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Allelopathic effect of aqueous leaf extracts of *withania somnifera* dual on germination and seedling growth of wheat

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Abstract
Allelopathic effect of leaves extract of Ashwagandha were examined on seed germination and seedling growth of four (Pusa Gold, GW 2008-156,HP-1731 & HD-2824) wheat varieties. The aqueous leaf extracts reduced per cent seed germination and seedling growth of wheat. On the basis of value of per cent reduction in seed germination and germination Relative Index (GRI), the inhibitory effect of different leaf extract was maximum in susceptible varieties (HP-1731 and HD-2824) followed by tolerant varieties (Pusa Gold and GW 2008-156). The shoot and root length and shoot and root dry weight declined as the concentration of the leaf extracts of Ashwagandha increased. Leaf extract of *Withania somnifera* showed inhibitory effect on sugar and protein and stimulatory effect on total amino acid content of wheat seedlings. The results indicated that the allelopathic effect of leaf extract *Withania somnifera* on tolerant varieties of wheat was maximum in terms of all physiological and biochemical parameters.

Keywords: Ashwagandha, aqueous leaf extracts, germination, wheat, seedling growth

Introduction
Withania somnifera (Ashwagandha) is a medicinal plant and has been used as an aphrodisiac, liver tonic, anti-inflammatory agent and astringent and more recently to treat bronchitis, asthma, ulcers, emaciation, insomnia and senile dementia.
Allelopathy is a biological phenomenon by which an organism produces one or more biochemical that influences the growth, survival and reproduction of other organism. Allelochemicals from plants are released into the environment by exudation from roots, leaching from stem and leaves or decomposition of plant material (Rice 1984; Rizvi and Rizvi 1992). Mathela (1994) reported that the secondary metabolities (flavonoids, glycosides, steroids and diterpenoids) of some medicinal and aromatic plants accounted for allelopathic activity likewise, Alagesaboopathi (2011) studies allelopathic effect of medicinal plants-*Andrographis paniculata* which showed inhibition in germination and seedling growth of lens culanaris.Allelopathic effect of Ashwagandha against the germinationand radicle growth of Cicer arietinum and *Triticum aestivum* (Chandra *et al.*. 2012) and allelopathic effects of leaf extract of kalmegh on seed germination and seedling growth of wheat (Mandal *et al*. 2016). The present study was thus aimed to determine the effect of aqueous leaf extracts of *Withania somnifera* on some physiological and biochemical changes during the seed germination and seedling growth.

Materials and Methods
The experiment was conducted during late kharif season 2014 in at Department of Botany and Plant Physiology, Faculty of Basic Sciences and Humanities, Rajendra Agricultural University Pusa, Samastipur, Bihar. There were shade dried for 10 days, then leaf powdered in grinders and sieved. For leaf extract 15g leaf powder was soaked in 100ml distilled water for 24 hours to get 15% extract. By dilutions with distilled water 2%, 4% and 6% concentrations of extracts were prepared. Seeds of *Triticum aestivum* were soaked in 0.1 per cent mercuric chloride for 2.0 minutes and thoroughly washed with deionized water. The seeds were germinated in distilled water (control) and leaf extracts (2.0%, 4.0% and 6.0%) in sterilized germinating boxes lined with blotting papers and kept at 25 ± 2^0C in BOD incubator under controlled conditions with three replications. The Germination Relative Index (GRI) was calculated as Sigma Xn(h-n) according to Srivastava Sareen (1972). Vigour Index (VI) was calculated according to Abdul Baki and Anderson (1973) as germination percentage x seedling length. Seven-day-old wheat seedlings were used for the extraction and determination of carbohydrate

as reducing sugar (Miller, 1959), soluble protein (Singh, 1980) and Lee and Takahashi (1996) for total amino acids.

Results and Discussion

Seed germination and Seedling growth

The values of germination and GRI of seeds after 7days old seedlings are presented in Table 1. With increase in the concentration of aqueous leaf extracts of Ashwagandha, the germination percentage of Pusa Gold, GW 2008-156, HP 1731 and HD 2824 varieties of wheat seeds significantly declined. Maximum decline in germination percentage was observed in susceptible varieties (HP 1731 & HD 2824) in comparsion to tolerant varieties (Pusa Gold and GW 2008-156). The GRI and Vigour Index (VI) of test varieties decreased with increase in concentration of leaf extracts. The interaction between treatments and varieties was found significant (P<0.05). Decreased germination and growth with increasing aqueous leaf extract have been observed in wheat (Abd EI Fattah *et al.* 2011. Arshad *et al.* 2011; Alagesaboopathi 2011) and Prasad *et al*. (2011) found similar results for germination of various crops with increase leaf extract concentration.

