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Using of Rhizo-Microbes as Bioherbicides for Weeds

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Abstract: The paper gives an overview of the efficacy evaluation process to some weed seed viability in some randomized compost sample and the approaches that can be used to address the biological control requirements for weed suppression.The bio-herbicidal potential of rhizo-microbes isolated from the rhizosphere of *Polyogon monspeliensis, Phalaris paradox and Convolvulus arvensis*, commonly present in compost samples, were bio-assayed under both laboratory and greenhouse conditions. The pre-emergence bioassay of *Pseudomonas syringae st.1, Pseudomonas syringae st.2* and *Colletotrichum sp*. metabolites revealed that the maximum inhibition *of* weed germination, shoot and root lengths was achieved by *P. syringae st.2* for both *P. monspeliensis* and *P. paradoxa* and by *P. syringae st.1* for *C. arvensis*. Phytotoxin metabolites were collected from microbial strains, extracted by ethyl acetate and subjected to bioassay. Microbial ethyl acetate extracts of *P. syringae st. 2* and *Colletotrichum sp* showed highly positive inhibitory effects at 120 µg ml^{-1} toward the target weed seedling where the reduction effect reached, 47.46 and 51.35 % for *P. monspeliensis* and *P. paradoxa,* respectively relative to its controls. Meanwhile, *C. arvensis* was slightly affected by most treatments except for *P. syringae st. 1* at120 µg ml^{-1} which reduced seedling biomass by 22.83%. Microbial metabolites phytotoxicity was bioassayed under greenhouse condition. *P. monspeliensis, P. paradoxa* and *C. arvensis* seedlings dry weight were reduced by (40, 46.4 and 32.6 %) for *P. syringae st.2*, (25, 43.2 and 21.4%) for *P.syringae st.1* and (31, 31.2 and 16.1%) for *Colletotrichum sp*. respectively, compared to control. Biologically-based weed suppression and High-Pressure Liquid Chromatography-mass spectrometry (Thermo Finnegan) electro spray analysis indicated the probably presence of coronatine and syringopeptin derivatives in the profile *of P. syringae st.1,* ferulic acids and syringopeptin derivatives in the *P.syringae st.2* profile and two ketopiperazine compounds and colletotrichins derivatives in phytotoxic profile of *Colletotrichum sp*. The study of microbial phytotoxins introduced the greatest chance of developing new bio-herbicides.

Key words: Bioherbicide · Phytotoxin · Weeds · Microbial metabolites

INTRODUCTION

In recent years biological methods have known as effective and appropriate ways in weed control. Phytotoxic secondary metabolites from pathogenic as well as non-pathogenic microorganisms as a biological product can be a valuable component in integrated weed control programs. Using microorganisms as a bioherbicide are uniquely capable of reducing invasive weed populations through highly specific impacts that are self-sustaining, contributing to the protection of natural ecosystems [1]. The potential for bioherbicides to control weeds provides advantages over herbicides because there is a decreased chance of bioherbicide resistance developing in the target weeds due to the multiple mechanisms involved [2], limiting the use and impact of chemical plant protection products on non-target organisms and absence of residue built up in the environment. In addition to, bioherbicides may be more specific for weed species they affect [3]. One group of microorganisms largely overlooked as biocontrol agents of weeds is the Deleterious Rhizobacteria (DRB) that can colonize plant root surfaces and able to suppress plant growth [4]. Many DRB are plant specific [5]. A major group of rhizobacteria with potential for biological control is the Pseudomonades [6]. Rhizobacteri and their metabolites have been evaluated as weed control agents in non-rice systems [6-10]. Live cultures of *Pseudomonas syringae* strain 3366 have sometimes reduced weed root growth in controlled-environments [11] and in field studies [6]. Ethyl acetate extracts from *Pseudomonas syringae* strain 3366 have dramatically reduced weed root

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and shoot growth under field conditions in the Pacific Northwest [12]. Two strains of *Pseudomonas spp.* consistently reduced density, growth and seed production of downy brome but did not affect density of winter wheat. Grain yield of winter wheat was significantly increased and attributed to the growth suppressive effects of the applied bacteria on downy brome, which allowed the wheat to be more competitive [6]. *Colletotrichum* species are known to synthesize an arsenal of phytotoxins which recovered only from the culture filtrates as; Colletotrichnins by *C. nicotianae* and *C. capsici*; Colletopyrone produced by *C. nicotianae*; Aspergillomarasmins produced by *C. gloeosporioides* and Ferricrocin produced by *C.* gloeosporioides [13-15]. However there are currently relatively few biological products used for weed control in Egypt. Compost added to the soil to improve the quality, the structure and texture of the soil enabling it to better retain nutrients, moisture and air for the betterment of plants. Incomplete composting, on the other hand, can result in the survival of weed seeds and/or plant pathogens. Improperly assembled and maintained piles or windrows may not reach high enough temperatures during the active phase of composting for killing all weed seeds and pathogens [16]. Several factors contribute to weed seed mortality during composting. The most important factors are the interaction between weed species, temperature, time and moisture [17-19]. Composting can kill all weed seeds only if properly managed, that high temperatures in compost piles kill weed seeds. Weed seeds will survive in any cool spots in the compost pile. This is why compost must be turned periodically to expose weed seeds to high temperatures [18]. The problem of weed competition with crops is of great economic importance in Egypt because it causes a 10-35% reduction in yield. So, managing weeds in composted fields requires timely weed control tactics, just as it does in non-composted fields.

