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Effect of spore suspension of pathogen causing diseases on *Cuscuta gronovii* parasitized on black gram and *lablab* bean

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Abstract
Cuscuta is a phanerogamic plant parasite and complete stem parasite of various cultivated pulses. In view to biological management of such serious plant parasite, *C. gronovii* parasitized on two pulse host plants such as black gram and *Lablab* bean were inoculated with different concentration of spore suspension of previously isolated pathogens. At 21 DAI, the maximum disease severity (85.33 per cent) on *C. gronovii* was recorded by *Alternaria dianthicola* @ 2 x 10^5 spores per ml concentration when *Cuscuta* parasitizing black gram. *Fusarium incarnatum* @ 2 x 10^5 which showed 84.67 per cent disease severity on *Cuscuta* parasitized on black gram and the host plant reaction was immune against pathogen. The results of present study proved that concentration of spore suspension (2×10^5) of *A. dianthicola* and *F. incarnatum* were very effective to cause diseases on *C. gronovii* particularly when *Cuscuta* parasitized on black 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 black gram. These concentrations of spore suspension of pathogens (fungi) act as a mycoherbicide or bioherbicide and applicable to control or manage *C. gronovii* parasitized on black gram plants effectively but in case of *C. gronovii* parasitized on *Lablab* bean, these concentrations of spore suspensions were not applicable to manage *Cuscuta* parasitized on *Lablab* bean plants due to susceptible host reaction.

Keywords: *Cuscuta gronovii*, Pulses, Spore suspension, *Fusarium incarnatum*, *Alternaria dianthicola*, *Curvularia pallescens*, mycoherbicide/bioherbicide 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. There are many reasons for low production of pulses. Out of them phanerogamic plant parasite i.e. *Cuscuta* infection is major problem.

Dodder is an obligate and holo stem parasite; it cannot complete its life cycle alone. Parasitic plants of the genus *Cuscuta* have no chlorophyll or only a reduced amount and are not usually photo-synthetically active (Garcia *et al*., 2014) [9]. In India, *Cuscuta* spp. causes a serious problem in pulses like black gram (*Vigna mungo* L.), especially in rice-fallows under rainfed as well as irrigated conditions. The yield reductions due to *Cuscuta* are reported to the tune of 60 to 87 per cent in different crops. In black gram 31-34% (Kumar and Kondap, 1992) [13] depending upon its intensity of infestation.

In Konkan region of Maharashtra, the *Cuscuta gronovii* was found to be parasitic on crops of *Rabi* season. In Raigad and Thane districts, it is serious problem on pulses like beans, black gram cow pea etc. in *Rabi* season. Its parasitic effects reduce the plant vigour and yield. In severe infestation the infested plants may die. While harvesting the pulses which are infected with *Cuscuta* leads to breathing and vomiting problems to labourers (Dalvi *et al*., 2014) [6].

Cuscuta spp. affects the growth and yield of infected plants. Losses occurred range from slight to complete destruction of the infested areas. In the production of crop seeds, the *Cuscuta* imposes a severe limitation because of difficulty of removal of their seeds when the crop is graded out thus, reducing the yield and quality. It is thought that seed can remain viable for many years. Thus, once a farm is infested, it can remain infested for many years. A single plant of *C. campestris* can produce 16000 seeds (Stevens, 1932) [17]. The weight of 1000 seeds is 0.77 – 0.87 g in *C. campestris* (Stevens, 1932 and Holm *et al*., 1997) [17, 11] and 0.3 g in *C. epithymum* (Kothekar, 1970). *Cuscuta* may survive at least 10 years in the field (Menke, 1954) [14] and up to 50 years or more in dry storage depending upon the species

(Gaertner, 1950; Dawson *et al*., 1984) [8, 7]. Hence it's a very difficult to control. Considering importance of the host plants and parasite, present study on *Cuscuta gronovii* parasitic on pulses was planned and conducted on biological management of Cuscuta.

