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Research Article

THE EFFECTS OF DIFFERENT CARRIER MATERIALS ON SOME MORPHOLOGICAL CHARACTERISTICS OF *RHIZOBIUM PHASEOLI*

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ABSTRACT. The study aimed to identify inexpensive and suitable carriers for microbial inoculants as an alternative to chemical fertilizers. The study investigated the effect of different carrier media on rhizobium bacteria's morphological properties. Alternative carrier media to peat were sought to enable longer viability of these bacteria and for more practical and easy agricultural application. Nine different materials (peat, perlite, pumice, mushroom compost, sawdust, barley powder, sludge, coal, K-Humate) and some combined mixtures of these materials (perlite + K-Humate, pumice + K-Humate, and sawdust + K-Humate) were used in the research. Peat was used as the reference carrier material. After the necessary procedures, rhizobium bacteria were inoculated onto the carrier materials and incubated for 6 months at 28±2ºC. After incubation, the rhizobium bacteria were isolated from the carrier media and grown in agar and broth culture media. Some morphological properties of the bacteria were determined on the YMA culture medium. After incubation, K-Humate, coal, barley powder, and sawdust materials were observed to support bacterial growth and were therefore identified as alternative carrier materials to peat in terms of the morphological properties of *Rhizobium* bacteria. Bacteria isolated from K-Humate showed weak growth at high salt concentrations (4%). All bacteria isolated from the carriers were observed to grow at 30°C and pH values ranging from 3 to 10. Bacteria isolated from sawdust, barley powder, coal, and K-Humate materials exhibited antibiotic resistance. The study demonstrated that K-Humate, coal, barley powder, and sawdust materials can be used as alternative carriers for bacteria instead of peat.

Keywords: *Inoculation, rhizobium, carrier material, morphological properties*

INTRODUCTION

Microorganisms that can be used as microbial fertilizers need to be easily producible, affordable, exhibit high metabolic activity, and have the ability to be stored for long periods. Research should be conducted to include multiple beneficial organisms in biological fertilizers, and there should be an emphasis on creating new combinations. Biological fertilizers can convert important nutrients from an unavailable form to an available form through biological processes [1]. Some bacteria are involved in the fixation of atmospheric nitrogen. In contrast, others, such as the heterotrophic, aerobic microorganism *Azotobacter* spp., widely distributed in different environments such as soil and water in free-living and non-symbiotic states, can fix nitrogen [2]. When planting in soils where natural rhizobium bacteria are absent or where nitrogen-fixing bacteria are scarce, inoculating legume seeds with a culture of nodulating bacteria is crucial for maximizing yield and saving on nitrogen fertilizer. The use of microbial inoculants as a biofertilizer increases crop yield, is environmentally friendly, and can be used as an alternative to or in conjunction with inorganic nitrogen fertilizers [3; 4]. Commercial legume inoculant formulations include powder or granular carriers, liquid culture, or liquid formulations [5]. Peat is a chosen carrier for agricultural applications [6]. An

