Research Article **AJAR (2016), Vol. 1, Issue 05**

American Journal of Agricultural Research
(ISSN:2475-2002)

Studies on interactions among bioagents colonized vermicompost, rhizospheric earthworms and stalk rot disease of sorghum caused by Erwinia chrysanthemi

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ABSTRACT

Bioagents colonized vermicompost can be a crucial alternative natural biological control of stalk rot diseases of sorghum because of the hazardous effects of agrochemicals on non-targeted organisms and soil health. To study the interaction among bioagents colonized vermicompost with stalk rot disease of sorghum caused by Erwinia chrysanthemi, and earthworms of rhizospheric soil, pre-plant soil application of bioagents colonized vermicompost @ 2.5 kg/4 m2 and chemicals viz. Blitox-50, Bleaching powder, Streptocyclin and Tetracyclin @ 2.5% were done under field conditions. In analysis of earthworm's population dynamics, maximum numbers of young earthworms per plant rhizosphere were observed with vermicompost colonized isolate Th-2 followed by vermicompost alone and vermicompost colonized isolate Psf-24. However, minimum number of young earthworms per plant rhizosphere was obtained with Bleaching powder. Maximum seed germination was recorded with vermicompost colonized isolate Th-2 followed by Th-R, Th-14, and Psf-12. Maximum number of earthworm's heap of cast per plant rhizosphere was obtained in plants treated with vermicompost colonized isolate Th-2 followed by Psf-19, Psf-12 and Psf-18. Significant increase in biomass yield of sorghum plants were observed among all the treatments. However, maximum biomass yield was observed with vermicompost colonized isolate Th-2 followed by Psf-24. Maximum reduction of disease severity was recorded with vermicompost colonized isolate Th-2 followed by Th-14, Th-R and Psf-3. Present investigation suggests the effect of bioagents colonized on the dramatic increase in earthworm's population (young and adult), earthworm's body length, sorghum plant biomass yield and reduction in stalk rot disease severity. Our experiments have shown that bioagents colonized vermicompost have considerable potential not only improving plant growth, suppression of stalk rot disease severity significantly but also increasing soil earthworms when used as pre-plant soil amendment.

Keywords:

Erwinia chrysanthemi, rhizospheric earthworms, sorghum, vermicompost, Trichoderma, Pseudomonas

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eSciencePublisher
eSciPub LLC, Houston, TX USA.
Website: http://escipub.com/

1. Introduction

The soft rot erwiniae are members of the Enterobacteriaceae, along with other plant pathogens such as *Erwinia herbicola, Erwinia trachiphila*, *Erwinia amylovora* and human pathogens such as *Escherichia coli*, *Shigella*, *Klebsiella*, *Proteus* and *Yersinia* (Pérombelon and Kelman, 1980). Stalk rot of sorghum is caused by a soil-borne, peritrichous, gram-negative, non-spore-forming, facultative anaerobe, straight rod (0.8-3.2×0.5-0.8 µm), and pectolytic entereobacterium *Erwinia chrysanthemi* Burkholder, McFadden, and Dimock (syn: *Dickeya dadantii*). The soft rot, *E. chrysanthemi* attacks the parenchymatous tissues (which comprised primarily of living cells without secondary wall thickening) of wide range of plants species (Bradbury, 1986; Clark and Moyer, 1988). The bacteria are distributed worldwide, with *E. chrysanthemi,* which has a higher optimum temperature for growth, being found primarily in the tropics or in temperate region (Pérombelon and Kelman, 1980). Saxena et al. (1991) reported this bacterium causing stalk and top rot of sorghum under natural conditions during 1987-88 crop season in sorghum field at Pantnagar (India) and disease was widespread and affected 60-80% of plants in different sorghum genotypes. Recently, the increased stalk rot disease severity ranging from 7.50-46.85% in Tarai region of Uttarakhand (India) have been shown (Kharayat and Singh, 2013). For management of soil-borne bacterial diseases chemical and non-chemical methods are used. The nonjudicious use of chemical pesticides to cure or prevent plant diseases has caused pest resistance, environmental pollution and detrimental effects in human health. Biological control of plant diseases has gained dramatic momentum in recent years primarily due to hazardous effects of agrochemicals on human beings, livestock, environment and drastic effects on non-targeted organisms etc. To mitigate this drastic problem, scientists have focused on an alternative solution such as biological control, using plant growth promoting rhizobacteria, fluorescent *Pseudomonas* strains and *Trichoderma* spp. It offers an environmentally friendly approach to the management of plant disease and can be integrated into an effective integrated disease management system. Several researchers have successfully employed antagonistic fungi, bacteria, *Streptomyces* and yeasts to control plant bacterial diseases (Ozaktan et al., 1999, Yesim et al., 2003; Lee et al., 2004, Hajhamed et al., 2007). Excessive use of chemical fertilizers and pesticide resulted in pollution of soil, water and air. There have been adverse effects on the health of human beings and cattle due to the residues of these agrochemicals in food products (Kumar and Bohra, 2006). Harmful effects of chemical fertilizers and pesticides have shifted the interests of researchers towards organic amendments like vermicompost which can increase the production of crops and prevent them from harmful pests without polluting the environment. Vermicompost is a finely-divided mature peat-like material which is produced by a non-thermophilic process involving interactions between earthworms and microorganisms leading to bioxidation and stabilization of organic material (Aira et al., 2000). It has been established that earthworms are one of the most useful and active agents in introducing suitable chemical, physical, and microbiologic changes in the soil (Hidalgo et al., 2006). Various reports suggested the presence of humic substances in vermicompost. Soil amendments which can manipulate physicochemical and microbiological environment of soil can be helpful for the suppression of soil borne pathogens (Sahni et al., 2008). Patriquin et al. (1995) reported that organically grown plants being more resistant to pathogens and pests. Either no or very little information is available on the effect of bioagents colonized vermicompost on earthworms, and the interaction of bioagents colonized vermicompost-earthworm-stalk rot disease of sorghum in an agro-ecosystem has not been studied so far. Therefore, the present study was aimed to evaluate the effects of interaction among bioagents colonized vermicompost, rhizospheric earthworm's population and stalk rot disease of sorghum.

