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COMPARATIVE ANALYSIS ON THE FUNGISTATIC ACTIVITY OF SALINE SOILS AND VERMICOMPOST AMENDED SOILS

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ABSTRACT

In conventional agriculture, incidence of pests and plant disease is generally curtailed through the use of pesticides and fungicides. Fungistasis, a phenomenon of wide spread occurrence has drawn the attention of workers around the world in understanding the concept leading towards the biological control mechanism. Organic amendments are generally effective, pathogens may gain resistance after repeated applications, thus jeopardizing future production and forcing farmers to depend on increasingly intensive control programs. The fungistatic behavior of natural/ vermicompost unamended as well as the soils amended with vermicompost were studied, employing soil emanation, agar disc and direct assay methods to compare their efficacy against *Fusarium solani* and *Alternaria alternata*. Varying level of sensitivity towards soil fungistasis was exhibited by *Fusarium solani* and *Alternaria alternata* after 10 days of vermicompost amendment. Statistically, assay methods and the concentration of vermicompost amendments significantly influenced the phenomenon of soil fungistasis as the 'F' ratio values obtained for these variables were found to be greater than their respective table values. *Fusarium solani* showed highest percent inhibition in its conidia germination in direct assay method followed by agar disc and direct assay method. It also showed a dose specific increase in fungistatic activity, as the minimum (68%) and maximum (84%) inhibition in conidia germination was observed in vermicompost unamended control and 25% vermicompost amended soils, respectively. *Alternaria alternata* also showed maximum percent inhibition in direct assay method followed by agar disc and direct assay method. The minimum (51%) and maximum (78%) inhibition in conidia germination was observed in vermicompost unamended control and 25% soils amended with vermicompost respectively.

Keywords: Fungistasis, Vermicompost and Saline Soils

INTRODUCTION

The term 'Fungistasis' was coined by Dobbs and Hinson in 1953. It is an important approach to control soil borne plant pathogen. Nearly all natural soils are fungistatic and it has been observed that in nature, not only fungi but bacteria (Brown, 1973) are also sensitive to fungistasis. Ko and Ho (1984) have reviewed fungistasis with the concept of microbiostasis. Organic certification standards also prohibit the use of such fungicides, leading growers to seek alternate strategies of disease control. Similarly in less developed countries, where chemical inputs are often not used due to high cost and limited availability, pathogens must be managed by natural techniques or amendments.

The application of mature compost colonized by mesophilic bacteria and fungi can result in increased biotic diversity in the soil. Microbial biodiversity can improve the resilience and stability of an agro-ecosystem by mitigating over-colonization by pathogenic populations (Bamforth, 1999; Altieri, 1995). Atiyeh *et al.*, (2001) found that dehydrogenase activity (a common indicator of microbial respiration) in 100% vermicompost was almost a hundred fold greater than metro mix. Masciandaro *et al.*, (2000) reported decreasing dehydrogenase activity in the soils amended with worm-degraded aerobic and anaerobic sludge. Tarkalson *et al.*, (1998) observed significant increase in mycorrhizal population of bean (*Phaseolus vulgaris*) roots in coarse-silty, mixed, mesic Durixerollic Calciorthid top soil and sub soil amended with manure and composted manure.

High population of beneficial microorganisms in compost and vermicompost allows the biological control of pathogenic fungi. Bio-control, or "general suppression," is carried out by the activities and interactions

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of soil microorganisms that compete with pathogens for nutrients or produce antibiotic chemicals (Baker, 1968). Generally, pathogen spores in compost amended soils are more densely covered with beneficial fungal and bacterial propagules, thereby limiting their infectivity. These beneficial populations often parasitize the hyphae of pathogenic fungi.

Additionally, by consuming the amino acids, carbohydrates, and volatile ethanols and aldehydes exuded from roots and seed tissue and decomposing plant residue, beneficial microbes may reduce resources required by pathogenic fungal spores for germination (Stone, 2002).

Tuitert *et al.*, (1998) observed suppression of *Rhizoctonia solani* in cucumbers (*Cucumis sativus*) planted in a potting mixture containing 20% mature household waste compost. However, in mixtures containing one month old immature compost, pathogen growth was stimulated.

