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PEST CONTROL POTENTIAL OF HALOPHYTE MEDICINAL PLANT *SALVADORA PERSICA* L. AGAINST WHEAT PEST *TRIBOLIUM CONFUSUM* JACQUELIN DU VAL.

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ABSTRACT

The research aimed to investigate he toxic nature of *Salvadora persica* against *Tribolium confusum* 6th instar larval and adult stages. Root and leaf extracts effectiveness was determined in terms of mortalities.50% mortalities were obtained with 0.014mL/g and 0.16mL/g of root extracts against larvae and adults, respectively. Whereas LD_{50} of leaf extracts was found to be 0.012mL/g and 0.12mL/g and 0.16mL/g against larval and adult stages, respectively. In comparison with the untreated control batch the protein contents depletion was observed up to 2% in larvae and 2.63% in adults.

Key words: Toxicity, *Salvadora persica*, stored grains pests, confused flour beetles, Total body protein.

INTRODUCTION

Protection of the agricultural products is equally important as storage of these products for the survival of any nation in world increasing population (Oni and Ogungbite, 2015). Almost 40 species of insects are known to attack on stored grains in Pakistan (Khan and Marwat, 2004) among them. *Tribolium* is the most common, injurious pest of stored grain, stands 2nd in pest ranking (Khan, 2014.) *Tribolium confusum* Jacquelin du Val commonly known as confused flour beetle. It is found in temperate areas (Tripathi *et al*., 2001). It is a cosmopolitan and polyphagous pest of stored grain products in home, grocery stores (Campbell and Runnion, 2003) where they cause heavy infestation by multiplying in to large populations. Its mode of feeding causes seed quality problem as it prefers to feed on germ part, endosperm and in the end broken kernels and grains, (Campbell and Reunion, 2003). Damaged products are of lower weight and nutritional value, bad taste results in economic loss which causes loss worth of millions of rupees in Pakistan(Khan & Ahmed 2003).) Various workers have worked on different aspects of *Tribolium* sp. They include Arthur *et al*. (2011); Lingampally *et al*. (2012); Vassilakos and Athanassiou (2012) and many more.

In Pakistan increasing demand of food and agricultural products lead to increased use of these pesticides as only way to meet requirements. On the other hand associated environmental problems including pollution, harm to beneficial plants and animals, soil degradation, ozone depletion and pest resurgence (Ali *et al*., 2010) are the most serious problems of pesticide use. In the present day world of intense environmental awareness ,any activity contributing towards its contamination, must be curbed. Unchecked pest control activity with excessive pesticides usage is one of the internationally identified culprits. In view of the severity of these problems an increasing concern has developed for the safe alternatives of these pesticides. (Aswalam *et al*., 2012).

Botanical or bio pesticides/phytopesticides are plant based pesticides and these are supposed to the safest alternate of synthetic pesticides and also of insect repellents, having good potential for insect control providing a cool breath as a new scientific front. *Salvadora persica* commonly known as peelu, meswak or tooth brush tree. It is a small tree or shrub with ever green leave, having pleasant fragrance and pungent taste. It belongs to family Salvadoraceae. It is a perennial halophyte and grows under extremely arid conditions. Miswak tree is always been a center of attention among researchers because of its pharmaceutical importance against many diseases (Akhtar *et al*., 2011; Khalil, 2006). Researchers worked on several aspects of this plant. It has adverse effects on male and female reproductive system and fertility (Dermani *et al.*, 2003).

The main aim of proposed study is to discover new bio pesticides from easily available local plant, that is environmental friendly as well as safe for human beings. It is expected that proposed research would be helpful to set a guideline in pest management. The research may enrich the existing knowledge on effective, safe and environmental friendly protection of stored grains by the indigenous plant.

