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Acaricidal Activity of Aqueous Extract of Some Indigenous Plants against *Rhizoglyphus Tritici* (Acaridae: Acari)

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Abstract: *Rhizoglyphus tritici* is a notorious pest of stored wheat grains rendering grains unfit for use. Laboratory studies were conducted to evaluate the acaricidal and biological activity of four indigenous plant extracts *viz*., *Melia azedarach*, *Datura stramonium*, *Citrus paradisi* and *Citrus reticulata*. Peels of *C. paradisi* and *C. reticulata*, leaves of *M. azedarach* and *D. stramonium* were used for extraction. 5 serial dilutions (100, 50, 25, 12.5 and 6.25%) along with a control were used for each extract. 20 adults of *R. tritici* were released in small petri dishes having treated concentration of each extract and number of dead specimens were recorded after 24, 48, 72 and 96 hours interval. With respect to present mortality, *D. stramonium* and *M. azedarach* gave highest mortality of *R. tritici* after all time intervals whereas *C. paradisi* and *C. reticulata* showed lowest mortality. Effectiveness of extracts was concentration and interval dependent. EC_{50} and ET_{50} values were also determined which demonstrated *D. stramonium* as most effective plant extract having lowest EC_{50} and ET_{50} followed by *M. azedarach*. Hence, it is concluded that *D. stramonium* and *M. azedarach* possess high toxicity against *R. tritici* and can be successfully used in future management strategies.

Key words: Indigenous Plants · Acaridae · Rhyzoglyphus Tritici · D. Stramonium · Aqueous Extracts

INTRODUCTION

Like under-developed nations, Pakistan's economy is also of agricultural based where wheat is main staple food and source of livelihood of most the rural community [1, 2]. Being cultivated on 8666000 ha, its annual production in country recorded is 23.5 million tons which contributes 2.6% to the total gross domestic product (GDP) and 12.5% to the value added in agriculture (Anonymous, 2011). Up to 10-25% losses are recorded all over the world from all biotic and abiotic sources including insect pest infestation which reduce quantity, quality as well as nutritional value of the grains [3]. In Pakistan, these losses ranged from 4 to 10 % [4]. Among arthropods, mites are important mainly present in large numbers covering low space in stored grains [5, 6]. *Rhizoglyphus tritici* (Acari: Acaridae) is an important member of stored grain mites which multiply progressively when temperature and moisture conditions are optimum, thus depriving the grains from starch and lowering their esthetic and nutritional balance [7]. It affects physical, chemical and flour quality characteristics of the stored grains. Its population reached maximum when moisture content of the grains increased above 15% [8] The use of contact acaricides and fumigants against stored grain mites is risky for humans and animals which make grains unfit for consumption. Moreover, resistance problems persist against number of these chemicals [9-11]. So need of the time is to adopt new tactics which should be eco-friendly and safe for environment. Botanicals are being tested for their toxic properties against number of insect pests which render good control against these pests [12]. Many plants are known to have acaricidal efficacy against mite pests of stored grains and field crops. Some of them are used are repellents while others as chemosterilants or antifeedants [13, 14]. Plant extracts have varying degree of toxicity to various insect pests of crops due to their different secondary metabolites [15].

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[16] documented suppression of aphids and mealy bugs due to the repellent property of three plants leaf extracts (*Azedarachta indica*; *Eucalyptus globules* L. and *Ocimum basilicum* L.). Many other plant extracts such as *Azedarachta indica, Allium sativum and Satorejahor tensis* are being applied against different mite species for the last two decades [12, 17-19]. Current studies were designed to investigate toxic properties of some indigenous plants such as *Melia azedarach, Datura stramonium, Citrus paradisi* and *Citrus reticulata* against *R. tritici.*

MATERIALS AND METHODS

Four indigenous plant species i.e. datura leaves (*Datura stramonium*), bakain leaves (*Melia azedarach*), kinnow peel (*Citrus reticulata*) and grapefruit peel (*Citrus paradisi*) were collected from the surroundings of University of Agriculture, Faisalabad for their toxic properties against *R. tritici* and tested under laboratory conditions. Leaves and peels of the above mentioned species were oven dried for 24 hours at 65°C to make them suitable for grinding. A fine powder was obtained after grinding with the help of blender.

Wheat: Chemical free grains of wheat variety Inqilab-91 were cleaned, autoclaved and maintained in the laboratory having moisture content up to 15% for obtaining fresh culture of the mites. Having ample quantity of *R. tritici*, nymphs and adults were released on 50 g of wheat grains kept in small plastic jars covered with muslin cloth under lab conditions of 27±2 °C temperature and 75±5 % relative humidity (R.H.).