appropriate carrier material for transporting microorganisms should have specific characteristics, such as high water-holding capacity, physical and chemical integrity, no toxic compounds to microbial strains, and environmental safety. At the same time, these materials should have an easily adjustable pH or be close to a neutral pH, and be locally abundant at an appropriate cost [7; 8]. Liquid inoculants do not require a carrier replacement or preparation, making the production process straightforward and easy to apply to seeds or fields. However, bacterial viability in such inoculants and inoculated seeds is worse due to the lack of carrier protection [9; 10]. Different types of composts have been found to provide an alternative to peat as a microbial carrier for organic and mineral materials [11; 12]. Three main factors affect bacterial viability in seeds: drying, toxic secretions from the seed coat, and high temperature [13]. Daza et al. [14] investigated the growth and viability of four rhizobium species as a bacterial inoculant using perlite as a carrier. Peat and perlite-based inoculants were evaluated in the study, and bacterial viability was similar for all strains in both carriers. In the end, perlit-based inoculants produced similar results to peat-based inoculants regarding nodule number, nodule dry weight, yield, and nitrogen content in bean and soybean plants. Ferreira and Castro [8] used waste from the mushroom industry as a carrier for producing legume inoculants, using a peat-based carrier as a control. High bacterial viability (approximately 10^9 bacteria g^{-1}) was found in all carriers 41 days after inoculation, and rhizobial viability remained constant during storage (450 days) (10^8-10^9). In conclusion, carriers are important for the production of rhizobium inoculants. Khavazi et al. [15] used perlite, malt waste, sugarcane bagasse, coal, and rice husk as carriers for soybean plants. They have also compared sterilization methods (autoclaving and gamma irradiation) in the study. They observed that all carriers supported the growth of rhizobium during the six months. Initially, the rhizobium population was found to be larger in the carrier treated with gamma irradiation than in the one treated with autoclaving. Still, after 6 months, this response was only significant in the mixture of perlite and sugar cane bagasse. They determined that simple sterilization and incubation-free production could produce high-quality and inexpensive inoculants using perlite-based carriers. Albareda et al. [16] used 6 different organic and inorganic carrier materials (grape pomace, mushroom compost, hydrated aluminum silicate, pumice, perlite, and amorphous silica) as alternatives to peat for rhizobium inoculation. Different liquid culture media were also evaluated with mannitol or glycerol as a carbon source. As a result, mushroom compost and perlite gave good results in supporting bacterial growth, similar to peat, and maintained the viability of the inoculated strains for a long time. In some liquid formulations, it was found that the growth and viability of soybean rhizobium strains were sufficient for at least 3 months of storage. Ashok et al. [17] used 4 carrier materials (bagasse, peat, charcoal, and coal) and 2 bacterial inoculants (*Rhizobium trifolii* (MTCC-905) and *Rhizobium meliloti* (MTTC-100)) in their research. Both bacterial strains were inoculated separately into all carriers. The bacterial population was determined in each carrier for up to 6 months of storage. Bagasse showed maximum population for both strains. The minimum population was observed in the carrier material coal. The present study suggests that bagasse can be used as an effective and inexpensive carrier material. Junofy Anto Rozarina et al. [18] used 4 materials (processed lignite, lignite mixed with 0.1% sludge, burnt rice husk, and vermiculite) in their research. They inoculated all carriers with rhizobium inoculants. They worked on population load, contamination level, pH, and moisture content at weekly intervals for 4 weeks. As a result, they determined that vermiculite can be used as a better carrier instead of processed lignite. Our study aims to develop cheap and suitable microbial inoculants to be used as an alternative to chemical fertilizers. For this purpose, we aimed to identify different carrier materials as an alternative to peat material for

bacteria by inoculating rhizobium bacteria into different carrier environments and determining the properties of the materials after six months of incubation.

MATERIAL AND METHOD

Carrier Materials

The materials used in the study were peat, perlite, pomza, mushroom compost, sawdust, barley powder, sludge sludge, coal, K-Humate, and combined applications, which were perlite+K-Humate, pumice+K-Humate, and sawdust+K-Humate. Peat material was used as a reference carrier.

Sterilization of Carrier Materials

The materials were air-dried, ground after being cleaned of debris and waste, and passed through a 250 μm sieve. The mixed materials were blended in a 1:1 ratio based on dry weight. All carrier materials' pH was adjusted to 6.5-7.5 (with H_2SO_4). After being moistened to a non-sloppy consistency, the carriers were placed into polyethylene bags (100 g) that were sealed with heat. Following a small puncture with a needle, they were sterilized for 2 hours at 121^{o}C in an autoclave.

Reproduction and Purification of Rhizobium Bacterial Strains

The strain used in the study was propagated on YMA (yeast mannitol agar) medium. For this step, 0.5 mL of the culture suspension was spread uniformly onto the YMA medium prepared in petri dishes (Ø 9 cm) and incubated at 28±2°C for 7 days. After this period, the smallest colony of *Rhizobium* spp. that had grown on the medium was transferred to tubes with its own medium, and the strain was purified by streaking it on the same medium [19].

