

# An Investigation On Sumateran Arthrobotrys Oligospora And Carbofuran Againsts Root-Knot Nematode (Meloidogyne Hapla) On Tomato (Solanum Lycopersicum Mill.)

Liana Dwi Sri Hastuti, Jane Faull

**Abstract:** A bioassay pot trial using three species of the nematode-trapping fungi isolated from Sumatera Utara Indonesia were tested for potential as biocontrol agents. The 10 ml conidial suspension of Arthrobotrys oligospora containing  $1 \times 10^7$  of conidia was added to the tomato media growth. Carbofuran® as chemical agent were also tested against Meloidogyne hapla on tomato (Solanum lycopersicum Mill.). Tomato plants were cultivated under growth cabinet conditions at temperatures between 22-25°C and 12 hours light and 12 hours dark cycle. An inoculant of M. hapla containing 3 egg masses was injected into the soil around the roots of 15 days old tomato plants. A. oligospora and Carbofuran® treated plants showed reduced numbers of infections by M. hapla in term of swollen of roots, sausage shaped and galls, moreover all treatments enhanced growth in terms of length, root length, fresh, and dry weight. Results from the biological agents such as A. oligospora and the chemical agent Carbofuran® as standard nematicide indicate that all offered disease reduction.

**Key Words:** Nematode Control Agent, Sumateran Nematode-trapping Fungi, Biocontrol agent, Root-Knot Nematode, M.hapla..

## 1 INTRODUCTION

About 2000 plants are susceptible to infection by root-knot nematodes and they cause more than 5% of global crop loss [1]. Based on information from the International Survey of Crop Losses (2008) the estimated number of yield losses for some crop due to nematodes are: tomato 20.6%, sweet potato 10.2%, potato 12.2%, tobacco 14.7% and eggplant (aubergine) 16.9 % per year and overall average yield losses for all crops in the world is 12.3% per year (USDA, 2008). According to East Seed Indonesia (2007), the tomato yield in Indonesia is below world average in quantity and quality. An average yield of tomato 11.89 – 15.55 tonnes/hectare is very low if compared to USA and Europe with an average of 100 tonnes/hectare [2]. The potential yield of tomato plants, under optimum cultivation, can average 20 to 30 tones/ha. Thus strategies that focus on improving the yield of tomato plants per unit area should be considered. There are many constraints to increasing the quality or quantity yield of tomato in Indonesia, which is partly due the presence of nematodes. Nematodes are often the hidden enemy of the crop, they are insidious, because the symptoms of infestation are often unspecific and easily misdiagnosed.

Above ground symptoms include stunting, chlorosis, yellowing, leaf drop, reduced vigor, wilting or curling and twisting of leaves and stems [3], [4]. The main symptoms of nematode infection are found on the roots of the plant as root swollen and formulation of root galls. The size of galls varies depending on species and should not be confused with leguminous root nodules [4]. Root nodules are attached to the root and can be removed easily, root-knot galls cannot. Despite M. hapla being identified as a northern RKN adapted to temperate climates, it is found in lower numbers in Indonesia, however, problems caused by this nematode can still be found in some crop plantations especially in plants grown in the tropical areas with high elevation. This has occurred due to natural adaptation to the conditions that exist in Indonesia (Prihanto, 2003). Tomato, potato, cabbage, cauliflower, carrot, spring onion, chili, and some others vegetables are cultivated in areas with high elevation in Indonesia (350-700 m sea level). In Indonesia those plants are known as mountain crops. In Indonesia, management to control of the number of root-knot nematodes has been conducted in various ways including cultural practices (crop rotation), the use of resistant cultivars of plant, and the use of chemicals (nematicides) [5]. The use of cultural control methods to manage root-knot nematodes is the most environmentally sustainable method for limiting root-knot nematode damage. However, root-knot nematodes have very large host ranges and without careful planning cultural control methods will be unsuccessful [6]. Due to this, the most common control method used in Indonesia is chemical. The nematicides used one from the carbamate group (N-methylcarbamate) and include Carbofuran®, Curater™ and Furadan 3G™. However, pesticides belonging to the group of carbamate such as carbofuran are suspected to have detrimental effects on the environment and poor worker-safety profiles and many have been restricted in use or withdrawn from the market [7]. In addition, granular nematicides such as carbofuran more easy to apply, and exert nematicidal activity. Those with systemic action are usually very effective and persistent. However problems may occur if residues accumulate in edible plants [8]. Thus, it is become imperative