Materials and Methods

Effect of spore suspension of pathogen causing diseases on *C. gronovii*:

Selection of test/host plants of phanerogamic parasite

Two cultivated pulses of economic and commercial importance comprising 2 species *viz*., black gram (*Vigna mungo* L.) and *Lablab* bean (*Lablab pupureus* L.) were 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 these pulse host plants were selected to test the effect of spore suspension of isolated pathogen of *C. gronovii* causing diseases on *Cuscuta*.

Isolation of pathogens causing diseases on *C. gronovii*

Fresh samples of diseased Cuscuta, showing the typical symptoms like browning or discoloured (blighted/necrotic) and shrivelling of vines were brought to the laboratory from different locations of Raigad and Thane districts of Konkan region of Maharashtra in 2015-16. These samples were washed with running tap water to remove extraneous material. Small pieces of the desired size were cut by taking care that each piece contained half infected and half healthy portion. Such pieces were then disinfected with 0.1 per cent mercuric chloride ($HgCl_2$) for 1 minute followed by three washings in distilled sterile water to remove the traces of mercuric chloride. These pieces were then placed on sterilized blotters for drying. Properly dried pieces were transferred aseptically in sterilized Petri plates containing sterilized, solidified PDA medium. The plates were incubated in BOD incubator at 25 ± 2 °C till the fungal mycelium fully covered the surface of the medium. This fungal growth was transferred to PDA slants and maintained as stock culture for further studies. On the basis of microscopic observations and cultural characters, the fungi were tentatively identified as Fusarium, Alternaria and Curvularia spp. The further identification at the species level was confirmed by the Chief Mycologist, Agharkar Research Institute, Pune and the fungi were identified as *Fusarium incarnatum* (Desm.) Sacc. *Alternaria dianthicola* Neerg. and *Curvularia pallescens* Boedijn.

Preparation of spore suspension of pathogens i.e. inoculum

Pure cultures of *A. dianthicola*, *C. pallescens* and *F. incarnatum* 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 different pulses plant parasitized by *C. gronovii*

Two pot experiments were conducted under glasshouse conditions. Seven treatments and two pulse 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	1. Black gram (*Vigna mungo* L.) 2. Lablab bean (*Lablab purpureus* 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 pulse plants *viz*., black gram and lablab bean were 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 pulse crops 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 host plant and rated weekly for 3 weeks. Disease was assessed on a scale of 1 to 10 (Cook *et al*., 2006) [4].

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 and 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 *et al*., 2006) [4].

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). The standard error and critical difference were worked out and the results obtained were compared statistically.

Results and Discussion

C. gronovii parasitized on different two pulse 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.

a) Black gram

It is apparent from the data presented in Table 2 & Fig. 1 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 31.67 %, 56.67 % and 85.33 %, respectively. There was no disease incidence on *Cuscuta* in control treatment. Black gram host plants were found to be immune to all treatments (PLATE I).

Table 2: Per cent disease severity on *C. gronovii* parasitizing on black 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	17.00 (24.33)*	39.33 (38.83)	70.67 (57.23)	I
T_2	*Fusarium incarnatum* @ 2×10^5	30.67 (33.62)	54.33 (47.49)	84.67 (66.99)	I
T_3	*Alternaria dianthicola* @ 5×10^4	18.67 (25.52)	43.67 (41.35)	71.00 (57.42)	I
T_4	*Alternaria dianthicola* @ 2×10^5	31.67 (34.23)	56.67 (48.83)	85.33 (67.50)	I
T_5	*Curvularia pallescens* @ 5×10^4	5.67 (13.73)	18.67 (25.59)	35.00 (36.27)	I
T_6	*Curvularia pallescens* @ 2×10^5	11.33 (19.65)	26.33 (30.85)	46.67 (43.08)	I
T_7	Control (sterile water)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	I
S.Em.±		0.95	0.55	0.63	
C.D. at 1%		4.00	2.33	2.63	

