

ORIGINAL ARTICLE

Field application of Clethodim herbicide combined with *Trichoderma* spp. for controlling weeds, root knot nematodes and *Rhizoctonia* root rot disease in two faba bean cultivars

Mahmoud A.T. El-Dabaa^{1*}, Hassan Abd-El-Khair², Wafaa M.A. El-Nagdi³¹ Botany Department, Weed Biology and Control, National Research Centre, Dokki, Cairo, Egypt² Plant Pathology Department, National Research Centre, Dokki, Cairo, Egypt³ Nematology Laboratory, Plant Pathology Department, National Research Centre, Dokki, Cairo, Egypt

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*Corresponding address:
eldabaam@yahoo.com

Abstract

Clethodim herbicide (Cle) and three *Trichoderma* strains (*Tri*) were applied either alone or in combination (Cle + *Tri*) for controlling weeds, root knot nematodes (*Meloidogyne arenaria*) and *Rhizoctonia* root rot disease (*Rhizoctonia solani*) as well as for evaluating their effects on total microbial count in the rhizosphere and the number of *Rhizobium* nodules on roots in two faba bean cultivars cultivated in naturally heavily infested fields. The evaluated characters were very similar for the two tested cultivars (Nubariya 1 and Sakha 3). Treatment with Cle alone highly reduced the fresh and dry matter of tested weeds (*Amaranthus viridis*, *Cynodon dactylon* and *Cenchrus ciliaris*), followed by Cle + *Tri* and *Tri* alone. Cle + *Tri* highly reduced nematode parameters viz. numbers of J_2 in soil or roots, females, eggs, galls and egg-masses when compared with each treatment alone. *Tri* alone caused a great decrease in *Rhizoctonia* root rot infection, followed by Cle + *Tri* and Cle alone. Total microbial count and *Rhizobium* nodules were affected only with Cle treatment. Plant growth parameters (shoot length, shoot fresh and dry weight and numbers of branches and leaves) and yield parameters (fresh pod and dry weight, seed number per pod, seed weight and ash pod weight of plant) were greatly improved for Cle + *Tri* treatments when compared with either *Tri* or Cle alone.

Keywords: faba bean, herbicide, root knot nematode, *Trichoderma* spp., yield parameters and weeds

Introduction

The legume, faba bean (*Vicia faba* L.) is grown worldwide as a protein source for food and feed. At the same time faba bean offers ecosystem services such as renewable inputs of nitrogen into crops and soil via biological nitrogen fixation and a diversification of cropping systems. Successful production of faba bean crops in the presence of a wide range of disease-causing fungi (*Rhizoctonia solani*), parasitic weeds, nematodes (*Meloidogyne incognita*), insects, mites and other pests depends on integration pest management by application of chemicals and biological treatments (Stoddard *et al.* 2010; Aboali and Saeedipour 2015). In Egypt, root-

knot nematodes (*Meloidogyne* spp.) are important plant parasites with a wide host range including faba bean, where the nematode has an increased population density in Behera governorate (Korayem *et al.* 2018).

Early application of herbicides for controlling weeds in faba bean is essential. Herbicides are not new and are highly efficient for controlling weeds, increasing yield, and improving quality (Arevalo *et al.* 1992). Clethodim is a selective, post-emergence grass herbicide, which provides broad spectrum control of annual and perennial grasses (Jordan 2011). Application of Clethodim and Simazine herbicides to faba bean provided

consistent and high levels of rigid rye grass control (96%) and increased faba bean yield from 723 to 1,046 kg · ha⁻¹ (Kleemann and Gill 2012). *Trichoderma* is a genus of fungi known for its ability to act as a bio-control agent against pathogenic soil borne fungi such as *Fusarium* spp., *Pythium* spp., *Rhizoctonia solani*, *Sclerotium rolfsi*, and *Macrophomina phaseolina*. El-Mougy and Abdel-Kader (2009) found that *Trichoderma viride* and *Trichoderma harzianum* could protect faba bean seeds against *R. solani* and *F. solani* infection under greenhouse and field conditions. Moreover, El-Shennawy (2011) reported that *T. harzianum* had highly significant protection against *Rhizoctonia* root-rot as well as increased plant height, fresh and dry weights of faba bean plants in greenhouse and field experiments. *Trichoderma* species caused significant reduction in *Rhizoctonia* damping-off incidence, increased seed germination and improved plant growth vigor of cauliflower in field application (Rehman *et al.* 2012). *Trichoderma* species have also been reported to control the root knot nematode *M. incognita*, where fungus could suppress nematode reproduction, reduce root galling and egg-masses as well as increase plant yield (Saxena *et al.* 2014).

Integration of chemical pesticides and *Trichoderma* spp. has been researched recently with *in vitro* tests. The potential value of bio-pesticides for controlling important plant pathogens requires investigation due to the general requirement for very low pesticide residues in foodstuffs and concerns over the increasing development of resistance to different classes of pesticides. The compatibility of *Trichoderma* species with chemical pesticides i.e. fungicides, insecticides and herbicides were tested for management of several soil borne pathogens. *Trichoderma viride* was more compatible with lambda cyhalothrin and can be safely used for any crop (Gampala and Pinna-maneni 2010). *Trichoderma viride* also showed a high compatibility with imidacloprid, followed by mancozeb and tebuconazole, while it was found to be fully compatible with contact fungicides *viz.*, pencyuron and propineb. *Trichoderma viride* was highly compatible with the herbicide imazethapyr, followed by 2,4-D sodium salt and oxyfluorefen (Madhav *et al.* 2011). Chlorantraniliprole 18.5% SC and chlorpyrifos 20% EC as insecticides were compatible with all tested *Trichoderma* isolates, while a varying level of inhibition, about 0.0–4.4%, was observed with the herbicides (Singh *et al.* 2014). Captaf, thiram, chlorothalonil and copper hydroxide as fungicides were compatible with *T. harzianum* up to 100 µg a.i. · ml⁻¹, and mancozeb, up to 250 µg a.i. · ml⁻¹. Monocrotophos, dichlorvos, profenophos and triazophos as insecticides were compatible up to 250 µl a.i. · ml⁻¹ and deltamethrin and quinalphos, up to 100 µl a.i. · ml⁻¹. Seven herbicides (2,4-D ethyl

ester, pretilachlor, aniliofos,alachlor, butachlor, fluchloralin and pendimethalin) were compatible with *T. harzianum* even at a higher concentration (250 µl a.i. · ml⁻¹) (Saxena *et al.* 2014).

