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Phytotoxicity of *Hyptis suaveolens* against *Cicer arietinum* L. (chickpea)

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Abstract
Chickpeas contain protein, carbohydrates, and the right amount of fat, making them highly nutritious. They are also rich source of calcium, iron, vitamin C (green plants), and vitamin B. It is used in various forms such as dal, chhole, sweets, flour, and other attractive dishes. Their leaves contain malic acid and citric acid, which are very helpful for stomach ailments and are the best blood purifier. The fungi causes several diseases in chickpea among them Fusarium wilt is common. *Fusarium oxysporum* f. sp. *ciceri*. was isolated from root of wilted plants of chickpea and extract of ten plant species were tested against wilt causing pathogen *Fusarium oxysporum* f. sp. *ciceri*. Among them *Hyptis suaveolens* was found most active against wilt pathogen. In the present investigation plant extract of *Hyptis suaveolens* has been selected for phytotoxicity against chickpea. The extract of *Hyptis suaveolens* had no adverse effect on the root - shoot lengths of the host plant because the root/shoot length ratios for treatment set was approximately equal to that of control set. When the seeds were treated with the extract of *Hyptis suaveolens* for 4h showed better results than 2h as 36 plants were found to be healthy on 50 days in 4h treatment while 27 plants were healthy in 2h treatment and all the plants were found to be completely wilted on 31 day in control set.

Keywords: Chickpea, fungi, *In vitro*, leaf extract, phytotocity

Introduction
The chickpea is an important Rabi season pulse crop in India. Seeds of chickpea regarded for its excellent nutritional value after dehulling, with protein concentrations ranging from 25.3 to 28.9% (Muehlbauer and Rajesh, 2008) [20]. Chickpea contains 3 percent ash, minerals such as calcium (202 mg), phosphorous (312 mg), iron (10.2 mg), vitamin C (3.0 mg), calorific value (360 cal), small amounts of B complex, fibre (3.9 g) and moisture (9.8 g). It doesn't have any anti-nutritional properties. Only one type of necessary amino acids that is lacking in chickpeas is sulphur containing amino acid; these may be added to the diet by eating grains.

On an average, chickpea contains 17-22 percent proteins, 60-64 percent carbohydrates and 3-4 percent fat (Sindhu *et al*., 1974). It is also a rich source of mineral component *viz*., phosphorus (340 mg/100 g), calcium (190 mg/100 g), iron (7 mg/100 g), zinc (3 mg/100 g) and vitamin B in considerable amount (Kousar *et al*., 2019) [14]. The essential amino acids found in chickpea are isoleucine, leucine, phenylamine, lysine and valine (Karim and Fattah, 2006) [13]. Chickpea can be used in a number of ways.

It can be fried, roasted and boiled or splitted into grains which are commonly known as dal or can be grounded into flour which is added to wheat flour to improve its protein content and can also be used to make sweets, dishes and snacks like pakodas, kadhi, namkeens and several other foods. Green leaves which are a rich source of oxalic and malic acid, are generally used as vegetable. Exudation of leaves possess medicinal value against constipation, bronchitis, cholera, digestive disorders and blood purification.

Germinated seeds are used in curing scurvy disease. They are also used as animal feed where the green or dry leaves and stems are fed to the livestock. Chickpea is also beneficial for soil. It aids in atmospheric nitrogen fixation and helps the crop to fix up to 140 kg N ha-1 from atmosphere (Saraf *et al*., 1998) [25].

Beside their dietary value and nitrogen fixing ability, chickpea also plays an important role in sustaining intensive agriculture by improving physical, chemical and biological properties of soil and are considered excellence crops for diversification of cereal based cropping system.

There are two main commercial types of chickpeas. *Cicer arietinum* is one of the most important leguminous crops worldwide and wilt disease is caused by soil-borne fungal pathogen, *Fusarium oxysporum* f. sp. *ciceri.* This pathogen enters in the vascular system and the plant where it may cause symptoms such as yellowing of leaves, wilting and plant death. Chickpea production is severely affected by *Fusarium* wilt, which causes large decreases in crop yield and quality. Typically, the disease is difficult to manage, because it can live long in the soil, thus limiting the effectiveness of traditional control measures like crop rotation or fungicide application.

