

Effect of Cropping Systems and Nitrogen on Wilt Incidence, *Fusarium udum* Population and Its Antagonistic Fungi in Pigeonpea Rhizosphere

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SUMMARY

Experiment was conducted in the wilt sick field to find out the comparative performance of organic, inorganic amendments, intercropping systems and their integration for the management of wilt in pigeonpea. Soil application of amendments through 20 kg N ha⁻¹ FYM + 20 kg N ha⁻¹ through KNO₃ (64%) caused maximum per cent inhibition followed by 40 kg N ha⁻¹ as KNO₃ (52%). Maximum reduction of pathogen populations (32%) and maximum antagonistic fungi (80%) was observed in the rhizosphere soil of plots amended with 40 kg N ha⁻¹ through KNO₃ over control. Integration of amendments with 40 kg N ha⁻¹ through KNO₃ with all cropping systems resulted in maximum reduction of fungal population and wilt incidence. Intercropping of sorghum + pigeonpea and soybean + sunflower found to reduce wilt incidence and *Fusarium* population. Of soil mycoflora enumerated from the rhizosphere, maximum levels of inhibition (71-83%) found with *Trichoderma viride* *Trichoderma harzianum* and *Aspergillus nidulans* spp. against *F. udum*. From the study, organic amendments, inorganic amendments and intercropping system proved best for the management of wilt.

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Vascular wilt (*Fusarium udum* Butler) is one the most important diseases of pigeonpea [*Cajanus cajan* (L.) Millsp.] causing loss upto 67% at maturity and complete loss in case of infection at prepod stage (Kannaiyan and Nene, 1981). To date, *Fusarium* wilt has been reported from 15 countries, but it is relatively more important in India and eastern Africa. Chemical control of the disease is difficult impractical and uneconomical. The wilt pathogen is reported to be present in infected plant stubbles in soil up to 3-4 years even in the absence of host crop ((Reddy *et al.*, 1992). It is now established that sorghum as a mixed or intercrop is an effective management approach for reducing wilt in pigeonpea (Natarajan *et al.*, 1985). Intercropping of pigeonpea with soybean, groundnut, sunflower and sorghum is a common practice in most of the areas in Andhra Pradesh. Cultural practices in addition to cropping pattern like organic amendments in pigeonpea (Raghuchander *et al.*, 1992) and inorganic fertilizers in muskmelon (Chattopadhyay and Sen, 1996) are reported to reduce wilt incidence as well as pathogen population. Enhancement of antagonistic fungal population by organic and inorganic fertilizers had been earlier reported. In view of the above, the present study was undertaken to assess the effect of intercropping systems with non host

crops and cultural practices on wilt incidence, population of pathogen and antagonistic fungi in pigeonpea rhizosphere.

MATERIALS AND METHODS

The experiment was laid out in two consecutive seasons of 1996-97 and 1997-98 at ICRISAT, Patancheru, AP. Field experiment was carried out in split design with three replication in a wilt sick plot with a *Fusarium* inoculum load of 46x10³ cfu/g soil with a wilt susceptible cultivar, ICP 2376. Sub plots consisting of cropping systems *viz.*, sole cropping of pigeonpea, pigeonpea + sorghum, pigeon pea + soybean intercropping system and sole crop of soybean. Six main treatments consisted of different nitrogen sources and levels along with recommended dose of fertilizer *i.e.* inorganic fertilizer, organic fertilizer and combination of inorganic + organic fertilizer at lower dose as 20 kg N ha⁻¹ through FYM, 20 kg N ha⁻¹ through KNO₃, 40 kg N ha⁻¹ through FYM and 40 kg N ha⁻¹ through KNO₃ and 20 kg N as FYM + 20 kg N kg as KNO₃. The recommended dose of fertilizer without any addition of amendment constituted control. The recommended NPK dose for intercropping was calculated based on the proportion of plant population of the component crops. Sowing was done in *kharif* in both the years of experimentation in a plot of 8x6 m² with a

