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Vermicompost either alone or with amendment can enhance the shelf-life of P and Zn mobilizing fungal inoculants used in sustainable agriculture

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Abstract

The shelf life of P solubilizing fungus *Aspergillus awamori*, Zn solubilizing fungus *Aspergillus niger* and AM fungus *Glomus mosseae* in enriched vermicompost was investigated. *A. awamori* and *A. niger* were mixed separately with vermicompost at 20% and 25% moisture levels and the population was enumerated periodically. The results showed that vermicompost supported population of *A. awamori* and *A. niger* to the level of 10^7/ g up to 360 days after storage (DAS) at 20% moisture level. The infective propagules (IP) of AM fungus *G. mosseae* mixed with vermicompost and in combination with agro products, coir pith and rice husk, was enumerated periodically. The results showed that vermicompost amended with rice husk (1:3) supported the propagation of *G. mosseae* better with highest number of IP 16 x 10^3/ g 405 DAS.

Keywords – Agro byproducts **–** *Aspergillus awamori* **–** *Aspergillus niger* **–** *Glomus mosseae*

Introduction

Bioinoculants are products containing live cells of efficient strains of microorganisms which, when applied to seed, plant surface or soil, colonize the rhizosphere and/or the interior of the plant and promote growth by increasing the supply or availability of primary nutrients to the host plant through biological processes like nitrogen fixation, mobilization of phosphorus, potassium, etc. Bioinoculants which include biofertilizers and biopesticides are expected to reduce the use of chemical fertilizers and pesticides (Vessey 2003, Mondal et al. 2015). The use of biofertilizers in crop plants is highly recommended in sustainable agriculture considering their positive effect on growth and yield (Woyessa & Assefa 2011). These microbes are also ecofriendly to soil and improve soil health. Bioinoculants are produced using effective strains which are mass cultured and then marketed either in liquid or solid form. For solid based bioinoculants the microbes are mixed with a carrier *viz.* peat, lignite or charcoal wherein the population of bioinoculants increase using the nutrients available in the carrier. In solid formulations, the carrier absorbs and retains the moisture and provides a surface for growth of the bacteria (Gandhi & Sivakumar 2010). Hence the carrier plays an important role in the shelf life of the inoculated

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microbial culture. At present, lignite powder is widely used as carrier material for most of the bioinoculant organisms which may result in poor availability in due course of time (or in future) as it is a natural resource deposit and is also being used as fuel by thermal power stations, etc. Availability of quality lignite powder is also in limelight because of adulteration by agents and improper mesh size in the pulverizing unit. Adding to all these drawbacks the shelf-life of most of the bioinoculants in these carriers are usually six months and there after the population of the added inoculant decreases mainly due to non-availability of nutrients in the carrier material. Further an alternative carrier which can increase the shelf life and proliferate the growth, survivability and economic status of the substrate is needed. In some countries compost has been used as a substrate to multiply biocontrol organisms that protect the plants against soil-borne plant pathogens and has been named as "suppressive compost" (Hoitink et al. 1999; Hadar & Papadopoulou 2012). Few reports have suggested compost (Margareth & Mangkoedihardjo 2010) or vermicompost (Gopinathan & Prakash 2014) can be a substitute as carrier material for bioinoculants which has to be validated through more research work; and in these studies researchers have multiplied microorganisms and then mixed with the compost/ vermicompost just before applying to the field. But research on mixing microbial inoculants with vermicompost as a carrier and studying shelf life of introduced microorganism on long term basis is not available; although few reports are made on enumerating population for a short period (Kumar & Singh 2001; Packialakshmi & Riswana 2014; Argal et al. 2015). Hence the present study was undertaken to investigate the role of vermicompost as carrier of bioinoculants with special reference to shelf life.

Materials and Methods

Production of vermicompost

Poly culture of earthworms *Eudrilus eugeniae, Eisenia fetida* and *Perionyx excavatus* were used for production of vermicompost. Heterogeneous waste as animal dung except poultry droppings, agricultural residues and weeds formed the source of substrate for earthworms. The earthworms were released into bins on the surface of substrate which moved downwards feeding on the mix of organic matter. They released the undigested material as their castings on the surface. Vermicompost thus collected (Nagavallemma et al. 2008) was air dried and used as the carrier material for three fungal inoculants.

