Table 2. Effect of extract of autoclaved soil amended with different crop residues on mycelial growth of some chickpea pathogens.

Crop	Percentage of inhibition (–) or stimulation (+) over control					
	A. rabiei	*R. solani*	*F. solani*	*F. oxysporum* f. sp *ciceris*	*F. equiseti*	*F. moniliforme*
Control	0.0 (2.23)[1]	0.0 (9.0)	0.0 (2.10)	0.0 (5.10)	0.0 (2.50)	0.0 (5.42)
Chickpea	+6.27 (2.37)	0.0 (9.0)	-12.38 (1.84)	-1.76 (5.01)	+3.2 (2.58)	+3.87 (5.63)
Lentil	+21.97 (2.72)	0.0 (9.0)	+14.28 (2.40)	+5.49 (5.38)	+10.0 (2.75)	+3.87 (5.63)
Rape	+10.31 (2.46)	0.0 (9.0)	+27.61 (2.68)	-0.98 (5.05)	+8.0 (2.70)	-0.73 (5.38)
Common vetch	0.0 (2.23)	0.0 (9.0)	+8.57 (2.28)	-2.15 (4.99)	+36.0 (3.40)	-0.36 (5.40)
Wheat	+2.24 (2.28)	0.0 (9.0)	+1.90 (2.14)	-2.15 (4.99)	+12.4 (2.81)	+2.58 (5.56)
Barley	-17.93 (1.83)	0.0 (9.0)	+42.85 (3.0)	-2.94 (4.95)	+36.0 (3.40)	+1.10 (5.48)
Oat	+19.28 (2.66)	0.0 (9.0)	+27.14 (2.67)	0.0 (5.10)	+16.8 (2.92)	+0.92 (5.47)
LSD ($P = 0.05$)	0.033	0.0	0.129	0.024	0.311	0.012

1. Figures in parentheses are actual mean values in mm.

growth of *A. rabiei* but it significantly stimulated the growth of *F. solani*. Lentil residue significantly stimulated growth of *F. oxysporum* f. sp *ciceris*, *A. rabiei*, and *F. moniliforme*. The colony growth of *A. rabiei* was stimulated by oat residue (Table 2).

Generally, the extracts of nonautoclaved soils amended with different crop residues inhibited the mycelial growth of the most of these pathogens. This results showed that toxic compounds produced during decomposition of crop residues by microorganisms in natural field soil have both inhibitory and stimulatory effects on the growth of some chickpea pathogens.

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Maintenance of Chickpea Viruses in Tissue Culture

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Chickpea is infected by several viruses. These include stunt, caused by bean leaf roll virus (BLRV) which is transmitted by the aphid vectors, *Aphis craccivora* and *Acyrthosiphon pisum* (Nene and Reddy 1987). Several luteoviruses and a leafhopper-borne geminivirus named chickpea chlorotic dwarf virus (CCDV) seem to be associated with chickpea stunt and "stunt-like" symptoms (Horn et al. 1996), but their precise role in the etiology of the disease is not yet clear. These viruses are phloem-limited, occur in very low concentrations in the plants, and are not mechanically transmissible; thus virus cultures can be maintained only through vector or graft transmission. Since the pioneering experiments of White (1934), plant tissue culture in vitro has been assayed as a potential system to study different aspects of the biology of plant viruses. This paper reports experiments to study the potential of callus cultures for maintenance of phloem-limited viruses infecting chickpea.

Chickpea plants showing "stunt-like" symptoms were collected from experimental fields in ICRISAT Asia Center, Patancheru, India. The plants were initially tested by double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) using antisera to CCDV, and a luteovirus infecting chickpea referred as chickpea luteovirus (CpLV) (Legumes Program, ICRISAT 1991, pp. 8–9). The protocol developed by Sheila et al. (1991) for micropropagation of chickpea was used for maintenance of chickpea viruses in tissue culture. The explants were surface sterilized with Clorox® (5.25% sodium hypochlorite), using Tween 20® (polyoxyethylenesorbitan monolaurate) as a surfactant, for 10 min. Ten explants plant^{-1} were cultured on modified L-6 medium containing 2.0 mg L^{-1} benzyladenine (BA) and 0.5 mg L^{-1} indole acetic acid (Sheila et al. 1991). Shoot proliferation occurred and callus was produced at the base of the explants. Shoots and callus were subcultured at 4-week intervals. The shoots were excised individually and then transferred onto fresh medium of the same composition. The callus was subcultured onto fresh medium of the same composition and on modified L-6 medium with 2.0 mg L^{-1} BA and 0.5 mg L^{-1} naphthalene acetic acid. Samples from shoots and callus cultures of each plant source were assayed for the presence of virus at 4-week intervals. In transmission studies, the vectors were given an acquisition feeding period of 24 h–the leafhopper *Orosius orientalis* on callus positive for CCDV and the aphid *Myzus persicae* on callus positive for CpLV–and then released onto healthy pea seedlings for inoculation feeding of 48 h. Three weeks after inoculation, pea seedlings were tested for the presence of CCDV and CpLV by DAS-ELISA as described earlier.

In DAS-ELISA tests, CpLV and CCDV were detected in shoots and callus cultures after 4 weeks and 8 weeks of initial culture. This indicates that these viruses multiply in the callus and can be maintained for up to 8 weeks. However, further studies are necessary to ascertain the maximum period of retention of different chickpea viruses in the callus. It has been shown that infectivity of the pumpkin mosaic virus in callus produced from infected leaves of *Cucurbita pepo* was retained for more than 6 months (Shankar and Nariani 1973). CCDV was successfully acquired from callus by the leafhopper vector and transmitted. Although CpLV was present in the callus, *M. persicae* failed to transmit the virus. This was in agreement with earlier observations that *M. persicae* is not an efficient vector of CpLV (Legumes Program, ICRISAT 1994, pp. 34–35). Nevertheless, the results indicate the potential value of tissue culture in studying the biology of phloem-limited viruses infecting chickpeas. Maintenance of viruses in callus provide a continuous source of inoculum at any time of the year, without having to depend on virus-infected plants from the field. Virus-infected callus can be produced in large masses in limited space, can serve as a good source for virus acquisition by the vector, and can be used for virus purification or extracting viral nucleic acids for molecular characterization. In addition, callus produced from virus-infected plants collected from different places and seasons can be maintained at one location, and used to study genetic diversity among these viruses.

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