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ETIOLOGY OF THE YELLOWING AND WILT DISEASE OF BLACK PEPPER (*PIPER NIGRUM L.*) INCLUDING BIOCONTROL[1]

B.A. Zaragoza,* M.B. Castillo and L.L. Ilag**
**Bicol Experiment Station, Department of Agriculture*
Camarines Sur, Philippines
***Department of Plant Pathology, University of the Philippines at Los Baños*

ABSTRACT

The cause of the yellowing and wilt disease attacking black pepper (*Piper nigrum* L.) observed in the Bicol Region was investigated and determined as *Meloidogyne incognita* Chitwood. The initial symptom was slight to general yellowing of leaves. Wilting occurred two to three months after heavy, continuous rains followed by sunny, warm and dry weather. The nature of the symptoms was similar to those caused by a wide range of biotic and abiotic factors.

A survey for the presence of *Meloidogyne incognita* in black pepper plants from 4 provinces, 12 towns and 14 barangays in the Bicol Region showed that 64.3% of the 70 samples had galls. Personal assessment of loss in stand was 10 to 65%. Suspect fungal pathogens particularly *Phytophthora* spp., were ruled out, despite a rapid isolation technique using pimaricin-vancomycin-pentachloronitrobenzene-hymexasol (PVPH) selective medium.

The nematode species was identified by the characteristic perineal patterns of the adult female nematodes. Inoculation tests on black pepper seedlings using different levels of egg mass and larval inocular produced galls. Differences in root and shoot weights in inoculated and uninoculated plants were not statistically significant at 5% level. Yellowing and wilting symptoms accompanied by severe root decay and rotting occurred six months after inoculation.

Chicken dung at 3.5 tons per ha and carbofuran at 2 kg ai per ha gave comparable control of the nematode while azolla at 2.0 tons per ha was ineffective.

Introduction

Black pepper (*Piper nigrum* L.) is a potential dollar-earning crop for the Philippines. Though an introduced crop to the Philippines from Siam (Thailand), India and Indonesia, it is fast gaining popularity among Filipino farmers as a cash

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crop in terms of production and acreage (1). Production, however, is hampered by attack of plant pests and diseases. One such disease that damaged several black pepper plantations in the Bicol Region, particularly Camarines Sur in the years 1988 and 1989, was not readily diagnosed and identified; thus this study was conducted. Damage was assessed as 10-65 loss in stand of four-year-old black pepper plants. Symptoms exhibited were wilting, slight to severe yellowing of leaves, stunting, browning and blackening of vines, stems and roots, premature falling of leaves and fruits of mature and fruit-bearing plants. These symptoms were also similar to those observed in black pepper plants with soil nutrient deficiencies and fungus infection like *Phytophthora* spp. (Figs. 1, 2, 3).

