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Histological and Control Studies on Aerial Gall Nematode Infecting Camphor Trees in Egypt

A. M. Kella

Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt.

Abstract

Histological studies on aerial gall nematode infecting camphor trees, *Cinnamomum camphora* in Egypt were conducted at the experimental station of the Nematology Department, Plant Pathology Research Institute, Agricultural Research Center. Transverse sections in the healthy and infected leaves with aerial gall nematode at early and late infection symptoms of camphor trees were done with the aid of electronic microtome. At early symptoms of infection with nematode, small galls with a light green color in the main axon of leaf were observed, meanwhile at late symptoms of infection, nematode galls transfer to dark green or light red color. Giant cells were characterized with thickened walls and necrosis. Histological preparations of the infected leaves cleared that, giant cells in the early symptoms infection contain different stages of the nematode, *Fergusobia* spp. and the insect, *Fergusonina* spp, while at the late symptoms infection it was found different pores in the galls where the emergency of the adult insect in the gall was done through it. Control experiments by the nematecide, Vydate 24% L. and the insecticide Pyridan 45% L. were applied spraying on the whole plant of camphor or applied in the irrigation water against both of nematode and insect, *Fergusobia* spp. and *Fergusonina* spp. The obtained results indicated that the mixed treatment of Vydate 24% L. at irrigation water and Pyridan 45% spraying on plants recorded the best results in controlling aerial nematode galls on camphor trees.

Key words: *Fergusobia*, *Fergusonina*, histological, camphor, nematode, insect, gall.

Introduction

Nematode, *Fergusobia* spp. shows dual parasitism of the insect *Fergusonina* spp. in aerial camphor galls. The highest frequency occurrence of nematode galls was recorded in the galls of camphor trees in summer and autumn season **(Kella and Bekhiet, 2011 a).** The transmitted insect adult female fly, *Fergusonina* spp. deposits its eggs singly inside the tissues of vegetative parts of camphor trees, also at the same time insects lay different stages of the nematode juveniles (*Fergusobia* spp.) inside the plant tissues. Galls, first take the green light color, then changed to green dark and finally become red light color **(Kella and Bekhiet, 2011 b).** *Fergusobia* nematode and fergusonina insect are live in a symbiosis relationship that cause of galls on shoot trees **(Giblin-Davis *et al.,* 2001 and 2004).** Several species of *Fergusobia* spp. are known to be parasitize of fergusonina flies and

aerial galls of *Eucalyptus* spp. **(Siddiqi, 1986 and 1997).**

The goal of this research was to throw the light on histopathological and control of the nematode, *Fergusobia* spp. and the insect, *Fergusonina* spp. induced aerial camphor galls.

Materials and Methods

Fifty camphor seedlings were transplanted to 50 cm diam plastic pots filled with sand clay soil 1:1. Each pot contained one camphor seedling.

Pots were classified to five groups:

1. Ten camphor seedlings sprayed by a handle sprayer with Vydate 24 % L at dose of 7 ml/liter water.
2. Ten camphor seedlings sprayed by a handle sprayer with Pyridan 45 % L at dose of one ml/liter water.
3. Ten camphor seedlings applied with Vydate 24 % L at dose of 7 ml/liter water added with the irrigation water.
4. Ten camphor seedlings treated with Vydate 24% L. at dose of 7 ml/ liter water were added with irrigation water and 1 ml/liter Pyridan 45% at dose of one ml/ liter water were sprayed on plants.
5. Ten camphor seedlings without any treatments were served as control treatment.

The treatments was replicated ten times. Observations were done one month after pesticide application, where the number of aerial camphor galls were recorded. Observations was continued during the period from May to October. To study the changes in the leaf structure during the period of experiment, healthy and infected leaf samples were taken to conduct the histo-pathological studies where leaves were kept in Formalin Acetic Acid (F.A.A.) solution for fixation. Dehydration was made in series of different concentrations of butanol and ethanol alcohols mixtures. Dehydrated galls tissues were then in filtrated in paraffin was at 50°C for ten days. Then, they were sectioned at thickness of 12 M by a rotary microtome, stained with safranin and fostgreen, and mounted in Canada balsam according to the procedures of **Sass (1964),** and recorded the changes by Leica image analysis. The histological studies were done in Cairo University Research Park, Faculty of Agriculture, Cairo university.

