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Null Hyper-Parasitism, a Threat for Successful Biological Control Management

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ABSTRACT

Null hyper-parasitism, a new term is coined by the author to define the hyper-parasitism by a microbial agent on another hyper-parasite. The novel phenomenon of null hyper-parasitism was discovered in the in vitro and in vivo experimentation, where the bio-control agent Trichoderma hamatum hyper-parasitic on Sclerotium *rolfsii*, a foot rot pathogen of groundnut, was hyper-parasitiosed by a microbial strain of *Aspergillus niger* and *Bacillus thermophillus*. Here *A. niger* and *B. thermophillus* as null hyper-parasite nullified the bio-control action of hyper-parasite *Trichoderma hamatum* on *Sclerotium rolfsii*. The *in vivo* experimentation suggest that such type of null hyper-parasitism exist in soil ecosystem, may be to maintain the natural microbial equilibrium and extinction of a microbial species from nature due to presence of hyper-parasite and its antagonistic or bio-control activity as evident in the above case of parasite/pathogen *S. rolfsii*, its hyper-parasite *T. hamatum* and null hypersite *A. niger* and *B. Thermophillus*. Now, therefore the success of the bio-control or hyper-parasitism of soil borne fungal plant pathogen by *Trichoderma sp* may be dependent on the non- existence of null hyper-parasite in the soil ecosystem where the hyperparasite has to be used.

Keywords: Null hyper-parasitism, hyper-parasitism, biocontrol agent, *Trichoderma sp*, *Sclerotium rolfsii*, *Aspergillus niger*, *Bacillus thermophillus*, soil ecosystem, Threat, biological control.

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I. INTRODUCTION

Presence of hyper-parasitic biocontrol agents for many fungal plant pathogens are known [1]-[3] and used to control different soil borne plant diseases [4]-[7]. However, the efficacy of these hyper-parasitic fungal bio-agents is not constant in all the ecosystem [8]-[11] limiting their use in the complete management of soil borne fungal plant diseases. The different reasons for this were stated in different scientific studies [12], [13]. However, in the present studies the phenomenon of null hyper-parasitism was discovered which seems to be one of the reasons for ineffective hyper-parasitism of soil borne plant pathogen in their management. The concept of null hyper-parasitism under in vitro and in vivo studies proved this phenomenon.

II. MATERIAL AND METHODS

A. Isolation of Sclerotium rolfsii, a soil borne foot rot pathogen of groundnut crop

Potato-dextrose-agar (PDA) medium (composition: peeled potato, 250 g., dextrose, 20 g., Agar, 20 g, distilled water to make 1 L medium, pH, 7.0) was used for the isolation of groundnut foot rot pathogen *Sclerotium rolfsii* from the infected diseased plant. The foot rot infected portion of plant sample was cut into small pieces and sterilised with mercurial chloride solution 0.1 percent, with subsequent three washing of distilled sterilised water. Such sterile infected portion was placed on sterilised solidified PDA media in the petri-plates. The isolation plates were incubated in BOD incubator at 28 °C temperature for 5 days. The mycelial growth radiating from the infected portion was picked up and transferred on another PDA plates and incubated further for 10 days in BOD incubator at the same temperature.

The formation of mustard shaped brown sclerotial bodies in the fungal mat was ascertain as the pure culture of *Sclerotium rolfsii* pathogen. The *sclerotium rolfsii* culture was used for its pathogenicity studies on groundnut seedlings under pot culture experimentation and in in vitro and in vivo interaction studies with its hyper-parasite *Ttrichoderma sp* and null hyper-parasite microbial culture.

B. Isolation of bio-control agent Trichoderma sp as hyper-parasite of Sclerotium rolfsii pathogen

A selective medium for *Trichoderma sp* (composition: $MgSO_4(7H_2O)$, 0.2 g., KH_2PO_4, 0.9 g., KCl, 0.14 g.,

NH_4NO_3, 1 g., anhydrous glucose, 3 g., Rose Bengal, 0.15 g., agar, 20 g., distilled water, 950 ml) was used for its isolation, and the isolated *Trichoderma sp* was used as biocontrol agent/hyperparasite against *Sclerotium rolsfii*.

