Al-Azhar J. Agric. Sci. Sector Res.,
Vol.3 : pp 155-164 (December)2007

INFLUENCE OF FUNGAL FILTRATES FROM GENUS *VERTICILLIUM* ON INVASION EFFICIENCY OF ENTOMOPATHOGENIC NEMATODE, *HETERORHABDITIS* (HETERORHABDITIDAE) TO INSECT HOST

***Mourad, M.H., **Mostafa, M.A. & **Anany, A.E.**

*The Regional Centre for Mycology and Biotechnology Al-Azhar Univ.
** Agric.Zoology&Nematology Department Fac. Agric. Al-Azhar Univ.

ABSTRACT:The effect of metabolite fungi of twelve species belonging to genus *Verticillium* on the average penetrating of infective juvenile stages of both entomophathogenic nematodes *Heterorhabditis bacteriophora* and *H.indica* against the last instar larvae of *Galleria mellonella* was tested under laboratory conditions. All concentrations of all *Verticillium* fungus species metabolites suppressed the invasion of juvenile stage of both entomophathogenic species against the last instar larvae of insect host. Standard concentration(s) of all metabolite fungal species tested exhibited the toxicity among all concentrations metabolite on both nematode species. Significantly decrease ($P \leq 0.05$) in the average number of both penetrated entomophathogenic nematode species was recorded in soil treated with secondary metabolite fungi as compared with untreated soil as a check. Negative relationship was found between dilutions of metabolites fungus and the decrease % of penetrated nematode species. The highest decrease percentages of both nematode species penetrated to the last instar larvae of *Galleria mellonella* was obtained in soil treated by metabolites of species *Verticillium bulbillosum*(185) with rates (87.6-67.3 and 56.1% and 89.2-61.9 and 42.1%), *Verticillium* sp. (3254) with rates (87.1-64.5 and 47.3% and 81-68 and 58.1%) , *V.fusisporum* (197) by rates (84.4-65 and 56% and 72.5-58.8 and 43.3%) , *V. leptobactrum* (201) by rates (77-69.8 and 59.7 and 77-58.8 and 38.9%) and *Verticillium* sp. (3168) with rates (75.8-71 and 55% and 72.5-60.4 and 42.8%) of all concentrations (s , s/10 and s/50) metabolites on both nematode species.

INTRODUCTION

Entomophathogenic nematodes in the genera *Steinernema* and *Hetrorhabditis* are important biological control agents of certain soil-dwelling insect pests. All life stages of these nematodes occur in the insect host, except the infective third stage juvenile (dour). The function

of the dour is to survive in the environment sense. The survival of Ijs in natural soil is limited by abiotic and biotic factor but indicate suppression of entomophathogenic nematodes by fungi and other entomophathogenic fungi (Van Sloun *et. al.,* 1990 and Timper *et. al.*, 1991). Many fungi are known to produce nematicial or nematistatic compounds (Anke *et.al.,* 1995; Anke and Sterner 1997; Chen *et. al.*, 2000; Meyer *et.al.*, 2000 and Kopck *et.al.*, 2001). Secondary metabolites from other endophetic fungi such as *Fusarium oxysporum* were toxic to *Meloidogyne inecognita* (Hallmann and Sikora 1996). Fungal endophytes of all fescue produced compounds including Loline alkaloids, pyrrolopyrazine and organic acids that may account for activity against some phytoparasitic nematodes (Bush *et.al.*, 1997). The fungus *Omphalotus olearius* produce omphatotin A.A as a nematidical compound that demonstrated greatest activity against many nematode species (Buchel *et. al.*, 1998 and Mayer *et.al.*, 1999). Susan *et.al.,* (2004) reported that many fungal isolates were produce active compounds against soybean cyst nematode and root-knot nematode.

The purpose of the present study was to test the role of metabolite fungi to effectiveness and hinder penetration of entomophathogenic nematodes to infect their insect hosts.

MATERIALS AND METHODS

A. Propagation of fungal metabolite (filtrate) :

Twelve fungal strains were kindly provided by the collection unit at Al- Azhar Univ. and used for this study.

