EFFECT OF CARRIER MATERIALS, COINOCULATION AND STERILIZATION ON SURVIVAL OF PLANT GROWTH PROMOTING MICROBES

ABSTRACT

The study was conducted to investigate best carrier material out of agricultural and environmental waste in maintaining the shelf life of plant growth promoting microbes (PGPM) at room temperature for locally produced inoculants in Malawi. Five different formulations divided into sterilized and unsterilizedwere prepared, using different carrier materials namely; rice bran plus plant extract (RBP), biochar plus plant extract (BP), Filter mud plus plant extract (FMP), rice bran, biochar and plant extracts (RBCP), filter mud, biochar and plant extracts (FMBP). Carrier materialswere packed in polyethylene pack (six per each treatment), thereafter each treatment was divided into sterilized and unsterilized. Each treatment was inoculated with either single or multiple inoculants. Survival of PGPM, was based on colony forming units (CFU) on specific selective media namely: modified yeast extract mannitol, pikovskaya's, Alexandria and basal media for nitrogen fixing microbes, phosphate, potassium and zinc solubilising media respectively. Results revealedthat encapsulated formulationof based combination formulationof RBCPin both single and multiple inoculants exerted high stable numbers of PGRM along the storage compared to other formulations. The results also show that unsterilizedformulations exert high numbers compared to sterilized which is as a result of hydrogen peroxide accumulation during sterilization. The study reveals thatfilter mud based formulations currently used in both single and multiple inoculantsis not favorable for local environments because microbial numbers decrease after 20 days at room temperature.This makes filter mud formulations usage not favorable for rural smallholder farmers with no refrigeration facilities.

Key words: carrier materials, agricultural and environmental waste, plant growth promoting microbes, inoculants

1. INTRODUCTION

The inoculants (biofertilisers)particularly rhizomicrobeshave synergistic interaction with roots hence provision ofregulatory effects on plant growth and development[1,2]. Biofertiliser have a positive implication on production costs, yield quality and quantity,crop stress regulation and bioremediation and [3,4].

Colony Forming Units (CFU) of inoculated plant growth promoting microbes (PGPM) to the soil may increase or decrease depending on environmental factors like moisture, temperature and type of carrier material[5,6,7]. Carrier materials is a substrate that carryviable cells of PGPMto be inoculated in the soil or seed. It has capability for the provision of conducive environment to PGPMto maintain the CFU above the standards. Biological, physical and chemical characteristics of carrier materials has properties similar to the indigenous environment where the microbes are isolated like moisture retention capacity, pH, organic matter content, nutrient levels, etc.[8,9].

Various organic materials such as perlite, biochar, maize bran, biochar, rice bran, karnolite, sodium alginate, peat, clay, begasse, saw dust, wood ashes and plant extracts are some of carrier materials used in inoculantsbut some of these are expensive, scarce and environmentally unfriendly[7,8,10,11]. Some carrier materials are amendedwith diverse additivesto improve seed adhesion, stabilization of the carrier material, survival of microbes in diverse environment, and easy to inoculate in the field [8,12,9]. Most additives use trade secrecy as intellectual property. These additives are used because of unique properties that make the product to have higher performance[13,14].The use of carrier materials and its additivesin biofertilisers as inoculation strategy is as old as the history of inoculation[10].

The efficacy of biofertilisers is dependent on several factors and one of which is CFU to be inoculated in the soil, compost or seed. This conforms to the principle of the survivability rateof carrier materials to ensure that thestandard CFUis maintained [10,15,16,8,17,18].

Many investigations have used peat as carrier material for PGPM, but less has been done of using site specific agricultural waste and plant extracts as carrier materials and additives respectively. In the present study, different agricultural and environmental waste were treated as amendment with plant extracts (a trade secret) to develop CM of PGPM consortium and survivability rate of PGPM inoculants.

2. Methodology

The experiment was carried out atthe LOGO-TECH Company. PGPM taken from LOGO-TECH were used in the experiment; *Bradyrhizobium*as nitrogen fixer, *K. pneumonia* as phosphorus solubiliser, *Pseudomonas* as potassium solubiliser, *A. calcoaceticus* as zinc solubiliserwere used for the developmentof single and multiple inoculantformulas.

2.1. Preparation of Carrier Material

Biochar, rice bran, filter mud and plant extracts were collected, milled in a motor millerinserted with 1 mm sieve. Five carrier materials namely; rice bran plus plant extract (RBP), biochar plus plant extract (BP), Filter mud plus plant extract (FMP), rice bran, biochar and plant extracts (RBCP), filter mud, biochar and plant extracts (FMBP) were used.Plant extracts are the recommended ingredient as a sticker andbiostimulant under trade secrecy of LOGO TECH Company. Polythene packswere used as packaging material covers. They were further subdivided into two (sterilized and unsterilized) for development of single and multiple inoculants. Sterilization of carrier material was done by autoclaving polythene bags sealed with carrier material at 121 °C, carrier material moisture content was adjusted to 40%.

