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Research Article

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Biocide effect of *Fusariumsolani* and *Penicillium spp* against the cereals cyst nematode "*Heteroderaavenae*"

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ABSTRACT

StrategicCultures byexcellence,cerealsare a valuablefoodin the world,unfortunatelythey are subject tovariouspest attacksinthe fieldsamongwhich cereals cyst nematodes "Heterodera" serious, parasite thatitsdamagegreatly reducesyields. In order to reduceits infestationsand preserve the healthof the consumer,we triedto usebiological controlusing twocultures filtrate of fungusnamelyFusariumsolani andPenicilliumsppagainst the nematode.After exposingthe eggsto various concentrationsof the twofiltratesfor a period of6, 24and 48 hours, the results are highly significantsand shows theovicidaleffect of the twofiltrates. This effect increases with the immersion time and the concentration of the two filtrates. A mortality rate of eggsof 93.62% and 82.45% is recordedrespectivelyforF solani andPenicilluimspp after48h ofexposure.By comparingthe efficiency ofthe two filtrates cultures onnematode,the results revealed thattheovicideaction ofpurefiltrate ofF.solani, reached94.39% after 48 hoursagainst(83.39%) for the purefiltrateof Penicilliumspp.So,theculture filtrate ofF.solaniis muchmore effective than thefiltrate ofPenicilluimspp.Alsoourexperimentationshave shown thatnematodeeggsare verysensitive to lowpH(3.5).

Key words: Fungi,*Heterodera avenae,*inhibitory effect.

INTRODUCTION

Cereals areconsideredthe mostcultivated cultures by manandhave longbeen prominentin human nutrition.Although these cultures have benefited from some development programsbut national production, did not know the expected improvement.The causes of this are mainly of technical orders, and phytosanitary. Of these,nematodeinduce10% loss of global production[38]. Forcereals, the complex *Heteroderaavenae*: cerealcystnematodeis by farthe most to fear.It is presentin temperatecerealregions,[25-26, 32]. This widedistribution followsthe expansion ofcerealsurfaces. TheHeteroderacomplex is represented bya twelve speciesin the world.However,thespecificcompositionvaries from one regionto another. In Algeria,*Heterodera*groupwas the subjectof severalinvestigations,the results of whichrevealedthe presence of severalspeciesin cerealplots, [24, 30]. Therethe use of syntheticnematicideswere unsatisfactoryin addition to theiradverse effects on theflora and faunathat have led to theirreduction. Thus, new solutions must befoundquicklyto solve thenematodes problems. Among these means, the use ofnatural antagonistsincludingfungalmetabolitesare an interestingway forward, easy to useandenvironmentally friendly. InthisconcordanceManyfungi are knownto producenematicidalscompoundsornématostatiques[3-4,6, 15, 21,23].Thussecondary metabolitesof *Fusariumoxysporum*are toxic to*Meloidogyneincognita*. Somanytoxins of *Fusariumspp*, reduce the viability of nematodes.[8, 3, 28]. Other studieshave shownthe inhibitory actionof hatchingeggs orNematicide fromsomefungiisolated fromoilnematodesand plants[1, 7, 5, 10, 15, 22, 27, 32, 35-36]. Our contributionaims tohighlight theeffect of toxic and biocidefiltrate of cultures of *Fusariumsolani*and *Penicilliumspp*bybio-assayssustainability of*Heteroderaavenae*eggs.

EXPERIMENTAL SECTION

The populations of the cereal cyst nematode "*Heteroderaavenae*" were taken in cereal plots in Mascara region (western Algeria). Soil samples thus recovered were dried prior to the extraction of cysts. For this, we used the technique FENWICK (1940), based on the floating cysts. This process involves separating the cysts of the mineral fraction of the soil. recovered cysts will be subject to a morphometric and anatomical study(microscopic observationofthe perinealregionrefers tovulvarconeand the constituents ofthe structuressuch asthepresenceor absence of thebullaeandorbridge, the shape of fenestraeandthelengthofthevulvarslit).both fungi used in this case *Fusariumsolani* and *Penicilliumspp* do not grow on cereals, They were obtained from the Laboratory of Plant Pathology.After incubation, the mycelial mass *Penicilliumspp* appears, on which we collected cuttings for aseptically drop the center of a Czapeck cast medium in Petri dishes. The cultures were thenincubated at 28 °C in an oven, in order to obtain pure cultures. Successivesubcultures ofthe previous cropare carriedagain on theCzapeckmedium.This strain is stored in a tube containing Czapeck medium set at a temperature of 4 ° C. Regarding the*Fusariumsolani*, we realized a culturebytaking asmall sample ofthe mycelial massofpure culturewe fileinto a tube containing10mlof sterile distilled water.When adjusted, the solution should contain about 100 spores per ml. A drop ofthis solution(afterstirring)is filedunder sterilewitha Pasteur pipetteon a PDAcast mediumin Petri disheswith sterileetaloir.Thisdropis spreadacross the surface.After 48 h of incubation at 28 °C, the spores witch have developed a mycelium, are transplanted one by one on a PDA medium[19].

