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Growth promotion of chickpea plant on treatment with native isolates of *Trichoderma* spp.

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Abstract
Trichoderma spp. (Hypocreales, Ascomycota) are free-living soil fungi that are commonly found root ecosystems. It is well reported as an efficient bio control agents against many soil borne pathogen of many crops. It is most popularly used as bio control agents due to its wide mechanism of parasitism i.e., Mycoparasitism and hyphal lysis, antibiosis, competition for nutrients and space. Apart from this, *Trichoderma* spp. are also well reported as a root colonizer which promote growth of plants significantly as compared to untreated plant. It can be applied as seed treatment and soil inoculation, which not only increase the germination percentage but also improves the root length, shoot length, dry weight, fresh weight, vigour index and vigour mass index. In chickpea cvs JG-14 and JG-24, maximum growth promotion observed with inoculation of *Trichoderma* isolate Tr- 7 followed by Tr- 6, Tr- 2 and Tr-1.

Keywords: *Trichoderma*, chickpea, Growth promotion, seed treatment, soil treatment

Introduction
Chickpea (*Cicer arietinum* L.) is a self-pollinated leguminous crop belongs to family *Fabaceae* and it's cultivation is mainly concentrated in semi-arid condition (Knights *et al.*, 2007) [11]. It is an important pulse crop i.e., ranked third after beans and pea. Chickpea seeds are rich source of carbohydrate (61.5%), protein (17-20%), vitamins (2.44%) and unsaturated fatty acid (4.5%) such as, linoleic and oleic acids. It also contains high quantity of minerals such as; phosphorus (340 mg/100 g), calcium (190 mg/100 g), magnesium (140 mg/100 g), iron (7 mg/100 g) and zinc (3 mg /100 g). Three important sterols, β-Sitosterol, campesterol and stigmasterol found in chickpea oil (Maiti, 2001) [13].
Trichoderma spp. (Hypocreales, Ascomycota) are free-living fungi that are cosmopolitan in soil, root ecosystems and on decaying wood and other forms of plant organic matter. This fungal agent is most popular due to its wide mechanism of parasitism i.e., Mycoparasitism and hyphal lysis, antibiosis, competition for nutrients and space and also promotion of plant growth (Das *et al*., 2006, Kumar *et al*., 2012, Patibanda *et al*., 2002, Tripathi and Khare, 2005 Jabbar *et al*., 2014 and Meher *et al*., 2018) [7, 12, 17, 19, 9, 15]. *Pseudomonas fluorescens* show great antagonistic activity against several soil-borne pathogens of economically important crops. Its bio-control activity is mediated through the production of antibiotics, lytic enzymes, siderophore and HCN as well as through competitive exclusion. Balasubramanian (2003) [2] had also reported that increased growth response in many crops such as beans, cucumber, pepper, Carnation, maize and wheat by *Trichoderma* species and other root colonizing fungi. Increase in height and dry weight of tomato and peachy seedlings grown in seedbeds treated with *Trichoderma* oven- dried starch pellets was also observed (Cuevas *et al*., 2005) [5]. Generally, population of PGPRs provide better resources for the improvement of plant growth promotion and bio-control ability, as different strains possess varied modes of action and survival in diverse environmental conditions (Ramesh *et al*., 2005) [18].

Materials and Methods
Soil samples from different locations of Jabalpur was collected to isolates *Trichoderma* spp., by serial dilution technique up to 10^{-4} dilution a as described by Meher *et al* (2017) [16]. The colonies of *Trichoderma* were identified by key based on branching of conidiophores, shape of phialides, emergence of phialides and spore characters (Gams and Bisset, 2002). Culture of *Pseudomonas fluorescens* was collected from JNKVV, Jabalpur. Mass multiplication of bio-agents were done in sorghum grains were pre-soaked in 2 per cent sucrose solution overnight, drained and boiled in fresh water for 30 minutes and drained again. This was transferred into 1000 ml flasks @ 400 g and autoclaved at 15 lb psi (121.6 ^{0}C) for 20 minutes. The flasks were allowed to cool at room temperature and inoculated with five mm discs of 3 to 4 days old

culture of *Trichoderma* grown on PDA. Pot culture studies were conducted to evaluate growth promotion activity of *Trichoderma* isolates and *Pseudomonas fluorescens in vivo*. by using two different application methodologies, i.e. seed treatment with 5 g/kg seed and soil application with 10 g/kg soil.