To isolate *Trichoderma sp* from soil samples, 10 g soil was suspended in 90 ml sterile water blank, shaken well and the soil particles were allowed to settle down to harvest the microbial population of Trichoderma present in the supernatant suspension. 1 ml aliquot of this supernatant suspension was transferred from stock solution to sterile water blank of 9 ml. The same procedure of serial dilution was repeated to make dilution of 10^{-1} to 10^{-10}. The different dilutions i.e. 10^{-5} to 10^{-10} were transferred to individual sterilized petri-plates and the sterilized selective medium was poured in these petri-plates. These plates were incubated for 3 days at 25 °C temperature in BOD incubator. Three plates of each dilution were taken for calculating average and total number of Trichoderma colonies to determine their population in a soil sample. The Trichoderma isolate was identified for their species.

C. Identification of Trichoderma species

The Trichoderma culture was identified by using the morphological, cultural, and microscopic structures of the fungi as per routine procedures described for identification of *Trichoderma species* [14], [15].

D. In vitro hyper-parasitism of Trichoderma hamatum on Sclerotium rolfsii

The efficacy of *Trichoderma hamatum as hyper-parasite* to control a fungus *Sclerotium rolfsii,* a foot rot pathogen of groundnut, was tested by dual culture technique on PDA medium in petri-plates. For this, the plates were divided in two halves and 5 mm disc of vigorously growing cultures of *Trichoderma hamatum* as hyper-parasite and the test pathogen *S. rolfsii* were inoculated at each halves face to face on the surface of the medium. The plates were incubated at 27± 1 °C in BOD incubator up to 5 days. The plates were observed for the zone of inhibition between the *Trichoderma hamatum* as antagonist/hyper-parasite on the test pathogen *S.rolfsii*.

E. In vivo (controlled pot condition) hyper-parasitism of Trichoderma hamatum on Sclerotium rolfsii

The steam sterilized soil with FYM was ¾ filled in medium sized plastic pots and the experiment was laid down with following treatments.

a. Groundnut seed sown in plain soil (as control).
b. Groundnut seed sown in soil having foot rot pathogen *S. rolfsii.*
c. Groundnut seed sown in soil having foot rot pathogen S. *rolfsii* + *Trichoderma hamatum* as hyper-parasite.

Each treatment had five replications. In each pot five groundnut seeds were sown. The experimental pots were watered as per requirement. The germination and growth of groundnut plants (healthy/ diseased foot rotted plants) were recorded up to 1month period to determine the hyper-parasitism of *T. hamatum* over *S. rolfsii*.

F. Isolation of associative microbes of Trichoderma sp from Trichoderma inhabited soil

1. Isolation of associative fungi

The associative fungi can be defined as the fungal species present in the soil in association with *Trichoderma sp* and survive in its presence.

To isolate the associative fungi of *Trichoderma sp*, the PDA medium was used. The same soil sample which yielded the *Trichoderma sp* during its isolation, was used to isolate the associative fungi.

10 g of this soil sample was suspended in 90 ml sterile water blank, shaken well and the soil particles were allowed to settle down to harvest the associative fungal flora present in the supernatant suspension. 1 ml aliquot of this supernatant suspension was transferred from stock solution to sterile water blank of 9 ml. The same procedure of serial dilution was repeated to make dilution of 10^{-1} to 10^{-10}. The different dilutions i.e. 10^{-5} to 10^{-10} were transferred to sterilized petri-plates and the sterilized PDA medium amended with 250 ppm streptomycin sulphate was poured in petri-plates. The plates were incubated for 3 to 4 days at 28±1 °C temperature in BOD incubator. Three plates of each dilution were taken for calculating average and total number of colonies and their types.

2. Isolation of associative bacteria

The associative bacteria can be defined as the types of bacterial species present in the soil inhabited by *Trichoderma sp* and survive in its presence.

