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Comparisons of the spore attachment of two populations of Pasteuria penetrans (Malakisi and Thika) on two different populations of root-knot nematode known as Hypericum and Navaisha

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Abstract *In-vitro* studies were conducted to point out most ignored points related to *Pasteuria penetrans* to achieve maximum number of spore and attachment to root-knot nematode isolates known as (Hypericum and Navaisha). The cuticles of root-knot nematodes have shown a high level of heterogeneity suggesting that this has an effect on the recognition and/or attachment process. Attachment levels of different *Pasteuria* populations developed from an original population of *P. penetrans* (the population known as Pp3) and a population that was developed from a single spore line of that original population are compared on a single egg mass line and a parent population of *M. javanica*. *Pasteuria penetrans* spore suspensions were prepared from previously soaked root powder containing endospores of *P. penetrans* (Malakisi and Thika). The attachment was compared between two populations of root-knot nematodes (Hypericum and Navaisha). Both types of spores were introduced to freshly hatched juveniles of the Hypericum and Navaisha populations so there should not be any differences in the vigour and condition of the nematodes. These attachment studies have proved that Malakisi population of *P. penetrans* has shown more significantly more number of spores attached to juveniles than Thika population. There was a significant ($P<0.05$) difference between both populations either in the number of spores per juvenile or percentage of spore encumbered juveniles. KEYWORDS: Root-knot nematodes (Hypericum and Navaisha), two populations of Pasteuria penetrans (Malakisi and Thika), attachment studies.

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1. INTRODUCTION

***Hypericum Calycinum*.**

Root-knot nematode (Hypericum) population was found from the infected roots of Hypericum calycinum. It is a prostrate or low-growing shrub species of the genus Hypericum (Hypericaceae). Widely cultivated for its large yellow flowers, its name as a garden plant include Rose of Sharon in Britain and Australia, and Aaron's beard, Great St-John's wort, and Jerusalem star. Grown in Mediterranean climates, widely spread in the Strandja Mountains along the Bulgarian and Turkish Black Sea coast. It is a low, woody shrub to about 1 m tall and 1–2 m wide but often smaller. The green, ovate leaves grow in opposite pairs. It is indigenous to southeast Europe and southwest Asia This hardy plant does not suffer from serious diseases or insect pests and is an excellent choice for a groundcover on poor soil, only root-knot nematodes can severely affect its growth and sometimes can reduce its cultivation if cultivated for longer. The following image of severely infected roots of the species could determine the severity of root-knot disease problem.

The Life Cycle of Root-Knot Nematodes

Plant parasitic nematodes go through six life stages including the egg, four juvenile stages, and the adult. Except the development of the egg into the first-stage juvenile, all stages are separated by a molt. The temperature and substrate quality have enormous effect on its Life cycles and vary from less than 5 days to more than a year depending upon the species. Egg production by nematodes is easily determined in species that deposit eggs in a sac, within plant tissues, or retain them within the female body. Quantifying egg production in the soil is extremely difficult and time consuming. Eggs are not only difficult to extract, but they are also difficult to identify (Barbercheck, and Kaya. 1991). However, several techniques have been developed that are effective for some genera. Root-knot nematodes (*Meloidogyne* spp.) are sedentary obligate endoparasites. The life cycle of *Meloidogyne* spp. can be affected by temperature (Davies *et al*., 2001), host (and nutrition. Adult females lay eggs within a gelatinous matrix, which forms an egg mass. Female javanica start laying eggs when they are 18 to 20 days old and can lay nearly 2000 eggs over a period of several weeks at 26°C (Tzortzakakis and Trudgill, 1996). The development and hatching of eggs takes about 11 days at that temperature (Trudgill, 1995). Egg hatching rate depends on many factors such as temperature, moisture, level of oxygen, age of females and presence of host plants. It has been reported that activity of certain enzymes like proteinase, collagenase and chitinase was associated with increasing the flexibility of eggshells before the eclosion of juveniles. The first stage juvenile develops inside the egg cuticle, as does the second stage, which hatches from the egg. After hatching, second stage juveniles migrate in soil