Obtained results were statistically analyzed by one way analysis of variance (ANOVA) and the means were separated using Duncan's multiple range test **(Duncan, 1955)** and the L.S.D 5% (least significant difference) according to the computer program, Costat Software **(Norman and Streiner, 1994).**

Results and Discussion

The transmitted insect adult female fly, *Fergusonina* spp. deposits its eggs singly inside the tissues of vegetative parts of camphor trees, also at the same time insects lay different stages of the nematode juveniles (*Fergusobia* spp.) inside the plant tissues (Fig. 1). The symposium relationship between *Fergusobia* nematode and *Fergusonina* insect led to producing galls in camphor leaves. Photographs in Fig. (2) show the different stages of gall formation on camphor vegetative parts compared with healthy camphor plants, where galls take a light green color at early symptoms and at final period take the dark green or light red color.

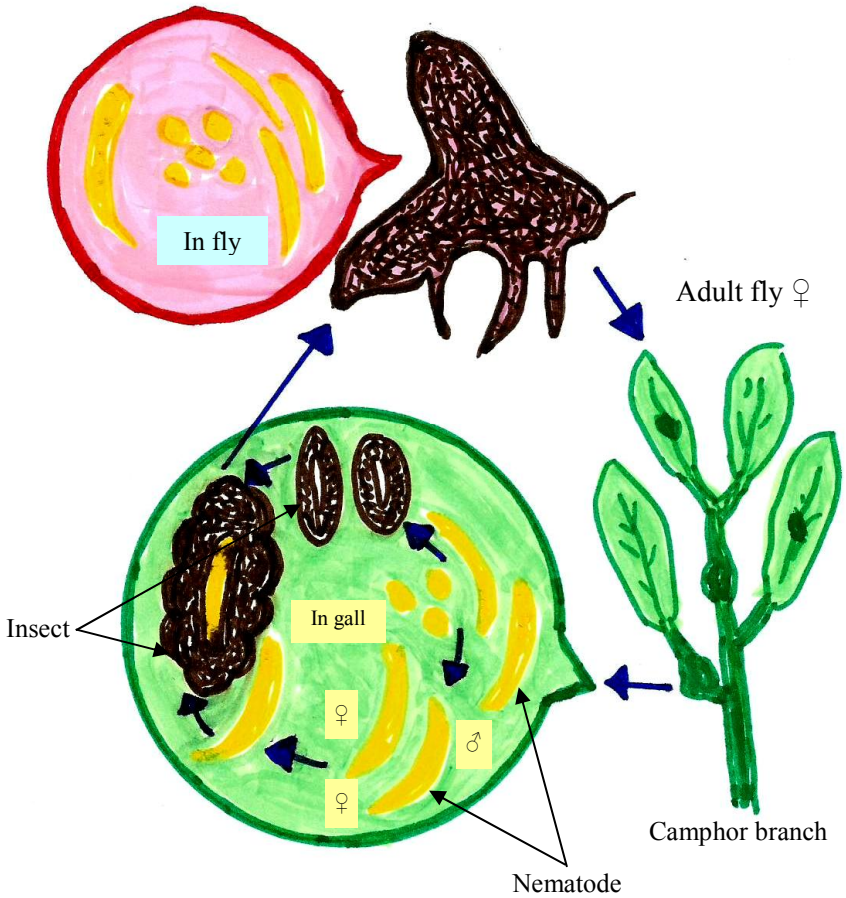

Fig. (1). Life cycle of *Fergusonina* insect and *Fergusobia* nematode in camphor trees.

Healthy without infection

Early symptoms

Late symptoms

Fig. (2). Aerial nematode galls in camphor trees.

Photographs in Fig. (3) show the histological changes in galls camphor infected with *Fergusobia* spp and *Fergusonina* spp where giant cells were found prolonged in vascular parenchyma cells with different shapes from circular to irregular shape. Giant cells were characterized with thickened walls. In transverse sections of galls camphor show the different between early symptoms and late symptoms infection compared with healthy without infection.

