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The definitive version is available at: <http://dx.doi.org/10.1016/j.jspr.2014.03.005>

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

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11

12 **Abstract**

13 A field study of the efficacy of a novel use of repellent plant material to protect stored
14 sorghum from pest damage was conducted in Kebbi State, Nigeria. A combination of
15 *Ocimum basilicum* (Sweet basil) and *Cymbopogon nardus* (Lemongrass) powdered dried
16 leaves (‘Lem-ocimum’) was found to be significantly more repellent to the most common
17 grain pest, *Tribolium castaneum*, when applied as a water-based paste between the layers of
18 double storage-bags at a dose of 1% w/w (plant powder/grain) than untreated double bags
19 (n=30, $P<0.001$). The efficacy of protecting a given percentage of grain in Lem-ocimum
20 treated double-bags was tested in 120 store-rooms, each of which contained 15-35 x 60 kg
21 single bags of sorghum that initially had moderate levels of beetle infestation (26-50 *T.*
22 *castaneum*/bag). After 5 months in storage, the percent change in grain weight and levels of
23 infestation by the two most prevalent pests, *T. castaneum* and *Rhyzopertha dominica*, inside
24 treated double-bags were significantly lowest in the store-rooms with the highest percentage
25 of all grain (4%) kept in treated double-bags ($P<0.01$, n=120 store-rooms). This result may

26 have been due to the mass fumigation effect of adding 400-900 g Lem-ocimum to each of the
27 store-rooms with 4% treated grain. Only the participant farmers that had stored 4% of their
28 grain in treated double-bags felt the treatment provided significant protection. The findings
29 suggest Lem-ocimum treated double-bags could improve the chances that a proportion of a
30 farmer's grain would be of good enough quality to sell in the market mid-way through the
31 storage season, when the price of grain would earn a good profit.

32

33 **Keywords: *Tribolium*, Synergist plant repellents, Treated double-bag, Sorghum, Small-**
34 **scale farmers.**

35

36 **1. Introduction**

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

51

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

72

73 The repellent plant species *Ocimum basilicum* L. (Sweet basil) and *Cymbopogon nardus* L.
74 (Lemongrass) were chosen due to their well-documented repellent properties and their local
75 availability; both plants are commonly cultivated for their culinary and medicinal properties,

76 and various sub-species of *O. basilicum* grow wild in the area. Several studies have
77 demonstrated the importance of plants from the genus *Ocimum* and *Cymbopogon* as grain
78 protectants (Regnault-Roger & Hamraoui, 1994; Parugrug & Roxas, 2008; Mishra *et al.*,
79 2012). Furthermore, *C. nardus* has been shown to be effective in reducing storage infestations
80 of a range of beetle species (Boeke *et al.*, 2004; Parugrug & Roxas, 2008; Manzoor *et al.*,
81 2011). Surprisingly, there appear to be no published reports of field research on the efficacy
82 of *O. basilicum*, one of the most commonly used repellent plants against *T. castaneum*, one of
83 the most common pests of stored grain (Lale & Yusuf, 2000 and Chimoya & Abdullahi,
84 2011) and the species most relevant to the study presented here.

85

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

94

95 Preliminary bioassay studies in the laboratory (Utono, 2013) demonstrated that the
96 combination of *O. basilicum* and *C. nardus* in a ratio of 1:1 by dry weight was significantly
97 more repellent (0.02 ± 0.007 proportion of beetles released in the bioassay arena that
98 penetrated into treated bags of grain) than equivalent weights of either plant on its own;
99 0.15 ± 0.022 and 0.06 ± 0.007 for *O. basilicum* and *C. nardus*, respectively (Analysis of
100 deviance: $\chi^2=304.130$, $df=5$, $P<0.01$, with the Bonferroni correction for multiple

101 comparisons). It was also shown that a water-based paste of the combination (Lem-ocimum)
102 applied between the layers of double bags with clean grain placed in the inner bag
103 significantly reduced the proportion of beetles released in the bio-assay arena that penetrated
104 into treated bags of grain (0.04 ± 0.021) as compared to a control untreated double bag
105 (0.33 ± 0.021 ; $F=20.927$, $df=4$, $P<0.001$) (Utono, 2013). However, it is essential that
106 laboratory results are verified under field conditions before recommending the new method to
107 farmers.

