Biological Forum – An International Journal **13(4): 571-576(2021)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Investigation of Liquid Formulation of *Trichoderma asperellum* against Fusarium Wilt of Chickpea

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(Received 02 September 2021, Accepted 03 November, 2021)
(Published by Research Trend, Website: www.researchtrend.net)

ABSTRACT: An investigation was undertaken to test the effect of liquid formulation of *Trichoderma asperellum* against Fusarium wilt of chickpea. Oil based formulation delivers organism in a physiologically dormant state and does not encourage the growth of contaminants during storage. Paraffin oil, soybean oil and groundnut oil were used as carrier material for oil based liquid formulation of *Trichoderma asperellum*. The population density of *T. asperellum* found in paraffin oil was 28.67 × 10^8 CFU/ml at 30 days whereas at 180 days it was 18.00 × 10^8 CFU/ml. Paraffin oil was found significantly superior over soybean oil and groundnut oil. The effect on percent growth inhibition of *Fusarium oxysporum* f. sp. *ciceri* was 74.44 percent. Efficacy of liquid formulation on growth parameter of chickpea revealed that paraffin oil based formulation of *T. asperellum* with combined seed treatment and soil application effectively increased the germination percentage (92.50%, 91.67%), shoot length (38cm, 32.16cm), root length (16.91cm, 14.75cm) and vigour index (5079.17, 4300.23) in variety Chafa 815 and JG 62 respectively. The application of *T. asperellum* showed percent wilt reduction of 68.12% and 34.37% over the absolute control in variety Chafa 815 and JG 62 respectively.

Keywords: *Trichoderma asperellum*, Liquid Formulations, Paraffin Oil, Fusarium Wilt, Chickpea.

INTRODUCTION

Trichoderma is a filamentous fungi which has attracted attention because of its multi prong action against various plant pathogens. The *Trichoderma* species are useful avirulent plant symbionts that act as biocontrol agents against phyto-pathogenic fungi via mechanisms of competition, competition for key nutrients, myco-parasitism, antibiotic (toxin) production, cell wall degrading enzyme production, induced resistance, induction of defense responses in plant, parasitism and promoting plant growth (Howell, 2003). The majority of *Trichoderma* species is antagonist of phyto-pathogenic fungi and had been broadly used as the most important biocontrol agent. *Trichoderma* spp. are widely studied and marketed as bio-pesticides, bio-fertilizers and soil amendments due to their ability to protect plants, enhance vegetative growth and to reduce pathogen population under numerous agricultural conditions. The commercial success of these fungal antagonists totally depends on cost effective formulations attributed to higher shelf life at the time of application with good coverage and retention after application in the field. Oil serves as the perfect medium to supply the inoculants in viable condition. Microorganism can be suspended in oil at high concentration in various degree of dehydration and remain viable. Liquid inoculants could be produced with minimum labour, space and energy and also the quantity of inoculums required is less as compared to solid based formulations. It is easier for farmers to handle. Liquid formulation could be considered as one potential strategy for improving the shelf life of bio-fertilizer. Unlike solid based bio-fertilizers, liquid formulations allow the manufacturer to include sufficient amount of nutrients, cell protectant and inducers responsible for spore formation to ensure prolonged shelf life. Liquid formulation has several advantages including high cell count, zero contamination, longer shelf life, greater protection against environmental stresses and increased field efficacy (Hegde 2002; Vendan and Thangaraju, 2006). Kolombet *et al.*, (2008) reported the extended shelf life of *Trichoderma asperellum* in liquid formulation.

