RESEARCH

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Discovery

Efficacy of different plant extract on packaging materials against *Tribolium castaneum*

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The present study was carried out to investigate the toxicity of plant extracts treated on three packaging materials against two stored product insect pests, *Tribolium castaneum*. Three plant extracts (*Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum*) were applied on three packaging materials (polyethylene, polypropylene and jute). Six concentrations of plant extracts (10, 15, 20, 25, 30 and 35%) were applied on each packaging material cut into circular pieces of petri dish size. After air drying, the treated pieces of packaging materials were placed in petri dishes. Then 30 adults of *Tribolium castaneum* was released in the petri dishes, which were tightly covered using rubber bands and were placed in incubator at optimum growth conditions. Mortality of insects were observed after 24, 48 and 72 hrs. According to the results of bioassay for evaluation of plant extracts, maximum mortality of *T. castaneum* at 35% conc. and 72 hours exposure time was 36.01% for *Azadirachta indica* treated on polyethylene packaging material, 32.95% for *Eucalyptus globulus* and 24.30% for *Ocimum basilicum* at the same packaging material, dose rate and exposure time.

INTRODUCTION

Insects are responsible for causing a lot of damage to stored products and stored grains, they cause up to 5-10% damage in stored products of temperate zone and about 20-30% damage in tropical zone (Nakakita, 1998). These insects are very significant in causing post-harvest losses, which leads up to 36% (Tefera, 2012). These insects also act as a vector of various diseases that are dangerous for the health of animals and human beings. For example, (Larson *et al.*, 2008) shows the evidence of enterococci in insects of stored products, which basically act as a reservoirs for various active genes and antibiotic resistant genes. Throughout the world the most injurious and damaging pests of stored products are *Tribolium castaneum* (Mondal, 1994). *T. castaneum* (Herbst) red flour beetle is one of the most important pest of stored grain (Shafique *et al.*, 2006; Hulasare *et al.*, 2003). Pest control in stored products is a very major problem in developing countries. Among the international pests of cereals, stored grains, fruit and nuts the red flour beetle, *Tribolium castaneum* (Herbst) are most important (Fedina and Lewis, 2007). Use of woven bags is one of the low-cost method of storing grains but this method needs to apply the insecticide (De Groote *et al.*, 2013; Maina *et al.*, 2016). In Sub-Sahara region for storing maize and some other crops hermetic storage bags were used for past ten decade (Baoua *et al.*, 2014; De Groote *et al.*, 2013). Many companies have applied package-testing programs in order to develop resistance against insect attack (Mullen, 1994). Purdue Improved Crop Storage (PICS) bags contains two layers of high density polyethylene (HDPE) surrounded by a polypropylene bag, they give an excellent protection against bruchid seed beetles to cowpea grain in West Africa (Baoua *et al.*, 2013; Baoua *et al.*, 2012; Murdock *et al.*, 2012).

Repellents, means having the ability to repel insect to enter or move across a surface which is treated with these repellents. The use of coating of these repellent on packages is helpful in order to prevent insect infestation at that portion where further research is to be conducted (Mullen *et al.*, 2012). Highland (1978) recorded the progress of repellent treatments as a priority. Different repellent formulations have been tested through the years. Natural and synthetic combinations were used in the studies of senior authors. They include insect growth regulators neem oil, methyl salicylate and DEET derivatives (Mullen *et al.*, 2012). EPA was accepted as Provision Gard™ in 2009, in which IGR methoprene was used and it is also used in many package applications now a days. Provision Gard™ is very useful in order to prevent the Indian meal moth entry.

Plant extracts are more effective in the way as they do not cause pollution, they produce less toxicity and are bio-degradable. Some plants naturally have the repellent effect against many stored grain pests (Behal, 1998). In many studies it was observed that *Eucalyptus camaldulensis* have fumigant potential which help in the control against *Tribolium castaneum* (Negahban and Moharramipour, 2007). According to the studies of last 20 years, in order to control some insect pests and pathogens by *Azadirachta indica* extract along with its compounds was proved best (Mwangangi and Mutisya, 2013). Different developmental

