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Original Paper

Vegetative Growth Responses to Applications of Compost from Biomass of the Same and Different Crop Species and in Vitro Test of Self-DNA Effects

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Abstract- Returning-back unharvested crop biomass into soil which is intended to maintain soil fertility status is now facing a challenge concerning self-DNA negative impacts related to the application of compost prepared from biomass of the same crop species at certain compost maturity stage. This study aimed at to evaluate the application effects of composts prepared from biomass of the same and different food crop species with different composting periods on the vegetative growth of rice, maize, and string beans and of DNA extracts from the applied composts on seed germination of the studied crops. The experiment applied CRD with 10 treatments [control, k (compost materials): rice (P), maize (J), and string beans (K) biomass, and composting periods: 0, 30, and 90 d]. DNA was isolated by the modified CTAB method. The results showed that application of mature compost supported the vegetative growth of the test crops, while kP30 and kJ30 treatments, stands respectively for the application of 30 days-composted rice and maize biomass, showed inhibitory effect to the vegetative growth of the same species (rice or maize compost applied to rice or maize cropping). However, in vitro self-DNA inhibition effect was inconsistent and therefore considered not occurred in this experiment.

Keywords: compost material, compost maturity stage, food crops, self-DNA effect

I. Introduction

Since the beginning of the history of agricultural cultivation, the application of organic matter is believed to have a positive effect on soil fertility and crop production by improving the physical, chemical, and biological properties of the soil. Various organic materials have been applied, both in the form of litter and harvested biomass as well as compost, manure, biochar and others. However, from several studies in the last ten years, contradictory results have been reported, that the application of organic matter can also have a negative effect in the form of growth inhibition, so that it has attracted the attention of researchers worldwide.

[1] Application of organic matter in the form of immature compost inhibited the proliferation of Lepidium sativum roots. The application of compost from the biomass of 16 plant species of forest ecosystems (grass, trees, legumes, and vines) with a composting time of less than 30 days (unripe compost) has an inhibitory effect on the growth of the same plant species (compost material in the form of the same plant biomass). This is because the decomposition process is still ongoing, so that N immobilization by microbes still occurs [2] and the formation of allelopathic compounds that can cause phytotoxicity if the levels are high. Over time, the levels of allelopathic compounds will decrease due to degradation. The compost decomposition process also produces DNA (Deoxyribo Nucleic Acid) due to slow degradation of plant tissue, so that over time it will accumulate in the soil. Extracellular DNA from this compost when absorbed by the same plant species will cause autotoxicity or is referred to as the Self-DNA effect [3] [4] [5].

The application of compost in agricultural cultivation in Indonesia still does not pay attention to the phenomenon of the Self-DNA effect. As an application of the concept of healthy agriculture, the movement of returning harvested biomass to land to maintain soil fertility is now faced with new challenges related to these negative effects. Until now, even internationally, the results of research on the effects of Self-DNA are still very few that have been published. Similar research for typical Indonesian seasonal crops is very necessary as an effort to overcome the leveling-off problem in monoculture national rice production and increase the production of other food crops and national horticulture.

This study aimed to evaluate the effect of application of compost from the same plant biomass and different species with different composting times or compost maturity on the vegetative growth of rice, maize, and long beans as well as the effect of DNA extract from compost applied to the germination of the seeds of the three test plants.

II. Materials and methods

A. Materials

The materials used in this study included rice straw, fresh biomass from harvested sweet corn and long beans, Inpari 42 rice seeds, Bimmo Jawara sweet corn seeds, and

Sabrina long bean seeds, and materials for extracting compost DNA which included: liquid nitrogen, ethanol, CTAB, Tris HCl, NaCl, Na_2EDTA, CH_3COONa, chloroform, isopropanol, isoamylalcohol, TE buffer, 1% agarose gel, TAE buffer, ethidium bromide, distilled water, alcohol, H_2O_2, and CaOCl.

