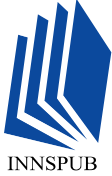

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# Evaluation of bio-wastes for multiplication of *paecilomyces lilacinus*

**Z. M. Mwathi*[1], W. M. Muiru[2], J. W. Kimenju[2], P. Wachira[3]**

[1] *Crop Science Department, Karatina University, Karatina, Kenya*

[2] *Department of Plant Science and Crop Protection, University of Nairobi, Nairobi, Kenya*

[3] *School of Biological Sciences, University of Nairobi, Nairobi, Kenya*

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## Abstract

Three agricultural based solid carriers namely barley, rice bran and rice husks were used in this experiment for continuous production of *Paecilomyces lilacinus*. The objective of this experiment was to establish the carrier that supported the highest growth of the fungi. A completely randomized design replicated four times was conducted in agriculture laboratory at Karatina University. The variable considered in this experiment was the colony forming units in each carrier at day 7, 14, 21 and 28 days after inoculation. Data collected was analyzed using Analysis of variance (ANOVA) with portable Genstat package. Separation of Means was done using Duncan's Multiple range test at $p<0.05$.In this experiment, barley exhibited the highest spore count of $3.38 \times 10^8$ on the 28$^{th}$ day of the experiment. Barley was followed closely by rice bran with a spore count of $2.99 \times 10^8$. With time there was an increment in the number of spores from week one of inoculation to the fourth week. Growth of this fungus was significant in barley carrier. This led to the conclusion that barley can be considered as a suitable carrier that can be used for continuous production of *Paecilomyces* spp. fungi for effective management of nematodes in bananas.

* **Corresponding Author:** Z. M. Mwathi ✉ zmuthoni@karu.ac.ke.

**Introduction**

Plant parasitic nematodes are known to cause high levels of economic loss in a range of agricultural crops. This contributes to huge yield losses in a variety of crops (Sikora &Fernandez, 2005). There has been a concern over the use of chemicals for pest and disease management and this has resulted to research in other control alternatives (Hutchinson *et al.*, 1999; Nico *et al.*, 2004; Kiewnick & Sikora, 2006). Toxicity in the nematicides currently in the market has been raising a lot of concerns due to risks of health hazards to the farmers, consumers and the environment at large (Gerhardson, 2002).

Biological control has attracted a lot of attention in the management of pests and diseases as an alternative to the chemical control (Mucksood & Tabreiz, 2010). This strategy has long been viewed a better alternative to use of synthetic nematicides for management of nematodes due to their safety to the user and the environment, their adaptability and also possibility of continuous production of biological control agents in soils with high organic matter content (Shanthi & Rajendran, 2006). A good number of scientists have carried out research work on various biological control agents. A research conducted by Sharon *et al* reported reduced root galling on tomato by *Meloidogyne javanica* (Sharon *et al.,* 2001; Kiewnick & Sikora, 2006) after soil pre-plant treatment with peat bran preparation in *Trichoderma harzianum*. Kerry and Hidalgo-Diaz Kerry & Hidalgo-Diaz, 2004) carried out a research on control of root-knot nematodes by use of nematophagus fungi *Pochonia chlamydosporia*. This method of pest and disease control has for a long time been taken as an alternative to inorganic control of nematodes. *Paecilomyces* sp. has the potential for biological control of nematodes (Amala & Naseema, 2012). This soil fungus has been reported to interfere with nematode population densities and has been gaining popularity due to its capability to manage plant parasitic nematodes. (Mucksood & Tabreiz, 2010). Studies have been carried out on plant parasitic nematodes where some strains of *P. lilacinus* have been used as biocontrol agents against nematodes (Khan *et al.,* 2003; Gortari *et al.,* 2008).

There is a need for research for better and effective biocontrol products for use for control of plant parasitic nematodes. Lack of reliable and readily available carriers (Mucksood & Tabreiz, 2010) for continuous production of the fungus has been a major challenge in this field of research. In order for entomopathogenic fungi to be assessed successfully in the field conditions, there is a need to produce it in bulk in a suitable carrier that would support its highest growth (Jagadeesh *et al*, 2008). Several studies have been performed on the successful utilization of agro-industrial residues successfully and by-products for mass production of *P. lilacinus* (Pandey *et al.,* 2000; Soccol & Vandenberge, 2003). Various carriers evaluated include different oil cakes and grains like (Gogoi &Neog , 2003); coconut oil cake, cow-dung, neem oil cake, vermin-compost, wheat grain tender nut husk, coir pith and sorghum (Gulsar *et al.,* 2006) sugarcane by-products (Somaseka *et al.,* 1998) and plant leaves (Sikora &Fernandez, 2005;Verma *et al.,* 2004). The main objective of this present study was to establish the best carrier that can be used for continuous production of the fungus.

**Materials and methods**

This experiment involved use of waste materials from agricultural products that are cheap to source. Three solid substrates were sourced from a local agrovets. Barley, rice bran and rice husks were assessed for continuous production of the fungi *Paecilomyces lilacinus*. Barley was sourced from Nyeri county.

The part of barley used was the seed which was blended to break it down in fine pieces to facilitate faster growth of the fungi. Rice bran and rice husks were sourced from Nice rice millers- based at Kirinyaga county. The rice husks were also blended to reduce it to fine particles for ease of fungus growth. Commercial Paecilomyces lilacinus fungus traded as Mytech WP a product of Dudutech Kenya was used in this study. This experiment was set up in a completely randomized design with four replications.

