CROP PROTECTION PROGRAMME

Promotion of sustainable approaches for the management of root-knot nematodes on vegetables in Kenya

R8296 (ZA 0568)

FINAL TECHNICAL REPORT

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1. Executive Summary

The project aimed to verify and promote sustainable approaches to the management of root-knot nematodes (*Meloidogyne* spp.) through the use of micro-organisms, cultural techniques and plant resistance. Through research participation smallholders have been acquainted with the production constraint and of potential ways of its allieviation. The novelty has been the inclusion of the naturally occurring biological control organisms within the cropping system preferred by (or acceptable to) the farmers. This will become an accepted practice when the national regulatory authority approves the use of "biopesticides", when organisations have the capability to mass produce these products at an acceptable price and when there are appropriate channels to deliver them to the smallholder grower community. Progress has been achieved with each of these stages ensuring the long term benefit to all sectors of the Kenyan vegetable-producing community.

The amendments to the pesticide legislation to include those defined as "biopesticides" such as *Pochonia chlamydosporia* and *Pasteuria penetrans* have been drafted and await final legislative ratification. This will then enable companies to produce and market these products.

Dudutech have refined the production of *P. chlamydosporia* and are producing 40 kg of formulated product per week; this can be scaled up to 100 kg /week when required. The estimated cost of treating 1 ha is £10 /1500 KSh (1 kg/ha).

There is no loss of pathogenicity in sequential batches of *P. chlamydosporia*, previously this had been observed with some isolates. The Standard Operating Procedures developed in collaboration with colleagues in CENSA, Havana, Cuba with support from Rothamsted International and DFID were applied in this project and appear to have obviated the problem of inoculum stability in mass culture. It is unlikely that a producer would sub-culture inoculum more than a few times. The stability of the inoculum means that a manufacturer of the fungus would need to conduct full inoculum quality tests infrequently and the development of more rapid assays is not necessary.

Methods of scaling-up the *in vivo* system of mass-producing *P. penetrans* are being investigated but as yet no consistent process has yet been developed because of the dependence on uniformly warm temperatures and large supplies of nematode hosts on which to produce the parasite. Spore yields of 3.26 – 5.6 x 10^6 per g of dried tomato root have been achieved (R8218).

P. chlamydosporia was applied through a drip system spores were not evenly delivered along the line of the drippers. This could be a technical problem relating to the size of chlamydospores. Distribution of spores of another biocontrol fungus, *Paecilomyces lilacinus,* through such a system is better, probably because spores of this fungus are smaller and consistent in size.

Approximately 100 farmers have participated in the evaluation of the nematode management strategy that has included biological control agents, nematode resistant varieties and crop rotations. Farmers' perceptions and preferences of some different tomato varieties show that a variety Monyala with root-knot nematode resistance was ranked best (of four) including the standard cultivar Cal J which is nematode susceptible.

These technologies are now available for wider use and will be of benefit to the poorer farmers growing nematode susceptible crops and indirectly to the wider community who buy these crops. In addition to improving the technology for the commercial export producers (a requirement of the European Union rules for harmonisation of use of pesticides [COLEACP]) commercial growers who grow crops on contract to the exporting companies will also benefit.

Two scientists (D. Karanja and J. Daniel) obtained their PhDs (2004) from work done on this project (and the preceding project R 7482)

2. Background

Root-knot nematodes (*Meloidogyne* spp.) are a concern to many smallholders and commercial producers involved in intensive vegetable production in Kenya. Although not all smallholders will recognise nematodes as a production constraint up to 20% of vegetable producers use nematicides when growing tomatoes (Oruko & Ndun'gu, 2001;see FTR for R7472) as up till the present, this is the only control strategy available other than not growing the crop. Nematodes are difficult to manage. They are soil-dwelling animals, too small to see without a microscope and cause symptoms on the root system which can be recognised only when a plant is lifted from the soil. For this reason smallholders, who are reluctant to dig up plants, usually overlook the presence of nematodes and attribute above-ground symptoms (small fruit, poor growth, yellow foliage, wilting during hot weather) to other biotic or abiotic constraints. This often results in use of inappropriate chemicals. Familiarisation of smallholders with nematodes has been a part of the objectives of this project since the desire to adopt biological and cultural control strategies will only result from an understanding of what nematodes are and how they damage crops.

All sectors of the vegetable industry are keen to adopt production methods based on low pesticide regimes. This change in attitude that is occurring worldwide is due to increased health and environmental conciousness of food production techniques. The impact of this in Kenya is, initially, on the commercial export producers who are attempting to meet European Union standards and codes of practice through the initiative on harmonisation of pesticide use COLEACP (www.coleacp.org) and the EurepGAP standards; an initiative of retailers belonging to the Euro-Retailer Produce Working Group (EUREP) a partnership of agricultural producers and their retail customers aiming to meet accepted standards and procedures for the global certification of Good Agricultural Practices (GAP) (www.eurep.org).

Because of the links between the commercial export sector and independent growers through the out-grower system, the new pest management technologies will also be introduced to the wider constituency of smallholder producers. In addition, locally based organisations and associations such as the Kenya Institute of Organic Farming (KIOF) represents a locally driven initiative of concerned farmers committed to organic production. During the course of the DFID RNRRS 1995-2005, the environment in which vegetables are produced has changed and the needs for biological systems of pest management have become greater. (See Lenné and Ward (2004) *Lesson learning study from the vegetable cluster with special emphasis on the links with the private sector.* Report of project ZA 0630, Crop Protection Programme.

Within this changing scene of improving systems of vegetable production, root-knot nematodes are one pest for which a sustainable management system needs to be developed. The opportunities for developing this have been strengthened with the collaboration of Dudutech (Kenya) Ltd. who have implemented a range of programmes of biological control including the mass production of *Pochonia chlamydosporia* and *Pasteuria penetrans* for managing root-knot nematodes.

The production and promotion of biological control agents for the wider farming community can be implemented only when the appropriate legislation on sale and use of biopesticides is in place. During the course of this project the amendments to the existing laws have been drafted and await approval.

Biocontrol agents of root-knot nematodes can be difficult to manipulate because of location-specific characteristics in terms of the pathogenicity of the natural pathogens and their interactions with abiotic and biotic soil factors.

Whilst overcoming the technical challenges, smallholders must be advised that the processes of biological control may take several crops for the bcas to take effect. The routes of promotion of a nematode control strategy based upon biological control agents will be through demonstrations and the more highly supervised production systems of the commercial export producers and their out-growers.

Through the successful collaboration with CENSA/Dudutech *Pochonia* production technology has been transferred to Kenya and a production system is now in place with the potential to produce 100 Kg of product per week. Dudutech are progressing the development of an *in vivo* production system for *Pasteuria penetrans* (Project R8218) that can be adapted to the local climatic conditions and is economically viable.

3. Project Purpose

The project aimed to verify and promote sustainable approaches to the management of root-knot nematodes through the use of micro-organisms, cultural techniques and plant resistance. Through research participation, smallholders have been acquainted with the production constraint and of potential ways of its allieviation. The novelty has been the inclusion of the naturally occurring biological control organisms within the cropping system preferred by (or acceptable to) the farmers. This will become an accepted practice when the national regulatory authority approves the use of “biopesticides”, when organisations have the capability to mass produce these products at an acceptable price and when there are appropriate channels to deliver them to the smallholder grower community. Progress has been achieved with each of these stages which will ensure the long-term benefit to all sectors of the Kenyan vegetable-producing community.

4. Research Activities

The project had advisory, technical and promotional components. Collaborators within this and other projects within the vegetable cluster have been involved in discussions with and in the provision of information to the authorities involved with the biopesticide registration process. The mass production of the two principal biocontrol agents for root-knot nematodes has been addressed and some detailed studies on the characteristics of isolates of *Pochonia chlamydosporia* and *Pasteuria penetrans* have been completed. The quality of sequential batches of *P. chlamydosporia* has been verified. On-farm demonstrations have introduced nematode management through the integration of biocontrol with use of resistant varieties and have been assessed by the participating farmers. Using participatory evaluation with farmer groups in NGO training schemes, a profile of the characteristics of those farmers most likely to adopt technologies has been devised. A link with CABI field schools and KIOF has been used to produce training and promotional materials including posters, instructional leaflets and media items for use by all collaborating organisations.

Where appropriate, the methods of the activities listed below are fully described as appendices.

4.1 Compare different isolates of the biological control agents being deployed

This research activity investigated differences in saprophytic growth in soil and the rhizosphere of eight selected isolates of *P. chlamydosporia* and in their virulence. The relationship between saprophytic competitiveness and virulence was studied to assess if there was a fitness cost for the most effective pathogens of nematodes.

4.2 Develop rapid methods to evaluate inoculum quality of *P. chlamydosporia* to assure quality control of sequential batches of mass-produced inoculum.

Research in this activity is aimed to develop rapid tests, which could be used by a manufacturer to assess the quality of inoculum produced and the development of attenuation. The sensitive measurement of virulence and rhizosphere colonization are the main objectives of these tests. Chlamydospore production can be readily assessed by extracting the spores and counting diluted aqueous suspensions in a haemocytometer. However, as some manufacturers' labs have access to a spectrophotometer, a more rapid assessment was tested.

4.3 Baiting technique for *P. chlamydosporia*

This method can be used to detect activity of the fungus in soil at population densities known to provide significant control of root-knot nematodes. It can be used to determine the need to apply further inoculum of the fungus; use of the assay would require evaluation in local conditions.

4.4 Evaluation of the mass production of the microbial agents

4.4.1 Production of *Pochonia chlamydosporia*

Production method

P. chlamydosporia var. *chlamydosporia* strain K12T2 (originally isolated and maintained by Rothamsted Research / CABI) was used for production purposes at Dudutech K. Ltd. Development of the Standard Operational Procedures (SOPs) for production was done in collaboration with Dr. Leopoldo Hidalgo of CENSA, as detailed under point 4.2, and was based on those originally developed by CENSA / Rothamsted. Small changes to the original SOPs were made as necessary to adapt the process to the use of the materials locally available in Kenya.

4.4.2 Other related activities for *Pochonia chlamydosporia*

Besides the activities related to the development of a production method for the standard strain of *P. chlamydosporia*, a survey was carried out in the attempt to isolate new and possibly more effective strains.

4.4.3 Mass production of *Pasteuria penetrans* (Pp)

A simple in vivo production system was established using tomato plants. At the beginning of the project this was done on an experimental scale, while in 2004 a dedicated production tunnel with an area of 650 m^2 was set up in Naivasha.

Experiments on root growth

In order to obtain a better root growth for potential host crops, (thus increasing the numbers of nematodes that might be infected with *P. penetrans*) various locally available substrates were tested using sweet pepper and melon as host crops.

4.5 Test isolates and isolate mixture of *P. penetrans* on Kenyan root-knot nematode populations.

Four Kenyan isolates of *Pasteuria penetrans* were tested for attachment capability to two root-knot nematode populations from Naivasha.

4.6 The usage of drip irrigation for dispersal of spores

In extending the crop protection project into the functional aspect of dispersal of biocontrol agents to the target soil, the idea of using drip irrigation as a modern medium was explored in the following experiments. This concept evolved from the existing system of application of fertilisers and chemical pesticides through drip irrigation. Drip irrigation precludes the use of fungal biocontrol agents in a form other than spores. This appears to be a deviation from the small-farmer focus of the Kenya project. But in a rain-fed system of cropping the use of drip irrigation itself is gaining importance in vegetable crops as an input that can improve water use efficiency and thus reduce costs. Using drip irrigation in crop protection activities can further reduce costs. It may also be seen as insurance against the failure of the seasonal rains or as a means of fitting one more crop within a year. This phenomenon is becoming acceptable even among small farmers, despite the initial costs. A reflection of the phenomenon is the promotion of drip irrigation among vegetable farmers in Kenya by the Kenyan Agriculture Research Institute with one of their own systems designed to be affordable by small farmers.

Several manufacturers of irrigation equipment now have products that cater to the small units. The system developed at KARI is fed by gravity. A slightly more sophisticated system manufactured and supplied by Netafim, an Israeli company is also gravity-fed, but has pressure compensated drippers that ensure more uniform dispersal of spores.

Experiment I

The objective of the experiment was to study the distribution of spores of *P. chlamydosporia* and *Paecilomyces lilacinus* through the drip irrigation system designed for small farmers.

Experiment II

To study the impact of the wetting pattern on the distribution of spores in the rhizosphere of the plants and to explore the advantages in splitting or reducing dosage of spores.

