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Compatibility of *Trichoderma asperellum* with Fungicides, Insecticides, Inorganic fertilizers and Bio-pesticides

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Abstract

Trichoderma is one of the most common soil inhabitants and extensively studied bio-control agents in the management of plant pathogens. Species of *Trichoderma* are being used for the management of fungal diseases of tropical tuber crops. The diseases, collar rot of elephant foot yam, tuber rot of cassava, yam anthracnose and taro leaf blight could be managed by this bio-agent. Many a time in field, *Trichoderma* is applied along with agrochemicals. It makes essential to assess the compatibility of *Trichoderma* to these chemicals in order to tap the potential of them in the integrated management system. Present study was conducted in ICAR- CTCRI, Sreekariyam, Thiruvananthapuram to assess the compatibility of *T. asperellum* with commonly used fungicides, insecticides, fertilizers and cassava based bio- pesticides developed by ICAR-CTCRI. Thirteen fungicides, eight insecticides, three chemical fertilizers and two bio-pesticides were tested at different concentrations *viz*., 100ppm, 200ppm, 400ppm and 800ppm. Among the fungicides, Metalaxyl M + Mancozeb (Ridomil Gold) followed by Mancozeb (Indofil M-45) were least inhibitory to *T. asperellum.* The fungicides containing carbendazim (Saaf, Bavistin, Sprint, Turf, Starbenz) were highly inhibitory to the mycelial growth of *Trichoderma* even at the lowest concentration tested (100 ppm). Compared to fungicides, insecticides showed less toxicity to *Trichoderma.* Even at the highest concentration of 800 ppm, none of the insecticides could completely inhibit the mycelial growth of *Trichoderma.* The growth inhibition by the inorganic fertilizers were minimum exhibiting >10% mycelium growth inhibition at 800 ppm. The results of this study will help in choosing suitable chemicals in Integrated Disease Management (IDM) for tropical tuber crops.

Key words: *Trichoderma,* compatibility, agrochemicals, metalaxyl, mancozeb

Introduction

The indiscriminate use of pesticides has resulted in accumulation of toxic compounds potentially hazardous to humans and environment (Cook and Baker, 1983). Due to the mounting concern over environment pollution and hazardous effects of chemical pesticides, biological management of diseases is getting more importance than chemical methods. Biological control offers an eco-friendly approach when applied either alone or in combination with other management practices without the demerits of chemical control (Papavizas, 1985). Bio-agents reduce excessive use and misuse of synthetic fungicide and development of fungicide resistance in pathogen. Members of the *Trichoderma* genus are referred to as imperfect fungi, fast growing in culture and produce numerous green spores. These occur worldwide and are commonly associated with root, soil and plant debris (Howell, 2003). Recent advances demonstrate that the effects of *Trichoderma* on plants, including induced systemic or localized resistance, are very important (Harman 2006). *Trichoderma* are being applied as soil incorporation/foliar application or along with planting material. In all the cases, the antagonist will be continuously exposed to different pesticides applied in the field either in soil or as foliar sprays and is likely to influence the efficacy of native or applied bio-control agent, *Trichoderma* (Ranganathswamy, 2012). To develop an effective disease management programme, knowledge about the compatibility of potential bio agents with commonly used agrochemicals is essential. Integration of compatible bio agents with fungicides may enhance the effectiveness of disease control and provide better management of soil borne diseases (Papavizas and Lewis, 1981). Combining antagonists with synthetic chemicals eliminates the chance of resistance development and reduces the fungicide application (Wedajo, 2015).

Tropical roots and tuber crops play a significant role in the global food security and constitute the third important food crops of mankind, after cereals and legumes. They contribute to the energy and nutrition requirements of more than two billion people in developing countries by providing about 6% of the world's dietary calories (Chakrabarti, 2016). Fungal diseases are one of the important factors which reduce the yield and quality of tropical tuber crops. Among the various fungal diseases, taro leaf blight, collar rot of elephant foot yam, tuber rot of cassava and anthracnose of yams are the most destructive ones which cause commendable loss to the crops. Integrative Disease Management (IDM) strategy involving cultural, chemical and biological control is adopted to mitigate the disease incidence and yield loss. *Trichoderma* is being used as a component in the integrated disease management of these diseases. Chemicals which possess inhibitory effect on the pathogen but no deleterious effect on the antagonists will be an ideal candidate in IDM. Hence the present study was conducted to determine the compatibility of locally isolated *T. asperellum* to group of contact fungicides, systemic fungicides, insecticides, chemical fertilizers and cassava based bio-pesticides. An understanding of the effect of these agrochemicals on the antagonist would provide information for the selection of chemicals and chemicals resistant antagonists.

