The Effect of Lentil-Tef Mixture on Occurrence of Adzuki Bean Beetle (*Callosobruchus chinensis*) and its Natural Enemy in Stored Lentil

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አህጽሮት

የምስር እና የጤፍ እህልን አቀሳቅሎ ማከማቸት በተራዮሬ ነቀዝ (Callosobruchus chinensis) እና በነቀዙ የተፈዋሮ ጠላት ላይ ያለውን ተፅዕኖ ለማወቅ የቤተ-ሙከራ ዋናት ተከናውኖ ነበር። ጤፉ (ቀይ ጤፍ) የተቀሳቀለው በመተካት መርህ ሲሆን መጠኑም 0% ጤፍ (ሙሉ ምስር)፣ የሙሉ ምስሩን ክብደት 5%፣ 10%፣ 15%፣ 20%፣ 25% ሕና 30% ቀንሶ የተቀነሰውን የምስር ሕህል መጠን ሕኩል በሆነ የጤፍ መጠን በመተካት ነበር፡፡ የሙሉ ምሥሩ መጠን በአንድ ከረጢት 10 ኪባ ሲሆን የሁሉም ተጠኝዎች የማከማቻ እቃ ደግሞ 40 ሳሜ በ 70 ሳሜ የሆነ የማዳበሪያ ከረጢት ነው። እያንዳንዱ ተጠኝ ሦስት ጊዜ ተደጋግሞ በመሉ-አድል ስርጭ ብሎክ ንድፍ (ራንደማይዝድ ኮምፐሊት ብሎክ ዲዛይን) በማስቀመጥ ነበር፡፡ ውህብ የተወሰደውም አንድ ወር በሚሆን ልዩነት ከኢየንዳንዱ ከረጢት 25 የምስር ፍሬዎችን በእድል በመምረጥ እና ሕየንዳንዱን ፍሬ በመመርመር የተራተሬ ነቀዝ እንቁሳል በቁተር፣ የተራተሬ ነቀዝ የወጣበት ቀዳዳ በቁተር እና የተፈዋሮ ጠላት የወጣበት ቀዳዳ በቁሞር በመመዝነብ ነበር። የምስር እህል ውስፑ የጤፍ እህልን እስከ 30 በመቶ አቀሳቅሎ ማከማቾት የፑራዋሬ ነቀዝ እንቁሳል ያለባቸውን ፍሬ ብዛት (%)፤ በአንድ ፍሬ ላይ ያለውን የፑራዋሬ ነቀዝ እንቁሳል ብዛት፣ የፑራዋሬ ነቀዝ የወጣባቸውን ፍሬዎች ብዛት (%)፣ የፑራዋሬ ነቀዝ የወጣባቸው ቀዳዳዎች ብዛት እና የነቀዙ የተፈዋሮ ጠላት የወጣበት ቀዳዳ ብዛት ሳይ ምንም ተፅዕኖ አልክገረውም፡፡ በዚህ アናት ጊዜ የተከሰቱት የፐራፑሬ ነቀዝ የተፈዋሮ ጠላቶች ሁለት ሲሆኑ ሳይንሳዊ መጠሪዮቸውም Dinarmus basalis እና Anisopteromalus calandrae ነው። D. basalis ኢትዮጵያ ውስዮ ለመጀመሪያ ጊዜ የታወቀ ነው። የተፈዋሮ ጠላቶቹ የሚያጠቁት የዋራዋሬ ነቀዝን እጭ ሲሆን ከ18 እስከ 66 በመቶ የሚሆነውን እጭ አጥቅተዋል። የጤፍ እህል ከምስር እህል ,ጋር ቢቀላቀልም ወይም ባይቀሳቀልም የዮራዋሬ ነቀዝ ምስርን መውረር፣ መራባት እና ብዙ ትውልዶችን ማፍራት ስለሚቸል አርሶ አደሮች የዮራዋሬ ነቀዝን ለመቆጣጠር በልምድ የሚጠቀሙት ምስርን እና ጤፍን የማቀሳቀል ዘዴ ፍቱን እንዳልሆነ እንዲያውቁ 98:29 BIAS ::