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to seek advantage alternative method by using the natural enemy such as Nematode-trapping Fungi to overcome the problems caused by chemical nematicide and the destructive pathogen such as root-knot Nematode. In this research, nematode\_trapping fungi originally from Indonesian soil *Arthrobotrys oligospora* has been used to determine the effectiveness in reducing the effect of the nematode infection as identified by swollen roots and root galls by the northern root-knot nematode *Meloidogyne hapla* in tomato plants (*Lycopersicon esculentum* Mill.). The first aim of this study is compare the efficacy among the NTF to reduce infection by *M. hapla*, and also compare them with Carbofuran® as the gold standard.

## 2 MATERIALS AND METHODS

### 2.1 Nematode-Trapping Fungi Preparation.

*Arthrobotrys oligospora* isolated from North Sumatera has been selected for further testing on the basis of preceding experimental results. They were produced as an inoculum ready for pot trialing. *A. oligospora* was cultured on Potato Dextrose Agar at 25°C for 14 days. After 14 days, for each strain, conidia were scraped from the culture with sterile glass rod and suspended in sterilized water to obtain a conidial suspension. The suspension was filtered through the gauze to remove mycelial fragments. The conidia suspension was pelleted by centrifugation at 3000 rpm for 5 minutes and the pellets were re-suspended in sterile distilled water. The concentration of each conidia suspension was adjusted to  $1 \times 10^7$  conidia/ml using the hemocytometer method. Conidial suspension (10 ml) of each strain containing  $1 \times 10^8$  conidia/ml was used as inoculum.

### 2.2 Pesticide Preparation

Carbofuran® was used as chemical control of nematodes as it is widely used in agriculture. Pesticide was diluted to concentrations used by 2 mg/10ml of solid Carbofuran® diluted in methanol to 200µg/ml or 2 mg/10ml liquid/suspension [9], [10]. Carbofuran® solution (10 ml) was poured on each pot, and controls received 10 ml of distilled water.

### 2.3 Root Knot Nematode Inoculant Preparation

Infected roots with galls were washed gently and cut into 1 cm section. Roots were vigorously shaken for 4 minutes in sodium hypochlorite solution (100 ml NaOCl 5-10%). Roots were poured and washed through a 250 µm of sieves. Roots containing galls in the sieve were placed in a crystallising dish, some petroleum jelly was spread on the borders of crystallising dish to prevent escape of nematodes and it was covered with a glass lid. The crystallising dish was incubated at 25° C temperature room for 4-7 days. A piece of tomato root was examined under the microscope to confirm infection, and the infected of tomato plant was used as a source of inoculum. Mature nematode egg masses were selected and 3 galls/egg mass (contain 400-600 per egg mass) were picked up with a pair of forceps. The egg mass was transferred into 15 ml tube containing sterile tap water. The 15 ml tube containing egg mass was left at room temperature to allow the juvenile (J2) to hatch. Most temperature species can be kept at 18-22°C and tropical and sub-tropical species at 25-30°C. Once hatched, the J2 was inoculated on to a previously transplanted tomato host. Preparation J2 of root-knot nematode used the combined technique described by Davies K.G. (University of

Nottingham), [11], [12].

### 2.4 Soil Preparation

Soil was dried for 4 days and sieved, and after air drying and sieving, soil (5000 grams per pot) was sterilized with dry sterilization program in 2000 ml beaker glass covered by aluminium foil. Soil contained in a glass beaker was autoclaved at 121°C for 15 minutes; this stage was repeated once more. Under sterile conditions, sterile soil was transferred to 8.5cm pots so that each pot contained a 100 grams of sterile soil. The soil was then treated with Carbofuran® and/or conidia suspension of nematode-trapping fungi, and distilled water (10 ml) was used as a control. There were three replicates of each treatment. In order to avoid contamination all treated pots were covered with para film on the top and bottom end of each pot. All treated pots were kept on the 25°C room for 3 days before receiving a tomato seedling.

