Management of *Tribolium castaneum* (Hbst.) (Coleoptera: Tenebrionidae) Using Neem (*Azadirachta indica* A. Juss) and Tumha (*Citrullus colocynthis* (L.))

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Abstract.- Studies were conducted in the Grain Research Training and Storage Management Cell, Department of Agri-Entomology, University of Agriculture, Faisalabad during 2006-07 to evaluate the effect of different doses (2.5%, 5%, 7.5% and 10%) of Neem (*Azadirachta indica* A. Juss) seed extract and Tumha (*Citrullus colocynthis* L.) fruit ethanol extracts on *Tribolium castaneum*. The effect of neem seed extract at tested concentrations against the test insect was dose dependent; as the 64.44%, 55.92%, 47.77% and 35.93% mortality recorded at 10%, 7.5%, 5% and 2.5% concentrations, respectively. The interaction effect of 10% neem seed extract and 72 h exposure gave 73.33% kill, which was significantly (P=0.05) more than the percent kill of the other concentrations and exposure time. Similarly, the *C. colocynthis* at 10% killed 44.07% *T. castaneum* which was significantly (P=0.05) more than the control and other concentrations of the same extract used in the experiment. The exposure of *T. castaneum* to the media treated with *C. colocynthis* for 72 h has a significant effect on its mortality. The interaction effect of 10% *Citrullus colocynthis* extract at 72 h exposure gave 50% kill, which was significantly more than the other concentrations and time of exposure. The insecticidal effect of these chemicals on the population build up of *T. castaneum* was dose and exposure time dependent.

Key words: *Azadirachta indica*, *Citrullus colocynthis*, *Tribolium castaneum*, stored grain pests, neem extract.

INTRODUCTION

In Pakistan cereals and pulses are stored by the producers in their homes and by the traders and the government agencies in godowns for one year or more. Wheat undergone heavy quantitative and qualitative losses in storages by several notorious insect pests. Jillani (1981) stated that among various factors contributing to losses in stored grain; insect pests are the most important. Ahmad et al. (1992) estimated more than 2.5% weight losses due to insect infestation. In China, Weifen *et al.* (2003) found 7 to 13% losses by insect pests in rural house hold storages. Singh and Yadav (1995) reported 2.03% losses in stored wheat by stored grain insect pests. Although, there are more than 40 different insect species that attack grains and grain products in stores, *Tribolium castaneum* (Hbst.) is one of the most serious insect pest of stored grain and grain products world wide. Both the larvae and adults cause damage. The greatest damage is during the hot and humid monsoon season which favors rapid build up of insect population. The larvae are negatively phototactic and are always found hidden in food.

The indiscriminate use of pesticides for the management of this obnoxious insect is not only hazardous to human beings but may affect the ecosystem as well. The use of these synthetic insecticides have resulted to resistance in *T. castaneum* toward these insecticides. Shakoori *et al.* (1998) and Lessard *et al.* (1998) reported resistance in *T. castaneum* against synthetic pyrethroids and some juvenile hormone analogues. Due to the hazardous nature of the conventional synthetic insecticides, it is imperative to evaluate botanicals against these insect pests, which are comparatively safe to man, other animals and non target organisms. Four medicinal plants have been used for their insecticidal activities against *T. castaneum* (Herbst.) (Jabilou *et al.*, 2006). Several such products from various floral species have been demonstrated to act as repellents, toxicants and antifeedants against a number of beetles that attack...
stored products (Papachristos and Stamopoulos, 2002; Tapondjou et al., 2002). More recently, researchers in the Western Hemisphere have begun to assess the use of neem and other plants’ essential oils as alternatives to fumigants and contact insecticides (Isman, 2000; Enan, 2001; Wang et al., 2001). Several plants materials have successfully been used as repellents, deterrent and anti-ovipositional against T. castaneum, (Jillani et al., 1993). Das et al. (2006) reported that Nimbicidine® commercial neem-based insecticide significantly inhibited egg hatching, pupation and adult emergence of T. castaneum. Rahim (1998) found that Azadirachitin was effective in reducing F1 progeny production by > 98% when used at 5 mg kg-1 on wheat against Rhzopertha dominica. The present study was undertaken to evaluate the insecticidal activities of Neem (Azadirachta indica A. Juss), Tumha (Citrus colocynthis) for the management of T. castaneum and to assess the residual activity of plant extracts in term of post treatment population buildup of T. castaneum.