Therefore, the aim of the present study was to evaluate the compatibility of Clethodim (Select Super 12.5% EC) with *Trichoderma* spp. including *T. harzianum*, *T. viride* and *T. vierns* for controlling weeds (*Amaranthus viridis*, *Cynodon dactylon* and *Cenchrus ciliaris*), root knot nematodes (*Meloidogyne arenaria*) and *Rhizoctonia* root rot disease (*R. solani*) in two faba bean cultivars. Their effects on the total microbial count in the rhizosphere, the number of *Rhizobium* nodules, growth and yield parameters of two faba bean cultivars were also studied.

Materials and Methods

Plant material

Faba bean seeds cvs. Nubariya 1 and Sakha 3 were obtained from the Legumes Crops Research Department, Agricultural Research Centre, Ministry of Agriculture, Giza, Egypt.

Primary detection of *Meloidogyne arenaria* and *Rhizoctonia solani*

The feeder root samples of faba bean, showing naturally occurring root knot nematodes and root rot symptoms, were collected from the National Research Centre (NRC) farm in Nubariya region, Behera Governorate, Egypt. Then the samples were transferred directly to the laboratory of the Plant Pathology Department, NRC, for isolation of *M. arenaria* (the causal agent of root-knot nematode) and *R. solani* (the causal agent of *Rhizoctonia* root rot disease) using standard nematode extraction methods and fungal isolation procedures, respectively.

Infected faba bean roots were washed with tap water and then surface sterilized by dipping in 2% sodium hypochlorite solution for 2 min. The roots were washed several times with sterile distilled water and then, dried between two filter papers before cutting into small pieces (1–2 cm). The small pieces of sterile roots were placed on the surface of potato dextrose agar medium in Petri dishes. The inoculated plates were incubated for 7 days at 30 ± 2°C. *Rhizoctonia solani* was identified according to characteristics outlined by Barnett and Hunter (1972).

Females of *M. arenaria* were isolated from infected faba bean roots collected from the Nubariya region. Adult females were used to identify the nematode species by the morphological characteristics of the female perineal pattern (Taylor and Sasser 1978).

Treatments

Clethodim (Cle) (Select super 12.5% EC) at the recommended dose ($2.38 \text{ l} \cdot \text{ha}^{-1}$) and *Trichoderma* spp. viz. *Th* (*T. harzianum*); *Tv* (*T. viede*) and *Tvr* (*T. vierns*) at 3×10^8 propagules $\cdot \text{ml}^{-1}$ were applied under field conditions. The applied treatments were as follows:

- untreated control,
- Cle only,
- *Th* only,
- *Tv* only,
- *Tvr* only,
- Cle + *Th*,
- Cle + *Tv*,
- Cle + *Tvr*.

Field experiment

The experiment was conducted during the 2015 and 2016 seasons to assess the protected activity of Clethodim and *Trichoderma* spp. as single or combined treatments for faba bean seeds against tested soil borne pathogens under field conditions. Based on previous surveys the authors chose a naturally heavily infested field with weeds, *M. arenaria* and *R. solani* at the NRC farm in Nubariya region, Behera Governorate, Egypt. The field experiment consisted of 64 plots each ($3 \times 2 \text{ m}^2$) in area, where 32 plots were used for each faba bean cultivar. Each plot was composed of 5 rows with 10 holes per row. Each row was 3 m in length, 20 cm in height and 40 cm in width with 20 cm spacing between plants within a row. Four replicates (plots) for each treatment in a completely randomized block design were used. The treatments were applied as soil treatment before sowing. Clethodim was applied at the recommended dose ($2.38 \text{ l} \cdot \text{ha}^{-1}$) and each *Trichoderma* spp. as a liquid formulation was adjusted at 3×10^8 propagules $\cdot \text{ml}^{-1}$ and applied at the rate of $100 \text{ ml} \cdot \text{m}^{-2}$. Then, surface disinfected faba bean seeds (cvs. Nubariya 1 and Sakha 3) were separately sown at the rate of two seeds per hole. Irrigation, recommended fertilizer levels and agronomical practices were used as usual in the reclaimed sandy soils (Abd-El-Khair et al. 2016). Effects of applied treatments were recorded on different parameters.

Weed masses

The fresh weight and dry matter of *A. viridis*, *C. dactylon* and *C. ciliaris* per m^2 were evaluated months after sowing.

Meloidogyne arenaria

Each soil sample was thoroughly mixed and 200 g soil, used to extract nematodes, was processed by sieving and decanting methods (Barker 1985). Roots from the same soil sample were gently washed to free them of

soil and an aliquot of 5 g per replicate was cut into 2 cm long pieces, placed in Petri dishes with distilled water and incubated under laboratory conditions ($25 \pm 5^\circ\text{C}$) for a week to extract *M. arenaria* J₂ and then examined under a stereoscopic microscope. Also, an aliquot of 5 g roots per replicate was blended at 3×10^3 rpm for 3 min to extract females and eggs from roots (Southey 1970).

Rhizoctonia root rot incidence

Rhizoctonia root-rot disease incidence (%) was recorded in treated and untreated bean plants 4 months after sowing. The percentages of infected bean root parts with *R. solani* were determined according to isolation procedures as mentioned before (El-Nagdi and Abd-El-Khair 2014).