Incubation of Carrier Materials

After sterilization, the bags were inoculated with 10 ml of rhizobium suspension ($1x10^8$ CFU) in a sterile environment without contamination. Following inoculation, the bags were shaken for a homogeneous distribution and then incubated at 28±2^{o}C for approximately 6 months.

Determination of Some Morphological Characteristics of Strains After Incubation

The quality control of the inoculants obtained in the study was carried out qualitatively and quantitatively during production and storage [20]. The qualitative control of the inoculants investigated whether there was any foreign microorganism in the inoculant [21].

Colony Morphology

The bacteria, inoculated onto YMA medium to obtain single colonies, were incubated at 28±2°C for 4-5 days until their growth was ensured. At the end of this period, the shape, color, and mucous formation of the bacteria grown on the petri dishes were examined [22].

Sodium Chloride Tolerance

The growth of bacteria isolated from carrier materials was examined in activated nutrient broth with different concentrations of NaCl (0%, 0.5%, 1%, 2%, 4%, and 8%) for 3-5 days at 28±2°C [22].

Temperature Tolerance

The growth of microorganisms in sterilized petri dishes containing activated nutrient medium with adjusted bacterial concentrations was examined at 3-5 days at 28±2ºC with the addition of different concentrations of NaCl, including 0%, 0.5%, 1%, 2%, 4%, and 8% [22]. Afterward, the bacteria were cultured on an activated nutrient medium with a specified bacterial count, and their growth was observed for seven days in incubators set to 4, 15, 30, and 40°C [23].

pH Tolerance

30 µl from each bacterial culture was inoculated into 3 ml liquid yeast mannitol broth (YMB) media tubes adjusted to pH values of 3, 4, 5, 6, 6.5, 7, 7.5, 8, 9, and 10. The growth of microorganisms was observed for 3-5 days at 28±2ºC [22].

Resistance to Antibiotics

Different amounts of erythromycin (30 and 66 µ/ml), streptomycin (40, 80, 100 µ/ml), and chloramphenicol (20, 50, 100, 200 µ/ml) were added to YMA medium that was cooled down to 45ºC. The medium was then filtered through a 0.45 µm disposable filter and poured into sterile petri dishes. Bacterial strains were streaked onto the medium and incubated at 28±2ºC for 7 days to observe their growth [24].

Resistance to Heavy Metals

YMA agar was cooled to 45°C and separately supplemented with $CuCl_2 \cdot 2H_2O$ at 50 and 100 µ/ml, $HgCl_2$ at 2.5, 5, and 10 µ/ml, and $CdCl_2$ at 5, 10, and 20 µ/ml, each passed through a 0.45 µm disposable filter, and poured into sterile petri dishes. Bacterial cultures were streaked onto the agar plates and incubated at 28±2°C for 3-4 days to observe their growth [24].

RESULTS AND DISCUSSION

Chemical Properties of Carrier Materials

The chemical properties of the carrier materials are given in Table 1. The pH values of the materials vary between 6.5 and 9.8, with the lowest pH value being determined in sawdust and the highest pH value in K-humate material. When considering the salt content, pumice and perlite have the lowest salt content (0.2 dSm^{-1}), while K-humate material has the highest salt content (24.9 dSm^{-1}). The water holding capacities of the tested materials also showed differences, with the highest in mushroom compost and the lowest in K-humate. While the highest organic matter content was determined in sawdust material, the highest organic carbon content was found in coal material. With regard to nitrogen and phosphorus content, sewage sludge is in the lead (Table 1).

Table 1. *Physical and chemical properties of some carrier materials used in the study*

