



# **Influence of 3-Methylthiopropionic Acid (MTPA) Produced by *Rhizoctonia solani* AG-3 on Yield and Dry Matter Accumulation of Potato**

**Frederick Kankam<sup>1,2\*</sup>, Elias Nortaa Kunedeb Sowley<sup>2</sup> and Qiu Huizhen<sup>1</sup>**

<sup>1</sup>College of Resources and Environmental Sciences, Gansu Agricultural University/Gansu Provincial Key Laboratory of Aridland Crop Science, Lanzhou 730070, China.

<sup>2</sup>Department of Agronomy, University for Development Studies, P.O.Box 1882, Tamale, Ghana.

## **Authors' contributions**

*This work was carried out in collaboration between all authors. Authors FK, ENKS and QH designed the study. Author FK performed the statistical analysis, wrote the protocol and first draft of the manuscript. Authors ENKS and QH managed the analyses of the study. Author ENKS managed the literature searches. All authors read and approved the final manuscript.*

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## **ABSTRACT**

Studies were conducted to investigate the effect of 3-methylthiopropionic acid (MTPA) produced by *Rhizoctonia solani* AG-3 on yield and dry matter accumulation of potato. The experiments were laid out in a completely randomized design with five treatments and five replications. The treatments were 0, 1, 2, 4 and 8 mM concentrations of MTPA. Results showed that the MTPA phytotoxin reduced plant growth and tuber yield, compared to the control. Plants treated with 8.0 mM MTPA significantly reduced aboveground fresh weight and belowground fresh weight by 260.14% and 395.95%, respectively, compared to the control. Plants treated with 8.0 mM MTPA significantly reduced tuber yield by 419.12% compared to the control. Application of 8.0 mM MTPA also significantly decreased fresh weight of individual tuber by 100.22% in comparison with control plants without MTPA phytotoxins. Treatment of plants treated with 8.0 mM MTPA increased biomass of

\*Corresponding author: E-mail: [fkankam@uds.edu.gh](mailto:fkankam@uds.edu.gh);

potato plants by 112.44%. Cankers were seen on the potato seedling stems treated with 3-methylthiopropionic acid with 8.0 mM MTPA producing the highest stolon and stem canker of 97.78% and 97.10%, respectively. Compared with untreated control, black scurf incidence of potato plants treated with the phytotoxin at a rate of 8 mM was increased by 94.59%. The study demonstrated that *R. solani* phytotoxin can induce stem canker and black scurf development resulting in yield reduction of potato plant.

**Keywords:** 3-Methylthiopropionic acid; toxin; *Rhizoctonia solani*; potato; biomass.

## 1. INTRODUCTION

The black scurf and stem canker diseases of potato (*Solanum tuberosum* L.) caused by the soil-borne fungus *Rhizoctonia solani* Kühn (teleomorph: *Thanatephorus cucumeris* (Frank) Donk) [1] is the most destructive disease in potato production. *R. solani* does not produce asexual spores, but survives as mycelium (hyphal growth form), sclerotia (dense asexual hyphal resting structures) or basidiospores [2]. Sclerotia of *R. solani* are compact bodies of accumulated melanised hyphae that aid the pathogen to persist under extremely severe conditions for long periods due to their high resistance to chemical and biological degradation [3]. The disease is common in potato growing areas. However, its severity is higher in temperate climates than in the tropics. The disease causes both quantitative and qualitative economic damage to the crop. Quantitatively, losses occur due to infection of stems, stolons and roots, which reduces tuber size and number. Qualitative losses occur because the disease is often associated with misshapen tubers and the discolorations described as black scurf. Potato is not the only plant affected by *R. solani* [4]. *Rhizoctonia* disease causes devastating effects to several plants [5] including rice [6,7], wheat and barley [8], cotton [9], chickpea [10], and soybean [11]. The stem canker is favoured by cool temperatures that delay emergence after planting. In severely infested fields, the aforementioned symptoms can result in death of sprouts and potato stems, leading to malformed tubers and reduced yields resulting from poor stand emergence [12].

