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Integration of botanicals and inert dusts with resistant varieties against *Sitophilus zeamais* (Motsch.) of stored maize (*Zea mays*) in Ethiopia

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Dosage rates of 0.625, 1.25 and 2.5% of the leaves powder of *Calpurnia aurea* and *Milletia ferruginea*, and red and white inert dusts that were admixed with the 100 g of three resistance maize varieties were tested as protectant against maize weevils under laboratory condition. Significantly ($p < 0.05$) higher parental adult weevil's mortality were recorded in all treatments of integration than untreated check at almost all days (except first day) after treatment. Mortality effects of the tested integrated components were also increased both with increased dosage and days after treatment. The mortality also became 100% in all treatments of integration applied at all rates, following 12 days of treatment application likewise the positive control. All treatments of integration applied at all rates also induced significantly ($P < 0.05$) higher (≥ 60.31%) protection of maize grains against F1 progeny emergence, grain damage (≤ 6.67) and weight loss (≤ 1.12) by *S. zeamais* than negative control. Besides, 100% inhibition of F1 progeny emergence, no percent grain damage and weight loss of maize grains were also observed in all of the three treatments of integration applied at dosage of 10% likewise also for that of the positive control. Therefore, integration of the aforementioned botanicals and inert dusts with resistant varieties applied at 2.5% and above (5 and 10%) rates were potent in preventing maize grains against maize weevils attack and could be used in the management of maize weevils as ecologically sound, safe and cheap weevil's management alternatives under farmer's storage conditions.

Key words: *Calpurnia aurea, Milletia ferruginea*, red inert dust, white inert dust, *Sitophilus zeamais*,, resistant varieties.

INTRODUCTION

In Africa, the bulk of grain is produced by small scale farmers and food security of these farmers depends on

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not only to their success to grow, but also to their success to store their staple food that they need for their families (Blum and Bekele, 2002). Accordingly, in developing countries like Ethiopia, lack of appropriate grain storage technologies leads to up to 20-30% post-harvest losses, particularly due to postharvest insect pests such as maize weevils (Tefera and Abass, 2012). In addition, estimations based on some limited observations indicated that grain losses in maize due to storage insect pests alone are about 30-100% (Tadesse, 2003; Demissie, 2006). Furthermore, storage insect pests are reported to be the most detrimental of even all factors (biotic and abiotic factors) that cause about 43% of the total physical and nutritional loss of grains such as maize occurring in the developing world (Chomchalow, 2003). As a result, post-harvest losses resulting from insects pests remain a huge challenge (Tefera et al., 2010).

Thus, crops loss by insect pests constitutes a great constraint to the realization of food security of farmers not only in Sub-Saharan Africa (SSA) including Ethiopia, but also worldwide (Obeng-Ofori, 2008). Accordingly, post-harvest losses reduction of food grains like maize due to storage insect pests is very crucial to ensure food security and to feed the ever-increasing population of the SSA countries (Dejene, 2004). However, less attention and resources have been allocated for this purpose over the past decades. These and other factors mentioned above, along with the current concerns of synthetic insecticides and the desire for residue-free grains by consumers, indicate the presence of great demand for searching and developing alternative management options such as an Integrated Pest Management (IPM). It has been reported that IPM give priorities to non-chemical methods such as botanicals, inert dusts, varietal resistance, etc. Thus, the current study was initiated with the following objectives: to study the effect of integration of *Calpurnia aurea* and *Milletia ferruginea,* and red and white locally available inert dusts with three resistant varieties of maize (Melkasa-6Q, MH-138Q and SPRH) at selected doses against *S. zeamais* under laboratory condition.

