies of the bruchids, which emerged from the cultures, were used for the experiment.

Preparation of Leaf Powders

Fresh leaves of neem, eucalyptus, and sugar apple were kept in a well-ventilated room away from sunlight at ambient temperatures 28º–34º C for 2 weeks. The dried leaves were then pounded to fine pounder using a motor and pestle. The resulting powder was sieved to obtain a fine and uniform pounder.

Application Rates and Infestation

Levels of leave powder formulations were applied at the rate of 2.5%, 5% and 10% w/w butter bean seeds with an untreated as control. Approximately 300 grams of healthy, fresh, clean and unbroken butter bean seeds were weighed and mix with various leaves pounder in accordance with the percentage rates, into clean and dry plastic jars (measuring 11 cm length, 11 cm width, and 16.5 cm high). The control did not receive any powder treatment. Each level was replicated three times.

Thirty unsex adult *Callosobruchus maculatus* (0–72 hours old) were added to each jar of treated and control seeds, and were covered with mesh cloth and held in place with rubber bands to prevent the contamination and escape of insects.

Data Collection

Evaluation on percentage adult mortality, percentage seed damaged and percentage weight lost were considered during the study.

Percentage Adult Mortality

The mortality of adult was recorded at 24, 48, 72 and 96 hours after treatment application. The number of dead insects in each jar was sieved and counted during each assessment. Mortality counts were corrected by using (Abbott's, 1925) formula.

$$(\%)\ \text{Adult mortality} = \frac{\text{Number of dead insects}}{\text{Total no of insects}} \times 100$$

Percentage Seed Damage (Infestation)

Percentage seed damage was assessed by the count method. One hundred seeds were randomly taken from each jar and the number of insect damaged and un-damaged grains was observed. The percentage seed damage was calculated according to Enobakhare and Law – Ogbomo (2002):

$$(\%)\ \text{seed damage} = \frac{\text{Number of damaged seeds}}{\text{Total no of seeds}} \times 100$$

Percentage Seed weight loss

Percentage weight loss was assessed at monthly interval and percentage weight losses determined using the formula of Adams and Schulter (1978) as follows:

$$(\%)\ \text{weight loss} = \frac{\text{Initial weight} - \text{final weight}}{\text{Initial weight}} \times 100$$

Statistical Analysis

Data collected were statistically analyzed using Analysis of variance (ANOVA) using SPSS computer software package (version 21) to ascertain the differences between means and least significant differences was used to separate treatment means that were significantly different at $p<0.05$.

Plate 1. Study Site (Meiktila University)

Results

Efficacy of different three plant leaf powders were evaluated on the basic of their effect on percentage adult mortality, percentage seed damage and percentage loss in seed weight.

Percentage Adult Mortality

The adult mortality was significant at ($p<0.05$) in the all treatments compared to untreated control butter bean seeds. However, there were no significant differences ($p<0.05$) between the effect of neem leaf powder and eucalyptus leaf powder in all concentrations, but sugar apple leaf powder showed significantly more effective than any treatment.

Percentage Seed Damage or Infestation

The seed damage was significantly ($p<0.05$) lower in the treatments containing plant materials than in the untreated control. In seed damage, significant differences ($p<0.05$) were observed in all dosages (2.5-10g/100g butter bean seed) of sugar apple leaf powder, followed by eucalyptus and neem. However, no significant differences ($p<0.05$) were observed between 5% and 10% sugar apple. The results showed that increase in the amount (%) of the leaf powders decreased the damage in seed stored.

Percentage Seed Weight Loss

It was observed that different concentration of plant leaf powders had a significant effect on the percentage in seed weight loss during one to six months after treatment. There were significant differences ($p<0.05$) among treatments and the untreated control in butter bean seed weight reduction. This showed the positive impact of the plant materials in protecting butter bean seeds against *C. maculatus* infestation.

