STUDIES ON INTEGRATED MANAGEMENT OF CHICKPEA WILT CAUSED BY *FUSARIUM OXYSPORUM* f. sp. *CICERI*

SUMMARY
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It is evidenced by various studies that chickpea wilt remains a major biotic constraint limiting the production of the crop. Prudent management of the disease calls for an amalgamation of various cultural practices and the exploration of new avenues which help in the sustainable development of agriculture and are also environment friendly. The present study was taken up to explore disease management through a combination of various approaches, such that it caters to the need of the farmers as well as the environment.

1. Periodic surveys during 2011-12 and 2012-13 cropping season across the six villages of *viz.* Mandhana, Rooma, Singhpur (Kanpur) and Magarwara, Singrossi, Sikandarpur (Unnao) and fields of IIPR Kanpur demonstrated that:

(a) (i) Wilt is a major disease affecting the chickpea crops, (ii) *Fusarium oxysporum*, f. sp. *ciceri* is largely associated with it, (iii) the losses encompassed ranged from (25.63-40.01%), (iv)There was no correlation between area, crop season, and the percentage of wilted plants. It was also noticed that the loss encumbered is on three fronts (i) loss of seeds sown, (ii) loss of productive space, (iii) the net loss of straw and seed productivity.

(b) Two basic patterns were observed on the basis of seeds sown by the farmers (i) broadcast crop and (ii) crop sown in rows. At a glance, the wilted plants occurred in small patches with somewhat circular outline in the former, while in the latter type the wilt disease appeared progressing along the lines.

The isolation, purification of cultures, subsequently proving of Koch's postulate confirmed the wilt causing fungus being *Fusarium oxysporum* f. sp. *ciceri* (Padw.) Snyder and Hansen.

2. Creation of symptoms artificially in pot cultures and the histopathological studies revealed drooping of seedlings from apices followed by loss in turgidity of leaflets

and petioles resulting in collapse of the entire plant. The leaves become chlorotic, turned yellow then straw colored or light brown. Most often the affected plant showed stunting. The collapsed seedlings when uprooted exhibited uneven shrinking of stem on both the sides of collar region, when splitted vertically downwards black discoloration of internal tissues was observed. In the fields when the wilt disease occurred at the flowering and podding stage, the above typical symptoms were noticed in one or two branches of individual plants. The pods were devoid of any seeds. The roots of such plants on isolation yielded the cultures of *F. oxysporum* f. sp. *ciceri*.

3. The histopathology of such affected plants depicted the presence of fungal mycelium in tracheids and vessels of xylem tissues in longitudinal sections of roots. In a few cases chlamydospores were found present but tyloses were very rare. The clogging of vessels by fungal mycelium in the tracheids was frequent but no macro or microconidia were observed.

4. (a) The isolates of *F. oxysporum* f. sp. *ciceri* after purification were grouped according to their discernible cultural and morphological characters into seven groups. The isolates if grouped according to their radial growth on PDA then isolate1, 3, 7 were medium growing and the rest were slow growing where the criteria for growth was fast growing if colony growth was greater than 70 mm, medium growing if growth was between 60-69 mm and slow growing, below 60 mm in 7 days.

(b) The observation on their morphological characters were (i) microconidia most frequent in isolate 4 and 5 and in others frequent (ii) macroconidia one septate most frequent in isolate 4 and in isolate 2, 3, 5, 6 frequent, rare in isolate 1 and very rare in isolate 7 and (iii) three septate macroconidia were frequent in all the isolates.

5. If reckoned on the basis of pathogenic behavior then isolate 7 was weakly pathogenic isolate 3 was highly pathogenic while the rest of the isolates were moderately pathogenic.

6. (a) It was observed that 31.4±1 percent water holding capacity and 43.5 percent pore space of the soil was the most conducive for wilting that was possible by adding 25% sand to the normal sieved soil (sandy loam) of Kanpur. The other soil factors like volume expansion of soil and absolute specific gravity were having no correlation to wilt development.

(b) The depth of inoculum in the pots proved significantly effective in altering the time lag of wilting. The immediate contact of inoculum to germinating seeds was the best suited for quick pathological screening as the plant wilted in 31 days at the temperature range of 21-28ºC.