Phytotoxic activities of 3-methylthiopropionic acid (MTPA) on various species of plants have been reported by various workers [13]. For example, there is one published report of carboxylic acid, including tiglic, phenylacetic, isovaleric, 3-methylthiopropionic and trans-3-methylthioacrylic acid, isolated from liquid cultures of the rice pathogen, *Xanthomonas*

*campestris* pv. *oryzae* [14]. There is a lot of information about the bioactive role played by several fungal toxins. Nonetheless, little information is available concerning the impact of *R. solani* toxins on potato stems, and the effects of 3-Methylthiopropionic acid (MTPA) on dry matter accumulation of potato. Knowledge on the effects of *R. solani* phytotoxin on potato, would lead to designing appropriate techniques to increase plant resistance. Furthermore, an understanding of the role of MTPA in the disease process would help in improving *in vitro* screening for *R. solani* resistance, thereby designing appropriate tools and strategies to manage the disease.

The objective of this study was to investigate the role of MTPA produced by *R. solani* on yield and dry matter accumulation on potato.

## 2. MATERIALS AND METHODS

### 2.1 Soil Sterilisation and Source of Seed

Sandy loam soil was collected from the surface of protected cultivation plots, in Lanzhou, China. Soil sterilisation was done using an autoclave at a temperature of 121°C and a pressure of 1.02 kg/cm<sup>3</sup> for 30 min. Seeds of potato cv. Leshu (susceptible cultivar) were obtained from the Gansu Provincial Key Lab of Aridland Crop Science, Gansu Agricultural University, Lanzhou, China.

### 2.2 Potato Stems Canker Response to MTPA

Six sprouted seeds of potato cv. Leshu were planted in plastic containers (10 cm radius, 7-cm deep) containing a 1:1 mixture of pasteurised peat and sand. The pots were placed in a 22 °C growth chamber. The source of light was fluorescent bulbs. The bulbs were set to a photoperiod of 14 h of light. The plants were watered as needed with distilled water for 2 weeks.

Aqueous solutions of MTPA were standardised to pH 2.5 and filter-sterilised. All solutions were prepared to a final total concentration of 0, 1, 2, 4 and 8.0 mM. Fifty microliters of each solution were applied to the base of the potato stems using an aseptic 1 cc syringe and 26-gauge needle. Control plants were treated with distilled water without MTPA solution. Each treatment was replicated five times. The experimental design used was randomized complete block design. The experimental plants were watered 24 h after phytotoxin treatment, with di-H<sub>2</sub>O. After 90 days, the plants were harvested from the pots and measurements on root and shoot length, number of lateral roots, incidence of root necrosis, length of stem canker and diseased stolon for each plant were recorded. The experiment was conducted twice.

### **2.3 Fungal Culture Filtrates and MTPA Toxin Production**

PDA plates were used to grow cultures of a virulent isolate of *R. solani* at 25°C for 7 days. Six millimeter (6 mm) diameter plugs of actively growing mycelium on PDA were cut with a cork borer. One disc was removed and later transferred into a 250 ml flask that contained 100 ml potato sucrose broth (PSB). Twenty-four flasks were placed on a rotary shaker (180 rpm) and incubated at 25°C for 3 days and then stored at 4°C. For production of toxin, two mycelium groups from PSB cultures were transferred into a 500-ml flask containing 200 ml of Richard's medium. There is one published report by Lu et al. [15] that *R. solani* releases toxins in this type of medium. The cultures were subsequently incubated under stationary conditions at 25°C on artificial vibration once a day for 18 days. Culture filtrates were obtained by passing the liquid through four layers of cheese cloth and Whatman No. 1 filter paper. The experiment was conducted twice. Fourier-transform infrared spectroscopy (FTIR), high performance liquid chromatography (HPLC) and <sup>1</sup>H and <sup>13</sup>C NMR spectral techniques were used to characterise the phytotoxin as 3-methylthiopropionic acid (3-MTPA) [16]. It had a molecular weight of 119.158 g/mol.