Percent germination was recorded 10 days after sowing and final count after 30 days of sowing. Height (cm), dry weight and fresh weight (gm) were recorded to calculate vigour index mass, vigour index percentage and seedling mortality up to 30 days by using following formulae given by (Kharb *et al.*, 1994) [10];

$$\text{Germination (\%)} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

Vigour index (%) = Germination percentage × Seedling length on the day of final count

Vigour index mass = Germination percentage × seedling dry weight

Result and Discussion

Screening for growth promotion of chickpea plant by seed and soil inoculation bacterial and fungal bio-agents

In this study, we had performed experiments to determine the effect of seed and soil inoculation bacterial and fungal bio-agents i.e., *Trichoderma* isolates and *Pseudomonas fluorescens* on different growth parameters like; shoot length, root length, dry weight, fresh weight vigour index and vigour index mass in two cultivars JG 14 and JG 24.

(i) Effect of seed treatment on percent germination and vigour index

Significant enhancement in shoot length, root length and vigour index of plant was observed on seed treatment with *Trichoderma* isolates and *Pseudomonas fluorescens* as compared untreated plant. Maximum plant vigour index was recorded on treatment with Tr- 7 i.e., 3383.3 with 26.5cm shoot length and 7.3cm root length followed by 3296.7, 3066.7 and 2791.2 with Tr- 6, Tr- 2 and Tr- 1 respectively in variety JG 14 as compared to 1589 and 2149.3 in pathogen treated control and untreated control respectively. Similarly, in variety JG 24 Maximum plant vigour index was recorded on treatment with Tr- 7 i.e., 3973.3 with 31.2 cm shoot length and 8.6cm root length was observed with Tr- 7 followed by 3639.9, 3543.4 and 3531 with Tr- 6, Tr- 2 and Tr- 1 respectively as compare to 1782 in pathogen treated control and 2324.6 in untreated control. Whereas, on seed treatment with *Pseudomonas fluorescens* vigour index of 2821.7 and 3284.8 observed in variety JG 14 and JG 24 respectively. The present findings are in agreement with the results reported on growth promotion with seed treatment of *Trichoderma* spp. by Conrath *et al*. (2002) [3] and Cuevas (2006) [6].

Table 1: Effect of seed treatment on vigour index of chickpea in Cvs JG 14 and JG 24

Verities	JG 14				JG 24			
Treatment	Germ. (%)	Shoot length (cm)	Root length (cm)	Vigour Index	Germ. (%)	Shoot length (cm)	Root length (cm)	Vigour Index
Tr- 1 isolate	93.33	23.6	6.3	2791.2	93.33	29.7	8.2	3531.0
Tr- 2 isolate	100	24.0	6.6	3066.7	93.33	29.8	8.2	3543.4
Tr- 3 isolate	93.33	23.0	6.1	2715.9	93.33	27.9	8.0	3117.2
Tr- 4 isolate	86.67	23.0	5.9	2501.9	86.67	27.2	7.8	3272.8
Tr- 5 isolate	93.33	23.2	6.0	2728.3	93.33	28.2	7.9	3372.3
Tr- 6 isolate	100	25.7	7.2	3296.7	93.33	30.7	8.3	3639.9
Tr- 7 isolate	100	26.5	7.3	3383.3	100	31.2	8.6	3973.3
Tr- 8 isolate	86.67	22.9	6.1	2516.3	86.67	28.7	8.1	3192.3
Pseudomonas fluorescens	93.33	24.1	6.2	2821.7	86.67	29.7	8.2	3284.8
Carbendazim +Thiram (1:1)	86.67	23.6	6.1	2577.0	80	29.4	8.1	3253.0
Inoculated soil +no treatment	66.67	19.4	4.4	1589.0	60	23.9	5.8	1782.0
Healthy soil + no treatment	80	21.0	5.9	2149.3	73.33	25.8	5.9	2324.6
SE m(±)	0.901	0.606	0.257	0.754	1.458	0.637	0.302	58.929
CD 5%	2.645	1.778	0.754	152.03	4.28	1.872	0.887	173.02

