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# INFLUENCE OF FUNGAL EXTRACTS, BOTANICAL EXTRACTS AND SYNTHETIC INSECTICIDE ON LARVAL MORTALITY AND REPELLENCY OF ANGOUMOIS GRAIN MOTH, *SITOTROGA CEREALELLA* (LEPIDOPTERA: GELECHIIDAE)

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**ABSTRACT**

A study was conducted to evaluate the repellent activity and growth inhabiting effects of botanical and fungal extracts and to compare the effect of oral toxicity with the contact applications against *Sitotroga cerealella*. It is concluded that *Melia azedarach* and *Aspergillus flavipes* both showed good performance in controlling the *Sitotroga cerealella* larvae. With the increase in storage duration larval mortality gradually increased while the maximum mortality test (oral toxicity) and repellency test both were recorded for *M. azedarach* and *A. flavipes* and revealed effective against the tested larvae. The biological activities of the fungal and botanical extracts revealed that the various compounds present in the extracts had repellent effect. Results revealed significant variation in larval mortality for botanical storage durations as well as their interactions. The chlorpyrifos performed highly toxic with the average larval mortality rate of 9.78, following *A. flavipes* (4.61), *M. azedarach* (4.08), Penicillium spp. (3.67) and *Polygonum hydropiper* (2.93) while the least toxicity was revealed by the control (0.03).

**Keywords:** *S. cerealella*, *M. azedarach*, *A. flavipes*, chlorpyrifos, Penicillium spp. *P. hydropiper*.

## INTRODUCTION

The Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) is a primary colonizer of the stored grain pest (Anon, 2001). Their distributions have been extended to Europe, Brazil, Australia, Indonesia, Benin, China, United States, Japan, and Samoa. It is commonly known with the name Angoumois, which was the name of a province of pre-revolutionary France and was primarily described scientifically by Olivier in 1789 (ABRS, 2008). The female moth set down eggs on grain kernels during the crib. Normally 40 eggs at a time are laid by the female that are glued to the kernel. From the eggs the emerging larvae with the help of kernel eat and feed upon the germ or endosperm. To break through the kernel by penetrating the larvae in a circular motion through the cocoon is used for leverage (Abraham and Basedow, 2009). The adult Angoumois grain moth is a small un-dyed to yellowish-brown moth. Its length is about one-third inch long having a wingspan of one-half inch. The wings are noticed to be a lighter color of the front as compare to the hind wing. When deposited the eggs are white that sooner turn red. Mature larvae are one-fifth inch in length and having white with a yellow color head. Below the head the diameter is slightly larger as compare to the posterior portion of the insect. The whole lifecycle normally completes in 5 weeks (Abraham and Basedow, 2009).

Synthetic insecticides are used for the control of insect pests, has been the most effective means of protection for stored products (Boshra, 2011). Synthetic insecticides are rapid in break down and act under the conditions of high grain moisture content with incidence of resistance appearing in strains of stored insects which belongs to Coleoptera and Lepidoptera (Javed *et al*., 2015). The increasing expenditure of synthetic organic chemicals and their threats to the environment substitute the control approaches and are being exploited. One of the alternative control approaches is the use of locally available inactive materials. Inactive materials such as wood ash and similar fine substances are used in the control of storage pests by filling the space between maize grains. The adverse effects of synthetic insecticides imposed the

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scientists and chemists all over the world to search for harmless pesticides (Feng *et al*., 2015; Zaidi *et al*., 2015).

The botanical products have been successfully exploited as insecticides, repellents and anti-feedings. Numerous plant extracts are known to possess insecticidal activity against several stored product insects (Emana, 2010). Botanical plant products are less expensive, readily available, biologically safe and less harmful in comparison to chemical insecticides (Copping and Menn, 2005). The main advantage of botanicals is that they are easily produced, locally available, broad spectrum and used by the farmers in small scale. There are about 2000 plant species reported to possess pest control properties and the application of simple plant materials like neem (*Azadirachta indica*), karanja (*Millettia pinnata*), mahogany (*Swietenia macrophylla*), nishinda (*Vitex negundo*), pithraj (*Aphanamixis polystachya*) and datura (*Datura stramonium*) in several cases proved to be very simple and highly effective against stored product insects. The plant products included oils, extracts, leaf powder and seed etc (Shaaya *et al*., 2013).

