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The Effect of Conventional and Nanoformulation Herbicide on *Sphagneticola Trilobata*

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Abstract

The conventional herbicide 2-methyl-4-chlorophenoxyacetic acid (MCPA) provide substantial risk to the environment such as contamination of soil and groundwater. The overuse application of conventional herbicide can leave harmful residues in soil and be washed off as runoff, thus causes toxic effect to nontarget organism. For instance, MCPA can cause toxicity to the freshwater organisms such as *Daphnia magna* and alga *Selenastrum capricornutum*. Zinc oxide as nanomaterials that are intercalated with 2-methyl-4-chlorophenoxyacetic acid (ZMCPA) herbicide may be useful to resolve the problems associated with MCPA. Nanoherbicide are known to provide a better penetration of active substances hence reduces the amount of herbicide to be applied. The nanostructured herbicide such as ZMCPA could substantially replace the conventional herbicide MCPA if it able to kill the targeted weeds efficiently and have a low risk to the environment. Therefore, it is important to compare the differences of herbicidal activity of MCPA and ZMCPA. The objective of this research is to determine the effects of MCPA and ZMCPA on growth and pigment content of *Sphagneticola trilobata*. The 2nd objective of this research is to measure the physiological effect and effectiveness of herbicidal activity between the conventional and nanoformulation against *Sphagneticola trilobata*. The preliminary study was conducted to determine a minimal concentration for MCPA to exert its effect on *Sphagneticola trilobata*. Then, in the main research, *Sphagneticola trilobata* was exposed to different concentrations of MCPA and ZMCPA in low, medium and high concentrations. The effect of MCPA and ZMCPA on growth and pigment content of *Sphagneticola trilobata* was observed at 7, 14 and 21 days, respectively. The results indicated there were no obvious differences in plant growth and pigment content observed between treatment of MCPA and ZMCPA at the same exposure concentration at 7, 14 and 21 days, respectively. MCPA showed higher herbicide efficacy than ZMCPA.

Keywords: Conventional herbicide, Nanoherbicide, MCPA, ZMCPA, *Sphagneticola trilobata*

Introduction

Herbicides are considered as the easiest method for controlling large scale weeds and conserving crops [1]. Weeds are the obnoxious plant that grows among crop plants and interferes with the management practice of the crops [2]. It has a similar trophic level as crop plants and compete with the crops in the terms of nutrients, water, space and light. Consequently, this causes in substantial crop yield loss. The quality and quantity of crops also decreases [3]. Hence, herbicides are commonly applied in various crops as the chemical weed control to eradicate and eliminate unwanted weeds [1].

MCPA is selective and post-emergence herbicides from synthetic auxins group that selective for broad-leaf weeds [4]. The mechanism of action of MCPA is as an auxin mimic which is acts by mimicking the hormone auxin in the plants. Auxins are growth hormones that exist in plants with function to promote cell elongation, cellular differentiation, cell expansion and inhibit apical dominance [1]. The indole-3-acetic acid (IAA) which is naturally occurring auxin able to stop the growth of the plant if used at high

concentration and can kill broad-leaf plants. MCPA has been used widely to certain weeds such as thistle and dock in pasture and cereal crops [5].

However, the continuous use of this conventional herbicides results in the development of resistance towards particular weeds. The population of weeds are able to survive under a normal capacity of herbicide exposure. According to the International Survey of Herbicide Resistance Weeds, there were 36 synthetic auxin herbicide-weed resistant. For instance, wild radish (*Raphanus raphanistrum L.*) in Australia that exhibits resistance towards conventional 2-methyl-4-chlorophenoxyacetic acid (MCPA) herbicide [6]. In addition, the overuse and repeated application of conventional herbicide can leave harmful residue in soil for a longer period of time. This residual is not active hence not available for plant uptake and able to decline the quality of soil [7]. The residue of MCPA herbicide is persistent in soil for about one month or lesser. Furthermore, if the high quantities of conventional herbicide are used, it also able to be washed off into rivers and streams as runoff thus induce toxic effect to nontarget organism. Thus, the residues of MCPA in the water may induce toxicity to humans and aquatic animals [4]. For instance, MCPA able to causes soil phytotoxicity to buckwheat (*Fagopyrum esculentum* var.Kora) and give negative effects on freshwater organisms such as *Daphnia magna*, *Thamnocephalus platyurus* and alga *Selenastrum capricornutum* [8].

Nevertheless, the use of herbicide is needed even though there are several problems associated with it. This is to prevent the growing of invasive weeds that able to cause serious threat in this global agriculture and significant problem to the farmers. Nevertheless, nowadays nanotechnology has offers advanced weed management approaches in the field of agrochemicals that can increase the effectiveness of conventional herbicides with the help of nanomaterials [9]. Nanoherbicides is the innovation and development of conventional herbicides to combat the problems caused by the conventional herbicide [10]. The uses of nanoparticles as the nanocarrier of bioactive substance will benefit both farmers and environment. The nano-encapsulated herbicide can improve weed control and lower the environmental risk [11,12]. This is due to the fact that nanoformulation provides controlled release herbicides through encapsulation. This controlled release characteristic can efficiently kill the targeted weed thus avoiding extensive use of herbicides [2].

The intercalation of nanotechnology material which is zinc layered hydroxide (ZLH) with 2-methyl-4-chlorophenoxyacetic acid (MCPA) formed a new composite which is ZMCPA. The ZMCPA nanohybrids were synthesized and utilizing the ion-exchange approach from the intercalation of MCPA into zinc oxide (ZnO) to formulate a new delivery system of herbicide that is more efficient than conventional herbicide. The intercalation process packages the active substance within a secondary material known as shell to form nano-capsules. Nanoparticles are a good carrier as a 'magic bullets' loaded with herbicides to deliver the active ingredient to the targeted weeds. Nanoherbicide are known to provide a better penetration, induce a slow and sustained release of the active substances thus improve its application. This resulting in a more proficient use of herbicide hence reduces the amount of herbicide to be applied as only minimal concentration needed, but the herbicide is still efficient to eliminate weeds. The ZMCPA could assist in reducing toxicity of MCPA to the environment and increase the optimal usage of herbicide to eliminate weeds [4].

Therefore, it is significant to study the comparison of herbicidal activity between the conventional and nanoformulation herbicide. This is because nanostructured herbicide such as ZMCPA could substantially replace the conventional herbicide MCPA. Hence, it is critical to investigate and compare the differences in the herbicidal activity of MCPA and ZMCPA to evaluate the performance of conventional and nanoformulation herbicide. It sets a benchmark for future weed management control. Furthermore, the research is important as the nano-agrochemicals has attained a great interest in the research field due to its enormous benefits to the modern agricultural system. For example, the study by Takeshita *et al.* [13] showed that nanoencapsulated metribuzin (nanoformulation) was efficient even though at lowest dose 48 $gha^{-}1$ compared to conventional metribuzin (non-nanoformulation) at dose 480 $gha^{-}1$ in inhibiting photosystem II activity and decreasing the pigment content of *Ipomea grandifolia.* The retention of nanometribuzin also was lower in the tested soils compared to conventional metribuzin hence causes a low risk of the groundwater contamination [13].

Sphagneticola trilobata was used as a model plant to compare the herbicide efficacy between the MCPA as conventional herbicide and the ZMCPA as nanoformulation herbicide. *Sphagneticola trilobata* is a member of the family Asteraceae in the sunflower and daisy family, with common name Singapore daisy or called as "Wedelia Kuning" in Malaysia [14]. It thrives in cropland, natural forests, wet roads, unused gardens, and oil palm plantations [15,16]. *Sphagneticola trilobata* is highly invasive and fast-growing weeds that is not easy to eliminate. Therefore, the effect of herbicide on *Sphagneticola trilobata* can prove the level of herbicide efficacy towards particular weed.

This research aims to establish the ability of nanoherbicide formulation to produce the desired effect and produce better results. The nanoformulation should have higher herbicide efficacy than the conventional herbicide. The objective of this research is to determine the effects of 2-methyl-4-chlorophenoxyacetic acid (MCPA) and zinc oxide intercalated with 2-methyl-4-chlorophenoxyacetic acid (ZMCPA) on the growth and pigment content of *Sphagneticola trilobata*. The 2nd objective of this research is to measure the physiological effect and effectiveness of herbicidal activity between conventional and nanoformulation against *Sphagneticola trilobata*.