4.7 Conduct ongoing farmer assessment of technologies, including participatory planning in the use of biological control agents (BCAs) and resistant varieties within their own farms/crop rotations and factors that encourage or constrain adoption

This activity aimed at verifying and promoting sustainable approaches for the management of root-knot nematodes (*Meloidogyne* spp.) through incorporation of naturally occurring microorganisms with cultural techniques such as crop rotation and use of resistant varieties.

On-farm trials were set up in Mwea and Kibirigwi. These areas are known for tomato production for both commercial and home consumption. The trials involved the use of *Pasteuria penetrans* and *Pochonia chlamydosporia*, crop rotation and tomato varieties with resistance to root-knot nematodes. The on-farm trials were participatory and involved farmers practising organic and inorganic farming methods. The first category consisted of farmers from Maragua practising organic farming under rain fed conditions. The second category was inorganic farmers from Kibirigwi practising production under rain fed and irrigated conditions and farmers from Mwea practising production under irrigation.

The farmer participation was aimed at ensuring sustainability of the technologies. This involved participation by the farmers in all aspects of assessment of the technologies. To this end, socio-economic assessments involving the farmers were undertaken.

4.7.1 Objectives of the assessments

1. To identify and score characteristics used by farmers when comparing/selecting tomato varieties
2. To assess the relative performance of specific tomato varieties
3. To describe the existing rotations and explore how BCAs/rotations could fit in
4. To enable farmers to assess the BCAs/rotation trials
5. To identify factors that encourage or constrain adoption of the technologies

4.7.2 Methodology (Appendix 6)

4.8 Using participatory evaluation with farmer groups in NGO training schemes, devise a profile of the characteristics of those farmers most likely to adopt technologies

4.8.1 Methods

The research process involved a two-staged interdependent data gathering process. Initially the outcome beliefs and social referent common to the target population regarding BCA application were identified through focus group discussions with tomato farmers in the different trial areas in Kenya. (A participative process.) The second stage was to incorporate the identified salient outcome beliefs and pertinent referents in a structured questionnaire, which was then applied to farmers that had either participated in or observed trials of the BCA for nematode control. The sampling process was restricted to those that had some degree of exposure to the BCA trials as the agent is not available as yet in the market place, nor known of amongst tomato farmers in general. In all 61, useable responses were acquired -a relatively small sample. The survey was conducted by face to face interviews. The surveys were carried out by CABI-Africa.

4.9 Knowledge Transfer

In 2004, Dr Leopoldo Hidalgo-Diaz from CENSA, Havana, Cuba spent three months at Dudutech Ltd., Naivasha, Kenya transferring the technology, including the SOPs for the production of *P. chlamydosporia*. Thus, there is now the ability to produce local isolates of the fungus in the quantities required for evaluation in the field in Kenya and elsewhere in East Africa. Dr Hidalgo- Diaz also visited Rothamsted Research to learn molecular diagnostic methods for the identification of specific isolates, which could also be used to determine the levels of contamination in mass-produced inoculum.

5. Outputs

5.1 Compare different isolates of the biological control agents being deployed

The growth of different isolates of *P. chlamydosporia* in sterilised and non-sterilised soils of different texture (compost, sandy loam and loamy sand) differed significantly (P < 0.001) between isolates (Fig. 1). Regardless of the soil type, the abundance (determined as the number of colony forming units (cfu) on the selective medium) of isolate Pc 309 was greatest whilst isolate Pc 60 was the least numerous isolate. In general, isolates significantly colonised compost soil more than (P < 0.001) sandy loam or loamy sand soils. Except for isolates Pc 399 and Pc 60 in loamy sand soil, all the isolates were significantly (P < 0.001) more abundant in sterilised soils compared to non-sterilised soils. The growth of different isolates was significantly different in different compartments (P < 0.001); the growth of all the isolates was greater in compartment A (closest to source of inoculum) while least on compartment C (farthest to the source). Irrespective of the soil type and condition, isolates Pc 309, Pc 399 and Pc 40 grew and spread consistently more (P < 0.001) compared to the other isolates in all the sections.

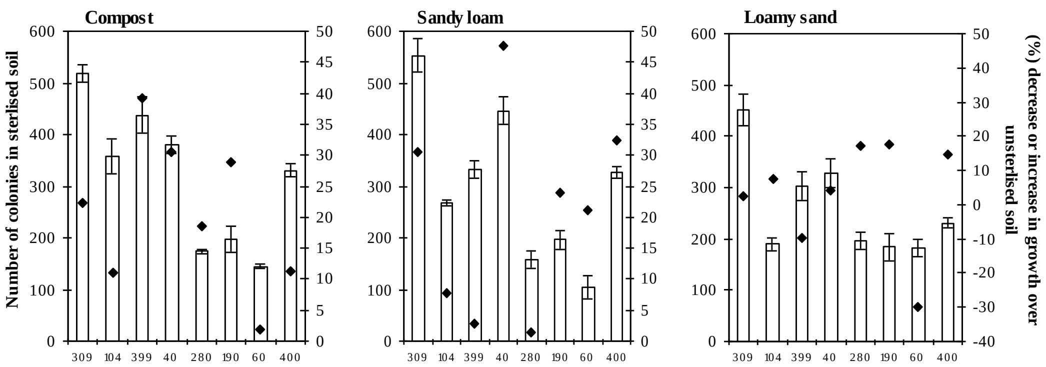


Fig. 1. Growth of different isolates of *Pochonia chlamydosporia* in sterilised soil and the increase/reduction in growth compared to that in non-sterilised soil [Columns represent the number of colonies on a selective medium while diamonds represent percentage decrease (+ve) or increase (-ve) in growth in un sterilised soil]

5.1.1 Estimation of fungal saprotrophic growth: Colonisation of eight isolates of *P. chlamydosporia* in the rhizosphere

Pochonia chlamydosporia isolates differed (P < 0.05) in their ability to colonise pea and mustard rhizospheres (Fig. 2). In general, isolates colonised the pea rhizosphere more (P < 0.001) than the mustard rhizosphere in both soils. Rhizosphere colonisation of the isolates was generally greater (P < 0.05) in compost soil compared to the sandy loam soil, regardless of plant species. When the colonisation of the isolates was compared, in the sandy loam soil, isolate Pc 40 colonised most extensively and Pc 280 least. A positive correlation between the densities of the isolates in the sterilised sandy loam soil and the colonisation of pea (r = 0.736; P = 0.037) and mustard (r = 0.719; P = 0.044) rhizospheres, was observed. Similar trends were recorded in sterilised compost soil (for pea, r = 0.769; P = 0.0258 and mustard, r = 0.706; P = 0.050).

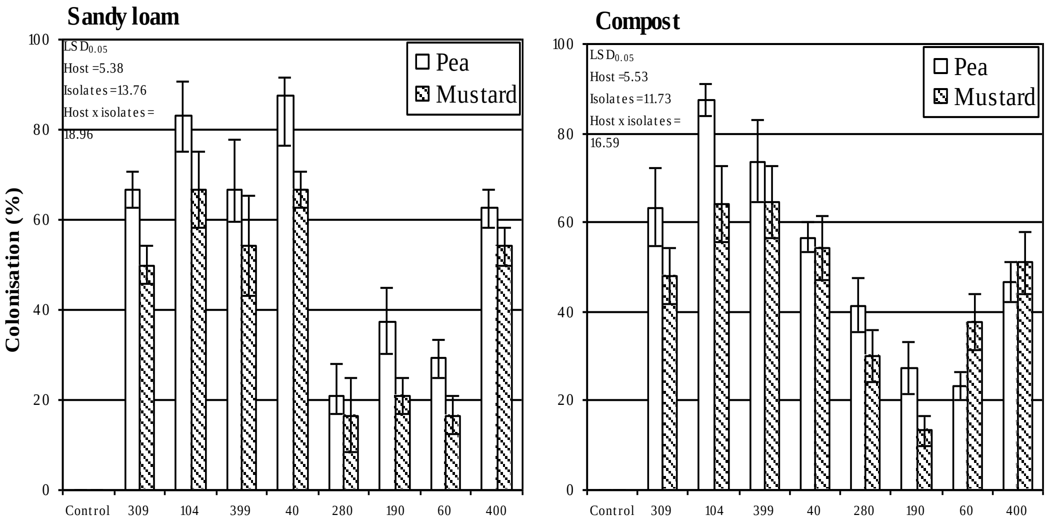


Fig. 2. Extent of colonisation of pea and mustard rhizospheres by different *Pochonia chlamydosporia* isolates in sterilised sandy loam soil and compost.

5.1.2 Estimation of fungal parasitic growth: virulence of eight isolates of *P. chlamydosporia* against eggs of *Meloidogyne* spp.

The method indicates that inoculation of 80-100 viable chlamydospores directly on egg masses results in limited egg infection by the fungal isolates (Fig. 3). There was a difference ($P < 0.05$) among isolates to parasitise *Meloidogyne* spp. eggs. Isolate Pc 280, Pc 399, Pc 190 and Pc 400 parasitized most eggs while isolate Pc 104, Pc 40 and Pc 60 least.

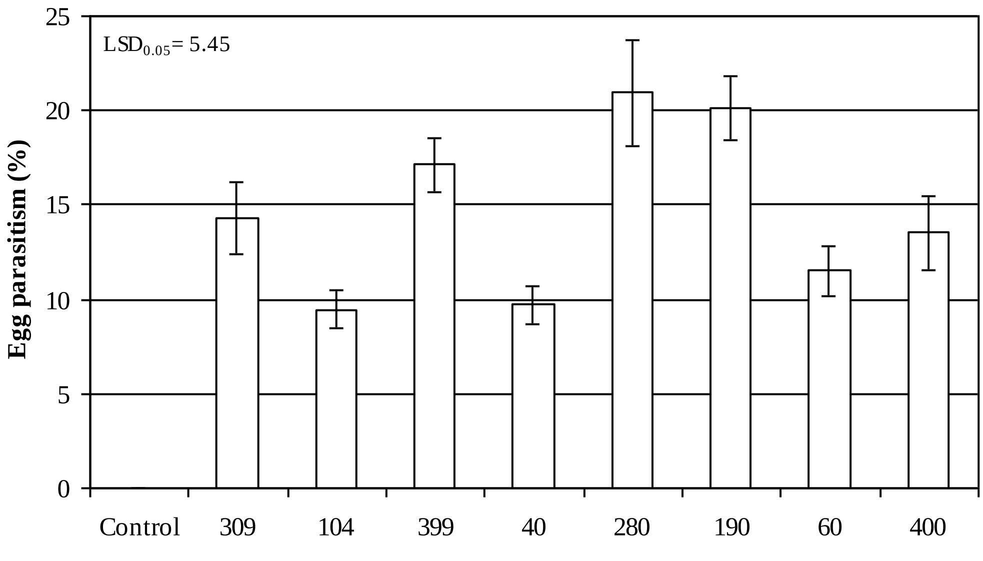


Fig. 3. Differences between *Pochonia chlamydosporia* isolates in their ability to parasitise *Meloidogyne hapla* eggs using a ninety-six-well microtitre plate technique. Error bars represent ±1 standard error.

General conclusions from activity 4.1

- Isolates of *P. chlamydosporia* differ significantly in their saprotrophic growth in soil and in the rhizosphere and in their pathogenicity against root-knot nematode eggs.
- There is a correlation between the ability of isolates to colonise soil and to colonise the rhizosphere.
- The fastest growing isolates in sterilised soil were not necessarily those that grew best in non-sterilised soil.
- Observations in these tests and in others conducted in our laboratory suggest that there may be a fitness cost to parasitism (see activity 4.2) and those isolates that are most pathogenic against nematode eggs are less successful colonisers of soil and rhizosphere compared to less virulent isolates. However, there are isolates that are good colonisers and virulent and these are the ones that should be selected as potential biological control agents.

These results indicate the need for careful selection of isolates of *P. chlamydosporia* as biological control agents. Such selection is not cheap to perform and in our experience approximately 5% of isolates screened meet the criteria required for chlamydospore production, rhizosphere colonisation and virulence. Such selection has, therefore implications for the cost of the development of the control agent if it has to be repeated for each country that only accepts the release of indigenous isolates.

5.2 Develop rapid methods to evaluate inoculum quality of *P. chlamydosporia* to assure quality control of sequential batches of mass-produced inoculum.