Pathogenicity test:
Almostthesaprophytic*Fusarium* lives in the soilandto differentiate them fromparasitic forms, morphological appearanceis not enough. Both formsdo not show anydifferences thatmaybe distinguished from eachother.Todothis,experimentbyinoculationisnecessary[11]. ThusHenzetomato varietyis sensitive to thestrain of *Fusariumsolani*. Threeweeks after planting, the young tomato plants(stageappearance of the firsttwo trueleaves)are dug; theirrootswere washed with water,thenfor 15 minutesinamistakenconidia suspension from anold culture4daysobtained byuniform spreadingon the surfaceof the medium ina Petri dish. The witnesses aremistakein distilled water forthe same duration. The inoculatedseedlings weretransplanted intopots of 30 cmdiametercontainingpotting soil. As of threetomato plantsis used toinoculateeachstrain, [17].

Estimation ofsymptoms:
External symptomsare estimatedtwiceper week duringtwo to threeweeks after inoculation[18]. In vitro testswe have doneare that described by[12, 16].

Methods of filtratecultures: A cuttingtaken fromthemycelialgrowth areaof a cultureof the antagonist is placed inflasks of 500 mlErlenMeyercontaining300mlGYPliquid nutrient medium. Theseflasks wereincubated for3weeksinthe dark at26° C. After this period,the myceliumis separated from theculture mediumbyfiltrationondifferent diameters(11, 12, 5, and 13), which avoidsthe passage of allcontaminatingspores

pHmeasurement
The pH is measured before the incubation of the fungus and adjusts to5.8. Thenematicidaleffectwas tested withthe filtratethendilutedin steriledistilledwaterto P/2, P/4and P/8. The pHof thetwofiltratesof the fungus ismeasured. Twocontrol mediaare usedforcomparison, one issterile distilledwater, the otheristhenon-inoculated culture medium,Adjusted tothe samepHasthetestfiltrate**.**

Test of filtrateson*H.avenae*eggs.
Eggs are depositedin hemolysistubes, eachtube receivesabout300 eggswhich we addeda dropletofliquidtested100µl.Eachtreatment was repeated5 times.The toxicityof the filtrateswas estimatedby the mortality percentageafter6,24 and 48hoursof immersion and aftercoloringeggswith newblue-R [13]. Dead eggsaredarkblue and viable eggsremain colorless. All Tests are carriedto ambient temperatureof laboratory.

Statistical analysis
All the results obtained are subjected to the treatment of the analysis of the variance to a critter of classification for all the parameters study with ugly of software Staticf, version 5.

RESULTS AND DISCUSSION

Heteroderaavenae:is a speciesenfeoffedcereals it is characterized bythepresenceofdenseandmassivebullae, twosemi-fenestrae widelyseparated bythevulvawhich has a length less 20µm. This lastis inthevulvarbridgewhich is freeof ramification. In this study,we confirmedthe presenceof this speciesin severalcerealplotsin theMascararegion (western Algeria).

