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ALLELOPATHIC POTENTIAL OF HARMAL

Faruukh Hussain, Rubina Nasrin and Yasmin

Department of Botany, University of Peshawar, Peshawar

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Harmal (*Peganum harmala* L.) is a weed and wasteland species in Pakistan. Aqueous extracts from shoots, soil collected beneath harmal, substance volatilizing from shoots and added shoot litter, besides being auto-toxic, significantly reduced the germination and radicle growth of *Pennisetum americanum, Brassica campestris, Lactuca sativa* and *Trifolium resupinatum* in various experiments. Paper chromatography indicated the presence of caffeic, ferulic, *p*-coumaric and *p*-OH-benzoic acids in shoot extracts.

The toxicity depended upon the test species used, soaking duration and physiological process involved. It is suggested that harmal is strongly allelopathic.

Key words: Peganum harmala, Weed, Allelopathy.

INTRODUCTION

Harmal (*Peganum harmala* L.), a small aromatic perennial herb, is common in Pakistan from plains upto 3500 m in dry habitats. Relatively few unhealthy plants grow in its association. The allelopathic suppression and exclusion of associated species by *Salvia* [12], *Adenostoma* [10], *Datura* [5], *Xanthium* [14], *Stachy parviflora* [9] and *Bakain* [15] has already been reported. Allelopathy plays an important role in controlling productivity and vegetation development in natural and agroecosystems [7,13,17]. Hussain and Nasrin [19] working on the germination behaviour of *Peganum* found that seed contains inhibitors that reduce and delay its germination. Keeping in mind the importance of allelopathy and lack of any such reference on the ecology of *Peganum,* the present investigation was, therefore, carried out to find its allelopathic potential.

MATERIALS AND METHODS

Mature harmal plants were dried in shade at room temperature (25-30°). The glassware was sterilized at 170° for 4 hours. Germination and radicle growth was recorded after 72 hour incubation at 26° unless otherwise stated. Results were statistically analysed using "Z and t tests" following Cox [1]. The preliminary preparations, storage and general bioassay techniques have been described by Hussain [3], Hussain, Gadoon [4] and Inam *et al.* [14].

Aqueous extract bioassay. Five g crushed shoots (leaves, stems) were soaked in 100 ml double distilled water for 12, 24, 48 and 72 hr at 25° and filtered. The extracts were tested against *Pennisetum americanum, Lactuca sativa, Trifolium resupinatum, Brassica campestris* and *Peganum harmala* using our standard bioassay techniques [11]. There were 10 replicates, each with 10 seeds.

Natural rain leachate bioassay. Fifteen g. shoots were placed over a sieve during rain on 1 meter high bench and leachate collected for 6 hr. A portion of this leachate (1X) was concentrated to half (2X) of its original volume at 50° These leachates alongwith rain water control were used against the same test species following our standard technique [5,6,14,19]. There were 10 replicates, each with 10 seeds.

Volatile inhibitors bioassay. (a). *Detached shoot voltaile (s) bioassay:* A dish containing 20 g fresh harmal shoots were placed in the centre of 15x6 cm containers. Twenty seeds of the test species were kept around it on twice folded Whatman Filter Paper No. 1. Control was similarly made by replacing shoots with 20 g moist pieces of filter papers. The seed-beds were moistened with equal volume of double distilled water. The large containers were closed with their lids to simulate the natural micro-environment. These containers alongwith the seeds and shoots were incubated at 26°. The germination and radicle growth of the same test species was recorded after 72 hr.

(b) *Intact shoot volatile (s) bioassay.* Twice folded Whatman No. 1 filter paper was spread in a polyethylene bag. A portion of the harmal plant growing in the field was introduced into the bag and loosely closed to allow gaseous exchange. After 96 hr, these filter papers were used as seed-beds for the aforementioned test species in a standard filter paper bioassay. Control was similarly made without harmal shoots.