Malt extract agar (MEA). Ingredients in one g/l Litre distilled water: malt extracts 20; glucose20; peptone 1; agar20. This medium was used for the regular subculture of the type strains. It was also used as a broth (without agar) for the cultivation of the strains to study their metabolite profiles. Yeast extract sucrose broth (YES).Intergradient in one g/l Litre g/l distilled water: yeast extract 20; sucrose 150; this medium was used for cultivation of the fungi strains for their secondary metabolite profiles studies. Each medium was prepared by dissolving the solid ingredients in 1 litre of cold distilled water and then heated to 60-70^{O}C with stirring. Media were sterilized by autoclaving at 121^{O}C for 15 minutes. The broth media were dispensed into flasks and sterilized; the flasks were then inculcated and incubate for 21 days at 28 ^{O}C. The flasks were then aseptically filtrated and the filtrate was concentrated using speed vacuum

device as standard (S) (Maxi Dry Plus). Each filtrate according to the type fungus was diluted with addition distilled water ten folds (S/10) and fifty folds (S/50).

B. Experimental studies.

The experiments were tested in plastic cups (4.5 cm height × 2.5 cm diameter) filled with sterilized sandy soil. The compared filtrates of twelve fungal species belonging to genus *Verticillium* were pipette on the dry soil at dilutions S, S/10 and S/50 , so that final moisture was standardized at 60% humidity field capacity. Three cups were pipetted by distilled water as a check. One hundred of infective juvenile stages of both entomopathogenic nematode species *Heterorhabditis bacteriophora* and *H. indica* were applied to each cup. Each treatment was replicated three times. All treatments were incubated at 25 ^{O}C. After 24 hrs. of nematode exposure to filtrate of tested fungi , one last instar larvae of *Galleria mellonella* was add to each cup. After 3-4 days, cadavers were dissected and the number of invading nematodes was counted. Data were then analyzed according to Duncan's multiply range test (1995) and (Steel and Torrie, 1980), and the percentage reduction of nematode penetration was calculated.

RESULTS AND DISCUSSION

The effect of metabolite fungi of twelve species belonging to genus *Verticillium* on the average penetrating number of infective juvenile stages of *Heterorhabditis bacteriophora* and *H. indica* invading last instar larvae of *Galleria mellonella* under laboratory conditions at 25^{O}C. The results indicated that, all dilutions of tested metabolite fungi of all *Verticillium* species were suppressed penetration rate of infective juvenile stages of both tested nematode species on the last instar larvae of *G. mellonella*. Negative relationships were found between dilution and the rate of nematode penetration. Standard concentration(s) of all metabolite fungal species exhibited the highest toxicity among all dilution metabolites on both nematode species, as compared with the check. Data presented in Table (1) and Fig. (1) showed that, the highest decrease percentages of the number of penetrated ijs of *Heterorhabditis bacteriophora* and *H. indica* penetration to last instar larvae of *G. mellonella* was obtained with metabolite of species *Verticillium bulbillosum* (185) with rates (87.6-67.3 and 56% & 89.2-61.9 and 42.1%) , *Verticillium* sp. (3254) with rates (87.1-64.5 and 47.3% &81-68 and

Table (1) Influence of different *Verticillium* fungal filtrates on invasion efficiency of two entomopathogenic nematode species on the last instar larvae of *Galleria*