### **2.4 Experimental Design**

The pots were arranged in a completely randomized design (CRD) comprising 5 treatments, each replicated 5 times. The treatments consisted of four levels of MTPA (1, 2, 4 and 8 mM) and a control (0 mM).

## **2.5 Data Collection**

Five plants were randomly sampled from the pots at 90 days after planting (DAP) to determine development of vegetative growth in potato plants. Tap water was used to wash the roots of various plants. Data were collected on the following parameters: stem diameter, diseased stolon, tuber weight, number of tubers per plant, tuber yield and stem canker indices.

### **2.5.1 Stem diameter**

This parameter was measured on the same plants whose heights were measured and at the same period after planting. The widths of the stems from the five tagged plants were measured using veneer calipers and their means calculated and recorded.

### **2.5.2 Number and weight of tubers**

The plants were harvested at 90 days after planting. The roots were then severed from the plants, washed carefully on a 2 mm sieve under a jet of tap water to remove any adhering soil and organic debris after which the tubers were detached from the roots and counted and the fresh tuber weights obtained by using an electronic weighing scale. Potato yield per plant was recorded on fresh tuber basis. Underground and above fresh weights were also determined using an electronic digital balance.

### **2.5.3 Plant biomass**

At 90 days after planting, the sampled plants were then dried separately at 80°C in an oven for 48 h to constant weights and the root and shoot dry weights were recorded. The various organs were thoroughly dried to obtain the biomass comprising of the tubers, roots, stems, and the leaves. Potato yield per plant was recorded on dry tuber basis. The weight measurements were done using an electronic digital balance. The ratio of root to shoot (RS) index was estimated by dividing the root biomass by the above-ground biomass. The harvest index (HI) was also determined by finding the ratio of tuber biomass to the whole plant biomass.

### **2.5.4 Disease severity assessment**

At 90 days after planting, plants were carefully removed from their pots and washed with running tap water to remove adhering sand. Disease symptoms assessed included stem

canker and stolon canker, necrosis of root and sclerotia on tubers. The infection on stems and stolon were assessed, on a scale of 0-5, as described by Tsrar and Peretz-Alon [17] where:

- 0 = healthy tissue
- 1 = several brown to black lesions
- 2 = up to 15% of the tissue is covered with lesions
- 3 = up to 30% of the tissue is covered with lesions
- 4 = up to 60% of the tissue is covered with lesions and
- 5 = > 60% of the tissue is covered with lesions

## 2.6 Statistical Analyses

All statistical analysis was performed with GenStat (Ninth Edition, 2007) using one-way ANOVA. Means were separated using the least significant difference (LSD) at  $P < .05$ . Duncan's multiple range tests was used when one-way ANOVA indicated significant differences ( $P < .05$ ). The relationship between the tuber yield and the stem canker severity or the ratio of root to shoot was analysed using Generalized Linear Model.

## 3. RESULTS

### 3.1 Stem Diameter

Generally, plants treated with MTPA were significantly shorter and had least stem diameter ( $P > 0.05$ ) than those of the control at 90 days after planting with 8 mM MTPA being the least (Table 1).

**Table 1. Effect of MTPA on stem diameter**

Treatment	Stem diameter (mm)
Control	6.50a
1 mM	5.00b
2 mM	4.10c
4 mM	3.64cd
8 mM	3.10d
LSD (0.05)	0.70
CV (%)	4.00

Means followed by the same letter(s) in a column are not significantly different ( $P > .05$ ).

