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# Biological control of *Fusarium* wilt of pigeonpea by *Pantoea dispersa,* a field assessment

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**Abstract -** *Fusarium* wilt is one of the major yield and growth-limiting factors of pigeonpea (*Cajanus cajan*). For an eco-friendly and sustainable management of such a disease, a novel mycolytic strain *Pantoea dispersa* was evaluated against fungal pathogen *Fusarium udum* that is known to be infecting the susceptible variety of pigeonpea (T-15-15), commonly prevalent in India. To study the efficacy of *P. dispersa* as a biocontrol agent in comparison with chemical fungicide Bavistin and antifungal biocontrol agent *Trichoderma* Monitor WP was studied in both pot and field experiments. *In vitro, P. dispersa* inhibits the growth of *F. udum*. In the pot experiment, *P. dispersa* the treated pigeon pea (T-15-15) seeds showed higher percentage of seed germination and decreased wilt incidence as compared to chemical fungicide; Bavistin and antifungal biocontrol agent *Trichoderma* Monitor WP treatments. Moreover, the root, shoot lengths and growth were also found to be higher. Due to high impact of *P. dispersa* in *in vitro* condition on controlling the *F. udum* growth, further study was conducted in field in a wilt-stricken plot. These studies were repeated for three cropping seasons (2004/2007). The seed dressing by *P. dispersa* reduced wilt incidence (47%) during field trials, which is greater than Bavistin (41%) and *Trichoderma* Monitor WP (36%) treatments. Hence *P. dispersa* was proven superior to *Trichoderma* Monitor WP and Bavistin; which are used as commercial *Fusarium* wilt control agents.

**Key words**: biocontrol, *Pantoea dispersa*, pigeonpea, Bavistin, *Trichoderma* Monitor WP, *Fusarium* wilting.

## INTRODUCTION

Agricultural food production is severely affected by phytopathogenic fungi, bacteria and nematodes. Among them fungal plant diseases are one of the major concerns to agricultural food production worldwide. Soil born plant pathogenic fungi such as *Pythium* sp., *Fusarium udum, Rhizoctonia* sp., *Phytophthora* sp., etc. attack most of the economically important crop plants either through seed root before germination or seedling after germination resulting in cumulative agricultural losses of billions of dollars (Giesler and Yuen, 1998; Hutson and Miyamoto, 1999; Morrissey *et al.,* 2004). The development of such a global system for sustainable food production by controlling diseases in agriculture is one of the greatest challenges (Emmert and Handelsman, 1990; Tilman *et al.,* 2002). Thus, there is a pressing need to control fungal diseases that reduce the crop yield, so as to ensure a steady and constant food supply to ever increasing world population. Conventional practice to overcome this problem has been the use of chemical fungicides, which have adverse environmental effects affecting non-target organisms and causing health hazards to humans (Huston and Miyamoto, 1999; Hoster *et al.,* 2005). Therefore there is need for an alternative ecofriendly and economical method for controlling fungal plant diseases.

Pigeonpea (*Cajanus cajan*) is one of the major leguminous crops of the tropical and subtropical regions. It is grown in many states of India, cultivated on 3816 ha of land and production is about 2876 thousand tons per annum. Wilt in pigeonpea, caused by *F. udum* Butler, is a serious threat to the crop, particularly in India and eastern Africa (Kiprop *et al.,* 2002). *Fusarium* wilt is the most destructive disease of pigeonpea. The annual loss due to wilt in pigeonpea in India and eastern Africa had been estimated to be 71 million and 5 million US $, respectively (Reddy *et al.,* 1990; Raju *et al.,* 1999; Gohel *et al.,* 2006b; Gwata *et al.,* 2006). Studies are therefore required to control such infection in pigeonpea.

The pathogen is soil borne and chemical control is impractical in established cases. Moreover, extensive use of synthetic compounds to improve crop productivity and disease control is a growing concern. At present, chemical fungicides such as Thiram, Bavistin and Benomyl are generally used to control *Fusarium* wilt (Vidyasekaran *et al.,* 1997; Meena *et al.,* 2002; Melent'ev *et al.,* 2006).

Mycolytic enzymes (chitinases, proteases and glucanase) producing microorganisms have great potential in solving such problems (Deshpande, 1999; Patel *et al.,* 2007). They play a unique role in the agricultural biotechnology because of the fungal mycelial lytic activity, which inhibits fungal development by degrading chitin and glucan, major components of the fungal cell wall (Hillocks *et al.,* 2000; Hoster *et al.,* 2005). Therefore a great deal of research has focused on the formulation of antagonists that produce mycolytic enzymes to control

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TABLE 1 - Experimental design for the determination of fungicide *in vitro*

| Plate | *Pantoea dispersa* (1.5-2.0 x $10^{10}$ CFU/ml; 1.0 ml/g seeds) | Bavistin (Carbendazim 50% WP; 2.0 mg/g seeds) | T-2 *Trichoderma* Monitor WP (3.0 mg/g seeds) | *Fusarium udum* (5.0 x $10^5$ spores/ml FU; 5.0 ml) | Sterile distilled water (ml) |
|---|---|---|---|---|---|
| A | - | - | - | - | 20 |
| B | - | - | - | + | 15 |
| C | + | - | - | - | 15 |
| D | + | - | - | + | 10 |
| E | - | + | - | - | 20 |
| F | - | - | + | - | 20 |
| G | - | + | - | + | 15 |
| H | - | - | + | + | 15 |

A: No treatment, B: *Fusarium udum* control, C: only *Pantoea dispersa* control, D: *P. dispersa* treatment with *F. udum*, E: Bavistin control, F: *Trichoderma* Monitor WP control, G: Bavistin treatment with *F. udum*, H: *Trichoderma* Monitor WP treatment with *F. udum.*

fungal plant pathogens (Deshpande, 1999; Vidhyasekaran *et al.,* 1997; Meena *et al.,* 2002).