108

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

114

115 **2. Materials and Methods**

116 The main aim of the field experiments presented here was to determine if locally available
117 repellent plant materials could deter enough target pest species to significantly reduce grain
118 losses when applied to bags of stored grain held in grain stores typical of the study area. The
119 basic approach was to place Lem-ocimum-treated double-bags of clean sorghum (i.e.,
120 initially free of insect pests) in local store-rooms that contained untreated bags of sorghum
121 with pre-existing moderate levels of beetle infestations (see Section 2.2). The levels of beetle
122 infestation and grain weight-loss in the treated double-bags were monitored monthly over 5
123 months. The study was conducted with the collaboration of 82 farmers who were consulted
124 before the field experiments began, as described in Section 2.2. By involving local farmers,
125 and responding to their perceptions of the outcome of the experiments, it is hoped that any

126 successful new methods that emerge from the study will be more readily acceptable within
127 the local community than if there were presented as a fixed set of recommendations.
128 Therefore, in addition to the field experiments, participant evaluations were conducted
129 throughout the study period to assess the views of 42 of the participating farmer as to how the
130 new approach to protecting sorghum grain compares with the other methods practiced in the
131 study area.

132

133 **2.1 Site of experiments**

134 Field experiments were conducted from September 2011 to March 2012 in three villages
135 (Tondi 11°36'46"N 3°35'54"E, Maga, near Dabai 11°28'23"N and Wasagu 11°22'4"N
136 5°48'36"E) in the southern area of Kebbi state, Nigeria. Preliminary surveys in 24 villages
137 across the whole of Kebbi state established that in the south of Kebbi the greatest proportion
138 of small-scale farmers relied on sorghum as the main grain crop and already used repellent
139 plant materials as grain protectants (Utono, 2013). Moreover, farmers in this area lost more
140 sorghum grain due to storage insect damage than farmers in the other regions of Kebbi state.
141 Thus, it was concluded that farmers in this area would have a greater understanding of the
142 problems associated with protecting stored sorghum from insect pests and would benefit the
143 most from participation in the field experiments.

144

145 **2.2 Selection of farmers and their stores for the experiments**

146 A survey was conducted in the three villages to identify the grain stores with the best
147 conditions for the planned experiments, based on the following criteria: sorghum was stored
148 in polypropylene bags in store-rooms, at least 15 x 60 kg bags of sorghum were stored per
149 store-room, all sorghum was stored in the threshed form, *T. castaneum* infestations were
150 already present in store-rooms and the levels of infestation were similar across a large enough

151 group of farmers to provide statistically meaningful results. The survey consisted of obtaining
152 a 1kg sample of sorghum from at least three different bags in each store-room, using a 50 cm
153 sampling spear (Gwinner *et al.*, 1990). The samples were sieved to count the number of live
154 adult *T. castaneum* present. Low infestations (1-25 *T. castaneum* per 1 kg sample of grain)
155 were found in 28 store-rooms, moderate infestations (26-50 *T. castaneum*) were found in 162
156 store-rooms and high infestations (≥ 50 *T. castaneum*) were found in 23 store-rooms. The
157 store-rooms with moderate infestations were chosen for the field experiments, because the
158 greatest number of store-rooms fell in this category. In each of the store-rooms selected, the
159 participating farmers kept 15–36 untreated 60 kg bags of threshed sorghum. Through
160 stakeholder meetings, the farmers had agreed in advance to do nothing to control insect pests
161 in their store-rooms during the experiments, and therefore, the untreated bags were
162 considered to present a reasonably standardized level of infestation pressure on the treated
163 double-bags. The participating farmers accepted the offer of being given the treated grain
164 used for the experiments in their respective store-rooms at the end of the experiments in
165 compensation for grain lost due to leaving their gain untreated during the experiments, .

