Using Plant growth-promoting fungi (PGPF), as a biofertilizer and biocontrol agents against *Tetranychus cucurbitacearum* on Nubian watermelon (*Citrullus lanatus* L.)

ABSTRACT

Plant growth-promoting fungi (PGPF) have attracted considerable interest as biofertilizers and biocontrol due to their multiple beneficial effects on plant quantity and quality as well as their positive relationship with the ecological environment. The objective of this study was to determine the efficacy of different concentrations 25, 50, 75 and 100% from cultural filtrate of *Trichoderma viride* and *T. harzianum* to induce the two-spotted spider mite, *Tetranychus cucurbitacearum* (*In vitro*), and their ability to improve the growth dynamics of Nubian watermelon plants in field experiment during two growing summer seasons of 2017 and 2018.

In general the effect of tested concentrations of *T. viride* were non effective on egg deposition by adult females after five days from treatment, while in *T. harzianum*, the concentration 75% was the most effect than the other concentrations. Also, egg hatchability % decreased with increased of two egg age (1 – 2 day old) at treatment by concentrations 25 and 50% of both fungal. In field experiment, the test compound (vertimec) was the more effective against egg stage of spider mite T. *cucurbitacearum* than motile stages of both *T. viride* and *T. harzianum* treatments. Also, plants inoculated with *T. harzianum* showed increases in vegetative growth parameters included numbers of leaves, Leaf dry weight, stem length and numbers of branches and biochemical analysis of leaves included chlorophyll content and percentages of NPK at 30 and 60 days from sowing during both seasons compared to uninoculated control plants. Also, enzymes activities, treatment T2 (inoculated with *T. viride*) recorded the highest values at all growth stages, which recorded 155.77, 257.29 and 114.62 mg TPF g^{-1} soil day^{-1} for dehydrogenase and 113.79, 201.03 and 115.24 mg $NH4^{+}$- N g^{-1} soil d^{-1} for urease at 30, 60 and harvest during 2017 growing season, respectively. The same trend was observed in total count of fungi during both seasons.

For fruit yield, *T. viride* (T2) had significantly the highest number of fruits per plant, number of seeds per fruit, fruit weight (g) and dry weight of 100 seeds (g) which recorded 1.92, 273.07, 1126 g and 16.29 g as compared to untreated control treatment, which attained 1.21, 185.08, 526.66 g and 14.41 g at 2017 season, respectively. Therefore, these results reflected to increase fruit yield (Kg/m^2), seed yield (g/m^2) and weight of yield (ton $fed.^{-1}$) during both seasons.

Key words: PGPF; *Trichoderma viride*; *Trichoderma harzianum*; *Tetranychus cucurbitacearum*; Nubian watermelon; yield

INTRODUCTION

Nowadays, agricultural sector has been facing the destructive activities of numerous pests like insects and weeds leading to significant decrease in many agricultural crops [1]. For example, in insect pests, the two-spotted spider mite, *Tetranychus cucurbitacearum*, consider a polyphase pest which can infest the plants and pierces the epidermis and feeds on the contents of mesophyll cells that results in chlorosis and due to a decrease in total chlorophyll content and an eventual loss of photosynthetic capacity and sometimes the plants will often die [2, 3].

To cope that pest, various approaches can be used such as chemical control which it is better to use some chemicals in controlling these pests. However, it considers expensive and not environmental friendly. So, many researchers have used biological control by use of living organisms to reduce damage caused by pests and diseases [4]. Recently, the use of biological control agents such as parasites, predators, bacteria or fungi has been encouraged and found to be an efficient method to reduce the harmful effects of pest insects on many crops. Therefore, it considers an effective and do not leave any harmful effect on environment [5, 6].

During the last 20 years, a series of studies about using of fungi as a Plant Growth Promoting Fungi (PGPF) such as species belonging to the genera *Trichoderma*, *Fusarium*, *Penicillium*, and *Phoma*. PGPF are nonpathogenic saprophytes and are reported to suppress fungal, bacterial diseases and insects of a number of crop plants [7] .The PGPF association with roots of various plant species and infection has also been shown to modulate growth, morphology, nitrogen assimilation, resource allocation and mineral uptake of the host plant and also improves host reproductive fitness by enhancing plant growth, increase biomass and grain yield of crop plants i.e. rice, wheat, maize and barley [8, 9, 10, 11]. For biocontrol agent on the two-spotted spider mite and it was observed significantly reduced plant damage. Fungal biocontrol agents are important natural regulators of insect populations which have potential as mycoinsecticide agents against diverse insect pests in agriculture. These fungi infect their hosts by penetrating through the cuticle, gaining access to the hemolymph, producing toxins, and grow by utilizing nutrients present in the haemocoel to avoid insect immune responses. The using of fungi as alternative to insecticide could be very useful for insecticide resistant management [12].