Szchech *et al.*, (1993) inoculated treatments of pure peat, pure cow manure vermicompost, and mixtures of peat with 10 and 20% vermicompost with *Phytophthora nicotianae*, *Fusarium oxysporum*, and *Plasmodiophora brassicae*.

Infection of tomato seedlings by *Phytophthora* was 75 to 300% less in vermicompost treatments than the control having only peat.

The vermicompost treatments also reduced and prevented club root disease (*Plasmodiophora*) development in cabbage (*Brassica oleracea capitata*).

Bulluck *et al.*, (2002) reported that southern blight (*Sclerotium rolfsii*) incidence was only 3% in field tomatoes amended with composted cotton gin trash as compared to 67% incidence in the plots amended with synthetic fertilizers.

Propagule densities of beneficial *Trichoderma spp.* and fluorescent *Pseudomonas spp.* were more than two-fold greater in plots amended with compost or swine manure than in the fields amended with inorganic fertilizer sources.

Consequently, *Fusarium spp.* population was significantly lower in the plots amended with compost.

Agrawal (1983) made a detailed investigation on the effect of oil cakes, fertilizers and chemicals on soil fungistatic activity employing three assay method and *Alternaria alternata*, *Curvularia lunata*, *Fusarium solani* and *polyschema spp* as the test organisms.

District Mathura, the chosen study site is having a vast area of land affected with salinity (nearly 5718 ha), as in major part of the area the water used for irrigation and other purposes is saline in nature.

It has been stated that organic materials together with inorganic amendments are found to be more cost effective, hasten the reclamation process and increase the yield (Dargan *et al.*, 1982). Therefore, their use should be encouraged.