	Properties	Sawdust	Barley Powder	Pumice	Mushroom Compost	K-Humate	Coal	Perlite	Sewage Sludge	Method
	pH	6.52	6.7	7.6	7.6	9.8	7.6	7.1	7.8	[25]
(dSm^{-1})	**EC**	0.34	4.3	0.2	12.8	24.9	0.4	0.2	5.8	[25]
%	**FC**	69.7	81.5	42.2	59.8	47.2	23.1	89	65.9	[26]
	FP	47.7	72.5	22	36.6	44.8	7.4	79.4	57.2	[26]
	OM	99.5	84.9	4.6	62.2	66.7	78	2.5	55	[27]
	OC	56.4	44.5	eseri	32.2	48.7	62.6	0.1	31.9	[27]
	TN	0.22	1.3	eseri	1.9	1.14	1.8	0.2	4.3	[27]
$mgkg^{-1}$ (WS)	**Ca**	1253	1803	743	40954	13740	1116	687	41397	[27]
	K	403	17986	282	19367	84761	220	175	12390	[27]
	Mg	135	333	78.8	4547	625	233	60	9563	[27]
	Na	171.7	583	210	1980	12237	459	288	1015	[27]
	P	50.8	1658	23.8	699	62.2	22.1	17.2	12811	[28]
	Cu	4.2	9.4	3.4	28.6	34.4	3.2	3.3	399	[29]
	Fe	12.9	49.3	117	45.5	7271	27.7	20.8	5320	[29]
	Mn	7.5	9.2	2.8	76.1	55.5	2.3	1.7	357	[29]
	Zn	3.5	14.8	1.9	13.7	20.8	1.6	1.5	2155	[29]

(FC: Field capacity; WP: Fading point; OM: Organic matter; OC: Organic carbon; TN: Total nitrogen; WS: water-soluble; T: total

Some Morphological Characteristics of Strains After Incubation

It sounds like you are describing an experiment that aimed to test different organic materials as potential carriers for rhizobium bacteria, which can help plants to fix nitrogen. Peat is a common carrier material for rhizobium bacteria, but the use of peat has environmental concerns, so alternative materials are being explored. In this experiment, the researchers tested various organic materials, such as pearlite, pumice, mushroom compost, sawdust powder, and barley powder, as well as waste sludge, coal, and K-Humate (a type of humic acid) as potential carrier materials for rhizobium bacteria. The researchers used a dilution process to check whether the bacteria could grow in these environments, and then they prepared petri dishes with a YMA medium to culture the bacteria. After 7-10 days of incubation, the researchers observed colony development in all of the organic carrier materials and combined applications they tested, including the ones with K-Humate added. This suggests that these materials could be viable alternatives to peat carrying rhizobium bacteria. It's worth noting that this experiment only tested the ability of the organic materials to support rhizobium bacteria growth in a lab setting. Further research would be needed to determine how effectively these materials promote plant growth in a real-world agricultural setting.

Colony Morphology

Rhizobium bacteria inoculated in different media were tested after a 6-month incubation period to determine alternative carrier materials to peat. Dilution was carried out initially by taking samples from these materials and checking whether the organism grew in these media. After dilution, YMA medium was prepared in petri dishes, and rhizobium bacteria were added to the petri dishes in a sterile environment. After inoculation, the Petri dishes were incubated at 28±2ºC for growth. Colony growth was observed after 7-10 days of incubation. It was observed that rhizobium bacteria grew in all organic carrier materials and combined applications (peat, perlite, pumice, mushroom compost, sawdust, barley dust, sewage sludge, coal, K-Humat, perlite+K-Humat, pumice+K-Humat and sawdust+K-Humat). When the colonies were examined, it was determined that bacteria isolated from peat, K-Humat, coal, barley powder, and sawdust powder were generally round colonies with 3-5 mm diameter.

Sodium Chloride Tolerance

Different concentrations of NaCl (0%, 0.5%, 1%, 2%, 4%, and 8%) were added to adjusted activated media to investigate the growth of bacteria at 28±ºC for 3-5 days [22]. In general, bacterial growth was not observed in concentrations of 4% and 8% salt in the carrier media isolates used in the study. The best growth of rhizobium bacteria was observed in media with salt concentrations of 0-0.5% and 1% at the applied doses. The best-growing bacteria in media with salt concentrations of 0%, 0.5%, and 1% were isolated from barley dust, sawdust, coal, and K-Humat (Table 2). On the other hand, weak growth of bacteria was observed in petri dishes when bacteria were isolated from perlite, pumice, mushroom compost, treated sewage sludge, and combined applications and inoculated into nutrient media with different salt concentrations (0%, 0.5%, 1%). When bacteria were inoculated into YMA media with increasing salt concentrations, their growth was generally weakened as the salt concentration increased. In contrast, it was determined that bacteria showed better growth in nutrient media with 0.5% NaCl concentration. It was determined that rhizobium bacteria isolated from wood shavings, barley powder, coal, K-Humate, and K-Humate-based combined applications showed better growth at a concentration of 0.5% NaCl (Table 2). Daza et al. [14] reported similar

results for both carriers regarding rhizobium viability in observations made for peat- and perlite-based inoculants.

