Field study of the repellent activity of 'Lem-ocimum'-treated double bags against the insect pests of stored sorghum, *Tribolium castaneum* and *Rhyzopertha dominica*, in northern Nigeria

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Abstract

A field study of the efficacy of a novel use of repellent plant material to protect stored sorghum from pest damage was conducted in Kebbi State, Nigeria. A combination of *Ocimum basilicum* (Sweet basil) and *Cymbopogon nardus* (Lemongrass) powdered dried leaves ('Lem-ocimum') was found to be significantly more repellent to the most common grain pest, *Tribolium castaneum*, when applied as a water-based paste between the layers of double storage-bags at a dose of 1% w/w (plant powder/grain) than untreated double bags (n=30, $P<0.001$). The efficacy of protecting a given percentage of grain in Lem-ocimum treated double-bags was tested in 120 store-rooms, each of which contained 15-35 x 60 kg single bags of sorghum that initially had moderate levels of beetle infestation (26-50 *T. castaneum*/bag). After 5 months in storage, the percent change in grain weight and levels of infestation by the two most prevalent pests, *T. castaneum* and *Rhyzopertha domin*ica, inside treated double-bags were significantly lowest in the store-rooms with the highest percentage of all grain (4%) kept in treated double-bags ($P<0.01$, n=120 store-rooms). This result may have been due to the mass fumigation effect of adding 400-900 g Lem-ocimum to each of the store-rooms with 4% treated grain. Only the participant farmers that had stored 4% of their grain in treated double-bags felt the treatment provided significant protection. The findings suggest Lem-ocimum treated double-bags could improve the chances that a proportion of a farmer's grain would be of good enough quality to sell in the market mid-way through the storage season, when the price of grain would earn a good profit.

Keywords: *Tribolium*, Synergist plant repellents, Treated double-bag, Sorghum, *S*mall-scale farmers.

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1. Introduction

Sorghum (*Sorghum bicolor* L.) grain is a staple food of small-scale farmers in Kebbi state, where a large proportion of the grain is stored after harvest in various traditional and modern storage structures. Stored grain is consumed by the family when other staple foods are scarce, and, when there is sufficient need, it is sold at market to provide cash for other socio-economic needs (KARDA, 2004; COA, 2009). However, grain losses due mainly to two storage beetles, *Tribolium castaneum* (Herbst) (Coleoptera: Tenobrionidae) and *Rhizopertha dominica* (F.), represent a threat to farmers in realizing these benefits (KARDA, 2004; Chimoye & Abdullahi, 2011). According to a current survey by Utono (2013), insect infestations in the study area cause weight and quality losses in stored grain that lead to a reduction in its market value toward the end of the storage season when grain prices increase dramatically as supplies decline. When farmers are unable to protect their grain from insect damage, but are in need of cash, they are forced to sell grain early in the season when the price is lowest (COA, 2009). Accordingly, damage due to storage insects affects the food security and income of small-scale farmer on a large scale (Manda & Mvumi, 2010).

Insecticides remain the most commonly used tools to control pests in developing countries (Udoh *et al*., 2000; Kamanula *et al*., 2011). However, due to the high cost and erratic availability of synthetic insecticides, small-scale farmers either leave their grain untreated or use dried repellent plant materials as grain protectants (Poswal & Akpa, 1991; Belmain & Stevenson 2001; Golob *et al*., 2002; Deng *et al*., 2009). Small-scale farmers in Kebbi rely largely on traditional approaches of protecting their stored grain, i.e., they mix their grain with a range of dried plant materials believed to be repellent, and store the mixture in single bags of woven polypropylene material. Unfortunately, the efficacy of this approach is inconsistent (KARDA, 2004; COA, 2009), probably due mainly to a lack of knowledge about variability between plant species and sub-species in the amount of active ingredient present and the detrimental effect of some methods used to dry and prepare plant materials on the strength and duration of their repellent properties (Belmain & Stevenson, 2001). Also, farmers tend to assume that storage bags serve as an effective barrier to insects, especially when repellent or pesticidal plant materials are added to the stored grain (Anwar *et al*. 2005; Hou *et al*., 2004; Koona *et al*., 2007), which is unfortunately not always true. Hence, the aim of this study was to determine whether grain could be better protected by double-bagging instead of single-bagging, thereby adding an additional physical barrier to insects, and by increasing the intensity of repellency by concentrating the distribution of repellent plant material in a continuous layer between the two bags instead of mixing it throughout the bag of grain.