The objective of the study was to evaluate the efficacy of selected rhizobacterial isolates and their metabolites (phytotoxins) as bioherbicides to suppress weeds most common in compost for potential development of an effective microbe-based weed control method.

MATERIALS AND METHODS

Weed Seed Viability in Compost Samples: Weed seeds isolated from composted sample by hand and flooding techniques were subjected to weed seed viability test according to Blackshaw and Rode [20]. Briefly, all weed seeds from each of compostedandcontrolweed seed bags were placed on moistened filter paper in petri-dishes in acontrolled environment chamber (temperature 20°C, relative humidity 40-50%) and allowed to germinate.Seeds that did not germinate were subjected to a tetrazoliumtest [21] by placing them in Petri-dishes on filterpaper moistened with a solution of 1% tetrazolium. After 48h at room temperature, the seeds were examined for red stainingat the growing point, an indication of respiration and henceviability. Seeds with a positive tetrazolium test were summedwith germinable seeds to give viable seeds. Viable seeds werethen expressed as a percent of total seeds to arrive at a percentviability. In some cases, viability was also expressed as apercent of thecontrolsample (control=100% viability) toaccount for the variation in the viability of thecontrol samplesamong weed species and between the same species in the two studies.

Isolation of Bacteria and Fungi from the Rhizosphere of Selected Weeds: Standard microbiological methods was attempted for the isolation of rhizo-microbes randomly from the rhizosphere of *Polyogon monspeliensis, Phalaris paradoxa* and *Convolvulus arvensis* infected plants in Maryout Research Station, Desert Research Center (DRC), during the period of November to March, 2009-2010. One gram of each soil sample was processed using the soil serial dilution method [22]. Aliquots (0.1mL) from these dilutions were plated on both Potato Dextrose Agar (PDA) and Nutrient Agar (NA) media for isolation of bacteria and fungi, respectively. After incubation at 28°C for 48h, isolated colonies were sub-cultured onto nonselective media using morphological characteristics to distinguish different strains. Thirty non-identified isolates were selected and stored.

Bioassay of Using Live Microbes on Total Biomass of Weed Seedlings: Seed samples were collected from faba bean field after the mature stage in Maryout Research Station, Desert Research Center (DRC) and deposited with my collection at the plant protection department (DRC), Cairo, Egypt. Seeds of *Polyogon monspeliensis, Phalaris paradoxa* and *Convolvulus arvensis* (the major weeds in compost samples) were subjected to surface-sterilization by soaking in 10% sodium hypochlorite solution for 5 min and then rinsed five times in sterile distilled water. For preconditioning, groups of 200-300 sterile seeds were sprinkled onto 9 mm moistened sterilized filter paper in petri-dishes and incubated at 25°C for 7days. The seven-day seedlings were transferred to sterile tube containing 3 ml MS basal medium [23]. Culture of each bacterial or fungal isolate was grown on nutrient broth

medium for two days or potato dextrose medium for five days, respectively. The concentration was =10^8 CFU/ml of each bacterial strain and =10^6 spore/ml for fungal strain. Then, the seven-day seedlings were inoculated with oneml of each broth medium. Controls were inoculated with oneml of sterile medium. Each strain was tested in three replicates. After 5days, the seedlings were removed and biomass measured. Only one fungal and two bacterial isolates, which cause a significant reduction of weed biomass were selected and subjected for further investigations. The recovered fungal isolate was identified to the genus based on cultural and morphological characteristics using the identification keys and observations described by Peerally [24] and Dugan [25]. And, the bacterial strains that inhibited the target weed plants under laboratory conditions were characterized using the biology system (Microlog Version 3.20), which is based on the differential utilization of a large number of organic compounds [26]. The two bacterial isolates were identified as *Pseudomonas syringae st.1 a*nd *Pseudomonas syringae st.2* and the fungal isolate was identified as *Colletotrichum sp.*

