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The efficacy of bioagents against *Fusarium oxysporum* f. sp. *udum* causing wilt disease of Pigeonpea

PH Ghante, KM Kanase, PA Gaikwad, SV Pawar and SJ Magar

Abstract
Fusarium oxysporum f. sp. *udum* is one of the most destructive soil-borne diseases of Pigeonpea. The main target of present investigation was to evaluate the antifungal activities of bioagents which can be used to control wilt disease of pigeonpea. The bioagents *viz. Trichoderma viride, T. harzianum, T. hamatum, T. longibrachiatum, T. (Gliocladium) virens, T. koningii, Aspergillus Niger, Trichoderma lignorum, Pseudomonas fluorescens and Bacillus subtilis* were screened *in vitro* for their antifungal activities against *F. udum* by using dual culture technique. *Trichoderma viride* was found most effective with least linear mycelial growth (10.50 mm). The second and third best antagonists were *T. koningii* and *T. harzianum*, which recorded less mycelial growth of 13.00 mm and 15.50 mm, respectively. They were followed by *Aspergillus Niger, T. (Gliocladium) virens, T. hamatum, T. longibrachiatum, T. lignorum, Bacillus subtilis* and *Pseudomonas fluorescens.*
In field evaluation under normal soil and semi sick soil condition, *Trichoderma viride* was shown minimum wilt incidence *i.e.* 19.24 % and 37.92 % and recorded maximum yield *i.e.* 773.78 and 531.20, respectively followed by *Trichoderma harzianum* shown 21.75 % wilt incidence with 709.59 kg /ha yield under normal soil and in semi sick soil condition, 40.14 % wilt incidence with 510.84 kg/ha yield recorded.
In various dosages of *T. viride* (15 g/kg seed) showed minimum wilt incidence i.e. 20.58, 37.22 and 61.25 %, respectively with maximum yield compared to other treatments i.e. 756.74, 508.18 and 316.51, respectively.

Keywords: Pigeonpea wilt, *Fusarium oxysporum* f. sp. *udum, in vitro*, bioagents, *Trichoderma viride* and *T. harzianum*

Introduction
Pigeonpea [*Cajanus cajan* (L.) Millspaugh] is known by more than 350 vernacular names, the most popular being arhar, yellow dhal, red gram, tur (India), congo pea, gandul, guandu (Brazil), angola pea (United Kingdom), catjang pea, ambrevade, pois d'angdie (French-speaking West Africa), quinochoncho (Venezuela). Archaeological finds of pigeonpea dating to about 3400 years ago (14th century BC) have been found at Neolithic sites in Karnataka state of India (Sanganakallu) and its border areas (Tuljapur Garhi in Maharashtra state and Gopalpur in Orissa state) and also the south Indian states such as Kerala, where it is called Tomara Payaru. From India it traveled to East Africa and West Africa. There, it was first encountered by Europeans, so it obtained the name Congo Pea. By means of the slave trade, it came to the American continent, probably in the 17th century.
The area of pigeonpea in Maharashtra is increased from 10.39 lakh ha to 15.33 lakh ha in 2016-17. Area of pigeonpea was highest in 2016-17 (15.33 lakh ha) while the production and productivity were highest during 2013-14 i.e.10.34 lakh tones and 906 kg/ha, respectively. In 2016-17 estimated production of pigeonpea in Maharashtra is 11.70 lakh tonnes. In Marathwada, area under pigeonpea was 5.95 lakh ha during 2016-17, while production and productivity were highest during 2013-14 i.e. 5.16 lakh tones and 933 kg/ha, respectively. Maharashtra contributes 30.29 % in terms of area with 28.29 % of production at national level (average of last ten years). Percentage of area increase during 2016-17 as compared to previous year (2015-16) is 27.25 %, 32.22 % and 33.64 % in India, Maharashtra and Marathwada, respectively. Area, production and productivity of pigeonpea cultivated in India, Maharashtra and Marathwada region are mentioned below:

Table 1: Area, production and productivity of pigeonpea cultivated in India, Maharashtra and Marathwada region are mentioned below

