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INTERACTION OF *FUSARIUM OXYSPORUM* WITH *MELOIDOGYNE INCOGNITA* ON ROSELLE

INTERAKSI FUSARIUM OXYSPORUM *DENGAN* MELOIDOGYNE INCOGNITA *PADA ROSELA*

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INTISARI

Pengujian patogenisitas empat puluh isolat Fusarium oxysporum *terhadap rosela (*Hibiscus sabdariffa *L. var.* sabdariffa*) telah dilakukan di rumah kaca. Isolat yang paling virulen kemudian digunakan untuk pengujian kompleks penyakit dengan nematoda puru akar* Meloidogyne incognita. *Tingkat kerusakan penyakit pada benih rosela yang diinokulasi dengan kombinasi jamur dan nematoda lebih tinggi daripada yang hanya diinokulasi dengan jamur atau nematoda saja. Benih yang diinokulasi dengan jamur dua minggu setelah inokulasi nematoda menunjukkan tingkat kerusakan penyakit yang paling tinggi dibandingkan dengan yang diinokulasi dengan nematoda dua minggu setelah inokulasi jamur atau yang diinokulasi dengan kedua patogen secara bersamaan. Hal tersebut menunjukkan bahwa infeksi akar oleh* M. incognita *meningkatkan kolonisasi* F. oxysporum *pada rosela dan kadang kala menyebabkan kerusakan yang lebih tinggi pada benih rosela. Tingkat kelayuan yang tinggi dengan keberadaan* M. incognita *dan* F. oxysporum *diduga disebabkan karena hubungan sinergistik di antara kedua patogen.*

Kata kunci: Fusarium oxysporum, Meloidogyne incognita, *rosela, kompleks penyakit*

ABSTRACT

Forty isolates of *Fusarium oxysporum* were tested for their pathogenicity to roselle (*Hibiscus sabdariffa* L. var. *sabdariffa*) in a plant house. The most virulent isolate was later used in a disease complex experiment with a root-knot nematode *Meloidogyne incognita*. Disease severity of roselle seedlings inoculated with a combination of fungus and nematode was higher than those inoculated with either fungus or nematode individually. Seedlings that were inoculated with fungus two weeks after nematode inoculation showed the highest disease severity compared to that inoculated with nematode two weeks after fungal inoculation or that inoculated simultaneously with both pathogens. It seems that root infections by *M. incognita* increased the colonization of roselle by *F. oxysporum* and subsequently caused higher damage to the roselle seedlings. The high wilt incidence in the presence of *M. incognita* and *F. oxysporum* may be due to the synergistic relationship between these two pathogens.

Key words: *Fusarium oxysporum, Meloidogyne incognita,* roselle, disease complex

INTRODUCTION

Vascular wilt caused by *Fusarium oxysporum* Schlecht. is a major problem in most roselle growing regions in Malaysia. From a field survey conducted at the beginning of 1995, diseases on roselle particularly vascular wilt could incur serious losses up to 35% (Salleh *et al.*, 1998). This soil-borne disease heavily infected roselle particularly on heavy soils for the second crop consecutively (Ooi and

Salleh, 1999; Ooi *et al.*, 1999). Fusarium wilt causing great loss to roselle planting farmers due to the decrease of product yield and the quality of roselle produced.

Root knot nematode *Meloidogyne incognita,* is associated with *F. oxysporum* in the development of Fusarium wilt in roselle (Hafiza, 1997). It has been known that *M. incognita* interacts with *F. oxysporum* and several other fungal pathogens in the soil (Powell, 1971; Mani & Sethi, 1987; Francl & Wheeler, 1993). *M. incognita* is reported to have the ability to increase the severity of Fusarium wilt on a number of economically important crops (Colyer *et al*, 1997; Dropkin, 1972; Mani & Sethi, 1987; Minton & Minton, 1966; Rao & Krishnappa, 1996; Starr *et al*, 1989).

The root-knot nematode-Fusarium wilt disease complex can result in plant mortality and suppression of plant growth. Both pathogens can damage plants independently, but losses are more severe when they occur together synergistically (Kappelman & Sappenfield, 1973; Martin *et al.*, 1956; Minton & Minton, 1966). The objective of this study is to examine the role of *M. incognita* in the severity of Fusarium wilt caused by *F. oxysporum* that forms a disease complex on roselle in Malaysia.

