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Effect of seed inoculation method with Rhizobium species on the germination of alfalfa seeds (Medicago sativa L.)

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Alfalfa Seeds
Charcoal Powder
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Gum Arabic
Rhizobium Bacteria
Sawdust

ABSTRACT

Inoculation alfalfa seeds with the right Rhizobium strain (nitrogen-fixing bacteria) can stimulate root nodulation and improve plant growth and yield. Different inoculation methods are used to inoculate alfalfa seeds; however, the effect of these methods on the germination of seeds is not well documented. In the present study, the germination of alfalfa seeds was evaluated after using different inoculation methods, i.e. Arabic gum solution and glucose solution as adhesive substances on the surface of the seeds, and charcoal powder and sawdust as seeds coating materials. The adhesive solutions and coating materials were tested separately and in combination. Three concentrations of Arabic gum solution and glucose solution were used (i.e., 5, 12.5, and 25%), alongside untreated seeds as control. Germination counts were started on days 3, 4, 6, 8, 10, and 12. On day 12, the experiment ended and the final germination percentage (FGP) and germination index (GI) were calculated. Application of sole Arabic gum and glucose solution of 5 and 12.5% on the surface of the seeds was beneficial to the germination of the inoculated seeds. The application of sole sawdust in the seedbed enhances germination significantly ($p < 0.05$) compared with coal powder. Moreover, using sawdust as a coating material in combination with Arabic gum (12.5%) gave significantly ($p < 0.05$) higher germination. The results demonstrate that using 12.5% of adhesive solution either with sawdust or charcoal powder as coating material did not harm the germination of alfalfa seeds.

أثر طريقة تلقيح البذور ببكتيريا العقد الجذرية على إنبات بذور البرسيم (Medicago sativa L.)

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الكلمات المفتاحية:
بذور البرسيم
بكتيريا العقد الجذرية
الصمغ العربي
محلول الجلوكوز
مسحوق الفحم
نشارة الخشب

الملخص

تلقيح بذور البرسيم ببكتيريا العقد الجذرية (البكتيريا المثبتة للنيتروجين) قبل الزراعة يعتبر مهما لتحفيز تكون العقد الجذرية والتي بدورها تحسن نمو النبات والإنتاجية. تستخدم العديد من الطرق لتلقيح بذور البرسيم ولكن تأثير هذه الطرق على إنبات بذور البرسيم يعتبر غير واضح. هدفت هذه الدراسة الى تقييم إنبات بذور البرسيم بعد التلقيح ببكتيريا العقد الجذرية باستخدام طرق تلقيح مختلفة، مثل محلول الصمغ العربي ومحلول الجلوكوز (سكر الطعام) كمواد لاصقة على سطح البذور، ومسحوق الفحم ونشارة الخشب كمواد حاملة للبكتيريا على سطح البذور. تم في هده الدراسة اختبار تأثير المحاليل اللاصقة والمساحيق الحاملة للبكتيريا بشكل منفصل

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وكذلك مع بعضها حيث استخدمت ثلاثة تراكيز من محلول الصمغ العربي ومحلول الجلوكوز وهي 5، 12.5، 25٪ هذا بالإضافة الى معاملة الشاهد (بذور غير ملقحة ولم تعامل بالمحلول اللاصق او المسحوق الحامل للبكتيريا). بعد زراعة البذور بدأ عد البذور النامية عند ملاحظة اول إنبات وكان في اليوم الثالث من الزراعة واستمر العد الى اليوم الرابع والسادس والثامن والعاشر، واخر عد كان في اليوم الثاني عشر بعد ذلك تم حساب نسبة الإنبات النهائية وكذلك مؤشر الانبات Germination Index (GI). أشارت النتائج الى ان استخدام الصمغ العربي ومحلول الجلوكوز بنسبة 5 او 12.5٪ كمادة لاصقة على سطح البذور بدون إضافة أي من المساحيق لم يؤثر سلبا على إنبات البذور مقارنة بالشاهد. كما ان استخدام نشارة الخشب بدون المادة اللاصقة (وضعها مباشرة في مهد البذرة) حسن الإنبات بشكل معنوي ($P < 0.05$) مقارنة بمسحوق الفحم. علاوة على ذلك، فإن استخدام نشارة الخشب كمادة حاملة للبكتيريا مع الصمغ العربي بتركيز 12.5٪ أعطى اعلى نسبة إنبات. خلصت الدراسة الى أن أفضل طريقة لتلقيح بذور البرسيم هوا استخدام محلول الصمغ العربي ومحلول الجلوكوز بتركيز 12.5٪ سواء مع نشارة الخشب أو مسحوق الفحم.