Comparing the two spore suspensions tested (Fig. 4), no significant differences were found by using either 0.1 % agar or 0.1% peg (ρ =0.979, df =1, r^2=0.8598). The regression line (Fig.4 and 5) shows a positive, significant relation between the variables, showing that it is possible to establish a relation between the absorbance and spore concentration (r^2=0.982 for peg, r^2=0.9703 for agar)

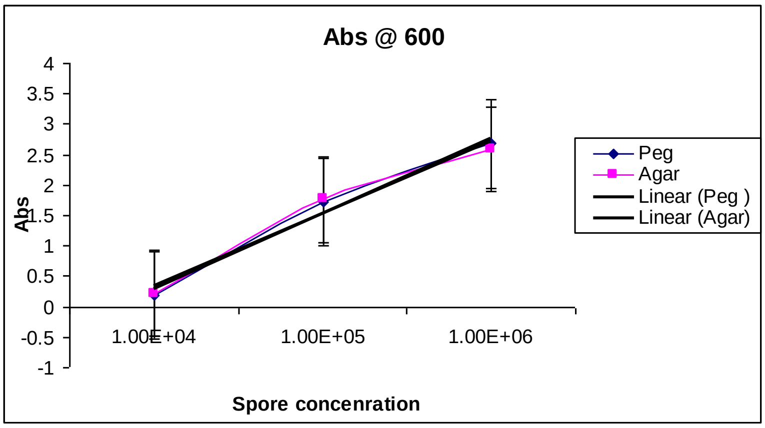


Fig. 4. Absorbance (Abs) of different spore concentrations of an isolate of *Pochonia chlamydosporia* suspended either in 0.1 % Peg or 0.1% agar.

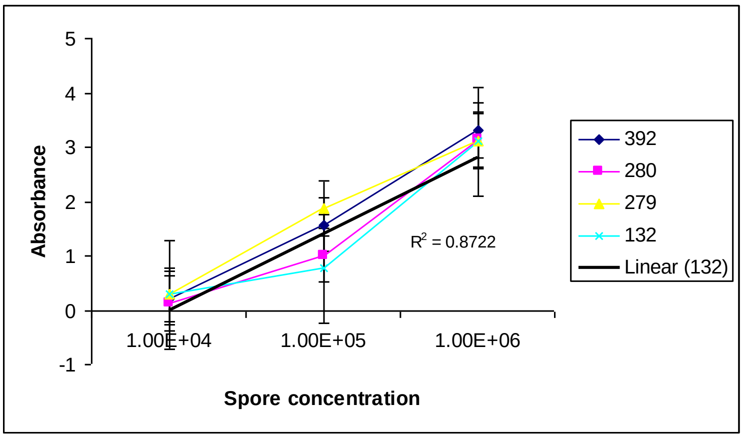
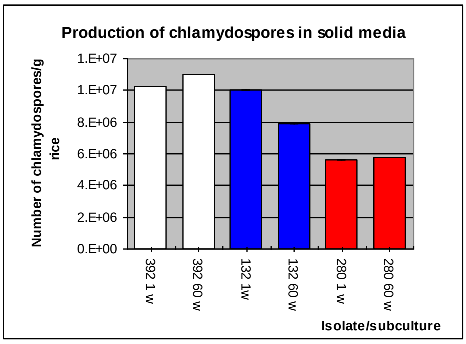


Fig. 5. Relation between absorbance level and chlamydospore concentrations of different isolates of *Pochonia chlamydosporia*. No differences between isolates were detected (ρ>0.05, df=3) using ANOVA single factor analysis.

When more isolates were tested (Fig. 5), the same positive and significant relation was detected. No significant differences between isolates were found (ρ = 0.861, df =3) when tested for all spore concentrations. The regression line traced in Fig 4. is for isolate Pc132 and was the lowest absorbance obtained for the group of isolates tested.

The results obtained have indicated a positive and significant relation between the two methods and so, the quantification of spore production by different isolates is possible by the determination of the absorbance level of a spore suspension. However, a standard curve of each isolate has to be calculated before subcultures are tested. Presumably, the chlamydospores produced by different isolates differ in size.

Subcultures from different isolates of *Pochonia chlamydosporia* (Pc 280, Pc 392 and Pc 132) were recovered from long term storage (freeze dried) and tested for possible changes in inoculum quality that could occur during maintenance of the fungus. Results showed no differences between the 1st and the 60 th weeks of subculturing, concerning differences in chlamydospore production in solid media (Figs 6 and 7).



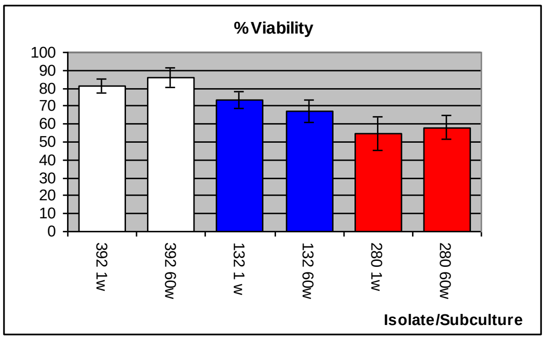


Fig. 6 and 7. Chlamydospore production in a rice medium and the viability of the chlamydospores of different isolates of *Pochonia chlamydosporia*.produced from inoculum derived from fresh cultures and those sub-cultured 60 times. No significant differences between the subcultures were found (p>0.05).

5.2.1 Saprophytic ability evaluation test

Significant differences were found when isolates were compared (ρ<0.001). Pc 400 was the best root coloniser, followed by Pc10 and then Pc 280 (Fig. 8). These differences were clearly seen on the agar plates; Pc 400 developed abundant mycelium and colonized 91 % of the roots on average after 12 days, Pc 10, 69% and Pc 280 only 11% (Fig 8). The root colonisation varied with time (ρ <0.001) and was greater than 80% after 16 days for two of the isolates (Fig. 8).

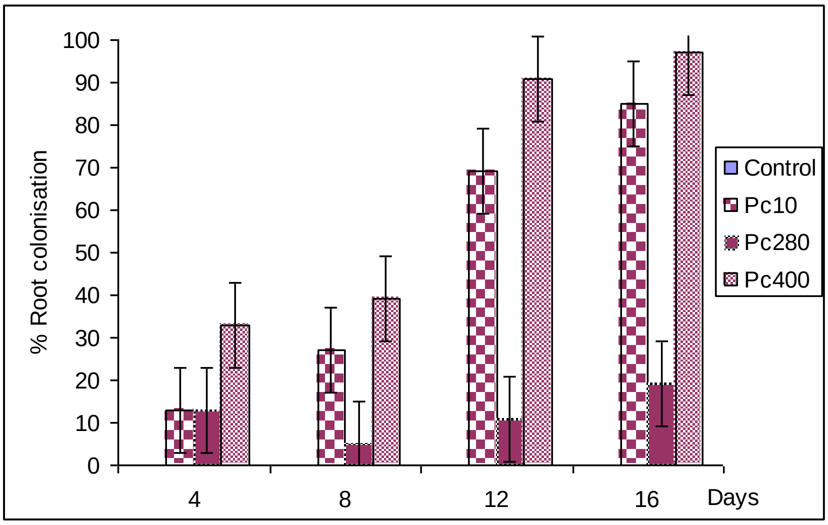


Fig. 8. Root colonisation evaluation by three isolates of *Pochonia chlamydosporia* inoculated using a suspension of chlamydospores.

Results have shown that the method could detect differences between isolates but the minimum time required to evaluate colonisation and pick differences within isolates has still to be determined. At present, it appears to be 8-10 days but this needs testing with more isolates.

Using this test, rhizosphere colonisation was compared for cultures of *P. chlamydosporia*, which were fresh (1 wk-old) or from freeze-dried cultures that had been sub-cultured on a weekly basis for 60 wk, as described above. There was a slight but insignificant reduction in the rate of colonisation of the rhizosphere by the inoculum derived from repeated sub-cultured material (Fig 9), which is unlikely to affect the performance of the fungus as a biological control agent.

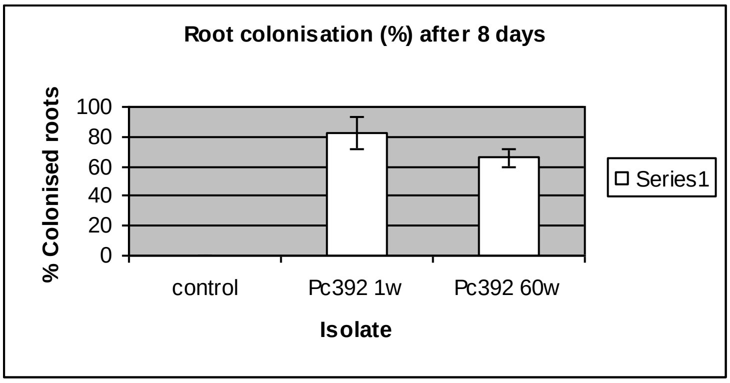


Fig. 9. Comparison of the rate of maize rhizosphere colonisation from seed applied inoculum of *P. chlamydosporia*, which was from a 1 week-old culture or a 60 week subculture.

5.2.2 Virulence test

Again, there were no significant differences in the proportion of eggs colonised in plates containing the different subcultures; all isolates significantly increased egg mortality compared to the uninoculated control plates (Fig. 10).

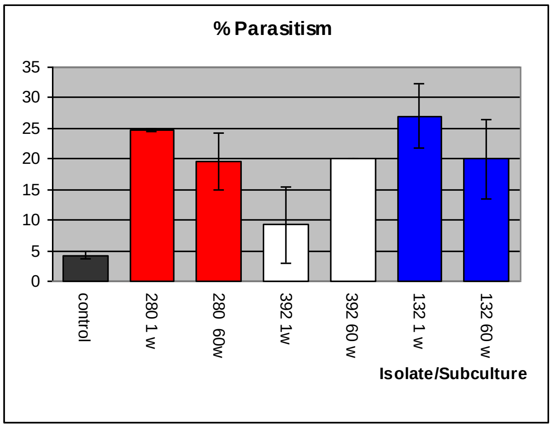


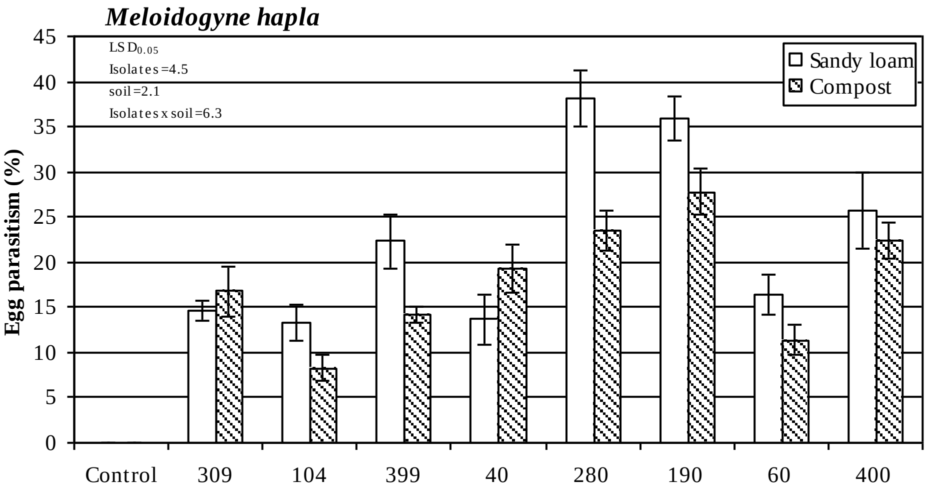
Fig. 10. Comparison of infection rates of *M. incognita* eggs by three isolates of *P. chlamydosporia*, which was from a 1 wk-old culture or a 60 wk subculture.

Conclusions from activity 4.2

- The isolates of *P. chlamydosporia* tested were stable in repeated mass-culture for the key selection criteria for potential biological control agents: Chlamydospore production, ability to colonise the rhizosphere and virulence.
- Related research has also demonstrated that the production of key enzymes is also not affected by the number of sub-cultures.
- Attenuated inoculum that lost the ability to produce chlamydospores had been observed with some isolates previously and made the fungus difficult to handle. The Standard Operating Procedures developed in collaboration with colleagues in CENSA, Havana, Cuba with support from Rothamsted International and DFID were applied in this project and appear to have obviated the problem of inoculum stability in mass culture. It is unlikely that a producer would sub-culture inoculum more than a few times.
- The stability of the inoculum means that a manufacturer of the fungus would need to conduct full inoculum quality tests infrequently and the development of more rapid assays is not necessary.

5.3 Baiting technique for *P. chlamydosporia* in soil

The virulence of isolates determined in terms of the parasitism of nematode eggs in a bait test indicated that there were marked differences ($P < 0.001$) in their ability to infect *M. hapla* and *G. pallida* eggs, regardless of the soil texture (Fig. 11). When parasitism in two soils was compared the parasitism of eggs of both nematode species was generally greater ($P < 0.001$) in sandy loam soil compared to compost soil. Interestingly, isolate Pc 280, an isolate from cyst nematode, colonised large number of eggs of both nematode species in both types of soils. In general, isolates from root-knot nematodes parasitised more of the eggs of *M. hapla* while isolates from cyst nematodes infected more eggs of *G. pallida*. A weak but non-significant negative correlation between soil population densities and virulence towards root-knot eggs was observed in both sandy loam and compost soils.