Another encouraging strategy with good potential to decrease the adverse effects of insecticides is utilizing entomo pathogenic fungi or other microbial control agents. The option of using fungal pathogens to reduce insects has been studied for several years, but less attention has been paid to the use of fungi as control agents against storage pests (Tunc *et al*., 2006).

## MATERIALS AND METHOD

The study was conducted at the Insecticide Toxicology Laboratory, Department of Plant Protection, The University of Agriculture, Peshawar to find out the toxicity of different fungal extracts (*Aspergillus flavipes* and Penicillium spp.), botanical extracts (*Melia azedarach* and *Polygonum hydropiper*) and synthetic insecticide (chlorpyrifos), against the Angoumois grain moth, *Sitotroga cerealella* (Olivier). The experimental conditions were 25±2$^0$C with relative humidity 65 ± 5% while the photoperiod of 16:8 D/L hours was allowed into the experiment.

### Insect rearing

The test insect (*Sitotroga cerealella*) was collected from the laboratory of Department of Plant Protection, The University of Agriculture Peshawar. The culture was then maintained under the definite laboratory conditions.

### Candidate insecticide

The candidate insecticide (chlorpyrifos) was bought from the market and tested into the experiment as and when needed while fungal and botanical extracts were made in the Department of Agricultural Chemistry, The University of Agriculture Peshawar by the following process.

### Fungal extract

Some bioactive mixtures were mined from Penicillium spp. and *Aspergillus flavipes* including the organic material Dimethyl sulfoxide (DMSO). The separation of aqueous solution and that of organic solvent was carried out with the help of a separator funnel, until separation was achieved by observing two separate layers. Organic solvent was received in the flask and the organic solvents were placed in sample flask and rotary evaporator then was switched on. In this process, the temperature remained at 45 $^0$C. Evaporated DMSO was collected in the receiving flask, and the crude was left in the sample flask. This crude was re-dissolved in 5 ml of DMSO and shifted to a vial (Javed *et al.,* 2015).

### Plant extract

The plant material, i.e., *Polygonum hydropiper* and *Melia azedarach* were collected from the New Developmental Farm, The University of Agriculture Peshawar. For drying, these plants were brought back to the laboratory. After shade drying, the material was milled with a grinder. After being dried in shade 100g of each sample was immersed in 200 ml of DMSO for 24 hours. The samples were then soaked and stirred with a magnetic stirrer, and then filtered. After filtration, the samples were taken to rotatory evaporator for formation of crude extract concentration with DMSO to be used in the experiment (Javed *et al.,* 2015).

### Procedure for experiment-1 (Repellency test)

The experiment was conducted in a completely randomized design (CRD) comprising of 5 treatments, which were repeated 5 times.

Wheat flour (0.3 g) was treated with 1 ml of the recommended dose of each chemical. Pellets (1.2 g) were made up of (wheat flour + pesticide) and dried at room temperature. Each pellet was positioned in its allowed quarter in a marked petri dish (9 cm diameter). *S. cerealella* larvae (20) were released at the center of the petri dish, so that each of the pellet is equally accessible. Care was taken while handling the petri dish and the pellets were not dislocated from their respective quarters. Repellency data was recorded on the basis of diet consumption of ten (10) days to the post exposure.

### Procedure for experiment-2 (Oral toxicity)

The experiment was conducted in a completely randomized design (CRD) comprising of six treatments, which were repeated 6 times.

Wheat flour (0.3 g) was treated with 1 ml of recommended dose of each chemical. Pellets (1.2 g) were made up of (wheat flour + pesticide) and dried at room temperature. A single pellet of each treatment was offered to *S. cerealella* larvae (10) in vials (10 ml).

Toxicity of the test chemicals were judged on the basis of insect mortality and diet consumption. Insect mortality data was recorded on daily basis for ten days post exposure. Diet consumption data was noted at the end of the experiment, i.e. ten days post exposure.

### Procedure for experiment # 3 (Contact application)

The testing arena (10 ml vial) was treated with 1 ml of each chemical. The treated vials were placed in a shaker for 10 minutes, to obtain uniform distribution of the chemicals. These vials were then dried at room temperature. *S. cerealella* larvae (10) was released in these vials along with diet (0.3 g wheat flour + 1 ml distilled water).

Toxicity of the test chemicals were determined on the basis of insect mortality and diet consumption. Insect mortality data was recorded on daily basis for ten days of post exposure. Diet consumption data was recorded at the end of the experiment.

**Data analysis**

The data was analyzed by using the computer based software Statistix 8.1 and LSD (Least significant difference) test was used for the mean comparison (Snedecor and Cochran, 1989).