Year	India			Maharashtra			Marathwada		
	A M. ha	P M. Ton	Y kg/ha	A L. ha	P L. Ton	Y kg/ha	A L. ha	P L. Ton	Y kg/ha
2009-10	3.53	2.46	697	10.93	9.19	841	4.55	3.72	817
2010-11	4.42	2.86	655	13.01	9.76	750	5.24	4.19	790
2011-12	4.04	2.65	656	12.33	8.71	706	5.10	3.57	691
2012-13	3.81	3.02	806	12.13	10.05	829	5.03	3.49	645
2013-14	3.88	3.17	849	11.41	10.34	906	5.23	5.16	933
2014-15	3.55	2.81	783	12.10	3.53	292	5.36	1.35	260
2015-16	3.79	2.56	673	10.39	3.95	380	3.85	0.78	203
2016-17*	5.21	4.23	826	15.33	11.70	764	5.95	4.47	759

* Second Advance Estimate (Source: Chief Statistician; Pune, 2017)

In general, there is low productivity due to susceptibility against pests and diseases. The crop is attacked by more than 100 pathogens (Nene *et al*., 1996) [11] including fungi, bacteria, viruses, phytoplasma like organisms and nematodes. However, only a few of them cause economic losses (Kannaiyan *et al.,* 1984) [9]. The diseases of considerable economic importance at present are sterility mosaic, *Fusarium* wilt, *Phytophthora* blight, *Macrophomina* root rot, stem canker and *Alternaria* blight.

Fusarium wilt is the most crucial disease of pigeonpea in India resulting in yield losses up to 67 per cent at maturity and 100 per cent in case of infection at pre-pod stage (Kannaiyan and Nene, 1981) [8]. The *Fusarium* wilt in pigeonpea was first reported from Bihar by Butler (1910) [2]. Surveys conducted for the disease by Kannaiyan *et al*. (1984) [9] have indicated it to be a major problem in the states of Bihar and Maharashtra (Reddy *et al*., 1990) [17]. *Fusarium* wilt characterized by wilting of the affected plants and characteristic internal browning or blackening of the xylem vessels extending from root system to stems. Partial wilting of the plants (Upadhyay and Rai, 1992) and patches of dead plants (Reddy *et al*., 1993) [17] were reported to be common in the fields during advanced stages of plant growth.

Fusarium udum is soil borne and is capable of saprophytic survival on crop residues in the soil for up to eight years (Nene, 1980). Chemical control of the disease is therefore difficult, impractical and uneconomical, as the large scale soil application of chemicals required is expensive, hazardous and disturbs the biological balance (Songa, 1990). Hence, efforts have to be made to curtail pathogen activity and restricting losses below economic threshold level by choosing alternative methods. Of late biocontrol methods involving manipulation of antagonistic rhizosphere microflora either by adding mycoparasites such as *Trichoderma* or by incorporating green manure, farm yard manure, plant residues, oil cakes or animal residues in the soil which increases antagonistic microflora are being extensively employed against soil borne plant pathogens. Considering economic importance of disease investigation was carried out to eco-friendly management (*in vitro*) of wilt disease of Pigeonpea causing *Fusarium oxysporum* f. sp. *udum.*

Materials and Methods

In vitro evaluation of bioagent

The experiment was conducted at Department of Plant Pathology, College of Agriculture Parbhani, VNMKV, Parbhani (M.S.). The pathogen was isolated from diseased leaves of Pigeonpea on PDA incubated at 27±2 ºC. The ten bioagents *viz. Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. longibrachiatum*, *T. (Gliocladium) virens*, *T. koningii*, *Aspergillus niger*, *Trichoderma lignorum*, *Pseudomonas fluorescens and Bacillus subtilis* were evaluated *in vitro* for their antifungal activities against wilt diseases of Pigeonpea, *Fusarium oxysporum* f. sp. *udum* by using dual culture technique (Dennis and Webster, 1971). The 20 ml of potato dextrose agar media was poured in sterilized petri plates and to be solidified. Fungal and bioagent disks of 5mm in diameter from 7 days old culture was placed at equidistance and exactly opposite with each other on solidified PDA medium under aseptic condition, incubated at 27±2 ºC for 7 days. Three replicated PDA plates with and without bioagents were measured from the bottom side of the petri dishes.