Materials and Methods

Trichoderma culture

Trichoderma was isolated from organically grown elephant foot yam field of ICAR-CTCRI. The identity of the organism was confirmed as *Trichoderma asperellum* by ITS and TEF region amplification.

Fungicides, insecticides, bio-pesticides and fertilizers

The list of chemicals used in the present study is given in Table.1

Table1. Details of fungicides, insecticides, bio-pesticides and inorganic fertilizers

Sl. No.	Brand name	Technical name
Fungicides		
1	Ridomil Gold	Metalaxyl-M(Mefenoxam) 4% + Mancozeb 64%
2	Saaf	Carbendazim12% + Mancozeb 63)
3	Bavistin	Carbendazim 50%
4	Sprint	Carbendazim 25% + Mancozeb 50%)
5	Indofil M-45	Mancozeb 75%WP
6	Turf	Carbendazim 12% + Mancozeb 63%
7	Trumpet	Metalaxyl-M (Mefenoxam)
8	Kavach	Chlorathalonil 75WP
9	Starbenz	Carbendazim 50% WP
10	Tilt	Propiconazole 250% EC
11	Avtar	Zineb 68% + Hexaconazol 4%
12	Contaf	Hexaconazole 5%
13	Samarth	Hexaconazole 2% EC
		Insecticides
14	Chlorpyrifos	Chlorpyrifos 50% EC
15	Rogor	Dimethoate 30% EC
16	Ekalux	Quinalphos 25% EC
17	Fenvalerate	Fenvalerate 20% EC
18	Vapona	Dichlorvos 76% EC
19	Karate	Lambda- cyhalothrin 2.5% EC
20	Malathion	Malathion 50% EC
21	Arrow	Bifenthrin 100EC
Bio-pesticides		
22	Nanma	
23	Shreya	
Fertilizers		
24	Urea	N
25	Rajphos	P_2O_5
26	MOP	K_2O

Mycelial growth inhibition

Compatibility was studied in terms of mycelial growth inhibition and the technique adopted was poisoned food technique (Zentmyer, 1955). Stock solutions of the chemicals were prepared in sterile distilled water and appropriate quantities were incorporated to autoclaved PDA medium to get final concentrations of 100,200,400 and 800ppm and dispensed into Petri dishes. Active ingredient of major chemical in the pesticide was taken into consideration while calculating the concentration of the chemicals. Two hexaconazole fungicides, Contaf and Samarth were tested at concentrations of 10, 20, 40 and 80 ppm in accordance with the low concentration of active ingredient in them (5% and 2% respectively). Making an allowance for the present recommendation, bio- pesticides were tested at the concentrations 1000, 2000, 4000 and 8000 ppm. In addition to these concentrations, based on the preliminary results, Mefenoxam at 10, 20, 40 and 80 ppm; Mancozeb + Carbendazim (Saaf) and Carbendazim (Bavisin) at 25 and 50 ppm were also tested.

T. asperellum was cultured on PDA for 48h and mycelial discs (1cm diam.) were cut from the growing edges and placed in the centre of plates containing PDA amended with various chemicals at different concentrations. PDA plates inoculated with *Trichoderma* mycelial disc without fungicide served as control. Three plates were maintained for each concentration. The plates were incubated at 28 ± 2°C. The growth of the colony was measured after 72 h. The radial growth of mycelium was measured at two points at right angle to each other from each of the three plates maintained for each concentration. The growth of the colony in control sets where no chemical was added was compared with that of various concentrations and the difference was converted into percent inhibition. The percent inhibition of *Trichoderma* isolates was calculated based on the diameter of growth of the colony by using the formula of Vincent (1947).

$$I = (C\text{-}T/C) \times 100$$

Where, I = Per cent inhibition
C = Growth of *Trichoderma* isolates in control
T = Growth of *Trichoderma* isolates in chemicals

Methods of data analysis

The statistical analysis of mycelia growth diameters of *T. asperellum* and per cent of inhibition were tested. Mean comparisons of different parameters were conducted using the procedures of SAS system. Mean separation was determined according to Duncan's multiple range test ($P < 0.05$).