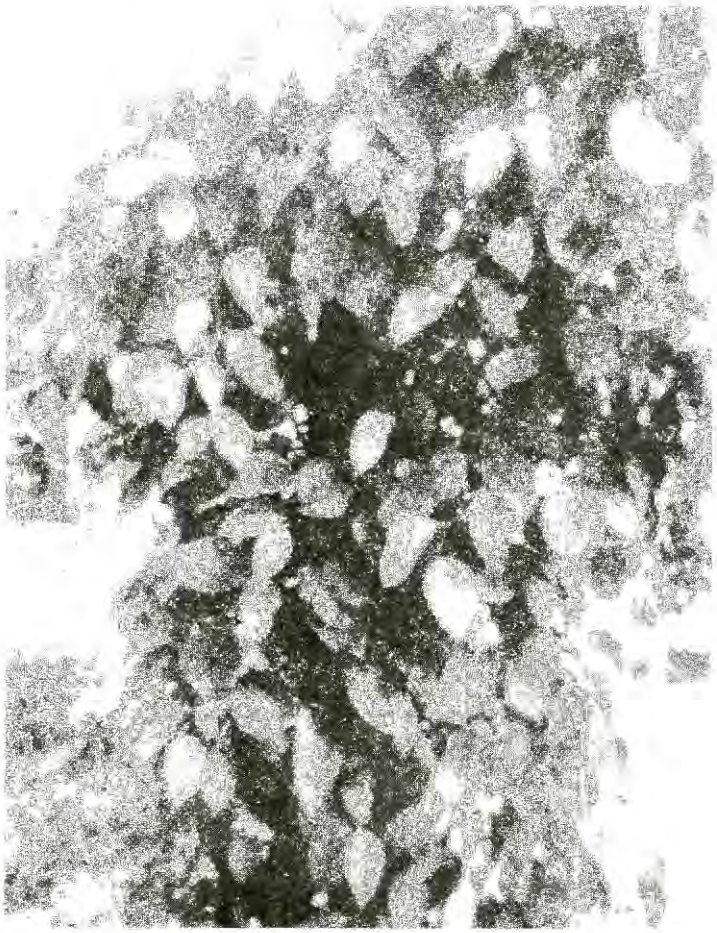

Figure 1. Naturally infected black pepper plant showing early symptom of yellowing of leaves (Tigaon, Camarines Sur). Photo by J. Cordero.

Proper and correct identification of the organism actually involved in a disease problem is important in formulating control measures and is useful for other studies.

Experimental evidence in the use of chicken dung and other organic materials to control root-knot nematode in black pepper is insufficient, though well-documented in other crops (2).

A greenhouse study was therefore designed to verify the effectiveness of chicken dung, azolla and carbofuran in controlling root-knot nematode in black pepper plants.

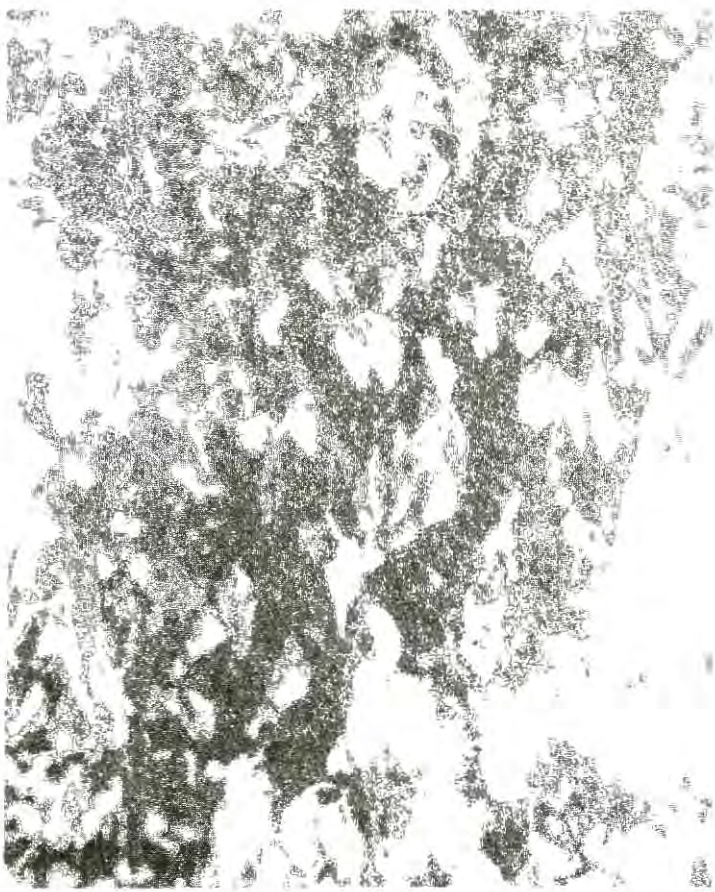

Figure 2. Naturally infected black pepper plants showing wilting, drying and blackening of leaves (Tigaon, Camarines Sur). Photo taken by J. Cordero.

Figure 3. Naturally infected black pepper plants showing later stage of yellowing and eventual wilting (Tigaon, Camarines Sur). Photo taken by J. Cordero.

Materials and Methods

Survey of Black Pepper Plantation and Collection of Diseased Plants and Infested Soil

Starting from the towns of Sipocot and Tigaon in Camarines Sur, where the report of the plant disease situation originated, other black pepper-growing areas in the Bicol Region were visited and surveyed for determination of prevalence and occurrence of the apparently new and unidentified disease. Diseased plants

showing symptoms and accompanying soil were collected for proper isolation and identification of the causal organism.