MATERIALS AND METHODS

The Test Insect's culture

S. zeamais adults were collected from maize grains stored in various farmers' traditional storage facilities of major maize producing localities Shashogo and Sankura districts of Southern Ethiopia. Then, the insects were brought to the laboratory of Addis Ababa University, Faculty of Life Science, insect science insectary of Zoological Science Department of Ethiopia. These test insects were cultured at 27 ± 3°C and 55-70% RH (Jembere et al., 1995; Kidane and Jembere, 2010). Shone variety of maize grains was also obtained from farmer's storages of the aforementioned districts. It was the most commonly grown hybrid in the region and considered to be susceptible to insect infestation. The grains were kept at -20 ± 2°C for 2 weeks to kill any infesting insects, cleared of broken kernels and debris and then graded manually according to size, and similar sized grains were selected and used for the experiment (Gemechu et al., 2013). Fifteen pairs of the adult of the test insects were placed in 12, 1 L glass jars containing 250 g disinfested seeds (Kidane and Jembere, 2010). The jars were then covered with nylon mesh and held in a place with rubber bands to allow ventilation and to prevent the escape of the experimental insects. The parent of the test insects were sieved out after an oviposition time of 13 days. Then, the jars were kept under laboratory condition until F1 progeny emergence. The F1 progeny, which emerged after 30 days, were sieved out and used for the experiment.

Botanicals

Plant materials (leaves) used for the study were collected from natural habitats of Hadiya zone, southern Ethiopia and the identities of the plants were confirmed into *C. aurea* and *M. ferruginea* species at the national herbarium of Life Science Faculty of Addis Ababa University.

Dried and ground materials

Following the methods by Gebre Selase and Getu (2009) the fresh plant materials (leaves) of a known weight were kept in a well-ventilated room under shade for 2-3 weeks depending on weather conditions and dried. Dried materials were ground to fine powder using mortar and pestles. Then they were sieved to remove larger material (Figure 3) (Tekie, 1999; Jembere, 2002).

Resistant varieties

The top three resistant varieties screened among 21 currently available varieties of maize obtained from (Figure 3) Bako, western Ethiopia in our other study were Melkasa-6Q, MH-138Q and SPRH respectively.

Inert dusts

Red and white inert dusts that have been used by local people for painting of houses in rural areas were obtained from rocky area of Hadiya zone of south Ethiopia (Figure 2). These were sieved to remove larger materials This inert dusts were tested in integration with others because, there was reported cases that indicate inert materials involving clays have been effective on stored-product insects at high rates and suggested as they might be viable protectants grain in underdeveloped countries (De Lima, 1987; Demissie et al., 2008). Besides, with current health, environmental and other concerns of insecticides as well as the desire for residue free food grains and their products by people, they have been receiving attentions. These facts have also been holds true for botanicals as well as resistant varieties.

Integration of botanicals and inert dusts with resistant varieties against *S. zeamais*

Following the methods by earlier researchers (Gebre selase and Getu; Kidane and Jembere 2010) 100 g of disinfected top three resistant varieties of maize grains (Figure 4), were put in 1 L glass jars. These were then treated differently with 0.625, 1.25 and 2.5% doses (rates) of the leaf powders of each of the test plants. Each plastic jar containing a total of 100 g of grains treated with respective concentrations of plant leaves powder was also admixed with the aforementioned selected rates each of the two colored inert dusts (red and white) following similar procedures adapted by Ibrahim (2017), and Ibrahim and Sisay (2012). Untreated grains of the aforementioned three varieties were kept under similar conditions and served as a negative control. Besides, Malathion (5%) dust at recommended dose of 0.05% (w/w) was served as positive standard check. The jars were then covered with nylon mesh and held in place with rubber bands to allow ventilation and to prevent the escape of the experimental insects. The corresponding glass jars were shaken well for 5 min in order to have a uniform mixture of treatments and were kept in the laboratory at 27 ± 5C and 55-70% RH (Jembere et al., 1995; Kidane and Jembere, 2010). 20 randomly picked newly emerged 3-7 days unsexed adult experimental insects were introduced to each treated and untreated jars and were kept for fourteen days of oviposition (Derera et al., 2001; Tadesse and Basedow, 2005). Each experiment was arranged in a completely randomized design with three replications. The adult mortality counts were performed after 1, 2, 3, 4, 5, 7 and 12 days post-exposure. All dead and live adults were taken away from the jars after the last count.

F1 progeny assessment

The treated and control grains were kept until emergence of F1 progeny under same experimental condition indicated in insect culture section. Then the numbers of F1 progeny produced by the experimental insects were counted. Counting were stopped after 56 days from the day of introduction to avoid overlapping of generation following similar procedures of previous scientists (Gebre Selase and Getu, 2009; Zewde and Jembere, 2010).

