Journal of Agriculture and Natural Resources (2021) 4(2): 86-97
ISSN: 2661-6270 (Print), ISSN: 2661-6289 (Online)
DOI: https://doi.org/10.3126/janr.v4i2.33674

Research Article

Effect of seed treatment using Mancozeb and Ridomil fungicides on *Rhizobium* strain performance, nodulation and yield of soybean (*Glycine max* L.)

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Received: June 01, 2020; Revised: October 30, 2020;
Received: June 01, 2020; Revised: October 30, 2020;
Accepted: December 15, 2020; Available online: January 01, 2021

ABSTRACT

The viability of commercial *Rhizobium* strains (SB-14 and SB-12) were inoculated and fungicides (Mancozeb and Ridomil) were used as seed dressed on soybean seed to investigate their effect on nodulation, plant growth and seed yield of soybean. Application of *Rhizobial* inoculants alone gave the highest nodulation and shoot dry weight performance as well as seed yield of soybean on both sites. SB-12 inoculant had significantly shown to be more effective than SB-14 inoculant in increasing nodulation and thus produced higher plant growth and seed yield. *Rhizobial* survival on the seeds was severely affected by both fungicides, resulting in decreased nodulation, plant growth and seed yield for both inoculants. However, Ridomil fungicide gave the lowest nodulation and seed yield when applied with either SB-12 or SB-14 Rhizobial strains. The strains differed in their sensitivity to Mancozeb fungicide that with strain SB-12 showed a slight effect or no effect on survival of rhizobium, nodulation and yield of soybean. Seed-dressing of mancozeb and ridomil resulted in reduction of seed yield by 882.8 kg ha^{-1} and 1154.7 kg ha^{-1}, respectively with SB-12 strain. The present results indicate that inoculated *Rhizobium* inoculants differ in their capacity to develop resistance to the two dressed fungicides. Seed treatment with Mancozeb in combination with SB-12 strain slightly affected the survival of the inoculated strain. Consequently, mancozeb fungicide may be compatable with survival of the inoculated SB-12 Rhizobia. The results also indicate that the suppressive effects of seed-applied fungicides on *Rhizobium* strains survival and nodulation development depend on specific strain and fungicide. Soybean seeds inoculated with SB-12 may not need management with fungicides or lower concentration of Mancozeb that could be compatible with SB-12 to suppress soil-borne pathogens for both Assosa and Begi sites, western Ethiopia.

Keywords: *Rhizobium* strain, fungicides, nodulation, soybean

Correct citation: Getachew, Z. & Abeble, L. (2021). Effect of seed treatment using Mancozeb and Ridomil fungicides on *Rhizobium* strain performance, nodulation and yield of soybean (*Glycine max* L.). *Journal of Agriculture and Natural Resources*, *4*(2), 86-97. DOI: https://doi.org/10.3126/janr.v4i2.33674

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INTRODUCTION

Inoculation of seeds with effective *Rhizobium* strain is a well known in enhancing nodulation, N uptake, growth and yield of soybean plants (Argaw, 2014). There is no doubt that specificity exists between *Rhizobial* strain and legume, and compatibility between the two is essential for successful nodulation (Zahran, 2000). Beyond this, to enchance the compatability and to improve the early plant emergence fungicidal seed dressing has been used by legume growers in different countries of the globe.

Soybean seed treatment with fungicides have expected as essential in the improved technology for increasing crop production due to its cost effectiveness against seed and soil borne pathogens (Labanya et al., 2017). Farmers are also treat soybean seeds with *Rhizobial* inoculants to promote effective biological nitrogen fixation and thereby for improved seed yield (Pudelko and Madrzak, 2004). In addition, fungicide seed treatment has been broadly practiced as cheap insurance against seed and soil-borne pathogens in legume producing areas. Even though seed treatment with fungicide is applying in the improved technology for increasing the growth and yield of crops for food production, some reports claim little damage (Curley and Burton, 1975). As reported by Kaur et al. (2007) studies showed considerable variation in toxicity of fungicides as well as sensitivity of *Rhizobium* to fungicides for seed dressing. However, the toxicity of most fungicides to different *Rhizobium* strains has often been underestimated (Campo et al., 2009). Fungicides can inhibit nodulation, nitrogen fixation and growth of various legumes and may affect negatively (Labanya et al., 2017). Various fungicide seed treatments tested on the survival of *Rhizobium* strains were investigsted for their effect on nodulation of soybean (Revellin et al., 1993; Martyniuk et al., 2002), bean (Ramos and Ribeiro, 1993), chickpea (Kyei-Boahen et al., 2001) and common bean (Guene et al., 2003); and detrimentally affected nodulation performance and thus found with poor nodulation. Fungicides affected bacterial survival on soybean seeds (Campo et al., 2009). Although reports are conflicting, several studies have conclusively shown that some of these chemicals are incompatible with *Rhizobium* (Pudelko and Madrzak, 2004). Bikrol et al. (2005) also reported fungicide applied to leguminous plants either as seeds dressing or soil drench reach the soil and may affect the symbiotic relationship. In addition, Schulz and Thelen (2008) found seed-applied fungicide to provide no widespread yield improvement with inoculation. On contrary, Yakubu et al. (2011) reported seed dressing with fungicides significantly enhanced growth performance of groundnut.