To isolate the associative bacteria of *Trichoderma sp*, the Nutrient-Agar (NA) medium (composition: peptone, 5 g., beef extract, 3 g., sucrose, 20 g., agar, 20 g., distilled water 1 L) was used. The same soil sample which yielded the *Trichoderma sp* during its isolation, was used to isolate the associative bacterial species.

10 g of this soil sample was suspended in 90 ml sterile water blank, shaken well and the soil particles were allowed to settle down to harvest the associative bacterial flora present in the supernatant suspension. 1 ml aliquot of this supernatant suspension was transferred from stock solution to sterile water blank of 9 ml. The same procedure of serial dilution was repeated to make dilution of 10^{-1} to 10^{-10}. The different dilutions i.e. 10^{-5} to 10^{-10} were transferred to sterilized petri-plates and the sterilized NA medium amended with 200 ppm aureofungin was poured in petri-plates. These plates were incubated after solidification of the medium for 3 to 4 days at 28±1 °C temperature in BOD incubator. Three plates of each dilution were taken for calculating average and total number of bacterial colonies and their types.

G. Interaction of associative microbes with Trichoderma hamatum

1. Interaction of associative fungi with Trichoderma hamatum

Mix growth culture technique was used to study the interaction effect of associative fungi with *T. hamatum*. 5 ml potato-dextrose broth (PDB) was suspended in each test tube and sterilized in autoclave at 15 lbs pressure for 30 minutes. A loop-full culture of each isolated test associative fungi was transferred separately in to PDB followed by transfer of a loop-full culture of *T. hamatum* and the tubes were shaken for mixing of the cultures. These test tubes were incubated for 72 hours at 25 °C temperature in BOD incubator.

Petri-plates containing sterilized solidified PDA medium was marked in to 4 divisions viz. B1, B2 and C1, C2. On B1 segment a suspended growth in broth of 72 hrs incubated test tube was plated, whereas on B2 segment a mycelial growth appearing on the top of broth was plated. On C1 segment pure culture of test associative fungus was plated while on C2 segment pure culture of T. hamatum was plated as control. The experimentation was repeated thrice for all the test associative fungi and their interaction with *T.hamatum*. These plates were incubated at 28± 1 °C in BOD incubator to observe the fungal growth on B1 and B2 segment and to compare it with the C1 and C2 fungal growth of associative fungi/*T. hamatum*.

2. Interaction of associative bacteria with Trichoderma hamatum

The same mix growth culture technique was used to study interaction effect of associate bacteria with *T. hamatum*. 5 ml sterilized PDB in test tubes was used for interaction studies. A loop-full of each isolated test bacterial culture was added in the broth in separate test tube. A loop-full of *T. hamatum* culture was added in each tube and mixed well. The interaction tubes were incubated at 27±1 °C for 72 hrs in BOD incubator. After incubation period, a small aliquot from each tube was plated on PDA medium and spread with the help of glass rod. These plates were incubated 27±1^{0}C in BOD incubator and observed after every 12 hrs to record the effect of interaction on growth of Trichoderma/ associative bacteria or both.

H. Identification of antagonist of Trichoderma hamatum hyper-parasite

1. Identification of antagonist fungal species to Trichoderma

The fungal culture was identified by using the morphological, cultural and microscopic structures of the fungi as per routine procedures described for fungus identification [16].

2. Identification of antagonist bacterial culture to T. hamatum

The bacterial culture was identified by its colony characteristic, gram reaction, odour of the culture and endospore formation and biochemical tests described for the identification of bacterial cultures [17].

I. In vitro antagonism of Aspergillus niger on T. hamatum hyper-parasite

The efficacy/ antagonism of *A. niger* on *T. hamatum* was tested by mix culture technique. For this, a loop-full culture of *T. hamatum* as well as *A. niger* was co- inoculated in potato-dextrose broth in 100 ml flasks in three replications. The inoculated flasks were incubated in BOD incubator at 29±1 °C temperature for 24 hours. A loop-full suspension from this broth was shaded on PDA medium in petri-plates at various places and the plates were incubated in BOD incubator at 29±1 °C temperature up to 4 days for observation of colonies of *Trichoderma* and *Aspergillus*.

