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Research Article

Effect of Glyphosate and Mancozeb on the Rhizobia Isolated from Nodules of *Vicia faba* L. and on Their N_2-Fixation, North Showa, Amhara Regional State, Ethiopia

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This study was designed to assess the effect of glyphosate and mancozeb on growth of *Vicia faba* rhizobia isolates in vitro and on their N_2-fixation performance. Hence, ten isolates were isolated using plant-soil trap method from soil samples collected from farm lands. Those isolates were morphologically characterized using YEMA medium and authenticated as nodulating rhizobia using sand culture. These isolates were treated with 100, 150, and 200 μg a.e. L^{-1} glyphosate, 100, 150, and 200 mg L^{-1} mancozeb, and their combinations. The result showed that almost all isolates were affected (only 4–10% survival) at lower (100 mg L^{-1}) concentration of mancozeb. However, 80% of isolates treated with higher concentration (200 μg a.e. L^{-1}) of glyphosate for 72 h formed colonies on YEMA medium. Moderate (40%) isolates also showed better (31–50% and 17–45%) survival within 100 : 100 and 150 : 150 combinations of glyphosate and mancozeb, respectively. For in vivo experiment, faba bean seedlings in sand culture were inoculated with four relatively in vitro test resistant and one sensitive isolates. The inoculated isolates were treated with field recommended concentration of glyphosate, mancozeb, and combinations. Thus, experimental plants almost all showed normal (61–124 nodule $plant^{-1}$) nodulation and N_2-fixation (90–109%) performance as compared to the control.

1. Introduction

The Ethiopian agriculture is mainly characterized by small-holding low-input traditional production system that feeds up on more than 85% of the population in the country [1]. The system includes crop rotation, mixed cropping, and agroforestry components with little or no agricultural inputs. Leguminous plants are important components of such systems for improving the soil fertility of the agroecosystem through biological nitrogen fixation.

Faba bean (*Vicia faba* L.) is one of the cool season leguminous crops which is widely grown in Ethiopia. It covers 370,000 hectares with an annual production of 450,000 tones [2]. Faba bean contains high protein (24%) and appreciable amount of minerals and vitamins, and thus it is part of the various popular Ethiopian dishes [3]. In addition, faba bean is one of the most effective nitrogen fixing plants by forming symbiotic association with *Rhizobium leguminosarum* bv. *viceae* and increases the nitrogen content in the soil [4]. Because of this, it is commonly integrated within crop rotation of traditional mixed low-input agricultural system in the highlands of the country.

Currently, to manage soil runoff in the highland part of the ecosystem, there is an interest in no-tillage agriculture in the country. This is very important initiative to minimize erosion and avoid soil organism disturbance through tillage. Under this circumstance, there is a tendency to use herbicides and fungicides to enhance crop production even under low-input agricultural set-up. For this situation, farmers might be

initiated to use the economical and broad spectrum type of agrichemicals.

Glyphosate (N-phosphonomethyl glycine) is one of the nonselective, broad spectrum type herbicides which is mostly applicable on no-tillage agricultural practice for cleaning up of the weeds from the farm land before sowing [5]. Likewise, mancozeb is also another commonly used fungicide to inhibit fungal pathogens and farmers apply it for pathogen control through spraying over plant vegetative part after seedling or by dressing seeds using recommended concentration before sowing [6].

Normally, glyphosate causes crop injury when it was applied directly on the broad leaved crops like *Vicia faba*. This is because of its inhibition effect on an enzyme 5-enolpyruvyl-shikimate-3-phosphate synthase (EPSPS) that is involved in shikimate pathway [7]. Such EPSPS inhibition disrupts the shikimate pathway and leads to general metabolic disruption and several associated metabolic disturbances, including the arrest of protein production and prevention of subsequent product formation like hormones, vitamins, lignins, alkaloids, and phenolics in plants and microorganism [8]. The shikimate pathway is also found in fungi and bacteria so that herbicides and fungicides may implicitly have a tremendous damage on nontarget soil microflora [9].