## RESULTS AND DISCUSSION

Data collected for *S. cerealella* larval mortality as affected by different fungal and botanical extracts at verging time interval/storage durations to 10 days are presented in Table 1-6. Upon analysis of variance results showed significant differences in larval mortality both for botanical storage durations and their interactions.

Maximum mean larvae mortality of 9.78 was observed for Chlorpyrifos, followed by 4.61 for *A. flavipes*, 4.08 for *M. azedarach*, 3.67 for Penicillium spp. and 2.93 for *P. hydropiper* whereas minimum larval mortality for 0.03 was recorded for control. These results revealed that botanical extracts varied significantly for their potency for larval mortality while chlorpyrifos was found the most effective. Results also indicated that with the increase in storage duration larval mortality gradually increase. Maximum mean larval mortality was 5.27 observed at day 10, while non-significantly in 5.19 and 5.05 at day 8 and 9 respectively. Day 7 showed larval mortality of 4.91 followed by 4.47 for days 6 which was statistically equal to 4.22 for days 5. Day 4 shows larval mortality of 3.88 that is statistically higher than that of 3.30 recorded at day 3. A minimum larval mortality of 2.61 is observed at day 1 that is statistically similar to that of 2.91 on day 2. It is clearly demonstrated that larval mortality increases significantly even with a gap of one day storage duration. The interactive effect of botanical and storage duration is also found (Table 1). A maximum larval mortality of 10 was observed for chlorpyrifos at day 6-10. While the minimum larval mortality of zero (0) was recorded for control at day 8. The results revealed that for botanical larval mortality increased significantly with the increase in storage duration or interval except control.

The data revealed (Table 2) that maximum consumption of 0.72g was recorded for control. Mean consumption of 0.46g for *P. hydropiper* was recorded that was statistically similar to 0.40g of Penicillium spp., while statistically higher than consumption observed in *M. azedarach* 0.27g. That is statistically similar to 0.21g for *A. flavipes*; minimum consumption of 0.05g was recorded for chlorpyrifos, which showed the minimum consumption compared to the other treatments.

Results for oral toxicity effects of different insecticides against larval mortality of *S. cerealella* are presented in (Table 3). Results indicate that maximum mean larval mortality of 8.78 was observed for Chlorpyrifos, followed statistically similar results of 5.08 and 4.95, respectively for *A. flavipes* and *M. azedarach* while 4.18 for *P. hydropiper* and 3.93 for Penicillium spp. whereas minimum larval mortality 0.21 was recorded for control.

Results also showed that with the increase in storage duration larval mortality gradually increases. It is clearly demonstrated that larval mortality increased significantly even with a gap of one day storage duration. Maximum mean larval mortality of 6.16 is observed at day 10, while day 9 and 8 shows mortality of 5.91 and 5.52, respectively which are statistically different. Day 7 shows larval mortality of 5.25 followed by 4.86 for days 6, 4.30 for days 5. Day 4 shows larval mortality of 3.97 that is statistically higher than that of 3.47 recorded at day 3. A minimum larval mortality of 2.69 is observed at day 1 that is statistically different to that of 3.08 on day 2.

The interactive effect of chemical and storage duration is also significant (Table 3). A maximum larval mortality of 10 is observed for Chlorpyrifos at day 7-10. Whereas the minimum larval mortality of zero (0) was recorded for control at day 7. It is clear from the results that for each fungal and botanical extract, the larval mortality increased with the increases in storage duration but not for control. This showed an increase in mortality at day 1 and so on.

Results obtained for consumption (g) as effected by fungal and botanical is presented (Table 4). Upon analysis of variances result showed maximum consumption of 0.44g for control that was statistically similar to 0.43g of Penicillium spp. while statistically higher than the rest of all treatments. 0.38g consumption observed for *P. hydropiper* followed by 0.34g *A. flavipes*, 0.29g for *M. azedarach* which was statistically different from the minimum consumption of 0.01g was recorded for chlorpyrifos. These results, whereas represented that Chlorpyrifos is rather most effective chemical showed minimum consumption as compared to other fungal and botanical extracts followed by *A. flavipes* and *M. azedarach*. This result indicated that fungal and botanical extracts showed various potency on the consumption caused by chlorpyrifos.