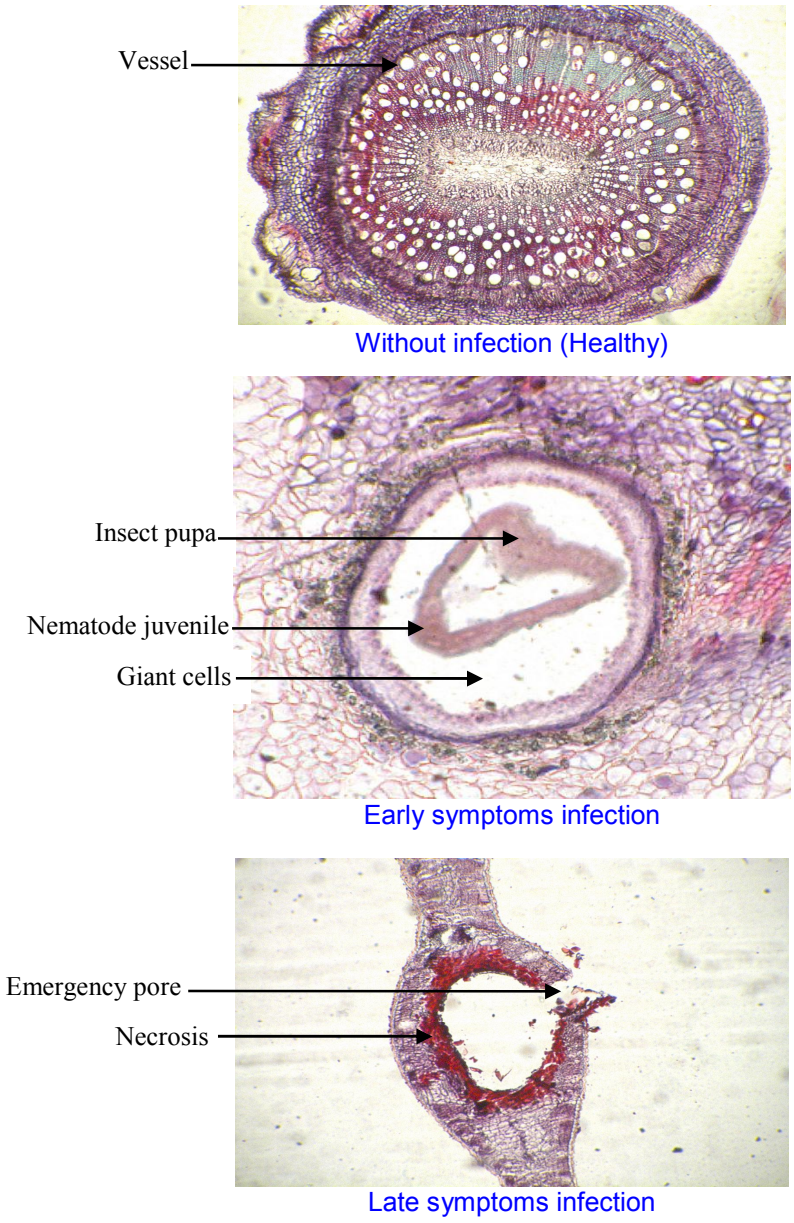

Fig. (3). Transverse sections of aerial gall camphor plants infected and non infected with *Fergusobia* spp. and *Fergusonina* spp.

Results in Table (1) indicated that the lowest average number of early symptoms and late symptoms aerial nematode galls in the treatment (Vydate irrigation in soil + Pyridan spraying in trees) recording 1, 2.5, 4, 10, 8 and 5.5 in May, June, July, August, September and October, respectively.

Also, results in Table (1) revealed that the higher average number of aerial nematode galls in all treatments recorded 27.1 in August while the lowest number recorded 5.3 in May.

Statistical analysis of data in Table (1) indicated that there were significant differences in the number of aerial nematode galls between months.

The obtained results in Table (2) revealed that the average numbers of early symptoms aerial nematode galls were decreased according to the application of (Vydate irrigation in soil + pyridan spraying in trees) or Vydate spraying trees recording 8.33 and 22.16, respectively. While the application of Vydate irrigation soil or Pyridan spraying trees recording 25.67 and 38, respectively.

Also, results in Table (2) reveal that the use of Vydate irrigation in soil + Pyridan spraying in trees recorded 84% reduction in numbers of total galls, while Vydate spraying in trees and Vydate irrigation in soil caused 61% and 58%, respectively compared with the control.

Statistical analysis of the data in Table (2) indicated that there were no significant differences, in the average numbers of early symptoms and late symptoms aerial nematode galls, between the treatments in the application of Vydate irrigation soil or Vydate spraying trees.