Furthermore, herbicides and fungicide adversely affect biological N_2-fixation including the host, the endosymbiont rhizobia, and the fixation process. Moorman [10] showed that herbicides directly affect the rhizobial symbiont and exert indirect effects on the physiology of the host plant. There are also reports concerning different fungicides such as captan, thiram, mancozeb, and darosal; those inhibit the number of rhizobial strains, even though the effect varies from species to species and chemical concentrations [11–13]. In addition, Mubeen et al. [12] also reported that the use of fungicides causes tremendous damage on legumes when they were used in conjunction with microbial inoculants with negative effect on root infection, nodule formation, and bacterial growth hormone production.

Thus, knowledge on the effect of herbicides and fungicides on rhizobia and symbiotic N_2-fixation has paramount importance in selecting resistant endosymbiont as microbial inocula for low-input, no-tillage agricultural application with the presence of toxic agrochemicals. Therefore, this study was targeted to assess the effects of glyphosate and mancozeb on the growth of indigenous faba bean (*Vicia faba* L.) nodulating rhizobial isolates in the laboratory and their N_2-fixation performance under greenhouse condition.

2. Materials and Methods

2.1. Sample Collection. Soil samples were collected from ten farm fields growing faba bean near the town of Checki, North Showa, Amhara Regional State, Ethiopia. From each study site, faba bean plants were selected and soil from 20 to 30 cm depth of the root was pooled and collected in alcohol sterilized (70%) plastic bags in September 2011. The soil samples were taken to Microbiology Laboratory, Departments of Microbial, Cellular and Molecular Biology, Addis Ababa University (AAU), for further work.

2.2. Isolation of Rhizobium from Nodules. Rhizobia were entrapped from collected soil samples by plant-soil trap method in pots [14] using Wolki variety of faba bean under greenhouse. After 45 days of plantation, the plants were uprooted and yellowish nodules were selectively picked, surface-sterilized, and crushed to release the rhizobia and 1 mL of suspensions was inoculated on the Yeast Extract Mannitol Agar (YEMA) medium (DIFCO) and incubated at 28°C for 5 to 7 days [15]. Those isolates were subsequently retransferred onto YEMA for purification. Then, purified isolates were preserved in YEMA slants, containing 0.3% (w/v) $CaCO_3$ and stored at 4°C of refrigerator [14]. For every experiment, isolates were routinely cultured at room temperature on rotary shaker operating at 120 rpm (revolution per minute) to standardize the inoculums (size of 10^8 cells mL^{-1}) for 72 h, unless stated otherwise.

2.3. Characterization of Isolates. The isolates were characterized on the basis of their morphological, cultural, and physiological characteristics [16] and authenticated as root nodule bacteria upon inoculating them into faba bean seedlings growing on sterilized sand pot culture irrigated with N_2-free medium under greenhouse [17]. The purified and authenticated isolates were then designated as AAUW with numbers (Table 1).

2.4. Effect of Glyphosate on Rhizobial Isolates. Liquid glyphosate (MONSANTO) was taken in full strength concentration according to the specification of the company and filtrated by using 0.45 μm pore size filter paper [12]. Filtered 100, 150, and 200 μg a.e. L^{-1} of glyphosate were separately mixed with sterilized 100 mL of YEM broth using 250 mL Erlenmeyer flasks. Then efficient 1 mL (10^8 cells mL^{-1}) of each isolate was inoculated into broth medium prepared with different concentrations of glyphosate and incubated on the shaker at room temperature for 72 h.

2.5. Effect of Mancozeb on Rhizobial Isolates. The stock solutions of mancozeb (Limin Chemical Co. Ltd.) were prepared by adding 10 g of mancozeb powder into 1000 mL of distilled water and filtrated by using 0.45 μm pore size filter paper [12]. Then 100, 150, and 200 mg L^{-1} of filtered mancozeb were separately added to sterilized 100 mL of YEM broth in 250 mL Erlenmeyer flasks. Then activated 1 mL (10^8 cells mL^{-1}) of each isolate was added to each concentration and incubated on shaker at room temperature for 72 h.

2.6. Effect of (Glyphosate + Mancozeb) on Rhizobial Isolates. In order to see the combination effect of both agrochemicals on isolates, three treatments, such as Treatment-1 (100 μg a.e. L^{-1} + 100 mg L^{-1}), Treatment-2 (150 μg a.e. L^{-1} + 150 mg L^{-1}), and Treatment-3 (200 μg a.e. L^{-1} + 200 mg L^{-1}), were prepared in 100 mL YEM broth using 250 mL Erlenmeyer flasks. The sizes of inoculum and incubation method were the same as the above experiments.