Introduction

Inoculation of legume seeds such as alfalfa with effective Rhizobium is essential for enhancing the growth and yield of legume crops [1]. Alfalfa (*Medicago sativa* L.) is a legume plant growing in a symbiotic relationship with Rhizobium bacteria [2]. Rhizobium is soil bacteria that form root nodules on the legume root to fix atmospheric N and in return, the legume plant provides Rhizobium bacteria with carbohydrates [3]. Inoculating legume seeds with the right rhizobia strain is required especially when the land is cultivated with a legume crop for the first time [4, 5]. Microbial inoculants can be applied directly to the soil or the plant by foliar spray or root dipping or to the seeds before sowing [6, 7, 8]. Application directly to the soil in the field can be done according to manufacturer's protocol and this requires a big amount of inoculants compared with the seed-inoculation to ensure the inoculant will reach the legume seed [6]. Seed inoculation is a simple and a well-known technique where effective rhizobia are added to the seeds prior to sowing [6]. Different methods can be used to inoculate legume seeds with Rhizobium bacteria by applying adhesive substances and coating materials [9]. The coating materials that can be used as a carrier of Rhizobium bacteria on the seed's surface are lime, rock phosphate, coal powder, molybdate and sawdust [1, 9, 10, 11, 12]. Whereas sticker materials are used to prepare adhesive solutions to hold coating materials on the seed surface, such as skimmed milk, sodium carboxymethyl, cellulose, sodium alginate, Arabic gum, and glucose [1, 4, 10, 12, 13].

These types of coating materials and adhesive solutions have different physical and chemical properties, which will provide an unnatural coat on the seeds and might influence their germination, especially small seeds such as alfalfa. Therefore, adhesive solution and coating material must be used without harming the ability of seeds to germinate. Most of the studies in this area have focused on the effect of the inoculation method on Rhizobium survival and plant nodulation, and not sufficient studies have been done to investigate the effect on seed germination. Besides, the effect of the adhesive solution concentration on seed germination is not well reported. However, different types of adhesive solutions with different concentrations have been used and reported in previous studies. For example, different concentrations of Arabic gum solutions were tested as carriers for inoculums i.e., 10, 20, 30, 40 and 45% [14, 15, 16] and also different concentrations of glucose solutions 10, 20, 25 and 50% [14, 17]. Therefore, the limitations in the literature on the effect of adhesive solution and coating materials on seed germination required more investigation to address the best inoculation method that can be used in sustainable agricultural systems. Consequently, the main objective of the present study was to investigate the effect of the inoculation method on the germination of alfalfa seeds (*Medicago sativa* L.) where different concentrations of adhesive solution were tested (Arabic gum and glucose solutions (i.e. 5, 12.5 and 25%)) in combination with charcoal powder and sawdust (coating materials to carry the inoculum).

Materials and methods

Soil collection and characteristics

Sandy soil was collected from the desert that located west of Sabha city in the southern part of Libya (22° 30′ N and 30° 00′ N and between the meridians of 10° E and 18° E). The soil site is an arid land that has not been cultivated or fertilized before. About 50 kg of totally dry soil was taken and transported to the laboratory and kept at lab temperature (23°C) until use. Before the germination experiment started, the soil was cleaned using 2-mm sieve. Physical and chemical properties of the used soil are presented in **Table 1.** For more details about the methods used in analyzing the soil properties, see Abubaker et al. [18].