Shoot and root length invariably decreased as the level of leaf extracts increased (Table 1). Maximum value of shoot and root length was observed in distilled water (control). Length of shoot and root was higher in tolerant varieties viz. Pusa Gold and GW 2008-156 as compared to susceptible varieties viz. HP 1731 and HD 2824 at 2%, 4% and 6% leaf extract concentration. Reduction in length of shoot and root with increasing levels of leaf extract concentration was reported by Mandal *et al*. (2005). Similar trend was followed shoot and root length in *Withania somnifiera*, a maximum reduction was recorded in susceptible varieties in comparison to tolerant ones. The shoot and root length of test crop were reduced significantly at P<0.05 by application of *Tithonia diversifolia* extract. The result was further supported by the work of Arshad *et al*. (2011) who supported that water and methanol extract of *Withania somnifera* markedly suppressed the germination, shoot and root growth of *Parthenium hysterophorus*.

Dry weight of shoot and root of different varities of wheat significantly decreased as the concentration of leaf extract of *Withania somnifera* increased. In each leaf extract concentration segment, maximum values of shoot and root dry weight were recorded for tolerant varieties of wheat followed by susceptible varieties of wheat (Table1). The per cent reduction in biomass was maximum in susceptible wheat varities viz. HP 1731 and HD 2824 as compared to tolerant varieties of wheat viz. Pusa Gold and GW 2008-156. An inhibitory effect was noticed on the dry weight of seedling with increase in leaf extract concentration. Effects of leaf extract on all four varieties were found highly significant. The greater inhibition shoot and root growth observed in this study, was in accordance with findings of Otusanya *et al.* (2012), Arshad (2011), Alam (1990) and Kaur and Rao (1998) have also reported such effects of leaf extracts. The maximum reduction in biomass occurred in susceptible varieties.

Biochemical parameters

The level of reducing and no- reducing sugar in wheat seedlings was more in control than aqueous leaf extracts. The level was higher in tolerant varieties as compared to susceptible varieties (Table2). On increasing aqueous leaf extracts of Ashwagandha, the sugar contents of different varieties of wheat declined. Minimum sugar content was found susceptible varieties as compared to tolerant varieties of wheat seedlings less sugar might be produced due to reduced starch hydrolysis with increasing leaf extract concentration. Mandal *et al*. 2003 & 2005 also observed that sugar content of Wheat seedling decrease with increase in leachate concentration of Dalbergia sisso, Acacia lenticularis, Bombax ceiba and Populus deltoides. Similar findings were reported by Padhay *et al*. (2000) also observed that sugar content of finger millet seedlings decreased with increase in leachate concentration of Eucalyptus.

As regards to soluble total proteins of 7 day-old seedlings, tolerant and susceptible varieties showed decreasing trend with increasing leaf extract concentration. However, protein value was higher in tolerant varieties as compared to susceptible varieties of wheat (Table-2). The protein content decreased linearly with increase in leaf extract concentration in the same way as reported by Mandal *et al*. (2003).The protrin content decreased and that of the amino acids increased with leaf extract concentration. Similar findings were reported by Ali and Mandal (2009). Decrease in protein might be due to disturbance in protein metabolism. The free amino acid content followed a reverse trend of increase at each leaf extract concentration in the all four varieties. The maximum value was found in tolerant group at 6% leaf extract concentration. The interactions between treatments and varieties for protein and total amino acids were highly significant (P<0.05).

The laboratory experiment results in the present study indicated the allelopathic effect of Withania somnifera on seed germination and seedling growth of different varieties of wheat. Analysis of toxic chemicals present in the leaf leachates and field experiments are however, necessary before any final conclusions are drawn on allelopathic effect of Ashwagandha species.

Table 1: Impact of aqueous leaf extracts of *Withania somnifera* Dunal of germination, growth and biomass of wheat at 7 days after sowing.