MATERIALS AND METHODS

The study was conducted in the Grain Research Training and Storage Management Cell (GRTSMC), Department of Agri-Entomology, University of Agriculture Faisalabad, during 2006-07 to study the response of T. castaneum to neem (A. indica A. Juss) seed kernel extract and fruit powder extract of Tumha (C. colocynthis L.). T. casataneum was collected from local godowns of the Punjab Food Department and cultured at 30±2°C and 60±5 R.H on whole wheat grains mixed with flour in 800 g capacity jars sterilized at 60°C for 60-90 minutes. Each jar was filled with 200 g wheat grains admixed with 20 g flour and 100 beetles were added to each jar assuming 50 males and 50 females. The jars were then covered with muslin cloth and tied with rubber bands to avoid the escape of beetles and the entry of other insects. Beetles were allowed for 3 days to oviposit in the medium. After 3 days, the insects were removed with the help of sieves using camel hair brushes and were added to another set of sterilized jars filled with 200 g wheat grains + 20 g flour for regular production of culture. The flour containing eggs was placed in other jar to get different larval stages of the test insects and the fresh adults. It takes approximately 28 days for reaching to 6th instars larvae and 39 days to become adult.

For the preparation of plant extracts and their concentrations one kg shade dried neem (A. indica) seed kernels and fruit of tumha (C. colocynthis) each was grinded with mechanical blender. A sample of 50 g of each sample was taken in a separate flask and 50 ml ethanol was added. Mouth of each flask was closed with cotton plug and aluminum coil. The flask was placed in rotary shaker at 320°C and 120 rmp for 24 h. Each extract was filtered with filter paper to obtain 100% filtrate. From the filtrate 2.5%, 5%, 7.5%, 10% concentrations were prepared for experimental purposes by taking 2.5, 5, 7.5 ml and 10 ml added with 97.5, 95, 92.5 and 90 ml acetone, respectively.

To test the toxic effect of neem seed extract and tumha fruit powder extract at different doses with different exposure time against the adults of T. castaneum, petri dishes with a filter paper were used as exposure chambers. Ethanol extracts of neem seed and fruit powder of tumha were tested at 2.5%, 5%, 7.5% and 10%. The toxicity of the plants extracts was recorded after 24, 48 and 72 h. All the treatments including control were replicated three times and data collected was analyzed statistically (M. Stat C software). The required concentration of each plant extracts was sprayed thoroughly on the filter papers placed in the Petri dishes by using micropipette. The Petri dishes were left exposed to open air for an hour to completely evaporate the solvent. Thirty adults of T. castaneum were released into each petri dish and the petri dishes covered. The mortality of the test insect was recorded after 24, 48 and 72 h and survived insects were released in fresh grains and flour mixture for further oviposition to see the post treatment population build-up of the test insect. For test, 200 g wheat grains and 20 g of wheat flour for each treatment were placed in separate glass jars of 800 g capacity. The jars were covered with muslin cloth and secured with rubber bands to prevent the entry of any other insects. The jars were placed in the controlled environment. The rearing temperature of the controlled environment was 30±2°C and 60±5 R.H with 12 h light. Data was recorded for population build up and finally data
was analyzed with Analysis of Variance (ANOVA) (M. Stat. C software) and means separated with LSD.