The repellent plant species *Ocimum basilicum* L. (Sweet basil) and *Cymbopogon nardus* L. (Lemongrass) were chosen due to their well-documented repellent properties and their local availability; both plants are commonly cultivated for their culinary and medicinal properties, and various sub-species of *O. basilicum* grow wild in the area. Several studies have demonstrated the importance of plants from the genus *Ocimum* and *Cymbopogon* as grain protectants (Regnault-Roger & Hamraoui, 1994; Parugrug & Roxas, 2008; Mishra *et al*., 2012). Furthermore, *C. nardus* has been shown to be effective in reducing storage infestations of a range of beetle species (Boeke *et al*., 2004; Parugrug & Roxas, 2008; Manzoor *et al*., 2011). Surprisingly, there appear to be no published reports of field research on the efficacy of *O. basilicum*, one of the most commonly used repellent plants against *T. castaneum*, one of the most common pests of stored grain (Lale & Yusuf, 2000 and Chimoya & Abdullahi, 2011) and the species most relevant to the study presented here.

The overall objective of this study was to increase the efficacy of a basic method of grain protection that farmers in the study area are already familiar with (i.e., the application of repellent plant material to bags of stored grain), by introducing a combination of plants that are synergistically more repellent when used together (Utono, 2013) and by enhancing the 'barrier' properties of storage bags by concentrating the repellent product between the layers of a double bag, thereby increasing the likelihood that beetles approaching the bag of grain from any direction would be deterred by the physical barrier of two layers of cloth and their behavioural responses to a relatively high dose of repellents.

Preliminary bioassay studies in the laboratory (Utono, 2013) demonstrated that the combination of *O. basilicum* and *C. nardus* in a ratio of 1:1 by dry weight was significantly more repellent (0.02±0.007 proportion of beetles released in the bioassay arena that penetrated into treated bags of grain) than equivalent weights of either plant on its own; 0.15±0.022 and 0.06±0.007 for *O. basilicum* and *C. nardus*, respectively (Analysis of deviance: χ^2=304.130, df=5, P<0.01, with the Bonferroni correction for multiple comparisons). It was also shown that a water-based paste of the combination (Lem-ocimum) applied between the layers of double bags with clean grain placed in the inner bag significantly reduced the proportion of beetles released in the bio-assay arena that penetrated into treated bags of grain (0.04±0.021) as compared to a control untreated double bag (0.33±0.021; $F=20.927$, $df=4$, $P<0.001$) (Utono, 2013). However, it is essential that laboratory results are verified under field conditions before recommending the new method to farmers.

Therefore, this study was designed to establish 1) how much extra protection from insect infestation and loss of grain is achieved by using a double bag compared to a single bag, 2) how much extra protection is provided if the double bags are treated with Lem-ocimum, compared to untreated double bags, and 3) if the grain-protectant properties of Lem-ocimum treated double-bags increases by treating a greater proportion of the grain in a store-room.

2. Materials and Methods

The main aim of the field experiments presented here was to determine if locally available repellent plant materials could deter enough target pest species to significantly reduce grain losses when applied to bags of stored grain held in grain stores typical of the study area. The basic approach was to place Lem-ocimum-treated double-bags of clean sorghum (i.e., initially free of insect pests) in local store-rooms that contained untreated bags of sorghum with pre-existing moderate levels of beetle infestations (see Section 2.2). The levels of beetle infestation and grain weight-loss in the treated double-bags were monitored monthly over 5 months. The study was conducted with the collaboration of 82 farmers who were consulted before the field experiments began, as described in Section 2.2. By involving local farmers, and responding to their perceptions of the outcome of the experiments, it is hoped that any successful new methods that emerge from the study will be more readily acceptable within the local community than if there were presented as a fixed set of recommendations. Therefore, in addition to the field experiments, participant evaluations were conducted throughout the study period to assess the views of 42 of the participating farmer as to how the new approach to protecting sorghum grain compares with the other methods practiced in the study area.

2.1 Site of experiments

Field experiments were conducted from September 2011 to March 2012 in three villages (Tondi 11°36'46"N 3°35'54"E, Maga, near Dabai 11°28'23"N and Wasagu 11°22'4"N 5°48'36"E) in the southern area of Kebbi state, Nigeria. Preliminary surveys in 24 villages across the whole of Kebbi state established that in the south of Kebbi the greatest proportion of small-scale farmers relied on sorghum as the main grain crop and already used repellent plant materials as grain protectants (Utono, 2013). Moreover, farmers in this area lost more sorghum grain due to storage insect damage than farmers in the other regions of Kebbi state. Thus, it was concluded that farmers in this area would have a greater understanding of the problems associated with protecting stored sorghum from insect pests and would benefit the most from participation in the field experiments.