2. Materials and methods

2.1 Source of bioagents

The isolates of bioagents *Trichoderma harzianum* and *Pseudomonas fluorescens* used during investigation were procured from Biological control laboratory, G.B. Pant University of Agriculture and Technology, Pantnagar.

2.2. Multiplication of T. harzianum and P. fluorescens isolates

Five isolates of *T. harzianum* (Th-2, 14, 19, 39 and Th-R) were multiplied on barnyard millet (*Echinocloa frumentacea,* vernacular: Jhangora) grains. Grains were soaked in water for 12 h and filled in 500 ml Erlenmeyer flask (@ 100 g/flask). These flasks were autoclaved at 121.1°C, 15 lbs psi for 20 min. After cooling to room temperature, the flasks were inoculated with 4-5 mycelial discs (7 mm) cut from freshly growing (2 days old) culture of *T. harzianum* isolates and incubated at 28 °C for 15 days. Fully colonized Jhangora grains were air dried in open shade and ground with the help of Willy Mill to get fine powder. This powder was passed through 50 to 80 mesh sieves to obtain a fine pure powder.

Five isolates of *P. fluorescens* (Psf-3, 7, 12, 18 and 24) were multiplied on King's B broth medium. Each isolate was inoculated in 250 ml flask containing 100 ml KB broth and incubated for 48 h at 28±1 °C. These multiplied isolates were used for colonization on vermicompost after mixing in talcum powder.

2.3. Colonization of vermicompost with T. harzianum and P. fluorescens isolates

Vermicompost of cattle's manure was prepared by using manure worm, *Eisenia foetida* and African Night crawlers *Eudrilus eugeniae*, obtained from Livestock Research Centre, Pantnagar, was used for colonization of *T. harzianum* and *P. fluorescens* isolates under shade conditions at room temperature. Polythene bags of 5 kg capacity were used for colonization of bioagents in which 3 kg of freshly prepared, unsterilized vermicompost was filled and was mixed with 5 gm (1×10^9 cfu/g) of talcum powder based formulation of each *T. harzianum* and *P. fluorescens* isolates. The polythene bags were left to colonize and kept slightly moist regularly with sterile water for 15 days. For control treatment, only vermicompost was filled in the bags.

2.4. Pre-plant soil application with bioagents colonized vermicompost

Pre-plant soil application (mixing) was done with bioagents colonized vermicompost @ 2.5 kg/4 m^2 at 10 cm depth in furrows just before seed sowing under field conditions. Three replications were maintained for each treatment.

2.5. Pre-plant soil drenching with chemicals

Pre-plant soil drenching with chemicals were also done using four chemicals viz. Blitox-50, Bleaching powder, Streptocyclin and Tetracyclin. Suspensions of these chemicals were applied @ 2.5% and drenched up to 10 cm depth in furrows just prior to seed sowing. Three replications were maintained for each treatment.

2.6. Studies on interaction between bioagents colonized vermicompost and earthworms of rhizospheric soil of sorghum

2.6.1. Population of earthworms in rhizospheric soil of sorghum

For counting numbers of earthworms a total 15 random samples (five from each of 3 replication of a treatment) from sorghum rhizospheric soil was dugout from 10 cm × 15 cm area by Trowel. All earthworms were collected in paper begs and kept in same rhizospheric soil at room temperature in laboratory upto observation. Earthworms were separated from rhizospheric soil manually and categorized into two major age groups i.e. young and adult. Counting of young and adult earthworms was done manually.

2.6.2. Measurement of earthworm's body length

Measurements of body length of young and adult earthworms were done manually by 30 cm ruler scale.

