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ORIGINAL RESEARCH ARTICLE

Evaluation of productivity and quality of *Withania somnifera* (L.) Dunal under different plant populations, irrigation levels and moisture conservation practices in sub-tropical plains of North India

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ABSTRACT

The roots of Withania somnifera (L.) Dunal is utilized to treat various ailments, including bronchitis, dropsy, stomach issues, lung inflammation, tuberculosis, asthma, skin conditions as well as male impotency. The demand for quality raw material of W. somnifera is increasing in the global market, which can be met through enhancing productivity with better quality by adopting appropriate agricultural practices. However, the complete information on input efficient agro-practices is still limited on this plant. Hence, the present study is planned to optimize the plant population, irrigation level and moisture conservation practice for enhancing the productivity and quality of its roots. Two field experiments were conducted at CSIR-Central Institute of Medicinal and Aromatic Plants farm, Lucknow, comprising eight plant populations (166,666, 200,000, 250,000, 333,333, 400,000, 500,000, 666,666, and 1,000,000 plants ha^{-1}) in first and having four irrigation levels (rainfed, 20±5% total available water (TAW), 40±5% TAW and 60±5% TAW) with three moisture conservation practices (dust mulch, distillation waste mulch, and control) in two consecutive years (2015-16 and 2016-17) in second. Results revealed that plant population of 400,000 plants ha^{-1} provided the maximum root yield (1030 kg ha^{-1}), total withanolide yield (3.64 kg ha^{-1}) and net return (88,453 INR ha^{-1}). In the second experiment, the irrigation at 20±5% TAW with dust mulch was recorded to be an appropriate combination for obtaining the highest biological yield (696 kg ha^{-1}) and yield of biologically active substances (3.62 kg ha^{-1}). Net return was recorded highest (90, 980 INR ha^{-1}) when irrigations were applied at 20±5% TAW with dust mulch. Thus, plant population at 400,000 plants ha^{-1}, providing irrigation at 20±5% TAW and using dust mulch for moisture conservation practice is recommended for obtaining higher yield and profit in the cultivation of Withania under subtropical plains of India.

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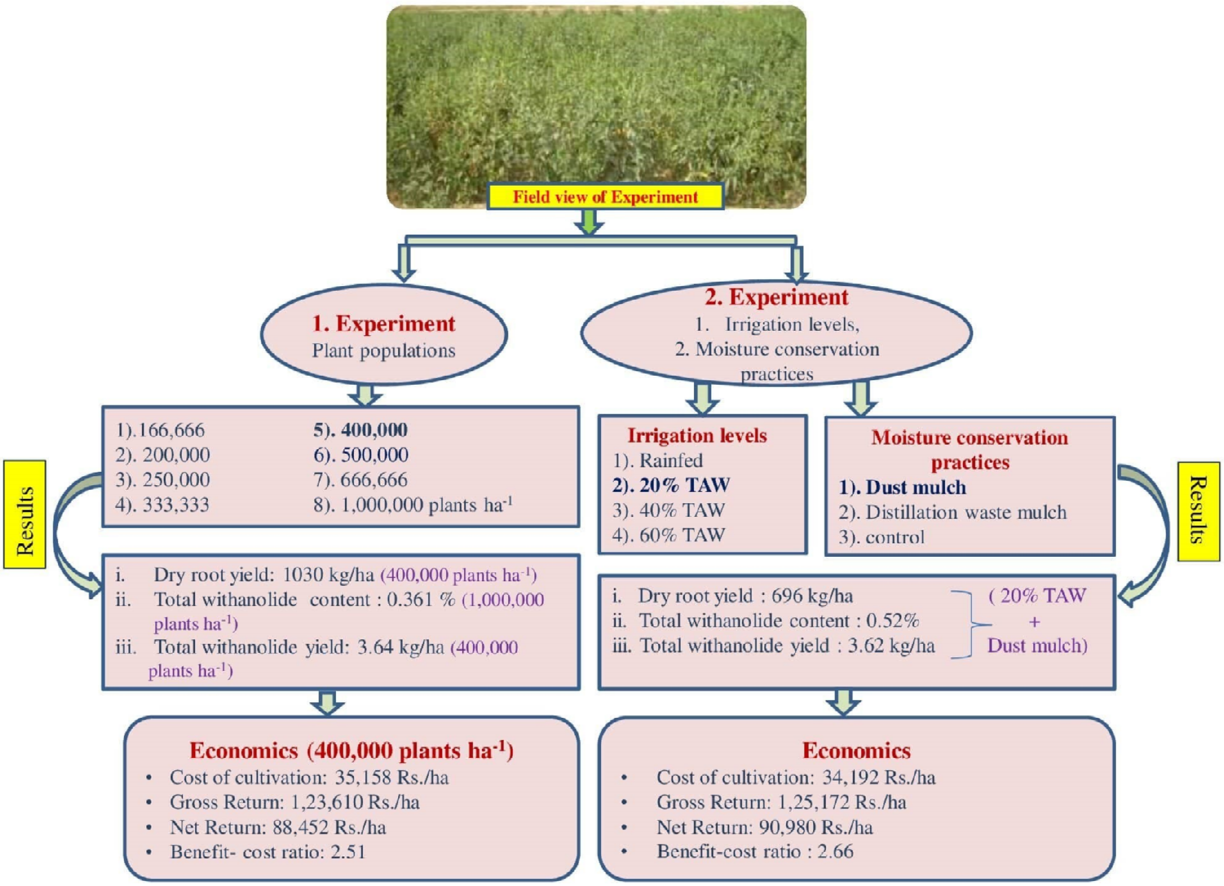
GRAPHICAL ABSTRACT



INTRODUCTION

The medicinal plant *Withania somnifera* (L.) Dunal, known as the 'winter cherry', Indian ginseng as well as ashwagandha, is classified under the family Solanaceae. This industrially important medicinal plant is cultivated worldwide in semi- arid and arid zone such as India, South-Africa, Morocco, Jordan, Pakistan, Baluchistan, Sind, Egypt, Afghanistan and distributed in the Mediterranean regions (Uddin et al., 2012). In India, its commercial production is done in the states like Gujarat, Madhya Pradesh, Punjab, Uttar Pradesh, Haryana, Maharashtra, Rajasthan and Telangana (Umadevi et al., 2012). Number of medicines is formulated from the roots of this plant, used for the treatment of several ailments such as hepatic, cardiovascular, immunological, neurological and metabolic disorders such as diabetes; also used as an energy booster and anti-stress tonic (Mishra et al., 2000).