Results and Discussion

Mycelial growth inhibition by fungicides

Eleven fungicides were tested at different concentrations viz., 100, 200, 400 and 800 ppm. Of these, Metalaxyl M-4% + Mancozeb 64% (Ridomil Gold) showed least inhibition to *Trichoderma* (Tab.2).

Table 2. Effect of fungicides on mycelial growth of *Trichoderma* asperellum

Sl. No	Brand name	Technical name/active ingredient	Mycelial inhibition (%) at 100 ppm	200 ppm	400 ppm	800 ppm
1	Ridomil Gold MZ 68 WG	Metalaxyl M-4% + Mancozeb 64%	0.00 [l]	1.80 [j]	16.40 [ij]	26.10 [ij]
2	Saaf	Carbendazim 12% + Mancozeb 63% WP	100.00 [a]	100.00 [a]	100.00 [a]	100.00 [a]
3	Bavistin	Carbendazim 50% WP	100.00 [a]	100.00 [a]	100.00 [a]	100.00 [a]
4	Sprint	Mancozeb 50% + Carbendazim 25% WS	94.40 [b]	100.00 [a]	100.00 [a]	100.00 [a]
5	Indofil M-45	Mancozeb	23.30 [j]	28.05 [i]	39.10 [h]	52.70 [fg]
6	Turf	Carbendazim 12 % + Mancozeb 63 % WP	100.00 [a]	100.00 [a]	100.00 [a]	100.00 [a]
7	Trumpet	Metalaxyl 35% WS	17.20 [k]	41.10 [h]	76.30 [d]	88.80 [c]
8	Kavach	Chlorothalonil 75 WP	48.80 [g]	54.40 [f]	62.50 [e]	72.50 [d]
9	Starbenz	Carbendazim50% WP	100.00 [a]	100.00 [a]	100.00 [a]	100.00 [a]
10	Tilt 250 EC	Propiconazole	100.00 [a]	100.00 [a]	100.00 [a]	100.00 [a]
11	Avtar	Zineb 68% + Hexaconazole 4% WP	76.4 [d]	88.8 [c]	100.00 [a]	100.00 [a]

In case of tropical tuber crops, Ridomil Gold is being recommended to combat taro leaf blight caused by *Phytophthora colocasiae*. The recommended dose of Metalaxyl + Mancozeb for taro leaf blight is 500 ppm. The percentage of Metalaxyl in Ridomil Gold is 4%, thus the actual concentration becomes 20 ppm. In the study, concentration of Mancozeb was taken into consideration and the effective concentration of Mefenoxam in the study was 6.25, 12.5, 25 and 50ppm corresponding to 100, 200,400 and 800 ppm of Mancozeb. The results showed that even at 800 ppm (50ppm of Metalaxyl M), Ridomil Gold inhibited only 26.1% mycelial growth (Fig.1). In field, the concentration gets further diluted and rarely the bio-agent faces the situation of facing the recommended dose of fungicide. Similarly, *Trichoderma* showed tolerance to another Metalaxyl fungicide, Trumpet (Metalaxyl 35% WS). Hence it is completely safe to use *Trichoderma* along with the fungicide, Metalaxyl + Mancozeb (Ridomil). Similar result was reported earlier by Sonavane and Venkataravanappa (2017), while studying compatibility of *T. harzianum* with Metalaxyl 35% WS. Thoudam and Dutta (2014) combined applications of BCAs followed by small quantities of fungicides and reported that fungicides like Metalaxyl M and Mancozeb, lower concentrations are less inhibitory to *Trichoderma* and can be safely used with these fungicides.

Mancozeb (Indofil M-45) showed 23.0% inhibition at 100 ppm and the inhibition rate positively correlated with the strength of the fungicide. Compared to all other fungicides barring Metalaxyl, Mancozeb was less inhibitory to the mycelial growth of *T. asperellum*. This is in agreement with the reports by earlier workers on compatibility of the fungicide with other species of *Trichoderma*. Wedajo (2015) tested fungicide, Sancozeb (Mancozeb 80 WP) at different concentrations viz., 100, 200, 400, 600, 800 and 1000 ppm on active ingredient basis to evaluate *Trichoderma* species viz., *Trichoderma harzianum* (AUT1) and *Trichoderma viride* (AUT2) in favour of tolerance to fungicides. The results of their study revealed the compatibility of *Trichoderma* isolates with sancozeb at 100 ppm and 200 ppm concentration and the incompatibility was observed from the concentration of 600 ppm. Similar results were reported by many workers in *T. viride* and *T. harzianum* (Bhai and Thomas, 2010; Madhavi et al., 2011 and Sonavane and Venkataravanappa, 2017).