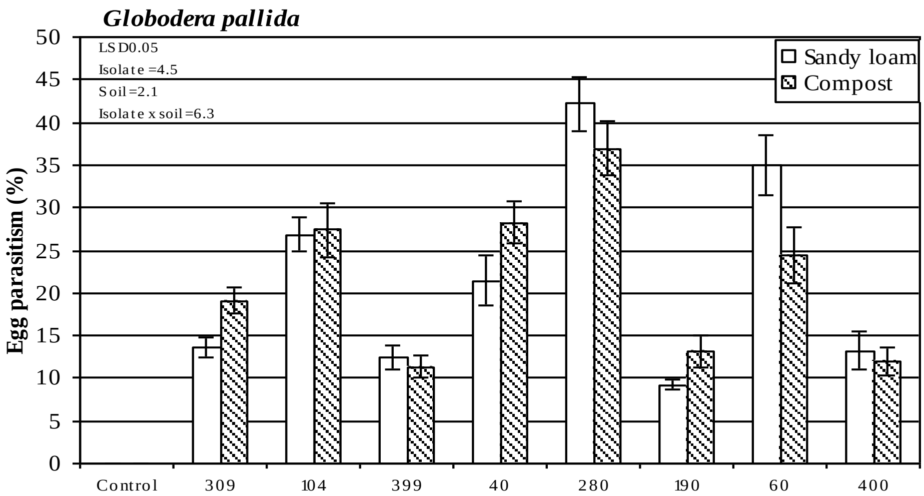
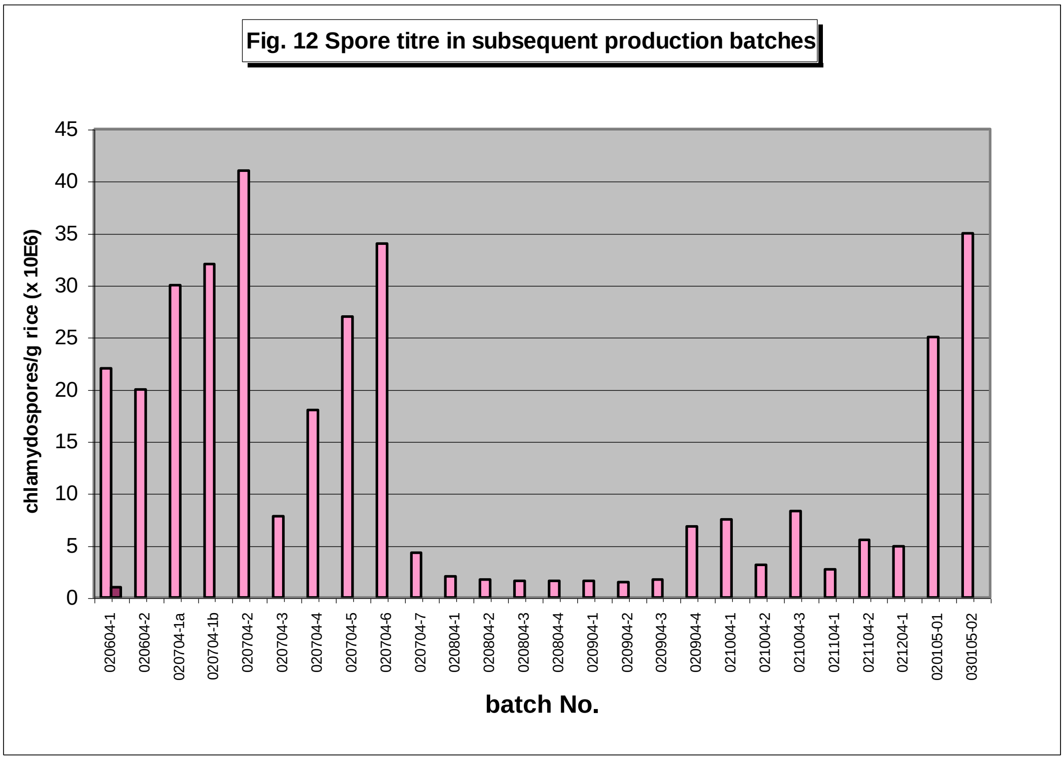


Fig. 11 Differences between *Pochonia chlamydosporia* isolates in their ability to parasitise *Meloidogyne hapla* and *Globodera pallida* eggs using a baiting technique. Data represent means of 10 replications obtained from two individual experiments each with five replicates. Error bars represent ±1 standard error.

5.4 Evaluation of the mass production of the microbial agents

5.4.1 Production of *Pochonia chlamydosporia*

In all, 28 production batches were produced, for a total of 312 kg. Spore production in the various batches ranged from 0.9 x 10^6 to 4.1 x 10^7 chlamydospores/g of dry rice (Fig. 12).

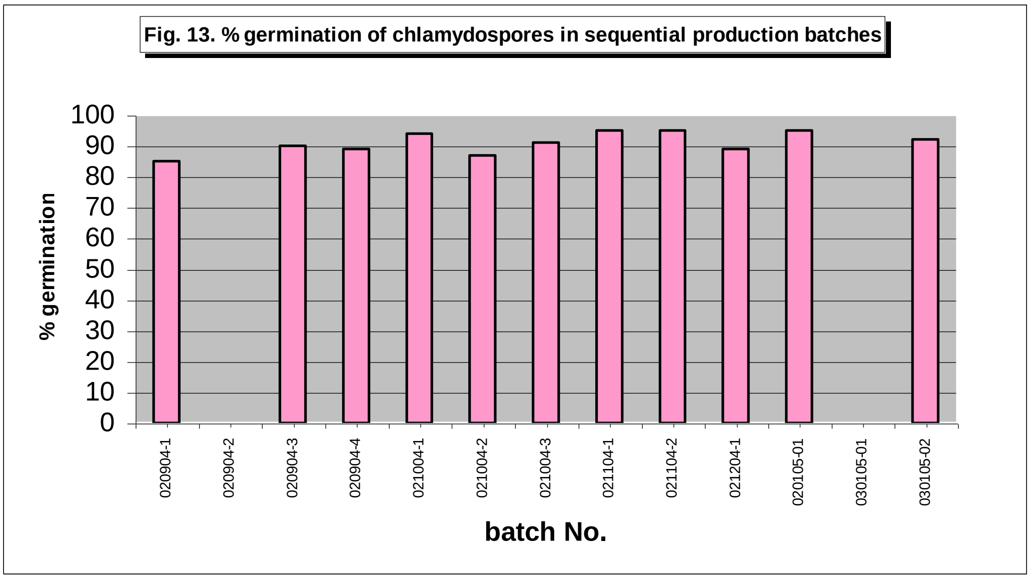


The drastic reduction in the number of spores observed starting with lot 020704-7 was caused mainly by the malfunctioning of the autoclave in Dudutech's new production laboratory, which prevented proper sterilization of the media hence frequent contamination of the production bags. Such problem was finally solved after some months, when spore titers rose again to the expected levels.

In an attempt to improve the production procedure and make it more amenable to scaling up, alternative production methods were also tested. With this purpose large (35 x 60 cm) autoclavable bags with an absolute filter (of the type used for spawn production in the mushroom industry) were tested. Using these bags it was possible to accommodate 2,5 – 3,5 kg of rice/bag, against the 250 g used in standard bags. This would obviously make the production process less labour intensive and more cost-effective. However, although in the filter-bags the initial colonization of the substrate appeared to be very good, the amount of chlamydospores obtained per unit of rice tended to be lower (around 1x10^7 spores/g) than with the smaller bags. Based on the preliminary observations carried out, it is thought that this was due to the fact that sporulation occurs mostly on the surface of the rice mass, but is limited within it. Besides, the large size of the filter caused the media underneath it to dry relatively fast, thus strongly limiting or inhibiting the growth of the fungus on a non-negligible part of the substrate. Unfortunately, these aspects could not be investigated more in detail due to the fact that the researcher in charge of the project resigned and could not be replaced immediately. For the same reasons the testing of other production methods (using trays, pellets, etc.) was started but could not be developed further.

5.4.2 Quality control

Moisture content of the final product, spore titer (chlamydospores/gram of dry product) and viability (expressed as % germination of chlamydospores) have all been used as QC parameters for each production batch with the exception of two. Spore germination varied between 85% and 95% (Fig.13), and was not dependent on the level of spores produced in the different batches.



5.4.3 Mass production of *Pasteuria penetrans*

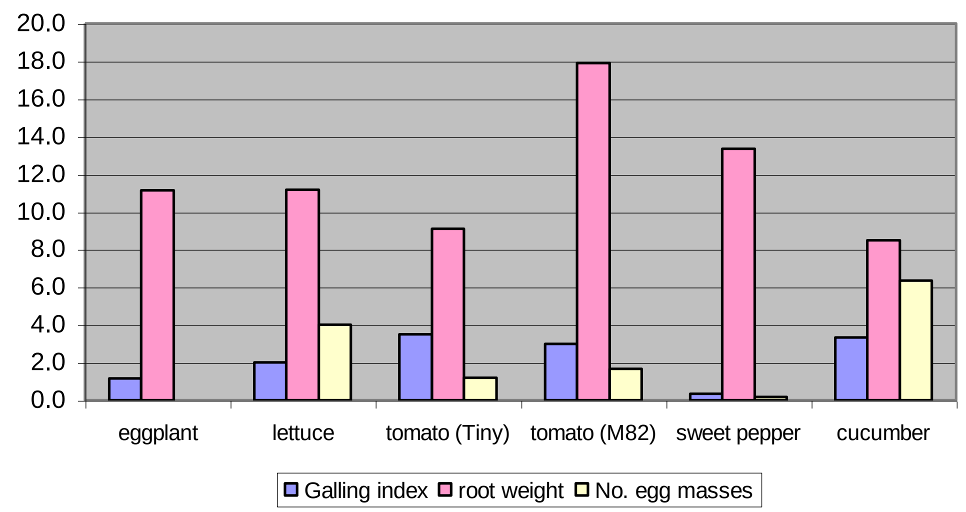
Plate 1. Pasteuria mass production tunnel at Kingfisher farm Naivasha.

By this simple method, at the end of February 2005 a total of 4,975 g of root powder had been obtained, with a titer of Pp spore ranging from 1.25 x10^5 to 7.97x10^6 spore/g of powder, depending on the batch of production. This material is presently being used to carry out semi-field and field trials. The very high variability in the results observed in different batches stresses the importance of developing a system with standardized conditions and not so dependent on climatic conditions. As a matter of fact, low mean greenhouse temperatures between the month of March and August (Appendix 2) resulted in a delayed scaling up of the production of Pp.

5.4.4 Experiment on host suitability

Cucumber has a mass of fibrous roots and had the greatest number of galls with egg masses (Fig. 14). The numerous galls were characterized by being small and discrete. There was secondary infection on the lower part of the root. The plant is a climber and required support for top growth. Lettuce also has a very fibrous root system, but also very weak small roots being easily broken and detached when uprooted or handled. Egg masses were many and well visible on the roots. Tomato var. Tiny Tim was noted to produce big galls. This can be partly due to the roots being bigger than those of lettuce and cucumber. There was secondary infection on the younger roots but not to the level of that observed on cucumber. Tomato variety M 82 was noted to have a bigger root mass than Tiny Tim. The galls on these roots were also noted to be big but not as much as those on variety Tiny Tim. Eggplant was noted to have strong roots and few galls that were difficult to observe. However, there were no egg masses on these roots. On the sweet pepper (*Capsicum*) roots there were few to no galls. The lack of galling in this crop may be an indicator of RKN resistant varieties in the market or that this species is not susceptible to the root-knot nematode population used at Naivasha.

Fig. 14 - Response of different plant hosts to RKN



5.4.5 Comments on *Pasteuria* production

The incorporation of vermicompost in some of the potting media caused up to 3-4-fold increase in the development of the root mass for the host plants tested. This would obviously be beneficial when attempting to develop a production system for both the RKN and *Pasteuria*. However, when juveniles of RKN were inoculated in media containing a high proportion of vermicompost, galling was much reduced, indicating a suppressive effect of vermicompost against these nematodes. This confirms the practical knowledge that addition of large amounts of organic matter to the soil aids in the containment of nematode populations. Additional experiments will be carried out to understand whether such effect is the result of a physical effect of vermicompost on nematode movement, or rather the antagonistic effect exerted by the many microorganisms present in the same.