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spacing of 30x15 cm for soybean, 60x15 cm for soybean + pigeonpea in 5:1 ratio, 60x15 cms for sorghum + pigeonpea in 2:1 ratio and 90x60 cms for sole pigeonpea (6 rows). The recommended dose of NPK fertilizers applied for sole crop as per the agronomic practices and the additional dose of nitrogen through FYM and KNO₃ was applied to all crops as basal dose. Wilt incidence was calculated at monthly intervals. Soil sample analysis for *F. udum* and antagonistic fungi was carried out at Crop Protection Division, ICRISAT. Fusarium population was estimated by sprinkling rhizosphere soil of 20 mg on Fusarium specific medium for *Fusarium*, Martin rose Bengal agar medium for *Aspergillus* spp. *Penicillium* spp. and *Trichoderma* spp. on Trichoderma specific medium as per the method standardised by Bhatnagar (1995). Counts of colonies of fungi were taken on the fourth day by colony counter. Antagonistic property of bioagents was tested against *F. udum* by dual culture technique (Naik, 1993). Three replications per treatment and suitable controls were maintained without antagonist. The plates were incubated at 28±2°C for 7 days. All the results were expressed in terms of per cent inhibition of mycelial growth over control. Data on wilt incidence, Fusarium population and antagonistic fungal population estimation were analysed statistically as per Panse and Sukhatme (1961)

RESULTS AND DISCUSSION

The results from Table 1 revealed that wilt incidence

and Fusarium population were significantly influenced by combination of both inorganic and organic sources of amendments. Per cent wilt decrease over control was 64% with 20 kg N ha⁻¹ through KNO₃ + 20 kg N through FYM and 52% with 40 kg N ha⁻¹ through KNO₃. This might be attributed that nitrate form of nitrogen fertilizer suppressed *Fusarium* due to lysis of fungal mycelium and low levels of chlamydospores in the nitrogen amended soil. Decreasing trend was observed with regard to Fusarium population with every ascending level of nitrogen in addition to recommended dose. Decrease in Fusarium population with 20 kg N ha⁻¹ through KNO₃ + 20 kg N ha⁻¹ through FYM and 40 kg N ha⁻¹ through KNO₃ over control was to the tune of 23 and 32%, respectively. Similar results were reported by Chaube and Singh (1990). Added potash application had a pronounced effect on the Fusarium population and wilt incidence. Added nitrogen tends to make the root zone less acidic (Mondal and Hyakumachi, 1998) and added potassium retards Fusarium population by reduction of pathogen penetration and multiplication (Woltz and Ebelhard, 1973). Combination of organic and inorganic sources even at lower doses decreases Fusarium population and increases antagonistic fungi (Table 1 and 2).

However, further decomposition of FYM releases various biochemical substances, by and large also served as food base for the multiplication of resident antagonistic soil micro organisms as observed in the present study by enumeration of fungi (Table 1). Significant increase in

Table 1: Effect of cropping systems and nitrogen on wilt incidence, Fusarium udum population and antagonistic fungi

| Treatments | Wilt incidence (%) | % Decrease over control | Fusarium udum population (cfu/g soil) | % Decrease over control | Antagonistic fungal population (cfu/g soil) | % Increase over control |
|--|--------------------|-------------------------|---------------------------------------|-------------------------|---|-------------------------|
| Cropping systems | | | | | | |
| Soybean | 23(27.4) | 68 | 7.4 (5.1) | 25 | 9.9 (7.5)** | 46 |
| Pigeonpea + Sorghum | 19 (26.2) | 73 | 6.3 (4.6) | 36 | 11.2 (8.5) | 64 |
| Pigeonpea + Soybean | 52 (58.3) | 28 | 7.8 (5.8) | 21 | 8.8 (6.4) | 29 |
| Sole pigeonpea | 73 (78.2) | - | 9.9 (7.4) | - | 6.8 (5.0) | - |
| N levels and forms | | | | | | |
| 20 kg through FYM + 20 kg N through KNO ₃ | 17 (24.2) | 64.4 | 7.16 (5.5) | 23 | 10.1 (7.5) | 55 |
| 40 kg N through KNO ₃ | 23 (19.2) | 52 | 6.3 (3.7) | 32 | 11.7 (8.7) | 80 |
| 20 kg N through KNO ₃ | 25 (28.9) | 47.9 | 7.75 (5.9) | 17 | 9.9 (7.2) | 52 |
| 40 kg N through FYM | 26 (30.5) | 45.8 | 8.05 (6.3) | 13 | 8.9 (6.7) | 36 |
| 20 kg N through FYM | 30 (36.9) | 37.5 | 8.96 (6.7) | 3.6 | 8.5 (6.3) | 30 |
| Control | 48 (54.1) | - | 9.3 (7.8) | - | 6.5 (4.4) | - |
| C.D. (P=0.05) | 2.01 | | 0.15 | | 0.39 | |