Total microbial counts

The total counts of spore forming bacteria, aerobic bacteria and fungi in the rhizosphere of faba bean were determined by the plate count technique using the dilution method on suitable media 4 months after sowing. Ten grams of each soil sample was separately shaken in 90 ml of sterilized distilled water in a 250 ml flask to give a dilution of 10^{-1} . The count of spore forming bacteria was determined after pasteurization of the dilution of 10^{-1} at 80°C for 20 min. Then, 1.0 ml of each 10^{-3} to 10^{-5} dilution were separately transferred onto sterilized Petri plates and filled with nutrient agar medium (NA: peptone 5 g, beef extract 3 g, glucose 20 g, agar 15 g, distilled water 1 l, pH 7). The count of aerobic bacteria was determined by separately adding 1.0 ml of each 10^{-5} to 10^{-7} dilution onto sterilized Petri plates and then filled with NA medium. After 2 days of incubation at 28°C , the resulting spore-forming and aerobic bacterial colonies were recorded. The count of total fungi was determined in 10^{-3} to 10^{-4} dilution using Martin medium (glucose 10 g, peptone 5 g, KH_2PO_4 1 g, MgSO_4 0.5 g, rose bengal 30 μg , agar 15 g; distilled water 1 l). Plates were incubated at $30 \pm 2^\circ\text{C}$ for 5 days and the resulting fungi were counted. The total count in all microorganisms was recorded as cfu $\cdot 10^{-1}$ soil sample (Bridson 1995).

Growth parameters

Four months after sowing, a random sample of six faba bean plants was separately taken from each treatment as well as the control. The growth parameters of plants, i.e. averages of shoot length, shoot fresh weight, shoot dry weight, the numbers of branches and leaves per plant were recorded.

Effect on yield parameters

At harvest, six faba bean plants were taken from each plot. The yield parameters of plants, i.e. averages of pod fresh weight, pod dry weight, the number of seeds $\cdot \text{plant}^{-1}$, seed weight and ash pod weight were recorded.

Statistical analysis

Data were subjected to analysis of variance by ANOVA using Computer Statistical Package User Manual Version 3.03, Barkley Co., USA, and mean values of each plant cultivar were compared according to Duncan's multiple range test at $p = 0.05$ level of significance (Snedecor and Cochran 1980).

Results

Effects on growth parameters of weeds

In cv. Nubariya 1 Clethodim combined with *Trichoderma* spp. (Cle + *Tri*) viz. Cle + *Th*, Cle + *Tv* and Cle + *Tvr* reduced the fresh and dry weights of *A. viridis* in the ranges of 16–30% and 15–30%, respectively, where Cle + *Th* and Cle + *Tvr* were significantly different from Clethodim alone. *Trichoderma* spp. (*Tri*) viz. *Th*, *Tv* and *Tvr* reduced the same weights of grass in the range of 1–5%, compared to 36% for Cle treatment. Cle + *Tv* exhibited a great reduction effect on fresh and dry weights of *A. viridis*, followed by

Cle + *Th* and Cle + *Tvr*. Treatments of Cle + *Tri* highly reduced the tested growth parameters of *C. dactylon* as fresh and dry weights in the ranges of 14–26% and 14–26%, respectively, compared to ranges of 7 and 1–7%, respectively, for *Tri* treatments and 43% with Cle. Treatments of Cle + *Tri* also highly reduced the fresh and dry weights of *C. ciliaris* by 17–28%, compared to 8–15% with *Tri* treatments and 49% with Cle. Cle + *Tv* also highly reduced the growth parameters of *C. dactylon* and *C. ciliaris*, followed by Cle + *Tvr* and Cle + *Th*, respectively (Table 1).

In cv. Sakha 3 the Cle + *Tri* treatments reduced the fresh and dry weights of *A. viridis* in the ranges of 13–14% and 13–15%, respectively, while *Tri* treatments reduced the weights of grass by 1–5%, compared to 15% with Cle. Treatments of Cle + *Tri* reduced the growth parameters of *C. dactylon* by 13–21%, compared to 2–9% with *Tri* treatments and 45% with Cle. The fresh and dry weights of *C. ciliaris* ranged from 18 to 34% with of Cle + *Tri* treatments, compared to 10 to 17% with *Tri* treatments and 38 and 39% with Cle, respectively. Cle + *Tv* also significantly reduced the growth parameters of three grasses, followed by Cle + *Th* and Cle + *Tvr*, respectively (Table 1).

Table 1. Effect of herbicide (Clethodim) combined with *Trichoderma* spp. on growth parameters of weeds associated with faba bean (cv. 1 and cv. 2) in field application

Treatments	Growth parameters of weeds											
	<i>Amaranthus viridis</i>				<i>Cynodon dactylon</i>				<i>Cenchrus ciliaris</i>			
	fresh weight		dry weight		fresh weight		dry weight		fresh weight		dry weight	
	[g]	red.* [%]	[g]	red.* [%]	[g]	red.* [%]	[g]	red.* [%]	[g]	red.* [%]	[g]	red.* [%]
Cv. 1 – Nubariya 1												
Untreated control	277.3 a*	–	28.6 a	–	292.7 a	–	88.7 a	–	307.7 a	–	53.0 a	–
Clethodim (Cle)	178.3 c	36	18.4 c	36	165.7 d	43	50.2 e	43	155.7 e	49	26.9 e	49
<i>Trichoderma harzianum</i> (<i>Th</i>)		5	27.1 a	5	272.0 ab	7	82.6 ab	7	260.3 c	15	44.9 b	15
<i>Trichoderma viede</i> (<i>Tv</i>)		1	28.2 a	1	271.0 ab	7	88.1 a	1	284.3 b	8	49.0 ab	8
<i>Trichoderma vierns</i> (<i>Tvr</i>)	262.3 a	4	27.5 a	4	272.7 ab	7	84.7 ab	5	266.3 c	14	46.2 b	13
Cle + <i>Th</i>	274.0 a	22	23.4 b	18	253.3 b	14	76.6 bc	14	255.5 c	17	44.1 bc	17
Cle + <i>Tv</i>	195.0 c	30	20.1 c	30	218.0 c	26	66.0 d	26	220.3 d	28	38.0 d	28
Cle + <i>Tvr</i>	234.3 b	16	24.2 b	15	240.7 bc	18	70.3 cd	21	225.5 d	27	38.9 cd	27
Cv. 2 – Sakha 3												
Untreated control	188.0 a	–	19.8 a	–	191.0 a	–	54.6 a	–	199.0 a	–	36.9 a	–
Clethodim (Cle)	160.0 b	15	16.8 b	15	105.0 d	45	30.0 d	45	122.7 d	38	22.7 d	39
<i>Trichoderma harzianum</i> (<i>Th</i>)	178.3 a	5	18.8 a	5	174.0 b	9	49.7 ab	9	165.3 bc	17	30.6 bc	17
<i>Trichoderma viede</i> (<i>Tv</i>)	186.7 a	1	19.7 a	1	188.0 a	2	53.7 a	2	179.7 b	10	33.3 b	10
<i>Trichoderma vierns</i> (<i>Tvr</i>)	184.0 a	2	19.2 a	3	175.7 b	8	50.2 ab	8	166.3 bc	16	30.8 bc	17
Cle + <i>Th</i>	163.3 b	13	17.2 b	13	167.0 b	13	47.7 bc	13	158.7 c	20	29.4 c	20
Cle + <i>Tv</i>	161.0 b	14	16.9 b	15	151.0 c	21	43.1 c	21	132.0 d	34	24.4 d	34
Cle + <i>Tvr</i>	163.3 b	13	17.2 b	13	167.0 b	13	47.7 bc	13	164.0 bc	18	30.4 bc	18