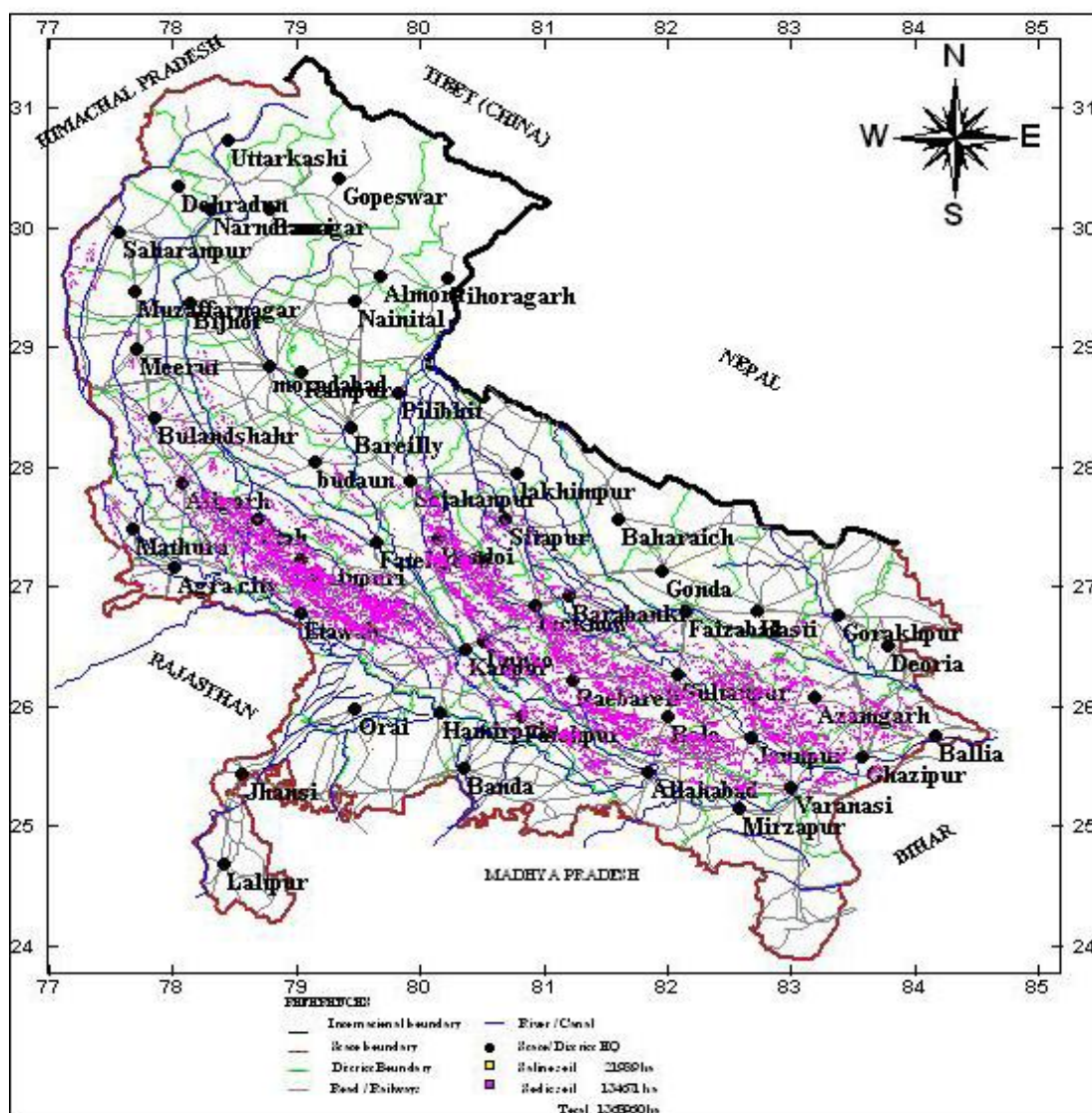
MATERIALS AND METHODS

The present study includes survey of selected sites, soil sampling, characterization of chosen sites, influence of organic amendments in maintaining fungistatic properties of salt affected/usal soils. The details of sites, geographical and climatic information of the location along with experimental set up are given below:

Survey of Selected Sites

Soil samples were collected from the six chosen sites of Raya village (Table - 1) situated in Mant Tehsil of Mathura district. The area is situated in the saline tract of Mathura district. Mathura district lies between 27° 14' and 27° 58' north latitude and 77° 17' and 78° 12' east longitude with an area of 3329.4 square kilometer. It is situated on Bombay-Delhi main Railway lines (WR, CR and NCR). It is linked with rest of the country with network of rails and roads. The district is bounded on North West by Gurgaon district of Haryana, north-east by Aligarh and for a short distance (13 km), in the east by Etah District.

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Salt Affected Soils in Uttar Pradesh State

Collection of Soil Samples

Soil samples were collected up to 0-6 cm depth from the surface with the help of sterilized iron borer, following the method of Johnson and Curl (1972). Simultaneously, small amount (10 g approx) of soil from each site was transferred to small tin containers which were previously weighed to a constant weight, for determination of soil moisture. For the collection of soil samples, six villages of Raya block were chosen and each was numbered as below. Soil samples from each site were collected in triplicate and were mixed together to make a composite sample.

Collection of Vermicompost

Vermicompost, the excreta of earthworm possesses high quantity of different nutrients such as nitrogen, potassium, phosphorous and C: N ratio etc. In the present study prepared vermicompost was collected from Shri Braj Gau Seva Trust, Vrindavan (Mathura). Such vermicompost has pH value of 6.5 - 7.4, organic carbon 27.43 - 30.3%, total nitrogen 1.80 - 2.05%, phosphorous 1.32 to 1.93%, potassium 1.28 to 1.50% , C:N ratio 14-15.1, calcium 3.0-4.5%, Magnesium 0.4 - 0.7% and sodium 0.2 - 0.3%. In addition to this such vermicompost also contains sufficient amount of bacteria, actinomycetes, fungi and growth hormones such as giberellins, auxins and cytokinins, that enhance the fertility level of saline/usar soils.

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Doses of Vermicompost Amendment and Preparation of Samples

To evaluate the role of organic enrichment on fungistatic activity of saline alkali soils, vermicompost was used. Freshly collected, air dried and sieved soil samples, weighed in equal amount, were taken in separate fresh polythene bags. To each polythene bag vermicompost was added separately in the ratio of 5, 10, 15, 20 and 25% (w/w), respectively.

After adding the respective doses, the samples were shaken and mixed thoroughly by hand manipulation and finally passed through the sieve for uniform mixing. Moisture status of the amended soil samples was maintained up to 60-70% water holding capacity by adding water. The samples were stored at room temperature. Detailed, analyses were made after 10 days of amendment. Natural soil samples without any amendment serving as control were also similarly maintained.