The standard procedures in diagnosis of plant diseases, determining their cause to serve as basis for devising methods for their control were followed (7, 12).

Background Information on the Field and Plants in the Area

A perspective of the problem in the field was obtained. The plants in the field were examined carefully and the grower interviewed as to the history of the field and the crops grown in it, particularly the symptoms exhibited by the diseased plants, the cultural practices performed and the source of the planting materials. Information on environmental conditions prevailing in the area during the incidence of the diseased condition as supplied by the farmer was verified at the local Agro-Meteorological and Weather Station at Pili, Camarines Sur.

After the field diagnosis, the next step was observation of the individual plants, first of the symptoms and signs of the causal organism. Careful examination included determining whether there was obvious physical damage or injury that might have been caused by machineries or farm tools. The location of the symptoms on the plant was noted. The plants were observed for signs of the pathogens. The signs and symptoms observed on the plants were compared with those reported for other crops and in standard reference works on the plant diseases.

Further work was done to ascertain the true pathogen. Individual plants were brought to the laboratory for more study. A dissecting microscope was used for making closer observations of signs of the disease. A better but slower technique was to make cultures from the affected tissue. To isolate and identify the organism and prove involvement in disease production, it was necessary to perform Koch's Postulates. In instances, however, where Koch's Postulates could not be rigidly followed as in obligate parasites such as nematodes, diagnosis by approximating Koch's Postulates was done by determining the precise involvement of nematode in disease production by greenhouse and laboratory experiments. As to the possibility of nutritional cause, chemical analysis of plant tissue was made and compared with normal healthy plants.

Source of Diseased Specimens for Fungal Isolations

Black pepper plants showing characteristic symptoms of wilting, yellowing and blackening of leaves and dry-rotting of roots were identified and marked to serve as source of diseased specimen. Infected roots and rootlets were collected by cutting through the rhizosphere with a sharp knife or scalpel. Infected aboveground parts like leaves and stems were also cut, collected and placed in plastic bags, properly labelled and brought to the laboratory for proper identification. Care in handling of the samples was taken by protecting the samples against

exposure to high temperature, prolonged handling and drying. Samples of *Phytophthora* or other fungi attacking fruit trees, vegetables and other crops may be light- and – temperature sensitive both to very low and high temperatures and should not even be kept in a refrigerator for a long time (personal communication, LeChi Tran-Gruber, BPI, RP-German Bio-control Lab.).

Detection and Isolation of Suspected Causal Fungus

Using the procedure and selective medium employed by Tsao (1970, 1987) and Tsao and Guy (1977) and provided by Dr. Tsao for this experiment, *Phytophthora* and other soil fungi were isolated. First, infected plant parts such as roots, rootlets, stems, leaves and bark of diseased black pepper were washed in tap water. Wtih a sterile scalpel, these plant parts were cut into 3 to 4 cm long pieces. The pieces were then laid individually in a clean paper towel to blot dry, picked with a sterile pair of forceps and placed in plated pimaricin-vancomycin-pentachloronitrobenzene-hymexazol (PVPH) and pimaricin (10 g) – vancomycin-pentachloronitrobenzene ($P_{10}VP$) selective media. Eight to 9 pieces of the smaller plant parts and 4 to 5 bigger pieces were arranged in a petri dish equidistant from each other to avoid overcrowding and overlapping of the fungal colonies that would develop after incubation. Two to 3 plates per medium were used. The plates were covered with black cloth and incubated for 3 days at 18°C. Black cloth would favor fungal development, especially for light-sensitive *Phytophthora* spp. Mycelial growth around the plated plant parts were observed. *Pythium* mycelial growth would be observed to develop faster but would appear more sparse than *Phytophthora* when viewed against the light. Microscopic examination would reveal *Phytophthora* hyphae to be gnarled or wavy or tortuous and much-branched, whereas those of *Pythium* would be straight and smooth.

After 2 or 3 days of incubation, the advancing mycelial growth in the agar medium were marked, picked and transferred to 3 or 4 plates containing PVPH and corn meal agar (CMA) media and incubated at 25°C under suspended fluorescent lights. These were then transferred anew to PVPH and CMA for further purification; then to CMA slants for storage of the culture for subsequent studies.

