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Efficacy of botanical and microbial extracts against Angoumois grain moth *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) under laboratory conditions

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Abstract
Larval mortality, food consumption and percent (%) repellency of *S. cerealella* was assessed for 10 days against the chemical (Chlorpyrifos @ 5000ppm), microbial (*Aspergillus*, *Penicillium* @ 10000ppm) and botanical (*Melia azedarach, Polygonum hydropiper* @ 10000ppm) extracts. Maximum mean larval contact mortality of 9.78 was observed for Chlorpyrifos with a minimum mean larval contact mortality of 0.03 for control. *S. cerealella* larvae showed maximum mean consumption of 0.72g for control with a minimum consumption of 0.05g for Chlorpyrifos during the assessment of consumption of the infested food with different treatments. Maximum mean larval oral mortality of 8.78 was observed for Chlorpyrifos whereas minimum mean larval oral mortality of 0.21 was noted for control. Results showed maximum consumption of 0.44g food for control and minimum consumption of 0.01g infested food for Chlorpyrifos during oral toxicity consumption (g). Maximum mean percent repellency for Aspergillus was 93.82%*Penicillium* with minimum of 50.02% for control.

Keywords: *Sitotroga cerealella*, *Botanical extracts, microbial extracts, mortality, consumption, repellency*.

Introduction
Cereals are said to be the dominant source of nutrition for one-third of the world's population especially in developing and underdeveloped nations of Sub-Saharan Africa and Southeast Asia. Among the cereals, rice, wheat and maize constitute about 85% of total global production [19]. Weevils (Curculionoidea), Angoumois grain moth *(Sitotroga cerealella)*, mites *Dermatophagoides pteronyssinus* and saw-toothed grain beetle *(Oryzaephilus surinamensis)* are the important storage pests of maize causing 37% to 88% germination loss and 14% to 29% weight loss in pulse grains and cereals [1]. Single larva of Angoumois grain moth per grain is responsible for 13-24 % economic losses in grain weight [12] along with the reduction in the nutritional importance of grain. Angoumois grain moth lays eggs on the grains which hatch into small white larvae and starts feeding on its content after boring [4]. Larval feeding of *S. cerealella* causes foul smell, decrease in grain quality and weight, and unsuitable for consumption. Infested corns also contains ears with minor holes on individual grains.

Control of pests by synthetic pesticides has been the most effective and sometime costly means of protection for stored products [6] but it may also act as pollutant and a pest may develop resistance against it [3, 10, 21, 22]. The adverse effects of synthetic insecticides imposed the scientists and chemists all over the world to search for harmless pesticides. In history, botanical products have been successfully exploited as insecticides, repellents and antifeedant. Numerous plant extracts are known to possess insecticidal activity against several stored product insects [5, 16-18]. Botanical plant products are less expensive, readily available, biologically safe and less harmful in comparison to chemical insecticides [15]. 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*, proved to be very simple and highly

Effective against stored product insects in several cases. The plant products included oils, extracts, leaf powder, seeds etc. [2].

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 [7].Keeping in view the fore-stated facts the present study was carried out with a purpose to evaluate the repellent activity, toxic/growth inhabiting effects of botanical and fungal extracts against *S. cerealella* and to compare the toxicity of oral and contact applications of the candidate insecticide.

Materials and Methods

During 2012-13, insecticide Chlorpyrifos was brought from the local market while *Aspergillus* and *Penicillium* were obtained from the Plant pathology department, the University of Agriculture, Peshawar. Bioactive compounds were extracted from *Aspergillus* and *Penicillium* along with organic solvent 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. Evaporated DMSO at 45 ºC was collected in the receiving flask through rotary evaporator and the left crude in the sample flask was re-dissolved in 5 ml of DMSO and shifted to a vial [9]. *Polygonum hydropiper* L. (Stems and leaves) and *Melia azedarach* L. (Droops) were collected from the New Developmental Farm (NDF) The University of Agriculture, Peshawar and were shade dried, and grinded in the laboratory. Then 100g of each sample was immersed in 200 ml of DMSO for 24 hours followed by soaking and stirring with a magnetic stirrer and filtrations. Then samples were taken to rotatory evaporator for formation of crude extract concentration with DMSO to be used in the experiment [9] and different doses of different treatments were made by Abid Unit calculator [24] (Table. 1).

Table 1: Name and doses of treatments used in the experiment.

S. No	Treatment/Chemical	Nature of chemical	Recommended concentration
1	Chlorpyrifos	Organophosphate insecticide	5,000 ppm
2	*Aspergillus*	Fungal Extract	10,000 ppm
3	*Penicillium*	Fungal Extract	10,000 ppm
4	*Melia azedarach*	Botanical	10,000 ppm
5	*Polygonum hydropiper*	Botanical	10,000 ppm

Repellency test

Wheat flour (0.3 g) was treated with 1 ml of the recommended dose of each treatment followed by Pellets (each of 1.2 g) making 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 pellets is equally accessible with extreme care for avoiding dislocation of the pellets from their respective quarters. Repellency data was recorded on the basis of diet consumption ten (10) days post exposure and percent (%) repellency was calculated by;

Percent (%) Repellency = Number of repellent pest / Total number of pest * 100

The experiment was conducted in a Completely Randomized Design (CRD) comprising of 5 treatments repeated 5 times.