Materials and methods

Plant growth

Sphageneticola trilobata plants were bought from nursery garden at Johor, Malaysia. Each pot was filled with 40 g of cocopeat soil and watered with 100 mL of water. The stem of *Sphagneticola trilobata* was cut below the nodes in 3 cm long at an angle of 45 ° by using scissors. The leaves from the cutting were removed but 2 leaves were remained on the upper stem. The leaves were removed to decrease the amount of water loss by transpiration and 2 leaves were remained for photosynthesis process. The cutting stem of *Sphagneticola trilobata* was planted in each pot. The end of each cutting was inserted into the medium soil and the soil were tamped around the stem. The nodes were buried in the soil. All plants were placed under sunlight and watered with 100 mL of water 2 times a day in the morning and evening. The experiment was set up in triplicate and the total *Sphagneticola trilobata* used in the study were 90 plants in which 18 plants used in the preliminary study and 72 plants used in the main study. The *Sphagneticola trilobata* plants were grown for 44 days.

Preparation of formulation

The chemicals used 99.5 % acetone solution and 97 % MCPA powder that was purchased from Sigma-Aldrich, 99 % ZnO powder BP Grade from R&M Chemical, 99.9 % dimethyl sulfoxide AP GRADE from Mega Prima Niaga and 0.1M ZMCPA provided by School of Chemistry and Environment, Faculty of Applied Sciences, Universiti Teknologi MARA (UiTM). Firstly, the MCPA stock suspension was prepared. 20 g of MCPA powder were diluted with 1 L of dimethyl sulfoxide. Then, for the preliminary study, the MCPA stock solution in the amount of the 0.1, 1, 10 µL, 0.1 and 1 mL was subsequently diluted with distilled water until reached 20 mL to obtain desired concentration of MCPA in 0.1, 1, 10, 100 mg/L and 1 g/L. Meanwhile, for the main study, the MCPA stock solution in the amount of 250 µL, 0.5 and 2.5 mL was diluted with distilled water until reached 100 mL to obtain desired concentration of MCPA in 50, 100 and 500 mg/L. Next, the ZMCPA formulation was prepared. The 0.1, 0.2 and 1 mg of ZMCPA powder was diluted with 50 mL of dimethyl sulfoxide and 50 mL of distilled water to obtain the desired concentration of ZMCPA in 50, 100 and 500 mg/L. Afterward, 0.1 M ZnO stock solution was prepared. The 8.14 g of ZnO powder was diluted with 1 L of dimethyl sulfoxide. Then, the ZnO stock solution in the amount of 50 mL was diluted with 50 mL of distilled water.

Preliminary study

In the preliminary study, the *Sphagneticola trilobata* were exposed to 5 treatments of MCPA in the range of 0.1 mg/L to 1 g/L. The *Sphagneticola trilobata* that were grown for 44 days were sprayed with the MCPA treatment in 5 concentrations of 0.1, 1, 10, 100 mg/L and 1 g/L. The blank control (water only with the equivalent volume as for other were set up in triplicate in which 15 plants were exposed to the treatment and 3 plants were set up as control (CK)). Each plant was sprayed with 3 mL of MCPA treatment for foliar uptake. All of the treatment were applied on the same day in the morning before 9 am. The frequency of spraying was only once. The performances of the plants were observed in terms of plant height, number of leaves, and chlorophyll content by chlorophyll meter (KONICA MINOLTA SPAD-502Plus) after 14 days.

Plant treatment

In the main study, the plants were submitted to the following treatments after grown for 44 days. The plants were sprayed with the formulation of polymeric ZnO nanocapsules without MCPA (NPC) as control, and blank control (CK). The plants were also exposed to the treatment of MCPA and ZMCPA in 50 mg/L represent lower concentration (MCPA-L and ZMCPA-L), 100 mg/L represent the medium concentration (MCPA-M and ZMCPA-M) and 500 mg/L represent high concentration (MCPA-H and ZMCPA-H). All of the treatments (CK, NPC, MCPA-L, MCPA-M, MCPA-H, ZMCPA-L, ZMCPA-M, ZMCPA-H) were set up in triplicate and were applied in the morning before 9 am. The treatments were set up in triplicate in which 54 plants were exposed to the treatment (MCPA-L, MCPA-M, MCPA-H, ZMCPA-L, ZMCPA-M,

ZMCPA-H) and 18 plants were set up as control (CK and NPC). Each plant was sprayed with 3 mL of MCPA, ZMCPA and ZnO treatment for foliar uptake. The frequency of spraying was only once. The plants were harvested after 7, 14 and 21 days of the 1st exposure to the formulation. The *Sphagneticola trilobata* were harvested and washed in water with care to wash off loose soil. Then, the plants were wiped out by using tissues to remove any moisture. Afterwards, the plants were inserted into the Ziploc bag and labelled (CK, NPC, MCPA-L, MCPA-M, MCPA-H, ZMCPA-L, ZMCPA-M, ZMCPA-H).

Measurement of growth parameters

Growth parameter observed in each plant in main study included plant mass, plant height, length of root and number of leaves. The fresh weight and dry weight of *Sphagneticola trilobata* were measured by using an electronic balance (ADAM PW214) (mg). The fresh weight of leaves, stem and roots were measured, and the plants were dried in a drying oven (LABTECH SFCN-302) at 50 °C overnight until it reached constant weight. Then, the dry weight of leaves, stem and roots were measured [17]. The plant height (cm) was measured from the soil's surface and base of the plant in the pots to the highest tip of leaves and apex of the plant. The root length was considered from root base to root tip (cm) [18]. All the number of healthy and damaged leaves were counted [19].

Measurement of pigment content

Leaf chlorophyll content was measured using a hand-held SPAD chlorophyll meter (KONICA MINOLTA SPAD-502Plus). The sample leaves were placed in between the sensors which are responsible for a measurement index of greenness. The amount of chlorophyll in a leaf was calculated by the index (SPAD value) [20]. Furthermore, the representative leaf of each plant was extracted to measure the pigment content. The *Sphagneticola trilobata* leaf of 0.2 g was crushed using a mortar and pestle. Then, 10 mL of 80 % acetone (Sigma-Aldrich) was added into the mortar and was homogenized. Afterwards, the mixture was transferred into the centrifuge tube and was centrifuged at 3,000 rpm for 10 min by using a benchtop centrifuge (CENTURION K240). Next, the supernatant was transferred to a 25 mL measuring cylinder and 80 % acetone was added to the supernatant until 25 mL. Then, the sample was transferred into cuvette (quartz). The optical density (OD) of chlorophyll α and b (chlα, chlb), and carotenoids (car) in the absorbance of 663, 645, and 480 nm, respectively was measured by UV/Vis Spectrophotometer (PG Instrument T80+). The chlorophyll and carotenoids content were calculated by using the formula [21].

$$\text{Chlorophyll } \alpha \text{ (mg/L)} = 12.7\times OD663 - 2.69\times OD645\times V/1{,}000(W)$$

$$\text{Chlorophyll b (mg/L)} = 22.9\times OD663 - 4.68\times OD645\times V/1{,}000(W)$$

$$\text{Total Chlorophyll (mg/L)} = 20.2\times OD645 + 8.02\times OD663\times V/1{,}000(W)$$

$$\text{Carotenoid (mg/L)} = OD480 + (0.114\times OD663) - (0.638\times OD645)\times V/1{,}000(W)$$

Data analysis

The collected data were entered into Microsoft Excel. The data were then transferred and analyzed using Statistical Package for the Social Sciences (SPSS) software. The collected data were analyzed using a paired sample t-test ($a < 0.05$) to analyze the significant difference between pretreatment and posttreatment for the parameters of plant height number of leaves and chlorophyll content (SPAD Value). The percentage differences before and after treatment for plant height, number of leaves, and chlorophyll content (SPAD Value) were calculated using the following formula:

$$\text{Differences} = \frac{\text{After treatment} - \text{Before treatment}}{\text{Before treatment}} \times 100$$

The data also was submitted to One-way Analysis of Variance (ANOVA) ($a < 0.05$) to investigate the significant differences between the treatments for parameter of fresh and dry weight of leaves, stem and roots, and length of root, and chlorophyll and carotenoids content. The post-hoc test used was Tukey's test to compare the significant differences between each treatment.

Results and discussion

Preliminary study

The preliminary study was conducted to determine the suitable and needed concentration for MCPA and ZMCPA to exert its effect on *Sphagneticola trilobata*. In this preliminary study, the plants were treated with 5 different concentrations of MCPA which were 0.1, 1, 10, 100 mg/L and 1 g/L of MCPA. The growth parameter and pigment content were analysed after 14 days.

Plant height

A 2-tailed, paired sample t-test with an alpha level of 0.05 was used to compare pre-treatment (M = 10.62, SD = 2.64) and post-treatment (M = 14.07, SD = 7.91) to the plant height. The difference was statistically significant, $t(18) = 2.256$, $p < 0.05$.

Table 1 Plant height (cm) ± SD before and after 14 days.