Production of starter culture of bioinoculants

The fungal inoculants used were *Aspergillus awamori* (P-solubilizer), *Aspergillus niger* (Zn-solubilizer) and *Glomus mosseae* (AM fungus). The three fungal inoculants used in the study were obtained from the culture collection at CNBRCD, Bangalore. The two fungal inoculants *A. niger and A. awamori* were maintained on potato dextrose agar slants and were multiplied on potato dextrose broth at 24^{o}C for 5 days. The P solubilizing ability of *A. awamori* was tested using Pikovskaya medium (Sharma et al. 2011) which showed clear hallow zones around their colonies. Similarly the Zn solubilizing ability of *A. niger* was tested using modified Pikovskaya medium (Bapiri et al. 2012). The mycorrhizal fungus *G. mosseae* used in the study was maintained on vermiculite:perlite:soilrite (1:1:1 v/v/v) mix as the substrate and Rhodes grass as the host. *G. mosseae* inoculum contained extra matrical chlamydospores, hyphae, infected root bits and substrate. The infective propagules present in the inoculum were 6.25 x 10^3/g which was determined based on most probable number (MPN) estimation (Porter 1979).

Physico-chemical properties of carrier material

The physical and chemical properties of vermicompost, rice husk and coir pith are presented in table 1.

Table 1 Physical and chemical properties of vermicompost, rice husk and coir pith

	Organic carbon (%)	Porosity (%)	pH	C/N ratio	N (%)	P (%)	K (%)
Vermicompost	15	80	8.41	15	2.30	1.60	1.50
Rice husk	19	80	5.20	22	0.50	0.05	0.02
Coir pith	15	75	6.10	26	1.08	0.19	0.20

Mixing of fungal inoculants with vermicompost and enumeration of population

A. awamori* and *A. niger : The fungal mats of *A. awamori* and *A. niger* were picked after 5 days growth from the culture (potato dextrose broth) flask carefully and were ground in a mixer for few seconds separately. Initial lab experiments performed using sterilized vermicompost to determine shelf-life of inoculated bioinoculants did not support the growth of inoculated organisms where the population of the organisms drastically reduced over a period of time and at 225^{th} day population was nil (unpublished). Hence each culture was then thoroughly mixed with 1kg of unsterilized vermicompost and the moisture adjusted to 20 and 25% using sterile water and sealed. The physico-chemical properties of unsterilized vermicompost used in the study is given in table 1. Two thin pin holes were made in the bags for aeration and the bags were kept at room temperature (20-26°C). Initial population of the two inoculant fungi mixed separately with the vermicompost was determined by serial dilution method. Later, initially once in every 15 days and later once in every 30 days the population of inoculants in the polythene bags was enumerated through serial dilution method. The last reading was taken at 810 DAS for *A. awamori* and 960 DAS for *A. niger*.

G. mosseae : Initial lab experiments conducted on shelf-life of *G. mosseae* with unsterilized and sterilized vermicompost showed less infective propagule numbers over a period of storage; and it was felt that it may be due to higher nutrient level in vermicompost. Hence an experiment amending unsterilized vermicompost (VC) with coir pith (CP) and rice husk (RH) as carrier substrate for *G. mosseae* at two different ratios 1:1 and 1:3 was carried out. The physico-chemical properties of coir pith and rice husk used in the study are given in table 1. *G. mosseae* inoculum (60g) with known number of infective propagules (6.25×10^3/g) was added separately to each of the polythene bags containing 540g of substrate mixture *viz.* VC+CP (1:1 ratio), VC+CP (1:3 ratio), VC+RH (1:1 ratio), VC+RH (1:3) and to VC alone. The mixture was shaken thoroughly to get 10^{-1} dilution. Sixty grams sample was drawn from each polythene bag and the initial IP numbers in all the 5 samples were determined by MPN method (Porter, 1979). All the 5 inoculated samples with *G. mosseae* in polythene bags were sealed and kept at room temperature (20-26°C). At 45 days interval the number of infective propagules of *G. mosseae* was determined by MPN method periodically and the results were recorded accordingly. The last reading was taken 405 DAS.

Results

The population of *Aspergillus awamori* steadily increased up to 75 days of storage and then showed a decline. In general, moisture level at 20% in vermicompost supported *A. awamori* population throughout the storage period up to 420 DAS i.e. 1.2×10^7/g. The population at 810 DAS was 0.0001 and 0.0001×10^7 /g at 20 and 25 % moisture levels respectively (Fig. 1). The population of Zn solubilizing fungus *A. niger* steadily increased up to 90 days and decreased slowly thereafter. Here 25% moisture level supported the population of *A. niger* better throughout the period compared to 20% moisture level. The population at 960 DAS was 0.1 and 0.3×10^7 /g at 20% and 25% moisture levels respectively (Fig. 2). The present results suggest that vermicompost supported the multiplication of fungal inoculants with population of 1.2×10^7/g and 0.009×10^7/g up to 420 days in *A. awamori* at 20% and 25% moisture level respectively. *A. niger* also had population of 1.0×10^7/g and 1.8×10^7/g up to 750 days at 20% and 25% moisture level respectively. Comparative results bring out overall 20% moisture supports both *A. awamori* and *A. niger*.