Bioassay of using Microbe Metabolites as a Pre-emergence Tool on Weed Control: Two bacterial strains were grown at 25°C for about 48 h on semi-synthetic King's B (KB) medium and supernatant was collected by centrifugation at 10000 rpm for 15 minutes. Fungal strain was grown on Potato Dextrose broth medium at 30°C for five days and fungal filtrate was collected. Microbial metabolites (bacterial supernatant or fungal filtrate) were added to the surface of water agar plates at the concentration of 500, 1000 and 2000 µL5 ml^{-1} sterile distilled water. Twenty surface sterilized seeds of each weed species were then placed on each plate. Plates in all experiments were sealed with parafilm and incubated in the dark at 20°C for 15days. Controls were inoculated with the sterile media. Each isolate was tested in 3 replicates. At the end of the incubation period, the germination was recorded, the seedlings were removed, root and shoot lengths were measured.

Bioassay of Using Microbe Ethyl Acetate Extracts on Total Weeds Biomass: Phytotoxin extraction was performed as described by Gealy *et al.* [12]. Briefly, equal volumes of ethyl acetate and each bacterial supernatant or fungal filtrate were combined and mixed overnight to extract the active compounds from the aqueous phase. The organic phase was separated from the aqueous phase and evaporated to dryness, re-suspended in a small volume of acetone (1ml) and placed in a glass vial. A sterile tube containing 3 ml of MS medium and seven-days seedling from each weed types was inoculated with 30,60,90,120 µg ml^{-1} of phototoxic solutions of each bacterial or fungal strain. Controls were inoculated with 30, 60, 90,120 µg ml^{-1} of sterile medium or methanol. Each strain was tested in three replicates. After 5days, the seedlings were removed and biomass measured.

Impact of Microbe Metabolites on Plant and Weeds Growth Parameters: Pot experiment was conducted in Desert Research Center to evaluate the herbicidal potential of *Pseudomonas syringae st.1, Pseudomonas Syringae st.2* and *Colletotrichum sp.* metabolites against the target weeds. Surface sterilized seeds of *Polyogon monospeliensis, Phalaris paradoxa, Convolvulus arvensis* and faba bean *(Vicia faba)* were planted into a 100mm pot containing a pasteurized sand soil. Microbial metabolites were prepared as described for the laboratory screening. Each pot was inoculated with 10 ml of microbial metabolites of an individual strain. After emergence, only 10 seedlings of each weed type and two seedlings of faba bean will remain in each pot. Pots had been initially watered two times weekly. There were three replicates of each treatment. Weed seedlings at 4-5 leaf stage of growth were sprayed to run-off with 100% concentration of microbial metabolites. After 6weeks the plants were harvested and plant fresh and dry weights were measured.

The Identification of Microbial Phytotoxins: Phytotoxin metabolites were submitted to ESIMS (Electro Spray Ionization with Mass Detector with a Thermo Finnigan LCQ instrument and a Fision VG Autospec apparatus) for identification and characterization at National Research Center, Cairo Egypt.

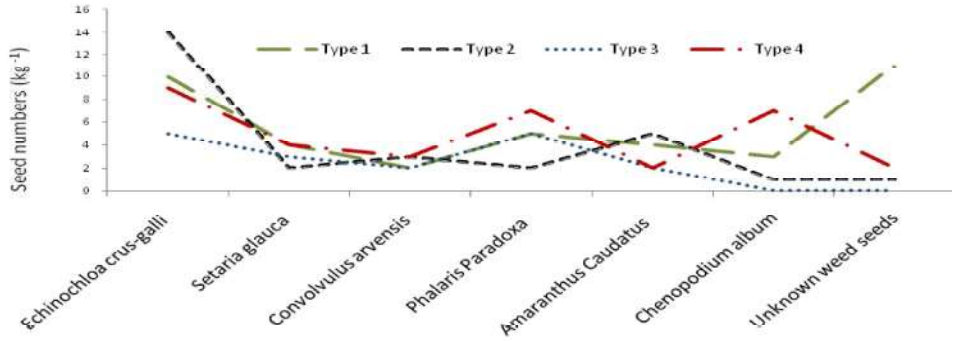
Statistical Analysis: Data were statistically analyzed by ANOVA, according to Snedecor and Cochran [27] and treatment means were compared by LSD test at 5% level of probability.

