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Effect of irrigation based on IW/CPE ratio and drip fertigation on secondary metabolites and antioxidant activity of cumin (*Cuminum cyminum*)

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Abstract
The aroma of cumin (*Cuminum cyminum* L.) is due to the presence of aromatic volatile compounds. The flavor of cumin is judged by its volatile oil content. Effect of irrigation based on IW/CPE ratio and drip fertigation on Essential oil chemical constituents and medicinal compounds of Cumin (*Cuminum cyminum*) were analyzed at Agricultural Research Station, Mandor, Jodhpur. The total phenolic content was ranged from 444.97 mg to 531.90 mg GAE 100 g^{-1} seed while total flavonoid content was ranged from 112.22 mg to 203.12 mg QE 100 g^{-1} seed. Highest total phenolic and flavonoid content was observed in drip fertigation at 0.8 IW/CPE ratio with 100% RDF. 2, 2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging antioxidant activity expressed as IC_{50} values ranged from 0.38-0.47 mg ml^{-1}. Lower IC_{50} value was observed in treatment with drip fertigation as compare to surface irrigation. Study results reveal that drip fertigation enhances the amount of secondary metabolites and antioxidant activity of cumin.

Keywords: Spices, cumin, drip fertigation, antioxidants

Introduction
Cumin (*Cuminum cyminum* L.), is a diploid plant of Apiaceae family. It is the most popular spice in the world after black pepper [1]. It is a tropical plant and cultivated in winter season in areas where atmospheric humidity remains low during the month of February and March. Cumin is one of the most important seed spice crop produced, consumed and exported from India and occupies significant place in Indian agriculture. There is great demand of value added products of cumin namely seed, powder, essential oil and oleoresin etc. in the domestic as well as in international market [2]. The aroma of cumin is due to the presence of aromatic volatile compounds [3].

Secondary metabolites or phytochemicals, naturally occurring in plants are biologically active and play important role in defense system of plants [4]. These phytochemicals have historically been used as pharmaceuticals, fragrances, flavor compounds, dyes, and agrochemicals [5]. *In vitro* studies reported that phytochemicals such as phenolic compounds have potential role against different diseases and used as anti-inflammatory, anti-mutagenic, antiviral and antibacterial, agents [6, 7].

In western Rajasthan water is one of the main constraint in crop production since the growing areas are deficit in annual rainfall and cumin crops is grown during winter season requires assured irrigation for successful production. Among many irrigation methods including surface irrigation, drip irrigation and sprinkler irrigation are being followed widely among growing areas depends on availability of water and micro irrigation facility. But considering the importance of water for future generation and also present need, it is most necessary to workout optimum irrigation level, critical stage of water requirement and method of irrigation in cumin to save water and to achieve higher productivity and quality. Drip fertigation is a highly efficient method for fertilizer application; minimize losses and adverse environment impact on crop production. Both water and nutrients applied through fertigation will be used by the plants for photosynthesis finally enabling plants to produce new tissues which have influence on growth and production of crops [8].

The present study was conducted at Agricultural Research Station, Mandor, (Agriculture University Jodhpur) during 2016-17 to analyze the Effect of irrigation based on IW/CPE ratio and drip fertigation on Essential oil constituents and medicinal compounds of cumin (*Cuminum cyminum*). Cumin variety GC-4 was used for this experiment. Drip irrigation based on climatological approach is promising with fertigation under scare water availability for higher productivity. However information for the cumin crop is not available. Hence the present investigation is undertaken.

Materials and Methods

Field trial details

A field experiment was conducted at Agricultural Research Station, Mandor, Jodhpur, Rajasthan during *Rabi*, 2016-17. Cumin variety GC-4 with seed rate of 25 kg ha^{-1} was planted using 3 levels of drip irrigation (0.4, 0.6, 0.8 IW/CPE) along with three fertigation level (60%, 80%, 100% RDF) in each irrigation schedule (Table-1).

