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Arthrobotrys sp.: Biology, Ecology and Biological Control Potential in Northern Thailand.

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Abstract: Numerous soil samples were collected from various crops grown plots located in the two main Royal Development Centre namely Khun Wang and Khae Noi under the Royal Project Foundation, Chiangmai Province. Nematophagous fungus was isolated by scattering 3ml soil onto water agar (WA) and baiting the 9cm diam. Petri-dish with 1 ml surface steriled nematode suspension. After 24 h, the hyphae grown unidirection from soil,within 48 h the nematode e.g. *Rhabidtis* sp. and *Meloidogyne javanica* juveniles were trapped by adhesive network. Seventy-two hours later the mycelium fully grown inside the nematode body. Some network was observed to trap the nematode time and again. After inoculating nematodes for 3 days, conidiophore and conidia were observed and 11 isolates were considered resembling to *Arthrobotrys oligospora* Fresenius. Comparison of radial growth ability on different culture media by isolate KN 01,which found less network traps, showed that media which soybean, mungbean, and brown rice used as ingredient mixed with dextrose agar provide good radial growth rates and showed equal hyphal density as compare to those cultured on corn meal agar (CMA).

Index words: Nematophagous fungus, *Arthrobotrys oligospora* Fresenius,soybean dextrose agar, corn meal agar (CMA).

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INTRODUCTION

The most common fungus act as natural enemies of nematode that have been isolated from field soils named *Arthrobotrys oligospora* Fresenius (Linford,1937). Information on fungus–nematode interaction are more limited until Duddington (1957), Boosalis and Mankau (1965), and Pramer (1965) had confirmed that some nematophagous fungi attack their prey by means of various trapping organelles while others are endozoic parasites. Numerous experiments have been done with the nematode–trapping fungi (Stirling, 1991) and some examples where a preparation containing these fungi is widely used for biological control purposes. In Thailand, a few literature have been published for a useful information on a diverse range of these fungal species in soil and there have been only few attempts to compare the performance of isolates. It is therefore the process used, the screening procedure, the trapping activity on agar and in soil should be investigated in details for providing a more realistic indication of biological control potential.

MATERIALS AND METHODS

Isolation

Soil sample collected from chrysanthemum *(Chrysanthemum hortorum* Hort.*)*, tomato *(Lycopersicon esculentum* Mill.*)*, chinese kale *(Brassica oleracea* L.*)* and gerbera *(Gerbera jamesonii* Hork.*)* plots. the soils were observed heavy infested with a root – knot nematodes *(Meloidogyne javanica* Treub,1855 Chitwood,1949*)*. These plots located at Khun Wang Development Centre, 1200 m above sea level. The others Development Centre named Khae Noi, 1010 m above sea level, soils were obtained from various plots grown with artichoke*(Cynara scolymus* L.*)*. Randomly collected of 300 ml soil from each sample,then it was extracting for nematodes by modified Cobb's sieving and Baermann's funnel method and 3 ml of each soil sample was kept for fungal isolation. Nematodes were surface steriled in 0.1% (W/V) NaOCl solution for 1 min and to 0.5% (W/V) streptomycin sulphate for 1 min then washed three times in sterile water. Nematophagous fungus was isolated by scattering 3 ml soil onto water agar (WA) and baiting the 9 cm diam plate with 1 ml surface steriled nematode suspension. All plates were examined for fungal radial growth, trapped and inactive network over a period of 2 weeks. The pure isolate was recovered by transferring conidia to potato dextrose agar (PDA) by single spore method.

Radial growth comparison in different culture media

Seven replicate 9 cm diam Petri-dishes containing 10 ml of each of eleven different media prepared from corn *(Zea may* L.*)*, glutinous rice seed *(Oryza sativa* L.*)*, brown rice *(Oryza sativa* L.*)*, wheat *(Triticum aestivum* L.*)*, black bean *(Vigna sinensis* Saviex*)*, and

soybean *(Glycine max* (L.) Merr.*)*, potato dextrose agar (PDA), potato carrot agar (PCA) and corn meal agar (CMA) were inoculated with a 5 mm diam disc of isolate KN 01 , which observed less network traps, taken from the edge of growing colonies. The fungal disc was placed upside down in the centre of particular Petri- dishes and incubated at 25 – 27 °C. Once the fungus had grown onto the agar, the dish was marked at the advancing edge of the colony. This process was recorded every day until the mycelium reach the rim of 9 cm diam petri-dish. The hyphal density and conidia size were also evaluated.

RESULTS

By the scattering method, agar surface was so cleaned and easily to investigate any event occurred onto. The hyphae were observed grown unidirection from soil of all plates within 24 h after inoculation. Fungus from chrysanthemum plot produced large numbers of trap (adhesive network) and captured *Meloidogyne javanica* juvenile (J2) (Figure1) within 48 h after inoculation. A large size free–living nematode, *Rhabditis* sp.,was found double trapped by this fungus derived from tomato plots (Figure 2). There was a few traps from gerbera site but still showed the effectiveness of the trap (Figure 3). In chinese kale plot, three conventional loops trapped nematode was also discovered (Figure 4). After 3 days of inoculation nematodes, conidiophore bearing conidia was observed from mycelium (Figure 5). The modified single spore method was used by means of capillary tube tip touched the conidium then transferred to PDA plates (in vitro). Four PDA plates were kept for pure culture isolation.