RESULTS AND DISCUSSION

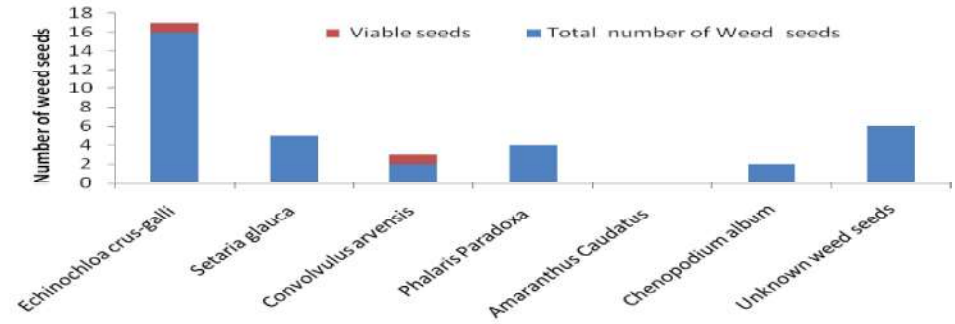
Weed Seed Viability in Compost Samples: A survey of weed seed species in six types of compost was done, whereas all seeds isolated by hand and flooding technique. After separation, the seeds were tested by germinability and tetrazolium tests. Meanwhile, the isolated weed species consisted of narrow leaves weeds, *Echinochloa crus-galli* (barnyard grass), *Setaria glauca* (foxtail), *Phalaris Paradoxa (*little seed) and three

broadleaves weed species, *Convolvulus arvensis* (bindweed), *Amaranthus Caudatus* (pigweed), *Chenopodium album* (Lambsquarters). Germination test pointed that there is no viable weed seeds in four compost types (Fig. 1A), however, the germinability and tetrazolium test revealed that the viable seeds in the other two compost samples ranged from 5.7 to 11.1% as compared to total weed count (Fig. 1B and 1C). The viable seeds were identified as barnyard grass, bindweed, little seed, pigweed and Lambsquarters.

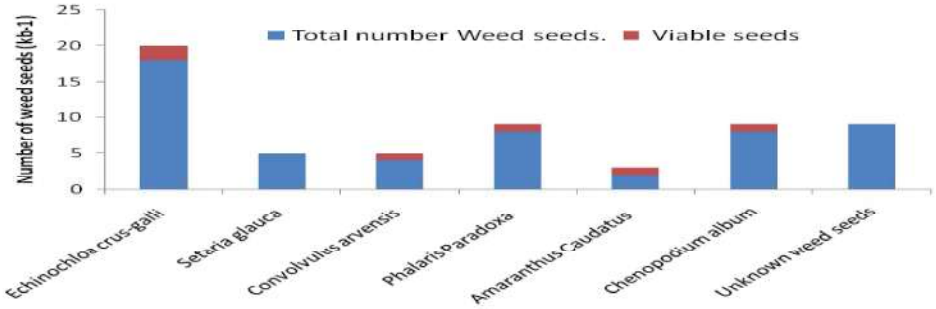
The Response of Weed Seedling Total Biomass to Microbe Application: The phytotoxicity of *P.syringae st.1, P. syringae st.2 and Colletotrichum sp*. against the target weed seedlings were tested by measuring the total biomass of weed seedlings in liquid media, which achieved a significant reduction in weed total biomass ranged from 27.45 to 36.93% (*Polyogon monspeliensis*), 34.93 to 37.33 % (*Phalaris paradoxa)* and 18.47 to 28.02% (*Convolvulus arvensis*), respectively, as compared with untreated control.



1A:Number of seeds in four type of composted sample.



1B : total number of weed seeds and some viable seed presented in Type 5.



1C : total number of weed seeds and viable seed presented in Type 6.

Fig. 1: Total and viable weed seeds count in 6 types of compost sample (Kg^{-1})

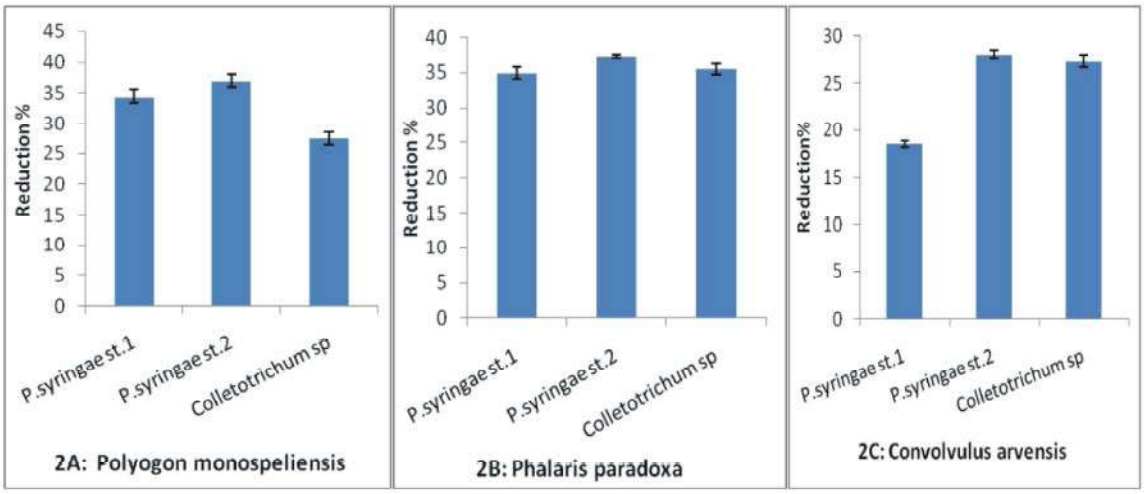


Fig. 2: Reduction percent of weed seedling total biomass as response of microbe application