Table 1. Properties of the soil used in the experiment.

Parameters	Values
Physical properties	
Sand (%)	97
Clay (%)	1.3
Silt (%)	1.7
Water-holding capacity (%)	21.6
Chemical properties	
pH_{paste}	8
ECe (ds m^{-1})	2.99
OM (%)	0.5
Total N (%)	0.1
Total C (%)	1
P (g kg^{-1}dw)	0.1
K (g kg^{-1}dw)	0.04
Na (g kg^{-1}dw)	0.25
Mg (g kg^{-1}dw)	0.04
Ca (g kg^{-1}dw)	0.16

Alfalfa seeds and Rhizobial strain

Alfalfa seeds (*Medicago sativa* L.) used in the germination experiment were obtained from an agricultural market located in Sabha city. The characteristics of the seeds are: - species: Alfalfa, variety: Victoria USA, germination: min. 80%, purity: min. 97%, producer: Semillas Fito. Before the alfalfa seeds were used, seeds vitality was tested according to Ellis et al. [19], which was 90 ± 2 %. The Rhizobium strain used in the germination experiment was isolated from the root nodules of *Medicago polymorpha* L. and *Trigonella stellate* L. [20]. Before using the isolated Rhizobium as inoculum, the ability of isolated Rhizobium to produce nodules on alfalfa (*Medicago sativa* L.) was tested in the lab and the results showed that this Rhizobium strain is able to establish a nitrogen-fixing symbiosis by forming nodules on alfalfa root (n = 4 plants; 20 ± 12) [10].

Coating materials and adhesive solutions

The coating materials used in the germination experiment were charcoal powder and sawdust (about 4 Kg were collected from each). Charcoal powder was obtained from a farm where charcoal is produced through the process of carbonization and torrefaction of windbreak tree branches (*Casuarina glauca*), by continuous earth pit kilns. Sawdust was taken from the factory of cupboard and wooden doors located in Sabha city, where beech wood is only use. After collection, both

coating materials were ground to a fine powder using a grinding machine and were then kept at room temperature (28 ± 3°C) until use. Arabic gum solution with different concentrations (5, 12.5, and 25%) was prepared by dissolving 10, 25, and 50 g of Arabic gum in 200 ml sterile distilled water on the heater at a temperature of 90°C. Different concentrations of glucose solution (5, 12.5, and 25%) were prepared by dissolving 10, 25, and 50 g of glucose powder in 200 ml sterile distilled water without heating [14].

Treatments and experiment setup

The experiment comprised 21 treatments (**Table 2**) with four replicates each, giving a total of 84 pots. The treatments used in the present study were divided into 4 groups as shown in Table 2. The first group consisted of 3 treatments where the adhesive solution was not added (only seeds were sowed as control (no inoculation), seeds sowed with sole charcoal powder (1 g added to the soil in the seedbed), and seeds sowed with sole sawdust (1 g added to the soil in the seedbed)). The second group consisted of 6 treatments where the adhesive solution was only added to the seed's surface, the seeds were treated with different concentrations of Arabic gum solution (5, 12.5, and 25%) and glucose solution (5, 12.5, and 25%) without coating with charcoal powder or sawdust. The third group consisted of 6 treatments, where the seed's surface was treated with 1 mL of Arabic gum solution (5, 12.5, and 25%) and coated with charcoal powder (1 g) and sawdust (1 g) and inoculated with Rhizobium (1 mL). The fourth group consisted of 6 treatments, where the seed's surface was treated with 1 mL of glucose solution (5, 12.5, and 25%) and coated with charcoal powder (1 g) and sawdust (1 g), and inoculated with Rhizobium (1 mL).

