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Effect of root exudates of chickpea cultivars on *Fusarium Oxysporum* F. Sp. *Ciceri* (Padwick) Synder and Hans

Hale SM, Patil MG, Chapke SM and Ambadkar CV

Abstract
Chickpea (*Cicer arietinum* L.) is an important pulse crop, which belongs to family Leguminaceae. Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* (FOC) is a soil borne and seed borne disease of chickpea which causes great annual yield losses. Successful management of the disease by a single mean including fungicides seems to be a difficult proposition, warranting new management approach. Plant roots serve a multitude of functions in the plant including anchorage, provision of nutrients and water, and production of exudates with growth regulatory properties. Root exudates containing root specific metabolites have critical ecological impact on soil macro and micro biota as well as on the whole plant. In context to this an *in vitro* experiment was conducted to study the effect of root exudates of eleven chickpea cultivars on spore germination and hyphal growth of *Fusarium oxysporum* f. sp. *ciceri* at VNMVV, Parbhani (M.S.). The root exudates of the susceptible cultivars JG 62 showed highest colony diameter *i.e.* 85.13 mm followed by root exudates of the cultivars Vijay showed 50.46 mm colony diameter. This shows that root exudates of both cultivar did not inhibit colony growth of Foc whereas, it was strongly inhibited by root exudates of resistant cultivar JG 315 which shows 17.33 mm colony diameter on 9th day which was highly inhibited than other cultivars. Results of spore germination depicted that the root exudates of JG 62 resulted higher per cent spore germination *i.e.* 84.70% and per cent spore germination by cultivar JG-315 representating significantly less spore germination *i.e.* 18.33%. The results led to conclusion that the resistance of chickpea to vascular wilt depends upon the antifungal activity of the root exudates clearly indicating that the resistant cultivar had a negative influence on fungal growth and spore germination whereas, the susceptible cultivar stimulated the fungal growth and germination.

Keywords: *Fusarium oxysporum* f. sp. *ciceri,* root exudates, mycelial growth, spore germination, chickpea

Introduction
Low yield of chickpea is attributed to its susceptibility to several fungal, bacterial and viral diseases; biotic stresses also reduce the productivity of chickpea, such as wilt, dry rot and collar rot caused by *Fusarium oxysporum* f. sp. *ciceri, Rhizoctonia bataticola* and *Sclerotium rolfsii,* respectively; out of these diseases, Fusarium wilt is causing tremendous losses in chickpea varying between 10 – 100% (Patel *et al*., 2011) [4].
The roots of JG-62 produce exudates that appears capable of stimulating spore germination, which may account for the extreme susceptibility of this cultivar (Haware and Nene (1984) [2]. Medicarpin and maackiain showed antifungal activity to *Fusarium oxysporum* f. sp. *ciceri* indicating that they have potential components of the resistance mechanism of chickpeas to fusarium wilt. It was reported that *Fusarium oxysporum* f. sp. *ciceri* was only inhibited by the root exudates of a wilt resistant chickpea genotype, indicating the genetic basis of the variation in the bioactivity of chickpea roots on soil fungi Stevenson *et al*. (1997) [9].
The present study is designed to characterize *Fusarium oxysporum* and parameters contributing to wilt resistance via, root exudates. Root exudates have been proposed as one of the major biochemical factor that confers disease resistance to varieties.

Materials and methods
Collection of seed
Chickpea seeds of different varieties (Kirpa, Virat, Vijay, Digvijay, Jaki, Saki-9516, BCP-10, BCP-160, Akash-797, and JG-315) were collected from ARS, Badnapur (M.S.) and seeds of JG-62 variety were collected from AICRP on chickpea, Sihore, (M.P.). The seeds were air dried and kept in paper bags and stored at room temperature for further studies.

Plant material and preparation of root exudates and extract

Seeds were placed on a floating polystyrene platform, in such a way that the protruding chickpea radical projected down through a hole in the platform into beaker containing 100 ml of sterilized water. The seeds of different cultivars of chickpea which varied in their resistance were sterilized in 2% hypochlorite solution and germinated on filter paper soaked in sterilized water, after 48 hr. The germinated platform floated on the surface of the water and incubated at 25^0 C under 1-2-hrs light: dark regime (Haware and Nene 1984) [2].

After seven days the polystyrene platform and chickpea seedling was removed and the resulting exudates solution filtered through sterilized Nalgene filters (0.45 um) under reduced pressure. The roots from each beaker were weighed so that the exudates from each cultivar can be diluted or concentrated to make exudates equivalent to 1 g root tissue per 10 ml of water. Sterilized root exudates of different cultivars were tested for their effect on conidial germination and mycelia growth.