The biologically active chemical constituents present in its roots are withanolides and withanone. Being an important medicinal plant and having vast applications, its demand in India is about 7000 tons per annum; however, only 1500 tons are being produced (Shinde et al., 2015). Hence, there is a necessity to enhance its cultivation and production at a large scale to fulfil the demand. Though the crop grows profusely up to 1m height and spreads fairly under natural conditions, the optimization of plant population in *W. somnifera* plays an essential role in enhancing the productivity and quality of its roots. Further, numerous associated factors are responsible for variation in plant population in different agro-climatic regions. Few experiments have been conducted to optimize the plant spacing in *W. somnifera* by some researchers like Pakkiyanathan et al. (2004), Nigam et al. (1984), Aggarwal et al. (2004) and Farooqui and Sreenivas (2001) and recorded significant impact on morphological features and root yield. However, optimization of the plant population of *W. somnifera*, especially to establish it as a winter crop is still lacking as this crop is mainly cultivated under rainfed conditions. Another essential area of research in *W. somnifera* is optimization of its irrigation requirement and moisture conservation practices because irrigation levels possess a significant effect on yield and morphological features as well as quality parameters. Organic mulches are used for different aromatic and medicinal crops *viz*., citronella java (Singh et al., 2001); geranium (Ram et al., 2003); mulberry (Shashidhar et al., 2008) and basil (Palada et al., 2000) which affect growth, yield and quality of these plants significantly. Different types of mulches have been used to obtain good crop growth and yield in other food crops like *Musa acuminata* (Ssali et al., 2003); *Lycopersicon esculentum* Mill. (Rahman et al., 2006); vegetables (Richard et al., 2002) and strawberry (Kumar and Dey, 2011). However, till date, no rigorous effort has been made to optimize the plant population and to test the interaction effect of moisture levels with mulching practices for *W. somnifera* in northern Indian plains. Considering these points, two field experiments were conducted, one for optimizing plant population, and another for optimizing irrigation level and moisture conservation practice to enhance the root yield and quality, increase water use efficiency and to reduce the cost of cultivation with enhanced net returns under sub-tropical conditions of northern Indian plains. These experiments are aimed to introduce a suitable package of practices to establish *W. somnifera* as a winter season crop in the plains of north India.

MATERIALS AND METHODS

Field sites descriptions

The two sets of field experiments were executed at CSIR-CIMAP, Lucknow (latitude 26.82Ú N, longitude 80.96Ú E with an altitude 120 m above sea level) for two consecutive years (2015-16 and 2016-17) from September to March. The experimental site witnesses sub-tropical climate with distinct summer, rainy and winter seasons. The soil was sandy loam, pH 8.4, having available N, P and K as 136, 45, and 140 kg ha^{-1}, respectively.

Experimental details

The seeds of *Poshita* cultivar of *ashwagandha* were sown in September in different line spacings to achieve various plant populations for the first experiment. Sowing was done at a uniform line spacing of 20×10 cm for second experiment. The recommended cultural practices for growing *ashwagandha* were followed, as suggested by Jat et al. (2015).

The details of two distinct field experiments are described below: -

Optimization of plant population

The experiment comprised of eight plant populations *viz*. a) 166,666 plants ha^{-1} (30×20 cm spacing); b) 200,000 plants ha^{-1} (25×20 cm spacing); c) 250,000 plants ha^{-1} (20×20 cm spacing); d) 333,333 plants ha^{-1} (15×20 cm spacing); e) 400,000 plants ha^{-1} (25×10 cm spacing); f) 500,000 plants ha^{-1} (20×10 cm spacing); g) 666,666 plants ha^{-1} (15×10 cm spacing); and h) 1,000,000 plants ha^{-1} (10×10 cm spacing). The experiment was conducted in a RBD (randomized block design) in triplicates during the early winter season of 2015-16 and 2016-17. The seeds of improved cv. Poshita, of CSIR-CIMAP were used for sowing in the first week of September and the harvesting was done in the first week of March in both the years.

Studies on irrigation level and moisture conservation practice

Twelve treatment combinations, comprising of four levels of irrigation *viz*. a) rainfed, b) 20±5% total available water (TAW), c) 40±5% TAW, and d) 60±5% TAW and three moisture conservation practices *viz*. a) dust mulch, b) distillation waste mulch and c) no mulch, were laid out in split-plot design with three replications. Dust mulch was created by shallow tilling (1-2 cm deep) of the upper soil layer through hand hoe. Distillation waste mulch was applied by using spent of aromatic grasses @2.5 kg m^{-2} (*Cymbopogon winterianus* Jowitt ex Bor., *Cymbopogon martini*, *Cymbopogon flexuosus*).

Thefollowing formula for optimization of irrigation levels (Estefan et al., 2013) was used:

$$\text{Total available water (TAW)\%} = \left(\frac{\text{FC} - \text{PWP}}{100}\right) \times \text{BD} \times \text{d} \qquad \ldots 1$$

Where FC= Field Capacity (%), PWP= Permanent Wilting Point (%), BD= Bulk Density (g cm^{-3}) and d= Depth of Root Zone (cm).

Observations and analysis

In both the experiments, harvesting was done at the start of flowering. The dry weight of shoot and root was recorded from each treatment. Properly dried roots were subjected to primary processing by cutting them into small pieces of 7-10 cm, then the roots were grinded into powder form and sieved through 0.2 mm sieve for quality analysis.

For *quality analysis* roots were dried in 100% shade for determination of withanolide A, withanone and total withanolide content (%). Processing was done as explained by Khajuria et al. (2004). Reverse HPLC analysis was performed to extract withanolide A, withanone and total withanolide content (Srivastava et al., 2018).