2.6.2. Counting of heaps of earthworm's casting

Numbers of heaps of castings made by earthworms were counted only from soil surface of 15 randomly selected sorghum plant's rhizosphere in 10 cm diameter area from each treatment. Only well structured heap of casting from the soil surface of sorghum's rhizosphere were taken into consideration for counting (Fig. 1).

2.7. Artificial inoculation of bacterium on sorghum plants

2.7.1 Leaf-whorl inoculation method

Artificial inoculation of bacterium *E. chrysanthemi*

Fig. 1. Heaps of earthworm's casts on soil surface of sorghum plant. Red circles showing structure of heaps of casts taken into considaration for countings.

Fig. 4. Soil as source of inoculum. Infection of *Erwinia chrysanthemi* through secondary roots of sorghum

was done to create high disease pressure however occurrence of stalk rot disease of sorghum is natural under field conditions at Pantnagar. Leaf-whorl inoculation method was adapted from Hartman and Kelman (1973) which they used in corn for inoculation of *Erwinia* spp. without causing injury. All the plants were artificially inoculated by spraying the bacterial suspension [0.7 % Tween-40 (v/v) + 2 × 10^8 cell/ml] in leaf whorls (2 ml/whorl) with the help of atomizer without causing any injury to 21 days old plants of susceptible sweet sorghum variety SPSSV 6. Plants sprayed with sterilized water served as control.

2.8. Observations

The experiment was conducted during *Kharif* season of the year 2012 at Live Stock Research Centre of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India). The observations on seed germination (%) were taken after 15 days of seed sowing however on stem diameter (cm), plant height (cm), and biomass yield (quintal/hectare) were taken after 65 days of sowing. The observations on number and body length (cm) of young and adult earthworms and number heap of castings made by earthworms were taken 45 days after sowing. Observation on disease severity (%) was taken after 45 and 65 days of sowing. Rating scale for severity of the disease was taken 0 to 5, which was modified from described by Muhammad (1983) used for evaluation of corn germplasm against *Erwinia* stalk rot disease as follows:

0 = No symptoms,

1 = Initial small necrotic areas/partial rotting at the base of the whorl/stalk,

2 = 25-49% dark brown, water-soaked, soft or slimy at the base of the whorl, disintegration of pith tissues at a single internode, premature of wilting uppermost leaves,

3 = 50-74%, decay spreading rapidly crossing 2-3 internodes in collapsed plant,

4 = 75-100% of tissue rotted with foul smell at the base of whorl/extensive necrosis/soft rotting with visible external symptoms,

5 = lodging accompanied by extensive necrosis/ rotting of leaf/stalk tissue usually having a very strong foul smell.

Calculations of (a) seed germination (%), (b) disease severity (%) and (c) biomass yield (quintal/hectare) were done using following formulae described by Kharayat, 2009.

a. $$\text{Seed germination (\%)} = \frac{\text{Total number of seed germinated}}{\text{Total number of seed sown}} \times 100$$

b. Percent disease severity (S) =

$$\text{Percent disease severity (S)} = \frac{\text{Sum of all numerical rating}}{\text{Total number of samples} \times \text{maximum rating grade}} \times 100$$

c. Biomass yield = $$\frac{\text{Weight of biomass (Quintal)}}{10{,}000\ \text{m}^2\ \text{area}}$$

2.9. Data analysis

Data were analyzed statistically for analysis of variance (ANOVA) in one-way by STPR software developed by computer centre of G.B. Pant University of Agriculture and Technology, Pantnagar and layout of experiment was done using Randomized Block Design (RBD). Fisher's least significant difference (LSD) test at 5 % probability level ($P < 0.05$) was used to detect the difference between mean of treatments.

3. Results and discussion

3.1 Effect of bioagents colonized vermicompost, vermicompost and chemicals on the population of sorghum rhizospheric earthworms

In the analysis of earthworm's population dynamics, it is evident from the data (Table 1 and Fig. 2 A, B, C & D) vermicompost colonized *T. harzianum* isolate Th-2 significantly increases number of young earthworms sorghum plant rhizospheric soil. Significant difference in number of young earthworms was observed with treatment like vermicompost colonized *Trichoderma harzianum*, *P. fluorescens* isolates and chemicals. No young earthworms were observed in three treatment viz. Psf-18 colonized vermicompost, Bliotox-50 and control. Similarly no significant difference in number of adult earthworms was observed among the treatments. However, significant in increase in number of adult earthworms in sorghum rhizospheric soil was observed with Th-2 colonized vermicompost. Significant increase in total number (young plus adult) of earthworms was also observed with colonized vermicompost Th-2. Minimum number of total earthworms per plant rhizosphere/ hectare was estimated with Bleaching Powder. It is evident from the data (Table 1) that when preplant soil drenching with chemicals viz. Blitox-50,

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Table 1 Effect of pre-plant soil application of bioagent colonized vermicompost and chemicals on population, average body length and number of heaps of casting of earthworms in rhizospheric soil of sorghum plants 45 days after sowing