Table 1: Reduction % of target weed seedling growth and germination as response to microbe's metabolites

		Polyogon monspeliensis			*Phalaris paradoxa*			*Convolvulus arvensis*		
	Conc. µL.5ml	*P.syringae*	*P.syringae*	*Colletotrichum*	*P.syringae*	*P.syringae*	*Colletotrichum*	*P.syringae*	*P.syringae*	*Colletotrichum*
Reduction %	water	*st.1*	*st.2*	*sp*	*st.1*	*st.2*	*sp*	*st.1*	*st.2*	*sp*
Shoot length (cm)	500	5.00	15.00	14.75	13.70	33.77	10.64	1.33	23.53	-12.86
	1000	16.67	30.00	16.39	14.16	53.95	21.28	13.89	24.71	8.57
	2000	30.00	35.00	16.39	34.15	82.46	43.35	33.33	38.24	10.71
LSD (0.05)		6.73			7.34			9.11		
Root length(cm)	500	66.67	0.00	73.33	11.60	40.27	16.36	12.50	44.00	-5.26
	1000	75.00	75.00	73.33	25.97	89.26	20.00	30.21	52.80	13.16
	2000	78.57	79.67	79.00	31.49	96.64	46.82	41.73	60.80	14.47
LSD (0.05)		4.56			5.23			8.95		
Germination %	500	17.86	3.85	11.54	9.09	13.04	13.64	8.70	0.00	4.55
	1000	21.43	15.38	15.38	18.18	39.13	22.73	21.74	13.64	0.00
	2000	28.57	30.77	30.77	27.27	78.26	25.00	43.48	36.36	13.64
LSD (0.05)		10.11			13.23			14.34		

It was noted that *P. syringae st.2* possessed the higher reduction activity for all weed types as compared with other pathogens, on the other hand, *Phalaris paradoxa* was more sensitive than other weed types (Fig. 2).

Bioassay of using Microbe Metabolites as a Pre-emergence Tool on Weed Control: The pre-emergence activity of *P.syringae st.1, P. Syringae st.2* and *Colletotrichum sp* crude extracts with four concentrations (0, 500, 1000 and 2000 µL) were tested to determine their inhibitory effects against the selected weed germination and seedling growth. *P.syringae st.1* metabolite with its all dilutions, significantly (p=0.05) inhibited *Polyogon monspeliensis* germination by 17.86, 21.43 and 28.57%, root length by 66.67, 75 and 78.57%, respectively, while only 1000 and 2000 µL reduced shoot length significantly by 16.67 and 30.0%, respectively as compared with its control (Table 1). For *P. syringae st.2*, both 1000 and 2000 µL significantly decreased *P. monspeliensis* (p= 0.05) germination by 15.38 and 30.77%, root length by 75.0 and 76.67% and shoot length by 30.0 and 35.0 %, respectively, than control. Finally, the reduction activity of 1000 and 2000 µL of *Colletotrichum sp.* metabolite reached (15.38 and 30.77%) for germination (73.33 and 80.0%) for root length and (16.39 and 16.39%) for shoot length, respectively as compared with control. Also, it was noted that root growth was always inhibited at lower concentrations than shoot growth or germination.