Treatments	Plant height (cm) before treatment	Plant height (cm) after treatment	Differences (%)
CK	11.6 ± 5.90	20.8 ± 15.13	+ 79 %
0.1 mg/L	10.9 ± 1.41	14.7 ± 2.61	+ 35 %
1 mg/L	10.9 ± 2.65	19.4 ± 2.74	+ 78 %
10 mg/L	9.8 ± 2.18	14.2 ± 2.74	+ 45 %
100 mg/L	10.5 ± 1.65	10.6 ± 4.45	+ 1 %
1 g/L	10.0 ± 2.16	4.7 ± 0.50	– 53 %

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases on percentage of plant height.

The plant height was statistically significant indicating there was a significant different in plant height between the pre-treatment and post-treatment. Based on **Table 1** the effects of MCPA to the plant height showed at concentration of 1 g/L in which the plant height decreases in 53 %. Meanwhile, the height increases despite exposed to 0.1, 1, 10 and 100 mg/L concentration of MCPA. However, the plant height increases only by 1 % for concentration of MCPA at 100 mg/L. The increment means the plants keep on growing.

Number of leaves

A 2-tailed, paired sample t-test with an alpha level of 0.05 was used to compare pre-treatment (M = 23.94, SD = 9.77) and post-treatment (M = 27.22, SD = 19.89) to the number of leaves. However, the difference before and after treatment of MCPA was not statistically significant, $t(18) = 0.957$, $p = 0.352$.

Table 2 Number of leaves ± SD before and after 14 days.

Treatments	Number of leaves before treatment	Number of leaves after treatment	Differences (%)
CK	25 ± 11.53	32 ± 11.14	+ 28 %
0.1 mg/L	21 ± 14.74	33 ± 22.48	+ 57 %
1 mg/L	31 ± 10.07	50 ± 14.57	+ 61 %
10 mg/L	25 ± 11.72	32 ± 14.00	+ 28 %
100 mg/L	24 ± 7.21	16 ± 12.50	– 33 %
1 g/L	17 ± 3.06	0 ± 0.00	– 100 %

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases on percentage of the number of leaves.

From **Table 2**, the effects of MCPA to the number of leaves showed at concentration of 100 mg/L and 1 g/L in which the number of leaves that exposed to the concentration of 100 mg/L and 1 g/L decreases after 14 days. Meanwhile, the number of leaves increases despite exposed to 0.1 mg/L, 1 mg/L and 10 mg/L concentration of MCPA. However, the number of leaves was not statistically significant indicating there were no significant decreases in number of leaves between the pre-treatment and post-treatment.

Chlorophyll content measured by chlorophyll meter

A 2-tailed, paired sample t-test with an alpha level of 0.05 was used to compare pre-treatment (M = 25.13, SD = 3.42) and post-treatment (M = 20.71, SD = 12.89) to the chlorophyll content. The differences were statistically significant, $t(18) = 2.256$, $p < 0.05$.

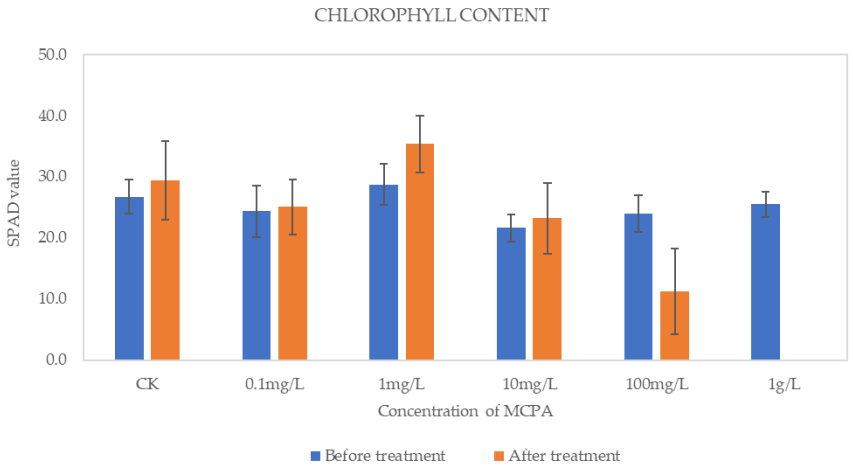

Figure 1 Chlorophyll content (SPAD value) ± SD before and after 14 days.

The differences in chlorophyll content were statistically significant. This showed that there were significant differences in chlorophyll content between the pre-treatment and post-treatment. **Figure 1** showed the chlorophyll content of the plants decreases when exposed to concentration of 100 mg/L and 1 g/L of MCPA. The concentration of MCPA that less than 100 mg/L does not affect the chlorophyll content of *Sphagneticola trilobata*.

Based on the observation of the preliminary study, it was found that the concentration that effectively showed the effect of the herbicide MCPA was 100 mg/L to 1 g/L. Therefore, further research was conducted to look for pronounced concentration of the herbicidal effect. 100 mg/L was established as the minimum concentration required for the herbicide MCPA to have an effect on *Sphagneticola trilobata*. At a concentration 100 mg/L and 1 g/L, symptoms such as leaf wilt, yellowing and stem bud formation were observed while at a concentration 1 g/L, the plant death was observed.

Main study

The concentration determined for MCPA and ZMCPA for low, medium, and high concentrations were 50, 100 and 500 mg/L. Based on the preliminary study the 100 mg/L concentration was set up as minimal concentration that the herbicidal activity is effective for MCPA. In the main study, the 50 mg/L was determined as low concentration (MCPA-L and ZMCPA-L), 100 mg/L as medium concentration (MCPA-M and ZMCPA-M) and 500 mg/L was determined as the high concentration (MCPA-H and ZMCPA-H).

Fresh weight of plants

The ANOVA for fresh weight of plants after 7 days was not statistically significant at $F(7,16) = 1.147$, $p = 0.384$. The fresh weight of plants after 14 days also was not statistically significant, $F(7,16) = 0.541$, $p = 0.792$ and after 21 days was not statistically significant at $F(7,16) = 2.645$, $p = 0.051$. This illustrated that there were no significant differences for the effect of different treatment to the fresh weight of plants. Nevertheless, based on **Figure 2** the plants treated with MCPA-H had lowest fresh weight of leaves compared to other treatment after 7 and 21 days.

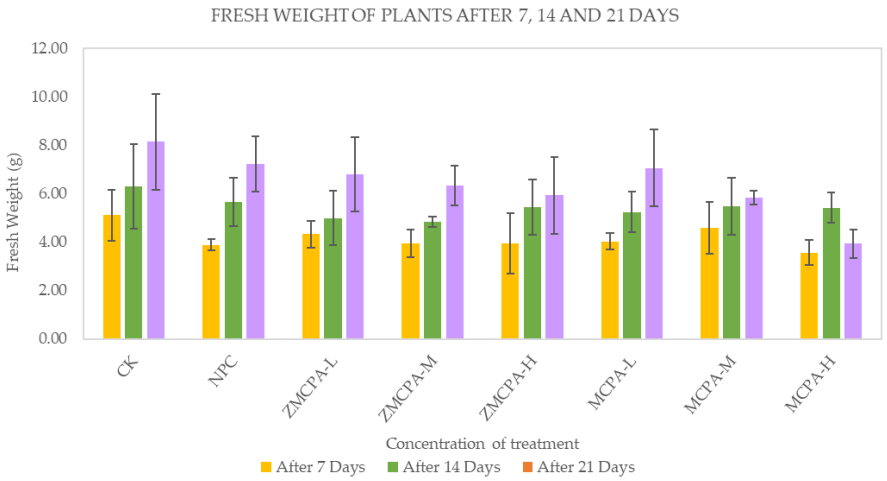

Figure 2 Fresh weight of plants (g) ± SD after 7, 14 and 21 days, respectively.

Dry weight of plants

The ANOVA for dry weight of plants after 7 days was not statistically significant at $F(7,16) = 0.517$, $p = 0.809$. The dry weight of plants after 14 days also was not statistically significant, $F(7,16)=1.215$, $p = 0.350$. However, the dry weight of plants after 21 days was statistically significant at $F(7,16) = 5.874$, $p < 0.05$. From the **Figure 3**, the MCPA-H had lowest dry weight of leaves compared to other treatment after 7 and 21 days.

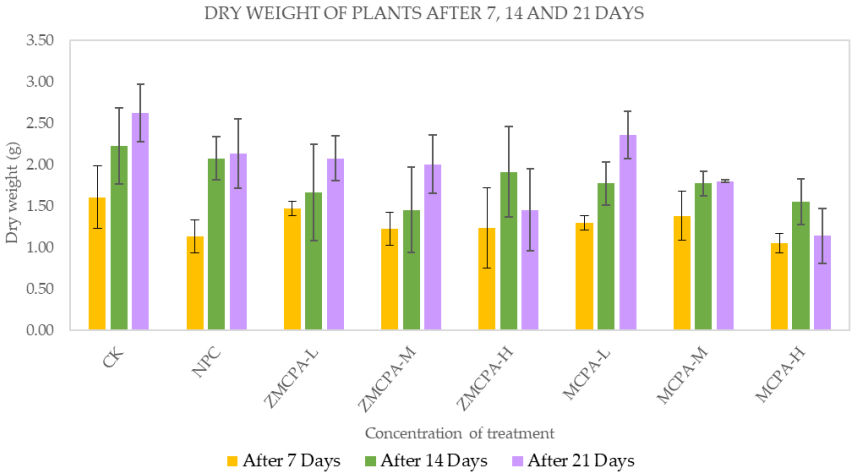

Figure 3 Dry weight of plants (g) ± SD after 7, 14 and 21 days, respectively.