The percent repellency value of the tested plant extract of *S. cerealella* larvae is shown in table 5. The results showed that the tested plants, and botanical extracts exhibited repellent effect on target species. Maximum percent repellency for *A. flavipes* was 93.82%, followed by *M. azedarach* (93.50%), Penicillium spp. (87.78%), while *P. hydropiper* revealed 75.02% and minimum % repellency is recorded for control (50.02%). The analysis of variance showed that the *A. flavipes* and *M. azedarach* were not statistically different. The biological activity of the fungal and botanical revealed that the various compounds present in the extracts had repellent effect which were similar.

The study showed that the highest mortality was noticed for chemical Chlorpyrifos, with a mortality rate up to 90 percent on the first day. Chlorpyrifos is considered an important insecticide for its effect on insects and also its usage mostly in the world as an effective insecticide. Due to its direct enhanced effect on insects, its repellency has not been taken in consideration. Mortality difference was noticed per contacting method between the Chlorpyrifos, as direct contact 100 % was recorded while by toxicity (nutrients) the mortality was decreased. Full mortality was concluded one day later than contact ($7^{th}$ day). These findings are in support with Sabry and El-Sayed (2011), whose research was to observe different chemicals (insecticide) effect on the larvae of insect. Their results showed that chlorpyrifos was more toxic to second instar larvae than lambda-cyhalothrin, cypermethrin, spinosad and buprofezin with $LC_{50}$ values of 1.78, 8.81, 26.9, 294.36 and 997.05 ppm, respectively. While LC50 of lambda-cyhalothrin was 0.04 ppm.

Fungal efficacy was measured with regard to its mortality by contact and toxicity on *S. cerealella* larvae. *A. flavipes* rate of mortality to *S. cerealella* was concluded to be fewer as well as

Penicillium spp. compared to chemical (Chlorpyrifos) treatment. The contact mortality was recorded to be less as compared to the toxicity (oral) which could be ideal because of the secondary metabolites (toxic) produce by these fungi. These results were in calibration with the findings of Misra *et al*., (1961) and Imura and Sinha (1984), who studied the contact of insect directly (inner surface) and indirectly (outer surface). The results indicated that the production of *A. flavipes* and Penicillium spp. to be side by side with *S. cerealella* in contact with the cells while direct contact of these fungi inside the moth had higher mortality efficacy.

The present study also includes plant extract efficacy on *S. cerealella*. The results indicate (Table 6) that *M. azedarach* and *P. hydropiper* had higher rates of toxicity compared to the scontact application technique. By contacting in ten days, the mortality was recorded to be approximately half of *M. azedarach* while *P. hydropiper* was unable to reach to half the mortality rate (mean mortality=4). On the other hand, the toxicity rate of the tested insect larvae was higher than the mortality which is concluded 6 in *P. hydropiper* while 6.8 in *M. azedarach*. These findings are in support with Saljoqi *et al*. (2006) and Islam and Akhtar, (2013) who examined ethanol extract of various plants as insecticide. The results concluded that *M. azedarach* (bakain drupes) was more effective compared to the *P. hydropiper* in percentage. These results ideally proved that the direct contact of these compounds (synthetic chemical and secondary metabolites) is highly responsible for mortality inside the insect instead of outer contact.

## CONCLUSION AND RECOMMENDATIONS

According to the results it is concluded that *M. azedarach* and *A. flavipes* was found the most effective in controlling the *S. cerealella* larvae. Maximum mortality and the maximum repellency of *S. cerealella* larvae were recorded for both *M. azedarach* and *A. flavipes*. The *Melia azedarach* (botanical) and *A. flavipes* (microbial) crude extract revealed the bioactivity so it is recommended to perform the bioassay analysis for different compounds. This will give us a better idea about bioactive secondary metabolites. It is also recommended to explore the botanicals and fungal flora of Pakistan for the secondary metabolites that can be used in agriculture.

## AUTHOR'S CONTRIBUTION

Uzair Ahmad: He has participated in the research analysis, analysis design, collected data, drafted and revised the paper.
Unab Begum: She has prepared the traps, participated in lab work, assembled the data, participated in research analysis, discussed the results, proof read and carefully revised the paper.