Abstract

Laboratory experiment was conducted to determine the effect of mixing lentil grain with tef grain on the occurrence of Adzuki bean beetle (ABB), Callosobruchus chinensis and its natural enemy in stored lentil. Brown-seed tef grain was mixed with lentil (cu. Derso) grain on replacement basis at the rate of 0% (full lentil), 5%, 10%, 15%, 20%, 25%, and 30% of the full lentil grain. The full lentil treatment was10 kg per bag (polypropylene bags (40 cm x 70 cm)), while for the remaining treatments the required amount of lentil grain was removed and replaced by equal amount of tef grain. Each treatment was replicated three times and was arranged in randomized complete block design. From each bag 25 lentil grain samples were randomly selected approximately at monthly intervals and examined for the presence or absence of ABB eggs, ABB emergence holes and its natural enemy emergence holes. Tef grain mixed (up to 30%) with lentil grain had no influence on the percentage of grains with ABB eggs (%); ABB eggs per grain; grains with emergence hole (%); ABB emergence hole per grain; and natural enemy emergence hole per grain. **Dinarmus basalis**, which is a new record to Ethiopia, and Anisopteromalus calandrae were the ectoparasitoids that attacked ABB larvae in stored lentil. The proportion of ABB larvae parasitized by the two natural enemies together ranged from 18% to 66%. Since ABB was able to infest, reproduce, and complete multiple generations on lentil irrespective of the amount of tef mixed,

farmer should be informed about the ineffectiveness of the traditional method they apply to control Adzuki bean beetle in stored lentil.

Introduction

The adzuki bean beetle (ABB), *Callosobruchus chinensis* (L) (Coleoptera: Chysomelidae) is pest of stored lentil throughout the world (Ghosh *et al.*, 2007). Although this insect pest is geographically widely distributed in many of lentil growing countries, the amount of damage it causes in stored lentil varies considerably from country to country or location to location within a country. For example, in Ethiopia, ABB is not a problem in highland areas (alt. > 2000 m a.s.l.) due to the cool temperature that exists throughout the year (Tadesse, 1992). However, in the mid-altitude (1500 to 2000 m a.s.l.) lentil growing areas, it infests stored lentil and the level of infestation in farmers store can reach up to 63% (Kemal and Tibebu, 1994).

However, farmers may or may not apply control measures to prevent infestation of lentil by ABB. Those farmers who apply control measures apply chemicals; rely solely on traditional pest management methods or use combination of both methods (Tadesse and Basedow, 2004; Hagstrum and Phillips, 2017). The most commonly used chemicals are gaseous fumigants such as Aluminium phosphide and residual contact insecticides like malathion and pirimiphos methyl, which are recommended by the Ministry of Agriculture to control storage insect pests in stored cereals and pulses (MOA, 2017). On the other hand, those farmers who apply traditional pest management methods mix grains with inert materials such as salt, sand, ash, or clay; or apply plant materials that may have insecticidal property (Boeke et al., 2004; Hagstrum and Phillips, 2017). Moreover, farmers in Ethiopia mix large sized grains and non-host small sized grains to protect the former from storage insect pest damage (Firdissa and Abraham, 1999; Girma et al., 2000; Tadesse and Basedow, 2004). For instance, to protect stored lentil from ABB infestation, farmers commonly mix lentil grain with tef (Eragrostis tef (Zucc.) Trotter) (Tebkew and Mohammed, 2006). Tef is a non-host small grain to ABB and has very small grains, which weigh about 19 to 34 mg per hundred seeds (Assefa et al., 2011). Blum and Abate (2000) believed that tef grain fills the inter-grain space; as a result, it obstructs insect movement and cools the storage. However, detailed information about the efficacy of indigenous grain storage techniques and storage pest management practices in general is lacking (Blum and Abate, 2000; Abate et al., 2000). According to Altieri (1984), scientific evaluation of indigenous knowledge is required to improve the small-scale farming systems and to guide the application of modern agricultural science in the system. Therefore, this study was undertaken to determine the effect of mixing lentil grain with tef grain on Adzuki bean beetle and its natural enemy occurrence.

