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Integrated Management of Root Knot Disease Complex of Brinjal by Using Fungal Bioagent

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ABSTRACT

In systematic survey a disease complex was found which caused disease complex resulting in heavy damage of brinjal crop caused by root knot nematode *Meloidogyne incognita* and root will fungus *Fusarium oxysporum f. sp. lycopersici*. Sevral sprophytic fungi were found associated with the rhizosphere soil viz. *Trichoderma hazarium, Aspergillus terreus, A. fumigatus, A. niger, Cladosporium oxysporum, Penicillium oxalium, Aspergillus* species which was not egg parasitic but exihibited high toxicity (85-100%) to second stage juveniles (J_2), *C. oxysporum* parasitized the egg by 84% but in effective against J_2, while the eggs and juveniles of *M. incoginita* get effected by *T. hazarium* by 80% and 72% respectively. The soil is amendment of vasicular mycorrhiza by talc based formulation of *T. hazarium* @8g Kg $^{-1}$ (2×10^6 spore load) along with leaf powder (0.05% w/w) of *Tinospora longifolia* and vasicular arbuscular mycorrhiza (*Glomus fasciculatum*) @ 100 chlamydospore per kg soil.

KEYWORDS: Antagonist, disease complex, Host tolerance, fungal biogents, Glomus fasciculatum

INTRODUCTION

Several worker reported the disease complex caused by root knot nemotode *Melidogyne incoginita* and wilt causing fungus *Fusarium oxysporum* on different crop. Brinjal is one the popularly and widely grown vegetable in the world. The aggravation and incident rate of disease development and severity to infection is purposed to fungal attack by root knows nematode. Eco-friendly management method with different V.A. mycorrhiza, fungal biogents and botanical antagonists were preferred over chemical to overcome the damage caused by the pathogens in present investigation an attempt has been made for eco-friendly management component for achieving healthier crop and soil.

MATERIALS AND METHODS

Continuous and systematic survey at the infected vegetable field, particularly brinjal is carried out while observing the uprooted plant heavy galling was observed due to root know nematode which also shows wilt symptoms on closer examination through perennial pattern and other character the galled root were observed to be *Melidogyne incognita* (Kofid & White) Chitwood. *F. oxysporum f. sp. lycopersici* and other saprophytic fungi were expressed when the soil adhere with the affected plant were subjected to soil dilution plate method which on incubation at 24 ± 2^0c in BOD incubator the work was carried out in four steps

1. Larvicidal hatching inhibition and egg. Parasitization capabilities in vitro evaluation of both rhizospere soil adhering the root and from egg masses of root knot nematode infecting brinjal plant and isolated against *M. incognita* mycoflora both.
2. By dual culture technique of isolated fungal bioagent against the pathogenic one
3. By common host and lastly establishment of disease complex by isolated pathogenic fungus and root knot nematode.

4. Eco-friendly management of above disease complex by saprophytic fungus with botanical antagonist.

In vitro test of larvicidal hatching and egg parasitazation capacities

The isolated mycoflora were allowed by grow in BOD incubator for 10 days at 24±2^{0}C and followed by filteration through whatmans filter paper not the isolated fungi viz. *A. fumigates, A. niger, A. terreus, F. oxysporum f. sp lycopersici, Trichoderma virens, T. hazarium, T. Viride, Clodosporium oxysporum and Paecilomyces lilacinus*. The 100 freshly hatched second stages Juveniles (J_2) of *M. incoginita* was added in culture filtrate of above fungi in stranded extract were used in 3 replicate of each. At an interval of 24, 48 and 72h the immobility of *M. incognita* juveniles exposed to each of the above fungal filtrates at standard extract was recorded. The three egg masses after surface sterilization were allowed to soak for 48h with the same concentration of each of the above fungi hatching inhibition test was also carried out followed by transferring them to sterilize after in which they were allowed to hatch. At the interval of 2-12 days the hatched juveniles were recorded. By introducing 5 fresh egg masses to the fungal mat of each of the above fungus incubating them for a weed of 24+^{0}c followed by the observation through stereo binocular the capacity of fungal bioagent for its infectivity was also recorded.