Galleria

Verticillium sp. & code number	Penetrated No. of entomopathogenic nematode / insect host					
	Heterorhabditis bacteriophora			*H. indica*		
	S	S/10	S/50	S	S/10	S/50
Verticillium albo-atrum (188)	13.0 de (c)	24.3 de(b)	29.0 ef(a)	8.0 e (d)	27.7 cd(ab)	29.7 de(a)
Verticillium bulbilloum(185)	7.7 f (c)	20.3 fg (b)	27.3 ef(a)	4.7 f (c)	16.7 ef(b)	25.3 f(a)
V. fungicola var. fungicola (132)	21.0 c (e)	26.3 d (d)	37.0 cd(a)	19.7 b(c)	30.0 c(c)	33.7 bcd(b)
V. fungicola var. flavidum (133)	23.7 bc(c)	30.0 c(ab)	34.0 cd(a)	20.0 b(c)	24.7 d(bc)	31.7 cd(a)
Verticillium fusisporum (197)	9.7 ef d)	21.7 efg(bc)	27.3 ef(a)	12.0 c(d)	18.0 e(c)	24.7 f(ab)
Verticillium lamellicolla (199)	26.0 b (c)	34.7 b(b)	40.3 c(a)	18.3 b(d)	35.3 b(b)	37.3 b(ab)
Verticillium leptobactrum (187)	14.3 d (d)	23.3 def(b)	29.7 ef(b)	21.0 b(c)	28.3 c(b)	35.7 bc(a)
Verticillium leptobactrum (201)	14.3 d(bc)	18.7 g(b)	25.0 f(a)	10.0 cde(c)	18.0 e(b)	26.7 ef(a)
Verticillium lindanimum (202)	16.3 d (e)	31.7 b(c)	46.3 b(a)	11.0 cd(f)	24.3 d(d)	37.0 b(b)
Verticillium sp.(130)	7.0 f (e)	12.0 h(cd)	41.0 c(a)	10.6 de(d)	15.3 f(c)	32.3 cd(b)
Verticillium sp.(3168)	15 d (bc)	18.0 g(b)	27.7 ef(a)	12.0 c(c)	17.3 ef(b)	25.0 f(a)
Verticillium sp.(3254)	8.0 f(d)	22.0 efg(b)	32.7 de(a)	8.3 de(d)	14.0 f(c)	18.3 g(bc)
Check	62.0 a	62.0 a	62.0 a	43.7 a	43.7 a	43.7 a
L.S.D. 0.05	3.45	3.60	4.87	2.67	3.32	3.74

Value in a column and/or (row) followed by the same letter are not significantly by ($p \leq 0.05$) according to Duncan's multiple-range test.

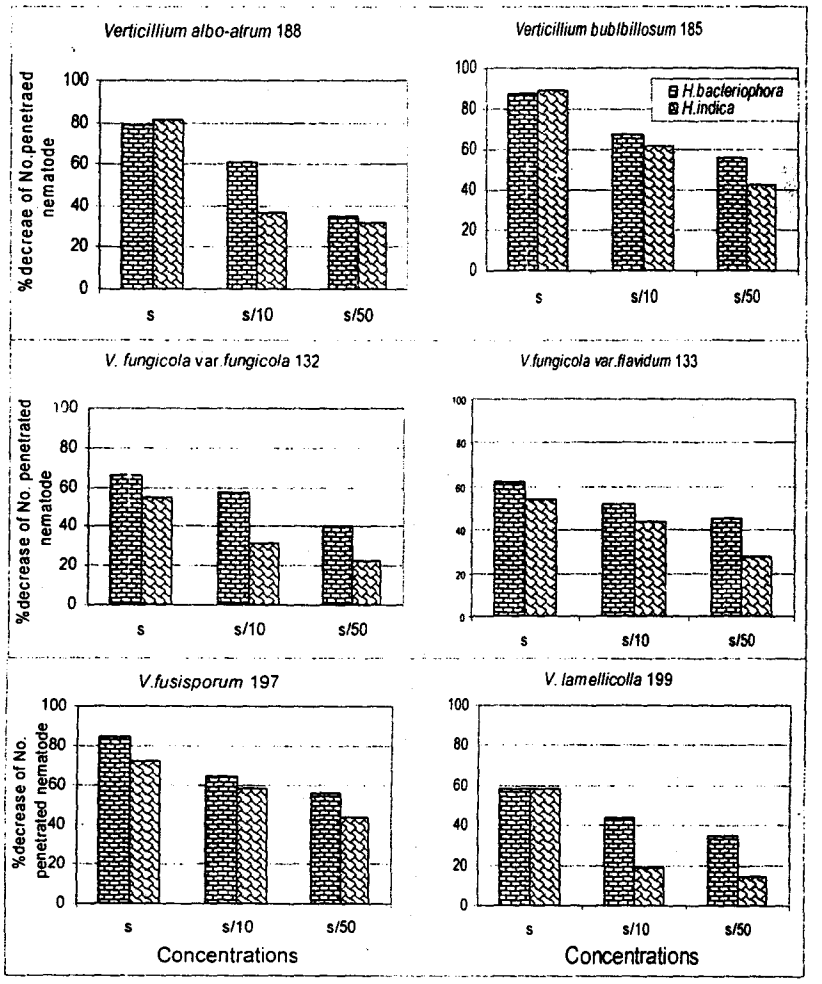

Fig.(1). Decraesa percentage of entomopathogenic nematode *Heterorhabditis bacteriophora* and *H.indica* numbers penetrated to last instar larval of *Galleria mellonella* as influenced by metabolite of different *Verticillium* fungus species.