166

167 **2.3 Interactive meetings with famers to determine how they would be involved in the** 168 **experiments**

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

175

176

177 **2.4 Preparation of experimental storage bags**

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

184

185 **2.5 Plant materials and grain treatment**

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

195

196 On the first day of an experiment, the leaves were ground to a powder with a mortar and
197 pestle used by local farmers. A 50:50 (by weight) combination of ground *O. basilicum* and *C.*
198 *nardus* was used to produce 1% w/w of 6 kg sorghum grain. This plant powder was mixed
199 with 10 g of starch per 100 mL of water to make a paste. The starch was used to ensure the
200 plant paste would adhere to the bags. The plant paste was spread all over the outside of the 6

201 kg bags and kept to dry in a room for 24 hours. The treated bags were loaded with grain and
202 then inserted into a second bag of the same size and sewn shut with string. The untreated
203 double and single bags used as controls were constructed in the same way, but they were not
204 treated with any plant materials.

205

206

207 **2.6 Experimental procedures**

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

213

214 **2.6.1 Experiment 1: How much extra protection from insect damage do double bags** 215 **provide compared to single bags? Does Lem-ocimum treatment significantly increase** 216 **the protection of grain stored in double bags?** Thirty store-rooms from the 162 store-

217 rooms identified with moderate infestations were chosen in Tondi and Maga (i.e., 15 store-
218 rooms in each); three 6 kg experimental bags of uninfested sorghum were prepared as
219 follows; one untreated single bag, one untreated double-bag and one Lem-ocimum treated
220 double-bag. One set of these three experimental bags was placed in each of the 30 store-
221 rooms. The experimental bags were positioned on top of the farmer's untreated single-bags,
222 with > 10 cm between each of the experimental bags (as for Fig. 1, except the experimental
223 bags were as described above). The range in number of farmers' untreated bags in each
224 store-room was 15-47 bags (mean=18.6 bags), and, therefore, the percentage of total grain

225 that was placed in experimental bags in each store-room was 0.5- 1.65%. The same
226 distribution of experimental bags was repeated for all 30 store-rooms in the two villages.

227

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

238

239 **2.6.2 Experiment 2: Does the percentage of stored grain kept in Lem-ocimum treated**
240 **double-bags affect the level of beetle infestations in treated bags?** The underlying

241 hypothesis associated with this experiment is that the greater the proportional amount of grain
242 protected by Lem-ocimum, the greater the overall level of protection is afforded to all treated
243 bags due to a cumulative effect of repellent volatiles emitted from the bulk of treated bags.

244 To test this, a variable number of 6 kg Lem-ocimum treated double-bags of grain were placed
245 in 120 store-rooms, distributed over the top of the farmers’ untreated bags, as described in
246 section 2.6.1 and the grain in treated bags was sampled each month to identify the levels of
247 beetle infestation and grain weight-loss.

248

249 To test if there is a ‘dose effect’ (i.e., the protection of grain stored in treated bags is
250 increased by treating a greater percentage of the grain in the store-room with Lem-ocimum
251 double-bags), 120 store-rooms were assigned randomly to three experimental groups of 40
252 store-rooms, with one of three levels of Lem-ocimum treated-double bags per room; low
253 (~1% *by weight* of all grain in the store-room, which amounted to 2 or 3 treated double-bags),
254 medium (~2% of all grain, 5-8 treated double-bags) or high (~4% of all grain, 9-18 treated
255 double-bags). The number of untreated single-bags of grain in each store-room varied (range
256 = 15-35 and mean = 22.2 bags/store-room). The calculation as to how many 6 kg treated
257 double-bags were to be added to each store-room was based on how many of the farmer’s 60
258 kg untreated bags of grain were present, and rounding to the nearest whole bag. Accordingly,
259 to set up a ‘low level’ (~1%) of grain in treated double-bags in a store-room with 30
260 untreated bags (i.e., 1800 kg grain), 3 treated double-bags (18 kg grain) were added to the
261 store-room and placed evenly over the top of the farmer’s untreated bags (Fig. 1). All treated
262 double-bags were sampled every 4 weeks for 5 months as for Experiment 1 (Section 2.6.1) to
263 monitor the levels of beetle infestation and grain weight-loss. The overall mean and SE was
264 calculated for each of the 40 store-rooms used to test the effect of each of the three ‘doses’ of
265 Lem-ocimum treated double-bags.