MATERIALS AND METHODS

Collection and rearing of test insects:

Initially *Tribolium confusum* was collected from GSRI, SARC, PARC (Pakistan agricultural and research center) university campus, Karachi. Collected insects were reared in the laboratory at controlled temperature 30 ± 2ºC and 65 ± 5 relative humidity. Sterilized wheat flour in sterilized glass jars was used as rearing medium of *Tribolium confusum.* Twenty five days old insects were used for the toxicological studies.

Preparation of plant extracts:

Salvadora persica roots and leaves were collected from University campus, Karachi. Root and leaf extracts of *S. persica* was prepared in a suitable organic solvent after Khan and Ahmed (2003) with some improvements.

Roots and leaves of *S. persica* were collected and washed and air dried and weighed 100g. 150 mL of ethyl alcohol was taken in two beakers each for leaves and roots, soaked separately in beakers for twenty four hours. After twenty four hours 15mL of ethyl alcohol was added in each beaker and left it again for twenty four hours. After total soaking time of 72 hours it was expected that all the contents are extracted, leaves and roots were ground. Grinded mixture was filtered with the help of filter paper and then homogenized at 1000 rpm for 15 minutes. Homogenized mixture was then centrifuged at 2500 rpm for twenty minutes. Supernatants were collected in separate flasks and left for evaporation at the rotary evaporator for 30 minutes at 40º C. After evaporation 50ml of root and leaves extracts were collected and stored at 4ºC for further use.

Toxicity testing:

Contact method was used for toxicity estimation. Prepared plant extracts (Root and leaf) were used as stock solutions. Different dilutions of stock solutions were prepared. Doses were applied with the help of pipette and micropipette to petri dishes. A control was kept for the determination of environmental effects on insects.30 adult insects were released in each petri dish separately and left for twenty four hours under completely controlled temperature and humidity kept starving, to check the toxicological effects of applied doses on released insects. Mortality was recorded after twenty four hours of dose application. Calculation after each experiment was done to obtain percent mortality in each dose and lethal dose and data were subjected to Biostat software. Alive insects from each LD_{50} were stored separately for further use in research.

Protein Contents Estimation:

Effects of *Salvadora* plant extracts on adults and larvae of *Tribolium confusum* were determined by calculating the total protein contents. Stored Alive insects from each LD_{50} were taken and crushed in mortar and pastel and then homogenization was made in 2mL of de ionized water for 15 minutes. Homogenates were collected and the centrifugation was done at 5000rpm.Supernatants were obtained for biochemical analysis. For the estimation of total protein contents Biuret method was applied using Randox kit: (115305). Spectrophotometric measurements were taken. Test tubes were prepared and marked as blank, standard and sample.6 samples were there to be tested i.e Root and leaf extracts against adult and larvae.1mL of biuret reagent was pipetted out in all test tubes, then 20µL standard and sample solutions were added in test tubes marked as standard and samples. Reagents were mixed and incubate for 22 ± 3º C for 30 minutes. Absorbance at 546nm wave length of samples and standard against blank reagent were measured.

RESULTS AND DISCUSSION

In the present research toxicity of *Salvadora persica* was observed against *Tribolium confusum*. Mortalities were observed of larvae and adults of insect by Root and leaf extracts presented in Table 1,3,5 and 7. Mortality rate was increased by increasing the dose of plant extracts. 24 and 66% are the lowest and highest mortalities observed of root extract. 30 percent and 62 percent are the lowest and highest mortalities caused by leaf extract. Percentile probit mortality analysis is presented in Table 2,4,6 and 8. Decrease in total protein contents of insects caused by plant extracts were observed in comparison with total protein contents of untreated insects in Table 9 which has shown toxic effects of the tested plant.