*reduction

Means in each column (for each cultivar) followed by the same small letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

Effect on *Meloidogyne arenaria*

In cv. Nubariya 1 the Cle + *Tri* treatments reduced the number of J_2 of *M. arenaria* in soil between 74–81%, compared to 55–65% for *Tri* treatments and 71% for Cle alone (Table 2). The same treatments significantly reduced the number of J_2 in roots between 72 and 78%, compared to 42–72% and 75%, respectively. Also, the Cle + *Tri* treatments significantly reduced the number of *M. arenaria* females by 77–81%, compared to 25–66% for *Tri* treatments and 69% for Cle. Cle combined with *Trichoderma* spp. reduced the number of nematode eggs by 83–92%, compared to 77–84% for *Tri* treatments and 83% for Cle. The reduction in the number of nematode galls ranged between 74 and 78% for Cle + *Tri* treatments, followed by 60–63% and 77% for *Tri* and Cle treatments, respectively. Meanwhile, these treatments reduced the number of egg-masses by 59–62%, 46–56% and 57%, respectively. As shown in Table 2, the greatest reduction in the numbers of J_2 in soil and eggs was obtained by Cle + *Tvr* treatment. Cle + *Tvr* produced the highest reduction in the J_2 in roots, as well as the numbers of females and galls. Cle + *Th* treatment highly reduced the number of egg-masses.

In cv. Sakha 3 data in Table 2 indicated that all treatments had almost the same trend. The Cle + *Tri* treatments reduced the J_2 in soil in the range of 68–73%, compared to the range of 52–74% and 63% with *Tri* and Cle, respectively. The reduction in the number of J_2 in roots ranged from 79–82%, 68–71% to 73% with Cle + *Tri*, *Tri* and Cle treatments, respectively. The reduction in the number of females ranged from 77 to 79%, compared to 69–73% and 70% with treatments of *Tri* and Cle, respectively. Cle + *Tri* treatments reduced the number of eggs by 84–91%, followed by 82–86% with *Tri* treatments, compared to 83% in Cle. The reduced number of galls ranged from 80–82%; 67–79% to 68% with treatments of Cle + *Tri*; *Tri* and Cle, respectively. The treatment of Cle + *Tri* reduced egg-masses by 63–69%, compared to 46–66% and 31% with *Tri* and Cle.

Effects on *Rhizoctonia* root rot incidence

In cv. Nubariya 1 *Rhizoctonia* root infection percentages ranged from 20.1 to 33.3% with Cle + *Tr* treatments, while it ranged from 6.7 to 20.0% with *Tri* treatments, compared to 53.3 and 73.3% with Cle and untreated control treatments, respectively. *Rhizoctonia* root infection percentages, in cv. Sakha 3, ranged from

Table 2. Nematicidal activity of herbicide (Clethodim) combined with *Trichoderma* spp. on *Meloidogyne arenaria* parameters in faba bean (cv. 1 and cv. 2) in field application

Treatments	log ₁₀ numbers and reduction of <i>M. arenaria</i> parameters											
	J ₂ in soil · 200 g ⁻¹		J ₂ in roots · 5 g ⁻¹		females		eggs		galls · 5 g ⁻¹ roots		egg masses · 5 g ⁻¹ roots	
	log ₁₀	red.* [%]	log ₁₀	red.* [%]	log ₁₀	red.* [%]	log ₁₀	red.* [%]	log ₁₀	red.* [%]	log ₁₀	red.* [%]
Cv. 1 – Nubariya 1												
Untreated control	2.44 a	–	2.73 a	–	2.64 a	–	3.10 a	–	1.66 a	–	1.32 a	–
Clethodim (Cle)	1.89 bcd	71	2.11 c	75	2.14 bc	69	2.35 b	83	1.02 c	77	0.95 bc	57
<i>Trichoderma harzianum</i> (<i>Th</i>)	1.98 bcd	65	2.49 b	42	2.52 a	25	2.37 b	82	1.26 b	60	1.05 b	46
<i>Trichoderma viede</i> (<i>Tv</i>)	2.03 bc	60	2.16 c	72	2.17 b	66	2.43 b	79	1.23 b	63	0.96 bc	56
<i>Trichoderma vierns</i> (<i>Tvr</i>)	2.07 b	55	2.39 b	54	2.19 b	65	2.31 b	84	1.23 b	63	0.98 bc	54
Cle + <i>Th</i>	1.83 bcd	74	2.16 c	72	1.99 cd	78	2.32 b	83	1.05 c	74	0.89 c	62
Cle + <i>Tv</i>	1.73 cd	81	2.12 c	75	2.00 cd	77	1.99 c	92	1.02 c	77	0.93 bc	59
Cle + <i>Tvr</i>	1.68 d	79	2.07 c	78	1.93 d	81	2.25 b	86	1.01 c	78	0.93 bc	59
Cv. 2 – Sakha 3												
Untreated control	2.45 a	–	2.74 a	–	2.65 a	–	3.11 a	–	1.67 a	–	1.33 a	–
Clethodim (Cle)	2.01 bcd	63	2.15 bcd	73	2.12 b	70	2.35 b	83	1.17 b	68	1.06 b	31
<i>Trichoderma harzianum</i> (<i>Th</i>)	2.11 b	52	2.23 b	69	2.09 b	73	2.32 b	84	1.18 b	67	0.93 bc	46
<i>Trichoderma viede</i> (<i>Tv</i>)	2.00 bcd	74	2.25 b	68	2.09 b	72	2.31 b	86	1.01 c	77	0.81 cd	61
<i>Trichoderma vierns</i> (<i>Tvr</i>)	2.01 bcd	60	2.20 bc	71	2.14 b	69	2.36 b	82	1.00 c	79	0.75 cd	66
Cle + <i>Th</i>	1.93 bcd	68	2.01 d	81	1.98 b	79	2.25 b	87	0.96 c	80	0.78 cd	65
Cle + <i>Tv</i>	1.87 d	73	1.99 d	82	2.02 b	77	2.06 c	91	0.92 c	82	0.79 cd	63
Cle + <i>Tvr</i>	1.89 cd	71	2.06 cd	79	2.03 b	77	2.32 b	84	0.92 c	81	0.70 d	69

