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Issue 0031: 2009	Issue 0032: 2009	Issue 0033: 2009	Issue 0034: 2009	Issue 0035: 2009	Issue 0036: 2009
Issue 0026: 2009	Issue 0027: 2009	Issue 0028: 2009	Issue 0029: 2009	Issue 0030: 2009	Issue 0031: 2009
Issue 0021: 2008	Issue 0022: 2008	Issue 0023: 2008	Issue 0024: 2008	Issue 0025: 2009	Issue 0026: 2008
Issue 0016: 2008	Issue 0017: 2008	Issue 0018: 2008	Issue 0019: 2008	Issue 0020: 2008	Issue 0021: 2008
Issue 0011: 2007	Issue 0012: 2007	Issue 0013: 2008	Issue 0014: 2008	Issue 0015: 2008	Issue 0016: 2007
Issue 0006: 2007	Issue 0007: 2007	Issue 0008: 2007	Issue 0009: 2007	Issue 0010: 2007	Issue 0005: 2007
Issue 0001: 2007	Issue 0002: 2007	Issue 0003: 2007	Issue 0004: 2007	Issue 0005: 2007	Issue 0006: 2007

Article #	WORLD ACADEMY OF SCIENCE, ENGINEERING AND TECHNOLOGY ISSUE 53 MAY 2011	Page #
1	Vertex Configurations and Their Relationship on Orthogonal Pseudo Polyhedra <i>Jefri Marzal, Hong Xie, Chun Che Fung</i>	1
2	Investigation on Some Ergonomics and Psychological Strains of Common Militarism Protective Clothing <i>A. Ashjaran, A. Rashidi, R. Ghazi-Saeidi</i>	9
3	Future Population Development in Kazakhstan <i>A. Jazybayeva</i>	12
4	WiMAX RoF Design for Cost Effective Access Points <i>Haruka Mikamori, Koyu Chinen</i>	15
5	The Effect of Selective Cyclooxygenase (COX) Inhibitors on Japanese Medaka (Oryzias latipes) Reproduction Parameters <i>Agata Kowalska, Radoslaw K. Kowalski, Zdzislaw Zakes</i>	19
6	Integration of Fixed and Variable Speed Wind Generator Dynamics with Multimachine AC Systems <i>A.H.M.A.Rahim</i>	24
7	A Comparative Study on Eastern and Western Wedding Ceremonies in Korean Films and Hollywood Films <i>Timothy Yoonsuk Lee, Joonhyoup Lee, Yoojin Chung</i>	32
8	A Confucianism Observed in Disaster Films of East Asia <i>Timothy Yoonsuk Lee, Jinhwan Yu, Somi Nah</i>	42
9	Power Control in a Doubly Fed Induction Machine <i>A. Ourici</i>	51
10	Extended Minimal Controller Synthesis for Voltage-Fed Induction Motor Based on the Hyperstability Theory <i>A. Ramdane, F.Naceri, S. Ramdane</i>	55
11	Passive Flow Control in Twin Air-Intakes <i>Akshay R. Paul, Pritanshu Ranjan, Ravi R. Upadhyay, Anuj Jain</i>	60
12	A Microcontroller Implementation of Model Predictive Control <i>Amira Abbes Kheriji, Faouzi Bouani, Mekki Ksouri, Mohamed Ben Ahmed</i>	68
13	Determinants Which Influence The Tourism Investments In Greece <i>Anastasios Alexandridis</i>	75
14	Extending the Conceptual Neighborhood Graph of the Relations for the Semantic Adaptation of Multimedia Documents <i>Azze-Eddine Maredj, Nourredine Tonkin</i>	80
15	Ethics in the Technology Driven Enterprise <i>Bobbie Green, James A. Nelson</i>	84
16	A Critical Study of Media Profiling on Society's Social Problems from a British Perspective <i>Cj Gleton Matthews Cn Jacobs, Kogilah Narayanasamy</i>	88
17	Towards An Extended International HRM Model for Emerging Multinational Enterprises <i>Connie Zheng</i>	92
18	The Association between the Firm Characteristics and Corporate Mandatory Disclosure the Case of Greece <i>Despina Galani, Anastasios Alexandridis, Antonios Stavropoulos</i>	101
19	Featured based Segmentation of Color Textured Images using GLCM and Markov Random Field Model <i>Dipti Patra, Mridula J</i>	108
20	Goodwill in the Current Greek Accounting Environment <i>Efthymios Gravas, Anastasios Alexandridis, Antonios Stavropoulos</i>	114
21	Computing a Time Based Effective Radius-of-Curvature for Roadways <i>Gary D. Cantrell, E. Alex Baylot</i>	120
22	Application-Specific Instruction Sets Processor with Implicit Registers to Improve Register Bandwidth <i>Ginsuan Li, Chiuyun Hung, Desheng Chen, Yiwen Wang</i>	132
23	Comparison of Performance between Different SVM Kernels for the Identification of Adult Video <i>Hajar Bouirouga, Sanaa El Fkihi, Abdeilah Jilbab, Driss Aboutajdine</i>	137
24	Some Applications of Grobner bases <i>Hassan Noori, Abdolali Basiri, Sajjad Rahmany</i>	141
25	Elucidating the Influence of Demographics and Psychological Traits on Investment Biases <i>Huei-Wen Lin</i>	145
26	Use Cuticular Hydrocarbons as Chemotaxonomic of The Pamphagidae Pamphagus elephas (Insecta, Orthoptera) of Algeria <i>M. Bounechada, F. Benia, M. Aiouaz, S. Bouharati , N. Djirar, H. Benamrani</i>	151
27	Towards the Creation of Adaptive Content from Web Resources in an E-Learning Platform to Learners Profiles <i>M. Chaoui, M-T. Laskri</i>	157
	Introduction of Hyperaccumulator Plants with Phytoremediation Potential of a Lead-	