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stages of larvae can be controlled by *A. indica* especially after emergence from the eggs, but it is not too much effective in controlling the mid stage and late stage instars (Xie *et al.*, 1995). The *A. indica* was proved too much effective by the researchers from various parts of the world, they experienced and noticed the various features and parts of the plant, used them to control *Tribolium castaneum* and then recorded the data that shows the success of *A. indica* plant extract to control the *T. castaneum* without causing any adversative effect on the surrounding environment (Saeed *et al.*, 2016). *Eucalyptus camaldulensis* essential oil shows a wide range of biological activity against pathogens including fungi, insects, bacteria, mites and also some weeds. It also provide a very simple, cheap and environmentally friendly (non-polluting and less or no toxicological concerns) alternate in order to control pest. Although its vapor forms are too much toxic towards a number of microorganisms as well as insects, hence they could commercially misused as fumigants for stored products and packaging materials, thus help in the prevention of insect infestation (Batish *et al.*, 2008).

The objective of present studies was; To evaluate the effectiveness of plant extract treated on packaging materials against *Tribolium castaneum.*

MATERIALS AND METHODS

The present studies were conducted at Grain Research, Training and Storage Management Cell, Department of Entomology, University of Agriculture Faisalabad during 2016-2017.

Collection and Rearing of Insects

Two insect species, of stored grains i.e. *Tribolium castaneum* was obtained from lab strain. Insect population of *Tribolium castaneum* was taken after 2-3 weeks post eclosion from laboratory cultures. Then provide optimum growth conditions in incubator, such as 30±2ºC temperature and 65±5% relative humidity. *T. castaneum* was dispose to sterilized flour. This uniform population was subjected for experimentation.

Packaging Materials

This experiments were conducted using three types of packaging materials i.e. Polyethylene, Polypropylene and jute bags. These packaging materials were cut into discs of petri dish size (9cm). These discs of packaging materials were treated with insecticides and plant extracts. After treatment these discs were placed in petri dishes and insects were released on these treated discs.

Preparation of Plant Extracts

Leaves of *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum* were collected from University of Agriculture Faisalabad, Pakistan. Leaves were washed and then air dried under shade. Powder of these air dried leaves was prepared using electrical grinder. To prepare crude extracts of these plant materials, 50 gm powder of each plant was mixed with 250 ml acetone in a conical flask. These conical flasks were placed on a rotary shaker (Waverly S3L-Pro LCD Linear Shaker) at 220 rpm for two days. After that, extract was filtered out. The filtrate was shifted to rotary evaporated (WG-EV311) to evaporate the acetone from the extract and to obtain pure crude extract of the respective plant.

Bioassay to evaluate the toxic effect of plant extracts treated on packaging materials against *Tribolium castaneum*

Three packaging materials (Polyethylene, Polypropylene and jute bags) were treated with six concentrations (10, 15, 20, 25, 30 and 35%) extracts of *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum.* The discs of packaging materials treated with plant extracts were transferred into petri dishes after air drying. Thirty beetles of *T. castaneum* were released in each petri dish. Each of six treatment combinations for each species and type of packaging were repeated three times. There was a control treatment for each plant extract. The mortality data were recorded after 24, 48 and 72 hours exposure time. If the beetles shows no movements on touching with brush they were considered dead.

Statistical Analysis

After the completion of all bioassays, corrected mortality of recorded data was calculated using Abbott's formula.

$$\text{Corrected Mortality (\%)} = \frac{\text{Mo(\%)} - \text{Mc(\%)}}{100 - \text{Mc(\%)}} \times 100$$

Mo = Observed mortality
Mc = Mortality in Control

STATISTICA 8 was used to analyze the data of % mortality and other treatments and their combinations. Significant treatments findings were compared with Tucky-HSD.

RESULTS AND DISCUSSION

Present study was planned to determine the effectiveness of plant extracts applied on different packaging materials against *Tribolium castaneum*.

Three types of packaging materials (Polyethylene, Polypropylene and jute bags) were used. Six concentrations (10, 15, 20, 25, 30 and 35%) of *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum* against *Tribolium castaneum.* Results are described in the following sub-headings.

Analysis of variance of data regarding % mortality of *Tribolium castaneum* using different concentrations of *Azadirachta indica* (10, 15, 20, 25, 30 and 35%) after 24 hours exposure time and different packaging materials (Polyethylene, Polypropylene and jute bags) are given in the table 1. This table shows the packaging materials and concentrations have significant effect on percent mortality of *T. castaneum*. The interaction between packaging material and concentration was also significant. The overall results show that % mortality of *T. castaneum* increase by increasing the concentrations while packaging materials have significant effect with each other.