Results in Fig. (4) illustrate that application of Vydate with irrigation water + Pyridan spraying on plants resulted in the lowest number of aerial galls in camphor, while the treatment Pyridan spraying trees only showed the highest number of aerial galls camphor compared with the control.

Finally, it could be concluded that the application of nematicides and insecticide for the control of fergusobia spp and Fergusonina spp infested camphor trees significantly decreased the severity of nematode and insect. Also, the relation between fergusobia nematode and fergsonina insect are symbiosis relationship which produce nematode galls, the higher number of aerial nematode galls in August. The obtained results are in agreement with those obtained by **(Kella and Bekhiet, 2011b); (Giblin-Davis et al., 2004) and (Siddqi, 1997).**

Table (1). Effect of used Vydate and Pyridan at different application methods on controlling aerial nematode galls on camphor trees.

Treatments	Average number of aerial nematode galls during active period																							
	May				June				July				August				September				October			
	Early symptoms	Late symptoms	Total	Mean	Early symptoms	Late symptoms	Total	Mean	Early symptoms	Late symptoms	Total	Mean	Early symptoms	Late symptoms	Total	Mean	Early symptoms	Late symptoms	Total	Mean	Early symptoms	Late symptoms	Total	Mean
Vydate spraying trees	5	0	5	2.5^{MN}	10	0	10	5^{KLM}	16	2	18	9^{GHI}	38	3	41	20.5^{DE}	36	5	41	20.5^{DE}	28	8	36	18^{DE}
Pyridan spraying trees	11	2	13	6.5^{JKL}	15	4	19	9.5^{GHI}	20	6	26	13^{F}	63	9	72	36^{B}	60	13	73	36.5^{B}	59	17	76	38^{B}
Vydate irrigation in soil	9	1	10	5^{KLM}	11	1	12	6^{JKL}	16	2	18	9^{GHI}	41	2	43	21.5^{D}	38	2	40	20^{DE}	39	2	41	20.5^{DE}
Vydate irrigation + pyridan spraying	2	0	2	1^{N}	5	0	5	2.5^{MN}	5	3	8	4^{LMN}	17	3	20	10^{FGH}	13	3	16	8^{HIJK}	8	3	11	5.5^{KLM}
Control	19	4	23	11.5^{FG}	25	10	35	17.5^{E}	32	18	50	25^{C}	70	25	95	47.5^{A}	64	30	94	47^{A}	60	30	90	45^{A}
Total	53				81				120				271				264				254			
Mean	5.3^{E}				8.1^{D}				12^{C}				27.1^{A}				26.4^{AB}				25.4^{B}			

L.S.D 5% for the average number of aerial nematode galls of months & treatments = 2.871

L.S.D 5% for the average number of aerial nematode galls of months = 1.284

Means of the rows and columns followed by the same letter(s) are not significantly different.

Table (2). Effect of Vydate and Pyridan on early and late symptoms of aerial gall nematode on camphor trees.

Treatments	Average number of early symptoms and late symptoms aerial nematode galls						% Reduction
	Total of early galls	Mean galls in months	Total of late galls	Mean galls in months	Grand total of galls	Grand mean of galls	
Vydate spraying trees	133	22.16^{D}	18	3^{G}	151	12.58^{C}	61
Pyridan spraying trees	228	38^{B}	51	8.5^{F}	279	23.25^{B}	28
Vydate irrigation in soil	154	25.67^{C}	10	1.67^{G}	164	13.67^{C}	58
Vydate irrigation + Pyridan spraying	50	8.33^{F}	12	2^{G}	62	5.17^{D}	84
Control	270	45^{A}	117	19.5^{E}	387	32.25^{A}	
Total of galls	835		208		1043		
Mean	27.83^{A}		6.93^{B}		17.38		

L.S.D 5% for the average number of grand mean in aerial nematode galls = 1.17

L.S.D 5% for the average number of early or late aerial nematode galls = 1.66

L.S.D 5% for the average number of total early or late aerial nematode galls = 0.74

Means of the rows and columns followed by the same letter(s) are not significantly different.