Damage and weight loss assessment

Two days after the last F1 count of 56 days, samples of 30 grains were taken randomly from each jar and the number of damaged (grains with characteristic hole) and undamaged grains were counted and weighed. Damaged grains were expressed as a percentage of the total number of seeds in each replicate. Percentage weight losses were calculated by count and weight method following similar procedures of previous scientists (Gebre Selase and Getu, 2009; Zewde and Jembere 2010) as:

$$\%\text{ Loss in weight} = \frac{UNd - Dnu}{U\,(Nd + Nu)} \times 100$$

Where U = weight of undamaged grain; D = weight of damaged grain; Nd = number of damaged grain and Nu = number of undamaged grain

Following similar procedures by Gebre Selase and Getu (2009), percent protection or inhibition rate in F1 progeny emergence (% IR) was calculated using the following formula:

$$\%\text{ IR} = (Cn-Tn) \times 100 / Cn$$

Where, Cn is the number of newly emerged insects in the untreated (control) jar and Tn is the number of insects in the treated jar.

Data analysis

Data on adult mortality, F1 progeny emergence, and grain damage and weight loss were managed with the Microsoft Excel version 2013 and then were subjected to analysis of variance (ANOVA) of SPSS Version 16. Then, the effect of integration on these parameters was analyzed using appropriate statistical method; Univariate (for the former one) and one-way analysis of variances (for the later ones). Significant differences between means of different treatments and time of exposure were separated using Tukey's studentized (HSD) test at 5% confidence interval. Standard errors (±se) are given following means in tables and as T-shaped beams in figures. Correlation between the treatments and the efficacy measuring parameters like weight loss and others were determined using Pearson's correlation of SPSS program of version 16.

RESULTS

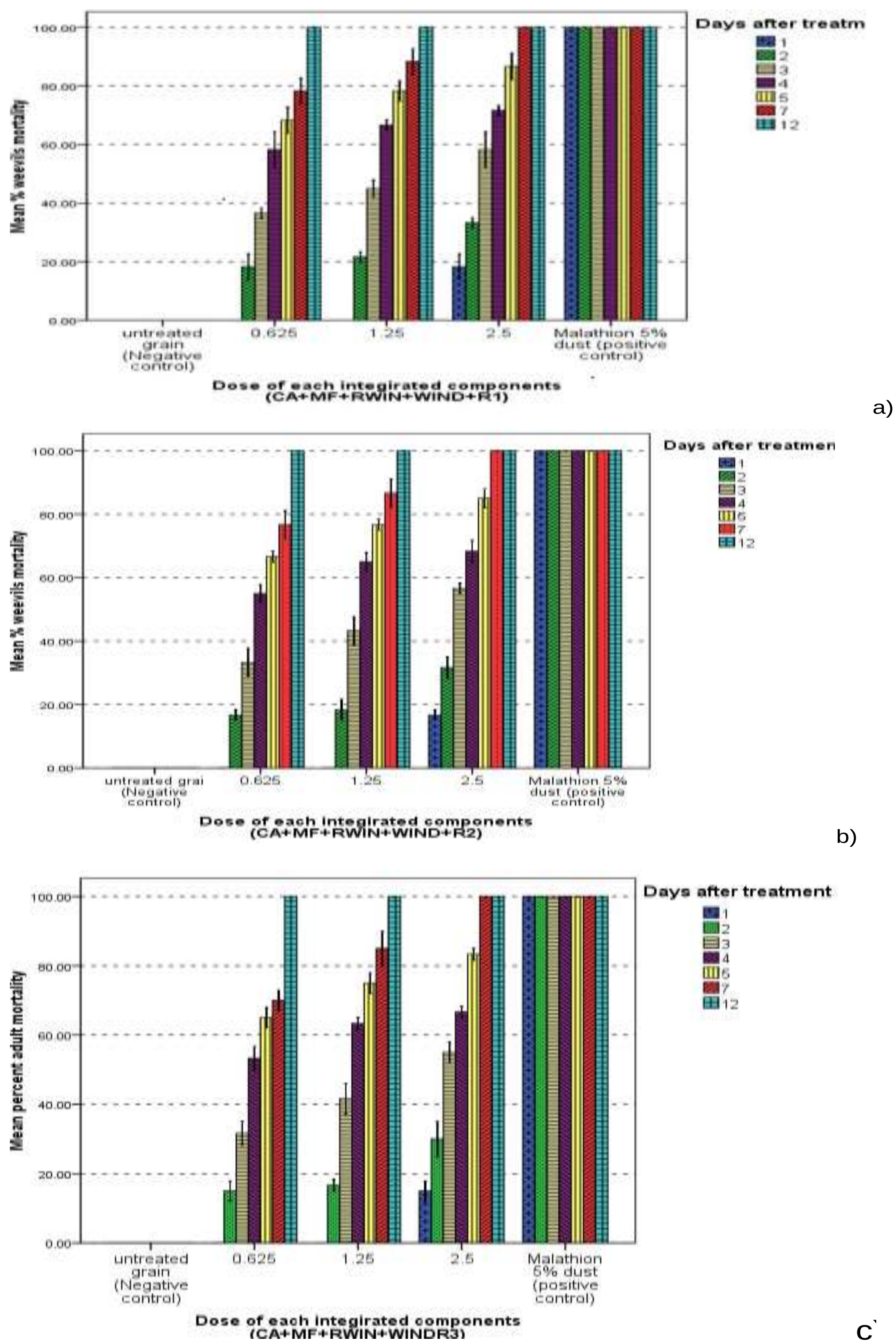
Integration of botanicals, inert dusts and resistant varieties on mortality of adult weevils