Effect on the mycelial growth of *Fusarium oxysporum* f. sp. *ciceri*

Pure culture of Foc isolates were represented different races/pathotypes transferred to PDA to study the effect of root exudates on the mycelia growth. Fifty ml of root exudates were added to 50 ml of PDA at round 50^0 C and mixed thoroughly. This was poured in Petri – dishes @ 15 ml per plate. PDA without root exudates served as control. Seven day old culture of *Fusarium oxysporum* f. sp. *ciceri* were representing different races grow on PDA used for inoculation. Three plates were kept for each treatment and incubated at 25^0 C for 10 days. Colony diameter was recorded on 5^{th}, 6^{th}, 7^{th} and 9^{th} day.

Effect on spore germination of *Fusarium oxysporum* f. sp *ciceri*

Pure culture of Foc isolates were transferred to PDA slant and kept in refrigerator at 4^0 C for further use. For the preparation of conidial suspensions, a block of the stock culture was transferred to PDA medium for 4 days at 28^0 C in the dark and then transferred to liquid potato dextrose medium for 4 days at 28^0 C in dark and shaken on the rotary at 150 rpm. The fungal culture was filtered under sterile condition through five layers of gauze, and the spore-suspension concentrated up to 1000 conidia per ml in 100 ml of water. Spore germination in presence of chickpea root exudates were tested on glass slide. Glass slides were uniformly covered with exudates (0.2 ml) on an area marked (2.5 × 2.5 cm) and dried. The conidial suspension (0.2 ml) was placed on treated slides. The slides were kept for incubation at 25^0 C for 48 hrs. Three microscopic fields examined under low power in each slide for conidial germination.

Results and Discussion

Effect of root exudates on fungal growth and spore germination of *Fusarim oxysporium* f. sp. *ciceri*

Effect of root exudates on fungal growth on *Fusarium oxysprum* f. sp. *ciceri*

Effect of root exudates of eleven chickpea cultivars on the growth of *Fusarium oxysporum* f. sp. *ciceri* were studied and presented in table 1. In order to study the effect of root exudates on fungal growth of *Fusarium oxysporum* f. sp. *ciceri* on PDA medium containing root exudates of all the 11 chickpea cultivar along with check was done by measuring the colony diameter after 5^{th}, 6^{th}, 7^{th} and 9^{th} days after incubation (DAI).

Table 1: Effect of root exudates of eleven chickpea cultivars on the growth of *Fusarium oxysporum* f. sp. *ciceri*.

Varieties	*Colony diameter in (mm)			
	5^{th}(DAI)	6^{th}(DAI)	7^{th}(DAI)	9^{th}(DAI)
JG-315	8.10	9.10	11.14	17.33
JG-62	52.20	60.10	67.26	85.13
Kirpa	9.36	10.30	12.21	19.33
Virat	13.33	14.26	15.16	20.50
Vijay	32.33	35.56	40.03	50.46
Digvijay	15.23	16.20	18.43	23.40
Jaki	17.40	18.56	20.33	26.50
Saki-9516	20.43	22.36	24.33	29.33
BCP-10	18.46	20.53	22.40	27.56
BCP-160	22.46	24.66	26.73	31.80
Akash	25.40	26.60	29.30	33.46
Control	55.13	60.46	70.36	88.30
SE	0.33	2.03	0.18	0.28
CD at 1%	1.31	1.32	0.72	1.12

* Mean of three replications **(**DAI): Days after Incubation

The data presented in above table revealed that the effect of root exudates of 11 chickpea cultivars on the growth of *Fusarium oxysporum* f. sp. *ciceri*. The colony diameter ranged from 17.33 mm to 88.30 mm on 9^{th} DAI. Highest colony diameter was recorded in the root exudates by variety JG-62 was 85.13 mm on 9^{th} DAI which was followed by the colony diameter in the root exudates of variety Vijay was 50.46 mm. Lowest colony diameter was recorded in the *Fusarium oxysporium* f. sp. *ciceri* by the root exudates of the variety JG-315 was 17.33 mm on 9^{th} DAI followed by colony diameter which was slightly higher by root exudates of variety Kirpa was 19.33 mm on 9^{th} DAI. The colony diameter of root exudates by the cultivar Jaki was 26.50 mm on 9^{th} DAI & colony diameter of root exudates by the cultivar BCP-10 was 27.56 mm on 9^{th} DAI were at par with each other.

Colony diameter of root exudates by the cultivar JG-62 was 85.13 mm on 5th DAI which was increased gradually on 6th, 7th & 9th DAI. The colony diameter of root exudates by the cultivar JG-315 was recorded lowest *i.e*. 8.10 mm on 5th DAI which was also increased gradually on 6th, 7th & 9th DAI. Colony diameter of root exudates by JG-315 was 8.10 mm & Kirpa was 9.36 mm which were at par to each other.