B. *Preparation of planting media*

The planting medium was prepared from Situ Gede Inceptisol topsoil and cow manure with a ratio of 3:1, weighing 8 kg/polybag. Compost material from rice straw and litter and biomass from corn and long beans harvest. The compost material is cut into small pieces and put into sacks, tightly closed, and placed in a room that is protected from sunlight. The compost was turned every 2 days and incubated for 0 (fresh), 30, and 90 days

C. *Seed sterilization*

Seed sterilization was carried out according to the [6] procedure, namely the seeds were soaked in a 10% H2O2 solution for 10 minutes, then washed with sterile distilled water 5 times (incubated for 5 minutes each time) with two replications. Then the seeds were soaked in 1% CaOCl solution for 1 hour and washed with sterile distilled water 10 times. Furthermore, the seeds are air-dried on filter paper until they are in their original condition. All steps are carried out in Laminar air flow

D. *Planting, compost treatment aplication and maintenance.*

The field experiment consisted of 90 experimental units with planting and compost application as presented in Table 1

Table 1 Planting and compost application treatment

No.	Planting			Replication
	Rice (P)	Sweet Corn (J)	Long bean (K)	
	Compost application treatment *			
1	P-control [a]	J-control	K-control	3x
2	P-kP0 [b]	J-kP0	K-kP0	3x
3	P-kP30 [c]	J-kP30	K-kP30	3x
4	P-kP90 [d]	J-kP90	K-kP90	3x
5	P-kJ0 [e]	J-kJ0	K-kJ0	3x
6	P-kJ30 [f]	J-kJ30	K-kJ30	3x
7	P-kJ90 [g]	J-kJ90	K-kJ90	3x
8	P-kK0 [h]	J-kK0	K-kK0	3x
9	P-kK30 [i]	J-kK30	K-kK30	3x
10	P-kK90 [j]	J-kK90	K-kK90	3x

* [a]rice cultivation without the application of compost; [b]rice planting with application of fresh straw; [c]rice cropping with straw compost application with composting age or time of 30 d; [d]rice plantations with the application of straw compost at the age of 90 d; [e]rice plantations with the application of fresh corn biomass litter; [f]rice crops with the application of corn biomass compost at the age of 30 d; [g]rice cropping with corn biomass compost application at 90 d; [h]rice plantations with the application of fresh long bean biomass litter; [i]rice cropping with the application of long bean biomass compost at 30 d; [j]rice plantations with the application of long bean biomass compost with 90 d of age; for corn and long beans, compost was applied with the same design as for rice cultivation.

Planting was carried out until the final vegetative phase, ie until the age of 49 DAP for rice and maize, and 35 DAP for long beans. Compost application was carried out 7 days before planting at a dose of 20 tons/ha [7]. The maintenance stages carried out included watering, weeding, controlling pests and diseases, as well as installing turus for long beans and observing the height of the test plants.

E. *DNA Isolation*

DNA isolation or extraction was carried out according to the modified CTAB method [8], in which 5 g of the compost sample were weighed and crushed using liquid nitrogen to a powder. Then the sample was put into a 50 mL centrifuge tube, added 20 mL of the extraction solution, and stirred evenly. The mixture was heated in a water bath at 60 ^{o}C for 15 minutes (the tube was inverted every 5 minutes), then cooled on ice for 10 minutes and added 10 mL of chloroform:isoamylalcohol (24:1), homogenized, and centrifuged at 2500 x g. 15 minutes at room temperature. 22 ^{o}C. The supernatant was transferred to a new centrifuge tube, added 2/3 volume of isopropanol supernatant and 1/10 volume of CH3COONa solution, then carefully homogenized. The solution was incubated on ice for 15 min and centrifuged at 4000 x g 15 min. The supernatant was discarded and resuspended using 1 mL TE buffer. The DNA content obtained was measured using nanodrop spectrophotometry and verified using agarose gel.

F. *In vitro test*

In vitro seed germination test was carried out according to the procedure of [3]; sterile seeds, compost DNA, filter paper, petri dishes, distilled water, and a sterile sprayer were prepared. A total of 9 sterile seeds were placed in a petri dish that had been lined with filter paper, 2 mL of the DNA solution was spread evenly, then incubated at 23 ^{o}C. Seeds were sprayed with sterile distilled water after 2 days of DNA solution application, incubated again for 7 days and root length was measured

III. Result and discussion

Preliminary analysis

Data on the initial properties of the growing media (Table 2) showed a high C/N 19 ratio, with a slightly acidic pH of 5.99.

The C/N ratio of rice, corn, and long bean compost (Table 3) decreased with increasing age or composting time. With 30 days of composting, the C/N ratio of rice (26) and corn (20) compost was higher than that of long bean compost (13). This indicates that the rice and corn compost is still immature and the decomposition process is still continuing due to the higher lignin content (Amin et al. 2015; Liang et al. 2015). The C/N ratio of 90-day-old rice, corn, and long bean compost showed mature compost with C/N < 20 characteristics, odorless, and black in color [9].