Integration of the botanicals and inert dusts with the resistant varieties had significant ($p< 0.05$) effect on percent mortality of adult weevils in comparison to the untreated check. Significantly ($p < 0.05$) higher parental adult weevil's mortality were recorded in all treatments of integration as compared to the untreated check at almost all days (except the first day) after treatment post exposure. Mortality effects of the tested integrated components were also increased both with increased dosage and days after treatment application (Figure 1).

Significant percentage weevil's mortality was not induced with all rates of the tested components of integration prior to four days post treatment exposure. However, significantly ($p < 0.05$) high weevil's mortality (≥ 53%) was caused by all treatments of integration applied at all dosages four days after post treatment exposure. Besides, the weevil's mortalities were significantly ($p < 0.05$) higher (≥ 60 and 65%) in the entire three treatments of integration applied at all doses, 5 and 7 days after treatment application respectively. The mortality also became 100% in all treatments of integration applied at all rates, following 12 days of treatment application likewise the positive control (Figure 1).

Botanicals, Inert Dusts and Resistant Varieties Integration in F1 Progeny, % Grain Damage and Weight Loss

The number of F1 progeny emerged, percent grain damage and weight were found to be significantly ($p < 0.05$) lower in all treatments of integration than untreated check. All the parameters measured were also significantly ($p < 0.05$) lower in all treatments of



CA = Calpurnia aurea powder, MF = Milittia ferruginea powder, RIN = Red inert dust, WIN = White inert dust, R1= the 1st, R2 = the 2nd, R3 = the 3rd resistant varieties of maize among the screened ones and the top three resistant varieties among screened varieties in in our other study were Melkasa-6Q, MH-138Q and SPRH, respectively.

Figure 1. Mean % mortality (mean ± SE) of adult weevils due to integration of botanicals, inert dusts and with the top three resistant varieties: a) Melkasa-6Q, b) MH-138Q and c) SPRH respectively applied at the rate of 0.65%, 1.25% and 2.5% each after days of post treatment exposure


a)

b)

Figure 2. Partial view of a) red and b) white locally available inert dusts.


a)

b)

c)

d)

Figure 3. Partial view of a) *C. aurea* leaf and b) *M. ferruginea* leaf dried under shade; c) leaf powder of *M. furginea* (upper one) and *C. aurea* (later one) and d) weight measurement through digital blance for powder treatment.

Figure 4. Partial view of maize varieties collected from Bako; western Ethiopia for screening against weevils.

Table 1. The effect of Integration of CA, MF, RIND and WIND with 3 resistant varieties on F1 emergence, % protection, and % grain damage and % weight loss.

