*Pak. J. Bot.*, 35(3): 409-414, 2003.

# SCREENING OF SUBSTRATES FOR MASS PRODUCTION OF BIOCONTROL AGENTS

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**Abstract**

Eleven different substrates viz., rice grain, sorghum grain, millet grain, cotton cake, mustard cake, wheat straw, rice straw, saw dust, sugarcane bagasse, sugarcane ash and wheat bran were used for the mass production of biocontrol agents viz., *Paecilomyces lilacinus, Trichoderma harzianum, Gliocladium virens and Rhizobium meliloti.* Rice grain, sorghum grain, millet grain and wheat bran were found suitable substrates for mass production of *P. lilacinus and R. meliloti.* Good growth of *T. harzianum* and *G. virens* was observed on sorghum grain followed by millet grain, rice grain, wheat bran, wheat straw, rice straw and sugar cane bagasse. Oil cakes, sugar cane ash and saw dust were found not suitable substrates for multiplication of biocontrol agents. The inoculum multiplied and stored in plastic bags remained viable for upto 360 days at 30°C.

## Introduction

The soilborne fungal pathogens play a major role in the development of root rot disease complex on many important field and horticultural crops which often results in the death of plants. Since soil applied pesticides are costly and produce environmental hazards, crop resistance to disease is the ideal means of controlling disease. Although many crops have little or no resistance to certain plant pathogens, use of microbiol antagonists in the biological control of plant disease is an alternative method for disease control that would also protect our environment from the use of hazardous chemicals (Lumsden & Locke 1989). Several fungi and bacteria have received considerable attention in the control of soilborne root infecting fungi like *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium* spp., and root knot nematodes (Ghaffar, 1978, 1988 and 1992). The main obstacle in the replacement of chemicals with biocontrol agents is their mass production. Studies were therefore carried out to develop simple, cheap and effective method for mass production of microbial antagonists for field application and plant disease control.

## Materials and Methods

Culture of *P. lilacinus* (KUCC 244) obtained from Dr. P. Jatala, Lima, Peru, *T. harzianum* (KUCC 155), *G. virens* (KUCC 464) isolated from soil and *Rhizobium meliloti* isolated from *Medicago sativa* (KUCC 139) obtained from USDA (3DOA-1) present in the Karachi University Culture Collection (KUCC) were used. Rice grain, sorghum grain, millet grain, cotton cake, mustard cake, wheat straw, rice straw, saw dust, wheat bran, sugarcane bagasse and sugarcane ash were used as organic substrate for mass multiplication of biocontrol agents. The substrates were soaked for 2 hrs in containers and 100g of grains and 50g of other substrates were transferred in polyethylene bags. The bags were sealed and then sterilized in an autoclave at 15psi for 20 minutes. The

substrates in polyethylene bags were inoculated by injecting with spore suspension of cultures of *P. litacinus. T. harzianum, G. virens* and *R. meliloti* @ 2 ml/150g substrate. There were 3 replicates of each treatment. The inoculated substrates were stored at 30°C and their population determined with the help of haemocytometer after 15, 30, 60, 90, 180 and 360 days interval.

**Results**

Initial population of *P. lilacinus* at 0-day was $1.3x10^7$ cfu/g on grains and $2.6x10^7$ cfu/g on other substrates. Of the 11 substrates used, growth of *P. lilacinus* was observed on rice grain, sorghum grain, millet grain and wheat bran only as compared to wheat straw, rice straw, sawdust, oil cakes, sugarcane bagasse and sugarcane ash (Table 1). Population of *P. lilacinus* increased with the increase in time.