Table 1: Names of villages from where soil samples collected

S. No.	Name of Villages	Soil Sampling Site
1	Deewana – Kalan	S1
2	Kherari	S2
3	Kumha	S3
4	Veerahana	S4
5	Sonai	S5
6	Pirasua	S6

Selection of Test Organism

For assaying the fungistatic activity of various soils, *Fusarium solani* and *Alternaria alternata* were selected as test organisms.

Assay Methods of Soil Fungistasis

The following methods were employed to evaluate the fungistatic behavior of unamended and vermicompost amended soil samples.

Direct Assay Method (Lingappa and Lockwood, 1963)

Air dried sieved soil (20 g), remoistened to 60% water holding capacity, was taken into a small Petri dish (50 x 15 mm). The surface of the soil was smoothened with a bent spatulum blade to obtain a flat, compact and smooth surface. It is required for the uniform distribution of spores and good contact with the soil surface. It also minimizes the removal of soil particles when spores are recovered. After this, the spore suspension was prepared in sterilized-distilled water in concentrations recommended by Lingappa and Lockwood (1963) and was applied uniformly in the form of drop over the soil surface. Spores were also placed on water agar plates for use as a control. The Petri dishes were covered with the lid and incubated for 24 h at room temperature. Following incubation, 2-3 drops of aqueous phenolic rose bengal solution (1% rose bengal, 5% phenol and 0.01% CaCl₂) was applied to the soil surface on the demarked area and was allowed to diffuse into the soil. After staining, test spores were recovered with the help of agar disc (3% water agar, and 7 mm in diameter) by lightly pressing it over the soil surface. The maximum number of spores was obtained by repeatedly pressing 3 or more agar disc over the soil surface. The germinated and ungerminated spores were then counted under the low power of microscope and finally the percent inhibition was calculated.

Agar Disc Method (Jackson, 1958)

It is a widely used indirect method to assay the fungistatic activity of the soil. To prepare the agar discs, 9 ml of melted 2% (w/w) distilled water agar was poured into a 85 mm Petri dish kept on a uniform surface for making a layer of 1.5 mm thickness. The disc was taken out from the agar with a flamed 7.5 mm diameter cork borer and was removed on the tip of a flamed scalpel.

Air dried soil (40 to 60 g), sieved and remoistened to 50% water holding capacity was packed into the sterilized Petri dish of 10 cm diameter. After smoothening the soil surface, 1 cm squares of sterile filter paper (Whatman No.1) were placed on it and an agar disc was placed on each square. Each disc was inoculated with a drop of spore suspension in distilled water. The control was similarly prepared, but the

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agar discs were placed on filter paper saturated with distilled water in Petri dishes without soil. The control and treated Petri dish with lid replaced were incubated at room temperature for 24 h. The agar discs were removed to microscopic slides and were covered with a drop of cotton blue in lactophenol, and a cover slip. The observations for germinated and ungerminated spores were made and the percent inhibition was calculated.

Soil Emanation Agar Method (Hora and Baker, 1970)

To determine the emanation of volatile inhibitors from soils, 50 g of air dried, sieved soil was packed in sterilized Petri dishes of 10 cm diameter. The soil was remoistened to make the moisture level at 60% water holding capacity. Agar disc (8 mm in diameter and 5-6 mm thick) prepared from 2% water agar was placed on the inner surface of a sterile cover of a Petri dish.

Spores of the test fungus in distilled water were transferred on to the discs and the Petri dishes with their lid were incubated at room temperature for 24 h. Agar discs exposed above distilled water in a Petri dish were used as control. After incubation, agar disc were observed for germinated and ungerminated spores and the percent inhibition was calculated.

RESULTS AND DISCUSSION

The fungistatic behaviour of natural/vermicompost unamended soils coupled with 5, 10, 15, 20 and 25% vermicompost amended soils was studied. Direct and indirect methods of soil fungistasis were used to evaluate the fungistatic activity in vermicompost unamended control soils as well as the soils amended with different doses of vermicompost. An attempt was also made to compare their efficacy of the vermicompost amendments against the test organisms, *Fusarium solani* and *Alternaria alternata*. All the fungistasis experiments were repeated at least twice.

After 10 days of vermicompost amendment, varying levels of sensitivity by *Fusarium solani* and *Alternaria alternata* towards soil fungistasis were observed. The soils amended with different doses of vermicompost, however, showed variable percent inhibition in fungistatic activity (Tables 2 and 3). In general, *Fusarium solani* and *Alternaria alternata* showed variable percent inhibition in different assay methods. The conidia germination in both the test fungi exhibited greater percent inhibition with the increase of the concentration of vermicompost in all the three methods adopted in the present study.