Data presented in the table are average of three replications

(ii) Effect of soil inoculation on percent germination and vigour index

From the data presented in Table 2, it was concluded that on soil inoculation with Tr- 7 highest plant vigour index of 3363.3 with 26.4cm shoot length and 7.3cm root length was recorded, followed by 3270, 2796.8 and 2796.8 with Tr- 6, Tr- 2and Tr- 8 respectively which is statistically significant as compared to 1589 in pathogen treated control and 2149.3 in untreated control in variety JG 14. Similarly, in variety JG 24 on soil inoculation with Tr- 7 highest plant vigour index of 3936.7 with 31cm shoot length and 8.3cm root length was observed with Tr- 7 followed by 3596.3, 3583.3 and 3493.7 with Tr- 6, Tr- 5 and Tr- 1 respectively as compared to 1782 in pathogen treated control and 2324.6 in untreated control. Whereas, on soil inoculation of *Pseudomonas fluorescens*, 2821 and 2986.7 plant vigour index were recorded in variety JG 14 and JG 24 respectively. The present findings are in agreement with the results reported on growth promotion with soil treatment of *Trichoderma* spp. had also been also reported by Alto *et al.* (1999) [1] and Cuevas *et al.* (2006) [6]. The variability among the isolate may be due to ability of particular *Trichoderma* spp. to produce growth hormones such as Auxins and Gibberellins which contributes to enhance the plant growth (Countreras-Cornejo *et al*., 2009 [4] and Martinez- Medina *et al*., 2011) [14]

Table 2: Effect of soil inoculation on vigour index of chickpea in Cvs JG 14 and JG 24

Verities	JG 14				JG 24			
Treatment	Germ. (%)	Shoot length (cm)	Root length(cm)	Vigour Index	Germ. (%)	Shoot length (cm)	Root Length (cm)	Vigour Index
Tr- 1 isolate	93.33	23.5	6.1	2760.1	86.67	29.1	8.1	3224.1
Tr- 2 isolate	93.33	23.6	6.3	2796.8	93.33	29.8	8.2	3540.3
Tr- 3 isolate	93.33	22.1	6.0	2625.7	93.33	27.1	8.0	3272.8
Tr- 4 isolate	93.33	22.8	5.7	2656.8	93.33	27.0	7.8	3251.0
Tr- 5 isolate	86.67	23.0	6.0	2510.5	100	28.0	7.8	3583.3
Tr- 6 isolate	100	25.6	7.1	3270.0	93.33	30.5	8.1	3596.3
Tr- 7 isolate	100	26.4	7.3	3363.3	100	31.0	8.3	3936.7
Tr- 8 isolate	80.00	22.8	6.1	2312.3	93.33	29.5	7.9	3493.7
Pseudomonas fluorescens	93.33	24.6	6.1	2821	80.00	29.2	8.2	2986.7
Carbendazim+ Thiram (1:1)	80.00	23.4	6.1	2357.3	80.00	29.2	8.0	2981.3
Inoculated soil +no treatment	66.67	19.4	4.4	1589.0	60.00	23.9	5.8	1782.0
Healthy soil + no treatment	80	21.0	5.9	2149.3	73.33	25.8	5.9	2324.6
SE m(±)	1.207	0.597	0.235	58.324	1.458	0.677	0.273	66.331
CD 5%	3.544	1.754	0.689	171.24	4.28	1.986	0.802	194.75

Data presented in the table are average of three replications

(iii) Effect of seed treatment on percent germination and vigour index mass

Significant enhancement in fresh weight, dry weight and vigour index of chickpea plant observed with the seed treatment of bio-agents as compared to control. On seed inoculation it was observed that, with inoculation of Tr- 7 maximum plant vigour index of 16.40 with 1.433g fresh weight and 0.164g dry weight was recorded, followed by 16.13, 15.77 and 14.62 with Tr- 6, Tr- 2 and Tr-1 respectively as compared to 8.24 in pathogen treated control in variety JG 14 and values were differing significantly. Similarly, in variety JG 24 with inoculation of Tr- 7 maximum plant vigour index of18.70 with 1.757g fresh weight and 0.187g dry weight was observed followed by 17.39, 16.99 and 16.86 with Tr- 6, Tr- 2 and Tr-1 respectively as compared to 9.50 in pathogen treated control. Whereas, on inoculation of *Pseudomonas fluorescens* plant vigour index of 14.65 and 14.48 observed in JG 14 and JG 24 respectively with seed treatment.