Litter mulching experiment. Two g crushed shoot was incorporated into the top layer of sterilized sand taken in 5x10 cm plastic pots. Fifty seeds in 5 replications of 10

each of the same test species were sown. Comparable control were made by replacing *Peganum* litter with filter papers. The pots were watered with half strength Hoagland's nutrient solution and incubated at 25°. After 4 days, germination was recorded and seedlings were thinned to 5 in each glass. After another two weeks fresh and drymass were determined. One seedling from each glass was used for the determination of total chlorophyll following Harborne [16].

Soil phytotoxicity bioassay. Test and control soil was sampled upto 15 cm depth from places with and without harmal thickets, respectively. The soils were dried and sieved through 2 mm mesh. These soils were tested against the same afore-mentioned test species using soil extract and soil-bed bioassay Hussain *et al.* [5] and Hussain and Gadoon [4].

Identification of phytotoxins. Ten percent aqueous extract from shoots was concentrated to 1/3 of original volume in rotavapour and acidified to pH 2.5 with 1 N HCl. To this extract double amount of ether was added and vigorously shaken in a separation flask. The mixture was allowed to stand to separation into etherial and aqueous layers. The former was treated for the second and third time with ether and then discarded. The etherial fractions were mixed and evaporated in rotavapour. The concentrate dried, and the residue dissolved in 2 ml ethanol and used for spotting. Whatman No. 1 chromatographic paper was used. The chromatograms were developed in 6% AA (6:94, acetic acid: water) initially, followed by BAW (10: 27: 63, n-butanol: Acetic acid: water) solvent systems in the second direction. The R_f, colour with UV and locating agents were known after Lodhi [20], Naqvi [21], Inam *et al.* [14] and compared with the standard.

RESULTS

Aqueous extract bioassay. Shoot extracts significantly inhibited germination and radicle growth of all the test species. *Lactuca* and *Peganum* seeds could not germinate in any of the extracts, except those growing in 12 hr. extract (Table 1). The toxicity depends upon soaking time and the test species used. Extracts obtained after 72 hr soaking were more inhibitory than those soaked for shorter duration.

Natural rain-leachate bioassay. The germination of *Lactuca* and *Brassica* in low concentration and those of all the test species in the concentrated rain leachate was significantly retarded (Table 2). Similarly, the radicle growth of all the test species declined in the rain leachate, especially in concentrated leachate (Table 2). The phytotoxicity enhanced in the concentrated leachates.

Volatile inhibitors bioassay. The germination and radicle growth of all the test species, except the germination of *Lactuca, Trifolium* and *Pennisetum,* were reduced by volatiles from harmal shoots (Table 2). This suggests the absorption and/or adsorption of some toxic volatile substances (s) on the filter paper seed beds. The toxicity was specific for different species.

Litter mulching experiment. The germination, fresh, dry mass and chlorophyll contents of all the test species decreased. *Pennisetum, Trifolium* and *Lactuca* were less affected than the other two species (Table 3).

Soil phytotoxicity bioassay. Germination and radicle growth of all test species, except that of *Brassica,* was significantly reduced by harmal affected soil (Table 2). *Peganum* followed by *Pennisetum* was the most susceptible species while *Lactuca* was the least susceptible . The inhibitory nature of affected soil suggested the presence, accumu-

Table 1. Effect of aqueous extract from shoots of harmal on the germination and radicle growth of test species. Each value is a mean of 10 replicates, each with 10 seeds, expressed as % of control.

Test species	Soaking durations (hr.)							
	12	24	48	72	12	24	48	72
	Germination				Radicle growth			
Pennisetum americanum	86.66	60.00	50.00	43.33	49.70	12.91	11.64	6.49
Lactuca sativa	24.99	00.00	00.00	00.00	23.95	00.00	00.00	00.00
Trifolium resupinatum	20.69	17.23	17.23	13.79	41.48	15.40	3.93	1.33
Brassica campestris	37.92	37.92	6.89	6.86	10.76	10.27	8.97	2.12
Peganum harmala	63.63	00.00	00.00	00.00	28.75	00.00	00.00	00.00

All the values are significantly different from control at P = 0.05

Table 2. Germination and radicle growth of test species in various bioassays. All values are expressed as percent of control. 1X and 2X represent the concentration of leachates.

