

Full Length Research Paper

Evaluation of *Stropharia* sp. 1.2052 nematicidal effects against *Meloidogyne incognita* on tomato

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To use unique nematicidal mechanism of *Stropharia*, the strain 1.2052 was evaluated for its potential to control the root-knot nematode *Meloidogyne incognita* on tomato. *In vitro*, the inhibition rate in 24 h of *Stropharia* sp. 1.2052 isolate was 100% for second-stage juveniles of *M. incognita* and *Panagrellus redivivus*, 41.81% for *Caenorhabditis elegans* and 99.25% for *Bursaphelenchus xylophilus*, respectively. In pot experiments, isolate 1.2052 reduced the root-knots to 68.16 to 84.19% in one month and 45.28 to 88.24% in two months after treatment, respectively. The reduction of nematodes in soil ranged from 26.39 to 61.18% compared to the negative control. There were significant efficacies for control of root knot nematodes on tomato.

Key words: *Stropharia*, *Meloidogyne incognita*, biological control, root-knot nematodes.

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp.) cause serious damages to agriculture and forest. It has been reported that plant parasitic nematodes cause annual losses of several billion of crops worldwide (Koenning et al., 1999; Perry et al., 2009). Various fungi have been tested and used to control root-knot nematodes during the last decades. The most studied fungi used and applied to control nematodes are *Hirsutella rhossiliensis* and *Pochonia chlamydosporia*. *H. rhossiliensis* is one of few endoparasitic fungi of mobile stage of nematode. Both species produce infective, adhesive conidia that may be attached to the cuticle of a passing nematode, and has shown great potential in soybean cyst nematode control (Li et al., 2008). *P. chlamydosporia* has a worldwide distribution, and has been found in nematode suppressive soils to parasitize nematode eggs (Manzanilla-Lo'pez et al., 2013). *Fusarium oxysporum* f. sp. *glycines* and *Sclerotium rolfsii* were studied for their effects on pathogenicity of *M. incognita* race 2 in soybean (Akinsanmi et al., 2003). Kiewnick et al. (2006) reported

that a *Paecilomyces lilacinus* strain was selected to control *M. incognita*. *Arthrobotrys dactyloides* was tested against *M. incognita* (J2) on Tomato (Kumar et al., 2006). *Muscodor albus* was evaluated as a potential biocontrol agent against four plant-parasitic nematodes of economically important vegetable crops (Riga et al., 2008). Biological control of root-knot nematode (*M. javanica*) by *Trichoderma harzianum* BI was also investigated in greenhouse and laboratory experiments (Sahebani and Hadavi, 2008).

Species of *Stropharia* (Strophariaceae, Basidiomycota) have purple-brown to tobacco brown spores and appear in woods, grasslands, compost piles, and animal dung. *Stropharia* cultures can produce unique stellate cells, called acanthocytes. Farr (1980) has made a detailed study of the morphology and development of acanthocytes, and considered the ability to form acanthocytes as a characteristic of the whole genus (Norvell et al., 2000). Luo et al. (2006) reported that *S. rugosoannulata* showed ability to immobilize the nematodes *Panagrellus redivivus*

and *Bursaphelenchus xylophilus* within hours on agar plates.

The nematode-attacking activity of this fungus is carried out by spiny acanthocytes and their mechanical force is an important factor in the process. Furthermore, the growth and nematode-attacking activity of the fungus in soil were also determined, suggesting that acanthocytes are functional in soil. In the present study, mechanism of *Stropharia* sp. 1.2052 activities against a population of the root-knot nematode *M. incognita* was evaluated on tomato, in greenhouse experiments.

MATERIALS AND METHODS

Fungal culture

Stropharia sp. 1.2052 was collected from forest in Yunnan, P. R. China. The pure culture was isolated from fruiting body, identified by Luo et al. (2006), and deposited in the culture collection of Key Laboratory for Conservation and Utilization of Bio-resource, Yunnan University, P. R. China.