266

267 **2.7 Follow-up survey to evaluate the perception of participating farmers on the efficacy** 268 **and acceptability of the new grain protection method**

269 The views of 42 participating farmers were assessed by a short survey at the end of the
270 experiments, based on their views of the efficacy of the new double bag method and their
271 readiness to adopt the new method. They were asked 1) whether they thought the new method
272 worked, 2) if they thought it protected the grain better than what they had done before, 3) if
273 they thought the new method reduced grain loss in their experience, 4) whether they thought

274 the infestations were reduced enough to encourage them to use the treated double-bag
275 method in the future, and 5) if the new method was too much work compared to the amount
276 of grain lost?

277

278 **2.8 Statistical analysis of data**

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

286

287 The slopes of the increase in numbers of insects of two target species (*T. castaneum*, *R.*
288 *dominica*) infesting the treated double-bags in each store-room over the 5 months of the
289 experiment were calculated, taking into account the use of repeated measures in the study
290 design (i.e., the same treated bags in the same store-rooms were sampled repeatedly over the
291 5 month experiment). Differences between slopes were tested by one-way ANOVA.

292

293 **3. Results**

294 **3.1 Experiment 1: Effect of adding double bagging and double bagging plus Lem-** 295 **ocimum on the level of insect infestations in bags of stored grain**

296 The most numerous insect species found infesting stored sorghum per store-room at the end
297 of the 5 months experiment were *T. castaneum* (mean±SD: 26.2±12.6) > *R. dominica*
298 (14.2±9.8).

299

300 The results in Fig. 2 show the trend in mean numbers of *T. castaneum* and *R. dominica* per
301 100 g grain sample per month obtained from untreated single-bags, untreated double-bags
302 and Lem-ocimum-treated double-bags. The results show a continuous monthly increase in the
303 number of beetles from the first month to the fifth month in all the experimental bags. The
304 increase was most rapid in untreated single-bags followed by untreated double-bags and
305 treated double-bags for both beetle species. The results in Table 1 show that the differences in
306 the rate of monthly increase in number of *T. castaneum* between the three treatment bags was
307 significant (ANOVA; $F=101.5$, $df=2,87$, $P<0.001$), and the difference between the means of
308 each of the three treatments was found to be statistically significant (Tukey HSD test;
309 $P<0.001$). Hence, these results suggest that double-bagging sorghum grain significantly
310 reduces *T. castaneum* infestations and, more importantly, the addition of Lem-ocimum
311 significantly enhances the deterrent properties of double bags against *T. castaneum*.

312

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

320

321

322 **3.2 Experiment 2: The effect of storing a variable number of 6 kg treated double-bags in**
323 **sorghum store-rooms on the rate of growth of insect infestations over time**

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

329

330 The results in Fig. 3 show there was a continuous increase in the mean number of *T.*

331 *castaneum* and *R. dominica* per month in store-rooms over the study period in all the stores.

332 The rate of increase in mean number of the beetles increased overall for all treatments, with a

333 distinct difference between the three levels of treated bags for both species of beetle. The

334 results in Table 2 indicate that the level of treated bags placed in each store-room had a

335 statistically significant effect on the rate of monthly increase in numbers of *T. castaneum*

336 found in the treated bags (ANOVA; $F=16.13$, $df=2,117$; $P<0.001$): the higher the level of

337 treated bags added to a store-room, the lower the rate of increase in numbers of beetles found

338 in the treated bags. The difference between the means of the three levels was found to be

339 statistically significant (Tukey HSD; $P<0.01$).

340

341 Similarly the results in Table 2 indicate that the levels of treated bags placed in each store-

342 room had a statistically significant effect on the rate of monthly increase in the numbers of

343 the *R. dominica* found in the treated bags (ANOVA; $F=5.52$, $df=2,117$, $P<0.01$). The

344 differences between the means of each of the three treatments was found to be significant

345 only between stores with high and medium or high and low levels of treated bags ($P<0.01$),

346 but not between stores with medium and low levels of treated double bags (Tukey HSD;

347 $P>0.05$), which suggests that the high levels of treated bags was most effective against this

348 beetle species.

349

350 The data for numbers of *T. castaneum* found in treated bags was analyzed in greater detail by
351 analyzing ‘live’ and ‘dead’ beetles separately to determine whether the beetles established
352 colonies within the treated bags, or tended to move through the bags without laying eggs.

