Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(2): 1805-1808
Received: 19-01-2019
Accepted: 21-02-2019

VR Bangar
Department of Plant Pathology, College of Agriculture, Dr. BSKKV, Dapoli, Maharashtra, India

JJ Kadam
Department of Plant Pathology, College of Agriculture, Dr. BSKKV, Dapoli, Maharashtra, India

HT Valvi
Department of Plant Pathology, College of Agriculture, Dr. BSKKV, Dapoli, Maharashtra, India

Correspondence
VR Bangar
Department of Plant Pathology, College of Agriculture, Dr. BSKKV, Dapoli, Maharashtra, India

Effect of spore suspension of pathogen causing diseases on dodder (*Cuscuta gronovii*) parasitized on green gram

VR Bangar, JJ Kadam and HT Valvi

Abstract
Pulses are cultivated in *Konkan* region during *Rabi* season after harvest of *Kharif* rice, the major threat on this pulses is plant parasite. *Cuscuta* causes a serious problem in pulses. In view to management of such serious plant parasite, we used different pathogenic suspension *viz. Fusarium incarnatum*, *Alternaria dianthicola* and *Curvularia pallescens* at diverse concentration. *Cuscuta gronovii* parasitized on green gram host plants were inoculated with different concentration of spore suspension of previous obtained pathogens. The maximum disease severity on *C. gronovii* were recorded by *A. dianthicola* @ 2 x 10^5 and *F. incarnatum* @ 2 x 10^5 concentrations parasitizing on green gram and the host plant reaction was found to be immune. At 21 DAI, *A. dianthicola* @ 2×10^5 and *F. incarnatum* @ 2×10^5 showed maximum disease severity 86.33% and 84.00% respectively. These concentrations of spore suspension of pathogens were act as a mycoherbicide or bioherbicide and applicable to control *Cuscuta* parasitized on green gram plants effectively.

Keywords: *Cuscuta*, pulses, pathogen, spore suspension, *Fusarium incarnatum*, *Alternaria dianthicola*, *Curvularia pallescens* and management

Introduction
India is the largest producer of pulses in the world, both in quantity and variety. Pulses are the primary source of protein for the poor and the vegetarians who constitute majority of Indian population. The traditional cropping pattern always included a pulse crop either as a mixed crop or in rotation, while the commercialization of agriculture has encouraged the practice of sole cropping. Cereal shortage in the mid-sixties and the green revolution accompanied with changes in the infrastructure and incentives including input supplies and price support systems in favour of major cereals, altered the traditional cropping pattern against pulses.
India ranks first in the world in terms of pulse production (25 per cent total worlds production), (FAOSTAT, 2014). Madhya Pradesh, Maharashtra, Uttar Pradesh, Andhra Pradesh, Karnataka and Rajasthan are the major states growing pulses in India. These six states contribute 80 per cent of total pulse production and area (Anonymous, 2014). Maharashtra had 1,125 thousand hectare area and 2,268 million tonnes total pulse production with 743 kg per hectare productivity of pulses (Anonymous, 2014). Pulses are cultivated in Konkan region during *Rabi* season after harvest of *Kharif* rice. There are many reasons for low production of pulses. Out of them phanerogamic plant parasite i.e. *Cuscuta* infection is major problem. In India, *Cuscuta* spp. causes a serious threat in green gram (*Vigna radiata* L.) especially in rice-fallows in the states of Andhra Pradesh, Chhattisgarh, Gujarat, Orissa, West Bengal and parts of Madhya Pradesh under rainfed as well as irrigated conditions. It reproduces mainly by seeds. The yield reductions due to *Cuscuta* are reported to the tune of 60 to 87 per cent in different crops. In green gram 31-34% (Kumar and Kondap, 1992) [3] depending upon its intensity of infestation. Ashton and Santana (1976) [12] used of *Alternaria* spp. to control dodder on alfalfa was effective (90%) and also used *Curvularia* species for control of dodder in Russia. Bewick *et al.* (1987) conducted an experiment and found that about 62.5% of the dodder was destroyed and 24 days after spraying, over 90% of the dodder was destroyed. Considering importance of the host plants and parasite, present study on *Cuscuta gronovii* parasitic on pulses was planned and conducted for biological management of Cuscuta as a mycoherbicide.