### 2.5 Host Preparation

Surface sterilised tomato seeds (Mr. Fothergill's Seeds, Tomato Alicante, Kenford, New Market, UK) were sown on wet tissue inside a crystallising dish. After 15 days, seedlings of tomato plants were transferred to the treatment pots and maintained in a Sanyo Fitotron at 22-25°C on a 12 hour light/dark cycle. One week after inoculation by pesticide and fungi, 1000 J2 [13] were added into holes made in the soil around the root of each plant. The pots were returned back into Fitotron for incubation.

### 2.6 Harvesting and Root Assessment

The invasion and development of J2 stages (swollen root, sausage stage, pear-like (kidney-shape) [14] and galls was assessed at 7 and 15 days and 30 days. Plants were harvested by pulling out the whole rooted plant from the soil. The soil attached on the root plant was removed by soaking the root in tap water in a bucket. The tomato plants were air dried and cut in line boundary between stem and the root. The length and weight of each stem and root were measured. Roots were stained with Pheloxin B for observation and number of swollen roots and egg masses (galls) were counted [15], [16]. At 7, 15 and 30 days the plants were harvested to evaluate the effect of treatments on plant production in term; stem height and root depth, stem and root fresh weight and stem dry weight.

### 2.7 Data Analysis

The record data was subjected to analysis variance Anova test and LSD test at 0.05 level of significance for comparing the difference among treatment means [17].

## 3 RESULT

### 3.1 Assessment I, 7 days after infected by *Meloidogyne hapla*

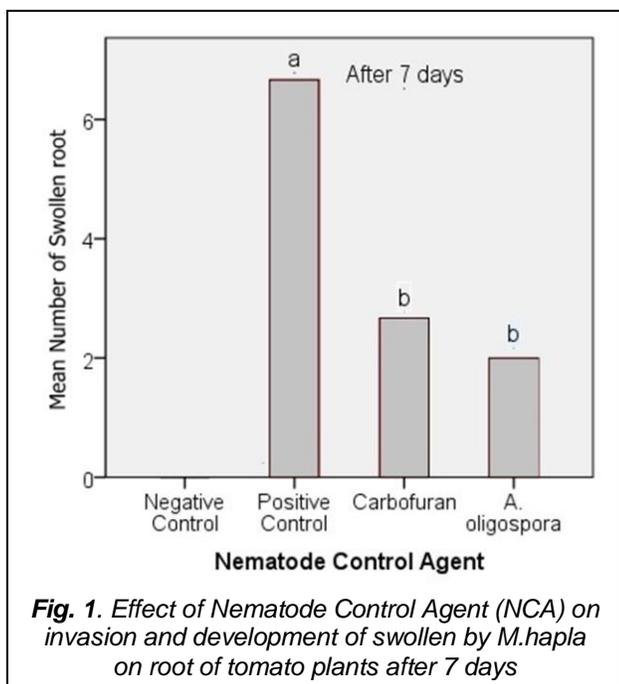
#### 3.1.1 Effect NCA to the swollen development after 7 days

After seven days the treatment were significantly different on the swollen with significant value lower than 0.05 (Table 1).

**TABLE 1: ANALYSIS OF VARIANCE FOR THE EFFECT OF NCA ON REPRODUCTION AND GALLS DEVELOPMENT ON TOMATO PLANTS AFTER 7 DAYS**

Test Statistics <sup>a,b</sup>	
	Swollen
Chi-Square	8.022
df	3
Asymp. Sig.	.046

a. Kruskal Wallis Test  
 b. Grouping Variable: Nematode Control Agent



The graph on fig.1 show that the minimum number of swollen is on the tomato plants treated by Sumateran *A. oligospora* followed by the tomato plants treated by Carbofuran if compared with Positive Control Plants.

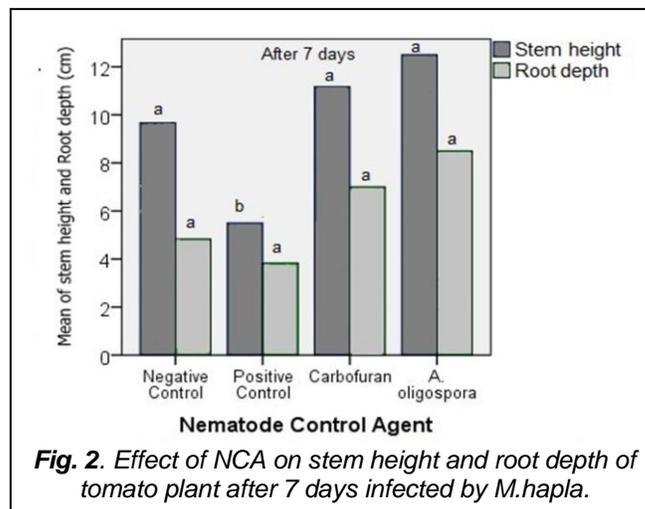
**3.1.2 Effect NCA to the stem height and root depth after 7 days**