Compatibility between *Rhizobial* inoculants and fungicides used for seeds treatment remains contentious and arguementative (Pudelko and Madrzak, 2004). The adverse effect of fungicides may depend on the survival potential of inoculated strain. Compatibility or survival of rhizobia on the treated seeds is a major concern when treating with fungicides (Van Kessel and Hartley, 2000). Hashem et al. (1997) reported that the compatibility of *rhizobium* and fungicides was uncertain as the tested fungicides differed in their effects on the survival and growth of rhizobium strains in soybean. Thus, it will be necessary to select superior N_2-fixing strains of *Rhizobium* that are tolerant of, if not resistant to or identifying alternate fungicides that are more compatible with rhizobia are mandatory. The formulation of inoculant influenced how fungicide affected nodulation in legumes (Kutcher et al., 2002).

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Siddiqi and Athar (2013) also found benomyl fungicide increased the relative abundance of nodules, the number of added rhizobia on the root, the total N content, and the percentage of N in soybean plants that were inoculated with a benomyl-resistant strain of *Bradyrhizobium*. The effect of fungicide on the strains also depended on the strains and concentration of fungicide applied (Hashem et al., 1997).

Several questions concerning the compatibility of soybean inoculants with these practices have arisen by legume growing farmers. On the other hand, there is lack of knowledge about the interaction between fungicide and *Rhizobium* inoculants in Ethiopia. Therefore, the aim of the present study is to evaluate the effect of the fungicides, Mancozeb and Ridomil, applied as seed dressing to the seeds of soybean alone or in combination with rhizobial strains on nodulation, growth and seed yield of soybean.

MATERIALS AND METHODS

Description of the study area

A field experiment was conducted during the 2016/17 cropping seasons at two locations, Assosa and Begi areas, western Ethiopia. Assosa is the capital city of Benishangul Gumuz regional state in western Ethiopia and lies on altitude of 1,480 m above sea level, and located at 09°58’41.7” N, 034°38’09.5” E coordinates. According to the FAO/UNESCO soil classification, the major soil type at Assosa area is Nitisols, but soils at Begi site are expecting to be Alfisols. The dominant soil types in Assosa Area are sandy clay loam and clay loam that are poor to medium in organic matter and poor nutrients because of the long cropping history without replenishment of nutrients.

Soil physico-chemical analaysis

Ten cores of soil samples (0 to 20 cm) were randomly took using a soil auger from each site two weeks before planting. The samples from each site combined into a composite sample and two sub-samples of the composite from each site were took to the laboratory for chemical analysis and averaged for all parameters (Table 1). Determination of particle size distribution was carried out by the Bouyoucos hydrometer method (Bouyoucos, 1962). Soil pH was measured in the supernatant suspension of 1:2.5 soil and water mixture using a pH meter. Soil organic carbon was determined by using Walkley and Black method (Walkley and Black, 1934). Total N of the soils was determined through digestion, distillation and titration procedures of the Micro-Kjeldahl method as described by (Nelson and Sommers, 1973). Available phosphorus was determined by the Bray II method (Bray and Kurtz, 1945). Cation Exchange Capacity (CEC) was determined by leaching the soil with neutral 1N ammonium acetate (pH=7) (Van Reeuwijk, 1993) and exchangeable K extracted by ammonium acetate method was determined using flame photometer.

