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PHYTOTOXIC, INSECTICIDAL AND CYTOTOXIC ACTIVITIES OF *ZIZIPHUS MAURITIANA* VAR. *SPONTANEA* EDGEW. AND *OENOTHERA BIENNIS* L.

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Abstract

The present study investigates phytotoxic, insecticidal and cytotoxic activities of samples extracted from *Ziziphus mauritiana* var. *spontanea* Edgew. and *Oenothera biennis* L. Good phytotoxic activity was measured by the ethanolic extract of *Z. mauritiana* var. *spontanea* and ethyl acetate extract of *O. biennis* against *Lemna minor*. Ethyl acetate samples of *Z. mauritiana* var. *spontanea* and *O. biennis* was very active against *Rhizopertha dominica* and *Sitophilus oryzae* respectively. Maximum activity against brine shrimps was noted for the ethanolic samples of *Z. mauritiana* var. *spontanea* and *O. biennis* ethyl acetate extract. Phytochemical screening of different solvent extracted samples of *Z. mauritiana* var. *spontanea* and *O. biennis* confirmed the presence of alkaloids, flavonoids, glycosides, saponin and tannins.

Key words: Oenothera biennis Ziziphus mauritiana, Cytotoxic, Insecticidal, Phytotoxic, Phytochemical screening.

Introduction

Medicinal plants are used in different countries around the globe as a source of medicine. Plants produce a wide variety of natural compounds making them as a rich source of remedies for the treatment of different health problems since ancient times (Shah *et al*., 2015). Excessive use of synthetic herbicides for eradicating weeds causes herbicide resistance and also produces harmful effects on environment. The synthetic herbicides should be replaced with phytotoxic plants which have growth inhibitory constituents and are eco-friendly and not expensive (Islam & Kato-Noguchi, 2014). Different synthetic chemicals are used from many years for controlling insects which produce harmful effects on other living organisms and environment. Scientists are trying their level best to produce plant based insecticides in order to minimize harmful effects of synthetic insecticides (Malau & James, 2008). Many scientists have observed the effects of plant extracts against different insects such as *Rhyzopertha dominica* (F), *Tribolium castaneum* (Herbst) and *Sitophilus oryzae* etc. *Tribolium castaneum* causes damage to store products. *Sitophilus oryzae* and *Tribolium castaneum* severely damage many cereals, flours, meal and grains etc. (Matthews, 1993). *Rhyzopertha dominica* is destructive insect and it destroys stored grains and causes serious ongoing problems in grain stocking at larval as well as adult stage (Ahmad *et al*., 2016).

Ziziphus mauritiana var. *spontanea* (Rhamnaceae) is a shrub or small tree known as Indian jujube (English), Ber (Hindi) and Badarh (Sanskrit) (Sivasankari & Sankaravadivoo, 2015). It is used to cure anaemia, bronchitis, diabetes, liver problems, stomach upset, skin infections, and urinary troubles (Mishra & Bhatia, 2014). Leaves are diaphoretic, astringent and can be used to treat typhoid in children. Bark is used to treat gingivitis, diarrhea and dysentery. Roots are used against headache and have soothing effect as well (Akhter *et al*., 2016). The active constituent of saponins is regarded as hypercholesterolemia, hyperglycemia, and antioxidant, anticancer, anti-inflammatory (Manjunatha, 2006). Tannins showed antimicrobial and antioxidant activities (Rievere *et al*., 2009).

Oenothera biennis (Onagraceae) is a biennial herb known as evening star or evening primrose. It occurs in temperate and subtropical regions and is native to eastern and central North America. Its seeds were imported from U.K. *O. biennis* is used for the treatment of diabetic neuropathy, atopic eczema, premenstrual syndrome, rheumatoid arthritis, arteriosclerosis, psoriasis and asthma (Subramoniam *et al*., 2013). Alkaloids are reported to have anti-inflammatory and analgesic function and help to develop resistance against diseases and endurance against stress and relieve pain. Flavonoids may have potentially significant application against microorganisms and antioxidant (Sudhira *et al*., 2015).