*Pantoea dispersa* is a Gram negative rod of *Enterobacteriaceae* family isolated from sea dumps containing crustacean wastes in Bhavnagar (India) (Gohel *et al.,* 2004a). It inducibly produces multiple forms of chitinases, proteases and glucanase when grown in media containing colloidal chitin as the sole carbon source (Gohel *et al.,* 2005b). Among the chitinases, total chitinase, endochitinase and chitobiase are the major chitinases detected in the culture supernatant of *P. dispersa* through enzymatic assay (Gohel *et al.,* 2006a; Gohel *et al.,* 2007). There is detailed information available on *P. dispersa* mycolytic enzymes and their effect on fungal inhibition (Gohel *et al.,* 2004a, 2004b). Therefore, this study was aimed at investigating the control of *Fusarium* wilting in *C. cajan* (T-15-15) by mycolytic *P. dispersa*, which also proved efficient in the management of chitinous waste earlier (Gohel *et al.,* 2005a, 2006b).

## MATERIALS AND METHODS

**Organisms and culture conditions.** The mycolytic bacterium, was isolated from sea dumps containing crustacean wastes, in Bhavnagar, India, and was identified as *Pantoea dispersa* on the basis of a biochemical analysis by using Rapid ID-32 E kit (manufactured by Biomerieux Company, France) (Gohel *et al.,* 2004a, 2006). The bacterium was cultivated on chitin agar medium (Monreal and Reese, 1969). The acid swollen chitin was prepared by the method of Hackman (1962).

An optimised medium was used for growth of mycolytic enzymes producing *P. dispersa* (Gohel *et al.,* 2004a). A five percent inoculum of *P. dispersa* was prepared as described previously (Gohel *et al.,* 2004a, 2005b). A five percent inoculum of *P. dispersa* (1 x $10^8$ CFU/ml) in log growth was added to 50 ml of liquid medium in 250 ml flasks and incubated for 72 h at 30 ± 2 °C on a rotary shaker (180 rpm). Subsequently the culture was collected at 72 h for seed dressing.

**Fungal inoculum preparation.** *Fusarium udum* was received from the Gujarat Agricultural University, India and was cultivated on Sabouraud's agar (Himedia, India) medium. *Fusarium udum* (5 x $10^5$ spores/ml) was grown in Sabouraud's broth (Himedia) for 7 days (Gohel *et al.,* 2007), after which fungal biomass was harvested, washed with sterile distilled water, and further used as pathogen to study the *in vitro* seed germination of *C. cajan* (T-15-15) and in pot experiment.

TABLE 2 - Protocol for the determination of biofungicide potential in pot experiments

| Pot | *Cajanus cajan* (T-15-15) seeds treatment with | | | |
|---|---|---|---|---|
| | *Pantoea dispersa* (1.5-2 x $10^{10}$ CFU/ml; 1 ml/g seeds) | Bavistin (Carbendazim 50% WP; 2 mg/g seeds) | T-2 *Trichoderma* Monitor WP (3 mg/g seeds) | *Fusarium udum* (5 x $10^5$ spores/ml; 50 ml/kg soil) |
| A | - | - | - | - |
| B | - | - | - | + |
| C | + | - | - | - |
| D | + | - | - | + |
| E | - | + | - | - |
| F | - | - | + | - |
| G | - | + | - | + |
| H | - | - | + | + |

Pot A: Control (untreated seeds sown in sterile soil); Pot B: *Fusarium udum* control (untreated seeds sown in *F. udum* spore inoculated soil); Pot C: *Pantoea dispersa* control (culture treated seeds sown in sterile soil); Pot D*: P. dispersa* treatment (treated seeds were sown in *F. udum* infected soil); Pot E: Bavistin treatment control (treated seeds were sown in sterile soil); Pot F: T-2 *Trichoderma* Monitor WP treatment control (treated seeds were sown in sterile soil); Pot G: Bavistin treatment (treated seeds were sown in *F. udum* infected soil); Pot H: T-2 *Trichoderma* Monitor WP treatment (treated seeds were sown in *F. udum* infected soil).
+: application of treatment, -: no application.

**Effect of *Pantoea dispersa*, Bavistin and *Trichoderma* Monitor WP on the control of infection in pigeonpea (*Cajanus cajan*) caused by *Fusarium udum*.**

In vitro *seed germination study.* Seeds of wilt susceptible cultivar of pigeonpea T-15-15 (*C. cajan*) were washed with sterile distilled water, surface sterilised by treating with 0.1% $HgCl_2$ in 0.05 N HCl for 2.0 min and washed again thrice with sterile distilled water. Seeds were further treated with *P. dispersa*, Bavistin-Carbendazim 50% WP powder (BASF India Limited, Mumbai, India) and *Trichoderma* Monitor WP powder-*Trichoderma viride* (Ocean Agro India Ltd., Vadodara, India) as indicated in experimental design (Table 1). Three-times experiments were conducted with four replications. In each treatment, ten seeds of pigeonpea T-15-15 (*C. cajan*) were used. The treated seeds were incubated in Petri-plates at 28 ± 2 °C up to six days to allow the germination. The percentage seed germination, length of root and plumule as well as weight of germinating seeds were measured after six days of germination.