Treatment	Number of earthworms in sorghum rhizospheric soil*			Total number of earthworms (young + adult) per hectare (10,000 m^2)*	Average body length (cm) of young	Average body length (cm) of adult earthworms*	Number of heaps of earthworms casting per plant rhizosphere (10 cm × 15 cm)*
	Number of young earthworms	Number of adult earthworms	Total number of earthworms (young + adult)				
Th-2	4.60^{a}	2.80^{a}	7.40	49.33×10^{4}	3.50^{hi}	15.58^{a}	17.80^{a}
Th-14	0.60^{dcf}	2.66^{a}	3.26	21.73×10^{4}	4.21^{fg}	11.44^{d}	9.75^{cd}
Th-19	0.40^{efg}	2.20^{ab}	3.30	21.99×10^{4}	4.80^{e}	12.78^{c}	11.55^{b}
Th-39	1.10^{bc}	1.70^{ab}	2.80	18.66×10^{4}	6.05^{b}	10.56^{e}	6.45^{g}
Th-R	0.90^{bcd}	1.90^{ab}	2.80	18.66×10^{4}	3.52^{hi}	14.35^{b}	5.56^{h}
Psf -3	0.26fg	2.20^{ab}	2.46	16.39×10^{4}	5.91^{bcd}	13.89^{b}	4.67^{i}
Psf-7	0.30^{fg}	1.80^{ab}	2.10	13.99×10^{4}	2.85^{i}	11.90^{d}	8.85^{e}
Psf-12	0.80^{cde}	1.80^{ab}	2.60	17.33×10^{4}	6.81^{a}	14.25^{b}	10.54^{c}
Psf-18	0.00^{h}	2.40^{ab}	2.40	15.99×10^{4}	0.00^{l}	9.85^{f}	9.68^{d}
Psf-24	1.33^{b}	1.90^{ab}	3.23	21.53×10^{4}	4.53^{ef}	9.36^{f}	7.54^{f}
Blitox-50	0.00^{h}	2.00^{ab}	2.00	13.33×10^{4}	0.00^{g}	7.94^{g}	4.55^{ij}
Bleaching Powder	0.10^{g}	1.70^{ab}	1.80	11.99×10^{4}	5.54^{d}	8.01^{g}	3.75^{jk}
Streptocyclin	0.53^{defg}	1.70^{ab}	2.23	14.86×10^{4}	3.05^{ij}	8.05^{g}	3.25^{k}
Tetracyclin	0.20^{fg}	1.90^{ab}	2.11	14.06×10^{4}	2.25^{k}	7.83^{g}	3.65^{k}
Vermicompost	1.36^{b}	1.30^{ab}	2.66	17.33×10^{4}	3.80^{gh}	12.63^{c}	6.55^{g}
Control	0.00^{h}	1.70^{ab}	1.70	11.33×10^{4}	0.00^{l}	7.55^{g}	0.00^{l}

The values in column with different letters are significantly different at 5% level according to Fisher's least significant difference (LSD) test

*Mean value of 15 random samples taken from each of treatment

Bleaching powder, Streptocyclin and Tetracyclin applied @ 2.5% done it did not significantly affect the numbers of natural resident earthworms in sorghum rhizospheric soil, as numbers of adult earthworms observed in all treatment including are statistically at par.

The present study revealed a dramatic increase in resident earthworm's population in sorghum plant rhizospheric soil. This increase may be interpreted by hypothetically as (a) there may be synthesis of a chemical stimulant that may be result from the interactions between sorghum plant root exudates and microbial activity of bioagents (*T. harzianum* and *P. fluorescens*) present in vermicompost attracting the resident earthworms toward the vermicompost applied sorghum plant rhizosphere, (b) induction of reproduction caused by availability nutrient in substrate (vermicompost), (c) induction of hatching of cocoons, (d) may help in increasing survival rate by reducing mortality rate of juveniles (as in case of Th-2 colonized vermicompost, the number of young earthworms per sorghum plant rhizosphere was found maximum (4.60), (d) microbial activity of bioagents may produce some chemicals which may be to toxic to microbes that may pathogenic or harmful to earthworm's cocoons and juveniles. Earthworms can modify soil microbial community structure depending on the types of organic matter presents (Enami et al., 2001). Their interactions have major impact on plant health in an agroecosystem because earthworms may shift the soil microbial community from plant pathogen to antagonist (Jack, 2011). The numbers of earthworms or density has been correlated inversely proportional to suppression of soilborne diseases by Elmer (2009). Earthworms have been shown to affect the overall microbial activity and diversity in the soil environment both directly, through feeding and secreting casts, and indirectly through various modifications of soil organic matter (Brown, 1995; Binet et al., 1998; Brown, et al., 2000).