The colony diameter of the fungus pathogen on medium was recorded and per cent inhibition was calculated by using following formula (Vincent, 1927).

$$\text{Per cent inhibition} = \frac{C - T}{C} \times 100$$

Where,
C= growth of the test fungus in untreated control plates
T= growth of the test fungus in treated plates

Field (*In vivo*) evaluation of bioagents

The experiment was conducted at Department of Plant Pathology, ARS, Badnapur, VNMKV, Parbhani (M.S.). during *Kharif* 2015 to evaluated the bioefficacy of seven bioagent *viz. Trichoderma viride*, *T. harzianum*, *T. hamatum*, *T. longibrachiatum*, *T. (Gliocladium) virens*, *T. koningii*, *Aspergillus niger*, *Trichoderma lignorum*, *Pseudomonas fluorescens and Bacillus subtilis* with randomized block design in three replications. Pigeonpea wilt susceptible variety ICP 2376 was sown at 90 cm x 20 cm spacing in Normal soil plot, Semi sick soil plot and Sick soil plot with inoculum load of *F. udum* (cfu / g of soil) 2.43 x 10^2, 3.14 x 10^4 and 2.87 x 10^7 respectively. The inoculum load of *F. udum* in the soils experimental sites was estimated before commencement of the field experiments by applying serial dilution and planting technique.

Field (*In vivo*) evaluation of various dosages of *T. viride*

The experiment was conducted at Department of Plant Pathology, ARS, Badnapur, VNMKV, Parbhani (M.S.) during *Kharif* 2015 to evaluated the bioefficacy of seven *T. viride* dosages *viz. T. viride* 2, 4, 6, 8, 10, 12, and 15g / kg seed respectively, with randomized block design in three replications. Pigeonpea wilt susceptible variety ICP 2376 was sown at 90 cm x 20 cm spacing in normal soil plot, semi sick soil plot and sick soil plot. The surface sterilized seeds of susceptible cv. ICP 2376 were treated with carrier based (Talc) formulation (2 x 10^7 cfu / g carrier) of *T. viride* at various dosages ranging from 2 to 15 g/ kg seeds. These *T. viride* treated seeds of pigeonpea were sown (90 cm x 20 cm) on dated 10 June 2015 having experimental plot size (Gross plot Size: 13 m x 40 m, Net plot Size: 12.8 m x 39 m, Block size per treatment: 3.6 m x 4 m with 4 rows and 20 plants / row).

Results and Discussion

Results revealed that all the bioagents evaluated, exhibited fungistatic/antifungal activity against *F. udum* and significantly inhibited its growth, over untreated control. Of the bioagents/antagonists tested, *Trichoderma viride* was found most effective with least linear mycelial growth (10.50 mm). The second and third best antagonists found were *T. koningii* and *T. harzianum*, which recorded less mycelial growth of 13.00 mm and 15.50 mm, respectively. They were followed by *Aspergillus niger*, *T.* (*Gliocladium*) *virens, T. hamatum, T. longibrachiatum, T. lignorum, Bacillus subtilis* and *Pseudomonas fluorescens.* All the treatments were significantly superior for fungistatic / antifungal action over untreated control.

The antagonists tested, *Trichoderma viride* was found most effective with the highest mycelial growth inhibition (88.33 %) of the test pathogen. The second and third most inhibitor antagonists found were *T. koningii* and *T. harzianum* with inhibition of 85.56 and 82.78 per cent, respectively. They were followed by *Aspergillus niger* (80.93 %), *T. (Gliocladium) virens* (80.74 %), *T. hamatum* (75.74 %), *T. longibrachiatum* (75.74 %), *T. lignorum* (66.67 %), *Bacillus subtilis* (49.63 %) and *Pseudomonas fluorescens* (44.63 %). Thus, the bioagents *viz*., *T. viride, T. koningii, T. harzianum* were found most potential antagonists against *F. udum*.

These results are in conformity with the earlier findings of those workers who reported that bioagents *viz*., *Trichoderma viride, T. koningii, T. harzianum* and *T. hamatum* had significantly inhibited mycelial growth of *F. udum* (Gaur and Sharma, 1991; Chauhan 1997; Raju *et al*., 2008; Niranjana *et al.*, 2009; Patel *et al*., 2011; Nehra *et al.*, 2012; Jadhav *et al.*, 2014; and Thaware *et al.*, 2017) [6, 3, 12, 13, 7, 19]. Results indicated that bioagents have potential to inhibit *F. udum* which is crucial for sustainable, eco-friendly and organic farming.