MATERIALS AND METHODS

Fungus inoculum. The fungal pathogen, *F. oxysporum* was isolated from roselle which showed symptoms of vascular wilt from various plots in three states (Penang, Terengganu and Perak) of the northern Malaysian Peninsula. *F. oxysporum* that isolated were transferred by a single spore isolation technique onto potato sucrose agar (PSA; Booth 1971). The cultures were then incubated for ten days in room temperature (28 ° ± 2 °C) under standard incubation conditions (Salleh & Sulaiman, 1984). All isolates are preserved in liquid nitrogen at the Fusarium Culture Collection Unit, Universiti Sains Malaysia (Salleh & Strange, 1988).

Nematode inoculum. Roots of roselle infected with *M. incognita* were collected from the plant house in the Universiti Sains Malaysia, Penang. The nematode was multiplied on the root of some highly susceptible 3 week-old tomato cultivars grown in 30 cm (diameter) earthen pots for two months.

Host. Apparently healthy and uncrack roselle seeds were surface-disinfected with 0.5% sodium hypochlorite (NaOCl) for 10 minutes followed by washing in three changes of sterile water. For the screening test with *F. oxysporum*, the seeds were planted in steam-sterilized soil in 28×38 cm plastic trays in the plant house. The two weeks old seedlings were thinned leaving 20 uniform and healthy seedlings in each tray. A total of 120 trays was prepared for 40 isolates of *F. oxysporum* (three trays for one isolate). For tests on disease complex, roselle seedlings were raised in 5 inch polythene bags containing approximately 1000 g sterile garden soil, one healthy seedling in each bag. Seventy-two bags were prepared for getting 12 replicates per inoculation type (with overall six types of inoculation).

Screening of **F. oxysporum.** Soil surrounding the seedlings was plunged with sterile sharp scalpel to cause injury on the healthy roselle roots. Then, 150 ml of the conidial suspension (2×10^6 conidia/ml) of *F. oxysporum* was poured into the soil per tray. Seedlings in the trays that were poured with sterile water served as controls. The suspension was prepared by harvesting the conidia with sterile distilled water from 10 day-old cultures (derived from monoconidial stock cultures) grown on PSA. The suspension was filtered through two thickness of sterile

lens tissue to eliminate mycelial fragments. The conidia were resuspended in sterile distilled water and the concentration was adjusted to 2 x 10^6 conidia/ml using a haemocytometer. Disease symptoms on individual plants were examined periodically and disease severity was assessed as stated by Ooi *et al.* (1999), eighth weeks after inoculation.

***Inoculation with* M. incognita.** Egg masses collected from tomato roots were incubated for 48 hours in sterile distilled water in a dark place at room temperature (28 ° ± 2 °C). The required numbers of juveniles (6000 nematode/ml water) in water were added to 2 cm deep holes made around the host (healthy roselle seedlings).

Evaluation of disease complex. Inoculum of the most virulent isolate of *F. oxysporum* obtained from the screening test was used in this evaluation. Six types of inoculation were employed, following a complete randomised design (CRD) with 12 replicates (in 12 polythene bags).

- ❶ no pathogens added, acted as controls (C).
- ❷ *F. oxysporum* alone (F).
- ❸ *M. incognita* alone (N).
- ❹ inoculation of both pathogens simultaneously (F+N).
- ❺ inoculation of *M. incognita* two weeks prior to inoculation with *F. oxysporum* (N - F).
- ❻ inoculation of *F. oxysporum* two weeks prior to inoculation with *M. incognita* (F - N).

The two week-old seedlings in inoculation type ❺ were inoculated with *M. incognita* by pipetting 6000 nematode larvae to each polythene bag. Whereas plants in the inoculation type ❻ were inoculated with 150 ml spore suspension (2 x 10^6/ml) of *F. oxysporum*. Both inoculations were to cause a predisposed injury onto the host plants two weeks prior to the next inoculation treatment.