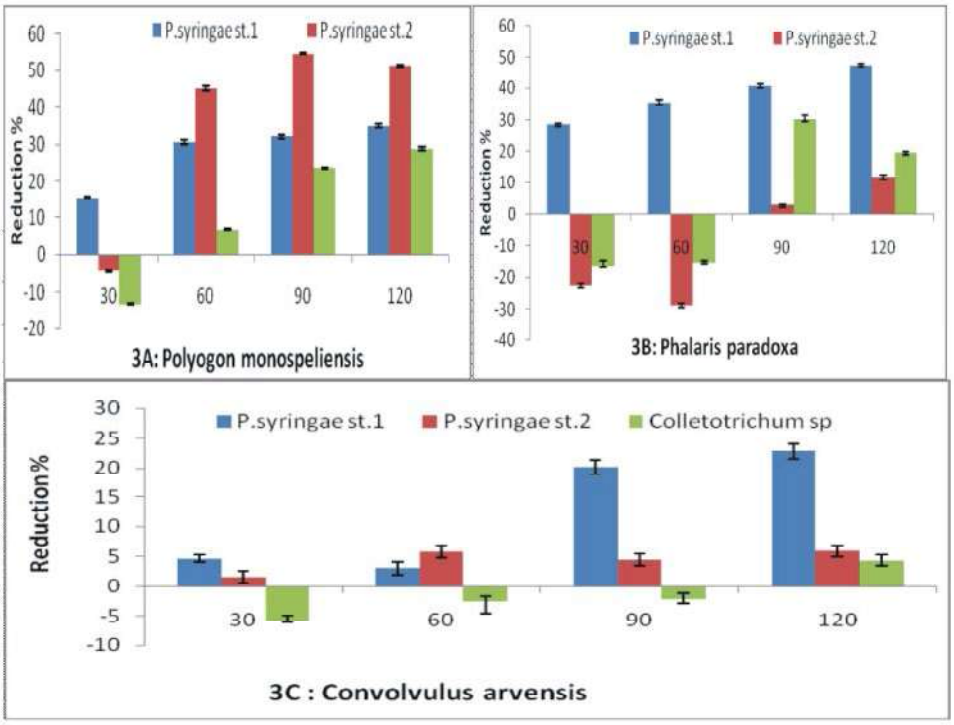


Fig 3: Response of target weed seedling biomass to microbe ethyl acetate extracts Impact of microbe metabolites on plant and weeds growth parameters.

Such results have agreement with those obtained by Gealy *et al.* [28] root growth was most sensitive in Katy, red rice and barnyard grass which was inhibited at least 67% at 40 mg L^{-1} of *P.syringae* 3366 extract.

Our bioassay showed that 2000 µL *P. syringae st.1* significantly decreased (p=0.05) *P. Paradoxa* by 27.27% (germination), 31.15% (root length) and 34.15% (shoot length) as compared with the control. For *P. syringae st.2* metabolit*e,* 1000 and 2000 µg ml^{-1} reduced *P. paradoxa* germination by 39.13 and 78.26%, root length by 89.26 and 96.64 % and shoot length by 53.95 and 82.46%, respectively than untreated control. Results showed that a significant (p=0.05) reduction achieved from *Colletotrichum sp*. metabolite with 1000 and 2000 µL by 22.73 and 25.0% (germination), 20.0 and 46.82% (root length), 21.28 and 43.35% (shoot length), respectively, than the control. It was noted in *C. arvensis* less response to metabolites, the highest reduction effect achieved from *P. syringae st.1* phytotoxin at 1000 and 2000 µL were 21.74 and 43.48% (germination), 30.21 and 41.73% (root length) and 13.89 and 33.33%(shoot length), respectively, than untreated control. The phytotoxic activity of *P. syringae st.2* metabolite at 1000 and 2000 µg ml^{-1} recorded 13.64 and 36.36% decreasing of *C. arvensis* germination, respectively. While all dilutions decreased *C. arvensis* shoot length by 23.53, 24.71 and 38.24 % and root length by 44.0, 52.8 and 60.8%, respectively, compared to untreated control. Finally, *Colletotrichum sp.* metabolites caused a significantly decreasing (p=0.05) only with the highest applied concentration (2000 µL) on *C. arvensis* germination, shoot and root length as compared with its respective control. These inhibitory effects of microbes metabolites toward target weeds are due to presence of the phytotoxic compounds. Natural herbicidal products are present in the culture broth from aerobic shake cultures of the rhizobacterium *Pseudomonas syringae* strain 3366[8]. *Colletotrichum spp* are known to synthesize an array of biologically active metabolites, phytotoxic in nature from liquid culture filtrates [29]. Also, the observed result did not have any significant effect on faba bean growth and germination (p=0.05) regardless of the type of the tested metabolites.

Phytotoxic Activity of Microbe Ethyl Acetate Extracts Against Weeds Seedling Development: The activities of microbial extracts were bioassayed against weed seedlings with four concentrations of 30, 60, 90 and 120 µg ml^{-1}. The applied concentrations achieved a significant reduction (p=0.05) of *P. monspeliensis* total biomass regardless of microbe types.

