

# Susceptibility of flexible plastic packaging for foodstuffs against the household ants *Monomorium indicum* Forel (Hymenoptera: Formicidae) (#88767)

1

First submission

## Guidance from your Editor

Please submit by **23 Aug 2023** for the benefit of the authors (and your token reward) .



### Structure and Criteria

Please read the 'Structure and Criteria' page for general guidance.



### Raw data check

Review the raw data.



### Image check

Check that figures and images have not been inappropriately manipulated.

If this article is published your review will be made public. You can choose whether to sign your review. If uploading a PDF please remove any identifiable information (if you want to remain anonymous).

## Files

Download and review all files from the [materials page](#).

12 Figure file(s)

1 Table file(s)

1 Raw data file(s)




# Structure and Criteria

---

## Structure your review

The review form is divided into 5 sections. Please consider these when composing your review:

1. BASIC REPORTING
2. EXPERIMENTAL DESIGN
3. VALIDITY OF THE FINDINGS
4. General comments
5. Confidential notes to the editor






 You can also annotate this PDF and upload it as part of your review

When ready [submit online](#).





## Editorial Criteria

Use these criteria points to structure your review. The full detailed editorial criteria is on your [guidance page](#).




### BASIC REPORTING

-  Clear, unambiguous, professional English language used throughout.
-  Intro & background to show context. Literature well referenced & relevant.
-  Structure conforms to [Peerj standards](#), discipline norm, or improved for clarity.
-  Figures are relevant, high quality, well labelled & described.
-  Raw data supplied (see [Peerj policy](#)).

### EXPERIMENTAL DESIGN

-  Original primary research within [Scope of the journal](#).
-  Research question well defined, relevant & meaningful. It is stated how the research fills an identified knowledge gap.
-  Rigorous investigation performed to a high technical & ethical standard.
-  Methods described with sufficient detail & information to replicate.

### VALIDITY OF THE FINDINGS

-  Impact and novelty not assessed. *Meaningful* replication encouraged where rationale & benefit to literature is clearly stated.
-  All underlying data have been provided; they are robust, statistically sound, & controlled.
-  Conclusions are well stated, linked to original research question & limited to supporting results.



The best reviewers use these techniques

## Tip

## Example

**Support criticisms with evidence from the text or from other sources**

*Smith et al (J of Methodology, 2005, V3, pp 123) have shown that the analysis you use in Lines 241-250 is not the most appropriate for this situation. Please explain why you used this method.*

**Give specific suggestions on how to improve the manuscript**

*Your introduction needs more detail. I suggest that you improve the description at lines 57- 86 to provide more justification for your study (specifically, you should expand upon the knowledge gap being filled).*

**Comment on language and grammar issues**

*The English language should be improved to ensure that an international audience can clearly understand your text. Some examples where the language could be improved include lines 23, 77, 121, 128 - the current phrasing makes comprehension difficult. I suggest you have a colleague who is proficient in English and familiar with the subject matter review your manuscript, or contact a professional editing service.*

**Organize by importance of the issues, and number your points**

1. Your most important issue
2. The next most important item
3. ...
4. The least important points

**Please provide constructive criticism, and avoid personal opinions**

*I thank you for providing the raw data, however your supplemental files need more descriptive metadata identifiers to be useful to future readers. Although your results are compelling, the data analysis should be improved in the following ways: AA, BB, CC*

**Comment on strengths (as well as weaknesses) of the manuscript**

*I commend the authors for their extensive data set, compiled over many years of detailed fieldwork. In addition, the manuscript is clearly written in professional, unambiguous language. If there is a weakness, it is in the statistical analysis (as I have noted above) which should be improved upon before Acceptance.*

# Susceptibility of flexible plastic packaging for foodstuffs against the household ants *Monomorium indicum* Forel (Hymenoptera: Formicidae)

Muhammad Junaid Iqbal<sup>1</sup>, Muhammad Waqar Hassan<sup>Corresp., 1</sup>, Ghulam Sarwar<sup>2</sup>, Moazzam Jamil<sup>3</sup>

<sup>1</sup> Department of Entomology, Faculty of Agriculture and Environment, Islamia University of Bahawalpur, Bahawalpur, Punjab, Pakistan

<sup>2</sup> Botany, Faculty of Chemical and Biological Sciences, Islamia University, Bahawalpur, Bahawalpur, Punjab, Pakistan

<sup>3</sup> Soil Science, Faculty of Agriculture and Environment, Islamia University, Bahawalpur, Bahawalpur, Punjab, Pakistan

Corresponding Author: Muhammad Waqar Hassan

Email address: waqar.hassan@iub.edu.pk

Plastics comprise major form of packaging of products due to benefits derived from plastic films. Ants belonging to species *Monomorium indicum* Forel (Formicidae: Hymenoptera) are ubiquitous insects and are commonly associated with household settings in Pakistan. Packaged foodstuffs are easily destroyed by household ants if packaging is of susceptible nature. Present research evaluated susceptibility of three common flexible plastic packaging materials namely opaque polyethylene, transparent polyethylene and polypropylene with thicknesses of 0.02 mm, 0.04 mm and 0.06 mm which were evaluated separately for their susceptibility against *M. indicum*. In order to simulate the household settings, experiments were conducted at faculty building of Agriculture and Environment during summer vacations when building is quiet. Different corners were selected near water source for maximum population of ants. Experimental cages used for experiment were built with wood and iron gauze of 2 mm to allow only ants to enter cages.

Experiments were run over three-time spans of fifteen days each from June 20<sup>th</sup> 2022 to August 15<sup>th</sup> 2022. Results showed all packaging materials were recorded susceptible against *M. indicum* at 0.02 mm thickness level. At higher level, polypropylene was susceptible at 0.04 mm thickness but resistant to ants at 0.06 mm thickness whereas polyethylene was susceptible to ants at higher thickness of 0.06 mm. Correlation of damages with weather factors showed temperature had positive relationship while relative humidity had negative association with *M. indicum* attack. Overall correlation of damages with packaging thickness for entire data showed thickness was also negatively associated with ants' damages to packaging. We studied mandibles of ants and three common stored product pests which usually attack foodstuff packaging. It was recorded that ants had maximum length of their mandible and frontal mandibular tooth compared with the

mandibles and frontal teeth of common stored product pests. Therefore, this study confirmed a greater pest status of household ants *M. indicum* for packaged foodstuffs relevant to common stored product pests. Although packaging thickness proved as a major factor causing resistance in flexible plastic packaging against household ants but current results recommend polypropylene as foodstuff packaging against household ants with a thickness of 0.06 mm compared with polyethylene packaging which were found susceptible at 0.06 mm thickness.