*reduction

Means in each column (for each cultivar) followed by the same small letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

13.3 to 33.3% with Cle + *Tri* treatments, while the percentages ranged from 6.7 to 26.7% with *Tri* treatments, compared to 60.0 and 80.0% with Cle and untreated control, respectively. In combination treatments; Cle + *Tvr* highly reduced the disease incidence, followed by Cle + *Tv* and Cle + *Th*. Applying *Tvr* significantly decreased the *Rhizoctonia* root incidence, followed by *Tv* and *Th* (Table 3).

Effects on *Rhizobium* nodules

Details of *Rhizobium* nodule as \log_{10} number are listed in Table 3. In cv. Nubariya 1, the number of *Rhizobium* nodules on roots of faba beans increased between 25 and 31% due to Cle + *Tri* treatments, while it increased between 29 and 34% with *Tri* treatments, compared with 23% for Cle. In cv. Sakha 3, the increase in *Rhizobium* nodules ranged from 24 to 25% with Cle + *Tri* treatments, while the increase ranged from 27 to 37% for *Tri* treatments, compared with 22% for Cle.

Effects on microbial counts

In cv. Nubariya 1 the spore forming bacteria count in the rhizosphere of faba bean with Cle + *Tri* treatments ranged from 4.52 to 4.55 \log_{10} cfu \cdot 10 g^{-1} soil, while the

bacterial count ranged from 4.51 to 4.53 \log_{10} cfu \cdot 10 g^{-1} soil with *Tri* treatments, compared to counts of 4.08 and 4.43 \log_{10} cfu \cdot 10 g^{-1} soil with Cle and untreated control, respectively. The count of aerobic bacteria ranged from 5.82 to 6.00 \log_{10} cfu \cdot 10 g^{-1} soil with Cle + *Tri* and it ranged from 6.07 to 6.13 \log_{10} cfu \cdot 10 g^{-1} soil with *Tri* treatments, compared to 5.88 and 6.15 \log_{10} cfu \cdot 10 g^{-1} soil with Cle and untreated control, respectively. The count of fungi ranged from 3.29 to 3.35 \log_{10} cfu \cdot 10 g^{-1} soil with Cle + *Tri*, while the count ranged from 3.60 to 3.83 \log_{10} cfu \cdot 10 g^{-1} soil with *Tri* treatments, compared to 3.05 and 3.16 \log_{10} cfu \cdot 10 g^{-1} soil with Cle and untreated control, respectively (Table 3).

In cv. Sakha 3 the count of spore forming bacteria ranged from 4.57 to 4.58 \log_{10} cfu \cdot 10 g^{-1} soil with Cle + *Tri* and ranged from 4.54 to 4.58 \log_{10} cfu \cdot 10 g^{-1} soil with *Tri* treatments, compared to 4.12 and 4.42 \log_{10} cfu \cdot 10 g^{-1} soil with Cle and untreated control, respectively. The count of aerobic bacteria was in the range of 6.01 to 6.07 \log_{10} cfu \cdot 10 g^{-1} soil with Cle + *Tri* treatments and ranges of 6.13 to 6.17 \log_{10} cfu \cdot 10 g^{-1} soil with *Tri* treatments, compared to 5.90 and 6.15 \log_{10} cfu \cdot 10 g^{-1} soil with Cle and untreated control, respectively. The count of fungi ranged from 3.31 to 3.35 \log_{10} cfu \cdot 10 g^{-1} soil with Cle + *Tri* treatments and ranged from 3.57 to 3.60 \log_{10} cfu \cdot 10 g^{-1} soil

Table 3. Effects of herbicide (Clethodim) combined with *Trichoderma* spp. on total microbial counts (cfu \cdot g^{-1}), *Rizoctonia* root rot incidence (%) and number of nodules in faba bean (cv. 1 and cv. 2) in field application