Nubian Watermelon (*Citrullus lanatus*) is one of the cucurbitaceae family which cultivated from early times in Egypt [13] and Egypt is the fifth country worldwide in the production [14]. Nubian Watermelon is a favorite and popular consuming crop and it can be used as animal feeds (green parts), and the seeds are used as snacks, as well as the residues are used as a source of heat energy for cooking [15]. Also, it can be used as a source of protein supplement to ruminant animals and a new source of vegetable oil [16, 17]. [18] Studied the relationship between the colonization of *Lotus japonicus* by plant growth-promoting fungi including *T. koningi*, *Fusarium equiseti*, and *Penicillium simplicissimum* and biosynthesis of the isoflavonoid phytoalexin vestitol and they found that only *T. koningi* colonized the roots long-term, suppresses isoflavonoid phytoalexin vestitol production and increased plant dry weight reached to 126% as compared to other tested fungi. [19] evaluated the role of *Fusarium solani* strain K (FsK) in altering plant responses to the two spotted spider mite *Tetranychus urticae* in tomato plants and they found that spider mite performance was negatively affected on FsK-colonized plants and feeding damage was lower on these compared to control plants. Also, FsK-colonization led to increased plant biomass to both spider mite-infested and un-infested plants and enhance indirect tomato defense as FsK-colonized plants attracted more predators than un-colonized plants.

So, the aim of this study was to determine the efficacy of different concentrations from cultural filtrate of *T. viride* and *T. harzianum* against *T. cucurbitacearum* (*In vitro*), and their efficacy of inoculation for improving vegetative growth and yield of Nubian Watermelon.

MATERIALS AND METHODS

Laboratory study:

Organism and culture conditions

Trichoderma viride and *Trichoderma harzianum* were provided from Bacteriology Laboratory, Sakha Agricultural Research Station, Kafr El-Sheikh. The fungal strains were cultured on potato dextrose broth (PDB) for 15 days at 25°C. Then, centrifuged at 10.000 rpm for 20 min, and the culture medium was discarded. Next, the supernatant was filtered by passing the culture broth through a sterile membrane filter (0.2 µm) [20].

Mite culture:

Colonies of the two-spotted spider mite, *Tetranychus cucurbitacearum* (Acarina. *Tetranychidae*), were collected from castor bean plants at the experimental farm of Sakha Agricultural Research Station, Kafr El-Sheikh Governorate and reared under laboratory conditions (25± 2 ºc and 65±5% RH) on potato leaves away from any contamination with pesticides .

Effect of cultural filtrates of fungal strains on *T. cucurbitacearum* egg laying zero time pretreated leaf discs during 5 days

The effects of tested cultural filtrates of fungal strains (*T. viride* and *T. harzianum*) to the spider mite, *T. cucurbitacearum* were evaluated by the leaf disc dip technique according to [21]. Four concentrations (25, 50, 75 and 100%) of the tested cultural filtrates were prepared. Four discs of cast or bean leaves were dipped in each concentration for 5 seconds and left to dry. Ten adult female mites were transferred on each disc after treatment zero time. Accumulative number of eggs laid was assesses 24, 48, 72, 96 and 120 hours later. All treatments were conducted at (25± 2º c and 65± 5% RH). Each treatment was replicated four times.

Effect of some/various concentrations of cultural filtrates of fungal strains on hatchability of *T. cucurbitacearum* egg at two ages

To get homogenous with the same age, five adult females of *T. cucurbitacearum* were transferred to potato leaves discs on wet cotton pad in petri-dishes to lay eggs for 24 hours, and then removed. Each treatment involved 120 eggs at age of 1 day old and 120 eggs of 2 day old in addition to the control with the same number. All treatments were dipped in four concentrations of each cultural filtrate of fungal isolates for 5 seconds as well as in water as control. The hatchability was assessed five days after treatments and the reduction in hatchability was counted for each cultural filtrate.

Field experiment:

An experiment was carried out at Sakha Agricultural Research Station farm country name to evaluate the effect of inoculation with *T. viride* and *T. harzianum* for growth promotion of Nubian watermelon plants during summer seasons of 2017 and 2018 and their effects to control motile stages and egg stage of *T. cucurbitacearum* with compare vertimec (Abamectin 1.8 EC) as acaricide compound during summer season of 2018.

Seeds of Nubian watermelon var. Colocynthoide were obtained from Horticultural Research Institute, ARC, Egypt. Area of the experimental plot was (42 m^2 each), and sown with seeds on 25/4/2017 for first season and 22/4/2018 for second season with spacing 40 cm. The inoculation treatments were prepared as peat-based inoculums, 15 ml of culture per 30 g of sterilized carrier and mixed with the seeds before sowing using a sticking material. Four replicates were used for each treatment in a complete randomized block design and the experimental soil analyses during the two growing seasons were shown in Table 1. Plants were thinned to two plants per hill after three weeks of planting. Treatments were as follows:

- T1: Control (no inoculation).
- T2: Inoculation with *T. viride* ($1x10^7$ spores mL^{-1}).
- T3: Inoculation with *T. harzianum* ($1x10^7$ spores mL^{-1}).

Acaricide compound (Vertimec) was sprayed using a Knapsack sprayer with one nozzle at the recommended rate (40 cm $fed.^{-1}$). All cultural practices for growing Nubian watermelon plants were performed as recommended by Egyptian Ministry of Agriculture for mineral fertilizers.

Plant analysis

Samples of ten Nubian watermelon leaves were collected at random from each plot before and 3, 7 and 14 days from spraying. The samples were kept in paper bags and transferred to the laboratory for examination by stereo-microscope binocular. Motile stages and egg stage of *T. cucurbitacearum* were counted and recorded. Percentage of reduction in population was assessed according to [22].

Table 1: Some physical, chemical and biological characteristics of the experimental soil during 2017 and 2018 growing seasons.