Baiting Technique in Fungal Isolation

Root fragments from infected black pepper plants and 30-cc soil sample were placed in a plastic cup and 150 cc sterile distilled water was added. The mixture was stirred with a glass rod to mix it thoroughly. Healthy whole black pepper leaves were then washed and allowed to float in the mixture. Sterile distilled water with healthy black pepper leaves was placed in a separate cup to serve as control. Three replications were done rather than using larger volume of soil per replicate to increase the chance of *Phytophthora* recovery (13). The bait assembly was incubated at 25°C at room temperature on top to the laboratory

counter. The cups were left uncovered. Lesions on the leaves were isolated and plated in $P_{10}VP$ and PVPH selective media. Potato dextrose agar (PDA) was also used as an additional medium to grow the organism in the advancing lesions.

For leaf-invading fungi other than *Phytophthora,* or other root pathogens, infected leaves were both directly plated in PDA after a 24-hour incubation period and indirectly, surface-sterilized first, then plated in PDA medium. Hyphae that grew out of the leaf tissue after 2 to 3 days were transferred to new PDA tubes or plates. Isolation by single spores was obtained by preparing a spore suspension on a microscope slide and removing individual spores with a capillary pipette (9). This pure culture was used for pathogenicity tests.

General Methods of Proving Host-Parasite Association and Involvement of Nematode in Disease Production

After pathogenicity tests using the isolated fungi proved negative, inoculation with root-knot nematode to prove host-parasite relationship was done. It was noted earlier that the root samples taken for fungal examination and isolation repeatedly showed presence of root galls or swellings.

Ten soil samples of about 300 cc each and about 2 g of roots were collected at random per site. The samples were collected near the rhizosphere by boring a hole around the roots of the black pepper plants about 10 cm deep using a garden trowel. These samples were then mixed thoroughly and two composite 300-cc samples were used for nematode analysis and examination. Each sample was placed in a plastic bag, labelled and tied with a rubber band. The samples were also protected from too much exposure to high temperatures to prevent killing of the nematodes. When not processed immediately, the samples were stored in a cold room (10-15°C). Analysis or processing was done in the laboratory.

Processing of Soil Samples and Staining Root Samples

The nematodes were extracted from the soil samples by using a combination of the Cobb's sifting and gravity and Baermann methods.

For extracting of adult immobile root-knot nematodes, the teasing out method was used. The nematodes from plant materials were dissected in a small amount of water with the use of dissecting needles and a binocular microscope.

To demonstrate the relationship of the root-knot nematodes is black pepper, the nematodes in plant tissue were stained. Root samples were stained right away but those that could not be stained immediately were washed gently under running water to remove the soil particles and fixed in vials containing formalin acetic acid (FAA – formation, 6 ml; 95 ethanol, 20 ml; glacial acetic acid, 1 ml; distilled water, 40 ml). The techniques in staining roots for identification and counting of nematodes conventionally for this purpose were used (4, 11). Lactophenol acid-fuchsin staining solution was prepared as follows: mix phenol, 20 g; lactic acid, 20 g; glycerin, 20 g; water, 20cc. To this mixture was added 5 cc of a solution made by dissolving 1 g of acid fuchsin in 100 cc of water.

Two grams of root material were washed in water to remove the soil particles. The staining solution was boiled and the root samples immersed for 3 to 5 minutes, then allowed to cool. The stained roots were removed from the staining solution and rinsed in water and then stored in a destaining solution (clear lactophenol solution, without acid-fuchsin) for several days.

Collection of Galled Swollen Roots of Black Pepper Plants

The root system of black pepper plants were dug and examined for the presence of galls or swelling. These galls were dissected under the binocular microscope in the laboratory for the presence of root-knot nematodes. Portions of roots with mature females were stained in hot acid fuchsin lactophenol, following the procedure of McBeth *et. al.*, 1941. From those mature females, fifty mounts of perineal patterns were prepared for identification of the species of *Meloidogyne*, the root-knot nematode. Before identification was done, the nematodes from the naturally-infected black pepper plants were first increased in susceptible tomato (*Lycopersicon esculentum* Mill.)