Table 2 Initial nature of planting media

Analysis Type	Unit	Value	Category*
C/N	-	19	High
pH H_2O	-	5,99	acidic

*Based on the criteria of the Soil Research Institute (2009)

Table 3 Compost C/N ratio based on material type and composting time

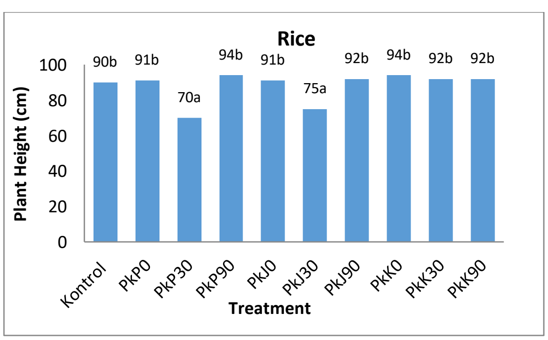
Type of compost material	Composting time (day)		
	0*	30	90
Rice Biomass	50	26	14
Corn Biomass	34	20	11
Long Bean Biomass	26	13	11

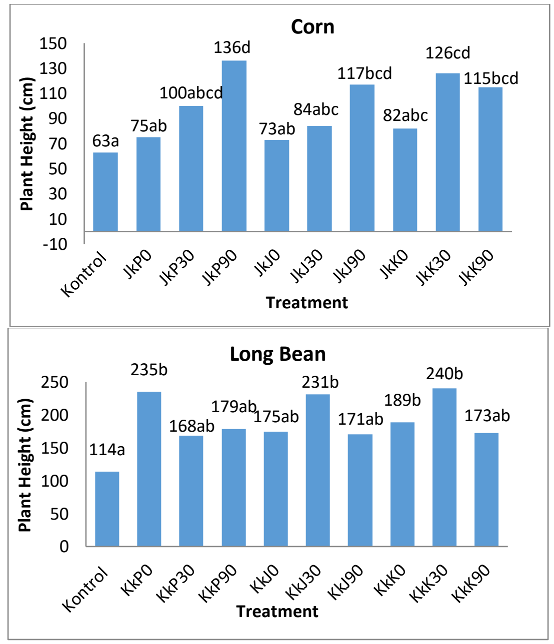
* Rice straw, corn biomass litter or fresh long bean biomass litter

Effect of compost application on the agronomic performance of the test plants

a. Plant Height

Figure 1 shows the lowest plant height that was significantly measured in rice cultivation with PkP30 and PkJ30 treatments. This is because the 30 days old rice (kP) and corn (kJ) compost is still immature (C/N > 20), so the decomposition process is still ongoing and N immobilization occurs [2] and allelopathic compounds are produced. which is a growth inhibiting factor.





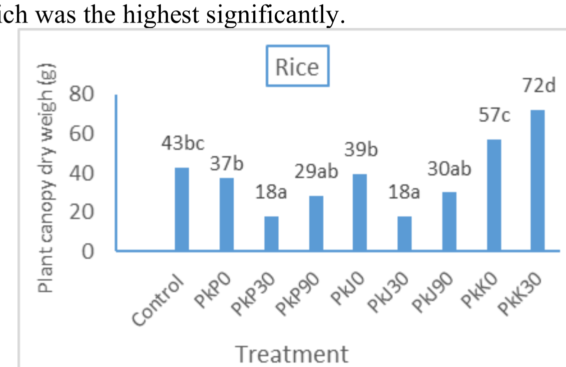
Description: Look at Table 1.

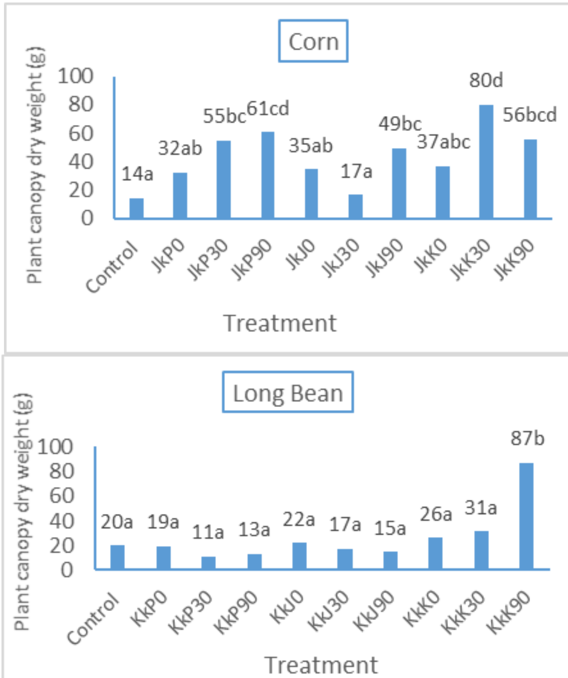
Figure 1 The height of the test plant in the late vegetative phase