Growth of isolates in different treatments was monitored through optical density measurement by using UV-7804C

Table 1: Resistance evaluation of rhizobial isolates towards various concentrations of glyphosate.

Isolates	Control	Inhibition effect of different concentrations of glyphosate (μg a.e. L^{-1}) on rhizobial isolates against control (OD 620 nm)					
		100 μg a.e. L^{-1}	%S	150 μg a.e. L^{-1}	%S	200 μg a.e. L^{-1}	%S
AAUW-1	0.417^{c}	0.091^{a}	**22**	0.082^{ab}	**20**	0.043^{c}	10
AAUW-2	0.377^{fe}	0.051^{c}	14	0.044^{d}	12	0.032^{d}	8
AAUW-3	0.457^{b}	0.062^{b}	14	0.058^{c}	13	0.044^{bcd}	10
AAUW-4	0.456^{b}	0.057^{b}	12	0.041^{d}	9	0.026^{d}	6
AAUW-5	0.343^{f}	0.064^{b}	**19**	0.057^{c}	**17**	0.054^{bc}	16
AAUW-6	0.467^{a}	0.094^{a}	**20**	0.090^{a}	**19**	0.085^{a}	8
AAUW-7	0.385^{d}	0.071^{b}	18	0.068^{b}	7	0.038^{d}	10
AAUW-8	0.411^{c}	0.017^{d}	4	0.012^{f}	2	0.008^{e}	2
AAUW-9	0.373^{f}	0.045^{c}	12	0.032^{e}	3	0.030^{d}	8
AAUW-10	0.414^{c}	0.092^{a}	**22**	0.086^{a}	**21**	0.077^{a}	19

Numbers in the same column followed by different letters are significantly different at $p = 0.05$ confidence level (Tukey's HSD). Letters in the columns (a, b, c, d, e, f, g, and h) are ranks of the mean; %S = percentage of survival; AAUW = Addis Ababa University Wolki variety.

spectrophotometer at 620 nm and compared with isolates grown on agrochemical-free control.

$$\%\ I = \frac{\text{OD of control} - \text{OD of treated}}{\text{OD of control}} \times 100, \quad (1)$$

where OD is optical density and % I is percentage of inhibition.

2.7. Cell Viability Test. The viability of the isolates in 100 and 200 μg a.e. L^{-1} glyphosate, 100 and 200 mgL^{-1} mancozeb, and their combinations is determined by culturing serially diluted suspensions of 72 h old culture onto YEMA plates. The 100 μL dilution (10^{-5} and 10^{-6}) from each treatment was transferred into the YEMA medium in duplicate, using Miles and Misra drop plate method [15], and incubated at 28°C for 48 h for plate counting.

2.8. Pot Experiments and Agrochemical Application. The pot experiments were conducted in greenhouse, College of Natural and Computational Sciences, AAU. For this purpose, thoroughly washed and autoclaved river sand was used and alcohol sterilized (70%); plastic pots were filled with 3 Kg of sterilized sand. The moisture content was adjusted to 75% through watering.

2.8.1. Treatment with Glyphosate. In the case of greenhouse application, 0.2 mL/m^2 or 2×0.036 g a.e. mL^{-1}/m^2 (0.072 g a.e. mL^{-1}/m^2) of glyphosate was diluted in 40 mL of water per square meter (sqm) [18]. Pots containing the sand were arranged in 1 sqm outside the greenhouse and diluted glyphosates were sprayed on the surface of sand filed within pots by using Tobor N-1 sprayer nuzzle and returned back to greenhouse and water was supplied to maintain the moisture at normal level.

Five sterilized seeds per pot were sown after 2 days of spraying to minimize direct contact of seeds with glyphosate. Then four (AAUW-3, AAUW-5, AAUW-6, and AAUW-10) relatively in vitro test resistant and one (AAUW-8) sensitive isolates were selected. Those isolates were activated and adjusted to inoculum size of 10^8 cells mL^{-1} and inoculated into each seedling after germination and thinned down into three.

The experimental control contains *Rhizobium* inoculated seedling but free from agrochemicals. Then all plants were fertilized with 100 mL pot^{-1} of N_2-free nutrients once a week [15] and supplied with 1000 mL pot^{-1} tap water every two days.