**RESULTS**

The results (Table I) showed that *Azadirachta indica* at 10% concentration exhibited the highest efficacy against *T. castaneum* followed by 7.5%, 5% and 2.5% in that order. The toxic effect of *A. indica* extract against the test insect was dose dependent and significantly (P=0.05) different from each other. It is obvious from the results that 2.5% *A. indica* extract killed significantly more *T. castaneum* than in the untreated check, therefore, the application of *A. indica* extract at 10% may be too high to be used against the test insect. The results (Table I) showed that the ethanol extract of *C. colocythis* significantly killed more *T. castaneum* at all level of concentrations as compared to control; however, *C. colocythis* at 2.5 and 5% concentrations killed the test insect statistically similar. *C. colocythis* at 10% ranked first with maximum kill of the test insect followed by 7.5% extract. The results (Table II) showed that when *T. castaneum* were released in the *A. indica* extract treated medium for different time intervals, the highest percent mortality was found at 72 h exposure of the test insect to the treated material which was significantly more compared to 48 and 24 h of the said material. The results (Table II) also showed that 72 h exposure time of *C. colocythis* had a positive effect on the mortality of the test insects than after 48 and 24 h exposure. The percent kill of the insect at 24 h exposure was statistically similar to the percent kill of the insect at 48 h exposure time. The results (Table III) showed that the interaction effect of the *T. castaneum* exposed to 10% *A. indica* extract treatment for 72 h resulted in 73.33% kill of the test insect which was significantly more than the other interaction effects in the experiment. All the *A. indica* extract concentrations at similar exposure time resulted into statistically different (P=0.05) percent mortality of the test insects. In all cases the interaction seemed to be dose dependent. However, the interaction effect of 10% at 24 h, 7.5% at 48 h and 5% at 72 h was not significantly different (P=0.05). Similarly, the interaction effect of 7.5% *A. indica* extract at 24 h exposure had no significantly difference effect to that of 5% at 48 h exposure against *T. castaneum*. The interaction effect of the 2.5% *A. indica* extract at 24 h exposure was lower than the same concentration after 48 h and 72 h exposure, but the last two were at par to each other. In general, the result showed that there was positive interaction effect between treatments and exposure time but was also dose dependent. No mortality was observed in untreated check. The interaction effect of *C. colocythis* extract at 10% concentration for 72 h showed that the 50% kill of the test insects, which was significantly more than the kill of the test insect exposed for 24 and 48 h at the same concentration. However, there was no significant difference in the interaction effect after 24 and 48 h exposure of *T. castaneum* at 10% concentration. Similar results were obtained in other tested concentrations after 24, 48 and 72 h exposure; as 44.44% kill of the test insect at 7.5% after 72 h was

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**Table I.** Mean percent mortality of *T. castaneum* at different doses of *A. indica* and *C. colocythis* seed kernel extract up to 72 h.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th><em>A. indica</em></th>
<th><em>C. colocythis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>2.50%</td>
<td>35.93 d</td>
<td>30.74 c</td>
</tr>
<tr>
<td>5.00%</td>
<td>47.77 c</td>
<td>31.85 c</td>
</tr>
<tr>
<td>7.50%</td>
<td>55.92 b</td>
<td>37.03 b</td>
</tr>
<tr>
<td>10%</td>
<td>64.44 a</td>
<td>44.07 a</td>
</tr>
<tr>
<td>Control</td>
<td>0.000 e</td>
<td>0.000 d</td>
</tr>
<tr>
<td><strong>LSD 0.05</strong></td>
<td><strong>2.391</strong></td>
<td><strong>2.393</strong></td>
</tr>
</tbody>
</table>

Means sharing similar letters are not significantly different from each other at P = 0.05

**Table II.** Mean percent mortality of *Tribolium castaneum* with *A. indica* and *C. colocythis* extracts at different time intervals.

<table>
<thead>
<tr>
<th>Exposure time (h)</th>
<th><em>A. indica</em></th>
<th><em>C. colocythis</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>24</td>
<td>35.55 c</td>
<td>25.33 b</td>
</tr>
<tr>
<td>48</td>
<td>41.33 b</td>
<td>27.11 b</td>
</tr>
<tr>
<td>72</td>
<td>47.55 A</td>
<td>33.77 A</td>
</tr>
<tr>
<td><strong>LSD 0.05</strong></td>
<td><strong>1.852</strong></td>
<td><strong>1.853</strong></td>
</tr>
</tbody>
</table>

Means sharing similar letters are not significantly different from each other at P = 0.05
Table III.- Interaction effect of A. indica and C. colocynthis extract concentrations and exposure time on the mean percent mortality of Tribolium castaneum.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>A. indica</th>
<th>C. colocynthis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>2.50%</td>
<td>26.67 f</td>
<td>38.89 e</td>
</tr>
<tr>
<td>5.00%</td>
<td>40.00 e</td>
<td>47.77 d</td>
</tr>
<tr>
<td>7.50%</td>
<td>46.67 d</td>
<td>54.44 c</td>
</tr>
<tr>
<td>10%</td>
<td>54.44 c</td>
<td>65.55 b</td>
</tr>
<tr>
<td>Control</td>
<td>0.000 g</td>
<td>0.000 g</td>
</tr>
</tbody>
</table>

LSD 0.05: 4.142 4.144

Means sharing similar letters are not significantly different from each other at P = 0.05

significantly more than the 32.22% and 34.44% mortality after 24 h and 48 h of exposure, respectively. Identical results were obtained in other (2.5% and 5%) concentration after the exposure of the test insect for 24, 48 and 72 h as mentioned above. The exposure time intervals and concentration of C. colocynthis had a good positive toxic effect on T. castaneum.