*Pot experimental study under controlled conditions.* Three-times pot experiments were performed using random block statistical design with four replications. Wilt susceptible cultivar of pigeonpea T-15-15 (*C. cajan*) seeds were surface sterilised as described earlier and 2.0 kg of sterile soil was added in each pot. A *Fusarium* wilt infection was developed in seven pots per replication by inoculating 50 ml spore suspension of *F. udum* (5.0 x $10^5$spores/ml) per 1.0 kg soil. Prior to sowing, the seeds of pigeonpea T-15-15 were treated with three different treatments which include dipping the seeds in solution containing *P. dispersa* [1.5 x $10^{10}$ to 2.0 x $10^{11}$ CFU/ml (0.1 ml/g seeds)], Bavistin (2.0 mg/g seeds) and *Trichoderma* Monitor WP [5.0 x $10^5$ spores/ml (3.0 mg/g seeds)] for 4 h at 30 ± 2 °C (Table 2). The ten seeds per replicate from each treatment were sown with appropriate spacing in pots, as described in experimental design (Table 2). The application of these commercial fungicides was as label recommendation. The population of *P. dispersa* on overnight dried seeds was about 1.2 x $10^9$ to 2.3 x $10^{10}$ CFU/seed at the time of sowing in pots. A total of ten seeds per replicate from each treatment were sown with appropriate spacing in pots, as described in experimental design (Table 2). Plants were watered regularly. The growth was monitored for a month, after which shoot and root lengths were recorded. The plantlets were dried in an oven at 50 ± 2 °C and their dry weights were measured until they were found to be constant.

*Filed experimental study.* The field trials of susceptible T-15-15 variety of *C. cajan* were conducted for three years (2004/2005 to 2006/2007) by using independent treatment with *P. dispersa* [1.5 x $10^{10}$ to 2.0 x $10^{10}$ CFU/ml (100 ml/kg seeds)], Bavistin (2.0 g/kg) and *Trichoderma* Monitor WP (4.0 g/kg) in a rain irrigated and well maintained *Fusarium* wilt affected plots at the Zonal Agricultural Research Station of Gujarat Agriculture University, Maktampur, Bharuch, India, during the monsoon season to control fungal infection. These seeds were incubated with each treatment at 30 ± 2 °C for 4 h and allowed to dry overnight. The population of *P. dispersa* of overnight dried seeds at the time of sowing in field was calculated using dilution method by inoculating the seed in the sterile distilled water and finally spread onto chitin agar medium (Gohel *et al*., 2004a). The total colony forming unit of *P. dispersa* was about 1.0 x $10^8$ to 3.0 x $10^9$ CFU/seed before sowing in field soil.

Three-year field trials were performed using random block statistical design with four replications. Prior to each field trial, the soil parameters of experimental field were analysed at the Gujarat State Fertilizers and Chemicals Ltd., Vadodara, India. An experimental field has deep black soil: $P_2O_5$, 8 kg/ac; $K_2O$, 288 kg/ac; S, 14 ppm; Zn, 1.86 ppm; Fe, 15.76 ppm; and Cu, 3.6 ppm. Fertilizers (NPK) were applied at a ratio of 20:40:00 kg/ha before or during field trials.

Treated seeds were sown manually in 20 rows in wilt sick plots at inter (90 cm) and intra row (20 cm) spacing at a depth of 2 to 3 cm. Untreated seeds were also sown. Plots were located in three soil strips that were separated from each other by 60 cm. Within each soil strip, plots were separated by bare soil strips (width 60 cm) to reduce cross contamination. The pigeonpea crops were harvested after four months. During the field trials, temperature and rainfall were monitored.

The percentage wilt incidence (PWI) was calculated according to the formula:

PWI = (Number of diseased plants/Total number of plants) x 100

Plant growth was evaluated by determining the height, number of branches and dry weight per plot on the day of harvesting. In addition, the number of pods per plant and grain yield was measured after harvesting.

**Statistical analysis.** All experiments were conducted three-times with four replications, and the data obtained were statistically analysed by using Indigenous software (Department of Statistics, B.A. College of Agricultural, Anand, India).

## RESULTS AND DISCUSSION

*Fusarium* wilt of pigeonpea has the potential to be a devastating disease for growers of pigeonpea in India especially in Gujarat District. Due to the soil borne nature of the disease, use of chemicals in controlling the pigeonpea wilt is hardly successful. Hence, an economical and feasible approach would be either to search for biological control agents. The biological control is the best alternative especially against soil borne pathogens such as *F. udum*. The limitations to biocontrol use are scarce knowledge on the ecology of rhizosphere and use of *in vitro* antagonism for selection of biocontrol agents. However, the advantages of using biocontrol include environmental friendly, cost and extent of protection (Gohel *et al.,* 2006b).