2.8.2. Treatment with Mancozeb. Faba been seedlings were inoculated with 1 mL (10^8 cells mL^{-1} $plant^{-1}$) of five selected isolates as previous experiment. Then, after 25 days of growth, rhizobium inoculated faba bean plants were sprayed with field recommended concentration (0.15 g mancozeb diluted with 75 mL of distilled water per sqm) of mancozeb using Tobor N-1 nuzzle.

2.8.3. Treatments with Glyphosate + Mancozeb. The combination treatment, that is, glyphosate treatments, is done at the beginning and 0.15 g of mancozeb sqm^{-1} was sprayed after 25 days of planting by taking pots out from the greenhouse.

Then all experimental pots with two replicates were arranged as a complete randomized block design and a photoperiod was 12/12 h with an average temperature of 25/18°C day per night. After 45 days of their planting, the whole plants were uprooted in order to determine nodule number, nodule dry weight, and shoot dry weight. N_2-fixation performances (fixation effectiveness) of the plants were checked by using the following formula:

$$\%\ \text{NFE} = \frac{\text{SDW of treated}}{\text{SDW of control}} \times 100, \quad (2)$$

where NFE is N_2-fixation effectiveness and SDW is shoot dry weight.

2.9. Data Analysis. Data analyses were made by one way of variance (ANOVA) using version 20 SPSS statistical program.

Table 2: Effect of mancozeb on the growth of rhizobial isolates.

Isolates	Control	Inhibition effect of different concentrations of mancozeb (mg L^{-1}) on rhizobial isolates against control (OD 620 nm)					
		100 mg L^{-1}	%S	150 mg L^{-1}	%S	200 mg L^{-1}	%S
AAUW-1	0.417^{c}	0.032^{d}	8	0.013^{dc}	3	0.011^{e}	3
AAUW-2	0.377^{f}	0.036^{ab}	9	0.028^{ab}	7	0.022^{b}	6
AAUW-3	0.457^{b}	0.041^{a}	9	0.031^{a}	7	0.026^{a}	6
AAUW-4	0.456^{b}	0.027^{ef}	6	0.015^{c}	3	0.012^{ef}	3
AAUW-5	0.343^{h}	0.032^{e}	9	0.019^{bc}	5	0.014^{de}	4
AAUW-6	0.467^{a}	0.023^{f}	5	0.023^{b}	5	0.020^{bc}	4
AAUW-7	0.385^{d}	0.030^{e}	8	0.021^{bc}	5	0.018^{c}	5
AAUW-8	0.411^{e}	0.018^{g}	4	0.012^{cd}	3	0.010^{f}	2
AAUW-9	0.373^{g}	0.036^{bcd}	10	0.023^{bc}	6	0.019^{bc}	5
AAUW-10	0.414^{c}	0.036^{b}	9	0.026^{bc}	6	0.022^{b}	5

Numbers in the same column followed by different letters are significantly different at $p = 0.05$ confidence level (Tukey's HSD). Letters in the columns (a, b, c, d, e, f, g, and h) are ranks of the mean; %S = percentage of survival; AAUW = Addis Ababa University Wolki variety.

Mean separation was calculated using Tukey's HSD test when the value was significant at $p = 0.05$.

3. Result and Discussion

All ten isolates were Gram's negative, rod shaped bacteria, similar in colony appearance with circular, transparent, raised, large mucoid, and fast growing nature with diameters between 2.5 and 4.8 mm after 3–5 days of growth on YEMA media (data not shown). They also changed BTB-YEMA medium into yellow to confirm isolates, and they were authenticated as nodulating rhizobia by inoculating them into faba bean seedlings grown within sand culture under greenhouse condition [16].

3.1. Effect of Agrochemicals on Rhizobial Isolates. All isolates were grown on YEM broth containing different concentrations of glyphosate and showed progressive reduction of $OD_{(620\,nm)}$ as compared to control through spectrophotometer. Isolates AAUW-1, AAUW-5, AAUW-6, and AAUW-10 showed relative resistance with 19–22% survival rate at 100 μg a.e. L^{-1} and 150 μg a.e. L^{-1} glyphosate concentrations as compared to the other isolates (Table 1).