Experimental Design and treatment arrangement

The experiment was carried out on experimental plots with 14.4 m^2 area in replicates each and soybean variety Belessa-95 was used in the experiment. Commercial *Rhizobium* strains such as SB-14 and SB-12 were used as inoculant and fungicides (Mancozeb and Ridomil) were used as seed dressed fungicides. The experiment was designed in RCBD with nine treatments (Table 1) using three replications.

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Table 1: Treatment arrangement

No.	Treatments
1	Inoculant (SB-14)
2	Inoculant (SB-12)
3	Seed dressing with Mancozeb
4	Seed dressing with Ridomil
5	Seed dressing with Mancozeb after inoculation of SB-14
6	Seed dressing with Mancozeb after inoculation of SB-12
7	Seed dressing with Ridomil after inoculation of SB-14
8	Seed dressing with Ridomil after inoculation of SB-12
9	Control (negative control)

Phosphorus from TSP was applied as a basal for all plots at 46 kg P_2O_5 ha^{-1}. Plot sizes were 3.6m*4.0m (14.4 m^2) consisted of six rows, two rows for nodulation and shoot dry weight parameters and the other rows excluding borders were harvestable rows. The plots were kept 1.0m apart with 1m spacing between blocks. Canals were prepared around each plot making the plots beds. Carrier-based inoculant was applied at the rate of 123 g inoculant for 15 kg seed (used to sow quarter ha). In order to ensure that all the applied inoculum stick to the seed, the required quantity of inoculant was suspended in 1:1 ratio in 10% sugar solution. The thick slurry of the inoculant was gently mixed with dry seed so that all the seeds received a thin coating of the inoculant. All inoculations were done just before planting under shade to maintain the viability of bacterial cells. Seeds were allowed to air dry for a few minutes and were then sown at the required rate and spacing. Plots with uninoculated seeds were planted first to avoid contamination. The inoculated and uninoculated seeds were then planted at a spacing of 10 cm between plants and 60 cm between rows making six rows per plot. Seeds were immediately covered with soil after sowing to avoid death of cells due to the sun's radiation. Fungicides Mancozeb and Ridomil were used in these experiments. The source of both fungicides were from commercial suppliers and chemically Mancozeb is Methyl-2-benzimidazole but Ridomil is Methyl N-(methoxyacetyl)-N-2,6-xylyl-D-alaninate.

Nodulation and shoot biomass

Ten plants were sampled randomly from the second border rows of each plot at mid flowering (50% flowering). The whole plant was carefully up-rooted using a spade to obtain intact roots and nodules for nodulation parameters and dry weight of plants. Uprooting was done by exposing the whole-root system to avoid loss of nodules. The adhering soil was removed by washing the roots with intact nodules gently with water over a metal sieve. The same ten plants from each plot were used to rate nodulation and to record number of nodules per plant and nodule dry weight. After nodules were taken, three plants from each plot were cut at the ground level for shoot dry weight determination. Total fresh shoot weight was measured using an electronic balance. Sub-samples of shoots were then kept in brown envelopes and oven dried at 70 ^oC for 48 hours. The dry materials were weighed and shoot dry weight recorded averaged as per plant.

Statistical analysis

The analysis of variance had carried using general linear model (GLM) procedure provided by SAS statistical software version 9.20 (SAS, 2008). Combined analysis across locations had performed for estimating variability of treatments for different locations. Analysis was

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carried out to evaluate treatments on nodulation, growth and seed yield of soybean. Means were separated using LSD procedure (P < 0.05).

RESULTS

Soil analysis for experimental sites

The soil of the experimental site (Assosa) was sandy clay loam in texture, while for Begi site it was clay loam. The average soil pH for Assosa was 5.46, which is strongly acidic. The pH of this site is characterstic of weathered soils which may be problematic for growing crops and need management for production of most field crops. Based on the results obtained for soil analysis, the average organic carbon (OC) and total nitrogen (N) were 1.86 % and 0.13 %, respectively.