Identification of Root-Knot Nematode Species Association with Black Pepper

The nematodes used in this study were recovered from diseased four-year-old black pepper plants owned and reported by Mr. J. Cordero of Tigaon, Camarines Sur. The heavily infected roots of black pepper from Tigaon, Camarines Sur were brought to the laboratory and the nematodes extracted from the galled roots. Egg masses were picked with a pair of pointed forceps and a single egg mass increased on tomato seedlings planted in plastic bags with heat-sterilized soil. The inoculated plants were grown in the greenhouse. The plants were watered carefully to prevent contamination. After one month, the plants were examined for the presence of galls. Infected plants were allowed to grow under controlled conditions in the greenhouse. Shade was provided in the greenhouse to simulate conditions in black pepper plantations under shade trees. After sixty days, galled roots of the inoculated tomato plants were gathered and washed preparatory to identification procedures. The rest of the infected tomato plants with abundant egg masses were used as source of inoculum for subsequent inoculation studies and host-range determination.

The most reliable basis for identifying the species of *Meloidogyne* is the morphology of the perineal patterns of adult female root-knot nematode (3, 5). This criterion was used in this study for the identification of the root-knot nematode attacking black pepper.

The method adopted from Thorne (1961) and Franklin (1960) of preparing perineal patterns was used in the present study. Fresh galled roots of inoculated tomato seedlings were stained immediately in boiling acid-fuchsin lactophenol. Thirty to fifty egg-laying female root-knot nematodes were placed in a plastic disposable petri dish with several drops of plain lactophenol. Each nematode was then individually cut with a thinly-bladed scalpel along the head or neck region,

to relieve the body pressure. The mid-region was cut and slight pressure applied to release the body contents or internal parts. Three-fourths of the body was cut, leaving the remaining fourth were the vulval and anal regions were located. The remaining cut portion was then transferred to a drop of lactophenol and the edges of the cup-like piece of cuticle were trimmed, leaving only a small piece bearing the perineal pattern. The cut sections were carefully cleaned of granular materials using a finely pointed bamboo splinter and 5 mm long eyebrow. The cleaned cut portion was transferred to a drop of lactophenol on a glass slide, mounted in an upside position and covered with a glass slip. The vulval ends were arranged in rows near the center of the drop with the dorsal view oriented upwards. The mount was sealed with clear nail polish using a small brush along the edges of the cover slip. The perineal patterns were examined carefully under the microscope using oil immersion lens and compared with the illustrations and descriptions of other known species. Photomicrographs of representative perineal patterns were taken. Identification of the species of *Meloidogyne* was made using the publications of Chitwood (1946), Thorne (1961), Franklin (1965) and Eisenback et al. (1981).

An experiment was conducted to test the effectiveness of organic soil amendments in minimizing infection induced by root-knot nemtodes.

Preparation of Test Plants

Two-month-old marcotted black pepper seedlings from apparently healthy mother plants were grown in 5" x 7" plastic bags with sterile soil. These seedlings in plastic bags were individually kept in the greenhouse with about 75% shade to simulate black pepper plantation under coconuts (14).

Inoculum Preparation

Larvae of the root-knot nemtode were used to inoculate the two-month-old seedlings used in the experiment. The inoculum level used was 4000 larvae of *Meloidgyne incognita* per seedling.

Root-knot Nematode Control Using Chicken Dung, Azolla and Carbofuran

The two-month-old marcotted seedlings were inoculated with 4000 larvae of *Meloidogyne incognita*. Ten days after inoculation, the treatments chicken manure, 3.5 tons per ha; dried azolla, 2.0 tons per ha; carbofuran, 20 kg ai per ha – were applied by incorporating them with the top soil just near the exposed roots of the seedlings. The calculations for the rate of the organic materials per potting materials in the greenhouse were based on the surface of the plastic bags as used by Karmacharya and Castillo (1986). The technical description of Furadan 3G is 2, 3-dihydro-2-dimethyl-carbamate (Carbofuran) as supplied by FMC International, S.A. The inoculated plants with the corresponding treatments were maintained in

the greenhouse with partial shade to approximate field conditions for the culture of black pepper.

Experimental Design and Disease Rating

Completely Randomized Design with three replications was used. Data on root galling, number of nematodes recovered from stained roots, root weight and shoot weight were gathered sixty days after inoculation.

The disease rating used for galling index was: 1 for no galling (0); 2 for light (1-25%); 3 for moderate galling (25-50%); 4 for severe galling (51-75%); 5 for very severe (more than 75%).