Materials and methods
Effect of spore suspension of pathogen causing diseases on *C. gronovii*
Selection of test/host plant
One cultivated pulses host of economic and commercial importance i.e. green gram

(*Vigna radiata* L.) was chosen from survey of host range of *C. gronovii* of Konkan region during 2015-16. On the basis of parasitism of *Cuscuta* on this host plants were observed in severe form particularly in pulse fields. Hence green gram as a host plant was selected to test the effect of spore suspension of isolated pathogen of *C. gronovii* causing diseases on *Cuscuta*.

Preparation of spore suspension of pathogens

Pure cultures of *Alternaria*, *Curvularia* and *Fusarium* species were used to test the effect of their spore suspension causing diseases on *Cuscuta*. Culture of all the pathogenic fungi were grown on PDA individually and placed under fluorescent lights with a 12 hour photoperiod until they sporulated. Seven to eight days old sporulated cultures were used to infect dodder (*C. gronovii*) growing on pulse crops in a controlled environment i.e. in a glasshouse. Spore suspension of the test pathogens was prepared by pouring the distilled sterile water in 7-8 days old culture plates. The resultant spore suspension was filtered through muslin cloth and filtrate obtained was suitably diluted with distilled sterile water to get desired inoculum concentration determined by using a haemocytometer. Different concentrations of the spore suspension were adjusted (Table 1).

Spore concentration was calculated by following formula

$$\text{Concentration (spores per ml)} = \frac{\text{Total spore counted}}{\text{No. of squares}} \times \text{Dilution factor} \times 10^4$$

Spraying of spore suspension of pathogens on green gram parasitized by *C. gronovii*:

A pot experiment was conducted under glasshouse conditions. Seven treatments and green gram host plants (Table 1) with three replications of each treatment were employed and arranged strictly under the Completely Randomized block Design (CRD) individually for each host plant.

Table 1: List of spore suspensions of pathogens tested against *C. gronovii* and its host plants

Tr. No.	Concentration of spore suspensions of pathogens used (Spores/ml)	*Cuscuta* parasitized on pulse host plants
T_1	*Fusarium incarnatum* @ 5×10^4	Green gram (*Vigna radiata* L.)
T_2	*Fusarium incarnatum* @ 2×10^5	
T_3	*Alternaria dianthicola* @ 5×10^4	
T_4	*Alternaria dianthicola* @ 2×10^5	
T_5	*Curvularia pallescens* @ 5×10^4	
T_6	*Curvularia pallescens* @ 2×10^5	
T_7	Control (sterile water)	

Seeds of *Cuscuta* were sown together with green gram plants was sown individually in 9 inch plastic pots containing desired potting mixture comprising of FYM and soil (1:2). The host-parasite system was allowed to grow for two weeks after parasitism had occurred. At this time, *Cuscuta* and host plants were inoculated with spore suspension of pathogen. Water with non-ionic surfactant 0.05% v/v was used as a carrier. The spore suspensions were applied with an atomizer. The plants were sprayed individually with 20 ml of the spore suspension. Both green gram plants and dodder were covered with uniform spore suspension of the test fungus. Control plants (un-inoculated controls) were sprayed with sterile distilled water. After the suspension had dried on the foliage, a fog system was used to provide surface moisture. The fog system was automatically timed to turn on for 25 seconds every one hr. for 1 day following inoculation.

Observations on disease incidence

Disease rating on host plants

The host reactions were determined by comparing the treatment reaction with the untreated control. After inoculation, observations on disease incidence were recorded on all the green gram host plants and rated weekly for 3 weeks. Disease was assessed on a scale of 1 to 10 (Cook, 2006).

Disease Rating	Description (Symptoms developed on host plant)	Reaction
1-2	None or few leaf spots observed, slight stunting	I
3-6	Hypersensitive response (HR), 25% of leaf covered in spots, stunting	R
7-10	Severe stunting, blighted, plant death	S

Where,

I = Immune, R = Resistant, S = Susceptible

Disease rating on *Cuscuta*

Observations were recorded weekly for 3 weeks after inoculation on dodder on the basis of development of disease symptoms. The disease severity was recorded in 0-5 scale as described below (Cook, 2006) [9].