2.2 Selection of farmers and their stores for the experiments

A survey was conducted in the three villages to identify the grain stores with the best conditions for the planned experiments, based on the following criteria: sorghum was stored in polypropylene bags in store-rooms, at least 15 x 60 kg bags of sorghum were stored per store-room, all sorghum was stored in the threshed form, *T. castaneum* infestations were already present in store-rooms and the levels of infestation were similar across a large enough group of farmers to provide statistically meaningful results. The survey consisted of obtaining a 1kg sample of sorghum from at least three different bags in each store-room, using a 50 cm sampling spear (Gwinner *et al*., 1990). The samples were sieved to count the number of live adult *T. castaneum* present. Low infestations (1-25 *T. castaneum* per 1 kg sample of grain) were found in 28 store-rooms, moderate infestations (26-50 *T. castaneum*) were found in 162 store-rooms and high infestations (≥50 *T. castaneum*) were found in 23 store-rooms. The store-rooms with moderate infestations were chosen for the field experiments, because the greatest number of store-rooms fell in this category**.** In each of the store-rooms selected, the participating farmers kept 15–36 untreated 60 kg bags of threshed sorghum. Through stakeholder meetings, the farmers had agreed in advance to do nothing to control insect pests in their store-rooms during the experiments, and therefore, the untreated bags were considered to present a reasonably standardized level of infestation pressure on the treated double-bags. The participating farmers accepted the offer of being given the treated grain used for the experiments in their respective store-rooms at the end of the experiments in compensation for grain lost due to leaving their gain untreated during the experiments, .

2.3 Interactive meetings with famers to determine how they would be involved in the experiments

A meeting was held in each village attended by an agricultural extension worker from the local government, the village head and the group of 82 farmers who owned the 162 store-rooms that had been chosen for the experiments to agree on how the farmers would participate. The new method of protecting stored sorghum with Lem-ocimum treated double-bags was explained and the aims of the field study were described. The farmers were encouraged to present their perspectives and ask questions.

2.4 Preparation of experimental storage bags

The participating farmers normally stored their grain in polypropylene bags that hold 60 kg of sorghum grain. Considering that the experiments would require 420 treated bags, it was not practical or affordable to use 60 kg bags for the study. Therefore, it was decided that 6 kg bags of sorghum would be the optimal size for experimental bags. Polypropylene bags that are normally used by farmers to store their grain were purchased from farmers and traders, and cut and sewn to a size small enough to contain 6 kg of sorghum grain.

2.5 Plant materials and grain treatment

Fresh bags of healthy sorghum were purchased from the King of farmers' store and fumigated with phosphine for 4 days prior to the start of the experiments to kill any live insects in them. Fresh leaves of *O. basilicum* and *C. nardus* were collected from various farmers in Tondi who grew the cultivated varieties of both plants, shade-dried for 3-4 days, packed in polypropylene bags and stored in a relatively cool, dark place for up to 7 days prior to the start of the experiments. One herbarium specimen of each plant sampled was deposited in the College of Agriculture, Zuru, Nigeria herbarium and another was deposited at Kew Gardens for identification and to add to their respective collections. Experts at Kew Gardens identified the species of both plants based on their physical attributes.

On the first day of an experiment, the leaves were ground to a powder with a mortar and pestle used by local farmers. A 50:50 (by weight) combination of ground *O. basilicum* and *C. nardus* was used to produce 1% w/w of 6 kg sorghum grain. This plant powder was mixed with 10 g of starch per 100 mL of water to make a paste. The starch was used to ensure the plant paste would adhere to the bags. The plant paste was spread all over the outside of the 6 kg bags and kept to dry in a room for 24 hours. The treated bags were loaded with grain and then inserted into a second bag of the same size and sewn shut with string. The untreated double and single bags used as controls were constructed in the same way, but they were not treated with any plant materials.

2.6 Experimental procedures

The following two field experiments were conducted; 1) to test the relative efficacy of single bags, double bags and Lem-ocimum treated double-bags in repelling beetle infestations and grain weight-loss, and 2) to test the effect of treating various proportions of the total grain kept in a store-room with Lem-ocimum treated double-bags on the level of protection from insect pests and grain weight-loss in the treated bags.