7. Production of cellulolytic and pectinolytic enzymes was observed throughout 7, 14 and 21 days of incubation. It was noticed that (i) there was more production of cellulolytic enzyme than pectinolytic enzyme (ii) maximum enzymatic activity was observed on the 14 day of incubation with cellulose registering 79.09% per cent loss in activity after 90 min while PG recorded 74.76% at the same time interval and day (iii) both the cellulolytic, Cellulase and pectinolytic Polygalacturonase (PG) enzymes showed a visible decrease in their activity on 21st day of inoculation..

8. Interaction of different isolates of FOC with the bioagents *T. harzianum T. viride* and *A. niger* was studied *in vitro* and *in vivo* separately and the interaction behaviour was recorded as per the terminology of Johnson and Curl (1972).

A Mutual intermingling of the two organisms.

B Mutual Inhibition on contact.

C Mutual inhibition at a distance.

D Inhibition on contact, the antagonist continues to grow, at unchanged or reduced rate though the colony of the inhibited organisms.

E Inhibition at a distance, the antagonist continues to grow resulting in a clear zone at an unchanged or reduced rate.

The dual culture of either of any antagonist with the 7 isolate inhibited the growth of the pathogen effectively to the level of 14.16-65.78 %. (a) The interaction pattern of *T. harzianum* with isolate 1, 2, 3, 5 and 7 was of D type whereas the isolate 4, 6 showed E type of interaction pattern *T. viride* revealed C type of interaction with the isolate 5, 6. D type of interaction with isolate 1, 3, 4 and 7 and E type with isolate 2. In case of *A. niger,* B type of interaction was shown by isolate 2, 3, 6 and 7. C type of interaction was depicted by isolate 1and 5. D type of interaction was seen with isolate 4 only while E type of interaction was not observed. The observation led to infer that (i) wherever the antagonist and the above pathogen was making a colony, the antagonist would neither intermingle nor parasitize the pathogen, though hampered the growth of the pathogen in most of the cases (ii) either of the pathogen isolates or the bioagents was juxtaposed to root zone the counter acting would never come nearer to it so if the pathogen was present on the rhizoplane of susceptible chickpea plant then the antagonist will be unable to save the same plant from fusarial attack.

(b) Hence the application of the antagonist should be in soil where the seeds were to be sown or the seeds should be dressed by the antagonist before sowing. So that the emerging nascent roots should come in contact with antagonist first.

(c) The efficacy of all the three bioagents were tested using 3 highly susceptible varities JG 62, Kanpur local and Ujjain local in pot cultures. *T. harzianum* was able to check the wilt incidence by 44, 18.6, 6.5, 00 percent while *T. viride* at same level of inoculums was able to control the incidence by 48.97, 38.29, 17.39, 00 percent of JG

62. However, 8 percent *T. viride* (w/w) proved cent per cent effective against wilt disease in all the varieties tested. The wilt incidence after the application of 2, 4, 6, 8, 10, per cent antagonist *A. niger* (w/w) was 45.8, 19.1, 16.3 and 6.25, 00 sequentially. Thus both the species of *Trichoderma* used were able to control wilt disease when applied at 8% concentration (w/w).Whereas the antagonistic *A. niger* checked the wilt incidence completely when applied at 10 per cent concentration (w/w).

9. The nutrients available in the crop debris and soil were sufficient enough for multiplication of pathogen. Therefore, the growth and sporulation of *F. oxysporum* f. sp. *ciceri* (isolate 1) was assessed on mustard, pigeon pea, chickpea, linseed, urid straw wheat straw and potato dextrose broth media. It was found that urid straw, wheat straw was the best suited for the growth as it yielded maximum dry weight mycelium (1060 mg) as compared to linseed straw which produced minimum biomass (360mg).

The microconidia produced per ml were minimum in chickpea and wheat straw, whereas it was maximum in potato dextrose broth followed by urid straw. Linseed straw produced maximum macroconidia ($9.6x10^2$). The pH in all the broth medium was shifted to alkalinity (pH 6-9). By the results it was inferred that the crop debris plays an important role in perpetuation and multiplication of the wilt inoculum. So, sanitation of the fields after harvesting in Rabi and Khariff crop season may help to control the wilt disease.

10. Control of wilt disease through botanicals (*viz.* plant extracts, seed powders and oilcakes) were assayed during the course of investigation.