### 3.2 Effect of MTPA Phytotoxin on Yield of Potato Plants

Tuber number and yield significantly declined as MTPA phytotoxin in plants increased. Plants treated with 8.0 mM MTPA significantly reduced aboveground fresh weight and belowground

fresh weight by 260.14% and 395.95%, respectively, compared with the control (Table 2). Plants treated with 8.0 mM produced the least number of tubers which were comparable to those produced by plants treated with 4.0 and 2.0 mM MTPA but significantly lower ( $P < .05$ ) than tubers produced by plants treated with 1 mM and the control (Table 2). Plants treated with 8.0 mM MTPA significantly reduced tuber yield by 419.12% compared with the control.

Results of the present study revealed that the application of MTPA phytotoxin affected fresh weight of individual tubers significantly ( $P < .05$ ). The phytotoxin increased stem canker and also significantly retarded potato growth and thus reduced the fresh weight of the tubers. The lowest fresh weight of individual tuber was found in the 8.0 mM MTPA treated plants, while the highest was found in the control plants which were without MTPA application (decrement of 100.22% in comparison with control). The fresh weight of individual tubers in all MTPA phytotoxin treatments was lower than those in the control plants in a rate dependent manner (Table 2).

### 3.3 Plant Biomass

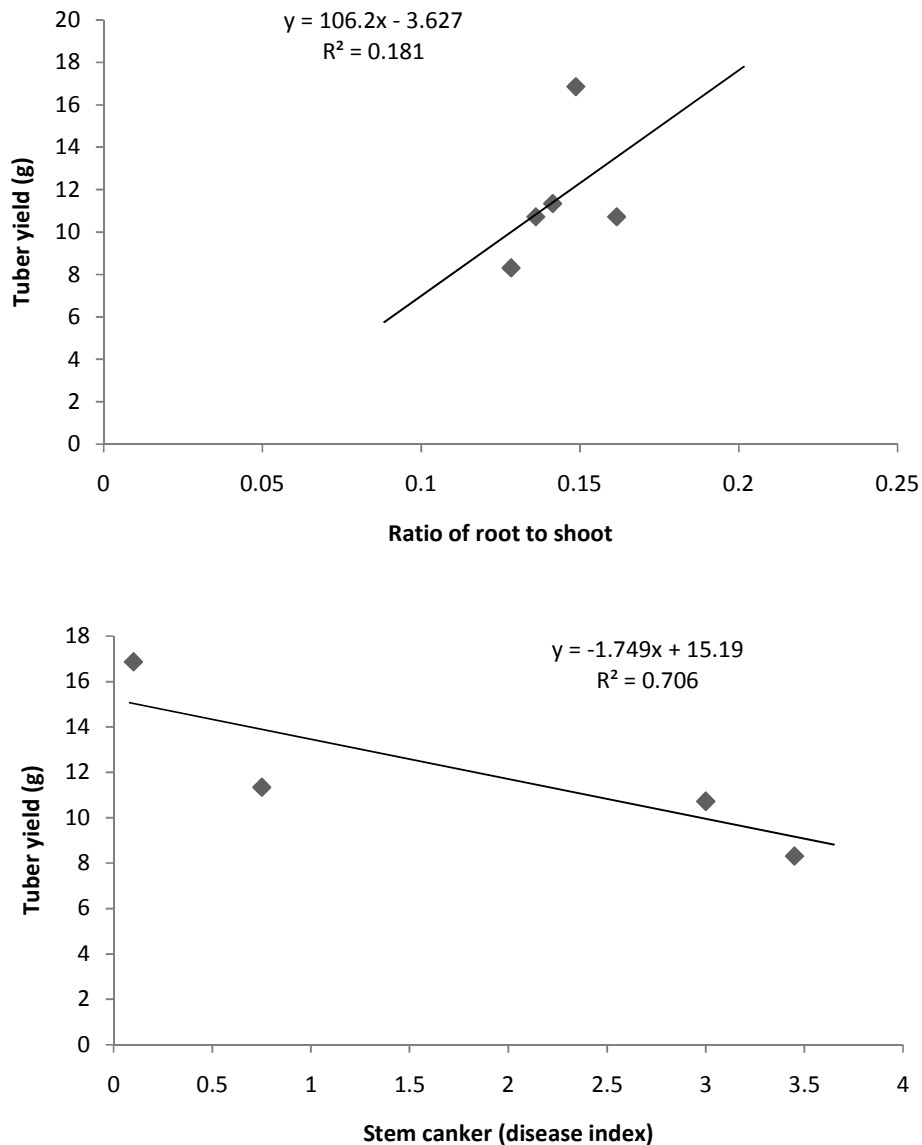
Biomass of the whole potato plants under MTPA phytotoxin treatment significantly decreased by 69.20% and 112.44% on 4.0 and 8.0 mM MTPA relative to control, respectively (Table 3). Biomass of different potato organs including tuber and stem as well as leaves under MTPA phytotoxin treatment all significantly decreased relative to the control especially potato roots decreasing the highest of 148.89% and 64.71% on 8.0 and 4.0 mM MTPA treated plants, respectively. Similarly, there were also significant differences ( $P < .05$ ) in root biomass under 4.0 and 8.0 mM MTPA treatments. Distinct difference in R:S value was observed between the control and MTPA phytotoxin treatments, and R:S value under MTPA phytotoxin treatment decreased by 7.98% on 4.0 mM MTPA and 15.90% on 8.0 mM MTPA compared with the control respectively, indicating that MTPA phytotoxin treatment significantly affected dry matter distribution between potato canopy and root system ( $P < .05$ ). Meanwhile, there was a significant positive correlation ( $R^2=0.1814$ ,  $P < .05$ ,  $n=24$ ) between root to shoot (RS) value and potato tuber yield (Fig. 1a). In addition, there were significant differences in harvest index (HI) between the control and MTPA phytotoxin treatments both on 4.0 mM and 8.0 mM MTPA ( $P < .05$ ). On the contrary, there was a significant negative