Table 2: *In vitro* efficacy of bioagents against *F. udum.*

Tr. No.	Treatments	Colony dia. of test pathogen * (mm)	Per cent Inhibition
T_1	*Trichoderma viride*	10.50	88.33 (70.07)
T_2	*T. harzianum*	15.50	82.78 (65.49)
T_3	*T. hamatum*	21.83	75.74 (60.53)
T_4	*T. longibrachiatum*	21.83	75.74 (60.53)
T_5	*T. (Gliocladium) virens*	17.33	80.74 (63.98)
T_6	*T. koningii*	13.00	85.56 (67.73)
T_7	*Aspergillus niger*	17.17	80.93 (64.13)
T_8	*Trichoderma lignorum*	30.00	66.67 (54.78)
T_9	*Pseudomonas fluorescens*	49.83	44.63 (41.86)
T_{10}	*Bacillus subtilis*	45.33	49.63 (44.77)
T_{11}	Control (untreated)	90.00	00.00 (00.00)
S.E. +		2.48	1.75
C.D. (P=0.01)		7.31	5.16

*****Mean of three replications, Dia: Diameter, Figures in parentheses are angular transformed values

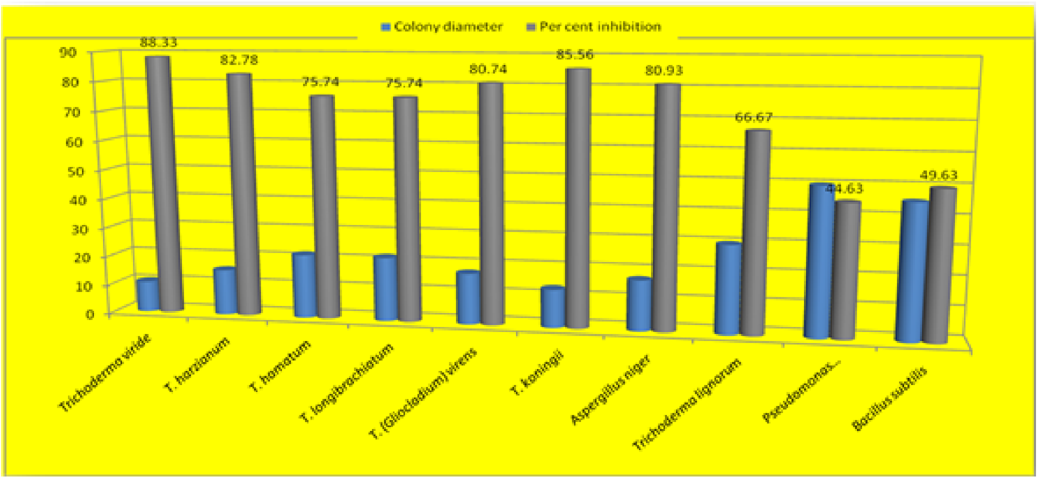

Fig 1: *In vitro,* efficacy of bioagents against *F. udum*

Field (*In vivo*) evaluation of bioagents

Under normal soil and semi sick soil condition, *Trichoderma viride* showed minimum wilt incidence *i.e.* 19.24 % and 37.92 % and recorded maximum yield *i.e.* 773.78 and 531.20, respectively compared to other treatments. Second best treatment was *Trichoderma harzianum* shown 21.75 % wilt incidence with 709.59 kg /ha yield under normal soil and in semi sick soil condition, 40.14 % wilt incidence with 510.84 kg/ha yield recorded. (Table 1, Fig. 2 and 3)

Maximum wilt incidence 43.08 % and 66.82 % were recorded in untreated seed T_8 with 504.64 and 290.39 kg/ha yield under normal and semi sick soil, respectively. Under normal soil condition, all the treatments were significantly superior over untreated control in both the cases (incidence and yield). These results are in conformity with the findings of those reported earlier by several workers (Gade *et al.*, 2007*;* Mahesh *et al.*, 2006; Prasad *et al.*, 2012) [5, 10, 14].

Table 3: Efficacy of seed treatments with various *Trichoderma* spp. on wilt incidence and seed yield in pigeonpea cv. ICP 2376 during *Kharif* 2015-16.