2.6.1 Experiment 1: How much extra protection from insect damage do double bags provide compared to single bags? Does Lem-ocimum treatment significantly increase the protection of grain stored in double bags? Thirty store-rooms from the 162 store-rooms identified with moderate infestations were chosen in Tondi and Maga (i.e., 15 store-rooms in each); three 6 kg experimental bags of uninfested sorghum were prepared as follows; one untreated single bag, one untreated double-bag and one Lem-ocimum treated double–bag. One set of these three experimental bags was placed in each of the 30 store-rooms. The experimental bags were positioned on top of the farmer's untreated single-bags, with > 10 cm between each of the experimental bags (as for Fig. 1, except the experimental bags were as described above). The range in number of farmers' untreated bags in each store-room was 15-47 bags (mean=18.6 bags), and, therefore, the percentage of total grain that was placed in experimental bags in each store-room was 0.5- 1.65%. The same distribution of experimental bags was repeated for all 30 store-rooms in the two villages.

The experimental bags were sampled every 4 weeks for 5 months to assess 1) the type and numbers of the two target beetle species present and 2) the amount of grain weight-loss that had occurred. All three experimental bags in each store-room were speared three times; the numbers of live and dead insects of each species were counted, and the average of the three counts was rounded to the nearest whole number and recorded. The numbers of live adults were recorded separately from the numbers of dead insects to find out whether the beetles that gained access to the bags might have reproduced within the treated bags or move through without laying eggs or dying. The sample was taken from a different corner of the bag each time. Weight loss was determined using the 'count and weigh' method of estimating weight loss as described by Adams & Schulten (1978).

2.6.2 Experiment 2: Does the percentage of stored grain kept in Lem-ocimum treated double-bags affect the level of beetle infestations in treated bags? The underlying hypothesis associated with this experiment is that the greater the proportional amount of grain protected by Lem-ocimum, the greater the overall level of protection is afforded to all treated bags due to a cumulative effect of repellent volatiles emitted from the bulk of treated bags. To test this, a variable number of 6 kg Lem-ocimum treated double-bags of grain were placed in 120 store-rooms, distributed over the top of the farmers' untreated bags, as described in section 2.6.1 and the grain in treated bags was sampled each month to identify the levels of beetle infestation and grain weight-loss.

To test if there is a 'dose effect' (i.e., the protection of grain stored in treated bags is increased by treating a greater percentage of the grain in the store-room with Lem-ocimum double-bags), 120 store-rooms were assigned randomly to three experimental groups of 40 store-rooms, with one of three levels of Lem-ocimum treated-double bags per room; low (~1% *by weight* of all grain in the store-room, which amounted to 2 or 3 treated double-bags), medium (~2% of all grain, 5-8 treated double-bags) or high (~4% of all grain, 9-18 treated double–bags). The number of untreated single-bags of grain in each store-room varied (range = 15-35 and mean = 22.2 bags/store-room). The calculation as to how many 6 kg treated double-bags were to be added to each store-room was based on how many of the farmer's 60 kg untreated bags of grain were present, and rounding to the nearest whole bag. Accordingly, to set up a 'low level' (~1%) of grain in treated double-bags in a store-room with 30 untreated bags (i.e., 1800 kg grain), 3 treated double-bags (18 kg grain) were added to the store-room and placed evenly over the top of the farmer's untreated bags (Fig. 1). All treated double-bags were sampled every 4 weeks for 5 months as for Experiment 1 (Section 2.6.1) to monitor the levels of beetle infestation and grain weight-loss. The overall mean and SE was calculated for each of the 40 store-rooms used to test the effect of each of the three 'doses' of Lem-ocimum treated double-bags.

2.7 Follow-up survey to evaluate the perception of participating farmers on the efficacy and acceptability of the new grain protection method

The views of 42 participating farmers were assessed by a short survey at the end of the experiments, based on their views of the efficacy of the new double bag method and their readiness to adopt the new method. They were asked 1) whether they thought the new method worked, 2) if they thought it protected the grain better than what they had done before, 3) if they thought the new method reduced grain loss in their experience, 4) whether they thought the infestations were reduced enough to encourage them to use the treated double–bag method in the future, and 5) if the new method was too much work compared to the amount of grain lost?

2.8 Statistical analysis of data

The data were analyzed using the R statistical software package (version 2.10.0) R Development Core Team (2012). A one-way ANOVA was used to test for significant effects of treatments in Experiment 1 (untreated single-bag, untreated double-bags and treated double-bags) and in Experiment 2 (three levels of treated bags; low, medium or high percentages of grain kept in treated double-bags) in store-rooms on the rates of beetle infestation and grain weight-loss. The differences between means of specific treatments were analysed for statistical significance using a Tukey HSD test.

The slopes of the increase in numbers of insects of two target species (*T. castaneum, R. dominica*) infesting the treated double-bags in each store-room over the 5 months of the experiment were calculated, taking into account the use of repeated measures in the study design (i.e., the same treated bags in the same store-rooms were sampled repeatedly over the 5 month experiment). Differences between slopes were tested by one-way ANOVA.