The results (Table IV) showed that all the A. indica extract concentrations significantly (P=0.05) reduced the post treatment population build-up of T. castaneum. A. indica extract at 10% ranked 1st in reduction of post treatment population build-up of T. castaneum followed by 7.5%, 5% and 2.5% extract. However, A. indica extract at 5% was statistically similar to 2.5% and 7.5% in post treatment population build-up of the test insect. The results (Table IV) showed that when the survived T. castaneum fed on A. indica and C. colocynthis extract at different concentrations for different time intervals were allowed to develop its population on untreated medium, the population build-up at higher concentrations was low as compared to lower concentrations and control. The population build-up at 10% C. colocynthis extract was significantly lower than all other concentrations used in the experiment. C. colocynthis extract at 2.5% and 5% were statistically at par with regard to the population build-up of the test insects after post treatment. The results (Table V) showed the post-treatment population build-up of T. castaneum exposed for 24, 48 and 72 h to treated media with A. indica and C. colocynthis extracts at different doses. The highest mean percent population build-up was found at 24 h exposure of the test insect which was significantly more when exposed for 48 and 72 h. It indicates that when more the exposure time the less will be the population build-up of T. castaneum.

Table IV.- Post-treatment population build-up of T. castaneum at different dose rates of A. indica and C. colocynthis seed extract.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>Mean population build-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A. indica</td>
</tr>
<tr>
<td>2.50%</td>
<td>721.9 b</td>
</tr>
<tr>
<td>5.00%</td>
<td>717.4 bc</td>
</tr>
<tr>
<td>7.50%</td>
<td>711.3 c</td>
</tr>
<tr>
<td>10%</td>
<td>696.6 d</td>
</tr>
<tr>
<td>Control</td>
<td>795.2 a</td>
</tr>
</tbody>
</table>

LSD 0.05: 6.954 3.665

Means sharing similar letters are not significantly different from each other at P = 0.05

Table V.- Post-treatment population build-up of Tribolium castaneum with A. indica and C. colocynthis seed extract at different exposure times.

| Exposure time (h) | Mean population build-up |
|------------------)|--------------------------|
|                 | A. indica | C. colocynthis |
| 24              | 744.8 a   | 764.1 a       |
| 48              | 729.5 b   | 759.8 b       |
| 72              | 711.1 c   | 755.3 c       |

LSD 0.05: 5.386 2.839

Means sharing similar letters are not significantly different from each other at P = 0.05.
Table VI.- Post treatment population build-up of Tribolium castaneum at various interactions between exposure times and concentrations of A. indica and C. colocynthis.

<table>
<thead>
<tr>
<th>Concentrations</th>
<th>A. indica Mean Percent Population Build-up</th>
<th>C. colocynthis Mean Percent Population Build-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>24 h</td>
<td>48 h</td>
</tr>
<tr>
<td>2.5%</td>
<td>735.2 de</td>
<td>709.7 gh</td>
</tr>
<tr>
<td>5.00%</td>
<td>734.9 de</td>
<td>700.3 hj</td>
</tr>
<tr>
<td>7.50%</td>
<td>733.6 de</td>
<td>697.6 ij</td>
</tr>
<tr>
<td>10%</td>
<td>734.9 de</td>
<td>700.3 hj</td>
</tr>
<tr>
<td>Control</td>
<td>782.2 c</td>
<td>794.4 b</td>
</tr>
<tr>
<td>LSD 0.05</td>
<td>12.04</td>
<td></td>
</tr>
</tbody>
</table>

Means sharing similar letters are not significantly different from each other at P = 0.05