Treatment	Dosage (g/100 g)	Mean number of F1 progeny	Percentage protection	Mean% grain damage	Mean% weight loss
	0.625 (2.5%)	8.67±1.67^{b}	72.62	4.67±0.88^{c}	0.79±0.27^{b}
T1 (CA+MF+RID+WID+R1)	1.25 (5%)	4.67±2.67^{ab}	85.25	2.33±0.33^{b}	0.35±0.27^{ab}
	2.5 (10%)	0.00±0.00^{a}	100.00	0.00±0.00^{a}	0.00±0.00^{a}
	0.625 (2.5%)	9.33±2.19^{b}	70.54	6.33±0.33^{c}	0.87±0.01^{b}
T2 (CA+MF+RE+WID+R2)	1.25 (5%)	5.33±1.67^{b}	83.17	2.67±0.88^{b}	0.42±0.00^{ab}
	2.5 (10%)	0.00±0.00^{a}	100.00	0.00±0.00^{a}	0.00±0.00^{a}
	0.625 (2.5%)	10.67±2.33^{b}	60.31	6.67±0.33^{c}	1.10±0.27^{b}
T3 (CA+MF+RE+WID+R3)	1.25 (5%)	5.67±1.73^{b}	82.10	3.33±0.88^{b}	0.52±0.47^{ab}
	2.5 (10%)	0.00±0.00^{a}	100.00	0.00±0.00^{a}	0.00±0.00^{a}
Untreated grain	0	31.67±0.88^{c}	0.00	11.67±0.33^{d}	5.39±0.33^{c}
Malathion dust 5%	0.05	0.00±0.00^{a}	100.00	0.00±0.00^{a}	0.00±0.00^{a}

Means followed by the same letter in a row are not statistically significantly different at $p < 0.05$. CA = Calpurnia aurea powder; MF = Milletia ferruginea powder; RIN = Red inert dust;WIN = White inert dust; R1= the 1st; R2 = the 2nd; R3 = the 3rd resistant varieties of maize among the screened ones T1 (CA+MF+RID+WID+R1) = IPM1; T2 (CA+MF+RE+WID+R2) = IPM2; T3 (CA+MF+RE+WID+R3) = IPM3; T=treatment and IPM=integrated components

Table 2. Correlation among efficacy determining parameters of integrated components.

Efficacy parameter	IPMC dose	F1IPM 1	F1IPM 2	F1IPM 3	GDIPM 1	GDIPM 2	GDIPM 3	WLIPM 1	WLIPMM 2	WLIPM 3
IPMC dose	**1**									
F1IPM1	-.850**	1								
F1IPM2	-.868**	-	1							
F1IPM3	-.883**	-	-	1						
GDIPM1	-.905**	.956**	-	-	1					
GDIPM2	-.939**	-	.952**	-	-	1				
GDIPM3	-.950**	-	-	.952**	-	-	1			
WLIPM1	-.790**	.965**	.969**	-	.944**	-	-	1		
WLIPMM2	-.799**	-	.971**	.963**	-	.904**	-	-	1	
WLIPM3	-.838**	.973**	.979**	-	-	-	.918**	-	-	1

Correlation coefficients with two asterisks (**) represent highly significant association at P values < 0.01 (2-tailed); with hyphen (-) represent no association and those without asterisk are non-significant. CA = Calpurnia aurea powder; MF = Milletia ferruginea powder; RIN = Red inert dust; WIN = White inert dust; R1= the 1st; R2 = the 2nd; R3 = the 3rd resistant varieties of maize among the screened ones T1 (CA+MF+RID+WID+R1) = IPM1; T2 (CA+MF+RE+WID+R2) = IPM2; T3 (CA+MF+RE+WID+R3) = IPM3; T=treatment and IPM=integrated components.

integration applied at rate of 5 and 10% as compared to those applied at lower dosage (2.5%). All of the three treatments of integration applied at all rates induced significantly ($P < 0.05$) higher (≥ 60.31%) protection of maize grains against F1 progeny emergence, grain damage (≤ 6.67) and weight loss (≤ 1.12) by S. zeamais than negative control. Besides, 100% inhibition of F1 progeny emergence, no percent grain damage and weight loss of maize grains were observed in all of the three treatments of integration applied at dosage of 10 % likewise with that of the positive control (Table 1`).

