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Effects of Nano Zero Valent Iron and Nano Carbon Particles on Germination and Growth of *Brachiaria ruziziensis*

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The research aimed to investigate effects of nano particles including nZVI and nC on germination and growth of *Brachiaria ruziziensis*. This study was divided into two parts: 1) the effects of nZVI and nC on germination rates for 7 days and 2) the effects of nZVI and nC on growth at day 15. The results demonstrated that the germination rates of nZVI with the concentration at 20-60 mg/100ml were higher than that of nC with the same concentrations. However, the germination rates of nC were higher than that of nZVI with concentration of 80-100 mg/100ml. The highest germination rate was found at 80 mg/100ml of nC. Regarding root elongation experiments, it was found that the elongation of nZVI was higher than that of nC with 20-60 mg/100ml. On the other hand, it was found that the elongation of nC was higher than that of nZVI with the concentration of 80-100 mg/100ml. The highest root elongation was found at 60 mg/100ml of nZVI. Regarding shoot elongation experiments, it was found that the elongation of nC was higher than that of nZVI with all concentrations. The highest shoot elongation was found at 100 mg/100ml of nC. This study indicated that the nano particles effected the germination, root elongation and shoot elongation with dependence on nano particle concentrations. In comparison with two types of nano particles, the optimal concentrations were 80 mg/100ml of nC, 60 mg/100ml of nZVI and 100 mg/100ml of nC for the germination, root elongation and shoot elongation, respectively.

Keywords: Nano Zero Valent Iron (nZVI), Nano Carbon (nC), *Brachiaria ruziziensis*, Germination.

Introduction

Currently, the cattle raisers and other farmers in rural areas face the problems about high production costs and shortage of forage during drought period. These are due to that natural pasture land as the main food source for most livestock is inadequate. As a result of this, the farmers have paid their

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attention to solve the problems by searching more forage. As one of their solutions, the farmers grow more pasture land with the varieties of grasses. It is regarded as a cheap source of animal food for feeding ruminants. It also helps to improve soil and soil erosion. The types of grasses include *Panicum maximum* cv. TD 58 (Purple guinea grass), *Brachiaria ruziziensis* (Ruzi grass), Paspalum atratum cv. Ubon (Ubon paspalum grass) and *Pannisetum purpureum* (Napier grass). *Brachiaria ruziziensis*, known as Ruzi grass, is a plant of monocots and belongs to the Poaceae family. It is a creeping perennial with soft hairy leaves. The height is up to 1-2 meters when fully grown (Schultze-Kraft and Teitzel, 1992). Their seeds are purple-green. Ruzi grass is drought-tolerant with high-yielding for feeding ruminants such as cattle, sheep, goats and buffaloes (Department of Livestock, 2015).

Nanotechnology is one of the interesting technology and increasingly used in many fields of the researches (Zhang and Elliott, 2008). It includes science, engineering and industry applications (El-Shall *et al.*, 1995). Nanoparticles are sometimes used for environmental experiments to remediate the contaminated environments or other purposes. Nano zero valent iron (nZVI) is an example of well-known nanoparticles that is wildly used for remediation of contaminated soil, water and air by Jiamjitrpanich *et al*. (2010), Jiamjitrpanich *et al*. (2012), Jiamjitrpanich *et al*. (2013), Kosanlavit *et al*. (2013) and Kosanlavit *et al*. (2016)

Objectives: The present research is a comparative study that focused on effects of nano zero valent iron (nZVI) and carbon nanoparticles (nC) on germination and growth of *Brachiaria ruziziensis* in terms of the germination rate and growth of roots and shoots. The experiments were carried out by soaking the seeds of *Brachiaria ruziziensis* with the varying nano particle concentrations and incubated in the Petri dish. The results were presented as averages or percentages.

Materials and methods

Materials

Seeds of *Brachiaria ruziziensis* (Ruzi grass) were obtained from Pakchong district, Nakhon Rachasima province, Thailand. Nano zero valent iron (nZVI) and Nano Carbon (nC) were used as the nano particles for the experiments. The synthesis of nZVI particles was prepared by the reductive precipitation process using two chemicals, namely, sodium borohydride ($NaBH_4$) and iron (III) chloride ($FeCl_3$). This procedure followed the method of C.B. Wang and Zhang (1997), Choe *et al*. (2001), Sun *et al*. (2007) . It was also modified and described by Jiamjitrpanich *et al*. (2010) and Jiamjitrpanich *et al*. (2012).

