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Laboratory experiment to test *Papaver pavoninum* Fisch. and C. A. Mey. allelophathic effect against test species maize and brassica

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Abstract

Papaver pavoninum Fisch. and C.A. Mey. (family Papaveraceae) was tested for its allelopathic effects on Maize and Brassica. The aqueous extracts from whole plant, litter and mulches in various experiments, invariably affected the germination, plumule growth, radical growth, Number of seminal roots and fresh and dry weight of both the test species. Phytotoxicity of extracts depended upon concentratin and soaking duration. Generally 48 hour extracts were inhibitorier. Hot water extract was inhibitorier than cold extracts. Added litter and mulching experiments also proved inhibitory, suggesting that *Papaver pavoninum* has strong allelopathic potential at least against the test species.

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Introduction

Allelopathy refers to the beneficial or harmful effects on one plant, both crop and weeds species by another plant through the release from plant parts by leaching, root exudation, volatilization, residue decomposition and other processes in both natural and artificial systems (Ferguson and Rathinasabapathi, 2009). Or simply defined as, "the release of phytotoxins by plants" (Bais *et al.*, 2003). The idea that plants affect neighboring plants by releasing chemicals in the environment has been well documented (Willis, 2004). It is the chemical mechanism of plant interference characterized by a reduction in plant emergence or growth, reducing their performance in the association (Bajva, 2007). Allelopathy along with competition in nature suppresses and finally exclude the susceptible associated species from the common habitat. Chemicals with allelopathic potential have been frequently observed in almost all plant parts which are released in to the habitat under specific environmental conditions (Hussain *et al.*, 2007; Singh *et al.*, 2003; Turk & Tawaha, 2003). Allelopathy can enhance the competitive success of the invader plant, since the release of phytotoxins in the environment may affect the growth and life processes of other community species (Callaway, 2002).

Weeds are unwanted plants that grow in association with agricultural crops and bring about significant decline in yield through their competition with crop plants for sunlight, space, nutrients etc. (Dangwal *et al.*, 2010). However, some weeds are also allelopathic in nature (Oudhia and Tripathi, 1997; 1998). There are innumerable reports on the inhibitory effects of weeds on crop plants (Bhowmik & Doll, 1992; Javaid *et al.*, 2007). Allelopathy is considered to be one of the possible alternatives for achieving sustainable weed management and pathogen reduction. (Xaun *et al*, 2005;Yongqing, 2005;Hussain *et al.*, 2007and Mehmood *et al.*, 2010).

Maize is the third important cereal after wheat and rice in Pakistan and ranks third of the most growing crop in the world with an area of more than 365 million acres with an annual production of about 750 million metric tons (Anon., 2008).

Oudhia (2002) reported the harmful allelopathic effects of *Argemone mexicana* on germination and seedling vigour of wheat, mustard, fenugreek, sorghum, finger millet, tomato, cucumber etc and its toxicity to animals, and cattle. The allelochemicals cinnamic and benzoic acid were identified as harmful chemicals responsible for inhibition of germination and seedling vigor. Shaukat *et al.*, (2002) examined the allelochemicals and nematicidal potential of *Argemone mexicana*. Mat *et al.*, (2003) studied the alkaloids and cytotoxicity of *Papaver dubium Subsp. Dubium* and *P. dubium Subsp. Laevigatum*. Bhattacharjee *et al.*, (2006) studied the sensitivity of two Gram positive and two Gram negative pathogenic multi-drug resistant bacteria against the crude extracts of leaves and seeds of *Argemone mexicana*. Saeed *et al.*, (2006) worked out the anticandidal activity of some culinary herbs including poppy seed *(Papaver somniferum)*. Qasem (2006) studied the parasitic weeds and allelopathy of various members of family Papaveraceae especially in *Argemone maxicana* and *Papaver*. Mohana *et al.*, (2009); Singh *et al.*, (2009) and Rahman *et al.*, (2009) separately studied the antibacterial activity of seed extracts of *Argemone mexicana* on some pathogenic bacterial strains. Review reveals that no such study was conducted on *Papaver pavoninum* commonly called Peacock poppy which is found wild from plains to 1900 meters and is a prominent weed of wheat crop especially in Malakand Division(Shinwari *et al.*,2006). It is an annual, 15-30 (-50) cm tall herb, native to Asia-temperate; Western Asia: Afghanistan, Iran; Middle Asia: Kazakhstan, Tajikistan, Turkmenistan, Uzbekistan; Asia-Tropical; Indian Subcontinent: Pakistan. Common on Stony slopes on low mountains, grasslands at field sides and arable lands. However as various other members of the family are known for their allelopathic properties, the present study was conducted to determine the allelopathic effects of *Papaver pavoninum* on Maize and Brassica.

Materials and methods

Papaver pavoninum was collected at flowering stage from the wheat fields of Batkhela, Malakand Agency in plastic bags, which was used for aqueous extraction. They were dried at room temperature (25-30°C), powdered and stored in paper bags. Glassware was thoroughly washed with water and sterilized at 170°C for 4 hours. There were 5 replicates, each with 10 seeds. The Petri dishes were incubated at 25°C for 72 hours.