Chickpea production is severely curtailed by Fusarium wilt caused by *Fusarium oxysporum* f. sp. *ciceri* in most chickpea growing areas of the world. Chickpea wilt account for 10 to 100% yield losses annually in India depending on varietal susceptibility agro climatic condition (Chand and Khirbat, 2009). It causes complete loss in grain yield if disease occur in the

vegetative and reproductive stages of the crop (Navas-Cortes 2000). Fusarium wilt is seed and soil born and it can persist in soil for long years and also grow in postharvest if grain is not dried properly. *Fusarium oxysporum* f. sp. *ciceri* is a facultative saphrophytic and it can survive as mycelium and chlamydospore in seed, soil and also on infected crop residues buried in the soil for upto five to six years in the absence of a host (Haware and Nene, 1982). Pathogen is both seed and soil born, hence drenching with fungicide is very expensive and impractical. Cultural methods including crop rotation are ineffective. Cultivation of resistant varieties is the best option but availability is limited owing to the high level of genetic variability in the pathogen. Therefore integrated disease management strategies are the only solution to maintain plant health and bioagent like *Trichoderma* as seed treatment and soil application found to be effective and eco-friendly against Fusarium wilt of chickpea.

MATERIALS AND METHODS

(i) Pure culture. Pure culture of *Trichoderma asperellum* was collected from Plant Pathology Section, College of Agriculture, Nagpur. The pure culture was mass multiplied on PD broth for further studies. *Fusarium* was isolated from the infected root part of chickpea plants. The pure culture of *Fusarium* was maintained on PDA slant.

(ii) Liquid formulation. Oil based liquid formulation of *Trichoderma asperellum* was prepared with additives such as glycerol, dispersant, suspender and surfactant. Mass multiplied *T. asperellum* was transferred into mixing tank to harvest the spore and mycelium. *T. asperellum* filtrate was poured into presterilized plastic bottles. Three oils viz., paraffin oil, soybean oil and groundnut oil were incorporated into in each plastic bottles as per the given in treatments from T1 to T6. Whereas, T7 was talc base departmental culture and T8 was liquid formulation market product. The bottles were packed with the help of caps and kept for storage for 180 days at (27±1) °C. CFU count was under taken at various interval by serial dilution followed by pour plate method.

(iii) Pot culture studies. Pot culture experiment was conducted in two sets with two varieties Chafa 815 and JG 62 with four replications to evaluate the performance of paraffin oil based liquid formulation *T. asperellum* against Fusarium wilt disease of chickpea. Seeds were collected from Pulses Research Unit, Dr. PDKV, Akola, (Maharashtra) India. Pot culture study was conducted to find out germination percent, mortality percent, wilt incidence and other parameters of chickpea.

(iv) Preparation of sick soil for pots. The field soil was sterilized by using 1% formalin solution. Surface sterilized plastic pots (2 kg capacity) were filled with sterilized soil. In pots, 15 cm upper layer of soil were mixed with inoculums of test pathogen F*usarium oxysporum* f. sp. *ciceri* which was multiplied on sand sorghum medium. 15 days old culture of *Fusarium* was used @ 10 g for 1 kg of soil prior to sowing before eight days.

(v) Liquid formulation treatment details. Each liquid formulation contained glycerol (10 ml) + dispersant (1 ml) + suspender (3 ml) + surfactant (3 ml) + *Trichoderma* filtrate (23 ml).

Tr. No.	Treatment details
T_1	Paraffin oil (60 ml)
T_2	Soybean oil (60 ml)
T_3	Groundnut oil (60 ml)
T_4	Paraffin oil (30 ml)+ Soybean oil (30 ml)
T_5	Paraffin oil (30 ml) + Groundnut oil (30 ml)
T_6	Soybean oil (30 ml) + Groundnut oil (30 ml)
T_7	Talc based culture (Departmental culture)
T_8	Liquid culture (Market product)
T_9	Control

Treatment details of pot culture experiment. Paraffin oil based liquid formulation of *Trichoderma asperellum* was applied in pot culture experiment according to treatment details as mentioned below:

Tr. No.	Treatment details	Concentration
T_1	Seed treatment	5 ml/kg of seed
T_2	Soil application	10 ml/kg of soil
T_3	Seed treatment + Soil application	5 ml +10 ml/kg
T_4	Control without *Fusarium*	Soil application @ 10 ml/kg
T_5	Control with *Fusarium*	—
T_6	Absolute control	—