Two weeks later, plants in inoculation type ❶ were treated with 150 ml sterile distilled water as control. Plants in inoculation type ❷ and ❺ were inoculated with 150 ml spore suspension (2 x 10^6/ml) of *F. oxysporum* while plants in inoculation type ❸ and ❻ were inoculated with nematode larvae (6000 larvae/bag). The plants in inoculation type ❹ were treated with both pathogens at the same time.

The assessment of the disease severity was done eight weeks after inoculation. External vascular wilt symptoms were observed as well as the symptoms of root-knot disease caused by *M. incognita.* The disease severity was assessed based on wilt symptoms by following a scale devised by Ooi *et al.* (1999).

Plants were taken out from the soil and washed with running tab water to remove the soil particle and debris. Observation was done to determine the present of galls on the roots of the plants. The number of galls or egg masses formed was used to estimate the survival population of nematode in the roots for different inoculation types. The size of the population was classified as follows:

Class	Symptom/Observation
0	No gall or egg masses
1	1-2 galls or egg masses
2	3-10 galls or egg masses
3	11-30 galls or egg masses
4	31-100 galls or egg masses
5	> 100 galls or egg masses

Then, the population size was estimated by calculating the index as follows:

$$P = \frac{(n \times 0) + (n \times 1) + (n \times 2) + (n \times 3) + (n \times 4) + (n \times 5)}{\text{Total plant assessed}}$$

where, P = Population index
n = Number of plants that showed the particular class.

The height of the plants was measured weekly after inoculation. On the eighth week, the plants were unearthed slowly and the roots were washed to eliminate soil particles. Fresh weights of the leaves, stems, fruits and roots were taken separately. Then, these parts of the plants were dried at 105° C for 24 hours to measure the dry weights.

RESULTS AND DISCUSSION

For the screening tests with *F. oxysporum*, disease severity as mean disease index was assessed eight weeks after inoculation. The most virulent isolate was No. P2883, obtained from stem of a wilted roselle plant in Penang. It gave the highest disease index of 3.30 (Ooi *et al.*, 1999). This isolate was subsequently used to represent *F. oxysporum* in the tests on disease complex.

The disease complex was evaluated from two aspects *i.e.* the Fusarium wilt and the root-knot disease. Fusarium wilt causes the symptoms of yellowing of the older leaves followed by defoliation and wilting. Similar symptoms are shown by the younger leaves at the later stages. The underlying tissues of the stem and root showed distinct vascular discoloration from which *F. oxysporum* can easily be isolated. Root-knot disease gives rise to the formation of galls on the roots with the diameter two to three times bigger than normal roots. The plants apparently retarded and wilt at noon but recover in the evening. Both diseases caused the declining of number and size of the leaves compared to the healthy control plants.

The disease index on plants inoculated with both pathogens (inoculation type ❹) was higher than that inoculated with the fungus alone (inoculation type ❷). However, roselle seedlings inoculated with nematodes two weeks prior to fungus inoculation (inoculation type ❺) showed the highest disease index value (Table 1). This showed that nematodes might cause the severe symptoms of wilt when it appeared before the fungus infection. The roots that exposed to nematodes intrusion might help in providing entrance for *Fusarium* infection.

Table 1. Average disease index of *Fusarium* wilt

Inoculation type	Average disease index
❶ Control (C)	-
❷ *F. oxysporum* (F)	2.2
❸ *M. incognita* (N)	-
❹ F + N	2.3
❺N - F	2.7
❻ F - N	2.4

When nematodes appeared simultaneously (inoculation type ❹) or two weeks after *F. oxysporum* (inoculation type ❻), it enhanced the synergism of the disease complex as the two pathogens interact positively with one and other on a host. It caused more serious damage than while they act individually. But when nematodes appeared two weeks before *F. oxysporum* (inoculation type ❺), the damage caused even greater than other inoculation types as the roots of the host plants were intensively injured by a great number of nematode causing wound and weakening the resistance of the plants to any other pathogenic invasions. Hence, the disease index recorded from inoculation type ❺ is the highest compared to two other types of pathogen combination treatment (inoculation type ❹ and ❻). The symptoms of root knot disease showed a formation of giant cells that caused by the wound made by nematode and so providing the entrance for the fungus thus, causing higher severity in a short time.