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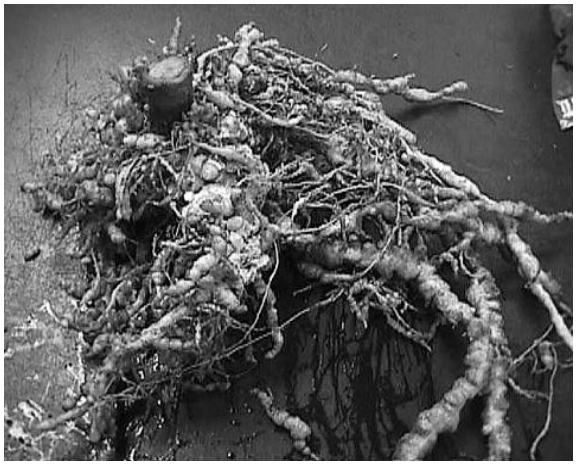
and are attracted by root diffusates. They are the only infective stage and usually enter roots just behind the root tip.. Then they become sedentary with their head in the edge of the phloem tissues and their tail in the root cortex. There they induce the formation of feeding cells, called giant cells due to their increased size. The cells around the feeding site also enlarge and increase in number thus causing a root swelling, the gall (1-10 mm diameter) The nutritional status of the host plant can affect the sex ratio; most *M. javanica* males were found in nitrogen deficient plants at high inoculation rates. Stressed roots also reduce the fecundity of females.



***Hypericum calycinum* roots severely infected with root-knot nematodes**.

NAVAISHA

The Naivahsa population of root –knot nematode was found from the area of lake Naivasha. It has not been mentioned yet the name of any crop or any ornamental plants or any wild plant species. Lake Naivasha is a freshwater lake in Kenya, lying northwest of Nairobi, outside the town of Naivasha. It is part of the Great Rift.

Root-Knot Nematodes And *Pasteuria Penetrans*.

The cuticles of root-knot nematodes have shown a high level of heterogeneity suggesting that this has an effect on the recognition and/or attachment process (Darban *et al*., 2004). In this study of attachment levels of different *Pasteuria* populations developed from an original population of *P. penetrans* (the population known as Pp3) and a population that was developed from a single spore line of that original population are compared on a single egg mass line and a parent population of *M. javanica*. *P. penetrans* has shown great variability of host specificity according to the origin of the isolated spores (Troter, *et al*, 2004). The spores of different populations of *P. penetrans* have different quantities of different types of proteins, which are associated with the attachment process (Davies *et al.,* 2001). It would be instructive to identify these proteins as a first step to understanding the basis of specificity and how *P. penetrans* might be better exploited as a biological control agent. The life cycle of *P. penetrans* from endospore to endospore was found to be highly dependent upon soil temperature. The temperature determines not only the duration of life cycle but also the number of endospores produced per parasitized female. Generally 25-30°C is a favorable temperature for all stages of the infection process and optimum for nematode development. The highest number of endospores recorded was produced at 25°C (2.2 x 10? per female). The control of root-knot nematodes is entirely dependent to the germination of spores inside the body of nematode host. Spore attachment to second stage juveniles is the first step in the establishing the host specificity of *Pasteuria* spp and determining their biological efficacies. Most of the techniques used to observe spore attachment rely on nematode movement through soil and in water (Darban *et al*, 2004). Stirling's findings showed the potential for mass producing spores in an in-vivo system is needed further studies on the relationship of host and soil temperature with spore production.