The nanoparticle powder of carbon (nC) was purchased from one of Thai distributors.

Research Methodology

This study was determined in terms of the germination rate and the growth of *Brachiaria ruziziensis*. The germination rate experiment involved seeds of *Brachiaria ruziziensis* which was incubated in the Petri dishes. The *Brachiaria ruziziensis* seeds were soaked in nano suspensions in a container for 24 h and germinated in Petri dish. Nano suspensions were divided into 6 concentrations.There were 0 (controls), 20, 40, 60, 80 and 100 mg/100 ml. The experiments were performed in triplicate. Two types of nanoparticles were used to prepare nano suspensions. There were nZVI and nC. The stock solutions of 1,000 mg/100 ml of nZVI and nC were prepared by using distilled water. After that, the stock was diluted to obtain the required concentrations (20, 40, 60, 80 and 100 mg/100 ml) by distilled water. Also, the distilled water without the nano particles was used as controls (0 mg/100 ml). *Brachiaria ruziziensis* seeds were soaked in one of nano suspensions in a container for 24 h. After that, the *Brachiaria ruziziensis* seeds were incubated in Petri dishes (each Petri dish contained 20 seeds) at room temperature. The germination rate was observed and calculated once a day with a period of 7 days.

The study of growth of *Brachiaria ruziziensis* were determined in terms of elongation of roots and shoots at an age of 15 days. The seeds were soaked in nano suspensions in a container for 24 h. Nano suspensions were divided into 6 concentrations. There were 0 (controls), 20, 40, 60, 80 and 100 mg/100ml (The experiments were done in triplicate) which the same preparation as the germination experiments. After that, the *Brachiaria ruziziensis* seeds were incubated in Petri dish with 20 seeds each. The lengths of roots and shoots were determined at day 15.

Results

The results of the present study were presented in terms of the germination rates and the average lengths of roots and shoots as shown below:

The effects of nZVI and nC on the germination rate of Brachiaria ruziziensis

The results of germination study were shown in (fig 1 and 2).

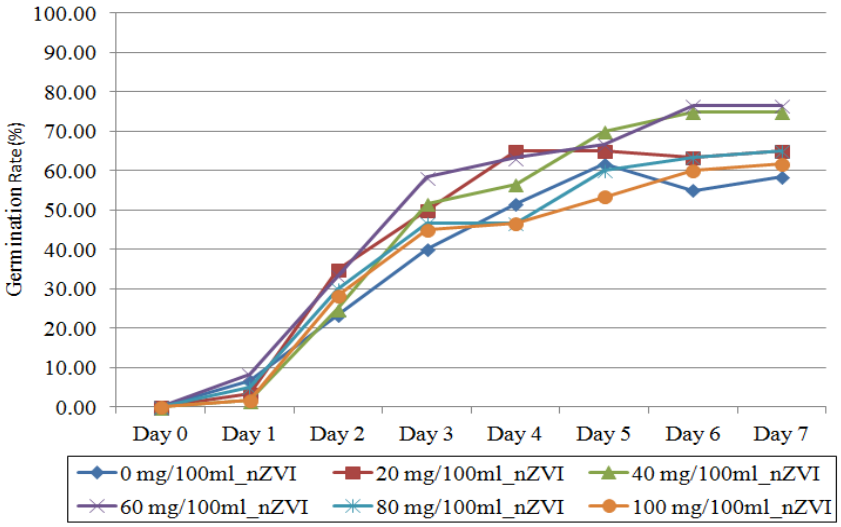

Fig. 1. The germination rates of *Brachiaria ruziziensis* seeds which were soaked in nZVI suspensions

The germination rates of *Brachiaria ruziziensis* seeds which soaked in nZVI suspensions for 1 h (Fig. 1) showed that the germination rates generally increased from day 0 to day 7. With regard to the time point of day 7, it found that the concentration of 60 mg/100ml nZVI showed the highest germination rate of 76.67 ±6.83%. It was followed by 40, 20, 80, 100 and 0 mg/100 ml nZVI (75.00 ±4.47, 65.00 ±7.75, 65.00 ±4.47, 61.67 ±11.25, and 58.33 ±5.16%, respectively). These results demonstrated at day 7 that the percentages of germination rates increased in accordance with the concentrations from 0 (controls) to 60 mg/100 ml. Though, after that, the rates decrease with the concentrations from 80 to 100 mg/100 ml.