Materials and Methods

The experiment was conducted between May 2018 and January 2019 under ambient temperature and relative humidity in a laboratory at Debre Zeit Agricultural Research Center using improved lentil cultivar Derso and brown-seed tef. Fresh harvested and uninfested lentil grain and the tef grain were obtained from the chickpea and lentil research program and tef research program, respectively, of the center. Tef grain was admixed with lentil grain on replacement basis at the rate of 0 (full lentil), 5, 10, 15, 20, 25, and 30% of the full lentil grain. For the full lentil, treatment 10 kg lentil grain was put in polypropylene bags (40 cm wide and 70 cm high), while for the remaining treatments the required amount of lentil grain was removed and replaced by equal amount of tef grain. After admixing the grains, the mouth of the bag was left open to equalize the chance of infestation by Adzuki bean beetle and its natural enemies. Each treatment was replicated three times and was arranged in randomized complete block design (RCBD). Except on 26 October 2018 and 20 December 2018 samplings on which samples were taken at the bottom of the bag, from each bag 25 lentil grain samples were randomly selected on the top surface approximately at monthly intervals and examined for the presence or absence of Adzuki bean beetle eggs, emergence holes and its natural enemy emergence holes. The size and shape of emergence (exit) hole is used as criterion to identify whether the hole is a host or natural enemy emergence hole (Dosdall et al., 2006; Veromann et al., 2011). Thus, grains with Adzuki bean beetle emergence hole had clear, large round holes (≈ 1 to 1.5 mm diameter), while natural enemy exit hole had $\approx < 1$ mm diameter. Moreover, natural enemy emergence holes were gently scraped with sharp blade and presence of remnant of larval integument in the hole was taken as additional criterion to confirm that the Adzuki bean larva was killed by a parasitoid. The number of emergence holes of Adzuki bean beetle and its natural enemy was an indicator of successful eclosion of adults from the grain and was considered as estimator of population of each insect type.

Data analysis

Before analysis all count data, which includes average number of eggs per seed, Adzuki bean beetle emergence hole, and natural enemy emergence hole were transformed to log (x + 1) scale; while the data on percentage of grains with eggs or holes were transformed to arc-sine scale. Both the original and transformed data were analyzed using Proc ANOVA of SAS but only original data are reported. When means are given, they are reported with their standard deviation (\pm SD).

Results and Discussion

Egg laying by Adzuki bean beetle was not affected (P > 0.05) by any of the treatments (Table 1). Thus, on 26 June 2018 sampling, in all the treatments there were on the average two to seven grains with eggs. Between 18 July 2018 and 18 September 2018 the number of grains with eggs continually increased and on 18 September 2018 on the average 13 to 22 grains had egg on them. On 26 October 2018 and 20 December 2018 at which sampling was taken at the bottom of the bag, the proportion of lentil grains with eggs was less than the preceding and succeeding sampling dates, while on 24 January 2019 nearly all sampled grains in all treatments had egg on them. As shown in Table 1, in most cases the standard deviation of mean was large relative to the mean, which indicates that the level of infestation was not uniform across replications. The increase in the percentage of lentil grain with eggs with increase in storage period suggests that the population of Adzuki bean beetle was not arrested by lentil-tef mixture treatments.