3. Results

3.1 Experiment 1: Effect of adding double bagging and double bagging plus Lem-ocimum on the level of insect infestations in bags of stored grain

The most numerous insect species found infesting stored sorghum per store-room at the end of the 5 months experiment were *T. castaneum* (mean±SD: 26.2±12.6) > *R. dominica* (14.2±9.8).

The results in Fig. 2 show the trend in mean numbers of *T. castaneum* and *R. dominica* per 100 g grain sample per month obtained from untreated single-bags, untreated double-bags and Lem-ocimum-treated double-bags. The results show a continuous monthly increase in the number of beetles from the first month to the fifth month in all the experimental bags. The increase was most rapid in untreated single-bags followed by untreated double-bags and treated double-bags for both beetle species. The results in Table 1 show that the differences in the rate of monthly increase in number of *T. castaneum* between the three treatment bags was significant (ANOVA; F=101.5, df =2,87, P<0.001), and the difference between the means of each of the three treatments was found to be statistically significant (Tukey HSD test; P<0.001). Hence, these results suggest that double–bagging sorghum grain significantly reduces *T. castaneum* infestations and, more importantly, the addition of Lem-ocimum significantly enhances the deterrent properties of double bags against *T. castaneum*.

Similarly the difference in the rate of monthly increase in numbers of *R. dominica* between the treatments was found to be statistically significant (ANOVA; F=10.37, df=2,87, P<0.001) (Table 1). However, the difference between the means of each of the three treatments was found to be significant only between untreated single- and treated double-bags, and untreated single- and untreated double-bags (P<0.001), but not between untreated double- and treated double-bags (Tukey HSD; P=0.341). This suggests that the repellent properties of Lem-ocimum have little effect on *R. dominica*.

3.2 Experiment 2: The effect of storing a variable number of 6 kg treated double-bags in sorghum store-rooms on the rate of growth of insect infestations over time

The main aim of this experiment was to determine how well treated double-bags protect clean (uninfested) grain from insects migrating into them from untreated bags. The untreated bags of sorghum in the farmers' store-rooms were considered to be the primary source of insect infestations. The variables tested for the effect on infestation levels and grain weight-loss were three levels of treated bags (low, medium or high) in a store-room.

The results in Fig. 3 show there was a continuous increase in the mean number of *T. castaneum* and *R. dominica* per month in store-rooms over the study period in all the stores. The rate of increase in mean number of the beetles increased overall for all treatments, with a distinct difference between the three levels of treated bags for both species of beetle. The results in Table 2 indicate that the level of treated bags placed in each store-room had a statistically significant effect on the rate of monthly increase in numbers of *T. castaneum* found in the treated bags (ANOVA; F=16.13, *df*=2,117; $P<0.001$): the higher the level of treated bags added to a store-room, the lower the rate of increase in numbers of beetles found in the treated bags. The difference between the means of the three levels was found to be statistically significant (Tukey HSD; $P<0.01$).

Similarly the results in Table 2 indicate that the levels of treated bags placed in each store-room had a statistically significant effect on the rate of monthly increase in the numbers of the *R. dominica* found in the treated bags (ANOVA; F=5.52, *d*f=2,117, $P<0.01$). The differences between the means of each of the three treatments was found to be significant only between stores with high and medium or high and low levels of treated bags ($P<0.01$), but not between stores with medium and low levels of treated double bags (Tukey HSD; $P>0.05$), which suggests that the high levels of treated bags was most effective against this beetle species.

The data for numbers of *T. castaneum* found in treated bags was analyzed in greater detail by analyzing 'live' and 'dead' beetles separately to determine whether the beetles established colonies within the treated bags, or tended to move through the bags without laying eggs. Table 3 shows the mean monthly increase in the number of live and dead adult *T. castaneum* per store-room per treatment. The level of treated bags had a significant effect on the rate of monthly increase in numbers of live *T. castaneum* (ANOVA; F=14.36, df = 2,117, P<0.001). The difference between the means of each of the three treatments was found to be significant only between high and medium levels of treated bags, and high and low levels of treated bags (Tukey HSD; P<0.001), but not between low and medium levels of treated bags (P=0.104). Hence, there were fewest live adults *T. castaneum* found in treated bags when the level of treated bags was highest. This suggests that there may have been a 'mass effect' of the presence of the Lem-ocimum repellent plant volatiles in store-rooms with the highest levels of treated bags.

Similarly the difference in the rate of increase in number of dead *T. castaneum* between the three treatments was found to be significant (ANOVA; F=15.92, df=2,117, P<0.001), and the difference between the means in each of the three treatment levels was also significant (Tukey HDS; P<0.001).