353 Table 3 shows the mean monthly increase in the number of live and dead adult *T. castaneum*
354 per store-room per treatment. The level of treated bags had a significant effect on the rate of
355 monthly increase in numbers of live *T. castaneum* (ANOVA; $F=14.36$, $df = 2,117$, $P<0.001$).

356 The difference between the means of each of the three treatments was found to be significant
357 only between high and medium levels of treated bags, and high and low levels of treated bags
358 (Tukey HSD; $P<0.001$), but not between low and medium levels of treated bags ($P=0.104$).

359 Hence, there were fewest live adults *T. castaneum* found in treated bags when the level of
360 treated bags was highest. This suggests that there may have been a ‘mass effect’ of the
361 presence of the Lem-ocimum repellent plant volatiles in store-rooms with the highest levels
362 of treated bags.

363

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

368

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

374 $P<0.0001$), which suggests the repellent plant materials reduces the likelihood of beetles
375 laying eggs in the treated bags.

376

377 **3.3 Effect of adding different levels of treated double-bags to store-rooms on grain** 378 **weight-loss due to insect infestations**

379 Figure 2C shows the trend in the amount of mean monthly weight loss of grain due to insect
380 species in store-rooms containing untreated single-bags, untreated double bags and Lem-
381 ocimum treated double-bags. These results show that there was a continuous monthly
382 increase in weight loss from the beginning of the experiment to the fifth month for store-
383 rooms with all three treatments. The difference in the rate of monthly increase in weight loss
384 between the three treatments was statistically significant (ANOVA; $F=23.5$, $df=2,87$,
385 $P<0.001$), and the differences between the means of the three treatments were also significant
386 (Tukey HSD; $P<0.001$). In the fifth month, grain weight loss from treated double-bags was
387 only $2.2\pm 0.38\%$, compared to $3.4\pm 0.39\%$ in untreated double-bags and $5.2\pm 0.45\%$ in
388 untreated single-bags, thus demonstrating that both increasing the physical barrier of bags
389 and treatment with repellent plant materials reduces grain weight loss.

390

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

398 percentage of grain in Lem-ocimum treated double-bags in store-rooms increases the efficacy
399 of the treatment.

400

401 **3.4 Effect of the number of untreated bags in a store-room on the rate of increase in** 402 **number of insects in the treated bags over time**

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

406

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

411

412 The results indicate that the greater the number of untreated bags in a store-room the lower
413 the rate of increase in the number of beetles in the treated bags. An analysis of covariance
414 found significant main effects for the untreated bag number covariate ($F=10.5$, $df=1,114$,
415 $P=0.0016$) and for the treated bag level factor ($F=21.4$, $df=2,114$, $P<0.0001$). The interaction
416 term was also significant ($F=6.0$, $df=2,114$, $P<0.01$), showing that the best fit model had
417 different slope parameters for the different levels of treated bags (Table 4).

418

419 **3.5 Evaluation of the perceptions of participating farmers on the effect of the new** 420 **method of protecting stored grain in repellent double bags**

421 Table 5 summarises the perceptions of the participating farmers on the effectiveness of the
422 new method tested in their store-rooms compared to their existing methods of mixing dried

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

434

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

448

449 4. Discussion

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

454

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

459

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

467

468 The efficacy of the double bag method is likely due to: a) the combination of two types of
469 repellent plant materials in Lem-ocimum (*C. nardus* plus *O. basilicum*), benefiting from a
470 wider range of active compounds, and b) the paste of dried plants applied in a layer between
471 the two bags formed a more concentrated barrier than if the same amount of material had
472 been scattered throughout the 5 kg of grain inside the inner bag, and c) the high infestation of

473 beetles found in the untreated single bags should not be a surprise, since the bags present the
474 least barrier to the beetles. The physical barrier of double bags without any repellent plant
475 materials may induce beetles to leave the double bag and end up moving into single bags.
476 Cline & Highland (1981) reported that storage pests such as *R. dominica*, *L. serricornis*, and
477 *T. castaneum* could enter packaging through openings less than 1.35 mm and their larvae can
478 enter even smaller openings. However, double bagging appears to be more difficult for
479 beetles to penetrate according to Mullen & Mowery (2002) and the results presented here.
480 Significantly, fewer beetles were found in untreated double bags than in single bags in
481 Experiment 1. Thus, the combination of a double barrier of potent repellent plant material and
482 a double layer of woven plastic may explain the significant reduction in infestation by *T.*
483 *castaneum*.