Table 1: Field trials details and treatment of irrigation along with drip fertigation

Treatment details and doses	
T_1	Standard Check (Surface Irrigation at 0.8 IW/CPE ratio with 100% RDF)
T_2	Drip Fertigation at 0.4 IW/CPE ratio with 60% RDF
T_3	Drip Fertigation at 0.4 IW/CPE ratio with 80% RDF
T_4	Drip Fertigation at 0.4 IW/CPE ratio with 100% RDF
T_5	Drip Fertigation at 0.6 IW/CPE ratio with 60% RDF
T_6	Drip Fertigation at 0.6 IW/CPE ratio with 80% RDF
T_7	Drip Fertigation at 0.6 IW/CPE ratio with 100% RDF
T_8	Drip Fertigation at 0.8 IW/CPE ratio with 60% RDF
T_9	Drip Fertigation at 0.8 IW/CPE ratio with 80% RDF
T_{10}	Drip Fertigation at 0.8 IW/CPE ratio with 100% RDF

RDF=Recommended Dose of Fertilizer

Chemicals and Reagent

The chemicals used in this study were procured from Loba Chemi (India) and Sigma-Aldrich (USA).

Total Phenolics Content (TPC)

The total phenolic content was determined using the Folin-Ciocalteu assay according to procedure describe by Dewanto *et al.* [9]. With slightly modification and the results were expressed as mg gallic acid g^{-1} seed. An aliquot of 1 ml of the crude seed methanol extract was mixed with 1 ml of the Folin-Ciocalteu reagent and 4 ml of a 20% sodium carbonate solution. Distilled water was added to a final volume of 25 ml. Following incubation for 30 min, the absorbance of the reaction mixture was measured at 765 nm using Lab India make spectrophotometer against a blank. Gallic acid was used as the standard. The amount of total phenolic was calculated by using the standard curve of Gallic acid drawn within a concentration range of 8.0 x 10^{-4} to 4.0 x 10^{-3} mg ml^{-1} having R^2 value 0.996 and was expressed as mg Gallic acid equivalents g^{-1} (mg GAE g^{-1}) seed.

Total flavonoid content (TFC): The total flavonoid content in methanol extract was determined using aluminium trichloride ($AlCl_3$) method protocol described by Chang *et al.* [10] with slightly modification. Briefly, 2 ml of 2% aluminium trichloride ($AlCl_3$) solution in methanol was mixed with the 2 ml of a diluted stock solution (0.01 or 0.02 mg ml^{-1}). Absorption readings were taken at 415 nm (Lab India spectrophotometer) after 10 min against a methanol blank, Quercetin was used as the standard. The total flavonoid content was determined using a standard curve of Quercetin drawn within a concentration range of 4.0 x 10^{-3} to 2.0 x 10^{-2} mg ml^{-1} having R^2 value 0.998 and was expressed as mg Quercetin equivalents g^{-1} seed (mg QE g^{-1} seed)

Antioxidant activity DPPH Assay: There are several methods commonly used to determine the antioxidant activity of natural products, we have choose the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) free radical reagent because it is an easy, precise, and accurate method. 2, 2-diphenyl-1-picryl-hydrazyl DPPH is a free radical, and produces a violet solution in alcohol. It is reduced in the presence of an antioxidant molecule. Antioxidant activity of the methanol extract of cumin seed and standard were assessed on the basis of the radical scavenging effect of the stable 2,2-diphenyl-1-picrylhydrazyl hydrate radical (DPPH). The diluted working solutions of the test samples were prepared in methanol. Gallic acid was used as the standard in solutions ranging from $5x10^{-4}$ to $4x10^{-3}$ mg ml^{-1}. 0.135mM DPPH solution was prepared in methanol. Then 2 ml of this DPPH solution was mixed with 2 ml of sample solutions (ranging from 1mg ml^{-1} to 8 mg ml^{-1}) and the standard solution were tested separately. These solution mixtures were kept in the dark for 30 min and optical density was measured at 517 nm using Lab India make spectrophotometer against methanol. 2 ml of methanol with 2 ml of DPPH solution was used as control[11, 12]. The optical density was recorded and percentage of inhibition was calculated using the formula given below:

$$\% \text{ of inhibition of DPPH activity} = (A–B/A) \times 100$$

Where, A is optical density of the control and B is optical density of the sample.

The IC_{50} values were calculated using linear regression of plots where the abscissa represented the concentration of the test solution and the ordinate was the percent of antioxidant activity (Figure-4).