The Post Hoc analyses with Tukey's HSD (using an α 0.05) stated that there were significance differences between treatment of ZMCPA-H and MCPA-H compared to CK after 21 days. The *Sphagneticola trilobata* treated with MCPA-H had lowest dry weight than all treatment for all 3 periods. There was no significant different between the treatment of ZMCPA-H compared to other treatment except CK. However, there was significant different between the treatment of MCPA-H compared to other treatment except ZMCPA-H. This showed that MCPA-H was more effective than ZMCPA-H. Nevertheless, the differences of effectiveness between MCPA-H and ZMCPA-H were not substantial.

Fresh weight of leaves

The ANOVA for fresh weight of leaves after 7 days was not statistically significant at $F(7,16) = 0.563$, $p = 0.775$. The fresh weight of leaves after 14 days also was not statistically significant, $F(7,16) = 0.541$, $p = 0.792$ and after 21 days was not statistically significant at $F(7,16) = 1.238$, $p = 0.339$. This indicated that there were no significant differences for the effect of different treatment to the fresh weight of leaves.

Nevertheless, MCPA-H had lowest fresh weight of leaves compared to other treatment for all 3 periods after 7, 14 and 21 days.

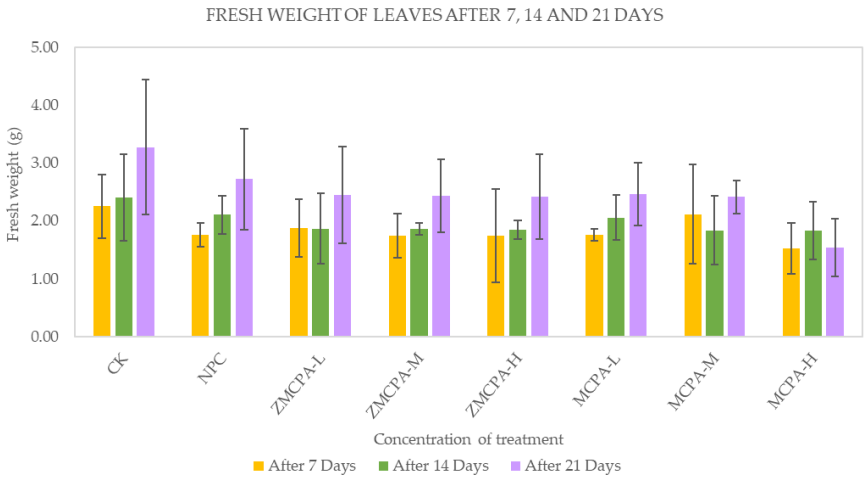

Figure 4 Fresh weight of leaves (g) ± SD after 7, 14 and 21 days, respectively.

Dry weight of leaves

The ANOVA for dry weight of leaves after 7 days was not statistically significant indicating there were no significant differences for the effect of different treatment to the dry weight of leaves, $F(7,16) = 0.368$, $p = 0.908$. The dry weight of leaves after 14 days also was not statistically significant, $F(7,16) = 0.641$, $p = 0.716$ and after 21 days was not statistically significant at $F(7,16) = 1.3439$, $p = 0.294$. This showed the treatment applied do not significantly affect dry weight of leaves of *Sphagneticola trilobata*.

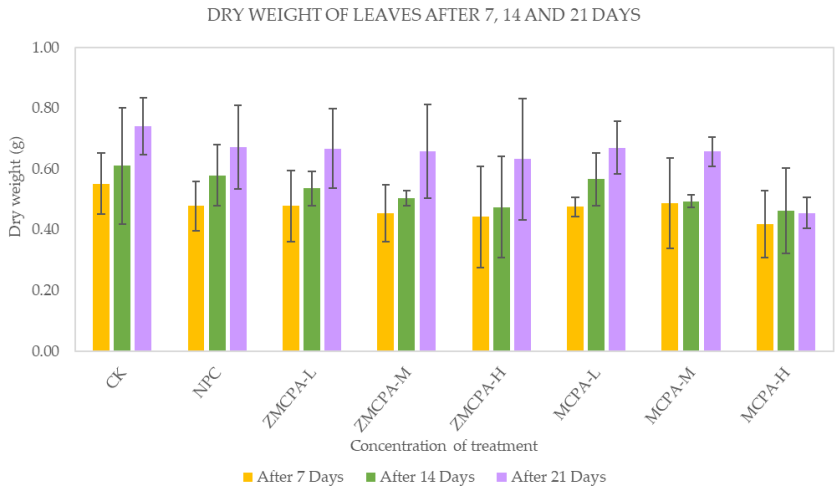

Figure 5 Dry weight of leaves (g) ± SD after 7, 14 and 21 days, respectively.

The MCPA-L and MCPA-M had lower reduction on dry weight than ZMCPA-L and the ZMCPA-M after 7 days. However, the dry weight of leaves treated with ZMCPA-L lower than MCPA-L after 14 days, with 0.54 g than 0.57 g. The dry weight of leaves treated with ZMCPA-L and MCPA-L had similar weight after 21 days with 0.67 g. Meanwhile, the treatment of MCPA-H had lower reduction in dry weight compared to other treatment for all periods after 7, 14 and 21 days.

Fresh weight of stem

The ANOVA for fresh weight of stem after 7 days was not statistically significant at $F(7,16) = 1.397$, $p = 0.273$. The fresh weight of stem after 14 days also was not statistically significant, $F(7,16) = 0.572$, $p = 0.769$. However, the fresh weight of stem after 21 days was statistically significant at $F(7,16) = 3.035$, $p < 0.05$.

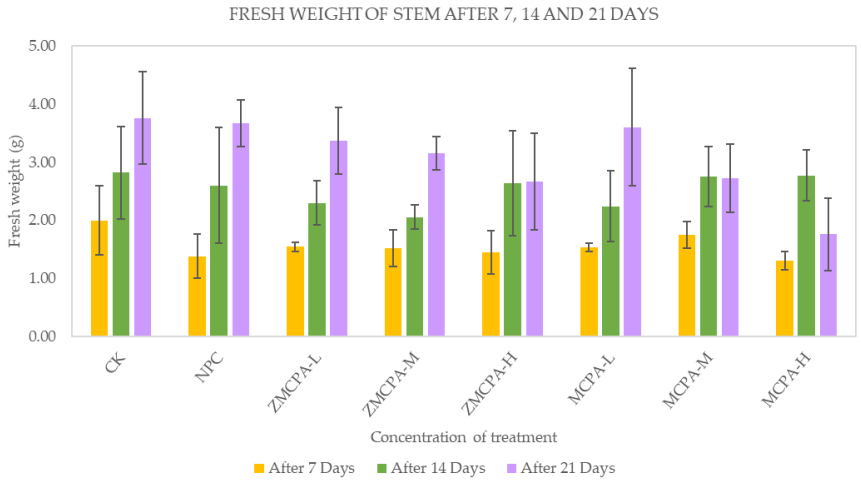

Figure 6 Fresh weight of stem (g) ± SD after 7, 14 and 21 days, respectively.

The Post Hoc analyses with Tukey's HSD (using an α 0.05) stated that there were significance differences between treatment of MCPA-H compared to CK on the fresh weight of stem after 21 days. However, there was no significant different between treatment of the ZMCPA-H and MCPA-H. The *Sphagneticola trilobata* treated with MCPA-H had lowest fresh weight of stem than all treatment after 7 and 21 days.

Dry weight of stem

The ANOVA for dry weight of stem after 7 days was not statistically significant at $F(7,16) = 0.959$, $p = 0.492$. The dry weight of stem after 14 days also was not statistically significant, $F(7,16) = 1.653$, $p = 0.191$. However, the dry weight of stem after 21 days was statistically significant at $F(7,16) = 5.235$, $p < 0.05$.