Inoculation with 90 µg ml^{-1} of *P.syringae st.2* extract caused a highest growth reduction reaching 54.81 %, followed by *P. syringae st.1* which caused 32.22 and 35.14% reduction at 90 and 120 µg ml^{-1}, respectively, relative to control. On the other hands, *Colletotrichum sp*. extract with 90 and 120 µg ml^{-1} concentrations possessed a slight inhibitory effect on *P. monspeliensis* total biomass and caused 23.70 and 28.83% reduction, respectively. For *P. paradoxa*, based on the extracts types, *P. syringaee st.1* extract with all dilutions possessed significant reduction (p=0.05) ranged from 28.42 to 47.46 % compared to control. Both *P. syringae st.2* and *Colletotrichum sp.* extracts have slight reduction activity against *P. Paradoxa* and only 120 µg ml^{-1} concentration recorded 11.86 and 19.49% reduction respectively, than the control. Furthermore, *C. arvensis* was slightly affected by most treatments. Only 90 and 120 µg ml^{-1} of *P. syringae st.1* ethyl acetate extract had a significant inhibition activity and decreased *C. arvensis* total biomass by 20.13 and 22.83% respectively, relative to control as shown in (Fig. 3). Chemical analysis of ethyl acetate extract of *P.syringae* 3366 revealed the presence of active phytotoxic compounds used in weed control [12].Yoshida *et al.* [30] isolated toxic compounds from the cell filtrate of *Colletotrichum dematium* by fractionating it with an equivalent volume of n-hexane and ethyl acetate. Jaya *et al.* [31] indicated the presence of the phytotoxic compounds in ethyl acetate extracted fraction of the cell free culture filtrate of *C. dematium* FGCC#20 as novel and lucrative source of potential herbicides for the management of weeds.

Phytotoxic Activity of Microbial Metabolites Against Weed Seedlings and Faba Bean under Greenhouse Condition: Greenhouse studies (Fig. 4) revealed that metabolites of selected strains showed significant reduction in both fresh and dry weights of weed seedlings compared to non sprayed controls. Crude metabolite of *P. syringae st.2* reduced *P. monspeliensis, P. paradoxa and C. arvensis* dry weights by 40, 46.4 and 32.6 %, respectively relative to controls and achieved the highest reduction activity than other treatments. Followed by *P.syringae st.1* at the same concentration which decreased *P. monspeliensis,* P. *paradoxa* and *C. arvensis* total biomass by 25, 43.2 and 21.4%, respectively. Finally, *Colletotrichum sp.* achieved a slight significant reduction (p=0.05) for *P. monspeliensis, P. paradoxa and C. arvensis* dry weights reaching 31, 31.2 and 16.1% respectively, than untreated control. Therefore, deleterious rhizobacteria (DRB) do not necessarily eradicate the problem weeds, but significantly suppress early growth of weeds and allow the development of crop plants to effectively compete with weakened weed seedlings [32]. Rhizobacteria are reported to reduce plant growth without obvious plant cell damage, an effect attributed to rhizobacterially produced metabolites being absorbed by roots [33, 34]. The results mentioned that, there was no reduction effect observed in the faba bean fresh and dry weights as compared with untreated control, this is compatible with that crude ethyl acetate extracts of *P.syringae* Strain 3366 in soil inhibited downy brome at different concentrations had little effect on winter wheat[10], *Colletotrichum spp*. are relatively ubiquitous in distribution and strains can be highly host-specific [35],and the hemibiotrophic infection makes this fungi a uniquely specific group of candidates for bioherbicides against annual weeds in annual crops [35, 36].

Identification of Phytotoxic Compounds in Microbe Ethyl Acetate Extracts: Biologically-based weed suppression indicated that there are many phytotoxin compounds in *Pseudomonas spp.* and *Colletotrichum sp*. profiles. The most promising phytotoxic metabolites in microbial extracts were identified by using High-Pressure Liquid Chromatography-mass spectrometry (Thermo Finnegan) electrospray analysis. For *Pseudomonas syringae st.1*, the first identified compound with molecular weight 319.58 deduced from m/z 320.58[M+1] might be coronatine which have the molecular formula $C_{18}H_{25}NO_4$. The second phytotoxic compound corresponding to molecular weight 1225.0 deduced as m/z 1226.0[M+1] might be syringopeptin derivatives. Coronatine, syringomycin, syringopeptin, tabtoxin and phaseolotoxin are the most intensively studied phytotoxins of *Pseudomonas syringae* and each contributes significantly to bacterial virulence in plants [37]. *Pseudomonas syringae st.2* phytotoxic profile presented many poly phenols metabolites such as catechol, 1-carboxy-cis, cis-muconic acids ($C_7H_6O_6$) and protocatechuate while first phytotoxic compound according to the review might be ferulic acids with molecular formula $C_{10}H_{10}O_4$ which established by EIMS, corresponding to peaks at m/z 195.2 [M + H], that have molecular weight of 194.04. The second phytotoxic compound corresponding to molecular weight 1225.0 deduced as m/z 1226.0[M+1] might be syringopeptin derivatives. *Colletotrichum sp*. phytotoxin profile was analyzed by nano spray-mass spectrometry using an LTQ ion trap instrument (Thermo Finnegan). Two ketopiperazine compounds might be present in the phytotoxic profile of *Colletotrichum,* the first compound