Tr. No.	Treatments	Normal Soil		Semi Sick soil	
		Incidence* (%)	Yield Kg/ha	Incidence* (%)	Yield Kg/ha
T_1	*Trichoderma viride*	19.24 (25.99)	773.78	37.92 (37.97)	531.20
T_2	*T. harzianum*	21.75 (27.73)	709.59	40.14 (39.27)	510.84
T_3	*T. hamatum*	26.35 (30.86)	691.45	44.37 (41.74)	478.97
T_4	*T. longibrachiatum*	29.70 (32.57)	677.28	48.33 (44.03)	435.36
T_5	*T. (Gliocladium) virens*	32.08 (34.48)	643.86	53.99 (47.29)	399.06
T_6	*T. koningii*	35.16 (36.32)	625.71	56.25 (48.57)	373.39
T_7	*T. lignorum*	35.83 (36.73)	620.84	59.17 (50.29)	324.25
T_8	Control	43.08 (40.99)	504.64	66.82 (54.82)	290.39
S.E. +		1.24	15.38	1.53	17.84
C.D. (P = 0.05)		3.80	47.09	4.68	54.64
CV		16.47	14.06	15.81	17.40

*: Mean of three replications Figures in parentheses are angular transformed values

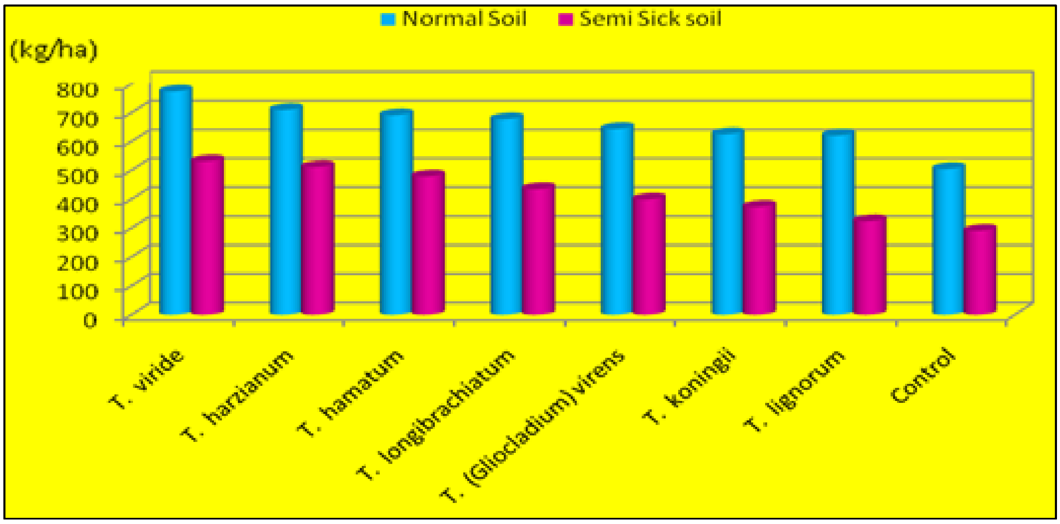

Fig 2: *In vivo*, efficacy bioagents on incidence of pigeonpea wilt (*F. udum*) Cv. ICP 2376, during *Kharif* 2015-16

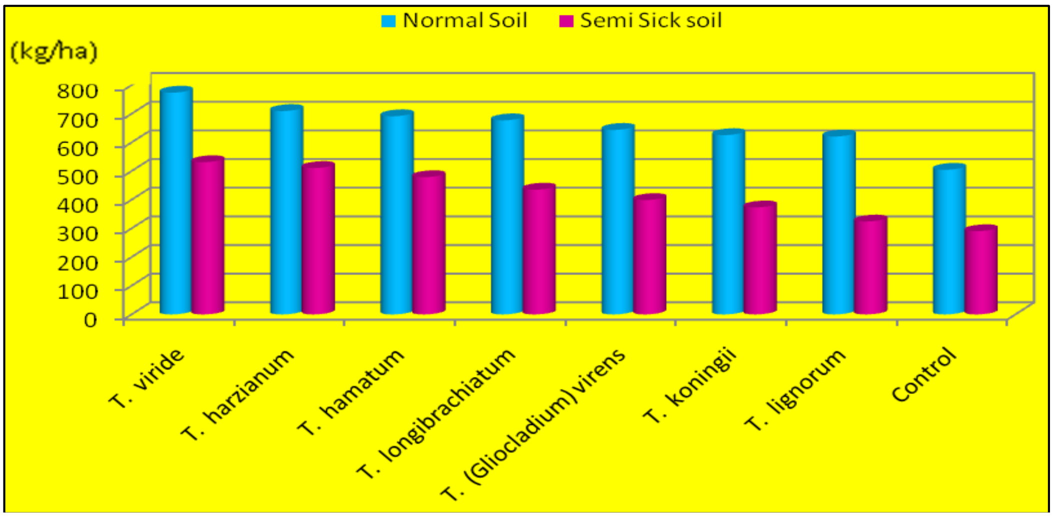

Fig 3: *In vivo*, efficacy bioagents on yield of pigeonpea Cv. ICP 2376, during *Kharif* 2015-16