**Table 1. Population of *Paecilomyces lilacinus* multiplied on different substrates during storage at 30°C.**

| | | Storage time (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Substrates | 0 | 15 | 30 | 60 | 90 | 180 | 360 |
| 1. | Rice grain | $1.3x10^7$ | $4.2x10^8$ | $8.0x10^8$ | $1.5x10^9$ | $3.6x10^9$ | $3.6x10^9$ | $2.5x10^8$ |
| 2. | Sorghum grain | $1.3x10^7$ | $2.0x10^8$ | $7.2x10^8$ | $4.0x10^8$ | $7.0x10^8$ | $2.7x10^8$ | $9.7x10^7$ |
| 3. | Millet grain | $1.3x10^7$ | $1.7x10^8$ | $5.0x10^8$ | $1.3x10^9$ | $2.2x10^9$ | $1.5x10^9$ | $1.5x10^8$ |
| 4. | Cotton cake | $2.6x10^7$ | - | - | - | - | - | - |
| 5. | Mustard cake | $2.6x10^7$ | - | - | - | - | - | - |
| 6 | Wheat straw | $2.6x10^7$ | - | - | - | - | - | - |
| 7. | Rice straw | $2.6x10^7$ | - | - | - | - | - | - |
| 8. | Saw dust | $2.6x10^7$ | - | - | - | - | - | - |
| 9. | Sugarcane bagasse | $2.6x10^7$ | - | - | - | - | - | - |
| 10. | Sugarcane ash | $2.6x10^7$ | - | - | - | - | - | - |
| 11. | Wheat bran | $2.6x10^7$ | $1.3x10^8$ | $3.8x10^8$ | $4.1x10^9$ | $1.8x10^9$ | $2.7x10^9$ | $8.7x10^8$ |

Similarly, growth of *T. harzianum* was observed on rice grain, sorghum grain, millet grain, wheat straw, rice straw, sawdust, sugarcane bagasse and wheat bran. Oil cakes and sugarcane ash were not found suitable substrates for the growth of *T. harzianum.* From an initial population of $1.4x10^7$ cfu/g on grains and $2.8x10^7$ cfu/g on other substrates, the population of *T. harzianum* increased with the increase in time. Highest population of *T. harzianum* was observed on sorghum grain ($2.1x10^9$ cfu/g) and millet grain ($1.3x10^9$ cfu/g) followed by wheat bran ($8.4x10^8$ cfu/g), rice straw, ($3.8x10^8$ cfu/g), rice grain ($2.4x10^8$cfu/g), wheat straw ($2x10^8$ cfu/g), sugarcane bagasse ($4x10^7$ cfu/g) and sawdust ($1.9x10^7$ cfu/g) after 60 days of storage. After 360 days of storage, population of *T. harzianum* increased to $6x10^9$ cfu/g on rice grain and $2.4x10^9$ cfu/g on sorghum grain, whereas the population on wheat straw was $4.6x10^7$ cfu/g, on rice straw $1.7x10^7$, on sugarcane bagasse $2.2x10^7$ cfu/g and zero on sawdust (Table 2). Of the 11 substrates used, sorghum grain, millet grain, wheat bran and rice grain were found more suitable substrates for the multiplication of *T. harzianum* during storage as compared to oil cakes and sugarcane ash.

**Table 2. Population of *Trichoderma harzianum* multiplied on different substrates during storage at 30°C.**

| | | Storage time (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Substrates | 0 | 15 | 30 | 60 | 90 | 180 | 360 |
| 1. | Rice grain | $1.4x10^7$ | $1.9x10^8$ | $1.6x10^8$ | $1.5x10^9$ | $2.9x10^8$ | $6.5x10^8$ | $6.0x10^9$ |
| 2. | Sorghum grain | $1.4x10^7$ | $2.0x10^8$ | $1.5x10^8$ | $4.0x10^8$ | $2.7x10^9$ | $5.5x10^9$ | $2.4x10^9$ |
| 3. | Millet grain | $1.4x10^7$ | $5.5x10^7$ | $1.1x10^8$ | $1.3x10^9$ | $2.5x10^9$ | $5.5x10^9$ | $8.2x10^8$ |
| 4. | Cotton cake | $2.8x10^7$ | - | | - | - | - | - |
| 5. | Mustard cake | $2.8x10^7$ | - | - | - | - | - | - |
| 6 | Wheat straw | $2.8x10^7$ | $5.5x10^7$ | $6.0x10^8$ | $2.0x10^8$ | $5.5x10^8$ | $5.0x10^8$ | $4.6x10^7$ |
| 7. | Rice straw | $2.8x10^7$ | $1.6x10^7$ | $1.6x10^8$ | $3.8x10^8$ | $4.5x10^7$ | $1.4x10^8$ | $1.7x10^7$ |
| 8. | Saw dust | $2.8x10^7$ | $8.0x10^6$ | $2.0x10^6$ | $1.9x10^7$ | $2.0x10^6$ | $5.6x10^7$ | - |
| 9. | Sugarcane bagasse | $2.8x10^7$ | $2.5x10^7$ | $5.0x10^7$ | $4.0x10^7$ | $1.7x10^7$ | $2.0x10^8$ | $2.2x10^9$ |
| 10. | Sugarcane ash | $2.8x10^7$ | - | - | - | - | - | - |
| 11. | Wheat bran | $2.8x10^7$ | $9.0x10^7$ | $2.0x10^7$ | $8.4x10^8$ | $2.7x10^8$ | $6.0x10^8$ | - |