Solid culture of this strain was prepared on wheat grains. Wheat was soaked 24 h with tap water and 1 h with boiling water, later mixed with CaCO₃, glucose and sawdust (wheat, 60%; sawdust, 36%; CaCO₃, 2%; glucose, 2% v/v). Such culture medium was transferred in 250 ml flask and sterilized at 121°C for 2 h. Inoculated cultures were incubated at 25°C for 30 days when more than 80% wheat was consumed.

Nematodes culture

M. incognita

the nematodes population was collected from tobacco in Yunnan, P. R. China. Tobacco roots with egg masses were dipped in water to remove adhering soil, cut into 0.5 to 1 cm pieces and agitated in 0.5% sodium hypochlorite solution for 2 to 3 min. Egg masses were rinsed with sterile water then placed in 0.5% sodium hypochlorite for 2 to 3 min, agitated and rinsed with sterile water (Kerry and Bourne, 2002). The eggs were incubated at 25 ± 1°C for three to five days to obtain second stage juveniles (J2) with modified Bearmann funnel method (Gray, 1984). The J2 were then used for *in vitro* and pot experiments.

Panagrellus redivivus

Nematodes were cultured on oatmeal medium (oatmeal: 20 g, water 80 ml) at 25°C for seven days, and then refrigerated at 4°C prior to use.

Bursaphelenchus xylophilus

Botrytis cinerea was cultured on a potato dextrose agar (PDA) plate at 25°C until the fungus was fully grown. The plate was then inoculated with the pine nematode, and then cultured until the fungal mycelia had been completely consumed.

Caenorhabditis elegans

The nematodes were multiplied in oatmeal medium at room tempe-

rate (21 to 26°C) for 6 to 7 days.

In vitro experiment

Washed nematodes (80 to 100) were transferred to the PDA culture plates containing abundant well-developed hyphae of *Stropharia* sp. 1.2052. The plates were incubated at 25°C, counting the numbers of active and inactive nematodes under a stereomicroscope (50x) at different times (24, 48, 72, 96, 120 and 144 h). The nematodes were considered dead if they showed no response to physical stimuli. Uninoculated plates were used as negative control, and avermectin (0.8 µg/ml) was used as positive control. Each treatment was replicated three times, and the mortalities (%) were calculated.

Pot assay

One-month-old tomato seedlings were transplanted into 15 cm-diameter pots containing 1000 g sterilized soil (loam soil : humus soil : sand, 1:1:1, v/v). A week after transplanting, the soil around the seedlings was inoculated with *M. incognita* at 2000 juveniles per plant. Pots were arranged in a randomized block in a greenhouse at 25 ± 2°C.

Four treatments were applied: three concentrations of strain 1.2052 (50, 75 and 100 g) were incorporated with soil around the seedlings. Avermectin (0.5%, 0.02 g/pot) was used as positive control, and untreated pots were used as negative control. The experiment was completely randomized with three replicates. All pots were kept under greenhouse conditions (25 to 32°C) and watered when needed.

After 30 and 60 days, the plants were harvested. Nematode damage was measured by rating root galls on the 0 to 5 scale (0 = complete and healthy root system, no infection, 1 = very few small galls only be detected upon close examination, 2 = 25% of root system severely galled, 3 = 25 to 50% of root system severely galled and not, 4 = 50 to 75% of root system severely galled, 5 = >75% of root system severely galled, nearly no healthy roots). In addition, population of nematodes in soil, plant heights, and shoot fresh weights were determined.

Statistical analysis

Results were tested with SPSS version 17.0 by one-way analysis of variance (ANOVA). Significantly differences between treatment means were separated by using *t* test at the *P*<0.05 (Sahebani and Hadavi, 2008).

RESULTS

Effect of fungi on nematode *in vitro*

Application of *Stropharia* sp.1.2052 significantly inhibited nematodes motility *in vitro*, when compared to control in water. At 24 h complete inhibition was observed for all the J2 of *M. incognita* and for *P. redivivus*, whereas lower values were found for *B. xylophilus* and *C. elegans*, which required a longer exposure to reach the same level of inhibition (Table 1).