Season	Clay %	Silt %	Sand %	Texture	pH	EC ($dS.m^{-1}$)	O.M (%)
2017	16.88	37.12	46.00	Loam	7.12	0.210	1.86
2018	16.23	38.77	45.00	Loam	7.22	0.290	1.88

Season	Cations (mq L^{-1})				Anions (mq L^{-1})				Macro-nutrient (ppm)		
	Na^+	Ca^{++}	Mg^{++}	K^+	CO_3^{--}	HCO_3^-	Cl^-	SO_4^{--}	N	P	K
2017	0.11	0.84	0.46	0.58	-	0.98	0.82	0.19	7.32	7.48	340
2018	0.12	0.85	0.46	0.60	-	0.95	0.86	0.22	8.90	7.86	370

Season	Total count of bacteria ($CFU\ g^{-1}$ dry weight)	Total count of fungi ($CFU\ g^{-1}$ dry weight)	Total count of actinomycetes ($CFU\ g^{-1}$ dry weight)
2017	$1.6X10^9$	$5.5X10^4$	$4.3X\ 10^6$
2018	$2.1X10^9$	$6.8X10^4$	$4.9X\ 10^6$

Physical, chemical and biological analyses of soil were determined by Department of Soil Chemistry and Department of Agriculture Microbiology, Soils, Water and Environment Research Institute, ARC.

Data Recorded

Growth characters:

A random sample of three plants from every experimental unit was taken after 30 and 60 days from sowing to investigate the following growth parameters: number of leaves per plant, stem length (cm), number of branches per plant and leaves dry weight per plant.

Biochemical analysis

Chlorophyll content (SPAD):

It was estimated in the fifth leaf from growing tip after 30 and 60 days from sowing by using chlorophyll meter Model-SPAD. 502 Minolta. Co., Japan [23].

Plant mineral content:

After 30 and 60 days from sowing the fifth leaf from the plant growing tip were dried at 70^oC then ground in a Willy mill. The dry material was wet digested with sulphuric acid-percholoric mixture as described by [24], as well as nitrogen, phosphorus and potassium percentages were determined according to the methods described by [25, 26, 27], respectively.

Microbial estimations:

In the rhizosphere of soil samples, total count of fungi was estimated by martin's medium according to [28] at 30 and 60 days from sowing as well as at harvest.

Enzyme activity:

At 30 and 60 days from sowing as well as at harvest, dehydrogenase and urease activities in the soil samples were determined as described by [29] and [30], respectively.

Fruit yield:

At the fruit stage, productivity was measured as the following: number of fruits per plant, number of seeds per fruit, fruit weight (g), dry weight of 100 seeds (g), fruit yield per m^2 (kg), seed yield per m^2 (g), and weight of seed yield (ton per fed.).

Statistical analysis:

All data obtained were subjected to analysis of variance and significant differences among means were determined at 5% level of significance according to [31].

Results and discussion

Effect of different concentrations of cultural filtrates of *T. viride* and *T. harzianum* on accumulative eggs deposited by adult females *T. cucurbitacearum* on zero time pretreated leaf discs during five days:

The data in Table (2) indicate the accumulative eggs deposited by adult females of *T. cucurbitacearum* and different concentrations of cultural filtrates of two fungal strains of *T. viride* and *T. harzianum* on zero time pretreated leaf discs during five days.

The data showed that all tested concentrations of fungi *T. viride* and *T. harzianum* were non-effective on egg deposition comparable with control treatment except concentrations 75 and 100 % through five days. On other hand the concentrations 75 and 100 % of cultural filtrates of fungi were the highest effect than the other concentrations from the first to third days after treatment, while other tested concentrations were gave the same effect on egg deposition by adult females of spider mite through 4th and 5th days comparable with control. In general the effect of different concentrations of cultural filtrates of two fungi indicated that the tested concentrations of fungi *T. viride* were non effective on egg deposition by adult females after five days from treatment, while in *T. harzianum*, the concentration 75% was the most effect than the other concentrations. The obtained results agree with those [32] they found the total mortality percentage caused by fungus *Cladosporium cladosporioides* (fresen) on bean (*Phaseolus vulgaris* L.) varied from 50.90 to 74.76 % and LT50 values ranged from 2.34 to 3.0 at 90 days . The results revealed that isolates of *C. cladosporioides* were effective against two spotted spider mite *Tetranychus urticae*.

Table 2. Accumulative eggs deposited by adult females *T. cucurbitacearum* of different concentrations of *T. viride* and *T. harzianum* on zero time pretreated leaf discs during five days.

Concentrations (%)	Mean No. of eggs deposited (day)				
	T. viride				
	1st	2nd	3rd	4th	5th
100	1.75 ^{b}	3.50 ^{c}	6.50 ^{b}	32.75 ^{a}	34.00 ^{a}
75	4.5 ^{a}	12.25 ^{b}	20.50 ^{a}	29.00 ^{a}	39.00 ^{a}
50	6.75 ^{a}	14.50 ^{ab}	23.00 ^{a}	28.50 ^{a}	37.50 ^{a}
25	4.75 ^{a}	9.25 ^{bc}	10.25 ^{b}	27.00 ^{a}	37.75 ^{a}
Control	6.75 ^{a}	20.00 ^{a}	29.75 ^{a}	31.00 ^{a}	43.20 ^{a}

Concentrations (%)	Mean No. of eggs deposited (day)				
	T. harzianum				
	1st	2nd	3rd	4th	5th
100	8.00 ^{ab}	18.00 ^{ab}	29.50 ^{b}	45.25 ^{a}	49.50 ^{ab}
75	9.00^{ab}	14.25 ^{b}	21.50 ^{bc}	43.50 ^{a}	41.75 ^{b}
50	12.75 ^{a}	22.00 ^{ab}	44.00 ^{a}	57.75 ^{a}	71.75 ^{a}
25	11.50 ^{ab}	27.50 ^{a}	40.50 ^{a}	51.75 ^{a}	54.33 ^{ab}

Control	6.75 [b]	26.00 [a]	19.75 [c]	51.00 [a]	53.25 [ab]

In a column means followed by a common letter are not significantly different at 1% level by DMRT.