Results

Field and Plant Diagnosis

Field investigation and interview with the black pepper grower in Tigaon, Camarines Sur who reported the plant malady or abnormality in his plants to the Bicol Experiment Station at Pili, Camarines Sur, in 1988 revealed the following informative data.

Distribution of the Infected Plants in the Field

Most of the infected plants appeared near one corner of the field and gradually progressed towards the main field. Actual count of visibly infected plants among 100 plants, showed 65 plants with the disease syndrome. The continuous drying and wilting of the plants resulted in loss in stand of the crop.

History of the Field

The field was originally planted to coconuts and still remains as a coconut plantation. Previous crops planted were sweet potato, and cassava that served as intercrops. To fully maximize land use, black pepper was planted in 1984, starting from a hectare and gradually expanding to more than three hectares. Land preparation was by means of animal power. Plants were set in furrows distanced 2.5 m between rows and 2.0 between hills. The grower usually cultivated and tilled the soil around the plants by using a wide-edged bolo, working from one plant to another throughout the field. A combination of organic and inorganic fertilizers was usually applied by ring application using a pointed wooden tool to dig around and near the roots of the plants and scatter the fertilizer around.

Source of Planting Materials

Information given by the farmer was that the black pepper seedlings usu-

ally planted in small plastic bags came from black pepper in Cavite, Batangas and Laguna. As he expanded his plantation, a new source of planting materials was his mature plants bearing runners.

Fungal Isolation and Inoculation to Black Pepper

Using the selective media for isolation of *Phytophthora* sp. and potato dextrose agar, no pathogenic fungal isolates were recovered. The two different media used by Tsao (1983) and Tsao and Guy (1977) yielded on the third day after inoculation colonies that were fast growing, had smoothedges, and raised growth on the plates. Upon microscopic examination, the mycelia observed were straight and rapidly growing, typical of *Pythium* and *Mortierella*.

Nonetheless, further attempts to grow the isolates in appropriate culture media, such as corn meal gar and oatmeal agar for production of reproductive structures of *Phytophthora* or other soil fungi, failed. Figure 4 shows unidentified structure or spores of Isolate D obtained.

Black pepper seedlings were inoculated with the fungal isolates obtained. The colonies were grown in corn meal agar for inoculation work. Inoculation by spraying of the leaves and soil drenching with the fungal suspensions failed to

Figure 4. Fungal isolate from black pepper roots and soil through baiting technique. (430 x)

produce any visible disease symptoms on the seedlings even after four weeks. No changes in leaf color and appearance of any lesions or spots or root-rotting was observed.

On inoculated detached leaves of seedlings kept in a moist chamber, no visible spotting or water-soaking was evident seven days after inocualtion.

Survey and Collection of Diseased Plant Samples

The survey and collection of diseased plants exhibiting the various symptoms of yellowing, stunting, and wilting and the consistent presence of galled roots was conducted in four provinces in the Bicol Region, namely Camarines Sur, Camarines Norte, Albay and Sorsogon. A total of 70 samples were collected from 4 municipalities and 6 barangays in Camarines Sur (Caraycayon, Panagan and Maligurong, Tigaon; Calagbangan, Sipocot; Manguiring, Calabanga; and San Agustin, Pili); 4 municipalities and 4 barangays in Albay (Culiat, Daraga; Buang, Tabacco; Tuburan, Ligao; and Banao, Guinobatan); 2 municipalities and 2 barangays in Sorsogon (San Antonio, Casiguran; Hacienda, Sorsogon); and 2 municipalities in Camarines Norte (Doongan, Vinzons; and Calagusan, Daet) (Table 1).

The galling indices used to indicate the degree of root-knot nematode infection were based on relative percentage of galled portions of the root systems as: 1 - no galls (0); 2 - trace infection (1=25 galled roots); 3 = slight infection (26-50%); 4-moderate infection (51-75%); 5-severe infection (75-100%).

The percentage of soil samples containing the nematodes was used as a measure of nematode prevalence.