Table VI shows the interaction effect of survived T. castaneum fed on A. indica and C. colocynthis extracts at different concentrations for different time intervals. The population build-up of the insect at 10% A. indica and C. colocynthis extract concentration for 72 h, was significantly lower than all other interaction effect in the experiment and also was significantly lower than population build-up at 24 and 48 h at the same concentration. However, there were no significant difference in the interaction of 2.5%, 5%, 7.5% and 10% concentration at 24 h in A. indica. Similarly 5% concentration of A. indica extract at 48 h was statistically similar to population built-up at 2.5% and 7.5% A. indica extract at the same exposure time. The population at 7.5% concentration for 48 h was statistically similar at 10% concentration for 48 h and at 2.5% concentration for 72 h. Identical results were obtained in the 5% and 7.5% concentration of the exposure of the test insect for 72 h. Table VI shows that the population of the test insect at 10% concentration of C. colocynthis for 24 h exposure interval is statistically similar to individuals exposed for 48 h at the same concentration. Similarly, population of the test insect at 7.5% concentration of C. colocynthis for 24 h exposure was statistically similar to individuals at 5% concentration for 48 h exposure. Identical results were obtained in 2.5% and 5% concentration at 48 h. Similarly there were no significant differences in population build-up at 2.5% and 5% concentration for 24 h. The exposure time interval and concentration of the A. indica and C. colocynthis extract has a negative effect on the population build-up of T. castaneum. Maximum population build-up was observed in the control at 72 h followed by 48 h and 24 h.

DISCUSSION

T. castaneum responded differently to the different concentration of neem seed extract and tumha fruit ethanol extract. All the tested concentrations of neem seed extract exhibited significant insecticidal effect against the red flour beetle than the control and the effect was dose dependent. The maximum mortality was recorded after 72 h exposure of the test insect to the treated media. Several other research workers obtained similar results when they used plant derivatives against stored grains and grain products insect pests. For example, Xie et al. (1995) reported that azadirachtin (98%) and 3 neem extracts (48%, 23% and 7% AZA) were toxic to T. castaneum. The F1 adults of the test insect in all treatments were significantly reduced as compared with the control. Athanassiou et al. (2005) evaluated NeemAzal at 50, 100 and 200 ppm against adults Rhyzopertha dominica (F.), Sitophilus oryzae (L.), and T. confusum and reported that mortality of the studied insects increased with increase in dose of the chemical and duration of exposure. Rahila (2006) reported that neem oil had growth inhibiting effect on T. castaneum, and synergistic effect of neem oil with turmeric oil in 1:1 and 9:1 combinations revealed a significant reduction in the number of F1 progeny of T. castaneum, compared with the control. Khattak et al. (2001) demonstrated that the
The insecticidal effect of 1000 ppm neem oil treatment against S. zeamais (M.) was lost 30 days after treatment but the 10,000 ppm neem oil retained its efficacy up to 60 days. El Nadi et al. (2001) found that A. indica show a remarkable toxicity against Trogoderma granarium (E.). The toxic effect was found to be dose and exposure time dependent. Chander et al. (1992) reported that the progeny of T. castaneum was suppressed by 60% and 84% with 2% turmeric powder and 8 ml/kg of mustard oil.

C. colocyntis although, was comparatively less toxic than neem extract against T. castaneum, it was significantly more insecticidal to T. castaneum at all levels of concentrations compared to the control. Plants derivatives have also been evaluated by other research workers against different stored grain insect pests. For instance, Raja et al. (2000) reported that the oil of Mentha spicata was most toxic to Callosobruchus maculatus (F.) followed by M. piperita, M. arvensis and Cymbopogon nardus and the oviposition and adult emergence of the insect was significantly low. Tripathi et al. (2002) also found that in T. castaneum, Curcuma longa leaf oil at 5.2 mg/cm² reduced oviposition and egg hatching by 72% and 80%, respectively. Ogendo et al. (2004) compared powders of two tropical plants (Lantana camara L. and Tephrosia vogelii Hook with synthetic insecticide at 1.0%, 2.5% and 5.0% each in stored maize for five months. The botanical treatments and synthetic insecticides were equally effective in reducing insect damage by 25% as compared to the control. In laboratory experiment, Jabilou et al. (2006) determined that good insecticidal activity against T. castaneum larvae and adults was achieved with extracts of Peganum harmala seeds followed by Ajuga iva, aristolochia baetica and Raphanus raphanistrum. They also inhibited the F1 progeny of the insect.

On the basis of results, it can be concluded that A. indica and C. colocyntis ensured good control of T. castaneum at 10% concentration in terms of percent mortality and restricted progeny development. Toxic effect of both plant extracts on T. castaneum was found to be dose and exposure time dependent. In both treatments the reduction of population buildup was dose and exposure time dependent. Seed extract of A. indica was superior in terms of the mortality and the population buildup of T. castaneum than C. colocyntis.

On the basis of results, it is suggested that A. indica and C. colocyntis at 10% could manage the T. castaneum in stores by direct mortality and reducing the further generation effectively.

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