Carbendazim (Bavistin) and carbendazim combination fungicides *viz*., saaf, turf, starbenz and propiconazole fungicide (tilt) completely inhibited the mycelial growth of *T. asperellum* (Fig.2). Even at the lowest concentration of 100 ppm, no mycelial growth of *Trichoderma* was observed suggesting non compatibility with the fungicide. The fungicides, mancozeb 50% + carbendazim 25% WS (Sprint), chlorothalonil 75 WP (Kavach) and zineb 68% + hexaconazole 4% WP (Avtar) did not completely inhibit the mycelial growth at the concentration of 100 ppm. A progressive increase in percent inhibition of radial growth in *Trichoderma* was observed as the concentration of all the fungicides increased. The fungicide, mancozeb

Fig.1. Mycelial growth of *T.asperellum* on different concentrations of metalaxyl-M + mancozeb (Ridomil Gold) amended medium

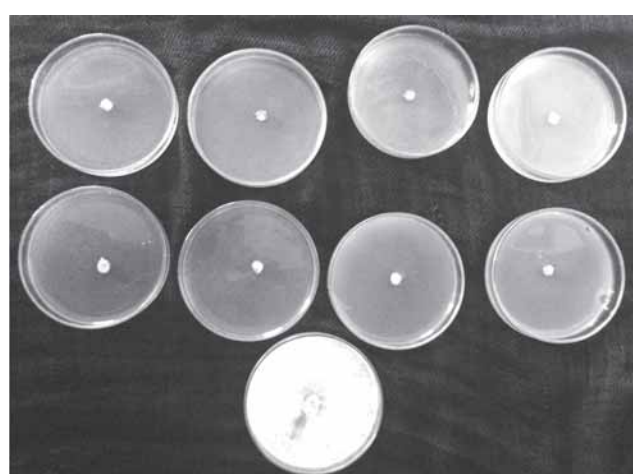

Fig.2. Mycelial growth of *T.asperellum* on different concentrations of carbendazim + mancozeb (SAAF) amended medium.

50% + carbendazim 25% WS (Sprint) is being used for the management of collar rot of elephant foot yam. Similarly, *Trichoderma* is also being recommended for reducing collar rot incidence. There is a fair chance of mixing the above mentioned items in an IDM strategy. Hence, the effect of lower concentrations of the fungicide (25 ppm and 50 ppm) on the growth of *Trichoderma* was also tested. It was found that they are not compatible even at lower concentrations. Nene and Thapliyal (1993) conducted a laboratory study and concluded that *T. viride* was not compatible with fungicide carbendazim. Fungicides, carbendazim and propiconazole completely inhibited mycelial growth of *Trichoderma* from 250 to 1000 ppm (Sonavane and Venkataravanappa, 2017). *T. viride* is highly incompatible with carbendazim and could not grow even 1mm at 1 ppm carbendazim (Malathi et al., 2002). Results of the studies conducted by many other workers also confirm high toxicity of carbendazim towards *T. viride* (Bhai and Thomas, 2010; Gaikwad et al., 2011; Madhavi et al., 2011 and Patil and Gupta 2011). Similarly, Arunasri et al. (2011) and Madhavi et al. (2011) studied the compatibility of *Trichoderma* spp. with propiconazole and revealed that the bio-agent was incompatible with the fungicide by showing 100% mycelial growth inhibition at tested concentration.

Two hexaconazole fungicides, hexaconazole 5% (Contaf) and hexaconazole 2% EC (Samarth) were also tested for their compatibility with *Trichoderma*. They were tested in concentrations, 10 ppm, 20 ppm, 40 ppm, 80 ppm on active ingredient basis. Both fungicides completely inhibited growth at all concentrations tested (Table 3). Non compatibility of *T. viride* with hexaconazole fungicides had been reported earlier by Nene and Thapliyal (1993) and Sonavane and Venkataravanappa (2017). The antifungal activity of hexaconazole has been reported to be the result of their ability to inhibit ergosterol biosynthesis in fungi.