(*Figures in parentheses indicate arc sin values) Where, I = Immune

Plate I: Disease incidence on *C. gronovii* parasitized on black gram and host reaction at 21 DAI

It is revealed from the data presented in Table 2 that, Seven DAI, the treatment *A. dianthicola* @ 2×10^5 was the most effective as it recorded maximum disease incidence (31.67%). It was at par with *F. incarnatum* @ 2×10^5 (30.67%). It was followed by *A. dianthicola* @ 5×10^4 (18.67%), *F. incarnatum* @ 5×10^4 (17.00%) and *C. pallescens* @ 2×10^5 (11.33%). *C. pallescens* @ 5×10^4 was the least effective treatment (5.67%). At 14 DAI, *A. dianthicola* @ 2×10^5 was the most effective treatment which showed recorded maximum disease severity (56.67%) on *Cuscuta*. It was followed by *F. incarnatum* @ 2×10^5 (54.33%), *A. dianthicola* @ 5×10^4 (43.67%), *F. incarnatum* @ 5×10^4 (39.33%) and *C. pallescens* @ 2×10^5 (26.33%). *C. pallescens* @ 5×10^4 was the least effective treatment (18.67%).

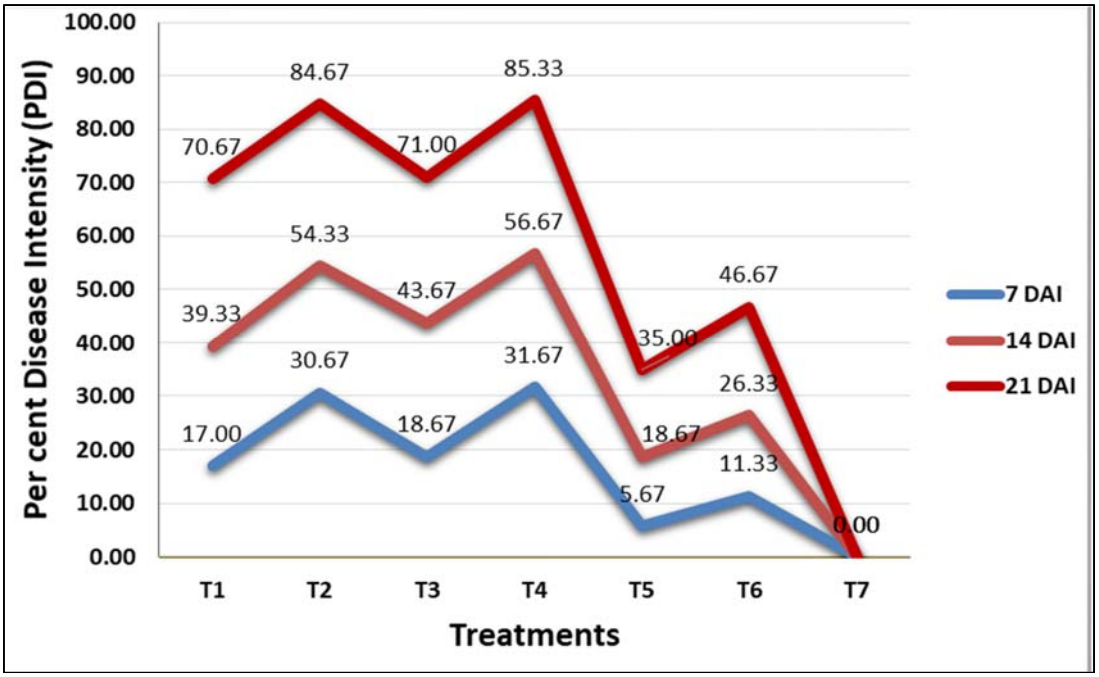

Fig 1: Effect of spore suspension of pathogen on *C. gronovii* parasitized on black gram