The emanation of inhibitory volatile components seemed to affect the germination of conidia of *Fusarium solani* because in almost all the soils amended with different doses of vermicompost, soil emanation method exhibited greater inhibition over the vermicompost unamended control soils. Maximum percent inhibition was observed in the soil amended with 25 and 15% vermicompost. However, the soil amended with 5% vermicompost showed only 2% inhibition in germination of the conidia. Similar results were also observed with *Alternaria alternata*. However, in *Alternaria alternata* maximum percent inhibition (10% each) was recorded in the soils amended with 20 and 25% vermicompost.

Similar emanation of inhibitory volatile components was also recorded in the soils amended with oil cakes (Agarwal, 1983). In this study an irregular sensitivity of *Fusarium solani* in response to different oil cakes amendments was also recorded. Lewis and Papavizas (1975) and Lockwood (1977) stressed that energy sources and other organic materials added to the soil may reduce or completely annul fungistasis initially. After a week or more, a long-term effect may become evident as manifested by an enhancement of fungistasis.

In the present study, the emanation of volatiles as a cause of inhibition of conidia germination in *Fusarium solani* may be due to higher nutritive content of vermicompost. Mac Millan (1956) has suggested that in the immediate vicinity of nitrogen rich substrate, a considerable amount of ammonia is released, which had inhibitory effect on the germination of fungal propagules.

It is evident from the data of Tables 2, 3 and Figure 1, 2 that percent inhibition of conidia germination in *Fusarium solani* and *Alternaria alternata* was found to be almost similar in soil emanation agar method as well as in agar disc method. Whereas maximum percent inhibition of conidia germination (10%) in *Alternaria alternata* was recorded in the soil amended with 25% vermicompost, *Fusarium solani*, however, showed its maximum percent inhibition of conidia germination (14% each) in the soils amended with 20

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and 25% vermicompost. Similar findings were also observed by Singh (2002), while working with contaminated/ degraded soils amended with rice husk compost. Composting is the best natural approach to reduce the plant pathogens and increase the crop yield (Mehta *et al.*, 2014).

As evident from the data of direct assay method that comparatively higher percent inhibition of conidia germination in *Fusarium solani* and *Alternaria alternata* was recorded in the soils amended with vermicompost over the vermicompost unamended control soils and fungistatic activity increased with increase in vermicompost concentration.

In case of direct assay method, *Fusarium solani* showed greater percent inhibition of conidia germination in comparison to *Alternaria alternata* in the soils amended with vermicompost over the vermicompost unamended control soils. *Fusarium solani* showed minimum percent inhibition (69%) of conidia germination in the soils amended with 5% vermicompost.

However, this value increased with the increase in the doses of vermicompost amendments and attained its maximum level (84%) in the soils amended with 25% vermicompost. *Alternaria alternata* showed maximum percent inhibition of conidia germination (78%) in the soils amended with 25% vermicompost. Chinn (1967), Kanaujia (1973), Pandey (1976) and Agrawal (1983) observed that fertilizer amendments of the soil resulted in enhancement of fungistasis.

Statistically, assay methods and the concentration of vermicompost amendments significantly influenced the phenomenon of soil fungistasis as the 'F' ratio values obtained for these variables were found to be greater than their respective table values. *F. solani* showed highest percent inhibition in its conidia germination in direct assay method followed by agar disc and direct assay method.

It also showed a dose specific increase in fungistatic activity, as the minimum (68%) and maximum (84%) inhibition in conidia germination was observed in vermicompost unamended control and 25% vermicompost amended soils, respectively. *A. alternata* also showed maximum percent inhibition in direct assay method followed by agar disc and direct assay method. The minimum (51%) and maximum (78%) inhibition in conidia germination was observed in vermicompost unamended control and 25% vermicompost amended soils, respectively.