MALAKISI

The Western Province of Kenya, bordering Uganda, is one of Kenya's seven administrative provinces outside Nairobi. It is west of the Eastern Rift Valley and is inhabited mainly by the Luhya people. Quakerism is widely practiced here. Kenya's second highest mountain, Mount Elgon is located in Bungoma District. The Kakamega Forest rainforest is part of the area. The climate is mainly tropical, with variations due to altitude. Kakamega district is mainly hot and wet most of the year, while Bungoma district is colder but just as wet. Busia district is the warmest, while the hilly Vihiga District is the coldest. The entire province experiences very heavy rainfall all year round, with the long rains in the earlier months of the year. 3Farming is the main economic activity in the province.

THIKA

Thika is an industrial town in Central Province, northeast of Nairobi, near the confluence of Thika River & Chania River. Thika is also known as a centre for light industry especially in food & horticulture processing.

2. MATERIALS AND METHODS

Studies on spore attachment of two *P. penetrans* populations (Malakisi and Thika).

Studies on spore attachment of two *P. penetrans* populations (Malakisi and Thika). *Pasteuria penetrans* spore suspensions were prepared from previously soaked root powder containing endospores two different populations of *P. penetrans* (Malakisi and Thika). 100 mg of root powder was diluted into 100 ml

of water. Then the suspensions were passed through 38 µm sieve and placed in universal glass bottles. Three aliquots were taken to estimate the number of mature endospores with the aid of a haemocytometer at x400 magnification. Suspensions of 200 juveniles from two different populations of root-knot nematode Hypericum and Navaisha were placed in 3cm diameter Petri dishes. To these were added suspensions of 50,000 spores from each population and were mixed with nematode suspensions. The total volumes of all suspensions/rep were made up to 4 ml by adding SDW as required. Then all Petri dishes were placed in an incubator at 28°C. The experiment was arranged in a completely randomised block design with three replicates with assessment of 20 juveniles per replicate.

3. RESULTS

These attachment studies have proved that Malakisi population of *P. penetrans* has shown more significantly more number of spores attached to juveniles than Thika population. There was a significant ($P<0.05$) difference between both populations either in the number of spores per juvenile or percentage of spore encumbered juveniles (Fig. 1a-b) Malakisi population has shown that attachment level was continuously increasing until the last time of assessment, which was 24 hours. However, both populations were remained slightly different in the percentage of encumbered juveniles, even after the time of 6 hours total number of encumbered juveniles did not increase significantly In (Fig.1a-b) both populations of *P. penetrans* have shown very low attachment and there was not any significant increase in either the percentage of encumbered juveniles or in the total number of spores per juvenile.

4. DISCUSSION

The attachment of two populations of *Pasteuria* (Malakisi and Thika) was compared between two populations of root-knot nematodes (Hypericum and Navaisha). Both types of spores were introduced to freshly hatched juveniles of the Hypericum and Navaisha populations so there should not be any differences in the vigor and condition of the nematodes. The results show that there was highly significant difference between the levels of spore attachment of both populations among the populations of nematodes. The relatively low attachment levels of Thika population persisted until 24h exposure time, even then there was a number of juveniles without spores and some juveniles thatremained without spores **(Fig. 1 a-b).** The spores from both populations did show significant differences of attachment to the juveniles of Navaisha population of nematode until 24h of incubation period **(Fig. 2 a-b)**. However, as compared to Hypericum population of nematodes (Fig. 1 a-b) the attachment level was found decreased either in percentage of encumbered juveniles or in total number of spores per juveniles. It is concluded that attachment of the spores of both population on the hypericum population of nematodes is more persistent, which creates more chances to increase infectivity of nematodes than on the Navaisha population of nematodes.