After seven days the treatments were significantly different on the stem height with significant value lower than 0.05 (Table 2).

**TABLE 2: ANALYSIS OF VARIANCE FOR THE EFFECT OF NCA ON STEM HEIGHT AND ROOT DEPTH OF TOMATO PLANTS AFTER 7 DAYS**

Test Statistics <sup>a,b</sup>		
	Stem height	Root depth
Chi-Square	7.876	6.958
df	3	3
Asymp. Sig.	.049	.073

a. Kruskal Wallis Test  
 b. Grouping Variable: Nematode Control Agent



Based on the graph on fig 2, the maximum stem height is on the tomato plants treated by *A. oligospora* followed by the tomato plants treated by Carbofuran.

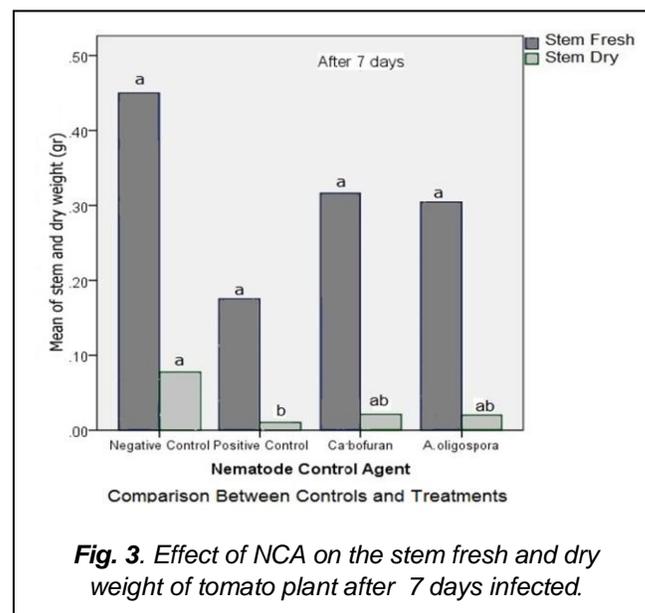
**3.1.3 Effect NCA to the stem fresh and dry weight after 7 days**

After seven days the treatment was significantly different on the stem dry weight with significant value  $\geq 0.05$  (Table 3).

**TABLE 3: ANALYSIS OF VARIANCE FOR THE EFFECT OF NCA ON STEM FRESH AND DRY WEIGHT OF TOMATO PLANTS AFTER 7 DAYS**

Test Statistics <sup>a,b</sup>		
	Stem Fresh Weight	Stem dry Weight
Chi-Square	7.205	7.667
df	3	3
Asymp. Sig.	.066	.053

a. Kruskal Wallis Test  
 b. Grouping Variable: Nematode Control Agent



Based on the graph on fig 3, the maximum stem dry weight is on uninfected tomato plants. The tomato plants treated by A. oligospora and tomato plants treated by Carbofuran were higher in stem dry weight than infected control plants.