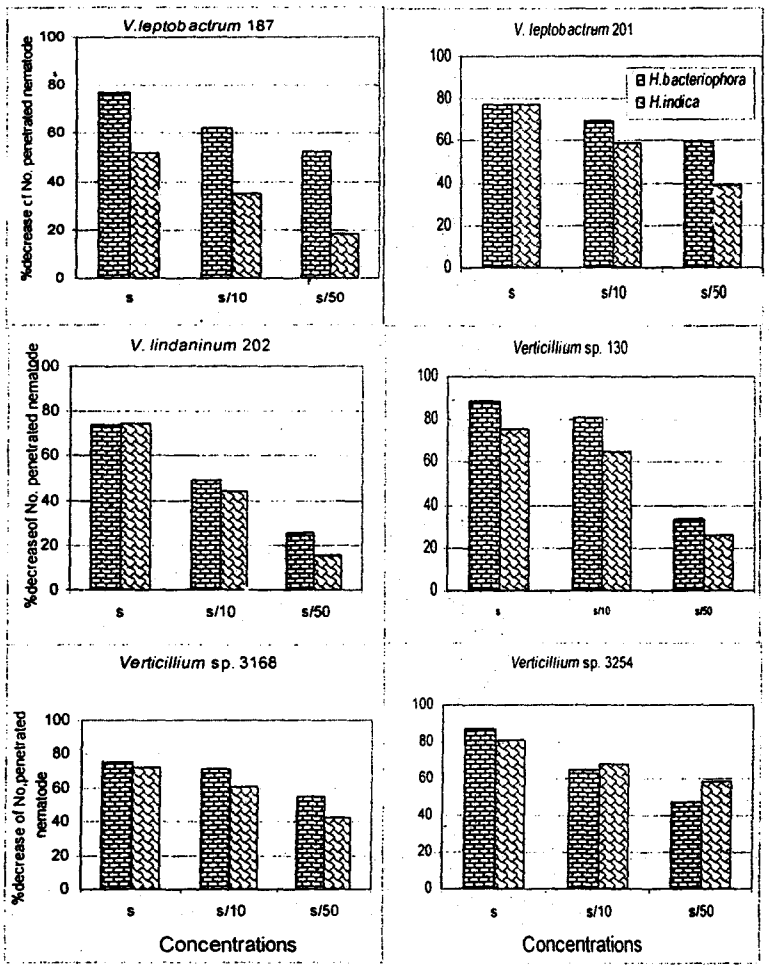

Fig.(1).Continued.

58.1%) , *V. fusisporum* (197) by rates (84.4-65 and 56% & 72.5-58.8 and 43.3%) , *V. leptobactrum* (201) by rates (77-69.8 and 59.7% & 77.5-58.8 and 38.9%) and *Verticillium* sp. (3168) with rates (75.8-71 and 55% & 72.5-60.4 and 42.8%) of all concentrations (S, S/10 and S/50) of metabolites on both nematode species *H. bacteriophor* and *H. indica* , respectively. Whereas, the lowest decrease percentages of penetrated nematodes were observed the metabolite fungal of species *V. lamellicolla* (199) with rates of (58.1-44.1 and 35% & 58.1-19.2 and 14.6%), *V. fungicola* var *flavidum* (133) with rates (61.8-51.6 and 45.2% & 54.2-43.5 and 27.5%) and *V. fungicola* var. *fungicola* (132) by rates (66.1-57.6 and 40.3% & 54.9-31.4 and 22.9%) on both nematode species, respectively.

Generally, the decrease percentages of the number of penetrated ijs of both nematode species in the haemocoel of *G. mellonella* larvae positively related to the concentrations of all test fungal metabolites.