2.2. Preparation of Microbial Consortium

PGRM formulations were prepared using modified yeast extract Mannitol, Alexandria Pikovaskaya'sand basal media for nitrogen fixing microbes, phosphate, potassium and zinc solubilising media in the respective order and incubated for 3-4 daysusing incubator with a shaker at 32 ^{0}C [19,20]). After incubation period each carrier material (sterilized and unsterilized) was mixed with 20% of broth as single microbe or in a mixture with other microbes with CFU of $1 * 10^{8}$ as documented by [21]. Multipleformulations were prepared by mixingnitrogen fixing microbes, potassium, phosphate and zinc solubilizing microbes same volume and CFU.

2.3. Storage and Survival Study

Survival rate of PGRM was enumeratedusing CFU (standard plate count method) of selective media at 0, 5, 10, 15, 20, 30, 90, 150and 360 days based on a method byIvan et al. (2009) with slight modifications followed by conversion to log10 CFU per gram.

3. Results and Discussion

Results showed that single inoculants had higher cells on log values for all microbes as shown in figure 2 and 3. Regardless of the decrease in CFU,some carrier materials have values above the set standards of one billion viable cells per gram. Thiscould lead to shift from single microbe inoculantsdue to economic value because same carrier material is used for several PGPM. This is in line with other studies which attribute economic implication of carrier material, method of application and co-inoculation in inoculant production [23,7,14].Results also showed no significant changes in viable cellsof PSM, KSM and ZnSMwithin the carrier material in single and multiple inoculants. There was high significant reduction in viable cells in multiple and single inoculants for NFM in FMP and FMBP (figure 2a and 2b) showing that filter mud provides low favorable conditions for PGPM as observed by other studies [9,11,6,24]. This is due to physical

and chemical parameters that have an implication on biological activity and also regulation of temperature [6].

The significant difference in survival of different microbes between single and multiple inoculants was mainly due to competition which in most of times include temperature, carrier materials and moisture. Diversity of microbes increased the number of microbes per gram which had an implication on temperature, nutrients and by products.

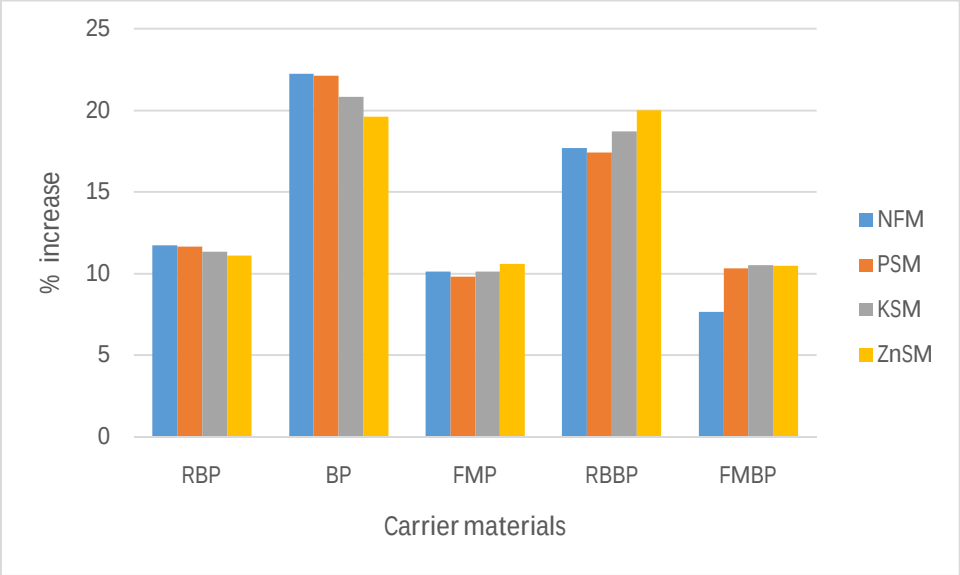

Figure 1: Average percentage differences between sterilized and unsterilized carrier materials

Results as shown in figure 2 and 3 also show percent fluctuation over time of cells on log values under different carriers in single and multiple inoculation is indicative of synergistic effects between microbes and is also dependent on environmental factors like carrier material [2526]. Ecologyof PGPMhas an implication on profitability of production methodology due to maximization of raw materials[27].

