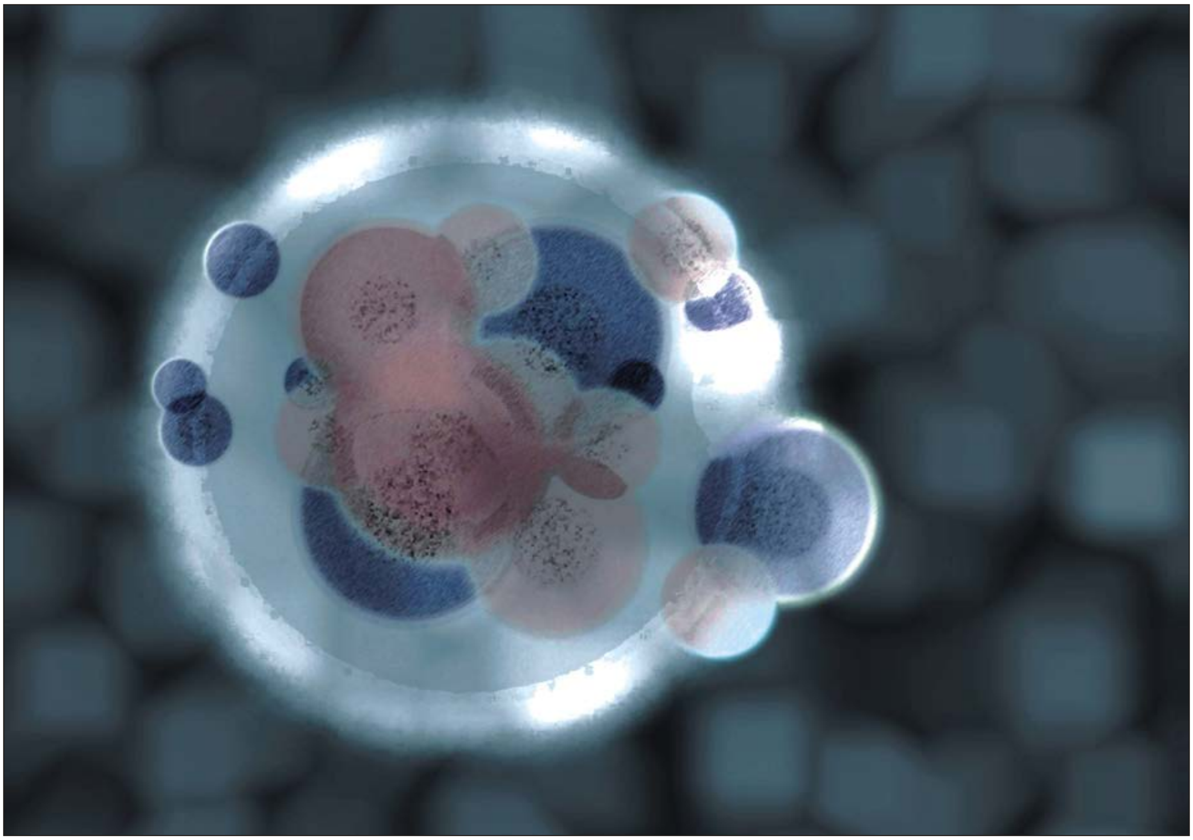

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Effect of Vermiwash from Different Sources (Bagasse, Neem, Paddy Straw, in Different Combinations) in Controlling Fungal Diseases and Growth of Tomato (*Lycopersicon esculentum*) Fruits in Guyana