**3.2 Assessment II, 15 days after infected by Meloidogyne hapla.**

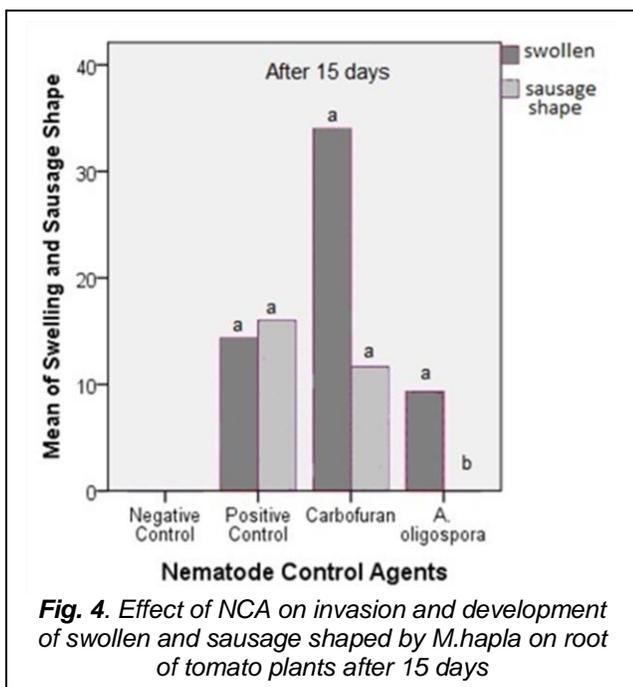
**3.2.1 Effect NCA to the swollen and sausage shaped development after 15 days**

After 15 days the treatment were significantly different on the swollen and sausage shaped with significant value lower than 0.05 (Table 4). However it is not significant different in the number of swollen with significant value more than 0.05

**TABLE 4: ANAYSIS OF VARIANCE FOR THE EFFECT OF NCA ON REPRODUCTION AND GALLS DEVELOPMENT ON TOMATO PLANTS AFTER 15 DAYS**

Test Statistics <sup>a,b</sup>		
	Swollen	Sausage Shaped
Chi-Square	6.550	9.739
df	3	3
Asymp. Sig.	.088	.021

a. Kruskal Wallis Test  
b. Grouping Variable: Nematode Control Agents



**Fig. 4. Effect of NCA on invasion and development of swollen and sausage shaped by M.hapla on root of tomato plants after 15 days**

Based on the graph in the fig. 4, show that the minimum number of the sausage shape is on the tomato plants treated by A.oligospora if compared with positive contro planst also with tomato plants treated by Carbofuran. In the second assessment the growth factors were not measurement. The assessment has been concerned in the effect of A.oligospora and Carbofuran and the galls development.

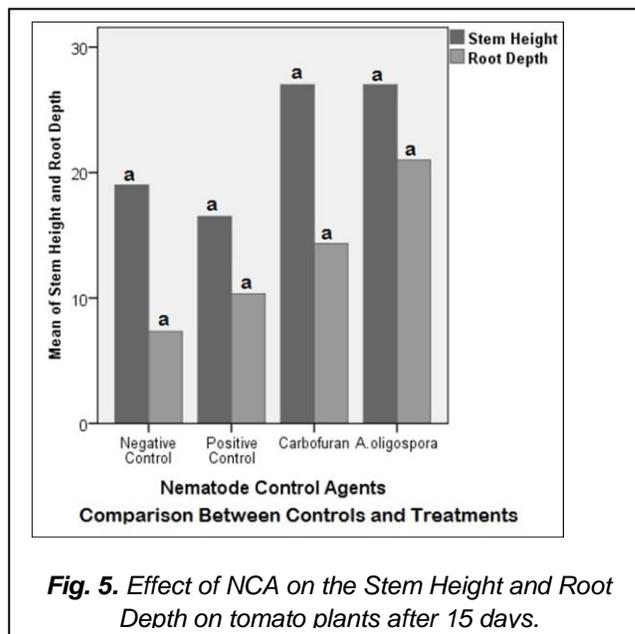
**3.2.2. Effect NCA to the stem height and root depth after 15 days**

After 15 days the treatment were not significantly different on the stem height and root depth with significant value bigger than 0.05 (Table 5).

**TABLE 5: ANAYSIS OF VARIANCE FOR THE EFFECT OF NCA ON STEM HEIGHT AND ROOT DEPTH ON TOMATO PLANTS AFTER 15 DAYS**

Test Statistics <sup>a,b</sup>		
	Stem Height	Root Depth
Chi-Square	4.867	5.955
df	3	3
Asymp. Sig.	.182	.114

a. Kruskal Wallis Test  
b. Grouping Variable: Nematode Control Agents



**Fig. 5. Effect of NCA on the Stem Height and Root Depth on tomato plants after 15 days.**

**3.2.3. Effect NCA to the stem fresh weight and stem dry weight after 15 days**

After 15 days the treatment were not significantly different on the stem fresh weight and stem dry weight of tomato plants with significant value bigger than 0.05 (Table 6).