According to treatment details, *T. asperellum* was applied in T_1 seed treatment @ 5 ml/kg seed, T_2 soil application @ 10 ml/kg soil, T_3 combined seed treatment and soil application @ 5 ml + 10 ml/kg. In T_4 control without *Fusarium*, soil application of *T. asperellum* @ 10 ml/kg soil was done but soil was not inoculated with *Fusarium*. In T_5 control with *Fusarium*, soil was inoculated with *Fusarium* but *T. asperellum* was not applied. In T_6 absolute control, neither soil inoculated with *Fusarium* nor *Trichoderma* was applied.

RESULTS AND DISCUSSION

(i) CFU count of different liquid formulations *of Trichoderma asperellum* (× 10^8 CFU/ ml) at various interval. It revealed from the data (Table 1) that there were significant differences in population density of *Trichoderma asperellum* at all the interval. The initial population of *Trichoderma asperellum* on 30 DAI found maximum i.e. 28.67 × 10^8 CFU/ml in T_1 (Paraffin oil). Paraffin oil was significantly superior over all treatments followed by T_7 (Talc based culture) was 26.33 × 10^8 CFU/ml. The observations recorded on 30 DAI in the treatments T_2 (12.33 × 10^8 CFU/ml), T_3 (11.00 × 10^8 CFU/ml) and T_9 (24.33 × 10^8 CFU/ml) was also significantly superior. At 180 DAI maximum population density of *T. asperellum* was observed in paraffin oil (18.00 × 10^8 CFU/ml) followed by talc based culture (16.33 × 10^8 CFU/ ml).

Similarly Rai and Tewari (2016); Mbarga *et al*., (2014); Chandra, (2011); Khan *et al*., (2011); Al-Taweil *et al*., (2010); Nadare *et al*., (2018); Mujtaba and Kulkarni,

(2017) revealed that the colony forming unit of *Trichoderma* were maximum in paraffin oil which was followed by soybean oil. The result correlates with the results of Reddy *et al*., (2017) who calculated *T. harzianum* in the form of CFU on 56th day of observation in paraffin oil (20×10^7) and in soybean oil (2.1×10^7) gave the best result of spore viability. Jeyarajan *et al*., (2006) worked on talc based formulations showing the results up to 4 month. Sathiyaseelan *et al.,* (2009) reported that application of paraffin oil increases the shelf life of *Trichoderma* which was used as a bio fungicide comparing to liquid formulation of *Trichoderma* was more effective to control phytopathogen.

(ii) Effect on percent growth inhibition of *Fusarium oxysporum* f. sp. *ciceri.* From data (Table 2) it revealed that *T. asperellum* was found significantly superior to checking the growth of *Fusarium oxysporum* f. sp. *ciceri.* Paraffin oil (T_1) showed 23.00 mm mean colony diameter against the control (90 mm) with percent inhibition of 74.44 at 8th DAI. It was followed by the treatment soybean oil (T_2), groundnut oil (T_3) and paraffin oil + soybean oil (T_4) with mean mycelial growth of the organism 26.66 mm in T_2, 28.66 mm in T_3 and 29.33 mm in T_4 with percent inhibition of 70.37 in T_2, 68.15 in T_3 and 67.41 in T_4 respectively against *Fusarium oxysporum* f. sp. *ciceri.*

Rajput *et al*., (2010); Srivastava *et al.,* (2012); Perveen and Bokhari, (2012); Sreeshma and Jose (2016); Adhikary *et al.,* (2017); Babychan and Simon (2017); Cherkupally *et al.,* (2017); Kumar and Mane, (2017); Patole *et al.,* (2017); Patra and Biswas, (2017); Nadare *et al*., (2018); Taral *et al*., (2018); Thaware *et al*., (2018) showed that the antagonistic activity of *Trichoderma* species against *Fusarium oxysporum* was excellent.