1           **Susceptibility of flexible plastic packaging for foodstuffs against the household ants**  
2                           *Monomorium indicum* Forel (Hymenoptera: Formicidae)

3   Muhammad Junaid Iqbal<sup>1</sup>, Muhammad Waqar Hassan<sup>1,\*</sup>, Ghulam Sarwar<sup>2</sup> and Moazzam Jamil<sup>3</sup>

4   <sup>1</sup>Department of Entomology, Faculty of Agriculture and Environment, Islamia University of  
5   Bahawalpur, Pakistan

6   <sup>2</sup>Department of Botany, Faculty of Chemical and Biological Sciences, Islamia University of  
7   Bahawalpur, Pakistan

8   <sup>3</sup>Department of Soil Science, Faculty of Agriculture and Environment, Islamia University of  
9   Bahawalpur, Pakistan

10   \*Author for correspondence email: [waqar.hassan@iub.edu.pk](mailto:waqar.hassan@iub.edu.pk); Phone: +92 629255471; Fax: +92  
11   629250232

12

13

14

15

16

17

18

19

20

21

22

23           **Susceptibility of flexible plastic packaging for foodstuffs against the household ants**  
24                           *Monomorium indicum* Forel (Hymenoptera: Formicidae)

25   **Abstract.** Plastics comprise major form of packaging of products due to benefits derived from  
26 plastic films. Ants belonging to species *Monomorium indicum* Forel (Formicidae: Hymenoptera)  
27 are ubiquitous insects and are commonly associated with household settings in Pakistan. Packaged  
28 foodstuffs are easily destroyed by household ants if packaging is of susceptible nature. Present  
29 research evaluated susceptibility of three common flexible plastic packaging materials namely  
30 opaque polyethylene, transparent polyethylene and polypropylene with thicknesses of 0.02 mm,  
31 0.04 mm and 0.06 mm which were evaluated separately for their susceptibility against *M. indicum*.  
32 In order to simulate the household settings, experiments were conducted at faculty building of  
33 Agriculture and Environment during summer vacations when building is quiet. Different corners  
34 were selected near water source for maximum population of ants. Experimental cages used for  
35 experiment were built with wood and iron gauze of 2 mm to allow only ants to enter cages.  
36 Experiments were run over three-time spans of fifteen days each from June 20<sup>th</sup> 2022 to August  
37 15<sup>th</sup> 2022. Results showed all packaging materials were recorded susceptible against *M. indicum*  
38 at 0.02 mm thickness level. At higher level, polypropylene was susceptible at 0.04 mm thickness  
39 but resistant to ants at 0.06 mm thickness whereas polyethylene was susceptible to ants at higher  
40 thickness of 0.06 mm. Correlation of damages with weather factors showed temperature had  
41 positive relationship while relative humidity had negative association with *M. indicum* attack.  
42 Overall correlation of damages with packaging thickness for entire data showed thickness was also  
43 negatively associated with ants' damages to packaging. We studied mandibles of ants and three  
44 common stored product pests which usually attack foodstuff packaging. It was recorded that ants  
45 had maximum length of their mandible and frontal mandibular tooth compared with the mandibles  
46 and frontal teeth of common stored product pests. Therefore, this study confirmed a greater pest  
47 status of household ants *M. indicum* for packaged foodstuffs relevant to common stored product  
48 pests. Although packaging thickness proved as a major factor causing resistance in flexible plastic  
49 packaging against household ants but current results recommend polypropylene as foodstuff  
50 packaging against household ants with a thickness of 0.06 mm compared with polyethylene  
51 packaging which were found susceptible at 0.06 mm thickness.

52   **Keywords:** consumer packaging, polyethylene, polypropylene, polyvinylchloride, food safety,  
53 house hold pests, integrated pest management, non-chemical control, entomology, agriculture

## 54 Introduction

55 More than 12,000 species of ants have been identified and these are a common group of  
56 insects that are frequently observable<sup>1</sup>. They often thrive in many types of environments and  
57 account for 15 - 25 % of all live land animals<sup>2</sup>. They are one of the most common household pests.  
58 These are social insects and live in the form of colonies. Their worker class scavenges food and  
59 brings it to a central nest, which is frequently built far from the food source<sup>3</sup>. They are common  
60 everywhere; they can obtain food and water<sup>4</sup>. The presence of ants in a residence infests all utensils  
61 and food, which, when consumed by people, causes illness<sup>5</sup>. The environment of Pakistan is  
62 conducive to the development of ants. It offers the ideal circumstances for their survival and  
63 growth<sup>6</sup>.

64 The widespread use of vulnerable packaging materials for food goods is crucial since losses  
65 from pest infestation of packaged foods equal the whole cost of cultivating, harvesting,  
66 transporting, preparing, and packing the food<sup>7</sup>. Any exhaustive examination of pest control in the  
67 food sector must consider the eradication or prevention of insect infestation. Many companies  
68 have implemented package-testing programs to improve resistance of packages to insect attack<sup>7</sup>.  
69 The most frequent method of preventing insect infestation without using insecticides or repellents  
70 is insect-resistant packaging<sup>8</sup>. Frequent causes of insect infestation include transportation-related  
71 issues or lengthy storage in suboptimal conditions at a warehouse or on a supermarket shelf.

72 **Insect resistant packaging can provide all in one solution to the damages caused by pest**  
73 **insects for packaged foodstuffs**. For example, foodstuff packaging derived from plastics like  
74 polypropylene with a thickness of 0.04 mm was resistant to insect penetrations or invasions against  
75 a major stored grain borer pest<sup>9</sup>. **Our earlier research about susceptibility evaluation** of flexible  
76 foodstuff plastic packaging films was about major stored grain insect pests' ability to tear plastic  
77 packaging and causing weight loss in packaged foodstuffs (10-15). However little or no research is  
78 available regarding susceptibility testing for commonly utilized flexible foodstuff plastic  
79 packaging films against household ants.

80 In the household settings, according to common observation, ants can be more threatening  
81 to a packaging material containing foodstuffs due to having their appearance out of nowhere and  
82 because of their ability to reach stored food materials through smallest possible openings. In



83 Pakistan ants are usually controlled in homes by insecticidal powders sprinkled along their trails  
84 and around the places of their origin. The use of chemical insecticides in residential places is riskier  
85 than in field crops even though pesticide labels claim those pesticide totally safe for indoor use.  
86 How much are these chemicals safe meant for use in human dwellings but safety criteria for  
87 pesticides should be entirely different and there should be no comparison in toxicity classification  
88 between pesticides being applied in field crops and those manufactured for household use.  
89 Although a number of social insect pests have been effectively managed by using baits<sup>16</sup> However,  
90 many bait-based initiatives failed because of pesticide resistance and insufficient appeal<sup>17</sup> and baits  
91 containing insecticides are also not without danger as for as their use in human residence is  
92 concerned.

93 Insect resistant packaging is an alternative method to prevent damage of food from insects.  
94 Insect resistant packaging of food material is the last line of defense for the producer against insect  
95 attack<sup>18</sup>. Therefore, packaging testing of different types and thickness levels against household  
96 ants is essential due to their ubiquitous nature. Different insect pests have significantly different  
97 ability of chewing substrate materials<sup>19</sup>. Stored product pests vary in their ability to contest  
98 packages<sup>20</sup>. Therefore, current study was designed to evaluate commonly utilized flexible  
99 foodstuff plastic packaging film types namely transparent polyethylene (low density), opaque  
100 polyethylene (high density) and polypropylene for their susceptibility in the form of small plastic  
101 pouches filled with fruit cake which is usually attacked by ants in household settings against the  
102 attack of household ants in natural way of occurrence for ants selecting their natural foraging  
103 places as the study sites.

104

105

106

107

108

109

110

## 111 **Material and methods**

### 112 **Ants' sources**

113 This research was conducted at faculty building of Agriculture and Environment, Baghdad  
114 campus in The Islamia University of Bahawalpur, Pakistan. Experiments were done during  
115 summer vacations when academic activities are limited and the academic building is usually quiet.  
116 There is abundant availability of ants at experimental location within the building of Agriculture  
117 and Environment. The ant specimens were preserved in ethanol and subsequently were identified  
118 as *Monomorium indicum* Forel, in Insect Biodiversity Laboratory, Department of Entomology,  
119 The Islamia University of Bahawalpur.