Despite the improvements obtained during this project, it is felt that the production of *P. penetrans* is still not satisfactory and represents the main constraints to the successful development of this bionematicide. For this reason, more intensive systems which are not so dependent on external conditions (i.e. climatic conditions) will be investigated in the future. One possibility which is envisaged is the use of tissue culture, and recently some steps have been taken in this direction using tomato calluses.

5.5 Test isolates and isolate mixture of *P. penetrans* on Kenyan root-knot nematode populations.

Attachment tests were done to compare the compatibility between 4 Kenyan *Pasteuria* isolates and two nematode populations.

All Pp isolates were able to attach to the Kenyan nematode populations, the isolate from Malakisi gave the best attachment with 100% of juveniles of both populations encumbered with 8 spores after 24 hours. Both nematode populations had similar attachment levels to all *Pasteuria* isolates, this may suggest that the nematode populations may be analogous.

A blend made from equal spore concentrations of the Pp isolates also gave 96 or 100% attachment to the nematodes population, however the mean spore attachment was 4 spores per nematode after 24 hours, lower than the attachment with Malakisi isolate. This might be explained by the smaller proportion of spores from the Malakisi isolate in the mixture. Nevertheless, the mixture gave better attachment on both nematodes than did the Thika, G11 and H22 isolates. When the spore concentration of the mixture was doubled, 100% attachment was achieved after 6 hours, after 24 hours, the mean attachment level on both nematode populations was 11 spores per juvenile.

The objective of making a mixture of isolates was to widen the genetic base of the *Pasteuria* to counteract possible selectivity and specificity of root-knot nematode populations, and these results uphold this hypothesis (Appendix 3)

5.6 The usage of drip irrigation for dispersal of spores

EXPERIMENT I

5.6.1 Flow rate of water

Between the two times of sampling, the difference in flow rate was barely significant (p=0.053). This difference was expected and is caused by the diminishing head of water over time (Table 1). Between flow rates in the 4 lanes highly significant differences were seen (p=<0.001) among Lanes 3 & 4 and Lanes 1 & 2. This highlights the drop in flow rate over distance from the water tank. The compounded effect of diminishing head and distance are seen as the significant differences (p=0.030) in the ‘time x lane’ interaction.

Table 1 Mean flow of water in millilitres in 5 minutes.

Time	Lane 1	Lane 2	Lane 3	Lane 4	Mean
At start	29.17	24.50	12.00	10.60	19.07
At midpoint	32.00	32.00	9.67	8.67	20.58
Mean	30.58	28.25	10.83	9.63	

Time l.s.d.=1.543, p=0.053
Lane l.s.d.=3.616, p=<0.001
Time x Lane l.s.d.=4.575, p=0.030

*5.6.*2 Distribution of spores of *P. lilacinus* through water

There were no significant differences in distribution of spores across time and across the lanes as evidenced by the absence of a significant difference in number of colony forming units counted from the samples (Table 2). However there was a significant difference in the interaction between time and distance of lane from the tank.

Table 2 Mean count of colony forming units of *P. lilacinus* from water samples

Time	Lane 2	Lane 4	Mean
At start	2013	1079	1546
At midpoint	966	1514	1240
Mean	1489	1296	

Time x Lane l.s.d.=924.3, p=0.022

5.6.3 Distribution of spores of *P. chlamydosporia* through water

There were no significant differences in distribution of spores across time and across the lanes as evidenced by the absence of a significant difference (P=>0.05) in number of colony forming units counted from the samples (Table 3).

Table 3 Mean count of CFU of *P. chlamydosporia* from water samples.

Time	Lane 3	Lane 4	Mean
At start	293	180	237
At midpoint	556	344	450
Mean	425	262	

5.6.4 Colonisation of roots

Root colonisation by the two bcas was different (Figs 15 & 16). The control plots showed a high level of contamination.

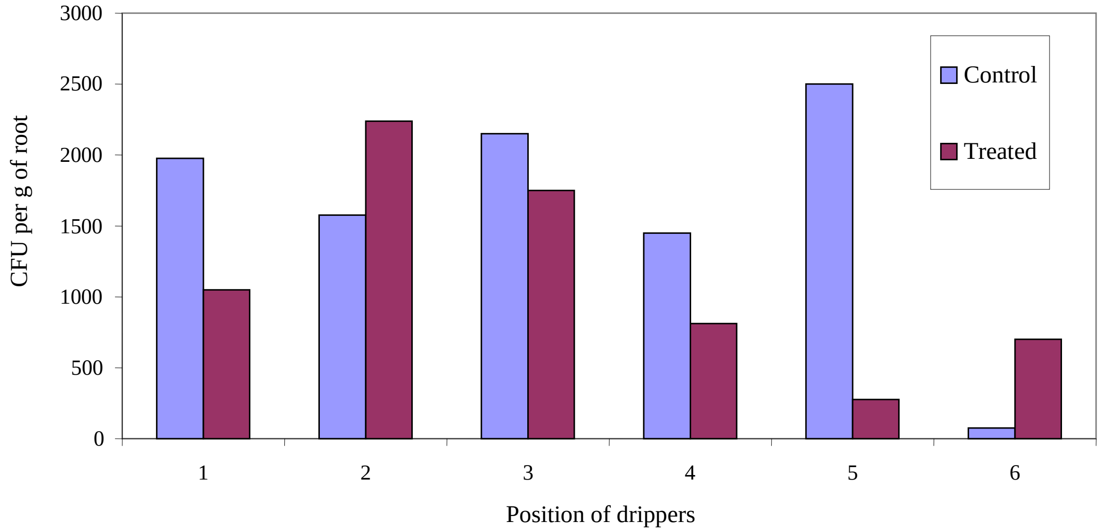


Fig. 15. Root colonisation by *P. chlamydosporia* corresponding to the position of drippers

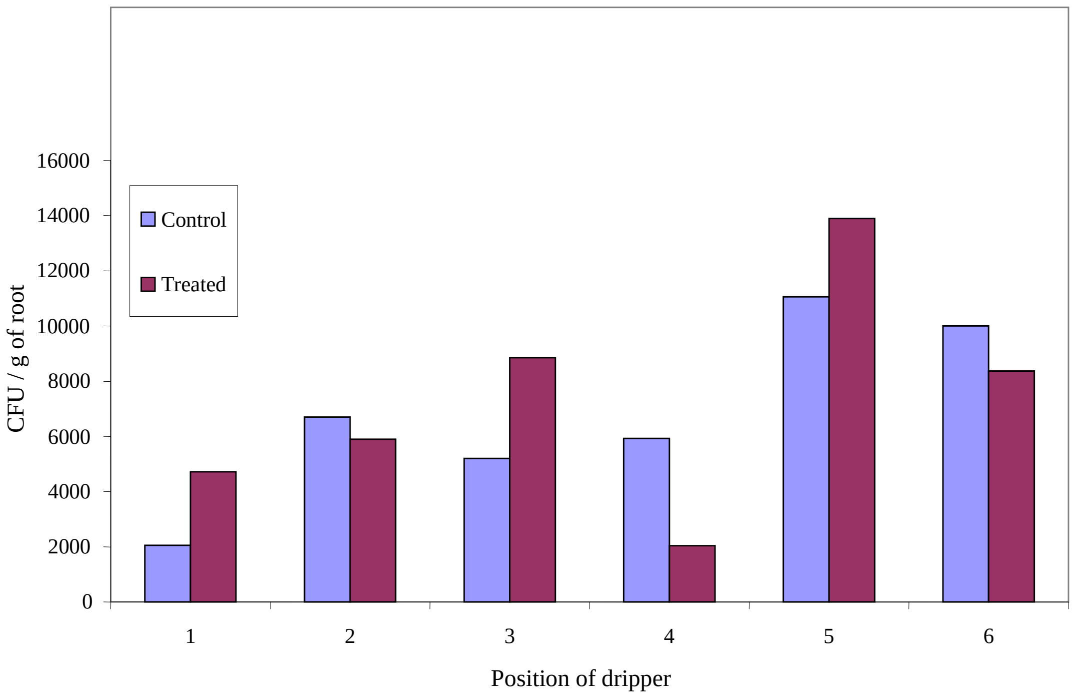


Fig. 16 Root colonization of *Paecilomyces lilacinus* corresponding to position of dripper.

5.6.5 Results and Discussion

EXPERIMENT I

The flow of water through the system is affected by the diminishing water column in the tank over time, and by distance. A similar trend was expected in the distribution of spores. However the movement of spores of *P. lilacinus* shows a similarity only in the interaction between time and distance while the spores of *P. chlamydosporia* have no similarity. The possible explanation may be that spores settle in the distribution lines and in the dripper units and make distribution erratic. As *P. lilacinus* seems to follow the pattern of distribution of water more closely than *P. chlamydosporia* it could be inferred that spores of *P. lilacinus* move more uniformly through the system. This phenomenon could be attributed to the fact that the spores of *P. lilacinus* used in this experiment come from a commercial formulation which uses glucose as a carrier, and which ensures a better dispersal as a suspension. *P. lilacinus* being smaller (spore size 2-3µ compared to 15-20µ of *P. chlamydosporia*) would probably pass through the system with a different sedimentation pattern compared to *P. chlamydosporia*. The dosage of *P. lilacinus* being higher (double) the resulting number of CFU is also seen to be higher. This experiment highlights the following issues related to mechanics of the irrigation system, formulation of the bio-pesticide and biological characteristics of the organisms.

1. A fully homogenous spore suspension in the mixing tank is essential for uniform distribution of spores. Such homogeneity can be achieved only by mechanical agitation of the suspension in the tank.
2. Efficiency in relation to precision of distribution of biocontrol agents may necessitate the use of pumps to maintain the discharge pressure constantly above the pressure at which the drippers are built to operate. Alternatively, because there is no fear of an overdose of spores (other than wastage) a second or third application could be a solution, to ensure the minimum dosage reaching all the target plots.
3. A suitable formulation is necessary to enhance the homogeneity of the suspension and to enable easier transportation down the irrigation lines. The laboratory method of production of spores of *P. chlamydosporia* delivers a final product that would contain starch particles which would quicken the settling of spores of in the irrigation lines. The glucose-based formulation of *P. lilacinus* has an advantage in this regard.
4. The viability of spores may have an effect on the settling time in water. A certain percentage of unviable spores are likely to occur in every batch produced. If there is a difference in mass between viable and nonviable spores, the distribution of viable spores may become less uniform. More studies are required to assess the variability that may occur.
5. The high level of contaminants in the control lines suggests that efforts should be made to thoroughly sterilise the equipment.

EXPERIMENT II

Colonisation of soil by *Pochonia chlamydosporia*, when applied as the split dose (2000 + 3000 spores/g soil) was not significantly different from the full dose of 5000 spores/g soil and neither was there a statistically significant difference in spore distribution at the three sampling points (P=>0.05). Similarly the distribution of spores of *Paecilomyces lilacinus* was also uniform in that there were no significant differences in the cfus at the three sampling points. (Data not presented)

The effects of the application of *Pochonia chlamydosporia* and *Paecilomyces lilacinus* in terms of nematode counts in root and soil were not statistically significant (P=>0.05) and data are not presented.

The levels of soil colonisation by both *Pochonia chlamydosporia* and *Paecilomyces lilacinus* did not show any significant difference between the sampling locations. This indicates that the spread of the fungal biocontrol agents is well distributed, allaying fears that proliferation may occur more at the point of delivery i.e. under the drippers and less away from it. This could either mean that spores in the soil move along with the water corresponding to “onion-shaped” wetting pattern in drip irrigation, or that the fungi can develop and spread within the duration of the crop. In hindsight, sampling of soils in the said locations immediately after application may have thrown light on the movement of spores in soil water. De Leij *et al.*, 1993 (*Nematologica* 39: 250-265) showed that overhead watering promotes better colonisation by *P. chlamydosporia* than moistening from below. Their study also revealed that in a 9-week period of weekly overhead irrigation, the movement of the fungus did not extend outside a 10 cm radius. With drip irrigation it seems that the spread could be better with 3-times-weekly drip irrigation because sampling points were 15 cm apart and the assessment of CFU done at 4 weeks did not vary significantly.