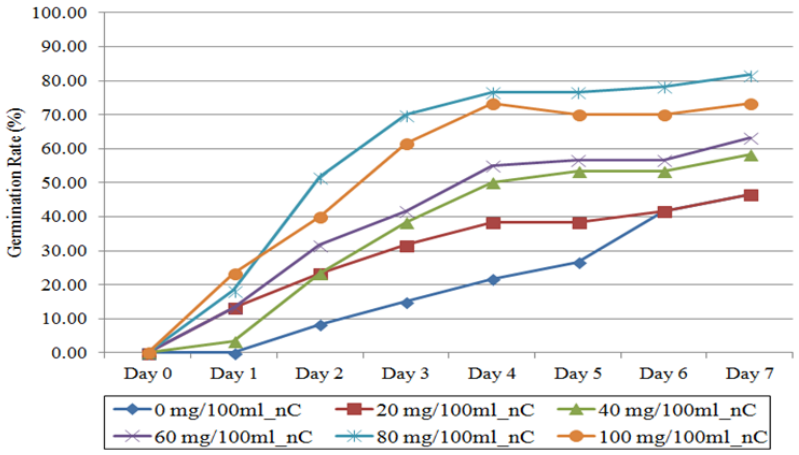

Fig. 2. The germination rates of *Brachiaria ruziziensis* seeds which were soaked in nC suspensions

The germination rates of *Brachiaria ruziziensis* seeds which were soaked in nC suspensions for 1 h (Fig. 2) showed that the germination rates generally increased from Day 0 to Day 7. With regard to the time point of day 7, it found that the concentration of 80 mg/100ml nC showed the highest germination rate of 81.67±9.31. It was followed by 100, 60, 40, 20, and 0 mg/100ml nC (73.33±2.58, 63.33±11.25, 58.33±5.16, 46.67±6.83, and 46.67±5.16%, respectively). These results demonstrated at day 7 that the percentages of germination rates increased in accordance with the concentrations from 0 (controls) to 80 mg/100ml. Though, after that, the rates decrease at the concentrations 100 mg/100ml.

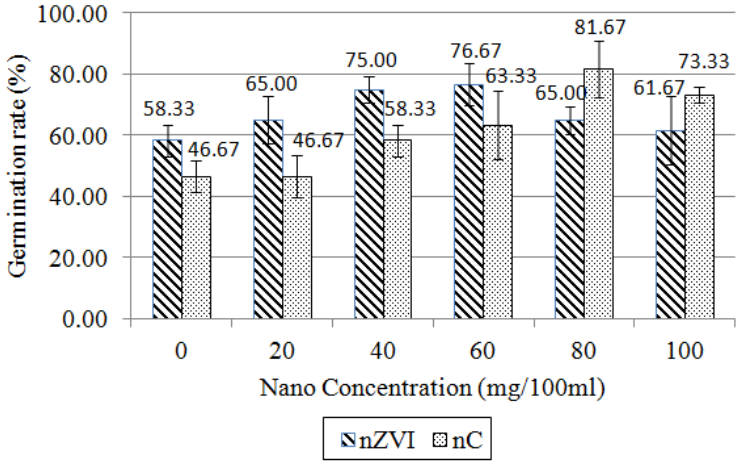

Fig. 3. The comparison of the germination rates between nZVI and nC

The results of the germination rates of nZVI experiments comparing to those of nC at the time point of the day 7 (Fig. 3). It found that nZVI showed the higher germination rates than those of nC with the concentrations of 0-60 mg/100ml. However, at the concentrations 80-100 mg/100ml, it found that the germination rates of nC were higher than those of nZVI. In addition, the highest germination rate found at 80 mg/100ml of nC with 81.67±9.31%.

The effects of nZVI and nC on growth of Brachiaria ruziziensis

The growth of *Brachiaria ruziziensis* were determined in terms of elongation of roots and shoots at the age of 15 days. The results of elongation were shown in (Fig. 4-7).