* Figures in parenthesis are angular transformed values,

** Figures in parenthesis are square root transformed values

FYM – Farm yard manure, KNO₃ – Potassium nitrate

Soybean/sunflower – intercropping system, Cfu/g soil – colony forming units per gram soil

Table 2: Interaction effect of cropping systems and N levels and forms on wilt incidence, *Fusarium udum* population and its antagonistic fungi

| Treatments | N levels and forms (kg ha ⁻¹) | | | | | |
|---|---|------------|---------------------|---------------------|--------------------------------|-----------------------------|
| | Control | 20 FYM | 40 FYM | 20 KNO ₃ | 40 KNO ₃ | 20 FYM+ 20 KNO ₃ |
| Wilt incidence (%) | | | | | | |
| Cropping systems | | | | | | |
| Sole soybean | 6 (13.3) | 6 (13.3) | 5 (12.4) | 2 (8.11) | 3 (9.33) | 1 (4.05) |
| Sorghum+Pigeonpea | 12 (20.8) | 8 (15.7) | 7 (15.1) | 11 (19.1) | 8 (15.7) | 6 (13.3) |
| Soybean+ Pigeonpea | 4 (10.6) | 3 (9.32) | 4 (10.6) | 3 (9.32) | 4 (10.6) | 1 (4.05) |
| Pigeonpea | 25 (29.5) | 12 (20.1) | 10 (18.6) | 9 (16.7) | 8 (15.7) | 9 (16.8) |
| <i>Fusarium udum</i> population (cfu/g soil) | | | | | | |
| Sole soybean | 9.5 (7.3) | 8.6 (6.4) | 5.5 (4.9) | 7.3 (5.6) | 7.0 (5.4) | 6.6 (4.7) |
| Sorghum + Pigeonpea | 5.4 (3.9) | 8.0 (6.3) | 6.9 (5.2) | 7.5 (5.7) | 2.9 (2.2) | 7.4 (5.5) |
| Soybean+ Pigeonpea | 10.2 (7.6) | 8.9 (7.0) | 9.5 (7.3) | 7.0 (5.4) | 6.3 (4.8) | 5.2 (3.9) |
| Pigeonpea | 12.0 (8.7) | 10.4 (7.8) | 9.8 (7.4) | 9.5 (6.9) | 8.9 (7.0) | 9.9 (7.6) |
| Antagonistic fungal population (cfu/g soil) | | | | | | |
| Sole soybean | 7.0 (5.5) | 9.2 (6.9) | 10.8 (8.1) | 11.2 (8.4) | 10.2 (7.6) | 11.4 (8.5) |
| Sorghum + Pigeonpea | 7.9 (6.6) | 7.5 (5.6) | 7.2 (5.4) | 9.0 (6.8) | 13.2 (9.8) | 10.1 (7.6) |
| Soybean+ Pigeonpea | 7.5 (5.6) | 11.0 (8.3) | 10.9 (8.1) | 11.8 (8.8) | 15.0 (10.5) | 11.1(8.3) |
| Sole pigeonpea | 4.0 (5.0) | 6.5 (4.8) | 6.8 (5.1) | 7.9 (6.6) | 8.3 (6.2) | 7.8 (6.2) |
| C.D. (P=0.05) | Wilt incidence | | Fusarium population | | Antagonistic fungal population | |
| | A-1.47 | | 0.23 | | 0.62 | |
| | B-1.08 | | 0.31 | | 0.17 | |

A For comparison of two sub treatments at the same level of main treatment

B For comparison of two main treatments at the same or different levels of sub treatments