correlation ( $R^2=0.7066$ ,  $P < .05$ ,  $n=24$ ) between stem canker severity and potato tuber yield (Fig. 1b). The results confirmed that MTPA phytotoxin could significantly reduce potato yield and plant biomass in potato production.

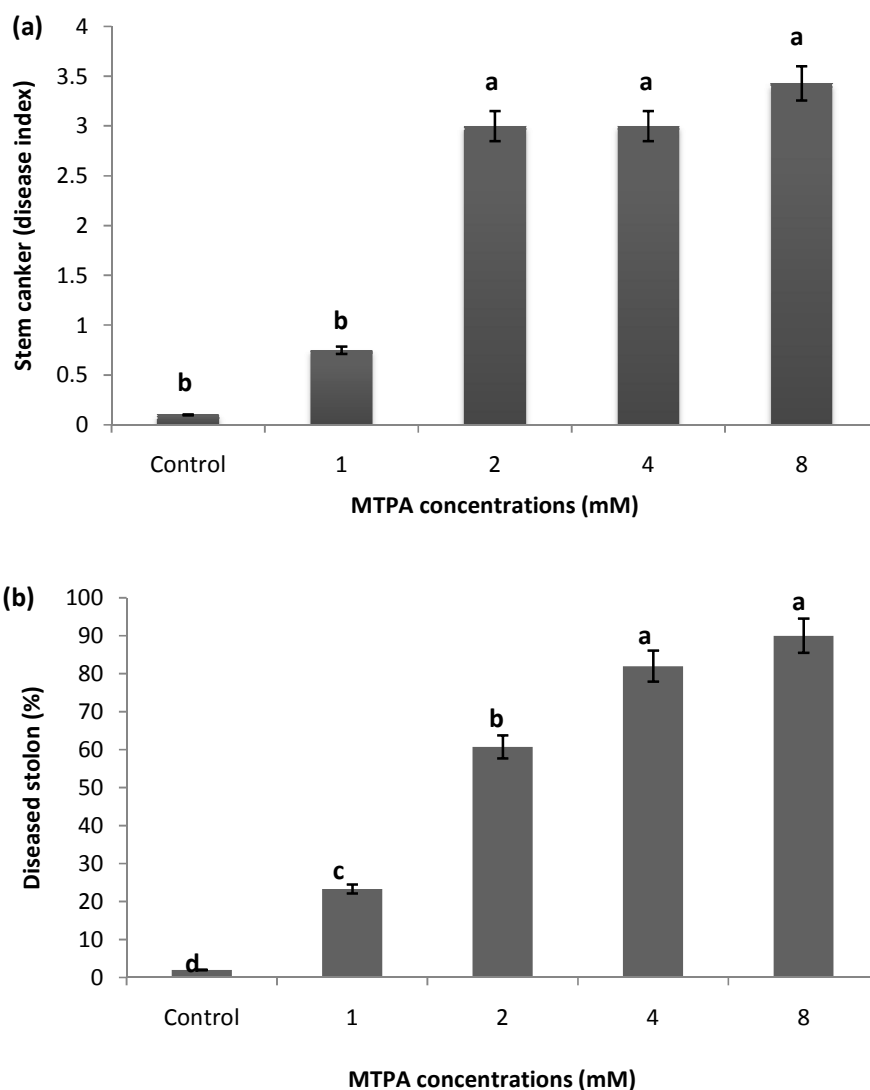
### 3.4 Disease Assessment of Potato Plants