Table 2. Some selected physical and chemical properties of soils for the experimental sites before planting

Type of soil analysis	Location	
	Assosa	Begi
Soil particle size (Texture)	Silt clay loam	Clay loam
pH	5.46	6.15
OC (%)	1.86	2.22
Total N (%)	0.13	0.24
Bray-II available P (mg kg^{-1})	11.50	23.20
Exchangeable K (g kg^{-1})	3.84	16.22
CEC (meq $100g^{-1}$)	15.30	27.80

Thus, According to the ratings suggested by Tekalign (1991), both parameter's value fall in the lower ranges for the study area (Assosa). In addition, soil available P was low (< 20 ppm) in accordance with the rating of Horneck *et al.* (2011). Cation Exchange Capacity was low (< 5 meq $100g^{-1}$ soil) according to Horneck et al. (2011), but moderate according to Hazelton and Murphy (2007). The exchangeable K was low according to Horneck *et al* (2011) and very low according to Berhanu (1980). The average total nitrogen (N) and available phosphorus (P) were low and may be not optimal for crop production. Generally, the total N and available P contents of the soil for Assosa might attributed to the relatively higher OC contents. Generally, the fertility status of Assosa site was very low (Table 2). On the otherhand, the average soil pH, organic carbon content and other nutrient contents of the soil for Begi site were good and ideal for the production of most field crops.

Two experiments were carriedout in 2016/17 cropping season in two locations of Benishangul Gumuz, Western Ethiopia in soybean growing soils. The effects of location and treatment influenced most of the parameters evaluated; however, the effect of treatment was more dominant than the other factors. Location x treatment interaction for most of the parameters evaluated were non-significant which showed the treatments performed equally likely for both locations. Therefore, data averaged over each location for 2016/17 cropping season had reported in the present study.

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Table 3. Effect of seed treatment with Mancozeb and Ridomil fungicides along with *Rhizobial* inoculation on growth, symbiotic parameters and yield of soybean under field conditions of Begi and Assosa areas

Variation	NN	NDW	SDW	PH	PPP	SPP	GY ($kg\ ha^{-1}$)
Location							
Assosa	20.51[a]	0.183	13.25[a]	66.72[b]	31.67	2.44[a]	1969.4[b]
Begi	13.42[b]	0.221	9.88[b]	74.91[a]	30.69	2.10[b]	2317.2[a]
LSD	5.08	NS	1.65	3.23	NS	0.071	261.61
Treatments							
Inoculant (SB-14)	30.0[ab]	0.438[a]	14.57[a]	63.36[de]	39.20[a]	2.30	2372.8[b]
Inoculant (SB-12)	35.2[a]	0.495[a]	14.00[a]	62.63[e]	40.73[a]	2.41	3069.3[a]
SD with Mancozeb	9.38[c]	0.125[bcd]	11.14[bc]	67.96[cde]	31.11[b]	2.35	1995.1[c]
SD with Ridomil	6.50[c]	0.071[d]	11.27[bc]	72.95[abc]	28.00[bc]	2.26	1913.7[c]
SD with Mancozeb and SB-14	6.94[c]	0.070[d]	9.80[c]	74.58[ab]	27.15[bc]	2.26	1841.8[c]
SD with Mancozeb and SB-12	21.9[b]	0.221[b]	12.56[ab]	76.26[a]	30.23[bc]	2.25	2186.5[bc]
SD with Ridomil and SB-14	9.66[c]	0.083[cd]	10.71[bc]	76.02[a]	25.50[c]	2.29	1909.4[c]
SD with Ridomil and SB-12	9.80[c]	0.141[bcd]	9.59[bc]	74.16[abc]	29.90[bc]	2.16	1914.6[c]
Control (negative control)	23.3[b]	0.175[cd]	12.48[ab]	69.38[bcd]	27.83[bc]	2.18	1917.9[c]
LSD	8.66	0.099	2.63	6.24	5.30	NS	366.65
F-test value							
Loc	15.05*	4.86[NS]	31.78**	49.52**	0.64[NS]	169.6***	13.62*
Trt	12.99***	21.06***	3.72**	5.83***	8.16***	1.71[NS]	9.16***
Loc x Trt	3.99**	5.44***	1.18[NS]	1.45[NS]	0.64[NS]	1.82[NS]	1.91[NS]
CV(%)	43.40	41.78	19.35	7.50	14.45	6.46	14.54

SD- Seed dressing, NN-nodule number, NDW- nodule dry weight, SDW- shoot dry weight, PH- plant height, PPP- pod per plant, SPP- seed per plant, GY- grain yield