RBP, BP, and RBCPformulation significantly maintained population density of microbes at the end of storage period at room temperature and combination rice bran and biochar had the highest values (figure 2 and 3). This is as a result of combined beneficial characteristics of biochar and rice bran. Biochar is a complex solid material (biological origin)which has made thorough biomass carbonization. It's designed for the reduction of greenhouse gases emission and carbon sequestration in soils for a long time hence its usage has a positive impact on climate change [28]. It's highly porous structure and has positive implication on diversity and interaction of PGPM by promoting mutualism and desiccation by reduced carbon as an energy source and mineral nutrients[29,30,31].Most of current studies have shown broad spectrum usage of biochar in agricultural land as a sustainable approach to bioremediation[32]. It increases plant growth, biostimulation, soil quality and yielding due to physical, biological and chemical positive changes when applied to soil [32,33,34]. Its effect is also dependent on raw materials and charring condition, application method and rate [28,30,35].

Carrier materials like RBP showing high viable cells in the study provide essential criteria of ideal carrier that are locally found[36,16,6]. Similar results of using different carrier in inoculant production were observed by other studies[37].

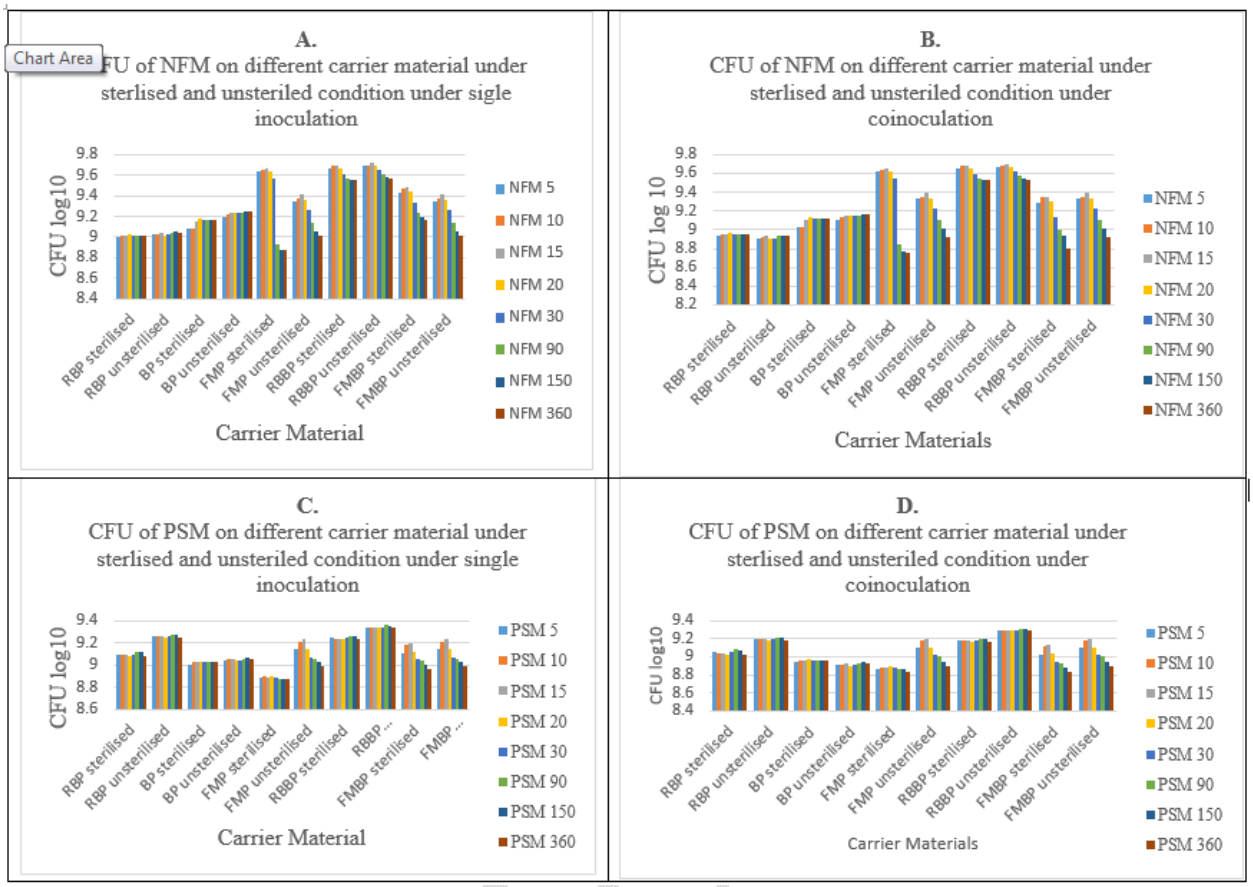

Figure 2: CFU of PGPM (NFM and PSM) on different carrier materials under sterilized and unsterilized conditions for single and multiple inoculants.