144	Yue-Yang Chen, Hui-Ling Huang, Wan-Yu Yu, Chung-Lun Wei Research of Potential Cluster Development in Pannonian Croatia Mirjana Radman-Funarić, Katarina Potnik Galic	782
145	Effect of Dietary Supplementation of Different Levels of Black Seed ( <i>Nigella Sativa</i> L.) on Growth Performance, Immunological, Hematological and Carcass Parameters of Broiler Chicks R. S. Shewita, A. E. Taha	788
146	Micro-aerobic, anaerobic and two-stage condition for ethanol production by Enterobacter aerogenes from biodiesel-derived crude glycerol Kanokrat Saisaard, Irini Angelidaki, Poonsuk Prasertsan	795
147	Workstation Design based on Ergonomics in Animal feed Packing Process Pirutchada Musigapong, Wantanee Phanprasit	799
148	Face Texture Reconstruction for Illumination Variant Face Recognition Pengfei Xiong, Lei Huang, Changping Liu	802
149	Talent Management and its Use in the Field of Human Resources Management in the Organization of the Czech Republic Petra Horváthová, Irena Durdová	809
150	Flexible Manufacturing System Peter Kostal, Karol Velisek	825
151	The Data Mining usage in Production System Management Pavel Vazan, Pavol Tanuska, Michal Kebisek	830
152	Big Bang – Big Crunch Optimization Method in Optimum Design of Complex Composite Laminates Pavel Y. Tabakov	835
153	Comparative Analysis of Tax Systems: Some Evidence from CEE Countries Marie Paseková, Jirí Strouhal, Lehte Alver, Arturs Praulīns	840
154	Prediction of Compressive Strength of Self- Compacting Concrete with Fuzzy Logic Paratibha Aggarwal, Yogesh Aggarwal	847
155	Image Analysis of Fine Structures of Supercavitation in the Symmetric Wake of a Cylinder Y. Obikane, M. Kaneko, K. Kakioka, K. Ogura	855
156	Using the V-Sphere Code for the Passive Scalar in the Wake of a Bluff Body Y. Obikane, T. Nemoto, K. Ogura, M. Iwata, K. Ono	859
157	Conservation and Repair Works for Traditional Timber Mosque in Malaysia: A Review on Techniques N.K.F. Mustafa, S. Johar, A.G. Ahmad, S.H. Zulkarnain, M.Y. A. Rahman, and A.I. Che Ani	862
158	Application of MADM in Identifying the Transmission Rate of Dengue fever: A Case Study of Shah Alam, Malaysia Nuraini Yusoff, Harun Budin, Salemah Ismail	868
159	A Framework for the Analysis of the Stereotypes in Accounting Nadia Albu, Catalin Nicolae Albu, Madalina Maria Gîrbina, Maria Iuliana Sandu	874
160	The Use of Acid-Aluminium Tolerant <i>Bradyrhizobium japonicum</i> Formula for Nisa Rachmania Mubarik, Tedja Imas, Aris Tri Wahyudi, Triadiati, Suharyanto, Happy Widlastuti	879
161	Design a single-phase BLDC Motor and Finite- Element Analysis of Stator Slots Structure Effects on the Efficiency Abdolamir Nekoubin	883
162	The Low-fertility problem in Hong Kong: Do Mainlanders' Births Help to Rejuvenate Low-fertility Problem? Nancy, Ling Sze Leung	891
163	Study of Microbial Critical Points of Saffron from Farm to Factory in Iran N. Khazaei, M. Jouki, A. Kalbasi, H. Tavakolipour, S. Rajabifar, F. Motamed. Sede, A. Jouki	898
164	Two Dimensional Model for Extraction Packed Column Simulation using Finite Element Method N. Outili, A-H. Meniai	902
165	Software Technology Behind Computer Accounting M. Župan, V. Budimir	907
166	Optimal Allocation Between Subprime Structured Mortgage Products and Treasuries MP. Mulaudzi, MA. Petersen, J. Mukudem-Petersen, IM. Schoeman, B. de Waal, JM. Manale	912
167	Micro Environmental Concrete M. Lánez, M.N. Oudjít, A. Bali	919
168	Regional Role of Higher Education Institutions in Croatia Mirjana Jelec Raguž, Barbara Pisker	924
169	Fatigue Properties of Steel Sheets Treated by Nitrooxidation M. Maronek, J. Barta, P. Palcek, K. Ulrich	930
170	Heart Rate Variability in Responders and Non- Responders to Live-Moderate, Train-Low Altitude Training Michael J. Hamlin, Apinan Manimmanakorn, Gavin R. Sandercock, Jenny J. Ross, Robert H. Creasy, John Hellermans	936
	Heart Rate-Determined Physical Activity in New Zealand School Children: A Cross-Sectional Study	943