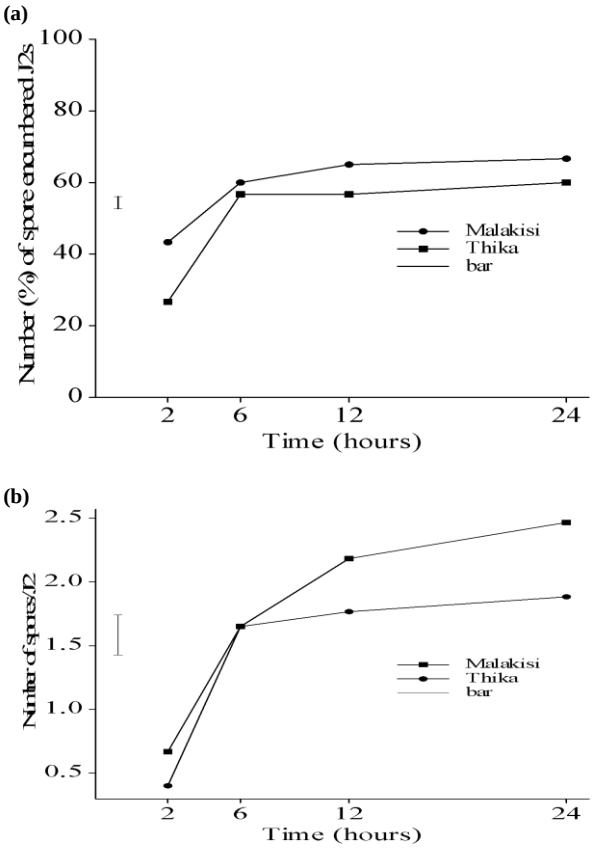
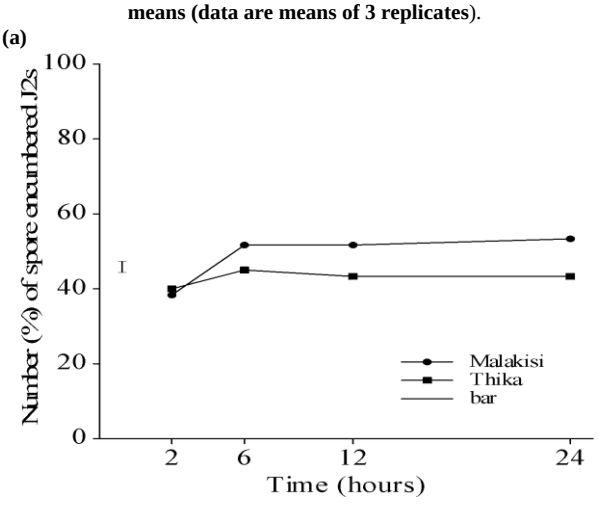


Fig.1 (a-b). Adhering efficacy of two populations of *P. penetrans* (Malakisi and Thika) on juveniles of the root-knot nematode (Hypericum). 20 J2s/replicate were examined to determine the percentage of encumbered J2s (figure-a) and no. of spores/J2 (figure-b). Error bars represent LSD of means (data are means of 3 replicates).



(b)

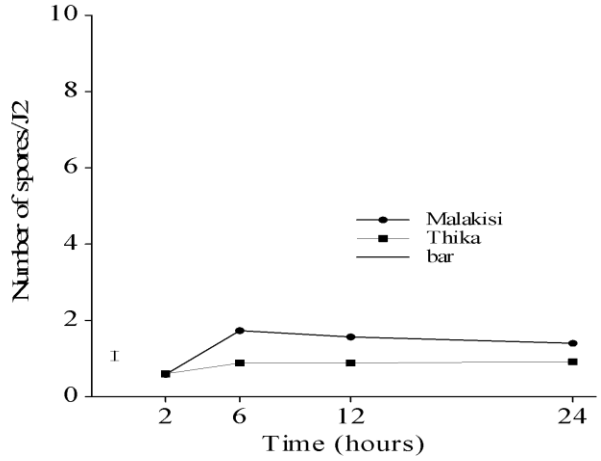


Fig. 2 (a-b). Adhering efficacy of two populations of *P. penetrans* (Malakisi and Thika) on root-knot nematode (Navaisha) population. 20 J2s/replicate were examined to determine the percentage of encumbered J2s (figure-a) and no. of spores/J2 (figure-b). Error bars represent LSD of means (data are means of 3 replicates).

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