**TABLE 6: ANAYSIS OF VARIANCE FOR THE EFFECT OF NCA ON STEM FRESH WEIGHT AND STEM DRY WEIGHT ON TOMATO PLANTS AFTER 15 DAYS**

Test Statistics <sup>a,b</sup>		
	Stem Fresh Weight	Stem Dry Weight
Chi-Square	3.821	1.351
df	3	3
Asymp. Sig.	.282	.717

a. Kruskal Wallis Test  
b. Grouping Variable: Nematode\_Control\_Agents

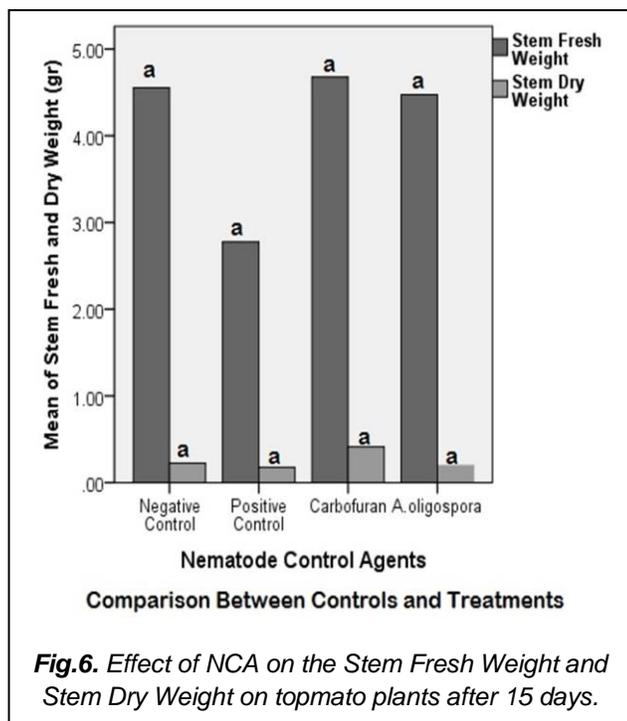


Fig.6. Effect of NCA on the Stem Fresh Weight and Stem Dry Weight on topmato plants after 15 days.

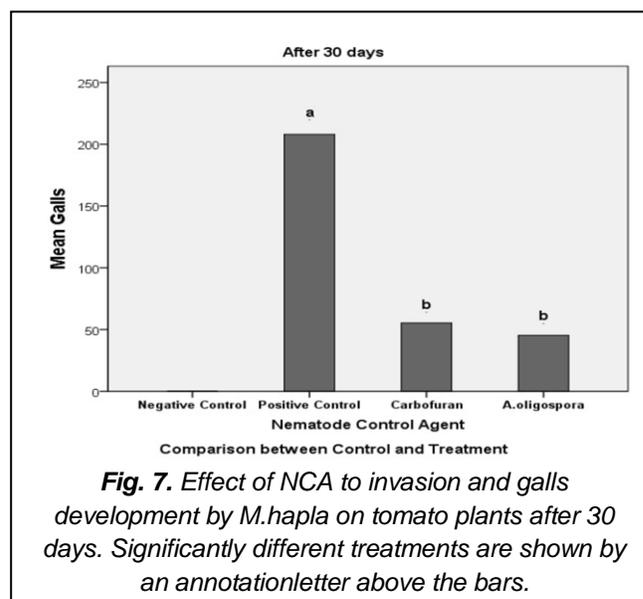


Fig. 7. Effect of NCA to invasion and galls development by M.hapla on tomato plants after 30 days. Significantly different treatments are shown by an annotation letter above the bars.

3.3. Assessment III, 30 days after infected by Meloidogyne hapla

3.3.1 Effect NCA to the sausage shaped and galls development.

After 30 days the treatment were significantly different on the sausage shaped and galls with significant value lower than 0.05 (Table 7).

TABLE 7: ANAYSIS OF VARIANCE FOR THE EFFECT OF NCA ON REPRODUCTION AND GALLS DEVELOPMENT ON TOMATO PLANTS AFTER 30 DAYS

Test Statistics <sup>a,b</sup>	
	Galls
Chi-Square	9.596
df	3
Asymp. Sig.	.022

- a. Kruskal Wallis Test
- b. Grouping Variable: Nematode Control Agent

3.3.2 Effect NCA to the stem height and root depth after 30 days

After 30 days the treatment were not significantly different on the stem and root length after 30 days infection by M. hapla with significant value higher than 0.05 (Table 8).