# The Use of Acid-Aluminium Tolerant *Bradyrhizobium japonicum* Formula for

Nisa Rachmania Mubarik, Tedja Imas, Aris Tri Wahyudi, Triadiati, Suharyanto, Happy Widiastuti

**Abstract**—Land with low pH soil spread widely in Indonesia can be used for soybean (*Glycine max*) cultivation, however the production is low. The use of acid tolerant soybean and acid-aluminium tolerant nitrogen-fixing bacteria formula was an alternative way to increase soybean productivity on acid soils. *Bradyrhizobium japonicum* is one of the nitrogen fixing bacteria which can symbiosis with soybean plants through root nodule formation. Most of the nitrogen source required by soybean plants can be provided by this symbiosis. This research was conducted to study the influence of acid-aluminium tolerant *B. japonicum* strain BJ 11 formula using peat as carrier on growth of Tanggamus and Anjasmoro cultivar soybean planted on acid soil fields (pH 5.0-5.5). The results showed that the inoculant was able to increase the growth and production of soybean which were grown on fields acid soil at Sukadana (Lampung) and Tanah Laut (South Kalimantan), Indonesia.

**Keywords**—*Bradyrhizobium japonicum*, acid-aluminium tolerant mutant, Tanggamus cultivar soybean, acid soils

## I. INTRODUCTION

LEGUME such as soybean could increase soil fertility and plant productivity. Inoculation of root nodule bacteria, such as *Bradyrhizobium japonicum*, on soybean plantation could enhance soybean quality and its productivity [1], [2]. A wide variation of *B. japonicum* tolerance to acid soil conditions have been reported on many agriculturally important legumes from various countries [3]. Bacteria can increase plant production because its ability to fix nitrogen and provide for plant. Atmospheric nitrogen ( $N_2$ ) was fixed by the bacteria into ammonium ( $NH_3$ ) in a nodule, so that the nitrogen can be used by the plant for its growth [4]. *Bradyrhizobium japonicum* is one of root nodule bacteria that can contribute on plant growth by providing fixed nitrogen in nodules of soybean plants [1]. Some strains of *B. japonicum* were tolerant on an acid condition, even at the pH level 4.0-4.5 [5]. Twenty five strains of *B. japonicum* had been selected for acid tolerance using either solid and broth medium [6]. In the Leonard bottle experiment, indigenous *B. japonicum* strain 11 (BJ 11) significantly could increase dry weight of the upper crop and nitrogen uptake of soybean

cultivar Slamet higher than standard strain from USA, USDA 110 [7]. Furthermore, pot experiments on acid soil pH 4.7 showed that inoculation of BJ 11 could increase plant height, shoot and root weight, number of flowers, pods, seeds, seeds dry weight, and shoot and seed nitrogen content [8]. This research was conducted to study to study the influence of acid-Al tolerant *B. japonicum* formula using peat as carrier on growth of Tanggamus and Anjasmoro cultivar soybean planted on field acid soil (pH 5.0-5.5).