*Procedure for substrates preparation*

A weight 65g of Sabouraud Dextrose agar (SDA) was suspended in 1 liter of sterile distilled water and heated to completely dissolve the media. The dissolved media was sterilized by autoclaving at 121 °C for 15 minutes (Thet and Saisamoin, 2012). The molten SDA was poured in sterile petri dishes and allowed to solidify in a laminar flow for about 45 minutes. Commercial nematophagus fungi (*Paecilomyces lilacinus*) were weighed at 12.5g. The fungi were mixed thoroughly with 50 ml of distilled water. The nematophagus suspension was serially diluted up to seventh dilution. A micropipette was used to transfer 100µl of the serial diluted suspension in the solidified media.

The fungi were allowed to grow in the SDA media in the incubator set at 25°C and was harvested on the 5th day for seeding. The three different substrates namely barley, ground rice husks and rice bran were weighed to obtain 50 g of each, put in 250 ml conical flasks and autoclaved at 121°C for 15 minutes Hutchinson *et al* .,1999; Thet and Saisamoin, 2012). Discs of the growing nematophagus fungi that had been cultured in petri-dishes were cut using a cork borer of 1 mm diameter (Thet and Saisamoin, 2012) and seeded in the sterilized carriers. Discs were cut at the center (Thet and Saisamoin, 2012) of the petri-dish and seeded at the centre of the conical flasks that contained the carriers. This gave uniform sized discs to ensure uniformity in population of the spores growing on the three different types of carriers. Conical flasks containing the 1mm seeded fungus discs were placed in an incubator set at 25°C. Observations were made on the 7$^{th}$, 14$^{th}$, 21$^{st}$ and 28$^{th}$ day to assess the rate of development and sporulation of the fungus. In each day of observation, analysis was done to determine the colony forming units in each substrate.

*Enumeration of the spores*

A weight of 0.25g of conidia was scooped from each of the conical flasks set in the incubator and mixed thoroughly with 250ml of sterile distilled water. The suspension was centrifuged at 4000 rpm for 5 minutes. The suspension was passed through a double layered muslin cloth. The filtrate was placed on a haemocytometer and later viewed using a microscope. The same procedure was repeated for the other three weekly observations and recorded on weekly basis for a period of one month.

*Statistical analysis*

Analysis of Variance (ANOVA) was done using Genstat 12 Portable. Separation of means was done using Duncan's Multiple Range Test.

**Results**

From this study it was observed that the *P. lilacinus* fungi was able to grow in the three substrates formed, but the rate of growth was influenced by the amount of nutrients in each carrier (Mucksood and Tabreiz, 2013).

**Table 1.** Mean sporulation of *P. lilacinus* in different substrates.

| | Spore count = x 10$^8$spores | | | |
|---|---|---|---|---|
| | 7th | 14th | 21st | 28th |
| Substrate | C.F.U/ML | C.F.U/ML | C.F.U/ML | C.F.U/ML |
| Rice husks | 123.0a | 141.5a | 255.7a | 256.8a |
| Rice bran | 245.0c | 265.0b | 290.5b | 299.5b |
| Barley | 129.2b | 256.5b | 306.8c | 338.0c |
| GM | 165.75 | 221.0 | 284.3 | 298.1 |
| LSD | 2.983 | 10.17 | 9.04 | 11.02 |
| CV | 1.0 | 2.7 | 1.8 | 2.1 |

N.B: CFU–Colony forming units, GM-Grand mean, LSD-Least significant difference, CV-Coefficient of variation. Data are means of four replications.

My findings indicate that growth was evident in the three substrates used, though there were no nutrients added. Among the three solid substrates evaluated, rice bran recorded the highest spore count in the first 7 and 14 days of $2.45x10^8$ spores/ml and $2.65x10^8$ spores/ml followed by barley with $1.29\ x10^8$ spores/ml and $2.56x10^8$ spores/ml and lastly rice husks with $1.23x10^8$ spores/ml and $1.41x10^8$ spores/ml respectively. A significant difference was recorded in all the sampling times in the colony forming units except on day 14 where rice bran and barley substrates did not differ significantly. In the first two weeks, rice bran differed significantly from barley and rice husks. In the 3rd (day 21) and 4th week (28), barley recorded the highest spore count 3.06x108 spores /ml and $3.38x10^8$ spores/ml) which was followed closely by rice bran ($2.90x10^8$ spores/ml and $2.99x10^8$ spores/ml) but differed significantly from rice husks (Table 1). From the results obtained, barley performed better than the other two substrates on the 28th day. Barley substrate was ranked top in supporting the highest sporulation of nematophagus fungi while rice bran was ranked second. The high numbers of spores achieved in barley substrate was due to the high nutrients content in the substrate that facilitated the highest growth rate of the fungus compared with the other two substrates. My findings concurred to results obtained by Humber (Humber, 2008), that indicated that growth of entomopathogenic fungi is influenced by the provision of nutrients.

## Conclusion

It can be concluded that barley was the preferred media for continuous production of *P. lilacinus* fungus which can be used within one month while retaining its effectiveness.

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