Water requirement, consumptive use of water and water use efficiency

The calculation of water requirement was done by using the formula (Eq. 2) by Kumar et al. (2021).

$$\text{WR} = \text{Depth of theirrigation applied (mm)} \times \text{numberof irrigation} + \text{effective rainfall (mm)} \ldots 2$$

The consumptive use of water between the periods of two successive irrigations was worked out by the formula (Eq. 3) given below:

$$\text{CU} = \frac{\text{M2} - \text{M1}}{100} \times \text{Asi} \times \text{Di} + \text{ER} \qquad \ldots 3$$

Where, CU=water in mm from the root zone (500 mm) between two successive sampling periods, M1= soil moisture (%) by weight before irrigation, M2= soil moisture (%) by weight after irrigation, Asi= Apparent specific gravity of the 50 cm deep layer of soil, Di= depth of root zone (45 cm), ER= Effective rainfall received throughout the crop growth duration in mm.

The Water use efficiency calculated by applying the formula Eq. 4 (Dastane, 1972).

$$\text{WUE} = \frac{\text{Root yield (kg ha}^{-1}\text{)}}{\text{Consumptive use of water (mm)}} \qquad \ldots 4$$

For the computation of gross income, prevailing market price of ashwagandha dry roots (Rs. 120 kg^{-1}) was considered and expressed in rupees (INR) per hectare.

The data was calculated separately for both the experimental years. According to the Panse and Sukhatme (1985), the average of both years was statistically analyzed with a significance level of 5% (P=0.05).

RESULTS AND DISCUSSION

Effect of plant population

The maximum dry shoot (5850 kg ha^{-1}) and root yield (1030 kg ha^{-1}) were recorded at plant population of 166,666 and 400,000 plants ha^{-1}, respectively (Table 1). The maximum dry root yield was at par with plant population of 500,000 plants ha^{-1} (1020 kg ha^{-1}). Statistically significant reduction in dry root yield was recorded when plant population was reduced from 400,000 to 166,666 plants ha^{-1} and enhanced from 500,000 plants ha^{-1} level. The root: shoot ratio was highest (0.23) at plant population of 400,000 plants ha^{-1}, which was statistically at par with 500,000 and 666,666 plants ha^{-1} whereas the minimum (0.10) root: shoot ratio was observed at plant population of 166,666 plants ha^{-1}.

The enhancement in the dry root yield with increasing plant population was due to increase in the number of roots, which could not be compensated by the greater thickness of roots

Table 1: Biological yield, content and yield of biologically active substances in *Withania somnifera* (L.) Dunal as influenced by plant populations

Plant population (plants ha^{-1})	Dry shoot yield (kg ha^{-1})	Dry root yield (kg ha^{-1})	Root:shoot Ratio	Withanolide A content (%)	Withanone content (%)	Total withanolide content (%)	Withanolide A yield (kg ha^{-1})	Withanone yield (kg ha^{-1})	Total withanolide yield (kg ha^{-1})
166,666	5850^{a}	610^{e}	0.10^{f}	0.280^{b}	0.054^{a}	0.334	1.71^{e}	0.33^{e}	2.04^{e}
200,000	5550^{a}	690^{d}	0.12^{e}	0.280^{b}	0.053^{a}	0.333	1.93^{d}	0.37^{d}	2.30^{d}
250,000	5370^{b}	810^{c}	0.15^{d}	0.285^{b}	0.053^{a}	0.338	2.31^{c}	0.43^{b}	2.74^{c}
333,333	5050^{b}	890^{b}	0.18^{c}	0.290^{b}	0.052^{a}	0.342	2.58^{b}	0.46^{b}	3.04^{b}
400,000	4550^{c}	1030^{a}	0.23^{a}	0.303^{a}	0.051^{b}	0.354	3.12^{a}	0.52^{a}	3.64^{a}
500,000	4810^{c}	1020^{a}	0.21^{b}	0.305^{a}	0.050^{b}	0.355	3.11^{a}	0.51^{a}	3.62^{a}
666,666	4040^{d}	850^{b}	0.21^{b}	0.310^{a}	0.048^{b}	0.358	2.64^{b}	0.41^{c}	3.04^{b}
1,000,000	3320^{d}	590^{e}	0.18^{c}	0.315^{a}	0.046^{c}	0.361	1.86^{d}	0.27^{f}	2.13^{d}
S Em±	109.3	18.9	0.004	0.006	0.001	0.007	0.06	0.01	0.08
CD at 5%	331.8	57.4	0.012	0.018	0.003	NS	0.16	0.03	0.23

Letters presenting the difference (P=0.05), NS= Non Significant

recorded under lower plant population. Due to differences in availability of light, moisture, and nutrients at different plant populations, variations in growth and dry matter accumulation were observed. The maximum dry root yield at plant population of 400,000 plants ha^{-1} is because of reduced interplant competition, better solar radiation, more absorption of soil moisture and nutrients. Similar results were also reported in *Mentha arvensis* L. (Kumar et al., 2021) and *Anethum graveolense* L. (Callan et al., 2007).

The withanolide A-content in roots was highest (0.315%) at plant population of 1,000,000 plants ha^{-1}, which was at par with 666,666, 500,000, and 400,000 plants ha^{-1}. The lowest (0.280%) withanolide A-content was observed at lower plant populations (166,666 and 200,000 plants ha^{-1}). The reverse trend was observed for withanone content which was reported maximum (0.054%) at plant population of 166,666 plants ha^{-1} and minimum (0.046%) at 1,000,000 plants ha^{-1}. However, total withanolide content was not affected significantly by plant population. The withanolide A yield was maximum (3.12 kg ha^{-1}) at plant population of 400,000 plants ha^{-1}, at par with 500,000 plants ha^{-1}, and lowest (1.71 kg ha^{-1}) at 166,666 plants ha^{-1}. However, maximum (0.52 kg ha^{-1}) withanone yield was recorded at plant population of 400,000 plants ha^{-1}, at par with 500,000 plants ha^{-1} (0.51 kg ha^{-1}). Similarly, the total withanolide yield was observed maximum (3.64 kg ha^{-1}) at plant population of 400,000 plants ha^{-1} which was at par with 500,000 plants ha^{-1} and the lowest(2.04 kg ha^{-1}) at 166,666 plants ha^{-1}. However, due to the low root yield in the low plant population treatments, the yield of these biologically active substancesis recorded low. Reddy and Krishna (1992) observed that optimum plant population produced higher solasodine yield than lower plant population in *Solanum viarum*. Similarly, Kumar et al. (2021) also worked on plant density in *Mentha arvensis* L. and reported that the optimum plant density plays an important role in more plant secondary metabolites yield.