Materials and Methods

Plant materials collection, identification and crude extract preparation: Plants of *Ziziphus mauritiana* var. *spontanea* were collected from the Palosi area of Peshawar KPK. Plants of *Oenothera biennis* were grown from the seeds imported from UK. The plants were identified and deposited via voucher specimen No Ambrin Bot. 33 (PUP) and Ambrin Bot. 34 (PUP) to the Herbarium of Department of Botany, University of Peshawar. The plants were shade dried for 20 days and grounded to powder. Fifty grams of powdered materials were soaked in 250 mL each of ethanol and ethyl acetate separately and filtered through Whatman filter papers# 1823 after one week of soaking. In order to concentrate the extracts it was subjected to rotary evaporator and kept at 4°C until used.

Phytoxic, insecticidal and cyto-toxic activity: Phyto-toxic, insecticidal and cyto-toxic activity was estimated by McLaughlin *et al*., (1991), Naqvi & Parveen (1991) and Meyer *et al*., (1982) respectively.

Phytochemical screening: Alkaloids, flavonoids and oils were detected by the methods of Trease & Evans (1989) and Kalaiselvi *et al*., (2016) respectively. Saponins were determined as described by Jamil *et al*., (2012). The procedures of Edeoga *et al*., (2005) were used for the estimation of glycosides and tannins.

Result

Ethanolic samples of *Z. mauritiana* var. *spontanea* reduced the growth of *Lemna minor* by 73.4% (FI_{50} =102.4µg/ml) followed by ethyl acetate samples (66.7%) at highest dose of 1000 µg/ml. Minimum growth retardation of 13.4% was shown by aqueous samples at 10µg/ml. Maximum phytotoxic activity of 73.4% was noted for ethyl acetate extract (FI_{50} =252.80µg/ml) of *Oenothera biennis* followed by ethanolic samples (66.7%) at 1000 µg/ml. The lowest activity of 16.7% was shown by an aqueous extract at 10 µg/ml (Table 1). Highest mortality of 90.0% was measured by ethyl acetate samples *Z. mauritiana* var. *spontanea* against *Rhizopertha dominica* followed by *Tribolium castaneum* (86.7%) and *Sitophilus oryzea* (80.0%) by ethanolic and ethyl acetate extract respectively (Table 2). Similarly, maximum mortality of 93.4% against *S. oryzea* was caused by ethyl acetate samples of *O. biennis* followed by *R. dominica* (90.04%) (Table 3).

Maximum cytotoxic activity (83.4%) was exhibited by ethanolic extract of *Z. mauritiana* var. *spontanea* followed by ethyl acetate extract (73.4%) at 1000 µg/ml dose. Minimum LD_{50}=107.68 µg/ml was noted for ethanolic extract (Table 4). Similarly, ethyl acetate extract of *O. biennis* revealed the highest mortality (93.4%) of brine shrimps followed by ethanolic extract (76.7%) at 1000µg/ml dose. The data showed that the lowest LD_{50}= 98.09 µg/ml was noted for ethyl acetate samples of *O. biennis* (Table 4). The results of both the studied plants revealed that mortality of brine shrimps increased with increase in extract concentration.

Phytochemical screening of different solvent extracted samples of *Z. mauritiana* var. *spontanea* confirmed the presence of alkaloids, flavonoids, glycosides, saponin and tannins. Likewise, phytochemical screening of ethanolic, ethyl acetate and aqueous extract of *O. biennis* noted the presence of flavonoids, oils, alkaloids, glycosides, saponin and tannins (Table 5).

Table 1. Phytotoxic activity of *Ziziphus mauritiana* var. *spontanea* L and *Oenothera biennis* L.

Plants	Extracts	Dose µg/ml	No. of fronds in test	No. of fronds in control (-ve)	% Inhibition	FI_{50} (µg/ml)
Ziziphus mauritiana var. *spontanea*	Ethyl acetate	10	20	30.00	33.4	127.3
		100	17		43.4	
		1000	10		66.7	
	Ethanol	10	25		16.7	102.4
		100	11		63.4	
		1000	8		73.4	
	Aqueous	10	26		13.4	1233.4
		100	20		33.4	
		1000	17		43.4	
Oenothera biennis	Ethyl acetate	10	18	30.00	40.0	252.8
		100	13		56.7	
		1000	08		73.4	
	Ethanol	10	22		26.30	431.2
		100	15		50.0	
		1000	10		66.7	
	Aqueous	10	25		16.7	720.6
		100	16		46.7	
		1000	13		56.7	

Table 2. Insecticidal activity of *Ziziphus mauritiana* var. *spontanea* Edgew.