(iii) Effect of liquid formulation of *Trichoderma asperellum* on vigour index. From data (Table 3) it observed that, maximum increase in shoot length, root length, germination percent and vigour index (VI) was 38.00 cm, 16.91 cm, 92.50%, and 5079.17 respectively in Chafa 815. While in JG 62, maximum increase in shoot length, root length, germination percent and vigour index (VI) recorded was 32.16 cm, 14.17 cm, 91.67% and 4300.23 respectively. Patil *et al*., (2015); Rai and Tewari (2016) studied that seed treatment with *Trichoderma* had improved germination (%) and increased vigour index significantly. Murthy *et al*., (2013) studied that *Trichoderma* with pathogen treated seeds increased root length, shoot length and vigour index. Meher *et al.,* (2018) studied that *Trichoderma* spp. as seed treatment and soil inoculation improves the root length, shoot length and vigour index

(iv) Effect of liquid formulation of *Trichoderma asperellum* on percent wilt incidence. It showed from data (Table 4) that minimum percent wilt incidence recorded by seed treatment + soil application (T_3) followed by seed treatment (T_1) followed by soil application (T_2) was 21.66%, 24.16% and 25.00% with percent wilt reduction of 68.12%, 57.98% and 56.52% respectively in Chafa 815. In JG 62 minimum percent wilt incidence recorded by seed treatment + soil application (T3) followed by seed treatment (T1) followed by soil application (T2) was 52.50%, 54.16% and 55.83% with percent wilt reduction of 34.37%, 32.30% and 30.20% respectively. All the treatment with *Trichoderma asperellum* shows significant effect on per cent wilt reduction over control.

Similarly, Kaur and Mukhopadhyay, (2008) studied soil application with different doses of *T. harzianum* gave 53.50-85.70% disease control in the glasshouse. Prasad *et al.,* (2012) recorded that *T. viride* seed treatment @ 5 g/kg + *T. viride* soil application @ 2.5 kg/ha in FYM with wilt incidence of 15.17%. Dubey *et al*., (2013) studied that combined soil application and seed treatment with *T. harzianum* was effective in reducing the wilt incidence. Rehman *et al*., (2013) studied that seed treatment with *T. viride* and *T. harzianum* reduce the wilt incidence significantly as compared to control. Bhagat and Pan, (2011) studied that seed and soil application of *Trichoderma* isolates significantly decreased wilt disease incidence. Ahamad *et al.,* (2020) observed that chickpea seeds treated with *Trichoderma viride* (soil application) recorded 21.50% wilt incidence followed by *Trichoderma viride* (seed application) 35.25%. While *Trichoderma viride* (soil application) gave 78.50% (highest) wilt disease control, followed by *Trichoderma viride* (seed application) 64.75% and least in check.

Table 1: CFU count of different liquid formulations of *Trichoderma asperellum* at various interval.

Tr. No.	Treatment	DAI (x 10^8 CFU/ ml)					
		30	60	90	120	150	180
T_1	Paraffin oil	28.67	24.00	22.67	21.33	20.67	18.00
T_2	Soybean oil	12.33	11.33	9.67	8.33	6.67	5.67
T_3	Groundnut oil	11.00	9.67	8.33	7.67	5.33	2.33
T_4	Paraffin oil + Soybean oil	9.33	8.00	7.33	6.33	5.00	1.67
T_5	Paraffin oil + Groundnut oil	8.33	7.33	7.00	6.00	4.33	1.33
T_6	Soybean oil + Groundnut oil	7.00	6.33	6.00	5.33	3.67	1.00
T_7	Talc based culture	26.33	24.33	20.33	19.67	18.33	16.33
T_8	Liquid culture	9.67	6.67	6.67	4.33	2.33	1.67
T_9	Control	24.33	21.67	18.33	10.67	9.33	2.67
	SE ± (m)	0.54	0.46	0.37	0.29	0.52	0.34
	CD (P=0.05)	1.62	1.39	1.11	0.86	1.56	1.03

Table 2: Effect of liquid formulation of *Trichoderma asperellum* on percent growth inhibition at 8th DAI.