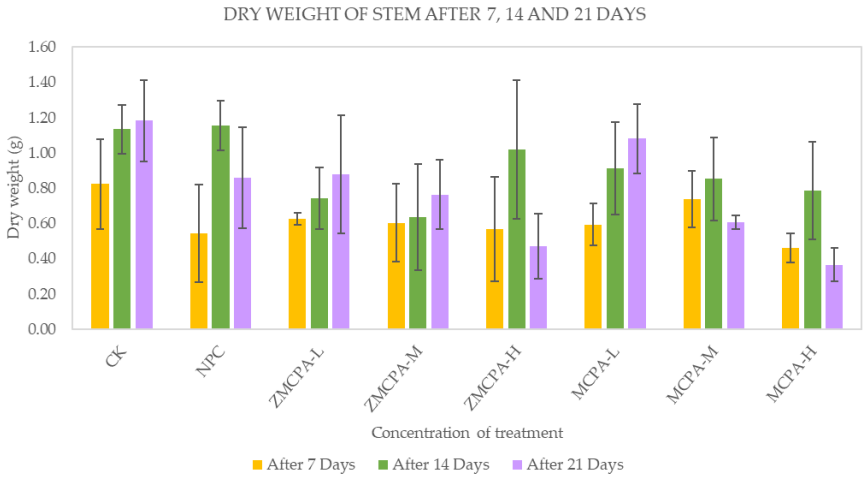

Figure 7 Dry weight of stem (g) ± SD after 7, 14 and 21 days, respectively.

The Post Hoc analyses with Tukey's HSD (using an α 0.05) stated that there were significance differences between treatment of ZMCPA-H and MCPA-H compared to CK on the dry weight of stem after 21 days. Nevertheless, the same pattern was analysed similar to the fresh weight of stem in which was no significant difference between treatment of the ZMCPA-H than the MCPA-H. Nonetheless, there was significant different between treatment of ZMCPA-H and MCPA-H compared to MCPA-L. The *Sphagneticola trilobata* treated with MCPA-H had lowest fresh weight of stem than all treatment after 7 and 21 days.

Fresh weight of root

The ANOVA for fresh weight of root was only statistically significant after 21 days at $F(7,16) = 4.497$, $p < 0.05$ but not statistically significant after 7 days at $F(7,16) = 0.501$, $p = 0.820$ and 14 days at $F(7,16) = 0.313$, $p = 0.937$.

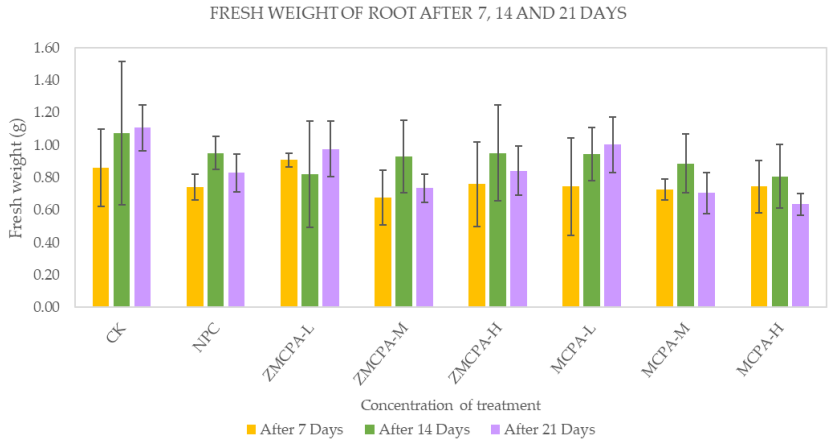

Figure 8 Fresh weight of root (g) ± SD after 7, 14 and 21 days, respectively.

The Post Hoc analyses with Tukey's HSD (using an α 0.05) stated that there were significance differences between treatment of ZMCPA-M, MCPA-H and MCPA-M compared to CK after 21 days. Nonetheless, there was no significant different between treatment of the ZMCPA-H and MCPA-H. The *Sphagneticola trilobata* treated with MCPA-H had lowest fresh weight of root than all treatment after 14 and 21 days.

Dry weight of root

The ANOVA for dry weight of root after 21 days was statistically significant at $F(7,16) = 4.874$, $p < 0.05$ but no statistically significant after 7 days, $F(7,16) = 2.338$, $p = 0.076$ and 14 days, $F(7,16) = 0.190$, $p = 0.983$.

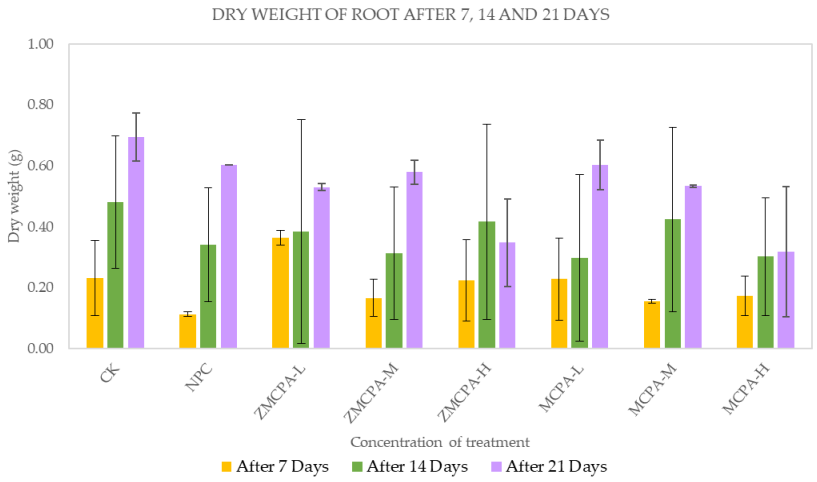

Figure 9 Dry weight of root (g) ± SD after 7, 14, 21 days, respectively.

The Post Hoc analyses with Tukey's HSD (using an α 0.05) stated that there were significance differences between treatment of the ZMCPA-H and MCPA-H compared to CK after 21 days. However, ZMCPA-H was no significantly different with MCPA-H. The *Sphagneticola trilobata* treated with MCPA-H had lowest fresh weight of root than all treatment after 21 days.

Plant height

A 2-tailed, paired sample t-test with an alpha level of 0.05 was used to compare pre-treatment (M = 14.73, SD = 0.84) and post-treatment after 7 days (M = 14.60, SD = 1.08) to the plant. The plant height decreases for treatment with MCPA-M and MCPA-H after 7 days compared to other treatment that showed increases in plant height or no increment on plant height. However, the difference was not statistically significant, $t(23) = 1.24$, $p = 0.229$.

Table 3 Plant height (cm) ± SD before and after 7 days.

Treatments	Plant height (cm) before treatment	Plant height (cm) after treatment	Differences (%)
CK	15.4 ± 1.25	15.9 ± 1.46	+ 3 %
NPC	14.3 ± 0.64	14.5 ± 0.36	+ 1 %
ZMCPA-L	14.9 ± 0.76	14.9 ± 0.76	No increment
ZMCPA-M	15.2 ± 0.40	15.2 ± 0.35	No increment
ZMCPA-H	14.1 ± 0.49	14.1 ± 0.49	No increment
MCPA-L	14.6 ± 1.62	14.7 ± 1.93	+ 0.2 %
MCPA-M	14.9 ± 0.42	14.1 ± 0.26	– 6 %
MCPA-H	14.3 ± 0.36	13.5 ± 0.91	– 6 %

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases and no increment as no changes occurred on percentage of plant height.

Meanwhile, the paired sample t-test show that the plant height before treatment (M = 21.18, SD = 1.80) and after treatment (M = 20.53, SD = 1.91) of 14 days was statistically significant, $t(23) = 5.64$, $p < 0.05$. The plant height decreases after exposed to treatment for ZMCPA-M, ZMCPA-H, MCPA-M and MCPA-H. However, the ZMCPA-L, MCPA-L and NPC showed no increment in plant height except CK.

Table 4 Plant height (cm) ± SD before and after 14 days.

Treatments	Plant height (cm) before treatment	Plant height (cm) after treatment	Differences (%)
CK	20.4 ± 0.76	21.0 ± 1.16	+ 3 %
NPC	20.0 ± 0.53	20.0 ± 0.53	No increment
ZMCPA-L	21.4 ± 2.02	21.4 ± 2.02	No increment
ZMCPA-M	21.9 ± 0.80	21.1 ± 1.54	– 3 %
ZMCPA-H	23.8 ± 0.90	22.4 ± 1.85	– 6 %
MCPA-L	19.8 ± 1.86	19.8 ± 1.86	No increment
MCPA-M	20.1 ± 1.81	18.5 ± 1.12	– 9 %
MCPA-H	22.1 ± 2.10	20.2 ± 3.27	– 9 %

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases and no increment as no changes occurred on percentage of plant height.

The paired sample t-test show that the plant height before treatment (M = 25.48, SD = 2.97) and after treatment (M = 24.88, SD = 3.05) of 21 days was not statistically significant, $t(23) = 0.827$, $p = 0.417$. The highest reduction of height occurred for plant that treated with conventional herbicide MCPA-H. However, the ZMCPA-L showed reduction 3 % than MCPA-L with no increment on plant height. The decreases of height were caused by stem epinasty in which plant bend downward because of wilting of leaf.