Test species	Natural rain-leachate bioassay		Volatile Inhibitors bioassay		Soil phytotoxicity bioassays	
	1X	2X	Detached shoots	Intact shoots	Soil extract	Soil-bed
Germination						
Pennisetum americanum	94*	82	86	95*	53	52
Lactuca sativa	78	57	89*	90*	90*	86
Trifolium resupinatum	98*	77	95*	95*	89*	75
Brassica campestris	83	56	82	85	90*	100*
Peganum harmala	98*	71	62	72	46	42
Radicle growth						
Pennisetum americanum	81	59	75	62	25	23
Lactuca sativa	80	42	53	73	43	62
Trifolium resupinatum	88*	66	54	68	40	48
Brassica campestris	88*	45	87	82	44	38
Peganum harmala	89*	75	40	65	26	25

All values are significantly different from control at P = 0.05 except those with astrike (*)

Table 3. Effect of harmal litter on the germination, growth and chlorophyll contents of test species. All values are expressed as per cent of control.

Test species	Germination	Fresh mass	Dry mases	Total chlorophyll
Pennisetum americanum	89	72	52	88
Lactuca sativa	75	69	56	80
Trifolium resupinatum	81	58	47	78
Brassica campestris	42	48	32	65
Peganum harmala	35	64	60	84

Values are significantly different from control at P = 0.05 except those with an astrike (8). The figures have been rounded off.

lation and effectivity of phytotoxins added by harmal to the habitat.

Identification of phytotoxins. Caffeic, ferulic, *p*-coumaric and *p*-hydroxybenzoic acids were identified as some of the phytotoxins. All of them are water extractable and proven allelopathic agents [10,13,17,20]. Some spots remained unidentified.

DISCUSSION

Many plants make the habitat undesirable for other species owing to allelopathy [12,13,17,20]. In the present study aqueous extracts, affected soil, litter, volatiles and natural rain leachates from *Peganum* reduced germination and early growth of various test species due to presence of caffeic, ferulic, *p*-coumaric and *p*-OH-benzoic acids. Non-palatability of harmal helps in the deposition of litter which releases phytotoxins. Aqueous extracts from *Salvia* [12], *Adenostoma* [10] and tobacco [6] inhibited the germination and growth of test species due to allelopathy. Hussain *et al.* [8,9] observed aqueous extracts of *Eragrostis* and *Stachys* to be inhibitory as also observed by us. The added litter proved toxic to the test species in the non-toxic growth medium. Similarly the affected soil reduced the growth and germination of test species. This is primarily due to the release of phytotoxins by harmal litter into the

soil. Soil phytotoxicity induced by many allelopathic plants has been confirmed [3-8, 12,13,17,20]. The volatile inhibitors might be absorbed on the wet soil surface as demonstrated in laboratory over filter papers. Volatile inhibitors from *Datura* [5], *Stachys* [9], *Salvia* [12], and many other aromatic plants [13,17] have similarly suppressed the growth of test species. Volatilization is an important mechanism manifesting allelopathy by aromatic plants. The test species exhibited poor biomass and chlorophyll contents. It has been reported that allelopathy reduces the growth of susceptible species by impairing the water and mineral uptake, reducing chlorophyll, protein and phytosynthetic activity. Hussain *et al.* [18], Rice [13] and Putnam and Tang [17] reported reduction in chlorophyll contents. The inhibitory effects were primarily due to the release of caffeic, *p*-coumaric, *p*-OH-benzoic and ferulic acids [13,17,20,21]. All of them being potential plant inhibitors. The reduction in chlorophyll, fresh mass, moisture contents ultimately affect the over all growth of seedlings which may die due to toxicity [15]. The findings reveal that the presence of relatively few unhealthy associated species in the vicinity of harmal thickets is are primarily due to allelopathy. Competition and other environmental physical factors, differentials species responses might also play a significant role in modifying the allelopathic activity of harmal [9,12,13,14,17]. As a weed, it decreaes the productivity of susceptible crops.

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