Many researchers have contributed their research in discovery of environmental friendly phytopseticides and their results showed toxicity of plants against stored grain pests. Application of different parts of plant extracts leaves different imapcts on insects varies according to the mode of action, part of the plant and the method of application (Neoliya *et al.,* 2007). Study on effect of plant extracts against stored product insects by Márcio *et al.*, 2007; Abdurrahman *et al.,* 2008; Javed *et al*., 2018) and against other insects like *Nezara viridula* by Durmusoglu *et al*. (2003) and *Psyttalia concolor* by Rehman *et al.* (2009) was done . Research reveals that plant based pesticides can easily increase the yield of crops and maintained safety of environment. Phyto pesticides also performed multiple functions against pests as Attractants in form of pigments and scents, deterrents (Repellents/Antifeedents),

as toxins effects growth of pests (Omar *et al*., 2012).). Root, leaves and bark extracts of *S.persica* studied for the malarial treatment and showed pharmacological properties for it (Innocent *et al*., 2016).

Table 1. Probit analysis of mortality data of 6th instar larvae of *Tribolium confusum* against *S.persica* root extract.

Doses (ml)	Insects exposed	Mean mortality%	Probit percent mortality	Difference	Chi-Square	Probit (y)
0.012	50	32	0.3431	-1.1535	0.0776	4.5327
0.014	50	50	0.4792	1.0381	0.045	5
0.016	50	63	0.5998	1.0091	0.034	5.305
0.018	50	70	0.6991	0.0443	0.0001	5.524
0.02	50	76	0.7771	-0.8554	0.0188	5.706

Table 2. Percentile probit mortality analysis of *Tribolium confusum* larvae under toxic effects of *S.persica* Root extract.

Percentile	Probit (Y)	Log 10[Dose(stimulus)]	Standard error	Dose (Stimulus)	Standard Error
1	2.6732	-2.2865	0.1173	0.0052	0.0014
5	3.3548	-2.1568	0.0864	0.007	0.0014
10	3.7183	-2.0877	0.07	0.0082	0.0013
16	4.0056	-2.0331	0.0573	0.0093	0.0012
20	4.1585	-2.004	0.0506	0.0099	0.0012
25	4.3258	-1.9722	0.0435	0.0107	0.0011
30	4.476	-1.9436	0.0372	0.0114	0.001
40	4.7471	-1.8921	0.0268	0.0128	0.0008
50	5	-1.844	0.0193	0.0143	0.0006
60	5.2529	-1.7959	0.0173	0.016	0.0006
70	5.524	-1.7443	0.0228	0.018	0.0009
75	5.6742	-1.7158	0.0279	0.0192	0.0012
80	5.8415	-1.6839	0.0343	0.0207	0.0016
84	5.9944	-1.6549	0.0406	0.0221	0.0021
90	6.2817	-1.6002	0.0528	0.0251	0.0031
95	6.6452	-1.5311	0.0689	0.0294	0.0047
99	7.3268	-1.4015	0.0996	0.0397	0.0092

Table 3. Probit analysis of mortality data of *Tribolium confusum* Adults against *S.persica* Root extract.

Doses (ml)	Insects exposed	Mean mortality%	Probit percent mortality	Difference	Chi-Square	Probit (y)
0.08	50	12	0.2449	-0.2439	0.0049	4.294
0.12	50	20	0.3902	0.4921	0.0124	4.7471
0.16	50	25	0.5053	-0.2645	0.0028	5
0.2	50	30	0.5948	0.2605	0.0023	5.2529
0.24	50	33	0.6646	-0.23	0.0016	5.412

The response of *Tribolium castaneum* to four plant extracts varied according to plant species. Larval growth was significantly inhibited when extracts incorporated to larval diet. Extract of *Peganum harmata* was the most potent followed by *Ajugaiva, Aristolochia baetica* and *Raphanus raphanistrum* (Jbilou *et al.,* 2006). Khan and Ahmed (2003) proved the toxic effect of extracts of neem fruit when tested against the insect pests *Tribolium confusum* and *Papilio demolus*. Many authors were interested to study the effect of plant extracts against stored product insects (Márcio *et al.,* 2007; Abdurrahman *et al*., 2008) and against other insects like *Nezara viridula* (Durmusoglu *et al*., 2003) and *Psyttalia concolor* (Rehman *et al*., 2009). Essential oils from plants were studied as fumigants against *Tribolium castaneum* and their efficacy proved them as good alternates of synthetic pesticides for resistance control too (Nattudurai *et al.,* 2016). *Melia azedarach* caused mortality and repellency of larvae and adults of *Tribolium castaneum* after exposing to leaves and fruit extracts of it (Sabiha *et al.,* 2017). Six

plant species used as admixtures to control *Tribolium castaneum* and their effectiveness results in higher mortalities suggesting their role as good alternates in future pest management programs (Ahmad *et al.,* 2018).