Overall, the rate of increase in live and dead *T. castaneum* was lowest when the level of treated bags was highest, which is what one might expect, since this treatment added the most repellent plant material overall to the store-rooms. It is interesting to note that the monthly rate of increase in live beetles was less than for dead beetles for every level of treated bags, and this increase was found to be statistically significant (ANOVA; F=27.4, df=1,234,

P<0.0001), which suggests the repellent plant materials reduces the likelihood of beetles laying eggs in the treated bags.

3.3 Effect of adding different levels of treated double-bags to store-rooms on grain weight-loss due to insect infestations

Figure 2C shows the trend in the amount of mean monthly weight loss of grain due to insect species in store-rooms containing untreated single-bags, untreated double bags and Lem-ocimum treated double-bags. These results show that there was a continuous monthly increase in weight loss from the beginning of the experiment to the fifth month for store-rooms with all three treatments. The difference in the rate of monthly increase in weight loss between the three treatments was statistically significant (ANOVA; F=23.5, df=2,87, P<0.001), and the differences between the means of the three treatments were also significant (Tukey HSD; P<0.001). In the fifth month, grain weight loss from treated double-bags was only 2.2±0.38%, compared to 3.4±0.39% in untreated double-bags and 5.2±0.45% in untreated single-bags, thus demonstrating that both increasing the physical barrier of bags and treatment with repellent plant materials reduces grain weight loss.

When many more Lemocimum-treated double-bags of grain were kept in farmers' store-rooms, the percent weight loss of grain was even lower; in store-rooms with 4% of grain kept in treated double-bags, grain weight loss was reduced to only 1.1±0.23% in the fifth month (Fig. 3C). The percentage grain kept in treated double-bags had a significant effect on the rate of increase in weight loss over time (ANOVA; F=44.77, df =2,117, P<0.001). Moreover, the difference between the means of each of the treatment levels was statistically significant between each level of treated bags (Tukey HSD; P< 0.01). Thus, keeping a greater percentage of grain in Lem-ocimum treated double-bags in store-rooms increases the efficacy of the treatment.

3.4 Effect of the number of untreated bags in a store-room on the rate of increase in number of insects in the treated bags over time

The untreated single-bags of grain in the farmers' store-rooms were considered to be the main source of insect infestations and the relationship between insect infestation in the treated bags and the number of untreated bags stored in the same store-room was investigated.

Figure 4 shows that, surprisingly, there was an **inverse** linear relationship between the numbers of untreated bags in a store-room and the rate of increase of *T. castaneum* in the treated bags. This relationship is dependent on the level of treated bags, only reaching statistical significance for high and medium levels (Table 4).

The results indicate that the greater the number of untreated bags in a store-room the lower the rate of increase in the number of beetles in the treated bags. An analysis of covariance found significant main effects for the untreated bag number covariate ($F=10.5$, $df=1,114$, $P=0.0016$) and for the treated bag level factor ($F=21.4$, $df=2,114$, $P<0.0001$). The interaction term was also significant ($F=6.0$, $df=2,114$, $P<0.01$), showing that the best fit model had different slope parameters for the different levels of treated bags (Table 4).

3.5 Evaluation of the perceptions of participating farmers on the effect of the new method of protecting stored grain in repellent double bags

Table 5 summarises the perceptions of the participating farmers on the effectiveness of the new method tested in their store-rooms compared to their existing methods of mixing dried repellent plant materials with their grain in single bags. The results indicate that the participating farmers generally had a positive impression that the new method was more effective than their existing methods. This view was given by 100% of respondents who tested a high level of treated double-bags in their stores, followed by those that tested a medium or a low level of treated bags, in rank order. However, some respondents who tested a medium or a low level of treated bags indicated that the effect of the new method was similar to their existing methods. Few of the respondents were not able to discern any differences between the methods. This difference in perception between participants that used different levels of treated bags suggests that the respondents experienced a range of effectiveness based on the level of treated bags used in their store-rooms and respondents who tested a higher level of treated bags experienced better efficacy.

Table 6 indicates how the respondents perceived the relative simplicity or difficulty in the preparation and application of the new method compared to their existing methods. More than half of the respondents who tested the high level of treated bags said that the new method was a bit easier than what they did currently. Only a few indicated that the method was harder than their existing methods. However, over 40% of the respondents who tested a low level of treated bags said that the level of difficulty was similar to their existing methods and only a few respondents indicated the new method to be more difficult. More than 50% of the respondents who tested a medium level of treated bags expressed the view that the new method was more difficult to implement. This suggests that farmers' views on the simplicity or difficulty of the new method depends on the efficacy they observed during the field experiment; more than half of the respondents who tested a high level of treated bags expressed the view that the method was more effective, which indicates they felt the implementation effort was worth the outcome.