Table 2. *Growth of bacteria isolated from some carrier materials at different salt concentrations*

	NaCl %					
	0	**0.5**	**1**	**2**	**4**	**8**
Peat	+	+	+	+	±	-
Perlite	±	±	±	±	-	-
Pumice	±	±	±	±	-	-
Mushroom Compost	±	±	±	-	-	-
Sawdust	+	+	+	±	-	-
Barley Powder	±	+	+	±	-	-
Sewage Sludge	±	±	-	-	-	-
Coal	+	+	+	±	-	-
K-Humate	+	+	+	±	±	-
Perlite+K-Humate	±	+	±	±	-	-
Pumice+K-Humate	±	+	±	±	-	-
Sawdust+K-Humate	±	+	±	±	-	-

+ : growth observed ± : weak observed - : no growth

Temperature Tolerance

Bacteria isolated from carrier materials were inoculated into sterilized petri dishes, and their growth was examined for seven days in incubators set to 4, 15, 30, and 40°C [23]. Since rhizobium bacteria effectively tolerate stress conditions, some growth was observed at all temperature levels. It was determined that the optimum temperature for growth of bacteria isolated from carrier materials was 30°C.

Table 3. *Growth of bacteria isolated from carrier materials at different temperatures*

	^{0}C			
	4	**15**	**30**	**40**
Peat	±	+	+	±
Perlite	-	-	±	-
Pumice	-	-	±	-
Mushroom Compost	-	-	±	-
Sawdust	-	±	+	±
Barley Powder	-	±	+	±
Sewage Sludge	-	-	±	-
Coal	-	+	+	±
K-Humate	±	+	+	±
Perlite+K-Humate	-	+	+	-
Pumice+K-Humate	-	±	+	-
Sawdust+K-Humate	-	±	+	-

+ : growth observed ± : weak observed - : no growth

On the other hand, the weakest growth was observed at 40°C. The best temperature at which bacteria could grow was determined as 30°C (Table 3). Bacterial growth of strains isolated from various carrier materials at different temperatures was investigated, particularly at 30°C, considered the optimal temperature for most microorganisms. It was determined that bacteria isolated from different carrier materials such as barley powder, sawdust powder, coal, K-Humate, and K-Humate mixtures showed the best growth at 30°C (Table 3). After being inoculated into petri dishes and incubated at 4°C, no growth

was observed in any carrier material, except for weak growth in bacteria isolated only from the K-Humate material. Ferreira and Castro [8] used mushroom industry waste as a carrier and found high bacterial viability in all carriers 41 days after inoculation.

pH Tolerance

Bacterial cultures isolated from different carrier media were inoculated with 30 µl from each into 3 ml YMB broth adjusted to pH 3.0, 4, 5, 6, 6.5, 7, 7.5, 8, 9 and 10, and incubated at 28±30ºC for 3-5 days to observe microbial growth [22]. The bacteria inoculated onto YMA medium generally exhibited better growth between pH 5 and 9, whereas weak growth was observed at other pH values. Rhizobium bacteria isolated from carrier materials showed better growth in petri dishes with pH between 6.5-7.5 (Table 4). Bacteria isolated from carrier materials such as sawdust, barley powder, K-Humate, Perlite+K-Humate, Pumice+K-Humate, and sawdust+K-Humate were found to grow well in media with pH ranges of 5, 6 and 7. However, when inoculated onto media with pH ranges of 3 and 4, bacteria isolated from perlite, pumice, mushroom compost, and sewage sludge did not show colony growth. Moreover, bacteria isolated from these materials exhibited weak growth in media with pH ranges of 5, 6, and 7. Bacteria isolated from K-Humate generally showed either growth or weak growth in petri dishes with pH ranges of 3, 4, 5, 6, 7, 9, and 10 (Table 4). Somasegaran and Hoben [19] determined that the optimal pH levels for rhizobium growth varied depending on the rhizobium species (pH 5.8-7.2). Rebah et al. [30] reported that water-holding capacity and pH were the primary factors affecting the survival of strains in carriers.