Extraction: 50 g powder of each plant extract was weighed and shaken in 300 mL distilled water on a rotary shaker for 24 h at 180 rpm. Whatman filter paper was used to filter the mixture under vacuum to obtain a pure liquid solution. It was considered 100% solution. Serial dilutions of 50%, 25%, 12.5% and 6.25% were prepared with distilled water for each botanical extract [8]. Five replicates were prepared of each dilution.

Bioassay: Bioassay studies were conducted using filter paper method. A round filter paper was fitted in a small petri dish covered with fine mesh. 50 µl of each concentration of plant extracts was sprayed on the filter paper with the help of micro applicator and allowed to dry for 5 minutes. After then, 20 adults of uniform age (one day old) were introduced to the treated filter paper with the help of fine camel hair brush. Very few flakes of wheat germ were added in each petri dish to serve as food for *R. tritici*. These treated petri dishes were kept in growth chamber at conditions of 27±2 °C temperature and 75±5 % relative humidity (R.H.). Percent mortality for each treatment was figured out with the help of binocular microscope by counting dead specimens on daily basis up to 96 hours of exposure. Whole experiment was replicated 5 times.

Statistical Analysis: Percent corrected mortality was carried out using Abbott's formula [20]. Analysis of Variance for mortality data was performed with Statistix version 9.0 [21] and after knowing significance of different concentrations, Least Significance Difference Test (LSD) was carried out to separate the mean mortality values of each treatment at 5% significance level. EC_{50} and ET_{50} values for each treatment were obtained by Probit analysis [22] using Minitab 17.0 statistical software. Plant extracts response was considered significantly different if their respective 95% fiducial limit did not overlap [23].

$$\%\ \text{Corrected Mortality} = \left(\frac{\%Morality\,in\,Treatmemt - \%Morality\,in\,Control}{100 - \%Morality\,in\,Control}\right) * 100$$

RESULTS

Analysis of variance (Table 1) showed highly significant different among all concentrations of tested extract of *M. azedarach* (F = 153, 233, 38.2 and 53.8 after 24, 48, 72 and 96 hours of exposure respectively) at 5 % level of significance. Similar statistical differences were acquainted with *D. stramonium* (F=98.5, 569, 223 and 9.77 for subsequent time intervals), *C. paradisi* (28, 119, 153 and 79.5 for four exposure times) and *C. reticulata* (21, 96.3, 138 and 41.8 after 24, 48, 72 and 96 hours of exposure respectively).

The adults of *R. tritici* were subjected to different concentrations of tested aqueous plant extracts. Results revealed that *M. azaderach* proved to be most effective against *R. tritici* giving 67.35 % mortality followed by *D. stramonium* at 100% concentration after 24 hours of exposure while *C. paradisi* and *C. reticulata* were least effective (36.35 and 26.80% mortality respectively) at same concentration after same exposure time (Table 2). Lowest mortality of only 3.50% was achieved by *C. reticulata* at 6.25 % concentration. After 48 hours of exposure, again maximum mortality (100%) was recorded by *M. azedarach* followed by *D. stramonium* at 100% concentration while *C. paradisi* and *C. reticulata* exhibited lowest percent moratlity (7.84 and 17.89% respectively) at 6.25% concentration.

Table 1: Analysis of Variance for efficacy of aqueous plant extracts against *R. tritici*

Plant Extract	Duration (Hours)	DF	SS	MS	F	P
M. azedarach	24	4	7176.16	1794.04	153	0.0000**
	48	4	16963.5	4240.89	233	0.0000**
	72	4	14211.7	3552.94	38.2	0.0000**
	96	4	8459.56	2114.89	53.8	0.0000**
D. stramonium	24	4	6118.63	1529.66	98.7	0.0000**
	48	4	13539.4	3384.84	569	0.0000**
	72	4	9049.95	2262.49	223	0.0000**
	96	4	1019.59	254.898	9.77	0.0017**
C. paradise	24	4	1683.62	420.904	28	0.0000**
	48	4	7158.8	1789.7	119	0.0000**
	72	4	14944.8	3736.21	153	0.0000**
	96	4	11777.8	2944.45	79.5	0.0000**
C. reticulata	24	4	1001.79	250.448	21	0.0001**
	48	4	4442.9	1110.73	96.3	0.0000**
	72	4	16713.1	4178.27	138	0.0000**
	96	4	17430.7	4357.68	41.8	0.0000**

* * Highly Significant at P=0.01

Table 2: Percent mortality of *R. tritici* by aqueous plant extracts after different time intervals