Sowing and germination attributes

The germination experiment was performed at the Research and Consulting Center of Sabha University, Libya. The experiment was done in plastic pots (7.5 cm diam. x 5 cm height) filled with pure soil. All pots were first filled with 200 g of sandy soil and sprayed with 50 ml of distilled water, and each pot was sowed with 20 seeds at a depth of 1 cm, deformed seeds (broken or wrinkled) were excluded. The inoculation with Rhizobium strain was done in a petri dish before sowing by wetting the seed's surface with Arabic gum or glucose solution and coating with charcoal powder or sawdust. After that, the seeds were kept in the lab for two hours before inoculation [21].

The inoculation was done by adding 1 mL of Rhizobium suspension that contains 105 CFU mL^{-1}. Uncoated seeds were used as control (C) where no inoculation was done to the seeds before sowing. The soil moisture in the pots was kept near 70% of its water-holding capacity during the experiment period by weighing and adding the water when needed by dropping. After sowing, all pots were placed on trolleys under field condition (13 to 15 h day, 11 to 9 h night, temperature 24 ± 3°C and air humidity 27% and 36%). The sowed pots were checked daily for germinated seeds. The germination counts were started and recorded on days 3, 4, 6, 8, 10 and 12. The final germination percentage (FGP) and germination index (GI) were calculated according to the method described by Dastanpoor et al. [22].

Table 2. Treatments used in the germination experiment.

	No. of treatments	Treatments	Abbreviations
First group (Just coating materials without adhesive substance)	1.	Control	C
	2.	Charcoal powder	Coal_P
	3.	Sawdust	SawD
Second group (Just adhesive substances)	4.	Arabic gum (5%)	A_Gum (5%)
	5.	Arabic gum (12.5%)	A_Gum (12.5%)
	6.	Arabic gum (25%)	A_Gum (25%)
	7.	Glucose (5%)	Glu (5%)
	8.	Glucose (12.5%)	Glu (12.5%)
	9.	Glucose (25 %)	Glu (25%)
Third group (Arabic gum+ coating + inoculation)	10.	Arabic gum (5%) + Charcoal powder + *Rhizobium*	A_Gum (5%)+ Coal_P+R
	11.	Arabic gum (12.5%) + Charcoal powder + *Rhizobium*	A_Gum (12.5%)+ Coal_P+R
	12.	Arabic gum (25%) + Charcoal powder + *Rhizobium*	A_Gum (25%)+ Coal_P+R
	13.	Arabic gum (5%) + Sawdust + *Rhizobium*	A_Gum (5%)+ SawD+R
	14.	Arabic gum (12.5%) + Sawdust + *Rhizobium*	A_Gum (12.5%)+ SawD+R
	15.	Arabic gum (25%) + Sawdust + *Rhizobium*	A_Gum (25%)+ SawD+R
Fourth group (Glucose + coating + inoculation)	16.	Glucose (5%) + Charcoal powder + *Rhizobium*	Glu (5%)+ Coal_P+R
	17.	Glucose (12.5%) + Charcoal powder + *Rhizobium*	Glu (12.5%)+ Coal_P+R
	18.	Glucose (25%) + Charcoal powder + *Rhizobium*	Glu (25%)+ Coal_P+R
	19.	Glucose (5%) + Sawdust + *Rhizobium*	Glu (5%)+ SawD+R
	20.	Glucose (12.5%) + Sawdust + *Rhizobium*	Glu (12.5%)+ SawD+R
	21.	Glucose (25%) + Sawdust + *Rhizobium*	Glu (25%)+ SawD+R

Statistical analysis

The data obtained from the experiment was analyzed using SPSS (WIN. Version 14). PROC GLM a two-way ANOVA followed by the Tukey (HSD) multiple comparison tests were used for repeated testing of paired differences between treatments of adhesive solutions (Arabic gam and glucose) and coating materials (charcoal powder and sawdust), and their interactions. Differences considered significant at the level of $p < 0.05$.

Table 3. A total germinated seeds at each inoculation method on days 3, 4, 6, 8, 10, and 12, the final germination percentage (FGP) and germination index (GI), where bold values with shaded cells indicate the days where maximum germination was reached. Reported values are the average ± standard deviation (n = 4) and the same letters within the same column indicate no significant difference (Tukey p = 0.05).