**Effect of *Pantoea dispersa*, Bavistin and *Trichoderma* Monitor WP on the control of infection caused by *Fusarium udum* in *Cajanus cajan* (T-15-15) during *in vitro* germination study**

*In vitro*, *P. dispersa* was previously shown to exhibit antifungal activity (Gohel *et al.,* 2004a). In order to determine efficacy of *P. dispersa* in conferring protection against *Fusarium* wilt in comparison with Bavistin and *Trichoderma* Monitor WP, a seed germination of *C. cajan* (T-15-15) was conducted. The percentage germination, dry weight, radical and plumule lengths were measured after six days of incubation and data were calculated statistically.

In absence of *F. udum* treatment, 95% seed germination was observed when seeds were treated independently with *P. dispersa*, Bavistin and *Trichoderma* Monitor WP. Whilst 100, 100 and 95% germination was observed in the presence of *F. udum* treatment in combination with *P. dispersa*, Bavistin and *Trichoderma* Monitor WP, respectively. 100% loss of seed germination was observed when seeds were treated only with *F. udum* (Table 3).

The plumule length (cm) of treated seeds with *P. dispersa*, Bavistin, *Trichoderma* Monitor WP, *P. dispersa* with *F. udum,*

TABLE 3 - Comparative effect of *Pantoea dispersa,* chemical and biological fungicides on the control of *Fusarium* wilt in *Cajanus cajan* (T-15-15) under pot study

| *Cajanus cajan* (T-15-15) seeds treatment | Germination (%) | Plumule length (cm) | Redical length (cm) | Dry weight (g) |
|---|---|---|---|---|
| Control | 100.00 | 2.80 | 2.72 | 0.22 |
| *Fusarium udum* | 0.00 | 0.00 | 0.00 | 0.00 |
| *Pantoea dispersa* | 100.00 | 3.07 | 2.66 | 0.25 |
| Bavistin | 95.00 | 1.94 | 1.57 | 0.19 |
| *Trichoderma* Monitor WP | 95.00 | 1.87 | 1.66 | 0.22 |
| *P. dispersa* + *F. udum* | 100.00 | 3.25 | 3.00 | 0.20 |
| Bavistin + *F. udum* | 80.00 | 2.09 | 1.84 | 0.17 |
| *Trichoderma* Monitor WP + *F. udum* | 95.00 | 2.21 | 1.56 | 0.15 |
| [A]S.Em | 0.51 | 0.058 | 0.018 | 0.001 |
| [B]C.D. at 5% | 1.572 | 0.179 | 0.056 | 0.002 |
| [C]CV % | 1.06 | 4.67 | 1.69 | 0.60 |

[A] Standard error of mean; [B] Critical difference; [C] Coefficient of variance.

Bavistin with *F. udum,* and *Trichoderma* Monitor WP with *F. udum* was found to be 3.07, 1.94, 1.87, 3.25, 2.21, and 2.09, respectively while the plumule length of treated seeds with sterile distilled water was 2.80 cm (Table 3).

Similarly the radical length (cm) of treated seeds with *P. dispersa*, Bavistin, *Trichoderma* Monitor WP, *P. dispersa* with *F. udum,* Bavistin with *F. udum,* and *Trichoderma* Monitor WP with *F. udum* was found to be 2.66, 1.57, 1.66, 3.00, 1.84, and 1.56, respectively while the radical length of treated seeds with sterile distilled water was 2.72 cm (Table 3).

The dry weight (g) of treated seeds with *P. dispersa*, Bavistin, *Trichoderma* Monitor WP, *P. dispersa* with *F. udum,* Bavistin with *F. udum,* and *Trichoderma* Monitor WP with *F. udum,* was found to be 0.25, 0.19, 0.22, 0.20, 0.17, and 0.15 g, respectively while the average dry weight of treated seeds with sterile distilled water was 0.22 g (Table 3).

The reduction in germination was observed when *C. cajan* (T-15-15) seeds were treated independently with Bavistin or *Trichoderma* Monitor WP or in presence of *F. udum* with Bavistin or *Trichoderma* Monitor WP with *F. udum* which resulted in a decrease in the length of plumule and root was observed in comparison with control. But dry weight was significantly affected with each treatment.

Previously, it had been reported that *P. dispersa* significantly inhibited the growth of *F. udum* by the production of mycolytic enzymes (Gohel *et al.,* 2004a*,* 2007). During *in vitro* germination experiments similar mechanism for inhibition was observed. Seed germination percentage was highest in seeds treated with *P. dispersa* and untreated control seeds. Also increase in growth of seeds by means of dry weight and lengths of plumule as well as root, was observed with treatment of *P. dispersa*. The present results are similar with the previous reports that indicated that biocontrol agent could produce growth factors resulting in increased the rate of seed germination (Benitez *et al.,* 1998). It has been also reported that treatment of biocontrol strains in several host pathogen systems did enhance the seed germination (Kumar and Dubey, 2001; Poddar *et al.,* 2004).

There was no adverse affect observed on seed germination when seeds were treated independently with *P. dispersa* and also in presence of *F. udum*. Furthermore, the dry weight, lengths of plumule and root were found to be increased, which indicates that *P. dispersa* is not only efficient in controlling the growth of *F. udum* but also increasing the length of plumule and root of pigeonpea in comparison with Bavistin and *Trichoderma* Monitor WP treatments.