Materials and Methods

Wilt causing pathogen *Fusarium oxysporum* f. sp. *ciceri*. was isolated from root of wilted plants of chickpea by single spore technique and extract of ten plant species *viz*. *Hyptis suaveoliens* Poir, *Ipomea fistula* Mart ex, *Lantana indica* Roxb., *Mentha arvensis* L. *Murraya koenigii* (L.) Spreng, *Nerium indicum* Mill., *Ocimum basilicum* L. *Parthenium hysterophorus* L., *Polygonum barbatum* L and *Putranjiva roxburghii* Wall. Were tested with use of "Poisoned Food Technique" (Grover and Moore, 1962) [8] against wilt causing pathogen *Fusarium oxysporum* f. sp. *ciceri*.

The phytotoxic effects of the leaf extract of the active plant *Hyptis suaveolens* was studied with respect to seed germination, seedling growth (root-shoot length), general health and morphology of *Cicer arietinum*.

1. Seed germination

The plant samples (dry leaves) were powdered with the help of sterilized pestle and mortar and sieved through 1 mm mesh. The powdered plant material dissolved in alcohol in 1:4 (w/v) ratio and kept for 24 hour and filtered through double layer muslin cloth. The extract was centrifuged at 5000 rpm for 10 minutes and the supernatant was used to assess the bioactivity.

For treatment sets, 10 g of surface sterilized seeds of *Cicer arietinum* were soaked in 5 ml of *Hyptis suaveolens* extract separately for 2 h and 4 h. Same amount of seeds were also soaked in sterilized water for the same period for control sets. Treated as well as control seeds were placed on three layers of moist filter paper for germination in separate Petri plates. Petri plates were incubated for germination at room temperature. The observations were recorded on 2nd, 3rd, 4th, 5th, 6th, 7th and 8th day and data are presented in Table - 1. Experiments were repeated twice and each set contained five replicates.

Table 1: Effect of the extract of *Hyptis suaveolens* on germination of *Cicer arietinum* seeds

Germination period (days)	Percent germination of seeds		
	Treatment		Control
	2h	4h	
2	25.0	35.5	23.0
3	71.3	85.2	68.9
4	80.5	92.0	85.0
5	89.8	95.0	88.0
6	100	100	100
7	100	100	100
8	100	100	100

2. Seedling growth (root-shoot length)

The leaf extract of the plant was prepared as in the previous experiment. Chickpea seeds were dressed for 4h with leaf extract of *Hyptis suaveolens*. Control sets were prepared by using the sterilized water in place of the extract. Treated as well as control seeds were placed on three layers of moist bloting papers in separate Petri plates. The Petri plates were incubated at room temperature. The observations were recorded on 4th, 5th, 6th, 7th and 8th day in terms of root-shoot lengths and data are presented as mean value of all replicates in Table - 2. Experiments were repeated twice and each set contained five replicates.

Table 2: Effect of the extract of *Hyptis suaveolens* on root and shoot length of *Cicer arietinum*

Age of seedlings (days)	Root-shoot length of seedlings (cm)			
	Treatment		Control	
	R	P	R	P
4	2.0	2.2	2.0	2.1
5	2.7	2.5	2.7	2.6
6	4.1	3.3	4.0	3.2
7	4.2	3.1	4.1	3.0
8	5.0	4.1	4.9	4.2

R = Length of radicle; P = Length of plumule

3. General health and morphology of the host plant

The extract of the plant was prepared as usual as in the last two experiments. The seeds dressed in the extract for 4h was used for treatment set. Control set was prepared by using the sterilized water in place of extract. Control and treated seeds were sown in sterilized soil in earthen pots separately. The general health and morphology of plants of treatment set were compared with those of control set at their different growth stages.

It was found that, there was no visual abnormality in general health and morphology of the plants of treatment set as compared to control set. The plants appeared in treatment set were found to be as healthy as of control set, indicating the non-phytotoxic nature of the extract.

4. Efficacy of *Hyptis suaveolens* leaf extract in the control of wilt of chickpea by seed treatment method

Efficacy of leaf extract *Hyptis suaveolens* selected as fungitoxic plant for control of wilt disease of chickpea was evaluated by seed treatment method of Mishra (1991) [18] within the following two condition

4.1 In plastic glass

The test organism - *Fusarium oxysporum* f. sp. *ciceri* was multiplied on soil - maize meal medium (190g field sieve soil, 10g of grinded maize meal and 70ml of sterilized water). The soil maize meal medium was prepared by the thorough mixing of the sieved soil (sieved through 2mm sieve) and maize meal, the mixture thus obtained was taken in a 500 ml conical flask, 70ml of sterilized water was then added to the flask and sterilized in an autoclave at 15 lb/sq inch pressure for 30 minutes for 2 consecutive days. Later the flask was inoculated with 3 discs (each of 5 mm diam) of the test fungus pre - grown on Potato Dextrose Agar medium and incubated at 28 ± 2° for 7 days for the growth of the fungus. Fungal inoculum thus prepared was used for the infestation of soil.