Seven isolates of nematode trapping fungi, at Khae Noi Centre, derived from artichoke plots. Each isolate produced few trap and showed inert activity compare to those from Khun Wang Centre (Figure 6). For example, isolate KN 02 allowed the prey passed about half a body length then trapped (Figure7) and only isolate KN 05 showed abundant traps (Figure8). Isolate KN 06 performed a high capability by trapping a new prey (see a corpse of the last below, Figure 9). The fungus was considered resemsling to *Arthrobotrys oligospora* Fresenius with prominent denticle on conidiophore (Figure10).

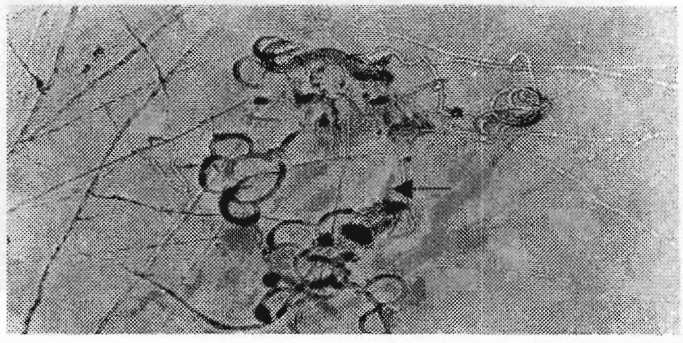

Figure 1 ***Meloidogyne javanica*** **(Treub,1885 Chitwood,1949) J2 juveniles (arrow) were trapped by adhesive network of** ***Arthrobotrys oligospora*** **Fres.**

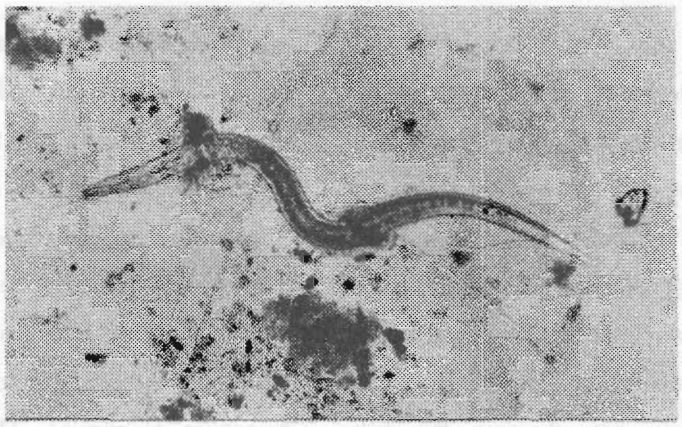

Figure 2 Free-living nematodes, *Rhabditis* sp., was double trapped by *Arthrobotrys oligospora* Fres. network at basal bulb of esophagous and about middle of body length.

Figure 3 A few trap but high capability of capturing a prey by *Arthrobotrys oligospora* Fres. from gerbera site.

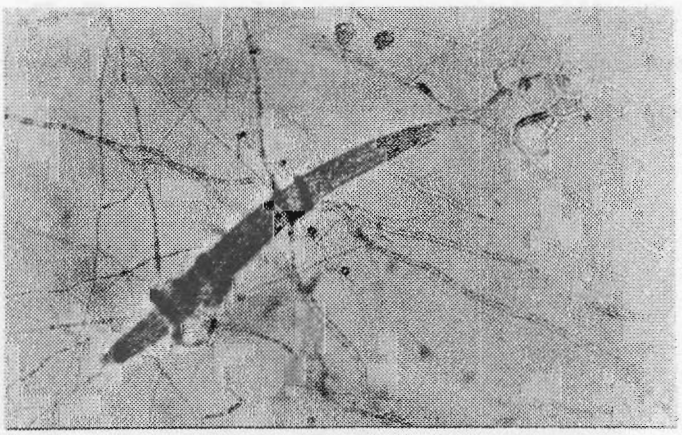

Figure 4 In chinese kale plot, a large size nematode was captured firmly. by three sets of *Arthrobotrys oligospora* Fres. loops.

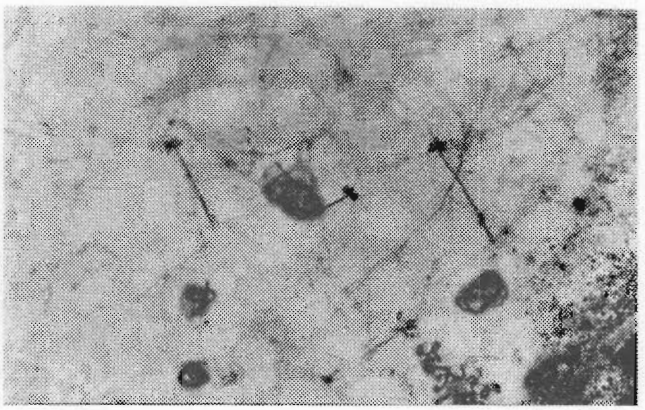

Figure 5 Conidiophore bearing conidia was observed within 3 days after nematode inoculation.