Effect of root exudates on spore germination on *Fusarium oxysprum* f. sp. *Cicero:* Effect of root exudates of eleven chickpea cultivars on spore germination of *F*usarium *oxysporum* f. sp. *ciceri* were studied and presented in table 2.

Table 2: Effect of root exudates of eleven chickpea cultivars on spore germination (%) of *Fusarium oxysporum* f. sp. *ciceri.*

Sr. No.	Varieties	*Spore germination (%) At 48 hrs.
1	JG-315	18.33
2	JG-62	84.70
3	Saki-9516	21.13
4	Digvijay	23.03
5	Vijay	42.66
6	Jaki	24.40
7	Virat	26.56
8	Kirpa	29.30
9	Akash	27.63
10	BCP-10	31.03
11	BCP-160	33.56
12	Control	85.30
SE (m)		0.58
CD @ 1%		2.31

* Mean of three replications

In order to study the effect of root exudates on spore germination of *Fusarium oxysporum* f. sp. *ciceri* on Potato Dextrose Broth medium containing root exudates of all the 11 chickpea cultivars along with control (where root exudates is absent). This study was done by measuring the per cent spore germination after 48 hrs. The spore germination per cent at 48 hrs. Ranged from 85.30% to 18.33%. The highest spore germination per cent was found in control treatment was 85.30% followed by spore germination per cent by the cultivar JG-62 was 84.70% and spore germination per cent by the cultivar Vijay was 42.66%. The lowest spore germination per cent found in JG-315 was 18.33% followed by root exudates by the cultivar Saki-9516 was 21.13% after 48 hrs. The spore germination per cent of root exudates by the cultivar Virat was 26.56% and Jaki was 24.40% were at par to each other after 48 hrs. The spore germination per cent of root exudates by the cultivar Kirpa was 29.30% and Akash was 27.63% were at par to each other after 48 hrs.

Similar results were agreement with Gupta *et al.* (1985) [1] reported that colony diameter of *Fusarium oxysporum* f. sp. *ciceri* on PDA mixed with root exudates of susceptible and resistant varieties. He clearly showed that minimum growth occurred on medium containing root exudates of susceptible variety JG-62.

The results were correlating with most of earlier scientists like Haware *et al*. (1994) [3] found that after 24 and 48 hours, spore germination and mean hyphal length was inhibited by the crude exudates of chickpea cultivars CPS1 and JG-62 as compared to control.

Stevenson *et al.* (1995) [8] reported that the inhibitory effects of the active exudates were negated when the apolar components of the exudates were removed by extraction with ethyl acetate. The root exudates of the susceptible cv. JG-62 and the late wilting cv. H-208 did not inhibit germination. The hyphal growth of germinated spores was also strongly inhibited by the concentrated exudates of CPSl and WR-315 and diluted exudates were less potent. The highest concentration of the exudate of the susceptible cv. JG-62 showed some inhibition of hyphal growth, whereas none of the exudates of H-208 were found to contain any antifungal activity. Shrivastava and Dube (2014) [7] reported that the germination of micro conidia of *Fusarium oxysporum* f. sp. *ciceri* started after 5 hours (100%) while macro conidia did not germinate up to 7 hours. The old concentrate mustard root leache with the increase in dilution of concentrate from 1.1 to 1.4, the spore germination started early i.e. after two hours (15.4% and 11.1% in micro and macro conidia, respectively).

Similar results were correlating with Patil (2015) [6] from the root exudates study concluded that among the four cultivars tested. Root exudates from resistant cultivar JG 315 had inhibitory effect on colony growth and spore germination of different isolates of *Fusarium oxysporum* f. sp. c*iceri* on the contrary, the root exudates from susceptible cultivar JG-62 and late wilting cultivar K-850 did not inhibit colony growth and spore germination of all isolates of Foc whereas; root exudates of JG-74 resulted in higher colony growth in all isolates except in I-20 (Race 4) where the inhibitory effect on colony growth and spore germination.