## II. MATERIALS AND METHODS

### Materials

Isolate acid tolerant *B. japonicum*, BJ 11, was maintained at IPB Culture Collection, Biology Department, Faculty of Science and Mathematics, Bogor Agricultural University. Tanggamus and anjasmoro cultivar soybean seeds were obtained from Research Institute for Food Crops and Genetic Resources. Peat as inoculant carrier was obtained from Indonesian Research Institute for Legumes and Tubers. Physical and chemical analysis of soils and peats was done in Indonesian soil Research Institute, Bogor.

### Medium and Formula Preparation

Isolates *B. japonicum* 11 (BJ 11) was grown on Yeast Mannitol Agar (YMA) that consist of mannitol ( $10\text{ g L}^{-1}$ ),  $K_2HPO_4$  ( $0.5\text{ g L}^{-1}$ ),  $MgSO_4 \cdot 7H_2O$  ( $0.2\text{ g L}^{-1}$ ),  $NaCl$  ( $0.2\text{ g L}^{-1}$ ), yeast extract ( $0.5\text{ g L}^{-1}$ ), added with congo red (0.0025%) and rifampicin ( $50\text{ }\mu\text{g mL}^{-1}$ ). The isolates were resistant to rifampicin [9]. The isolates were incubated for about 7-8 days at the room temperature. Then they were inoculated into Yeast Mannitol Broth (YMB) on an Erlenmeyer, and incubated for five days with an 125 rpm shaker. Formula contained  $10^8\text{-}10^{10}$  cell/gram BJ 11 mixed with peat, gum arabic, limestone, and packed in plastic (0.5 Kg/plastic pack).

### Field Trial

The seeds were coated with the formula before sowing. Seeds were sown by hand in each hole and planted 3 seeds per hole at a depth of 3 cm, distance of hole 20 cm x 40 cm. Fertilizer was placed at other hole besides of seeds hole. Watering was carried regularly if no rain. Removal of weeds or grasses were done as far as possible. Soybean seed were sown by hand in a hole at soil. There are three seeds per polybag. Soybean seeds were selected based on the same size and healthy (able to shoot). Some treatments were done to soybean seed as follows: 1. inoculated by *B. japonicum* galur BJ 11, 2. inoculated by BJ 11 and application with 100 % N fertilizer; 3. inoculated by *B. japonicum* galur BJ 11 and application with 50 % N fertilizer + 50% compost; 4. Control: without inoculant, without inoculant + 100% N fertilizer, without inoculant + 50 % N fertilizer + 50% compost. Each

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treatments were done at 150-200 m<sup>2</sup> land replicated two times per each. As for mineral fertilization treatment 100 % N consisted of 100 Kg ha<sup>-1</sup> urea + 200 Kg ha<sup>-1</sup> TSP + 100 Kg ha<sup>-1</sup> KCl. As for 50% N consisted of a half dose of urea + 200 Kg ha<sup>-1</sup> TSP + 100 Kg ha<sup>-1</sup> KCl + compost 1000 Kg ha<sup>-1</sup>. Compost spread out at land surface one week before seeds planting. Urea used twice at one planting period i.e. a half dose at seeds planting and the rest at 30 days after planting (DAP). Growth parameters such as plant height at 30 days after planting (DAP), number of pods at 90 DAP, total number of seeds, total of seed weight, and weight of 100 seeds numbers of pods compare to control were determined. Growth parameters were measured from 10 plants per treatments. Data were analyzed using completely randomized design and the means at p (0.05) level of significance.

## II. RESULTS AND DISCUSSION

### Isolate Growth and Formula of Inoculants

Isolate BJ 11 was able to grow on YMA which were added with 0.0025% congo red and 50 µg/ml rifampicin after 7 days incubated on room temperature. Morphology of *B. japonicum* colonies were mucoid, not quite able to reserve congo red, and curve elevated (Fig. 1a). Formula of BJ 11 on peat as carrier contained 10<sup>9</sup>-10<sup>10</sup> cell/gram BJ 11 and packed in 0.5 Kg/plastic pack (Fig. 1b).