At 21 DAI, *A. dianthicola* @ 2×10^5 was the most effective treatment which recorded maximum disease severity (85.33%) on *Cuscuta* and was at par with *F. incarnatum* @ 2×10^5 (84.67%). This was followed by *A. dianthicola* @ 5×10^4 (71.00%), *F. incarnatum* @ 5×10^4 (70.67%), *C. dianthicola* @ 2×10^5 (46.67%) and *C. pallescens* @ 5×10^4 (35.00%). *C. pallescens* @ 5×10^4 was the least effective treatment. The findings of present study are also in close conformity with Shakir *et al*. (1999) [16], Bewick *et al*. (2000) [3] and Cook *et al*. (2009) [5].

b) *Lablab* bean

It is apparent from the data presented in Table 3 that, all the treatments of spore suspension were significantly superior over control. The disease severity on *C. gronovii* was recorded at 7, 14 and 21 DAI ranged between 0.00 to 31.33, 60.67 and 100 per cent, respectively. There was no disease incidence on *Cuscuta* in control treatment.

Table 3: Per cent disease severity on *C. gronovii* parasitizing on *Lablab* bean 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	19.00 (25.82)*	47.67 (43.66)	92.00 (73.73)	S
T_2	*Fusarium incarnatum* @ 1.2×10^5	30.33 (33.41)	55.33 (48.07)	94.33 (76.24)	S
T_3	*Alternaria dianthicola* @ 5×10^4	21.00 (27.26)	50.00 (45.00)	100.00 (90.00)	S (death)
T_4	*Alternaria dianthicola* @ 1.2×10^5	31.33 (34.03)	60.67 (51.17)	100.00 (90.00)	S (death)
T_5	*Curvularia pallescens* @ 5×10^4	4.67 (12.46)	13.00 (21.10)	25.00 (29.99)	R
T_6	*Curvularia pallescens* @ 1.2×10^5	11.00 (19.32)	35.33 (36.47)	70.00 (56.82)	S
T_7	Control (sterile water)	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)	I
S.Em.±		0.45	0.46	0.54	
C.D. at 1%		1.88	1.95	2.26	

(*Figures in parentheses indicate arc sin values) Where, S = Susceptible, R = Resistant and I = Immune

At 7 DAI, the treatment *A. dianthicola* @ 2×10^5 was the most effective which showed maximum disease incidence (31.33%) on *Cuscuta* and was at par with *F. incarnatum* @ 2×10^5 (30.33%). It was followed by *A. dianthicola* @ 5×10^4 (21.00%), *F. incarnatum* @ 5×10^4 (19.00%) and *Curvularia pallescens* @ 2×10^5 (11.00%). *C. pallescens* @ 5×10^4 was the least effective treatment which showed 4.67 % disease incidence on *Cuscuta*. Fourteen DAI, the maximum disease severity (60.67%) on *Cuscuta* was observed in the treatment of *Alternaria dianthicola* @ 2×10^5. It was followed by *F. incarnatum* @ 2×10^5 (55.33%), *A. dianthicola* @ 5×10^4 (50.00%), *F. incarnatum* @ 5×10^4 (47.67%), *C. pallescens* @ 2×10^5 (35.33%). *C. pallescens* @ 5×10^4 was the least effective treatment against *Cuscuta* which showed 13.00 % disease incidence. At 21 DAI, the maximum disease incidence (100%) on *Cuscuta* was observed in treatment of *A. dianthicola* @ 2×10^5 and *A. dianthicola* @ 5×10^4. This was followed by *F. incarnatum* @ 2×10^5 (94.33%), *F. incarnatum* @ 5×10^4 (92.00%), *C. pallescens* @ 2×10^5 (70.00%). *C. pallescens* @ 5×10^4 was the least effective treatment against *Cuscuta* which showed 25.00 % disease incidence.