The fungistatic activity as observed in various assay methods can be attributed in the following order: direct assay method > agar disc method > soil emanation agar method. These results were also supported by the findings of earlier workers (Dixit, 2002; Singh, 2002). Fungistasis at initial doses of vermicompost amendment was positively correlated with the microbial population. Contrary to the present findings, an annulment of fungistasis has been widely observed by the addition of organic substances or energy rich compounds to the soil (Chin and Ledingham, 1957; Powelson and Patil, 1963; Agnihotri and Vaartaja, 1967; Papavizas and Adams, 1969; Hsu and Lockwood, 1973; Lockwood, 1977; El-Abyad and Ismail, 1984; Singh, 2002; Dixit, 2002). It has been proved that vermicompost act as an organic fertilizer as well as biological control agent by suppressing many plant borne pathogens and pests also (Yurdagul, 2011).

Table 2: Fungistatic activity (in terms of % inhibition) of chosen soils amended with vermicompost after 10 days of amendment against *Fusarium solani*

Doses of amendments (%)	Test Fungi <i>Fusarium solani</i>		
	SEA	AD	DA
5	2	4	69
10	5	8	79
15	10	8	79
20	12	14	80
25	15	14	84
Control	0	0	68

SEA - Soil emanation method

AD - Agar disc method

DA - Direct assay method

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Analysis of Variance

Sources of variation	S.S.	d.f.	M.S.S.	F _(observed)	F _(tabulated)	
					5%	1%
Concentration (%)	281.73	4	70.43	20.83**	3.84	7.01
Methods	15871.6	2	7935.8	2347.8**	4.46	8.61
Error	27.07	8	3.38			
Total	16180.4	14				

Table 3: Fungistatic activity (in terms of % inhibition) of chosen soils amended with vermicompost after 10 days of amendment against *Alternaria alternata*

Doses of amendments (%)	Test Fungi		
	<i>Alternaria alternata</i>		
	SEA	AD	DA
5	2	3	53
10	5	6	58
15	7	6	62
20	10	8	68
25	10	10	78
Control	0	0	51

