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Rotylenchus buxophilus and *Xiphinema diversicaudatum* and their Control with Chitin Formulation in Palm Oil Rhizosphere in South Sumatra, Indonesia

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SUMMARY

Plant parasitic nematodes are one of the major factors limiting agricultural productivity world-wide, and in South Sumatra, Indonesia. Recently, nematicides used to control plant nematodes have been reported to affect human health and pollute the environment. This study investigated the control of plant parasitic nematodes using chitin formulation in palm oil plantations in South Sumatra, Indonesia. Plant parasitic nematodes were first isolated and identified from the rhizhosphere of palm plantation. Experiments were then conducted on the control of the plant parasitic nematodes using formulation consisting of chitin, neem, urea and carbofuran. The result showed that the plant parasitic nematodes were dominated by *Rotylenchus buxophilus* and *Xiphinema diversicaudatum*. The *Rotylenchus buxophilus* population was suppressed by chitin formulation 250gr to 46.8 nematodes, carbofuran 150gr to 55.8 nematodes and to 116 nematodes by formulation without chitin and carbofuran. The *Xiphinema diversicaudatum* population was reduced by chitin formulation 250gr to 44.6, by carbofuran 150gr to 53.2 nematodes and to a mean of 112.2 nematodes by formulation without chitin and carbofuran. The results indiacte that chitin formulation could supress plant parasitic nematode population. The treament could increase the population of microbes that are antagonist to plant parasitic nematodes.

INTRODUCTION

Palm oil (*Elaeis guineensis Jacq.*) is an important source of cooking oil, margarine, components of many soaps, washing powders and personal care products, and biofuels. The African Oil Palm was introduced to Sumatra and the Malaya area in the early 1900s; many of the largest plantations of oil palms are now in this area, with Malaysia growing over 20,000 square kilometres. Malaysia claims that in 1995 it was the world's largest producer with 51% of world production. Malaysia and Indonesia produce crude palm oil with total production of 1,8 million ton and 7,5 million ton in year 2001, respectively (Sastrosayono, 2005).

Oil palms are grown for their clusters of fruit, which can weigh 40-50 kg. Upon harvest, the drupe, pericarp and seeds are used for production of soap and edible vegetable oil; different grades of oil quality are obtained from the pericarp and the kernel, with the pericarp oil used mainly for cooking oil, and the kernel oil used in processed foods. For each hectare of oil palm, which is harvested year-round, the annual production averages 10 tonnes of fruit, which yields 3,000 kg of pericarp oil, and 750 kg of seed kernels, which

yield 250 kg of high quality palm kernel oil as well as 500 kg of kernel meal. The meal is used to feed livestock. Some varieties with even higher productivities are being considered for production of biodiesel.

The African oil palm is reported to tolerate high pH, laterite, low pH, savanna, virus infections, and waterlogging (Duke, 1978). African Oil Palm is monoecious and cross-pollinated, and individual palms are very heterozygous. Three varieties are distinguished: those with orange nuts which have the finest oil but small kernels; red or black nut varieties have less oil, but larger kernels.

Deli Palm (Dura type) originated in Sumatra and Malaya, and they give high yields in the Far East, but are not well suited to West Africa. Dumpy Oil Palm, discovered in Malaya among Deli Palms, is low-growing and thick stemmed. Breeding and selection of oil palm has been aimed at production of maximum quantity of palm oil and kernels per hectare, and resistance to disease. Recently, much attention has been directed at cross-breeding with *E. oleifera* for short-trunk hybrids, thus making harvesting easier. Zeven (1972) elucidates the center of diversity, and discusses the interactions of some important oil palm genes (2n = 32,36)

Plant parasitic nematodes are among the most widespread pests limiting agricultural productivity world wide (SASSER & CARTER, 1985). They infect a wide range of crop plants including many vegetables, fruit crops, ornamental plants, cereal and industrial plants (TAYLOR & SASSER, 1978). It causes economic damage by reducing crop yield and quality. Crop damage is not just limited to the direct plant-nematode interaction itself, but very often nematode infected plants are more susceptible to pathogenic fungi, bacteria and virus in causing root disease complexes (ABAWI & CHEN, 1988).

Nematicides used to control plant nematode are known to cause human health and environmental problems. Consequently, control measures focus on preventing the build up of large population densities of root knot nematodes by applying crop rotation, chemical control, resistant varieties, and biological control measures. If done properly, crop rotation can limit damage by nematodes (KIRKPATRICK & SASSER, 1984). However, a major constraint is the presence of more than one key nematode species in a field, particularly those with wide host ranges such as nematode virus vector (RIGGS, 1995).