From the four provinces surveyed, with Catanduanes and Masbate excluded, 64.3% of the samples collected contained the root-knot nematode. Stained root samples collected from Tigaon, and Sipocot and in Casiguran, Sorsogon, were heavily infected with *Meloidogyne*. Further examination of stained root samples showed presence of *Meloidogyne* females in roots from all the inoculation surveyed.

Identification of the Root-Knot Nematode Species

The morphology of the posterior cuticular or perineal patterns of the adult female egg-laying nematode constitutes the most reliable characteristic for the identification of *Meloidogyne* (10). In this study, the main criterion for identification of the *Meloidogyne* isolates from black pepper plants was the morphology of the perineal patterns from artificially inoculated plants (Fig. 5).

The general characteristics of the perineal pattern of mature egg-laying female nematode obtained from black pepper roots conformed with those descriptions set by Chitwood (1949) and the pictorial key (2) as *Meloidogyne incognita* Chitwood.

Table 1. Occurrence, distribution and population of *Meloidogyne* (root-knot nematode) in black pepper plants in the Bicol Region.

Locality	*No. of Samples*	*Area (ha)*	*No. Female Meloidogyne*[1]	*Galling Index*[2]
Camarines Sur				
Tigaon, Caraycayon	20	5	270 (13)[3]	5
Panagan	3	6	37 (2)	4
Maligurong	6	15	240 (6)	4
Calagbangan, Sipocot	8	3	67 (3)	5
Manguiring Calabanga	4	1	30 (3)	3
San Agustin, Pili	8	1	86 (6)	5
Albay				
Tuburan, Ligao	1	1	0	1
Culiat, Daraga	1	1	0	1
Buang, Tabaco	5	2	20 (3)	4
Banao, Guinobatan	3	1	31 (2)	4
Sorsogon				
San Antonio, Casiguran	6	1	129 (5)	5
Hacienda, Sorsogon	3	5	20 (1)	3
Camarines Norte				
Doongan, Vinzons	1	1	0	1
Calagasan, Daet	1	1	7	4

[1]Two-gram stained roots

[2]Galling index to denote degree of infection: 1-no galls (0); 2-trace (1-25% galled roots); 3-slight (26-50%); 4-moderate (51-75%); 5-severe (75-100%)

[3]Number of samples containing the nematodes

Effect of Chicken Dung, Azolla and Carbofuran on Infection Induced by Root-Knot Nematode

Table 2 shows the effect on galling index, number of nematodes recovered and root and top weight, takes sixty days after inoculation. On gall formation, treatments with chicken dung rated 2.5 and did not differ significantly with carbofuran at 2.7 rating. The number of female nematodes recovered in the azolla treatment was highest at 40.0 per two-gram roots; followed by chicken dung and

Figure 5. Artificially inoculated black pepper plant showing yellowing and wilting.

furadan at 29.0 and 36.0, respectively, with no statistical difference at five level of confidence, but significantly different with azolla and the untreated check. For number of egg masses, chicken dung had the lowest count, 5.0 and significantly different with azolla and carbofuran and the untreated check with 41.0. Root weights of chicken dung and azolla did not statistically differ but were significantly different with root weights of carbofuran. The difference between the top weights of chicken measure, azolla and the checks were not significantly different from each other.

Discussion

Evidences showed the involvement of the root-knot nematode, *Meloidogyne incognita* Chitwood in the yellowing and wilt diseases of black pepper in the Bicol Region. A survey of black pepper plantations revealed that 64.3% of soil and plant samples from 4 provinces, 12 towns and 14 barangays of the Bicol Region were infected by the nematode. The presence of root galls containing feeding *Meloidogyne* females was consistently observed in stained roots.

The occurrence of root-knot on black pepper was first reported in the Philippines by Valdez (1968) and later confirmed in a survey and subsequent

Table 2. Effect of chicken dung, dried azolla and carbofuran on black pepper inoculated with *Meloidogyne incognita* under greenhouse conditions.

Treatment and Application Rate	Galling Index[1]	No. of Females[2]	No. of Eggmasses[2]	Root Weight[2] (g)	Top Weight[2] (g)
Chicken Manure 3.5 tons/ha	2.5 b[3]	29.0 b	5.0 b	7.0 b	36.0 a
Azolla 3.5 tons/ha	3.3 c	40.0 c	19.0 c	7.3 b	30.0 a
Furadan 2 kg ai/ha	2.7 b	36.0 b	16.0 c	6.0 a	24.5 b
Untreated Check	3.8 c	53.0 d	41.0 d	8.3 c	33.3 a
Check	1.0 a	0 a	0 a	9.3 d	39.0 a

[1]Indicates no galling (0); 2-light (1-25%); 3-moderate galling (25-50%); 4-severe galling (51-75%); 5-very severe (more than 75%).