The results regarding mean % mortality of *Tribolium castaneum* after 24 hours exposure time using different packaging material are given in the table 1.1. This table describe that the highest mortality was showed at the polyethylene packaging material was 20.78% and lowest % mortality was 13.48% found in jute material.

Data showed in table 1.2 the % mortality of *Tribolium castaneum* at several concentrations of *Azadirachta indica* (10, 15, 20, 25, 30 and 35%). This table showed the % mortality of *T. castaneum* increase by increasing the concentrations of *Azadirachta indica*. The highest mortality shown 35% concentration of the *Azadirachta indica* was 25.84%. The % mortality of *T. castaneum* at the other concentrations (10, 15, 20, 25 and 30%) was 8.61, 12.36, 14.98, 18.35 and 22.09% respectively. The results regarding the % mortality of *T. castaneum* using different concentrations (10, 15, 20, 25, 30 and 35%) of *Azadirachta indica* applied at three packaging material (Polypropylene, polyethylene and jute) are given table.4.10.3 which shows that the 35%

concentration on polyethylene packaging material caused the highest mortality (29.21%). The packaging material of jute bag at 10% concentration caused the least mortality (6.74%). The overall results showed that by increasing the concentrations of *Azadirachta indica* there is an increase in the % mortality of *T. castaneum;* while polypropylene packaging material shows highest % mortality *T. castaneum*.

Analysis of variance of data regarding % mortality of *Tribolium castaneum* using different concentrations of *Azadirachta indica* (10, 15, 20, 25, 30 and 35%) after 48 hours exposure time and different packaging materials (Polyethylene, Polypropylene and jute bags) are given in the table 2. This table shows the packaging materials and concentrations have significant effect on percent mortality of *T. castaneum*. The interaction between packaging material and concentration was non-significant. The overall results showed that % mortality of *T. castaneum* increase by increasing the concentrations while packaging material have significant effect with each other.

The results regarding mean % mortality of *Tribolium castaneum* after 48 hours exposure time using different packaging materials are given in the table 2.1. This table describe that the highest mortality was showed at the polyethylene packaging material was 23.67% and lowest % mortality was 16.10% found in jute material.

Data showed in table 2.2 the % mortality of *Tribolium castaneum* at several concentrations of *Azadirachta indica* (10, 15, 20, 25, 30 and 35%). The highest mortality was showed that at 35% concentration of the *Azadirachta indica* was 29.16%. The % mortality of *T. castaneum* at the other concentrations (10, 15, 20, 25 and 30%) was 10.96, 14.77, 17.42, 20.83 and 25.00% respectively.

Analysis of variance of data regarding % mortality of *Tribolium castaneum* using different concentrations of *Azadirachta indica* (10, 15, 20, 25, 30 and 35%) after 72 hours exposure time and three packaging materials (Polyethylene, Polypropylene and jute bags) are given in the table 3. This table shows the packaging materials and concentrations have significant effect on percent mortality of *T. castaneum*. The interaction between packaging material and concentration was Non-significant. The overall results showed that % mortality of *T. castaneum* increase by increasing the concentrations while packaging materials have significant effect with each other.

The results regarding mean % mortality of *Tribolium castaneum* after 72 hours exposure time using different packaging materials are given in the table 3.1. This table describe that the highest mortality was showed at the polyethylene packaging material was 30.07% and lowest % mortality was 21.26% found in jute material.

Data showed in table 3.2 the % mortality of *Tribolium castaneum* at several concentrations of *Azadirachta indica* (10, 15, 20, 25, 30 and 35%). The highest mortality shown at 35ppm concentration of the *Azadirachta indica* was 36.01%. The % mortality of *T. castaneum* at the other concentrations (10, 15, 20, 25 and 30%) was 15.71, 20.69, 23.37, 26.82 and 30.65% respectively.

Analysis of variance of data regarding % mortality of *Tribolium castaneum* using different concentrations of *Eucalyptus globulus* (10, 15, 20, 25, 30 and 35%) after 24 hours exposure time and three packaging materials (Polyethylene, Polypropylene and jute bags) are given in the table 4. This table shows the packaging materials and concentrations have highly significant effect on percent mortality of *T. castaneum*. The interaction between packaging material and concentration was non-significant. The overall results showed that % mortality of *T. castaneum* increase by increasing the concentrations, while packaging materials have significant effect with each other.