In maize and long beans, the lowest plant height was significantly obtained in the control treatment. The application of compost from the same or different species biomass with a composting time of 90 days had a positive effect on the height of all test plants.

b. Plant canopy dry weight

The dry weight of the canopy is the net accumulation of CO_2 assimilation during the process of plant growth and development [10] Figure 2 shows that the lowest rice canopy dry weight was significantly obtained in the PkP30 and PkJ30 treatments. The lowest corn canopy dry weight was obtained in the JkJ30 treatment, although it was not significantly different from the control. Compost from rice straw and corn biomass with a composting time of 30 days is classified as immature, so it has the opportunity to inhibit plant growth. The effect of compost treatment was not significantly different on the dry weight of long bean canopy, except for the KKK90 treatment which was the highest significantly.



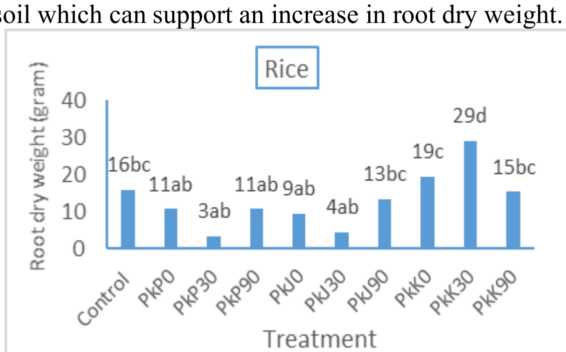


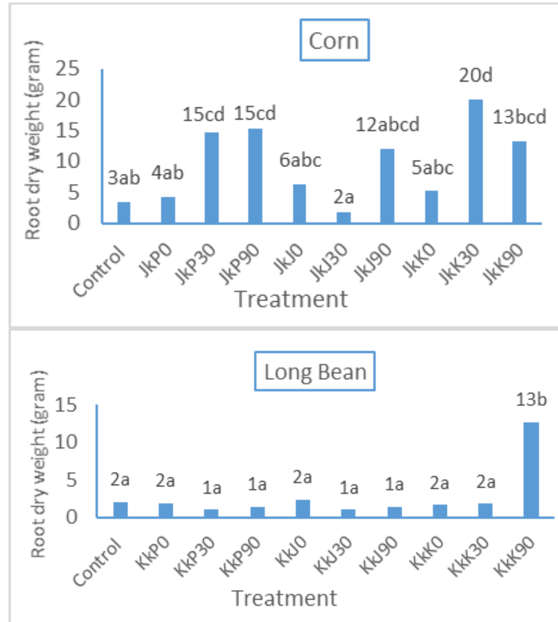
Description: Look at Table 1.

Figure 2 Dry weight of the crown of rice, corn and long beans in the late vegetative phase

c. Root dry weight

The lowest dry weight roots of rice and corn was significantly obtained in the PkP30 and JkJ30 treatments. Compost kP30 and kJ30 are immature, so they have the opportunity to inhibit plant growth (Figure 3). The highest dry weight roots of rice and maize were significantly obtained in the kK30 treatment (PkK30 and JkK30, Figure 3), while for long beans in the KKK90 treatment. Compost kK30 and kK90 are classified as mature, so their application has a positive effect on soil fertility through the provision of nutrients and the process of loosening the soil which can support an increase in root dry weight.