TABLE 8: ANAYSIS OF VARIANCE FOR THE EFFECT OF NCA ON THE STEM HEIGHT AND ROOT DEPTH ON TOMATO PLANTS AFTER 30 DAYS

Test Statistics <sup>a,b</sup>		
	Stem Height	Root Depth
Chi-Square	1.616	5.109
df	3	3
Asymp. Sig.	.656	.164

- a. Kruskal Wallis Test
- b. Grouping Variable: Nematode Control Agent

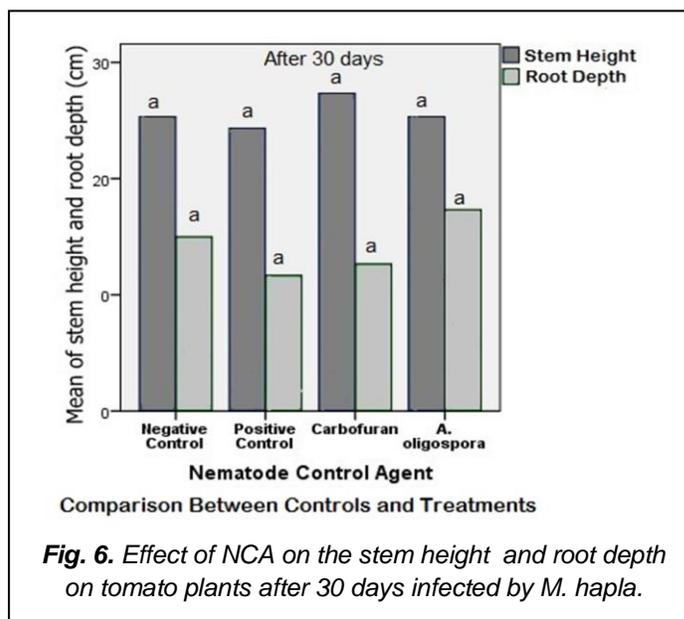


Fig. 6. Effect of NCA on the stem height and root depth on tomato plants after 30 days infected by M. hapla.

### 3.3.3 Effect NCA to the stem fresh weight and stem dry weight after 30 days

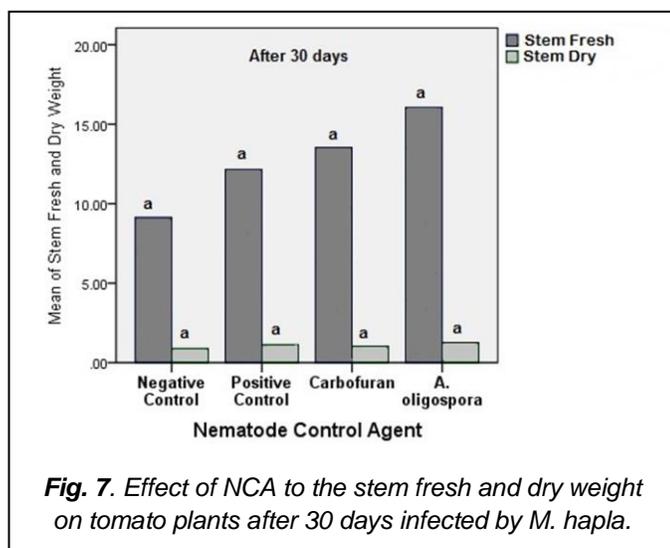
After 30 days the treatment were not significantly different on the stem fresh weight and stem dry weight after 30 days infection by *M. hapla* with significant value higher than 0.05 (Table 7).