Isolates AAUW-5 and AAUW-10 were maintained at 16% and 19% survival at 200 μg a.e. L^{-1} of glyphosate, respectively (Table 1). Other reports showed up to 87.5% reduction of *Bradyrhizobium* population at lower concentration of 43.2 μg a.e. L^{-1} [19] and 67% reduction at 450 μg glyphosate $disk^{-1}$ [13] indicating that the local isolates were relatively resistant to the agrochemicals. However, isolates AAUW-7 and AAUW-8 were completely inhibited by 100 μg a.e. L^{-1} of glyphosate as determined from cell viability test (Table 4). Likewise, Zablotowicz and Reddy [20] reported that USDA 110, 123, and 138 *B. japonicum* strains were completely inhibited at 5 mM and caused rapid cell death at 10 mM of glyphosate. This indicates that the sensitivity difference of rhizobia is associated with the inherent chemical resistance of the isolates and geographical areas from where they were isolated.

The in vitro inhibition tests of mancozeb also showed that isolates were very sensitive with drastic cell reduction of (90–96%) even at the low concentration (100 mg L^{-1}) as compared to the control (Table 2). This is similar to the reports of Cevheri et al. [21] that rhizobial isolate (V5) from *Vicia palaestina* was inhibited by 98% at field recommended rate of mancozeb in vitro. Even the 50% reduction of two rhizobial isolates (isolate USDA 3187 and one strain from peanut) at lowest (0.2 mg L^{-1}) concentration of mancozeb [11] indicates the sensitivity of root nodule bacteria to the fungicide mancozeb.

However, it is interesting to note that isolates were more resistant to the mixture of the two chemicals than they were to the individual ones. Many of the isolates (70%) were resistant to Treatment-1 (100 : 100) combinations with survival rates between 21 and 50% where isolate AAUW-4 was the most resistant (50% survival) (Table 3). The isolates, AAUW-1 and AAUW-3, are consistent in their resistance for all tested combined concentrations. This is contrary to the report of Cevheri et al. [21] that showed a 97% inhibition of *Rhizobium* isolate (V5) from *Vicia palaestina* exposed to a mixture of mancozeb and carbendazim in a field recommended concentration. The resistance of isolates to the combined chemicals may be attributed to the buffering effect of chemical reactions between each other. Furthermore, one or both chemicals may add the nutritional value for the rhizobial isolates. In fact, glyphosate was reported as the sole source of phosphorus under P-limiting conditions to grow several members of Rhizobiaceae including *Rhizobium* spp. and *Agrobacterium* spp. in liquid culture [22]. Moreover, the cell viability plate count data confirmed the existence of several colonies on the combined chemical treatments when they are transferred into YEMA medium (Table 4).

Conversely, isolates treated with mancozeb only do not form any colony when they were transferred onto YEMA

Table 3: The combination effect of glyphosate plus mancozeb on the growth of rhizobial isolates.

Isolates	Control	$OD_{620\,nm}$ of pesticide combinations of glyphosate plus mancozeb in deferent concentration in YEMB (μg a.e. L^{-1}) + (mg L^{-1})					
		100 : 100	%S	150 : 150	%S	200 : 200	%S
AAUW-1	0.417^{c}	0.155^{c}	37	0.147^{a}	35	0.098^{a}	23
AAUW-2	0.377^{f}	0.112^{f}	30	0.103^{cb}	27	0.029^{c}	8
AAUW-3	0.457^{b}	0.186^{b}	41	0.113^{b}	25	0.049^{b}	13
AAUW-4	0.456^{b}	0.230^{a}	**50**	0.079^{d}	17	0.018^{d}	4
AAUW-5	0.343^{h}	0.113^{e}	33	0.016^{gh}	5	0.011^{e}	3
AAUW-6	0.467^{a}	0.147^{d}	31	0.030^{e}	6	0.021^{d}	4
AAUW-7	0.385^{d}	0.043^{f}	11	0.022^{f}	6	0.022^{d}	6
AAUW-8	0.411^{e}	0.036^{fg}	9	0.035^{e}	8	0.013^{e}	3
AAUW-9	0.373^{g}	0.031^{g}	8	0.015^{h}	4	0.012^{ed}	3
AAUW-10	0.414^{c}	0.088^{f}	21	0.034^{e}	8	0.011^{e}	3

Numbers in the same column followed by different letters are significantly different at $p = 0.05$ confidence level (Tukey's HSD). Letters in the columns (a, b, c, d, e, f, g, and h) are ranks of the mean; %S = percentage of survival; AAUW = Addis Ababa University Wolki variety.