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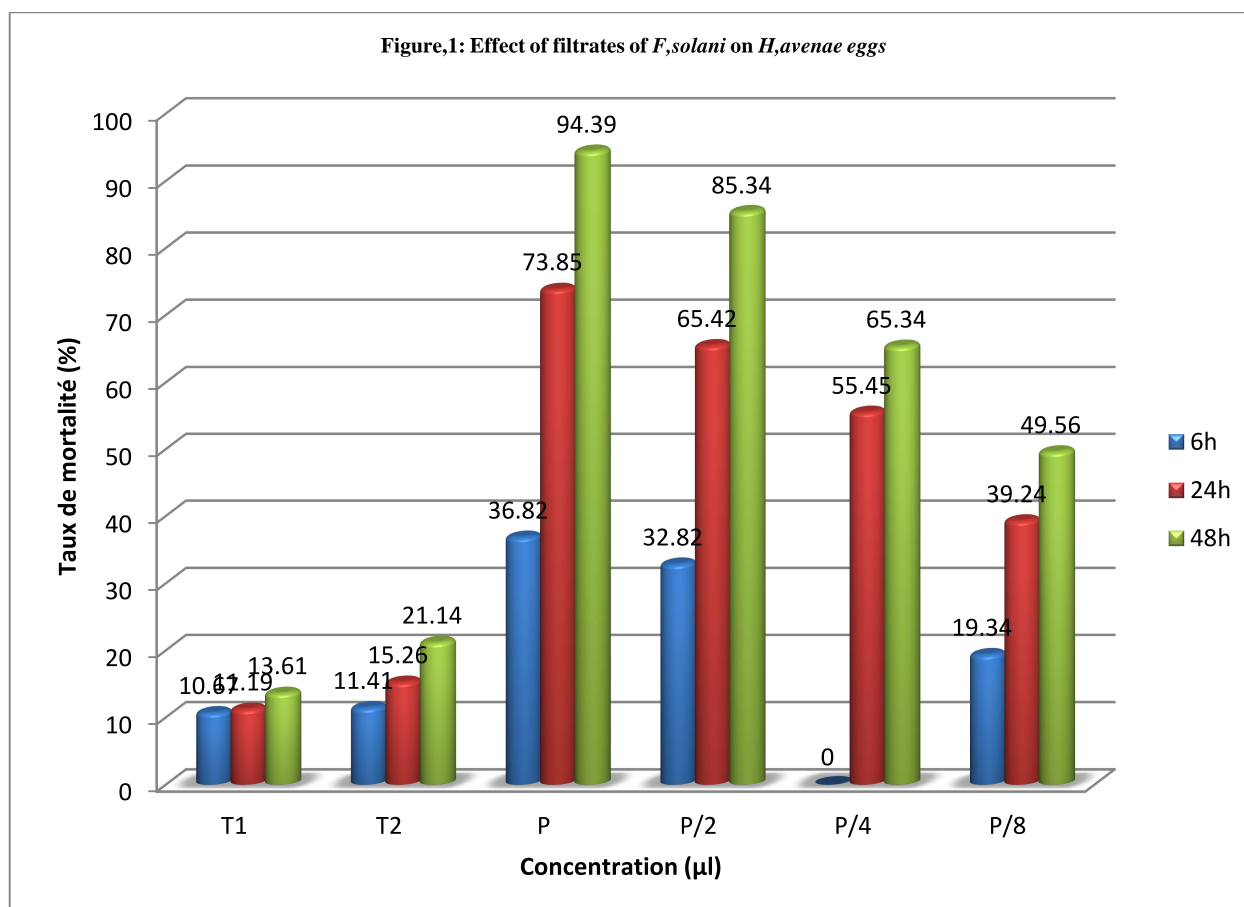
Studyof pathogenicity test:
Pathogenicityof*Fusariumsolani*isestimated usingexternal symptomsexpressedas follows:Healthy plantanddiseased plant.Thus, afterthree weeksof incubation,it appears thatourstrain used isnon-pathogenicfortomato.If we Comparedpotsinoculatedwith the witness,we findthat allplantsare identicals to thecontrol plants.To confirmthe absenceof the fungusinthe plant,were seededsmall fragments ofplants inoculatedon PDA.After 7 days ofincubationat 28 °C, microscopic observationrevealedthe absenceof*Fusariumsolani*withinthe plant.The use ofsaprophyticstrainsis necessaryto avoid the riskof havingadditionaldamage tothe plant. Therefore ourstrainissaprophyteforthetomato.We appreciatethe influenceof agingon the pathogenicity ofculture of *F.solani.* Forthestrain*Penicilliumspp*, used in this test, itisalsosaprophytefortomato.