Table 3: Effect of seed treatment on vigour index mass of chickpea in Cvs JG 14 and JG 24

Verities	JG 14				JG 24			
Treatment	Germ. (%)	Fresh weight (gm)	Dry weight (gm)	Vigour Index mass	Germ. (%)	Fresh weight (gm)	Dry weight (gm)	Vigour Index mass
Tr- 1 isolate	93.33	1.390	0.1567	14.62	93.33	1.670	0.181	16.86
Tr- 2 isolate	100	1.380	0.1577	15.77	93.33	1.713	0.182	16.99
Tr- 3 isolate	93.33	1.353	0.1553	14.50	93.33	1.687	0.177	15.31
Tr- 4 isolate	86.67	1.357	0.1543	13.38	86.67	1.627	0.177	16.49
Tr- 5 isolate	93.33	1.317	0.1553	14.50	93.33	1.660	0.178	16.58
Tr- 6 isolate	100	1.410	0.1613	16.13	93.33	1.733	0.186	17.39
Tr- 7 isolate	100	1.433	0.1640	16.40	100	1.757	0.187	18.70
Tr- 8 isolate	86.67	1.337	0.1500	13.00	86.67	1.647	0.180	15.60
Pseudomonas fluorescens	93.33	1.400	0.1570	14.65	86.67	1.710	0.181	14.48
Carbendazim +Thiram (1:1)	86.67	1.390	0.1557	12.45	80	1.703	0.180	14.40
Inoculated soil +no treatment	66.67	1.157	0.1237	8.24	60	1.403	0.158	9.50
Healthy soil + no treatment	80	1.230	0.1383	10.14	73.33	1.513	0.167	12.22
SE m(±)	0.901	0.025	0.002	0.203	1.425	0.034	0.002	0.158
CD 5%	2.645	0.073	0.007	0.596	4.184	0.099	0.006	0.464

Data presented in the table are average of three replications

(iv) Effect of soil inoculation percent germination and on vigour index mass

As per the data indicated in Table 4, it was revealed that on soil inoculation of different isolates of *Trichoderma* spp. In variety JG 14, with inoculation of Tr- 7 maximum plant vigour index of 16.47 with 1.443g fresh weight and 0.1647g dry weight was recorded, followed by 16.23, 14.87 and 14.75 with Tr- 6, Tr- 2 and Tr- 1 respectively as compared to 8.24 in pathogen treated control and 10.14 in untreated control. Similarly, also in variety JG 24 highest vigour index mass of 18.81 with 1.777g fresh weight and 0.188g was observed with Tr- 7 followed by 17.87 with Tr- 5 isolate and 17.45 with Tr- 6 as compared to 9.50 in pathogen treated control and 12.22 in untreated control. Whereas, on inoculation of *Pseudomonas fluorescens* plant vigour index of 14.96 and 14.59 observed in JG 14 and JG 24 respectively with soil inoculation.

Table 4: Effect of soil inoculation on vigour index of chickpea in Cvs JG 14 and JG 24

Verities	JG 14				JG 24			
Treatment	Germ. (%)	Fresh weight (gm)	Dry weight (gm)	Vigour Index mass	Germ. (%)	Fresh weight (gm)	Dry weight (gm)	Vigour Index mass
Tr- 1 isolate	93.33	1.390	0.1580	14.75	86.67	1.683	0.184	15.95
Tr- 2 isolate	93.33	1.393	0.1593	14.87	93.33	1.723	0.182	16.99
Tr- 3 isolate	93.33	1.383	0.1567	14.62	93.33	1.690	0.177	16.52
Tr- 4 isolate	93.33	1.377	0.1557	14.53	93.33	1.630	0.178	16.58
Tr- 5 isolate	86.67	1.380	0.1557	13.49	100.00	1.687	0.179	17.87
Tr- 6 isolate	100	1.420	0.1623	16.23	93.33	1.747	0.187	17.45
Tr- 7 isolate	100	1.443	0.1647	16.47	100.00	1.777	0.188	18.81
Tr- 8 isolate	80.00	1.357	0.1520	12.16	93.33	1.657	0.182	16.99
Pseudomonas fluorescens	93.33	1.397	0.1603	14.96	80.00	1.717	0.182	14.59
Carbendazim +Thiram (1:1)	80.00	1.393	0.1593	12.75	80.00	1.710	0.181	14.48
Inoculated soil +no treatment	66.67	1.157	0.1237	8.24	60.00	1.403	0.158	9.50
Healthy soil + no treatment	80	1.230	0.1383	10.14	73.33	1.513	0.167	12.22
SE m(±)	1.207	0.025	0.002	0.201	1.458	0.032	0.002	0.181
CD 5%	3.544	0.074	0.007	0.590	4.28	0.095	0.006	0.531