Table 2: Economic (In Rupees) of *Withania somnifera* (L.) Dunal as influenced by plant populations

Plant population (plants ha^{-1})	Cost of cultivation (Rs. ha^{-1})	Gross return (Rs. ha^{-1})	Net return (Rs. ha^{-1})	Benefit-cost ratio
166,666	33969	73215^{e}	39246^{f}	1.16^{e}
200,000	34340	82803^{d}	48463^{e}	1.41^{d}
250,000	34712	97223^{c}	62511^{d}	1.80^{c}
333,333	34935	106812^{b}	71877^{b}	2.06^{b}
400,000	35158	123610^{a}	88452^{a}	2.51^{a}
500,000	35307	122421^{a}	87114^{a}	2.47^{a}
666,666	35530	101980^{b}	66451^{c}	1.87^{b}
1,000,000	35976	70836^{e}	34786^{g}	0.97^{f}
S Em±	708	1843	1181	0.03
CD at 5%	NS	5597	3590	0.11

*Letters presenting the difference (P=0.05), NS= non-Significant

For growing *ashwagandha*, maximum cost of cultivation (35,976 INR ha^{-1}) was recorded at plant population of 1,000,000 plants ha^{-1} followed by 666,666 plants ha^{-1} (35,530 INR ha^{-1}) while the lowest cost of cultivation (33,969 INR ha^{-1}) was calculatedat plant population of 166,666 plants ha^{-1}. The reason for higher cost of cultivation for higher plant population is due to higher seed requirement and higher money and time incurred for weeding and harvesting operations. The gross and net returns were recorded highest (1,23,610 and 88,452 INR ha^{-1}) at plant population of 400,000 plants ha^{-1}. The lowest gross and net returns (70,836 and 34,786 INR ha^{-1}) were computed at plant population of 1,000,000 plants ha^{-1}. The plant population of 400,000 plants ha^{-1} indicated the highest (2.51) benefit-cost ratio, which was closely followed by 500,000 plants ha^{-1} (2.47) while, 1,000,000 plants ha^{-1} registered minimum (0.97) B:C ratio. Similarly, Singh et al. (2003) found that growing *W. somnifera* is more economical at (100×10^3 and 200×10^3 plants ha^{-1}) because planting at an ideal density with a suitable spacing is an imperative thought to make strides in the efficiency of the crop.

Effect of irrigation levels and moisture conservation practices

Biological yield (dry shoot yield, dry root yield and root: shoot ratio)

A significant impact of different irrigation levels and moisture conservation practices on the dry shoot and dry root yield of ashwagandha was observed (Table 3). 20±5% total available water (TAW) recorded maximum (1210 kg ha^{-1}) dry shoot yield and minimum yield (744 kg ha^{-1}) was found at 60±5% TAW. The highest dry shoot yield (950 kg ha^{-1}) was recorded in dust mulch over control and distillation waste mulch. The interaction effect of treatments indicated that providing irrigation at 20±5% TAW with dust mulch yielded highest dry shoot yield of 1255 kg ha^{-1}.

Similarly, maximum (607 kg ha^{-1}) dry root yield was recorded at 20±5% TAW, and it was significantly superior over dry root yield of other irrigation levels. Irrespective of irrigation levels, the highest dry root yield (499 kg ha^{-1}) was recorded in dust mulch. Interaction effect of treatments showed that maximum (696 kg ha^{-1}) dry root yield was obtained when irrigation was applied at 20±5% TAW with dust mulch was created.

The maximum (0.50) root: shoot ratio was recorded when irrigation was applied at 20±5% TAW and recorded minimum (0.44) at highest irrigation level (60±5% TAW). Among different moisture conservation practices, maximum (0.52) root: shoot ratio was recorded in dust mulch. Interaction effect of irrigation level and moisture conservation practices showed that maximum (0.55) root: shoot ratio was recorded at 20±5% TAW with dust mulch. Higher production of root at 20±5% TAW is because every crop shows significant growth and fair yield at optimum moisture level when proper aeration occurs.

Ashwagandha is a hardy crop that can grow in limited total available water but providing scheduled irrigation enhanced its productivity. In contrast irrigation levels 60±5% and 40±5% TAW was not found appropriate because more water creates an anoxic condition near the root zone, which causes poor growth of the plants and roots. Nilofer et al. (2018) reported that maximum biological yield was recorded when irrigation was applied at 20% ASM in the medicinal plant *Cassia angustifolia* Vahl. It is general observation that in rainfed condition, crop growth is not luxuriant. The increase in the number of irrigations in Faba bean appeared to be directly related to an increase in yield (Ahmed et al., 2000). Further Halepyati et al. (2002) reported that the growth parameters and fresh weight of tuberose plant are increased under optimum moisture condition.

Dust mulch provided better aeration which helped in root proliferation in comparison to organic mulch and created an environment that allows greater nutrient uptake along with enhanced root growth and also the potential regeneration of the damaged root system. Dust mulch breaks the soil capillarity and thus reduces the loss of soil moisture. Kumar and Dey (2011) observed that mulch was effective in conserving the soil moisture. Pirboneh et al. (2012) reported that yield and yield

Table 3: Biological yield, content and yield of biologically active substances in *Withania somnifera* (L.) Dunal as influenced by irrigation levels and moisture conservation practices