Plant Extract	Conc.	24 hours	48 hours	72 hours	96 hours
M. azedarach	T1 (100%)	67.35±2.63 a	100.00±0.00 a	100.00±0.00 a	100±0.00 a
	T2 (50%)	36.25±2.92 b	79.17±2.08 b	100.00±0.00 a	100±0.00 a
	T3 (25%)	21.83±0.38 c	45.83±2.08 c	75.61±6.23 b	100±0.00 a
	T4 12.5%)	12.76±1.95 d	22.92±4.16 d	43.71±8.29 c	74.17±1.58 b
	T5 (6.25%)	5.45±0.09 e	10.42±2.08 e	22.81±6.88 d	64.64±7.93 b
D. stramonium	T1 (100%)	67.34±2.63 a	100.00±0.00 a	100.00±0.00 a	100.00±0.00 a
	T2 (50%)	43.66±0.77 b	85.78±1.71 b	100.00±0.00 a	100.00±0.00 a
	T3 (25%)	27.19±2.71 c	59.19±1.81 c	100.00±0.00 a	100.00±0.00 a
	T4 12.5%)	18.12±1.46 d	28.55±1.85 d	60.07±1.46 b	96.67±3.33 a
	T5 (6.25%)	10.81±2.95 e	24.51±0.49 d	42.31±3.84 c	78.81±5.68 b
C. paradisi	T1 (100%)	36.35±1.62 a	74.02±1.76 a	100±0.00 a	100±0.00 a
	T2 (50%)	27.19±2.71 b	65.93±2.41 b	100±0.00 a	100±0.00 a
	T3 (25%)	19.98±1.69 c	50.00±1.69 c	80.98±3.01 b	100±0.00 a
	T4 12.5%)	14.52±1.72 c	25.98±1.76 d	43.16±5.03 c	77.78±5.55 b
	T5 (6.25%)	5.36±3.04 d	17.89±3.18 e	21.58±2.51 d	27.78±5.55 c
C. reticulata	T1 (100%)	26.80±0.48 a	56.86±1.96 a	100±0.00 a	100±0.00 a
	T2 (50%)	19.59±1.46 b	43.13±1.96 b	81.62±2.37 b	100±0.00 a
	T3 (25%)	12.37±3.41 c	33.33±1.96 c	49.79±4.05 c	91.07±4.49 a
	T4 12.5%)	8.86±1.65 cd	19.60±1.96 d	26.07±4.70 d	50.00±12.37 b
	T5 (6.25%)	3.50±1.75 d	7.84±1.96 e	10.47±2.46 e	13.69±0.59 c

Means sharing similar letters are not statistically different at p=0.05

The order changed after 72 hours of exposure as *C. paradisi* and *C. reticulata* recorded 100% mortality along with *M. azaderach* and *D. stramonium*. Similar trend was of efficacy by plant extracts was noticed after 96 hours of exposure. The bioactivity of each aqueous extract was increased with the increasing exposure interval. So, highest percent mortality was achieved after 96 hours of exposure but lowest percent mortality was recorded by all extracts after 24 hours.

With respect to effective concentration to kill 50% population of the tested *R. tritici* adults, *M. azaderach* having lowest EC_{50} value (83.22%) proved to be most effective after 24 hours followed by *D. stramonium* (44.25%). After 48 hours, *D. stramonium* was most effective among all other extracts having lowest EC_{50} value of 12.07%. *D. stramonium* also possessed stronger acaricidal activity to kill 50% population after 72 and 96 hours followed by *M. azedarach. C. paradisi*

Table 3: EC_{50} values of different aqueous plant extracts against *R. tritici*

Plant extracts	Time (Hours)	EC_{50} (% Solution)	Fiducial Limit	P-Value	Regression Equation
M. azedarach	24	83.2271	42.41-799.65 abc	0.003	Y= -1.86781+0.422431x
D. stramonium	24	44.2509	26.13-114.48 ab	0.000	Y= -1.86926+0.493225x
C. paradisi	24	224.553	1.44-66.21 a	0.039	Y= -1.57361+0.290650x
C. reticulata	24	189.502	76.85-18416.3bcd	0.006	Y= -2.27962+0.434678x
M. azedarach	48	15.1279	9.96-21.06 abc	0.000	Y= -2.32653+0.856430x
D. stramonium	48	12.0745	7.30-17.08 a	0.000	Y= -2.05816+0.826209x
C. paradisi	48	17.8795	8.414-30.311 ab	0.000	Y= -1.45508+0.504596x
C. reticulata	48	41.9614	22.83-137.95 bcd	0.002	Y= -1.58986+0.425465x
M. azedarach	72	7.09666	3.51-10.19 ab	0.000	Y= -1.88756+0.963228x
D. stramonium	72	5.47672	2.21-7.78 a	0.000	Y= -2.03657+1.19762x
C. paradisi	72	7.00777	3.60-9.88 ab	0.000	Y= -2.01822+1.03657x
C. reticulata	72	9.19449	4.47-13.71 abc	0.000	Y= -1.66241+0.749306x
M. azedarach	96	2.84543	0.01-5.37 a	0.019	Y= -1.06449+1.01795x
D. stramonium	96	2.84543	0.01-5.37 a	0.019	Y= -1.06449+1.01795x
C. paradisi	96	3.12626	0.01-5.39 ab	0.033	Y= -1.42375+1.24909x
C. reticulata	96	3.79327	0.52-6.58 abc	0.002	Y= -1.16598+0.874556x