Table 4. *Growth of bacteria isolated from various carrier materials at different pH values*

	pH									
	3	**4**	**5**	**6**	**6.5**	**7**	**7.5**	**8**	**9**	**10**
Peat	±	±	+	+	+	+	+	+	±	±
Perlite	-	-	-	±	±	±	±	-	-	-
Pumice	-	-	±	±	±	±	±	-	-	-
Mushroom Compost	-	-	-	±	±	±	±	±	±	-
Sawdust	-	-	+	+	+	+	+	±	±	-
Barley Powder	-	±	+	+	+	+	+	±	±	-
Sewage Sludge	-	-	-	±	±	±	±	-	-	-
Coal	-	±	±	+	+	+	+	+	+	±
K-Humate	±	±	+	+	+	+	+	±	±	±
Perlite+K-Humate	-	±	+	+	+	+	+	±	±	-
Pumice+K-Humate	-	-	+	+	+	+	+	-	-	-
Sawdust+K-Humate	-	±	+	+	+	+	+	±	±	-

+ : growth observed ± : weak observed - : no growth

Resistance to Antibiotics

The determination of bacterial resistance to antibiotics was performed using the disk diffusion test. YMA medium was prepared and bacterial inoculation was performed using a sterile loop. Prior to application of antibiotic-soaked disks, petri dishes were left for five minutes to allow absorption of the inoculated bacterial suspension and evaporation of excess moisture from the surface, with a waiting time not exceeding 15 minutes. Disks were placed on the agar surface 15 minutes after inoculation. The disks were pressed down onto the agar surface to ensure complete contact. Within 15 minutes after disk

placement, the petri dishes were placed in an incubator set at 28±2°C with the lids facing down. After incubation, the diameter of the area showing complete inhibition, called the "zone diameter," was measured by using a ruler or caliper from the underside of the petri dish, including the disk zone diameter. The antibiotic resistance of bacteria isolated from some carrier materials, such as sawdust, barley powder, mushroom compost, perlite, pumice, coal, K-Humate, perlite+K-Humate, pumice+K-Humate, sawdust+K-Humate, and sewage sludge, showed different effects (Table 5). The bacteria isolated from the reference culture showed different effects on some antibiotics. The measured zone diameters in petri dishes were determined as 8 mm for streptomycin antibiotic and 7 mm for chloramphenicol antibiotic. Resistance of rhizobium bacteria to erythromycin antibiotic was observed.

Table 5. *Resistance of bacteria isolated from some carrier materials to different antibiotics*

	Antibiotics		
Carrier Materials	**Erythromycin**	**Streptomycin**	**Chloramphenicol**
Peat	+	+	+
Perlite	±	-	-
Pumice	±	-	-
Mushroom Compost	±	-	-
Sawdust	+	+	+
Barley Powder	+	+	+
Sewage Sludge	±	-	-
Coal	+	+	+
K-Humate	+	+	+
Perlite+K-Humate	±	±	-
Pumice+K-Humate	±	±	-
Sawdust+K-Humate	±	±	±

+ : growth observed ± : weak observed - : no growth

After six months of incubation, the resistance of different bacteria isolated from carrier materials to some antibiotics (erythromycin, streptomycin, and chloramphenicol) was measured. According to the research results, the bacteria isolated from carrier materials such as sawdust, barley powder, coal, and K-Humate showed growth in nutrient media containing erythromycin antibiotic. On the other hand, the bacteria isolated from carrier materials such as perlite, pumice, mushroom compost, sewage sludge, and K-Humate combinations showed weak growth in nutrient media containing erythromycin antibiotic (Table 5). Bacteria isolated from perlite, pumice, mushroom compost, and sewage sludge did not show growth in nutrient media containing streptomycin antibiotic. However, weak or moderate growth was observed in other carrier materials. It was determined that bacteria isolated from carrier materials such as sawdust, barley powder, coal, and K-Humate showed growth in nutrient media containing chloramphenicol antibiotic, while bacteria isolated from other materials did not show any growth.