Score/ Grade	Disease severity (%)	Description (Symptoms developed on *Cuscuta*)
0	0	No symptoms.
1	1-10	Tip necrosis; stems starting to wilt and become necrotic.
2	11-35	Slightly more stem necrosis; flowers starting to senesce.
3	36-65	Over half of the stems are dead or dying; clusters of flowers senescing.
4	66-90	The majority of the stems and flowers are dead or dying; some healthy flowers and stems may still be present.
5	91-100	*Cuscuta* vine death.

Statistical analysis

The data obtained were statistically analysed by the methods suggested by Gomez and Gomez (1986). Three replications of Each treatment were employed and arranged strictly under the Completely Randomized block Design (CRD). The standard error and critical difference were worked out and the results Obtained were compared statistically.

Results and Discussion

C. gronovii parasitized on green gram host plants were inoculated with different concentration of spore suspension of 3 fungi consisting 6 treatments and another control treatment in greenhouse condition to test the effect of spore suspension of pathogens infecting the *Cuscuta*. The data obtained on the effect of different spore suspension of pathogens on disease severity or incidence on *Cuscuta* and host reaction was recorded weekly up to 3 weeks and results obtained are presented in (Table 2).

Table 2: Per cent disease severity on *C. gronovii* parasitizing on green gram and its reaction

Tr. No.	Inoculation of spore suspension (spores/ml)	Mean Per cent Disease severity on *C. gronovii*			Overall Host Reaction
		7 DAI	14 DAI	21 DAI	
T_1	*Fusarium incarnatum* @ 5×10^4	18.67 (25.56)*	38.33 (38.24)	71.33 (57.66)	I
T_2	*Fusarium incarnatum* @ 2×10^5	30.33 (33.41)	54.00 (47.30)	84.00 (66.50)	I
T_3	*Alternaria dianthicola* @ 5×10^4	19.33 (26.05)	40.33 (39.42)	73.33 (58.94)	I
T_4	*Alternaria dianthicola* @ 2×10^5	31.33 (34.04)	56.33 (48.65)	86.33 (68.44)	I
T_5	*Curvularia pallescens* @ 5×10^4	6.00 (14.15)	16.00 (23.57)	33.00 (35.05)	I
T_6	*Curvularia pallescens* @ 2×10^5	10.00 (18.42)	24.33 (29.53)	43.33 (41.16)	I
T_7	Control (Sterile water)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	I
S.Em.±		0.45	0.56	0.76	
C.D.at 1%		1.88	2.35	3.19	

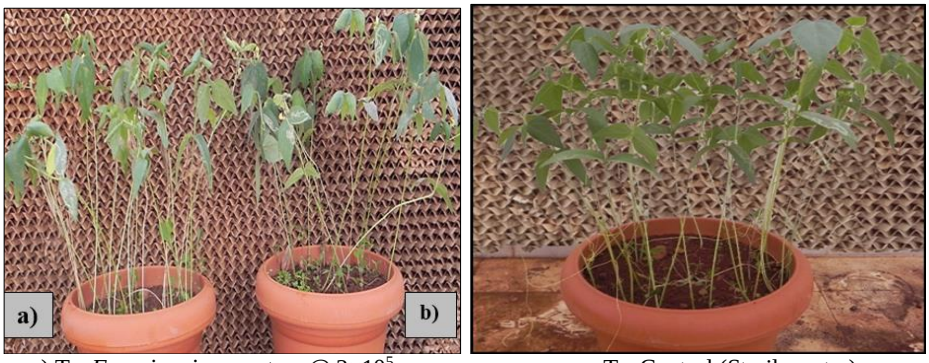
(*Figures in parentheses indicate arc sin values)

Where, I = Immune/Incompatible

It is apparent from the data presented in Table 2 that, all the treatments of spore suspension were significantly superior over control. The disease severity on *C. gronovii* was recorded at 7 DAI, 14 DAI and 21 DAI ranged between 0.00 to 31.33%, 56.33% and 86.33%, respectively (Fig. 1). There was no disease incidence on *Cuscuta* in control treatment. Green gram host plants were found to be immune to all treatments (PLATE I).