Data presented in the Table are average of three replications

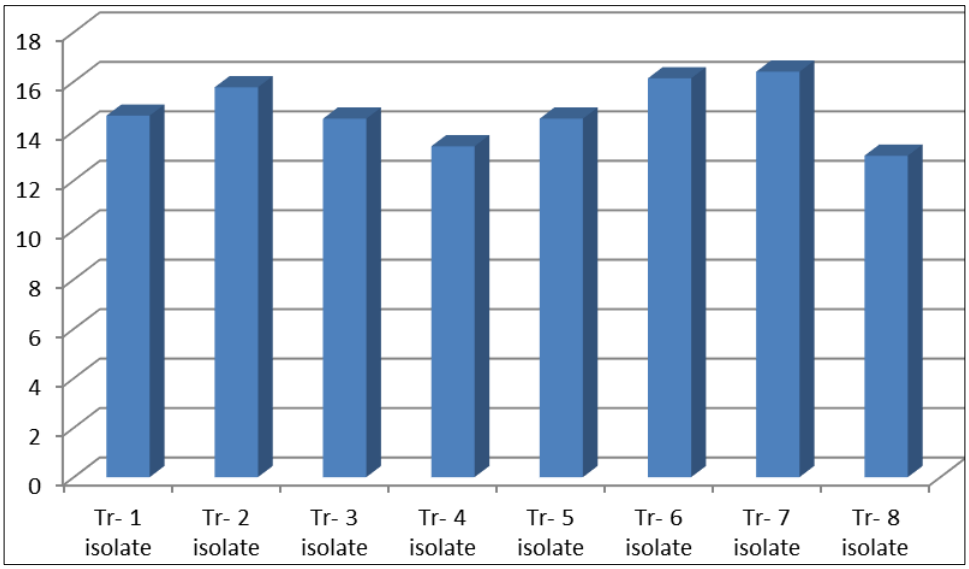

Fig 1: Efficacy of different isolates of *Trichoderma* spp. as seed treatment on vigour index of chickpea in Cv JG 14

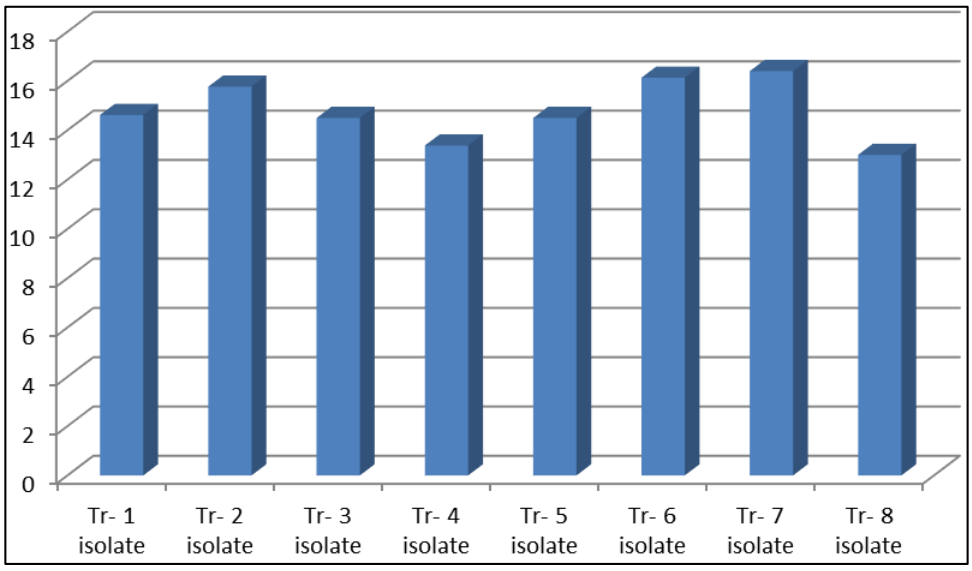

Fig 2: Efficacy of different isolates of *Trichoderma* spp. as seed treatment on vigour index of chickpea in Cv JG 24