Result and Discussion

Total Phenolics Content (TPC)

The colorimetric method using the Folin-Ciocalteau reagent is frequently used for total phenolic content estimation. A blue colour complex forms due to the reaction of Folin-Ciocalteau reagent and phenols that allow quantification. The total phenolic content was ranged from 444.97 mg to 531.90 mg GAE 100 g^{-1} seed. Figure-2 represents the comparative total phenolics content among different treatments.

Highest total phenolic content was observed in treatment T_{10} followed by T_3, T_8 while lowest total phenolic content was found in T_4 (Table-2). Surface Irrigation at 0.8 IW/CPE ratio with 100% RDF contains low total phenolic content (445.79) as compare to drip fertigation. Study results reveal that drip fertigation at 0.8 IW/CPE ratio with 100% RDF enhances total phenolic content in cumin.

Moghaddam *et al.* [13]. analyzed the total phenolic content in cumin seeds at different maturity stages and reported 25.52-40.0 GAE g^{-1} seed which were lower as compared to the present findings. Ereifej *et al.* [14] analyzed Jordan cumin seed phenolics content in methanol, acetone and ethanol solvent and reported phenolics 19-43.8 mg GAE 100 gm^{-1} seed. Polyphenols content in Serbian cumin seed post destillation waste material were ranged from 30.1-47.5 mg GAE g^{-1} seed [15]. Abdelfadel *et al.* [16]. analyzed cold and hot water extract of Egyptian cumin seed and reported total phenolics 270.3-299 mg GAE 100 ml^{-1}. Current study reveals that drip fertigation enhances the amount of total phenolics.

Total flavonoid content (TFC)

Total flavanoids content was estimated by aluminium chloride method. Quercetin dihydrate was taken as standard flavonoid and results were calculated as means of triplicate and represented as mg Quercetin equivalent g^{-1} seed (mg QE $g^{-1)}$ with standard deviation.

The total flavonoid content was ranged from 112.22 mg to 203.12 mg QE 100 g^{-1} seed. Highest total flavonoid content was observed in treatment T_{10} followed by T_8, T_9 while lowest

total phenolic content was found in T_2 (Table-2). Figure-3 represents the comparative total flavanoids content among different treatments.

As similar to total phenolics, flavonoids content were also found more in treatment T_{10}. As total phenolics drip fertigation contains higher flavonoids as compare to surface irrigation. Dubey *et al.* [17]. and Rebey *et al.* [18]. studies revealed that total flavonoid content in cumin seed extract were ranged from 28 to 36 mg QEg^{-1} seed and 2-5 mg QE g^{-1} seed, respectively. Current study reveals that drip fertigation also enhances the amount of total flavonoids.

Antioxidant activity DPPH Assay

The determination of the antioxidant activity of cumin seed extract was based on the DPPH radical scavenging activity through the IC_{50} parameter, which represents the concentration of the material necessary to inhibit 50% of free radicals. Thus, a lower IC_{50} value shows a superior ability to neutralize free radicals and potential antioxidant content.

The scavenging ability of methanol seed extract of cumin represented as IC_{50} of DPPH radical are shown in Table-2. IC_{50} values ranged from 0.38-0.47 mg ml^{-1}. Figure-3 represents the inhibition of DPPH radical by different concentration of cumin seed extracts (T1-T10) for prediction of IC50 Value. DPPH radical scavenging % by cumin seed extract was presented in Table-3. Lower IC_{50} value was observed in treatment with drip fertigation as compare to surface irrigation. Study results reveal that drip fertigation enhances the antioxidant acitivity.

Khan *et al.* [19]. Reported 14-16 mg/ml antioxidant activity expressed as IC_{50} value in western Rajasthan growing two genotypes of cumin. Dubey *et al.* [17]. Reported 15.31-15.38 mg ml^{-1} antioxidant activity of cumin seed in Agro-Ecological Sub Regions, India.