Nodulation and shoot dry weight

Seed treatments with agrochemicals (fungicides) and inoculation of strains significantly affected nodulation and shoot dry weight parameters evaluated. Inoculation of *Rhizobial* strains (SB-12 and SB-14) had significantly improved nodulation, while seed treatment with fungicides significantly ($p < 0.05$) reduced the average number of nodules per plant and dry weight of nodules (Table 3). Besides the influence of fungicide treatment on nodulation varied with the type of strain used for inoculation. Seed treatment with fungicide Ridomil alone had significantly reduced nodule number (78.3 and 81.5% reduction compared with inoculation treatments of SB-14 and SB-12 strains, respectively). Combined use of inoculation and fungicide significantly reduced the nodulation; especially the effect was significantly stronger for strain SB-14 with Mancozeb (76.8% and 5 fold reduction of the nodule number and dry weight) than inoculation of SB-14 alone (Table 3). Comparatively, the effect of Mancozeb on nodulation of soybean was to some extent good with strain SB-12 that showed fungicide effect differed with the bacterial strain used for inoculation.

Soybean seed yield and yield traits

The results showed that treatments differed significantly ($P<0.05$) in their responses to inoculation of strains and seed dressing of fungicides for soybean plant with respect to all the parameters measured (Table 3). Soybean seed yield and pod per plant significantly ($P<0.05$) affected by treatment in the experiment except seed per pod (Table 3). Rhizobia inoculation alone significantly increased pod per plant but not when applied in combination with the fungicides. Inoculation of both SB-12 and SB-14 inoculants showed significantly higher

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number of pods and increased pod number of soybean by 31.7 and 29.0% over non-inoculated control. On the other hand, inoculation of SB-12 alone significantly increased pod number by 25.7 and 26.5% over simultaneous use of SB-12 with Mancozeb and Ridomil fungicides, respectively. In addition, inoculation of SB-14 increased number of pods for soybean by 30.7 and 35.0% over combined use of SB-14 with Mancozeb and Ridomil fungicides respectively. Seed dressing of both fungicides reduced soybean pod yield especially more reduction observed when seeds had treated with fungicides after inoculation with SB-14 strain.

The results also showed that treatments significantly ($P < 0.05$) differed in their effect on seed yield of soybean. Seed inoculation significantly increased seed yield of soybean over the non-inoculated control and treatments consisted seed-dressed fungicides alone or applied in combination with inoculation (Table 3). Inoculation of SB-12 and SB-14 significantly increased seed yield of soybean by 37.51 and 19.17% over the non-inoculated control. In addition, application of fungicide on rhizobia inoculated seeds significantly reduced seed yield because of seed-dressed fungicides. Therefore, inoculation of SB-12 increased seed yield of soybean by 28.7 and 37.6% over combined use of SB-12 with Mancozeb and Ridomil fungicides respectively. Seed dressing of both fungicides reduced soybean seed yield especially more reduction observed when SB-14 inoculated seeds had treated with Ridomil fungicide. Inoculation of SB-14 increased soybean seed yield by 22.4 and 19.5% over combined use of SB-14 with Mancozeb and Ridomil fungicides respectively. The two inoculants significantly increased seed yield compared to the control and fungicide treatments with or without inoculant. However, SB-14 inoculant responded negatively to seed dressing of both fungicides. Significantly higher and improved seed yield of soybean had found at Begi compared to Assosa.

DISCUSSION

Two commercial fungicide seed treatments had evaluated for their possible effect on the survival of *Rhizobium strains* and on the nodulation and yield of soybean under field condition. Results showed that combined use of *Rhizobial* inoculant and fungicide significantly reduced nodulation; especially the effect was significantly stronger for strain SB-14 with Mancozeb (76.8% and 5 fold reduction of the nodule number and dry weight) than inoculation of SB-14 alone (Table 3). The two fungicides react differently on the two inoculants. Comparatively, the extent of Mancozeb fungicide effect on nodulation of soybean was good with strain SB-12 that showed fungicide effect differed with the bacterial strain used for inoculation. Inoculated treatments accumulated significantly the highest nodule dry matter compared to combined application of inoculation with seed dressing fungicide. However, fungicide seed dressing alone and in combination with inoculation of strain depressed nodulation in soybean. Simultaneous use of *Rhizobial* strains with seed dressing of fungicides caused the reduction of the nodules size formed on the plant root, however the extent of that effect differed with the bacterial strain used for inoculation. Seed dressing of Mancozeb after inoculation of SB-14 reduced drastically the occurrence of inoculated strains in nodules number and dry weight; while the same seed applied fungicide, with SB-12 inoculation had slightly affected the survival of the inoculated strain.