Table 4: Cell viability of isolates after exposure to agrochemicals.

Isolates	Control	100 μg a.e. L^{-1} glyphosate		200 μg a.e. L^{-1} glyphosate		Combination 100 : 100		Combination 200 : 200	
	Log	Log	%cfu	Log	%cfu	Log	%cfu	Log	%cfu
AAUW-1	2.56	0.50	20	0.39	15	1.23	48	0.67	26
AAUW-2	2.80	0.41	17	0.33	12	1.60	57	1.23	44
AAUW-3	2.42	0.45	19	0.33	14	1.43	59	0.72	30
AAUW-4	2.56	0	0	0	0	1.29	50	0.26	10
AAUW-5	3.01	0.41	18	0.37	12	1.33	44	0.48	16
AAUW-6	3.22	0.45	19	0.41	13	0.97	30	0.45	14
AAUW-7	3.05	0.09	0	0	0	0.50	16	0.42	14
AAUW-8	1.58	0.08	0	0	0	0.22	10	0.16	10
AAUW-9	1.73	0.19	11	0	0	0.28	17	0.21	12
AAUW-10	2.58	0.41	16	0.35	14	0.34	13	0.27	11

cfu = colony forming units; Log = logarithmic growth of isolates.

(data not shown). Similarly, Fawole et al. [23] experienced significant reduction of rhizobial cfu at 2.34 mg L^{-1} of mancozeb. Drouin et al. [13] also reported that mancozeb at 450 μg $disk^{-1}$ concentration affects cfu of *Mesorhizobium* and *Bradyrhizobium* strains by 67% and 91%, respectively. Although 60% of the isolates showed 16–20% and 12–15% cfu growth at 100 μg a.e. L^{-1} and 200 μg a.e. L^{-1} of glyphosate, respectively, the highest (30–59%) cfu growth was observed at a combination (100 : 100) concentration of the two agrochemicals. The isolates AAUW-1, AAUW-2, and AAUW-3 also showed the further resistance towards the combination concentration (200 : 200) by maintaining their cfu of 26%–44% (Table 4).

3.2. Effect of Glyphosate, Mancozeb, and Their Combinations on N_2-Fixation Performance of Isolates under Greenhouse. Based on the findings from in vitro test, four relatively resistant (AAUW-3, AAUW-5, AAUW-6, and AAUW-10) and one sensitive (AAUW-8) isolates were selected for in vivo experiment. The isolates inoculated plants showed significant difference on their parameters with better record of SDW with agrochemical treatments.

All isolates except AAUW-5 showed highest nodulation range (72–113 NN p^{-1}) with their SDW between 1.63 and 2.95 g $plant^{-1}$. The experimental plants showed unaffected height and green leave character without any effect. They also showed effective (65%) to highly effective (100–109%) symbiotic N_2-fixation performance with filed recommended concentration of all glyphosate, mancozeb, and combinations over the control (Table 5). Similarly, reports are indicating that glyphosate in field experiment with 1.12 and 3.36 kg a.e. ha^{-1} [24] and mancozeb in field recommended concentration [11] has no significant effect on shoot and nodule dry mass and nodule numbers. For instance, soya bean plants were relatively well nodulated and nodule biomass was relatively unaffected by glyphosate with two applications of 2.52 kg ha^{-1} as compared to the control [25]. The normal nodulation and better nitrogen fixation performance of isolates may result by which agrochemicals might lose their toxicity to isolates through adsorption or degradation [26]. In addition, isolates

Table 5: Effect of glyphosate, mancozeb, and their combination on the N_2-fixation performance of isolates in greenhouse condition.