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ABSTRACT

The demand for products and technologies based on plants to control plant pathogens has increased in recent years due to the concern about the use of hazardous pesticides. The present study was carried out during the year 2011-12 at University of Guyana, Guyana with the objective of using vermiwash produced from different plant sources against tomato fruit fungus and growth of tomatoes. Vermiwash was produced using bagasse, neem and paddy straw in different combinations (T1 to T9) and was used as foliar spray on tomato plants with respective treatments. Plant growth parameters and effect of vermiwash produced from different plant materials were used against tomato fruit fungus *Alternaria alternata* were monitored during the study. The findings from this experiment indicated that vermiwash with combination of neem+earthworm+cattle dung in T3, T6 and T7 were very effective depending on their concentration or percentage to get rid of pathogens and improve yield and growth parameters of tomato fruit. The treatment T1, T2, T6, T7, T8 and T9 at 10% dilution of vermiwash were effective against *A. alternata* affecting tomato fruits. At 5% dilution there was no inhibition of *A. alternata*, T7 had a maximum of 12 colonies and T1 on the other hand had a maximum of 5 colonies. At 10% dilution growth of *A. alternata* were killed for most treatments except T3, T4 which consisted of two colonies each and T5 a colony count of 6. At 15% dilution there was growth of *A. alternata* in all treatments except T8. Maximum growth was observed on petri plates containing T1 and T2 with a count of 8 colonies and a minimum growth of one colony present in T7. At 20%, growth of *A. alternata* was observed in all treatments. The maximum growth was seen in T1 with 27 colonies and minimum growth in T5 with 1 colony. This indicated that at a concentration of 10% vermiwash would more likely to inhibit growth or kill the *A. alternata*.

Key words: Earthworms, vermiwash, neem, vermitech, tomato, *Alternaria alternata*

INTRODUCTION

Modern agriculture produces plentiful food, at a reasonable price, all year round. They are expected to be harmless and nutritious particularly fruits and vegetables, not having any blemishes. Over the years farmers and growers have changed the way they produce food in order to meet the expectations of consumers, supermarkets and Governments (Ansari and Hanief, 2013). In achieving such goal, farmers make changes to their farming technique such as the use of pesticides.

Human activities produced colossal quantities of solid biodegradable waste that range from domestic to agricultural and industrial wastes. Our water ways are often clogged up with waste that poses a serious treat on our environment and can therefore lead to flooding, resulting in a lot of health issues (Hartenstein and Hartenstein, 1981). Vermicompost has high microorganisms content that enhances plant productivity and converts mineral nutrients to plant available forms. Foliar spray is produced from vermicomposting and can be used against plant diseases and as a fertilizer. Vermicompost contains 1.2-6.1% more nitrogen, 1.8-2.0% more phosphate and 0.5-0.75% more potassium compared to farm yard manure. It also contains hormones like auxins and cytokinins, enzymes, vitamins and useful microorganisms like bacteria, actinomycetes, protozoans, fungi etc. (Melgar *et al*., 2009).

Fruit fungus *A. alternata* is serious problem in tomatoes and effective control is needed. The use of vermiwash against such fungal disease could be an effective solution to grow tomatoes organically without the use of chemical pesticides. Therefore this experiment was carried out in order to have sustainable production of tomatoes.

MATERIALS AND METHODS

Present investigations were carried out during the year 2011-2012 at the University of Guyana, Georgetown focusing on recycling organic waste using vermiwash from vermitech unit from different combinations (Table 1) for exploring the effects on productivity of tomato plants and its ability to inhibit or kill microorganisms such as fungus causing fruit rot on tomatoes in Guyana.

The vermiwash units were set up using plastic buckets (in triplicate). A tap was fixed close to the bottom of the buckets to facilitate the collection of vermiwash. About 10 cm of broken pebbles were placed in the bottom of the buckets then 10 cm layer of coarse sand was put on the pebbles. Water was then allowed to flow through these layers to enable the settling of the basic filter unit. A layer of 10-15 cm of loam soil was placed on top of the filter bed. Fifty earthworms were introduced into the soil. Dried grass and cattle dung were placed on top of the soil. Water was sprinkled into the buckets thrice per week to keep the units moist. Vermiwash was collected after 60 days of decomposition.

Vermiwash as foliar spray and vermicompost as soil application were applied on tomato plants cultivated in plant pots (in triplicate) with treatments from T1 to T9 (Table 1). Application of vermiwash at the rate of 100 mL (10% dilution) was sprayed per week. Vermicompost from T1 to T9, respectively, at the rate of 100 g per pot was applied at the start of planting of seedlings followed by flowering stage and fruit setting stage. The plant growth parameters (shoot length, number of leaves, number of flowers and fruits, average weight of fruit and total biomass) were recorded during the course of the experiment. The experiment was completed in 6 weeks.