Treatments	Day 3	Day 4	Day 6	Day 8	Day 10	Day 12	FGP (%)	GI
C	11±1	14±4	14±4	14±4	**15±4**	15±4	73±18 ^{d}	23±5 ^{c}
Coal_P	10±1	10±1	**11±1**	11±1	11±1	11±1	55±5 ^{b}	17±1 ^{c}
SawD	13±2	15±2	16±3	**17±3**	17±2	17±2	85±10 ^{de}	26±3 ^{b}
A_Gum (5%)	10±3	12±3	14±2	14±2	14±2	**15±2**	73±11 ^{d}	21±3 ^{c}
A_Gum (12.5%)	10±2	13±1	13±1	13±1	14±1	14±1	70±5 ^{d}	21±2 ^{c}
A_Gum (25%)	8±2	8±1	9±2	**10±2**	10±2	10±3	52±15 ^{d}	15±3 ^{a}
Glu (5%)	10±4	14±2	**16±2**	16±2	16±2	16±2	80±9 ^{d}	23±2 ^{c}
Glu (12.5%)	8±4	14±1	**15±1**	15±1	15±1	15±1	73±6 ^{d}	21±1 ^{c}
Glu (25%)	9±2	10±2	12±1	**13±0**	13±0	13±1	67±3 ^{a}	19±1 ^{c}
A_Gum (5%)+ Coal_P+R	10±2	15±2	16±1	16±1	**17±2**	17±1	87±3 ^{d}	25±1 ^{c}
A_Gum (12.5%)+ Coal_P+R	8±4	13±2	13±4	15±2	**16±2**	16±2	80±9 ^{d}	22±2 ^{c}

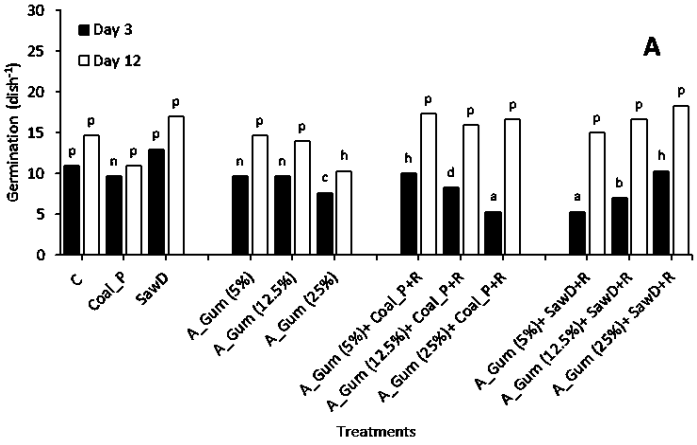
A_Gum (25%)+ Coal_P+R	5±2	13±1	15±2	15±2	16±1	**17±2**	83±8 ^{d}	21±1 ^{c}
A_Gum (5%)+ SawD+R	5±1	12±1	13±1	14±3	**15±3**	15±4	75±18 ^{d}	19±3 ^{c}
A_Gum (12.5%)+ SawD+R	7±2	14±1	16±2	**17±1**	17±1	17±1	83±6 ^{d}	23±1 ^{c}
A_Gum (25%)+ SawD+R	10±2	14±2	18±2	**18±2**	18±3	18±3	92±14 ^{df}	25±2 ^{c}
Glu (5%)+ Coal_P+R	11±2	15±1	17±1	17±2	**18±2**	18±2	88±10 ^{dg}	25±1 ^{c}
Glu (12.5%)+ Coal_P+R	13±3	15±2	16±1	17±1	**18±2**	18±2	92±10 ^{df}	27±3 ^{c}
Glu (25%)+ Coal_P+R	9±1	12±2	14±1	15±2	**16±2**	16±1	80±5 ^{d}	22±1 ^{c}
Glu (5%)+ SawD+R	7±2	7±2	9±2	10±2	**11±2**	11±2	57±7 ^{c}	15±3 ^{a}
Glu (12.5%)+ SawD+R	12±4	14±2	17±1	17±1	**18±1**	18±1	88±3 ^{dg}	25±2 ^{c}
Glu (25%)+ SawD+R	9±4	11±5	14±2	**16±2**	16±2	16±1	82±3 ^{d}	22±4 ^{c}