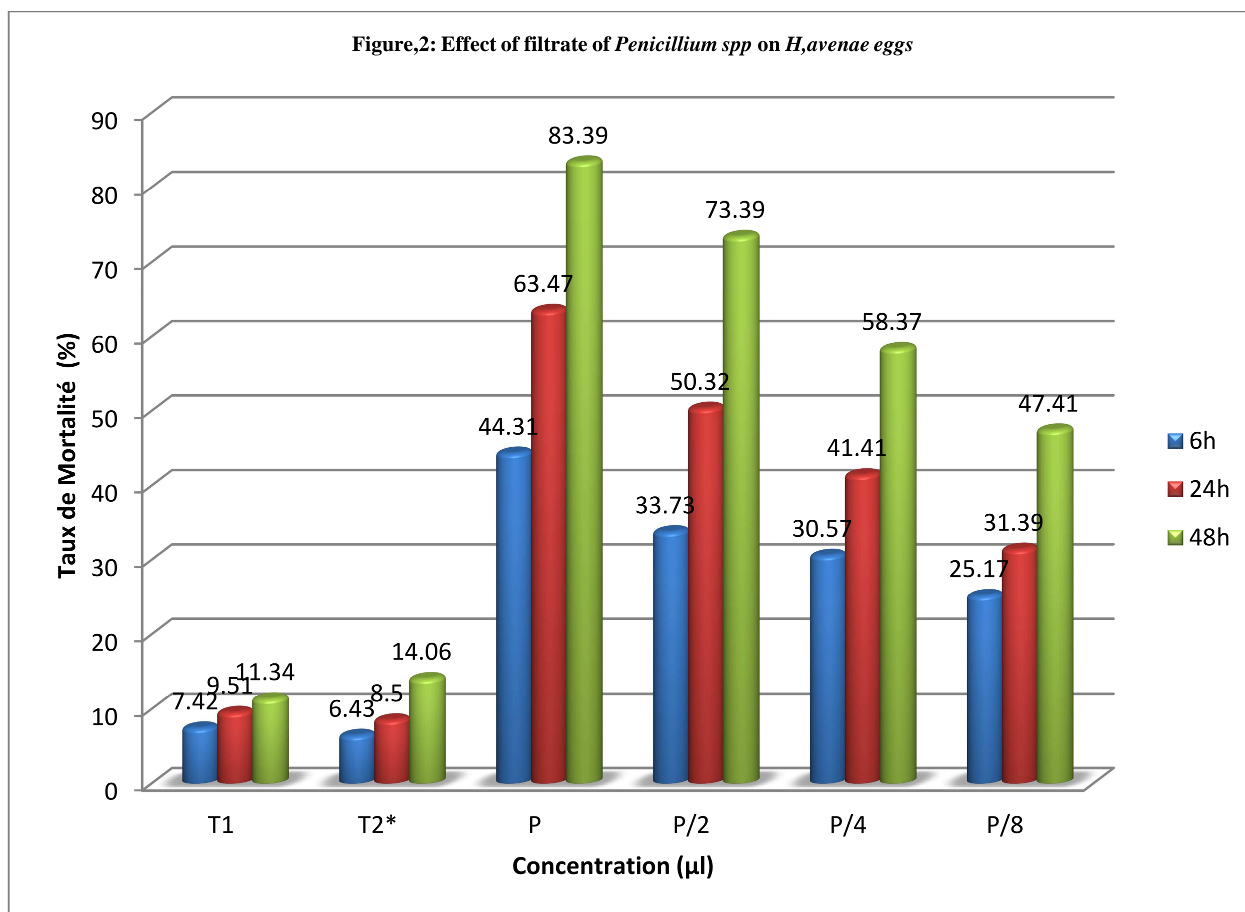
Effect offiltrateculture of*F.solani*on eggsof *Havenae*:According to the results(Figure1),we find thatthe percentage ofdeadeggsincreases with the concentrationof the filtrate andthe immersion time.After6 hoursof immersion, there has been a death rateof35.33% with purefiltrateof *F.solani*.This ratereached72.20% after 24 hoursand100%after48hours.Regardingthe filtratedilutedto 50%(P/2),the mortality rateincreases withtheeggsimmersion time (85.34%) after 48 hours.Even atlower dilutions(P/ 8), the filtrate is still effective, themortality percentagesare19.34,39.24and49.56%, respectively, after 6 hours, 24 hoursand 48 hours ofimmersion.These resultsare highly significant(Fc=31.59 and Fth=2.32)compared to controlsafter48 hoursof immersion(distilled water: 13.61%) and (uninoculated medium: 25.14%).