By showing high CFU within 15 days of storage at room temperature using filter mud and constant CFU if kept at 4 ^{0}c ^{6} which indicates that filter mud provides conducive environment for commercial farmers with cooling systems [9,38,39]. The quality of carrier material is dependent on deliveringstandardundistorted number of viable cells, adoptability and economic implications in production. The use of local organic materials like biochar and bran havinghigherand stable CFU for longtime over filter mud which has been used since 1981is welcome development for smallholder rural farmers in Malawi[31,40,28,30].

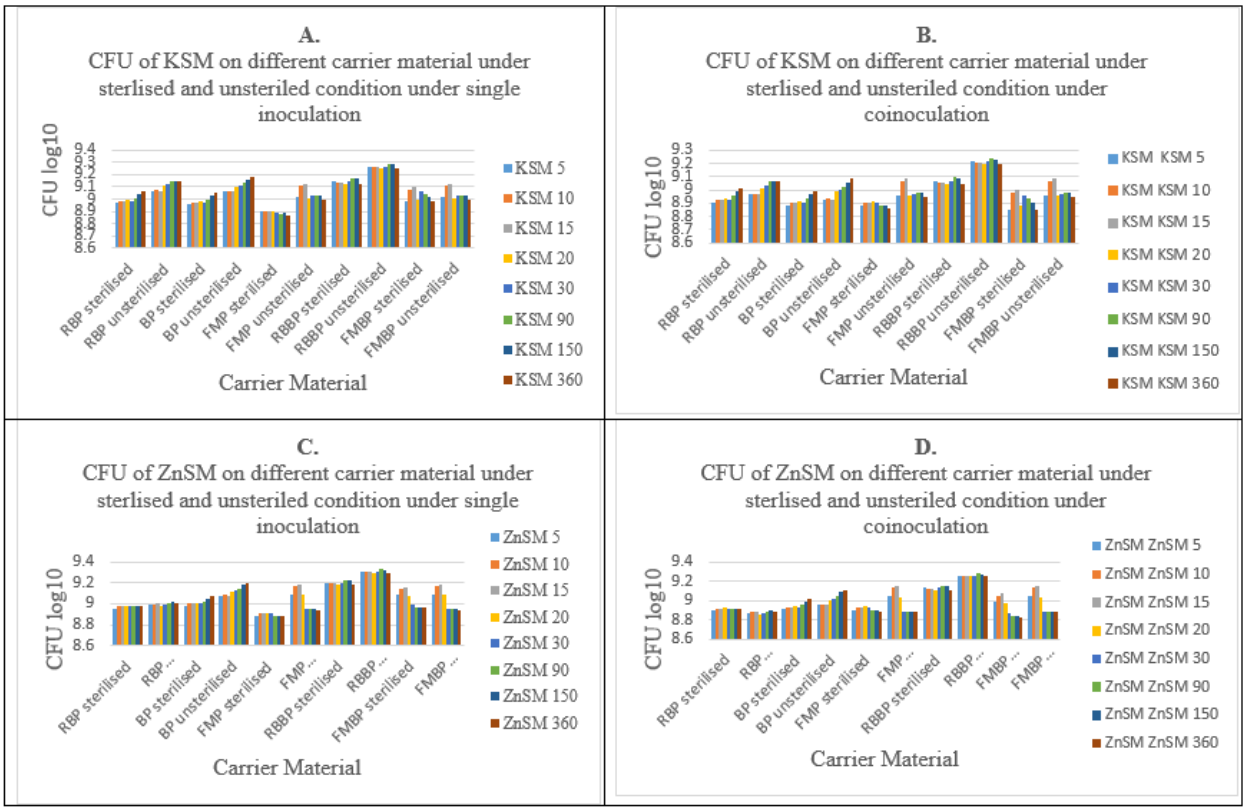

Figure 3: CFU of PGPM (KSM and ZnSM) on different carrier materials under sterilized and unsterilized conditions for single and multiple inoculants.

Beside carrier material and consortium development the study also revealed the implication of sterilization in carrier development. The results showed that sterilization had no significant effect on CFU of intended PGPM. Lack of contaminationcould be attributed to the heat in the motor mill when milling carrier materials which needs additional study. The study results are similar to those obtained by other researchers doing commercial research[24,41]who found that sterilization processes is used for efficiency in inoculant production while carrier material has an implication on quality and quantity of PGPM.

The study concludes that RBBP is a potential carrier material for inoculant and that sterilization has no implication on contamination but decrease in CFU.

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