484

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

498

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

506

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

510

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

515

516 Generally, the perception of the respondents of the new method, as tested in their respective
517 stores, was positive based on its efficacy, ease of application and cost effectiveness. A few
518 participants, however, expressed concern about the low efficacy, difficulty and cost of
519 additional materials. These views were influenced by the level of treated double-bags that
520 were tested in their respective store-rooms. The positive impression given by all the
521 respondents who tested high levels of Lem-ocimum treated bags in their store-rooms
522 indicates that they were impressed by how efficacious the method was. The impression of a

523 few respondents of those who tested low or medium percent levels of treated double bags,
524 that the new method produced similar results to their own methods, may indicate that the
525 method was less effective in their store-rooms than in those with a high level of treated
526 double-bags. This suggests that farmers' interests can be influenced by the demonstrated
527 efficacy of a control method. Belmain & Stevenson (2001), Mugisha-Kamatenesi *et al.*
528 (2008) and Deng, *et al.* (2009) report that farmers' perceptions and choices of botanical
529 pesticides as control agents are influenced by efficacy, availability and cost effectiveness,
530 indicating that these could affect farmer acceptance and uptake. Hence, when introducing a
531 new method of grain protection to farmers, the efficacy, cost and availability should be
532 discussed to encourage acceptance by farmers, although this may depend on the particular
533 circumstances of farmers and their locality.

534

535 **5. Conclusions**

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

548

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

561

562 **Acknowledgements**

563 The authors thank the Kebbi State Government, Nigeria for the studentship support that
564 resulted in this research outcome. Thanks should be given to Stephen Young (NRI
565 statistician) for the statistic advice and Kebbi Agricultural and Rural Development Authority
566 (KARDA) for their support during the field work.

567

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679

680 **Figure Legends**

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

687
688 **Fig. 2 Comparison of the grain protection provided by three types of storage bag; an**
689 **untreated single-bag (light grey), an untreated double-bag (dark grey) and a Lem-ocimum**
690 **treated double-bag (black).** Trend in the mean±SE number of A) *Tribolium castaneum* and B)
691 *Rhyzopertha dominica* beetles found in grain samples (100 g /bag/month) taken from the
692 three experimental bags of initially uninfested sorghum grain and C) grain weight-loss,
693 estimated by the 'count and weigh' method (Adams & Schulten, 1978) with 100 g
694 samples/bag/month. Experimental bags had been placed in store-rooms containing untreated
695 single-bags that initially had moderate levels of *T. castaneum* infestations (n=30 store-
696 rooms/treatment/month). Each sample is the mean of three spear samples of grain taken from
697 each experimental bag.

698
699 **Fig. 3 Comparison of the effect of 'dose' of Lem-ocimum treated double-bags on the**
700 **trend in mean±SE numbers of A) *Tribolium castaneum* and B) *Rhyzopertha dominica* beetles**
701 **found in grain samples (100 g /bag/month) and C) grain weight-loss, estimated by the 'count**
702 **and weigh' method (Adams & Schulten, 1978) with 100 g samples/bag/month. A 'Low'**
703 **percent (1% of total grain weight in store-rooms kept in treated bags; light grey), 'Medium'**
704 **percent (2%; dark grey) or 'High' percent (4%; black) of treated bags were placed in store-**

705 rooms containing farmers' untreated single-bags that initially had moderate levels of *T.*
706 *castaneum* infestations (n=40 store-rooms/treatment/month).

707

708 **Fig. 4 Correlation between 'percent grain in 6 kg Lem-ocimum treated double-bags'**

709 **and 'number of 60 kg untreated bags in store-rooms' on the mean monthly rate of**

710 **increase in numbers of adult *Tribolium castaneum* per 100g sample of grain over the five**

711 **month experiment. Overall, there was a significant inverse relationship ($P < 0.001$) in the rate**

712 **of increase in number of beetles in the treated bags as the number of untreated bags**

713 **increased. There was a significant main effect for untreated bag number, percent treated bags**

714 **and their interaction ($P < 0.01$, ANCOVA).**

715

716