Effect of fungi on *M. incognita* in pot assay

Significant control of root-knot nematodes was observed

Table 1. The inhibition of *Stropharia* sp. 1.2052 on nematodes tested *in vitro*.

Test time (h)	<i>M. incognita</i> (%)	<i>P. redivivus</i> (%)	<i>C. elegans</i> (%)	<i>B. xylophilus</i> (%)	Avermectin (%)	Negative control (%)
4	46.97 ± 2.96*	25.15 ± 7.46*	8.33 ± 1.43	26.01 ± 12.13	82.28 ± 9.01**	0
12	77.47 ± 22.06*	58.68 ± 6.17**	17.64 ± 1.82	54.32 ± 2.75*	91.87 ± 14.08**	2.71 ± 0.85
24	100 ± 3.45	100 ± 5.67	41.81 ± 1.88**	99.25 ± 1.29**	93.03 ± 12.06**	6.04 ± 1.24
36	100 ± 2.78	100 ± 6.77	96.81 ± 3.13**	100 ± 2.33	93.54 ± 11.19**	9.37 ± 1.89

Means of three replicates ± SD. Values in each column followed by * one and ** two asterisks were significantly different from water control at P≤0.05 and P≤0.01, respectively.

Table 2. The control efficacy of strain 1.2052 against the *M. incognita* in pot assay.

Treatment	Dosage (g)		30 days			60 days		
	Gall index	Biocontrol efficacy (%)	J2/g of plant root	J2 in soil (% in control)	Gall index	Biocontrol efficacy (%)	J2/g of root	J2 in soil (% in control)
Negative control	2	0	29.33 ± 3.45	0	2	0	86.96 ± 6.76	0
Avermectin	0.02	0	2.33 ± 0.15	98.63	0	96.18	1.33 ± 0.45	93.01
Treat1	50	1	7.67 ± 3.78*	26.39	1	45.28	24.58 ± 2.13	61.18
Treat2	75	0	4.00 ± 2.60*	41.72	0	88.14	17.03 ± 1.33	56.77
Treat3	100	0	5.35 ± 2.34	27.88	0	67.32	20.48 ± 4.53	45.77

Means of three replicates ± SD. Values in each column followed by * one and ** two asterisks were significantly different from water control at P≤0.05 and P≤0.01, respectively.

in pots. A dose response relationship was observed for treatments with *Stropharia* isolate 1.2052 which caused the greatest decrease of galls in one and two months, compared to positive controls. Application of *Stropharia* at lower doses resulted in lower damage reduction in one and two months, respectively (Table 2).

There were significant reductions of the *M. incognita* J2 (Table 2), however a dose-response relationship was less evident as the treatment with 75 g/pot caused the highest reduction of J2 from roots, either in one and two months, compared to negative control. Application of *Stropharia* sp. 1.2052 at 50 and 100 g had 8 and 5/g roots in one month, 25 and 21/g roots in two months, respectively. The percent reduction of nematodes in soil compared to the negative control appeared dose-dependent, and was higher than avermectin.

Differences among treatment were observed for plant height and biomass. The plants showed an increase in height after two months, with tallest plants recorded for treatment with *Stropharia* sp. 1.2052 at 75 g/pot and the shortest from the positive control. The fungus application significantly improved shoot weight over the inoculated treatment, the highest weight recorded for treatment at 75 g/pot. A dose-response relationship was also observed for *Stropharia* sp. 1.2052 applied at 50, 75 and 100 g/pot that increased the fresh root weights, compared to positive

and negative controls, respectively (Table 3).