Results

Seeds germination

Analysis of variance showed that adhesive solution and coating materials and their interactions significantly ($p < 0.05$) affected seeds germination. The germination record on days 3, 4, 6, 8, 10 and 12 is shown in **Table 3**. The first germination was observed 3 days after sowing in all treatments with different numbers of germinated seeds among them ranging between 5±1 and 13±3 (**Fig.1 A, B**). On day 3, both types of adhesive solutions of 5% resulted in higher seeds germination either with or without coating with charcoal powder and ranged from 10±2 to 11±2.

At the same concentration, both adhesive solutions resulted in lowering germination significantly ($p < 0.05$) in the treatments of sawdust coated seeds (5±1 and 7±2). At 12.5%, glucose solution with charcoal powder or sawdust resulted in higher seeds germination (13±3 and 12±4, respectively) compared with Arabic gum solution treatments and the control. At 25%, seeds germination number was mostly lower in all treatments with or without coating materials in comparison with treatments of 12.5%. In 25% Arabic gum treatment, seeds germination significantly ($p < 0.05$) reduced in charcoal powder coated seeds (5±2) in comparison with sawdust coated seeds (10.3±2.1). On day 12, the germination increased significantly ($p < 0.05$) compared to day 3. However, the germination pattern was similar to what was observed on day 3, which means it was higher in the same treatments that are reported on day 3 (**Fig. 1A, B**). Applying sole sawdust without adhesive solution enhances seeds germination on days 3 (13±2) and day 12 (17±2) in comparison with the control i.e. 11±1 and 15±4, respectively (**Fig. 1A, B**). Whereas applying sole charcoal powder reduced germination significantly ($p < 0.05$) on day 3 and insignificantly on day 12. Applying 25% sole adhesive solution (Arabic gam and glucose) on the seed's surface without coating reduced germination significantly ($p < 0.05$) on day 3 and on day 12 in comparison with the control.



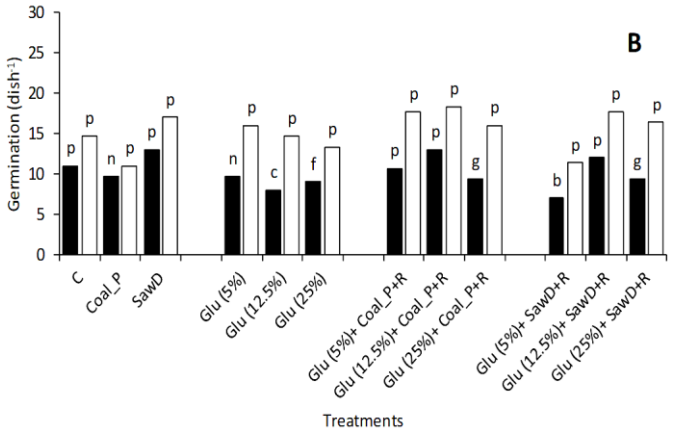


Fig. 1. A total germinated seeds on days 3 and 12 after inoculation using different inoculation methods. Figures (A) and (B) show the germination after applying Arabic gum and glucose solution as an adhesive substance on the seeds, respectively, with and without coating with charcoal powder or sawdust. The same letters on bars in each figure indicate no significant differences among them (Tukey p = 0.05).