**Table 1**

Larval mortality of angoumois grain moth, *Sitotroga cerealella* through contact application of fungal and botanical treatments at various intervals.

| Chemicals | Days | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| Chlorpyrifos | 9 | 9.50 | 9.66 | 9.83 | 9.83 | 10 | 10 | 10 | 10 | 10 | 9.78 |
| *Aspergillus flavipes* | 2.00 | 2.33 | 3.00 | 4.00 | 5.00 | 5.16 | 5.66 | 6.16 | 6.33 | 6.5 | 4.61 |
| Penicillium spp. | 1.16 | 1.50 | 2.16 | 3.16 | 3.66 | 4.16 | 5.00 | 5.00 | 5.33 | 5.50 | 3.67 |
| *Melia azedarach* | 2.00 | 2.50 | 3.00 | 3.66 | 4.00 | 5.16 | 5.16 | 5.33 | 5.33 | 5.50 | 4.08 |
| *Polygonum hydropiper* | 1.50 | 1.66 | 2.00 | 2.66 | 2.83 | 3.16 | 3.66 | 3.83 | 4.00 | 4.00 | 2.93 |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.1 | 0.16 | 0.03 |
| Mean | 2.61 | 2.91 | 3.30 | 3.88 | 4.22 | 4.47 | 4.91 | 5.05 | 5.19 | 5.27 | |

LSD (0.05) for Treatments = 0.27, LSD (0.05) for days = 0.34 and LSD (0.05) for treatments x days = 0.85

**Table 2**

Effect of contact toxicity of different insecticides on wheat flour consumption by angoumois grain moth, *S. cerealella* larvae.

| Treatments | Consumption (g) |
|---|---|
| Chlorpyrifos | 0.05 |
| *Aspergillus flavipes* | 0.21 |
| Penicillium spp. | 0.40 |
| *Melia azedarach* | 0.27 |
| *Polygonum hydropiper* | 0.46 |
| Control | 0.72 |

LSD (0.05) for consumption = 0.07

**Table 3**

Oral toxicity effect of different insecticides against larval mortality of angoumois grain moth, *Sitotroga cerealella* at various intervals.

| Chemicals | Days | | | | | | | | | | Mean |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | |
| Chlorpyrifos | 6.50 | 7.16 | 7.50 | 8.16 | 8.83 | 9.66 | 10.00 | 10.00 | 10.00 | 10.00 | 8.78 |
| *Aspergillus flavipes* | 2.33 | 3.00 | 3.83 | 4.33 | 4.66 | 5.50 | 6.50 | 6.66 | 7.00 | 7.00 | 5.08 |
| Penicillium spp. | 1.83 | 2.00 | 2.83 | 3.33 | 3.66 | 4.50 | 4.00 | 4.83 | 5.66 | 6.16 | 3.93 |
| *Melia azedarach* | 3.00 | 3.66 | 3.83 | 4.33 | 4.66 | 5.16 | 5.50 | 6.00 | 6.50 | 6.83 | 4.95 |
| *Polygonum hydropiper* | 2.50 | 2.66 | 2.83 | 3.66 | 4.00 | 4.33 | 5.00 | 5.33 | 5.50 | 6.00 | 4.18 |
| Control | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.33 | 0.83 | 1.00 | 0.21 |
| Mean | 2.69 | 3.08 | 3.47 | 3.97 | 4.30 | 4.86 | 5.25 | 5.52 | 5.91 | 6.16 | |

LSD (0.05) for Treatments = 0.17, LSD (0.05) days = 0.22 and LSD treatments x days = 0.56

**Table 4**

Effect of oral toxicity of different insecticides on wheat flour consumption by angoumois grain moth, *Sitotroga cerealella* larvae.

| Chemicals | Consumption (g) |
|---|---|
| Chlorpyrifos | 0.01 |
| *Aspergillus flavipes* | 0.34 |
| Penicillium spp. | 0.43 |
| *Melia azedarach* | 0.29 |
| *Polygonum hydropiper* | 0.38 |
| Control | 0.44 |

LSD (0.05) for consumption = 0.03

**Table 5**

Repellency of angoumois grain moth, *Sitotroga cerealella* larvae toward different fungal and botanical insecticides.

| Pesticides | Mean % repellency |
|---|---|
| *Aspergillus flavipes* | 93.82 |
| Penicillium spp. | 87.78 |
| *Melia azedarach* | 93.50 |
| *Polygonum hydropiper* | 75.02 |
| Control | 50.02 |

LSD (0.05) for repellency = 0.39

**Table 6**

Mortality and toxicity rate of angoumois grain moth, *Sitotroga cerealella* toward different fungal and botanical insecticides.

| | Contact method | *M. azedarach* | *P. hydropiper* |
|---|---|---|---|
| Mortality rate | 4 | 8 | 2 |
| Toxicity rate | - | 6.8 | 6 |

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