Name of insects	Extracts	Dose (µg/ml)	Total no. of insects	No. of insects in control	No. of insects survival	No. of dead insects	Percent mortality	LD_{50} (µg/ml)
Tribolium castaneum (Herbst)	Ethyl acetate	10	30	30	16	14	46.7	97.15
		100	30	30	10	20	66.7	
		1000	30	29	07	23	76.7	
	Ethanol	10	30	30	19	11	36.7	185.8
		100	30	30	12	18	60.0	
		1000	30	30	04	26	86.7	
	Aqueous	10	30	30	22	08	26.7	100.0
		100	30	30	18	12	40.0	
		1000	30	30	15	15	50.0	
Rhyzopertha dominica (F)	Ethyl acetate	10	30	30	13	17	56.7	18.2
		100	30	30	08	22	73.4	
		1000	30	30	03	27	90.0	
	Ethanol	10	30	30	18	12	40.0	491.7
		100	30	30	15	15	50.0	
		1000	30	30	10	20	66.7	
	Aqueous	10	30	30	20	10	43.3	10253.2
		100	30	30	16	13	46.7	
		1000	30	30	14	16	53.4	
Sitophilus oryzea (L)	Ethyl acetate	10	30	30	15	15	50.0	84.1
		100	30	30	12	18	60.0	
		1000	30	30	06	24	80.0	
	Ethanol	10	30	30	19	11	36.7	326.9
		100	30	30	13	17	56.7	
		1000	30	30	09	21	70.0	
	Aqueous	10	30	30	24	06	20.0	962.8
		100	30	30	18	12	40.0	
		1000	30	30	15	15	50.0	

Table 3. Insecticidal activity of *Oenothera biennis* L

Insects	Extracts	Dose (µg/ml)	Total no. of insects	No. of insects in control	No. of insects survival	No. of dead insects	Percent mortality	LD_{50} (µg/ml)
Tribolium castaneum (Herbst)	Ethyl acetate	10	30	30	21	09	30.0	708.5
		100	30	30	17	13	43.4	
		1000	30	29	11	19	63.4	
	Ethanol	10	30	30	18	12	40.0	115.4
		100	30	30	09	21	70.0	
		1000	30	30	06	24	80.0	
	Aqueous	10	30	30	23	07	23.0	962.8
		100	30	30	19	11	36.0	
		1000	30	30	13	17	56.0	
Rhyzopertha dominica (F)	Ethyl acetate	10	30	30	12	18	60.0	8.04
		100	30	30	08	22	73.4	
		1000	30	30	03	27	90.0	
	Ethanol	10	30	30	15	15	50.0	17.89
		100	30	30	10	20	66.7	
		1000	30	30	07	23	76.7	
	Aqueous	10	30	30	26	04	13.4	1500.08
		100	30	30	22	08	26.7	
		1000	30	30	16	14	46.7	
Sitophilus oryzea (L.)	Ethyl acetate	10	30	30	09	21	70.0	0.044
		100	30	30	05	25	83.4	
		1000	30	30	02	28	93.4	
	Ethanol	10	30	30	13	17	56.7	9.3
		100	30	30	09	21	70.0	
		1000	30	30	05	25	83.4	
	Aqueous	10	30	30	18	12	40.0	192.6
		100	30	30	13	17	56.7	
		1000	30	30	08	22	73.4	

Table 4. Cytotoxic activity of *Ziziphus mauritiana* var. *spontanea* Edgew. and *Oenothera biennis* L.