It is revealed from the data presented in Table 2 that at 7 DAI, the treatment *Alternaria dianthicola* @ 2×10^5 was the most effective as it recorded maximum disease incidence (31.33%) and was at par with *Fusarium incarnatum* @ 2×10^5 (30.33%). It was followed by *A. dianthicola* @ 5×10^4 (19.33%), *F. incarnatum* @ 5×10^4 (18.67%) and *C. pallescens* @ 2×10^5 (10.00%). *C. pallescens* @ 5×10^4 was the least effective treatment which showed 6.00% disease incidence on *Cuscuta*.

At 14 DAI, *Alternaria dianthicola* @ 2×10^5 was the most effective treatment as it recorded maximum disease severity (56.33%) on *Cuscuta* and was at par with *F. incarnatum* @ 2×10^5 (54.00%). It was followed by *A. dianthicola* @ 5×10^4 (40.33%), *F. incarnatum* @ 5×10^4 (38.33%) and *C. pallescens* @ 2×10^5 (24.33%). *C. pallescens* @ 5×10^4 was the least effective treatment (16.00%).



a) T_2- *Fusarium incarnatum* @ 2×10^5
b) T_4 -*Alternaria dianthicola* @ 2×10^5
T_7- Control (Sterile water)

PLATE 1: Disease severity on *C. gronovii* parasitized on green gram and host reaction at 21 DAI

At 21 DAI, *A. dianthicola* @ 2×10^5 was the most effective treatment which showed maximum disease severity (86.33%) on *Cuscuta*. It was at par with *F. incarnatum* @ 2×10^5 (84.00%). It was followed by *A. dianthicola* @ 5×10^4 (73.33%), *F. incarnatum* @ 5×10^4 (71.33%) and *C. pallescens* @ 2×10^5 (43.33%). *C. pallescens* @ 5×10^4 was the least effective treatment showed disease incidence (33.00%) on *Cuscuta*. The results are in accordance with those reported by Ashton and Santana (1976) [12]. They found that use of *Alternaria* spp. to control dodder on alfalfa was effective (90%) and also used *Curvularia* spp. for control of dodder in Russia. Similarly, Bewick *et al.* (1987) conducted an experiment in which spore suspension (5×10^5) of each fungi *viz*., *Fusarium tricinctum* and *Alternaria* spp. were applied individually by surface spraying to field plots where actively growing in a cranberry marsh were parasitized by *C. gronovii* Willd. (Swamp dodder) and observed that 10 days after spraying, about 62.5% of the dodder was destroyed and 24 days after spraying, over 90% of the dodder was destroyed. In another experiment conducted by Bewick *et al.* (1987) reported that different economic crops *viz*., cranberries, carrots, celery, mint, alfalfa and potatoes were found to be

immune to *Alternaria* spp. ATCC 20831 and *Fusarium tricinctum* ATCC 20832.The findings of present study are also in close conformity with Shakir *et al.* (1999) [11] and Cook *et al*. (2009) [10].