Oral toxicity

The experiment was conducted in a Completely Randomized Design (CRD) comprising of six treatments repeated 6 times. Wheat flour (0.3g) was treated with 1 ml of recommended dose of each chemical followed by Pellets (each of1.2 g) making and dried at room temperature. A single pellet of each treatment was offered to *S. cerealella* larvae (10) in vials (10ml).Toxicity of the test chemicals was judged on the basis of insect mortality and diet consumption. Insect mortality data was recorded on a daily basis for ten days post exposure. Diet consumption data was noted at the end of the experiment, i.e. ten days post exposure.

Contact Application

For determination of contact application, the testing arena (10ml vial) were treated with 1 ml of each treatment. The treated vials were placed in a shaker for 10 minutes to obtain uniform distribution of the chemicals and were dried at room temperature. *S. cerealella* larvae (10) were released in these vials along with diet (0.3 g wheat flour + 1 ml distilled water). Toxicity of the test chemicals was determined 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 were recorded at the end of the experiment.

Analysis

Data was analyzed by using computer based software statistix 8.1 and LSD (Least Significant Difference) test was used for mean comparison [20].

Results

Repellency test

The result shows that the tested plants and microbial extracts exhibited repellent effect on the pest. Maximum repellency of 93.82% was recorded for *Aspergillus* with a minimum of 50.02% repellency for control (Table. 2)

Table 2: Repellency of different treatments against Angoumois grain moth, *Sitotroga cerealella* (Oliver) larvae under laboratory conditions.

Pesticides	Mean% Repellency
Aspergillus	93.82a
Penicillium	87.78b
Melia azedarach	93.50a
Polygonum hydropiper	75.02c
Control	50.02d

*Mean followed by same letters (column) is non-significantly different = * significance at $p < 0.05$

LSD (0.05) for repellency = 0.39

Oral Toxicity

Oral toxicity results indicate that maximum mean larval mortality of 8.78 was observed for Chlorpyrifos with a minimum mean larval mortality of 0.21 for control. Maximum mean larval mortality of 6.16 is observed at day 10 and a minimum mean larval mortality of 2.69 was observed at day 1 when studied day wise. The interactive effect of chemical and storage duration showed that maximum larval mortality of 10 is observed for Chlorpyrifos at day 7-10. While the minimum larval mortality of zero (0) is recorded for control at day 7.

(Table. 3.). Consumption results indicate that Chlorpyrifos is rather most effective chemical showing minimum consumption (0.01g) as compared to other botanicals and microbial with maximum consumption of 0.44g for control (Table. 4.)

Table. 3. Oral toxicity effect of different insecticides at various intervals against larval mortality of Angoumois grain moth, *Sitotroga cerealella* (Oliver)

Chemicals	Days										Mean
	1	2	3	4	5	6	7	8	9	10	
Chlorpyrifos	6.50fgh	7.16de	7.50d	8.16c	8.83b	9.66a	10.00a	10.00a	10.00a	10.00a	8.78a
Aspergillus	2.33wxy	3.00uv	3.83rst	4.33pqr	4.66nop	5.50jkl	6.50fgh	6.66efg	7.00def	7.00def	5.08b
Penicillium	1.83y	2.00xy	2.83uvw	3.33tu	3.66st	4.50opq	4.00opq	4.83mnop	5.66ijk	6.16ghi	3.93d
Melia azedarach	3.00uv	3.66st	3.83rst	4.33pqr	4.66nop	5.16klmn	5.50jkl	6.00hij	6.50fgh	6.83ef	4.95b
Polygonum hydropiper	2.50vwx	2.66vw	2.83uvw	3.66st	4.00qrs	4.33pqr	5.00lmno	5.33klm	5.50jkl	6.00hij	4.18c
Control	0b	0b	0b	0b	0b	0b	0b	0.33ab	0.83za	1.00za	0.21f
Mean	2.69j	3.08i	3.47h	3.97g	4.30f	4.86e	5.25d	5.52c	5.91b	6.16a	

*****Mean followed by same letters (row wise) is non-significantly different = * significance at $p < 0.05$
LSD (0.05) for Chemicals = 0.17, LSD (0.05) days = 0.22. LSD chemicals x days = 0.56

Table 4: Effect of oral toxicity of different insecticides on the Wheat flour consumption by the larvae of Angoumois grain moth, *Sitotroga cerealella* (Oliver)