**Effect of *Pantoea dispersa*, Bavistin and *Trichoderma* Monitor WP on the control of infection caused by *Fusarium udum* in *Cajanus cajan* (T-15-15) during pot experiments**

After knowing through seed germination analysis, *P. dispersa* treatment was enhancing the length of plumule and radical of pigeonpea and controlling the growth of *F. udum* in the Petri-plates. An attempt was made to study effectiveness of *P. dispersa* in comparison with Bavistin and *Trichoderma* Monitor WP treatment on growth of pigeonpea and *F. udum* when *P. dispersa* treated *C. cajan* seeds (T-15-15) were soaked in the soil harbouring *Fusarium* wilt. For this, pot experiments were conducted.

Initially, the populations of *F. udum* in soil were maintained at levels of 5.0 x $10^5$ spores/ml (50 ml/Kg soil) in one set of pot experiment whilst in another set of experiment *F. udum* was not added in the soil as described in the materials and methods. The seeds were then treated independently with *P. dispersa*, Bavistin and *Trichoderma* Monitor WP and were soaked in the respective pots as described in Table 2.

The root, shoot lengths and dry weight were measured by pulling out plantlets from the pots after a month (Fig. 1). The shoot lengths of every plantlet in each pot were also monitored every week (Fig. 2). The seeds treated with *P. dispersa* (pot-D) were not only protected from *F. udum* infection in pigeonpea but also effective in increasing plantlets growth in comparison with treatments of Bavistin (pot-G, Fig. 1) and *Trichoderma* Monitor WP (pot-H, Fig. 1), as indicated by measuring the shoot length of each treatment (Fig. 2).

In addition, the dry weight, ratio of shoot to root of *P. dispersa* treatment plantlets was also higher in comparison with Bavistin and *Trichoderma* Monitor WP treatments which indicate that *P. dispersa* significantly conferred protection to *C. cajan* against *F. udum* (Fig. 3). However, Bavistin (pot-G) was also found to impart similar protection against *F. udum*, like *P. dispersa* (pot-D), whereas *Trichoderma* Monitor WP showed less protection compared to *P. dispersa* treatment. When seeds were infected with *F. udum* (pot-B), they failed to germinate and hence no plantlet was observed (Fig. 1).

Bora *et al.* (2004) stated that seed-bacterisation with strain of *P. putida* was more suppressive against *Fusarium oxysporum* f. sp. *melonis* than an application of the fungicide benomyl. In some biocontrol treatments against soilborne plant pathogens, seed protection with biocontrol bacteria was better than the fungicide application (Vidhyasekaran and Muthamilan, 1995; Mathre *et al.,* 1999). Similar suppressive nature of *P. dispersa* against *F. udum* was found higher than Bavistin treatment during *in vitro* germination and pot experiments. Furthermore, *Trichoderma* sp. treated seeds were found to be significantly enhanced the length of shoot and root in the pot experiment analysis (Dubey *et al.,* 2007).

By *in vitro* seed germination and *in planta* pot experimental studies, it was found that the *P. dispersa* seed dressing of *C. cajan* provided better protection against *Fusarium* disease in pigeonpea in comparison with commercially available fungicides like Bavistin and *Trichoderma* Monitor WP. The *P. dispersa* also influences the growth of plantlets in respect of the host pathogen systems.


FIG. 1 - Effect of *Pantoea dispersa*, Bavistin and T-2 *Trichoderma* Monitor WP on the control of infection caused by *Fusarium udum,* in *Cajanus cajan* (T-15-15) during pot experiment. A: No treatment, B: *F. udum*, C: *P. dispersa*, D: *P. dispersa* + *F. udum*, E: Bavistin, F: T-2 *Trichoderma* Monitor WP, G: Bavistin + *F. udum*, H: T-2 *Trichoderma* Monitor WP + *F. udum*.

### Effect of *Pantoea dispersa*, Bavistin and *Trichoderma* Monitor WP on the control of infection caused by *Fusarium udum* in *Cajanus cajan* (T-15-15) in field trials

After successful *in vitro* studies of *P. dispersa* on controlling *Fusarium* diseases in pigeonpea, further experiments were performed in the wilt sick plot upto three years (2004-2005 to 2006-2007) in order to study effectiveness of *P. dispersa* in controlling *Fusarium* wilt in pigeonpea (*C. cajan*) at field level.

When treated pigeonpea seeds of T-15-15 susceptible variety were sown in wilt sick plot at the Zonal Agricultural Research Station of Gujarat Agriculture University, Maktampur, Bharuch, India. Wilt incidence was lowest (43.62%) in plots sown with seeds treated with *P. dispersa*. This was superior to wilt incidence (93.94%) in untreated seeds but it was statistically on par with treatments of Bavistin and *Trichoderma* Monitor WP during the year 2004-2005. In the year 2005-2006, the lowest (36.73%) wilt incidence was recorded with treatment of Bavistin, but it was on par with the rest of the treatments. During 2006-2007 the wilt incidence (45.40%) was lowest in *P. dispersa* treatment but it was on par with *Trichoderma* Monitor WP treatment, but this treatment was on par with other treatments. The pooled analysis indicated that the incidence of wilt was reduced by 47, 41 and 36 % by *P. dispersa*, Bavistin and *Trichoderma* Monitor WP, respectively, as compared to untreated seeds (Table 4). These results indicate that *P. dispersa* has more potentiality in controlling the wilting disease in pigeonpea than Bavistin and *Trichoderma* Monitor WP commercially available fungicides.