Tr. No.	Treatment	Mycelial growth (mm)	% Growth inhibition over control
T_1	Paraffin oil	23.00	74.44
T_2	Soybean oil	26.66	70.37
T_3	Groundnut oil	28.66	68.15
T_4	Paraffin oil + Soybean oil	29.33	67.41
T_5	Paraffin oil + Groundnut oil	31.33	65.18
T_6	Soybean oil + Groundnut oil	34.00	62.22
T_7	Talc based culture	35.00	61.11
T_8	Liquid culture	40.00	55.55
T_9	Control	30.66	65.93
	Control	90.00	100
	SE ± (m)	0.42	
	CD (P=0.05)	1.26	

Table 3: Effect of liquid formulation of *Trichoderma asperellum* on vigour index.

Tr. No.	Treatment	Chafa 815				JG 62			
		Shoot length (cm)	Root length (cm)	Germination (%)	Vigour index at 90 DAS	Shoot length (cm)	Root length (cm)	Germination (%)	Vigour index at 90 DAS
T_1	Seed treatment	36.25	15.83	86.66	4513.25	31.75	14.00	88.33	4041.09
T_2	Soil application	35.50	15.25	87.50	4440.62	31.50	13.92	86.66	3936.09
T_3	Seed treatment + soil application	38.00	16.91	92.50	5079.17	32.16	14.75	91.67	4300.23
T_4	Control without Fusarium	37.00	16.25	89.17	4748.30	31.66	14.08	90.00	4116.60
T_5	Control with Fusarium	28.00	11.50	80.00	3160.00	27.17	11.25	80.83	3105.48
T_6	Absolute control	31.92	12.17	85.00	3747.65	28.08	11.92	84.16	3366.40
	SE ± (m)	0.37	0.23	1.06		0.41	0.16	1.31	
	CD (P=0.05)	1.12	0.71	3.21		1.23	0.49	3.96	

Table 4: Effect of liquid formulation of *Trichoderma asperellum* on percent wilt incidence at various interval.

Tr. No.	Treatment	Chaffa 815		JG 62	
		Average wilt incidence (%)	Percent wilt reduction over absolute control at 90 DAS	Average wilt incidence (%)	Percent wilt reduction over absolute control at 90 DAS
T_1	Seed treatment	17.29	57.98	33.54	32.30
T_2	Soil application	18.96	56.52	35.62	30.21
T_3	Seed treatment + soil application	15.41	68.12	29.36	34.37
T_4	Control without *Fusarium*	17.08	60.86	32.50	35.42
T_5	Control with *Fusarium*	58.12	-	67.71	-
T_6	Absolute control	41.87	-	59.58	-
	SE ± (m)	2.68		1.84	
	CD (P=0.05)	8.10		5.57	

CONCLUSION

Thus it can be concluded that CFU count of *Trichoderma asperellum* was maximum in treatment containing paraffin oil. The incorporation of liquid formulation of *T. asperellum* can reduce wilt incidence and also enhanced plant growth in both chickpea varieties.

Acknowledgment. I express deepest sense of gratitude to my advisor Dr. Damayanti D. Guldekar and Dr. S. R. Potdukhe. I am thankful to Professor and Head, Plant Pathology Section, College of Agriculture, Nagpur for providing facilities to conduct the research work.

Conflict of Interest. None.

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How to cite this article: Patil¸ S.S.; Guldekar, D.D.; Potdukhe, S.R. and Khobragade, H.M. (2021). Investigation of Liquid Formulation of *Trichoderma asperellum* against Fusarium Wilt of Chickpea. *Biological Forum – An International Journal, 13*(4): 571-576.