The results regarding mean % mortality of *Tribolium castaneum* after 24 hours exposure time using different packaging materials are given in the table 4.1. This table describe that the highest mortality was showed at the polyethylene packaging material was 17.41% and lowest % mortality was 10.11% found in jute material.

Data showed in table 4.2 the % mortality of *Tribolium castaneum* at several concentrations of *Eucalyptus globulus* (10, 15, 20, 25, 30 and 35%). The highest mortality was showed that at 35% concentration of the *Eucalyptus globulus* was 22.47%. The % mortality of *T. castaneum* at the other concentrations (10, 15, 20, 25 and 30%) was 5.24, 8.98, 11.61, 14.98 and 18.72% respectively.

Analysis of variance of data regarding % mortality of *Tribolium castaneum* using different concentrations of *Eucalyptus globulus* (10, 15, 20, 25, 30 and 35%) after 48 hours exposure time and three packaging materials (Polyethylene, Polypropylene and jute bags) are given in the table 5. This table shows the packaging materials and concentrations have significant effect on percent mortality of *T. castaneum*. The interaction between packaging material and concentration was Non-significant. The overall results shows that % mortality of *T. castaneum* increase by increasing the concentrations, while packaging materials have significant effect with each other.

The results regarding mean % mortality of *Tribolium castaneum* after 48 hours exposure time using different packaging material are given in the table 5.1. This table describe that the highest mortality showed at the polyethylene packaging material was 20.26% and lowest % mortality was 12.50% found in jute material.

Data showed in table 5.2 the % mortality of *Tribolium castaneum* at several concentrations of *Eucalyptus globulus* (10, 15, 20, 25, 30 and 35%). The highest mortality shown at 35% concentration of the *Eucalyptus globulus* was 25.75%. The % mortality of *T. castaneum* at the other concentrations (10, 15, 20, 25 and 30%) was 7.75, 11.36, 14.01, 17.42 and 21.21% respectively.

Analysis of variance of data regarding % mortality of *Tribolium castaneum* using different concentrations of *Eucalyptus globulus* (10, 15, 20, 25, 30 and 35%) after 72 hours exposure time and three packaging materials (Polyethylene, Polypropylene and jute bags) are given in the table 6. This table shows the packaging materials and different concentrations have significant effect on percent mortality of *T. castaneum*. The interaction between packaging material and concentration was non-significant. The overall results shows that % mortality of *T. castaneum* increase by increasing the concentration, while packaging material have significant effect with each other.

The results regarding mean % mortality of *Tribolium castaneum* after 72 hours exposure time using different packaging materials are given in the table 6.1. This table describe that the highest mortality was showed at the polyethylene packaging material was 26.82% and lowest % mortality was 17.81% found in jute material.

Data showed in table 6.2 the % mortality of *Tribolium castaneum* at several concentrations of *Eucalyptus globulus* (10, 15, 20, 25, 30 and 35%). The highest mortality shown at 35% concentration of the *Eucalyptus globulus* was 32.95%. The % mortality of *T. castaneum* at the other concentrations (10, 15, 20, 25 and 30%) was 12.26, 17.24, 19.92, 23.37 and 27.20% respectively.

Analysis of variance of data regarding % mortality of *Tribolium castaneum* using different concentrations of *Ocimum basilicum* (10, 15, 20, 25, 30 and 35%) after 24 hours exposure time and three packaging materials (Polyethylene, Polypropylene and jute bags) are given in the table 7. This table shows the packaging materials and concentrations have significant effect on percent mortality of *T. castaneum*. The

Table 1 Analysis of variance of data regarding % mortality of *Tribolium castaneum* against *Azadirachta indica* in treated packaging materials after 24 hours exposure time

SOV	DF	SS	MS	F Value
Packaging materials (Pm)	2	480.99	240.49	36.87**
Concentrations (C)	5	1817.28	363.46	55.73**
(Pm)x (C)	10	66.91	6.69	1.02*
Error	36	234.81	6.52	
Total	53	2599.99		

* (Significant) ** (Highly Significant)