Effect of different concentrations of cultural filtrates of *T. viride* and *T. harzianum* on hatchability of *T. cucurbitacearum* egg at two ages:

Effect of four concentrations of cultural filtrates of two fungal strains of *T. viride* and *T .harzianum* on hatchability of *T. cucurbitacearum* egg at two ages (1 –and 2 day old) is shown in Table 3. It is apparent that egg hatchability decreased with increased of two egg age at treatment by concentrations 25 and 50% of both fungal , while other tested concentrations (75 and 100%) gave the same effect where the reduction in egg hatchability was 100% at two egg age . In these results may be consider the two fungal *T. viride* and *T. harzianum* as good ovicides or biocides to control of spider mite *T. cucurbitacearum.* [33] they used different concentrations of successive extracts of brown alga (*petalonia fascia*) (Muller) against adult females and egg stage of *T. urticae* (Koch) for 7 days which they found 0.1 g / ml concentration gave 100 % mortality in all extracts, the egg stage was more susceptible to diethyl ether extract and using lower concentration of different extracts, the number of eggs laid by the females was drastically decreased especially when using diethyl either extract.

Table 3. Effect of various/different concentration of cultural filtrates of fungal strains of *T. viride* and *T. harzianum* on hatchability and reduction (%) of *T. cucurbitacearum* egg at two ages

Concentrations (%)	Egg age (day)							
	T. viride				*T. harzianum*			
	1 – day old (%)		2 – day old (%)		1 – day old (%)		2 – day old (%)	
	H.	R.	H.	R.	H.	R.	H.	R.
100	0.0	100	0.0	100	0.0	100	0.0	100
75	0.0	100	0.0	100	0.0	100	0.0	100
50	6.98	93.03	0.0	100	8.70	91.31	0.0	100
25	8.70	91.31	0.0	100	9.75	90.25	0.0	100
Control	100	0.0	100	0.0	100	0.0	100	0.0

H. = Hatchability (%); R. = Reduction (%).

Effect of vertemic on population density of the spider mite on Nubian watermelon leaves as affected by inoculation with fungal strains of *T. viride* and *T. harzianum* in the field experiment:

Intensity of infestation with the spider mite *T. cucurbitacearum* presented in Table (4) as counts through (30) days beginning from the 30 up to 60 days after sowing this period which are expected to intensity infestation with the mites .

The data indicated that the highest reduction of motile stages and egg stage was recorded after three days from application of *T. harzianum* after that all treatments recorded reduction 100 % of both motile stages and egg stage after 7 and 14 days from application. The average percent reduction ranged between (77.88 – 93.94 %) of motile stage while egg stage recorded 100% reduction of all treatments. The obtained results agree with those [34] found that the Biafly exhibited mean reduction

76.42 % in motile stages of spider mite *T. urticae* and 81.55 % mean reduction in egg stage on soybean plants in the field conditions .

Table 4. Population density of the spider mite *T. cucurbitacearum* on Nubian watermelon leaves as affected by inoculation with *T. viride* and *T. harzianum* in the field experiment during 2018 season

Treatment	Reduction at indicated days (%)								Mean reduction (%)	
	before treatment		3		7		14			
	Mite	Eggs	Mite	Eggs	Mite	Eggs	Mite	Eggs	Mite	Eggs
T. viride	44.67	37.67	33.64	100	100	100	100	100	77.88	100
T. harziamum	55.33	46.0	81.82	100	100	100	100	100	93.94	100
Control	50.67	57.0	45.33*	52.0*	25.33*	30.33*	13.67*	23.33*	28.11*	35.22*

*Mean number of *T. cucurbitacearum* motile and egg stages in control through experiment period.

Vegetative growth:

Growth parameters included number of leaves, Leaf dry weight, stem length and numbers of branches were estimated at 30 and 60 days from sowing during 2017 and 2018 seasons.

Data of Table 5 indicate that in most cases, plants inoculated with *T. viride* and *T. harzianum* had the highest values of leaves number / plant during both growth periods (30 and 60 days after sowing), compared to un-inoculated plants (control). The maximum values were obtained from plants inoculated with *T. harzianum* which recorded 27.66 and 30.33 for the first season and 206.66 and 214.00 for the second season. The differences were significant in both seasons.

Concerning leaf dry weight and stem length, data of Table 5 showed that increasing effect on the leaf dry weight and stem length during different growth periods of both seasons. The increased rate was obtained from plants inoculated with *T. harzianum* which recorded 31% at 30 days and 33% at 60 days from sowing compared to control treatment during 2017 season. The same trend was observed at 2018 season. For number of branches / plant, all inoculation treatments with studied fungi (*T. viride* and *T. harzianum*) showed significantly higher number of branches / plant during both seasons and different growth periods reached to 10.66 and 12.33 at 60 days from sowing for inoculation treatment with *T. harzianum* during 2017 and 2018 seasons, respectively.