Treatments	<i>Rizoctonia</i> root rot incidence [%]	Nodules no.		\log_{10} of total microbial counts (cfu \cdot g^{-1})		
		\log_{10}	increase [%]	total spore-forming bacteria (10^3)	total aerobic bacteria (10^5)	total fungi (10^2)
Cv. 1 – Nubariya 1						
Untreated control	73.3 a	1.22 c	–	4.43 b	6.15 a	3.16 de
Clethodim (Cle)	53.3 ab	1.50 b	23	4.08 c	5.88 bc	3.05 e
<i>Trichoderma harzianum</i> (<i>Th</i>)	20.0 c	1.60 ab	31	4.51 ab	6.13 a	3.67 a
<i>Trichoderma viede</i> (<i>Tv</i>)	13.3 c	1.57 ab	29	4.53 ab	6.11 ab	3.60 ab
<i>Trichoderma vierns</i> (<i>Tvr</i>)	6.7 c	1.64 a	34	4.52 ab	6.07 ab	3.48 bc
Cle + <i>Th</i>	33.3 bc	1.58 ab	30	4.54 ab	5.99 abc	3.39 c
Cle + <i>Tv</i>	26.7 bc	1.52 b	25	4.52 ab	6.00 abc	3.35 cd
Cle + <i>Tvr</i>	20.0 c	1.58 ab	34	4.55 a	5.82 c	3.29 cd
Cv. 2 – Sakha 3						
Untreated control	80.0 a	1.22 c	–	4.43 b	6.15 a	3.14 c
Clethodim (Cle)	60.0 ab	1.49 b	22	4.12 c	5.90 b	3.03 d
<i>Trichoderma harzianum</i> (<i>Th</i>)	26.7 c	1.57 b	29	4.54 a	6.15 a	3.60 a
<i>Trichoderma viede</i> (<i>Tv</i>)	6.7 c	1.67 a	37	4.58 a	6.13 a	3.60 a
<i>Trichoderma vierns</i> (<i>Tvr</i>)	6.7 c	1.55 b	27	4.56 a	6.17 a	3.57 a
Cle + <i>Th</i>	33.3 bc	1.52 b	25	4.58 a	6.07 ab	3.35 b
Cle + <i>Tv</i>	33.3 bc	1.51 b	24	4.57 a	6.03 ab	3.34 b
Cle + <i>Tvr</i>	13.3 c	1.52 b	25	4.58 a	6.01 ab	3.31 b

Means in each column (for each cultivar) followed by the same small letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

with *Tri* treatments, compared to 3.03 and 3.14 log₁₀ cfu · 10 g⁻¹ soil with Cle and untreated control, respectively (Table 3).

Effects on growth parameters of faba bean

In cv. Nubariya 1 the shoot length of faba bean plants increased from 38 to 58% with Cle + *Tri* treatments, while it increased from 9 to 26% with *Tri* treatments, compared to 31% with Cle. The combined treatments increased the shoot fresh weight of plants by 469–618%, compared to increases of 135–235% with *Tri* treatments and 261% with Cle, when compared with untreated control. The shoot dry weight increased by 469–620%, while it ranged from 135 to 234% with *Tri* treatments, compared to 226% with Cle. The Cle + *Tri* treatments increased the numbers of branches and leaves by 100–135% and 207–245%, while the same parameters increased by 75–90% and 93–124% with *Tri* treatments, compared to 117% with Cle, respectively. In combination treatments; Cle + *Tvr* significantly increased the shoot length, shoot fresh weight and shoot dry weight, while Cle + *Th* and

Cle + *Tv* significantly increased the numbers of branches and leaves, respectively. In single treatments, *Tvr* significantly increased the vegetative parameters, compared to *Tri* treatments (Table 4).

In cv. Sakha 3 the Cle + *Tri* treatments increased the shoot length of plants in the range of 29 to 31%, while the same parameter ranged from 6 to 20% with *Tri* treatments, compared to 22% with Cle. The shoot fresh weight increased in the range of 317 to 508% with combined treatments, compared to 114–287% with *Tri* treatments and 226% with Cle. The increase in the shoot dry weight ranged from 371 to 500% and from 112 to 285% with *Tri* treatments, compared to 250% with Cle. The Cle + *Tri* treatments increased the numbers of branches and leaves by 50–85% and 172–187%, while *Tri* treatments increased the same parameters by 40–50% and 112–126%, compared to 50 and 119% with Cle, respectively. In combination treatments, Cle + *Th* also significantly increased the shoot length and number of branches, Cle + *Tv* significantly increased the shoot fresh weight and shoot dry weight, while Cle + *Tvr* increased the number of leaves, respectively. In single treatments, *Th* significantly increased

Table 4. Effects of herbicide (Clethodim) combined with *Trichoderma* spp. on growth parameters of faba bean (cv. 1 and cv. 2) in field application

Treatments	Vegetative growth parameters									
	shoot length		shoot fresh weight		shoot dry weigh		branches		leaves	
	[cm]	increase [%]	[g]	increase [%]	[g]	increase [%]	no.	increase [%]	no.	increase [%]
Cv. 1 – Nubariya 1										
Untreated control	44.8 h	–	20.3 h	–	3.5 h	–	2.0 f	–	23.2 h	–
Clethodim (Cle)	58.7 d	31	73.2 d	261	11.4 e	226	3.8 d	90	48.4 e	109
<i>Trichoderma harzianum</i> (<i>Th</i>)	50.2 f	12	47.7 g	135	8.2 g	134	3.8 d	90	44.7 g	93
<i>Trichoderma viede</i> (<i>Tv</i>)	49.0 g	9	50.3 f	148	8.7 f	149	3.5 e	75	47.7 f	106
<i>Trichoderma vierns</i> (<i>Tvr</i>)	56.2 e	26	68.0 e	235	11.7 d	234	3.8 d	90	52.0 d	124
Cle + <i>Th</i>	62.0 c	38	130.2 b	541	22.4 b	540	4.7 a	135	71.8 b	210
Cle + <i>Tv</i>	66.7 b	49	115.5 c	469	19.9 c	469	4.3 b	115	80.0 a	245
Cle + <i>Tvr</i>	70.8 a	58	145.8 a	618	25.2 a	620	4.0 c	100	71.3 c	207
Cv. 2 – Sakha 3										
Untreated control	47.5 h	–	18.8 h	–	3.4 g	–	2.0 f	–	20.7 h	–
Clethodim (cle)	58.0 d	22	61.2 f	226	11.9 e	250	3.1 c	55	45.3 e	119
<i>Trichoderma harzianum</i> (<i>Th</i>)	56.8 e	20	72.7 d	287	13.1 d	285	3.0 cd	50	44.7 f	116
<i>Trichoderma viede</i> (<i>Tv</i>)	50.3 g	6	40.2 g	114	7.2 f	112	2.9 de	45	43.8 g	112
<i>Trichoderma vierns</i> (<i>Tvr</i>)	55.7 f	17	65.8 e	250	11.8 e	247	2.8 de	40	46.7 d	126
Cle + <i>Th</i>	66.0 a	39	78.3 c	317	16.0 c	371	3.7 a	85	56.7 b	174
Cle + <i>Tv</i>	62.3 b	31	114.3 a	508	20.4 a	500	3.5 b	75	56.3 c	172
Cle + <i>Tvr</i>	61.2 c	29	89.0 b	373	17.3 b	409	3.0 cd	50	59.5 a	187