Table 1.1 Mean mortality (%) of *Tribolium castaneum* against *Azadirachta indica* after 24 hours exposure time by treating different packaging materials

Packaging materials	Mean mortality± S.E
Jute	13.48±1.27 c
Polypropylene	16.85±1.70 b
Polyethylene	20.78±1.54 a

Table 1.2 Comparison of mean values of the data regarding percent mortality of *Tribolium castaneum* using different concentrations of *Azadirachta indica* after 24 hours exposure time

Concentrations (%)	Mean mortality± SE
10	8.61±0.87 e
15	12.36±1.23 d
20	14.98±1.35 cd
25	18.35±1.56 c
30	22.09±1.42 b
35	25.84±1.46 a

Table 2 Analysis of variance of data regarding % mortality of *Tribolium castaneum* against *Azadirachta indica* in treated packaging materials after 48 hours exposure time

SOV	DF	SS	MS	F Value
Packaging materials (Pm)	2	520.38	260.19	44.77**
Concentrations (C)	5	2019.54	403.91	69.51**
(Pm) x (C)	10	55.52	5.55	0.95^{NS}
Error	36	209.18	5.81	
Total	53	2804.62		

Table 2.1 Mean mortality (%) of *Tribolium castaneum* against *Azadirachta indica* after 48 hours exposure time by treating different packaging materials

Packaging materials	Mean mortality± S.E
Jute	16.10±1.35 c
Polypropylene	19.32±1.72 b
Polyethylene	23.67±1.63 a

Table 2.2 Comparison of mean values of the data regarding percent mortality of *Tribolium castaneum* using different concentrations of *Azadirachta indica* after 48 hours exposure time

Concentrations (%)	Mean mortality± S.E
10	10.98±0.88 e
15	14.77±1.27 d
20	17.42±1.36 cd
25	20.83±1.58 c
30	25.00±1.39 b
35	29.16±1.47 a

Table 3 Analysis of variance of data regarding % mortality of *Tribolium castaneum* against *Azadirachta indica* in treated packaging materials after 72 hours exposure time

SOV	DF	SS	MS	F Value
Packaging materials (Pm)	2	700.62	350.31	33.85**

Concentrations (C)	5	2361.22	472.24	45.63**
(Pm)x (C)	10	49.76	4.98	0.48^{NS}
Error	36	372.55	10.35	
Total	53	3484.15		

Table 3.1 Mean mortality (%) of *Tribolium castaneum* against *Azadirachta indica* after 72 hours exposure time by treating different packaging materials

Packaging materials	Mean mortality± S.E
Jute	21.26±1.55 c
Polypropylene	25.28±1.74 b
Polyethylene	30.07±1.90 a

Table 3.2 Comparison of mean values of the data regarding percent mortality of *Tribolium castaneum* using different concentrations of *Azadirachta indica* after 72 hours exposure time

Concentrations (%)	Mean mortality± SE
10	15.71±1.29 e
15	20.69±1.28 d
20	23.27±1.38 cd
25	26.82±1.60 bc
30	30.65±1.67 b
35	36.01±2.23 a

Table 4 Analysis of variance of data regarding % mortality of *Tribolium castaneum* against *Eucalyptus globulus* in treated packaging materials after 24 hours exposure time

SOV	DF	SS	MS	F Value
Packaging materials (Pm)	2	480.99	240.49	36.87**
Concentrations (C)	5	1817.28	363.46	55.72**
(Pm)x (C)	10	66.91	6.69	1.02^{NS}
Error	36	234.81	6.52	
Total	53	2599.99		

Table 4.1 Mean mortality (%) of *Tribolium castaneum* against *Eucalyptus globulus* after 24 hours exposure time by treating different packaging materials

Packaging materials	Mean mortality± S.E
Jute	10.11±1.27 c
Polypropylene	13.48±1.7 b
Polyethylene	17.41±1.54 a

Table 4.2 Comparison of mean values of the data regarding percent mortality of *Tribolium castaneum* using different concentrations of *Eucalyptus globulus* after 24 hours exposure time

Concentrations (%)	Mean mortality± S.E
10	5.24±0.87 e
15	8.98±1.25 d
20	11.61±1.35 cd
25	14.98±1.56 c
30	18.72±1.42 b
35	22.47±1.48 a