### 120 **Experiment cages**

121 Cages used for experiment were built with wood and iron gauze. The size of single cage  
122 was  $8 \times 8$  square inches. In total there were nine such cages to retain three replicates per each  
123 thickness type. The wire gauze was 2 mm size which is used in all boxes. Cages were built with  
124 the purpose that only the ants should enter in box but no other damaging pests (rodents, lizard, cats  
125 and squirrels) could enter.

### 126 **Packaging materials**

127 There are specific flexible plastic packaging types being used in Pakistan for food stuff  
128 packaging which include opaque polyethylene (high density), transparent polyethylene (low  
129 density) and polypropylene at the level of 0.02 mm thickness. These plastic materials were  
130 purchased from wholesale plastic market in Lahore at rate of 400 rupees per Kg. Mean thickness  
131 of the different packaging materials was identified using a digital micrometer (Mitutoyo  
132 Corporation, Kawasaki, Japan). Thickness levels of these plastic packaging was 0.02 mm. At 0.04  
133 mm and 0.06 mm thickness level available flexible packaging are transparent polyethylene and  
134 polypropylene but not high density or opaque polyethylene.

135 For this purpose, these plastic packaging films were purchased accordingly and were used  
136 in the experiments to evaluate their susceptibility against house hold ants, *M. indicum*. For this  
137 purpose, small bags of these plastic films ( $8 \times 10$  cm) were prepared in the laboratory using a pair  
138 of scissors and an impulse (heat) sealer.

## 139 **Packaged food**

140 Fresh fruit cake was selected as food source inside packaging to check the susceptibility of  
141 packaging types against the house hold ants. Fruit cake was purchased from local market. Eighteen  
142 g fresh fruit cake slice was weighed on an electrical weighing balance and packed in prepared  
143 plastic bags for different packaging types and thickness levels. After packing this fruit cake, plastic  
144 bags were sealed with heat sealing machine.

## 145 **Experimental setup**

146 Three types of plastic bags *i.e.*, opaque polyethylene (high density), transparent  
147 polyethylene (low density) and polypropylene at 0.02 mm thickness level were filled with fruit  
148 cake and sealed with impulse heater. There were no prior vents in bags for entry of insects. These  
149 three types of packaging containing fruit cake inside and sealed thereafter were placed inside a  
150 cage. Three similar packaging types but without fruit cake (control treatments) were also placed  
151 in that experiment cage. Then cage was closed with lock to restrict entry of any foreign objects.  
152 Other two cages were prepared in same manner for keeping three replications per treatment for  
153 0.02 mm thick packaging. Similar method was used for evaluation of 0.04 mm and 0.06 mm  
154 thickness packaging in which only transparent polyethylene and polypropylene plastic bags were  
155 placed both with and without food.

156 Three cages for each thickness of packaging testing were placed at three different places  
157 near water source where ants' movement was usually detected at faculty building of Agriculture  
158 and Environment. In all there were nine such cages for three thickness levels of packaging  
159 evaluation placed at nine different locations. This experiment was under observation for whole  
160 study period to reduce any disturbance from outside. These cages were visited daily and data  
161 regarding number of holes in packaging was collected after every five days till fifteen days for this  
162 experimental setup. First experiment lasted from 20<sup>th</sup> June to 5<sup>th</sup> July 2022.

163 After every five days cages were opened bags were removed and then observed externally  
164 to observe any damage in the form of holes. Packaging displaying any sealing defects were  
165 replaced immediately with similar type of packaging to avoid ants' entry into packaging not  
166 because of holes created by ants in packaging which should be otherwise be termed as invasions  
167 <sup>24</sup>(Mullen *et al.* 2012). If there was hole in packaging together, we noticed ants' presence during

168 this time in the cage then this damage was recorded as one hole and so on. It was followed by  
169 opening of bags to measure weight loss in fruit cake caused by ants by using following formula.

170

$$171 \quad \% \text{ age weight loss} = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \times 100$$

172 This setup of experiment was maintained for fifteen days for data recording regarding  
173 number of holes and weight loss in all packaging types with respect to three thickness levels.  
174 Following first experiment, this experiment setup was repeated from 10<sup>th</sup> July to 25<sup>th</sup> July 2022  
175 (second experiment) and finally from 1<sup>st</sup> August to 15<sup>th</sup> August 2022 (third experiment). Each time  
176 for the second and third experiments there were new packets used along with newly packed fruit  
177 cake (18 g) for each packaging type and thickness.

## 178 Data analysis

179 Data was analyzed statistically using SPSS software<sup>21</sup> (Version 2016). Data was analyzed  
180 separately for each thickness level using 1-Way ANOVA in which different packaging types both  
181 with and without food served as independent variable to see the effect of packaging types regarding  
182 each thickness level on number of holes and percent weight loss in packed fruit cake which  
183 therefore served as dependent variables. Similarly, to see the effect of experiment dates for the  
184 three experiment dates, on number of holes and weight loss in packed fruit cake, experiment dates  
185 served independent variable while number of holes and percent weight loss in fruit cake served as  
186 dependent variables. Mean values were separated post hoc at 5 % level of probability using Tukey  
187 HSD test. For each thickness level, correlation (Pearson) was also done between damages (holes)  
188 created by *M. indicum* and weather data regarding temperature and relative humidity along three  
189 experiment dates to see the effect of these factors on damages by *M. indicum* and to see the overall  
190 effect of packaging thickness on damages a correlation was done on entire data between holes in  
191 all thickness levels (omitting high density polyethylene in 0.02 thickness to standardize data along  
192 three thickness levels) and packaging thickness. Finally, to statistically compare the measured  
193 mandible of *M. indicum* with measured mandibles of three common stored product pests namely  
194 *Rhyzopertha dominica*, *Tribolium castaneum* and *Trogoderma granarium* an analysis of variance  
195 1-Way ANOVA was also done in which lengths of mandible and frontal tooth of *M. indicum* and

196 three storage pests served as dependent variables while insect types served as independent  
197 variables. Means values were separated post hoc by Tukey HSD test at 5 % level of probability.

#### 198 **Study of insect mandibles**

199 Three specimens of each insect type were selected, head region was separated by using fine  
200 forceps and surgical blade no 14, which was then mounted on a clean glass slide in glycerin (50  
201 %). Mandibles were oriented under camera (Model HD 1500 T, Meiji, TECHNO, Saitama, Japan)  
202 fitted trinocular stereoscope microscope (Labomed, CXR3, Labo America, Inc., Fremont,  
203 California, USA) with installed software (T Capture Version 3.9 digital software<sup>22</sup> (T Capture  
204 2017) on Laptop computer (DELL Core i3, 10<sup>th</sup> Gen). The mandibles of concerned insect  
205 specimens were orientated for proper measurements and visual comparisons, captured and saved  
206 with proper labelling for future reference. The images were opened with T Capture software and  
207 software was calibrated by using the micrometer scale (1mm) captured with those pictures. The  
208 mandibles as well as mandibular frontal tooth of three specimens for each insect type under study  
209 were measured. The images along with measurements were saved and the respective values were  
210 tabulated in Microsoft excel 2021 (Microsoft Corporation Version 2019) for further data analyses.