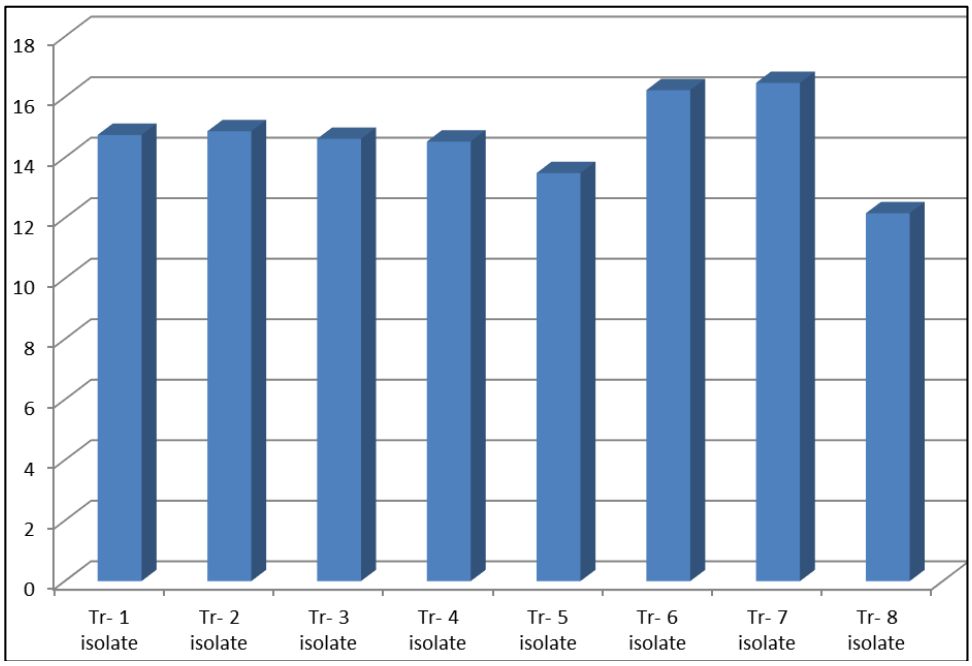

Fig 3: Efficacy of different isolates of *Trichoderma* spp. as soil inoculation on vigour index mass of chickpea in Cv JG 14

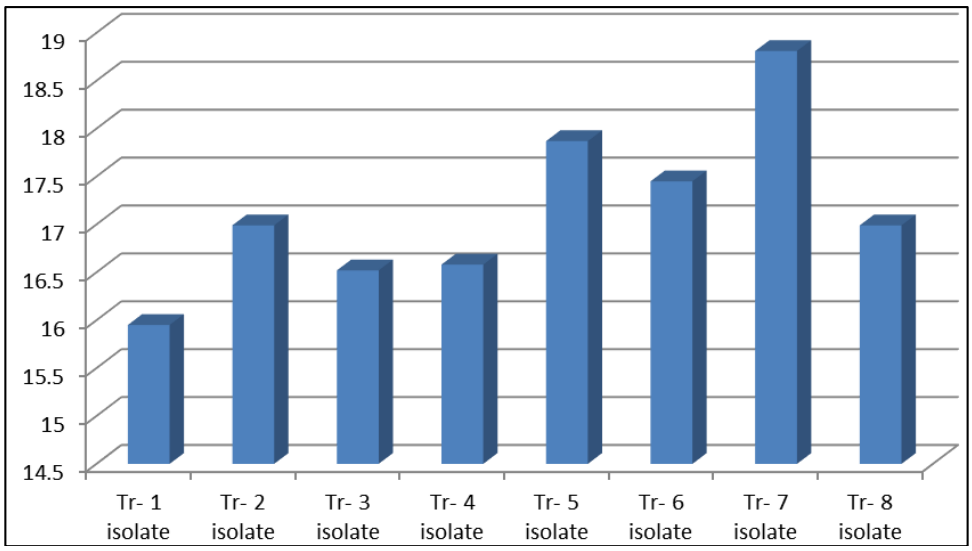

Fig 4: Efficacy of different isolates of *Trichoderma* spp. as soil inoculation on vigour index mass of chickpea in Cv JG 24

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