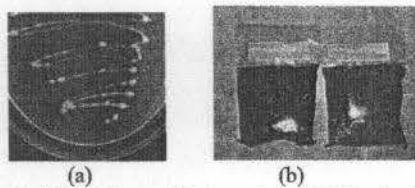


Fig. 1 (a) Colony of *B. japonicum* BJ 11 after 7 days of incubation on YMA + 0.0025% congo red + 50 µg/ml rifampicin, (b) formula of BJ 11 on peat as carrier.

*Bradyrhizobium japonicum* strain BJ 11 categorized as slowly growth bacterium because its colony on solid medium was seen after 7 days of incubation. The isolate could not absorb congo red at yeast mannitol agar (YMA) added with congo red [1]. BJ 11 also secreted exopolysaccharides in a large amount of mannitol [10], the colony was appeared sticky and mucoid colony. Role of exopolysaccharide to resistance at acid condition [11].

### Field Trial

A field trial was conducted to examine the efficiency of BJ 11 on the growth, nodulation and yield of soybean cultivar Tanggamus and Anjasmoro. Tanggamus is one of leading cultivar which can adapt to dry acid soil, Anjasmoro also showed good adaptation on paddies fields. The experiments took place at Sukadana (Lampung) and Tanah Laut (South Kalimantan). Chemical properties of the soil showed at Tabel 1. There were not found indigenous *B. japonicum* on field trial location and compost before treatments (Table 2 & 3).

TABLE I  
CHEMICAL PROPERTIES OF SOIL AT THE TRIAL LOCATIONS

Parameter	Soil contents	
	Lampung	South Kalimantan
C (%)	0.73	2.54
N (%)	0.11	0.20
P (%)	0.0142	0.0749
Mg (%)	0.01	0.02
K (%)	0	0.01
Ca (%)	0.14	0.67
C/N (%)	6.64	12.7
Al-dd	0.88	0.15
Capacity of cation exchange	2.45	7.14
pH : aquadest	5.56	6.18
pH :KCl	4.76	4.81

TABLE II  
TOTAL PLATE COUNT OF CELL BACTERIA AND INDIGENOUS *B. JAPONICUM* IN FIELD LOCATION

Field location	Numbers of cell (cfu/ml)	Numbers of <i>B. japonicum</i> (cfu/ml)
Sukadana -Lampung	5.9 x 10 <sup>5</sup>	0
Tambang Ulang -South Kalimantan	7.4 x 10 <sup>5</sup>	0

TABLE III  
COMPOSITION BACTERIA ON COMPOST USED IN THIS EXPERIMENTS

No	Microbes	Cell (cfu/ml)
1	<i>Lactobacillus</i>	0
2	Phosphate solubilization bacteria	320
3	<i>Rhizobium/Bradyrhizobium</i>	0
4	Actinomycetes	0
5	Yeast	0

Analysis data showed there were significant effect of *B. japonicum* inoculation for soybean grown at Tanah Laut-South Kalimantan and Sukadana - Lampung compared to control, without inoculants and fertilizer (Table 4 & 5). Inoculation BJ 11 formula showed better response on soybean growth than control, treatment without fertilizer and inoculant.

There were an association between soybean and root nodule bacteria which were played an important role in increasing the plant growth. Nitrogen fixation by the bacteria provided fixed nitrogen to the plants and then support growth and development of plants. Plants inoculated with BJ 11 isolate approximately had higher plant height, number of pods, and seeds, weight of 100 seeds. Inoculation of *Rhizobium* effectively influenced the formation and development of pods [11]. Pods that were already formed then were filled with phosynthate to form seeds. Numbers of seeds were effected by the number and size of pods. Higher number of pods were also gave higher numbers of seeds [8].