Vermiwash (T1 to T9) was tested against *A. alternate*, *Fusarium*, *Trichoderma* and *Cerosporium* using their cultures in petri dishes. The colony forming units were recorded to check the effectiveness of respective verimwash on the growth of different fungi.

Table 1: Experimental treatment for the production of the vermiwash

Symbol	Treatments
T1	Cattle dung only (control treatment No.1)
T2	Neem +Rice straw +Bagasse +cattle dung without earthworms (control treatment No. 2)
T3	Neem +cattle dung+earthworms
T4	Rice straw +cattle dung+earthworms
T5	Bagasse +cattle dung +earthworms
T6	Neem +Rice straw+cattle dung+earthworms
T7	Neem +Bagasse +cattle dung+earthworms
T8	Rice straw +Bagasse +cattle dung+earthworms
T9	Neem +Rice straw +Bagasse +cattle dung+earthworms

Physicochemical analyses of the vermicompost and vermiwash: Both vermiwash and vermicompost were analyzed according to the method of AOAC (2012):

- Total salts
- Organic carbon
- Nitrogen, carbon
- Nitrogen
- Phosphorus
- Potassium
- pH

Microbial analysis: Microbial analysis was done to check the Colony Forming Units (CFU) for the following species:

- Bacteria
- *Trichoderma*
- *Fusarium*
- *Rhizopus*
- *Aspergillus*

Statistical analysis: The data was subjected to statistical tool ANOVA to determine the significance of various treatments.

RESULTS AND DISCUSSION

The physico-chemical properties of vermicompost analysis (Table 2) was carried out on T3-T9 for total salts (ppm) resulting in T7 having a maximum value of 6845 ppm and T4 has a minimum value of 3513 ppm. T3-T9 was also tested for pH and the test indicated that T9 had a pH of 6.78 as such being close to neutral or slightly alkaline. This is as a result of the decomposition of organic substrates by microbial activity resulting in the production of CO_2 and other intermediate species of organic acid in vermicomposting (Ansari, 2008a, b; Sinha *et al*., 2010). The shift in pH to higher acidity was as a result of the mineralization of nitrogen and phosphorous into nitrite/nitrate and orthophosphate. Slightly acidic pH is a characteristic of good quality compost

Table 2: Physico-chemical analysis of vermicompost for the soil nutrients at different treatment

	Physico-chemical analysis of vermicompost						
Treatments	pH	Total salts (ppm)	Organic carbon (%)	Nitrogen (%)	C:N	Phosphorous (%)	Potassium (%)
T3	6.02	4025	23.21	1.80	12.89	0.003	0.098
T4	5.33	3513	23.53	1.00	23.53	0.002	0.072
T5	6.58	5120	22.90	1.72	13.31	0.003	0.120
T6	6.48	3775	23.85	2.53	9.43	0.002	0.089
T7	6.41	6845	32.12	2.06	15.59	0.003	0.110
T8	6.48	4620	27.67	1.43	19.35	0.003	0.110
T9	6.78	3820	25.76	1.66	15.52	0.003	0.100