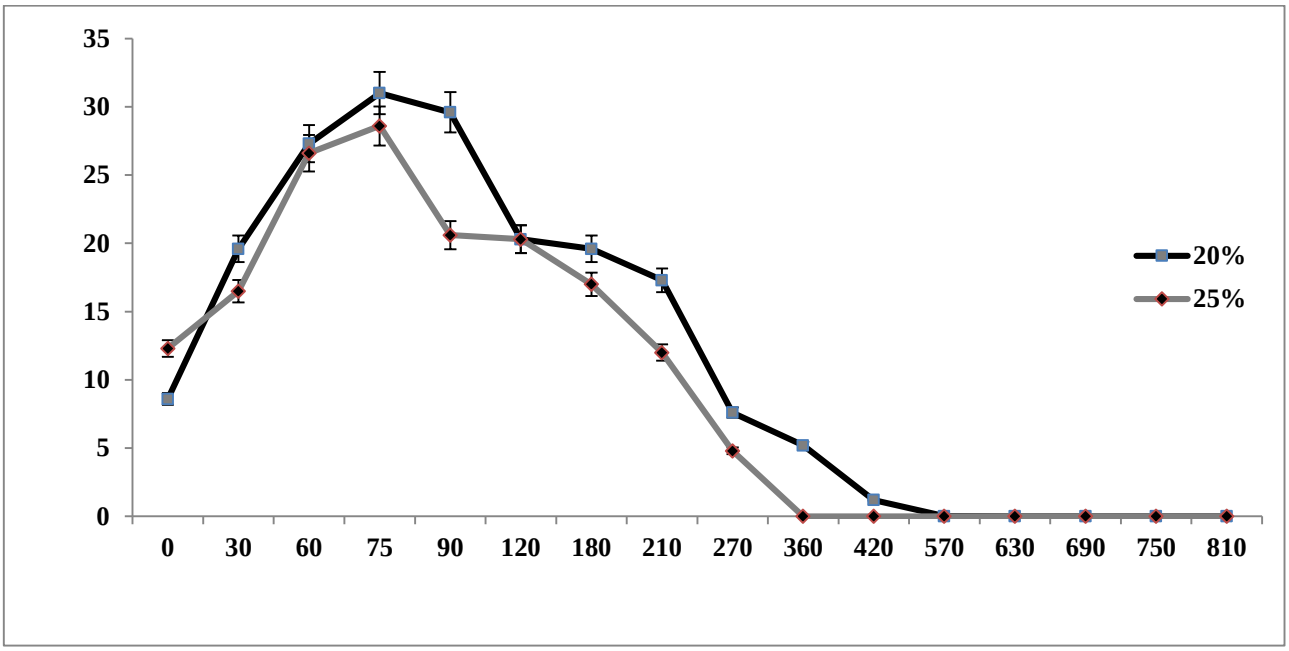

Fig. 1 – Population of *A. awamori* in vermicompost at 20% and 25% moisture level at different days after storage (DAS)