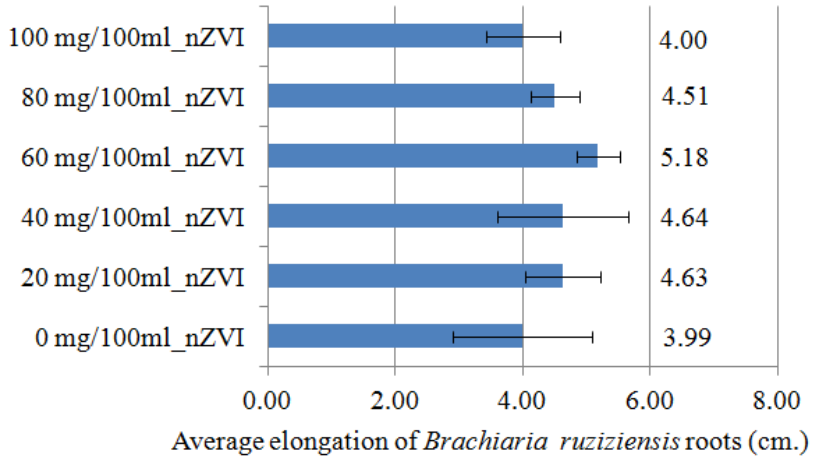

Fig. 4. The effects of nZVI on elongation of *Brachiaria ruziziensis* roots

Figure 4 showed the effects of nZVI on elongation of *Brachiaria ruziziensis* roots. It found the elongation of *Brachiaria ruziziensis* roots was highest at 60 mg/100ml nZVI. There were 5.18±0.34 cm of roots. It was followed by 40 mg/100ml nZVI (4.64±1.03 cm of roots) and 20 mg/100ml nZVI (4.63±0.59cm of roots).

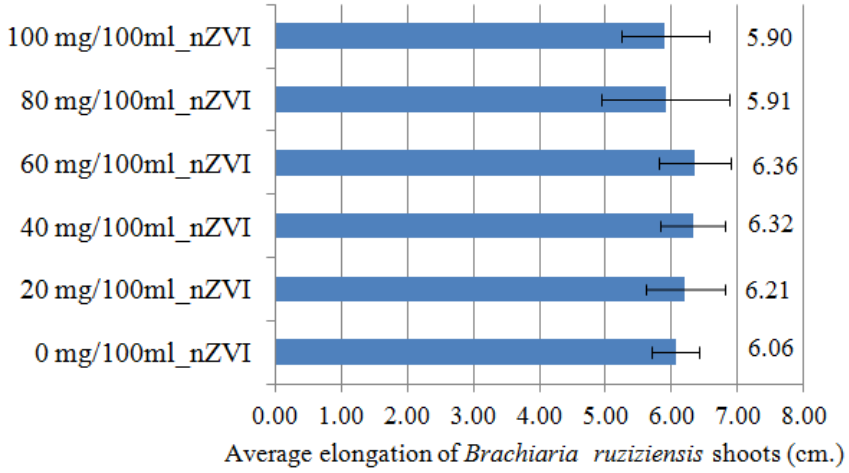

Fig. 5. The effects of nZVI on elongation of *Brachiaria ruziziensis* shoots

Figure 5 showed the effects of nZVI on elongation of *Brachiaria ruziziensis* shoots. The results of elongation for both roots (Fig. 4) and shoots (Fig.5) demonstrated in the same manner with regard to the nano concentrations. It found the elongation of *Brachiaria ruziziensis* shoots was highest at 60 mg/100ml nZVI. There were 6.36±0.54 cm of shoots. It was followed by 40 mg/100ml nZVI (6.32±0.49 cm of shoots) and 20 mg/100ml nZVI (6.21±0.59 cm of shoots). Moreover, it found that the concentration of 80 and 100 mg/100ml nZVI only demonstrated that elongation of *Brachiaria ruziziensis* shoots were less than that of controls (6.06±0.36 cm). It was also noted that the elongation of shoots with experiments of 80 and 100 mg/100ml nZVI was less than that of control (6.06±0.36 cm).