211

212

213

214

215

216

217

218

219

220

221

## 222 Results

### 223 Effect of packaging types on holes in packaging and weight loss in packed fruit cake caused 224 by house hold ants *M. indicum*

225 Fig 1 shows effect of packaging types with 0.02 mm thickness on damages (holes in  
226 packaging and percent weight loss in fruit cake) caused by household ants, *M. indicum*. Results  
227 showed ants damaged and created maximum holes (2.00) in polyethylene high density followed  
228 by number of holes in low density polyethylene bags (1.56) and least in polypropylene (1.22) but  
229 none in the packaging types without food material ( $F_{5, 53}: 1.832; P: .124$ ).

230 Weight loss in packed fruit cake was recorded in packaging where holes were created by  
231 ants. Percent weight loss due to ants feeding was maximum in polyethylene high density (39.64  
232 %) followed by weight loss in fruit cake in polypropylene (21.03 %) and minimum in polyethylene  
233 low density (18.56 %) with zero weight loss recorded in packaging without holes ( $F_{5, 53}: 2.762; P:$   
234  $.028$ ).

235 In 0.04 mm thick packaging maximum average holes were recorded as 0.11 in  
236 polypropylene with fruit cake while no holes occurred in polyethylene packaging and packaging  
237 without fruit cake ( $F_{3, 35}: 1.000; P: .405$ ). Similarly, percent weight loss was recorded only in  
238 polypropylene packaging 8.09 % with fruit cake but not in packaging without holes ( $F_{3, 35}: 1.000;$   
239  $P: .405$ ) (Fig 2).

240 In 0.06 mm thick packaging maximum average holes were recorded as 0.44 in polyethylene  
241 packaging with fruit cake while no holes occurred in polypropylene packaging and packaging  
242 without fruit cake. Similarly, percent weight loss was recorded only in polyethylene packaging  
243 5.36 % with fruit cake but not in packaging without holes ( $F_{3, 35}: 1.000; P: .405$ ) (Fig 3).

### 244 Effect of experiment dates on holes in packaging and weight loss in packed fruit cake caused 245 by house hold ants *M. indicum*

246 Dates of experiments regarding packaging evaluation showed at 0.02 mm thickness level,  
247 ants were able to cause damages on all three dates of experiments ranging from 25<sup>th</sup> June to 5<sup>th</sup>  
248 July, 15<sup>th</sup> July to 25<sup>th</sup> July and from 5<sup>th</sup> August to 15<sup>th</sup> August during 2022 (Fig 4). In these ranges,  
249 maximum holes were recorded 1.28 in first dates followed by numbers of holes in 1.06 in second

250 experiment dates and least numbers of holes were recorded .06 in third experiment dates ( $F_{2, 53}$ :  
251 1.806;  $P$ : .175).

252 Maximum percent weight loss in packed fruit cake was 23.72 % in first experiment  
253 followed by the percent weight loss 11.78 % in second experiment dates and least weight loss was  
254 4.11 % in third experiment dates ( $F_{2, 53}$ : 1.818;  $P$ : .173).

255 Dates of experiments regarding packaging evaluation showed at 0.04 mm thickness level,  
256 holes created by *M. indicum* were recorded .08 in first experiment dates however no holes were  
257 created in 0.04 mm thick packaging in second or third dates of experiments. Similarly, weight loss  
258 in packed fruit cake in these packaging was recorded 6.06 % in first dates of experiment but not in  
259 second or third dates of experiments ( $F_{2, 35}$ : 1.000;  $P$ : .379) (Fig 5).

260 Dates of experiments regarding packaging evaluation showed at 0.06 mm thickness level,  
261 holes created by *M. indicum* were recorded .33 in first experiment dates however no holes were  
262 created in 0.06 mm thick packaging in second or third dates of experiments. Similarly, weight loss  
263 in packed fruit cake in these packaging was recorded 5.36 % in first dates of experiment but not in  
264 second or third dates of experiments ( $F_{2, 35}$ : 1.000;  $P$ : .379) (Fig 6).

### 265 **Correlation of damages caused by *M. indicum* with weather factors and packaging thickness**

266 The correlation with weather factors showed in all three thickness levels, temperature had  
267 strong positive relationship with damages to packaging caused by *M. indicum* while relative  
268 humidity usually had strong negative effect on damages. Correlation of overall data for all  
269 thickness levels effect showed packaging thickness had negative correlation with damages caused  
270 by *M. indicum* (Table 1).

### 271 **Study of *M. indicum* mandibles in relation to mandibles of major stored grain insect pests**

272 Fig 7 shows comparison of mandibular length and frontal tooth length comparison between  
273 household ant *M. indicum* and three major stored product pests. Results showed *M. indicum* had  
274 significantly more length of their mandible compared with mandibles of three common stored  
275 product pest ( $F_{3, 11}$ : 94.551;  $P$ : < 0.001). Maximum mean length of mandible was 400.67  $\mu\text{m}$  for  
276 *M. indicum*. It was followed by mandibular length of 241.67  $\mu\text{m}$  for *R. dominica* adult and 201.33  
277  $\mu\text{m}$  for *T. castaneum* while least length of mandible was 174.33  $\mu\text{m}$  for *T. granarium* larva.  
278 Mandibular frontal tooth length was maximum in *M. indicum* (124.00  $\mu\text{m}$ ) followed by frontal

279 tooth length of 81  $\mu\text{m}$  in *R. dominica* and *T. castaneum* and least length was 32.00  $\mu\text{m}$  in *T.*  
280 *granarium* larva ( $F_{3,11}$ : 68.601;  $P$ : < 0.001).

281

282

283

284

285

286

287

288

289

290

291

292

293

294

295

296

297

298

299

300

301



## 302 Discussion

303 This research was conducted to check susceptibility of different available flexible plastic  
304 packaging films which are normally used as foodstuff packaging against the household ants *M.*  
305 *indicum*. Miniature size bags of relevant available packaging films related to 0.02 mm, 0.04 mm  
306 and 0.06 mm thicknesses were created and checked for their susceptibility against the naturally  
307 foraging household ants. According to our results at a thin level of 0.02 mm thickness, highest  
308 susceptibility was in high density opaque polyethylene films followed by low density transparent  
309 polyethylene and polypropylene in descending order while no damages or holes were recorded in  
310 these packaging without fruit cake. Among the damaged packaging, weight loss was significantly  
311 more in opaque polyethylene followed by polypropylene and least in transparent polyethylene.

312 No attack to packaging without fruit cake might be because ants could distinguish between  
313 packaging with and without fruit cake and there might odours coming out of these fruit cake  
314 packaging. It has been reported that food odors may be prevented from escaping the package  
315 through the use of barrier materials, resulting in a package that is invisible to invading insect<sup>23</sup>.  
316 Furthermore, some authors<sup>24</sup> also emphasized the importance of odor barriers to prevent insect  
317 infestation in packaged foodstuffs<sup>24</sup>.

318 The packaging tested in this setup were of 0.02 mm thickness to which foraging ants could  
319 distinguish and created holes in them and attacked on fruit cake subsequently. Regarding the  
320 thickness level of packaging materials these results can be compared with those of<sup>25</sup> which showed  
321 that thickness of plastic packaging is one of the important factors for insect damage to food.  
322 According to their results more penetrations by insects were in packaging with less thickness.  
323 Similar results were also recorded about thickness effect on penetration by larvae<sup>26</sup>.