* Figures in parenthesis are angular transformed values

** Figures in parenthesis are square root transformed values

antagonistic fungal population was observed with single application of 40 kg N ha⁻¹ through KNO₃ followed by 20 kg N ha⁻¹ of KNO₃ + 20 kg N ha⁻¹ of FYM in addition to the recommended dose. Singh *et al.* (2002) observed that FYM effectively reduced chickpea wilt disease. In present study, both nitrogen sources found superior in reducing wilt incidence. The amendments were attributed to inhibit the soil borne disease either by antibiosis or by competition or by increasing the saprophytic soil microbial population (Zakaria and Lockwood, 1980). The decomposition of any organic matter provides the food source in which the antagonistic fungi thrive and multiply rapidly because of continuous supply of nutrients from the substrate (Paulitz and Baker, 1987) and due to less available nitrogen in less acidic soil due to added nitrate fertilizers in rhizosphere (Chaube and Singh, 1990). Carbon to nitrogen ratio (13.5) used in the present study might result in reduction of *Fusarium* population. Similar results have been reported by Hoitink *et al.* (1997). Population increase of some rhizosphere fungi might be due to addition of organic and inorganic amendments. Antagonistic property against *F. udum* with some fungi

against pathogen was estimated by dual culture technique. Perusal of the data from Table 3 showed significant differences in the reduction of mycelial growth of *F. udum* with antagonistic fungi, of which maximum levels of inhibition (71-83%) was observed with *T. viride*, *T. harzianum* and *A. nidulans* might be due to antibiosis and overgrowth. However, *A. niger* and *P. penophyllum* showed moderate levels of inhibition. The mycelial growth

Table 3: Per cent inhibition of *Fusarium udum* by antagonistic fungi

| Sr. No. | Antagonistic fungi | Inhibition (mm) | Per cent inhibition over control |
|---------------|--------------------------------|-----------------|----------------------------------|
| 1. | <i>Trichoderma viride</i> | 1.5 | 83.3 |
| 2. | <i>Trichoderma harzianum</i> | 2.5 | 77.7 |
| 3. | <i>Aspergillus niger</i> | 4.2 | 53.3 |
| 4. | <i>Aspergillus flavus</i> | 5.0 | 44.4 |
| 5. | <i>Aspergillus nidulans</i> | 2.6 | 71.1 |
| 6. | <i>Penicillium citrinum</i> | 5.1 | 43.3 |
| 7. | <i>Penicillium decumbens</i> | 2.9 | 67.7 |
| 8. | <i>Penicillium penophyllum</i> | 4.5 | 50.0 |
| 9. | Control | 9.0 | - |
| C.D. (P=0.05) | | 0.06 | |

inhibition might be due to secretion of volatile and non volatile substances or cell wall degrading enzymes like glucanases by the antagonists. Such effective antagonism against *F. udum* has been reported also.

Subplot treatments comprising, cropping systems also found effective on Fusarium population and wilt incidence. Per cent decrease in wilt incidence was maximum in sorghum + pigeonpea (36%) followed by soybean + pigeonpea (21%). Intercropping of pigeonpea + sorghum resulted in maximum reduction of Fusarium population and maximum increase in antagonistic fungi. The decrease in wilt incidence might be due to influence of root exudates of sorghum and soybean on Fusarium conidial germination which decreases Fusarium population. These findings are in agreement with that of Natarajan *et al.* (1985). Recovery of Fusarium population in non host crops in the rhizosphere might be affected due to volatile substances (Kalpana Sastry and Chattopadhyay, 1999) and root exudates effect of these plants which substantially increases microbial activity in the rhizosphere (Bais *et al.*, 2006).

Maximum antagonistic fungal population was recorded with 40 kg N ha⁻¹ through KNO₃ in pigeonpea + sorghum intercropping system which was significantly higher over all other treatments and control. This might be attributed to qualitative and quantitative changes in root exudates which increased antagonistic activity and release of volatile substances like hydrocyanic acid as in sorghum. Results of interaction of cropping systems and N levels and sources (Table 2) showed that 40 kg N ha⁻¹ and 20 kg N ha⁻¹ through KNO₃ + 20 kg N ha⁻¹ FYM in all cropping systems resulted in reduction of wilt, Fusarium population and maximum antagonistic fungal population.

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