Table 4: Time mortality response of *R. tritici* against aqueous plant extracts

Plant extracts	ET_{50} (Hours)	St. Error	Fiducial Limit	χ^2	P-Value	Regression Equation
M. azedarach	37.2609	4.531	26.93-46.16 ab	1.2860	0.000	Y=-5.38492+1.48839x
C. paradisi	45.9613	4.7991	35.79-56.22 abc	2.1867	0.000	Y=-5.98574+1.56376x
C. reticulata	37.3271	4.0677	28.28-45.35ab	0.6360	0.000	Y=-6.17336+1.70548x
D. stramonium	31.7702	3.3443	24.19-38.28 a	4.2765	0.000	Y=-6.87043+1.98652x

and *C. reticulata* were least effective to kill 50% tested population of *R. tritici* as these two extracts possessed highest EC_{50} values at each time interval. Chi square values for *R. tritici* mortality tests exhibited no heterogeneity in all the experiment (Table 3).

In terms of effective time to kill 50% population of the subjected *R. tritici*, *D. stramonium* took minimum time (31.77 hours) to kill 50 % population followed by *M. azedarach* (37.26) and *C. rericulate* (37.32 hours). Longest time was taken by *C. paradisi* (45.96 hours) to kill 50% population (Table 4).

DISCUSSION

Plant extracts evaluated in current study showed great toxicity to *R. tritici* under laboratory conditions. The present results revealed that mortality trend of plant extracts after 24, 48 and 72 hours was Bakain (*Melia azedarach*) = Datura (*Datura stramonium*) > Grapefruit (*Citrus paradisi*) > Kinnow (*Citrus reticulata*) while after 96 hours mortality trend of plant extracts was almost similar. The most effective extract in our study were *D. stramonium* and *M. azrdarach* having lowest EC_{50} and ET_{50} values which recorded 100% mortality at almost each exposure interval except at 24 hours as supported by various earlier scientists [24, 25]. EC_{50} values of *M. azedarach* and *D. stramonium* ranged from 2.84-83.22% and 2.84-44.25% while ET_{50} values calculated were at 37.26 and 31.77 hours for *M. azedarach* and *D. stramonium*. These results are in conformity with those of [8] who found that *A. indica, D. stramonium, M. azedarach* and *C. colocynthis* extracts were highly and equally effective. Our results somewhere demonstrated 100% mortality as reported by Tuni and ªahinkaya [26] who recorded 100% mortality of *T. Cinnabarinus* and *A. gossypii* Glover when essential oils of *Cuminum cyminum, Pimpinella anisum* and *Origanum syriacum* were used in greenhouse conditions. Yanar *et al.* [27] evaluated acaricidal effects of different plant parts extracts on two-spotted spider mite (*T. urticae* Koch) and reported that *M. azedarach* recorded 76.45% mortality of *T. urticae* after 24 hours interval of application. This anomaly in results may be attributed to difference in plant part used. Antonius *et al.*; Kumral *et al.* [28, 29] also confirmed fitness of our results who reported that ethanolic extracts of *D. stramonium* leaves and seeds exhibited acaricidal, ovicidal and repellent activities against two-spotted spider mite, *T. urticae* (Koch). Furthermore as reported by Khan and Marwat [30], bark powder of *M. azedarach* exhibited more repellency than leaves and bark of *Calotropis procera* against *R. dominica*. Similarly, Bounechada and Fenni [31] reported *M. azedarach* 2% oil extract exerted higher mortality than all other extract and decrease food consumption against *Ocnervidia volxemi bolivar* adults.

CONCLUSION

Aqueous extracts of tested *M. azedarach* and *D. stramonium,* exhibit more acaricidal activity as compared to *C. paradisi* and *C. reticulata*. So, we recommend implementation of former extracts in future IPM programs to control mite pests of stored commodities. However, their efficacy may be due to single or multiple ingredients. So there is need to further expand these studies to know the actual ingredient.

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