Treatment	Isolates	Parameters			%NFE	Score
		NN p^{-1}	NDW g p^{-1}	SDW g p^{-1}		
GBRI	AUWR-3	86^{ab}	0.107^{a}	1.63^{de}	65	E
	AUWR-5	23^{c}	0.04^{b}	1.78^{d}	106	VE
	AUWR-6	113^{a}	0.066^{b}	2.95^{a}	108	VE
	AUWR-8	95^{ab}	0.106^{a}	2.84^{b}	107	VE
	AUWR-10	72^{b}	0.119^{a}	2.80^{c}	108	VE
MARI	AUWR-3	79^{a}	0.81^{b}	2.6^{a}	104	VE
	AUWR-5	61^{c}	0.07^{c}	1.64^{c}	98	VE
	AUWR-6	75^{b}	0.05^{c}	2.55^{b}	93	VE
	AUWR-8	68^{b}	0.05^{c}	2.65^{a}	100	VE
	AUWR-10	76^{ab}	0.94^{a}	2.73^{a}	105	VE
GBMARI	AUWR-3	76^{a}	0.13^{a}	1.98^{b}	79	E
	AUWR-5	65^{a}	0.10^{a}	1.73^{b}	103	VE
	AUWR-6	87^{a}	0.07^{b}	2.73^{a}	100	VE
	AUWR-8	56^{a}	0.11^{a}	2.68^{a}	101	VE
	AUWR-10	59^{a}	0.12^{a}	2.65^{ab}	102	VE
Control	AUWR-3	103^{a}	0.07^{b}	2.50^{b}		
	AUWR-5	64^{c}	0.08^{a}	1.68^{c}		
	AUWR-6	102^{ab}	0.06^{c}	2.73^{a}		
	AUWR-8	91^{b}	0.05^{cd}	2.65^{ab}		
	AUWR-10	102^{ab}	0.06^{c}	2.60^{ab}		

GBRI = glyphosate application before isolates inoculation; MARI = mancozeb application after isolates inoculation; GBMARI = combination of glyphosate application before isolates inoculation and mancozeb after inoculation; NN p^{-1} = nodule number per plant; NDW = nodule dry weight; SDW = shoot dry weight; numbers in the table are the mean of two plants per pot; g p^{-1} = gram per plant; E = effective; VE = very effective; NFE = N_2-fixation effectiveness.

Table 6: Summery on N_2-fixation performance of isolates with agrochemical stress.

Isolates	Control	GBRI		MARI		GBMARI	
	SDW	SDW	SP%	SDW	SP%	SDW	SP%
AUWR-3	2.50^{b}	1.63^{de}	65	2.60^{a}	104	1.98^{b}	79
AUWR-5	1.68^{c}	1.78^{d}	106	1.64^{c}	109	1.73^{bc}	105
AUWR-6	2.73^{a}	2.95^{a}	108	2.55^{b}	93	2.73^{a}	100
AUWR-8	2.65^{ab}	2.84^{b}	107	2.65^{a}	100	2.68^{a}	101
AUWR-10	2.60^{ab}	2.80^{c}	108	2.73^{a}	105	2.65^{ab}	102

%SP = symbiotic performance of isolates; SDW = shoot dry weight in gram/plant.

and/or faba bean plant might get the nutritional value from glyphosate and mancozeb degraded substrate components for growth as well as nutrient exudates for microorganism to activate and change their physiological function [27, 28].

4. Conclusion

From this study, it can be concluded that the results obtained under in vitro experiment and in vivo investigation were negatively correlated with each other. The isolates treated with glyphosate and mancozeb under in vitro experiment showed reduction of cell population in respect to concentration. This is the evident that accumulations of high agrochemical concentration are more destructive to beneficial microbes as compared to less concentration. Some agrochemicals like mancozeb also affect *Rhizobium* even at lower concentration. On the other hand, isolates which are treated with combination of the two agrochemicals showed better cell survival. This may have resulted because of both chemicals buffering ability with each other and minimizing the toxicity or both/one of chemicals may have nutritional value for rhizobial isolates. Furthermore, the results under in vivo investigation showed unaffected nodulation, plant growth, and N_2-fixation performance of faba bean inoculated with *Rhizobium* isolates although treated with field recommended concentration of glyphosate, mancozeb, and their combinations in greenhouse condition as compared to control (Table 6). It is expected that the applied agrochemicals may be absorbed by plants/sand or degraded by isolates.

This is promising result to identify and obtain agrochemical resistant inoculum for more N_2-harvest under no-tillage agriculture system, but further actual field experiment will be required.

Conflicts of Interest

The authors declared that they have no conflicts of interest.

Authors' Contributions

Most works are done by the first author and methodology modification and several rounds of paper editing were done by the second author.

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