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Both fungicides reduced soybean nodulation, especially in the presence of SB-14 strain. The chemical dressings further reduced the survival of the symbiotic bacterium on the stored soybean seeds as compared to untreated seeds (Martyniuk et al., 2002). In the present study, SB-12 strain responded to Mancozeb fungicide than Ridomil, but SB-14 strain responded negatively to both fungicides. The two strains differ in their sensitivity to both fungicides. Similar to our results, Hashem et al. (1997) also investigated two fungicides (Benomyl or Vitavax) tested differ in their effect on the growth and survival of *Bradyrhizobium* strains depending on the type of strains. They found that the growth and survival of strain USDA 3384 on peanut seed was inhibited, while strain USDA 3456 resisted both fungicides under laboratory. With other fungicide, Ahimed et al. (2007) also found that strains of *Rhizobium or Bradyrhizobium* whether introduced or locally isolated differ greatly in their sensitivity to Captan fungicide under laboratory condition. Kuar et al. (2007) also found that from the two tested fungicides (captan and carbendazin) carbendazin was toxic to nodule bacteria. On contrary, Campo et al. (2009) reported that all seed-applied fungicides tested severely affected bacterial survival and reduced nodule number.

To achived effective nodulation and highest N_2 fixation requires large number of viable rhizobia colonizing the rhizosphere (Deaker et al., 2004). Therefore, compatibility or survival of rhizobia on the agrochemical treated seeds is considered so essential (Ramos and Ribeiro, 1993; Kutcher et al., 2002). Even though, the applied fungicides were incompatible with survival of the inoculated rhizobium strains, but more reduction in nodulation as well as seed yield of soybean found with SB-14 strain for both seed dressed fungicides. Unfortunatly, these fungicides may be incompatable with survival of the inoculated *Rhizobial* strains. Depression in nodulation due to seed dressing of fungicides along with inoculation of strain for different legumes such as common bean, green gram and lablab has been found by Muthomi et al. (2007). A study conducted in Brazilian soil by Zilli *et al*. (2009) also showed that seed treatment with Thiram and Cabendazin reduced nodulation in soybean inoculated with *Bradyrhizobium* stain.

The inoculated strains showed a lower nodulation performance when treated with both Mancozeb and Ridomil fungicides. In addition, fungicide treatments alone had a direct effect which was observed from the leaves color during half growth stage, were yellowish rather than healthy deep green color. May be these type of fungicides themselves can be toxic to microorganisms and may affect the symbiotic relationship between soybean and *Rhizobial* strain. The presence of agrochemicals in the rhizosphere may interfere with the infection process by inhibiting bacteria-induced root hair deformation (Andre´s et al., 2012). Annapurna (2005) also reported that survival of *Bradyrhizobium japonicium* inoculated to soybean seed with dressing of both bavistin and mancozeb fungicides separately was hindered. The author suggested it may due to fungicide treatment decreased the viable rhizobia on the seed. Castro et al. (1997) found a 50 % decrease in growth of two *Rhizobium* strains due to seed dressing of mancozeb fungicide. Mancozeb treated seeds had found with significantly less nodules, both in terms of numbers and mass, as well as lower fresh and dry weight of shoots as compared to the plants grown from the control seeds and other fungicides tested (Martyniuk et al., 2002). Ramos and Ribeiro (1993) on the otherhand also found a drastic mortality of two strains (CIAT 652 and CPAC 1135) on bean seeds treated with Benlate and Ridomil under laboratory. Ridomil might not be compatible with seed applied strain SB-14 due to lose of their ability to induce nodulation and were generally toxic to

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rhizobia. Measurements of compatibility should include not only ability to produce colonies but also ability to induce nodulation and to fix nitrogen. Slight effect of Mancozeb seed dressing after with strain SB-12 may be due to compatibility between the inoculant and fungicide, which was proved through induced nodulation and deep green leaves during vegetative stage. The reduction on nodulation and root growth of plants due to seed dressing of agrochemicals may be attributed to reducing in nitrogenase activity in active strains as suggested by Niewiadomska and Klama (2005).