In comparing the progressive effect of the split doses with a single full dose of *P. chlamydosporia*, there seemed to be no significant difference in the levels of soil colonisation. It may be inferred that 2000 spores/g of soil applied at the first instance colonizes the soil the same as 5000 spores applied a month later. But the growth has been mostly in ‘saprophytic’ conditions because there were no nematode egg masses at the beginning. The 2000 spores applied in the first instance would have had less competition for space and nutrients, compared to the 5000 spores applied later. This confirms earlier reports that *P. chlamylosporia* multiplies fastest at lower rates of application (de Leij *et al.*, 1992, *Nematologica* 38:112-122). From the levels of nematode control achieved it may be tempting to say that the splitting of doses has had no differential effect, but because *P. chlamydosporia* is an egg parasite, a true judgment can be made only after a sequence of crops that would have allowed the root-knot nematode to go through several life cycles in varying conditions. From a practical point of view this experiment highlights the ease of application using drip irrigation. Further research may be required to study the movement of root-knot nematodes in relation to the wetting pattern in drip irrigation, which may in turn become an important factor for consideration in biocontrol.

5.7 Evaluation of resistant tomato varieties

Plate 2

Cal J (susceptible)

Monyala (resistant)

5.7.1 Identification and scoring of characteristics for selecting tomato varieties (Appendix 5)

5.7.2 Preliminary assessment of the relative performance of different tomato varieties

Table 4: Preliminary performance of different tomato varieties

Characteristics	Cal J		Monyala		Caltana		Nemonetta		Weight factor
	Raw Score	Weighted score	Raw Score	Weighted score	Raw Score	Weighted score	Raw Score	Weighted score	
Size (4.00)	4	0.88	5	1.1	4	0.88	3	0.66	0.22
Shape (2.67)	1	0.15	3	0.45	3	0.45	5	0.75	0.15
Maturity period (2.67)	2	0.30	4	0.60	3	0.45	2	0.30	0.15
Vigour (2.33)	2	0.26	3	0.39	3	0.39	2	0.26	0.13
Weight (2.00)	3	0.33	2	0.22	4	0.44	3	0.33	0.11
Disease and pest resistance (4.33)	3	0.72	2	0.48	1	0.24	3	0.72	0.24
Weighted average	-	2.64	-	3.24	-	2.85	-	3.02	-
Rank	4		1		3		2		-

Note: The relative importance of each characteristic is given by the scores in the brackets. The corresponding weight factors are ratios of the scores of each characteristic to the total number of scores for all of the characteristics. The weighted scores are the product of the weight factors and the raw scores.

From the initial assessments conducted by the farmers the variety that performed best was Monyala and the worst performer was Cal J.

5.7.3 Final assessment by farmers of the relative performance of different tomato varieties

5.7.3.1 Assessment by Nyagithambo Organic farmers

According to the assessments Monyala emerged as the best variety and Cal-J was rated the worst performer (Table 5).

Table 5: Matrix scoring of the varieties by the Nyagithambo organic farmers

Characteristic	Cal-J	Nemonetta	Monyala	Caltana
Yield (4)	1	3	4	2
Size of tomato (3)	2	2	4	3
Firmness (5)	4	1	2	3
Colour (2)	1	4	3	2
Resistance to disease and pest (6)	1	4	3	2
Shape (1)	2	1	4	3
Total scores	40	56	66	51
Rank	4	2	1	3

Note: The relative importance of each characteristic is given by the scores in the brackets. The total scores are a summation of the product of the characteristic scores and scores for individual varieties.

5.7.3.2 Assessment by Mwea and Kibirigwi inorganic farmers (Table 6)

Table 6: Matrix scoring of the varieties by Mwea and Kibirigwi farmers

Characteristic	Cal-J	Nemonetta	Monyala	Caltana
Yield (4.00)	1	2	4	2
Marketability (4.67)	1	2	4	2
Size of tomato (4.00)	1	4	2	3
Firmness (3.00)	1	4	3	2
Colour (2.67)	1	2	4	3
Resistance to disease and pest (4.33)	2	3	4	1
Shape (2.67)	2	1	4	3
Harvesting frequency (1.67)	1	4	3	2
Total scores	34.01	73.02	95.37	59.03
Rank	4	2	1	3

Note:The relative importance of each characteristic is given by the scores in the brackets. The total scores are a summation of the product of the characteristic scores and scores for individual varieties.

The four groups of farmers had the same ranking for the varieties. Monyala had the best rank while Cal-J had the worst performance in terms of ranking. After ranking, discussions were held with the farmers to elicit their perception about the varieties. The farmers' views were that:

1. Nemonetta was good but it was growing too high and had very large fruits. They suggested that it would be better to breed for medium height and size for this particular variety. It would be better for home consumption or kitchen gardening. It was also noted that it is a sweet variety.
2. Monyala was rated best overall. It appeared long lasting and heavy yielding according to the farmers.

Farmers did not raise any issues about Caltana and Cal-J other than noting that these were not as good as Nemonetta and Monyala. Cal-J had worst performance.

5.7.3.3 Traders (middlemen) assessment of different tomato varieties

According to the middlemen's assessment Monyala was rated the best while Cal-J was the worst (Table 7).

Table 7: Variety assessment by middlemen

Characteristics	Weight factor	Cal-J		Nemonetta		Monyala		Caltana	
		Raw score	Weighted score	Raw score	Weighted score	Raw score	Weighted score	Raw score	Weighted score
Consumer preference (1)	0.05	2	0.10	1	0.05	4	0.20	3	0.15
Size (medium) (5)	0.24	2	0.48	1	0.24	4	0.96	3	0.72
Firmness (shelf life) (3)	0.14	1	0.14	4	0.56	3	0.42	2	0.28
Weight (6)	0.28	1	0.28	4	1.12	3	0.84	2	0.56
Colour (red) (4)	0.19	1	0.19	3	0.57	4	0.76	2	0.38
Shape (oval) (2)	0.10	2	0.20	1	0.10	4	0.40	3	0.30
Weighted average	-	-	1.39	-	2.64	-	3.58	-	2.39
Rank	-	4		2		1		3	

Note: The relative importance of each characteristic is given by the scores in the brackets. The corresponding weight factors are ratios of the scores of each characteristic to the total number of scores for all of the characteristics. The weighted scores are the product of the weight factors and the raw scores.

The middlemen noted that the tomatoes are usually put in five grades, in which the top grade fetches the highest amount. The farm gate price for the top grade, which is grade 1, was an average of Ksh. 500 per crate, while the average price for the lowest grade (5) was Ksh. 100 per crate (Table 8). In most cases the middlemen do not separate the tomatoes according to the varieties. The tomatoes are graded depending on the specified characteristics and especially size and weight but not on the basis of varieties. The current field exercise has enlightened the middlemen. Their understanding now is that by purchasing Monyala they are likely to get higher net profits.

Table 8: Farm gate prices for the various tomato grades per crate (60 kg) (13/8/2004)

Tomato grade	Farm gate prices (Kshs.)	Price in Nairobi (Kshs.)
1	500	1300
2	400	1200
3	300	1100
4	200	1000
5	100	900

Most of the middlemen sell their tomatoes in Nairobi, although a few middlemen noted that they sell their tomatoes in Mwingi District. The transaction costs, including transport and other levies imposed on the tomatoes, varied depending on where each middleman would sell their tomatoes. The middlemen noted that they always ensured that they purchase tomatoes and sell to generate at least a reasonable profit margin to allow continuity of their business.

5.7.4 Harvesting, yields and root-knot nematode gall indices of tomato varieties

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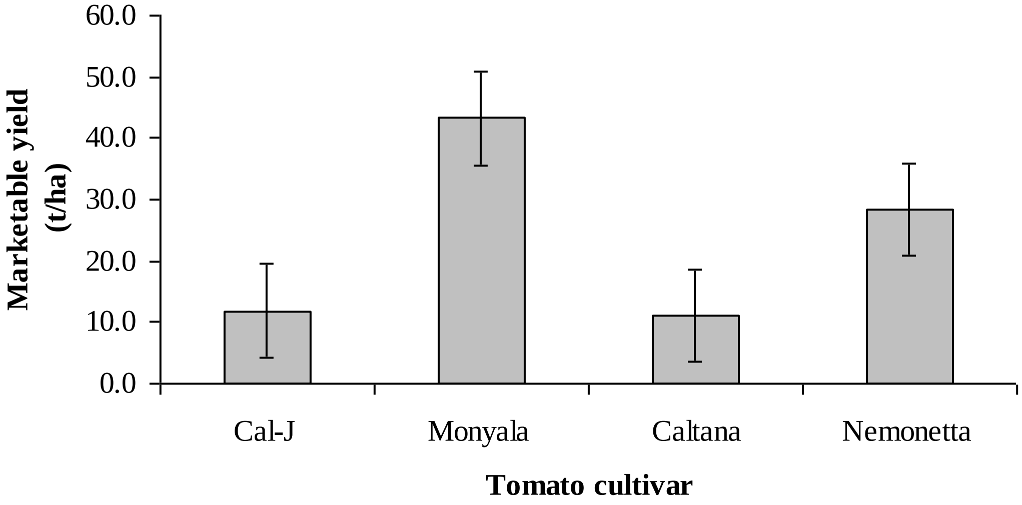

Figure 17: Marketable yield of different tomato cultivars evaluated for resistance to root-knot nematodes at Mwea, Kenya.

Monyala performed best and and Nemonetta had the second highest yield. An important finding is that there is an inverse relationship between susceptibility to nematodes and marketable yield. The mean gall indices for Cal-J and Caltana were higher while for Monyala and Nemonetta were low (Figure 18). The actual level of infestation (x) is an average computed from ten plants.

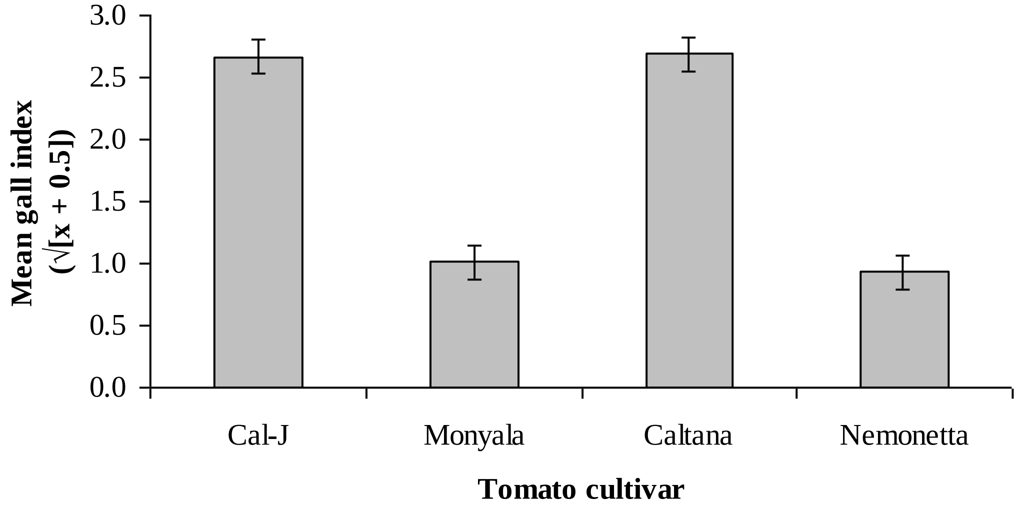

Figure 18: Root-knot nematode gall index on different tomato cultivars at Mwea, Kenya.

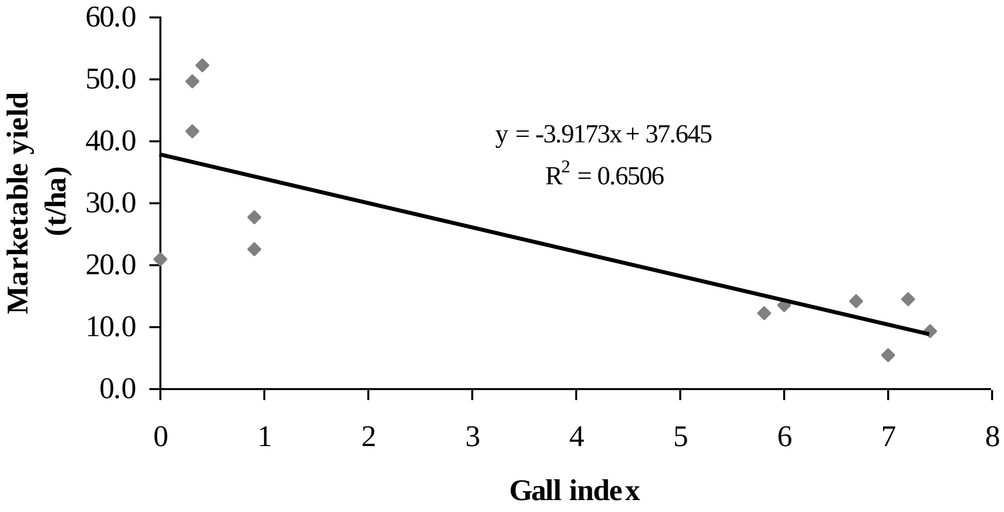

Figure 19: Correlation between gall index and marketable yield of tomatoes infested by root-knot nematodes at Mwea, Kenya.