3.2 Effect of bioagents colonized vermicompost and chemicals on body length of sorghum rhizospheric earthworms

The studies of vermicompost colonized bioagents and chemicals on body length earthworm reveled that there is a significant increase in body length of young and adult earthworms (Table 1). Maximum increase in body length of young earthworms was observed with *Pseudomonas fluorescens* isolate Psf-12 followed by *Trichoderma* isolate Th-39 and Psf-3. However, maximum increase in body length of adult earthworms was observed in vermicompost colonized *T. harzianum* isolate Th-2 followed by Th-R and *Pseudomans* isolate Psf-12. Significant increase in body length can be correlated primarily with the presence of vermicompost and microbial activity of bioagents and secondarily by other microbes present in vermicompost as unsterilized vermicompost was used for colonization of bioagents. Chemical treatments significantly did not affected increases the body length of both young and adult earthworms.

3.3 Effect of bioagents colonized vermicompost, vermicompost and chemicals on heap of casting of sorghum rhizospheric earthworms

Number of earthworm's heap of casts produced by earthworms on surface of per plant rhizospheric soil (Table 1, Fig. 3 B & C) were observed mmaximum with vermicompost colonized isolate Th-2 followed by Psf-19, Psf-12 and Psf-18. Minimum number of earthworm's heap of casting per plant rhizosphere was obtained in plants treated with Streptocyclin.

Higher number of castings can be correlated with higher activity of earthworms in rhizospheric soil of plants. As Th-2 colonized vermicompost produced maximum number of earthworm's heaps of cast, maximum numbers of earthworms and micro- and macro-pores (Fig. 3 C) in heaps which creates a proper aeration to the roots zone of plants. This treatment also shown to produce maximum biomass yield which may be synergistic effect of soil engineering by earthworms and microbial activity of vermicompost colonized bioagents plus other vermicompost colonizing microbes. It has been shown that earthworms influence soil structure by forming macropore; which allow O_2 to enter the soil whereas micropores between the aggregates give good water-holding capacity (Willems et al., 1996). The casts have been reported having high nutrient values for the growth of the plants. Castings are also rich in calcium humate, a binding agent (Edwards and Arancon, 2004) that reduces desiccation of individual castings and favors the incubation and proliferation of beneficial organisms, such as *Trichoderma* spp. (Tiunov and Scheu, 2000),

Table 2 Effect of pre-plant soil application of bioagents colonized vermicompost and chemicals on seed germinations, stem diameter, plant height, disease severity and biomass yield under field conditions

Treatment	Seed germination (%)	Stem diameter (cm)	Plant height (cm)	Biomass yield (q/ha)	Disease severity (%)			Reduction in disease severity (%)
					Days after sowing		Mean	
					45	65		
Th-2	88.66^{a}	1.60^{a}	231.36^{ab}	357.50^{a}	23.70^{a}	32.70^{a}	28.20	32.37
Th-14	86.83^{abc}	1.70^{a}	235.50^{a}	330.00^{c}	24.50^{ab}	33.90^{ab}	29.20	29.97
Th-19	87.46^{ab}	1.43^{a}	224.86^{de}	310.00^{e}	25.90^{bcd}	37.60^{bcde}	31.75	23.86
Th-39	84.73^{bcde}	1.30^{a}	231.40^{ab}	330.00^{c}	26.40^{cd}	39.10^{defg}	32.75	21.46
Th-R	87.26^{abc}	1.50^{a}	230.50^{bc}	320.00^{d}	24.90^{abc}	34.10^{bc}	29.50	29.25
Psf-3	82.76^{d}	1.46^{a}	221.83^{ef}	300.00^{g}	25.40^{bcd}	36.10^{abcde}	30.75	26.25
Psf-7	83.50^{cdef}	1.33^{a}	225.46^{de}	277.50^{j}	25.90^{bcd}	36.30^{abcde}	31.00	25.65
Psf-12	85.76^{abcd}	1.33^{a}	222.76^{de}	285.00^{i}	26.90^{de}	38.90^{def}	32.90	21.10
Psf-18	80.90^{fg}	1.30^{a}	220.70^{f}	272.50^{k}	27.10^{def}	38.40^{cdef}	32.75	21.46
Psf-24	81.73^{ef}	1.50^{a}	226.80^{cd}	335.00^{b}	26.60^{cde}	35.40^{abcd}	31.00	25.65
Blitox-50	75.33^{h}	1.50^{a}	224.60^{de}	332.50^{bc}	28.26^{efg}	40.90^{fg}	34.58	17.07
Tetracyclin	70.70^{i}	1.23^{a}	218.46^{f}	305.01^{f}	28.80^{fgh}	40.40^{fg}	34.60	17.02
Bleaching Powder	71.50^{i}	1.40^{a}	221.40^{ef}	310.02^{e}	29.30^{gh}	42.20^{fg}	35.75	14.26
Streptocyclin	69.80^{i}	1.23^{a}	217.66^{f}	292.53^{h}	30.30^{h}	43.60^{g}	36.95	11.39
Vermicompost	77.63^{gh}	1.20^{a}	224.70^{de}	265.02^{l}	27.50^{def}	39.60^{def}	33.55	19.54
Control	64.86^{j}	1.20^{a}	215.56^{g}	222.51^{m}	33.80^{i}	49.60^{h}	41.70	00.00
The values in column with different letters are significantly different at 5% level according to Fisher's least significant difference (LSD) test								