Means in each column (for each cultivar) followed by the same small letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

the vegetative parameters, with the exception of the number of leaves (Table 4).

Effects on yield parameters

In cv. Nubariya 1 the pod fresh weight of faba bean increased from 54 to 91% with Cle + *Tri* treatments, while the weight ranged from 30 to 52% with *Tri* treatments, compared to 17% with Cle. The pod dry weight ranged between 88 and 129% for Cle + *Tri* treatments, compared to increases of 23–40% with *Tri* treatments and 11% with Cle. The seed number in pods increased by 56–64% and ranged from 11 to 40% for *Tri* treatments, compared to an increase of 9% with Cle. The Cle + *Tri* treatments increased both seed and ash pod weights by 28–48% and 57–71%, compared to 7–27% and 9–22% with *Tri* treatments respectively, and compared to 7% for seed and 4% for ash pod weights with Cle treatment. The Cle + *Tv* in combination treatment and *Tv* in single treatments highly increased the yield parameters of faba beans (Table 5).

In cv. Sakha 3 the Cle + *Tri* treatments increased the pod fresh weight by 41–63% compared to an increase of 11–23% with *Tri* treatments and 9% with

Cle. The pod dry weight increased in the range of 109 to 141% with Cle + *Tri* treatments, compared to 37–53% with *Tri* treatments and 4% with Cle. The seed number increased by 37–39%, while it ranged from 25 to 31% with *Tri* treatments, compared to 5% with Cle. Cle + *Tri* treatments increased both seed and ash pod weights and ranged from 33 to 72% and from 95 to 110%, while it increased by 14–19% and 13–45% with *Tri* treatments, compared to 4 and 8% with Cle, respectively. In combination treatments, Cle + *Tv* also highly increased yield parameters, such as, averages of pod fresh weight, pod dry weight, seed number/plant and seed weight. In single treatments, *Tv* highly increased the seed number and weight, *Tvr* increased the pod dry weight and ash pod weight, while *Th* increased the pod fresh weight (Table 5).

Discussion

Faba bean is a poor competitor to weeds, particularly during the seedling stage. Control of perennial weeds by use of clean seeds is important for successful production. A number of diseases also that infect faba

Table 5. Effects of herbicide (Clethodim) combined with *Trichoderma* spp. on yield parameters of faba bean (cv. 1 and cv. 2) in field application

Treatments	Averages of yield parameters									
	Pod fresh weight		Pod dry weight		Seed no./pod		Seed weight		Ash pod weight	
	[g]	increase [%]	[g]	increase [%]	count	increase [%]	[g]	increase [%]	[g]	increase [%]
Cv. 1 – Nubariya 1										
Untreated control	11.25 b	–	2.08 c	–	2.08 c	–	0.75 b	–	0.58 d	–
Clethodim (Cle)	13.20 b	17	2.30 c	11	2.27 ab	9	0.80 b	7	0.60 cd	4
<i>Trichoderma harzianum</i> (<i>Th</i>)	15.45 ab	37	2.56 abc	23	2.59 ab	25	0.80 b	7	0.63 bcd	9
<i>Trichoderma viede</i> (<i>Tv</i>)	14.64 ab	30	2.92 bc	40	2.91 ab	40	0.95 ab	27	0.71 b	22
<i>Trichoderma vierns</i> (<i>Tvr</i>)	17.09 ab	52	2.70 bc	30	2.31 ab	11	0.87 ab	16	0.67 bc	16
Cle + <i>Th</i>	17.36 ab	54	3.90 ab	88	3.24 a	56	1.09 a	45	0.91 a	57
Cle + <i>Tv</i>	21.45 a	91	4.76 a	129	3.41 a	64	1.11 a	48	0.99 a	71
Cle + <i>Tvr</i>	20.45 a	82	4.18 ab	101	3.38 a	63	0.96 ab	28	0.92 a	59
Cv. 2 – Sakha 3										
Untreated control	8.53 d	–	1.89 e	–	2.22 c	–	0.72 f	–	0.40 d	–
Clethodim (Cle)	9.28 cd	9	1.97 de	4	2.32 c	5	0.75 ef	4	0.43 cd	8
<i>Trichoderma harzianum</i> (<i>Th</i>)	10.48 c	23	2.59 cd	37	2.78 b	25	0.82 def	14	0.45 cd	13
<i>Trichoderma viede</i> (<i>Tv</i>)	10.37 c	22	2.78 c	47	2.91 ab	31	0.91 bcd	26	0.56 bc	40
<i>Trichoderma vierns</i> (<i>Tvr</i>)	9.49 cd	11	2.90 c	53	2.86 ab	29	0.86 cde	19	0.58 b	45
Cle + <i>Th</i>	12.06 b	41	4.37 ab	131	3.05 ab	37	0.96 bc	33	0.78 a	95
Cle + <i>Tv</i>	13.92 a	63	4.56 a	141	3.25 a	46	1.24 a	72	0.84 a	110
Cle + <i>Tvr</i>	12.61 b	48	3.95 b	109	3.08 ab	39	1.02 b	42	0.83 a	108

Means in each column (for each cultivar) followed by the same small letter are not significantly different according to Duncan's multiple range test ($p = 0.05$)

beans can reduce yield and quality. The use of herbicides, nematicides or fungicides to kill weeds and for controlling soil borne pathogens is costly and produces environmental and health hazards to men and affects beneficial microorganisms in soil. Plant pathologists tend to use alternative methods to control plant diseases. Integrating chemical and biological control agents can benefit growers not only by reducing pesticide residues on food, but also by decreasing the development of herbicide resistance. Therefore, this work involved field application of the herbicide Clethodim combined with *Trichoderma* spp. for controlling soil borne pathogens. Many researchers have studied the compatibility of pesticides with *Trichoderma* spp. *in vitro* tests (Saxena et al. 2014). *Trichoderma* spp. are considered to be effective bio-control agents against several soil-borne pathogens of many important crops and they can play an important role in integrated disease management practices.