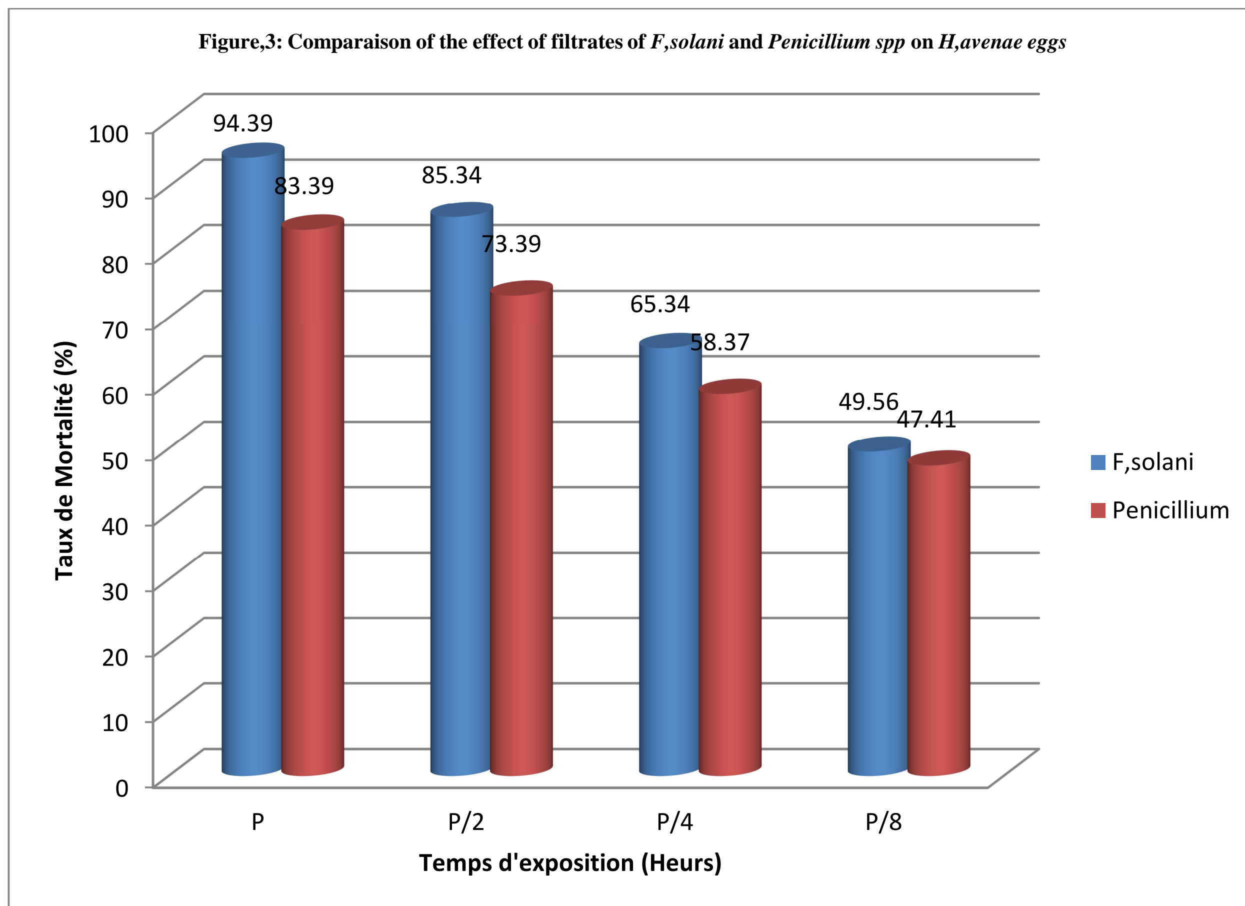
Effect offiltrateculture of *Penicilliumspp*on eggsof *H.avenae*:
The results of the*Penicilliumspp*effecton eggsof*H.avenae*are mentioned inthe (Figure 2).And dice the first hoursof immersion(6h), pure filtratewas effectivewith a mortalityrate of44.31%.This value reaches 83.39% after 48 h, very significant result with (Fca=55.72 and Fth=2.32).Even withdilutions (P/2, P/4,and P/8), the filtrate remains effectiveagainstegg viability,ThustheInhibit rateof73.39%is achievedafter 48 hoursunder the effectofthe lowestdilution(P/8),Compared tothe two witnesseswith whomwe recordedweakinhibition rate10.24 and13.49% respectively for distilled waterانduninoculated medium.

Our resultsare consistentwith those of[8, 3, 28],whichhighlightedthe toxicity ofsecondary metabolites produced byendophyticfungi such as*Fusariumoxysporum*on*Meloidogyneincognita*and other toxinsfrom various*Fusariumsp*, which can reduce the viability of nematodes.