Table 5 Analysis of variance of data regarding % mortality of *Tribolium castaneum* against *Eucalyptus globulus* in treated packaging materials after 48 hours exposure time

SOV	DF	SS	MS	F Value
Packaging materials (Pm)	2	545.34	272.67	42.23**
Concentrations (C)	5	1984.46	396.89	61.47**

(Pm) x (C)	10	64.13	6.41	0.99^{NS}
Error	36	232.43	6.46	
Total	53	2826.36		

Table 5.1 Mean mortality (%) of *Tribolium castaneum* against *Eucalyptus globulus* after 48 hours exposure time by treating different packaging materials

Packaging materials	Mean mortality± S.E
Jute	12.50±1.35 c
Polypropylene	15.91±1.72 b
Polyethylene	20.26±1.63 a

Table 5.2 Comparison of mean values of the data regarding percent mortality of *Tribolium castaneum* using different concentrations of *Eucalyptus globulus* after 48 hours exposure time

Concentrations (%)	Mean mortality± S.E
10	7.57±0.87 e
15	11.36±1.27 d
20	14.01±1.36 cd
25	17.42±1.58 c
30	21.21±1.65 b
35	25.75±1.47 a

Table 6 Analysis of variance of data regarding % mortality of *Tribolium castaneum* against *Eucalyptus globulus* in treated packaging materials after 72 hours exposure time

SOV	DF	SS	MS	F Value
Packaging materials (Pm)	2	732.32	366.16	42.64**
Concentrations (C)	5	2434.54	486.91	56.70**
(Pm) x (C)	10	57.69	5.77	0.67^{NS}
Error	36	309.13	8.59	
Total	53	3533.69		

Table 6.1 Mean mortality (%) of *Tribolium castaneum* against *Eucalyptus globulus* after 72 hours exposure time by treating different packaging materials

Packaging materials	Mean mortality± S.E
Jute	17.81±1.55 c
Polypropylene	21.84±1.74 b
Polyethylene	26.82±1.92 a

Table 6.2 Comparison of mean values of the data regarding percent mortality of *Tribolium castaneum* using different concentrations of *Eucalyptus globulus* after 72 hours exposure time

Concentrations (%)	Mean mortality± SE
10	12.26±1.29 e
15	17.24±1.28 d
20	19.92±1.38 cd
25	23.37±1.60 bc
30	27.20±1.67 b
35	32.95±2.15 a

Table 7 Analysis of variance of data regarding % mortality of *Tribolium castaneum* against *Ocimum basilicum* in treated packaging materials after 24 hours exposure time

SOV	DF	SS	MS	F Value
Packaging materials (Pm)	2	132.13	66.06	7.67**
Concentrations (C)	5	187.66	37.53	4.36**
(Pm) x (C)	10	56.38	5.63	0.65^{NS}
Error	36	309.90	8.60	
Total	53	686.09		

Table 7.1 Mean mortality (%) of *Tribolium castaneum* against *Ocimum basilicum* after 24 hours exposure time by treating different packaging materials

Packaging materials	Mean mortality± S.E
Jute	1.51±0.66 b
Polypropylene	1.32±0.70 b
Polyethylene	4.73±0.93 a

Table 7.2 Comparison of mean values of the data regarding percent mortality of *Tribolium castaneum* using different concentrations of *Ocimum basilicum* after 24 hours exposure time

Concentrations (%)	Mean mortality± S.E
10	0.36±0.82 c
15	1.13±0.80 bc
20	3.41±0.98 abc
25	3.79±1.24 ab
30	1.89±1.10 abc
35	5.30±1.36 a

Table 8 Analysis of variance of data regarding % mortality of *Tribolium castaneum* against *Ocimum basilicum* in treated packaging materials after 48 hours exposure time

SOV	DF	SS	MS	F Value
Packaging materials (Pm)	2	383.55	191.77	10.75**
Concentrations (C)	5	855.18	171.03	9.59**
(Pm) x (C)	10	261.13	26.11	1.46^{NS}
Error	36	642.04	17.83	
Total	53	2141.93		

Table 8.1 Mean mortality (%) of *Tribolium castaneum* against *Ocimum basilicum* after 48 hours exposure time by treating different packaging materials

Packaging materials	Mean mortality± S.E
Jute	5.75±0.88 b
Polypropylene	5.55±1.01 b
Polyethylene	11.30±1.98 a