Clethodim herbicide has a high potency against weeds *viz.* *A. viridis*, *C. dactylon* and *C. ciliaris* in two cultivars of faba beans, where the herbicide treatment led to the loss of fresh and dry weights of weeds, as compared to treatments of the herbicide combined with *Trichoderma* spp. alone. This may be due to the fact that Clethodim herbicide inhibits the synthesis of some amino acids by interfering with the acetolactate synthase enzyme (ALS), which causes a rapid lack of cell division. Clethodim does not provide control of broadleaf weeds such as *A. viridis*. However, the loss of fresh and dry weights of this weed may be due to possible intraspecific plant competition.

The lack of effectiveness of the pesticide in mixed treatments may be because *Trichoderma* spp. degrade the herbicide. These results are in agreement with those reported by Peer et al. (2013). They mentioned that integrated control of weeds by using the pendimethalin herbicide 1.5 kg · ha⁻¹ gave better soybean seed yield.

The combined treatments of Clethodim with *Trichoderma* spp. showed efficacy in controlling nematode parameters *viz.* numbers of J₂ in soil and roots, females, eggs, galls and egg-masses, compared with each treatment alone, but non-significant differences were recorded. This shows that it is possible to rely on these treatments in the control of nematodes. The efficiency of *Trichoderma* (*T. harzianum*) in reducing the incidence of the root-knot nematode *Meloidogyne javanica* in tomato was recorded by Naserinasab et al. (2011). Also, applying fungi, such as *Streptomyces avermitilis*, *Bacillus thuringiensis* and *Serratia marcescens*, by soaking faba beans or soil treatment, reduced the population density of *M. incognita* while increasing the measured plant growth (El-Nagdi and Youssef 2004).

In contrast, treatment with *Trichoderma* spp. alone was highly efficient for controlling *Rhizoctonia solani*, compared to combined treatments or herbicide

treatment only. *Trichoderma vierns* has shown high efficiency against *Rhizoctonia* root rot, either alone or in mixed treatment. These results agree with those recorded by Habtegebriel and Boydom (2016). They suggested that *Trichoderma* spp. had strong biological control activity against *F. solani* *in vitro* and *in vivo* pot experiments. *T. harzianum*, *T. hamatum*, *T. viride*, *T. polysporum* and *T. koningii* may act on the wilt disease complex of chickpea caused by *Fusarium oxysporum* f. sp. *ciceri* and *R. solani* under field conditions (Khan et al. 2014). Application of seed dressing or soil application treatment reduced the incidence of faba bean *Fusarium* root rot. Application of *T. harzianum* with seed soaking could be used for controlling soil-borne diseases *F. oxysporum* and *R. solani* in addition to improving growth and productivity of lentils (Abd El-Hai et al. 2017).

Application of herbicide combined with *Trichoderma* spp. as well as the antagonists only showed an increase in the number of *Rhizobium* nodules on roots. Such results are indirectly in accordance with those reported by Saber et al. (2009). They reported that the dual inoculation of seeds with a mixture of *R. leguminosarum* and *T. viride* enhanced nodulation, nitrogenase activity and the nitrogen fixing bacterial population in the rhizosphere and improved plant growth and yield. In contrast, our results indicated that application of herbicide with *Trichoderma* spp. or antagonistic only treatments did not affect the total numbers of spore-forming bacteria or fungi. The treatment of Clethodim herbicide only led to a decrease in the total number of these organisms. Treatment with the herbicide combined with *Trichoderma* spp. decreased the total number of aerobic bacteria compared to herbicide only. The most frequently isolated bacteria from herbicide treated soil are *Bacillus* (spore forming bacteria), while *Aspergillus* spp., *Rhizopus* sp. and *Penicillium* spp. were the most frequently isolated fungi from treated soils. The results revealed that herbicide has a significant effect on the soil microbial community (Oyeleke et al. 2011).

Our study showed that the combined treatments were clearly effective in improving the growth parameters *viz.* shoot length, shoot fresh and dry weights and numbers of branches and leaves of faba bean plants when compared to the herbicide or *Trichoderma* spp. only. The same combined treatment also significantly improved the yield parameters of faba beans *viz.* pod fresh and dry weights, seed number, seed weight and ash pod weight. These results are agreement with those obtained by Farfour and Al-Saman (2014). They suggested that application of *T. harzianum* enhanced the plant growth of faba bean and controlled root rot disease caused by *R. solani* in greenhouses.

From the above results, it can be reported that the combined treatments improved the efficiency of the root

system of the faba bean plants, through good integrated control of weeds, nematodes and pathogenic fungus. Ten *Trichoderma* spp. isolates (three of *T. harzianum*, three of *T. viride*, one of *T. virens* and three of *Trichoderma* spp.) were compatible with thiophanate-methyl, mancozeb, metalaxyl M + mancozeb, pencycuron and flutolanil, while it was incompatible with carbendazim and thiram + tolclofos-methyl. All *Trichoderma* spp. isolates also had antagonistic effects against *Fusarium solani*, *F. oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina* and *Sclerotinia sclerotiorum* in agar assays (Elshahawy *et al.* 2016). Therefore, seed treatment with *Trichoderma* with a compatible herbicide may be used as a common practice among farmers for economic and effective management of seed and soil-borne pathogens (Saxena *et al.* 2014).

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