J. In vitro antagonism of Bacillus sp on T. hamatum hyper-parasite

The efficacy of *Bacillus sp* as antagonist to *T. hamatum* hyper-parasite was tested by mix culture technique. For this, a loop-full culture of T. hamatum and bacillus was co-inoculated in nutrient broth in 100 ml flasks in three replications. The inoculated flasks were incubated in BOD incubator at 29±1 °C temperature for 24 hours. A loop-full suspension from this broth was spread on NA medium with curved glass rod and the plates were incubated in BOD incubator at 29±1 °C temperature for 72 hours for observation of colonies of Trichoderma and bacillus.

K. In vivo (controlled pot experimentation) null hyper-parasitism of Aspergillus niger on Trichoderma hamatum nullifying its biocontrol/hyper-parasitic action on Sclerotium rolfsii

The steam sterilized soil with FYM was ¾ filled in medium sized plastic pots and the experiment was laid down with following treatments.

a. Groundnut seed sown in plain soil (as control).
b. Groundnut seed sown in soil having foot rot pathogen *S. rolfsii*.
c. Groundnut seed sown in soil having foot rot pathogen *S. rolfsii* + *T. hamatum* as hyper-parasite.
d. Groundnut seed sown in soil having foot rot pathogen *S. rolfsii* + *T. hamatum* as hyper-parasite + *A. niger* as null hyper-parasite.
e. Groundnut seed sown in soil having foot rot pathogen *S. rolfsii* + *T. hamatum* as hyper-parasite + *Bacillus thermophillus* as null hyper-parasite.
f. Groundnut seed sown in soil having foot rot pathogen *S. rolfsii* + *A. niger* as null hyper-parasite
g. Groundnut seed sown in soil having foot rot pathogen *S. rolfsii* + *B. thermophillus* as null hyper-parasite.
h. Groundnut seed sown in soil having *A. niger*
i. Groundnut seed sown in soil having *B. thermophillus*.

Each treatment had five replications. In each pot five groundnut seeds were sown. The experimental pots were watered as per requirement. The germination and growth of groundnut plants (healthy/ diseased foot rotted plants) were recorded up to 1month period to determine the hyper-parasitism of *T. hamatum* and null hyper-parasitism due to *A. niger*/ *B. thermophillus* over *T. hamatum.*

III. Result and Discussion

The culture of *Trichoderma sp* isolated from the soil sample was identified as *Trichoderma Hamatum*. It was tested for its antagonistic/hyperparasitic activity against the *Sclerotium rolfsii* pathogenic on groundnut causing foot rot disease. In vitro, the antagonistic effect of isolated *T. hamatum* as hyper-parasite on the foot rot pathogen *S. rolfsii* was studied by employing dual culture technique, where it was observed that *T. hamatum* was effective in the control/antagonism of *Sclerotium rolfsii*. It could not allow *S. rolfsii* to grow and the whole space in interaction plate was covered by *T. hamatum*. Therefore, for control of *S. rolfsii* pathogen of groundnut in soil, *T. hamatum* should be used. Various workers reported different species of *Trichoderma* as effective in controlling different soil borne plant pathogens. T. harzianum is known to be antagonist against Fusarium oxysporum, Rhizoctonia solani and *Sclerotium rolfsii* [1], [18] – [21]. Singh and Dwivedi [22] reported partial control of *S. rolfsii* by T. viride and T. harzianum. Therefore, based on our results the T. hamatum should be used in the control of *S. rolfsii* which gave cent percent control of the pathogen rather than using T. harzianum. The T. viride was reported antagonist against Fusarium oxysporum, Pythium debaryanum and Rhizoctonia solani [23], [24]. Jayalaxmi [25] reported that *Trichoderma species* viz. T. viride, T. Koningii and T. hamatum gave antagonistic effect against Fusarium udam. Kapoor [3] found *Trichoderma species* effective in inhibiting rhizoctonia solani.

The results on associative microbiota of *Trichoderma* in the soil under the present investigation are indicative that certain fungal colonies were always associated or present where *Trichoderma* population was available in the soil. Therefore, these microbial isolates were termed as associative microbial flora of *Trichoderma species*. At least 6 such fungal isolates having different morphological characteristic were present with *Trichoderma* population as associative fungi in *Trichoderma* inhabited soil.