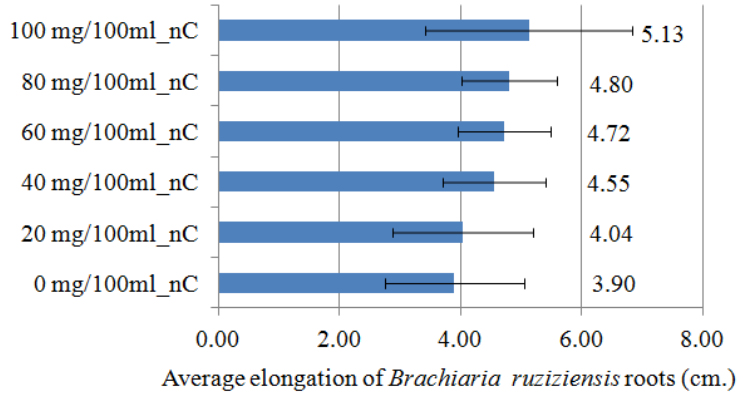

Fig. 6. The effects of nC on elongation of *Brachiaria ruziziensis* roots

Figure 6 showed the effects of nC on elongation of *Brachiaria ruziziensis* roots that the highest efficiency of nC on the elongation of roots was at concentration of 100 mg/100ml and the highest lengths was 5.13±1.70 cm. It is followed by 80, 60, 40, 20, and 0 mg/100ml nC which the lengths of roots were at 4.80±0.79, 4.72±0.77, 4.55±0.85, 4.04±1.17, and 3.90±1.15 cm, respectively.

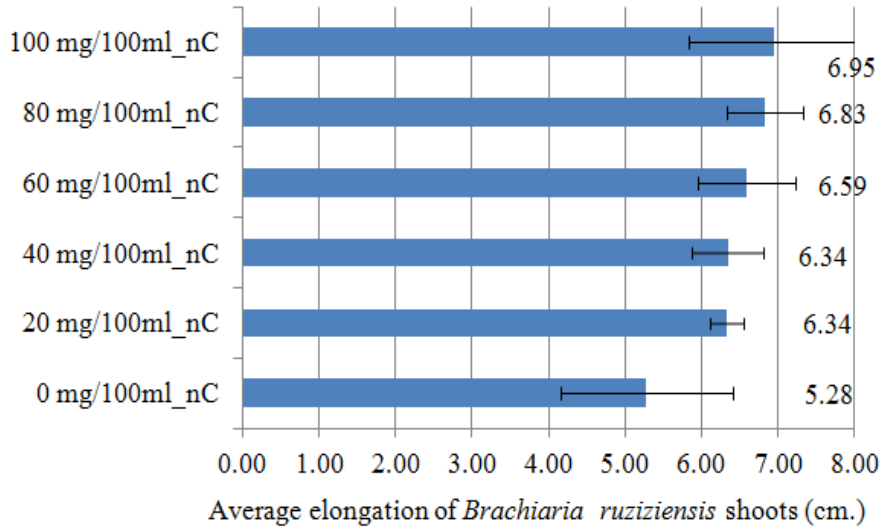

Fig. 7. The effect of nC to shoots elongation of *Brachiaria ruziziensis*

Figure 7 showed the effects of nC on elongation of *Brachiaria ruziziensis* shoots that the highest efficiency of nC on the elongation of shoots was at concentration of 100 mg/100ml and the highest lengths was 6.95±1.12 cm. It is followed by 80, 60, 40, 20, and 0 mg/100ml nC which the lengths of shoots were at 6.83±0.50, 6.59±0.64, 6.34±0.47, 6.34±0.22, and 5.28±1.13 cm for 80, 60, 40, 20, and 0 mg/100ml nC, respectively.

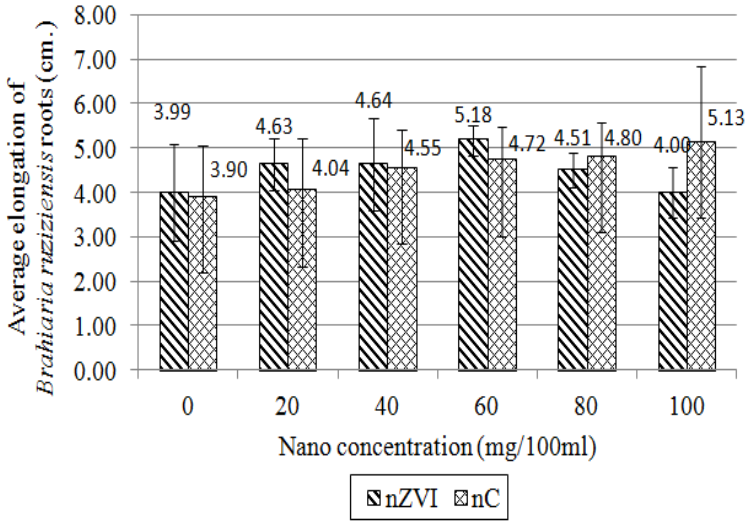

Fig. 8. The comparison of *Brachiaria ruziziensis* root elongation treated by nZVI and nC

The comparative study on the effects of nZVI and nC to *Brachiaria ruziziensis* root elongation (Fig. 8). It found that the effects of nZVI on root elongation were higher than those of nC at the concentration of 0-60 mg/100ml. However, the root elongations of experiments with nC were higher than those of nZVI at the concentration of 80-100 mg/100ml. In addition, the highest elongation (5.18±0.34 cm) was found with the experiment of 60 mg/100ml nZVI.