Plants treated with 8 mM MTPA had the highest number of diseased stolon and severe stem

canker indices followed by 4, 2, and 1 mM MTPA (Fig. 2). However, the control plants without MTPA phytotoxin had the least disease infection. There was significant difference between 8 mM MTPA and the control ( $P < .05$ ). Cankers resulted from the injection of potato seedling stems with 8.0 mM MTPA producing the highest stolon and stem canker of 97.78% and 97.10%, respectively (Fig. 2a and 2b).



**Fig. 1. Relationship between tuber yield per plant and ratio of root to shoot or stem canker severity; (a) Correlation between tuber yield and ratio of root to shoot ( $P < .05$ ,  $n=24$ ) (b) Correlation between tuber yield and stem canker severity ( $P < .05$ ,  $n=24$ )**



**Fig. 2. Effect of *R. solani* phytotoxins on diseased stolon, stem canker and black scurf severity; (a) stem canker length (b) diseased stolon**

**Table 2. Effect of *R. solani* phytotoxins on yield and yield components of potato**

Treatment	Aboveground fresh weight (g)	Underground fresh weight (g)	Tuber number per plant	Tuber yield per plant (g)	Average fresh weight of individual tuber (g)
Control	104.80a	104.05a	3.63a	101.02a	27.83a
1 mM	91.30b	51.00b	2.40b	48.62b	20.26b
2 mM	63.60c	33.26c	1.80c	31.75c	17.64bc
4 mM	40.00d	27.00cd	1.71cd	25.65d	15.00cd
8 mM	29.10e	20.98d	1.40d	19.46e	13.90d
LSD (0.05)	1.66	6.56	0.33	5.73	2.76
SED	0.72	2.85	0.10	2.49	1.20
CV (%)	1.30	3.70	8.00	2.60	7.70

Means within the same column which share the same letters are not significantly different at  $P > .05$