The increase metabolic activity of the giant cells stimulated mobilization of photosynthesis products from shoots to roots and in particular to the giant cells, where the products were removed and

utilized by the feedings nematodes (Bird & Loveys, 1975; Dropkin, 1972). The abnormal growth of roots caused the reduction of root surface area and limited the capacity of the root system to explore throughout the soil. Thus the primary cause of poor nutrient uptake and suppressed growth of infected plants could be related to the reduced root system (Hunter, 1958). Growth of root tips invaded by *M. incognita* juveniles was frequently temporary inhibited. The resumption of growth of the root tips and the formation of galls might create a drain on the plant resources in the shoot tissue, resulting in suppression of shoot growth (Wallace, 1971).

The temporary inhibition of root growth probably also interrupts the synthesis of cytokinin and gibberelin which were produced in root apexes. The decrease in synthesis of these growth regulators in young plants, when they are most vulnerable to nematode damages probably had a profound influence on plant growth. Furthermore, the entrance or injury predisposed by nematode also causing the breakdown of the host resistance to *F. oxysporum* thus enhanced the fungal entrance.

The resistance breakdown was believed to be caused by the failure of root knot infected plants to develop tyloses (Beckman *et al.*, 1972) which will inhibit *F. oxysporum* from penetrating the whole plants through the xylem vessels. *F. oxysporum* induced tyloses formation in the xylem vessels by the balloon-like expansion of xylem parenchyma cells through the xylem pits. Xylem parenchyma cells that are modified to form giant cells or are physiologically influenced by adjacent giant cells are unlikely to produce tyloses.

The combination of two pathogens (inoculation type ❹) gave no significant different on the number of galls formed compared to nematode alone (inoculation type ❸). In fact, when nematode was inoculated two weeks after *F. oxysporum* (inoculation type ❺), the galls formed were less (Table 2). This might due to the decline of nematode population in the soil. Presence of fungus along with nematode resulted in significant reduction in nematode population, more so when fungus was inoculated prior to nematode (Rao & Krishnappa, 1996). According to Powell (1971), in combined infection, the fungus component of an interaction often has a real effect on nematode population and generally population of sedentary forms like *Meloidogyne* spp. are reduced because they remain sedentary at one place and therefore are subjected to influences by changes in the host system, as a result of fungal infections. However, when nematode was inoculated before the fungus (inoculation type ❺), the number of galls formed reached the highest value of average three to ten galls or egg masses on the roots (Table 2). The nematode was inoculated to the plants two weeks earlier than those of other inoculation types. The longer the nematode presents in the rhizosphere the higher it stands a chance to multiply and cause infection on the plants.

Table 2. Average population index of nematode in the root system of roselle for the six inoculation type

Inoculation type	Average population index
❶ Control (C)	-
❷ *F. oxysporum* (F)	-
❸ *M. incognita* (N)	1.8
❹ F + N	1.8
❺ N - F	2.8
❻ F - N	1.6

The effect of the six treatments on the growth of roselles was observed by the measurement of plants height (Figure 1). The control plants showed normal growth in height while all pathogen treated plants had lower height compared to the healthy controls. This means that the infected plants were retarded and reduced in growth. Roselles inoculated with nematode two weeks prior to fungus inoculation were the shortest among all others.

Generally, roselles inoculated with both pathogens gave less average dry weight compared to roselles inoculated with either one pathogen alone (figure 2). Control plants had the highest value in dry weight of all parts of the plants measured (root, stem and fruit).