Effect of aqueous extracts

Five and Ten g of dried powdered plant material was separately soaked in 100 ml distilled water at 25°C for 24 and 48 h and filtered to get aqueous extracts. These extracts were tested against Maize and Brassica. Ten seeds each were placed on 3-folds of filter paper in petri dishes which were moistened with the respective extracts or the distilled water in case of control. Five replicates were used for each treatment. After 72 hours, germination, growth of plumule and radical were noted. Twenty seedlings were randomly taken out for fresh weight determination. These seedlings were dried at 65°C for 72 h and dry weights were determined.

Effect of hot water extracts

Five g dried plant parts was boiled in 100 ml water for 5 minutes and filtered, cooled at room temperature and was applied against the same test species.

Effect of litter

Five g of crushed litter was placed in Petri dish and topped with single sheet of filter paper. The dishes were provided with 5 ml distilled water. In control treatment fine pieces of filter paper were used to keep it moist throughout the bioassay. The bioassay was run as before.

Effect of mulching

Five g crushed dried plant material was placed in plastic pots containing sterilized sand. There were five replicates, each with 10 seeds. Control consisted of fine pieces of filter paper. The plastic pots were incubated at 25°C. Germination, plumule and radical growth were measured after 24 days. Twenty seedlings were randomly taken out for the determination fresh, dry weight and moisture contents.

All the results were statistically analyzed in one way ANOVA.

Results and discussion

Effect of aqueous extract

Five g aqueous extracts significantly reduced the germination, plumule and radical length (No. of seminal roots in Maize only) of both Maize and Brassica at both the soaking durations (Table-1). Increasing soaking duration and concentration enhanced inhibition. Similar inhibitions were reported for *Dodonaea viscosa* (Barkatullah *et al.*, 2010), *Lactuca sativa* (Chon *et al.*, 2005), *Broussonetia papyrifera* (Hussain *et al.*, 2004), *Tamarindus indica* (Parvez *et al.*,2003) and *Anagalis arvensis* (Rebaz *et al.*, 2001). The five g 48h extract completely inhibited germination of Brassica, while ten g extract at both the soaking durations completely inhibited the germination of both the test spps similar to the phytoxicity of sorghum + sunflower water extracts (Mehmood *et al.,* 2010). These results suggest increase in phytotoxicity with increasing soaking duration and concentration.

Fresh weight and dry weight of both the test species also showed reduction (Table 1). These results agree with Kaul & Bansal (2002), who reported that *Ageratina adenphora* litter reduced growth of *Lantana camara*. Similarly, Maciel *et al.*, (2003) also observed similar results. However the moisture contents of the test species increased as compared to the control. This is in contradiction to the findings of

Table 1. Effect of Aqueous extract of *Papaver pavoninum* on percent germination, plumule and radical growth (mm), No. of seminal roots, Fresh and dry weight (mg), and percent moisture contents of Maize and Brassica. Each value is a mean of five replicates, each with 10 seeds.

Treatment	Germination (%)		plumule growth (mm)		Radicle growth (mm)		No. of seminal roots		Fresh weight (%of control)		Dry weight (%of control)		Moisture content (% of control)	
	Maize	Brassia	Maize	Brassia	Maize	Brassia	Maize	Brassia	Maize	Brassa	Maize	Brassia	Maize	Brassia
Control	86	100	37.58	28.64	39.86	8.42	4.32	-	-	-	-	-	-	-
5g/24 hrs soaking	92	18***	5.78***	1.2***	15.02***	2.36***	3.32	-	71.25	64.11	66.66	43.32	111.04	96.42
5g/48 hrs soaking	38***	0	2.02***	0	4.32***	0	1.12	-	60.00	0	43.33	0	161.60	0
10g/24 hrs soaking	0	0	0	0	0	0	0	-	0	0	0	0	0	0
10g/48hrs soaking	0	0	0	0	0	0	0	-	0	0	0	0	0	0

*significantly different from control at alpha 0.050 in one way ANOVA

**Highly significantly different from control at alpha 0.050 in one way ANOVA

*** Very highly significantly different from control at alpha 0.050 in one way ANOVA

Table 2. Effect of Hot Water extract & litter of *Papaver pavoninum* on percent germination (%), plumule and radical growth (mm), No. of seminal roots, Fresh and dry weight (mg), and percent moisture contents of Maize and Brassica. Each value is a mean of five replicates, each with 10 seeds

Treatment	Germination (%)		plumule growth (mm)		Radicle growth (mm)		No. of seminal roots		Fresh weight (%of control)		Dry weight (%of control)		Moisture content (% of control)	
	Maize	Brassia	Maize	Brassia	Maize	Brassia	Maize	Brassia	Maize	Brassa	Maize	Brassia	Maize	Brassia
Control	86	96	37.58	66.26	39.86	30.2	3.96	-	-	-	-	-	-	-
Hot extract	0	100	0	44.1**	0	25.8***	0	-	0	39.62	0	7.07	0	132.01
Litter	0	0	0	0	0	0	0	-	0	0	0	0	0	0

*significantly different from control at alpha 0.050 in one way ANOVA

**Highly significantly different from control at alpha 0.050 in one way ANOVA

*** Very highly significantly different from control at alpha 0.050 in one way ANOVA

Table 3. Effect of Mulch of *Papaver pavoninum* on percent germination, plumule and radical growth (mm), No. of seminal roots, Fresh and dry weight (mg), and percent moisture contents of Maize and Brassica. Each value is a mean of five replicates, each with 10 seeds.