SEA=Soil emanation method

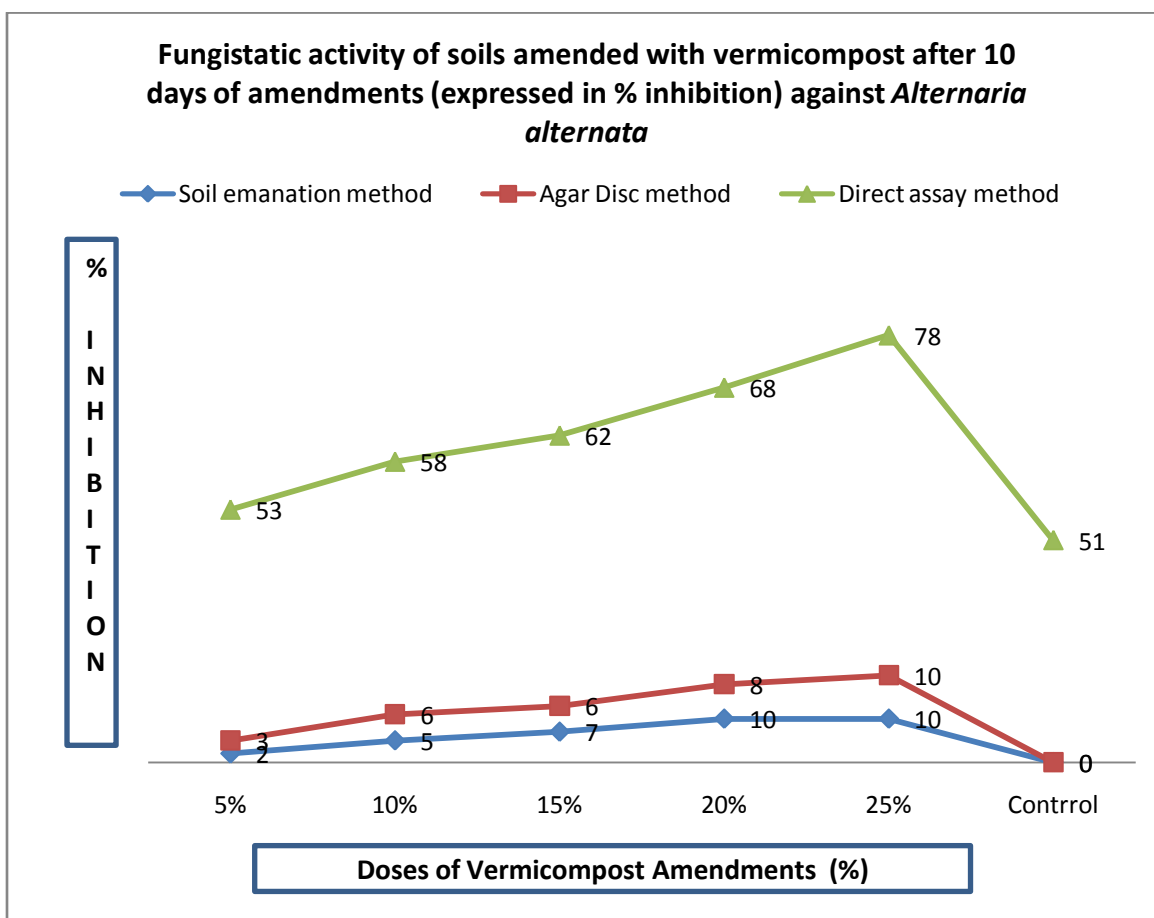
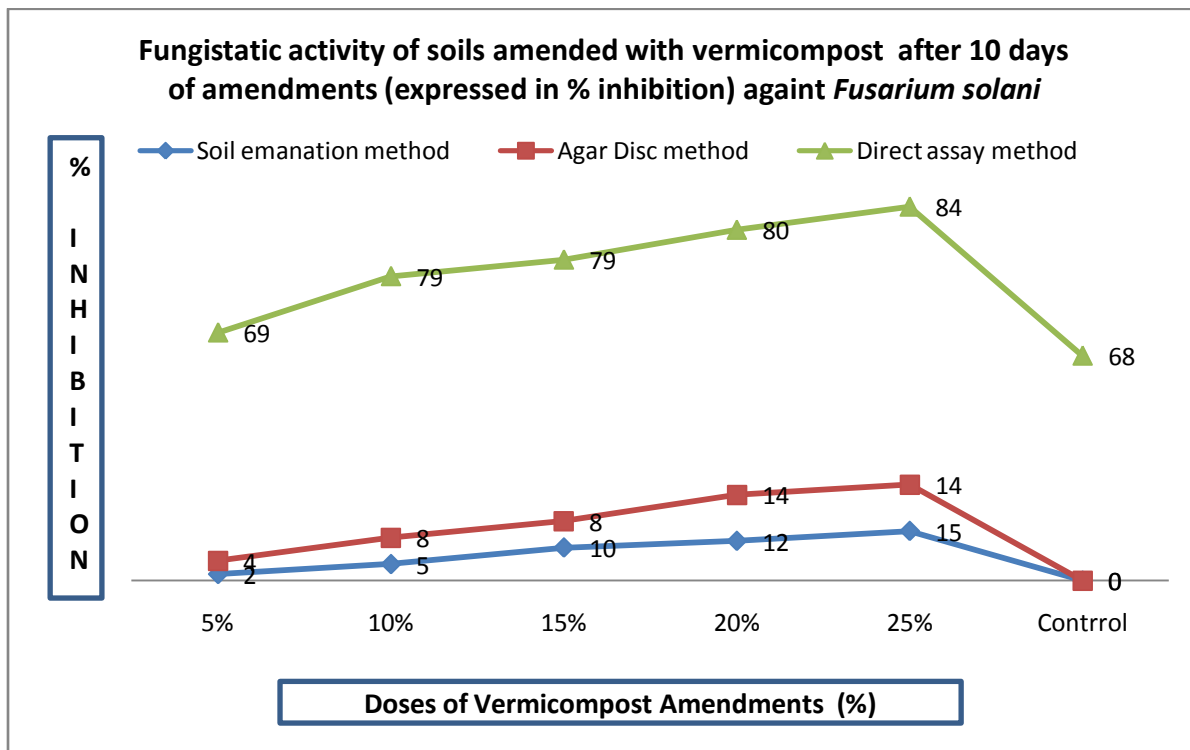
AD=Agar disc method

DA=Direct assay method

Analysis of Variance

Sources of variation	S.S.	d.f.	M.S.S.	F _(observed)	F _(tabulated)	
					5%	1%
Concentration (%)	316.94	4	79.23	4.88*	3.84	7.01
Methods	10868.14	2	5434.07	334.8**	4.46	8.65
Error	129.86	8	16.23			
Total	11314.9	14				

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