* Mean value of 5 random samples taken from each of treatment

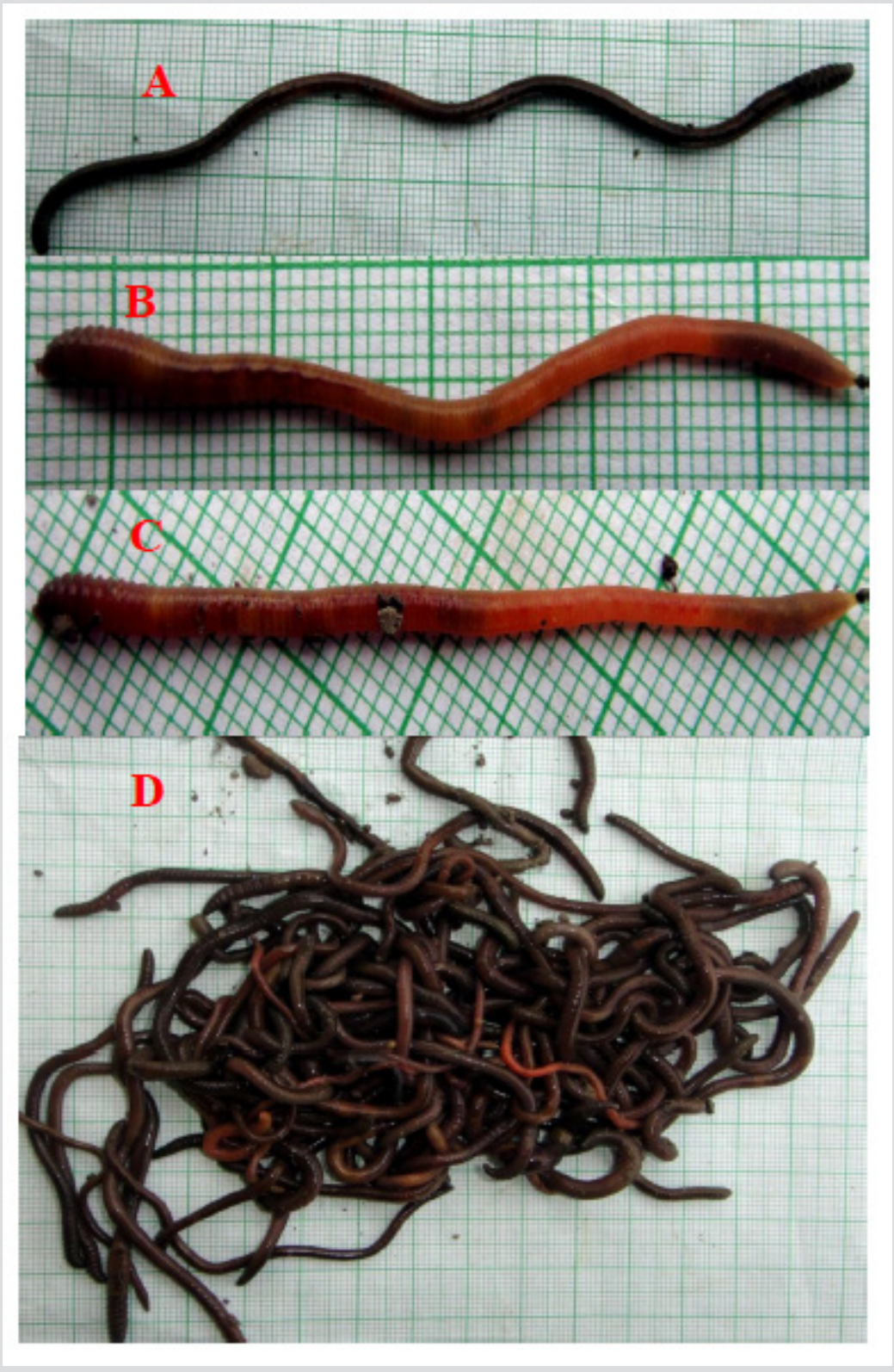

Fig.2. Population dynamics of earthworms. A. T. harzianum isolate Th-2 colonized vermicompost, B. Pseudomonas fluorescence isolate Psf-18, and C. Vermicompost alone, D. Young and adult earthworms in mass collected from rhizospheric soil of sorghum treated with Th-2 colonized vermicompost 45 days after sowing