These results agree with (Djian *et. al.*, 1991) reported that acetic acid was an active component from culture filtrates of *Paecilomyces lilacinus* and *Trichoderma longbrachiatum*. The wood–rotting basidiomycete *Pleurotus ostreatus* produced the nematotoxin Trans-2- decanedioic acid (Kwok *et. al.*, 1992). Linolic acid was identified as a nematotoxic compound from the nematophagus fungi *Arthrobotrys conoides* and *A. oligospora* (Anke *et. al.*, 1995). Filtrates from culture of *A. ochraceus, F. oxysporum, P. lilacinus, T. viride* and *P. chlamydosporia* were toxic to population of *Meloidogyne incognita* juveniles in soil and plants (Hallman and Sikora 1996; Khan 1999; Sharma 1999; Wang *et. al.*, 1999; Costa *et. al.*, 2000 and 2001 and Randhawa *et. al.*, 2001).Culture filtrates from isolates of *P.chlamydospora* produced toxic activity varying with culture medium and with species of fungus (Chen *et. al.*, 2000 and Sun *et. al.*, 2002).

REFERENCES

Anke, H. and Sterner, O. (1997): Nematicial metabolites from higher fungi. Current organic chemistry. 1,361-374.

Anke, H., Stadler, M., Mayer, A. and Sterner, O. (1995): Secondary metabolites with nematicidal and antimicrobial activity from nematophagous fungi and Ascomycetes. Canadian J.Botany.73,5932-5939.

Buchel, E.,Mayer, A.,Martin, U., Anke, H. and Sterner, O. (1998): Structure elucidation of omphalotin a cyclic dodecapeptide with

potent nematicidal activity isolated from *Omphalotus olearius*.Pesticide Science. 54,309-311.

Bush,L.P.,Wilkinson,H.H. and Schardl,C.L.(1997): Bioprotective alkaloids of grass- fungal endophyte symbioses. Plant Physiology.114, 1-7.

Chen, S.Y., Dickson, D, W. and Mitchell, D.J. (2000): Viability of *Heterodera glycines* exposed to fungal filterates.J.Nematology. 32. 190-197.

Costa, M.J.N., Campos, V.P.,Pfenning , L. H. and Oliveira, D.F. (2000): Pathogenicity and reproduction of *Meloidogyne incognita* in tomato plants (*Lycopersicon esculentum*) with application of fungal filtrates or plant and animal manure extracts. Nematologia Brasileria 24, 219-226.

Costa, M.J.N., Campos, V.P.,Pfenning , L. H. and Oliveira, D.F. (2001): Fungus filtrates toxicity to *Meloidogyne incognita.* Fitopathologia Brasileira. 26, 749-755.

Djian, C., Pijarowski, L.Ponchet, M., Arpin, N. and Favre-Bonvin, J. (1991): Acetic acid is a selective nematicidal metabolite from culture filtrate of *Paecilomyces Lilacinus* (Thom) Samson and *Trichoderma* Longibrochiatum Rifai. Nematologica. 37, 101-112.

Duncan, D.B. (1955): Multiple ranged F-test. Biometrics, II; 1-47.

Hallmann, J. and Sikora, R.A. (1996): Toxicity of fungal endophyte secondary metabolites to plant parasitic nematodes and soil-born plant- pathogenic fungi. European J. plant Pathology. 102, 155-162.

Khan, T.A. (1999): Studies on the toxic effect of culture filtrate of some fungi on root-knot nematode. Bionotes 1, 38-39.

Kopcke, B., Wolf, D., Anke, H. and Sterner, O. (2001): New natural products with nematicidal activity from fungi. British Mycological Society International Symposium. Bioactive Fungal Metabolites-Impact and Exploitation. UW,Swansea, UK, 22-27 April 2001. p.72 [Abstr].

Kwok, O.C.H., Plattner, R., Weisleder, D. and Wicklow, D.T. (1992): A nematodicidal toxin from *Pleurotus ostreatus* NRRL-3526. Journal of chemical Ecology 18, 127-136.

Mayer, A., Kilian,M., Hoster, B., Sterner, O. and Anke, H.(1999): in-vitro and in-vivo nematicidal activities of the cyclic dodecapeptide omphalotin A. Pesticide Science 55, 27-30.

Mayer, S.L.F., Massoud, S.I., Chitwood, D.J. and Roberts, D.P. (2000): Evaluation of *Trichoderma virens* and *Burkholderia cepacia* for antagonistic activity against root-knot nematode, *Meloidogyne incognita*. Nematology 2, 871-879.

Randhawa, N., Singh, P., Sandhu, K.S. and Bhatia, A. (2001): Effect of culture filtrates of soil fungi on hatching of *Meloidogyne incognita*. Plant Disease Research 16. 280-282.