Treatment	Germination (%)		plumule growth (mm)		Radicle growth (mm)		No. of seminal roots		Fresh weight (%of control)		Dry weight (%of control)		Moisture content (% of control)	
	Maize	Brassia	Maize	Brassia	Maize	Brassia	Maize	Brassia	Maize	Brassa	Maize	Brassia	Maize	Brassia
Control	80	96	34.02	66.26	75.5	30.2	3.84	-	-	-	-	-	-	-
Mulch	12***	0	35.24	0	13.9***	0	2.70	0	73.71	0	56.25	0	142.43	0

*significantly different from control at alpha 0.050 in one way ANOVA

**Highly significantly different from control at alpha 0.050 in one way ANOVA

*** Very highly significantly different from control at alpha 0.050 in one way ANOVA

Pervez *et al.*, (2003), Hussain *et al.*, (2004), Hussain & Ilahi (2009), Samreen *et al.*, (2009) and Barkatullah *et al.*, (2010). This might be due to excessive absorption of water by the cells in order to aquire an appropriate osmotic potential.

Effect of hot water extract

Hot water extract from the whole plant significantly inhibited the germination and seedling growth of Maize. It was also seen that hot water extracts has more inhibitory effect than the cold water extracts (Table-2). Chung *et al.*, (2007), Peneva (2007), Hussain *et al.*, (2004), Hussain & Ilahi (2009) and Barkatullah *et al.*, (2010) also reported similar results for hot water extracts against test species. However the plumule and radical growth significantly increased in Brassica which might be due to denaturation of some chemicals (toxic to Brassica), by heat. The use of hot water extract is unnatural but it reduces the time period for extraction of allelochemicals. Fresh weight, dry weight and moisture contents of test plant seedlings were generally reduced in various treatments. However, the extent of inhibition was specific to test species.

Effect of Litter

Plant litter generally increases soil fertility after decay but it has been seen that many species release phytotoxic substances before decay. It was observed that litter of *Papaver pavoninum* when used as growth medium totally inhibited the germination of both test spps (Table-2).

Effect of mulching

Papaver pavoninum mulch in experiments significantly inhibited the germination, plumule and radical growth and no. of seminal roots in Maize. While complete inhibition of germination occurred in Brassica. Fresh weight and dry weight of Maize also got retarded (Table 3).Allelopathic substances released by the plants accumulate in the soil to physiological activity level (Hussain *et al.*, 2004; Hussain and Ilahi, 2009; Samreen *et al.*, 2009). Inderjit & Duke (2003) stated that plants release phytochemicals from dead tissues, and their incorporation to the soil could be accelerated by leaching thus facilitating their harmful effects in the field. These findings agree with those of (Rebaz *et al.*, (2001) Hussain *et al.*, (2004), Eppard *et al.*, (2005) and Barkatullah *et al.*, (2010) who also observed similar phytotoxicity by other plants. However the moisture content increased as in case of aqueous extract.

The mode of action of allelochemicals spans over a wide range of actions including cell lysis, blistering or growth inhibition (Wu *et al.,* 2003). *Papaver pavoninum* contains N^2-methyl-1,2,3,4-tetrahydro-β-carboline as the dominant alkaloid, along with minor amounts of the tertiary bases protopine, allocryptopine, corydine, isocorydine and corytuberine and quaternary bases, coptisine, magnoflorine and an unidentified alkaloid PP 1 (Taborska *et al.*, 1988). Lagar *et al., (*2003) also reported presence of alkaloids in *Papaver pavoninu*, which are poisonous to mammals. These alkaloids might be responsible for its phytotoxicity also.

The present study suggests *Papaver pavoninum* to be allelopathic plant, which is capable of suppressing the germination and growth of both the test species. Hot and cold aqueous extracts, litter and mulches of various concentrations from entire plant of *Papaver pavoninum* exhibited allelopathic stress against the germination, seedling growth, fresh and dry weight of tested species.

Allelophathic effect also depended upon the test species, as Brassica is found to be more sensitive than Maize. Germination and growth were affected independently. Although the present results are laboratory based, yet it indicates the capability of *Papaver pavoninum* to release allelopathic substances. It is quite possible that the *Papaver pavoninum* might be one of the causes for reduction in our crop yields, due to its allelopathy. However, further study is needed to explain allelopathic mechanism and to identify the allelopathic principle. It may also be investigated to test its efficacy as a weeds, pests and disease control agent

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