Table 2: Secondary metabolites and Antioxidant Activity of cumin

Treatment	TPC mg 100g^{-1} Seed	TFC mg 100g^{-1} Seed	IC_{50} Value mg ml^{-1}
T_1	445.79 ± 3.34	124.24 ± 1.33	0.47
T_2	444.97 ± 2.08	112.22 ± 1.53	0.39
T_3	530.64 ± 2.13	136.78 ± 3.25	0.40
T_4	461.35 ± 2.75	123.17 ± 0.48	0.45
T_5	461.58 ± 3.87	117.62 ± 1.29	0.44
T_6	472.96 ± 3.46	120.27 ± 1.69	0.39
T_7	478.48 ± 3.17	172.81 ± 1.24	0.43
T_8	498.92 ± 1.17	199.61 ± 0.75	0.49
T_9	495.79 ± 4.30	196.63 ± 0.16	0.38
T_{10}	531.90 ± 2.86	203.12 ± 2.78	0.39

Table 3: DPPH radical scavenging % by cumin seed extract

Treatment	DPPH radical scavenging %		
	0.1 mg ml^{-1}	0.5 mg ml^{-1}	1.0 mg ml^{-1}
T_1	26.48	53.78	80.37
T_2	32.46	59.25	82.42
T_3	25.48	63.01	88.18
T_4	29.00	55.65	78.77
T_5	27.26	59.82	79.22
T_6	31.74	68.04	82.36
T_7	30.79	54.34	82.19
T_8	26.54	51.83	79.22
T_9	28.48	65.75	82.65
T_{10}	29.45	60.96	85.48

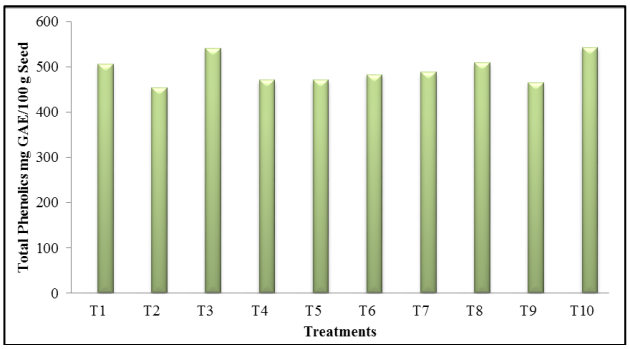

Fig 1: Total Phenolics content in cumin seed expressed as gallic acid equivalent 100 g^{-1} seed

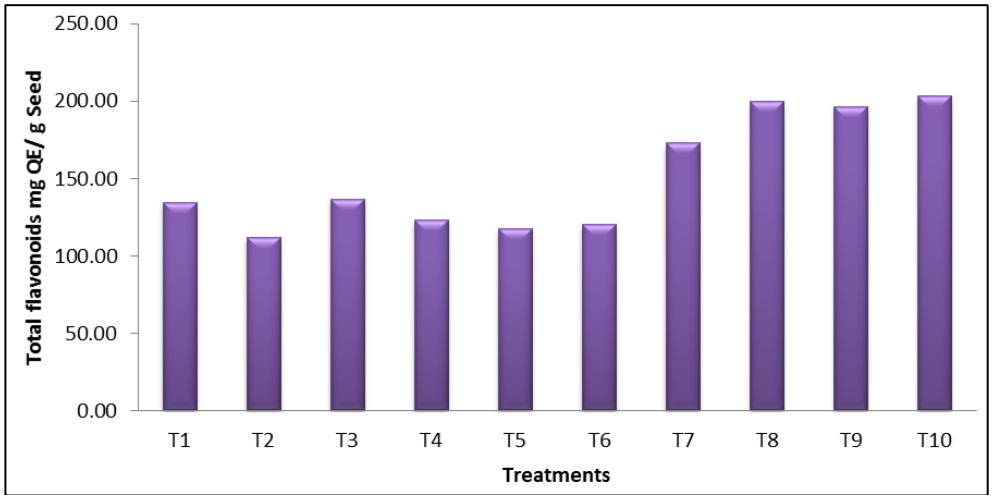

Fig 2: Total flavonoid content in cumin seed expressed as quercetin equivalent 100 g^{-1} seed