Biological yield												
	Dry shoot yield (kg ha^{-1})				Dry root yield (kg ha^{-1})				Root:shoot Ratio			
Moisture conservations (M) Irrigation levels(I)	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean
Rainfed	857^{c}	891^{b}	897^{b}	882^{b}	368^{b}	492^{b}	438^{b}	432^{b}	0.43^{b}	0.55^{a}	0.49^{b}	0.49^{a}
20 % TAW	1134^{a}	1255^{a}	1242^{a}	1210^{a}	522^{a}	696^{a}	602^{a}	607^{a}	0.46^{a}	0.55^{a}	0.49^{b}	0.50^{a}
40 % TAW	922^{b}	912^{b}	890^{b}	908^{b}	386^{b}	469^{b}	463^{b}	439^{b}	0.42^{c}	0.51^{c}	0.52^{a}	0.48^{b}
60 % TAW	775^{d}	743^{c}	715^{c}	744^{c}	329^{c}	340^{c}	303^{c}	324^{c}	0.42^{c}	0.46^{b}	0.42^{c}	0.44^{c}
Mean	922	950	936		401^{c}	499^{a}	452^{b}		0.43^{c}	0.52^{a}	0.48^{b}	
	S Em±	CD at 5%			S Em±	CD at 5%				S Em±	CD at 5%	
Irrigation level (I)	9.16	27.22			6.91	20.53				0.01	0.03	
Moisture conservation (M)	12.53	NS			7.42	29.12				0.01	0.04	
I ×M	15.87	47.14			11.97	35.56				0.02	0.05	
Content of biologically active substance.												
	Withanolide A content (%)				Withanone content (%)				Total withanolide content (%)			
Moisture conservations (M) Irrigation levels (I)	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean
Rainfed	0.24	0.24	0.24	0.24^{b}	0.19	0.20	0.20	0.20^{b}	0.43	0.44	0.44	0.44^{b}
20 % TAW	0.25	0.29	0.25	0.26^{a}	0.20	0.24	0.21	0.22^{a}	0.45	0.52	0.46	0.48^{a}
40 % TAW	0.22	0.25	0.24	0.23^{c}	0.18	0.20	0.19	0.19^{c}	0.40	0.45	0.43	0.43^{c}
60 % TAW	0.22	0.23	0.21	0.22^{d}	0.18	0.19	0.18	0.18^{d}	0.40	0.42	0.39	0.40^{d}
Mean	0.23^{c}	0.25^{a}	0.24^{b}		0.19^{b}	0.21^{a}	0.19^{b}		0.42^{c}	0.46^{a}	0.43^{b}	
	S Em±	CD at 5%			S Em±	CD at 5%			S Em±	CD at 5%		
Irrigation level (I)	0.008	0.023			0.004	0.010			0.007	0.020		
Moisture conservation (M)	0.004	0.016			0.003	0.010			0.006	0.024		
I ×M	0.013	NS			0.006	NS			0.012	NS		
Yield of biological active substances.												
	Withanolide A yield (kg ha^{-1})				Withanone yield (kg ha-^{1})				Total withanolide yield (kg ha^{-1})			
Moisture conservations (M) Irrigation levels(I)	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean
Rainfed	0.86^{b}	1.20^{b}	1.06^{b}	1.04^{b}	0.71	0.98^{b}	0.87^{b}	0.85^{b}	1.57^{b}	2.18^{b}	1.93^{b}	1.89^{b}
20 % TAW	1.29^{a}	1.99^{a}	1.52^{a}	1.60^{a}	1.06	1.63^{a}	1.25^{a}	1.31^{a}	2.35^{a}	3.62^{a}	2.76^{a}	2.91^{a}
40 % TAW	0.85^{b}	1.15^{b}	1.09^{b}	1.03^{b}	0.70	0.94^{b}	0.89^{b}	0.84^{b}	1.54^{b}	2.09^{b}	1.98^{b}	1.87^{b}
60 % TAW	0.72^{c}	0.78^{c}	0.65^{c}	0.71^{c}	0.59	0.64^{c}	0.53^{c}	0.59^{c}	1.31^{c}	1.41^{c}	1.18^{c}	1.30^{c}

Letters presenting the difference (P=0.05), NS= Non Significant, I=Irrigation level, M= Moisture conservation, and TAW= Total Available Water

contributing traits improved in brinjal by using straw mulch. In another experiment, seed yield in ashwagandha was significantly affected when mulching was done with black plastic foil (Obidoska et al., 2004) and the percentage of germination of seeds was also enhanced significantly. Kumar et al. (2014) found that utilizing poplar and sick oak leaf mulches to increase soil nutrient availability is crucial and that stevia plants respond well to these mulches.

Quality content of biologically active substances (withanolide A, withanone and total withanolide content)

Results presented in Table 3 revealed that all these constituents were recorded maximum [withanolides A (0.26%), withanone (0.22%) and total withanolide content (0.48%)] at 20±5% TAW, followed by rainfed condition and lowest values were reported in highest irrigation level. Maximum content of biologically active substances [withanolide A (0.25%), withanone (0.21%) and total withanolide content (0.46%)] were observed where dust mulch was created.

Interaction of irrigation levels and moisture conservation practices showed that maximum withanolide A content (0.29%), withanone (0.24%) and total withanolide content (0.52%) was recorded at 20±5% TAW and dust mulch. The possible reason is the moderate moisture provided, which created stress condition and thus, plant synthesized more secondary metabolites under 20±5% TAW condition. However, some plants synthesize more metabolite under higher availability of water, whereas some perform better under moderate moisture. In continuation with this, Liu et al. (2011) observed that secondary metabolites accumulation in plants enhanced under stress conditions as a defence mechanism whereas Nilofer et al. (2018) reported a higher sennosides under rainfed condition in *Cassia angustifolia* Vahl. Numerous reports shows increased production of secondary metabolites during water stress condition in some medicinal plants such as *Papaver somniferum* (Szabo et al., 2003), *Bupleuri radix* (Zhu et al., 2009), *Scrophularia ningpeoensis* (Wang et al., 2010).

Table 4: Water requirement, Consumptive use of water and Water use efficiency as influenced by irrigation levels and moisture conservation practices

	Water requirement (mm)				Consumptive use of water (mm)				Water use efficiency (kg ha^{-1} mm^{-1})			
Moisture conservations (M) Irrigation levels (I)	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean
Rainfed	104.5	104.5	104.5	104.5^{d}	104.5^{d}	104.5^{d}	104.5^{d}	104.5^{d}	3.52^{a}	4.71^{a}	4.19^{a}	4.14^{a}
20 % TAW	440.2	440.2	440.2	440.2^{c}	330.0^{c}	247.5^{c}	231.0^{c}	269.5^{c}	1.58^{b}	2.81^{b}	2.61^{b}	2.33^{b}
40 % TAW	578.8	578.8	578.8	578.8^{b}	450.5^{b}	337.8^{b}	315.4^{b}	367.9^{b}	0.86^{c}	1.39^{c}	1.47^{c}	1.24^{c}
60 % TAW	690.0	690.0	690.0	690.0^{a}	470.0^{a}	352.5^{a}	329.0^{a}	383.8^{a}	0.70^{d}	0.97^{d}	0.92^{d}	0.86^{d}
Mean	453.4	453.4	453.4		338.7^{a}	260.6^{b}	245.0^{c}		1.66^{c}	2.47^{a}	2.30^{b}	
	S Em±	CD at 5%			S Em±	CD at 5%			S Em±	CD at 5%		
Irrigation level (I)	6.52	19.38			2.92	8.68			0.04	0.12		
Moisture conservation (M)	5.91	NS			3.75	14.71			0.03	0.11		
I ×M	11.30	NS			5.06	15.03			0.07	0.20		