Effectiveness of inoculation with *Trichoderma* treatments in improvement of growth were evident from the initial stages itself wherein number of leaves, Leaf dry weight, stem length and numbers of branches were improved over the control during the two growing seasons. These results suggest that *Trichoderma* strains have a strong capacity to mobilize and increase plants nutrient uptake, thus making it more efficient and competitive than many other soil microbes to promote growth and development [35]. A number of mechanisms for plant growth promotion by *Trichoderma* which lead to the increase in nutrient availability and its uptake, resulting in the efficient nutrient absorption and thereby promoting plant growth have been reported by [36]. These traits were considered as the basis for how *Trichoderma* exerted beneficial effects on plant growth and development. However, now it is becoming increasingly clear that certain strains have substantial direct influence on plant growth and development and on crop productivity with multiple mode of action [37]. Similar improvement of different vegetative growth by strains of *T. harzianum* has been reported earlier in sunflower [38], bean [39], cotton [40], tomato [41] and chilli [42] sunflower [43].

Table 5. Effect of inoculation with PGPF (explain) on number of leaves, Leaf dry weight (g), stem length (cm) and numbers of branches of Nubian watermelon plants at 30 and 60 days from sowing during 2017 and 2018 summer seasons

Treatments	No. leaves plant^{-1}		Leaf dry weight (g)		Stem length (cm)		No. branches	
	2017							
	30	**60**	**30**	**60**	**30**	**60**	**30**	**60**
T1	17.33 c	151.33 c	0.044 b	0.093 c	42.33 c	196.00 b	3.33 b	6.33 c
T2	24.00 b	167.66 b	0.054 a	0.109 b	55.00 b	231.66 a	4.33 ab	8.33 b
T3	27.66 a	206.66 a	0.058 a	0.124 a	64.00 a	216.00 b	5.33 a	10.66 a
LSD 0.05	1.48	13.10	0.004	0.006	4.66	9.32	1.15	1.63
Treatments	**No. leaves plant^{-1}**		**Leaf dry weight (g)**		**Stem length (cm)**		**No. branches**	
	2018							
	30	**60**	**30**	**60**	**30**	**60**	**30**	**60**
T1	10.33 c	153.66 c	0.047 b	0.100 c	45.33 c	201.33 c	3.66 b	6.66 c
T2	25.66 b	173.66 b	0.057 a	0.110 b	58.33 b	219.00 b	4.66 ab	8.33 b
T3	30.33 a	214.00 a	0.060 a	0.130 a	65.66 a	236.00 a	5.33 a	12.33 a
LSD 0.05	1.63	8.04	0.003	0.005	2.57	7.14	1.15	1.15

In a column means followed by a common letter are not significantly different at 5% level by DMRT. T_1: Control; T_2: inoculation with *T. viride* and T_3: inoculation with *T. harzianum*.

Biochemical analysis of leaves:

Biochemical analysis of leaves was determined as the total chlorophyll content (SPAD) and the content of nitrogen (N), phosphorus (P) and potassium (K) as percentage of leaves dry weight.

Data in Figure 1 cleared that Nubian watermelon plants inoculated with *T. harzianum* had the highest chlorophyll content followed by that inoculated with *T. viride* in both seasons, while the lowest values were obtained from non-inoculated plants (control). The application of the different fungi strains revealed that there is an increase in nitrogen, phosphorus and potassium percentage. The highest N content was found in treatments 3, where it recorded 1.75 and 2.92% followed by 1.68 and 2.88% for treatment 2 at 30 and 60 days from sowing compared to control treatment during 2017 season, respectively. The same trend was observed in 2018 season (Fig. 1). In case of P content (%), the results showed that the influence of the studied bio-inoculants on the leaves P content had a similar trend. T3 treatment gave, 0.22 and 0.23% with regard to control (T1) which exhibited 0.16 and 0.17% at 60 days of 2017 and 2018 seasons, respectively. For K content (%), the results showed an increase of K content of leaves recorded 1.39 and 2.48% for T3 (inoculated with *T. harzianum*), followed 1.25 and 2.39% for T2 (inoculated with *T. viride*), compared to 1.11 and 2.18% for control treatment (T1) at 30 and 60 days during the first growing season. The differences were significant in both seasons (Fig. 1).

These results may be refer to that inoculation treatments influences absorption and translocation of magnesium [44], which plants contain more Mg in their leaves than the non-inoculated ones and that may interpret their higher chlorophyll content. Also, the increase in the chlorophyll content attributed can be ascribed to the presence of rhizomicrobes in the rhizosphere influencing the crop roots to secrete growth promoting substances.

The increase in N% in Nubian watermelon plants at 30 and 60 days from sowing lead to the increase in nutrient availability and its uptake and these increased due to single or combined inoculation of microorganisms which has been documented on different crops such as sunflower [43] soybean [45] common bean [46] and cowpea [47]. For phosphorus, mineralization process through microorganisms as well as plants have many potential mechanisms to increase P uptake from soil including regulation of phosphate membrane transport systems, the increased growth of root hairs, the release of phosphatases, changes in root architecture and the release of organic acids which all of them due to the availability of nutrient phosphate in the soil that eventually will be uptake by plants. Similarly, fungal strains (*T. viride* and *T. harzianum*) are known to produce some organic products such as citric acid, oxalic acid that are mainly known to decompose or solubilize natural silicates and help in removal of metal ions from the rocks and soils and this process was more effective in the alkaline soils and lead to increase K uptake to plant [48, 49].