Table 4. Percentile probit mortality analysis of *Tribolium confusum* adults under toxic effects of *S.persica* Root extract.

Percentile	Probit (Y)	Log10[Dose(Stimulus)]	Standard Error	Dose(Stimulus)	Standard Error
1	2.6732	-1.7965	0.2547	0.016	0.0099
5	3.3548	-1.5051	0.1808	0.0313	0.0134
10	3.7183	-1.3496	0.1419	0.0447	0.0149
16	4.0056	-1.2268	0.1117	0.0593	0.0154
20	4.1585	-1.1614	0.096	0.069	0.0154
25	4.3258	-1.0899	0.0795	0.0813	0.015
30	4.476	-1.0256	0.0654	0.0943	0.0143
40	4.7471	-0.9097	0.0448	0.1231	0.0127
50	5	-0.8016	0.0387	0.1579	0.0141
60	5.2529	-0.6934	0.0504	0.2026	0.0236
70	5.524	-0.5775	0.0734	0.2646	0.0449
75	5.6742	-0.5133	0.0879	0.3067	0.0625
80	5.8415	-0.4417	0.1048	0.3616	0.0882
84	5.9944	-0.3763	0.1207	0.4204	0.1184
90	6.2817	-0.2535	0.1511	0.5579	0.198
95	6.6452	-0.098	0.1901	0.798	0.3606
99	7.3268	0.1934	0.2642	1.5611	1.0093

Table 5. Probit analysis of mortality data of 6th instar larvae of *Tribolium confusum* against *S.persica* leaf extract.

Doses (ml)	Insects exposed	Mean mortality%	Probit percent mortality	Difference	Chi-Square	Probit (y)
0.004	50	30	0.28	0.9998	0.0714	4.476
0.008	50	38	0.4175	-1.876	0.1686	4.695
0.016	50	50	0.5043	-0.2169	0.0019	5
0.018	50	58	0.5661	0.6972	0.0172	5.2015
0.02	50	62	0.6129	0.3541	0.0041	5.305

Table 6. Percentile probit mortality analysis of *Tribolium confusum* 6th instar Larvae under toxic effects of *S.persica* Leaf extract.

Percentile	Probit (Y)	Log 10 [Dose (Stimulus]	Standard Error	Dose (Stimulus)	Standard Error
1	2.6732	-3.7994	0.6827	0.0002	0.0004
5	3.3548	-3.2517	0.481	0.0006	0.0008
10	3.7183	-2.9596	0.3742	0.0011	0.0011
16	4.0056	-2.7287	0.2907	0.0019	0.0013
20	4.1585	-2.6058	0.2469	0.0025	0.0015
25	4.3258	-2.4714	0.2	0.0034	0.0016
30	4.476	-2.3507	0.1595	0.0045	0.0017
40	4.7471	-2.1328	0.0965	0.0074	0.0016
50	5	-1.9296	0.078	0.0118	0.0021
60	5.2529	-1.7263	0.1195	0.0188	0.0052
70	5.524	-1.5085	0.189	0.031	0.0139
75	5.6742	-1.3878	0.2307	0.0409	0.0228
80	5.8415	-1.2533	0.2784	0.0558	0.0383
84	5.9944	-1.1304	0.3225	0.0741	0.0602
90	6.2817	-0.8995	0.4064	0.126	0.1359
95	6.6452	-0.6074	0.5135	0.2469	0.3649
99	7.3268	-0.0597	0.7154	0.8716	2.179

Table 7. Probit analysis of mortality data of *Tribolium confusum* adults against *S.persica* Leaf extract.