Furthermore, during the three years of experimental period, significant grain yield was recorded. The highest grain yield (1136 Kg/ha) was obtained with *P. dispersa* treatment during the year 2004-2005 but it was on par with Bavistin treatment and Bavistin treatment was on par with *Trichoderma* treatment. Whereas in the year 2005-2006 highest grain yield (684.0 Kg/ha) was recorded in Bavistin treatment, but it was on par with *P. dispersa* treatment. During this period effect of *Trichoderma* treatment was not significant on the grain yield.

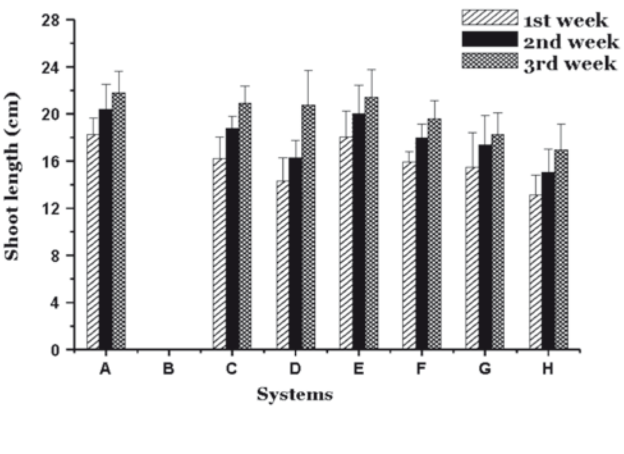


FIG. 2 - Effect of *Pantoea dispersa*, Bavistin and T-2 *Trichoderma* Monitor WP on shoot length of *Cajanus cajan* (T-15-15) during the pot experiment. A: no treatment, B: *Fusarium udum*, C: *P. dispersa*, D: *P. dispersa* + *F. udum*, E: Bavistin, F: T-2 *Trichoderma* Monitor WP, G: Bavistin + *F. udum*, H: T-2 *Trichoderma* Monitor WP + *F. udum.*

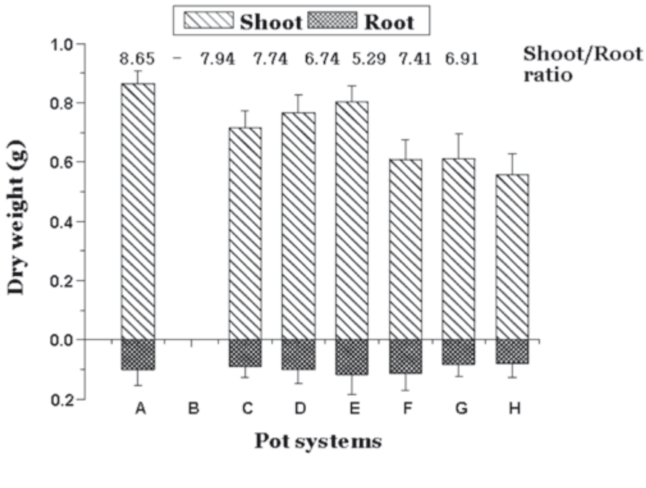


FIG. 3 - The ratio of shoot to root dry weight of treated plantlet of pot experiments. A: no treatment, B: *Fusarium udum*, C: *Pantoea dispersa*, D: *P. dispersa* + *F. udum*, E: Bavistin, F: T-2 *Trichoderma* Monitor WP, G: Bavistin + *F. udum*, H: T-2 *Trichoderma* Monitor WP + *F. udum*, (Figures in parenthesis indicates shoot to root dry weight ratio; N.D.: not detectable).

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TABLE 4 - Comparative effect of *Pantoea dispersa,* chemical and biological fungicides on the control of *Fusarium* wilt in *Cajanus cajan* (T-15-15) under field trials