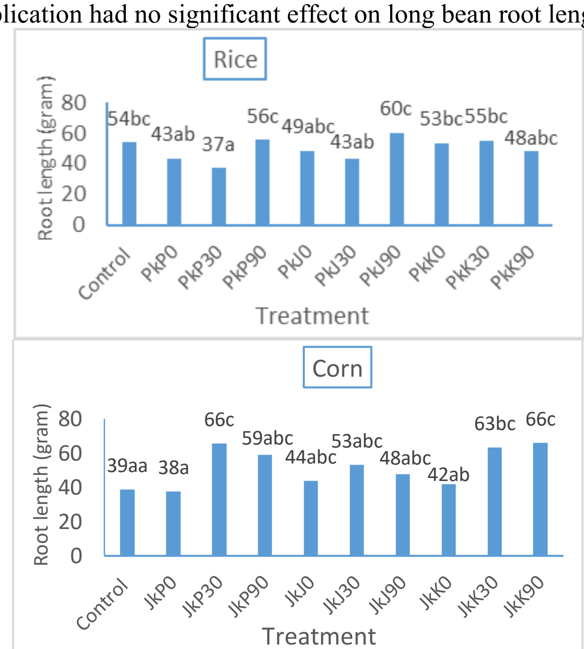


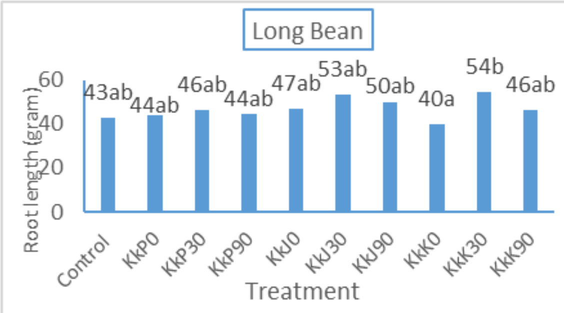
Description: Look at Table 1.

Figure 3 Dry weight of roots of rice, corn and long beans in the late vegetative phase

d. Root length

Root length is one of the morphological characters of plants that can be used to determine the extent of root reach in nutrient and water absorption and drought resistance [11]. Figure 4 shows that the PkP30 treatment gave the lowest rice root length significantly, while in maize it was obtained in the JkP0 treatment although it was not significantly different from the control. Both are due to immature compost. Compost application had no significant effect on long bean root length.

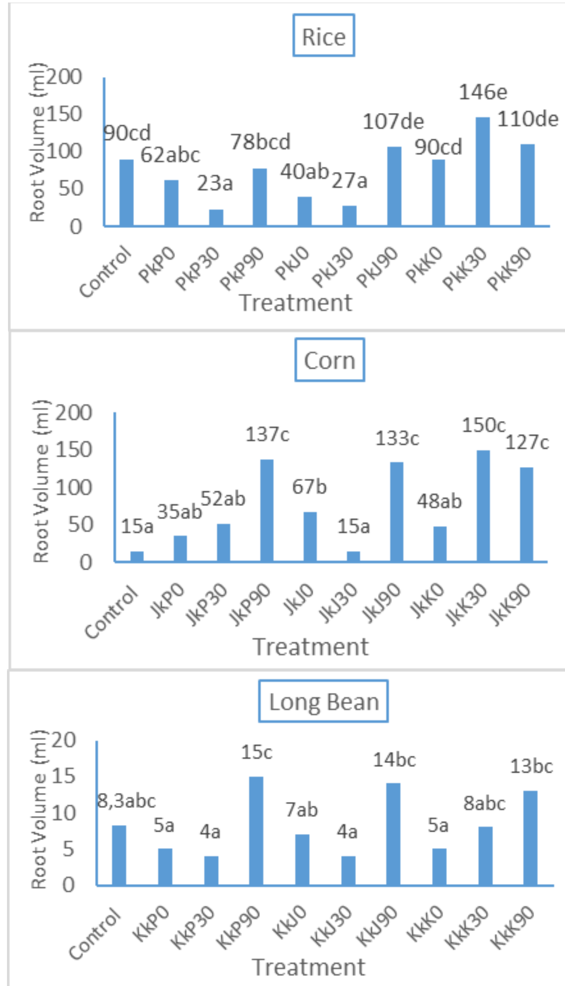




Description: Look at Table 1.
Figure 4 Root length of rice, maize and long beans in the late vegetative phase