Pseudomonas spp. (Schmidt et al., 1997), and mycorrhizal spores (Doube et al., 1995). Earthworms feed on soil and on a wide range of decaying organic substances, and excrete wastes in the form of casts. Variability number and pore space of casting has been observed with different substrate like sheep, horse and cattle manure and also correlated with plant growth. Substrates with greater pore space produced plants with greater dry weight and root development than substrates with less air space (Matta et al., 2008). Production of different type of structure of heap of cast depends on different species of earthworms. Chaudhuri et al. (2009) have been reported that three different forms of casts are produced by earthworm species of rubber plantation. These are tower-like casts (*Metaphire houlleti*, *Eutyphoeus gammiei*, *Eutyphoeus gigas*, *Eutyphoeus scutarius*, *Eutyphoeus assamensis*, *Eutyphoeus callosus*, *Eutyphoeus comillahnus*, *Eutyphoeus turaensis*, *Eutyphoeus* sp.), composite irregular casts (*Pontoscolex corethrurus*, *Drawida papillifer papillifer*) and large globoid mounds (*Kanchuria* sp.). Cast production by earthworms has been shown significant positive correlation with soil temperature, soil moisture and rainfall and week correlation with both pH and organic matter content of the soil. Total cast production is an indicator of burrowing and soil turnover, because 99.9% of ingested material is egested as casts (Chaudhuri et al., 2009). Earthworms are soil dwellers that have profound effects on soil ecosystem. Their feeding and burrowing activities help to incorporate soil particles with organic matter and redistribute them back into the soil via casts. The casts produced enhance microbial activities in soil that promote nutrient cycling (Teng et al., 2012). Earthworms can modify soil structure and nutrient availability, and hence alter conditions for plant growth through their burrowing and casting activities (Arnone and Zaller, 2014).

3.4 Effect of bioagents colonized vermicompost, vermicompost and chemicals on seed germination

Maximum seed germination was recorded with Th-2 followed by Th-R, Th-14, and Psf-12. It can be assumed from the present study, pre-plant soil mixing with bioagent colonized vermicompost can enhance seed germination by three possible ways; first, the bioagents itself forms a protective cover over the seed surface which directly shields the seed from the destructive soilborne pathogens, second, by the synergistic effect of vermicompost colonized bioagents plus other seed exudates colonizing microbial antagonist of *E. chrysanthemi* present in vermicompost, third, as vermicompost harbor the microbial community which can increase competition to soilborne pathogens for occupying space/substrate and nutrients. Pre-plant soil mixing with vermicompost alone not significantly increases seed germination however when soil mixing with vermicompost colonized bioagents were done, significantly these treatment increases seed germination percentage of sorghum e.g. vermicompost colonized *T. harzianum* isolate Th-2 (Table 2). However, *Pseudomonas fluorescens* isolates also observed superior in increasing percent sorghum seed germination over chemicals and control. Enhancement of sorghum seed germination has been shown when seed biopriming plus two foliar sprays with *Trichoderma harzianum* isolates (Th-32, Th-38 and Th-43) were done by Kharayat and Singh, 2012. Jack and Nelson (2008) have been shown that seed-colonizing microbes from vermicompost chemically modify cucumber seed exudates, thus interrupting the directional swimming of *Pythium* zoospores so that they fail to reach and infect their host.

3.5 Effect of bioagents colonized vermicompost, vermicompost and chemicals on stem diameter

No statistical significant differences in stem diameter were observed among all treatments. Maximum stem diameter was recorded with isolate Th-14 followed by Th-2. However minimum was with vermicompost. Previous studies has been also reported that there were no significant differences on stem diameter when seed-biopriming of sorghum and two foliar sprays with *T. harzianum* isolates (Th-32, Th-38, Th-39 and Th-43) and *Pseudomonas fluorescens* isolate Psf-28 were done (Kharayat and Singh, 2012; Meena et al., 2012).

3.6 Effect of bioagents colonized vermicompost, vermicompost and chemicals on plant height

It is evident from data (Table 2) vermicompost colonized bioagent and chemical increases plant height over control. Maximum plant height was recorded with Th-14 followed by Th-2, while min-

imum was recorded with Streptocyclin. However, there were no significant differences in plant height among the treatment. Only *T. harzianum* isolate Th-14 significantly increases plant height. Growth promotion and increase in height of sorghum plant with *T. harzianum* isolates have been already shown (Kharayat and Singh, 2012).

3.7 Effect of bioagents colonized vermicompost, vermicompost and chemicals on biomass yield

Significant increase in biomass yield of sorghum plants were observed among all the treatments (Table 2). Maximum biomass yield was observed with Th-2 followed by Psf-24. It is evident from results that pre-plant soil mixing with vermicompost colonized and pre-plant soil drenching with chemicals significantly increased biomass yield. Joshi et al. (2013) studied the effect of vermicompost on growth, yield and quality of wheat and compared it with application of chemical fertilizers. Previous studies have shown that vermicompost have considerable potential for improving growth and yield in terms of biomass of sorghum plant significantly when used as amendment to soil (Hameeda et al., 2007). Compost and vermicompost have been reported to enhance the growth of a wide range of plant species which can be expected because of the supply of nutrients (Edwards, 2004; Grigatti, 2007).