Chemicals	Consumption (g)
Chlorpyrifos	0.01 e
Aspergillus	0.34 c
Penicillium	0.43 a
Melia azedarach	0.29 d
Polygonum hydropiper	0.38 b
Control	0.44 a

Mean followed by same letters is non-significantly different = significance at $p < 0.05$ LSD (0.05) for consumption 0.03

Contact application
Maximum mean larval mortality of 9.78 was observed for Chlorpyrifos with a minimum mean larval mortality of 0.03 for control during the assessment of contact application. Maximum mean larval mortality of 5.27 was observed at day 10 while a minimum larval mortality of 2.61 was observed at day 1 when studied day wise. The interactive effect of botanical and storage duration shows that 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 (Table. 5.). Results of larval consumption revealed that Chlorpyrifos was an effective chemical with minimum consumption (0.05g) as compared other botanical, microbial insecticides and control (maximum consumption of 0.72g) (Table. 6.).

Table. 5. Larval mortality of Angoumois grain moth, *Sitotroga cerealella* (Oliver) through contact application of different treatments at various intervals.

Chemicals/Treatment	Days										Mean
	1	2	3	4	5	6	7	8	9	10	
Chlorpyrifos	9b	9.50ab	9.66ab	9.83a b	9.83ab	10a	10a	10a	10a	10a	9.78a
Aspergillus	2.00opqr	2.33mnopq	3.00klmn	4.00ij	5.00fgh	5.16fg	5.66cdef	6.16cde	6.33cd	6.5c	4.61b
Penicillium	1.16qr	1.50pqr	2.16nopq	3.16jklm	3.66ijkl	4.16 hi	5.00fgh	5.00fgh	5.33ef	5.50def	3.67d
Melia azedarach	2.00opqr	2.50mnop	3.00klmn	3.66ijkl	4.00ij	5.16fg	5.16fg	5.33ef	5.33ef	5.50def	4.08c
Polygonum hydropiper		1.66pqr	2.00pqr	2.66mno	2.83lmno	3.16jklm	3.66ijkl	3.83ijk	4.00ij	4.00ij	2.93e
Control	0s	0s	0s	0s	0s	0s	0s	0s	0.16s	0.16s	0.03f
Mean	2.61f	2.91f	3.30e	3.88d	4.22cd	4.47c	4.91b	5.05ab	5.19ab	5.27a	

*Mean followed by same letters (row wise) is non-significantly different = * significance at $p < 0.05$ LSD (0.05) for Chemicals = 0.27, LSD (0.05) days = 0.34, LSD chemicals x days = 0.85

Table. 6. Effect of different contact insecticides on the larvae of Angoumois grain moth, *Sitotroga cerealella* (Oliver) by the wheat flour consumption.

Chemicals	Consumption (g)
Chlorpyrifos	0.05 d
Aspergillus	0.21 c
Penicillium	0.40 b
Melia azedarach	0.27 c
Polygonum hydropiper	0.46 b
Control	0.72 a

Mean followed by same letters is non-significantly different = significance at $p < 0.05$ LSD (0.05) for consumption 0.07

Discussion
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 worldwide usage. 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 (7th day). These findings are in support with the Sabry [13] who observed Chlorpyrifos as a highly toxic chemical against the 2nd instar larvae of green lace wing when compared with other chemicals.

Fungal efficacy was measured with regard to its mortality by contact and toxicity on *S. cerealella* larvae. *Aspergillus* rate of mortality to *S. cerealella* was concluded to be fewer as well as *Penicillium* compare to chemical (Chlorpyrifos) treatment although some species like *Aspergillus flavus* and *A. parasiticus* produce potent mycotoxins (Ochratoxins, aflatoxins[11] and kojic acid [25]), having insecticidal and repellent (only some) properties [25]. 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. Imura [8] studied that mean percent group infestation of *Aspergillus glaucus* does not changed until week 10 and increased at week 15 causing a maximum infestation of 100% in *S. cerealella*, supporting our findings. The results indicated that the production of *Aspergillus* and *Penicillium* 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* which indicate that *M. azedarach* and *P. hydropiper* had higher rates of toxicity compare to contact procedure. 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 was higher of *S. cerealella* larvae whose mortality was concluded up to 6 in *P. hydropiper* and 6.8 in *M. azedarach*. These findings were in support of the result of Saljoqi [14] and Islam [23] who's examined ethanol extract of various plants as insecticide. The results concluded that *M. azedarach* (bakain drupes) was more effective compare to *P. hydropiper* in percentage. These results, ideally prove that direct contact of these compounds (a synthetic chemical and secondary metabolites) is highly responsible for mortality inside the insect instead of outer contact.

Conclusion

M. azedarach and *Aspergillus* were found to be the most effective in controlling the *S. cerealella* larvae by recording maximum mortality and the maximum repellency for both. The *M. azedarach* (botanical) and *Aspergillus* (microbial) crude extract revealed the bioactivity and should be further explored.

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