Resistance to Heavy Metals

Heavy metal-added nutrient agar media were poured into sterilized petri dishes. Line inoculations were made on the petri dishes from bacterial cultures, and their growth was examined at 28±2^{o}C for 3-4 days (Rodriguez-Navarro et al., 2000). After examination, weak growth was observed in petri dishes containing heavy metals. The resistance of different bacteria isolated from various carrier materials to some heavy metals (Cu, Hg, Cd) also varied. Generally, bacteria isolated from carrier materials showed weak growth or no growth in nutrient agar media containing certain heavy metals. However, rhizobium bacteria did not grow in the 2nd and 3rd doses of heavy metals (Table 6). Bacteria isolated

from sawdust, barley powder, coal, K-Humate, and K-Humate combinations generally showed weak growth in the presence of heavy metals at the first dose (Cu (50 µ/ml), Hg (2.5 µ/ml), Cd (5 µ/ml)) (Table 6). Yakanto and Shutsrirung [31] evaluated the potential use of solid-based inoculants, namely perlite, vermiculite, and a mixed medium, as carriers for Rhizobium CIAT899 and Azospirillum VAs087 in this study. The results showed that different carriers had varying effects on the growth and survival of CIAT899 and VAs087 during a 120-day incubation period. The results demonstrated that using mixed media as carriers was suitable for producing bacterial inoculants.

Table 6. *Resistance of Bacteria Isolated from Some Carrier Materials to Different Heavy Metals*

Carrier Materials	Cu (µ/ml)		Hg (µ/ml)			Cd (µ/ml)		
	50	100	2.5	5	10	5	10	20
Peat	±	-	+	±	-	±	-	-
Perlite	-	-	-	-	-	-	-	-
Pumice	-	-	-	-	-	-	-	-
Mushroom Compost	-	-	-	-	-	-	-	-
Sawdust	±	-	±	-	-	±	-	-
Barley Powder	±	-	±	-	-	±	-	-
Sewage Sludge	-	-	-	-	-	-	-	-
Coal	±	-	±	-	-	±	-	-
K-Humate	±	-	±	-	-	±	-	-
Perlite+K-Humate	±	-	±	-	-	±	-	-
Pumice+K-Humate	±	-	±	-	-	±	-	-
Sawdust+K-Humate	±	-	±	-	-	±	-	-

+ : growth observed ± : weak observed - : no growth

CONCLUSION

New alternative solutions have been developed to reduce the negative effects of chemicals on human and environmental health. Biological fertilization has gained importance in reducing chemical fertilization in agricultural production. The microorganisms to be used as biological fertilizers should be easy to produce, inexpensive, show high metabolic activity, and provide the possibility of long-term storage. Due to its protective properties, rhizobium bacteria are generally preferred to multiply and store in peat material. However, in some countries, such as ours, natural peat resources are scarce, or peat mines are protected by environmental laws in some countries, and their use for various purposes is prohibited. Due to these restrictions, alternative rhizobium carrier materials that are cheaper and easier to find have been investigated in different countries. Different carrier materials have shown the potential to replace the standard medium and peat, widely used in inoculant production. Although these materials' presence, cost, and breeding limit their use in inoculation, they provide an alternative for recycling materials that support sustainable agriculture. Generally, different carrier materials have shown that coal, sawdust, and barley powder can be used as carrier materials instead of peat as alternatives in terms of pH, salinity, and organic matter. As a result, it was observed that carrier materials such as sawdust, barley powder, K-Humate, and coal provide results as good as peat in terms of supporting bacterial growth. Balanced biological fertilization is important for obtaining quality products, soil reclamation, and the health of humans who consume these products.

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