Treatment	26-Jun-18	18-Jul-18	16-Aug-18	18-Sep-18	26-Oct-18	27-Nov-18	20-Dec-18	24-Jan-18
Full lentil	12.0 ± 6.9	28.0 ± 6.9	56.0 ± 6.9	77.3 ± 26.6	42.7 ± 6.1	53.3 ± 6.1	36.0 ± 17.4	89.3 ± 18.5
Lentil + 5% tef	8.0 ± 8.0	33.3 ± 26.6	73.3 ± 12.2	62.7 ± 11.6	44.0 ± 22.3	66.7 ± 6.1	50.7 ± 16.7	94.7 ± 6.1
Lentil + 10% tef	25.3 ± 28.4	42.7 ± 24.1	54.7 ± 29.5	88.0 ± 4.0	53.3 ± 42.8	66.7 ± 16.2	48.0 ± 32.0	94.7 ± 9.2
Lentil + 15% tef	21.3 ± 8.3	41.3 ± 18.0	57.3 ± 2.3	74.7 ± 34.0	42.7 ± 26.6	61.3 ± 2.3	65.3 ± 16.2	98.7 ± 2.3
Lentil + 20% tef	20.0 ± 10.6	52.0 ± 46.1	72.0 ± 10.6	72.0 ± 22.3	14.7 ± 4.6	61.3 ± 8.3	52.0 ± 28.8	100.0 ± 0.0
Lentil + 25% tef	26.7 ± 12.2	33.3 ± 12.9	64.0 ± 24.3	53.3 ± 11.6	18.7 ±18.5	42.7 ± 23.1	52.0 ± 24.3	100.0 ± 0.0
Lentil + 30% tef	17.3 ± 10.1	41.3 ± 12.9	70.7 ± 22.7	68.0 ± 0.0	41.3 ± 29.4	50.7 ± 2.3	41.3 ± 22.0	88.0 ± 12.0
Tukey (5%)	NS	NS	NS	NS	NS	NS	NS	NS

Table 1. The effect of lentil- tef mixture on percentage (n= 25) of lentil grains with Adzuki bean beetle

Mixing tef grain with stored lentil had no influence (p > 0.05) on the average number of eggs per grain (Fig.1). Thus, the Adzuki bean beetle laid eggs irrespective of the amount of tef grain mixed with lentil grains. Between 26 June 2018 and 16 August 2018 the average number of eggs per grain increased continually on all treatments and on the average there were 0.75 to 1.25 eggs per grain. Moreover, on 18 September 2018 sampling, the average number of eggs per grain was greater than one on most treatments. The exceptions were lentil +5% tef and lentil +25% tef treatments on which the average number of eggs was less than one per grain. On the other hand, on 26 October 2018 sampling the average number of eggs per grain was less than one on all treatments; while on 27 November 2018 sampling the average number of eggs was greater than one per grain except on lentil + 25% tef and lentil + 30% tef mixture, which had an average of 0.73 eggs per grain. On 20 December 2018, the average number of eggs per seed was similar to 26 October 2018 sampling except on treatments lentil + 15% tef and lentil + 20% tef, which had greater than one egg per grain. On 24 January 2019, sampling the average number of eggs was greater than one per grain on all treatments.

Because natural infestation was used to infest lentil grains within a bag, the numerical differences in the number of eggs per grain might be due to the difference in the time of infestation i.e. all bags might have not been infested at equal time. Except in January 2019, the average number of eggs per grain was generally less than one, which might be attributed to the egg laying behavior of Adzuki bean beetle. This beetle and its relative *C. maculatus* are known to evenly distribute their eggs among available grains (Sakai *et al.*, 1986; Wilson 1988). Thus, in January 2019 the number of eggs was greater than one egg per grain in all treatments which might be due to the absence of egg free lentil grains.