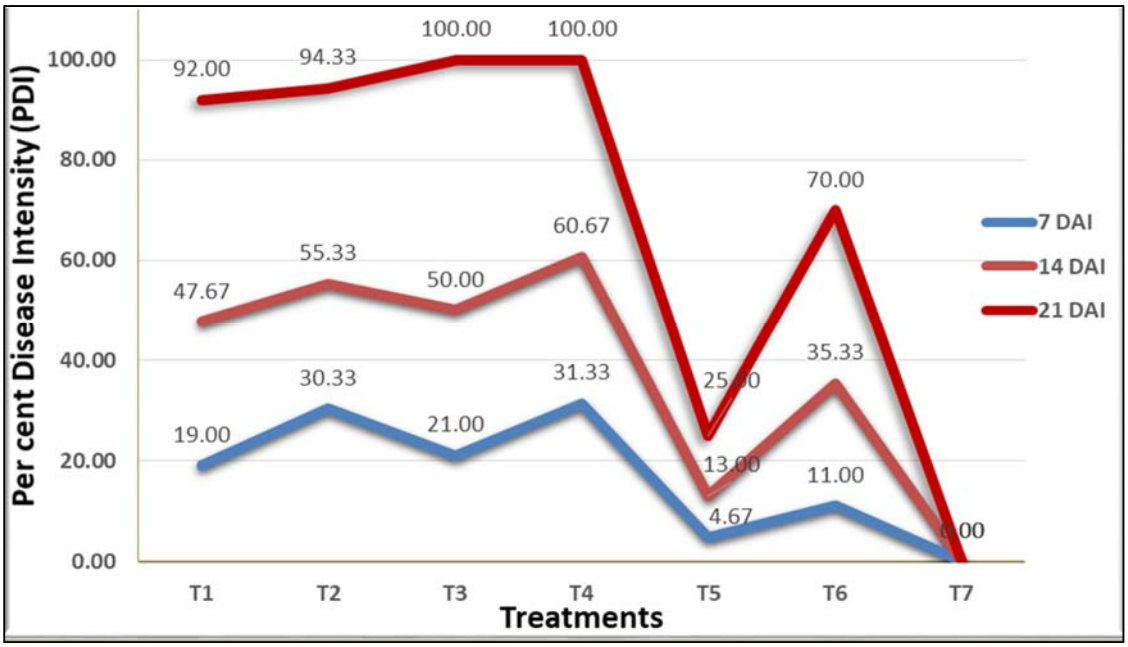

Fig 2: Effect of spore suspension of pathogen on *C. gronovii* parasitized on *Lablab* bean

It is revealed from the data presented in Table 3 & Fig. 2 that all the treatments showed the severe pathogenic effect on both *Cuscuta* and *Lablab* bean plants (PLATE II). At 21 DAI, the maximum disease severity was observed on *Cuscuta* and host plants were found susceptible (wilting) to all the treatments except T_5 (*C. pallescens* @ 5×10^4) due to severe pathogenic effect to host plants.

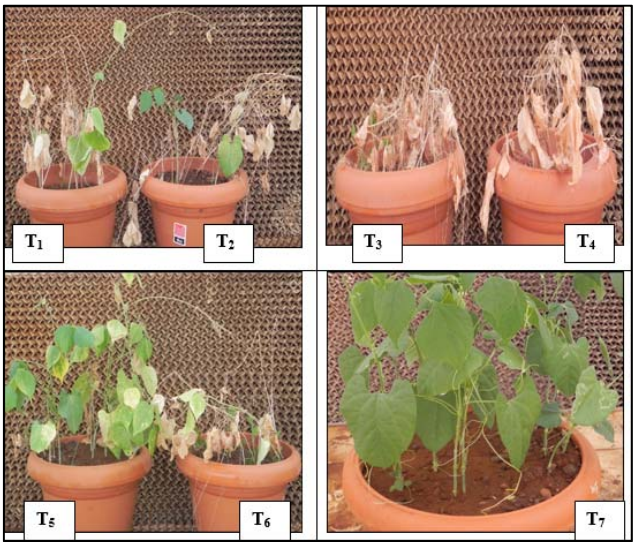

PLATE II: Disease incidence on *C. gronovii* parasitized on *Lablab* bean and host reaction at 21 DAI