Mycelial growth inhibition by insecticides

Compared to fungicides, insecticides showed less toxicity towards *Trichoderma*. Quinalphos 25% EC was most toxic to *T.asperellum* followed by Malathion 50% EC. The least toxic one was Bifenthrin 100EC followed by Dimethoate 30% EC (Table 4). The most frequent recommended dose of pesticide is 0.05% to 0.075% and even with highest concentration of 0.075% with 100 EC, highest concentration in spray fluid will be 750 ppm and it gets diluted in field condition due to many factors. So, the chemicals Dimethoate 30% EC, Fenvalerate 20% EC, Lambda - cyhalothrin 2.5% EC and Bifenthrin 100EC may be safe to use along with *Trichoderma* in field condition. Singh et al., (2012) reported that insecticides, Imidacloprid 17.8% SL, dimethoate 30% EC, monocrotophos 36% SL and deltamethrin 2.8% EC are compatible with *T. harzianum* while some insecticides, quinalphos 25% EC, carbosulfan 25% EC inhibits the growth of *T. harzianum*. The insecticide, Lambda cyhalothrin (second-generation synthetic pyrethroid), was more compatibile than conventional pesticides monocrotophos and quinalphos under field conditions Gampala and Pinnamaneni (2010).

Mycelial growth inhibition by inorganic fertilizers

Fertilizers have played a vital role in the success of India's green revolution and consequent self-reliance in food grain production. The use of inorganic fertilizers is a common practice for supplementing plant nutrients that get depleted during long term mono-cropping and land cultivation. Among the chemicals tested, fertilizers are least inhibitory to *Trichoderma* and it is very clear that *Trichoderma* is compatible with all the fertilizers tested (Table 5).

The NPK recommendation for cassava is100:50:150 kg/ha. When we calculate the concentration of potassium in soil following the recommendation, it is 66.9 µg/g soil. Hence, *Trichoderma* can be safely applied to the soil where inorganic fertilizers are being applied. Shylaja and Rao, 2012 conducted *in vitro* studies on compatibility of *T.harzianum* with different concentrations of commonly used inorganic fertilizers, urea, single super phosphate (SSP), muriate of potash (MoP) and calcium ammonium

Table 3. Effect of hexaconazole fungicides on mycelial growth of *Trichoderma*

Brand name	Technical name/active ingredient	Mycelial inhibition (%)			
		10 ppm	20 ppm	40 ppm	80 ppm
Contaf	Hexaconazole 5%	100	100	100	100
Samarth	Hexaconazole 2% EC	100	100	100	100

Table 4. Effect of insecticides on mycelial growth of *Trichoderma*

Brand name	Technical name/active ingredient	Mycelial inhibition (%) 100 ppm	200 ppm	400 ppm	800 ppm
Chlorpyrifos	Chlorpyrifos 50% EC	30.4(*0.58)^{o}	38.56(0.67)^{n}	54.7(0.83)^{l}	60.5(0.89)^{k}
Rogor	Dimethoate 30% EC	15.97(0.41)^{q}	25.2(0.53)^{p}	65.0(0.94)^{ij}	73.6(1.03)^{efg}
Ekalux	Quinalphos 25% EC	84.4(1.17)^{c}	87.7(1.21)^{b}	91.1(1.27)^{a}	92.2(1.29)^{a}
Fenvalerate	Fenvalerate 20% EC	39.7(0.68)^{n}	60.3(0.89)^{k}	73.3(1.02)^{fg}	79.2(1.10)^{d}
Vapona	Dichlorvos 76% EC	48.3(0.77)^{m}	55.5(0.84)^{l}	61.6(0.90)^{jk}	68.6(0.98)^{hi}
Karate	Lambda – cyhalothrin 2.5% EC	36.4(0.65)^{n}	53.8(0.82)^{l}	76.6(1.07)^{def}	88.3(1.22)^{b}
Malathion	Malathion 50% EC	61.1(0.90)^{jk}	70.8(1.00)^{gh}	77.2(1.07)^{de}	84.4(1.17)^{c}
Arrow	Bifenthrin 100EC	4.9(0.22)^{s}	11.1(0.40)^{r}	51.3(0.80)^{lm}	76.1(1.06)^{def}