TABLE IV  
GROWTH OF ANJASMORO AND TANGGAMUS CULTIVAR SOYBEAN PLANTS ON  
TREATMENT WITH ACID-ALUMINIUM TOLERANT *B. JAPONICUM* FORMULA ON  
ACID SOIL AT SUKADANA- LAMPUNG

Anjasmoro					
Treatments	Plant height at 30 DAP (cm)	Number of pods at 90 DAP	Total number of seeds	Total of Seed weight (g)	Weight of 100 seeds (g)
BJ 11 + 1 N	46.7 a	34.8 bc	73.9 a	9.5 ab	16.1 a
BJ 11 + 1/2 N + C	40.6 b	58.2 a	66.2 ab	7.2 bc	11.2 ab
BJ 11	25.3 d 43.2 ab	67.7 a 27.9 cd	35.2 d 53.8 bc	3.1 de 7 c	8.8 b 11.7 ab
1 N	40.9 b	27.2 cd	57.4 bc	6.4 c	10.1 ab
1/2 N + C Without fertilizer and inoculants	33.6 c	15.6 d	42.9 cd	4.1 d	9.6 b

Tanggamus					
Treatments	Plant height 30 DAP (cm)	Number of pods 90 DAP	Total number of seeds	Total of seed weight (g)	Weight of 100 seeds (g)
BJ 11 + 1 N	31.1 c	51.6 a	128.5 a	9.7 b	7.8 a
BJ 11 + 1/2 N + C	36.1 a	58.3 a	130.1 a	10.1 b	7.4 a
BJ 11	26.3 d	21.3 b	43.3 bc	3 c	7.1 a
1 N	33 abc 32.3 bc	69.1 a 60.4 a	145.3 a 129 a	16.1 10.6 b	7.3 a 7.9 a
1/2 N + C Without fertilizer and inoculants	29.7 c	35.2 b	73.1 b	5.5 bc	7.8 a

TABLE V. GROWTH OF ANJASMORO AND TANGGAMUS CULTIVAR SOYBEAN PLANTS ON TREATMENT WITH ACID-ALUMINIUM TOLERANT *B. JAPONICUM* FORMULA ON ACID SOIL AT TAMBANG ULANG-SOUTH KALIMANTAN

Treatments	Plant height 30 DAP (cm)	Number of pods 90 DAP	Total number of seeds	Total of seed weight (g)	Weight of 100 seeds (g)
BJ 11 + 1 N	53 a 44.3 b	34.7 bc 58.2 a	81 bc 75.9 c	14.1 bc 12.3 cd	15 abc 15.3 ab
BJ 11 + 1/2 N + C	40.9 cd	67.7 a	101.1 ab	17.5 ab	13.3 c
1 N	53.4 a	27.9 cd	46.3 d	6 e	16.1 a

1/2 N + C Without fertilizer and inoculants	35.8 e	27.2 cd	63.1 cd	9.2 de	13.3 c
	41 cd	15.6 d	40.6 d	5 e	14.6 abc

Tanggamus					
Treatments	Plant height 30 DAP (cm)	Number of pods 90 DAP	Total number of seeds	Total of seed weight (g)	Weight of 100 seeds (g)
BJ 11 + 1 N	33.8 ab	41.1 ab	74.9 a	6.9 a	11.4 ab
BJ 11 + 1/2 N + C	35 a 33.9 ab	45.1 a 31.6 cd	64.9 ab 36.7 d	6.8 a 3.5 c	10.9 ab 12.1 a
BJ 11	34.9 a 32.4 bc	21.7 e	38.8 d	5.8 ab 4.1 c	10.3 b 11.4 ab
1 N	28.8 d	26.3 de	50.3 bcd	4.4 bc	10.6 b

BJ 11 = BJ 11 inoculant formula; N = 100 Kg.Ha<sup>-1</sup> urea + 200 Kg. Ha<sup>-1</sup> TSP and 100 Kg. Ha<sup>-1</sup> KCl); ½ N = 50 Kg.Ha<sup>-1</sup> urea + 200 Kg. Ha<sup>-1</sup> TSP and 100 Kg. Ha<sup>-1</sup> KCl; C = compost.

Based on experiments, weight of 100 seeds of Anjasmoro cultivar tent to higher than Tanggamus cultivar (Table 5). Using recommended fertilizer, Tanggamus and Anjasmoro cultivar commonly had about 12 g/100 seeds and 15 g/100 seeds, respectively. In this experiment, treatment with BJ 11 + 1/2 N + C using Anjasmoro cultivar achieved 15.3 g/100 seeds, and treatment only with BJ 11 achieved 12.1 g/seed using Tanggamus cultivar. In conclusions, inoculation BJ 11 was able to increase the growth and production of soybean plants which were grown on acid soil fields.

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