Table 1. Mean values of adult mortality caused by *C. maculatus* on butter bean seeds admixed with different plant leaf powders at different rates at 24 hours to 96 hours after storage

Treatment	The rate of dosage (% w/w)	(%) Adult mortality (mean ± SD) (hours after treatment)			
		24h	48h	72h	96h
Control		0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]	0.00±0.00[a]
Neem	2.5	7.78±1.92[b]	15.56±1.92[b]	30.00±3.33[b]	36.67±3.33[b]
	5	11.11±1.92[c]	23.33±3.33[c]	53.33±3.33[c]	64.44±1.92[c]
	10	18.89±3.85[d]	28.89±1.92[d]	58.89±5.09[d]	70.00±6.67[d]
Eucalyptus	2.5	8.89±1.92[b]	14.44±1.93[b]	28.89±3.85[b]	38.89±3.85[b]
	5	15.56±3.85[c]	22.22±1.92[c]	54.44±5.09[c]	63.33±3.33[c]
	10	20.00±3.33[d]	28.89±3.85[d]	57.78±6.94[d]	68.89±5.09[d]
Sugar apple	2.5	11.11±1.92[e]	21.11±1.92[e]	36.67±3.33[e]	44.44±5.09[e]
	5	24.44±5.09[f]	46.67±3.33[f]	75.56±6.94[f]	100.00±0.00[f]
	10	40.00±6,67[g]	72.22±6.94[g]	100.00±0.00[g]	100.00±0.00[f]

Superscripts of different letter in the same column are significantly different ($p<0.05$)

Table 2. Mean values of seed damage by *C. maculatus* on butter bean seeds admixed with different plant leaf powders at different rates at one to six months after storage

Treatment	The rate of dosage (% w/w)	Mean monthly seed damage (%) (mean ± SD)					
		1 Month	2 Month	3 Month	4 Month	5 Month	6 Month
Control		22.67±1.53[a]	88.33±0.58[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]	100.00±0.00[a]
Neem	2.5	12.33±0.58[b]	17.67±2.52[b]	42.00±3.61[b]	86.67±1.53[b]	91.00±1.00[b]	92.33±2.08[b]
	5	5.00±0.00[c]	12.33±1.15[c]	33.00±4.58[c]	80.67±5.13[c]	86.00±1.73[c]	89.33±0.58[c]
	10	2.33±0.58[d]	6.67±1.53[d]	20.67±4.16[d]	75.00±5.20[d]	79.33±0.58[d]	80.33±0.58[d]
Eucalyptus	2.5	9.33±3.08[b]	32.33±4.93[b]	38.67±2.08[b]	84.00±4.36[b]	86.33±4.16[b]	88.00±6.08[b]
	5	4.67±0.58[c]	26.00±5.13[c]	27.67±2.52[c]	76.00±6.08[c]	80.67±0.58[c]	84.33±1.55[c]
	10	2.67±1.15[d]	10.33±2.08[d]	20.67±1.53[d]	70.67±2.52[d]	75.00±4.00[d]	76.67±0.58[d]
Sugar apple	2.5	11.00±6.56[e]	17.33±4.51[e]	28.00±3.00[e]	48.33±6.11[e]	67.67±2.52[e]	75.67±4.16[e]
	5	0.33±0.58[f]	0.00±0.00[f]	0.00±0.00[f]	0.00±0.00[f]	0.00±0.00[f]	0.00±0.00[f]
	10	0.00±0.00[f]	0.00±0.00[f]	0.00±0.00[f]	0.00±0.00[f]	0.00±0.00[f]	0.00±0.00[f]

Superscripts of different letter in the same column are significantly different ($p<0.05$)

Table 3. Mean values of seed damage by *C. maculatus* on butter bean seeds admixed with different plant leaf powders at different rates at one to six months after storage