Letters presenting the difference (P=0.05), NS= Non Significant, I=Irrigation level, M= Moisture conservation, and TAW= Total Available Water

Table 5: Economics of *Withania somnifera* (L.) Dunal as influenced by irrigation levels and moisture conservation practices

	Cost of cultivation (Rs. ha^{-1})				Gross return (Rs. ha^{-1})				Net return (Rs. ha^{-1})				Benefit-cost ratio			
Moisture conservations (M) Irrigation levels(I)	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean	Control	Dust Mulch	Distillation waste mulch	Mean
Rainfed	27056	31813	37611	32160^{d}	66154^{c}	88453^{b}	78790^{c}	77799^{c}	39098^{b}	56639^{b}	41179^{b}	45639^{b}	1.44^{b}	1.78^{b}	1.10^{b}	1.44^{b}
20 % TAW	29435	34192	39915	34514^{c}	93879^{a}	125172^{a}	108447^{a}	109166^{a}	64444^{a}	90980^{a}	68532^{a}	74652^{a}	2.19^{a}	2.66^{a}	1.72^{a}	2.19^{a}
40 % TAW	33449	38206	43929	38528^{b}	69424^{b}	84365^{c}	83324^{b}	79038^{b}	35976^{c}	46159^{c}	39395^{c}	40510^{c}	1.07^{c}	1.21^{c}	0.90^{c}	1.06^{c}
60 % TAW	35158	39915	45639	40237^{a}	59167^{d}	61248^{d}	54558^{d}	58324^{d}	24083^{d}	21333^{d}	8845^{d}	18087^{d}	0.68^{d}	0.53^{d}	0.19^{d}	0.47^{d}
Mean	31274^{c}	36031^{b}	41773^{a}		72156^{c}	89809^{a}	81280^{b}		40900^{b}	53778^{a}	39488^{c}		1.35^{b}	1.55^{a}	0.98^{c}	
	S Em±	CD at 5%			S Em±	CD at 5%			S Em±	CD at 5%			S Em±	CD at 5%		
Irrigation level (I)	491	1459			1181	3509			590	1754			0.02	0.06		
Moisture conservation (M)	602	2364			1115	4377			977	3833			0.03	0.12		
I ×M	850				2046	6078			1023	3038			0.03	0.10		

Letters presenting the difference (P=0.05), NS= Non Significant, I=Irrigation level, M= Moisture conservation, and TAW= Total Available Water

Yield of biologically active substances (withanolide A, withanone and total withanolide)

Table 3 revealed that maximum (1.60 kg ha^{-1}) withanolide A yield was recorded at 20±5% TAW and dust mulch (1.28 kg ha^{-1}). Interaction effect revealed that maximum (1.99 kg ha^{-1}) withanolide A yield was recordedat 20±5% TAW under dust mulch condition. Similarly, maximum (1.31 kg ha^{-1}) withanone yield was recorded at 20±5% TAW over other irrigation levels and in dust mulch (1.05 kg ha^{-1}). Interaction effect revealed that withanone yields were maximum (1.63 kg ha^{-1}) when irrigation was applied at 20±5% TAW under dust mulch condition. The total withanolide yield was highest (2.91 kg ha^{-1}) at 20±5% TAW. Dust mulch was proven to be best treatment and gave highest (2.33 kg ha^{-1}) yield of total withanolide. The interaction effect of irrigation levels and moisture conservation practices was also significant and maximum (3.62 kg ha^{-1}) yield was observed at 20±5% TAW under dust mulch condition, whereas lowest (1.18 kg ha^{-1}) was recorded in 60±5% TAW under distillation waste mulch. Further root yield was also reported maximum under the same treatment due to which yield was reported maximum under treatment combinations. It was reported in several studies that higher biological yield results in higher secondary metabolites yield. Nilofer et al. (2018) reported in senna that maximum yield of sennoside was found under 20% ASM condition. Mohapatra et al. (1999) reported that mulching with plastic foil plays an important role in enhancement of productivity in several crops of Solanaceae.

Water requirement (mm), consumptive use of water (mm) and water use efficiency

Minimum WR (104.5 mm) was recorded under rainfed condition, whereas, maximum (690.0 mm) water requirement was observed when irrigation was applied at 60±5% TAW (Table 4). Irrigation levels significantly affected the CUW. The water use was higher (383.8 mm) at 60±5% TAW, whereas lowest (104.5 mm) water was received by rainfed treatments. Similarly, different moisture conservation practices also affected the CUW and maximum (338.7 mm) was observed in control. Further, the WUE decreased significantly with increase in irrigation levels. Maximum WUE (4.14 kg ha^{-1} mm^{-1}) was calculated in rainfed condition followed by 20±5% TAW (2.33 kg $ha^{-1}mm^{-1}$). In different moisture conservation practices, maximum (2.47 kg ha^{-1} mm^{-1}) WUE was found in dust mulch. WUE is affected by water requirement and yield of the particular treatment. As the water requirement decrease, WUE was enhanced automatically. Saxena and Singh (1996) reported that crop needed 300 to 400 mm of water at Irrigation Water/ Cumulative Pan Evaporation ratio (IW/CPE ratio) under shallow water table condition (62 to 119 cm). Behera et al. (2014) reported that application of irrigation at 100% PE (Pan Evaporation) increased CU and decreased the WUE. In Northern China, straw mulching has significant effect on soil evaporation loss with enhanced WUE of winter wheat (Zhang et al., 2005)

Economics

The maximum cost of cultivation (45,639 INR ha^{-1}) in distillation waste mulch when irrigation was applied at 60±5% TAW and the minimum cost of cultivation (27,056 INR ha^{-1}) was found in rainfed condition in controlled condition (because the expenditure on irrigations and mulches were nil).