The plastic pots of 15 cm size were washed with water and dipped in 5 percent NaOCl (Sodium hypochlorite) solution. The pots were thoroughly rinsed with sterilized water before use, the field soil (sieved through 2 mm mesh, dried under sun and autoclaved for two consecutive days) was filled alongwith maize meal and fungal inoculum (8:1:1 w/w/w) upto 10 cm height in the plastic pots.

The mixture of each pot covered on the top with sterilized soil upto 2 cm height, given light irrigation and the pots were incubated for 7 days at room temperature. Both the treatment as well as the control sets were prepared similarly.

The plant samples (dry leaves) were powdered with the help of sterilized pestle and mortar and sieved through 1 mm mesh. The powdered plant material dissolved in alcohol in 1:4 (w/v) ratio and kept for 24 hour and filtered through double layer muslin cloth. The extract was centrifuged at 5000 rpm for 10 minutes and the supernatant was used for seed treatment. The treatment of seeds of Chickpea (*Cicer arietinum*).

10 g of sterilized seeds of Chickpea were dressed in 5 ml extract for 2 and 4h. For control set, sterilized water was used in place of extract for the same period. Five treated Chickpea seeds were sown equidistantly in sterilized soil layer of each pot. The water treated seeds for same period were sown (5 seeds/pot) in sterilized soil of each pot containing pathogen infested soil, served as control. The treatment and control sets were placed in green house and the pots were watered, as and when required. The experiments were repeated twice with 10 replicates each.

The observations were recorded after 6 days as symptoms developed in Chickpea plants for wilting up to 50 days and data are presented in Table-3 to 5.

Table 3: Symptoms of wilting in Chickpea plants (seeds dressed with the extract of *Hyptis suaveolens* for 2h) grown in plastic pots containing the soil mixed with fungal inoculums

Age of the plant (days)	Symptoms present in plants				
	A	B	C	D	E
7	50	-	-	-	-
9	50	-	-	-	-
11	50	-	-	-	-
13	50	-	-	-	-
15	50	-	-	-	-
17	50	-	-	-	-
19	47	3	-	-	-
21	45	5	-	-	-
23	43	6	1	-	-
25	40	6	2	2	-
27	35	10	2	3	-
29	35	6	5	4	-
31	30	4	6	4	6
33	30	2	6	6	6
35	30	-	9	5	6
37	30	-	8	6	6
39	30	-	3	8	9
41	30	-	3	7	10
43	29	-	2	9	10
45	28	-	4	8	10
50	27	-	3	10	10

A: Absence of any symptom;
B: Drooping of leaves
C: Chlorosis of leaves
D: Wilting of rachis and leaflets
E: Complete wilting of plants

Table 4: Symptoms of wilting in chickpea plants (seeds dressed with the extract of *Hyptis suaveolens* for 4h) grown in plastic pots containing the soil mixed with fungal inoculum

Age of the plant (days)	Symptoms present in plants				
	A	B	C	D	E
7	50	-	-	-	-
9	50	-	-	-	-
11	50	-	-	-	-
13	50	-	-	-	-
15	50	-	-	-	-
17	50	-	-	-	-
19	47	2	1	-	-
21	45	4	1	-	-
23	41	3	2	2	2
25	38	2	5	3	2
27	37	1	3	5	4
29	36	-	4	6	4
31	36	-	5	4	5
33	36	-	4	5	5
35	36	-	5	4	5
37	36	-	4	4	6
39	36	-	2	6	6
41	36	-	2	5	7
43	36	-	2	6	6
45	35	-	1	7	7
50	35	-	1	7	7

A: Absence of any symptom
B: Drooping of leaves
C: Chlorosis of leaves
D: Wilting of rachis and leaflets
E: Complete wilting of plants

Table 5: Symptoms of wilting in chickpea plants grown in the plastic pots containing sterilized soil mixed with fungal inoculum (Control set)