Field (*In vivo*) evaluation of various dosages of *T. viride*

In normal soil, semi sick soil and sick soil, *Trichoderma viride* (15 g/kg seed) showed minimum wilt incidence *i.e.* 20.58, 37.22 and 61.25 %, respectively with maximum yield compared to other treatments *i.e.* 756.74, 508.18 and 316.51, respectively compared to other treatments. (Table 2, Fig.3 and 4) Second best treatment was *Trichoderma viride* (12 g/kg seed) which has shown 21.25, 39.17 and 61.67 % wilt which was at par with treatment T_7 and produced grain yield 744.79, 504.20 and 315.40 kg/ha in normal, semi sick and sick soil, respectively. Maximum wilt incidence was recorded in untreated seed T_8 (untreated control) with no yield in sick soil

Under normal soil condition, all the treatments were significantly superior over untreated control in case of reduction of wilt incidence but T_1 treatment was at par with T_8 (untreated control) and rest were significantly superior over untreated control in case of yield.

Under semi sick soil condition, T_1 treatment was at par with T_8 (untreated control) and rests of treatment were significantly superior over untreated control in case of reduction of wilt incidence. Whereas, in case of yield, all the treatments were significantly superior over untreated control.

Table 4: Efficacy of various dosages of seed treatments of *T. viride* against wilt of pigeonpea under varied inoculum potential of *F. udum*

Tr. No.	Treatments	Normal soil		Semi sick soil		Sick soil	
		Incidence* (%)	**Yield Kg/ha***	**Incidence* (%)**	**Yield Kg/ha***	**Incidence* (%)**	**Yield Kg/ha***
T_1	*T. viride* 2 g / kg	37.92 (37.99)	459.27	63.75 (52.98)	333.11	90.39 (72.12)	122.62
T_2	*T. viride* 4 g / kg	34.17 (35.75)	532.53	52.08 (46.18)	412.12	87.05 (69.14)	136.12
T_3	*T. viride* 6 g / kg	31.67 (34.23)	591.40	49.35 (44.61)	407.70	81.25 (64.39)	183.04
T_4	*T. viride* 8 g / kg	29.71 (32.99)	607.12	47.92 (43.79)	424.07	78.66 (62.47)	202.30
T_5	*T. viride* 10 g / kg	26.25 (30.77)	673.74	43.50 (41.24)	466.79	72.29 (58.25)	253.65
T_6	*T. viride* 12 g / kg	21.25 (27.41)	744.79	39.17 (38.72)	504.20	61.67 (51.73)	315.40
T_7	*T. viride* 15 g / kg	20.58 (26.93)	756.74	37.22 (37.56)	508.18	61.25 (51.48)	316.51
T_8	Untreated seed	41.67 (40.18)	421.20	65.42 (54.04)	304.33	100.00 (90.00)	00.00
S.E.+		0.60	22.40	1.37	7.27	1.48	6.24
C.D. (P = 0.05)		1.85	68.60	4.19	22.26	4.54	19.11
C.V.		13.14	16.48	15.27	12.99	13.95	15.65

*: Mean of three replications Figures in parentheses are angular transformed values