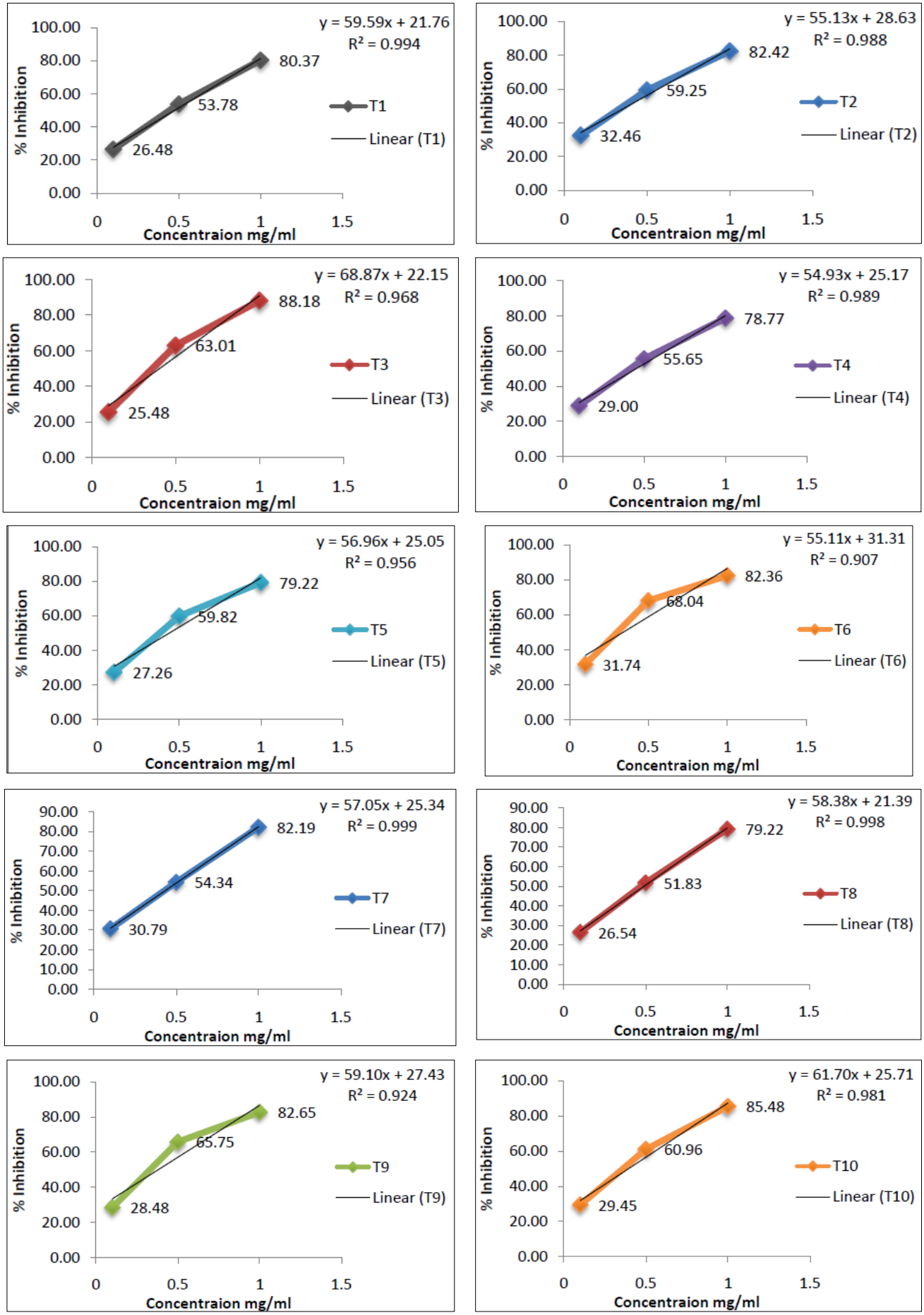

Fig 3: Inhibition of DPPH radical by different concentration of cumin seed extracts (T_1-T_{10}) for prediction of IC_{50} Value

Conclusion

Effect of micro irrigation and fertigation on secondary metabolites and antioxidant activity of cumin (*Cuminum cyminum*) has been identified. The result reveals that drip fertigation enhances total phenolics, total flavonoids and antioxidant activity in cumin. Micro irrigation and fertigation techniques enhances cumin quality, flavor and fragrance along with water saving.

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