| Treatments | (%) Wilt incidence[A,B] | | | | Grain yield (kg/ha) | | | | Plant height (cm) | | | | Branches/plant | | | | Pods/ five plants | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 2004/05 | 2005/06 | 2006/07 | Pooled mean | 2004/05 | 2005/06 | 2006/07 | Pooled mean | 2004/05 | 2005/06 | 2006/07 | Pooled mean | 2004/05 | 2005/06 | 2006/07 | Pooled mean | 2004/05 | 2005/06 | 2006/07 | Pooled mean |
| *P. dispersa* | 41.22 (43.63) | 39.03 (39.90) | 42.32 (45.40) | 40.86 (42.97) | 1136.11 | 657.99 | 756.94 | 850.35 | 159.50 | 131.25 | 127.25 | 139.33 | 18.03 | 9.25 | 11.50 | 12.93 | 270.65 | 61.80 | 117.20 | 149.88 |
| Bavistin | 43.95 (48.22) | 37.19 (36.73) | 49.49 (57.62) | 43.55 (47.52) | 993.40 | 684.03 | 857.64 | 845.02 | 159.75 | 126.25 | 128.50 | 138.17 | 18.70 | 8.85 | 11.20 | 12.92 | 252.25 | 66.40 | 117.80 | 145.48 |
| *Trichoderma* Monitor WP | 49.52 (57.66) | 38.89 (40.02) | 48.81 (56.59) | 45.74 (51.42) | 767.71 | 548.61 | 809.03 | 708.45 | 160.00 | 129.75 | 129.50 | 139.75 | 18.48 | 9.30 | 11.43 | 13.10 | 257.50 | 68.475 | 120.0 | 148.65 |
| Control [No seed treatment] | 76.01 (93.94) | 56.80 (69.68) | 61.51 (77.04) | 64.77 (80.22) | 347.22 | 461.81 | 513.89 | 440.97 | 159.25 | 132.25 | 126.00 | 139.17 | 18.05 | 8.13 | 10.75 | 12.31 | 295.30 | 67.72 | 114.25 | 159.09 |
| [C]S.Em | 4.08 | 4.18 | 2.54 | 2.27 | 106.63 | 36.66 | 37.31 | 83.84 | 1.14 | 2.24 | 0.94 | 0.946 | 0.88 | 0.45 | 0.47 | 0.339 | 14.80 | 4.12 | 2.68 | 105.53 |
| [D]C.D. at 5% | 13.04 | 13.37 | 8.13 | 6.55 | 341.09 | 117.26 | 119.34 | 290.12 | NS | NS | NS | NS | NS | NS | NS | NS | NS | NS[F] | NS[F] | NS[F] |
| [E]CV % | 15.48 | 19.45 | 10.06 | 15.09 | 26.29 | 12.47 | 10.16 | 19.28 | 1.43 | 3.45 | 1.47 | 2.23 | 9.65 | 10.15 | 8.40 | 9.90 | 11.01 | 12.45 | 4.57 | 9.34 |

[A] Arc sin transformed values; [B] Figures in parentheses are original values; [C] Standard error of mean; [D] Critical difference; [E] Coefficient of variance; NS: not significant.

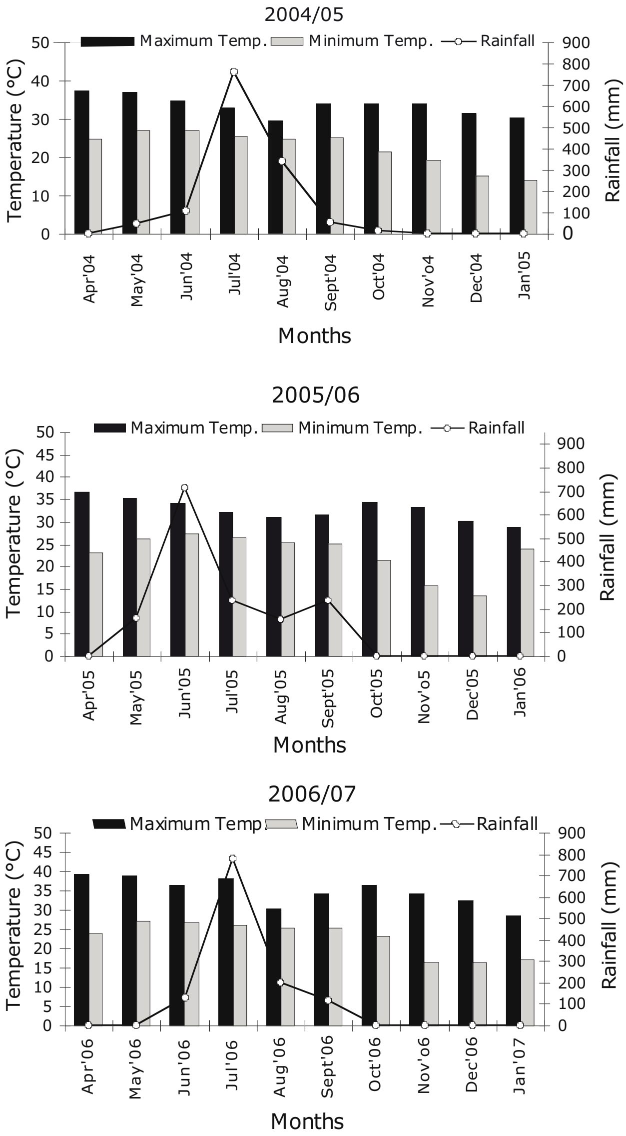


FIG. 4 - Monthly mean temperatures (°C) and total rain fall for field trial in years 2004/2005, 2005/2006, 2006/2007.

In year 2006-2007, the better grain yield 858 and 850 Kg/ha was recorded in the Bavistin and *P. dispersa* treatments, respectively but it was on par with the *Trichoderma* treatment. When compared statistically, significant difference was observed for wilt incidence and grain yield in each treatment. These results indicate that each treatment has its own effect on grain yield and wilting reduction, while there were no significant effects observed in plant height, and number of branches and pods (Table 4).

Weather conditions during the three years field trials were recorded at the Zonal Agricultural Research Station of Gujarat Agriculture University, Maktampur, Bharuch, India (Fig. 4). The vegetation period in 2004-2005 and 2006-2007 started with the rainy season in June during both years. The initial rainfall was quite sufficient for sowing pigeonpea seeds. These periods were followed by low rainfall during the second week of September 2004 and September 2006. During the flowering period and harvest, the minimum temperature between November 2004 and January 2005 was generally lower when compared to October 2004. The same was observed in the year 2006.