4. Discussion

The field experiments demonstrated that Lem-ocimum-treated double-bags can significantly reduce the rate at which beetles infest bags of grain, and, more importantly, the higher the percentage of grain kept in Lem-ocimum treated double-bags, the greater the protective effects of the treatment.

Although there are cost implications of storing grain in Lem-ocimum-treated double-bags, the results of this study show that this method may be cost effective for farmers to keep a certain proportion of their grain as free from pest damage as possible, as long as possible, to ensure they have clean grain to sell late in the season.

This study demonstrates that the method has potential for protection of stored grain longer than the farmers' existing methods do. For example, it indicates that the resulting repellent effect of the method has an effect on the overall number of beetles after 5 months, i.e., with just 6 *T. castaneum* per sample of grain from the store-room with a high level of treated double-bags. This result is highly favourable, compared to the high levels of infestation in the surrounding untreated single bags (baseline 26-50 *T. castaneum* per sample) and the grain weight loss was only 0.9%±0.11%.

The efficacy of the double bag method is likely due to: a) the combination of two types of repellent plant materials in Lem-ocimum (*C. nardus* plus *O. basilicum*), benefiting from a wider range of active compounds, and b) the paste of dried plants applied in a layer between the two bags formed a more concentrated barrier than if the same amount of material had been scattered throughout the 5 kg of grain inside the inner bag, and c) the high infestation of beetles found in the untreated single bags should not be a surprise, since the bags present the least barrier to the beetles. The physical barrier of double bags without any repellent plant materials may induce beetles to leave the double bag and end up moving into single bags. Cline & Highland (1981) reported that storage pests such as *R. dominica*, *L. serricorne*, and *T. castaneum* could enter packaging through openings less than 1.35 mm and their larvae can enter even smaller openings. However, double bagging appears to be more difficult for beetles to penetrate according to Mullen & Mowery (2002) and the results presented here. Significantly, fewer beetles were found in untreated double bags than in single bags in Experiment 1. Thus, the combination of a double barrier of potent repellent plant material and a double layer of woven plastic may explain the significant reduction in infestation by *T. castaneum*.

The greater repellent efficacy demonstrated by the stores treated with a high level of Lem-ocimum double-bags could be as a result of the mass fumigant effect of adding more Lem-ocimum to the group of treated bags. Mikhaiel (2011) and Mishra *et al*. (2012) reported that more repellent volatiles from many sources lead to greater deterrence of insects. This may not be the case in store-rooms with small or medium levels of treated bags, where the bags were sparse and at distance from each other. The mechanism could be that when there is a greater number of treated bags placed next to each other in a store-room, there is also a greater concentration of volatile repellent compounds emanating from the treated bags, seeping down and sideways into the untreated bags beneath the treated bags and causing a local area repellent effect, moving beetles out of the untreated bags and further away from the treated bags. The store-room itself would become fumigated with repellent volatiles, and mask the ability of the beetles to perceive the presence of grain odour and repelled out of the store-room altogether.

Experiments 1 and 2 demonstrated that the efficacy of the different treatments varied with insect species. The effect was much less impressive for *R. dominica* than *T. castaneum.* The first species occurred at much lower levels and are not considered to cause as much damage as the later. This may suggests that some insect species are more susceptible to certain treatments than other species, which may be due to differences in responses to chemical compounds in plants. Isman (2006) reported that some plant substances that deter one pest can be tolerated or even an attractant to other pests.

The finding that the monthly rate of increase in live beetles was less than for dead beetles for every level of treated bags suggests that the live beetles did not establish colonies (i.e. lay eggs) in the treated bags, although this point still needs to be investigated directly.

When a new method of grain protection is developed and tested among local participants, it is important to evaluate the perception of the participants about the new method tested. This may provide information about what participants think about the method, what they appreciate most and where there are needs for improvement for better acceptance and uptake.