324 In packaging testing for more thickness like 0.04 mm fairly less damages in the form of  
325 holes in packaging and weight loss in packed fruit cake occurred due to ants. At this thickness  
326 level, a few holes were only recorded in case of polypropylene packaging than in polyethylene  
327 packaging. Again, no attacks were recorded in packaging without fruit cake.

328 In case of 0.06 mm thickness level fewer holes and weight loss in packed fruit cake was  
329 recorded in polyethylene packaging than in polypropylene packaging. Firstly, due to more  
330 thickness there might be less food odor emission through packaging films. Secondly packaging  
331 thickness also prevented ants from damaging the packaging. These results are in agreement with  
332 earlier reports which stated that when packaging was used with extra cover these were resistant to

333 insect penetration than when used alone (Mullen and Mowery 2000). Therefore, packaging  
334 thickness proved as major factor to cause resistance in packaging against household ants.

335 Compared with polypropylene, polyethylene proved more susceptible due to having holes  
336 in them at a higher thickness level of 0.06 mm. <sup>27</sup>Marouf and Momen (2007) in a comparative  
337 study among polyethylene, polypropylene and polyvinylchloride packaging, found polypropylene  
338 with comparatively less thickness as an ideal liner of bags to resist insect penetrations.

339 These resulted can be compared with our earlier study results which showed that  
340 polypropylene packaging proved resistant to damage caused by insects like punctures, holes and  
341 penetrations compared with polyethylene <sup>9</sup>(Hassan et al. 2016). According to <sup>28</sup>Pacheco and  
342 Wiendl (1989) polypropylene is an effective wrapper for packed beans to stave off common bean  
343 weevil penetration.

344 Although there are many factors known to affect insect pests' ability to tear packaging but  
345 one of them would be the smooth surface or texture of packaging films. It has been reported that  
346 smooth surfaces of plastic bags are known to affect insect walking <sup>29</sup>(Domingue et al. 2022) and  
347 it might be one of the reasons behind polypropylene packaging resistance against pest insect  
348 chewing. It has been reported that polypropylene has more slippery surface compared with  
349 polyethylene in this regard (<sup>30</sup>Cline, 1978, <sup>31</sup>Jassim et al. 2022).

350 Effect of dates of experiments showed ants damages to packaging and packed fruit cake in  
351 0.02 mm thick packaging were more in first experiment dates during late June to early July than  
352 in later dates while in higher thickness testing levels of 0.04 mm and 0.06 mm ants damages were  
353 only recorded in first experiment dates compared with later two dates. Correlation with weather  
354 factors for thickness level study showed temperature had positive relationship with ants' damages  
355 to packaging and fruit cake. However relative humidity had negative relationship with ants'  
356 damages to packaging and packed fruit cake. These data showed ants infestations were usually  
357 more in hot and drier periods of the season during which time packaged foodstuffs are faced with  
358 relatively more attack from foraging ants.

359 These results are in agreement with study findings of <sup>32</sup>Barbani (2003) which stated similar  
360 relationship of ants' foraging activity with weather factors. The more is the foraging activity by  
361 ants the more are packaging exposed to them and packaging forte come under a greater challenge.

362 Our results about packaging susceptibility showed ants are more harmful to foodstuff  
363 packaging than majority of stored grain pests against a packaging thickness of 0.04 mm proved

364 resistant. Therefore, to confirm this we compared mandibles of ant species *M. indicum* with three  
365 common stored product pests which we earlier studied in our research project namely *R. dominica*,  
366 *T. castaneum* and *T. granarium*. According to microphotography of mandibles, mandibles of *M.*  
367 *indicum* were significantly larger than three common stored products. Similarly frontal tooth  
368 length was also maximum in ants compared with these pests' species which therefore confirms  
369 that *M. indicum* is more hazardous against foodstuff packaging than common stored grain pests  
370 and as per current study finding it is recommended to use polypropylene packaging for foodstuffs  
371 at a thickness of 0.04 mm to prevent the attack of household ants particularly against *M. indicum*.

372

373

374

375

376

377

378

379

380

381

382

383

384

385

386

387

388

389

390

391

392

393

394

## 395 REFERENCES

- 396 1. Hammond, P., Atlas of the World's Strangest Animals. Marshall Cavendish Square Publishing,  
397 LLC. New York, 2011.
- 398 2. Schultz, T.R. 2000. In search of ant ancestors. *PNAS*, 2000, **97**, 14028–14029.
- 399 3. Beatson Campbell S.H., Ants: Formicidae, Hymenoptera." Ecology and management of food  
400 industry pests, Gorham JR (Ed.), Association of Official Analytical Chemists, Arlington,  
401 Virginia, FDA. *Tech. Bull.*, 1991, **4**, 207-215.
- 402 4. Li, H., Medina, F., Vinson, S.B. and Coates, C.J., Isolation, characterization, and molecular  
403 identification of bacteria from the red imported fire ant (*Solenopsis invicta*) midgut.  
404 *J. Invertebr. Pathol.*, 2005, **89**, 203–209.
- 405 5. Garcia, F.R.M., Ahlert, C.C., de Freitas, B.R., Trautmann, M.M., Tancredo, S.P. and Lutinski,  
406 J.A., Ants (Hymenoptera: Formicidae) in five hospitals of Porto Alegre, Rio Grande do Sul  
407 State, Brazil. *Acta Scientiarum - Health Sci.*, 2011, **33**, 203–209.
- 408 6. Máximo, H.J., Felizatti, H.L., Ceccato, M., Cintra-Socolowski P. and Beretta, A.L.R.Z., Ants  
409 as vectors of pathogenic microorganisms in a hospital in São Paulo County, Brazil. *BMC Res.*  
410 *Notes*, 2014, **7**, 1–5.
- 411 7. Mullen, M.A. and Mowery, S.V., Insect-resistant packaging. *Int. Food Hyg.*, 2000, **11**, 13-14.
- 412 8. Mullen, M. A. and Highland., Package defects and their effect on insect infestation of instant  
413 dry non-fat milk. *J Package Technol Res*, 1988, **2**, 266-267.
- 414 9. Hassan, M.W., Gulraize, Ali, U., Fazal, U.R., Najeeb, H., Sohail, M., Irsa, B., Muzaffar, Z.  
415 and Chaudhry, M.S., Evaluation of standard loose plastic packaging for the management of  
416 *Rhyzopertha dominica* (F.) (Coleoptera: *Bostrichidae*) and *Tribolium castaneum* (Herbst)  
417 (Coleoptera: *Tenebrionidae*). *J. Insect Sci.*, 2016, **16**, 91.
- 418 10. Qasim, M.U., Hassan, M.W., Wang, J.J., Jamil, M., Iqbal, J., Hassan, M.U., Management of  
419 *Tribolium castaneum* (Coleoptera: Tenebrionidae) with phosphine fumigation in relation to  
420 packaging materials and food types. *Pak. J. Zool.* 2013, **45**, 1639–1640.
- 421 11. Hassan, M. W., Qasim, M. U., Iqbal, J., Jamil M., Study of penetration ability by *Tribolium*  
422 *castaneum* (Herbst.) (Coleoptera: Tenebrionidae) through different loose plastic packaging. *J.*  
423 *Pure App. Sci.*, 2014, **24-33**, 17–20.
- 424 12. Yar, M.A., Hassan, M.W., Ahmad, M., Ali, F. and Jamil, M., Effect of packaging materials  
425 and time period for damage in packaging and weight loss in packed wheat flour (*Triticum*