Major rainfall was recorded between June 2004 and August 2004, where as 60% rainfall was recorded during the period July 2006 and August 2006. The amount of rainfall from June 2005 to October 2005 was higher when compared to other years.

Since there was heavy rainfall in June 2005, it was not favourable for sowing; therefore sowing was done during the second week of July 2005. Wilting was observed during all the three years in November and December, except in the year 2005-2006, when wilting was observed during December 2005 and January 2006, due to a gradual decrease in temperature that boosted the growth of *F. udum*

A visual study of the longitudinal section of the shoot and the root of infected and healthy plants revealed *F. udum* infection in the vascular system of infected plants but not in healthy plants (Fig. 5).

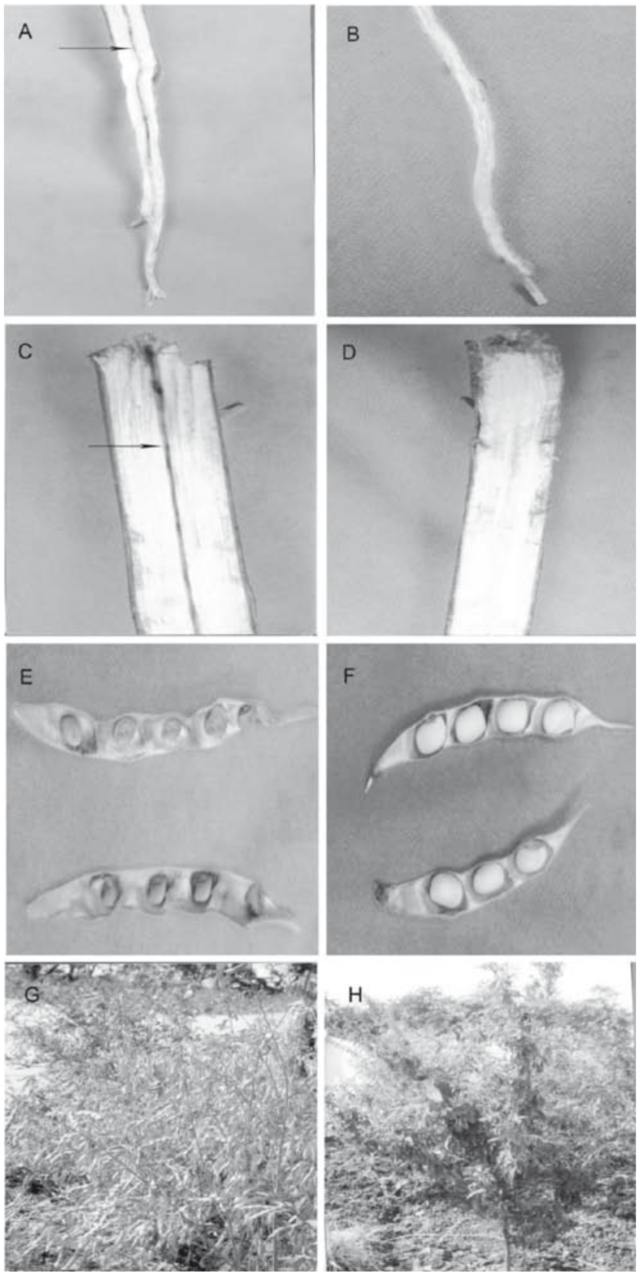


FIG. 5 - Root, shoot, seeds and plants of uninfected and wilt infected *Cajanus cajan* T-15-15. A: Infected root, B: unifected root, C: infected shoot, D: uninfected shoot, E: seed pod of infected plant, F: seed pod of uninfected plant, G: infected plant, H: uninfected plant.

It has been reported that *Pseudomonas fluorescens* was effective in reducing the *Fusarium moniliforme* infection in five different cultivars of sorghum (Raju *et al.,* 1999). When cotton seedlings were inoculated with strains of *Bacillus cereus*, *Bacillus subtilis* and *Bacillus pumilus*, disease incidence by *Rhizoctonia solani* was reported to be reduced by 51, 46 and 56%, respectively (Pleban *et al.,* 1995). *Stenotrophomonas maltophilia* strain C3 was reported to reduce 80% foliar necrosis (brown patch disease) caused by *R. solani* in Tall fescue (Giesler and Yuen, 1998). The antagonist *Bacillus brevis* was reported to control *Fusarium* wilt in the pigeonpea in the pot and field conditions (Bapat and Shah, 2000).

Furthermore Larena *et al.,* (2003) demonstrated that non-formulated *P. oxalicum* conidia, or conidia applied together with the substrate of production, reduced tomato wilt. Unfortunately, these conidia were (relatively) insoluble in water, and they quickly became unviable (Larena *et al.,* 2003). But our *P. dispersa* isolate was found to be effective in all respects of controlling *Fusarium* wilt and enhancing the plant growth.

*Pantoea dispersa* showed significant protection to infection caused by *F. udum,* in susceptible variety of *C. cajan* (T-15-15) under plate, pot and field trials throughout three years. *P. dispersa* was comparable in efficacy to chemical fungicide Bavistin in controlling wilting but this was found to be better than biological fungicide *Trichoderma* Monitor WP. Thus our mycolytic strain *P. dispersa* can be used as a biocontrol agent for controlling infection caused by fungal plant pathogens.

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