**Table 3. Effect of *R. solani* phytotoxins on dry matter contents of potato plants**

Treatment	Whole plant (g.plant-1)	Root (g.plant-1)	Stem (g.plant-1)	Leaf (g.plant-1)	Tuber (g.plant-1)	(RS)	(HI)
Control	34.67a	2.24a	10.42a	5.15a	16.86a	0.1487a	0.4863b
1 mM	25.92b	1.70b	8.00b	4.00b	11.34b	0.1414a	0.4375c
2 mM	23.58c	1.52b	7.52b	3.82b	10.72b	0.1361a	0.4546c
4 mM	20.49d	1.36b	5.50c	2.98c	10.72b	0.1616a	0.5232a
8 mM	16.32e	0.91c	5.00c	2.10d	8.31c	0.1283a	0.5092a
LSD (0.05)	0.83	0.43	0.97	0.55	1.68	0.0346	0.0179
SED	0.36	0.19	0.42	0.24	0.73	0.0150	0.0078
CV (%)	3.20	6.10	11.10	1.30	7.80	10.20	3.30

The values in this table are biomass for the whole plant or different organs. Means within the same column which share the same letters are not significantly different at  $P > .05$

#### 4. DISCUSSION

This study was sought to determine the concentrations of MTPA phytotoxins required to influence yield and dry matter accumulation and disease symptom development on potato. The phytotoxin had a significant effect on the overall development of the plant in the trials, with decreased stem girth and lower tuber yields than control plants grown in untreated MTPA medium. Plants treated with 8 mM MTPA produced the shortest and poorly developed canopies compared to the other treatments and the control. The application of MTPA reduced aboveground fresh weight, tuber weight and number of tubers per plant compared to the control. The results showed that the tuber yield of plants treated with 8 mM MTPA decreased by 419.12% relative to the control plants (Table 2). The poor performance of MTPA treatment with respect to growth and yield could be due to the pathogenic effect of *R. solani*, producing symptoms of stem canker on underground stems, stolon and tuber-borne sclerotia [18]. The production of a higher number and heavier tubers in control plants without MTPA phytotoxin could be due to a better translocation of water and nutrients to the shoots [19] than the MTPA treated plants whose underground stems had stem canker, leading to a reduction in crop vigor as a result of expenditure of seed energy used to produce secondary sprouts to compensate for damage to primary sprouts reported by [20].

The results also showed that the phytotoxin treatments substantially reduced biomass of potato plants compared with the control (Table 3). Meanwhile, the incidence of stem canker and diseased stolon significantly increased on 8.0 mM MTPA treatment compared with the control (Fig. 2a and 2b). The results confirmed findings of Mao and other workers [21], that phytotoxin

can induce the growth of *R. solani*. In addition, 8.0 mM MTPA treatment also significantly reduced the ratio of root to shoot (RS) in potato plants by 15.90% compared with the control treatments (Table 3). The increase in RS value of crops was considered as the basal strategy in response to the bio- or abiotic-stress environment such as continuous monoculture practice, which would greatly consume assimilation products and limit the biomass filling into potato tuber. Linear correlation analysis between RS value and tuber yield, and biomass of potato plants also confirmed the shift in RS value was related to tuber yield decline. In this study, it was considered that the significant decline of RS value of potato plants treated with MTPA phytotoxin compared with plants phytotoxins might represent the alleviation of environment stress caused by *R. solani* phytotoxin and the normal distribution of assimilation products performed by potato plants, which were probably connected with the relative reaction of phytotoxin supplied.

The higher incidence of diseased stolon and stem canker in MTPA treated plants could be due to the production of toxic metabolites, as reported by Robeson and Cook [22]. It could also be attributed to the effect of the phytotoxins which induced pathogenicity in the potato plant, resulting in chlorotic lesions and stem canker [23].

#### 5. CONCLUSION

The study revealed that all the MTPA concentrations reduced stem diameter, aboveground fresh weight, and tuber weight, number of tubers per plant, plant biomass and diseased stolon. Increasing the MTPA phytotoxin concentration resulted in corresponding increase in diseased stolon and stem canker severity. The

reductions in growth parameters and stem canker indices were found to be proportional to the MTPA phytotoxin level. MTPA phytotoxin is a key component in the pathogenicity of *Rhizoctonia* disease of potato and the damage is most severe at 8 mM MTPA.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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