In the past, nematicides were applied extensively in order to use the same fields to produce a preferred crop each year (JONHSON & FELDMESSER, 1987). However, nematicides are toxic and must be used properly. Furthermore, nematicides are not selective and also kill beneficial nematode species which contribute to nutrient cycling or serve as biological control agents of plant pests (STIRLING, 1991; YEATES & WARDE, 1996). Over the past decade, several nematicides have been removed from the market due to environmental and health concerns.

The use of host plant resistance, if available, has proved to be an excellent method for the control of plant parasitic nematodes (ROBERTS, 1992). Resistant plants can completely prevent nematode reproduction. Their use requires little or no technology and is cost effective. However, resistance is only available for a few crops and sometimes breaks down at higher temperature such as for the Mi gene in tomato. Races of some nematode species are also now known that can break resistance in this crop (SIKORA et al., 1972).

Based on these reports, successful management of plant parasitic nematodes with organic amendments provides an alternative strategy which can be combined with other control strategies in an integrated approach. However, further research is needed on how to reduce the amount of organic amendments required and how they can be specifically added to the soil ecosystem to stimulate soil micro-organisms towards increased antagonism against plant parasitic nematodes. In the present study, chitin formulation based on renewable raw materials were used in low amounts to control plant parasitic nematodes. The main objectives were to evaluate the effect of a chitin formulation for the control

of various plant parasitic nematodes on palm oil.

MATERIALS AND METHODS

Nematode sampling and isolation: The soil samples were collected from Palm oil plantation in 14 regencies and 92 districts sampling locations with diverse habitats such as undisturbed forest, disturbed forest, shrub, perennial crops, vegetable crops, and food crop. Soil samples were taken 3 to 16 km apart within 200 m of a road. Each sample site was 2-4 m^2, and 5 sub samples (10x10x15cm each) were collected with a small hand shovel (Campbell *et al.*, 1998). The subsamples were combined resulting in ca. 800 ml of soil which was placed in a plastic bag and kept cool (10 to 15^oC). If any insects were observed, they were examined for nematode infection.

Morphometric studies: Identification of nematodes to genus level was attempted by making temporary mounts of ten infective juveniles, male and female for each isolate. The nematodes were killed and fixed in 4% hot formaldehyde. Fixed nematodes were transferred to anhydrous glycerin according to Seinhorst`s rapid method as modified by De Grisse (20 ml Ethanol 96%, 1 ml Glycerol and 79 ml distilled water). Permanent slides were prepared according to Cobb. All measurements were made using a drawing tube attached to a light microscope.

All observations and measurements were performed within a week after collection. For light microscope observation, 20 males and females and 25 infective juveniles were examined live. Additional specimens of different stages were killed in warm water (40^oC), and fixed in either TAF (10 ml Formalin 40%, 2 ml Triethanolamine , 88 ml and distilled water) or lactophenol. These nematodes were used when more observations were needed to confirm the morphology or variation of some structures. Nematodes fixed in TAF were processed to glycerin using the Seinhorst method. Type specimens were mounted in glycerin. Coverglass supports were used in all cases to avoid flattening of specimens. Measurements were carried out using micrometer ocular and objective within different magnification 40x, 400x and 1000x.

The identification of the specimens of this study is based on systematic, morphological and morphometrical characters following the keys given by Poniar (1992); Nguyen & Duncan (2002); Kaya & Stock (1997) and Adams & Nguyen (2002). The following abbreviations have been used in the text L= total body length; EP = excretory pore position; ES = oesophagus length; MBW = maximum body width; NR = nerve-ring position; ratio a= L/MBW; ratio b= L/ES; ratio c= L/TL; SpL = spicule; TL = tail length.

RESULTS AND DISCUSSION

A. Species and populations of plant parasitic nematodes in oil palm

Rotylenchus buxophilus Steiner

Female description: Body shape varying from one and a half helix to open C, rarely almost fully stretched, gradually tapering from base of oesophagus anteriorly and from immediately anterior to anus posteriorly. Head conical, not set off from rest of body, truncated anteriorly; Cephalic framework, stylet and smaller than anterior spermatheca. Tail short, almost hemispherical, less than anal body.

Males description: Body gradually tapering from base of oesophagus anteriorly and from 1-2 body widths anterior to anus posteriorly, its shape varying from open to almost closed C. Head conical, not set off from rest of body, truncated anteriorly; cephalic framework well developed, Stylet and stylet knobs considerably less well developed than in female. Stylet knobs narrow, flat to slightly indented anteriorly. Opening of the dorsal oesophageal gland close to base of stylet. Median bulb of oesophagus reduced. Oesophageal glands overlapping intestine further than in female and dorsal gland overlapping intestine more than sublateral glands. Excretory pore and hemizonid distinct, Testis short, generally reaching no further than about half the distance to base of oesophagus. Tail about one body width long, with distinct hyaline tail peg, number of annules on tail peg 6-7. Phasmids small, located near level of anus. Bursa well developed, enveloping tail terminus.