**Table 3. Population of *Gliocladium virens* multiplied on different substrates during storage at 30°C.**

| | | Storage time (days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Substrates | 0 | 15 | 30 | 60 | 90 | 180 | 360 |
| 1. | Rice grain | $1.7x10^7$ | $6.7x10^9$ | $5.7x10^8$ | $1.5x10^9$ | $1.2x10^9$ | $7.7x10^9$ | $2.6x10^8$ |
| 2. | Sorghum grain | $1.7x10^7$ | $2.5x10^8$ | $4.5x10^8$ | $7.5x10^9$ | $1.8x10^9$ | $1.6x10^9$ | $2.5x10^8$ |
| 3. | Millet grain | $1.7x10^7$ | $2.2x10^8$ | $3.7x10^8$ | $3.0x10^9$ | $2.3x10^9$ | $6.7x10^9$ | $3.5x10^8$ |
| 4. | Cotton cake | $3.4x10^7$ | - | - | - | - | - | - |
| 5. | Mustard cake | $3.4x10^7$ | - | - | - | - | - | - |
| 6 | Wheat straw | $3.4x10^7$ | $1.4x10^8$ | 2.0x10 | $9.5x10^7$ | $2.2x10^8$ | $1.6x10^8$ | $8.6x10^7$ |
| 7. | Rice straw | $3.4x10^7$ | $2.1x10^8$ | 2.0x10 | $5.5x10^7$ | $1.4x10^8$ | $2.1x10^8$ | $7.2x10^7$ |
| 8. | Saw dust | $3.4x10^7$ | - | - | - | - | - | - |
| 9. | Sugarcane bagasse | $3.4x10^7$ | $1.2x10^8$ | 1.4x10 | $2.5x10^8$ | $1.9x10^8$ | $9.5x10^8$ | $2.1x10^7$ |
| 10. | Sugarcane ash | $3.4x10^7$ | - | - | | - | - | - |
| 11. | Wheat bran | $3.4x10^7$ | $4.0x10^8$ | $2.0x10^8$ | $4.5x10^8$ | $4.2x10^8$ | $6.7x10^8$ | - |

**Table 4. Population of *Rhizobium meliloti* multiplied on different substrates during storage at 30°C.**

| | | Storage time(days) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Substrates | 0 | 15 | 30 | 60 | 90 | 180 | 360 |
| 1. | Rice grain | $1.5x10^7$ | $3.8x10^9$ | $2.6x10^9$ | $2x10^9$ | $2.3x10^8$ | $2.6x10^7$ | $1.0x10^7$ |
| 2. | Sorghum grain | $1.5x10^7$ | $6.0x10^8$ | $1.3x10^8$ | $1.8x10^8$ | $1.9x10^8$ | $2.8x10^7$ | $1.0x10^7$ |
| 3. | Millet grain | $1.5x10^7$ | $9.1x10^8$ | $1.1x10^8$ | $9x10^7$ | $1.9x10^8$ | $2.3x10^7$ | $2.7x10^7$ |
| 4. | Cotton cake | $3.0x10^7$ | - | - | - | - | - | - |
| 5. | Mustard cake | $3.0x10^7$ | - | - | - | - | - | - |
| 6 | Wheat straw | $3.0x10^7$ | - | - | - | - | - | - |
| 7. | Rice straw | $3.0x10^7$ | - | - | - | - | - | - |
| 8. | Saw dust | $3.0x10^7$ | - | - | - | - | - | - |
| 9. | Sugarcane bagasse | $3.0x10^7$ | - | - | | - | - | - |
| 10. | Sugarcane ash | $3.0x10^7$ | - | - | | - | - | - |
| 11. | Wheat bran | $3.0x10^7$ | $3.2x10^8$ | $6.9x10^8$ | $6.0x10^8$ | $2.2x10^8$ | $2.8x10^8$ | - |