Aqueous Leaf Extract concentration	Wheat varieties	Germination (%)	GRI	Vigour Index	Shoot length (cm)	Root length (cm)	Shoot dry weight (mg)	Root dry weight (mg)
	Pusa Gold	100.0	300.0	2491.0	12.7	12.1	149.0	86.0
Control (0%)	GW 2008156	98.0	298.3	2743.0	14.0	13.8	145.0	84.5
	HP1731	100.0	300.0	2587.0	13.0	12.8	144.0	85.2
	HD2824	98.3	297.6	2759.1	14.1	13.9	144.5	83.9
	Mean	99.o	298.9	2645.0	13.4	13.1	145.6	84.9
2%	Pusa Gold	96.6	278.3	2226.3	11.9	11.0	138.9	76.8
	GW2008-156	95.0	276.0	2436.7	13.0	12.6	133.0	74.0
	HP1731	93.3	269.6	2263.7	10.6	9.9	129.5	72.2
	HD 2824	91.6	265.0	2251.4	11.4	10.4	128.0	70.9
	Mean	94.1	272.2	2294.5	11.7	10.9	132.3	73.4

4%	Pusa Gold	91.6	262.3	1968.1	11.1	10.3	130.5	72.1
	GW2008-156	90.0	258.6	2140.2	12.1	11.6	126.8	69.1
	HP 1731	88.3	244.3	1819.5	9.3	8.6	121.8	65.7
	HD 2824	85.0	239.6	1882.7	9.7	9.2	120.0	62.5
	Mean	88.7	251.2	1952.6	10.5	9.9	124.7	6.3
6%	Pusa Gold	88.3	236.3	1661.8	9.8	8.9	118.0	63.5
	GW 2008-156	86.6	230.0	1788.0	10.6	9.9	113.9	59.3
	HP 1731	80.0	198.3	1417.6	7.4	6.9	103.9	51.4
	HD 2824	76.6	193.6	1433.7	7.4	7.2	99.5	48.3
	Mean	82.8	214.5	1575.2	8.8	8.2	108.8	55.6
	CD (0.05)							
	Stress (s)	0.4	13.7	112.3	0.7	0.6	5.2	1.1
	Genotypes (G)	0.4	13.7	112.3	0.7	0.6	5.2	1.1
	Interaction (S X G)	0.9	27.5	224.7	1.4	1.3	10.3	2.2

Table 2: Impact of aqueous leaf extracts of *Withania somnifera* Dunal sugar,protein and free amino acid wheat at 7-days after sowing.

Aqueous leaf extract conc. (%)	Wheat genotypes	Reducing sugar (mg/g.d.w.)	Non reducing sugar (mg/g.d.w.)	Total Sugar (mg/g.d.w.)	Soluble protein (mg/g.d.w.)	Free amino (mg/g.d.w.)
Control (0%)	Pusa Gold	24.8	28.7	53.5	38.6	19.9
	Gw 2008-156	24.7	28.6	53.3	38.5	20.0
	HP1731	24.8	29.5	54.3	38.5	19.8
	HD2824	24.7	29.7	54.4	38.5	19.7
	Mean	24.7	29.1	53.8	38.5	19.8
2%	Pusa Gold	23.4	26.8	50.2	35.6	22.2
	GW2008-156	23.1	26.7	49.8	35.2	22.4
	HP1731	20.7	25.4	46.1	33.8	23.4
	HD2824	20.4	25.5	45.9	33.4	23.5
	Mean	21.9	26.1	48.0	34.5	22.8
4%	Pusa Gold	21.7	24.8	46.5	33.6	23.8
	GW 2 008-156	21.3	24.8	46.1	33.2	24.0
	HP1731	17.2	18.0	35.2	30.8	25.6
	HD 2824	16.9	17.8	34.7	30.4	25.8
	Mean	19.2	21.3	40.6	32.0	24.8
6%	Pusa Gold	19.4	21.4	40.8	29.8	25.3
	GW2008-156	19.1	21.0	40.1	29.5	25.2
	HP1731	13.4	16.5	29.9	24.7	27.8
	HD2824	12.9	16.2	29.2	24.2	29.1
	Mean	16.2	18.7	35.0	27.0	26.6
	CD(0.05)					
	Stress (s)	0.9	0.9	0.7	0.8	0.8
	Genotypes(G)	0.9	0.9	0.7	0.8	0.8
	Interaction (S X G)	1.8	1.9	1.4	1.6	1.6

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