Table 5 Plant height (cm) ± SD before and after 21 days.

Treatments	Plant height (cm) before treatment	Plant height (cm) after treatment	Differences (%)
CK	22.7 ± 3.91	25.9 ± 4.01	+ 14 %
NPC	25.7 ± 2.25	25.7 ± 2.25	No increment
ZMCPA-L	24.8 ± 1.35	24.0 ± 1.92	– 3 %
ZMCPA-M	27.9 ± 1.15	27.0 ± 2.11	– 3 %
ZMCPA-H	25.9 ± 2.10	25.5 ± 2.47	– 2 %
MCPA-L	22.3 ± 2.48	22.3 ± 2.48	No increment
MCPA-M	25.5 ± 3.59	24.8 ± 4.59	– 3 %
MCPA-H	28.9 ± 1.13	24.8 ± 4.37	– 14 %

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases and no increment as no changes occurred on percentage of plant height.

Root length

The ANOVA for length of root after 7 days was not statistically significant $F(7,16) = 0.250$, $p = 0.965$. However, the length of root after 14 days was statistically significant, $F(7,16) = 4.441$, $p < 0.05$. Meanwhile, after 21 days the length of root was not statistically significant at $F(7,16) = 1.834$, $p = 0.149$.

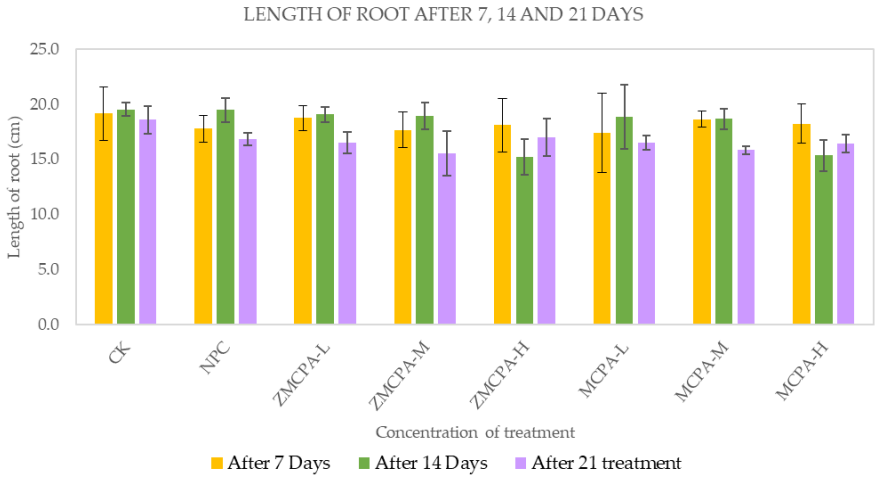

Figure 10 Length of root (cm) ± SD after 7, 14 and 21 days, respectively.

The Post Hoc analyses with Tukey's HSD (using an α 0.05) stated that there were significance differences between treatment ZMCPA-H and MCPA-H compared to CK on the length of root after 14 days. However, there was no significant different between treatment of the ZMCPA-H than MCPA-H.

Number of leaves

A 2-tailed, paired sample t-test with an alpha level of 0.05 was used to compare pre-treatment (M = 14.73, SD = 0.84) and post-treatment (M = 14.60, SD = 1.08) after 7 days to the number of leaves. The t-test showed that the difference was statistically significant, $t(23) = 6.93$, $p < 0.01$ after 7 days of the treatment. All MCPA and ZMCPA treatment showed reduction in number of leaves. However, the MCPA-H had highest reduction to the number of leaves with 20 % reduction.

Table 6 Number of leaves ± SD before and after 7 days.

Treatments	Number of leaves before treatment	Number of leaves after treatment	Differences (%)
CK	19 ± 4.62	19 ± 4.62	No increment
NPC	16 ± 5.29	14 ± 4.04	+ 13 %
ZMCPA-L	17 ± 4.16	14 ± 4.04	– 18 %
ZMCPA-M	17 ± 3.61	15 ± 4.16	– 12 %
ZMCPA-H	18 ± 7.21	16 ± 6.66	– 11 %
MCPA-L	15 ± 4.16	13 ± 3.06	– 13 %
MCPA-M	23 ± 12.86	20 ± 11.15	– 13 %
MCPA-H	15 ± 5.03	12 ± 4.51	– 20 %

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases and no increment as no changes occurred on percentage of number of leaves.

In addition, the paired sample t-test showed that the number of leaves before treatment (M = 23.83, SD = 7.62) and after treatment (M = 17.21, SD = 6.20) after 14 days also was statistically significant, t(23) = 2.563 $p < 0.05$. The MCPA-H also showed highest reduction number of leaves after 14 days. However, the ZMCPA-L showed more reduction of number of leaves than MCPA-L, with 26 % than 11 %.

Table 7 Number of leaves ± SD before and after 14 days.

Treatments	Number of leaves before treatment	Number of leaves after treatment	Differences (%)
CK	23 ± 10.26	23 ± 10.26	No increment
NPC	31 ± 8.33	23 ± 8.33	– 26 %
ZMCPA-L	19 ± 7.57	15 ± 5.03	– 26 %
ZMCPA-M	19 ± 1.15	13 ± 3.06	– 32 %
ZMCPA-H	25 ± 1.15	16 ± 1.53	– 40 %
MCPA-L	19 ± 4.16	17 ± 3.51	– 11 %
MCPA-M	25 ± 4.16	16 ± 5.29	– 36 %
MCPA-H	33 ± 10.26	15 ± 7.27	– 52 %

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases and no increment as no changes occurred on percentage of number of leaves.

In addition, the paired sample t-test showed that the number of leaves before treatment (M = 32.88, SD = 11.14) and after treatment (M = 22.75, SD = 7.89) after 21 days also was statistically significant, t (23) = 5.243, $p < 0.01$. The similar pattern showed with after 14 days in which the MCPA-H showed highest reduction number of leaves and the ZMCPA-L showed more reduction of number of leaves than MCPA-L, with 27 % than 23 %. The **Table 8** also demonstrate that the number of leaves decreases for all treated plant and also control zinc oxide. The highest decreases occurred on conventional herbicide of MCPA for all 3 periods. However, after 14 and 21 days the nanoformulation ZMCPA-L has the highest reduction in number of leaves than MCPA-L.

Table 8 Number of leaves ± SD before and after 21 days.

Treatments	Number of leaves before treatment	Number of leaves after treatment	Differences (%)
CK	23 ± 4.16	26 ± 7.21	+ 13 %
NPC	31 ± 9.87	25 ± 11.02	– 16 %
ZMCPA-L	30 ± 14.00	22 ± 11.85	– 27 %
ZMCPA-M	26 ± 9.17	21 ± 9.02	– 19 %
ZMCPA-H	39 ± 18.15	22 ± 11.50	– 44 %
MCPA-L	30 ± 9.17	23 ± 6.43	– 23 %
MCPA-M	42 ± 4.00	27 ± 4.16	– 38 %
MCPA-H	42 ± 7.37	17 ± 4.16	– 62 %

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases on percentage of number of leaves.

Chlorophyll content measured chlorophyll meter

A 2-tailed, paired sample t-test with an alpha level of 0.05 was used to compare pre-treatment and post treatment. The paired t-test showed that the pre-treatment (M = 20.88, SD = 3.71) and post-treatment (M = 19.33, SD = 5.30) after 7 days to the chlorophyll content. The t-test was statistically significant, t(23) = 1.78, $p < 0.05$ after 7 days of the treatment. The MCPA-H had lowest chlorophyll content than other treatments.

Table 9 Chlorophyll content (SPAD value) ± SD before and after 7 days.

Treatments	Chlorophyll content (SPAD value) before treatment	Chlorophyll content (SPAD value) after treatment	Differences (%)
CK	24.8 ± 0.40	26.1 ± 2.91	+ 5 %
NPC	23.0 ± 2.35	22.5 ± 2.16	– 2 %
ZMCPA-L	22.1 ± 3.41	21.2 ± 3.83	– 4 %
ZMCPA-M	17.9 ± 1.39	16.5 ± 1.31	– 7 %
ZMCPA-H	21.8 ± 2.87	20.2 ± 3.42	– 7 %
MCPA-L	20.2 ± 2.57	19.9 ± 2.46	– 2 %
MCPA-M	15.3 ± 4.82	14.6 ± 4.96	– 5 %
MCPA-H	21.9 ± 2.40	13.6 ± 8.15	– 38 %

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases on percentage of chlorophyll content.

In addition, the paired sample t-test showed that the chlorophyll content before treatment (M = 23.59, SD = 1.91) and after treatment (M = 18.80, SD = 4.15) after 14 days also was statistically significant, t(23) = 5.91, $p < 0.01$. As the concentration of MCPA and ZMCPA increases, the chlorophyll content decreases. However, the MCPA-H had lowest chlorophyll content than other treatments.