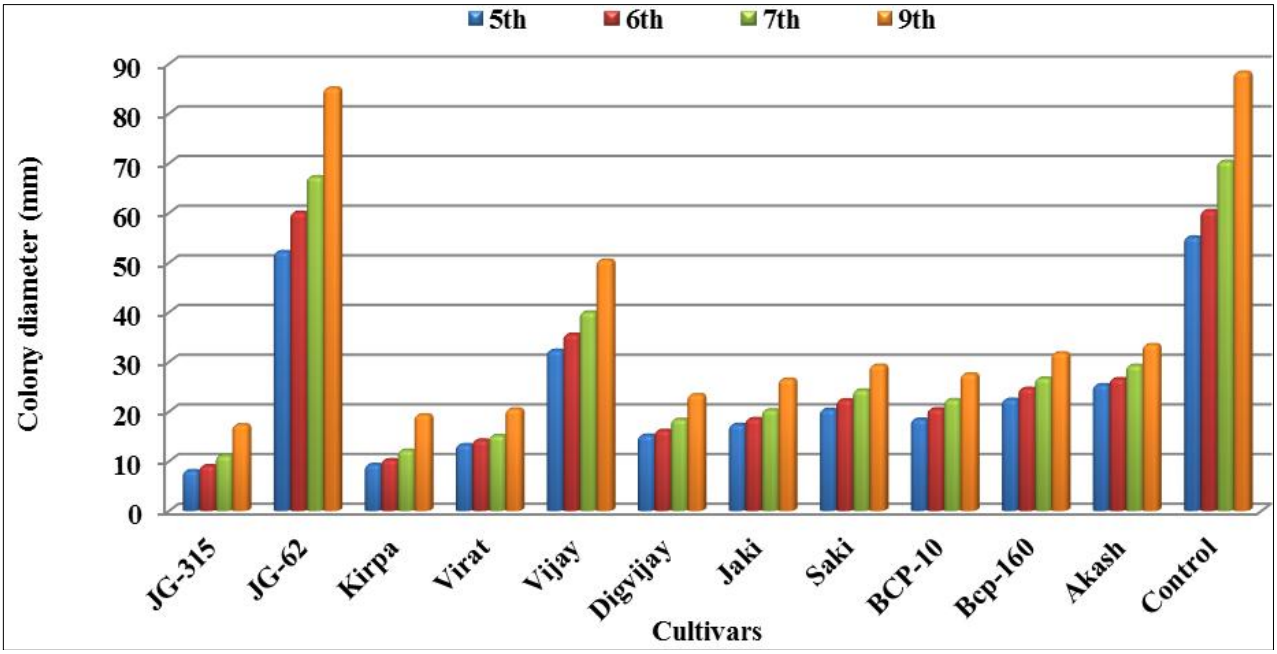

Fig 1: Effect of root exudates of eleven chickpea cultivars on the mycelial growth of *Fusarium oxysporum* f. sp. *ciceri* at different intervals

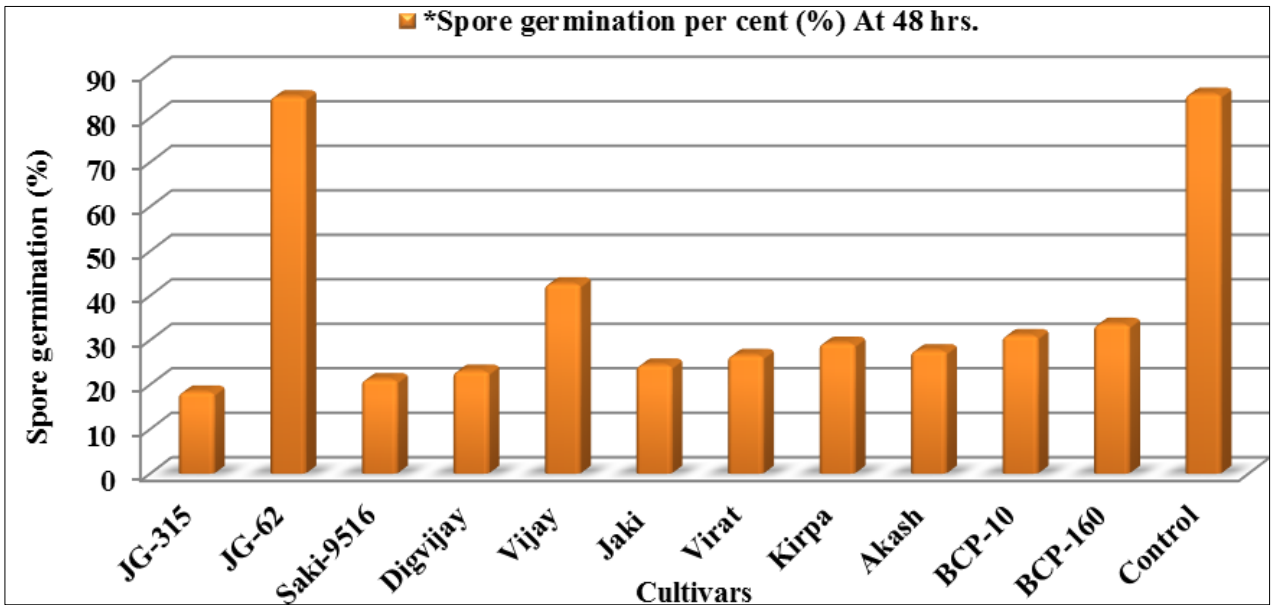

Fig 2: Effect of root exudates of eleven chickpea cultivars on spore germination of *Fusarium oxysporum* f. sp. *ciceri* at 48 hrs.

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