Generally, the perception of the respondents of the new method, as tested in their respective stores, was positive based on its efficacy, ease of application and cost effectiveness. A few participants, however, expressed concern about the low efficacy, difficulty and cost of additional materials. These views were influenced by the level of treated double-bags that were tested in their respective store-rooms. The positive impression given by all the respondents who tested high levels of Lem-ocimum treated bags in their store-rooms indicates that they were impressed by how efficacious the method was. The impression of a few respondents of those who tested low or medium percent levels of treated double bags, that the new method produced similar results to their own methods, may indicate that the method was less effective in their store-rooms than in those with a high level of treated double-bags. This suggests that farmers' interests can be influenced by the demonstrated efficacy of a control method. Belmain & Stevenson (2001), Mugisha-Kamatenesi *et al*. (2008) and Deng, *et al*. (2009) report that farmers' perceptions and choices of botanical pesticides as control agents are influenced by efficacy, availability and cost effectiveness, indicating that these could affect farmer acceptance and uptake. Hence, when introducing a new method of grain protection to farmers, the efficacy, cost and availability should be discussed to encourage acceptance by farmers, although this may depend on the particular circumstances of farmers and their locality.

5. Conclusions

The results of this study support the conclusion that Lem-ocimum treated double-bags could provide better, longer-lasting protection for sorghum grain from infestation by beetles than the existing methods of grain storage used by farmers in Kebbi, and, therefore, could ensure a better reserve of clean grain for farmers. Although the experiments tested 6 kg experimental bags, which are just 10% of the weight of standard farmers' bags of grain (60 kg), the outcome was positive, and demonstrated that it would be worthwhile to undertake a larger field trial with standard sized farmers' bags. The impact of this method needs to be tested over a long period of storage, i.e., at least a year, to establish the duration of efficacy. This study suggests that the slow rate of increase in infestations with treated double-bags may be enough to maintain a low enough level of infestation over the maximum period of sorghum storage i.e., 7- 12 month (Adejumo & Raji, 2007), which would have obvious implication for food security and marketing of grain within the study area.

The main aim of this research was to help farmers improve their storage practices, and ensure they can keep a proportion of their grain of good enough quality to sell for a better profit than previously. Farmers can also benefit from this new technology since it reduces the burden of winnowing required by their traditional method of mixing grains with repellent plant materials. Farmers are already conversant with the use of bags and plant materials and the materials are all available in the study area. It may be possible to identify new and more effective repellent plant materials to apply to the outside of the inner double-bag, plants which have not been used traditionally due to their bitter flavour and/or toxicity when mixed with stored grain. These products could be used with the double bagging method since grain will have no direct contact with the plant materials. However, future research needs to be conducted on the effect of plant residues that may remain in the grain when stored over long periods of time.

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Figure Legends

Fig. 1 Lem-ocimum treated double-bags (5 x 6 kg bags) containing uninfested sorghum, placed on top of 60 kg bags of farmers' untreated single bags of sorghum. Untreated bags were infested with moderate levels of *Tribolium castaneum* beetles (26-50 *T. castaneum* beetles per 1 kg sample of grain). A similar arrangement of bags was used to compare the amount of grain protection of untreated single and double bags and treated double-bags (see text).

Fig. 2 Comparison of the grain protection provided by three types of storage bag; an untreated single-bag (light grey), an untreated double-bag (dark grey) and a Lem-ocimum treated double-bag (black). Trend in the mean±SE number of A) *Tribolium castaneum* and B) *Rhyzopertha dominica* beetles found in grain samples (100 g /bag/month) taken from the three experimental bags of initially uninfested sorghum grain and C) grain weight-loss, estimated by the 'count and weigh' method (Adams & Schulten, 1978) with 100 g samples/bag/month. Experimental bags had been placed in store-rooms containing untreated single-bags that initially had moderate levels of *T. castaneum* infestations (n=30 store-rooms/treatment/month). Each sample is the mean of three spear samples of grain taken from each experimental bag.

Fig. 3 Comparison of the effect of 'dose' of Lem-ocimum treated double-bags on the trend in mean±SE numbers of A) *Tribolium castaneum* and B) *Rhyzopertha dominica* beetles found in grain samples (100 g /bag/month) and C) grain weight-loss, estimated by the 'count and weigh' method (Adams & Schulten, 1978) with 100 g samples/bag/month. A 'Low' percent (1% of total grain weight in store-rooms kept in treated bags; light grey), 'Medium' percent (2%; dark grey) or 'High' percent (4%; black) of treated bags were placed in store-rooms containing farmers' untreated single-bags that initially had moderate levels of *T. castaneum* infestations (n=40 store-rooms/treatment/month).

Fig. 4 Correlation between 'percent grain in 6 kg Lem-ocimum treated double-bags' and 'number of 60 kg untreated bags in store-rooms' on the mean monthly rate of increase in numbers of adult *Tribolium castaneum* per 100g sample of grain over the five month experiment. Overall, there was a significant inverse relationship ($P<0.001$) in the rate of increase in number of beetles in the treated bags as the number of untreated bags increased. There was a significant main effect for untreated bag number, percent treated bags and their interaction ($P<0.01$, ANCOVA).