These 6 associative fungal isolates were tested by employing dual culture technique for their interaction with *Trichoderma hamatum* a hyper-parasite for groundnut foot rot pathogen *S. rolfsii*. The interaction results (Table 1) of associative fungal isolates indicated that the isolate No.3 and 5 were complementary to *Trichoderma hamatum* i.e. both the fungi (associative fungal species and *Trichoderma hatamun*) grew well in the presence of each other. However, *T. hamatum* did not allowed the fungal isolate No 2, 4 and 6 to grow in its presence. Interestingly, the fungal isolate No.1 has got the ability to overgrow on the colonies of *T. hatamum*, though it did not completely kill the hyper-parasite *T. hamatum*.

TABLE.1: INTERACTION OF *T. HAMATUM* WITH ASSOCIATIVE FUNGAL ISOLATES

Associative Fungal (AF) Isolate No.	Interaction Result
1	Overgrew on the colonies of *T. hamatum*. Did not produced any antifungal substance for inhibition of *Trichoderma* growth.
2	*T. hamatum* did not allow the AF isolate to grow.
3	AF isolate and *T. hamatum* was complementary to each other for their growth.
4	*T. hamatum* did not allow AF isolate to grow, however it could not kill it
5	AF isolate and *T. hamatum* was complementary to each other for their growth.
6	*T. hamatum* did not allow AF isolate to grow, however it could not kill it

As the associative fungal isolate No.1, which overgrew on *Trichoderma* colonies and did not allow the *Trichoderma* to grow, this associative fungal isolate was identified on the basis of its colony morphology and microscopic studies [16]. The fungal colonies on PDA at 25 °C was initially white, quickly becoming black coloured with conidial production. The reverse side of colonies was pale yellow. Microscopically, the fungal hypha was septate and hyaline, conidial heads radiated initially splitting into columns at maturity. Conidiophores were long, smooth, hyaline becoming darker at apex and terminating into globose vesicle. The conidia were brown to black, very rough globose. Based on its characteristic the fungal isolate was identified as *Aspergillus niger*.

As *Aspergillus niger* was an associative fungus with Trichoderma in soil, its interaction effect on *T. hamatum*, a known hyper-parasite on *S. rolfsii* was studied under in vitro experiments. The results indicated that the associative fungi *A. niger* colonized the colonies of *T. hamatum* i.e it overgrew and covered the colonies of *T. hamatum* (Fig. 1). No such report of interaction of associative soil fungi with Trichoderma hyper-parasite is available. However, *A. niger* did not colonies the *T. harzianum* colonies as evident in the figure.

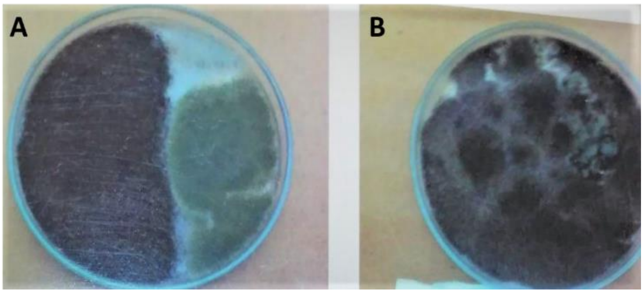

Fig.1. a. Interaction of T. harzianum with A.niger where both grew in their growth spaces.,
b. Interaction of T. hamatum with A. niger where A.niger overgrew on the colonies of T.Hamatum.

Similarly, the 8 associative bacterial isolates different in their colony morphology, colour and growth characteristic were also tested in interaction studies for their effect on *T. hamatum*. The interaction results (Table 2) of associative bacterial isolates with *T. hamatum* indicated that all the eight associative bacterial isolates were complementary with Trichoderma for their growth. No report of interaction of associative bacteria with Trichoderma, a hyper-parasite is available. Therefore, a known bacterial isolate of Bacillus thermophillus as antifungal antagonist [26] was included in the present studies.