Initial population of *G. virens* at 0-day was $1.7x10^7$ cfu/g on grains and $3.4x10^7$ cfu/g on other substrates. After 60 days of storage, population of *G. virens* was highest on sorghum grain ($7.5x10^9$ cfu/g) followed by millet grain ($3x10^9$ cfu/g), rice grain ($1.5x10^9$ cfu/g), wheat bran ($4.5x10^8$ cfu/g), wheat straw ($9.5x10^7$ cfu/g) and rice straw ($5.5x10^7$ cfu//g). Oil cakes sawdust and sugarcane ash were not found suitable substrates for multiplication of *G. virens*. After 90 and 180 days storage, the population of *G. virens* showed an increase on wheat straw, rice straw and sugarcane bagasse. After 360 days of storage, population of *G. virens* decreased on all the substrates used (Table 3). Of the 11 substrates used for multiplication of *G. virens*, sorghum grain, millet grain, rice grain and wheat bran were found more suitable substrates as compared to oil cakes, sawdust and sugarcane ash.

Initial population of *R. meliloti* was $1.5x10^7$ cell/g on grains and $3.0x10^7$ cell/g on other substrates. Growth of *R. meliloti* was observed on rice grain, sorghum grain, millet grain and wheat bran. Population of *R. meliloti* after 30 days incubation was highest on rice grain followed by millet grain, sorghum grain and wheat bran (Table 4). Population of *R. meliloti* decreased with the increase in time on rice grain, sorghum grain and millet grain whereas it reduced to zero on wheat bran (Table 4). Of the 11 substrates used for multiplication of *R. meliloti*, rice grain, sorghum grain and millet grain were found suitable substrates for multiplication of *R. meliloti*.

## Discussion

A biocontrol formulation should possess several desirable characteristics such as ease of preparation and application, stability, adequate shelf life, abundant viable propagules and low cost of production (Churchill, 1982; Lisansky, 1986). In the present studies on mass production of biocontrol agents, a low quality of broken rice grain were used. Rice grain, sorghum grain and millet grain were found suitable substrates of mass multiplication of *P. lilacinus* as compared to cotton cake, mustard cake, wheat straw, rice straw, sugarcane bagasse and sugarcane ash whereas Sharma & Trivedi (1987) observed maximum and rapid growth of *P. lilacinus* on oil cakes of sesamum followed by cotton, linseed, mustard and groundnut oil cake. Jatala (1981, 1985) and Bansal *et al.*, (1988) also reported that rice grain was a good substrate for multiplication of *P. lilacinus*. Similarly maize, millet, sorghum, chopped lucerne, sesame oil cake, mungbean husk have been found suitable for multiplication of *P. lilacinus* (Davide, 1985; Hasan, 1988; Sharma & Trivedi, 1986, 1987). Stephan & Al-Din (1987) reported that peeled rice grains were the best growth medium for *P. lilacinus* followed by unpeeled rice grains, wheat and barley grains. Sporulation of *P. lilacinus* was maximum on rice grains than on rice straw, wheat straw or sorghum grains (Shahzad & Ghaffar, 1989) which is similar to the results of the present study. There are also reports where sporulation of *P. lilacinus* was enhanced when gram was used as substrate compared to other substrate (Zaki & Bhatti, 1991).

In the present study good growth of *T. harzianum* was observed on sorghum grain and millet grain followed by sugarcane bagasse, rice grain, wheat straw and rice straw with lowest population on saw dust. Such similar reports have been made by Papavizas & Lewis (1981) and Papavizas (1985) where grain seeds and meals, bagasse, straws, wheat saw dust individually or in combination were found suitable for multiplication of

biocontrol agents, *T. harzianum* was produced in large quantities which maintained good viability without specialized storage system (Harman, 1996).

The biocontrol product which contain living organisms when formulated must also have an acceptable shelf life without special storage requirements so that their viability is maintained. Population of biocontrol agents increased with the increase in time after multiplication on different suitable substrates with maximum population of *P. lilacinus* found on rice grain, millet grain and sorghum grain, *T. harzianum* on sorghum grain and millet grain and *Gliocladium virens* on sorghum grain, millet grain and rice grain *R. meliloti* on rice grain, millet grain and wheat bran. The inoculum can be multiplied in polyethylene bags in small quantities on a mass scale and sold in the market for use by the farmers in the control of plant diseases instead of using pesticides which are hazardous.

**Acknowledgement**

This work was carried out under the grant of Pakistan Science Foundation (PSF) which is sincerely acknowledged.

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(Received for publication 30 December 2002)