and such reduction is advantageous in retaining nitrogen as this element lost as volatile ammonia at high pH values (Sinha *et al*., 2010). Wastes treated with *Eisenia fetida* showed reduction in organic carbon levels following the order T5, T3, T4, T6, T9, T8 and T7. The reduction of carbon in vermicomposting resulted from respiration and mineralization of organic matter produced by microorganisms and earthworms. Earthworms through their fragmenting action modify the substrate conditions which consequently increase the surface area for microbial action (Ansari and Sukhraj, 2010). Nitrogen (N) was calculated as percentage which indicated that T6 consisted a maximum value of 2.53% and T4 a minimum of 1.00%. Chauhan and Joshi (2010) reported a considerable rise in total nitrogen in vermicomposting. Earthworms play a crucial role in enhancing and improving the nitrogen content of waste by addition of mucus, nitrogenase casts and by facilitating microbial mediated nitrogen mineralization through decomposition of earthworm tissues (Ananthakrishnasamy *et al*., 2009). The C:N ratio was calculated for T3 to T9 and revealed that T4 had a maximum C: N of 23.53 and T6 a minimum C: N of 9.43. All treatments except T4 maintained the acceptable C:N limits of below 20, a C:N ratio below 20 is an acceptable maturity level while a ratio of 15 or lower is highly preferable for agronomic purpose (Zularisam *et al*., 2010). Phosphorus (P) level among the treatments indicated a progressive increase that all treatments within a close range the minimum value being that of T4 and T6 with a value of 0.002 and T3, T5, T7, T8 and T9 with a maximum value of 0.003. The rise of phosphorus was due to the action of earthworms, due to the presence of earthworms' phosphatases and phosphorus solubilizing microorganisms. Potassium (K) showed that T5 contained a maximum value of 0.12% and T4 a minimum value of 0.072. Larger amount of symbiotic micro flora present in the gut and cast of earthworms and water might increase the degradation of ingested organic matter and release of available metabolites. These metabolites enhance the enrichment of vermicompost with exchangeable potassium. Morais and Queda (2003) and Muthukumaravel *et al*. (2008) reported lower levels of potassium in vermicompost than the initial substrate.

Table 3 gives the details of vermiwash analysis. Based on the obtained results, maximum pH value was seen in T6, a pH of 7.73 slightly alkaline, T7 having a neutral pH of 7, control T1 having a pH of 6.22 which indicated that it is more acidic than neutral and T8 having a pH of 5.63. The decrease in pH from alkaline to slightly acidic in some treatments may be due to the decomposition of organic substrates by microbial activity which results in the production of organic acid and CO_2 (Ansari and Sukhraj, 2010). Maximum increase in EC was observed in T5 having a value of 7.71 and a minimum value was found in T2 to be 1.76 msec cm^{-1}. Treatments tested for organic carbon T1 had maximum value of 0.20% whereas T3, T4, T6 and T8 had minimum values of 0.02% among the samples tested. The organic carbon in vermiwash releases nutrients slowly and steadily into the soil and enables the plant to absorb the available nutrients (Ansari, 2008a, b; Tharmaraj *et al*., 2010; Tomati *et al*., 1988). Maximum nitrogen content was observed in T8 with a value of 0.67 and minimum value in T6 with a value of 0.24. Phosphorus concentration, T7 had a minimum value of 0.08 whereas T6 having a value of 0.37 and tests potassium revealed that T3 had maximum value of 25.49% and T2 minimum value of 5.45%. The vermiwash and vermicompost were found to improve the trace element content in the soil. The combination of these bio-fertilizers was found to be more effective in improving soil nutrient content (Ansari, 2008a).

The microorganisms present in T1-T9 (Table 4) included Bacteria, *Trichoderma*, *Fusarium*, *Rhizopus* and *Aspergillus*. The microbial count of each of these microorganisms was accounted for based on Colony Forming Unit (CFU). T5 accounted for 94 colonies of bacteria formed as the maximum colonies formed. T1, T2, T3, T4 and T6 there were no colony formation whereas T7, T8 and T9 accounted for 75, 10 and 2 colonies, respectively.

T1-T9 was tested against *A. alternata* at concentrations of 5, 10, 15 and 20% vermiwash (Table 5). Observation revealed that there were growth of *A. alternata*, *Fusarium*, *Trichoderma* and *Cerosporium* as counted in CFU. At 5% there was no inhibition of *A. alternata*. T7 had a maximum of 12 colonies and T1 on the other hand had a maximum of 5 colonies. At a concentration of 10% growth of *A. alternata* were killed for most treatments except T3, T4 which consisted of two colonies each and T5 a colony count of 6. At 15% there was growth of *A. alternata* in all treatments except T8. Maximum growth was observed on petri plates containing T1 and T2 with a count of 8 colonies and a minimum growth of one colony present in T7. At 20% growth of *A. alternata* was observe in all treatments. The maximum growth was seen in T1 with 27 colonies and minimum growth in T5 with 1 colony. Therefore, at a concentration of 10% vermiwash would be more likely to inhibit or kill the growth of *A. alternata*.