FGP and GI

The adverse effect of the inoculation method on seeds germination was significantly ($p<0.05$) confirmed by the reduction in FGP and GI. Low GI indicates slower germination and higher values mean faster germination, and the results are shown in **Table 3**. Germinations reached their maximum at different times among the treatments and were mostly reached on day 10 (values shown in **Table 3** as bold font with shaded cells). Treatment of sole sawdust gives the highest FGP and GI compared to the treatment of sole charcoal powder and the control. Furthermore, in the treatments of low concentration of sole Arabic gum (5%) and glucose solution (5%), FGP and GI were significantly ($p < 0.05$) higher in comparison with high concentrations (12.5% and 25%). The combination of Arabic gum with sawdust increased FGP and GI significantly ($p < 0.05$) compared with treatment of Arabic gum and coal powder. The combination of glucose solution (5% and 12.5%) with charcoal powder significantly ($p < 0.05$) increased FGP and GI more than 25% glucose solution did. In the case of the combination of glucose solution with sawdust, the highest FGP and GI were obtained in the treatment of 12.5%.

Discussion

Inoculating alfalfa seeds with the right Rhizobium bacteria strain prior to planting is an important step to enhance root nodulations and improve alfalfa growth and yield. In the present study, germination of alfalfa seeds (*Medicago sativa* L.) was evaluated after inoculation with local Rhizobium strain using different adhesive solutions and different coating materials. The results showed that using a high concentration of sole Arabic gum and glucose solution (25 %) as an adhesive solution on the seed's surface without coating reduced seed germination and final germination percentage. A similar effect was observed in one of our previous studies where coating alfalfa seeds with Arabic gum (25%) and charcoal powder resulted in lowering the final germination percentage compared with uncoated seeds (control) [10]. This adverse effect may be explained by the fact that Arabic gum contains a high percentage of complex sugars [23], and the white sugar consist of simple carbohydrates, which will increase the viscosity of the adhesive solutions and will ultimately hinder the emergence of seeds. Arabic

gum and white sugar are natural materials, the first exudates by the stems and branches of Acacia Senegal and consists mainly of high molecular weight polysaccharides [23], while the second is made from refined sugar cane [24].

Using lower concentrations of Arabic gum or glucose solutions (i.e. 5 and 12.5 %) gave better germination, which confirms the principle of viscosity in reducing seed germination. Moreover, a high concentration of sole Arabic gum and glucose solution might lead to an increase in the hardness of the seed coat and blog seed hilum resulting in a delay the germination. However, in previous studies concentrations of 45 and 40 % of Arabic gum has been used for inoculation [11, 15] and none of these studies noticed any adverse effect on germination. Furthermore, the combination of adhesive solutions with charcoal powder and sawdust as coating materials improved seeds germination even in adhesive solution treatments of high concentration. This was probably due to the fact that these coating materials absorb part of the adhesive solution on the seed's surface prevent clogging the hilum of the seeds, and reduce the effect of the adhesive solution on vigor. It has been reported that charcoal and sawdust have high adsorbent ability [25, 26]. The enhancement of seeds germination after the application of coating materials was more pronounced in Arabic gum + sawdust treatment (25%) and charcoal powder + glucose solution (12.5%) treatment. The application of coating materials on the seed's surface may control the germination time and emergence by influencing seed imbibition or altering seedbed water availability and gaseous exchange [27]. Furthermore, the particles size of the charcoal powder used in the present study is smaller than sawdust, which will probably will manipulate seed coat and seedbeds, and also affect germination. It has been reported that coating the seed's surface with small particles will provide higher physical resistance and limited gas and water exchange, whereas larger particles increase porosity, but reduce mechanical integrity and coat resilience [28, 29]. The efficiency of sawdust in absorbing water may explain higher seed germination in sawdust treatment, as the high moisture that sawdust provides around the seeds may stimulate rapid germination.

Conclusion

The method used to perform alfalfa seed inoculation with Rhizobium bacteria should be chosen carefully to ensure high seed germination and establishment. From the results of this study, it is recommended to use adhesive solutions Arabic gum of 5 and 25% with charcoal powder and sawdust, respectively. Whereas using glucose solution concentration of 12.5% is recommended either with charcoal powder or sawdust. Further investigation is needed to identify the best seed inoculation methods to achieve an effective and a successful symbiosis in the same soil type used in the present study.

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