It is interesting and important to note that the order in which the resistant varieries were ranked by the middlemen and by famers (at both stages of growth) and the levels of yields and reduced infection measured for the varieties all agreed. The findings this far indicate that promotion efforts should target Monyala.

5.7.5 Results of description of existing rotations and exploration of could how BCAs/rotation could fit in (Table 9).

Table 9: Responses on rotations, constraints and nematode control from different locations

Location	Crop rotations	Production constraints	Nematode control
Mwea (irrigated)	French beans, potatoes and field beans	Bacterial wilt, nematodes, blight, lack of capital marketing, impure seeds, low pesticide efficacy, mites, cut worms, whiteflies, thrips and rust	Main control is use of ash and trash burning. Mexican Marigold and *Crotalaria* spp. are also used. There is very limited use of nematicides.
Kibirigwi (irrigated)	Cabbages, maize, other vegetables, sweet potatoes, French beans and onions.	Nematodes, bacterial wilt, canker, blight, whiteflies, thrips, spider mites and blossom-end rot	No control and /or limited use of ash.
Kibirigwi (rain fed)	Maize, sweet potatoes and beans.	Bacterial wilt, nematodes, blight, rust, white flies,	No control. In most cases, there is limited control by

		aphids, thrips and red spider mites.	ash and trash burning
Nyagithambo (organic)	Cabbage, onions, field beans, maize, sweet potatoes, cow peas and egg plant,	Bacterial wilt, nematodes, blight, marketing, boll worms, birds, yellowing of leaves and blossom-end rot.	Main control is ash. Trash burning, crop rotation, hot water treatment, Mexican marigold, double digging (solarization) and pymac.

The production constraints for all the categories of farmers were listed as bacterial wilt, nematodes, bacterial canker, spider mites, viruses, whiteflies, aphids, *Fusarium* wilt, thrips, blight (early and late), blossom-end rot, marketing problems, lack of capital and poor quality seeds. Bacterial wilt was noted to be the most serious constraint, followed by nematodes, under rain fed conditions in Kibirigwi and undr organic farming and irrigated conditions in Mwea. Under irrigated conditions in Kibirigwi, farmers ranked nematodes as the most serious constraint in tomato production, followed by bacterial wilt. This underscored the importance of efforts to forestall the deleterious effects of nematodes on tomatoes. Farmers explained how they control nematodes and other pests and diseases. This involves the use of fungicides, insecticides, regular watering, crop rotation with maize and use of ash. Nematodes were controlled using ash, trash burning, pyrethrum extract (pymac) and trap crops such as *Crotalaria* spp. and Mexican marigold. The use of Mexican Marigold was reported by some organic farmers and some Mwea farmers that had participated in an earlier CABI project. Ash is applied after planting or is incorporated with the manure in the seed bed before sowing. A few chemicals such as Mocap® are used but in general chemical control is rarely used. This is because the chemicals are either banned or have low efficacy. For example, Furadan has restricted use because of toxicity, while Nemacur is considered as having declining efficacy. Farmers indicated that the indigenous control method, which involves using ash, was not effective. It was clearly apparent that nematode control was a problem and farmers were eager to obtain alternative control methods.

An overview of the practices being promoted by the project was provided to elicit farmers' interest and indicate the need for mutual participation in the drive to reduce the root-knot nematode problem. In addition, this was to help farmers to identify how these practices fit into their current production practices. The technologies being considered are use of BCAs (*Pasteuria penetrans* and *Pochonia chlamydosporia)* and crop rotation using cabbages.

Farmers had several suggestions on how the BCAs / rotations should be undertaken in order to maximize the benefits from the research activities. In addition to cabbages they suggested use of maize, sweet potatoes and French beans as alternative rotation crops. Sweet potatoes required less labour and were noted to be less susceptible to nematodes meaning that no control was needed. Maize was thought of as a rotation crop because it is a major food crop. Maize is also preferred because it uses residual fertilizers, hence no fertilizers are applications are made. No reasons were attributed to the use of French beans, but the research team discouraged the use of French beans because it is a host for nematodes. Farmers also requested information on alternative rotation crops that would mature within three months or generate sufficient income. It was indicated that the crop to be used as a rotation crop should be one that encourages proliferation of the BCAs. Regarding the BCAs, the farmers expressed concerns about availability, accessibility and the cost implications. Whereas they indicated appreciation of the BCAs, they requested to be told how the BCAs could be found and the costs involved. This raises questions regarding BCA production, the requisite amounts and costs. The need for computation of input costs, especially seeds, was also pointed out. Exchange visits were also requested in order to facilitate sharing of information. Farmers indicated the need for proper communication channels or links between researchers/ extension staff and the farmers themselves. Similarly, the dissemination materials such as books and leaflets require to be simplified. Rethinking and remodeling issues as suggested by the farmers in a participatory manner may be a way forward in ensuring that farmers reap maximum benefits from the practices being promoted.

5.7.6 Results of assessment of the biological control agents (BCAs) trials

Table 10: Ranking of BCAs vs. normal farmer practices

Characteristic	**Organic farmers ranking**		**Inorganic farmers ranking**	
	BCAs score	Normal practice score	BCAs score	Normal practice score
Yield	2	1	2	1
Resistance to pests	2	1	1	1
Vigour of plants	2	1	2	1
Harvesting period	2	1	2	1
Shape of the fruit	1	1	1	1
Colour	1	1	1	1
Weight	2	1	2	1
Resistance to diseases	1	1	2	1
Size of the fruit	2	1	2	1
Shelf life	1	1	1	1
Total scores	**16**	**10**	**16**	**10**

A score of 2 meant that the production practice was superior in terms of providing the specified characteristic and 1 meant that it was inferior in terms of proving the specified characteristic.

On average the farmers prefer the BCAs trials to their own production practices. They are however indifferent about their own practices and the BCAs with respect to certain characteristics. Among these are resistance to pests, shape of the fruit, colour of the tomatoes, and shelf life.

5.7.7 Factors that encourage or constrain adoption the technologies

Individual farmer interviews were used to identify the factors that affect the adoption of nematode control technologies. A random sample of 57 farmers was interviewed to provide the data. The farmers were selected from the administrative lists of the households located within the vicinity of the on-farm trials. The selected farmers were interviewed using structured questionnaires to provide information on tomato production practices, production constraints, level of education and willingness to try the technologies. Details of the data collected are as in the questionnaire appended to this report. Data collected were analyzed using descriptive statistics.

5.7.8 Factors that encourage or constrain adoption of BCAs / rotations

Most of the farmers (80%) grow tomatoes for commercial purposes. As a consequence they need to maximize returns to continue being in business, improve their livelihoods and meet other financial obligations. The production of tomatoes is reduced by nematode infestation.

A majority of the farmers (96.5%) know that nematodes exist and are aware of the tomato losses attributed to nematodes. The ability to forestall the losses is weakened by the inability of farmers to control the nematodes. The existing nematode control methods are not able to curtail nematodes. The current approaches for controlling the nematodes include crop rotation, trash burning, use of ash and limited application of nematicides.

The high costs of nematicides and the low efficacy of some nematicides will encourage farmers to adopt the new technologies, which have a relatively low cost. Most of the farmers do have adequate capital to purchase the nematicides, which are now considered to be the most appropriate approaches. Most of the farmers (86.1%) do not control nematodes at all.

Farmers indicated that they lack a suitable and affordable control method for the nematodes. Currently some farmers use double digging but they report that this approach is labour intensive and whenever they undertake trash burning it affects the entire plot or farm against their interests. Farmers have interest in trying the technologies.

There is an increasing trend in occurrence of nematodes in the farms as reported by 89.5% of the farmers. The appreciation of an increase in nematode population in the farms is likely to encourage adoption of technologies that can help alleviate the problem.

Farmers acknowledge that there is an increase in production due to the new varieties. 84.2% of the farmers interviewed indicated that they expected an increase in production due to the new varieties.

Buyer preference for some of the varieties may encourage adoption of the technologies. For instance the ranking by the traders indicated their preference for onyala. Since most of the tomato farmers undertake the exercise for commercial purposes it is likely that they may take up Monyala earlier than any other variety.

Technical know-how and level of education will encourage adoption of the technologies. The level of farmer willingness to try the nematode resistant varieties and the BCAs increases with the level of formal education of the farmer.

Gender of the household head has direct implications for adoption. Male farmers were more likely to try the new technologies. This may be attributed to the existing extension system that is biased in favour of the male farmers and the fact that more male farmers are involved in commercial farming.

Tomato production involves the use of various inputs and at the same time tomatoes are very perishable commodities. This means that timing of activities is very crucial. Some of the inputs are very costly. The seeds for the nematode resistant varieties are noted to be costly meaning that the high seed costs are likely to stop farmers from adopting the technologies. This is likely to be an issue given that already some farmers (59.6%) are already indicating that the costs of the seeds of existing tomato varieties are high.

Marketing of the tomatoes is major problem to most of the farmers (61.4%). The problems associated with marketing include low prices and the selective behaviour of the middlemen. Similarly, the transportation cost to the key marketing centre (Nairobi) is high. Given that most of the farmers grow tomatoes for commercial purposes a ready market is of immense importance. In this regard information on market prices in various market places and the availability of marketing middlemen is crucial. Farmers are rational decision makers and are unlikely to enter into production of new commodities unless a market is assured. The practical implication here is that all stakeholders will have to encourage and promote the varieties through the use of appropriate approaches and systems.

Availability of the seeds and the BCAs is a factor that will curtail adoption. As of the time of the final evaluation of the technologies it was still not apparent as to how the BCAs will be made available or the cost implications. The seeds of the nematode resistant varieties are as yet not readily available.

Performance of the varieties and the BCAs being promoted will influence adoption. 68.4% of the farmers indicate that Nemonetta is better than their varieties, while 87.7% of the farmers say that Monyala is better than their varieties. 47.4% of the farmers indicate that Cal-J and Caltana are worse than the varieties that they are now growing. Regarding the use of BCAs, 45.6% of the farmers indicated that they are better than their current practices, while the remainder of the farmers were indifferent between BCAs and their own practices.

Awareness regarding the technologies may affect adoption. Whereas 84.25% of the farmers are aware of the nematode resistant varieties, only 49.1% are aware of the BCAs. This means that the promotion efforts would be very crucial to ensure adoption of these technologies, especially the BCA.

Farmers' perceptions and willingness to try the new varieties and the BCAs is variable. Out of all the farmers interviewed only 47.4% indicated that they would try the BCAs if they are made available. The percentages of farmers willing to try Monyala, Nemonetta, Caltana and Cal-J were 86.0%, 68.4%, 8.8% and 7.0% respectively. It therefore likely that given availability of the seeds and the BCAs the farmers would start by trying the Monyala variety. This assertion is also attributed to the fact that the buyers and by implication the consumers have a preference for Monyala.

5.7.9 Conclusions

The participatory farmer assessments of the technologies together with the exploration of farmers' intentions regarding adoption and factors that encourage or constrain this, were conducted to facilitate adoption, ensure that the technologies were appropriate to farmers practices and systems and to improve understanding of factors likely to influence adoption. Farmers appeared to assess the technologies from two perspectives. These were the farmers' own demand for specific technology characteristics and the ability of the technologies to supply the stated characteristics. Assessment of the factors that constrain or encourage adoption elicited individual and group views.

According to the farmers Monyala had the best performance while Cal-J had least performance. Nemonetta was the second in ranking while Caltana was the third.

Interestingly the results of the assessment from the farmers agreed with those from the traders. This is as expected because most of the farmers (80%) produce tomatoes for commercial purposes. Farmers' preferences agreed with yields which in turn coincided with levels of resistance evident from assessing infestation. The levels of willingness to try the new varieties ascertained from the survey reflected the above as well and showed very high proportion of farmers wanting to try Monyala and Nemonetta. These should now be promoted in the area and could improve production significantly. The farmer willingness to adopt BCAs was lower compared to the nematode resistant varieties but still important at just under 50%. It is likely that the resistant varieties would be adopted faster than the BCAs.

A number of factors could to interfere with the farmers' intentions to produce the new varieties. Among these are the high costs and availability of the seeds. To facilitate adoption it will be necessary to make the seeds more readily available and packaged in different quantities including small quantities that would be more affordable for the various categories of farmers. Regarding the BCAs farmers are not aware of where to find them and the costs involved. This needs to be addressed and again packaging in small quantities for farmers to try and that are affordable will be important. The technical know-how regarding use of BCAs/rotations also has to be provided given that it is a new approach. These findings have important implications for seed suppliers, agrochemical suppliers, extension services and NGOs and need to be addressed as a high proportion of farmers are willing to adopt one or more of the technologies and they have the potential to improve tomato production and farmers livelihoods. Further important issues regarding factors influencing adoption and implications for promotion are given in the other socio-economic report from this project (McKemey, Appendix 6) which explores potential uptake.