- 426 *aestivum* L.) by red flour beetles *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae).  
427 *J. Agri. Sci.*, 2017, **9**, 242–247.
- 428 13. Akram W, Hassan MW, Sajjad A, Arshad J., Evaluation of different plastic packing materials  
429 and food substrates on efficacy of phosphine fumigation against larvae of *Tribolium castaneum*  
430 (Herbst) (Coleoptera: Tenebrionidae) and *Trogoderma granarium* (Everts) (Coleoptera:  
431 Dermestidae). *Plant Prot.*, 2018, **2**, 9–15.
- 432 14. Hussain, S., Hassan, M.W., Ali, U., Sarwar, G., Evaluation of plastic packaging for prevention  
433 of damage to wheat by *Trogoderma granarium* (Coleoptera: Dermestidae), and suitability of  
434 phosphine fumigation. *Fla. Entomol.*, 2019, **102**, 531–537.
- 435 15. Waheed, H.W., Hassan, M.W., Sarwar, G., Jamil, M., Laboratory Evaluation of Storage Bags  
436 for Infestations in Wheat Caused by *Rhyzopertha dominica* F. (Coleoptera: Bostrichidae) and  
437 *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and Their Control Using Phosphine  
438 Fumigation. *Insects.*, 2022, **13**, 955. <https://doi.org/10.3390/insects13100955>
- 439 16. Bennett, N., Croke, B., Guariso, G., Guillaume, J., Hamilton, S., Jakeman, A.J., Marsili-  
440 Libelli, S., Newham, L., Norton, J., Perrin, C., and Pierce, H., Characterizing performance of  
441 environmental models. *Environ. Model. Softw.*, 2013, **40**, 1–20.
- 442 17. Krushelnycky, Paul D., and Rosemary G.G., Compositional and functional stability of  
443 arthropod communities in the face of ant invasions. *Ecol Appl.*, 2008, **18**, 1547-1562.
- 444 18. Hou, X., Fields, P. and Taylor, W., The effect of repellents on penetration into packaging by  
445 stored-product insects. *J. Stored Prod. Res.*, 2004, **40**, 47–54.
- 446 19. Hassan, M.W., Sarwar, G., Farooqi, M.A. and Jamil, M., Extent and pattern of damage in  
447 wheat caused by three different species of storage insect pests. *Int. J. Trop. Insect Sci.*, 2021,  
448 **41**, 593–599
- 449 20. Arthur, F. and Phillips, T.W., Stored Product Insect Pest Management and control, eds. Y. H.  
450 Hiu et al. 341 New York, Marcel Dekker Inc. 2003.
- 451 21. SPSS Inc., SPSS for Windows, Version 16.0. SPSS Inc., Chicago, Illinois, USA. 2007.
- 452 22. T Capture., Software Version 3.9, build 5001 in 2017. Tucsen Photonics Co. Ltd., Fuzhou,  
453 Fujian, China. 2017.
- 454 23. Sacharow, S. and A. L. Brody., Packaging: An Introduction. Harcourt Brace Jovanovich  
455 Publications, Inc. Duluth, MN. 508 pp. 1987.

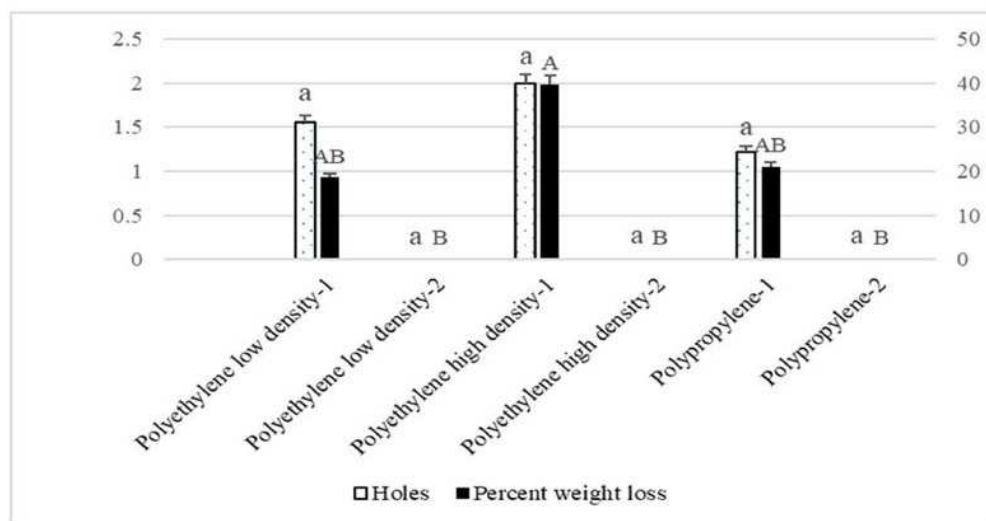
- 456 24. Mullen, M. A., Vardeman, J.M., Bagwell, J., Insect-resistant packaging. In: Hagstrum, D.W.,  
457 Philips, T.W., Cuperus, G. (Eds.), *Stored-Product Protection*. Kansas State University,  
458 Manhattan, Kansas, USA, pp. 135-142. 2012.
- 459 25. Chung, S. K., Seo, J. Y., Lim, J. H., Park, H. H., Kim, Y. T., Song, K. H., Park, S. J., Han, S.  
460 S. and Park. Y. S., Barrier property and penetration traces in packaging films against *Plodia*  
461 *interpunctella* (Hubner) larvae *Tribolium castaneum* (Herbst) adults. *J. Stored Prod. Res.*,  
462 2011, **47**, 101–5.
- 463 26. Li, S.H., Kwon S.J., Lee S.E., Kim, J.H., Lee, J.S., Na, J.H. and Han J.. Effect of type and  
464 thickness of flexible packaging films on perforation by *Plodia interpunctella*. *Korean J. Food*  
465 *Sci. Technol.*, 2014, **46**, 739–42.
- 466 27. Marouf, A. and Momen, R. F., An evaluation of the permeability to phosphine through  
467 different polymers used for the bag storage of grain. International Conference on Controlled  
468 Atmosphere and Fumigation in Stored Product. Gold-Coast Australia. FTICLtd. Publishing,  
469 Israel. 2007.
- 470 28. Pacheco, I. A. and Wiendl, F. M., Resistencia de materiais utilizados para embalagens a  
471 perfuracao por *Acanthoscelides obtectus* (Say, 1831) e *Zabrotes subfasciatus* (Boh., 1833)  
472 (Coleoptera: *Bruchidae*). Estimativa de perda de peso de feijao decorrente da infestacao por  
473 esses carunchos. *Coletanea Do Instituto De Tecnologia De Alimentos*, 1989, **19**, 165–72.
- 474 29. Domingue, M. J., Scheff, D. S., Leva, N. and Myers, S. W., Climbing ability of *Trogoderma*  
475 *granarium* larvae on artificial household and insecticide-treated materials. *J. Stored Prod. Res.*,  
476 2022, **95**, 101922, <https://doi.org/10.1016/j.jspr.2021.101922>.
- 477 30. Cline, L. D., Clinging and climbing ability of larvae of eleven species of stored-product insects  
478 on nine flexible packaging materials and glass. *J. Econ Entomol.*, 1978, **71**, 689–  
479 691. <https://doi.org/10.1093/jee/71.4.689>
- 480 31. Jassim, S. H., Mubark, A. A and Falih, A. I., Mechanical and thermal properties of PP and PE  
481 blend reinforced with nano particle for industrial applications. Thesis, University of Babylon.  
482 2022.
- 483 32. Barbani, L. E., Foraging activity and food preferences of the odorous house ant (*Tapinoma*  
484 *sessile* Say) (Hymenoptera: Formicidae). M. S. Thesis, Virginia Polytechnic Institute and State  
485 University, Blacksburg, Virginia, 2003.