TABLE 2: INTERACTION OF ASSOCIATIVE BACTERIA AND *B. THERMOPHILLUS* WITH *T. HAMATUM*

Associative Bacterial (AB) Isolate No.	Interaction Results
1 to 8	Complementary with *Trichoderma hamatum* growth
Antifungal Bacillus thermophillus bioagent	Inhibited the growth of *Trichoderma hamatum*

Similar results were observed for the interaction of the bacterium B. thermophillus with *Trichoderma hamatum* in the interaction plates. The B. thermophillus completely inhibited the hyper-parasite *T. hamatum* and no fungal colonies of Trichoderma appeared in the interaction plates.

The interaction results of *A. niger* and B. thermophillus with *T. hamatum* obtained under in vitro studies were used for their confirmation under in vivo studies under pot culture experimentation. The results (Table 3) showed that the treatment No. 1 i.e. groundnut seed without *S. rolfsii* inoculum gave rise to good growth of groundnut seedling; whereas the treatment No. 2 i.e. groundnut seed sown in *S. rolfsii* inoculated soil failed to germinate and rotted due to *S. rolfsii* infection. In treatment No. 3 where *T. hamatum* was incorporated to the groundnut seed rhizosphere having *S. rolfsii*, there was good germination and growth of seedling, indicating that *T. hamatum* acted as hyper-parasite on *S. rolfsii* and was effective in controlling the foot rot pathogen and its disease. However, in treatment No. 4 and 5 where *T. hamatum* and *S. rolfsii* interaction took place in groundnut rhizosphere in the presence of *A. niger* and *B. thermophillus*, there was poor seed germination and subsequent stunted plant growth indicating that A. niger and B. thermophillus did not allowed *T. hamatum* to fully antagonise *S. rolfsii* to give disease free healthy good growth of groundnut seedling. This indicated that *A. niger* and *B. thermophillus* acted as null hyperparasite on T. hamatum thereby reducing its efficacy in controlling *S. rolfsii* where the stunted growth of plant was due to partial hyperparasitism of *S. rolfsii* with *T. hamatum* (Fig. 2). In treatment No. 6 and 7 where *S. rolfsii* was present without its hyper-parasite, but was having null hyper-parasite, the *S. rolfsii* exerted its pathogenic effect on groundnut germination, indicating that null hyper-parasite did not inhibit the pathogen *S. rolfsii* from exerting its pathogenic potential. Similarly, the null hyper-parasite alone, in this case, did not affect the germination of groundnut seed. The effect of null hyper-parasite is only there, where the hyper-parasite is present.

Fig. 2. Healthy seedling in pot 1 (effect of hyper-parasitism of *T. hamatum on S. rolfsii*)., Stunted seedling in pot No. 2(is due to effect of null hyper-parasitism of *A. niger* on *T. hamatum*) and No seedling in pot No3(is due to infection of *S. rolfsii* on groundnut seedling in absence of hyperparasite *T. hamatum).*

TABLE 3: IN VIVO INTERACTION OF *A. NIGER* AND *B. THERMOPHILLUS* AS NULL HYPERPARASITE ON HYPERPARASITISM OF *TRICHODERMA HAMATUM* ON *S. ROLFSII* FOOT ROT PATHOGEN IN GROUNDNUT