The present study showed use of *Rhizobium* inoculants significantly increased grain yield. On the other hand, chemical dressing applied to seed of soybean alone or in combination with inoculated of *Rhizobium* had also significant effect on seed yield and yield traits. Inoculation is important to provide sufficient numbers of viable and effective rhizobia that will induce fast colonization of the rhizosphere to enable rapid nodulation, which may led to produce optimum yields at harvest. Birah et al. (2014) also suggested that inoculation of efficient *Rhizobium* strain enhances the nitrogen fixing capability of legume crops and their productivity. Literally in our study, application of fungicide on rhizobia inoculated seeds significantly reduced seed yield due to seed-dressed fungicides. Therefore, inoculation of SB-12 increased seed yield of soybean by 28.7 and 37.6% over combined use of SB-12 with Mancozeb and Ridomil fungicides respectively. Seed dressing of both fungicides reduced soybean seed yield especially more reduction observed when SB-14 inoculated seeds had treated with Ridomil fungicide. Our result indicated that perhaps with the use of more efficient strain without fungicide, or with seed dressing of compatable fungicide, responses to nodulation and higher seed yield. However, Schulz and Thelen (2008) reported that fungicide and rhizobium inoculant combinations increased soybean yield in situations where fungicide had no impact on the inoculant.

Absence of effective indigenous rhizobia in the soils of the study areas may contributed for the inoculantion treatments to equitably increase in nodulation and yield. Inoculation of effective strain has been found to be useful not only in enhanced BNF but also to trigger improved plant vigor and growth, which consequently enriched crop performance. Seed dressing of both fungicides reduced soybean seed yield especially more reduction observed when SB-14 inoculated seeds had treated with both fungicides. But little damage associated with a little reduction in seed yield for Mancozeb fungicide with SB-12 strain may be due to difference for the strains in their sensitivity to fungicides. These findings are in agreement with those obtained by Curley and Burton (1975) who reported variation of *Rhizobium* strains in their sensitivity to different fungicides. Revellin et al. (1993) also reported that several commercial fungicides were evaluated under greenhouse and field conditions and some of them found with a decreased in *Rhizobium* survival, nodulation and yield of soybean. Thus cannot be considered compatible with soybean seed inoculation. It would suggest that a combination of *Rhizobium* and a suitable fungicide may provide better protection of roots from invasion by root infecting fungi and also enhance root nodulation thus resulting in healthy plant growth and seed yield. Significantly higher and improved seed yield of soybean had found at Begi compared to Assosa, which may reveal the considerable variation in nutrient status of the two sites. Major causes of nutrient depletion in Assosa area are farming without replenishing nutrients over time, and/or chemical imbalance issues such as soil acidity leading to P fixation often driven by continuous cropping of cereals, removal of crop

ISSN: 2661-6270 (Print), ISSN: 2661-6289 (Online)
DOI: https://doi.org/10.3126/janr.v4i2.33674

residues, leaching and low levels of fertilizer usage. Therefore, lower inherent soil fertility problem in soils of Assosa area largely accounts for lower crop yields.

CONCLUSION

In the present study, the effect of two fungicides on *Rhizobial* inoculants for soybean growth had investigated. The two fungicides (Mancozeb and Ridomil) applied as seed dressing to the seeds of soybean alone or in combination with inoculants significantly reduced the nodulation, growth and seed yield. Mancozeb fungicide was observed to be less toxic to SB-12 strain with respect to plant growth and soybean yield. While SB-14 strain significantly affected by either of seed dressed fungicides.

Our results also revealed that when adverse situations to seed and soil borne pathogens occur, it might be possible to use inoculation with lower concentration of Mancozeb fungicide; even though some degree of yield reduction may be expected. However, more information is necessary to draw conclusion on the concentration of Mancozeb that could be compatible with *Rhizobium* strain to inhibit pathogen and cost effective to farmers in wetern Ethiopia.

Acknowledgments

Ethiopian Institute of Agricultural Research (EIAR), Assosa agricultural research institute, supported the implementation of this research work. Therefore, this organization is acknowledging for the financial support.

ACKNOWLEDGEMENTS

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Authors' Contributions

Zerihun Getachew design and conduct the experiment as well as write the manuscript. Lejalem Abeble did lab analysis and gave comments on writing the manuscript. All authors read, revised and accepted the manuscript for journal publication.

Conflicts of Interest

The authors declare that there is no conflict of interest among authors for manuscript publication

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ISSN: 2661-6270 (Print), ISSN: 2661-6289 (Online)
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Journal of Agriculture and Natural Resources (2021) 4(2): 86-97
ISSN: 2661-6270 (Print), ISSN: 2661-6289 (Online)
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