486

## Figure 1

Effect of packaging types with 0.02 mm thickness on holes in packaging and weight loss in packed fruit cake caused by *M. indicum*

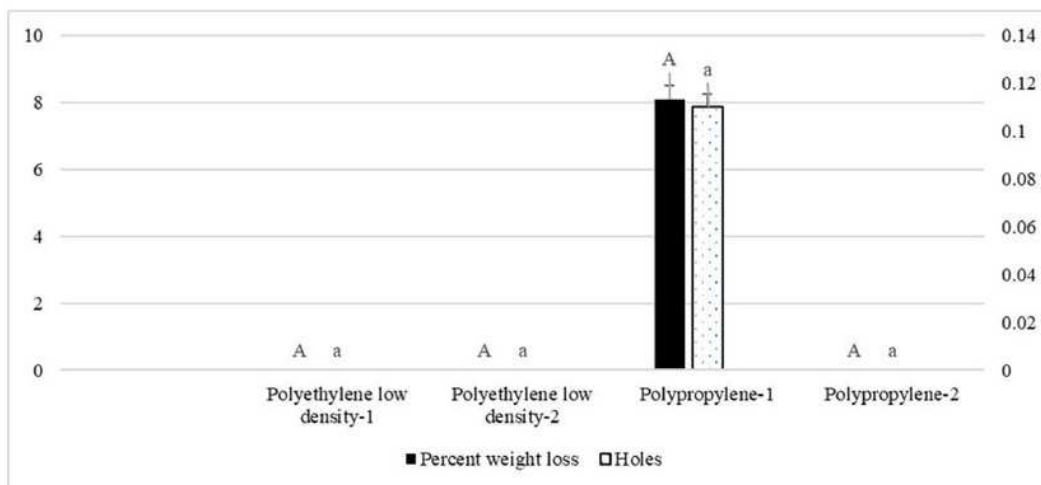
Fig 1.1: Packaging with food material, 2: Packaging without food material. Means comparison by Tukey HSD test at 0.05 level. Different letters along error bars show significant difference between mean. Small letters lie along error bars for number of holes, capital letters lie along percent weight loss bars.



## Figure 2

Effect of packaging types with 0.04 mm thickness on holes in packaging and weight loss in packed fruit cake caused by *M. indicum*.

Fig 2.1: Packaging with food material, 2: Packaging without food material. Means comparison by Tukey HSD test at 0.05 level. Small letters lie along error bars for number of holes, capital letters lie along percent weight loss bars.

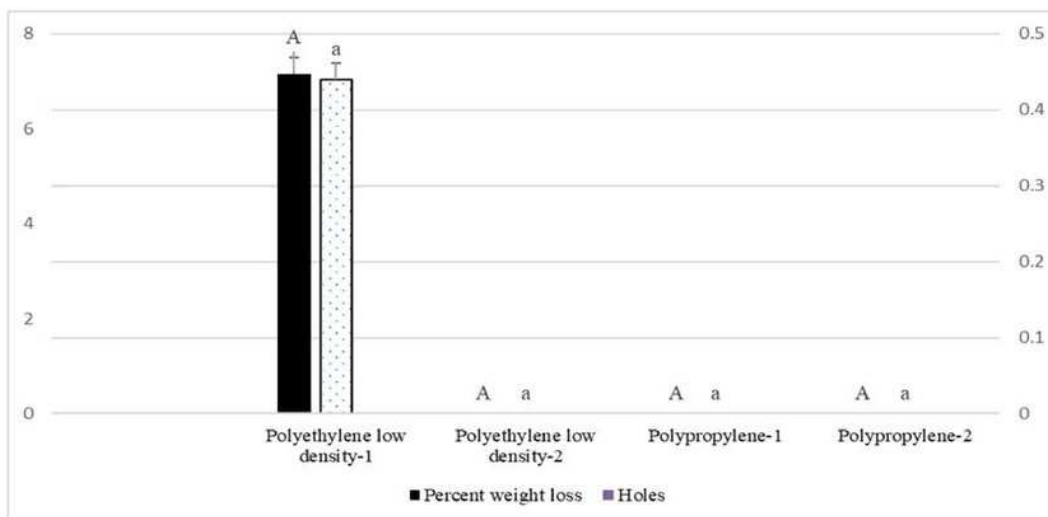




## Figure 3

Effect of packaging types with 0.06 mm thickness on holes in packaging and weight loss in packed fruit cake caused by *M. indicum*.

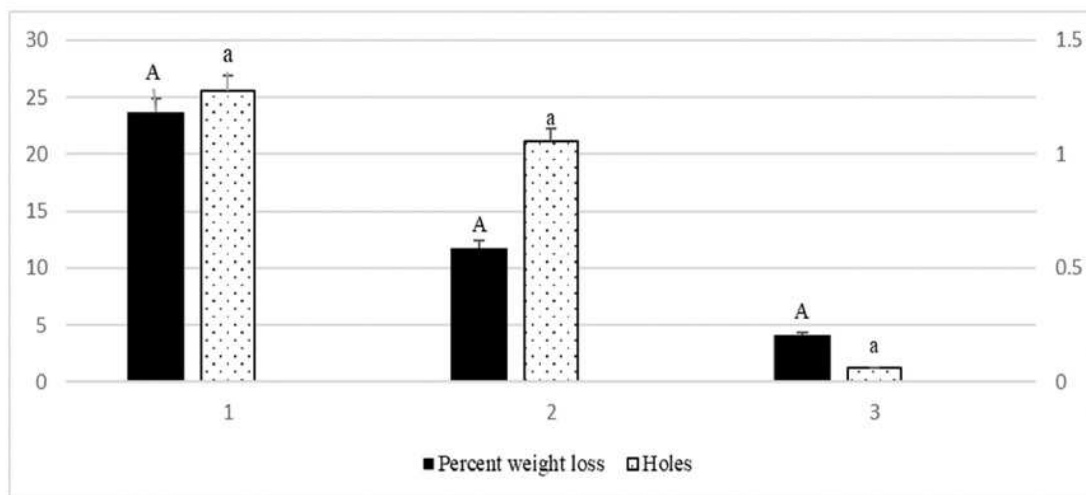
Fig 3. 1: Packaging with food material, 2: Packaging without food material. Means comparison by Tukey HSD test at 0.05 level. Small letters lie along error bars for number of holes, capital letters lie along percent weight loss bars.



## Figure 4

Effect of experiment dates on holes in packaging and weight loss in packed fruit cake in 0.02 mm thick packaging caused by *M. indicum*.