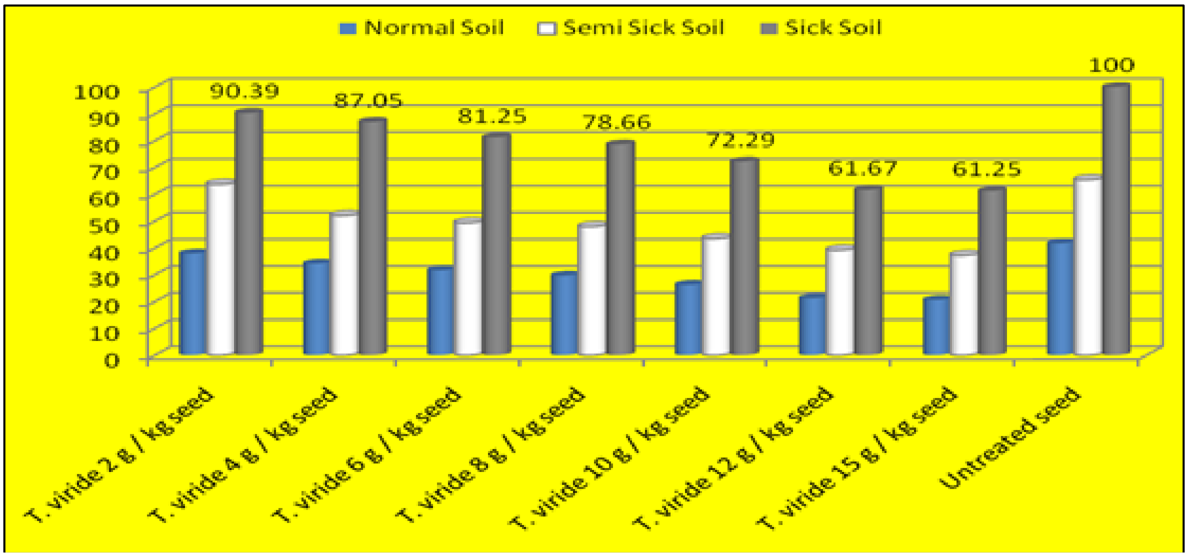

Fig 4: Efficacy of various dosages of *T. viride* (Seed Treatments) on incidence of pigeonpea wilt under varied inoculum potential of *F. udum*

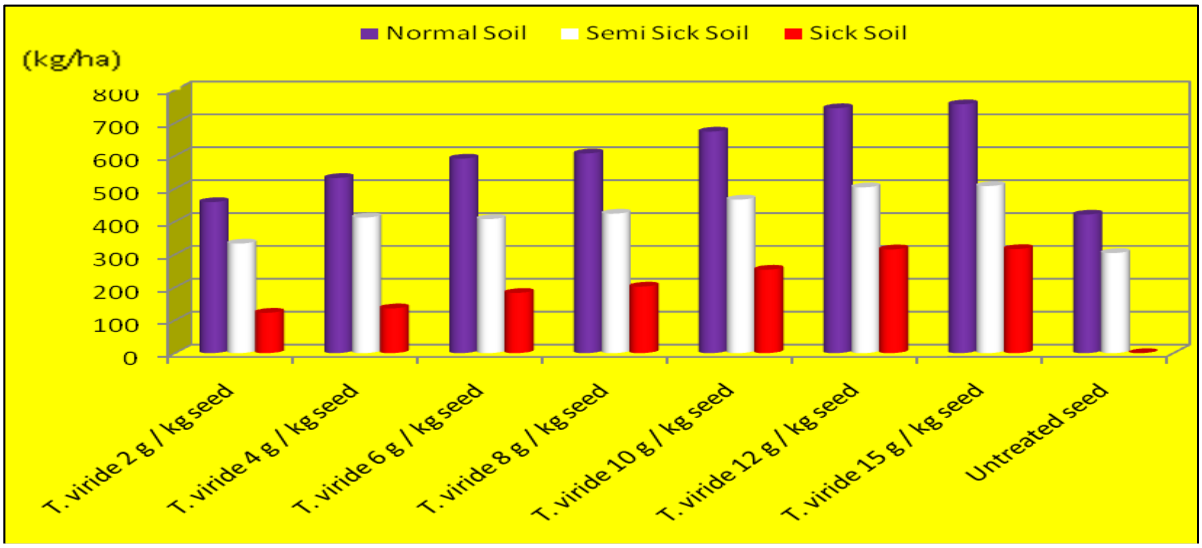

Fig 5: Efficacy of various dosages of *T. viride* (Seed Treatments) on yield of pigeonpea under varied inoculum potential of *F. udum*

Under sick soil condition, all the treatments were significantly superior over untreated control in both the cases (reduction of wilt incidence and increasing yield parameter).

Similarly, Rajendran *et al*. (2014) [15] tested the talc based formulation of two bioagents (Pf and Tv) and its mixture against *Fusarium* wilt in carnation and gerbera under protected cultivation. The mixture of two biocontrol agents performed better than the individual i.e. 7.8 as well as 3.3 % wilt incidence during first year and 9.5 as well as 7 % wilt incidence during second year in carnation and gerbera, respectively which were at par with carbendazim treatment.

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