Table 10 Chlorophyll content (SPAD value) ± SD before and after 14 days.

Treatments	Chlorophyll content (SPAD value) before treatment	Chlorophyll content (SPAD value) after treatment	Differences (%)
CK	26.0 ± 0.96	26.2 ± 0.38	+ 0.8 %
NPC	25.2 ± 2.78	22.2 ± 4.76	– 12 %
ZMCPA-L	22.6 ± 2.36	18.1 ± 1.65	– 20 %
ZMCPA-M	24.5 ± 6.21	17.6 ± 1.08	– 29 %
ZMCPA-H	22.5 ± 1.90	15.1 ± 1.64	– 33 %
MCPA-L	21.5 ± 1.57	19.6 ± 2.55	– 9 %
MCPA-M	22.4 ± 1.67	16.9 ± 2.10	– 24 %
MCPA-H	23.9 ± 2.48	14.6 ± 1.18	– 39 %

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases on percentage of chlorophyll content.

The results also showed that after 21 days the difference was statistically significant for chlorophyll content before treatment (M = 29.23, SD = 2.87) and after treatment (M = 23.95, SD = 4.54) with the significant $t(23) = 4.302$, $p < 0.01$. The MCPA-H still had lowest chlorophyll content than other treatments. Nevertheless, as the chlorophyll content decreases as the concentration of MCPA and ZMCPA increases.

Table 11 Chlorophyll content (SPAD value) ± SD before and after 21 days.

Treatments	Chlorophyll content (SPAD value) before treatment	Chlorophyll content (SPAD value) after treatment	Differences (%)
CK	29.4 ± 0.75	31.3 ± 2.26	+ 7%
NPC	27.0 ± 1.36	26.9 ± 0.64	– 0.4%
ZMCPA-L	26.7 ± 3.56	23.9 ± 3.36	– 11%
ZMCPA-M	30.3 ± 2.06	22.7 ± 2.63	– 25%
ZMCPA-H	30.0 ± 1.61	21.5 ± 2.71	– 28%
MCPA-L	26.3 ± 2.29	25.3 ± 1.40	– 4%
MCPA-M	31.7 ± 2.80	22.0 ± 3.11	– 31%
MCPA-H	32.5 ± 0.95	18.1 ± 5.30	– 44%

Values are expressed as mean ± standard deviation (n = 3). The differences between before and after treatment of + expressed as increases and - expressed as decreases on percentage of chlorophyll content.

This showed that for all 3 periods there were significant differences in the reduction of chlorophyll content before and after treatment. The number of leaves decreases for all treated plant after 7, 14 and 21 days. The chlorophyll content also decreases when exposed to NPC after 7 and 21 days. The highest decreases occurred on conventional herbicide of MCPA-H for all 3 periods. However, the ZMCPA-M showed higher reduction of chlorophyll content than MCPA-M after 7 and 14 days. Meanwhile, after 14 and 21 days the chlorophyll content showed decreases on treatment with ZMCPA-L than MCPA-L.

Chlorophyll content by spectrophotometer

The ANOVA was statistically significant indicating there was significant differences for the effect of different treatment to the chlorophyll α after 7 days $F(7,16) = 8.61$, $p < 0.01$. The total of chlorophyll α after 14 days was statistically significant, $F(7,16) = 3.51$, $p < 0.05$. after 21 days also was statistically

significant at $F(7,16) = 29.47$, $p < 0.01$. This showed that the treatment applied affect the total of chlorophyll α. The MCPA-H had lowest chlorophyll α content than all treatment for all periods.

Table 12 Chlorophyll α (mg/L) ± SD after 7, 14 and 21 days.

Treatments	Chlorophyll α (chlα) (mg/L) after 7 days	Chlorophyll α (chlα) (mg/L) after 14 days	Chlorophyll α (chlα) (mg/L) after 21 days
CK	0.14 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
NPC	0.13 ± 0.01	0.12 ± 0.02	0.12 ± 0.02
ZMCPA-L	0.13 ± 0.00	0.11 ± 0.03	0.11 ± 0.03
ZMCPA-M	0.10 ± 0.01	0.11 ± 0.01	0.09 ± 0.01
ZMCPA-H	0.11 ± 0.00	0.08 ± 0.03	0.04 ± 0.03
MCPA-L	0.11 ± 0.01	0.12 ± 0.01	0.11 ± 0.01
MCPA-M	0.10 ± 0.01	0.10 ± 0.02	0.09 ± 0.02
MCPA-H	0.08 ± 0.03	0.07 ± 0.02	0.03 ± 0.02

Values are expressed as mean ± standard deviation (n = 3).

The similar pattern also showed for chlorophyll b. The total of chlorophyll b was statistically significant after 7 days at $F(7,16) = 2.86$, $p < 0.05$. after 14 days at $F(7,16) = 11.00$, $p < 0.01$. after 21 days also was statistically significant at $F(7,16) = 54.00$, $p < 0.01$. This showed the treatment applied had effect to the to the chlorophyll b content.

Table 13 Chlorophyll b (mg/L) ± SD after 7, 14 and 21 days.

Treatments	Chlorophyll b (chlb) (mg/L) after 7 days	Chlorophyll b (chlb) (mg/L) after 14 days	Chlorophyll b (chlb) (mg/L) after 21 days
CK	0.11 ± 0.02	0.13 ± 0.02	0.13 ± 0.02
NPC	0.11 ± 0.02	0.12 ± 0.01	0.11 ± 0.01
ZMCPA-L	0.09 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
ZMCPA-M	0.08 ± 0.02	0.07 ± 0.00	0.07 ± 0.00
ZMCPA-H	0.07 ± 0.02	0.08 ± 0.01	0.06 ± 0.01
MCPA-L	0.10 ± 0.01	0.09 ± 0.02	0.08 ± 0.02
MCPA-M	0.07 ± 0.02	0.07 ± 0.01	0.06 ± 0.01
MCPA-H	0.06 ± 0.02	0.07 ± 0.01	0.06 ± 0.01

Values are expressed as mean ± standard deviation (n = 3).

The total chlorophyll content was statistically significant after 7 days at $F(7,16) = 5.95$, $p < 0.05$ and after 14 days at $F(7,16) = 18.46$, $p < 0.01$. The chlorophyll content after 21 days also was statistically significant at $F(7,16) = 45.35$, $p < 0.01$. This showed the treatment applied had effect to the to the chlorophyll content.

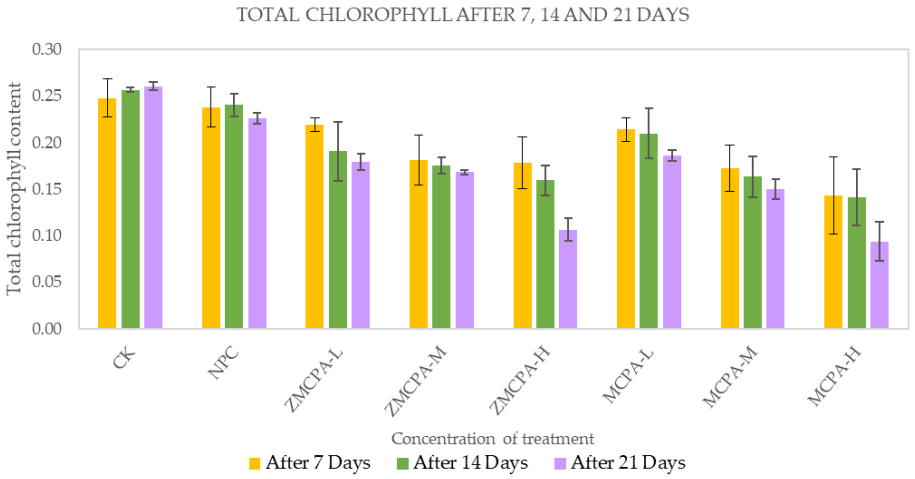

Figure 11 Total chlorophyll (mg/L) ± SD after 7, 14 and 21 days, respectively.

The Post Hoc analyses with Tukey's HSD (using an α 0.05) stated that there were significance differences between the MCPA-M and MCPA-H compared to the CK after 7 days at $p < 0.05$. The pattern from highest to lowest followed by CK, NPC, ZMCPA-L, MCPA-L, ZMCPA-M, ZMCPA-H, MCPA-M, and MCPA-H. Meanwhile, after 14 days all treatment with ZMCPA and MCPA have significance differences $p < 0.001$ compared to the CK. However, after 14 days the pattern changes in which the plants treated ZMCPA-L had lower chlorophyll content than MCPA-L. The pattern from highest to lowest followed by CK, NPC, MCPA-L, ZMCPA-L, ZMCPA-M, ZMCPA-H, MCPA-M and MCPA-H. The pattern shown after 14 days also were similar as after 21 days in which ZMCPA-L exert more effect to the chlorophyll content of *Sphagneticola trilobata* than MCPA-L. However, the chlorophyll content of plants treated with ZMCPA-H lower than MCPA-M after 21 days. There were significant different between all treatment of ZMCPA, MCPA and NPC compared to CK. The *Sphagneticola trilobata* that exposed to the treatment with MCPA-H has the lowest chlorophyll content compared to all treatment for all 3 periods. Nevertheless, there were no significant different between ZMCPA-H and MCPA-H after 21 days at $p = 0.930$.