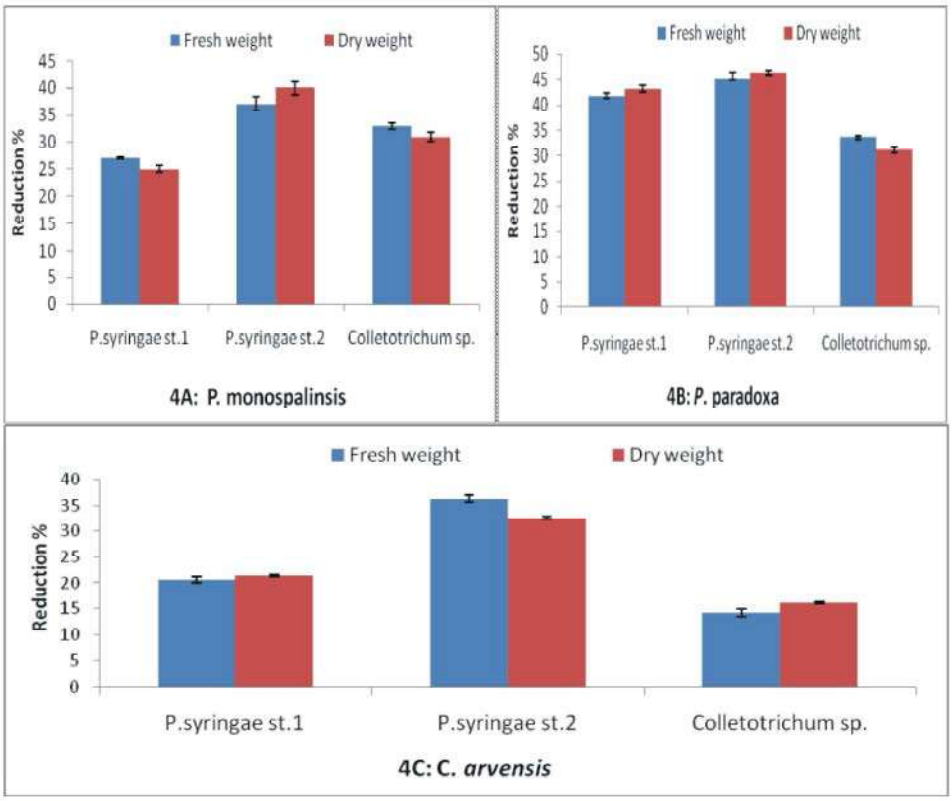


Fig. 4: Reduction % of target weeds seedling fresh and dry weights as response to microbes metabolites

corresponding to m/z 197.0 [M + H] have molecular weight of 196.04 and molecular formula $C_{14}H_{16}N_2O_2$. The second compound bears the molecular formula $C_{14}H_{16}N_2O_3$ and molecular weight of 259 deduced as m/z 260 [M+1] by the quasimolecular ions at m/z 282.7 [M + Na]. The last phytotoxic compound corresponding to m/z 505 [M+2] might be colletotrichins derivatives with molecular weight calculated by 502.11. *Colletotrichum* species produce several toxins including colletotrichins produced by *C. nicotianae* [14] and *C. capsici* [38, 39].

Generally, using microbe metabolites against weed seeds and seedlings were more efficiency than live microbes regardless of microbe types. So, microbial metabolites have become the focus of attention of researchers searching for natural product alternatives to conventional herbicides biologically-based weed tests revealed that *Pseudomonas spp.* metabolites were more phytotoxic than *Colletotrichum sp*. metabolites. According *to* weed sensitivity, *Phalaris paradoxa* was more sensitive to microbes and their phytotoxins than other weed types presented in the compost. Active metabolites (phytotoxins) isolated and identified from *Pseudomonas syringae st.1* ethyl acetate extract as coronatine and syringopeptin were more efficiency than other *Pseudomonas syringae st.2* and *Colletotrichum sp.* metabolites against weed seed growth and germination[40]. Therefore, further phytochemical studies are required to identify a wide range of secondary metabolites found in microbe phytotoxic profile. It has been suggested that remarkable phytotoxicity of microbes metabolites in depressing weed seedling growth under laboratory condition was more than that under greenhouse condition. In addition, the selected microbes metabolites did not show any harmful effects on faba bean growth in both laboratory and greenhouse condition at the selected concentration and this may allow the application of these toxins in faba bean weed management in the future. However the further studies about their application in natural field condition seem to be necessary.

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