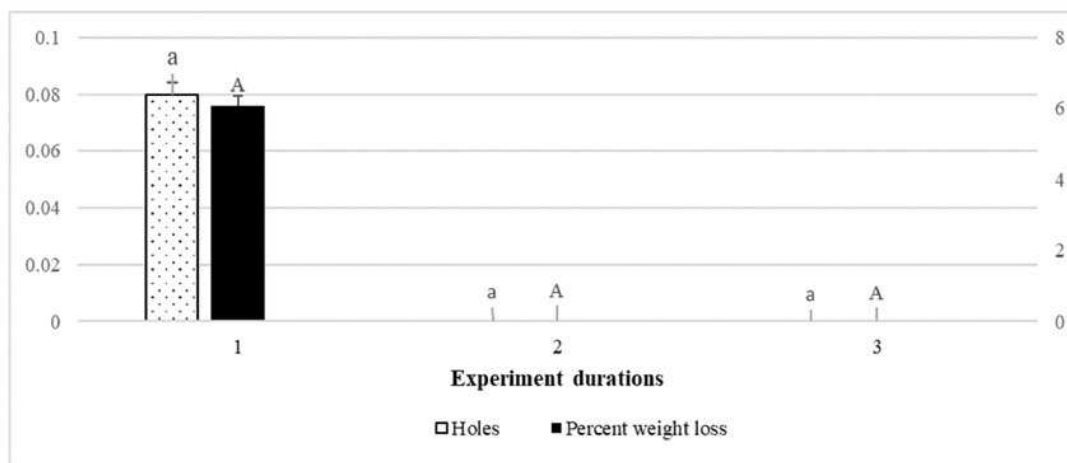
Fig 4. 1: first experiment (20<sup>th</sup> June to 5<sup>th</sup> July 2022), 2: (10<sup>th</sup> July to 25<sup>th</sup> July), 3: (1<sup>st</sup> August to 15<sup>th</sup> August). Means comparison by Tukey HSD test at 0.05 level. Small letters lie along error bars for number of holes, capital letters lie along percent weight loss bars.



## Figure 5

Effect of experiment dates on holes in packaging and weight loss in packed fruit cake in 0.04 mm thick packaging caused by *M. indicum*.

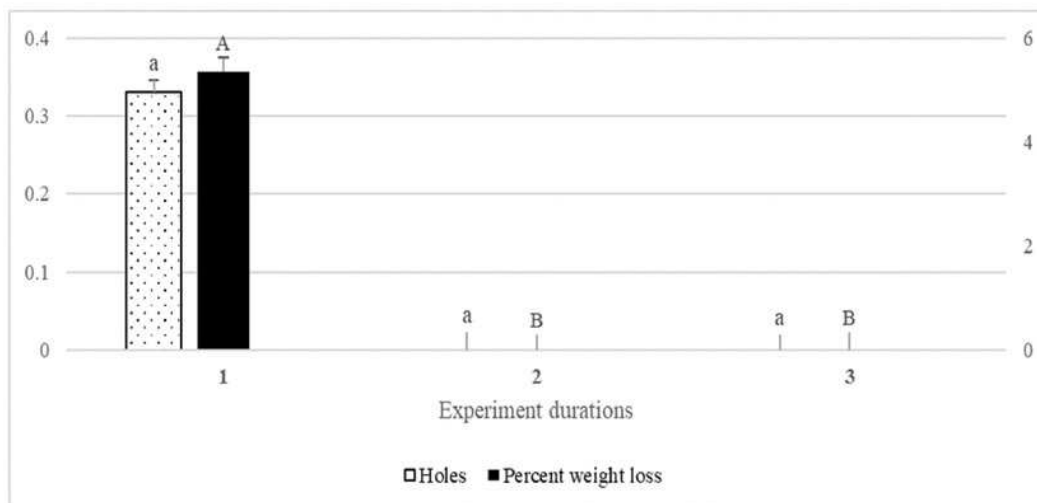
Fig 5. 1: first experiment (20<sup>th</sup> June to 5<sup>th</sup> July 2022), 2: (10<sup>th</sup> July to 25<sup>th</sup> July), 3: (1<sup>st</sup> August to 15<sup>th</sup> August). Means comparison by Tukey HSD test at 0.05 level. Small letters lie along error bars for number of holes, capital letters lie along percent weight loss bars.



## Figure 6

Effect of experiment dates on holes in packaging and weight loss in packed fruit cake in 0.06 mm thick packaging caused by *M. indicum*.

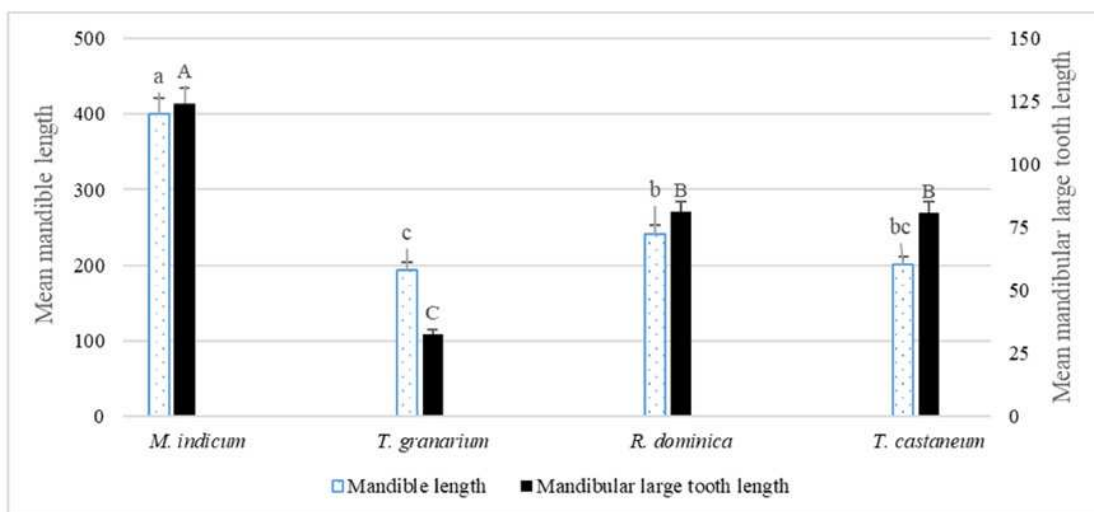
Fig 6. 1: first experiment (20<sup>th</sup> June to 5<sup>th</sup> July 2022), 2: (10<sup>th</sup> July to 25<sup>th</sup> July), 3: (1<sup>st</sup> August to 15<sup>th</sup> August). Means comparison by Tukey HSD test at 0.05 level. Small letters lie along error bars for number of holes, capital letters lie along percent weight loss bars.



## Figure 7

Mean mandibular and mandibular largest teeth lengths of *M. indicum* and three common stored product pests.

Fig 7. Means comparison by Tukey HSD test at 0.05 level. Different letters along bars show significant difference between means. Small letters are alongside mandibular length bars while capital letters accompany frontal tooth length bars.



**Table 1** (on next page)

Correlation of damages in packaging with weather factors and packaging thickness

1 **Table 1. Correlation of holes and percent weight loss in packaging with weather factors**  
 2 **and packaging thickness**

Thickness Factors	Correlation of weather factors and packaging material thickness with damages					
	0.02 mm		0.04 mm		0.06 mm	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>	<i>R</i>	<i>P</i>
Temperature	0.5083	0.6606	0.9066	0.2774	0.9066	0.2774
Relative humidity	-0.6003	0.5901	-0.9476	0.207	-0.9476	0.207
Thickness effect	<i>r</i> -0.2662; <i>P</i> : 0.0517					

3