Table 3: Physico-chemical properties of Vermiwash

	Parameters					
Treatments	pH	Electrical conductivity (msec cm^{-1})	Organic carbon (%)	Nitrogen (%)	Phosphorous (%)	Potassium (%)
T1	6.22	1.87	0.20	0.290	0.240	6.460
T2	6.65	1.79	0.03	0.260	0.120	5.450
T3	7.20	7.64	0.02	0.300	0.095	25.49
T4	7.07	7.22	0.02	0.260	0.200	22.66
T5	7.30	7.71	0.03	0.396	0.091	23.92
T6	7.73	5.31	0.02	0.240	0.370	19.15
T7	7.00	5.32	0.04	0.470	0.080	20.82
T8	5.63	7.60	0.02	0.670	0.170	13.84
T9	7.22	6.48	0.03	0.560	0.096	21.53

Table 4: Microbial analysis of vermiwash from different sources

	Microbial analysis of vermiwash (CFU)					
Treatments	Bacteria	*Trichoderma*	*Fusarium*	*Rhizopus*	*Aspergillus*	Total
T1	0	84	7	0	0	91
T2	0	34	0	65	0	99
T3	0	38	0	67	0	105
T4	0	89	0	3	0	92
T5	94	13	0	8	0	115
T6	0	56	0	0	2	58
T7	75	5	0	0	4	84
T8	10	36	0	0	0	46
T9	2	82	0	0	6	90

Table 5: Effect of different concentration of vermiwash from different sources against tomato fruit fungus (*Alternaria alternata*)

	Concentration (%)															
	A. alternata				*Fusarium*				*Trichoderma*				*Cerosporium*			
Treatments	5	10	15	20	5	10	15	20	5	10	15	20	5	10	15	20
T1	5	0	8	27	0	0	6	0	15	23	2	0	0	0	0	0
T2	8	0	8	0	0	0	0	0	12	15	7	8	0	5	0	0
T3	9	2	3	3	0	0	0	0	0	0	33	25	0	0	0	0
T4	5	2	2	23	0	0	0	0	19	7	12	0	0	0	0	0
T5	10	6	3	1	0	0	0	2	15	12	27	20	0	0	0	0
T6	2	0	2	10	0	0	0	0	23	9	28	29	0	0	0	0
T7	12	0	1	15	0	0	2	0	25	17	10	26	0	0	0	0
T8	8	0	0	6	0	0	4	0	17	5	51	12	0	0	0	0
T9	10	0	2	10	0	0	0	0	0	15	5	18	0	0	0	0

Fusarium was found as a form of contamination among some of the treatments. At 5 and 10% vermiwash there was no growth of *Fusarium* in any of the treatments. At 15% growth of *Fusarium* was seen in T8 with maximum growth of 4 and T7 with minimum growth of 2 and finally at 20% growth of two colonies of *Fusarium* was seen in T5 only.

Trichoderma had a maximum of 25 which was observed in T7 and a minimum growth of 12 colonies in T2. Zero growth was observed in T3 and T9. At concentrations of 10% maximum growth was observed in T1 consisting of 23 colonies and minimum growth in T8 consisting of 5 colonies, however, no growth was seen in T3. At concentrations of 15% maximum growth was obtained in T3 having 33 colonies and minimum growth in T1 consisting of 2 colonies. And at 20% vermiwash maximum growth of 29 colonies was observed in T6 and minimum growth T2 having a total of 8 colonies; whereas T1 and T4 had no growth of *Trichoderma* at a concentration of 20%. Growth of *Cerosporium* was found in T2 only at a concentration of 10% as a result of contamination. In contrast all treatments were effective against *Cerosporium* at all concentration except T2 at 10%.

ANOVA test was used to determine the significance of the data and it revealed that between treatments $F_{cal} = 2.086$ and $F_c = 2.077$; hence, the values were significant between treatments. The removal of pathogens may be due to release of antibiotics by earthworms, responsible to kill pathogenic organisms, inhibit and render it virtually sterile (Hartenstein and Hartenstein, 1981). The worms also release coelomic fluids that have anti-bacterial properties which may destroy all pathogen in reactors as indicated by Ansari and Hanief (2013). On the contrary absolute removal of pathogens is difficult to achieve, some may survive even after vermistabilization and hence it is advisable to apply treatment twice to insure complete sterilization.