The maximum gross return (1,25,172 INR ha^{-1}) was recorded when irrigation was applied at 20±5% TAW, and dust mulch was applied. Whereas, minimum gross return was recorded in 60±5% TAW (54,558 INR ha^{-1}) with distillation waste mulch. Economic analysis of different irrigation levels and moisture conservation practices in ashwagandha reflects the superiority of the interaction effect of 20±5% TAW and dust mulch as higher net return (90, 980 INR ha^{-1}) and benefit-cost ratio (2.66) was recorded in this treatment combination whereas, minimum net return (8,845 INR ha^{-1}) and B:C ratio (0.19) was recorded in 60±5% TAW with distillation waste mulch. Higher net return and B:C ratio in 20±5% TAW is due to the higher gross return in this irrigation level, and low cost of cultivation due to comparatively less number of irrigations was applied at 20±5% TAW, and the expenditure in creating dust mulch is less in comparison with the cost of distillation waste mulch.

CONCLUSION

The results of present study indicated that the root yield of *ashwagandha* can be enhanced by optimizing plant population, irrigation level sand by adopting moisture conservation practices. The present study revealed that different plant population had significant influence on root yield, root content and yield of biologically active substances in ashwagandha. The dry root yield (1030 kg ha^{-1}) and total withanolide yield (3.64 kg ha^{-1}) were significantly higher at plant population of 400,000 plants ha^{-1}. The maximum net return (88,452 INR ha^{-1}) was obtained with 400,000 plants ha^{-1}. The higher dry root yield (696 kg ha^{-1}), withanolide A content (0.29%), withanone content (0.24%), total withanolide content (0.52%) and the yield of secondary metabolites were reported maximum when irrigations were applied at 20±5% TAW and dust mulch was provided as moisture conservation practice. Net return was highest (90,980 INR ha^{-1}) when irrigations were applied at 20±5% total available water (TAW) under dust mulch condition. Therefore, using plant population of 400,000 plants ha^{-1} by maintaining 25×10 cm spacing, providing irrigations at 20±5% TAW and creating dust mulch after each irrigation is recommended for getting high productivity and profitability of *W. somnifera* grown under subtropical plains of north India.

Declaration of Competing Interests

Authors state that for the manuscript submission, there are no conflicts of interest.

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REFERENCES

Aggarwal M, Singh P, Agarwal MK. 2004. Effect of sowing dates and spacing on yield attributes and root yield of ashwagandha. *J Med Arom Pl Sci* **26**: 473-474.

Ahmed FE, Samia OY, Elsiddig AE, Elsheikh EA. 2000. Effects of mycorrhizal inoculation and phosphorus application on the nodulation, mycorrhizal infection and yield components of faba bean grown under two different watering regimes. Khart. *J Agric Sci* **1**: 137–151.

Behera MS, Mahapatra PK, Singandhupe RB, Kundu DK, Kannan K, Mandal KG., Amarpreet S. 2014. Effect of drip fertigation on yield, water use efficiency and water productivity of Mint (*Mentha arvensis* L.). *J Agr Phys* **14**: 37-43.

Callan NW, Johnson DL, Westcott MP, Welty LE. 2007. Herb and oil composition of dill (*Anethum graveolens* L.): effects of crop maturity and plant population. *Ind Crops Prod* **25**: 282–287. https://doi.org/10.1016/j.indcrop.2006.12.007.

Dastane NG. 1972. Practical manual for water use research in agriculture. NovbharatPrakashan, Pune, Maharastra. 120 p.

Estefan G, Sommer R and Ryan J. 2013. Methods of soil, plant, and water analysis. A manual for the West Asia and North Africa region, 3, pp.65-119.

Farooqui AA, Sreenivas BS. 2001. Aromatic and Medicinal Plants, IBH Publications, New Delhi. Pp. 27-34.

Halepyati AS, Sujatha K, Prabhakar M. 2002. Growth, yield and water use of summer tuberose as influenced by irrigation levels and plant population. *J Orna Hortic New Ser* **5**: 47–50.

Jat RS, Reddy RN, Bansal R, Manivel P. 2015. Good agricultural practices for Ashwagandha. Directorate of Medicinal and Aromatic Plants Research.

Khajuria RK, Suri KA, Gupta RK, Satti NK, Amina M, Suri OP,Qazi GN. 2004. Separation, identification, and quantification of selected withanolides in plant extracts of *Withaniasomnifera* by HPLC UV (DAD)–Positive ion electrospray ionisation–mass spectrometry. *J Sep Sci* **27**: 541-546. https://doi.org/10.1002/jssc.200301690

Kumar R, Sood S, Sharma S, Kasana RC, Pathania VL, Singh B, Singh RD. 2014. Effect of plant spacing and organic mulch on growth yield and quality of natural sweetener plant stevia and soil fertility in western Himalayas. *Int J Plant Prod* **8**: 311-334.

Kumar S, Dey P. 2011. Effect of different mulches and irrigation methods on root growth, nutrient uptake, water use efficiency and yield of strawberry. *Sci Hortic* **127**: 318-324. https://doi.org/10.1016/j.scienta.2010.10.023

Kumar D, Kumar R, Singh AK, Verma K, Singh KP, Nilofer, Kumar A, Singh V, Kaur P, Singh A, TM Anandkumar, Khare P, Singh S. 2021**.** A novel and economically viable agro-technique for enhancing productivity and resource use efficiency in menthol mint (*Mentha arvensis* L.). *Ind Crops Prod*162https://doi.org/10.1016/j.indcrop.2020.113-233.

Liu H, Wang X, Wang D, Zou Z, Liang Z. 2011. Effect of drought stress on growth and accumulation of active constitutuents in *Salvia milriorrhiza* Bunge. *Ind crops Prod* **33**: 146–151. https://doi.org/10.1016/j.indcrop.2010.09.006

Mishra LC, Singh BB, Dagenais S. 2000. Scientific basis for the therapeutic use of *Withaniasomnifera*. (Ashwagandha): A review. *Alt Med Rev* **5**:334–346.