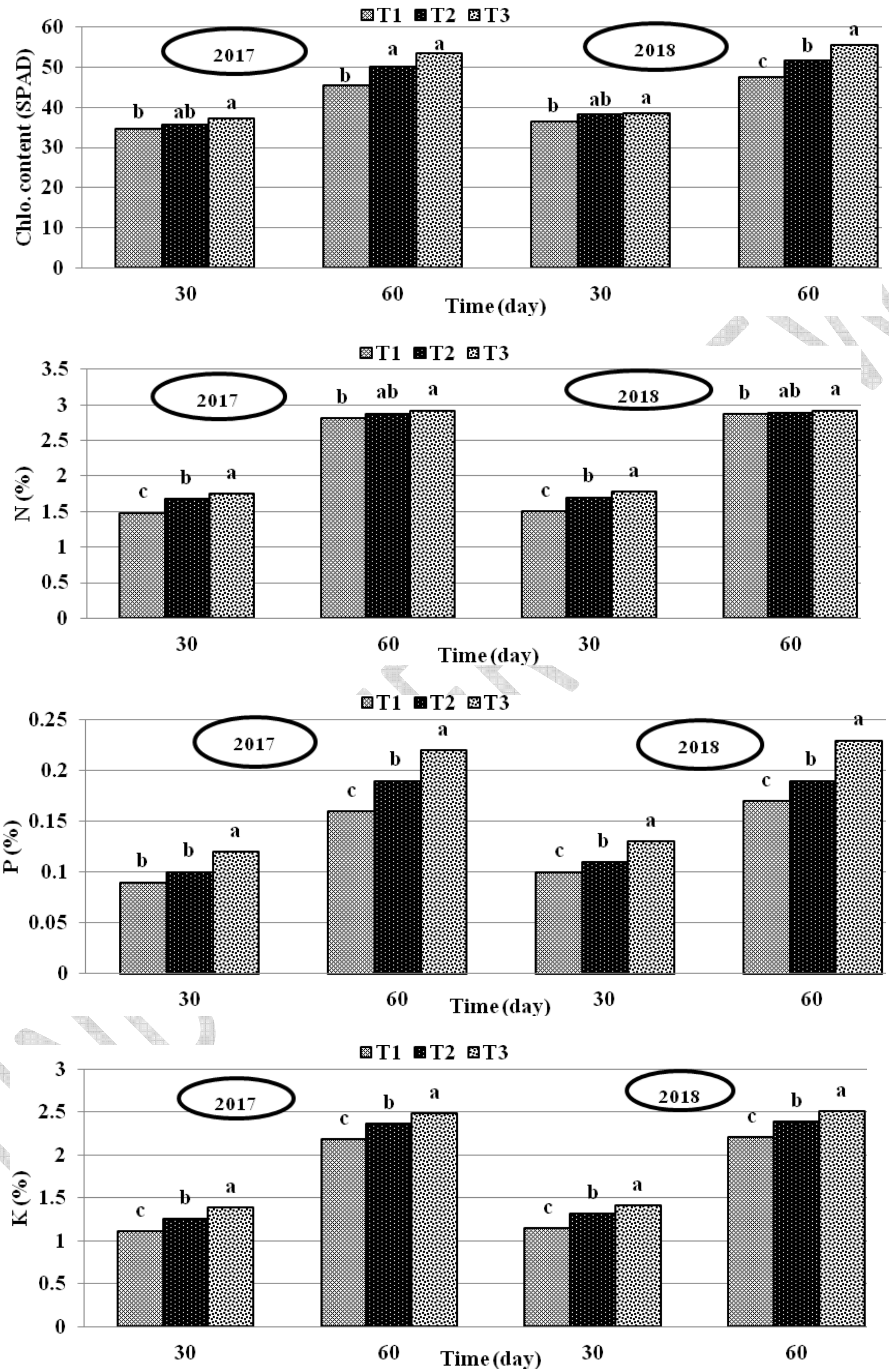

Fig. 1. Effect of inoculation with PGPF on chlorophyll content and percentages of NPK of Nubian watermelon plants at 30 and 60 days from sowing during 2017 and 2018 summer seasons

Enzyme activity:

Data of Table 6 revealed an increase in dehydrogenase and urease activities with the application of the different inoculation treatments. The dehydrogenase activity was noted to increase then decreasing in the harvest stage. In general, the treatment T2 (inoculated with *T. viride*) recorded the highest values at all growth stages, which recorded 155.77, 257.29 and 114.62 mg TPF g^{-1} soil day^{-1} at 30, 60 and harvest during 2017 growing seasons, respectively. The same trend was observed in the second growing season (2018).

On the other hand, urease activity was shown to rise in 60 days from sowing due to inoculation treatments compared to the respective control. Also, it is clearly showed that urease activity levels decreased with the increase in plant age (harvest). T2 treatment showed significant maximum urease activity (201.03 and 211.95 mg $NH4^{+}$- N g^{-1} soil d^{-1}) followed by T3 (188.59 and 201.22 mg $NH4^{+}$- N g^{-1} soil d^{-1}) compared to control (156.27 and 164.18 mg $NH4^{+}$- N g^{-1} soil d^{-1}) at 60 days from sowing during 2017 and 2018 growing seasons, respectively .