e. Root volume

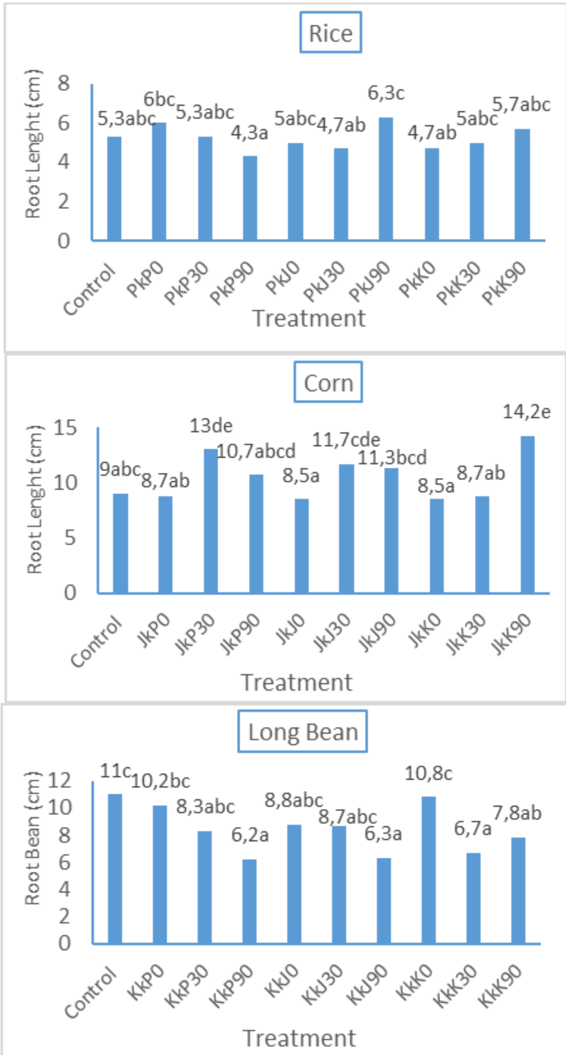
Root volume can be used as an indicator of groundwater availability. An increase in root volume indicates a morphological response of plants to water shortage conditions [12]. Figure 5 shows that the lowest rice root volume was significantly obtained in the PkP30 and PkJ30 treatments. The kJ30 treatment also gave the lowest root volume significantly in maize (JkJ30). In the long bean plantation, the lowest root volume was significantly obtained in the KkP0, KkP30, KkJ30, and KkK0 treatments. Compost kP30 and kJ30 immature so it can inhibit the growth of plant roots. On the other hand, mature compost had a positive effect on increasing root volume in the three test plants, one of which was related to increasing soil capacity to hold water and loosening soil [13]



Description: Look at Table 1.
Figure 5 Root volume of rice, corn, and long beans in the late vegetative phase

The effect of the application of compost DNA on the germination of the test plant seeds

Figure 6 shows that the highest in vitro germination of rice seeds was obtained in the PkJ90 treatment and only significantly different from the lowest germination in the PkP90 treatment. The highest corn seed germination was obtained in the JkK90 treatment and significantly different with the lowest germination in the control treatment, JkP0, JkP90, JkJ0, JkK0, and JkK30. The highest germination of long bean seeds was obtained in the control treatment and KkK0 and significantly different with the lowest germination in the KkP90, KkJ90, KkK30, and KkK90 treatments.



Description: Look at Table 1.

Figure 6 In vitro root germination of rice, maize, and long beans

From the results of this study, it could not be concluded that the inhibition of root germination of rice, maize and string beans by DNA extracts of mature and immature compost from the biomass of the same species (treatments PkP90, JkJ0, KkK30, and KkK90) was self-DNA effect because it was inconsistent. in terms of compost maturity and perhaps more so in terms of compost maturity. The self-DNA effect occurs when extracellular DNA enters the cell and inhibits gene expression in the germination phase [3][4]. On the other hand, in the PkJ90 and JkK90 treatments, there was an increase in root length due to the positive effect of heterospecific compost DNA application (compost DNA from different species of biomass) [14].

The results of this study indicated that the effect of DNA application of compost extract on seed germination of the test plants was inconsistent. Thus, the inhibitory effect of self-DNA on rice, maize, or string bean germination in vitro related to the application of compost from the same species (specific) biomass with different compost maturity levels was not or has not been successfully demonstrated in this study.

CONCLUTION

The application of compost from the same plant biomass and different species with the test plants of rice, corn and long beans with different compost maturity levels had a significant effect on the vegetative growth of the three test plants. Treatment of rice compost and corn compost at the age of 30 days showed a negative effect on the agronomic performance of rice and corn plants. Mature compost had a positive effect on the growth of the test plants. The effect of self-DNA on the germination of rice, corn, and string beans in vitro was inconsistent based on the compost material and the maturity level of the compost used.

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