DISCUSSION

Many mushrooms, previously considered only as saprobes, are actually considered capable of taking advantage of other organisms present in their microcosm, exploited for nutritional purposes (including nematodes, plants, other fungi, and bacteria). There is a special group of nematophagous basidiomycetous fungi provided with hyphal appendages that are indispensable for their nematode-attacking ability. The appendages within the various species include hourglass-shaped adhesive knobs on members of the genus *Nematoctonus* (Drechsler, 1949, 1954), and its teleomorph *Hohenbuehelia* (Barron and Dierkes, 1977). Other appendages include the secretory cells of *Pleurotus* spp. (Barron and Thorn, 1987), the secretory appendages of *Conocybelactea* (Hutchison et al., 1996), the stephanocysts on *Hyphoderma* spp. (Burdson, 1969; Tzean and Liou, 1993) and the spiny balls produced by *Coprinus comatus* (Luo et al., 2004). Thapa et al. (1987) reported that *Stropharia rugoso-annulata* was resistant to the mycophagous nematode species *Aphelenchoides sacchari* and *Ditylenchus myceliophagus*. However,

Table 3. Plant growth promotion of strain 1.2052 on tomato.

Treatment	Dosage (g)	Height increasing rate (%)	Fresh shoot weight (g)	Fresh root weight (g)
Negative control		78.9	12.78 ± 0.13	5.38 ± 0.34
Avermectin	0.02	74.0	8.66 ± 0.87	4.00 ± 0.29
Treat1	50	84.4	26.21 ± 1.07	10.13 ± 1.89
Treat2	75	78.8	34.25 ± 1.67*	12.68 ± 2.89
Treat3	100	79.6	32.67 ± 0.96*	19.56 ± 1.13*

Means of three replicates ± SD. Values in each column followed by * one and ** two asterisks were significantly different from water control at P≤0.05 and P≤0.01, respectively.

Grewal (1990) found that the mycophagous nematode *Aphelenchoides composticola* was able to feed on *S. rugoso-annulata*. *Stropharia* species also have been reported to be able to immobilize the nematode *P. redivivus* and *B. xylophilus* with spiny acanthocytes and the results shown that the acanthocytes are functional in soil (Luo et al., 2006).

In the present study, a series of experiments were undertaken to further investigate the effect of *Stropharia* sp. 1.2052 against nematodes. In the *in-vitro* assay, this isolate inhibited more than 80% of motile stages of *M. incognita*, *P. redivivus* and *B. xylophilus*, except *C. elegans*. Application of *Stropharia* sp. 1.2052 to infested tomato plants in the greenhouse reduced the number of galls and final nematode population of *M. incognita* with highest efficacy either at one and two months. Such results are similar to the nematode control effects reported for many other fungi. In controlled experiments on tomato, a pre-planting soil treatment with *P. lilacinus* strain 251 was reported to reduce root galling by 66% (Kiewnick et al., 2006). When *F. oxysporum* was inoculated on soybean, the mean number of nematodes that penetrated roots decreased by 75%, when the soil was treated with *S. rolfisii* the number decreased by 78% (Akinsanmi et al., 2003). Greenhouse study shows that *M. albus* caused significant reduction (91 and 100%) of *Paratrichodorus allius* in soil, and 100% for *Pratylenchus penetrans* in bean roots and soil; 85 and 95% for *Meloidogyne chitwoodi* in potato roots; 100% for *Meloidogyne hapla* both in pepper roots and soil (Riga et al., 2008). The trapping fungus *A. dactyloides* was applied with cow dung manure, which reduced the number of root knots by 61.7 to 66.6% and of juveniles by 68.1 to 88.0% (Kumar et al., 2006).

Conclusion

Stropharia sp. 1.2052 provided effective control of nematode comparable to those reported for other fungal species. As *Stropharia* spp. are common saprophytic basidiomycete in soil, they have less chances to be

pathogenic to plants and human beings, compared to other fungi. The main problems in application of *Stropharia* are related to its cultivation and form of delivering into soil. Its use as symbiont of seeds or plant roots could also be considered for future application, as well as its development in the integrated management of root-knot nematode.

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