3.8 Effect of bioagents colonized vermicompost, vermicompost and chemicals on disease severity

Maximum reduction in disease severity was recorded with Th-2 followed by Th-14, Th-R and Psf-3 while Bleaching powder was found to be least effective in reducing disease severity. As *Erwinia chrysanthemi* is a soil bacterium and it causes infection from primary roots and secondary roots both (Fig. 4). This is evident from the data (Table 2) chemicals did not stimulate or affect the growth of earthworms in sorghum rhizosphere. The heap of casting was also found less in number in comparison to bioagent colonized vermicompost in rhizospheric soil. The chemicals were also found less effective in reduction of disease severity as compared to bioagent colonized vermicompost and vermicompost alone. The plausible reason for this could be unavailability of nutrients for earthworms and microbial activity in the plant soil drenched with chemicals. In addition to increasing soil organic matter, the highly diverse microflora of composts and vermicomposts can suppress plant diseases when used as soil amendments. Disease-suppressive composts and vermicomposts have been reported (Hoitink and Fahy 1986; Weltzien 1989; Hoitink et al., 1991; Litterick et al., 2004). As vermicompost is rich in microbial activity and contains antagonistic organisms to control plant pathogens, therefore it is an effective biocontrol agent. Singh and Chauhan (2009) applied vermicompost, FYM and chemical fertilizers alone as well as in combination to study their effect on growth and yield of French bean (*Phaseolus vulgaris*) under irrigated conditions. The growth and yield of garlic (*Allium sativum*) on application of vermicompost and farm yard manure was studied by Suthar (2009). Synergistic effect of vermicompost colonized bioagents and microbiota of vermicompost have been reported. There are evidences that microbiota of vermicompost helps in biocontrol when microbiota of vermicompost was killed by autoclaving, such vermicomposts were no longer effective to suppress the pathogen (Szczech and Smolinska 2001; Simsek-Ersahin et al., 2009). Suppression of various plant diseases through cattle manure vermicompost has been reported by various workers (Kannangara et al., 2000, Rodriguez et al., 2000; Szczech and Smolinska 2001; Asciutto et al., 2006; Edwards and Arancon, 2004; Zaller, 2006, Simsek-Ersahin et al., 2009). Vermicompost not only serves as source to supply nutrient to plant and microbial community but also as substrate for bioagents to applied at field level against soilborne plant pathogens. Chakravarty and Kalita (2011) have reported that vermicompost and farmyard manure used as substrate carrier in conjunction with carboxymethyl cellulose as adhesive in the formulations provided better nutrient sources and congenial microenvironment required for proper growth and subsequent longer shelf life of *P. fluorescens.* The effectiveness of agricultural residue derived vermicompost in providing protection against various plant diseases, especially against soil-borne plant pathogens has been studied extensively (Dominguez, 2004). In the previous studies effective control of soilborne plant pathogen infections was observed on application of vermicompost. Most of the research is focused on elucidating the mechanism of soilborne pathogen suppression and the potential types of interactions between micro-flora of

Fig. 3. Effect of soil applied bioagents colonized vermicompost on earthworms in rhizospheric soil of sorghum plants. A. Earthworm with rhizospheric soil adhered on sorghum plants, B. Earthworm casting on surface of the rhizospheric soil, and C. Structure of earthworm castings with macropore (red arrow) and micropore (blue arrow)

vermicompost and the pathogens (Dominguez, 2004).

4. Conclusion

This is the first finding demonstrating the bioagent colonized vermicompost on earthworm, sorghum plants, *Erwinia* stalk rot disease. The study suggests the effect of bioagents on the dramatic increase in earthworms' population (young and adult), earthworm's growth (length), plant biomass yield and reduction in percent disease severity. Our finding also suggests that bioagent colonized vermicompost may be used as an important alterative in soil health maintenance, allowing resident earthworm population to naturally increase over time and suppression of *Erwinia* stalk rot disease of sorghum. Bioagent colonized vermicompost can be an effective, economic, ecofriendly and logical methods to manage not only stalk rot of sorghum but also to manage other destructive soilborne diseases because it may have possible multisite of action against diseases viz. (1) harbor biocontrol agents (antibiosis, parasitism, competition, Induced Systemic Resistance and Systemic Acquire Resistance), (2) harbor antagonistic microbes and plant growth promoting rhizobacteria (PGPR), (3) nutrient for plant which can keep to plant in good health (4) soil engineering through soil engineer (earthworms), earthworms make macro and micro-pore which supply O_2 in different soil layers or root zones of plants which can inhibit growth of anaerobic bacterial plant pathogens however *Erwinia chrysanthemi* is facultative anaerobes, (5) can increase resident earthworm populations and earthworms activity rhizospheric soils. Thus, bioagent colonized vermicompost can be an integral component for the development of a more sustainable agriculture. Our study also suggests vermicompost colonized bioagent can be effective biologically based soilborne plant disease management practice and also points out directions for future research efforts.

Acknowledgment

The authors thank Dr. Karuna Vishunavat, Head, Department of Plant Pathology, G.B. Pant University of Agriculture & Technology, Pantnagar for providing necessary facilities during the course of investigation.

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