[2]Taken 60 days after inoculation

[3]Numbers in a column followed by a similar letter are not significantly different at P = 0.05 level with Duncan's Multiple Range Test.

studies by Castillo (1974, 1976). The present study reports for the first time the occurrence and parasitism of root-knot nematode on black pepper in the Bicol Region. This study further confirms the reports of Lamberti and Evanayake (1983) in Sri Lanka and of Huang and Manso (1984) in Brazil of root-knot nematode on black pepper and other horticultural and special crops.

Based on the morphology of the perineal patterns of adult and egg-laying female *Meloidogyne*, in this study *M. incognita* was identified as the associated species on black pepper. Earlier, Valdez (1968) made a similar report; however, Castillo (1974) reported *M. acrita* and *M. javanica* as the species associated with black pepper. This conflicting report is related to the difficulty in differentiating between *M. incognita* and *M. acrita* based on perineal patterns alone. It was resolved by considering all collection bearing *acrita* type under *incognita* (2), based on the findings and suggestions of Traintaphyllou and Sasser (1960). In a later report, Eisenback (1981) consolidated the species of *Meloidogyne* into 4 distinct species, namely, *incognita, javanica* and *hapla*.

Chicken dung used in this experiment gave a significant control of root-knot nematode inoculated on black pepper comparable to carbofuran. This confirms the effectiveness of chicken dung as a promising nematicide (6, 8). Aside from its nematicidal effects and low cost, its nutritive value is high and may enhance the yield of crops.

Summary and Conclusion

The cause of the yellowing and wilt diseases of black pepper (*Piper nigrum* L.) observed in the Bicol Region was investigated and determined as *Meloidogyne incognita* Chitwood.

The initial symptom of the disease was slight to general yellowing of the leaves. Wilting occurred two to three months later after heavy, continuous rains followed by sunny, warm and dry weather. The nature of the symptoms was similar to those caused by biotic and abiotic factors. Although the symptoms exhibited by the affected plants were similar to those caused by other biotic and abiotic factors, root-knot nematode was proved to be ecologically and etiologically associated with the diseased black pepper plants. Therefore, care should be exercised in determining the causal organism based on symptoms alone, especially with root pathogens. It is suggested that other causes producing similar symptoms and other diseases on black pepper be further studied. Farmers will thus be benefited in solving their crop protection and management problems.

A survey for the presence of *Meloidogyne incognita* Chitwood, a root-knot nematode was conducted in 4 provinces, 12 towns and 14 barangays of the Bicol Region. The results showed that 64.3% of the 70 samples collected showed presence of the root-knot nematode. Loss in stand of black pepper plants ranged from 10-65% based on personal assessment. Swollen, galled roots and adult egg-laying female nematodes were consistently observed in the affected black pepper plants.

Initial investigation for the possible association of suspect fungal pathogens, particularly *Phytophthora* spp. gave negative results, despite the rapid isolation technique using the selective medium, pimaricin-vancomycin-pentachloronitrobenzene-hymexazol (PVPH) and the common culture medium, potato dextrose agar (PDA).

Identification of the *Meloidogyne* species that affected the black pepper plants was based on the posterior cuticular patterns of the adult female nematodes. Fifty perineal patterns of adult, egg-laying root-knot nematodes revealed a characteristic belonging to *Meloidogyne incognita* Chitwood, which is a distinct high squared-off arch composed of smooth to wavy striae that fork near the lateral lines.

Chicken dung, dried azolla and carbofuran were compared with respect to their effectiveness in minimizing gall formation in black pepper seedlings. Chicken dung at 3.5 tons per ha and carbofuran at 2 kg ai per ha were comparable in

controlling the nematodes while azolla at 2.0 tons per ha was not effective. More studies should be conducted, however, in combination with other methods to determine the most effective yet economical and practical control of the nematodes.

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