Carotenoids content

The ANOVA for carotenoids content after 7 days was statistically significant indicating there were significant differences for the effect of different treatment to the carotenoids content, $F(7,16) = 10.55$, $p < 0.01$. The carotenoids content after 14 days was statistically significant, $F(7,16) = 18.46$, $p < 0.01$ and after 21 days also was statistically significant at $F(7,16) = 22.89$, $p < 0.01$. This proved that there the treatment applied also able to affect the carotenoids content.

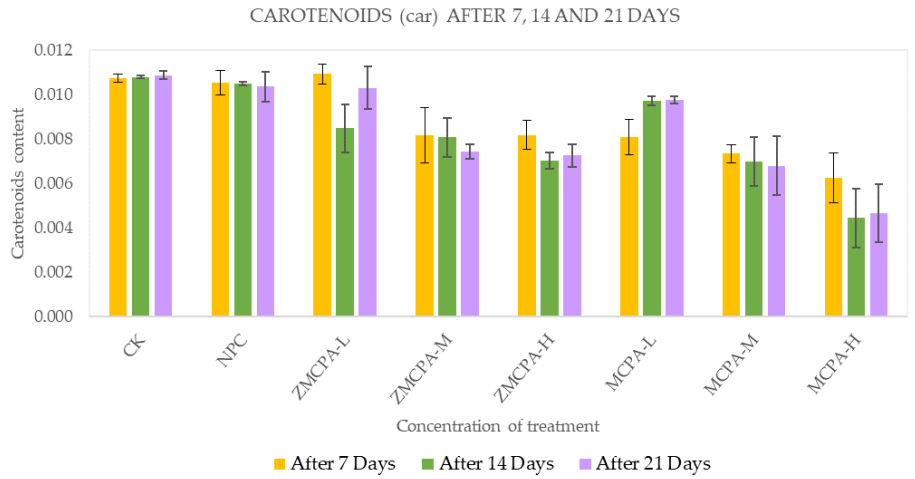

Figure 12 Carotenoids content (mg/L) ± SD after 7, 14 and 21 days, respectively.

The Post Hoc analyses with Tukey's HSD (using an α 0.05) stated that there were significance differences between all treatment of ZMCPA and MCPA, except treatment with ZMCPA-L in comparison with CK after 7 days. Meanwhile, there were significance differences between all treatment of ZMCPA and MCPA, except for the ZMCPA-L and MCPA-L in comparison to CK after 14 days. The same pattern also showed for carotenoids content after 21 days in which there were no significant differences of ZMCPA-L and MCPA-L compared to CK. As the concentration increases, the carotenoids content decreases for treatment with ZMCPA and MCPA. Nevertheless, the MCPA-H had lowest carotenoids content than all treatment for all 3 periods.

The herbicidal activity of MCPA and ZMCPA was effective against *Sphagneticola trilobata*. The symptoms of MCPA and ZMCPA toxicity observed in this study were the same at all concentration such as wilting of leaves, yellowing and epinasty of stems. Based on Zimdahl (2018) the application of the synthetic auxin-herbicide can cause leaf wilting and chlorosis. The results also showed that there was an effect of MCPA and ZMCPA on growth and pigment content in response to treatment with MCPA and ZMCPA and NPC ZnO [22]. The ZnO also has a toxic effect on the plants equivalent to that of herbicide. The results of Rajput *et al.* [23] showed that ZnO can damage the structural organization and reduce the photosynthetic activities of plants. There was a lower number of chloroplasts per cell observed in the *Hordeum sativum* leaf cells treated with ZnO NPs as compared to the non-treated plants [23]. The ZMCPA and MCPA can affect the photosynthetic activity of the plants by significantly reducing the pigment content. The research by Ghani *et al.* (2018) also showed that the ZnO treatments significantly inhibited the root and shoot growth and decreased the content of chlorophylls α and b of *Solanum lycopersicum* [24].

This study indicated no significant differences between MCPA and ZMCPA. The results showed that the conventional and nanoformulations herbicides inhibit the growth of *Sphagneticola trilobata* to the same extent after 7, 14 and 21 days. The efficacy was about the same. However, MCPA showed higher efficacy than ZMCPA in affecting plant growth and pigment content. Reduction in leaf number and chlorophyll and carotenoid content were enhanced with increasing concentration of ZMCPA and MCPA and increasing in duration. Nevertheless, the efficacy of ZMCPA-L was higher than that of MCPA-L after 14 and 21 days, which may be due to the controlled release the herbicide in nanoformulation that allows slow and prolonged release of the active ingredient [2]. However, MCPA-M and MCPA-H were more effective than ZMCPA-M and ZMCPA-H at 7, 14 and 21 days, respectively.

We expected the higher efficacy of ZMCPA than MCPA in inhibiting the growth and reducing pigment content of *Sphagneticola trilobata*. Nanoformulated herbicide should improve the solubility and adhesion of active ingredients to the biological membranes of plants. This increasing penetration, coverage, and uptake of active ingredient by the targeted plants that eventually enhance the controlled release of herbicide [1].

The findings of this study contraindicated with other studies. Other research illustarted that the nanoformulation has higher efficacy than conventional herbicide. For instance, research by Sousa *et al.* (2020) showed that nanoatrazine was more effective than conventional atrazine in reducing weight of *Digitalis insularis* at both dosages of the 1,000 and 2,000 gha^{-1} [25]. Furthermore, the research by Takeshita *et al.* [13], showed that the nanometribuzin able to effectively inhibiting pigment content of *Ipomea grandifolia* at lower dose 48 gha^{-1} than the conventional metribuzin 480 gha^{-1} [13]. The research by Wu *et al.* [26], showed that nanoatrazine was more effective to induce toxic effect to photosynthetic activity of *Lactuca sativa* than conventional atrazine at high concentration of 2.7 ± 0.5 mg/kg soil of nanoformulation and 3.2 ± 0.3 mg/kg soil of conventional formulation after 6 weeks [26]. In addition, the study by Sousa *et al.* [27] indicated that nanoatrazine causes greater reduction in *Bidens pilosa* than conventional atrazine at the same concentration of 2,000 gha^{-1} [27].

Nevertheless, the positive results of ZMCPA toxicity to the treated plants indicated that the polymeric capsules ZnO does not altering the efficacy of MCPA as herbicide. The successful uptake of MCPA and ZMCPA through foliar absorption showed that the herbicide can be absorbed through the leaves and stomata. The epinasty of stems may be due to the mobility of herbicide that was translocated to the meristems of the plant [28]. Therefore, this causes the unsustainable growth of *Sphagneticola trilobata*. This study proves that ZMCPA can be used as alternative method for inhibiting weed growth.

Further studies on MCPA and ZMCPA are needed to compare the properties of nanoformulations and conventional formulations of herbicides. This is necessary to improve the design of nanoparticles to increase herbicide efficacy, since the purpose of a nanoformulation is to effectively control the targeted weed species by using the lower amounts of active ingredient required. The study on the effects of MCPA and ZMCPA on protein content or nutrients of the *Sphagneticola trilobata* can be conducted to investigate other physiological effects of the herbicides. Further studies are also needed to determine the optimal dose of MCPA and ZMCPA herbicides required for high herbicide efficacy. The effect of MCPA and ZMCPA

on other plants such as non-target plants and crops, can also be studied. Herbicide efficacy should also be linked to the study of the environmental impact of the nanoherbicide to prove that nanoherbicides are able to reduce environmental risk compared to the conventional herbicides.

Conclusions

In summary, the objectives of this study were achieved. The expectation of ZMCPA should be more effective than MCPA was contraindicated with the results of this research. Nevertheless, this research has successfully determined the effects of MCPA and ZMCPA on the growth and pigment content of *Sphagneticola trilobata*. In addition, the physiological effect and effectiveness of herbicidal activity between the conventional and nanoformulation against *Sphagneticola trilobata* also were successfully measured. Our research provides important information for the development of new nanoherbicides. The uses of herbicide cannot be avoided because they are backbone of agriculture and required for healthy crop yield. This nanoformulation can be used for commercial purpose and serve as alternative tool in weed control management. The nanoformulation must compete with existing conventional herbicide in terms of their performance and economic feasibility [28].

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