Treatment No	Treatment details	Interaction effect (at 15 DAS)	Interaction effect (at 25 DAS)	Remark
1	Groundnut seed sown in plain soil (as control).	Seed germination and growth of seedling	Growth of healthy seedling	
2	Groundnut seed sown in soil having foot rot pathogen *S. rolfsii*.	No germination	Seed rot due to foot rot pathogen	*S. rolfsii* killed the germinating seed of groundnut
3	Groundnut seed sown in soil having foot rot pathogen *S. rolfsii* + *T. hamatum* as hyper-parasite.	Seed germination and growth of seedling	Growth of healthy seedling	*T. hamatum* was effective as hyperparasite in controlling *S. rolfsii* infection
4	Groundnut seed sown in soil having foot rot pathogen *S. rolfsii* + *T. hamatum* as hyper-parasite+ *A. niger* as null hyper-parasite.	Poor seed germination	Stunted growth	*A. niger* acted as null hyper-parasite on *T. hamatum* thereby reducing the efficacy of trichoderma in controlling *S. rolfsii*. This caused stunted growth of plant due to *S. rolfsii*
5	Groundnut seed sown in soil having foot rot pathogen *S. rolfsii* + *T. hamatum* as hyper-parasite + *B. thermophillus* as null hyper-parasite.	Seed germination	Stunted seedling growth	*B. thermophillus* acted as null hyperparasite on trichoderma thereby reducing the efficacy of trichoderma in controlling *S. rolfsii*. This caused the stunted growth of plant due to *S. rolfsii*
6	Groundnut seed sown in soil having foot rot pathogen *S. rolfsii* + *A. niger* as null hyper-parasite	No seed germination, seed rot due to *S. rolfsii*	Seed rot due to *S. rolfsii*	*S. rolfsii* killed the germinating seed of groundnut. *A. niger* had no effect on *S. rolfsii*
7	Groundnut seed sown in soil having foot rot pathogen S.rolfsii + *B. thermophillus* as null hyper-parasite.	No seed germination, seed rot due to *S. rolfsii*	Seed rot due to *S. rolfsii*	*S. rolfsii* killed the germinating seed of groundnut. *B. thermophillus* had no effect on *S. rolfsii*
8	Groundnut seed sown in soil having *A. niger*	Seed germination and growth of seedling	Growth of healthy seedling	*A. niger* did not have any effect on seed germination
9	Groundnut seed sown in soil having *B. thermophillus*	Seed germination and growth of seedling	Growth of healthy seedling	*B. thermophillus* did not have any effect on seed germination

Such antagonism of *Trichoderma species* by fungal and bacterial antagonist is reported by different workers. Bin [27] reported Pseudomonas fluroscence while Marnyye et.al [28] reported Bacillus species particularly Penibacillus polymyxa as inhibitory to *T. harzianum*. Hubbard et.al [29] also demonstrated that seed colonizing Pseudomonads were largely responsible for failure of *Trichoderma species*. Varshney et.al [30] observed that in co-inoculation studies of *T. harzianum* and fluroscent pseudomonads strains, fluroscent pseudomonas were highly inhibitory to Trichoderma. These results are indicative that some bacillus strain and pseudomonas strains are antagonist to

Trichoderma species. However, these workers referred these antagonistic bacterial strains to *Trichoderma species* as hyper-parasite of Trichoderma. Generally, *Trichoderma species* are known to be hyper-parasite of many soil borne fungal plant pathogens [31]-[33] as these reduced the growth or parasitized the plant pathogenic fungi, while some other workers also named the bacterial species antagonistic to *Trichoderma species* as hyper-parasite [29]. In our present investigation we found that T. hamatum was hyper-parasite of *S. rolfsii* while the efficacy of this hyper-parasite was reduced by antagonist *A. niger* and *B. thermophillus* and therefore a new term was coined for such antagonist who decrease the efficacy of hyper-parasite as null hyper-parasite. The significance of null hyper-parasite is that these may be essential to maintain the natural microbial equilibrium in soil ecology and also to restrict the extinction of a microbial species from nature due to presence of its hyper-parasite and its antagonistic or bio-control activity. Now, therefore the further success of the bio-control or hyper-parasitism of soil borne fungal plant pathogen by *Trichoderma sp* may be dependent on the non- existence of null hyper-parasite in the soil ecosystem. Therefore, it looks important to ascertain the existence/non-existence of null hyper-parasite in the soil to make a hyper-parasitism of plant pathogen by *Trichoderma species* a more success story.

IV. Conclusion

Null hyper-parasite signifies its importance in the management of plant pathogens. Before releasing or utilizing any hyper-parasite for its biocontrol activity in the soil ecosystem against a soil borne plant pathogen, it seems to be mandatory to ascertain the presence of any null hyper-parasite in the ecosystem so as to derive the requisite benefit of the utilised hyper-parasite against the pathogen.

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