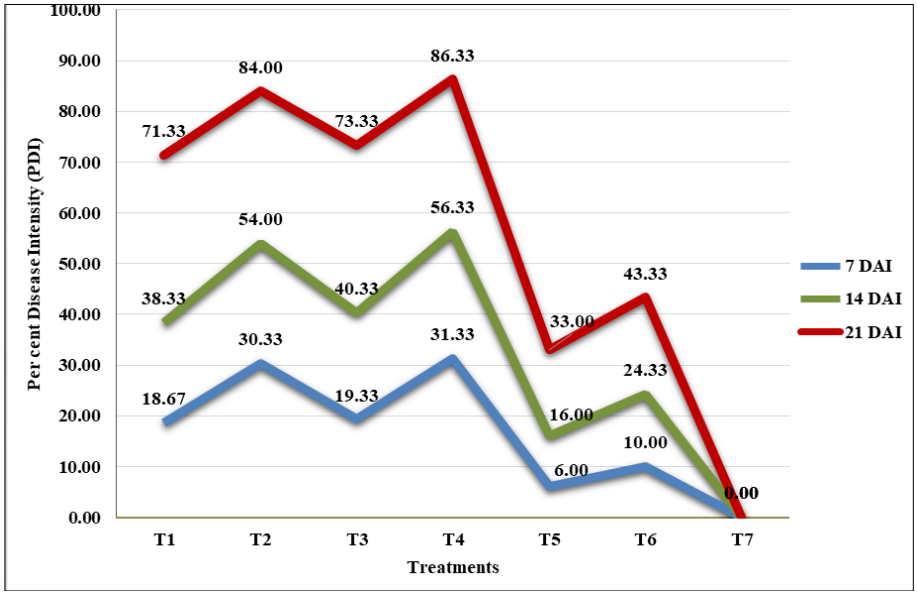


Fig 1: Effect of spore suspension of pathogen on *C. gronovii* parasitized on green gram

Conclusion

The results of present study proved that concentration of spore suspension (2×10^5) of *A. dianthicola* and *F. incarnatum* are very effective to cause diseases on *C. gronovii* particularly when *Cuscuta* parasitized on green gram host plant. This is probably due to the fact that, fungi like *A. dianthicola* and *F. incarnatum* are capable or potential to infect *C. gronovii* i.e. pathogenic to *Cuscuta* but non-pathogenic to green gram as a host plants. These concentrations of spore suspension of pathogens are act as a mycoherbicide or bioherbicide and applicable to control or manage *C. gronovii* parasitized on green gram plants effectively.

References

1. Anonymous. Production of pulses, 2014. www.agropedia.com.
2. Awatigeri MB, Hosmani MM, Shetty RA, Vijaya KN. Curr. Res. *(Monthly Newsletter)*, Univ. Agril. Sci. Bangalore. 1975; 4:47-48.
3. Kumar RM, Kondap SM. Response of greengram and blackgram cultivars to *Cuscuta* infestation. Ind. J Plant Protec. 1992; 21:167-171.
4. Tosh GC, Patro GK, Misra A. Effect of pronamide and chlorpropham on *Cuscuta* stem parasite in Niger. Abst. Papers of Weed Sci. Conf., Ind. Soc. of Weed Sci., Hyderabad, India, 1977, 275-276.
5. Moorthy BTS, Mishra JS, Dubey RP. Certain investigations on the parasitic weed *Cuscuta* in field crops. Ind. J Weed Sci. 2003; 35:214-216.
6. Marambe B, Wijesundara S, Tennekoon K, Pindeniya, D, Jayasinghe C. Growth and development of Cuscuta chinensis Lam. and its impact on selected crops. Weed Biol. Manage. 2002; 2:79-83.
7. Narayana L. Management of *Cuscuta* in alfalfa. M. Sc. Thesis, Andhra Pradesh Agril. Univ. Hyderabad, India, 1989.
8. Dalvi MB, Joshi MS, Chavan LS. Control of Dodder Parsitic on Pulses. Associate Director of Research, Regional Agril. Research Centre, Karjat, Dist. Raigad. Project Report Submitted to Project Director, ATMA, Alibag, Raigad, 2014.
9. Cook JC. Integrated control of Dodder (*Cuscuta pentagona* Engelm.) using Glyphosate, Ammonium sulfate and the biological control agent *Alternaria destruens* Simmons, sp. nov. Ph. D. Dissertation presented or submitted to the Graduate School, University of Florida, 2006.
10. Cook JC, Charudattan, R, Zimmerman TW, Rosskopf EN, Stall WM, MacDonald GE. Effects of *Alternaria destruens*, Glyphosate, and Ammonium Sulfate Individually and Integrated for Control of Dodder (*Cuscuta pentagona*). Weed Technology. 2009; 23(4):550-555.
11. Shakir AS, Iqbal MZ, Sahi ST. First report on Association of Some Fungal Organisms with Dodder (*Cuscuta*) Blight in Pakistan. Pakistan Journal of Biological Sciences. 1999; 2(3):991-992.
12. Ashton FM, Santana D. *Cuscuta* spp. (Dodder): A literature review of its biology and control. Division of Agril. Sciences. University of California. Bulletin. 1976; 1880:22.