Although, the sample of farmers was relatively small, the findings indicate the categories of farmer likely to apply the BCA agent to both seedbeds and field, if it is made available. The research also indicates what expectations are influencing their decisions to apply the agent. Social referents are identified that have influence on the decision making process with the different categories of farmer more open to social persuasion. The findings can therefore be used to inform the future promotion of the BCA application, indicating key messages and appropriate channels of communications.
Overall the finding suggests that farmers are very likely to apply the BCA to both seedbed and field if made available, although seedbed application is more likely.

- The categories of farmer that appear most likely to apply the BCA to the seedbed are those that feel able to manage the risk, have the greatest dependency on farm income, that are using furrow irrigation systems and that have a serious current problem with nematodes.
- In contrast those categories that registered the weaker intent to apply the BCA to seedbeds are those that do not have a current problem with nematodes, that perceive the risk as low and those that depend on rain-fed systems.

When the issue of applying the BCA to the field directly is considered, the categories of farmer most and least likely to apply the agent change, i.e.

- Those mostly likely to apply the agent to the field are those that are not members of agricultural organisations, those using furrow irrigation and those receiving a higher proportion of the farm income from tomato sales.
- Those registering the weaker intent were those with overhead irrigation systems, that do not have a current problem with nematodes and those deriving a lower proportion of their farm income from tomatoes.

In general the decision to apply the BCA will be governed by the farmers' own experience and knowledge (attitudes). However, in the case of those using overhead irrigation, perceived social pressure may have the greater influence on their decision process.

Those expectations that appear to be driving the decision to apply the agent to the seedbed were in order of influence:

1. *Vigorous seedling growth*
2. *Improved yields*
3. *BCA influence will be long lasting*
4. *Non toxic agent complimenting organic production*
5. *Ability to continue cropping invested fields*
6. *Will protect the seedlings after transplanting*
7. *Increased profit*
8. *Effective control of nematodes*

- The final outcome attitude re nematode control is implicit in all the other ranked expectations.

With respect to the application of the BCA directly to the field, the expectations that appear to have the positive influence in rank order are:

1. *Improved yields*
2. *Ability to continue cropping worm infested fields*
3. *e toxic -compatible with organic systems*
4. *Field application will provide protection to directly sown crops*
5. *The effect of the BCA will last -for several seasons*
6. *Increased profit*

The issue that is distinct to field application intent is the protection provided to directly sown crops.

The issue of seedling vigour is a key expectation regarding seedbed application. This may reflect a belief that a vigorous seedling will be able to withstand attacks once transplanted, rather than depending on the transfer of the BCA agent from seedbed to field.

In general these expectations will need to be reinforced in any promotional strategy. However, some of these current expectations may be beyond the capability of the BCA. For example that the agent once applied will have a residual impact over several seasons, the ability to continue cropping infested fields and that if applied to the seedbed the agent will continue to provide effective protection once the plant has been transplanted. Also the BCA does not necessarily encourage seedling vigour. These are key influential outcome expectations that are driving the current positive response. However, if any of these are proved wrong, it will lead to a weakening of the current positive intent. If some of these are false expectations then they need to be addressed before the farmers find out through trial and error. These 'possible' errors in perception should be challenged but at the same time those expectations that are correct should be reinforced so as to counter-balance any negative response.
In the case of seedling vigour it may be appropriate to add a fertiliser to the BCA so as to help insure a more vigorous seedling response as well as providing protection

against nematodes. This would have the effect of meeting the farmers' expectation and thus help to ensure continued use of the BCA.

With those not facing a current nematode problem the expectation of having to change the method of transplanting to ensure effective transfer of the agent to the field was acting as a cognitive barrier to the future application of the BCA to the seedbed. This was the only expectation that was acting as a deterrent and was only relevant to those not confronting a nematode problem. However, the issue of insuring the effective transfer of the agent from seedbed to field during transplanting is a key underlying issue.

The most influential social referents regarding the application of the BCA are other farmers, local radio, and promotional publications and materials as well as workshops/seminars. This indicates the importance of identifying the influential local farmers and enlisting their support and the use of local radio in any promotional strategy when addressing farmers in general.

- However, those most dependent on the farm income are also influenced by the agricultural research stations. Therefore the research institutions need to target this category of farmer when promoting the BCA application.
- Preventative strategies that focus on encouraging those not currently experiencing a nematode problem should consider enlisting the support of the agro-chemical suppliers. These suppliers appear to have influence on those not currently exposed to nematode infestation.
- Care should be taken regarding enlisting Government agricultural extension officers, as they appear to have a negative influence on the farmers' decision to apply the BCA agent.

In general the majority of farmers is very aware of the nematode problem and appears keen to apply anything that will prove effective. Cost was not the most influential factor. The prospects for an initial uptake of the BCA agent appear very positive. However, this positive intent may be based on some false assumptions and these should be identified and addressed prior to the release of the product. In doing so it will be important to reinforce those correct influential expectations.

5.8 Link with CABI field schools and KIOF to produce training and promotional materials including posters, media items and instruction materials for FFS.

A leaflet entitled; “Underground menaces” was developed to create awareness of the root-knot nematode damage. The leaflet, which was originally drafted in English, was translated into a local language (Kiswahili). Two thousand copies (1000 copies for each of the English and Kiswahili versions) were produced. In addition, a poster (electronic copy attached) entitled, “Root-knot nematodes (*Meloidogyne* spp.) and their management in tomato fields” was developed, and 2000 copies produced for dissemination (Appendix 7).

Nine agricultural extension staff (from the Ministry of Agriculture): three in Athi river, two in Kibirigwi and one from each of the Gitura (Karigu-ini), Nyagithambo, Nyathuna and Ruiru locations, were involved in participatory field training sessions with farmers in Central and Eastern Provinces of Kenya. In addition, a local Diploma (Agriculture) student on internship at the divisional agricultural extension office in Kibirigwi, a PhD student studying information flow on IPM in tomatoes (from the University of Hohenheim, Germany - on research attachment at CABI - Africa Regional Centre), a training officer from Kenya Institute of Organic Farming (KIOF) and the chairperson of Kenya Organic Farmers Association (KOFA), participated in the participatory training with farmers.

Through the linkage with KOFA, the project established contacts with a new initiative known as the Kenya Organic Agricultural Network (KOAN). The chairperson of KOFA is a member of KOAN, hence provided a strategic linkage with the activities of the network.

Plate 3. A farmer participatory training session on nematode management in Central Kenya

5.8.1 Provide materials to all collaborating organisations for use in their training

The posters (2000 copies) and leaflet (2000 copies) were disseminated to all collaborating institutions in Kenya (CABI, KARI, Dudutech and KIOF) and UK (University of Reading and Rothamstead Research). Dissemination of the leaflets to farmer's groups (i.e. Karigu-ini Organic farmer's Group; Nyagithambo Organic Farmers Group; Taisei-Kirui-ini self-help Group (TAKI); Kibirigwi Irrrigation Farmers Cooperative (KIFCO); Nyangati-Gikumbo horticultural self-help group; Nyamukia fruits and vegetable growers; Gitimuki horticultural group; Kang'otora self-help group) and individual farmers (not belonging to any farming organisation) who participated in the activities of the current (R8296) and previous (R7571: Management of virus diseases of vegetable crops in Kenya; R7472: Management of root-knot nematodes on vegetables in Kenya; R7449: Development of biorational brassica IPM in Kenya; R7403: Pest management in horticultural crops in Kenya) CPP funded projects was done. At least 30 individual farmers in each of the following locations: Athi River (Machakos District), Ruiru (Thika District), Karigu-ini and Nyagithambo (Maragua District), Nyathuna (Kiambu District), Kibirigwi and Mwea (both in Kirinyaga District), were issued with the posters and leaflets.

Thirty copies of each of the leaflet and poster were disseminated to the Ministry of Agriculture, agricultural extension division, in each of the above locations, for use by staff in their training programmes, and dissemination to farmers' field schools operating within their designated areas of operation. Each farmer was issued with three posters, one for him/herself and two for dissemination to other neighbouring farmers. In addition, the posters were disseminated to representatives from Kenya Plant Health Inspectorate Services (KEPHIS), Lagrotech Co. Ltd. (a registered seed merchant based in Western Kenya) and Central Science Laboratory (CSL, UK) for display in their company's offices, and use as training materials. The posters and the leaflets were well received by all.

The leaflet and poster will also be reproduced and used as training materials in FFS in Kenya, Uganda and Tanzania during training workshops on, integrated production and pest management of tomato to be held later this year. The training will be coordinated under the activities of the current CPP funded project i.e. Accelerated Uptake of CPP Research Outputs in Kenya, led by CABI. This project is directly linked to the Kenya component of the East African Sub-Regional Pilot Project for Farmer Field Schools on Integrated Production and Pest Management, funded by IFAD (International Fund for Agricultural Development) through FAO (Food and Agriculture Organisation). Electronic copies of the poster and the leaflet have already been issued to the co-ordinator of the project in the region (East Africa). This provides an opportunity for ongoing promotion and dissemination of the root-knot nematode awareness leaflet and poster.

Plate 4. Dissemination of training materials on the management of root-knot nematodes to farmers and agricultural extension staff in Central Kenya.

The poster and leaflet will also be distributed and discussed at the Gatsby Nematology Course to be held at ILRI 4 July-20 August 2005.

5.9 Biopesticde approval

A small team at the Ministry of Agriculture studied the Biopesticides regulations and suggested that some minor corrections (not fundamental changes) be made. However, a new legal officer at the Ministry was appointed and this has delayed the ratification process.
Nevertheless, data for registering several biopesticides have been presented and have been accepted for the registration process without any problems.

See Warbule, M N., Ngaruiya, P N., Kimmins, F K, and Silverside, P J., 2004 *Registration for Biological Control Agents in Kenya.* Proceedings of the PCPB/KARI/DFID CPP Workshop Nakuru Kenya 14 –16 May 2003. KARI/PCPB Nairobi Kenya and Natural Resources International Ltd., Aylesford, UK 233pp

6. Contribution of Outputs to developmental impact

An environmentally safe strategy for managing root-knot nematodes is now closer which need not include a hazardous chemical such as carbofuran (a product used by some of the commercial producers supplying tomatoes to the Nairobi market).

Dudutech have demonstrated their capacity to mass-produce both the biological control agents; the formulation of the fungus *Pochonia chlamydosporia* is of a consistent quality. Dudutech will still need to refine the methodology for mass-producing *Pasteuria penetrans.*

These "products" are now available for wider use and will be of benefit to the poorer farmers growing nematode susceptible crops and indirectly to the wider community who buy these crops. In addition to improving the technology for the commercial export producers (a requirement of the European Union rules for harmonisation of use of pesticides [COLEACP]) commercial growers who grow crops on contract to the exporting companies will also benefit. The knowledge and information leaflets produced will be promoted to organisations representing the commercial growers such as Fresh Produce Exporters Associations of Kenya (FPEAK) and the Kenya Flower Council, at farmer field schools and training courses for regional scientists such as that being funded by the Gatsby Charitable Foundation.

Farmers have participated in the evaluation of the nematode management strategy that has included biological control agents, nematode resistant varieties and crop rotations.
One scientist (D. Karanja) obtained his PhD (2004) from work done on this project (and the preceding project R 7482)

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KERRY, B. (2004). Interactions in the rhizosphere and the biological control of root-knot nematodes using *Pochonia chlamydosporia. Advances in applied biology; providing new opportunities for consumers and producers in the 21st century. Centenary meeting of the Association of Applied Biologists, 15-17 December, 2004, Oxford, UK. (Abstract).*

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PEMBROKE, B., DARBAN, D.A. and GOWEN, S. R. (2004) *Pasteuria penetrans* a tritrophic intereaction? *IOBC Bulletin*, 27(1) 229-234.

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LEAFLET. Underground Menaces. CABI Africa Regional Centre, Nairobi, Kenya.

Biometricians Signature

The projects named biometrician must sign off the Final Technical Report before it is submitted to CPP. This can either be done by the projects named biometrician signing in the space provided below, or by a letter or email from the named biometrician accompanying the Final Technical Report submitted to CPP. (Please note that NR International reserves the right to retain the final quarter's payment pending NR International's receipt and approval of the Final Technical Report, duly signed by the project's biometrician)

I confirm that the biometric issues have been adequately addressed in the Final Technical Report:

Signature:
Name (typed):
Position:
Date: