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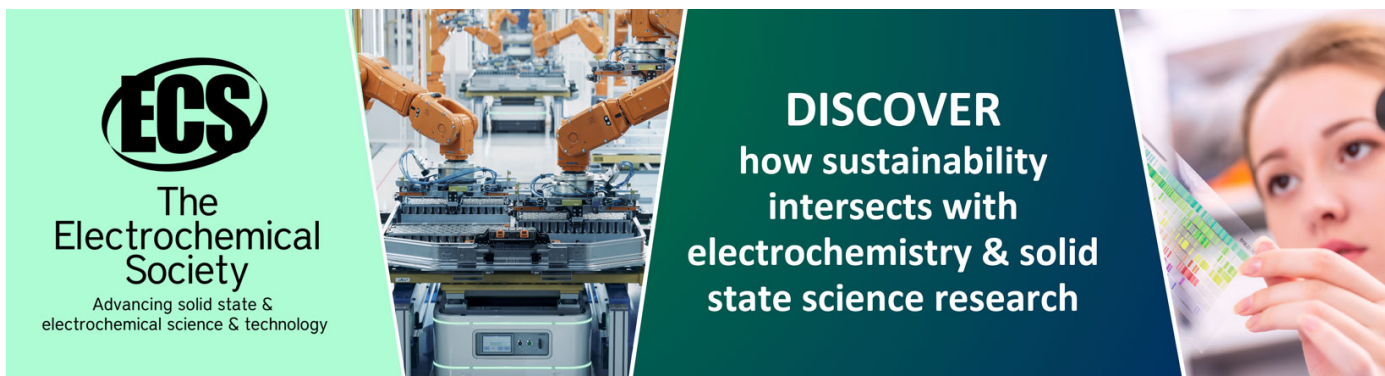
Sustainable Control process of SOME WEEDs BY SOME OF bio - agents Fungi

To cite this article: Nihad. H. Mutlag *et al* 2020 *J. Phys.: Conf. Ser.* **1660** 012075

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Sustainable Control process of SOME WEEDs BY SOME OF bio - agents Fungi

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Abstract. This study was reported to assessment the impact of bio-control fungi [*Trichoderma harzianum* . Rafai (*T. h. t*) and *Chaetomium elatum* (*C. e*)] isolates in controlling *Echinochloa crus-galli* . Gali weeds which growing with the rice- class Yassamine cultivation. The first laboratorial experemments were consisted isolation, purification of fungi in rice fields (AL-Mishkhab at agricultural season 2018), and comparing their pathogenicity, promotional abilities through putting a rice and *Echinochloa* seeds with studied bio-control fungi (their biomass and filterates), as well as the second experiments were acheived by using petri dishes which treated with studied fungi and their filtrates, in order compare the seeds germination rate, plumiles lengths (cm), plumiles fresh and dry weights (gm) of rice and *Echinochloa* seedlings, these experiments was designed with Completely Randomized Design (C. R. D) The results were as follow;

1-Treatments with the biological control fungi, *T. h. t* and *C. e* caused 95. 23, 94. 21, 91. 29 % germination rate of rice seedlings.

2- The *T. h. t.* fungi and its filtrates gave an significant results in controlling of *Echinochloa* weeds which were cultivated in petri dishes, *Echinochloa* germination rates in petri dishes reached 7. 11 % and its filterates gave 11. 49% in petri dishes. Also *T. h. t.* and their filtrates were appeared a significant results in combating *Echinochloa* germination rates by decreasing plumiles lengths, plumiles fresh and dry weight that reach 1. 84, 0. 68 % and 0. 077, 0. 019 cm and 0. 019, 0. 009 cm, respectively in compare with control treatment which were 4. 70, 1. 29% and 0. 209, 0. 031cm and 0. 038, 0. 024 cm, respectively.

Keywords. *Trichoderma harzianum*, Yassamine, *Echinochloa crus-galli* (L.) Beauv, Bio-control, Germination percentage.

1. Introduction

The rice (*Oryza sativa*. L) is one of the major cereal crops in the world, It occupies the second rank after the wheat. , from the economic side it is fed by half of the world's population and is the main resource for millions of people in Asia [18]. Rice is grown in more than 113 countries and gives 27% of the energy needed for humans and 20% of protein in developed countries. Its contains a high amounts of carbohydrates and proteins, its nutritional importance comes from the high content of easily digestible carbohydrates that humans need for energy supply, rice protein has a equivalent content of initial amino acids, such as lysine [11]. it consists 75-80% starch, 13. 3% water, 6. 7-8% protein, and 0. 4% fat and



and 0.9% of metals such as calcium, phosphorus, chlorine, iron (salts and metals) and vitamin A, B. The rice in Iraq comes in a second rank after the wheat crop in terms of economic importance and production, it is a strategic crop in food security [1]. Annual Report on Agricultural Development in the Arab World, the area planted with rice in Iraq was about 1.39 million hectares with a production rate of 2102.7 kg per hectare

The rice is very sensitive to the weeds, especially in the early stages of growth. The results of the previous studies pointed that the decreases in the production due to the weeds were ranged between 63%-85% according to the density of the weeds, so the researchers in the weeds control field used chemical fertilizers and obtained very good results in reducing its impact and eliminating it, that reflected on the increasing of productivity per unit area [5], this has contributed to the thinking of modern ways to combat the bush, including the use of some biocontrol fungi in the fight against the weed and at the same time increase the productivity of acres of rice crop [32], while the addition of herbicides that accumulate frequently, caused environmental, health problems, and appearance of some weeds species show resistance to these herbicides [31]. Bio-control is one of the oldest methods of pest control, but it is currently one of the most complex and advanced methods in the field of pest control, because the maximum use depends on good knowledge of the life and environmental information of both the pest and its associated organisms within the agricultural ecosystem [27]. In order for bio-control to be effective, all its ingredients must be available. Bio control agents due to its considerable abilities to produce enzymes that help to analyze the host's cells and can be used as a food source [6] as well as the ability to occupy the pathogens sites and works to remove the composition of secondary colonies [10], also the use of bio control fungi significantly reduced plant susceptibility to seed rot disease, seedling fall and wilt caused by pathogenic soil fungi such as *Fusarium*, *Pythium*, *Phytophthora* and *Rhizoctonia*.

Chaetomium spp fungus is Ascomycetes fungi, which comprises more than 100 species [2], some of them very common in nature such as *C. elatum*, *C. globosum* and *C. murorum* [36]. Non-pathogenic species of *Chaetomium* fungus have been used in the biological control of some pathogens. [32] found that growth-promoting fungi, including *C. globosum*, had reduced the growth of pathogenic fungus *F. o. l.* That soil treatment with *C. globosum* increased the germination rate of rice seeds and increased weights and lengths of both vegetative and root parts [32]. The promoting fungi strains (nonpathogenic) were used in destroy cellulosic components or increasing microbial mass, so the protein was increased in the treated remains by fungi [39], than these products will be free from toxins.

Trichoderma discovered by Person in 1794 that belongs to the department of true fungi Eumycota, under the section Deuteromycotina, relying on the asexual phase that isolated it from soil and organic matter), Rifai cultivated *T. harzianum* mushrooms in 1969, Weindling was the first to discover the importance of *Trichoderma* spp. in biological control. *Trichoderma* is one of the most common fungi used in this field because of its easy isolation and rapid growth on the natural agricultural media and the lack of special nutritional requirements [22].

This important role of *Trichoderma* spp in the control of many pathogens, also improving growth and productivity of plant, that led many companies and research institutions to move towards the manufacture of biocides from the fungus spores for use in control [32], the work of the fungus to reduce the rate of infection *Fusarium* spp, In potato, peas, tomato, rice and wheat crops [20].

To achieve and explain the ability of *Trichoderma harzianum* in controlling *Echinochloa* weeds which grow with rice crops, a following themes is important:

1. Isolation and diagnosis the fungi from rice soils.
2. Antagonistic efficiency of biocontrol agents on P. D. A. against some of pathogenic fungi
3. Ability of some fungi in combating *Echinochloa* weeds.

Therefore the researchers tried to use of boil sustainable methods to control the weeds in order to decrease the side effects of chemical herbicides, and the costs to the lowest possible levels, these methods consist the use of bio control fungi .

2. Materials and Methods:-

The laboratorial studies implemented in science college laboratories, while the field experimental study conducted in Al Mishkhab research stations –Najaf governorate .

Isolation and diagnosis of some fungi from the rice soils .

Soil samples were collected randomly from the studied rice soils for a depths of 0-15 cm in order to hold physical, biological and chemical analyses, the fungi were isolated by dilutions methods on P. D. A medium, then isolate fungal genus and species in 1 gm soils, the petri dishes were incubated at a temperature of 25 ± 2 C°.

The colonies were purified on P. D. A, W. A medium in order to diagnosis according to the classification keys[30; 28; 8; 16].

Test antagonistic efficiency of some bio-control fungi against pathogenic fungi *F. oxysporum* , *F. pseudogrameniarum* and *R. solani*

Double transplant technique used in petri dishes contained sterilized P. D. A, to test the ability of *T. h. t* . *T. h. a* and *C. e* isolates against pathogenic fungi *R. solani* and *F. oxysporum*, petridish was divided into two equal parts, a disk of diameter (0. 5) cm from pathogenic fungi was mediated first section center near the edges of petri dish, while another disk from biocontrol agents was put in the second half of petri dish, each treatment repeated with in three replicates. also control treatment was carried out by putting adisk of each of biocontrol agents in the center of petri dish individually [13]. All petri dishes were incubated at a temperature (25 ± 2)C°, then after seven days of dual transplant, then the growth rate of pathogenic fungi and bio control fungi were measured, inhibition distance were calculated according to equation (Aghighi and others, 2004).

The impact of bio-control fungi on the seedlings germination rates and plumile lengths, fresh and dry weight of rice and *Echinochloa* seedlings in petri dishes.

One disk of each of *T. harzianum* (*T. h. a*) (*T. h. t*) and *C. elatum* *R. solani*, *F. oxysporum*, *F. pseudogrameniarum*, fungi isoltes (prepared previously) were transplanted (with in three replicates)in the center of petri dishes (contain P. D. A medium), which took from colonies of those isolates and incubated at a temperature of 25 ± 2 C°. After 48 hours, all petri dishes were planted with rice and *Echinochloa* seeds that sterilized by sodium hypo chloride (concentration 4%) for 4-5 minutes (25 seeds per each dish), just 1 cm from the edge of the dish [9].

After 14 days, the seed germination rates seedling lengths, plumile fresh and dry weight for of rice and *Echinochloa* seedlings in petri dishes were calculated .

The impact of studied fungi filterates on the seedlings germination rates and plumile lengths, fresh and dry weight of rice and *Echinochloa* seedlings in petri dishes. .

Filterates of each of *T. harzianum* (*T. h. a*) (*T. h. t*) and *C. elatum*, *R. solani*, *F. pseudogrameniarum*, *F. oxysporum*, fungi isoltes were poured (with in three replicates)in the petri dishes (contain filter papers), which took from flasks of those isolates that incubated at a temperature of 25 ± 2 C° for 30 days. After 1 hours, rice and *Echinochloa* seeds that sterilized by sodium hypo chloride (concentration 4%) for 4-5 minutes petri dishes were distributed (25 seeds per each dish) [9].

After 14 days, the seed germination rates seedling lengths, plumile fresh and dry weight for of rice and *Echinochloa* seedlings in petri dishes were calculated .

The laboratory experiments were designed as Randomized Completely Design (R . C. D), treatment means has been compared according to less significant difference (L. S. D) at 0. 05.

3. Results and Discussion

Physical and Chemical Properties:-ok

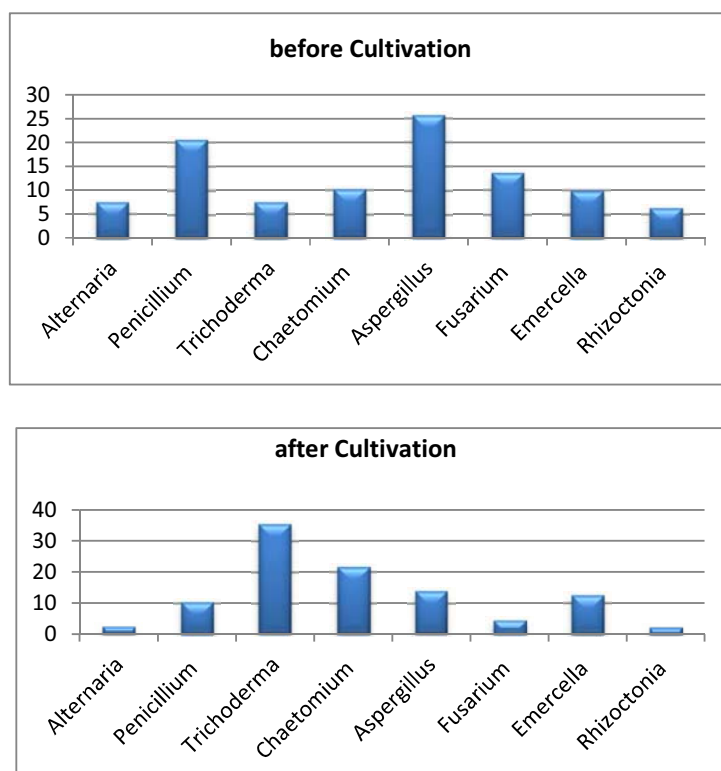
(Table 1)explain that the Al-Mishkhab rice research center soils were silty clay loam texture, The bulk density values were 1. 36 gm. Cm⁻³, while the value pH, ECe were 7. 64, 2. 55 ds. m⁻¹respectively, these values indicated to good values due to the suitable drainage processes[21].

Table 1. Some physiochemical properties of studied soils in the Al-Mishkhab rice research center, for agriculture seasons 2018.

Property	AlMishkhab
Soil Texture	Silty-clay-Loam
Bulk density	1.36
pH	7.64
ECe ds. m ⁻¹	2.55

Isolation, diagnosis of fungi from the rice soils samples.

The Figure (1) data of isolation and diagnosis fungi genus from the rhizosphere region of studied soils referred to 8 genus of fungi, which were *Chaetomium*, *Rhizoctonia*, *Alternaria*, *Fusarium*, *Penicillium*, *Aspergillus* and *Trichoderma* Figure (1), these results had agreed with previous studies [33]. *Aspergillus* genus percentage were 25.53% with the most frequency species of fungi before planting, while *Trichoderma* genus were 35.30% as the most frequency fungi genus in the end of season.

**Figure 1.** The genus of isolated fungi (%) in studied rice soils (1 gm dry soil) before and after cultivation.**Antagonistic efficiency of some fungi isolated on P. D. A. Against *F. oxysporium*. and *F. pseudogrameniarum* and *R. solani***

The bio-control agents *T. h. a*, *T. h. t* and *C. e*. showed a high antagonistic ability against the two important pathogenic fungus *F. o.* and *R. s* table(2), the inhibition distances were 2.48, 2.29 and 1.12 cm respectively against the fungus *F. o.* these results are agreeable with the previous researches [35, 3]. The fungus *T. h.* has affected the pathogenic fungus *F. o.* through the secretion of toxins such as

fumitermoginB, pencillic acid, verruculogen, viridicatumtoxin, ethyleacetate, citrinine, patulin, ochratoxin [10][34]

The results shown in the table (2) a high inhibition distances between the bio-control fungi against *R. s.* isolate which amounted 2. 39, 2. 18 and 0. 41 cm respectively, which referred that the efficiency of *T. h.* fungi to discourage the growth of the pc Chitinase enzymes, which stricke the glucans walls and break it down in the pathogenic fungus cells [26] this results consisted with the results of [37], as well as consistent with the results of [23] indicated that the mechanism that are used by *T. h.* through microscopic views in double culture appear the mycelium of *T. h.* wrap spirally around the pathogenic fungus *R. s.* mycelium, in same time *R. s.* secrete amaterial riched with galactose sugar which is fed by the *T. h.* fungus, as well as containment walls of *R. s.* of chitin as N-Aectyl glucosamine, which is induced *T. h.* to secrete an enzyme Chitianse leading to the analysis of the walls of the *R. s.* mycellium [18], [14] had pointed that there is a relationship between the production of pyrone by the fungus *T. h.* And its ability to antagonist with *R. s.* And *F. o. C. e.* fungi appeared lower inhibitory ability in compared with the previous, it is due to lack of toxic secretions to this isolates [10][34].

Table 2. Antagonistic effect of biocontrol agent on diametric growth of pathogen fungi *R. s* and *F. o* on P. D. A at 25 ± 2 C⁰

Fungi	<i>F. oxysporum</i>	<i>R. solani</i>
	Inhibition Distance (cm)	
<i>C. elatum</i>	1. 12	0. 41
<i>T. harzianum . a.</i>	2. 48	2. 39
<i>T. harzianum . t.</i>	2. 29	2. 18

The impact of bio- control fungi on the seedlings germination rates and plumile lengths, fresh and dry weight of *Echinochloa* and rice seedlings in petri dishes.

Table (3) appear that *T. h. a.*, *T. h. t.* and *C. e* isolates gave a significant differences in germination rate of rice seedlings that reached 95. 23, 94. 21 and 91. 29%, respectively in compare with control treatment which was 86. 39%, this is, may be due to secretion an encouragement material that help germinate and grow like indole acetic acid (decompose the outer crust of the grains and facilitates the process of germination), [20][32] that pointed the significant effect on the promoting germination rate and growth characteristics of wheat seedlings by bio-control fungi.

Also noticed that the (*R. s*) fungus gave a notable germination rate which was 79. 05% and gave an increase in plumile lengths of rice seedlings of 6. 92cm, also in fresh and dry weight which reach 0. 168 and 0, 078 respectively, because of previous research which points that the (*R. s*) fungus has a high pathogenic -capacity, this may be due to genetic differences among them [5].

As well as, the results showed that the *T. h. t.* , *F. o* and *R. s.* isolates were decreased germination rate of *Echinochloa* seedlings, that reached (7. 11, 11. 22, 15. 44 %), respectively, in compared with control treatment which gave 83. 09 %. Also (*T. h. t.* *R. s.* and *F. o*) appears a significant decreasing in plumile lengths (cm), plumile fresh and dry weights (gm) of *Echinchola* seedlings (table3), this may be due to *F. pg.* and *R. s* isolates pathogenicity, or secretion of toxins such as Fusaric acid Zearalenone and Trichothecin, that destroy seeds embryos directly [25][17].

The impact of *T. h. t.* , *F. o.* , and *R. s.* isolates, on plumile length of *Echinochloa* seedlings, reached 1. 84, 1. 60, 1. 38 cm, respectively, in comparison with control treatment, that gave 4. 75 cm, while *T. h. a* and *C. e* isolates achieved a significant increasing in plumile lengths which reach 6. 90, 6. 78 cm respectively at comparison with control treatment, which was 4. 70 cm. So, it was noticed that the *T. h. t* and *R. s* isolates were attained a significant decreasing of the percentage of seeds germination with significant impacts in the controlling of this weeds, this may be attribute, to genetically modification among these isolates.

Table 3. Impact of bio-control species in the germination rate, plumile lengths (cm), plumile fresh and dry weights (gm) of *Echinochloa* and rice seedlings in a petri dish.

Fungi isolates		germination rate %	Plumile lengths (cm)	plumile weights (gm)	
				Fresh	Dry
<i>C. elatum</i>	R	91. 29	9. 87	0. 357	0. 087
	E	90. 33	6. 78	0. 272	0. 071
<i>F. oxysporum</i>	R	15. 34	2. 56	0. 129	0. 020
	E	11. 22	1. 60	0. 066	0. 017
<i>T. harzianum . t.</i>	R	94. 21	10. 01	0. 360	0. 090
	E	07. 11	1. 84	0. 077	0. 018
<i>T. harzianum . a.</i>	R	95. 23	10. 09	0. 367	0. 101
	E	91. 13	6. 90	0. 276	0. 079
<i>R. solani</i>	R	79. 05	6. 92	0. 168	0. 078
	E	15. 44	1. 38	0. 056	0. 013
Control	R	86. 39	7. 38	0. 306	0. 061
	E	83. 09	4. 70	0. 209	0. 038
L. S. D. 0. 01	R	5. 710	2. 470	0.	0.
				0477	0149
	E	5. 322	2. 097	0.	0. 033
				0619	

Whereas R= Rice, E=Echinochloa Seedlings

The impact of bio-control fungi filterates on the seedlings germination rates and plumile lengths, fresh and dry weight of Echinochloa and rice seedlings in petri dishes. .

The result of table (4) refers to significant differences of germination rate caused by the *T. h. a.*, *T. h. t.* And *C. e* filtrates clarify of rice seedlings, which were 93. 76, 92. 40 and 92. 01%, respectively, in comparison with the control treatment, that reached 84. 21%, This accentual due to the possibilities of these isolates to excrete some regulators or by raising the nutritional availability [19]. Also *T. h. a.*, *T. h. t.*, and *C. e* filtrates contributed in the increasing of the plumile lengths in comparison with the control treatment, that were 3. 08, 3. 00, 2. 59 cm respectively. In addition, the result appear that the *F. o* isolate significantly reduced the germination rates of rice seedlings which reach 23. 32 %, in compared with the control treatment, which was 84. 21%. , . Also, *F. o* isolate decrease plumile lengths, that was 0. 38 cm, in comparison with the control treatment, which gave 1. 81 cm, may be according to its abilities to secrete toxins and inhibit the plumile [12]. While *R. s.* isolate attained a germination rate that reached 80. 49% and increasing a plumile lengths, plumile fresh and dry weight of rice seedlings which were 1. 71 cm, 0. 038gm and 0. 019 gm, respectively, that inconsistent with what refers to secretion enzymes and toxins and cause agenetic changes [15], or may be according to genetic alteration with in isolates [5].

The results also displayed that filtrates of *T. h. t.*, *F. o* and *R. s.* isolates caused significantly reducing the germination rates of *Echinochloa* seeds, which were 11. 49, 13. 23, 16. 49 respectively, in comparison with control treatment that was 84. 41%. Also *T. h. t.*, *R. s.*, *F. o* fungi filtrates were decreased plumile lengths of *Echinochloa* significantly, that reached 0. 68, 0. 46, 0. 32 cm, respectively, in comparison with control treatment, which was 1. 29 cm, as well as they were reduced significantly the plumile fresh and dry weight for of *Echinochloa*, which attained 0. 019, 0. 010, 0. 010 gm and 0. 009, 0. 007, 0. 006gm respectively (table 4).

T. h. a. And *C. e.* isolates attained significant increasing in germination rates of *Echinochloa* that were 94. 04, 92. 44%, respectively, while they gave a significant increasing in the plumile lengths, that was 2. 80, 2. 59 cm respectively, in comparison with control treatment, which attained 1. 29 cm .

It also gave a significant differences in plumile fresh and dry weights for, that were 0. 080, 0. 067 gm and 0. 050, 0. 029 respectively, because these fungi able to protect seedlings from pathogens.

Table 4. Impact of bio-control fungi filtrates in the germination rate, plumile lengths (cm), plumile fresh and dry weights (gm) of Echinochloa and rice seedlings in a petri dish.

Fungi isolates		germination % rate	Plumile lengths (cm)	plumile weights (gm)	
				Fresh	Dry
<i>C. elatum</i>	R	92. 01	2. 92	0. 078	0. 031
	E	92. 44	2. 59	0. 067	0. 029
<i>F. oxysporum</i>	R	23. 32	0. 38	0. 026	0. 010
	E	13. 23	0. 32	0. 010	0. 006
<i>T. harzianum . t.</i>	R	92. 40	3. 00	0. 196	0. 075
	E	11. 49	0. 68	0. 019	0. 009
<i>T. harzianum . a.</i>	R	93. 76	3. 08	0. 226	0. 086
	E	94. 04	2. 80	0. 080	0. 050
<i>R. solani</i>	R	80. 49	1. 71	0. 038	0. 019
	E	16. 49	0. 46	0. 010	0. 007
Control	R	84. 21	1. 81	0. 100	0. 037
	E	84. 42	1. 29	0. 031	0. 024
L. S. D. 0. 01	R	3. 119	1. 088	0. 087	0. 041
	E	43. 420	1. 031	0. 0223	0. 0188

Whereas R= Rice, E=Echinochloa Seedlings

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