(a) Plant extracts

Control of wilt disease by applying cold water and alcoholic extracts of 11 species of medicinal plants were studied by assaying them using food poison technique *in vitro.*

It was inferred that (i) in all the cases the efficacy of alcoholic extracts in inhibiting the pathogen was more as compared to water extracts and (ii) as the percentage of concentration of phytoextracts increased, percentage inhibition also increased that was somewhat in a proportionate manner.

There were 3 plant species whose extract inhibited the growth of FOC to the extent of more than 90%, at 500 ppm, these notable plant species were *Aegle marmelos, Azadirachta indica* and *Callistemon lanceolatus*. There was only 1 plant species *Syzygium cumini* whose water extract checked the growth below 30%, while all the other water extracts inhibited the growth above 30 % at the same concentration mentioned above.

(b) Among the seed powders tested both *Piper nigrum* and *Cuminum cyminum* showed excellent result in checking the radial colony growth of the pathogen at all concentrations. Both of them at 30 % concentration controlled the growth to 90.53 and 74.38% respectively.

(c) Among the three oilcakes tested *viz.* mustard, til and neem at three concentrations (10, 20, 30 %) all of them significantly checked the growth of the pathogen. At higher concentrations all the oilcakes showed superior result in controlling the growth of the pathogen. The most notable among them was mustard oil cake showing 80.25% inhibition followed by neem 68.74 and til 66.75%.

11. 9 Essential oils were extracted and assayed at 250, 500 and 1000 ppm. 4 plant species inhibited the pathogen greater than 60% and these were *Tagetes erecta, Eucalyptus citriodora, Ocimum sanctum* and *Cymbopogon jwarancusa* at 1000 ppm. Inhibition of the pathogen by C*ucurbita pepo* and *Daucus carota* were new findings in the present investigation.

12. The efficacy of five fungitoxicants *viz.* (Bavistin) carbendazim, Captan,

diniconazole (FOB), mancozeb (Indofil M- 45) and Zineb (Indofil Z-78) were evaluated on the mycelial growth of FOC. They were tested in infested pot using highly susceptible variety JG 62. Carbendazim was found to be the most efficacious both *in vivo* and *in vitro* as it inhibited the mycelial growth by 92.64% and reduced the plant mortality to 4%. The next was captan that resulted in 81.31% inhibition of colony of FOC and checked the wilt in infested pots upto 92%.

13. Integrated management trial using botanicals and fungitoxicant in six treatment sets were assayed *viz.* T1: *Cuminum cyminum* extract (10%) + Carbendazim (0.3%); T2: *Cuminum cyminum* extract (20%) + Carbendazim (0.3%); T3: *Cuminum cyminum* extract (30%) + Carbendazim (0.3%); T4: *Piper nigrum* extract (30%) + Carbendazim (0.1%); T5: *Piper nigrum* extract (30%) + Carbendazim (0.2%); T6: *Piper nigrum* extract (30%) + Carbendazim (0.3%). All the treatments set were extremely effective in controlling the pathogen both *in vitro* and under infested pot conditions. All the treatment were cent per cent effective except for treatment set1 comprising of *Cuminum cyminum* (10%) + carbendazim(0.3%) which showed an inhibition of 87.72% and mortality % of 3.03.

The present study brings to take off level (i) with clear identity of the pathogen involved in wilting of chickpea (ii) existing variability in *Fusarium oxysporum* f. sp. *ciceri*. (iii) cellulolytic and pectinolytic enzyme secretions by the pathogen (iv) efficient disease management with botanicals, bioagents essential oils and fungitoxicant. (v) sanitation as an important exercise proved experimentally, (vi) integrated approach of managing the disease as superior to the above management practices when done singly.

RECOMMENDATIONS

The findings of the present study reveal that use of integrated management trials either singly, like use of alcoholic extracts of plants *viz. Callistemon lanceolatus, Azadirachta indica, Aegle marmelos*; oilcakes, like mustard, neem and bioagents like *Trichoderma harzianum, Trichoderma viride*. *Aspergillus niger;* or in combination like using botanicals and fungitoxicants *viz. Piper nigrum* and *Cuminum cyminum* extracts along with carbendazim were potent in checking the growth of the pathogen and can be looked upon as viable options to control the wilt menace effectively in fields.