Plant	Extracts	Dose (µg/ml)	Total no. of larvae	No. of survival larvae	No. of death of larvae	% mortality	LD_{50} (µg/ml)
Ziziphus mauritiana var. *spontanea*	Control		30	30	0	0	-
	Ethyl acetate	10	30	20	10	33.4	453.6
		100	30	16	14	46.7	
		1000	30	08	22	73.4	
	Ethanol	10	30	16	14	46.7	107.6
		100	30	12	18	60.0	
		1000	30	05	25	83.4	
	Aqueous	10	30	23	07	23.4	984.0
		100	30	20	10	33.4	
		1000	30	15	15	50.0	
Oenothera biennis	Ethyl acetate	10	30	17	13	43.4	98.09
		100	30	11	19	63.4	
		1000	30	02	28	93.4	
	Ethanol	10	30	19	11	36.7	267.3
		100	30	13	17	56.7	
		1000	30	07	23	76.7	
	Aqueous	10	30	26	4	13.4	1342.6
		100	30	23	7	23.4	
		1000	30	18	12	40.0	

Table 5. Phytochemical screening of *Ziziphus mauritiana* var. *spontanea* Edgew. and *Oenothera biennis* L.

Chemical constituents	*Ziziphus mauritiana*			*Oenothera biennis*		
	ETAC	EtOH	Aq	ETAC	EtOH	Aq
Alkaloids	+	+	+	+	+	+
Oils	-	-	-	+	+	-
Flavonoids	+	+	+	+	+	+
Saponins	-	+	+	-	+	+
Glycosides	+	-	+	+	+	-
Tannin	-	+	-	+	-	+

Key: ETAC = Ethyl acetate, EtOH = Ethanol, Aq = Aqueous
+ = Presence; - = Absence

Discussion

The phytotoxic activity showed that ethanolic samples of *Z. mauritiana* var. *spontanea* were more active against *Lemna minor* when compared with ethyl acetate samples. Adenike *et al*., (2015) and Rehmanullah *et al*., (2014) reported similar results when studied *Anthocleista vogelli* and *Euphorbia* species. Similarly, ethyl acetate samples of *O. biennis* also showed good phyto-toxic activity against *L. minor* when compared with ethanolic samples. These results are in line with Rahman *et al*., (2016) and Khuda *et al*., (2012) who reported similar results in case of *Picea smithiana* and *Duchesnea indica*. The probable reasons for the phytotoxic activity could be due to the presence of different phytochemicals such as glycosides, alkaloids, saponins, flavonoids and tannins in these plants (Zeb *et al*., 2014; Otusanya & Ilori, 2012).

Insecticidal activity revealed that ethyl acetate samples of *Z. mauritiana* var. *spontanea* showed good results against *Rhyzopertha dominica* with LD_{50}=18.22. The higher LD50 values for *Tribolium castaneum* and *Sitophilus oryzea* revealed that these insects showed resistance to the extract doses. These results are in line with Khan *et al*., (2013) who reported similar results against *R. dominica* by ethyl acetate samples of *Desmodium elegans*. Likewise, ethyl acetate samples of *O. biennis* showed good activity against *Sitophilus oryzea* (LD_{50}=0.044). Thambi & Cherian (2015) reported similar highest mortality of *Sitophilus oryzea* by the ethyl acetate extract of *Manihot esculenta*. The present study also revealed that *Rhyzopertha dominica* and *T. castaneum* were resistant to the sample concentration. These results agree with Ahmad *et al*., (2016) and Ajayi *et al*., (2012). Maximum toxicity to insects was due to ethanolic samples of *Z. mauritiana* var. *spontanea* and ethyl acetate extract of *O. biennis* as compared to aqueous samples.

Ethanolic samples of *Z. mauritiana* var. *spontanea* showed highest mortality against brine shrimps as compared to ethyl acetate and aqueous samples. Socorro *et al*., (2014) reported similar results when studied ethanolic samples of *Piper betle* at highest dose. Ethyl acetate samples of *O. biennis* were more potent against brine shrimps as compared to ethanolic and aqueous samples. Rashid *et al.*, (2013) and Billacura *et al*., (2017) reported similar mortality by the samples of *Fagonia olivieri* and *Crescentia cujete*. The profound cytotoxic activity of both the studied plants against brine shrimps might be due to the presence of chemical constituents. Musa *et al*., (2012) and Misonge *et al*., (2015) reported that natural substances such as phenols, alkaloids, steroids, glycosides, flavonoids and terpenoids were accountable for cytotoxic activity.

Conclusion

It can be concluded from the present study that good phytotoxic, insecticidal and cytotoxic activity was found in both the studied plants. The current study revealed the presence of different phytochemicals in both plants. Phytotoxic, insecticidal and cytotoxic activity could be due to the presence of different bio-active compounds in the studied plants.

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