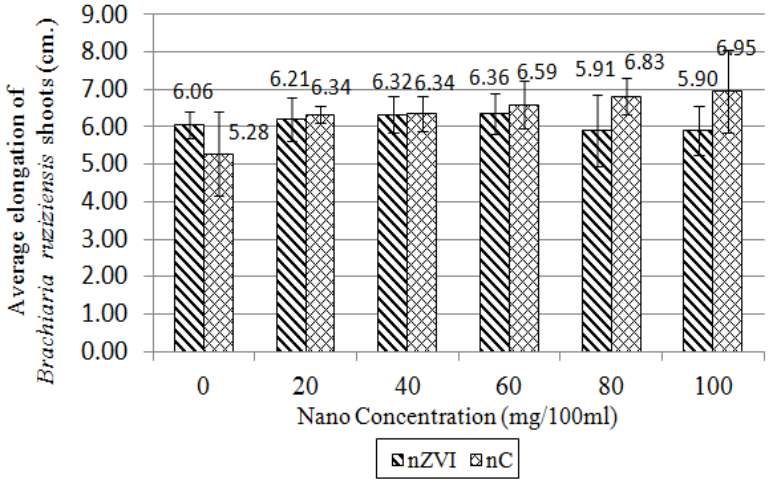

Fig. 9. The comparison of *Brachiaria ruziziensis* shoot elongation treated by nZVI and nC

The comparative study on the effects of nZVI and nC to *Brachiaria ruziziensis* shoot elongation (Fig. 9). It found that the effects of nC on shoot elongation were higher than those of nC at the concentrations of 20-100 mg/100ml. Though, the control of nC experiments was lower than that of nZVI experiments. In addition, the highest elongation (6.95±1.12 cm) was found with the experiment of 100 mg/100ml nC.

Discussions

With regard to the germination rates of nZVI experiments comparing to those of nC at the time point of the day 7, it found that nZVI showed the higher germination rates than those of nC with the concentrations of 0-60 mg/100ml. However, at the concentrations 80-100 mg/100ml, it found that the germination rates of nC were higher than those of nZVI. In addition, the highest germination rate found at 80 mg/100ml of nC with 81.67±9.31%. The study on the effects of nZVI and nC to *Brachiaria ruziziensis* root elongation was found that the effects of nZVI on root elongation were higher than those of nC at the concentration of 0-60 mg/100ml. However, the root elongations of experiments with nC were higher than those of nZVI at the concentration of 80-100 mg/100ml. In addition, the highest elongation was found with the experiment of 60 mg/100ml nZVI. The study on the effects of nZVI and nC to *Brachiaria ruziziensis* shoot elongation was found that the effects of nC on shoot elongation were higher than those of nC at the concentrations of 20-100 mg/100ml. Though, the control of nC experiments was lower than that of nZVI experiments. In addition, the highest elongation was found with the experiment of 100 mg/100ml nC. The results of the present study were consistent with the study by Tongkom (2010). It was reported that the carbon nanotube showed positive effects to growth of tomatoes in Thialand. Also, Khodakovskaya and Biris (2010) reported that carbon nanotubes effected growth of tomato seeds. It was found that carbon nanotubes could promote the germination and the growth of tomatoes, significantly. These were due to carbon can promote and be useful for increasing biomass of the plant. It can be applied for development of fertilizer in the future.

In conclusion, this present research demonstrated that nZVI and nC effected on germination and growth of *Brachiaria ruziziensis*. This study indicated that the effects were dependent on nano particle concentrations. In comparison with these nano particles, the optimal concentrations were 80 mg/100ml of nC, 60 mg/100ml of nZVI and 100 mg/100ml of nC for the germination, root elongation and shoot elongation, respectively.

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