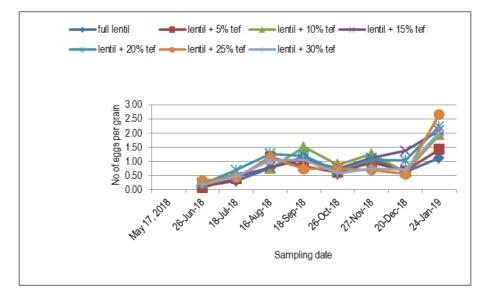


Fig. 1. The effect of lentil-tef mixture on the average number of eggs per grain laid by adzuki bean beetle

Table 2 depicts the percentage of lentil grains with both Adzuki bean beetle and its natural enemy emergence hole. There was no statistically significant difference (p > 0.05) among the different treatments in the percentage of lentil grains with emergence holes. Thus, on 26 June 2018, sampling, only one grain from lentil + 10% tef treatment had one Adzuki bean beetle emergence hole but in the remaining treatments lentil grains had no emergence hole. Similarly, on 18 July 2018 sampling there was no lentil grain with emergence hole in all but lentil + 5% tef and lentil + 20% tef treatments in which 4 and 1 grain, respectively, had Adzuki bean beetle emergence hole. On 16 August 2018 sampling, there were lentil grains with Adzuki bean beetle, its natural enemy or both emergence holes in all the treatments. In the rest of the sampling dates, different proportions of grains had holes of Adzuki bean beetle and its natural enemy holes. The pattern of increment of number of grains with emergence hole fluctuated throughout the experimental period, which is partly ascribed to the sampling position in the bag. However, the emergence of Adzuki bean beetle and its natural enemy in the absence or presence of tef indicates that tef did not affect the lifecycle of both insects.

The average number of Adzuki bean beetle emergence holes was not affected (p > 0.05) by lentil-tef mixture (Fig. 2). Up to mid-July (18 July 2018 sampling), the number of Adzuki bean beetle emergence hole was extremely rare. Thus, only in lentil + 5% tef mixture there was an average of 0.04 hole per grain. Between 16 August 2018 and 27 November 2018, the average number of Adzuki bean beetle emergence holes continually increased on all treatments and on 27 November 2018, there were an average of 0.2 to 0.45 holes per grain. On 20 December 2018 sampling the average number of Adzuki bean beetle emergence hole tended to decline but it increased thereafter (24 January 2019 sampling).

Dinarmus basalis (Rondani) and *Anisopteromalus calandrae*, which belong to the Order Hymenoptera and Family Pteromalidae, were the ectoparasitoids that attack Adzuki bean beetle in stored lentil. *A. calandrae* was previously reported by Abraham (1996) in stored maize in western Ethiopia, while *D. basalis* is a new record to Ethiopia. Dependent up on the sampling date and lentil-tef mixture treatments, the proportion of Adzuki bean beetles parasitized by the two natural enemies together ranged from 18% to 66%.

Lentil- tef mixture did not affect (p > 0.05) the occurrence of the natural enemies of Adzuki bean beetle (Fig.3). Natural enemy emergence holes were initially detected on16 August 2018 in all but full lentil and lentil + 30% tef mixture. However, on 18 September 2018, all treatments not only had natural enemy emergence holes but also had the maximum number of natural enemy emergence holes. The average number of natural enemy emergence holes. The average number of natural enemy emergence holes. This is attributed to factors such as unsuitable host size; intraspecific competitions among individuals of a species; and interspecific competitions that are known to reduce the effectiveness of *A. calandrae* and *D. basalis* in stored lentil (Qumruzzaman and Islam, 2005).

Treatment	26-Jun-	18-Jul-	16-Aug-18	18-Sep-18	26-Oct-18	27-Nov-18	20-Dec-18	24-Jan-18
	18	18						
full lentil	0.0	0.0	8.0 ± 4.0	30.7 ± 16.2	22.7 ± 18.5	38.7 ± 8.3	12.0 ± 14.4	40.0 ±30.2
lentil + 5% tef	0.0	4.0	17.3 ± 12.9	33.3 ± 12.9	21.3 ± 18.0	34.7 ± 15.1	24.0 ± 12.0	41.3 ± 14.1
lentil + 10% tef	1.3	0.0	5.3 ± 6.1	37.3 ± 12.9	37.3 ± 38.9	45.3 ± 18.0	34.7 ± 19.7	62.7 ± 18.9
lentil + 15% tef	0.0	0.0	14.7 ± 2.3	22.7 ± 12.2	17.3 ± 2.3	26.7 ± 2.3	28.0 ± 4.0	56.0 ± 6.9
lentil + 20% tef	0.0	1.3	9.3 ± 2.3	24.0 ± 17.4	5.3 ± 2.3	32.0 ± 0.0	13.3 ± 12.9	57.3 ± 4.6
lentil + 25% tef	0.0	0.0	12.0 ± 20.8	13.3 ± 10.1	5.3 ± 4.6	20.0 ± 14.4	17.3 ± 9.2	42.7 ± 2.3
lentil + 30% tef	0.0	0.0	6.7 ± 6.1	16.0 ± 6.9	21.3 ± 16.2	20.0 ± 6.9	8.0 ± 4.0	44.0 ± 20.8