*Values in parentheses are arcsine transformed

Table 5. Effect of inorganic fertilizers on mycelial growth of *Trichoderma*

Trade name	Active ingredient	Mycelial inhibition (%) 100 ppm	200 ppm	400 ppm	800 ppm
Urea	N	0^{e}	3.6^{c}	5.5^{b}	8.05^{a}
Rajphos	P_2O_5	0^{e}	1.1^{d}	2.7^{c}	3.6^{c}
Muriate of Potash (MOP)	K_2O	0^{e}	0.8^{de}	2.7^{c}	5.0^{b}

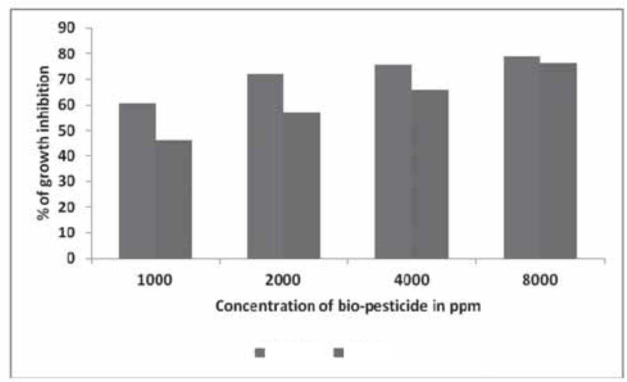

Fig.3. Effect of cassava based bio-pesticides on mycelial growth of *Trichoderma*

nitrate (CAN) at concentrations of 100, 200, 500, 1000 and 2000 ppm. Urea and MOP increased the growth of *T. harziaum* at all concentrations tested while SSP and CAN inhibited the growth. Similar trend had been reported by Gampala and Pinnamaneni (2010). They studied compatibility of chemicals with *T. viride* and found that *T. viride* was more compatible with fertilizers and pesticides, and can be safely used with chemical fertilizers that give major nutrients for any crop. Kumhar et al., (2017) also found that muriate of potash and urea are compatible with the antagonist, *T. harzianum*.

Mycelial growth inhibition by cassava based bio-pesticides

The bio-pesticide developed from cassava is gaining popularity. It has been found that apart from insect management, it can be used for management of post harvest diseases in tuber crops. Hence the sensitivity of *Trichoderma* to bio-pesticides, *Nanma* and *Shreya* was also studied (Fig.3).

Trichoderma was found highly sensitive to *Nanma* rather than *Shreya*. The neem oil content in *Nanma* is higher than in *Shreya*. Neem oil is known to possess antifungal property and this may be inhibiting the growth of *Trichoderma*.

Conclusion

Organic measures to combat pests are the need of the hour and highest priority has been given for this. However, bio-agents have not yet attained efficiencies matching those of currently available fungicides. Integration of pesticides with bio-control agents will be a better option for improving the efficiency of bio-control agents. Combined application of chemical and bio- agents will help in extending the period of active disease control as well as reducing the cost of crop protection. In addition, the use of inorganic fertilizers is a common practice for supplementing plant nutrients that get depleted during long term mono-cropping and land cultivation. Hence it is necessary to give importance to compatibility of bio-agents and agrochemicals while making decision on integration of management options. In case of fungal diseases of tuber crops, *T. asperellum* is being recommended to combat collar rot of elephant foot yam, tuber rot of cassava, yam anthracnose and taro leaf blight. Compatibility studies of *T. viride* and *T.harzianum* with various agrochemicals had been done by many workers. The present study confirms that the compatibility of *T. asperellum* with various agrochemicals is matching with compatibility profile of other species of *Trichoderma* viz., *T. viride* and *T. harzianum*. Metalaxyl and mancozeb fungicides are compatible with *T. asperellum* and it's safe to be applied together in the system. *Trichoderma* being a fungus, it is affected by most of the fungicides. Hence utmost care may be taken while applying incompatible combination of fungicides and bio-agents or a safe interval must be provided while application. Inorganic fertilizes as well as many insecticides will not significantly hinder the growth and establishment of bio-agents. To draw final conclusions, the effects of agrochemicals on *T. asperellum* must be investigated under field conditions.

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