Treatment	The rate of dosage (% w/w)	Mean monthly loss in weight (%) (mean ± SD)					
		1 Month	2 Month	3 Month	4 Month	5 Month	6 Month
Control		17.53±0.31^{i}	20.09±0.96^{i}	29.73±1.30^{a}	34.55±0.85^{i}	46.78±0.31^{a}	57.75±0.91^{a}
Neem	2.5	10.74±0.63^{h}	16.25±0.48^{h}	19.67±0.61^{h}	24.41±0.91^{h}	38.90±0.77^{b}	41.26±1.31^{b}
	5	7.66±0.71^{c}	10.82±1.05^{g}	17.62±1.16^{c}	21.56±0.87^{g}	30.29±0.67^{c}	34.53±0.75^{c}
	10	3.50±0.22^{d}	5.74±0.45^{d}	9.77±1.77^{d}	13.79±0.95^{f}	16.29±0.40^{e}	21.59±1.44^{e}
Eucalyptus	2.5	11.17±1.61^{h}	15.34±1.28^{h}	19.52±0.49^{h}	22.72±0.27^{h}	38.13±0.56^{b}	40.40±0.57^{b}
	5	7.43±0.81^{c}	10.87±0.81^{g}	16.83±0.81^{c}	20.60±1.00^{g}	23.04±0.79^{c}	34.11±1.25^{c}
	10	2.85±0.29^{d}	5.12±0.65^{d}	8.83±1.35^{d}	12.42±1.14^{f}	15.80±0.66^{e}	21.59±0.34^{e}
Sugar apple	2.5	2.95±0.40^{e}	3.49±0.26^{e}	4.86±0.69^{e}	7.16±0.83^{e}	10.39±0.39^{e}	17.11±0.82^{e}
	5	0.000±0.00^{f}	0.000±0.00^{f}	0.000±0.00^{f}	0.000±0.00^{h}	0.000±0.00^{f}	0.000±0.00^{f}
	10	0.000±0.00^{f}	0.000±0.00^{f}	0.000±0.00^{f}	0.000±0.00^{h}	0.000±0.00^{f}	0.000±0.00^{f}

Superscripts of different letters in the same column are significantly different ($p<0.05$)

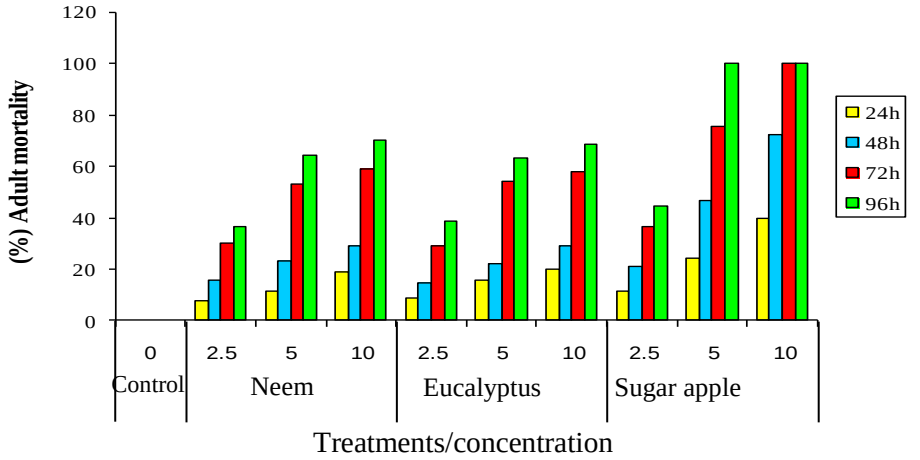

Figure 1. Mean values of adult mortality caused by *C. maculatus* on butter bean seeds admixed with different plant leaf powders at different rates at 24 hours to 96 hours after storage