The correlations among the treatments of integration applied at different dosage and the efficacy parameters measured (the number of F1 progeny emerged, percentage grain damage and weight loss) were highly significant and strongly negative (r was in the range between -0.95 and -0.790). However, they were strongly positive between F1 progeny produced, and percent grain damage (r ≥ 0.923) and weight loss (r ≥ 0.960) of all the three treatments of integration (Table 2).

DISCUSSION

The current study has showed significantly mortality of weevils following 7 days and 100% mortality following 12 days as a result of different treatments of integration. It has also indicated significantly higher botanicals and inert dusts with resistant varieties could in weevils management. This suggests that integrating inhibition of F1 progeny emergence, higher reduction in grain damage and weight loss due to different treatments of integration, and hence, the effectiveness of integration enhance their potency in managing weevils. This in turn might be probably because of the existence of great possibility of synergism of morality factors in integration than those used alone. This finding is in line with those of the finding of preliminary studies conducted by Demissie (2006) at Bako of Ethiopia in which the combined use of minimum rates of *Chenopodium* plant powder, botanical triplex, silicosec and filter cakes with weevil tolerant varieties has reduced grain damage. Ibrahim (2017) also indicated that integrating neem seed and Mexican tea powder provided significant protection to maize from the maize weevil. Besides, it was also indicated that the powders of *P. glandulosus* and *A. indica* at their different proportions of their binary combinations generally caused significant mortality to adult *C. maculatus* and *S. zeamais* compared to the controls (untreated grain) (Katamssadan, 2016). It was also reported that combinations of different rates of Malathion (5%) dust and neem seed powder caused higher weevils mortality than the untreated control (Ibrahim and Sisay, 2012). Ibrahim (2015) also indicated that the rates of mortality of weevils in all of the combinations of Malathion dust and Mexican tea powder were significantly higher than that of untreated check. It was also shown that the combination of neem seed powder and Malathion at the proportions of 40+20% and 50+10% on maize were additive with respect to the mortality caused to *Sitotroga cerealella* Olivier (Ibrahim, 2014).

Being in direct association of the mortality effects of the integrated components with dose applied and post treatment exposure days against the weevils in the present study was also reported by previous researcher (Bayih, 2014).

The present study has also shown that the integration of the different management tactics tested induced significantly higher protection of maize grains against weevils attack than untreated check. This might be due the enhanced mortality effect in integrated treatments, which in turn might be probably due to the presence of great possibility of synergism of morality factors in integration. Similarly, it was also reported that for the reason of their synergetic effects, integration of one management method with other sustainable method could provide long lasting solution to losses in storage (Dobie, 1977). Besides, it was also reported that combining (integrating) of different botanicals has enhanced their potency in controlling stored grain insect pests than those applied unitarily (Agona and Muyinza, 2003; Bayih, 2014). In his preliminary study, Demissie (2006) also reported that the combined use of minimum rates of *Chenopodium* plant powder, botanical triplex, silicosec and filter cakes with weevil tolerant varieties has reduced grain damage at Bako of Ethiopia. Ibrahim (2017) also indicated that integrating neem seed and mexican tea powder provided significant protection to maize from the maize weevil. It was also reported that combinations of different rates of Malathion (5%) dust and neem seed powder caused higher weevils mortality than the untreated control (Ibrahim and Sisay, 2012).

Conclusion

In the current study, it is possible to see significantly higher mortality of weevils following 7 days and 100% mortality following 12 days of treatment application of integrated components. It is also possible to see significantly higher inhibition of F1 progeny emergence, higher reduction in grain damage and weight loss due to different treatment integrated components and hence, the effectiveness of integration in weevils management. This confirmed that integration of the different tactics (botanicals and inert dusts with resistant varieties) at all rates (2.5%) and above (5 and 10%) were potent in preventing maize grains against maize weevils attack. Thus, integration of the aforementioned components at all rates (2.5%) and above (5 and 10%) could be used in managing maize weevils as ecologically sound, safe and cheap weevils management alternative under subsistence farmer’s storage conditions in Ethiopia and elsewhere with similar pest problems.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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