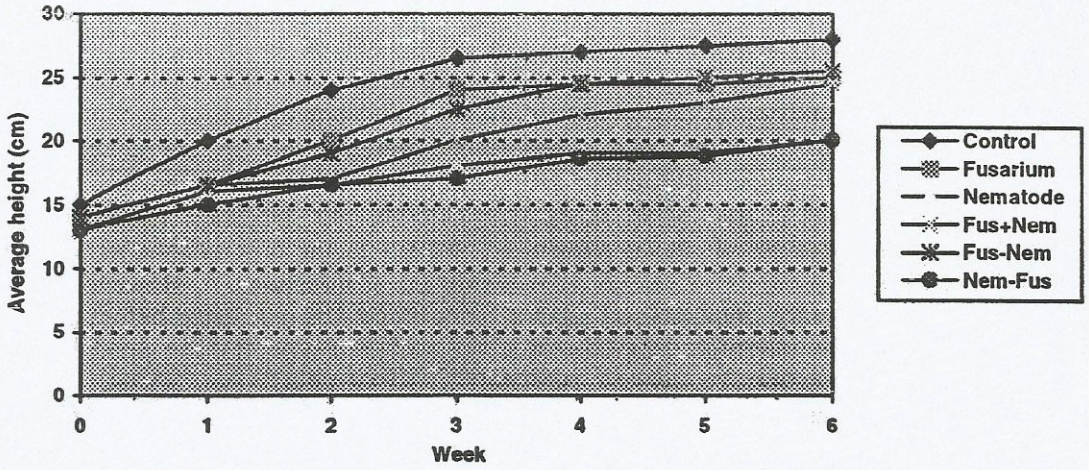

Figure 1. Average height of roselles treated with six types of inoculation

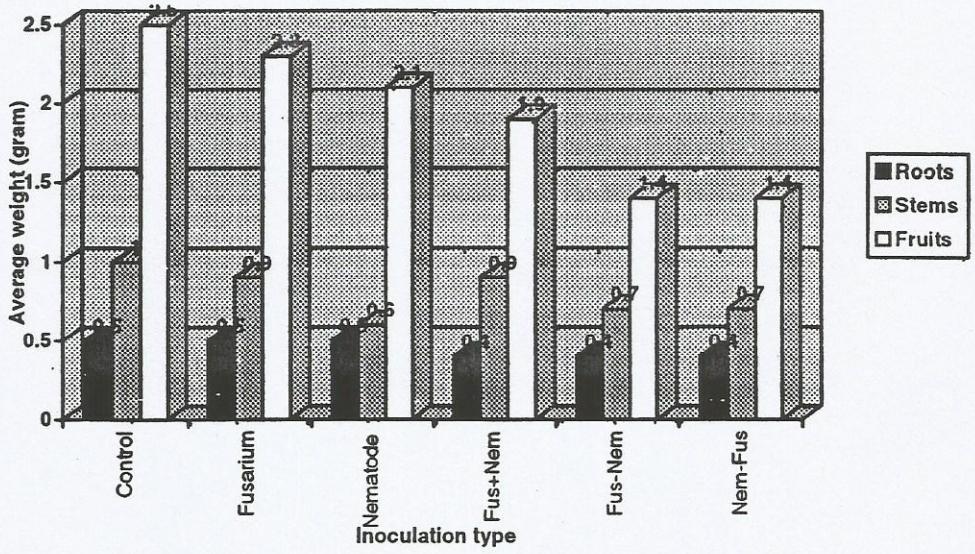

Figure 2. Average dry weight of roselles treated with six types of inoculation.

Statistical test on fruit dry weight showed that all kinds of combined inoculation gave different dry weight value at $P_{0.01}$ compared to control plants and those inoculated with either fungus or nematode alone. This proved that the disease complex caused a significant decrease in plant biomass.

The interaction of *F. oxysporum* and *M. incognita* on roselle caused similar symptoms like each individual disease occurs separately on roselle, but only to cause higher severity in both Fusarium wilt and root-knot disease that incurred in one particular plant. This synergistic interaction also found out to cause earlier expression of symptoms by the combined treated plants compared to plants inoculated with either pathogen alone as most of the wilting and root-knot symptoms could be observed on the second week after inoculation with both pathogens while the first symptom could only be observed three to four weeks after inoculation with either pathogen alone. The synergism also had effect on plant growth. The height, the number and size of the leaves and fruits as well as the weight of plants treated with combined pathogens were generally less than those treated with either pathogen.

Disturbance caused by *M. incognita* in plant roots obviously has a significant impact on the physiology of the whole plant. These nematodes directly and indirectly affect the translocation pattern of substances between the shoots and the roots, which may ultimately affect the yield of plants. Nevertheless, as Dropkin (1972) suggests, the greatest effect of *M. incognita* on plant health may be through predisposition of the roots to secondary infections and subsequent damages by other soil-borne organism. This synergistic phenomenon could increase the severity of Fusarium wilt on roselle.

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