Table 8.2 Comparison of mean values of the data regarding percent mortality of *Tribolium castaneum* using different concentrations of *Ocimum basilicum* after 48 hours exposure time

Concentrations (%)	Mean mortality± S.E
10	2.30±0.81 d
15	3.45±0.81 cd
20	6.13±0.76 bcd
25	11.11±2.68 ab
30	8.81±1.91 abc
35	13.41±2.25 a

Table 9 Analysis of variance of data regarding % mortality of *Tribolium castaneum* against *Ocimum basilicum* in treated packaging materials after 72 hours exposure time

SOV	DF	SS	MS	F Value
Packaging materials (Pm)	2	333.93	166.97	4.97**
Concentrations (C)	5	3814.55	762.91	22.73**
(Pm) x (C)	10	688.15	68.81	2.04*
Error	36	1208.65	33.57	
Total	53	6045.27		

Table 9.1 Mean mortality (%) of *Tribolium castaneum* against *Ocimum basilicum* after 72 hours exposure time by treating different packaging materials

Packaging materials	Mean mortality± S.E
Jute	16.67±2.95 ab
Polypropylene	14.53±1.55 b
Polyethylene	20.54±2.74 a

Table 9.2 Comparison of mean values of the data regarding percent mortality of *Tribolium castaneum* using different concentrations of *Ocimum basilicum* after 72 hours exposure time

Concentrations (%)	Mean mortality± S.E
10	0.84±5.421 d
15	1.35±5.79 d
20	12.40±0.69 cd
25	30.62±3.41 ab
30	20.15±3.31 bc
35	24.3±2.30 a

Table 9.3 Mean mortality (%) of *Tribolium castaneum* after 72 hours exposure time against different concentrations of *Ocimum basilicum* applied on different packaging materials

Packaging materials	Concentrations (%)	Mean mortality± S.E
Jute	10	8.14±2.32 ef
Jute	15	5.81±2.01 f
Jute	20	11.63±1.16 def
Jute	25	36.04±8.13 a
Jute	30	12.79±2.01 cdef
Jute	35	25.58±4.65 abcde
Polypropylene	10	6.98±1.16 f
Polypropylene	15	10.46±3.07 def
Polypropylene	20	11.63±1.62 def
Polypropylene	25	22.09±1.62 abcdef
Polypropylene	30	17.44±4.19 bcdef
Polypropylene	35	18.60±3.07 abcdef
Polyethylene	10	6.98±1.16 f
Polyethylene	15	10.46±1.16 def
Polyethylene	20	13.95±1.16 cdef
Polyethylene	25	33.72±4.02 ab
Polyethylene	30	30.23±5.32 abc
Polyethylene	35	27.91±3.07 abcd

interaction between packaging material and concentration was non-significant. The overall results shows that % mortality of *T. castaneum* increase by increasing the concentrations, while packaging materials have significant effect with each other.

The results regarding mean % mortality of *Tribolium castaneum* after 24 hours exposure time using different packaging materials are given in the table 4.16.1. This table describe that the highest mortality was showed at the polyethylene packaging material was 4.73% and lowest % mortality was 1.32% found in polypropylene material.

Data showed in table 7.2 the % mortality of *Tribolium castaneum* at several concentrations of *Ocimum basilicum* (10, 15, 20, 25, 30 and 35%). This table showed the % mortality of *T. castaneum* increase by increasing the concentrations of *O. basilicum*. The highest mortality was showed that at 35% concentration of the *O. basilicum* was 5.30%. The % mortality of *T. castaneum* at the other concentrations (10, 15, 20, 25 and 30%) was 0.37, 1.13, 3.41, 3.79 and 1.89% respectively.

Analysis of variance of data regarding % mortality of *Tribolium castaneum* using different concentrations of *O. basilicum* (10, 15, 20, 25, 30 and 35%) after 48 hours exposure time and three packaging materials (Polyethylene, Polypropylene and jute bags) are given in the table 8. This table shows the packaging materials and concentrations have significant effect on percent mortality of *T. castaneum*. The interaction between packaging material and concentration was non-significant. The overall results shows that % mortality of *T. castaneum* increase by increasing the concentration, while packaging material have significant effect with each other.