Figure,1: Effect of filtrates of *F,solani* on *H,avenae eggs*


Figure,2: Effect of filtrate of Penicillium spp on H,avenae eggs
Taux de Mortalité (%)
90
80
70
60
50
40
30
20
10
0
7.42
9.51
11.34
6.43
8.5
14.06
44.31
63.47
83.39
33.73
50.32
73.39
30.57
41.41
58.37
25.17
31.39
47.41
T1
T2*
P
P/2
P/4
P/8
Concentration (µl)
6h
24h
48h


Figure,3: Comparaison of the effect of filtrates of F,solani and Penicillium spp on H,avenae eggs
Taux de Mortalité (%)
100
90
80
70
60
50
40
30
20
10
0
94.39
83.39
85.34
73.39
65.34
58.37
49.56
47.41
P
P/2
P/4
P/8
Temps d'exposition (Heurs)
F,solani
Penicillium

	T1	T2	P	P/2	P/4	P/8
6h	10.67	11.41	36.82	32.82	32,8229,69	19.34
24h	11.19	15.26	73.85	65.42	55.45	39.24
48h	13.61	21.14	94.39	85.34	65.34	49.56

	T1	T2*	P	P/2	P/4	P/8
6h	7.42	6.43	44.31	33.73	30.57	25.17
24h	9.51	8.5	63.47	50.32	41.41	31.39
48h	11.34	14.06	83.39	73.39	58.37	47.41

	F,solani	Penicillium
P	94.39	83.39
P/2	85.34	73.39
P/4	65.34	58.37
P/8	49.56	47.41

The effect of pHon the mortality ofeggs:
According tothe results, we note that the low pH(3.5)forthe witness (T2) influencesthe mortalityof*H.avenae*eggsunder the action of*F.solani*after48 hours,by registering amortality rate of21.14%,butbypH= 5 (T2) under *Penicilliumspp*,we noticeda very smalleffect on theviability of the eggs(mortalityrate after48 hourswas14.21%),thisrateisnearlysimilar to thatobtainedunder the effectofthedistilledwater(11.34%).Ourresults agree withthoseof [6], whoshowed thattheculture filtratesare toxicon nematodesat a lower ph.

Comparison of theeffect of the twofiltrates:The twofiltratesculture of*F.solani*and *Penicilliumspp*.exhibit aprominenttoxicity*onH.avenae*eggs.This toxicityincreases with boththe immersion time andwith the differentconcentrations(P,P/2, P/4 and P/8).

The ovicide action of pure filtrate of *F.solani*, reached 94.39% after 48 hours against (83.39%) for the pure filtrate of *Penicillium spp*. at lower dilutions P/8, the two filtrates also showed efficacy with inhibitory rate of hatching (49.56 and 47.41%) respectively for the culture filtrate of *F.solani* and *Penicillium spp*. Eggs of *Heterodera* appear to be more sensitive to culture of *F.solani* filtrate but statistically there is no significate difference (Fcal=0.12 and Fth=4.19) (Figure 3).

CONCLUSION

Thephytoparasitics nematodeshave a verysignificant economic impacton a global scale,much more in developingcountries thanindustrial countries.Considering theirextreme resistance, due to theirgreat physiologicalvariability andthe fact that theyaresoil pests, it is very difficultto combat them.In this context,thenematode problemswill not besolved bythe adoptionof a single methodbut bythe combination ofall available meansused together:crop rotation, resistant's varieties, nematophagous fungi, mycorrhizalplants,nematicidesplants, organic amendments...That set of measurescanonlyreplacethe destructiveperformance offered bysyntheticnematicides.This is whyresearch workson othernatural'snematicidesmoleculeshave helpedto identify toxic substancesproduced by fungithat can acton nematodes.Thesesubstances are presentin the filtrates.

In that regardour testallowed us toconfirm the highsignificant efficacyof the two filtrates on *H avenae*eggs. Ovicideaction after 48 hours of emersion reached 94.39% and (83.39%) respectively for purefiltrate of*F.solani*, and pure filtrateof *Penicillium spp*. All the resultsobtainedshows that it isnow possibleto consider abiological controlagainstcyst nematodes"Heterodera" through *F. Solani*and*Penicilliumspp*while respecting certainprecautions:Evaluationof thegrowth of the fungusso that theyhave time tosecretesubstancesmetaboliteandtobeeffectivelyagainstthe eggs ofphytophagousnematodes.Regardingsoil

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protection, we are moving towardsthe use ofsaprophytic fungi(*F.solaniandPenicillium spp*),Thenthe soil pHshould bebelow thepH5.The safeguarding of the food value of this invaluable food product is very important thus any attempt at study in this field by the use of natural substances and fungi filtrates is very justified.

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