It is well known that enzymes play a key role in the transformation, recycling and availability of plant nutrients in soil. They are likely to be influenced by fertilizers and manures. [50] Showed that the increase in dehydrogenase activity was mainly due to the higher microbial population and the earlier studies revealed that the enzyme activities are often used as indices of microbial growth rather than the microbial number, which further may reflect the microbial respiration and the potential capacity of soil to perform biological transformations of importance to soil fertility. Also, the variation in the urease was little influenced by different inoculation treatments. More than the microbial population, the enzyme activities are regulated by the soil characters like organic carbon, pH and nutrient status [51] The observations are in accordance with [45, 46, 49, 52].

Table 6. Effect of inoculation with PGPF on dehydrogenase and urease activities at different growth stages of nubian watermelon plants during 2017 and 2018 summer seasons

Treatments	Dehydrogenase activity (mg TPF g^{-1} soil d^{-1})					
	2017			2018		
	30	60	harvest	30	60	harvest
T1	106.86 c	140.45 c	92.37 c	113.74 c	129.10 c	86.22 c
T2	155.77 a	257.29 a	114.62 a	159.84 a	248.46 a	121.15 a
T3	127.53 b	234.36 b	106.10 b	144.74 b	237.47 b	106.21 b
LSD 0.05	7.55	9.32	6.44	7.29	5.18	3.95
Treatments	**Urease activity (mg $NH4^{+}$- N g^{-1} soil d^{-1})**					
	2017			2018		
	30	60	harvest	30	60	harvest
T1	91.85 c	156.27 c	105.81 c	101.47 c	164.18 c	107.34 c
T2	113.79 a	201.03 a	115.24 b	122.02 a	211.95 a	137.15 a
T3	108.18 c	188.59 b	124.84 a	111.39 b	201.22 b	132.01 b
LSD 0.05	2.79	3.90	3.49	3.64	4.48	3.77

In a column means followed by a common letter are not significantly different at 5% level by DMRT. T_1: Control; T_2: inoculation with *T. viride* and T_3: inoculation with *T. harzianum*.

Fungal estimations:

Response of Nubian watermelon plants to the inoculation with *T. viride* and *T. harzianum* strains and their activities in the rhizosphere of soil samples with different times after application during 2017 and 2018 growing seasons are presented in Figure 2. The differences were significant in both seasons.

Concerning untreated control, results showed few increasing in counts of fungi with different days after treatment. However, the treated soil showed an increasing trend of log number from 30 day (6.49 and 6.26), to 60 day (7.36 and 7.09), after treatment then gradually decreases and found to be minimum on harvest (5.71 and 5.39), for inoculation with *T. viride* and *T. harzianum* treatments at 2017 season, respectively. Similar trend was also exhibited in treated soil at 2018 season.

The application of inoculation with plant growth promoting fungi *T. viride* and *T. harzianum* may be lead to significant changes in microbial populations and activities influencing microbial ecological balance affecting soil fertility. Also, the gradual increase in fungi counts may be attributed to their ability to temporarily mineralize and use soil organic carbon as energy source and this greater count of fungi in the rhizosphere at 30 and 60 days from sowing leading to greater release of plant nutrients in soil for enhancement the growth and yield of crops [53, 54, 55].

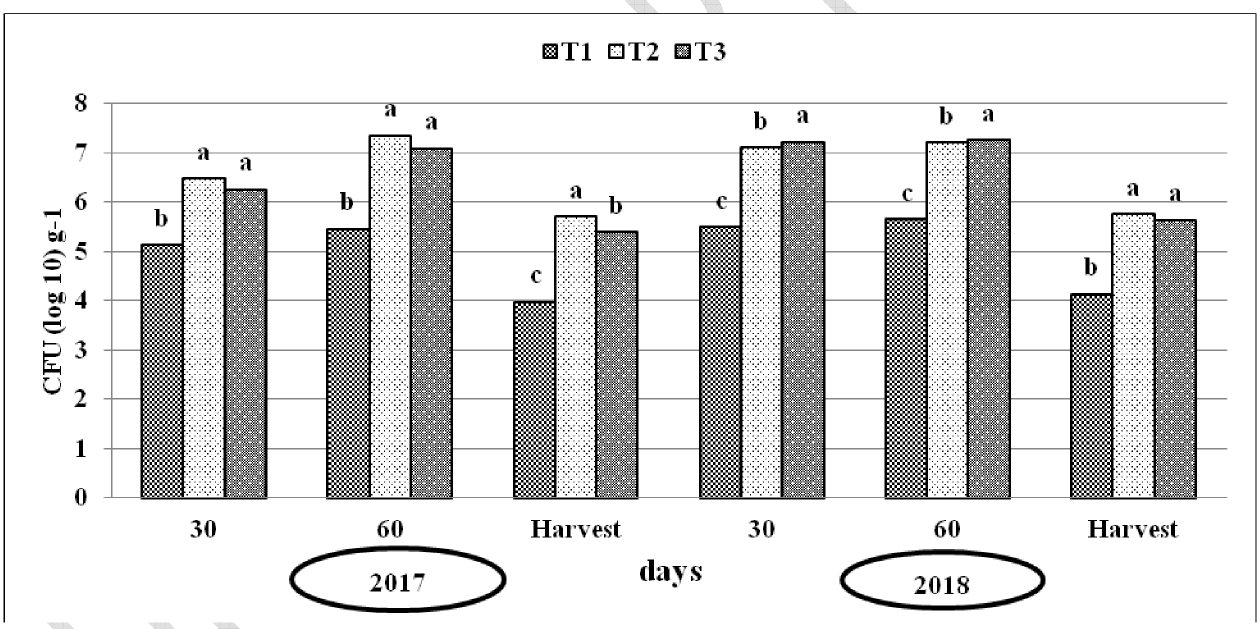

Fig. 3. Effect of inoculation with PGPF on total count of fungi (CFU log10 g^{-1}) at different growth stages of nubian watermelon plants during 2017 and 2018 summer seasons