By dual culture technique of isolated fungal bioagent against the pathogenic one

The pathogenic fungus, *F. oxysporum f. sp. lycopersici* was carried out in sterilized periplates contain potato dextrose agar medium for the mycotoxicity test of fungal bioagent. The bioagent viz *T. Hazarium, P. lilacinus, A. niger, A. tarreus* was introduced separately introduced under laminar flow and pathogenic fungus at opposite end which is *Fusarium oxysporum f. sp. lycopersici* 3 replication was maintained which were transferred to BOD, incubator at 24+2^{0}C where they allow to grow each of above bioagent pathogenic combination.

The study of interaction between the two disease causing agent M. incognita and *Fusarium oxysporum f. sp. lycopersici* which cause disease complex on brinjal plant accomplished by six treatments-
(a) Fungus alone (F) (b) Nematode alone (N) (c) F+N simultaneously (d) F prior to N after 10 days
(e) N prior to F after 10 days (f) control (Un-inoculated).

Three replication ever maintained for each treatment Earthen pots of diameter 20 cm were filled with sandy lomy soil and a healthy seedling of brinjal which is three week old was transplant in each pot Inoculation was done after 1 week of transplantation, observation were recorded after about 45 days of incolulation @ 2 J_2/g soil for *M. incognita* and 2g fungal mycelia mat per kg soil for *F. oxysporum f.sp. lycopersici*.

Eco-friendly integrated management of disease complex

In randomized block design of five replicates with nine treatments. The three week old healthy brinjal plant were transplanted in to earthen pots (20 conida) were filled with autoclaved sandy loamy soil the three plant antagonists *T. longifolia, G. fasciculatum and T. hazarium* were used in the experiment. The shade dried leaf powder @ 0.05% was amended to soil two week prior to transplantation at the time of transplantation both *M. incognita and F. oxysporum f.sp. lycopersici* in required inoculums level two days prior to sowing the talc based formulated product of *T. hazarium* (with spore load of 2×10^8) and chlamydospore of *G. fascitulatum* @100kg^{-1} soil was amended to soil the treatment were (i) T. harzianum (T.h) + F+N (ii) Tinospora longifolia (T.l.) + N + F (iii) G. fasciculatum (VAM) + N + F (iv) T. l + T.h. + N+ F (v) T.l. + VAM + N + F (vi) VAM + T.h. + N+F (vii) T.l. + T.h. + VAM + N+F (vii) Control (N + F) (ix) Control the observation was recorded 45 days after inoculation on the nemotode multiplication, wilt percentage and mycorrhizal colonization percentage (MCP), plant growth character.

RESULTS AND DISCUSSION

The hatching inhibition test of M. incognita from table (1) the toxic fungus which caused maximum inhibition of hatching M. incognita J_2 from egg masses is a culture of *A.niger* culture filtrate. When the culture filtrate of pathogenic fungus. *F. oxysporum f. sp. lycopersici* is treated with egg masses the maximum number of J_2 was recorded the J_2 hatched only 56 and 29 when treated with bioagent *T. viride and T. harzianum* also expressed hatching inhibition properties the earlier worked has reported that *C. oxysporum* was established to be a eggparasitic fungus and have also confirmed the toxic nature of *A. niger*. The disease complex which are established on common host brinjal by the root know nematode and will causing fungus is presented in table the root know disease complex due to m. incoginita and *Fusarium oxysporam f. sp. lycoperici* clearly showed that for the fungal attach which predisposed on *M. incognita* caused synergistic effect. The disease complex factor on host by *M. incognita and F. oxysporum* was earlier established number of worker.

The mortality test of *M. incognita* exposed to all other fungal culture filtrate showed moderate efficacy which was between 26-48% after 24-72h exposure. Larvicidal test showed that M. incognita J_2 after 72h followed by *A. fumigates and A. terreus* which immobilized 89 and 92% *T. hazarium* after same time of exposure exhibited 80% larval motility and *A. niger* killed 100% J_2. The pathogenic fungus slowed less immobility (30%) on the J_2 of M. incognita due to the both toxic and egg parasitic nature which reduces the proneness and incidence of pathogen on the host the fungal bioagent T. *hazarium* was preferred over other fungal bioagent for the present integrated management studies of disease complex. The outstanding performance in minimizing the disease occurrence with improving the plant vigour the combination of all other management component with *T. hazarium*. The fungal bioagent showed mycotoxicity against pathogenic fungus F. *oxysporum f. sp. lycopersici* was maximum with 100% inhibilition by A. niger followed by *T. hazarium* (77.2%) the bioagent *T. harzarium* with high egg parasitization capacity proved better than *A. niger* however since *A. niger* completely lack egg parasitization capacity of *Melidogyne incognita* the cumulative performance of *T. longifolia T. hazarium* and G. *fasciculatum*, was found superior to any other treatment which made the host most tolerant to fungal attach in the integrated management of disease complex each management component reduce root know disease complex incidence and improve the plant growth compare to control this increased brinjal root biomass alleviated root knot, increased mycorrhizal colonization and root rot as well thereby reducing the cumulative effect of both the pathogen. When any of the three components used alone does not give better result than dual application. May be due to nematotoxic and egg parasitization capacity the reduction in disease severity, increase in biomass and mycorrhizal colonization. As well as mycotoxic effect of fungal bio control agent and result in better host tolerance and production and host growth harmones. Apparently before recommendation to farmer the VA *mycorrhiza, T. longifolia* and *T. Hazarium* it deserves the integration in multilocational trials in farmer field.