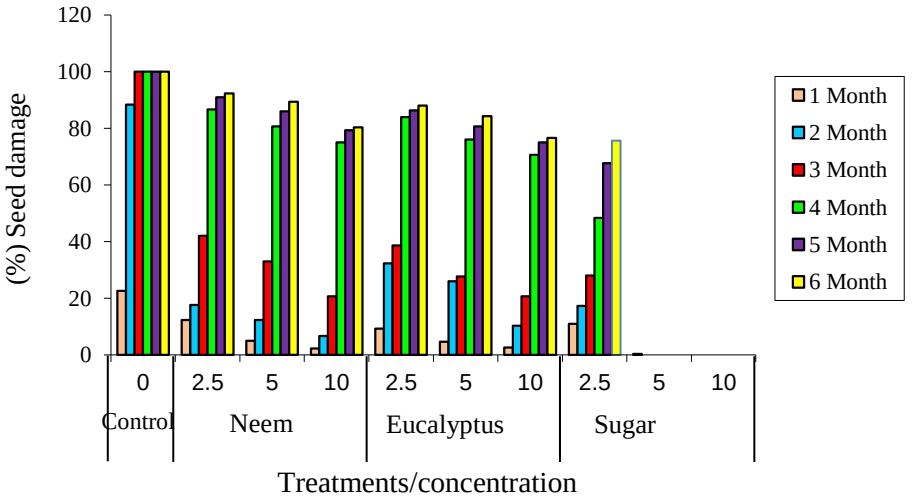

Figure 2. Mean values of seed damage caused by *C. maculatus* on butter bean seeds admixed with different plant leaf powders at different rates at one to six months after storage

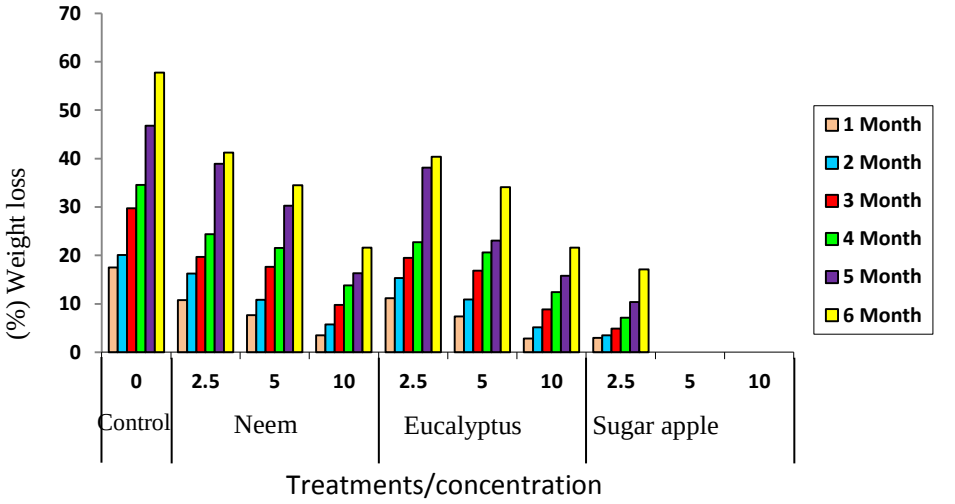

Figure 3. Mean values of weight loss caused by *C. maculatus* on butter bean seeds admixed with different plant leaf powders at different rates at one to six months after storage

Plate 3. Preparation for the experiment

Plate 4. Untreated and treated butter beans at 10% concentration sugar apple leaf powder for six months storage

Discussion

The present study was conducted to determine adult mortality, percentage seed damage and percentage weight loss of beans infested with *Callosobruchus maculatus* by way different concentrations of leaf powders at various exposure time. At all the different powders, adult mortality was decreased with the passage of time while no mortality was observed in untreated control beans. Seed damage and weight loss increase with the passage of time in both control and treated seeds. Seed damage and loss of weight were high in seeds treated with neem leaf powder, followed by those treated with eucalyptus leaf powder and the minimum was found in beans treated with sugar apple leaf powder. No previous work was found in any literature where the same types of plant extracts, were used against the *C. maculatus.* However, results of some of the previous research work may be comparable with the present findings.

Chandrakala *et al.* (2013) reported that all the treatments were statistically analyzed and proved to be significant and superior than in control in minimizing the pest infestation. Similarly, in the present study adult mortality, seed damage and seed weight loss was significant con 9 to the control.

In conclusion, the results of the present study indicated that among the three-different leaf powder tested, sugar apple (*Annona squamosa*) proved to be the most effective in the protection of butter bean against the infestation of the pest, *Callosobruchus maculatus*. So, it can be said that leaf powder of sugar apple has great potential to use as a stored grain protectant. It was followed by the leaf powder eucalyptus (*Eucalyptus globulus*) and neem (*Azadriachta indica*) which showed almost same results and with no significant differences. Therefore, it is suggested that instead of relying on the chemical control method of controlling the insect pests, which rather poses a threat to the living organisms, people should be encouraged to avert to the use of botanical products which are easily available, easy to use, no residual effects on stored commodity environmentally friendly.

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