Measurements: Female: N (20) ; P = 365,00 $\pm$ 70,45 (240-480) μm; L = 17,10 $\pm$ 2,63 (12-20) μm; E = 58,20 $\pm$ 6,32 (50-78) μm; N = 59,30 $\pm$ 6,37 (50-74) μm; EP = 69,20 $\pm$ 9,70 (60-92) μm; T = 31,70 $\pm$ 4,41 (26-40) μm; V = 10,5 $\pm$ 1,93 (8-14) μm; Sty = 14,40 $\pm$ 2,01 (12-18) μm.; a = 21,91 $\pm$ 5,92 (15-34); b = 6,35 $\pm$ 1,46 (5-8); c 5,04 $\pm$ 1,47 (3-6).

***Xiphinema diversicaudatum* Cobb, 1913**

Male description: Body curved, head region continous or offset. Stylet very long consisting of an anterior odontostyle which needle like and has a forked base and a posterior odontophore with three prominent basal flanges. Stylet guiding ring located in posterior half of odontostyle. Oesophagus consisting of a long, narrow, procorpus and a short, glandular, bulb. Spicules very powerful, acruate. Vental supplements form a pre-cloacal row.
Female description: vulva usually at 40-50% but may be more anterior. Usually two genital tracts, but when the vulva is more anterior only the posterior tract remains. Tail very variable from short and rounded to long filiform.
Measurements (female n = 20) ; L = 2083,00 ± 258,19 (1700-2600) µm; W = 39,00 ± 6,41 (20-50) µm; E = 272,00 ± 39,15 (200-300) µm; N = 296.50 ± 31,83 (250-370) µm; EP = 311,50 ± 31,67 (260-320) µm; T = 42,20 ± 5,94 (34-50) µm; V = 44,50 ± 10,99 (30-60) µm; Sty = 114,00 ± 10,46 (100-130) µm; a = 55,10 ± 14,62; b = 7,72 ± 0,85; c = 6,71 ± 0,73.
Measurements (male: n =5)) ; L = 1896,00 ± 139,57 (1700-2020) µm; W = 34,00 ± 5,48 (30-40) µm; E = 240,00 ± 26,46 (210-280) µm; N = 296.0 ± 27,02 (250-320) µm; EP = 304,00 ± 11,40 (290-320) µm; T = 36,00 ± 3,74 (30-40) µm; Sty = 118,00 ± 13,04 (100-130) µm ; Spy = 44,80 ± 3,28 (42-48) µm; a = 57,37 ± 2,48; b = 7,97 ± 0,99; c = 6,41 ± 0,50.

Control of nematodes with chitin formulation: The number of nematodes before and after treatment is shown in Tabel 1 and 2. The data showed a three fold reduction of nematode populations during the experimental period. The reduction occured on treatments with nematicide and chitin formulation. After chitin application for two weeks the number of nematodes was reduced to 25,4 for *X.diversicaudatum* and 26,2 for *R.buxophilus.*

Tabel 1: Population of *Xiphinema diversicaudatum* in various treatments.

Treatments	**Average number of nematodes**		
	Before application	**1 week after application**	**2 weeks after application**
Control	87,9	112,2	122,3
Nematicide	79,4	53,2	35,4
Chitin formulation	89,1	46,8	25,4

Tabel 2: Population of *Rotylenchus buxophilus* in various treatments.

Treatments	**Average the number of nematodes**		
	Before application	**1 week after aplication**	**2 weeks after aplications**
Control	91	116	135
Nematicide	83,4	55,8	36,4
Chitin formulations	87,4	48,5	26,2

The observation of the number of nematode in 100g soil showed that the treatments with chitin formulation had the lower nematodes. At one week after application the number of nematodes was 46/100g soil for *Xiphinema diversicaudatum* and 48,5/100 gr soil for *Rotylenchus buxophilus.* At two weeks after application there was a reduction of the nematodes to 25,4/100g soil for *Xiphinema diversicaudatum* and 26,2/100g soil nematodes for *Rotylenchus buxophilus.* We observed that the formulation of chitin and urea 250g can decrease the number of nematode in the soil and reduce the root loss due the nematodes. The results show that the chitin formulation could suppress the population of plant parasitic nematodes.

REFERENCES
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