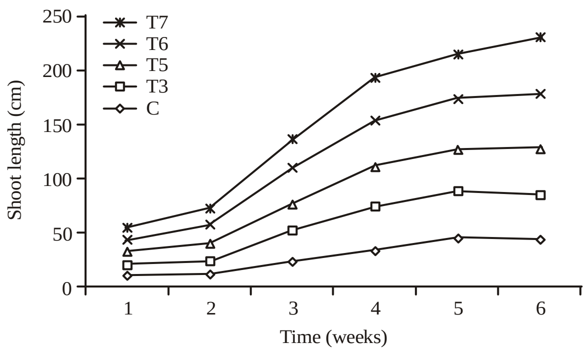

Fig. 1: Shoot length for the different treatments

Growth parameters of tomato plants and its effect against vermiwash from different sources (its effect on initial and final shoot and root length): Initially for shoot length as recorded in centimeters indicated that T7 had a maximum of 12.17 cm and T2 a minimum of 7.83 cm at the time of replanting (Table 6 and Fig. 1). The final shoot length taken at the time of harvest for maximum average shoot length T7 accounted for 52.33 cm and T3 with a minimum average of 41.33 cm. In shoot length T6 had the highest overall increase of 40.33% and T3 on the other hand with an increase of 30.83%. Plants treated with vermiwash and vermicompost increase plant height (Chauhan and Joshi, 2010). The vermiwash is a major contributor of micro-nutrients to soil. Vermicompost and vermiwash are also enriched in certain metabolites and vitamins that belong to the B group and provitamin D which also help to enhance plant growth (Tomati *et al.*, 1988). Earthworm cast and vermicompost influence the development of plant and promote root length (Edwards and Bohlen, 1996). Shoot lengths were recorded significant except T7 with a t_{crit} value < 4.3, $p \leq 0.05$).

Initially maximum number of leaves (Table 7) was found to be 49±7.00 in T2 and minimum average of leaves found in T9 equal 29.67±8.08. Final average number of leaves indicated that T1 yield a maximum of 203.33±110.75 and a minimum value of 91.00±12.17 as an average value of T9. Maximum percentage increase was calculated to be 79.01% among the control treatments and a minimum value of 56.84% for those of T5 (Table 7). Earthworm cast and vermicompost also increase the number of leaves (Tomati *et al.*, 1988) as was indicated by this study.

Fruits found on T7 plant had maximum average weight of 41.9 g and T3 a minimum weight of 14.2 g (Table 8). Presence of flowers, T2 and T8 had no flowers at the time of harvesting. T7 had a maximum average of 10.50±13.44 and T5 with a minimum value of 1.00±1.73 (Table 8). The number of fruits present revealed that T2 had a maximum value of 9.00±1.41 and T5 with minimum value of 3.33±2.31. That vermiwash of different vermicomposts have time and dose dependent significant effect on the growth, flowering period and productivity of crops (Nath and Singh, 2010).

T7 had a maximum wet weight of 103.58±28.70 g and T1 with a minimum wet weight of 71.63±6.52 g. Dry weight on the other hand indicated that T3 had a maximum decrease in fresh weight with a value of 20.22±0.41 g and T1 with minimum decrease of 27.32±14.10 g (Table 9). T1 having a percentage of 62.07 indicated that most of the moisture content among the three treatments decreased at a higher rate as

Table 6: Shoot length and its increase in terms of percentage

	Shoot length (cm)			
Treatments	Initial	Final	Increase	Increase (%)
C	10.17±1.61	43.67±1.53	33.50	76.71
T1	11.50±1.00	43.67±2.08	32.17	73.67
T2	7.83±1.26	42.33±1.15	31.50	81.50
T3	10.50±1.32	41.33±1.15	30.83	74.59
T4	9.33±1.89	45.33±3.51	36.00	79.42
T5	11.50±0.50	43.50±2.12	31.75	73.56
T6	10.33±0.29	50.67±5.03	40.33	79.61
T7	12.17±2.47	52.33±3.51	40.17	76.74
T8	9.50±0.50	45.33±0.58	35.83	79.04
T9	9.83±0.58	48.33±4.51	38.50	79.66