**TABLE 7: ANALYSIS OF VARIANCE FOR THE EFFECT OF NCA ON STEM FRESH AND STEM DRY WEGHT ON TOMATO PLANTS AFTER 30 DAYS**

Test Statistics <sup>a,b</sup>		
	Stem Fresh Weight	Stem Dry Weight
Chi-Square	6.795	4.846
df	3	3
Asymp. Sig.	.079	.183

a. Kruskal Wallis Test

b. Grouping Variable: Nematode Control Agent



**Fig. 7. Effect of NCA to the stem fresh and dry weight on tomato plants after 30 days infected by *M. hapla*.**

## 4. Discussion

In this study the comparison between tomato plants treated by Nematode Control Agent: Sumatran NTF *Arthrobotrys oligospora* as biocontrol agents and Carbofuran as a chemical control agent and un-treated plants, demonstrated that biocontrol could be achieved using fungi in a newly developed pot bioassay. At 7 days, positive control plants were exhibiting significantly higher numbers of swollen roots than any of the NCA treatments. By 15 days nematode infection had progressed in the infected control to significantly more sausage shaped roots than in *A. oligospora* treated plants, however no other NCA treatments showed a significant reduction in the number of sausage shaped roots. After 30 days galls had developed in the infected control plant roots, but in Carbofuran and *A. oligospora* treatment plants gall development was significantly lower. In this pot trial, inoculation of *A. oligospora* onto the roots of tomato seedling proved as effective as Carbofuran® treatment in reducing the numbers of infections as assessed by the development of galls. Recently experiment done by researcher known that some secondary metabolite compounds produced by Nematophagous fungi are attractant and non-attractant to nematodes. Among of those compounds the oligosporon compounds (4', 5'-dihydrooligosporon) are nematicidal activity

most likely derived from *A. oligospora* [18]. This compound causes the nematodes to be attracted approach to the trapping organ. When the body wall of nematode merely touches the trapping organ, the body wall will stick to the trap-forming cell. The haustoria hyphae will penetrate the body wall of the nematode and the digest process will begins. [19]. The effects of infestation of tomato plants with nematodes on plant growth parameters including plant height, root depth, and fresh and dry weight of the stem can be seen by looking at the progressive changes in these parameters over the sample period. Based on the data shown in measurements demonstrated that the roots of the tomato treated by either Carbofuran or *A. oligospora* showed increase the growth of tomato plant until the end of assessment on the plant height, stem fresh weight and stem fresh weight parameter. However, NCA neither *A. oligospora* or Carbofuran show growth promotion until the end of experiment. Plants treated by *A. oligospora* were seen to have the best growth parameters in terms of plant height, root depth and stem fresh and dry weight of tomato plants especially in the first week after infection. It can conclude that all the growth of tomato plants treated by either Carbofuran® or *A. oligospora* were only faster in the first 15 days of the experiment, and slowing in the subsequent 15 days until the end of the assessment. Similar research conducted by [19] using two levels of concentration of inoculum of *Monacrosporium ellipsosporium* against RKN demonstrated that *Monacrosporium ellipsosporium* improved tomato plant growth and reduced infestation of *M. incognita* on the root of tomato plants. Furthermore, research using (Pc123) [20] proved that *Pochonia chlamydosporia chlamydosporia* also had plant growth promoting effects. The precise reason for the plant growth promoting effects of the NTF is unknown. The growth promoting effects of fungal treatment inhibited may be because at the same time as the lesion occurred in the root of tomato plants. Lesion formed by the stylet injection of the second stage juvenile of *M. hapla* can cause simultaneous entry by other pathogenic organisms or synergistic effects of NTF. Similar experiment using *A. oligospora* to control root knot disease of tomato caused by *M. incognita* and root rot disease by *Rhizoctonia solani* were also made by [21]. In their research proved that *A. oligospora* reduced number of *M. incognita* and disease caused by *R. solani* and also enhance growth of tomato plants. It can assume that *A. oligospora* has a potential as bio-protection agents against *M. incognita* and *R. solani* also growth promoting agent to the host plant. Recent experiment also done by Mostafanezhad et al., 2014 [22]. Based on their research, proved that *A. oligospora* also potential as bioprotection in control number of *M. javanica* and show a growth promoting in tomato plant.

## 4 CONCLUSION

*A. oligospora* isolate from North Sumatera proved comparable with Carbofuran as gold standard in suppress number of infections by Root-Knot Nematode on tomato plants. It must be considered their potential as bioprotection agents against *M. incognita* also as growth promoting effect as an advantage method to replace conventional pesticide contain hazardous compound. Natural barriers to the use of Sumatran Nematode trapping fungi must be broken down and developed especially in the farming area in North Sumatera, Indonesia, since the impact on the environment and the farmer is minimal.

## 5. Acknowledgments

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