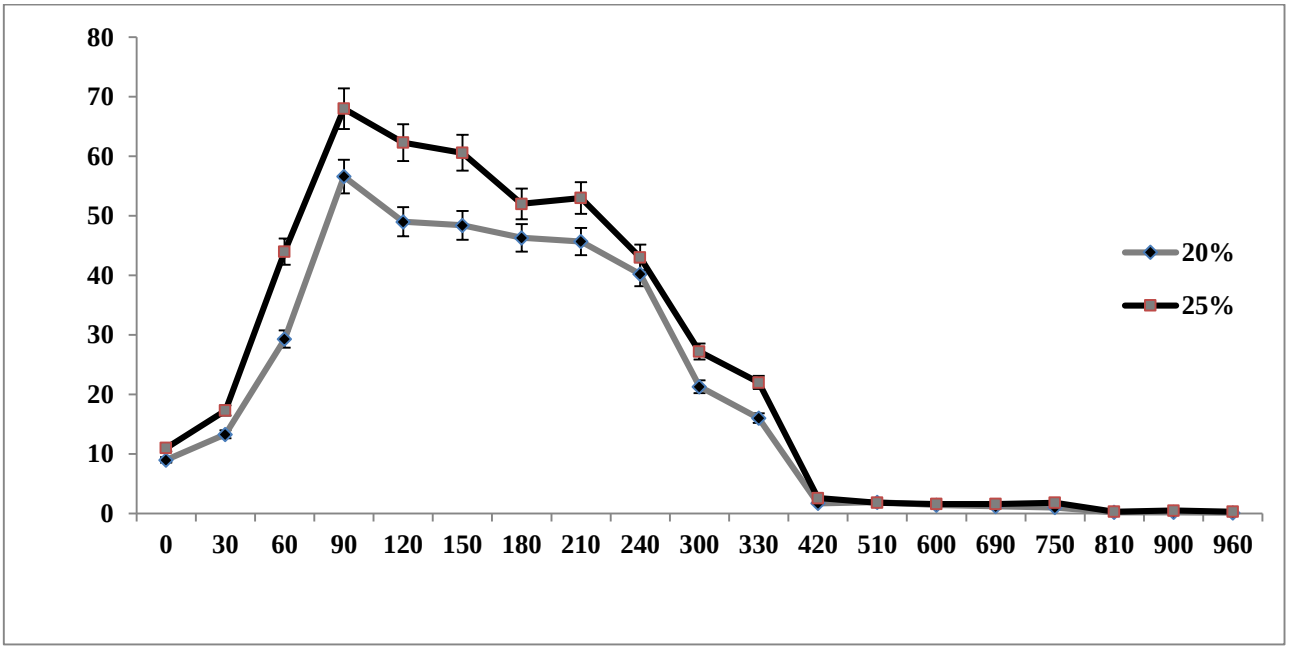

Fig. 2 – Population of *A. niger* in vermicompost at 20% and 25% moisture level at different days after storage (DAS)

Regarding the AM fungus *G. mosseae*, vermicompost + RH at 1:3 ratio supported higher IP numbers of 16.0 x 10^3/g, 405 days after storage. It was also noted the initial IP number of 35.0 x 10^3/g was high in VC+RH (1:3) compared to other substrate combinations and even though it reduced over a period it stabilized at 180 DAS with 16.0 x 10^3/g IP numbers. Other combination of substrates did not support the survivability from initial period itself and reduced IP numbers drastically (Table 2).

Table 2 Infective propagule (IP) numbers of *G. mosseae* at different days after storage (DAS)

DAS/ Substrate	Infective Propagules (IP x 10^3/g) at different days after storage									
	0	45	90	135	180	225	270	315	360	405
Vermicompost + Rice husk (1:1)	0.28	0.11	0.14	0.28	0.04	0.05	0.04	0.04	0.03	0.01
Vermicompost + Coir pith (1:1)	9.2	2.2	0.28	0.22	0.22	0.28	0.28	0.28	0.22	0.04
Vermicompost + Rice husk (1:3)	35.0	28.0	28.0	22.0	16.0	16.0	16.0	16.0	16.0	16.0
Vermicompost + Coir pith (1:3)	1.6	1.7	2.8	1.4	0.49	0.22	0.30	0.30	0.16	0.09
Vermicompost	0.28	0.01	0.02	0.005	0.002	0.002	0.005	0.002	-	-

Discussion

The beneficial role of vermicompost in crop production is well documented. But the information on enriching vermicompost with bioinoculants is meager, which was contemplated in this study. Vermicompost is used as a substitute for farm yard manure/ compost because of its rich nutrient content and comparatively lesser quantity needed for application to the field. It also increases soil pore space, water holding capacity, cat ion exchange capacity and soil health (Chanda et al. 2011; Manivannan et al. 2009). Application of vermicompost in reducing the amount of chemical fertilizer usage has also been reported (Mondal et al. 2015). Plants absorb a small amount of nutrients from chemical fertilizers especially phosphorus added to the soil and most of the added phosphorus fertilizers are fixed in the soil to a form unavailable for plant growth. Phosphorus (macronutrient) is a component of the complex nucleic acid structure of plants, which regulates protein synthesis and is therefore important in cell division and development of new tissue. It is also associated with complex energy transformations in the plant. P deficiency leads to stunted growth and often shows dark bluish-green colour in leaves and stem becoming purplish. (http://goo.gl/XyxoMU). Zinc (micronutrient) is used by plants in the formation of chlorophyll and is apparently linked with iron and manganese in this process. Typical zinc deficiency symptoms of field crops are chlorosis of the interveinal tissue and shortening of internodes which makes the plant appear stunted (http://goo.gl/ewJ7K5). Production of organic acids, chelating materials, inorganic acids and phosphatase enzyme are mechanisms used by fungal bioinoculants for dissolution of insoluble organic and inorganic phosphates (Rodriguez 2006). Numerous reports are available on increased yield in crops due to inoculation with bioinoculants (Selvakumar et al. 2012; Umesha et al. 2014, Saeed et al. 2015).