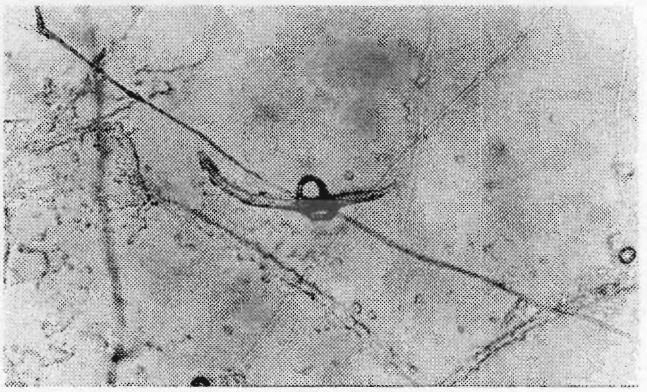

Figure 6 Active adhesive network trapped a prey about basal part of esophagous.

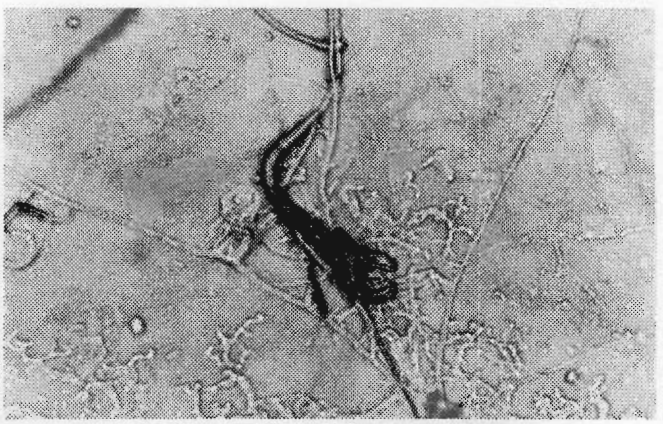

Figure 7 An inactive network captured nematode about posterior part instead of anterior part of the body.

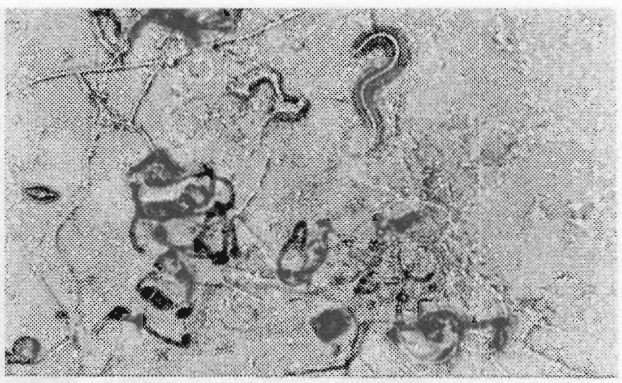

Figure 8 A cluster of *Arthrobotrys oligospora* Fres. network from isolate KN 05.

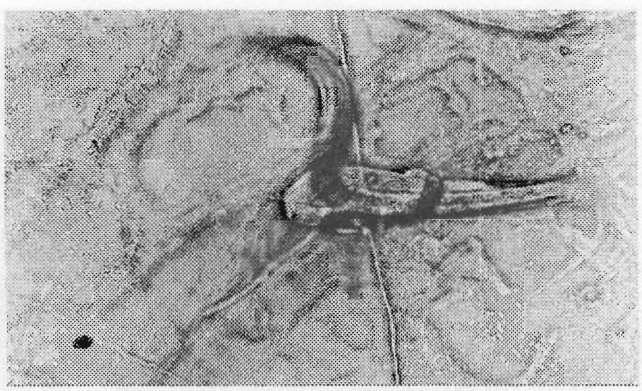

Figure 9 *Arthrobotrys oligospora* Fres. loop re-trapped a new living nematode while a corpse of the last still located below.

Figure 10 A prominent denticle (arrow) on conidiophore is only prominent evident to verify this fungus as *Arthrobotrys oligospora* Fres.

DISCUSSION

The aim of this study is to find out the proper biological control agents in relation to its efficacy in controlling the nematodes such as root knot species (*Meloidogyne* sp.) or kidney (*Rotylenchulus reniformis*) nematodes and/or another serious plant-parasitic nematode species which had found caused a tremendous damages to various crops grown in the vicinity of 34 Development Centres under the Royal Project Foundation. It is necessary to understand the biology including identification, ecology and the method for screening its potential in capturing nematodes. The present experiment revealed that soybean, mungbean and brown rice can be used as essential component, with low price and gain a good mycelial density, compare to corn meal from abroad, However, the fungus *Arthrobotrys oligospora* Fres. was selected because its abundance and ease to study in the laboratory, further more, soils still harbor numerous component of microfauna which have potential as biocontrol agents. Additional studies on other high potencial nematophagous fungi are required.

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