Table 2: The effect of lentil- tef mixture on percentage (n= 25) of lentil grains with Adzuki bean beetle and it natural enemy emergence holes

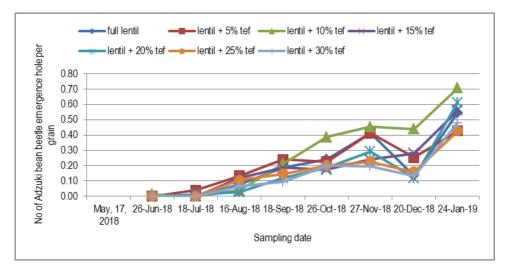


Fig. 2. The effect of lentil-tef mixture on the number of Adzuki bean beetle emergence holes

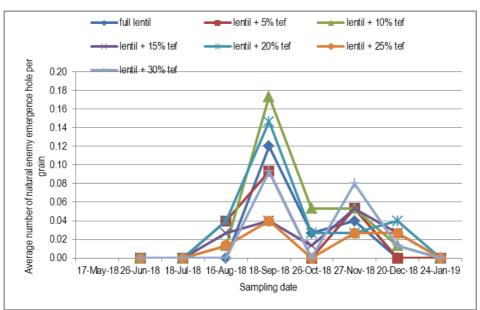


Fig. 3. The effect of lentil-tef mixture on emergence of Adzuki bean beetle natural enemy emergence

The current study revealed that mixing tef up to 30% (w/w) on replacement bases with lentil grain had no effect on occurrence of Adzuki bean beetle and its natural enemies. This result is similar to the finding of Tadesse and Basedow (2005) who affirmed that mixing maize grain with tef at the rate of 33% does not protect maize from damage by maize weevil, *Sitophilus zeamais*. The physics of both grains, which includes flowability of grains; formation of arches (with empty space below) by grains; and non-homogeneous grain size that form non-uniform force network, might be responsible for the ineffectiveness of tef grain in preventing Adzuki bean beetle

occurrence and associated damage. First Derso is a small seeded lentil cultivar with 1000 seed weight of 32 to 37 g. According to Amin et al. (2004), lentil seed that weighs 20 g per 1000 seed and at moisture content of 10% (w/w) had 34% intergranular space. On the other hand, grains of tef are minute in size and their diameter ranges from 0.5 to 1.0 mm (Assefa et al., 2011). Moreover, grains have no cohesive force that binds them together (Jaeger and Nagel, 1992). Therefore, the tef grains easily trickled down through the inter-granular space and accumulated at the bottom of the bag. Second, the amount of tef used was not sufficient to fill the inter-granular space found within stored lentil grains. Ukeh et al. (2010) stated that if host and nonhost grains are mixed, the non-host provides protection to the host through emission of deterrent or repellent volatile compounds. Therefore, the other reason for the occurrence of Adzuki bean beetle and its natural enemies within mixture of lentil and tef grains could be attributed to the absence of volatile compounds emitted by tef that may repel or deter both insect species. In conclusion, since Adzuki bean beetle was able to infest, reproduce, and complete multiple generations on lentil irrespective of the amount of tef mixed, farmer should be informed about the ineffectiveness of the traditional method they apply to control Adzuki bean beetle in stored lentil.

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