Sharma, D.D. (1999): Effect of culture filtrates of biocontrol agents on larvae mortality of *Meloidogyne incognita*, in comparison with Rugby 10 G. Indian J. of Sericulture 38, 152-154.

Steel, R.G.D. and Torrie, J.D. (1980): Principle and Procedures of Statistics. Mcgrow- Hill Book, Co., New York. 481 pp.

Sun, M.H., Liu. X.Z. and Jin, Z.B. (2002): Effect of *Paecilomyces lilacinus* on egg hatching and juvenile mortality of *Heterodera glycines*. Acta phytophlactica sinica, 29, 57-61.

Susan, L.F.M., Robin, N.H., Xing, Z.L., Richard, A.H. Jean, J.and James, K.N.(2004): Activity of fungal culture filtrates against soybean cyst nematode and root-knot nematode egg hatch and juvenile mortality. Nematology 6(1)23-32.

Timper, P., Kaya, H.K.and Jaffee, B.A. (1991): Survival of entomogenous nematode in soil infested with the nematode parasitic fungus *Hirsutella rhossiliensis* (Deuteromycotina: Hyphomycetes): Biol Contr. 1, 42-50.

Van Sloun, P.,Nicolay, R., Lohmann, V. and Sikora, R.A. (1990): Anfalligkeit von entompathogenen Nematoden gegenuber nematodenfangenden and endoparasitaren. Pilzen. J. Phytopathol. 129, 217-227.

Wang, L.F., Yang, B.J. and Li, C.D. (1999): Evaluation of pathogenicity of parasitic fungi to root-knot nematodes. Scientia Silva Sinicae. 35, 41-47.

تاثير راشح فطريات جنس الفيرتيسيلوم على قدرة نيماتودا الحشرات (هيتيرورابد تيدا) لاختراق العائل الحشرى

***محمد حسنى السيد مراد ؛** مصطفى عبد اللطيف مصطفى**

**** عبد المنعم السعيد عنانى**

*المركز الاقليمى للفطريات وتطبيقاتها – جامعه الازهر – القاهرة
** قسم الحيوان الزراعى والنيماتودا –كليه الزراعه- – جامعه الازهر – القاهرة

تعتبر الفطريات من الكائنات الحيه الموجودة بالتربه عامل من العوامل البيئيه والحيويه والتى تحيط بنيماتودا الحشرات بالتربه وقد تؤثر هذه الفطريات على استخدام نيماتودا الحشرات فى المكافحه البيولوجيه للافات الحشريه بالتربه.

لذلك استهدف هذا البحث دراسه تاثير الرشح الفطرئ (نواتج الايض الثانويه) لاثنى عشر نوعا من الفطريات الفيرتيسيلوم وذلك بثلاث تركيزات لكل نوع عاى كفاءة اختراق الاطوار المعديه لانواع النيماتودا *Heterorhabditis bacteriophora* and *H. indica* على يرقات العمر الاخير لدودة الشمع الكبيرة *Galleria mellonella* وذلك بعد تعريض الاطوار المعد يه لكل من نوعى النيماتودا السابقه فى تربه معامله بالراشح *وتركيزاته المختلفه* لاثنى عشر نوعا من الفطريات الفيرتيسيلوم المختبرة وذلك تحت الظروف المعمليه .

وقد اوضحت النتائج ما يلى :

ان كل التركيزات للراشح لكل انواع الفطريات المستخدمه قد ادى وجودها فى التربه الى انخفاض كبير فى متوسط اعداد الاطوار المعديه لكل من نوعى النيماتودا والتى اخترقت يرقات العمر الاخير لدودة الشمع الكبيرة .

وقد اوضحت النتائج ايضا انه توجد علاقه ايجابيه بين زياده التركيز والزيادة فى النسبه المئويه للنقص فى معدل الاختراق لكل من نوعى النيماتودا وقد كانت اعلى نسبه مئويه للانخفاض فى اعداد النيماتودا المخترقه لجسم العائل الحشرى فى التربه المعامله براشح الفطريات لكل من الانواع الاتيه: الفيرتيسيلوم بلبيلوزم ١٨٥ و جنس الفيرتيسيلوم٣٢٥٤ و الفيرتيسيلوم فيوزيبورم ١٩٧ و الفيرتيسيلوم لبتبكيرم ٢٠١ و جنس الفيرتيسيلوم ٣١٦٨ .