Table 7: Average number of leaves at the time of harvest

	Average number of leaf		
Treatments	Initial	Final	Increase (%)
C	42.67±7.37	203.33±110.75	79.01
T1	48.33±11.59	139.00±26.96	65.23
T2	49.00±7.00	123.00±12.58	60.16
T3	39.00±7.00	142.67±60.05	72.66
T4	43.67±10.21	195.67±33.61	77.68
T5	47.33±0.58	109.67±28.04	56.84
T6	42.67±2.31	133.33±14.43	68.00
T7	39.67±11.24	138.33±26.08	71.32
T8	47.67±6.11	120.00±9.540	60.28
T9	29.67±8.08	91.00±12.17	67.40

Table 8: Average number of flowers and fruits recorded at the time of harvesting along with average weight of fruit

Treatments	Flowers	Fruit	Fruit weight (mean)
C	0.00±0.00	5.50±4.95	15.9
T1	4.00±6.93	4.67±1.53	26.5
T2	0.00±0.00	9.00±1.41	20.5
T3	4.00±4.58	6.67±1.15	14.2
T4	6.33±3.79	5.00±3.00	21.4
T5	1.00±1.73	3.33±2.31	00.0
T6	3.67±3.21	5.00±1.00	22.8
T7	10.50±13.44	3.50±0.71	41.9
T8	0.00±0.00	7.67±1.15	27.3
T9	2.00±3.46	6.00±2.65	33.6

Table 9: Total biomass of plant

	Total biomass (g)		
Control	Fresh weight	Dry weight	Moisture (%)
C	76.79±37.74	20.50±6.65	69.82±11.47
T1	71.63±6.52	27.32±14.1	62.07±17.92
T2	83.95±33.98	21.42±5.13	73.03±5.59
T3	74.24±27.24	20.22±0.41	70.36±9.96
T4	90.74±12.07	23.22±5.67	74.41±5.53
T5	80.70±20.85	21.41±1.01	72.73±5.80
T6	85.35±8.90	26.19±0.29	69.09±3.28
T7	103.58±28.70	21.46±4.24	78.51±5.31
T8	91.78±20.09	22.78±3.75	74.85±3.77
T9	76.00±6.86	22.02±1.91	71.01±0.43

compared to T2-T9 and T7 with a percentage of 78.81% indicated that T7 among the three treatment had more moisture content as compared to the other treatments.

Overall growth parameters: The percentage increase of all the growth parameters for each treatment was calculated based on composite index and the treatment with the least value was assigned rank 1 and those with maximum value was assigned rank 8 among 11 treatments. T4 was rank 1 followed by T6 and T7 (Rank 2) whereas T5 was ranked least (Rank 8) followed by C and T1 (Rank 7). Hence, this indicated that in terms of growth parameters T4 showed the maximum increase in overall growth parameters. Excellent plant growth in vermicompost was possible due to plant growth promoters present in vermicompost (Suthar, 2009; Ansari and Jaikishun, 2010; Ansari and Ismail, 2001).

CONCLUSION

From the results obtained it was concluded that the vermiwash of different combination of animal, agro and kitchen waste have enhance growth and productivity of tomato plants. It also works well in inhibiting the growth of *A. alternata* at 5, 15 and 20% and works best for kill the growth of *A. alternata* at 10% for T1, T2, T6, T7, T8 and T9. However, for overall effect in improve plant productivity and inhibition of fungus T3, T6 and T7 were found to be considerably effective. As a result this form of organic farming was proven to be beneficial to plants and a healthy method of farming. In effect vermiwash is less expensive than chemical fertilizers, easily producible, eco-friendly and one of the best organic manure for foliar spray on different crops. Vermicompost on the other hand has similar effect on plant activity and it also improves soil nutrients.

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