Doses (ml)	Insects exposed	Mean mortality%	Probit percent mortality	Difference	Chi-Square	Probit (y)
0.004	50	18.	0.3646	-0.2303	0.0029	4.642
0.008	50	21.	0.4232	-0.1613	0.0012	4.7985
0.016	50	25.	0.4836	0.82	0.0278	5.
0.032	50	27.	0.5444	-0.2176	0.0017	5.1002
0.064	50	30.	0.6041	-0.2042	0.0014	5.2529

Table 8. Percentile probit mortality analysis of *Tribolium confusum* Adults under toxic effects of *S.persica* Leaf extract.

Percentile	Probit(Y)	Log10[Dose(Stimulus)]	Standard Error	Dose (Stimulus)	Standard Error
1	2.6732	-6.3069	3.6214	0.	0.001
5	3.3548	-4.9617	2.5475	0.	0.0019
10	3.7183	-4.2444	1.9762	0.0001	0.0027
16	4.0056	-3.6773	1.5262	0.0002	0.0035
20	4.1585	-3.3754	1.2878	0.0004	0.0041
25	4.3258	-3.0453	1.0289	0.0009	0.0048
30	4.476	-2.7489	0.7994	0.0018	0.0055
40	4.7471	-2.2139	0.4086	0.0061	0.0066
50	5.	-1.7147	0.2396	0.0193	0.0112
60	5.2529	-1.2155	0.5171	0.0609	0.0909
70	5.524	-0.6805	0.9203	0.2087	0.856
75	5.6742	-0.3841	1.1516	0.4129	2.9126
80	5.8415	-0.054	1.4115	0.8831	11.3723
84	5.9944	0.2479	1.6505	1.7697	39.5461
90	6.2817	0.8149	2.1009	6.5303	411.9212
95	6.6452	1.5323	2.6726	34.065	8,013.9727

Table 9. Effects of *Salvadora persica* extracts on Total protein contents of *Tribolium confusum* adults and larvae.

	Extract of *S.persica*	Observed protein contents of Larvae (mg/mL)	Observed protein contents 0f Adult (mg/mL)
	Leaf extract	Untreated 7.693(mg/mL)	Untreated 8.55(mg/mL)
		Treated 5.62(mg/mL	Treated 6.64(mg/mL)
	Root extract	Untreated 7.693(mg/mL)	Untreated 8.55(mg/mL)
		Treated 5.693(mg/mL)	Treated 5.92(mg/mL)

Alteration in total proteins of insects after treatment with plant extracts and essential oils was reported previously (Neoliya *et al.*, 2007; Renuga and Shayaraj, 2009; Al Qahtani *et al.*, 2010).

Al Qahtani *et al.* (2012) worked on *Oryzaephilus surinamensis* which is regarded as the most dangerous pest recorded in Saudia Arabia. They discovered the lethal effect of three plants named ginger (*Zingiber afficinale*), hail (*Elettaria cardamomum*) and shammer (*Foeniculum vulgare*) on it. All three plants applied in the form of dried powder and alteration in protein contents were recorded after their application. This research also favours the present research in which Salvadora extracts also reported to alter protein contents.

The most fundamental constituent of numerous plant extricates or their basic oils are monoterpenoids possessed fumigant properties which make them a decent component in pest control programs (Konstantopoulou *et al*., 1992; Regnault-Roger and Hamrouni, 1995; Ahn *et al.*, 1998). Plant extracts reported to hinder the electron transport amid

respiratory process (Nicholson *et al*., 1995; Márcio *et al*., 2007). The plant separate material, surangin B gave off an impression of being a strong inhibitor of mitochondrial electron transport and delivers decrease in ATP in vivo when tried as plant extricate against pests of stored products (Nicholson *et al*., 1995). We can reason that *Salvadora persica* plant is of insecticidal properties with a correlation with certain proteins depletion and it can be incorporated as a strong components in pest control and management programs.

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