The results regarding mean % mortality of *Tribolium castaneum* after 48 hours exposure time using different packaging material are given in the table 8.1. This table describe that the highest mortality was showed at the polyethylene packaging material was 11.30% and lowest % mortality was 5.55% found in polypropylene material.

Data showed in table 8.2 the % mortality of *Tribolium castaneum* at several concentrations of *Ocimum basilicum* (10, 15, 20, 25, 30 and 35%). This table showed the % mortality of *T. castaneum* increase by increasing the concentrations of *O. basilicum.* The highest mortality shown at 35% concentration of the *O. basilicum* was 13.41%. The % mortality of *T. castaneum* at the other concentrations (10, 15, 20, 25 and 30%) was 2.30, 3.45, 6.13, 11.11 and 8.81 % respectively.

Analysis of variance of data regarding % mortality of *Tribolium castaneum* using different concentrations of *Ocimum basilicum* (10, 15, 20, 25, 30 and 35%) after 72 hours exposure time and three packaging materials (Polyethylene, Polypropylene and jute bags) are given in the table 9. This table shows the packaging materials and concentrations have highly significant effect on percent mortality of *T. castaneum*. The interaction between packaging material and concentration was significant. The overall results shown that % mortality of *T. castaneum* increase by increasing the concentrations, while packaging material have highly significant effect with each other.

The results regarding mean % mortality of *Tribolium castaneum* after 72 hours exposure time using different packaging materials are given in the table 9.1. This table describe that the highest mortality was showed at the polyethylene packaging material was 20.54% and lowest % mortality was 14.53% found in polypropylene material.

Data showed in table 9.2 the % mortality of *Tribolium castaneum* at several concentrations of *Ocimum basilicum* (10, 15, 20, 25, 30 and 35%). This table showed the % mortality of *T. castaneum* increase by increasing the concentrations of *O. basilicum.* The highest mortality was showed that at 20% concentration of the *O. basilicum* was 30.62%. The % mortality of *T. castaneum* at the other concentrations (10, 15, 20, 30 and 35%) was 7.36, 8.91, 12.40, 20.15 and 24.03 % respectively.

The results regarding the % mortality of *T. castaneum* using different concentrations (10, 15, 20, 25, 30 and 35%) of *Ocimum basilicum* applied at three packaging materials (Polypropylene, polyethylene and jute) are given table.9.3 which shows that the 25% concentration on jute packaging material caused the highest mortality (36.04%). The packaging material of jute bag at 15% concentration caused the least mortality 5.89%. The whole results showed that by increasing the concentrations of *O. basilicum* there is an increase the% mortality of *T. castaneum*; while polypropylene packaging material shows highest % mortality *T. castaneum*.

DISCUSSION

After 24 hours exposure time data regarding maximum mean % mortality was 25.84, 22.47 and 5.30% in *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum* respectively at conc. 35%. While packaging materials treated with *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum* showed maximum % mortality in polyethylene was 20.78, 17.41 and 4.73% respectively. After 48 hours exposure time data regarding maximum mean % mortality was 29.16, 25.75 and 13.41% in *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum* respectively at conc. 35%. While packaging materials treated with *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum* showed maximum % mortality in polyethylene was 23.67, 20.26 and 11.30% respectively. After 72 hours exposure time data regarding maximum mean % mortality was 36.01, 32.95 and 24.30% in *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum* respectively at conc. 35%. While packaging materials treated with *Azadirachta indica*, *Eucalyptus globulus* and *Ocimum basilicum* showed maximum % mortality in polyethylene was 30.07, 26.82 and 20.54% respectively. According to (Islam and Talukder, 2005) our results revealed that leaf powder of *Azadirachta indica* showed a certain degree of toxicity against *Tribolium castaneum*. Plant derivatives, neem seed extract (100 µg/insect) showed higher direct toxicity (53.13 % mortality) towards red flour beetles. In the present study it was revealed that polyethylene material is better than other materials use. This was confirmed according to Chung *et al.* (2011) observed that in comparison of different packaging materials, polyethylene is much more resistant against *Tribolium castaneum*.

CONCLUSION

The overall maximum percent mortality of *T.castaneum* at 35% conc. and 72 hours exposure time was 36.01% for *Azadirachta indica* treated on polyethylene packaging material, 32.95% for *Eucalyptus globulus* and 24.30% for *Ocimum basilicum* at the same packaging material, dose rate and exposure time.

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