It was observed that complete death of *Lablab* bean plants in both treatments *A. dianthicola* and *A. dianthicola* @ 5×10^4 and ultimately *Cuscuta* was died. The weakening of the host plants due to the pathogenic effect of inoculants and parasitism of parasite. The treatment *Curvularia pallescens* @ 5×10^4 which showed symptoms like small necrotic spots or lesions on the leaves, yellowing and chlorosis (blighted) and stunted plants as a resistant host reaction. Host plants were found to be immune in control treatment where no disease incidence was observed on *Cuscuta* as well as host plants. This concentration of spore suspension was not applicable to manage or control of *C. gronovii* when parasitized on *Lablab* bean. The findings of present study are close as well as differ with Cook *et al*. (2006) [4] who tested some plants belongs to family Fabaceae with spore suspension of *Alternaria destruens* (1.5×10^5 spores per ml) were found to be immune as well as resistant. Ashton and Santana (1976) [2] found that use of *Alternaria* spp. to control dodder on sugar beets was less effective.

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 black 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 black gram. These concentrations of spore suspension of pathogens (fungi) are act as a mycoherbicide or bioherbicide and applicable to control or manage *C. gronovii* parasitized on black gram plants effectively but not applicable to control *C. gronovii* parasitized on Lablab bean due to severely pathogenic to both Cuscuta and *Lablab* bean as a host plants.

References

1. Anonymous. Production of pulses, 2014. www.agropedia.com.
2. 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.
3. Bewick TA, Porter JC, Ostrowski RC. Field trial results with Smolder: a bioherbicide for dodder control. In Proceedings of North eastern Weed Science Society, Woodstown, NJ: NEWSS. 2000, 66.
4. 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.
5. 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.
6. 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.
7. Dawson JH. A vegetative character that separates species of *Cuscuta*. Eds. Proc. 3rd Intern. Symp. on Parasitic Weeds. The International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria. 1984, 184–187.
8. Gaertner EE. Studies of seed germination, seed identification, and host relationships in dodders, *Cuscuta* spp. Cornell Exp. Sta. Mem. 1950; 294:1-56.
9. Garcia MA, Costea M, Kuzmina M, Stefanvoic S. Phylogeny, character evolution and biogeography of *Cuscuta* (Dodders; Convolvulaceae) inferred from coding plastid and nuclear sequences. Am. J Bot. 2014; 101:670-690.
10. Gomez KA, Gomez AA. Statistical procedures for agricultural research. 2nd ed. Willey, New York, USA, 1984, 880.
11. Holm L, Doll J, Holm E, Pancho J, Harbinger J. World Weeds: Natural Histories and Distribution. John Wiley & Sons, NY, USA, 1997.
12. Kothekar V. A handbook of pests, diseases and weeds of quarantine significance. Agric. Res. Serv. US Dept. Agric. Amerind Publishing Co. Ltd., New Delhi, India, 1970, 206–224.
13. Kumar RM, Kondap SM. Response of greengram and blackgram cultivars to *Cuscuta* infestation. Ind. J. Plant Protec. 1992; 21:167-171.
14. Menke HF. Dodder infestation can halt certified seed production. Western Feed and Seed. 1954; 9:36–37.
15. Mishra JS. Biology and Management of *Cuscuta* spp. Indian J. Weed Sci. 2009; 41(1-2):1-11.
16. 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.
17. Stevens OA, The number and weight of seeds produced by the weeds. Am. J Bot. 1932; 19:784–794.