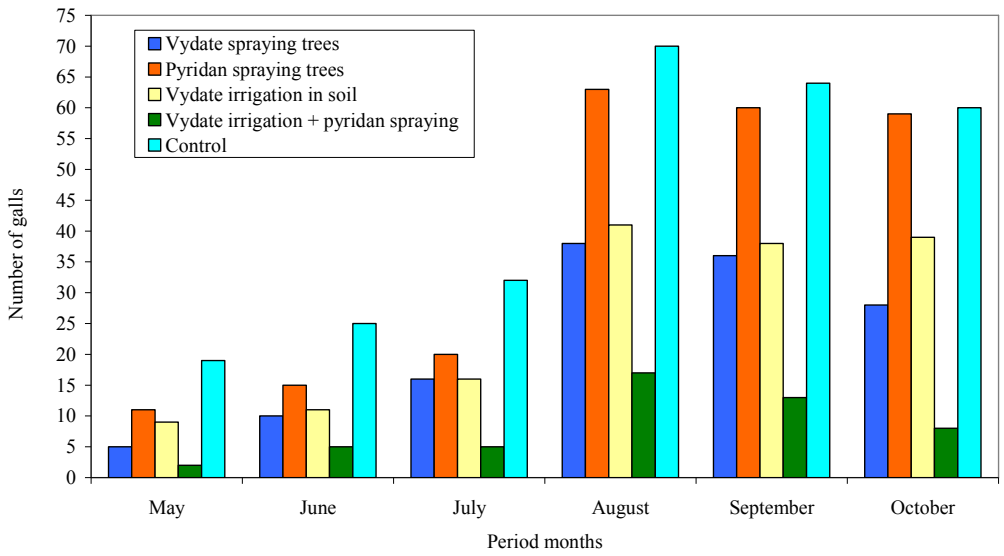

Fig. (4). Effect of vydate and pyridan on number of early symptoms aerial galls on camphor trees.

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الملخص العربي

دراسات هستولوجية ومكافحة على نيماتودا العقد الهوائية لأشجار الكافور في مصر

عاطف محروس كيلة

معهد بحوث أمراض النبات – مركز البحوث الزراعية – الجيزة – مصر

تم إجراء دراسة هستولوجية على العقد الهوائية الموجودة على المجموع الخضري لأشجار الكافور وذلك بعمل قطاعات في الأجزاء النباتية ذات حالات مختلفة (غير مُصابة – مُصابة إصابة متقدمة – مُصابة إصابة متأخرة) بهدف المقارنة بين القطاعات الثلاثة من حيث وجود المُسبب المرضى، وأيضًا الاختلافات في خلايا النسيج النباتي بين القطاعات المختلفة. أيضًا تم إجراء مكافحة بالمبيد النيماتودى الفايديت ٢٤٪ سائل والمبيد الحشري بيريدان ٤٥٪ سائل وذلك عن طريق استخدام أكثر من طريقة تطبيق وقد أظهرت النتائج أن :

- أظهرت الدراسات الهستولوجية أن الأجزاء المصابة إصابة مُبكرة يُوجد بداخل النسيج خلايا مُغذية كبيرة الحجم مستدير أو غير منتظمة الشكل ذات جدار سميك وموت موضعي للخلايا ويُوجد بداخلها النيماتودا والحشرة لاستكمال دورة حياتهما، وعند تقدم الإصابة والتي من خلالها تكون الحشرة قد استكملت دورة حياتها وصلت إلى الطور الكامل نجد ثقب في العقدة والتي من خلالها تخرج الحشرة الكاملة مُحملة بأطوار النيماتودا لتقوم بنقل الإصابة لأجزاء أخرى من أشجار الكافور وذلك بالمقارنة في النسيج الغير مُصاب.
- عند إجراء المكافحة باستخدام المبيدات النيماتودية والحشرية أظهرت النتائج أن أفضل المعاملات التي أدت إلى خفض نسبة الإصابة في شتلات الكافور وأيضاً عدم إتمام الحشرة لدورة حياتها كان في المعاملة بإضافة مُبيد الفايديت مع ماء الري على التربة بالإضافة لرش مبيد البيريدان على المجموع الخضري لنباتات الكافور في نفس التوقيت.
- وأظهرت النتائج أنه لا يُوجد فرق معنوي بين متوسط أعداد عقد النيماتودا عند استخدام مبيد الفايديت رش على النباتات أو استخدامه ري على سطح التربة. أيضًا تبين من الدراسة أن أعلى مستوى إصابة لأشجار الكافور كان خلال شهر أغسطس حيث أن متوسط أعداد العقد بلغ ٢٧.١ بينما كان أقل مستوى إصابة خلال شهر مايو حيث كان متوسط أعداد عقد النيماتودا الهوائية ٥.٣.