Age of the plant (days)	Symptoms present in plants				
	A	B	C	D	E
7	50	-	-	-	-
9	50	-	-	-	-
11	50	-	-	-	-
13	50	-	-	-	-
15	44	6	-	-	-
17	36	8	4	2	-
19	27	4	9	8	2
21	15	13	8	5	9
23	11	11	9	7	12
25	7	10	11	7	15
27	-	10	4	11	25
29	-	-	-	5	45
31	-	-	-	-	50

A: Absence of any symptom
B: Drooping of leaves
C: Chlorosis of leaves
D: Wilting of rachis and leaflets
E: Complete wilting of plants

Table 6: Efficacy of leaf extract of *Hyptis suaveolens* for the control of wilt disease of chickpea by seed treatment (Plastic pot)

Sets	Period of seed treatment (h)	Percent wilt control
Treatment	2	54
(*Hyptis suaveolens*)	4	70
Control	2	00
(Sterilized water)	4	00

Results and Discussion

Among ten tested plant species *Hyptis suaveolens* was found to most active. *Hyptis suaveolens* contain phytochemicals and having fungicidal activity against wilt pathogen *Fusarium oxysporum* f. sp. *ciceri*. The seeds were dressed with the extract of *Hyptis suaveolens* for 2h and 4h had no adverse effect on the seed germination (Table-1) and root -shoot lengths of the host plant because the root/shoot length ratios for treatment set was approximately equal to that of control set (Table-2).

When the seeds treated with the extract of *Hyptis suaveolens* for 4h showed better results than 2h as 36 plants were found to be healthy on 50 days in 4h treatment while 27 plants were healthy in 2h treatment (Tables - 3 and 4). But all the plants

were found to be completely wilted on 31 day in control set (Table - 5).

On the basis of above observations, finally the efficacy of leaf extaract of the plant against wilt disease in Chickpea was calculated and data are presented in term of percent wilt control of Chickpea (*Cicer arietinum*) plants in Table-6. This table indicates that in control set there was no wilt control, however in treatment set the extract of *Hyptis suaveolens* controlled the wilt disease upto 54 and 70 percent.

An ideal fungi toxicant should be pathocidal (Killing pathogen) but not phytocidal (plant killing) i.e., it must be free from ill effects to the host plant.

According to Fawcett and Spencer (1970) [7] antifungal substances from higher plants have been proved to be less phytotoxic than the synthetic. Besides these substances are also biodegradable in nature (Beye, 1978) [1] and regulate the host metabolism. Recently plants and their products have been found to be nonphytotoxic in nature (Tripathi *et al*., 1978; Dikshit *et al*., 1979; Singh *et al.*, 1993; Singh and Tripathi, 1999; Singh and Tripathi, 2000, Chand and Singh, 2005; Chawla and Gangopadhyay, 2009; Jibhkate and Waghmare, 2010; Nisa and Ahmed, 2011; Kamdi and Thakur, 2012; Minz *et.al* 2012; Hossain and Rahman, 2013; Pal and Kumar, P, 2013; El-Mohamedy and Hassan, 2014; Mishra and Sharma, 2014; Mahmood, 2015; Patil, 2019; Shukla, 2019; Jamil and Ashraf, 2021; Muche and Yamala, 2022; Faima, 2023; Navneet and Rao, 2024) [29, 28, 17, 4, 2, 3, 11, 22, 12, 16, 9, 23, 5, 17, 15, 24, 26, 10, 19, 6, 21].

In the present investigation, the leaf extracts of *Hyptis suaveolens* did not show any adverse effect on the host plant - *Cicer arietinum* with respect to the seed germination, seedling growth and general health and morphology of the plant, indicating its non-phytotoxic nature.

Conclusion

Chickpea (*Cicer arietinum* L.) holds significant importance as a pulse crop during the Rabi season, playing a vital role in supplying protein to the vegetarian population of the country. However, it is susceptible to various disease. The conventional use of synthetic fungicides has led to the emergence of numerous challenges, including environmental pollution, residue accumulation in grains, and harm to non-target organisms. To address these challenges, this study delves into exploring alternative management strategies for Fusarium wilt and the plant diseases. By investigating the efficacy of plant extracts against *Fusarium oxysporum* f. sp. *ciceri*, the research aims to provide insights into sustainable approaches for disease management.

It is noteworthy that the proposed plant would constitute a cheap, readily available, indigenous, safe and economical source of disease control.

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