Fruit yield:

Fruit yield was determined as number of fruits per plant, number of seeds per fruit, , fruit weight (g), dry weight of 100 seeds (g), fruit yield per m^2 (kg), seed yield per m^2 (g), and weight of seed yield (ton $fed.^{-1}$).

Data in Table 7 indicated that plants treated with *T. viride* (T2) had significantly the highest number of fruits per plant, number of seeds per fruit, fruit weight (g) and dry weight of 100 seeds (g) which recorded 1.92, 273.07, 1126 g and 16.29 g as compared to untreated control treatment, which attained 1.21, 185.08, 526.66 g and 14.41 g at the first growing season (2017), respectively. The same trend was observed in the second growing season (2018).

Data presented in Table 8 cleared that the highest fruit yield per m^2 was found in treatments 2, where it recorded 7.33 and 7.01 followed by 4.71 and 5.22 for treatment 3 compared to control treatment during 2017 and 2018 growing seasons, respectively. In case of seed yield per m^2 (g), the results showed that the influence of the studied bio-inoculants had a similar trend. T2 treatment gave, 222.00 and 226.00 g with regard to control (T1) which exhibited 107.33 and 121.66 at 2017 and 2018 seasons, respectively (Table 8). For weight of seed yield (ton $fed.^{-1}$) the results showed an increase of seed yield of Nubian watermelon plants recorded 0.93 and 0.95 ton $fed.^{-1}$ for T2 (inoculated with *T. viride*), followed 0.76 and 0.76 ton $fed.^{-1}$ for T3 (inoculated with *T. harzianum*), compared to 0.45 and 0.51 ton $fed.^{-1}$ for control treatment (T1) at the first and second growing seasons, respectively. The differences were significant in both seasons (Table 8).

It is clear from the above mentioned data that plants inoculated with PGPF had higher values for most fruit yield parameters compared to control [56, 57]. Also, the root system of Nubian watermelon plants mostly had very strong root system [58] it is often capable of absorbing water and nutrients more efficiently than non-inoculated plants and may serves as a good supplier of endogenous plant hormones [59, 60]. These results are in harmony with those obtained by several researches on cucumber [61, 62], watermelon [63, 64], Nubian watermelon [65].

Table 7. Effect of inoculation with PGPF on number of fruits / plant, number of seeds / fruit, fruit weight (g) and dry weight of 100 seeds (g) of nubian watermelon plants during 2017 and 2018 summer seasons

Treatments	number of fruits / plant	number of seeds / fruit	fruit weight (g)	dry weight of 100 seeds (g)
		2017		
T1	1.21 c	185.08 c	526.66 c	14.41 b
T2	1.92 a	273.07 a	1126 a	16.29 a
T3	1.54 b	233.00 b	939.66 b	14.29 b
LSD 0.05	0.06	8.19	55.30	0.89
Treatments		**2018**		
T1	1.24 c	193.61 c	764.66 c	13.16 b
T2	1.99 a	298.16 a	1132 a	14.11 a
T3	1.47 b	270.83 b	1009.33 c	13.06 b
LSD 0.05	0.09	13.38	33.36	0.21

In a column means followed by a common letter are not significantly different at 5% level by DMRT. T_1: Control; T_2: inoculation with *T. viride* and T_3: inoculation with *T. harzianum*.

Table 8. Effect of inoculation with PGPF on fruit yield (kg/m^2), seed yield (g/m^2), and weight of seed yield (ton fed.$^{-1}$) of nubian watermelon plants during 2017 and 2018 summer seasons

Treatments	fruit yield (kg/m^2)	seed yield (g/m^2)	Weight of seed yield (ton fed^{-1})
		2017	
T1	2.22 c	107.33 c	0.45 c
T2	7.33 a	222.00 a	0.93 a
T3	4.71 b	181.00 b	0.76 b
LSD 0.05	0.48	8.42	0.03
Treatments		**2018**	
T1	3.56 c	121.66 c	0.51 c
T2	7.01 a	226.66 a	0.95 a
T3	5.22 b	183.00 b	0.76 b
LSD 0.05	0.15	13.18	0.03

In a column means followed by a common letter are not significantly different at 5% level by DMRT. T_1: Control; T_2: inoculation with *T. viride* and T_3: inoculation with *T. harzianum*.

CONCLUSION

The results of the present investigation confirmed that application of inoculation with PGPF (*T. viride* and *T. harzianum*) at the time of planting could be recommended for controlling the two-spotted spider mite, *Tetranychus cucurbitacearum* of nubian watermelon plants/crop as well as increased the activities of most soil enzymes, especially dehydrogenase and urease and enhancement the vegetative growth and seed yield.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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