In general the results showed that vermicompost supported higher population of the two fungal bioinoculants i.e. *A. awamori* up to 420 days with 1.2 x 10^7 cfu/ g and *A. niger* up to 750 days with 1.0 x 10^7 cfu/ g at 20% moisture level. This reveals that vermicompost is a good carrier material for inoculating bioinoculant organisms. The positive interaction between inoculated organisms and vermicompost not only supported the fungal inoculants but also helped in multiplication of their population. Vermicompost is suitable as a carrier because of sufficient availability of nutrients, good water-holding capacity (Ferreira & Castro 2005) and producing substances that stimulate and regulate plant growth (Tomati et al. 1988) which is a favourable environment for bioinoculants to survive and multiply. In another study this enriched vermicompost was also tested on tomato and marigold under microplot conditions and the results showed enhanced yield in tomato fruits by 29.82 % and with increased number of flowers by 8% in marigold compared to the yield in plants treated with vermicompost alone (Ashwin et al. 2013, 2014).

Initial study on shelf life of *G. mosseae* with vermicompost alone which did not support the survival of higher infective propagules lead to another experiment with amending unsterilized vermicompost (VC) with coir pith (CP) and rice husk (RH) as carrier substrate at two different ratios 1:1 and 1:3. The results showed good response when vermicompost was amended with coir pith and rice husk rather than using vermicompost alone. Vermicompost as such proved to be bad carrier for

G. mosseae where in the IP numbers declined from the initial phase itself to as low as $0.002x10^3$/g at 315 DAS. This is probably because of higher nutrient content present in vermicompost. The *G. mosseae* IP numbers were highest in VC+RH at 1:3 at both initial and last sampled level (405 DAS) with $35.0x10^3$/g and $16.0x10^3$/g respectively. Interestingly the same substrate VC+RH at 1:1 ratio had $0.01x10^3$/g at 405 DAS. Rice husk is composed mainly of silica and lignin and is also an insulation material for grains. It has low moisture content, bulk density, high porosity and good water absorption capacity. The possibility of AM fungus *G. mosseae* surviving with high IP numbers at 1:3 ratio might be because of rice husk protected the AM fungus like an insulating material from deleterious effects of environment with the optimum level of nutrients available from the amended vermicompost. This supports the work of Ogbo and Oda (2011) who reported that rice husk supports survival and growth of biofertilizer microbes. On the other hand coir pith did not support the shelf-life of *G. mosseae* at both ratios studied. The IP numbers declined to 0.28 $x10^3$/g (1:1 ratio) and 2.8 $x10^3$/g (1:3 ratio) 90 DAS. Coir pith is mainly composed of cellulose and lignin and failed to support the shelf-life of *G. mosseae* in comparison with rice husk.

According to the Fertilizer (Control) Order 1985 (2015) notification from Government of India which regulates quality of biofertilizers, P and Zn solubilizing microorganisms should contain a viable count of 5 x 10^7 viable cells/ g and no contaminants at 10^{-5} dilution of solid substrate. The enriched vermicompost in our study had more than 5 x 10^7 viable cells/ g at 360 DAS and no contaminants at 10^{-5} dilution of the substrate. The quality control prescribed for AM fungus is that the product should have a minimum of 1200 IP numbers/ g. In the present study the infective propagule numbers of *G. mosseae* in vermicompost + rice husk (1:3) substrate was 16 x 10^3 IP/ g at 405 DAS. Most of the biofertlizer products in the market have shelf life of not more than 6 months. Here our results clearly showed vermicompost can be used as a substitute, for currently being used carriers, in biofertilizer products with shelf life of 12 months for P and Zn solubilizing fungi and a shelf life of 13 months for AM fungi.

Conclusion

It can be concluded that in future vermicompost can be used conveniently as a carrier material which supports longer shelf life of 1 year for fungal inoculants like *A. awamori* and *A. niger*, in comparison with substrates used at present which supports shelf life of 6 months. Regarding *G. mosseae*, VC with rice husk in the ratio of 1:3 has supported adequate infective propagule numbers for nearly 13 months. Further vermicompost enriched with beneficial microorganisms can be easily applied to the field by farmers adopting sustainable agriculture and get increased crop yield as evidenced by earlier studies with marigold and tomato (Ashwin et al. 2013, 2014).

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