Mohapatra BK. 1999. Efficiency of plastic mulching in tomato. *Environ Eco* **17:** 775-776.

Nigam KB, Rawat GS, Prasad B. 1984. Effects of methods of sowing, plant population and fertility levels on Ashwagandha. *South Ind Hort* **32**: 356-359.

Nilofer, Singh AK, Singh A, Singh S. 2018. Impact of sowing and harvest times and irrigation levels on the sennoside content of *Cassia angustifolia*Vahl. *Ind Crops Prod* **125**: 482–490. https://doi.org/10.1016/j.indcrop.2018.09.025.

ObidoskaG, Sadowska A, Rumowska M. 2004. The effect of growing factors on the yield of seeds of ashwagandha (*Withaniasomnifera* (L.) Dun.) cultivated in polish climatic conditions. *Folia Hortic* **16**: 57-63.

Pakkiyanthan K, Pasha YN, Reddy YN, Sathe A. 2004. Effect of spacing and phosphorus level on growth and root yield of Ashwagandha (*Withaniasomnifera*Dunal.). *Ind J Hort* **61** :195-197.

Palada MC, Crossman SMA, Kowalski JA, Collingwood CD. 2000. Evaluation of organic and synthetic mulches for basil production under drip irrigation. *J Herbs Spices Med Pl* **6**: 39-48. https://doi.org/10.1300/J044v06n04_05.

Panse VC, Sukhatme PV. 1985. Statistical methods for Agricultural Workers, Indian Council of Agricul. Res. New Delhi, India.

Pirboneh H, Ghasemi M, Gohari AA, Bhari B, Bazkiyaei ZB. 2012. Effect of irrigation and straw mulch on yield and yield components of Eggplant (*Solanum melongena* L.). *Int Res J Appl Basic Sci* **3**: 46-51.

Rahman MJ, Uddin MS, Bagum SA, Mondol ATMAI, Zaman MM. 2006. Effect of mulches on the growth and yield of tomato in the coastal area of Bangladesh under rainfed condition. *Int J Sustain Crop Prod* **1**: 6-10.

Ram M, Ram D, Roy SK. 2003. Influence of an organic mulching on fertilizer nitrogen use efficiency and herb and essential oil yield in geranium (*Pelargoinum graveolens*). *Bioresource Technol* **87**: 273-278. https://doi.org/10.1016/S0960-8524(02)00232-8

Reddy GS, Krishna R. 1992 Yield response of diploid and induced autotraploids of *Solanum viarum* under high density planting. *Ind J Agr Sci* **62**: 551-553.

Richard PL, Bracy RP, Rosendale RM. 2002. Use of plastic mulch for successive crops. *J Veg Crop Prod* **8**: 63-70. https://doi.org/10.1300/J068v08n01_08

Saxena A, Singh JN. 1996. Yield and nitrogen uptake of Japanese mint (*Mentha arvensis*) under various mulch regimes mulch application and nitrogen fertilizer. *J Med Arom Pl Sci* **18**: 477-480.

Shashidhar KR, Bhaskar RN, Priyadharshini P,Chandrakumar HL. 2008. Effect of different organic mulches on pH, organic carbon content and microbial status of soil and its influence on leaf yield of M5 mulberry (*Morus indica* L.) under rainfed condition. *CurrBiot* **2**:405-413.

Shinde A, Pankaj G, Kumar RS. 2015. Conservation and sustainability of Ashwagandha: A medicinal plant. *Int J Biol Sci* **3**: 94-99. DOI: 10.7897/2321-6328.03220

Singh A, Singh M, Singh S. 2001. Effective utilization of distillation waste as organic mulch for weed management in the aromatic grass, Citronella java. *Int J Pest Manag* **47**: 253-257. https://doi.org/10.1080/09670870110046777

Singh S, Khanuja SPS, Singh A, Singh M, Singh UB. 2003. Potential and economics of ashwagandha (*Withaniasomnifera* L. Dunal) in overlapping cropping system under rainfed condition of tropical north India. *J Spices AromCrop* **12**: 101-106.

Srivastava A, Gupta AK, Shanker K, Gupta MM, Mishra R, Lal RK. 2018. Genetic variability, associations, and path analysis of chemical and morphological traits in Indian ginseng [*Withaniasomnifera* (L.) Dunal] for selection of higher yielding genotypes. *J Ginseng Res* **42**: 158-164. https://doi.org/10.1016/j.jgr.2017.01.014

Ssali H, McIntyre BD, Gold CS, Kashaija IN, Kizito F. 2003. Effect of mulch and mineral fertilizer on crop, weevil and soil quality parameters in highland banana. *NutrCyclAgroecosys* **65**: 141-150.

Szabo B, Tyihak E, Szabo LG, Botz L. 2003. Mycotoxin and drought stress induced change in alkaloid content of *Papaver somniferum* plantlets. *Acta Bot Hung* **45**:409-417. https://doi.org/10.1556/abot.45.2003.3-4.15

Uddin Q, Samiulla L, Singh VK, Jamil SS. 2012. Phytochemical and pharmacological profile of *Withania somnifera* Dunal: A review. *J Appl Pharm Sci* 2: 170-175.

Umadevi M, Rajeswari R, Rahale CS, Selvavenkadesh S, Pushpa R, Kumar KPS, Bhownik D. 2012. Traditional and Medicinal uses of *Withania somnifera*. *The Pharma J* Vol. **1**:102-109.

Wang DH, Du F, Liu HY, Liang ZS. 2010. Draught stress increases iridoid glycosides biosynthesis in the roots of *Scrophularia ningpoensis* seedling. *J Med Pl Res* **1020**: 2691–2699.https://doi:10.5897/JMPR09.338

Zhang X, Chen S, Liu M, Pei D, Sun H. 2005. Improved Water Use Efficiency associated with cultivars and agronomic management in the north china plain. *Agron J* **97**:783-790. https://doi:10.2134/agronj2004.0194

Zhu ZB, Liang ZS, Han RL, Wang X. 2009. Impact of fertilization on drought response in the medicinal herb *Bupleurum Chinense* DC: Growth and Saikosaponin production. *Ind Cro Prod* **29**: 629–633. https://doi.org/10.1016/j.indcrop.2008.08.002