Table 1: Immobility of M. incognita juveniles exposed to fungal filtrates

Fungal filtrates	**% immobility**		
	24h	**48h**	**72h**
A. *fumigates*	76	84	96
A. niger	96	98	100
A. terreus	68	74	86
C. oxysporus	24	26	36
P. lilacinus	25	27	46
T. harzianum	69	79	82
F. *oxysporum f. sp. lycopersici*	22	28	32
Control	0	3	7
$CD_{5\%}$	6	9	10

Table 2. By exposing to different fungal filtrates Hatching inhibition test of M. Incognita

Fungi	Number of eggs hatched						Total
	2d	4d	6d	8d	10d	12d	
A. *Fumigatus*	30	20	29	25	18	17	139
A. *Niger*	30	31	26	25	24	20	156
A. *Terreus*	31	30	28	25	24	20	158
C. *oxysporus*	81	78	78	70	51	46	404
P. *lilacinus*	99	87	80	77	69	50	462
T. *viride*	71	67	61	59	58	51	367
T. *virens*	114	110	100	93	85	80	58
T. *Harzianum*	48	41	40	38	31	23	221
F. *oxysporum f. sp. Lycopersici*	31	19	26	23	21	16	36
control	198	89	198	126	74	10	895
$CD_{5\%}$	10	9	8	8	6	7	48

Table 3: Egg parasitization % of fungal bioagents

Fungi	Egg parasitization (%)
A. Fumigates	0
A. Niger	0
A. Terreus	0
C. oxysporus	88
P. lilacinus	63
T. viride	49
T. virens	62
T. harzianum	74
Control	0
$CD_{5\%}$	8

Mean of three replications

Table 4: Effect on growth of tomato and nematode multiplication alone and in combination of M. incognita and *F. oxysporum f. sp. lycopesici*

Treatment	Shoot Length	Dry shoot weight	Galls per Plant	Number egg masses	Egg per eggmass*	Soil nematode population
Control	46.3	3.1	-	-	-	-
Fungus alone	37.8	2.0	-	-	-	-
Nematode alone	42.6	2.9	83.6	46.6	123.4(10.9)	5463.8(69.6)
F 10 days before N	40.8	2.9	59.8	37.1	89.3 (9.3)	29358.7(54.4)
F+N days before N	28.5	1.8	43.1	42.6	97.7(9.8)	3367.0(58.8)
N 10 days before F	32.5	1.5	46.5	42.3	107.9(10.4)	3108.8(55.3)
C.D. @ 5%	4.3	0.3	2.0	5.9	(4.7)	(7.9)

Mean of three replication; * figures in parenthese are angular transformed values; ** Data indicated in parenthesis are $\sqrt{n+0.5}$ transformed values

Table 5: In dual culture technique (after 20 days) Interaction between *F. oxysporum f. sp. lycopersici* and fungal bioagents

Fungal bioagents	Reaction	Radial growth of *F. oxysporum f. sp. Lycopersici* (mm)	Radial growth of bioagents (mm)	Inhibition %=(C-T/C)×100
A. *fumigalus*	+	14.5	76.5	84.1
A. *niger*	+	0.0	80.0	100.0
A. *terreus*	+	6.0	85.0	96.2
C. *oxysporum*	+	43.3	45.7	53.1
P. *lilacinus*	+	35.5	54.5	52.3
T. *harzianum*	+	14.6	73.2	87.8
CD5%	+	5.5	5.8	7.9

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